
Porous silicon flow-through membrane: application to the optical detection of bacteria from their lysate

Roselien Vercauteren

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Dissertation committee

Prof. Laurent A. Francis (UCLouvain, advisor)
Prof. Jacques Mahillon (UCLouvain, advisor)
Prof. Sophie Demoustier (UCLouvain)
Prof. Denis Flandre (UCLouvain)
Prof. Michael J. Sailor (UCSD, USA)
Dr. Thierry Leïchl  (Georgia Tech, USA and CNRS, France)
Prof. Christophe De Vleeschouwer (UCLouvain, chair)

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ABSTRACT

It has been predicted that by 2050, bacteria will make more victims than cancer. One way to combat pathogenic bacteria and their growing danger is to develop tools that enable an early diagnosis and allow for an effective treatment to eliminate the microbes. Fast bacterial detection methods not only have an impact on the public and preventive health, but also on the food industry and the environment.

In this work, we developed a biosensor for the indirect detection of bacteria via their lysate. The developed sensor is based on porous silicon membranes, which are known for their many attractive optical and physical properties. Porous silicon has indeed been widely studied for sensing applications, mainly because of its large surface area, rendering it sensitive to minute changes in its environment. Unlike traditional porous silicon biosensors, the selectivity of the bioassay presented in this work does not rely on bio-probes attached to the sensor surface; the selectivity is added in the analyte itself, by the addition of lytic enzymes that target only the desired bacteria. The resulting bacterial lysate is then able to penetrate into the porous silicon sensor and affects its optical properties, while intact bacteria accumulate on top of the sensor.

The first part of this work concentrates on the design of the sensor, studying both the analyte and the sensor, in order to optimise the detection protocol. In the second part, a first prototype is fabricated and its performance is compared to traditional porous silicon layers. The third part of this thesis then focuses on the optimisation of this first

prototype, via the addition of atomic layer deposited metal oxide layers inside the porous membranes. These metal oxides improve the sensor's stability in aqueous media and enhance its optical properties. The full performance of the sensor, in terms of sensitivity, selectivity and versatility is then demonstrated using both *Bacillus cereus*, targeted by the phage-coded PlyB221 endolysin, and *Staphylococcus epidermidis*, lysed by the commercial lysostaphin.

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LIST OF PUBLICATIONS AND CONFERENCES

Journal Publications

- [RV1] Clara Whyte Ferreira, Roselien Vercauteren, and Laurent A. Francis. Passivated porous silicon membranes and their application to optical biosensing. *Micromachines*, 13(1), 2022.
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- [RVp3] Clara Whyte Ferreira, Roselien Vercauteren and Laurent A. Francis. Fabrication of mesoporous silicon membranes passivated with atomic layer deposited metal oxides. In *Micro and Nano Engineering Conference*, Turin, Italy, 2021.

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- [RVt1] Roselien Vercauteren, Clara Whyte Ferreira, Audrey Leprince, Jacques Mahillon, Laurent A. Francis. Silicon nanotechnology for life science applications: an example of a silicon-based biosensor for the detection of bacteria. In *SINANO Equality, Inclusion and Diversity Plan Event*, Udine, Italy, 2022.
- [RVt2] Laurent A. Francis, Roselien Vercauteren, Audrey Leprince, and Jacques Mahillon. Stable porous silicon membranes for fast bacterial detection. In *1st International Conference on Micromachines and Applications (ICMA 2021)*, Online conference, 2021.
- [RVt3] Roselien Vercauteren, Audrey Leprince, Jacques Mahillon, and Laurent A. Francis. Optical Detection of Bacterial Lysate on a Porous Silicon Membrane. In *19th National Day on Biomedical Engineering*, online conference, 2020.
- [RVt4] Roselien Vercauteren, Audrey Leprince, Jacques Mahillon, and Laurent A. Francis. Porous silicon biosensor for the detection of bacteria through their lysate. In *The 1st International Electronic Conference on Biosensors*, online conference, 2020. MDPI.
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LIST OF ACRONYMS

The next list describes several acronyms that will be later used within this thesis manuscript.

Al ₂ O ₃	Aluminium oxide
ALD	Atomic Layer Deposition
AMR	Anti-microbial Resistance
AOB	Ammonia Oxidising Bacteria
APTES	(3-aminopropyl)triethoxysilane
APTES	(3-aminopropyl)dimethylethoxysilane
BSA	Bovine Serum Albumin
CBD	Cell Binding Domain
CCD	Charge-Coupled Device
CFU	Colony Forming Unit
CMOS	Complementary Metal-Oxide-Semiconductor
DI water	De-Ionized water
DNA	DesoxyriboNucleic Acid
DRIE	Deep Reactive Ion Etching
EDX	Energy-Dispersive X-ray spectroscopy
EIS	Electrochemical Impedance Spectroscopy
ELISA	Enzyme-Linked ImmunoSorbent Assay
EOT	Effective Optical Thickness

FET	Field-Effect Transistor
FFT	Fast Fourier Transform
FoM	Figure of Merit
HF	Hydrofluoric acid
HfO ₂	Hafnium oxide
HRP	Horseradish Peroxidase
IAW	Interferogram Average over Wavelength
ICP	Inductively Coupled Plasma
KOH	Potassium hydroxide
LB	Lysogeny Broth
LFA	Lateral Flow Assay
LoD	Limit of Detection
MACE	Metal-Assisted Chemical Etching
MALDI	Matrix-Assisted Laser Desorption Ionization
MEMS	Microelectromechanical systems
MS	Mass Spectrometry
NP	Nanoparticle
PBS	Phosphate Buffered Saline
PC	Photonic Crystal
PCR	Polymerase Chain Reaction
PECVD	Plasma-Enhanced Chemical Vapor Deposition
PL	Photoluminescence
PSi	Porous Silicon
PSiO ₂	Oxidized Porous Silicon
QCM	Quartz Crystal Microbalance
RBP	Receptor Binding Protein
RF	Radio Frequency
RI	Refractive Index
RIE	Reactive Ion Etching
RIFTS	Reflective Interferometric Fourier Transform Spectroscopy
RIU	Refractive Index Unit
RNA	RiboNucleic Acid
RPM	Rotation Per Minute
SB	Saline Buffer

SEM	Scanning Electron Microscopy
SERS	Surface Enhancement Raman Spectroscopy
SIMS	Secondary Ion Mass Spectrometry
SLIM	Spectroscopic Liquid Infiltration Method
SNR	Signal to Noise Ratio
SPR	Surface Plasmon Resonance
TDMAHf	Tetrakis(dimethylamido)hafnium
TMA	Trimethylaluminum
TMM	Transfer Matrix Method
TOF	Time-Of-Flight
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
XPS	X-ray Photoelectron Spectroscopy
XRD	X-Ray Diffraction

CONTEXT & THESIS OBJECTIVES

Context

The second decade of the 21st century has proven one thing: diseases can spread fast and wide in our globalised world. Moreover, they can have disastrous consequences not only for global health, but also for our society, for the economy and for the environment. While viruses have mainly taken all the spotlight in recent years due to the COVID-19 pandemic, pathogenic bacteria are also responsible for several millions of deaths each year. The treatment and prevention of infectious diseases are thus of the utmost importance. The discovery of antibiotics in the late 1920s was a big step forward towards curing infectious diseases, but the emergence of antimicrobial resistance (AMR) some decades later put a damper on this advancement of medicine [1]. AMR is a natural process present in bacteria, but it has grown at an alarming rate over the years, helped by the widespread use of broad-spectrum antibiotics, and has now become a pressing issue. According to the World Health Organisation, AMR is part of the top ten threats to global health, and it has been estimated that by 2050, more people will die from AMR-related complications, than from cancer [2, 3].

One way to decelerate the AMR growth is to bring forward new solutions for the detection of pathogens. Indeed, the lack of fast diagnosis solutions is one of the main causes of the AMR increase, as it has encouraged the use of broad-spectrum antibiotics as immediate response

to infection [3]. Conventional methods for the detection and identification of pathogens require several culturing steps to isolate a single bacterial colony, which are time-consuming and can last several days. These long delays enable the spread of the diseases and prevent a correct therapeutic response, using the appropriate antibiotic. That is why the scientific community has focused towards the development of cheap, fast, specific and sensitive diagnosis solutions [4]. *Biosensors* are such detection platforms; they are measurement systems that combine selective bio-recognition elements and transducers [5]. Usually, they are devices that detect biomolecules, such as proteins, nucleic acids or even whole cells. Biosensing experiments combine both biology and technology (e.g. electronics or optics) and involve several components, that need to be adapted to each other (see Figure 1).

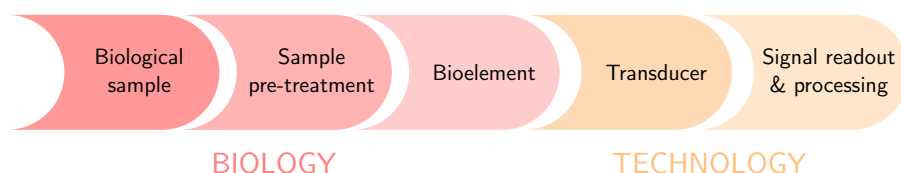


Figure 1: Illustration of the conceptual components of a bioassay.

The *biological sample* is composed of a matrix, which contains target pathogens. The nature of the matrix can greatly influence the complexity of the biosensing experiment. Indeed, matrices such as water, food, blood, urine, air or waste solids, have different physical properties, chemical compositions and microbial diversity [4].

The *sample pre-treatment* can therefore be of a great importance depending on the matrix. It may involve mixing, homogenisation in a stomacher, filtering, among other processes, to make the sample compatible with the detection method. Further pre-treatment steps include the removal of inhibitory substances in order to reduce parasitic effects and increase the homogeneity of the sample and the concentration of the target biomolecules [4].

The *bio-recognition element* can be of various natures: antibodies, enzymes, aptamers or bacteriophages. It allows the biosensor to be specific to one bacterium. The bio-receptors can be added in the sample volume or on the sensor surface. By correctly selecting and optimising this recognition element, both the sensitivity and reliability of the biosensor can be improved, reducing the risk of false positives and false negatives [5].

The *transducer* or sensor converts the bio-recognition phenomenon into a readable signal. This can be deciphered in changes of weight, in optical or electrical properties, or even in the emission of light or fluorescence. The transducer is designed to be as sensitive as possible to the measured changes [5].

The *signal readout & processing* is a unit or interface that converts the transducer output into a readable signal. To obtain a readable signal, amplification and noise reduction might be needed. The readable signal is then compared to positive or negative controls to establish a diagnosis. This diagnosis can be qualitative (the targeted bacteria are present or not) or quantitative (the concentration of the targeted bacteria) [5].

Biosensors could have a significant impact in areas where traditional detection methods are not sufficient. For example, they can provide a rapid diagnosis in case of epidemics or food-borne infections, in order to isolate the infected and prevent the spread of the disease, but also to look for the source of the infection. Other environments, such as developing countries where the access to a sterile laboratory might be difficult or even impossible, could also benefit from this detection method.

Thesis objectives

Based on all the advantages developed above, biosensors seem to be a pertinent solution for the detection of bacterial cells. *Biosensor* is however a wide term, as each of its components presented in Figure 1 can be very diverse. In this thesis, an optical biosensor based on a porous silicon (PSi) transducer is combined with lytic proteins.

On the one hand, the choice of an optical transducer was made mainly because this type of detection is easy to set up and not influenced by the dielectric properties of the sample matrix. On the other hand, PSi is a substrate that has shown many promising applications [6], especially for biosensing [7, 8]. PSi sensors have many interesting physical and optical properties: they are for example characterised by a large surface-to-area ratio, which makes them reactive to even minute changes in the surrounding environment. Moreover, their morphology can easily be controlled by their straightforward fabrication route [6]. While PSi biosensors have already shown a lot of promising results for bacterial detection [9], we believe that improvements are still possible in terms of sensitivity, specificity or response time. One limitation of the PSi

biosensors' performance is the diffusion of the species to the pore surface; to overcome this hindrance, it was decided to fabricate PSi membranes instead of PSi layers. The added value of PSi membranes has already been verified for the detection of other bio-molecules, as the flow-through operation mode enables an increase in sensitivity and response time [10].

Moreover, as opposed to other PSi-based biosensors, the method of detection presented in this work does not rely on a bio-recognition layer fixed on the transducer surface. In our protocol, the bioelement is added to the analyte volume as pre-treatment. This novelty eliminates the need for functionalisation steps during the fabrication of the process, and reduces the requirements on the use and storage conditions of the biosensors. More specifically, the bioelement is a selective lytic agent that digests the cell wall of the targeted bacteria and releases its internal content into the sample volume. This internal content can then be captured inside a PSi membrane, through which the sample is flown. Upon capture, the bacterial debris will affect the optical properties of the PSi membrane, which can easily be measured with a simple optical setup.

In this thesis, *Bacillus cereus* was selected as the target for our biosensor as it is a common contaminant found in the food industry. Moreover, *Staphylococcus epidermidis* was also used as the second target, in order to demonstrate the versatility of the detection platform.

Thesis outline

This thesis is organised in three parts. The first part focuses on the fundamentals of bacterial detection methods (Chapter 1), the concept of biosensors (Chapter 2) and an overview on porous silicon technology, especially with regard to PSi membranes and biosensors (Chapter 3).

The second part of the manuscript relates to the results produced during this research and is divided as follows:

- Chapter 4 studies the analyte and the transducer of the proposed biosensors with the purpose of optimising the design of the detection platform. The bacterial lysate is observed using several microscopy techniques and a size distribution is established. Based on these observations, the morphology of the porous silicon transducer is adapted and studied accordingly. The impact of fabrica-

tion parameters on the properties of the porous silicon is identified. The performance of the biosensor is then further optimised by modelling both the filtration and the optical response.

- Chapter 5 studies the proof-of-concept of the biosensor designed in the previous chapter. The fabrication route of the PSi membranes is detailed, and their biosensing performance is compared to traditional porous silicon layers. The sensitivity, selectivity and versatility of the detection platform are also verified.
- Chapter 6 focuses on an optimised prototype of the biosensor presented in the previous chapter. The first prototype indeed suffers from chemical instability, which leads to a signal drift. This issue is addressed by using atomic layer deposition to passivate the pore walls and prevent their dissolution when in contact with aqueous media. Several metal oxide coatings are tested and characterised. PSi/TiO₂ is selected as the most promising candidate for bacterial detection. The sensitivity of the second prototype is vastly improved. The selectivity and versatility of the biosensor remain unchanged.

The final part of this thesis offers a general discussion on the presented biosensor, comparing its features to traditional bacterial detection methods, other bacterial biosensors discussed in the literature and finally other PSi bacterial biosensors. The performance of the biosensor as an optical sensor is also weighed against other optical sensors in the literature. Moreover, the advantages and drawbacks of porous silicon as transducer for biosensing applications are also discussed. The passivation and integration challenges are overviewed, and their impact on the industrialisation of PSi transducer is assessed. Finally, the major scientific findings of this thesis are summarised and a series of perspectives are proposed.

Part I

Background on bacterial detection, biosensing and porous silicon

CHAPTER 1

INTRODUCTION TO BACTERIAL DETECTION

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Abstract

This chapter provides a short introduction to bacterial detection, overviewing the concepts of pathogenic bacteria and bacterial infections. The traditional methods for bacterial detection are also outlined; their benefits and drawbacks are reviewed.

1.1 Fundamentals on pathogenic bacteria

Bacteria are prokaryotic microorganisms, meaning they are single-celled and without nuclei. They can have a spherical, rod or spiral shape and their size does not exceed a few micrometres. They can organise themselves in specific cellular arrangements, like biofilms for example [11,12].

The common bacterial structure contains the following features: a cytoplasm, with a nucleoid, ribosomes and often plasmids, flagella, a cell membrane, a cell wall and for the Gram-negative ones (see below) an outer membrane (See Figure 1.1). The external structures (e.g. pili, flagella or glycocalyx) serve for cell motility, attachment and protection. The cell wall determines the shape of the bacterial cell and prevents its lysis, but is also a criterion for bacterial classification: Gram-positive bacteria have a thick cell wall, while Gram-negative bacteria have a thinner cell wall surrounded by an outer membrane [11].

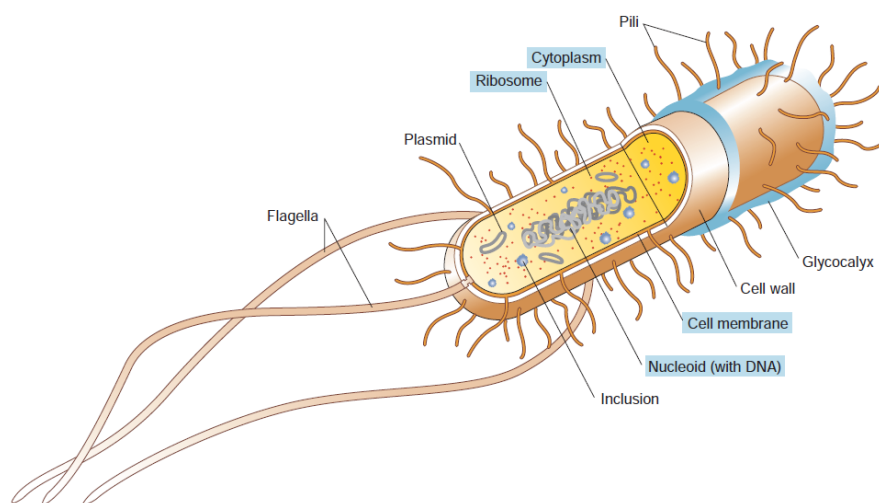


Figure 1.1: Schematic representation of common features in an "idealised" bacterial cell (Reproduced from [11])

Bacteria can multiply by binary fission, at rates as fast as 20 min, and their growth is typically following four phases: (1) a lag phase of several minutes to several hours, during which the bacteria adapt to the surrounding environment; (2) an exponential growth phase, during which the bacteria divide at their optimal rate; (3) a stationary phase, as the

lack of available nutrients limits the growth and (4) a death phase when the nutrients are disappearing [11].

Bacteria are highly adaptable and can grow in soil, water, plants and animals. Most of them are inoffensive or even beneficial, but some are qualified as pathogenic. As per definition, pathogenic bacteria can cause infectious diseases with different degrees of severity, by disrupting tissues and/or releasing toxins within their host (e.g. humans, animals or plants). In the human body, bacteria can cause diseases such as food-borne infections and intoxication, pneumonia, tuberculosis or cholera [11].

1.2 Sources of infection

Bacterial infections can have multiple sources and multiple routes of infection. For human pathogens, infection can be via inhalation, ingestion, abrasion to the skin or via an insect bite. The route of infection depends on the bacteria, and may also affect the outcome of the disease; some diseases are more severe depending on the route (e.g. inhalation for anthrax) [4].

As bacteria are naturally present in nature, such as in the soil, on animals or in water, sources of natural infection are present everywhere. Sources of infection can be divided in three categories [4]:

- **Food and water-borne infections**

While these infections usually result in mild to severe gastrointestinal symptoms, they can also have life-threatening complications. Common food-borne pathogens are *Escherichia coli*, *Salmonella spp.*, *Listeria monocytogenes*, *Staphylococcus aureus* or members of the *Bacillus cereus* group. The danger of these infections is the low number of bacteria needed to cause severe illnesses, which requires sensitive and precise detection methods.

- **Hospital-acquired infections**

Also called nosocomial infections, these are one of the most common causes of patient morbidity. The usual culprits for these infections are *S. aureus*, *enterococci* or *enterobacteriaceae*. This type of infection has become even more concerning with the emergence of antibiotic-resistant microbes.

- **Intentional infection**

This source of infection can be used for biological warfare or bioterrorism. Bacteria such as *Bacillus anthracis* or *Yersinia pestis* are among the agents that could be used for a large-scale aerosol attack. Fast and sensitive diagnosis solutions are therefore essential here to prevent the spread of such possible attacks.

1.3 Traditional methods for the detection of bacterial pathogens

Rapid and sensitive detection methods for pathogenic bacteria are essential for the prevention and treatment of infectious diseases. The main industries relying on pathogen identification are the food industry, the clinical area and the water and environment system [13]. Various types of samples must thus be analysed, from food to blood samples, each requiring their own detection level [4]. Some examples of typical guideline limits of detection (LoD) or limits of infection are given in Table 1.1. These limits are expressed in Colony Forming Units (CFU), which estimates the number of viable bacteria cells.

Pathogen	Matrix	Infection limits	Ref.
<i>B. cereus</i>	Ready-to-eat food	10^3 CFU/g	[14]
<i>L. monocytogenes</i>	Ready-to-eat food	10^2 CFU/g	[15]
<i>Mycobacterium tuberculosis</i>	Aerosol	10 CFU/inhalation	[16]
<i>S. aureus</i>	Blood	1 CFU/mL	[17]
<i>E. coli</i>	Urine	10^5 CFU/mL	[18]
<i>Enterococcus</i> spp.	Urine	10^5 CFU/mL	[18]

Table 1.1: Typical guideline limits for bacterial infection or detection

Many detection methods have been developed over the years, most of them used in laboratory environments. Of all the developed techniques, the most prominent ones are culture and colony counting, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR) and matrix-assisted laser desorption ionisation time-of-flight mass spectroscopy (MALDI-TOF MS). Their pros and cons are discussed hereafter, and summarised in Table 1.2 [13, 14, 19].

	Selectivity	LoD (CFU/mL)	Assay time	Cost
Culture	Low	1	5-14 days	Low
ELISA	High	1-10	≥ 2 h	Medium
PCR	Very high	<1	≥ 2 h	High
MALDI-TOF	High	10^5	20 min*	High

Table 1.2: Conventional methods of bacteria detection [5, 13, 14, 19] (* after sample preparation).

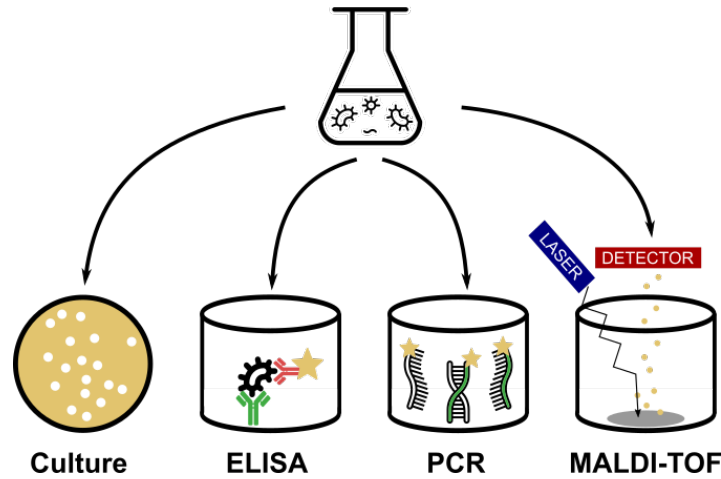


Figure 1.2: Schematic representation of four conventional techniques for the identification of bacteria.

1.3.1 Culture and colony counting

Culturing and colony counting methods are regarded as the gold standard for pathogen detection and have been so since the 19th century. The typical workflow for microbial identification via culturing includes sample preparation, enrichment, dilution, plating, enumeration and isolation of single species colonies for further analysis. Pathogen identification requires additional characterisation when generic media are used for bacterial growth [19]. For instance, morphology observations, Gram staining and biochemical testing can be used to identify the studied bacteria [20]. Specific and differential growth media can also be used during the plating, suppressing the growth of competitive bacteria or allowing given bacteria to express specific features such as colour for their easy identification [13].

Culture and counting methods are popular due to their efficiency, sensitivity, reliability and wide range of applications. They are also rather inexpensive and simple, while enabling the screening for the cell viability and antibiotic resistance. On the downside, these methods can be laborious and time-consuming because of the sample preparation, the incubation time needed for bacterial growth and the execution of additional characterisation tests. While typical culturing and counting methods take between 2 and 7 days, the delay can go up to 15 days for the confirmation of positive results. Moreover, these methods require specialised labs, where aseptic techniques must be used to avoid contamination [5, 14].

1.3.2 ELISA

ELISA is an immunology-based detection technique, meaning it is based on antibodies. It was developed in 1971 and is one of the most established methods for bacterial identification. ELISA uses the specific interaction between antibodies and antigens present on a bacterial cell surface or toxins produced by pathogens. The most common configuration of the assay is the "sandwich ELISA", which is illustrated in Figure 1.2. In this configuration, a primary antibody, specific to the target antigen, is immobilised at the bottom of the test well. The test sample is then added to the well; if the target reacts with the antibody, it is retained after the well is washed. A secondary antibody, also target-specific but labelled with an enzyme, is then added to the test well and a "sandwich" structure is formed. Finally, a substrate is added that will react with the enzyme, creating a coloured by-product. This by-product can be quantitatively measured by optical density scanning of the well plate. If the target antigen is present in the sample and reacted with the primary antibody, the ELISA assay will be coloured and therefore positive. If the antigen is not recognised, the test is colourless and hence negative, as no antibody-enzyme conjugate is bound [20].

ELISA is a very sensitive and specific technique, but also user-friendly. It can produce both qualitative and quantitative results. Modern tools can complete an assay in 2 hours or less, in an automated manner. On the downside, the possible targets are limited by the commercially available antibodies, and detection limits are very dependent on the used antibody. Moreover, ELISA is very prone to potential interferences from contaminants inside the samples, such as salts or acids [14].

1.3.3 PCR

Polymerase chain reaction (PCR) is a detection method invented in the early 1980s, based on the nucleic amplification of deoxyribonucleic acid (DNA) present inside the bacterial cytoplasm and/or bacterial lysate. Amplification only focuses on a DNA target sequence and is achieved by the repetition of a three-step cycle: denaturation, annealing and extension, as illustrated in Figure 1.3.

In the denaturation step, the DNA containing the target sequence is denatured by heat into two single strands. In the second step, the temperature is lowered and synthetic DNA primers, with sequences identical to those surrounding the targeted sequence, hybridise with the DNA strands. Then, during the extension step, DNA polymerases present in solution extend the primers into copies of the target DNA.

This means that one single DNA molecule is amplified into two copies at the end of the cycle, resulting in an exponential growth when this cycle is repeated multiple times, reaching one million copies after 20 cycles, for example. Once the number of target DNA copies in solution is high enough, its presence can be detected by gel electrophoresis, that separates DNA sequences based on their size, via the application of an electrical field [20]. As an alternative, real-time PCR adds a fluorescently labelled probe to the reaction mixture, enabling the quantitative measurement of the amplification during the exponential growth [13].

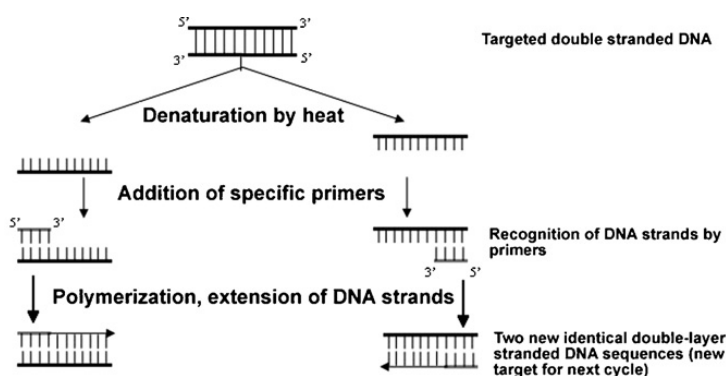


Figure 1.3: Schematic illustration of one PCR cycle, which includes three steps: denaturation, annealing and extension. Reproduced from [13].

All types of PCR amplification methods have the advantage of being sensitive, selective, reliable and most of all, fast; they can provide results in

only a few hours. Some PCR methods can provide quantitative results, and multiplexing is also possible. There are however some drawbacks: the risk of cross-contamination is amplified, and the overall cost of the method remains high. Indeed, specialised labs and staff are needed, and, even if large high-end equipments can perform many analyses at once, their cost remains high. Moreover, PCR cannot distinguish between live or dead bacterial cells and a prior knowledge of the suspected pathogens is necessary to select the correct DNA primers [14, 19].

1.3.4 MALDI-TOF MS

Matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectroscopy is a rather recent but very popular method. In this technique, the sample is treated with an energy-absorbent matrix, which rapidly heats up under laser ablation and helps to ionise the sample. The created ions are then separated based on their mass to charge ratio, and measured using a time-of-flight analyser. A mass spectrum is generated, as illustrated in Figure 1.4 for *B. cereus*, forming a "fingerprint" of the sample that can then be compared to a database in order to identify species [19].

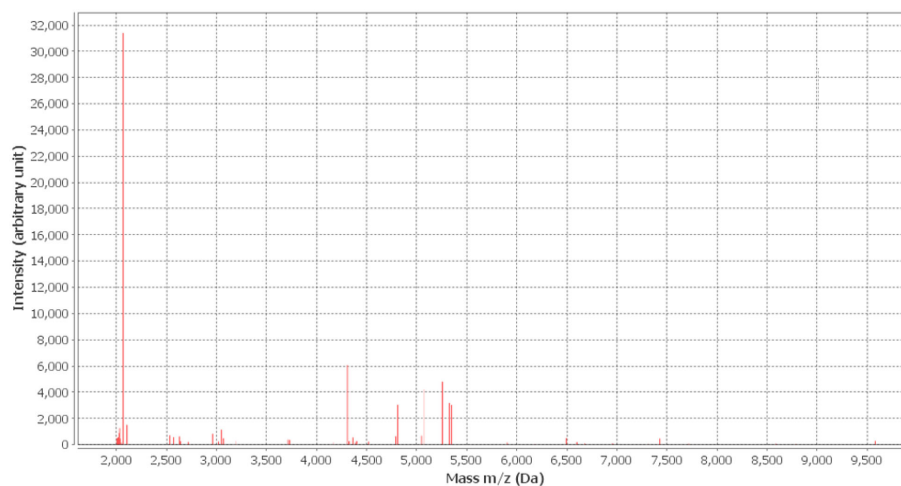


Figure 1.4: MALDI spectrum obtained from direct analysis of intact bacterial cells of *B. cereus*. The spectrum was acquired in linear mode using a time-of-flight mass spectrometer (Vitek MS Plus, BioMerieux). Reproduced from [14].

MALDI-TOF mass spectroscopy is a sensitive and reliable method to identify microbes, as long as reference spectra are available in the databases.

But most of all, it is a rapid method, with results available in a matter of minutes. The initial investment cost is high, but sample runs are relatively inexpensive. The main drawback of this method is the inability to differentiate between closely related species, hence the need to first isolate and culture the bacteria on agar before identification. This means that a pathogen detection can easily take more than a day, even though the identification itself is very fast [14].

CHAPTER 2

FUNDAMENTALS ON BIOSENSORS

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Abstract

In this chapter, the concept of biosensor is defined. Each of its components is reviewed, namely the target analyte, the biosensing element, the transducer and the signal read-out. When discussing the biosensing element, the notion of bacteriophages and their use in biosensing is introduced. For the transducing component, many options are listed, and more information is given about optical transducers. Finally, some general figures of merit, that enable to compare biosensors, are defined.

2.1 Biosensors for bacterial detection

Traditional methods of bacterial detection show good potential, but further improvements are still required. The combination of the following parameters could be optimised [21]:

- Assay time: the detection should be as fast as possible;
- Sensitivity: the limit of detection should be as low as possible, reducing the instances of false negatives;
- Selectivity/specificity: the selectivity should be as high as possible, reducing the risk of false positives;
- Portability: the detection should be possible anywhere, without the need of a specific lab environment;
- Sample volume: the assay should use as little sample volume as possible;
- Cost: the cost per assay should be as low as possible;
- Sample preparation: the sample preparation should involve as little to no steps, or should be as fast as possible.

To answer to all these criteria, the scientific community has defined the concept of a *biosensor*. As per definition, a biosensor is "*a device that produces a measurable signal proportional to the concentration of the target analyte, usually incorporating a biological sensing element and measuring signals derived from biological interactions* [22]". This definition highlights the main components of a biosensor which are illustrated in Figure 2.1 and will be described in further details with regard to bacterial detection.

2.2 Target analyte

While the end goal of bacterial detection is to resolve the presence/absence of bacteria, the target analyte is not necessarily whole-cell bacteria. The presence of bacteria can also be detected using nucleic acids or by-products of the bacteria, such as bacterial proteins (e.g. toxins) or metabolites [4]. The detection of whole microorganisms is often more challenging than the detection of molecular analytes, not only because

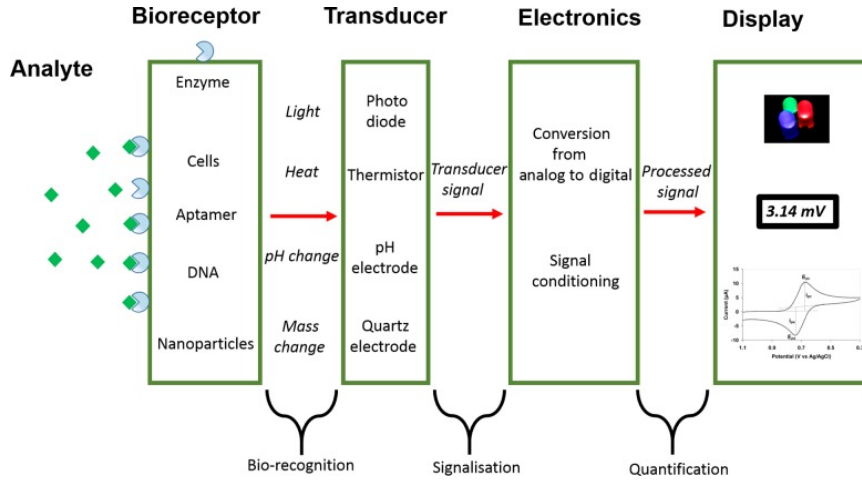


Figure 2.1: Schematic representation of the components of a biosensor; Reproduced from [23].

of their larger size and varying shapes, but also because whole cells are more prone to non-specific interactions [24].

Based on the nature of the target, the analyte sample may require handling for the sensing to reach a high performance. For example, to detect whole-cell bacteria in low concentration samples (e.g. 1 CFU/mL), pre-concentration steps are needed to increase the likelihood of the detection. Pre-concentration can be performed via different techniques [4]:

- *Centrifugation* — The bacterial cells form a solid pellet after this step; the remaining liquid, called supernatant, can be removed and replaced by another medium of lower volume.
- *Immunomagnetic separation* — Paramagnetic particles, coated with specific ligands, are added to the sample volume and bind to the target bacteria; by applying a magnetic field, a bacterial pellet is formed, that can then be re-suspended in smaller volumes.
- *Electrokinetic forces* — The sample is subjected to DC or AC voltages that attract bacteria to the region of interest, such as the sensor surface.

For other targets, such as nucleic acids, further sample preparation is required; indeed, the nucleic acids must be released for the bacterial cell. This can be achieved by inducing the cell lysis using physical methods

(e.g. heating the sample up) or biochemical methods (e.g. adding specific lytic proteins to the sample volume) [4].

2.3 Biological sensing element

The biological sensing element is responsible for the recognition and identification of the target analyte. By its high affinity to the analyte, the biological sensing element will provide selectivity to the biosensor. When the bioelement interacts with the target, which is often called the bio-recognition phenomenon, a signal is generated. This signal can be of various natures, as for example in the form of light, heat or a change in mass, in pH or in conductivity. Selectivity can be added to the biosensor either via the sensor surface or via the analyte sample volume [23].

Most often, the former is preferred and the bioelement is added to the sensor surface in order to capture and bind the analyte. The most common bio-probes are antibodies, proteins, DNA or ribonucleic acid (RNA) aptamers, carbohydrates or bacteriophages (and their proteins) [4, 24]. These surface-based bioelements have, however, several drawbacks. First of all, the functionalisation process that binds the probes to the sensor surface is laborious; it must be carried out before sensor encapsulation and put constraints on further processing. It also puts limitations on the storage and usage conditions of the sensors [25]. Moreover, only targets close to the sensor surface bind to the bio-probes, resulting in a low overall capture percentage [26].

For volume-based biorecognition elements, the number of interactions is increased. Among these elements, lytic enzymes and bacteriophages have been increasingly studied in recent years [4, 25, 27–30]. The advantages of bacteriophages will therefore be detailed in the following section.

2.3.1 Bacteriophages for biosensing

2.3.1.1 General features

Bacteriophages are viruses that target bacteria as hosts. They can only replicate inside living bacteria, and use the intercellular machinery to reproduce. This reproduction is however only possible within the proper host, meaning that bacteriophages are highly specific. In terms of morphology, they are vastly diverse, but their most common structure is a

protein capsid that holds the viral genome. Most phages also belong to the *Caudovirales* order, whose morphology is also characterised by a tail, which is attached to the capsid. The *Caudovirales* order is itself subdivided into three families, based on the contractility of the tail: (1) *Myoviridae*, with a contractile tail, (2) *Podoviridae*, which have no baseplate and are short-tailed and (3) *Siphoviridae* with a long non-contractile tail as illustrated in Figure 2.2.

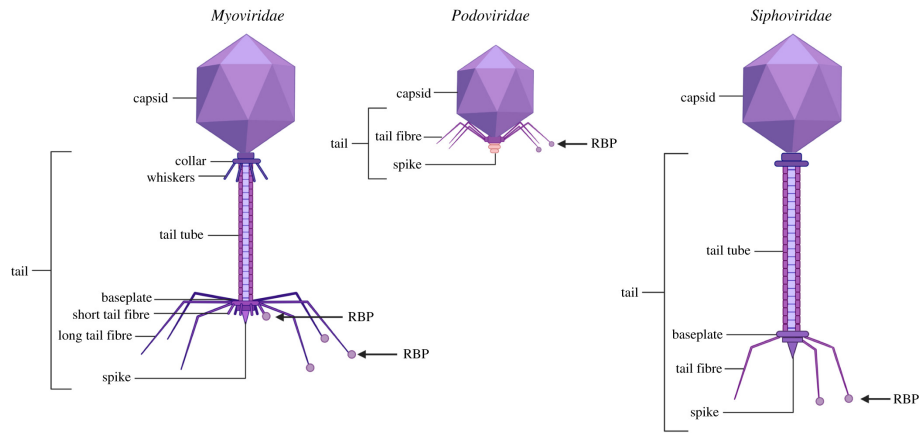


Figure 2.2: Tailed bacteriophages from the *Caudovirales* order, which consists of three families: *Myoviridae*, *Podoviridae* and *Siphoviridae*; Reproduced from [31]

The phage tail participates in the host recognition and genome injection. The host recognition is a multistep process, which also involves specific receptor binding proteins (RBPs). These RBPs recognise and adsorb onto the receptors of the host cell surface. Following this interaction, the viral genome is injected into the host cell. At this point, the phage can undergo two different cycles: the virulent phage will enter a *lytic cycle*, while the temperate phage will undergo a *lysogenic cycle*. The lytic cycle is illustrated in Figure 2.3: once its viral genome is injected, the phage will use the internal metabolism to replicate and produce lytic proteins. The bacterial cell will eventually disrupt and release the newly assembled virions (single particle of bacteriophage). In the lysogenic cycle, the viral genome either integrates with the host DNA or behaves as an independent plasmid molecule. This viral genome will replicate alongside the bacteria DNA for many generations, until a shift to the lytic cycle is triggered [25, 28].

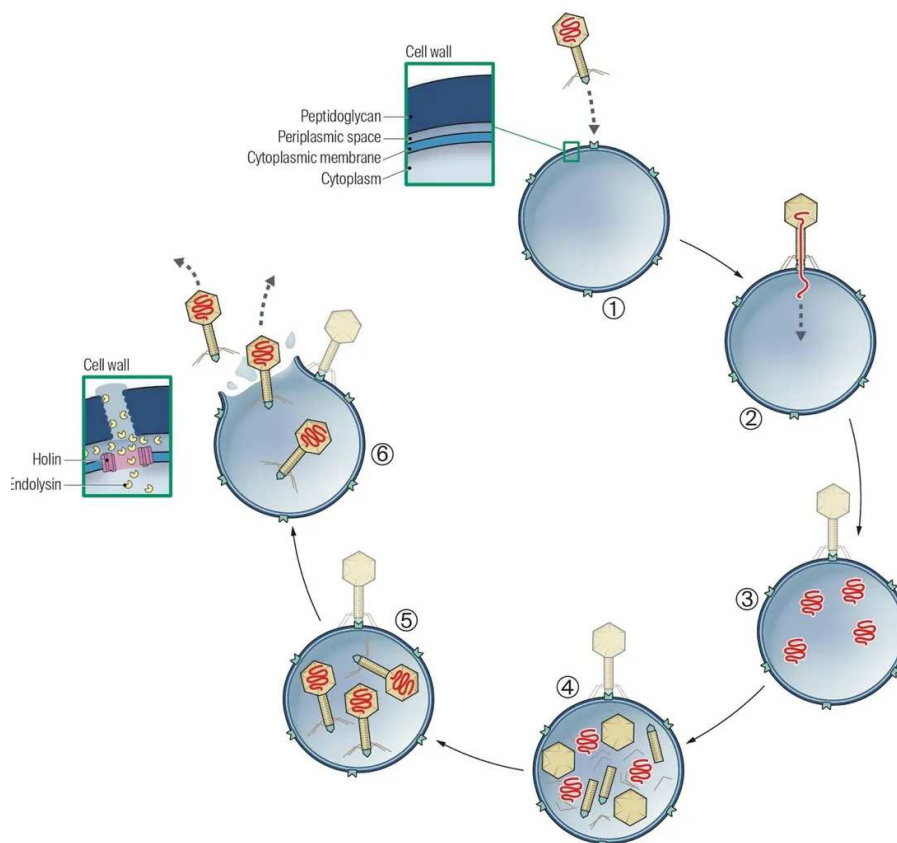


Figure 2.3: Illustration of the lytic cycle of bacteriophages; Reproduced from [32].

2.3.1.2 Phages as bioelement for bacterial detection

Bacteriophages have many advantages for biosensing. They are efficient and specific against host bacteria. There is an abundance of phage types, who could theoretically target and therefore detect every bacterial strain. They also allow the distinction between live and dead cells, as only viable hosts can ensure their multiplication. Another advantage of phages is their production mechanisms: phages can be produced in large quantities and properly purified very quickly and cheaply thanks to their short replication time. Some phages are also robust, retaining their activity even after being exposed to high temperatures or other critical conditions, such as organic solvents [25,30].

Bacteriophages can be used in different bacterial detection strategies, as illustrated in Figure 2.4:

- *Infection of bacteria as signal trigger* — The infection of bacteria by phage leads to cell lysis. Upon lysis, both the cell content and the progeny phages are released. Biosensing strategies may therefore rely on the detection of bacterial metabolites [33]. Other strategies rely on changes in physical or chemical properties upon lysis, such as increases in conductivity [34]. Finally, progeny phages can also be the target of a biosensing assay, such as the indirect detection of bacteria [35, 36].
- *Infection of bacteria for gene expression* — Genetic modifications can be introduced into the phage genome, coding fluorescent proteins for example, that are expressed inside the host cell upon infection. The infected bacteria can therefore be easily detected [37, 38]. Modified phages are however challenging to obtain and often less infectious [30].
- *Bacteriophage-based sensing layer* — Phages can be immobilised to the transducer surface and used as bio-recognition element for the detection of bacteria. Using the correct transducer, the capture of the targeted bacteria by the phages can be translated into a readable signal. Transducers can measure changes in optical properties (e.g. surface enhanced Raman spectroscopy sensors [39, 40]), dielectric properties (e.g. Electrochemical Impedance Spectroscopy sensors [41, 42]) or even changes in mass (e.g. magneto-elastic sensors [43–45]).
- *Bacteriophages as capture elements* — Phages can be bound to micro- or nanoparticles and form bioconjugates that capture, separate and/or detect the targeted bacteria. The nature of the nanoparticles will dictate the mode of action: for example, conjugated gold nanoparticles can be used for colorimetric detection [46] and conjugated magnetic nanoparticles can be used for separation [47, 48].

There are however some disadvantages to biosensors using whole phages as bioelement. One of them is the large size of the virions, usually comprised between 50 and 200 nm, which limits the miniaturisation. In case of a phage-based sensing layer, the binding event may therefore also be too far from the transducer surface, hindering the signal detection.

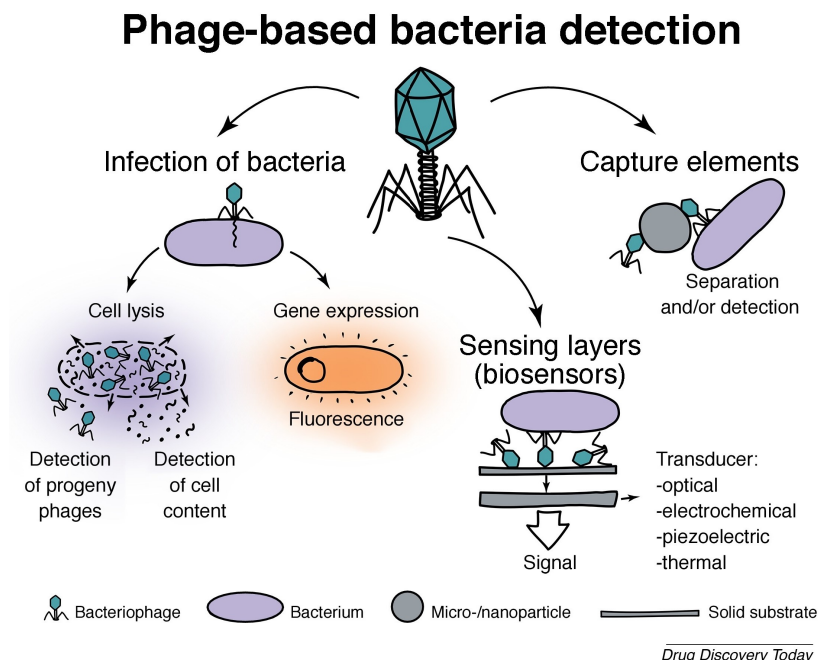


Figure 2.4: Bacteriophage-based modes of action for bacterial detection; Reproduced from [28].

For this case, another challenge is the correct orientation of the phages. Finally, the biggest disadvantage is that all bacteriophages eventually cause cell lysis, limiting the period of analysis [30]. These challenges encouraged the scientific world to utilise only part of the phages for the strategies detailed above. The used parts are the components that naturally show attraction to host cells, which include receptor-binding proteins, lysins and/or their cell-binding domains (CBDs).

- RBPs are responsible for the host recognition and binding. Their binding ability has been used for the capture and magnetic separation of bacteria when conjugated to magnetic nanoparticles [49,50]. The captured bacteria can then be labelled and detected using colorimetric [50] or fluorescence assays [51].
- Also called endolysins or murein hydrolases, lysins are enzymes produced by bacteriophages at the end of their lytic cycle. They are able to cleave the bacterial cell wall by degrading the peptidoglycan, and therefore enable the virions to disrupt their hosts and

be released in the surrounding environment [52]. Most endolysins encoded by phages with Gram positive hosts have separate cell wall binding and enzymatic domains. The CBDs confer the specificity to host cells but do not induce lysis, making them perfect candidates for biosensing [52]. CBDs have for example been used as sensing layer for the electrochemical impedance detection of *Listeria* cells [53]. CBDs can also be conjugated with magnetic beads and serve as capture agents, as well as combined with tags in order to enable bacteria detection via a sandwich fluorescence assay [54] or via a colorimetric assay [55]. Finally, CBDs have been used as bioelements in lateral flow assays (LFAs), either conjugated to gold nanoparticles for colorimetric detection of *B. cereus* [56] or functionalised onto the nitrocellulose substrate for the Electrochemical Impedance Spectroscopy (EIS) of *Bacillus thuringiensis* [57].

2.4 Transducers

The transducer translates the recognition event between the bioelement and its target into a readable signal. This signal can either simply indicate the presence of bacteria (qualitative measurements) or be proportional to the number of bacteria detected (quantitative measurements). The main types of transducer deal with either optical or electrochemical sensing, as will be detailed in sections 2.4.1 and 2.4.2. Other types of transducer however exist:

- *Mass-based transducers or acoustic transducers* — They respond to small changes in mass upon the capture of the target. Piezoelectric and quartz crystal microbalance biosensors for example vibrate at a specific frequency depending on the mass and the applied current; a binding event will therefore create a shift in the resonant frequency [58]. The same principle applies to micro-cantilevers: they oscillate at their resonance frequency, which changes due to the mechanical bending induced by the binding of the target [24]. Magneto-elastic sensors, on the other hand, rely on the principle of magnetostriction: in response to both an alternating and static magnetic field, the micro-elastic transducer vibrates at its resonance frequency, which shifts when small extra mass is applied [59].
- *Thermal transducers* — They exploit the heat generated or ab-

sorbed by a biological reaction. This biological reaction is often an enzymatic catalysis, which can be monitored with a thermistor or a thermopile. Microelectromechanical systems (MEMS) thermal sensors have also been used recently, as they can easily be miniaturised and show improved thermal isolation properties [58].

- *Electronic transducers* — They rely on field-effect transistors (FETs). These consist of two electrodes, the source and the drain, which are the starting and the end point of the charge carriers, joined through a gate electrode. A sensing channel, such as a silicon nanowire or graphene layer, connects the source and the drain and is functionalised by bioelements. When a target is recognised on the channel, the surface potential changes and alters the current between the source and drain terminals [58, 60].

2.4.1 Electrochemical transducers

Electrochemical transducers are the most widely studied and used sensors. They exploit the biochemical reaction that occurs when the analyte binds to the bio-receptors fixed on the sensor's surface. This reaction is converted into a detectable signal, such as changes in voltage, current, impedance or capacitance. This signal is proportional to the analyte concentration, enabling a quantitative detection. The three main types of electrochemical transducers rely on amperometry, potentiometry and electrochemical impedance spectroscopy (EIS).

- *Amperometric transducers* rely on the direct measurement of the current induced by the oxidation or reduction of species generated by the interactions between the bioelement and the analyte. The amperometric sensors are composed of two or three electrodes and are easy to miniaturise. They offer an excellent sensitivity, reaching limits of detection as low as 1 CFU/mL [24]. They however lack in selectivity, as other redox-active species may interfere with the signal [58].
- *Potentiometric transducers* exploit changes in the potential of the analyte solution upon the recognition of the target by the bioelement. This bioelement is fixed on an ion-selective working electrode, and the potential is measured relative to a reference electrode [58]. Potentiometric sensors however lack specificity and sensitivity for the detection of large targets such as bacteria; reasonable limits of detections, in the 10^3 CFU/mL range, were however

possible [24].

- *Impedimetric transducers* employ EIS to characterise biological interactions. They are based on two- or three-electrode systems, to which a small-amplitude frequency-dependent alternating voltage or current is applied. The thus-produced electrical impedance is affected by the interactions between the analyte and the bio-receptor; these interactions cause changes in capacitance and electron transfer resistance across the working electrode surface [58]. Impedimetric sensors are highly sensitive, with LoDs as low as 1 CFU/mL, and do not require enzymatic reaction or electro-active species such as the other electrochemical detection methods [24]. They however lack in reproducibility, which can be linked to non-specific binding [61].

Electrochemical sensors in general are so attractive because their inexpensive electrodes can easily be integrated with simple electronics, making easy-to-use and low-cost portable detection platforms. Another big advantage of these transducers is their low response time, which is often expressed in minutes or hours. Moreover, the emergence of new nanomaterials such as carbon nanotubes or graphene enables the lowering of the limit of detection. One drawback of these sensors is that they can easily be influenced by the electric and dielectric properties of the analyte matrix [62].

2.4.2 Optical transducers

Optical biosensors rely on changes in optical properties upon analyte binding events, which are translated into measurable signals by the transducers. They exploit modifications of the absorbance, the transmission, the reflection, the refraction, the phase, the amplitude, the frequency and/or the polarisation of light. Fluorescence, luminescence or plasmonic effects can also be exploited. Fluorescence and luminescence are often generated by labels, which results in an indirect detection of the analyte. In label-free sensing, on the contrary, the signal is generated by direct interaction of the analyte and the transducer [58, 63].

2.4.2.1 Label-based optical transducers

Fluorescent-based optical detection relies on the labelling of the analyte with fluorescent tags. These tags emit light of a specific wavelength when

excited with a light of a different wavelength. This light emission can then be detected with a fluorescent microscope or photospectrometer. The nature of the fluorescent tag can be varied: dyes, quantum dots, nanoparticles or even fluorescent proteins [58]. Limits of detection as low as 10 CFU/mL have been reached with this technique [64].

Luminescence-based biosensors exploit the emission of light induced by a reaction [58]. This photon-emitting reaction can be triggered by chemical reagents, such as isoluminol or acridinium esters. Most often, enzymes are used as labels, such as horseradish peroxidase (HRP). HRP is a label enzyme that catalyses the reaction of hydrogen peroxide with certain organic substrates to yield coloured products, which provide a quick and visible confirmation of the presence of bacteria. Bioluminescence is also a possibility, with the chemiluminescent reaction occurring in living organisms catalysed by enzymes or photoproteins. The emitted luminescence can be detected by a plethora of devices, such as photomultiplier tubes, charger-transfer detectors, colour Charge-Coupled devices (CCDs) or even Complementary Metal-Oxide-Semiconductor (CMOS) imagers [65].

2.4.2.2 Plasmon-based transducers

The most common plasmon-based transducer relies on *Surface Plasmon Resonance* (SPR). A SPR sensor measures changes in the refractive index caused by interaction on a thin metal surface. Surface plasmons are resonant oscillations of conduction electrons. They are induced by the illumination of a thin conducting surface, at the interface between two media of different refractive indices, by a polarised light at a given angle. SPR requires the use of a coupling device such as a prism; the light therefore enters the prism at a certain angle, known as the resonance angle, and then exits at another angle which is related to the refractive index, as illustrated in Figure 2.5. At the resonance angle, light is absorbed by the electrons in the thin metal film, inducing the surface plasmons. The SPR phenomenon is translated by the decrease in intensity of the reflected light compared to the incident light and takes the form of an intensity dip in the SPR reflection intensity spectrum, as depicted by the Sensorgram inset in Figure 2.5. The shape and angular position of this intensity dip can be used to convey information about the sensor's surface. When an analyte binds to the surface, the refractive index is modified, which induces a change in the angular position of the intensity dip. This shift in angle is proportional to the mass of the

bound analyte, and is therefore monitored overtime [58,63,66]. SPR is a powerful tool for the detection of bacteria, reaching LoDs in the order of 1 CFU/mL [64].

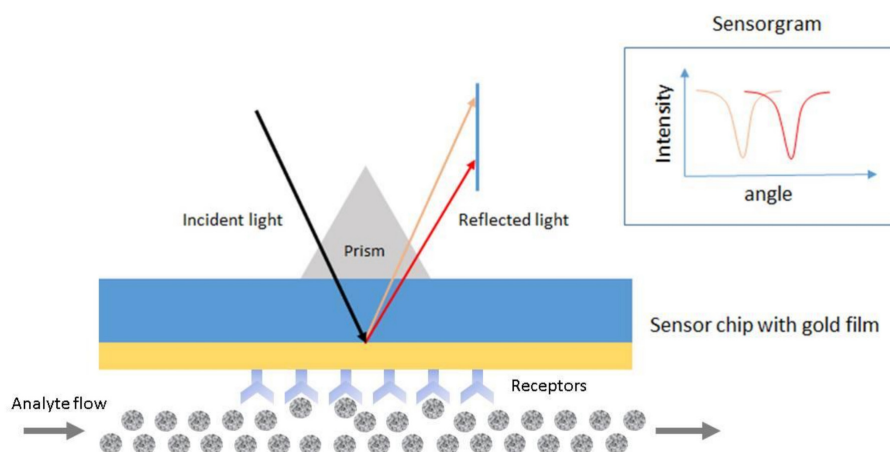


Figure 2.5: Schematic illustration of a surface plasmon resonance (SPR) biosensor, reprinted from [66].

When the surface plasmon is confined in metal-based nanoparticles (NPs) with dimensions similar to the incident wavelength, *localised surface plasmon resonance (LSPR)* arises. The conducting electrons of the NPs are excited and oscillated with the incident light. The frequency of the plasmons shifts with changes of the refractive index surrounding the NPs.

LSPR also plays a role in *Surface Enhancement Raman Spectroscopy (SERS)*. Raman scattering is the inelastic scattering of photons via the interaction with molecular vibrations or rotations, phonons or other excitations. Since Raman scattering is typically very weak, this process is enhanced in SERS via two mechanisms - an electromagnetic mechanism and a chemical one. The electromagnetic enhancement relies on LSPR; the excitation of localised surface plasmons enhances the local electric field, which amplifies the absorption of phonons and therefore increases the intensity of the Raman signal. The chemical enhancement mechanism relies on charge transfers from the Fermi level of the metal surface to the analyte molecules energy levels [66,67]. SERS is a powerful tool, able to detect single molecules and cells [68].

2.4.2.3 Optical Waveguide

An *optical waveguide* is depicted in Figure 2.6 and consists of an optically transparent guiding layer and surrounding cladding layers, whose refractive indices are lower than the guiding one. By carefully selecting the guiding layer material and design, light can be confined within the guiding layer and can propagate along the layer via total internal reflections at the interfaces with the cladding layers. At each reflection point, an evanescent field (i.e. a standing wave of the electromagnetic field) is created and penetrates into the cladding layer, as illustrated in Figure 2.6. This wave decays exponentially as the distance from the waveguide surface increases and therefore acts a surface-sensitive probe: when an analyte is bound to the waveguide surface, the refractive index of the cladding is modified and affects the evanescent field. The modification of the evanescent field can be detected in various ways: (1) the evanescent wave induces a fluorescent signal at the surface of the waveguide, which can be monitored; (2) the refractometric changes at the surface of the waveguide can be detected using interferometry; or (3) the attenuation of the guided wave due to absorption or scattering can be monitored via spectroscopy [69, 70]. The limit of detection of optical waveguides with bacteria as target is in the 10^3 cells/mL range [70].

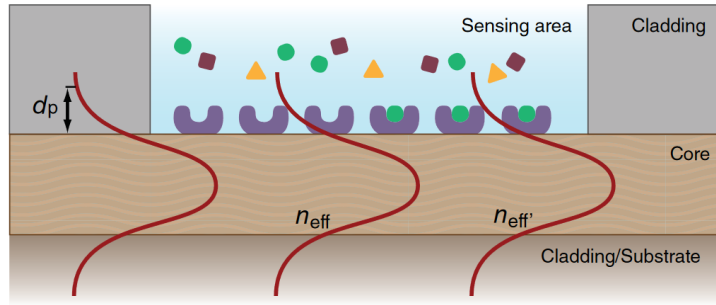


Figure 2.6: Schematic illustration of an optical waveguide transducer, reproduced from [69].

2.4.2.4 Optical Resonator

An *optical resonator* is designed to confine light and induce its interference with itself; in other words, the light waves continuously travel between two or more interfaces, or circulate within the resonator structure. As a result, only specific frequencies, i.e. the resonance frequencies, are

supported inside the resonator without suffering significant losses. The other frequencies are inhibited by destructive interferences. The material and geometry of the optical resonator will determine the resonant frequency. Changes in refractive index, temperature or even pressure of the medium surrounding the resonator also affect the resonant frequency, which are translated by a change of light intensity [71, 72].

There are various types of optical resonator: Fabry-Pérot interferometers, with a simple optical cavity; whispering gallery mode resonators, which confine light waves in any radial or polygonal enclosed surface; photonic crystal microcavities; or even optical fibre based interferometers [72]. Optical resonators are easy to fabricate and have shown promising results, detecting concentrations as low as 20 bacteria cells per mL [73].

2.4.2.5 Photonic Crystal

Photonic crystals consist of micro- or nanostructures in which two or more materials with different dielectric constants are periodically arranged and provide a high reflection of the light at specific wavelengths. The periodicity of the photonic crystals can be in one (1D), two (2D) or three dimensions (3D). A 1D photonic crystal, for example, is composed of a multi-layered stack and is also known as a Bragg mirror or an optical filter. The spatially periodic variations of refractive index induce a wavelength band in which light propagation is prohibited, meaning there is no optical signal visible for this photonic band gap in the transmission or reflection spectra. The periodic structure can be changed locally by introducing a defect mode, resulting in a sharp peak appearing within the photonic band gap. The position of this peak is strongly influenced by the refractive index in and around the defect, meaning that it can be monitored for binding events in case of biosensing applications [58, 74, 75]. While photonic crystals are most often used to detect small molecules, such as proteins, they have also been used for the detection of bacteria, reaching limits of detection as low as 200 CFU/mL [76].

2.5 Signal read-out & interpretation

The signal read-out interface strongly depends on the type of transducers. For electrochemical transducers, bulky analysers such as potentiostats used at the early stage of the biosensor development can easily

be miniaturised to integrated circuits. For optical transducers, the setup - often composed of lamps, spectrometers and lenses - can also be miniaturised, but not at the integrated levels; external read-out units are still needed.

2.5.1 Figures of merit

To compare a biosensor's performance to current techniques or to other biosensors, figures of merits (FoM) must be defined. A schematic illustration of a typical sensor output and some FoMs is illustrated in Figure 2.7.

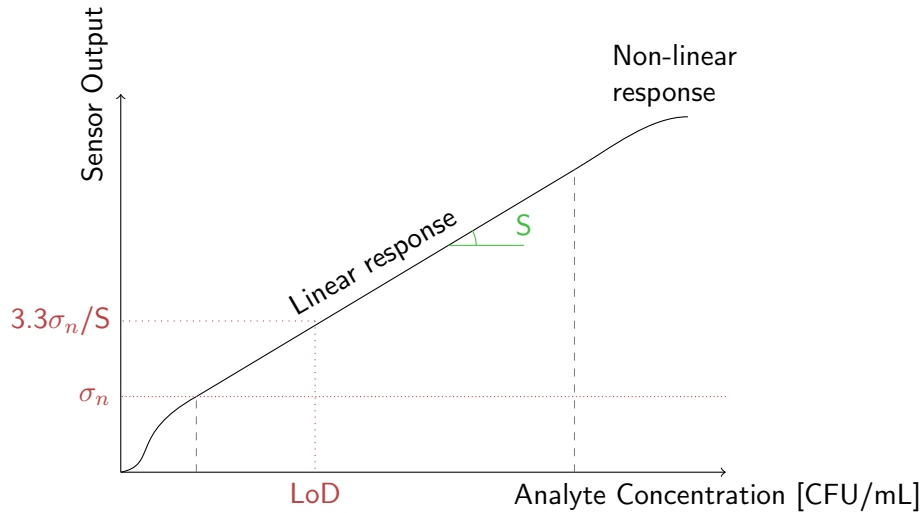


Figure 2.7: Diagram of a typical sensor output for the detection of bacteria, highlighting the noise level σ_n , the limit of detection (LoD) and the sensitivity (S).

Signal-to-noise ratio (SNR) The signal-to-noise ratio (SNR or S/N) quantifies how much signal has been corrupted by noise. It compares the level of desired signal and the level of background noise. The SNR is calculated as the ratio of signal power to the noise power, often expressed in decibels. High SNR values translate signal with less noise, as illustrated in Figure 2.8, and are therefore often desired when developing a biosensor.

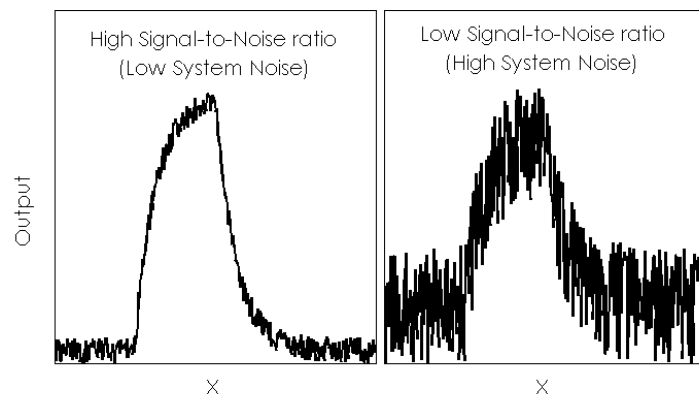


Figure 2.8: Schematic illustration of a signal with a high SNR and a noisy signal with a low SNR, reproduced from [77].

Sensitivity (S) The sensitivity translates the shift in sensor output per variation unit of analyte concentration. It corresponds to the slope of the sensor output in case of a linear response. For optical sensors, the sensitivity is often expressed in terms of refractive index units (RIU), as it enables to compare sensors regardless of the target analyte. The RIU change can then be linked to the analyte concentration for specific applications. Sensitivity is one of the most important FoM for sensors: the higher the sensitivity, the lower the odds for false negatives sensing tests.

Limit of Detection (LoD) This FoM translates the minimal detectable concentration of bacterial cells contained in the sample. It is expressed in CFU/mL or in cells/mL. It can be calculated in several manners [78].

- *Blank determination* — According to International Union of Pure and Applied Chemistry (IUPAC) ¹, the LoD depends on the mean of blank measures (μ_{blank}), the standard deviation of blank measures (σ_{blank}) and a numerical factor (k) chosen to express the

¹IUPAC. Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). Online version (2019-) created by S. J. Chalk. ISBN 0-9678550-9-8. <https://doi.org/10.1351/goldbook>.

confidence level.

$$\text{LoD} = \mu_{\text{blank}} + k \cdot \sigma_{\text{blank}} \quad (2.1)$$

The value of k is typically set to 3, which corresponds to a confidence value of 0.00135.

- *Linear regression* — In case of a linear calibration curve, the LoD can be related to the response standard deviation (σ) and the sensitivity (S). The response standard deviation can be obtained from blank measures or based on the residual or estimated standard deviation of the regression line.

$$\text{LoD} = \frac{3.3\sigma}{S} \quad (2.2)$$

- *Limit of blank* — The limit of blank (LoB) is defined as the *highest apparent analyte concentration expected to be found when replicates of a sample containing no analyte are tested* [78]. It is calculated based on the mean of blank measures (μ_{blank}), the standard deviation of blank measures (σ_{blank}).

$$\text{LoB} = \mu_{\text{blank}} + 1.645\sigma_{\text{blank}} \quad (2.3)$$

The LoB is related to the LoD via the standard deviation of a low concentration sample response (σ_{response}).

$$\text{LoD} = \text{LoB} + 1.645\sigma_{\text{response}} \quad (2.4)$$

Response time A biosensor does not change its output instantly when brought into contact with the analyte. Especially when the recognition of the biological target is based on binding, the diffusion of the target molecules to the receptor and the binding event itself may require some time. The *response time* is therefore defined as the time required for the sensor to go from its initial state to its final settle state. Generally, this response time should be as short as possible in order to inform the user of the presence or absence of the analyte.

Selectivity The selectivity, also called specificity, of the biosensor translates its ability to detect the target molecules in samples contain-

ing many other species. This FoM is often attributed to the bioelement of the sensor. The selectivity is closely related to the matrix complexity, which will influence the sensor's response. A high specificity will translate into less false positive sensing results. The selectivity can be quantified by the *reproducibility* of the biosensing. This terminology defines the sensor-to-sensor variation that is often attributed to non-specific binding or randomly positioned receptors on the sensor surface.

Robustness The robustness relates to the stability under flow and the lifetime of the biosensor. Indeed, the bioelement must withstand biofouling and, in case of binding events, the kinetics must be constant. Likewise, the transducer response must not be altered during long-term applications by corroding or biological effects. In other words, no signal drift must be observed, or this drift must be calibrated its contribution to the signal. Finally, the sensor performance must also remain constant after a defined period of storage.

CHAPTER 3

POROUS SILICON MEMBRANES FOR
BIOSENSING APPLICATIONS

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Abstract

This chapter introduces the field of porous silicon and is partly adapted from [RV3]. After an overview of the fundamental concepts on the fabrication and characterisation of porous silicon, emphasis is put on porous silicon membranes. Finally, the application of porous silicon for biosensing is reviewed.

3.1 Fundamentals on porous silicon

Silicon (Si) technology has dominated microelectronics for more than half a century. Not only is silicon the second most abundant element on earth, but it is also characterised by properties that ideally balance performance and stability [79]. In recent decades, microelectronics has permeated to other areas of applications: it is no longer limited to digital circuits but is included in so-called More-than-Moore devices. These devices are used, among others, for sensing, optics, Radio Frequency (RF), MEMS or power electronics. To enable this diversification of applications, the functionality of silicon technology was increased, and the porosification of silicon presented a simple and cheap way to allow its expanded use. Porous silicon (PSi) indeed exhibits properties that are remarkably different from bulk silicon and which can be fine-tuned by adapting the porous morphology. To fabricate PSi, conventional techniques such as lithography and dry etching can be used. Other approaches however exist, a well-established one being the use of electrochemical etching.

PSi was first discovered in the mid-1950s by Arthur Jr. and Ingeborg Ulhir [80]. The Ulhirs were initially studying new techniques for polishing silicon surfaces when they accidentally fabricated the first porous silicon film. Their discovery was however more or less forgotten until the late 1980s and early 1990s, when PSi was found to exhibit properties of quantum confinement [81, 82]. This breakthrough spurred the scientific communities' interest in PSi and its many properties. Among the topics that emerged back then was the fabrication of porosified silicon membranes [83, 84]. Membranes can be defined as interfaces, separating two phases and restricting the transport between those two phases. Porous silicon membranes (PSiMs) are therefore permeable barriers, formed by detaching a porous layer from the underlying bulk silicon substrate. Pores become open-ended channels, enabling a flow-through applications. Obvious applications for PSiMs therefore include separation, adsorption or transport of molecules, ions or small particles. PSiMs are also important tools for the characterisation of PSi, as its properties can be studied in isolation, without the subjacent silicon substrate. The flexibility of the porosification process enables the fabrication of PSiMs with a wide variety of structural features: PSiMs can be ultra-thin or up to 1 mm thick; they can be qualified as micro-, meso- or macroporous depending on the pore size and their porosity can vary at will within a

single membrane.

3.1.1 PSi fabrication

The global interest of the scientific community in porous silicon can be explained by its wide range of features. The multiple fabrication processes enable the creation of porous silicon with various structural attributes. Pore size, for example, can vary from a few nanometres to tens of microns. Likewise, the film thickness can be expressed both in nanometres or in microns. Other parameters, like porosity, pore distribution and pore shape can also be controlled by the fabrication process.

Several approaches are therefore used to fabricate porous silicon, mainly relying on dry etching or on electrochemistry. The dry etching technique requires the use of a hard mask to define the holes that will be anisotropically formed by plasma etching. For the electrochemical approach, two principal methods can be distinguished: electrochemical etching and stain etching. The latter relies on the spontaneous electroless etching of silicon when placed in an oxidising hydrofluoric acid (HF)-containing solution [6]. When combined with metal films or nanoparticles, this technique becomes Metal-Assisted Chemical Etching (MACE) [85]. As to date no recent works concerning MACE-fabricated PSiMs have been found by the authors, this review will not include this fabrication technique and mainly focus on electrochemical and dry etching.

Electrochemical etching, which is also called the *anodisation* method, relies on the wet etching of monocrystalline silicon under the application of an electric current, using an electrolyte containing HF [86]. The current method can be carried out in either a double bath etching cell, with two platinum electrodes, or in a single bath etching cell where the silicon wafer serves as anode and a platinum wire acts as cathode. The etching cells are fabricated in HF-resistant materials, like Teflon[®] or Polyether ether ketone (PEEK). A schematic illustration of a single etching cell is represented in Figure 3.1.

Two regimes can be observed within the electrochemical process: pore formation and electropolishing. The transition between those two regimes depends on the substrate's properties, like doping or crystalline orientation, and the fabrication parameters, such as electrolyte concentration and temperature, but is mostly governed by the ratio between the applied current density and the concentration of fluorine ions, as depicted in Figure 3.2. The formation of pores in silicon is controlled by en-

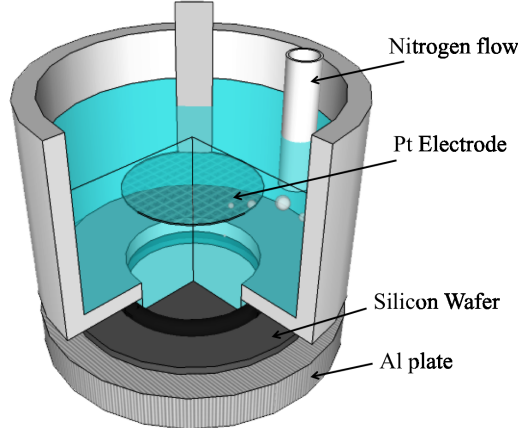


Figure 3.1: Illustration of a single-bath electrochemical etching cell, Reprinted from [77].

ergy bonds. By applying a current, electron holes reach the surface and weaken the Si-H bonds. These bonds can then be substituted by Si-F bonds, which are strongly polarised. Si-Si bonds are then weakened and attacked, resulting in the release of SiF_4 molecules and the creation of a gap in the silicon surface. After pore nucleation, current flows preferentially at the tip of the pore. A space-charge region is also created at the pore walls, preventing lateral etching, as illustrated in Figure 3.3. By increasing the current density, this space charge region decreases. When the pores start to merge, the electropolishing regime is reached.

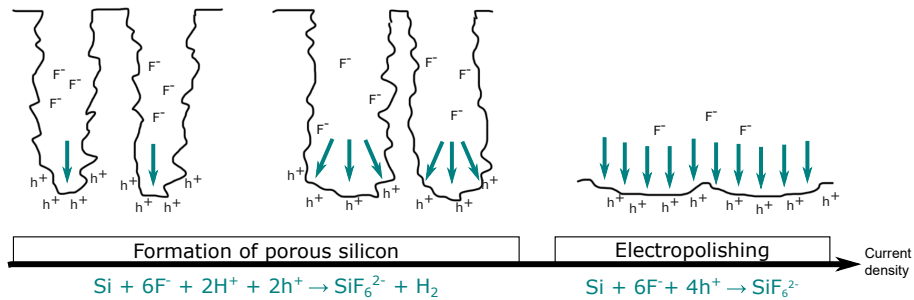


Figure 3.2: Mechanism of pore formation with increasing current densities. The holes reaching the pore tips weaken the Si-H surface bonds and allow the dissolution of the silicon. By increasing the current density, the pore walls become thinner, until the pores merge in the electropolishing regime. Adapted from [77].

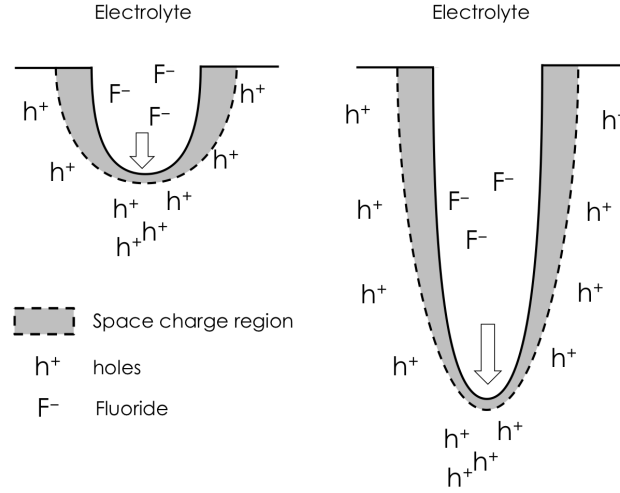


Figure 3.3: Illustration of the pore growth via the formation of a space charge region that tends to passivate the pore wall and induce the etching of the pore tip. Reprinted from [77].

3.1.2 PSi characterisation

3.1.2.1 Pore size

PSi is often classified based on the *pore size*: microporous PSi has an average pore diameter inferior to 2 nm; mesoporous PSi is characterised by a pore size ranging from 2 to 50 nm; and macroporous PSi has a pore size larger than 50 nm. The pore size is mainly influenced by the doping of the silicon substrate, as described in Table 3.1.

Wafer type	Doping conc.	Pore size
n-doped Si	$< 10^{18} \text{ cm}^{-3}$	wide range, going from nanometres to micrometres
p-doped Si	$< 10^{15} \text{ cm}^{-3}$	large pores in the μm -range with nanometric pores within
p+ Si	$10^{15}\text{-}10^{18} \text{ cm}^{-3}$	1 to 10 nm
p++ & n++ Si	$> 10^{19} \text{ cm}^{-3}$	from 10 to 100 nm

Table 3.1: Effect of wafer doping concentrations on the PSi pore size, adapted from [87].

The pore size can however be fine-tuned by selecting the appropriate current density and electrolyte composition. Lower current densities lead to smaller pores, for example [87–89]. Pore size can be imaged using

transmission electron microscopy (TEM), scanning electron microscopy (SEM) or atomic force microscopy (AFM) [90–92].

3.1.2.2 Pore depth & porosity

Another feature of PSi is the *pore depth* or *layer thickness*. The thickness of the layer is directly correlated to the duration of the electrochemical etching. The etch speed is however related to the current density, the electrolyte composition and the substrate, as summarised in Table 3.2. It is important to highlight that, for long etching times, the etch rate can vary due to the local change in HF concentration at the pore tip caused by diffusion limitation.

The *porosity* of the porous layer is another important parameter and is defined as the ratio of empty volume to the total volume of the porous region [90]. One can however distinguish open porosity (i.e. the volume of pores that are accessible to a given analyte) and the closed porosity (i.e. the volume of pores that are inaccessible), whose sum results in the total porosity. The porosity is strongly dependent on the current density, increasing with higher current values (Table 3.2) [90].

Increase of	Porosity	Etching rate
HF concentration	Decreases	Decreases
Current Density	Increases	Increases
Etching Time	Increases	-
Temperature	-	-
Doping (<i>p</i> -type)	Decreases	Increases
Doping (<i>n</i> -type)	Increases	Increases

Table 3.2: Effect of electrochemical etching parameters on the PSi formation, adapted from [93].

Both the layer thickness and the porosity can be characterised using the gravimetric method. This method relies on the selective etching of the porous silicon, which is much more reactive to wet etching solution such as KOH or NaOH than monocrystalline silicon because of its large surface area. It measures the weight of the sample before porosification (m_1), after porosification (m_2) and finally after the removal of the PSi layer (m_3). These values, as well as the etched surface A and the density of elemental silicon δ_{Si} , can then be used to express the layer porosity

P and the layer thickness L .

$$P = \frac{m_1 - m_2}{m_1 - m_3} \quad (3.1)$$

$$L = \frac{m_1 - m_3}{A\delta_{\text{Si}}} \quad (3.2)$$

The drawback of the gravimetric method is that it is a destructive method. Cross-section SEM, which is often used for the characterisation of the layer thickness, is also a destructive method. A non-destructive technique for the characterisation of both porosity and layer thickness is the *spectroscopic liquid infiltration method* (SLIM), which is based on Reflective Interferometric Fourier Transform Spectroscopy (RIFTS). These techniques rely on the optical response of the porous layer in the visible light range. The reflectance spectrum of the porous silicon layer is acquired using a light source and a spectrometer, coupled with an optical fibre, as illustrated in Figure 3.4, a. The spectrum is acquired twice, at the exact same spot, using two different filling media; usually the first spectrum is acquired in air, and for the second spectrum the PSi layer is filled with a solvent. Because there is a double reflection on the top and bottom of the PSi layer, the reflectance spectra exhibit Fabry-Pérot fringes, which result from destructive and constructive interferences (Figure 3.4, b). When applying a Fourier transform to each spectrum, a frequency peak is observed (Figure 3.4, c). The position of each peak translates the effective optical thickness (EOT), which is related to the layer thickness L and the average refractive index of the filled porous layer n such that $\text{EOT}=2nL$. The EOT values for both filling media are fed into a two-component Bruggeman or Looyenga equation, that enables the computation of both the thickness and the porosity of the PSi layer [90].

3.1.2.3 Morphology

The *morphology* of the pores is also affected by the anodisation parameters, as well as by the doping and the crystal orientation of the silicon substrate: pores can be straight or branched, cylindrical or pyramidal. A large variation in microstructure exists, as illustrated in Figure 3.5. PSi can also have a layer structure, where different morphologies appear within the depth of the layer.

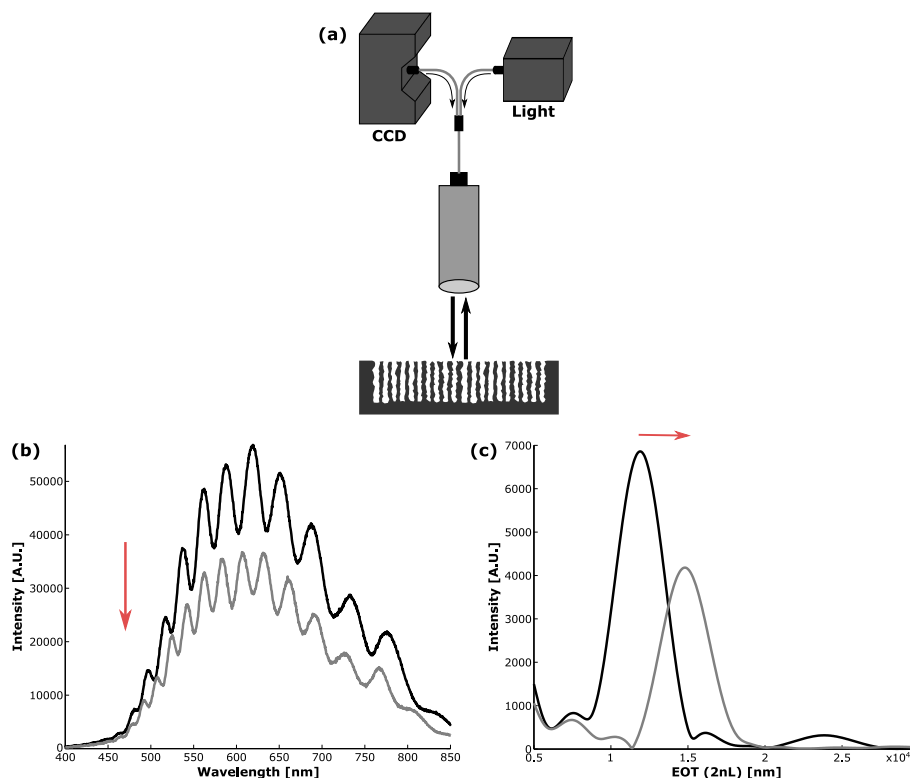


Figure 3.4: Schematic illustration of the working principle of SLIM using white light reflectance interferometry. (a) The surface of PSi is illuminated perpendicularly using a halogen light source. The light source is coupled with a CCD spectrometer using a bifurcated optical fibre, which acquires the light reflected from the PSi surface. (b) The double reflection at the interfaces of the PSi layer creates interference fringes that can be observed in the reflectance spectra; the black and grey traces are the spectra for PSi in air and ethanol, respectively. (c) Fourier transforms of the reflectance spectra from (b) resulting in a peak whose position translates the effective optical thickness, $2nL$. Reprinted from [94].

3.1.3 PSi nanostructures

When adapting the current density overtime during a single electrochemical etch, porous multi-layers with different pore sizes and porosities can be created. Examples of the different structure that can be obtained using specific current wave forms are illustrated in Figure 3.6.

A PSi double layer is obtained by lowering the current density at a

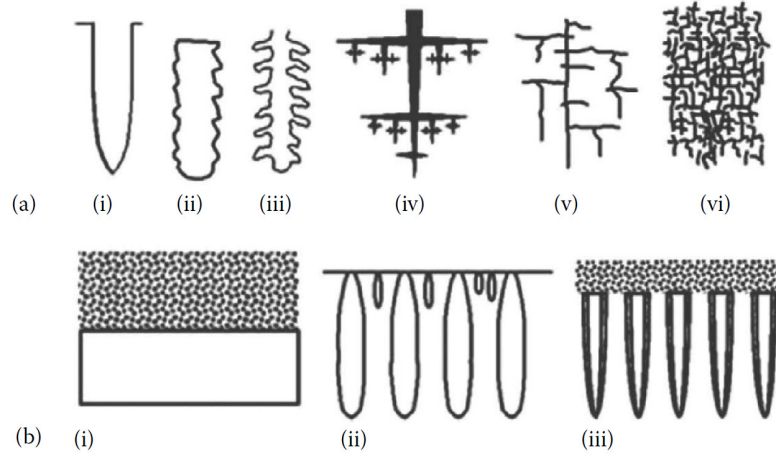


Figure 3.5: Illustration of various pore microstructures : (a) variants in branching; (i) smooth pore wall; (ii) branches shorter than diameter; (iii) second level branches only; (iv) dendritic branches; (v) main pores with second and third level branches; (vi) dense, random, and short branched; (b) depth variation of PSi layer; (i) single layer of microporous Si; (ii) single layer of macro-porous Si with smaller pores near the surface; (iii) a layer of microporous PSi on the top of macroporous PSi. Reprinted from [95].

given time during the porosification; the resulting structure is a stack, with large pores and a high porosity on top, and small pores with a lower porosity on the bottom. This type of structure is particularly interesting to discriminate between molecules of different sizes, as bigger molecules may not be able to penetrate the bottom layer [96, 97].

The second PSi nanostructure, illustrated in Figure 3.6, is the Bragg stack, also called Bragg mirror or Bragg reflector. This structure is achieved by alternating between two different current densities for specific amounts of time. The result is a periodic stack of low and high porosity regions. Each region is characterised by a given refractive index n and layer thickness L , such that the nL products of each region are equal to each other and to one fourth of the target wavelength to be reflected by the stack. The reflectance spectrum of the Bragg stack indeed exhibits a large high reflectivity region, centred around the target wavelength [90].

A microcavity is a variation of the Bragg stack, into which a defect layer has been introduced. This defect layer is often a wide high porosity

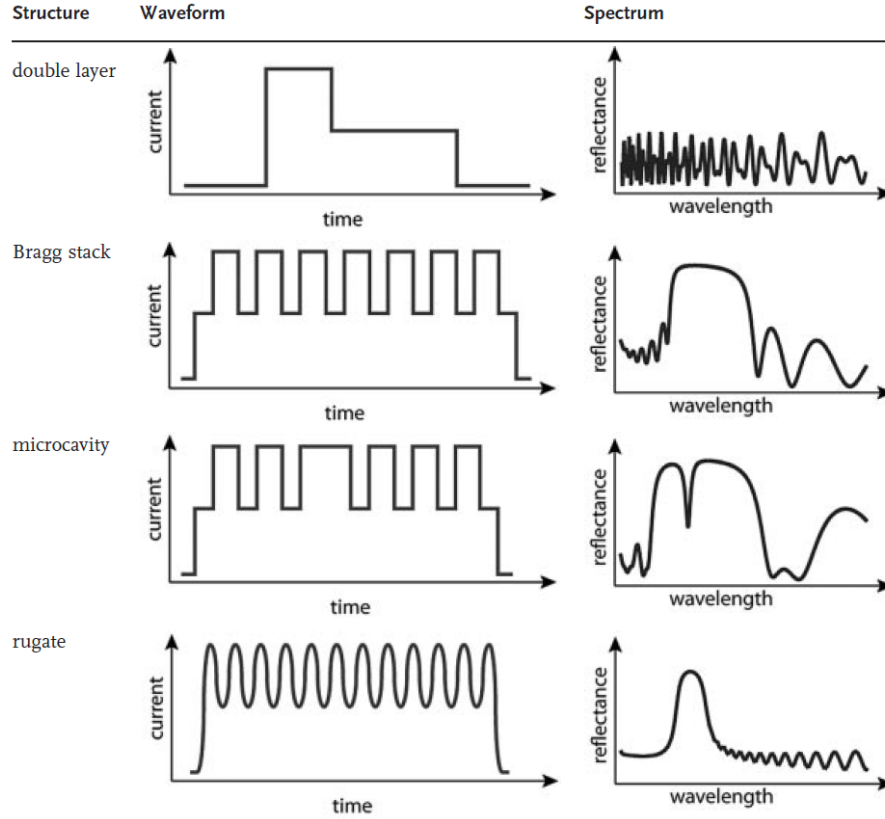


Figure 3.6: Illustration of various PSi nanostructures obtained by varying the current density during the electrochemical etching. Reprinted from [90].

layer, whose EOT corresponds to one-half of the target wavelength of the Bragg stack and which creates a sharp dip in the high reflectivity region of the Bragg stack spectrum. The position of this peak depends on the position of the defect layer and is very sensitive to changes of the refractive index inside this defect layer. Coupled with the fact that it is a small spectral feature, it is often used for sensing applications [98].

A rugate filter also exhibits a small spectral feature like the microcavity, but is more similar to the Bragg stack: it is a periodic stack of porous silicon, but whose pore size and porosity are dictated by a sinusoidal current waveform. The reflectance spectrum of a rugate filter therefore exhibits a high reflectivity peak centred around a central wavelength. The position of the peak depends on the both the current densities

and the frequency of the sinusoidal waveform; the width of the peak depends on the refractive index contrast between the extreme current density values; and the peak height depends on the number of periods in the sinusoidal waveform [90, 98].

3.2 Porous silicon membranes

3.2.1 Porous membranes fabrication techniques

PSiMs have been fabricated using many different strategies, but all of them can be divided into two categories: the ones relying on electrochemical etching to form the pores, and the ones relying solely on other microfabrication techniques. A summary of PSiM fabrication techniques is presented in Figure 3.7.

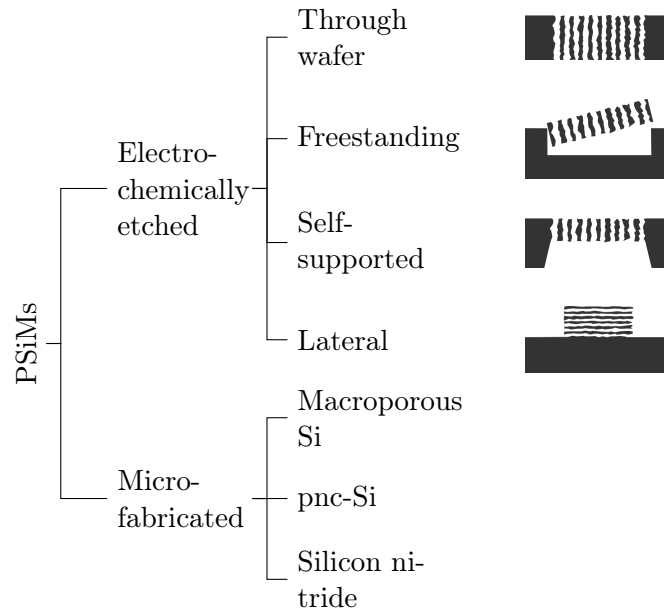


Figure 3.7: Summary of porous membranes fabrication techniques.

For the electrochemically etched PSiMs, four different routes can again be distinguished: (1) the anodisation through the entire silicon wafer of standard thickness [99–103]; (2) the anodisation of part of the silicon wafer and "lift-off" of the porous matrix, creating freestanding PSiMs (see Section 3.2.1.1); the fabrication of self-supported PSiMs us-

ing anodisation and microfabrication techniques (see Section 3.2.1.2); and (4) the creation of lateral PSiMs using anodisation and microfabrication techniques (see Section 3.2.1.3). Each approach has its strengths and weaknesses. Through wafer thickness anodisations are very time-consuming and result in non-uniform membranes, freestanding membranes are easy to produce but difficult to handle; the fabrication of lateral and self-supported membranes requires the access to microfabrication techniques but their integration into devices is straightforward.

Other approaches that do not rely on electrochemical etching are used to fabricate porous membranes with highly controlled pore geometries. They require techniques such as lithography, dry or wet etching, deposition or annealing. Three different types of non-anodised porous membranes can also be distinguished: macroporous silicon membranes, nanoporous silicon nitride membrane and porous nanocrystalline silicon membranes (pnc-Si). All three types are discussed in further details in Section 3.2.1.4.

3.2.1.1 Freestanding PSiMs

Freestanding PSiMs are fabricated by electrochemically etching a silicon substrate over a specific area and then consecutively lifting off the porous region. Several approaches exist for the lift off of the membrane. The most common one is the electropolishing of a small cavity below the porous region, by applying one or multiple high current pulses at the end of the electrochemical etch. In order to decrease the amplitude of these current pulses, the HF content of the electrolyte can be lowered [104]. In another approach, Preobrazhenskiy *et al.* used an alkaline potassium hydroxide (KOH)-based solution for the membrane detachment as it enables an increase in porosity as well [105]. Mechanical detachment is equally possible, by fixing the porous region on tape [106] or polydimethylsiloxane (PDMS) [107], as illustrated in Figure 3.8.

Additionally, Figure 3.8 points out another advantage of freestanding membranes: the possible recycling of the silicon substrate. It is indeed possible to reuse the same silicon wafer for the fabrication of multiple membranes [106, 108]. It is important to note that freestanding PSiMs may, however, present a strong spatial anisotropy in terms of morphological features. Due to the directionality of the electrochemical etching and the carrier depletion for long etching times, porosity gradients can be observed, either on the surface or deeper in the membrane.

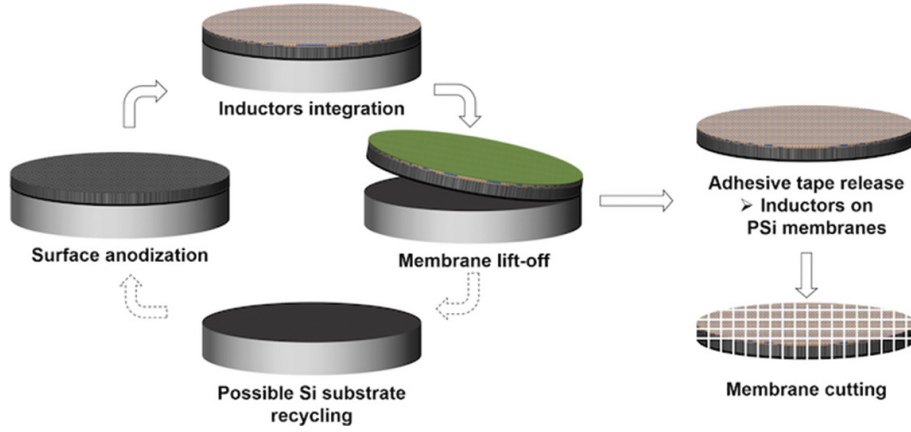


Figure 3.8: Illustration of the mechanical detachment of a PSi membrane and the possible recycling of the silicon substrate. Reprinted from [106].

The integration possibilities of freestanding PSiMs are numerous. To establish electric contacts, metals can be deposited on the porous membrane, as illustrated in Figure 3.9,a. These metals include vacuum evaporated gold [109], electroplated copper [105,110] or sputtered indium tin oxide (ITO) [109,111]. For microfluidic integration, PDMS can be cast on top of the still attached PSi films, which then serves as supporting material during detachment [104,112–114]. This method is depicted in Figure 3.9,b. Another integration approach consists simply in the deposition of the membrane on top of a specific substrate. This substrate can be another PSi layer [115], a gold slide [116], an ITO slide [117], a glass slide [118–120] or poly(ethylene glycol) diacrylate (PEGDA) matrices [121,122] (see Figure 3.9,c).

Composite structures can also be fabricated using freestanding PSiMs. Cench *et al.* constructed an Anodic Aluminium Oxide (AAO)-PSi hybrid membrane, relying on capillary forces to firmly attach the PSiMs to the alumina [123]. Other composite structures rely on the deposition of specific materials inside the porous matrix, like the electroless deposition of silver nanoparticles [104,112,113] or the chemical vapour infiltration of graphene [124]. Furthermore, PSiMs can serve as templates for the growth of nanowires, as illustrated by the work of De La Luz-Merino *et al.* [125] in which CuInSe_2 was electrodeposited onto glass/transparent conductive oxide (TCO)-supported PSiMs in order to develop novel material for solar cell applications.

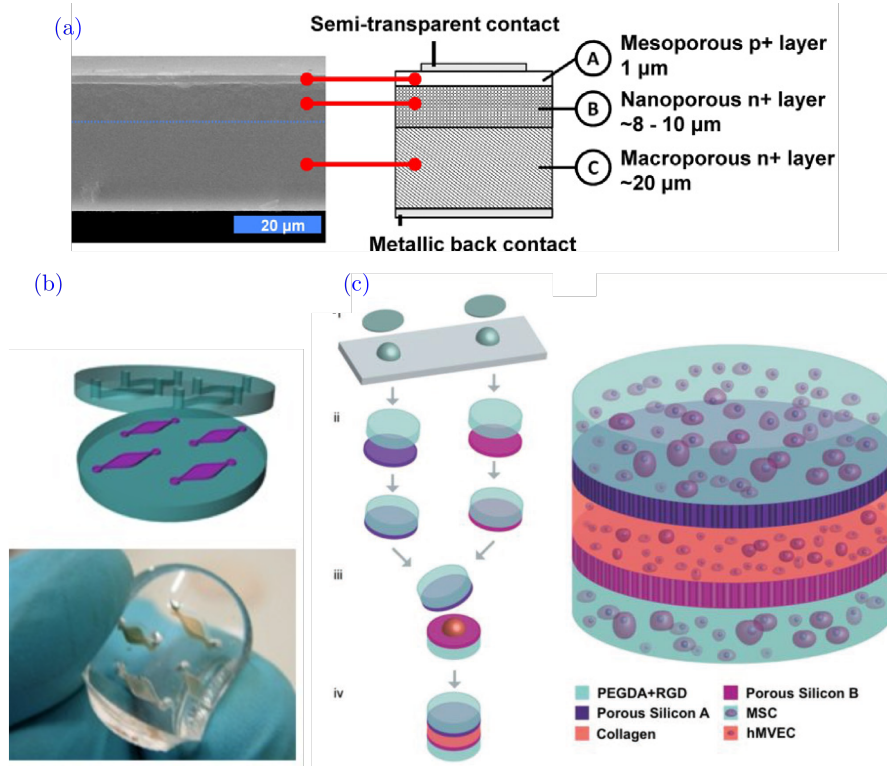


Figure 3.9: Examples of freestanding PSi membranes: (a) Integration of a PSiM using vacuum evaporated gold, aluminium and sputtered ITO, reprinted from [109]; (b) PDMS-integrated PSiM, reprinted from [112]; (c) Integration of two PSiMs with a cell-laden hydrogel and two PEGDA matrices, reprinted from [122].

3.2.1.2 Self-supported PSiMs

Self-supported PSiMs were developed when the need for robust structures with a high optical quality arose [126]. Freestanding or "etch-through" membranes could not fulfil these needs, as they are often characterised by strong spatial anisotropies and porosity gradients. In the "self-supported" manufacturing approach, microfabrication techniques are used to create cavities in the silicon substrate before or after the electrochemical etching. Depending on the doping of the silicon substrate, wet or dry etching techniques are employed.

Figure 3.10 illustrates a process employed for the fabrication of self-supported PSiMs using the wet etching on a standard silicon wafer: (a-

b) both sides of the wafer are coated with silicon nitride (Si_3N_4) layers and covered with photoresist; (c-d-e-f) the SiN layers are patterned using photo-lithography and buffer oxide etching; (g) the cavity is etched using KOH wet etching until only a few micrometres of silicon remain; (h) the remaining silicon nitride is removed using buffer oxide etching; and (i) electrochemical etching is carried out in order to form the pores. Similar processes have been used in multiple works [127–132] and the resulting membranes are comparable to the ones represented in Figure 3.11, a-b.

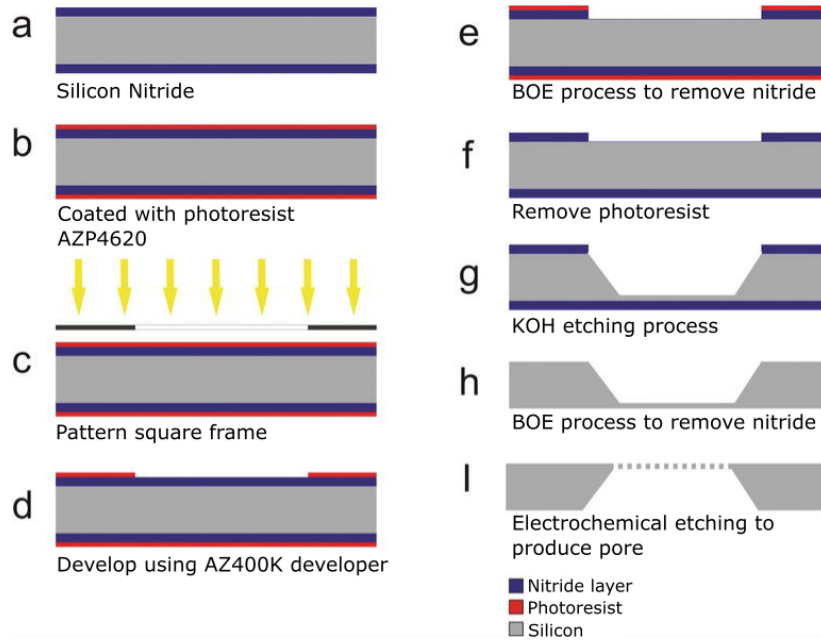


Figure 3.10: Fabrication processes for self-supported PSiMs using combinations of dry, wet and electrochemical etching on standard silicon substrates, adapted from [130].

A sacrificial porous silicon layer at the backside can also be used as an alternative to the wet etching [133]. An additional step that is often added to this process is the use of dry etching after the anodisation in order to obtain a fully porous membrane [134]. Indeed, only a few pores open the rear side of the membrane after the electrochemical etching. This can be explained by the fact that, once the first channels reach the backside of the membrane, the current goes through these paths of least resistance and the process stops [135].

Other processes first anodise the silicon wafer over a certain thickness

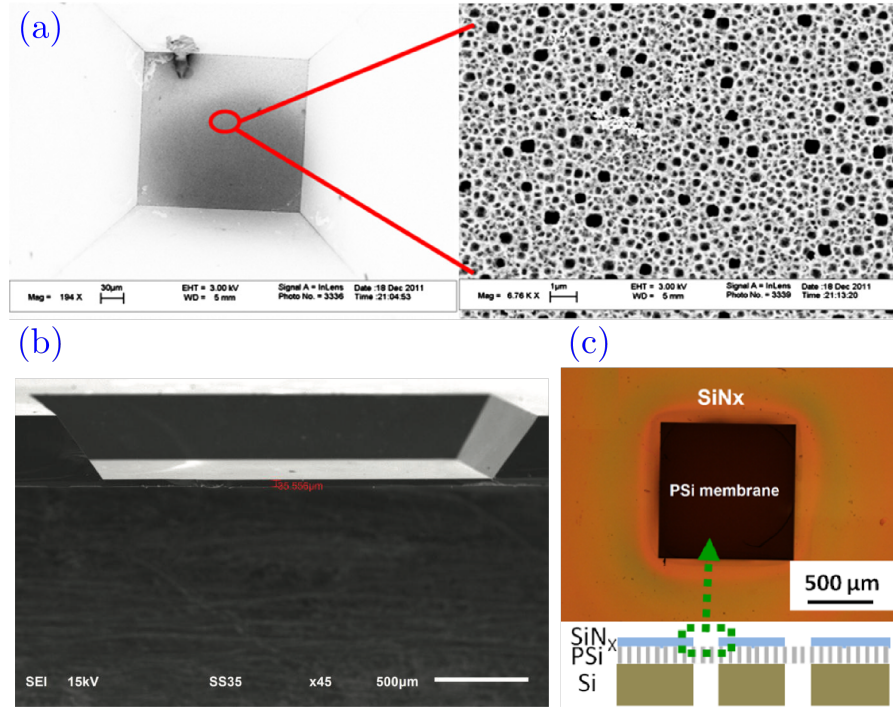


Figure 3.11: Examples of self-supported PSiMs (a) Top view of a mesoporous membrane created via wet etching reprinted from [136]; (b) Top view of a PSiMs created via wet etching, reprinted from [130]; (c) Top view and schematic view of a PSiMs fabricated using DRIE after anodisation, reprinted from [10].

and area, before opening the membranes from the backside. This can be achieved using dry etching techniques like Deep Reactive Ion Etching (DRIE) [10, 126, 137–140], as shown in Figure 3.11,c. For this type of process, the oxidation of the PSi prior to the etching is desirable, as this may reduce the etch rate of the porous matrix and create a more uniform membrane. Mechanical grinding is another method used to open the membrane [141–145].

In some works, Silicon-On-Insulator (SOI) substrates are preferred to standard bulk Si, and the top thin Si layer, also called device or active layer, serves as membrane. The handle, which is the thick Si layer, can be opened using wet or dry etching techniques prior to the electrochemical etch [146–148], as depicted in Figure 3.12. In this Figure, a single nanopore is formed in the device of the SOI substrate using the following steps: (a-d) fabrication of a Si_3N_4 mask and KOH wet etching of the

handle; (e) wet etching of the SiO_2 layer; (f-h) deposition and patterning of a new Si_3N_4 mask; (i-j) patterning of the Si_3N_4 on the device side of the wafer; (k) pre-patterning of the pore using KOH wet etching; and finally (l) the electrochemical etching of the single nanopore. Similar processes can be used to etch membranes with multiple pores [149,150]. The pre-patterning step on the front-side using KOH is also often employed for the creation of macropores, as it induces very regular pore shapes and sizes.

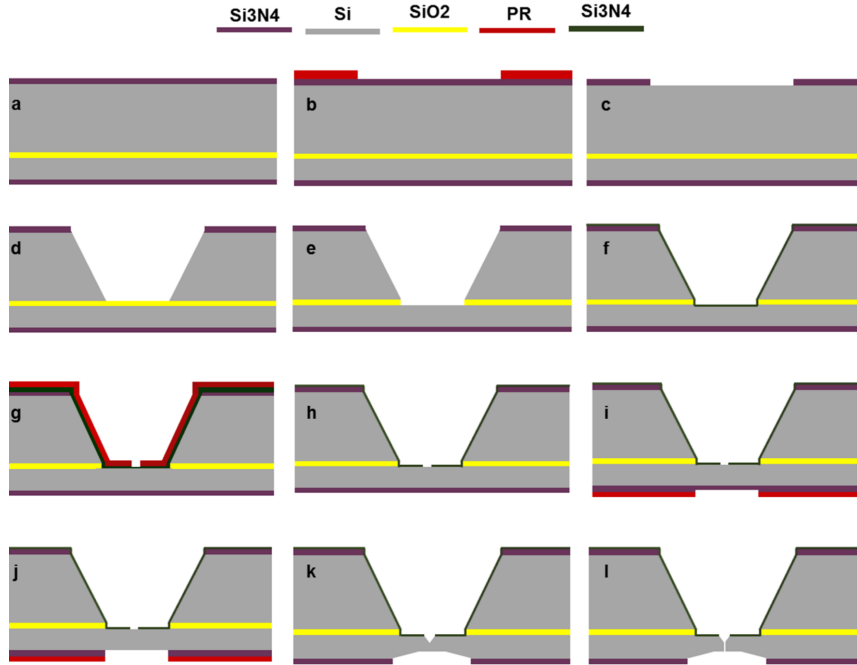


Figure 3.12: Fabrication processes for self-supported PSiMs using combinations of dry, wet and electrochemical etching on SOI substrates, reprinted from [146].

The integration of self-supported PSiMs into devices is more straightforward than for freestanding PSiMs. As they are easier to handle, they can be incorporated into reusable custom-built setups that can include chambers, channels and electrodes [127, 137, 138, 142, 143, 150]. For individual integration solutions, PDMS is a good alternative [10, 126, 128, 129]. Other possible substrates are glass slides [151], carbon-paper electrodes [141] or poly(methyl methacrylate) holders [131, 132]. Self-supported PSiMs can also be used as substrates onto which the devices are trans-

ferred [133].

Hybrid structures can be achieved by depositing materials inside the pores, including by the chemical vapour infiltration of graphene [144] or the growth of carbon nanotubes using thermal chemical vapour deposition [152]. Metals can be coated inside the porous matrix as well. Platinum was immersion plated on PSi to serve as a catalyst for micro fuel cell electrodes; since it was deposited before opening the PSi membrane from the backside of the silicon wafer, it also served as an etch-stop layer during the plasma etching [153, 154]. Other methods which induce electrical conductivity in fuel cell electrodes include the sputtering and electrodeposition of titanium, nickel and gold layers [155].

3.2.1.3 Lateral PSiMs

In order to facilitate the integration of PSiMs, lateral porous membranes have been fabricated using SOI substrates, with pores parallel to the substrate surface [156]. The building process for these lateral PSiMs was developed by *Leïchlé* and co-workers and can be divided in four major steps: (1) the micro-channels surrounding the membrane are created using dry etching; (2) the Cr/Au working electrode is deposited and patterned where the anodisation will be initiated; (3) the PSi membrane is created by electrochemical etching, using the deposited metal as anode and a Pt wire as cathode; (4) the Cr/Au is removed via wet etching, the fluidic inlet and outlet are made by sandblasting and the chip is encapsulated via anodic bonding.

The resulting device is depicted in Figure 3.13. As can be seen in the SEM picture in this figure, the pores are horizontal. This can be explained by the fact that, after their initiation on the membrane sidewall, the pores propagate following the current lines; these current lines are horizontal, since a metal electrode has been deposited on the opposite side wall.

To improve the process described above and allow the use of standard silicon substrates, ion-implantation can be used in order to define the region of the future membrane [157]. This however puts constraints on the height of such a membrane, which is limited by the thickness of the doping diffusion layer.

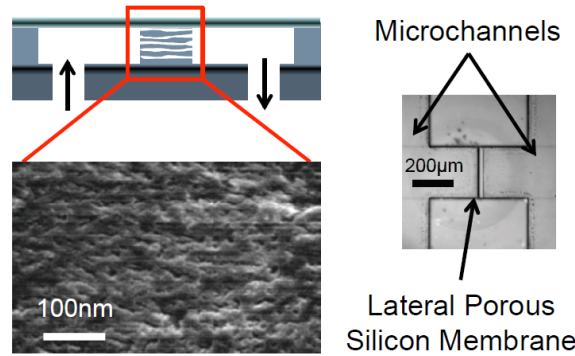


Figure 3.13: Schematic view and SEM images from a lateral PSiM, reprinted from [156]. In terms of physical properties, the obtained membrane is about $20\text{ }\mu\text{m}$ -high and the pores are $10\text{ }\mu\text{m}$ -long.

3.2.1.4 Microfabricated porous membranes

Porous silicon membranes are also prepared without the use of electrochemical etching. To create PSiMs with highly defined geometries, a combination of photolithography and Reactive Ion Etching (RIE) may be used [158–162]. The advantage of this type of process is that the pore size and periodicity can be controlled by the photolithographic pattern, as depicted in Figure 3.14,a. Pore diameters as low as 130 nm can be reached by using a combination of e-beam lithography and DRIE [163–165]. Beside photolithographic masks, block-copolymers have also been used as templates, iron oxide nanodots then serving as "resist mask" to create the pattern via DRIE [166, 167]. This method however resulted in many defects in the pore arrangement and pore size distribution.

Directly patterning the channels onto the silicon substrate is nonetheless very time-consuming, especially when using e-beam lithography, which has spurred the interest in alternative processes. One of these alternatives is the spontaneous creation of pores during the rapid thermal annealing of amorphous silicon. The thus-formed membranes are called porous nanocrystalline silicon membranes (pnc-Si). The pores originate from the nucleation of nanocrystals inside the amorphous silicon during the rapid thermal anneal [168, 170]. As can be seen in Figure 3.14,b, very thin mesoporous membranes can be created using this technique.

A last type of microfabricated porous membranes, which could be consid-

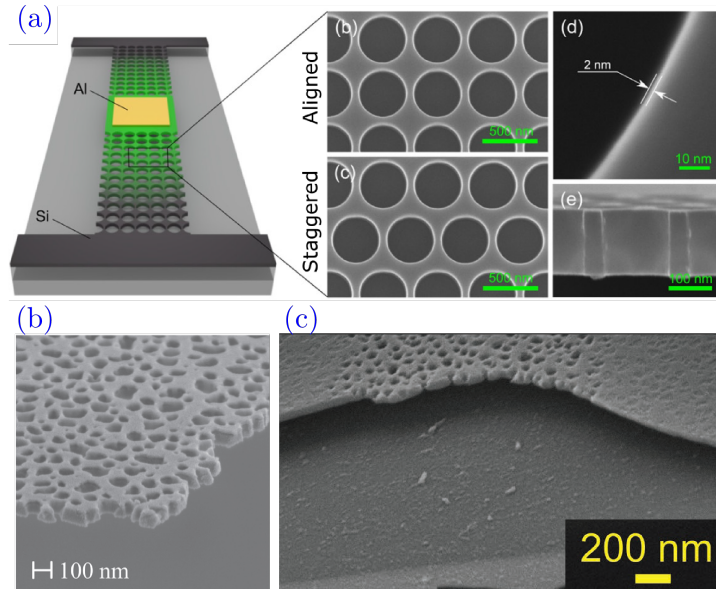


Figure 3.14: Microfabricated PSiMs: (a) macroporous membrane prepared using standard microfabrication techniques like e-beam lithography and RIE, reprinted from [164]; (b) pnc-Si membrane, reprinted from [168]; (c) nanoporous silicon nitride membrane, reprinted from [169].

ered as a hybrid form of PSiM, is nanoporous silicon nitride membranes. These membranes can be fabricated using the same microfabrication techniques as plain silicon membranes, such as optical lithography and dry etching [167, 171–173], as depicted in Figure 3.14,c. Focused ion beam (FIB)-milling can be used as an alternative to plasma etching in order to probe holes into the membrane [174, 175]. Finally, nanoporous silicon nitride membranes can also be combined with pnc-Si membranes, where the latter serves as a template for the dry etching of the nitride layer [168, 169, 176].

3.2.2 Properties

The immense appeal of PSi lies in the alteration of the properties of bulk silicon by the introduction of nano- and microscale porosity into the material. Moreover, these properties are highly tuneable, mostly by fine-tuning the structural features of the porous matrix. By carefully selecting certain morphological parameters, such as porosity, pore size or layer thickness, scientists have been able to adjust the mechanical,

electrical, thermal, and biochemical properties of PSi. By detaching PSi layers from their bulk substrate, properties can be altered further, as the effect of the underlying substrate is removed. While the removal of the underlying Si substrate has no effect on the intrinsic properties of PSi, it has an impact on the properties of PSi-based devices. The scope of this impact is detailed in the present section.

3.2.2.1 Mechanical properties

Monocrystalline silicon has exceptional mechanical properties: it is fairly hard, characterised by a Knoop hardness of 8.5 GPa (versus 6.5 GPa for steel) [177]; it is remarkably strong but very brittle, which is reflected by a fracture toughness of approximately $1 \text{ MPa}\sqrt{\text{m}}$ [178]; its yield strength of 7 GPa is higher than that of steel, which ranges from 2 to 4 GPa depending on the variety [79]. However, when silicon is porosified, its mechanical properties are altered. This change depends on the three main morphological features that characterise porous silicon: thickness, porosity, and average pore size. Understanding and optimising the mechanical properties is important for the fabrication of PSiM-based devices, as deformations of the internal structure impact the chemical reactivity and physical properties of the PSi, and as the cracking of the membrane affects the durability and operability of the devices. Mechanical properties of PSi and PSiMs must be considered in all the integration schemes developed in Section 3.2.1, as the fragility of PSi curtails the number of possible process steps after the porosification.

The stiffness of PSi is often studied by simulating or fabricating PSi membranes, in order to remove the impact of the underlying bulk Si [179–183]. Both experimental and theoretical results highlight the dependence of the Young’s modulus, which quantifies the stiffness, on morphological features: the modulus decreases with increasing porosity and is affected by the pore shape, especially by the presence of branches inside the pores. The evolution of the Young’s modulus with porosity is illustrated in Figure 3.15, in which results from several experimental and finite-element method (FEM)-based studies are compared. A fitting function of Young’s modulus E of PSi and PSiMs has been proposed and takes the form of a power law of porosity ϕ , defined as:

$$E_{PSi} = E_{Si}(1 - \phi)^m \quad (3.3)$$

The value of parameter m depends on the geometrical features but is

often chosen as $m = 1$ in simulations [168,175]. Other studies prefer the $m = 3$ [184,185] when considering mesoporous silicon. Gong *et al.* [183] modelled this parameter for several types of pore shape, with resulting values fluctuating around $m = 1$ and $m = 2$.

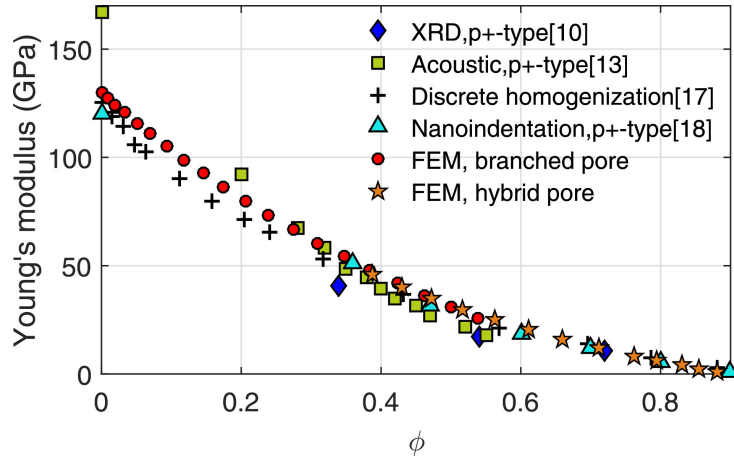


Figure 3.15: Evolution of PSiMs Young's modulus with porosity ϕ , obtained both experimentally and via simulations; reprinted from [183].

Additional experimental studies concentrate mainly on the residual mechanical stress inside PSiMs [168,185,186]. In Guider *et al.*'s work [186], it was observed that tensile stress is accumulated in the porous silicon layer during the electrochemical etching process. This tensile stress is partially compensated by the underlying substrate. However, when the PSi layer is lifted off the substrate, this stress is released and causes the bending of the PSiM. To quantify tensile residual stress, single bulge test measurements have been used on both pnc-Si and silicon nitride membranes: the fitted values of residual stress are in the order of 10-100 MPa, depending on the type of membrane [168]. Dariani *et al.* [185] extended the study of residual stress by using X-ray diffraction measurements to inspect the crystal lattice expansion and the perpendicular strain in PSiMs. The internal stress in supported PSi layers was attributed to the slight expansion of the crystal lattice inside PSi and the constraint of the perpendicular planes to the same interatomic spacing as the substrate. When the PSi layer is detached from its substrate, this constraint is released and the perpendicular strain is decreased.

The strength of PSiMs is often analysed via FEM-based simulations of the deflection and Von Mises stress [175,187,188]. It was observed

that deflection and stress are dependent on the thickness of the PSiMs: for thick membranes, the deflection and stress are nearly constant with respect to the externally applied pressure; thinner membranes exhibit both higher deflection and stress, which increases with the applied pressure. Reported values of Von Mises stress are in the order of 10 MPa for membranes which are a few tens of nanometres thick [187,188]. The total area of the PSiM also has an effect: smaller membranes accumulate less stress and withstand higher pressures. Additionally, pore size and porosity can be tuned to improve mechanical strength, by selecting smaller pore diameters and densities. These theoretical observations were verified experimentally on pnc-Si and nanoporous silicon nitride membranes [168,175].

3.2.2.2 Mass transport properties

One of the motives behind the development of open-ended PSiMs was to overcome the diffusion challenges attributed to PSi layers. To verify the added value of membranes, several studies compare the sensitivity of close- and open-ended PSi sensors [10,114,126,189]. The sensing scheme of these studies relies on the adsorption of analyte. Both experimental data and simulations show the increased sensitivity of PSiMs, which means that there is an increase in adsorption. Analyte adsorption has two limiting factors: the transport of the analyte and the adsorption kinetics. In PSi layers, the transport is diffusion-limited, causing a low sensitivity and slow sensor response time. For PSiMs, the transport of mass is influenced by both diffusion and convection, as the analyte flows through the pores, and can be described by the convection-diffusion equation:

$$\frac{\partial c}{\partial t} = \nabla \cdot (D \nabla c - c \mathbf{u}) \quad (3.4)$$

where c is the analyte concentration, D is the diffusivity and $\mathbf{u}$ is the flow velocity.

An increase of transport leads to an increase of adsorption, which itself translates in a high sensitivity and rapid response time. The increase in adsorption capabilities of PSiMs with respect to PSi layer is illustrated in Figure 3.16, where the average surface concentration of analyte was simulated for both a PSi layer in flow-over operation mode and a PSiM in flow-through operation mode [10].

The effects of physical properties, such as pore size, membrane thick-

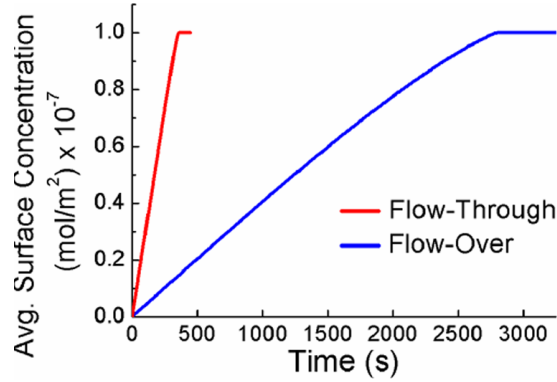


Figure 3.16: Simulated average surface concentration of analyte with the same diffusivity, captured by close- and open-ended PSi sensors as a function of time; reprinted from [10].

ness and porosity, on the transport of mass inside PSiMs remain to be investigated. Nonetheless, there is a lot of available knowledge concerning mass transport and diffusion on porous materials in general, which might apply to PSiMs as well [190, 191].

3.2.2.3 Optical and optoelectronic properties

The peculiar optical properties of PSi have been the subject of multiple studies and applications [6, 192]. These optical properties can be tuned in a wide range, depending, for example, on the pore size, which can be smaller than the optical wavelength. One of the main optical parameters of PSi, namely its refractive index, can be fine-tuned by adapting the porosity. Variations of the refractive index within a PSi membrane create specific structures, like Bragg stacks, microcavities and rugate filters [107, 119–121, 123, 193, 194]. Photoluminescence is also another major optical property of PSi, which is conserved when fabricating PSiMs [117]. The study of the optical properties of PSi and PSiMs often involves reflection and transmission measurement techniques. An accurate assessment is however not as straightforward since surface roughness and morphological inhomogeneities must be considered [192].

Reflection and transmission techniques can also be used to study optoelectronic properties of PSi and PSiMs, such as free carrier lifetime. One of these techniques is time resolved pump-probe transmission and reflection measurements, which have been used to study the dielectric

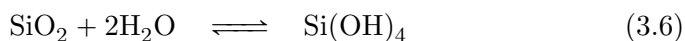
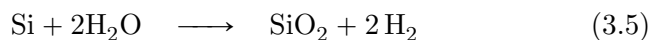
function and the carrier dynamics inside PSiMs [195–199]. Pump excitation induces the optical activation of free carriers, which then modify the dielectric function of the silicon skeleton of the membrane. This modification is however non-uniform, which can be easily explained: the laser pulse intensity drops when it propagates into the depth of the membrane; since free carriers reproduce the absorption of this pulse, the optical response is also depth-dependent. A model was developed by He *et al.* [195] to retrieve the change of the dielectric function based on the membrane depth. This model was then used in later work in order to map the time-dependent spatial distribution of free carriers [199]. It was found that Shockley-Read-Hall and Auger recombination governs the carrier's dynamics, while diffusion is insignificant. Constants related to excited charge transport, such as the recombination time and the carrier diffusion constant were also been modelled and studied [196, 199].

Zakar *et al.* [197, 198] complemented these findings and refined the analysis of the Auger recombination mechanism, which was found to amplify at the wavelength matching the infrared (IR)-active vibrational modes of the molecular impurities present on the pore's surface. These uncoverings were partly supported by Park *et al.* [200], as they found that a PSiM exhibits high transparency properties in the mid-wavelength infrared (MWIR) spectrum, while being strongly absorptive for shorter and longer wavelengths. The absorptive properties are linked to vibrational mode from surface impurities. The transparency of the PSiM could be modulated by external optical excitation, demonstrating the possible application of PSiMs as optical intensity modulators [198, 200]. Upon external optical excitation, excited free charge carriers interact with the MWIR signal, and transmittance is reduced. This decrease can reach up to $\sim 50\%$ and last from a few tens to a few hundreds of pico-seconds, all depending on the porosity, pore size and thickness.

3.2.2.4 Biochemical properties

PSi is often used in biomedical applications because of its biocompatibility [6]. Freshly etched PSi is naturally biodegradable in physiological solutions. The hybrid silicon structure covering the surface of the pore is very reactive and quickly oxidises when in the presence of water. Depending on the pH, this oxide can then react with water to form silicic

acid, as described in the following reactions:



This oxidative hydrolysis reaction is stable at low pH, but highly corrosive at high pH [201]. At physiological pH, there is only a slow dissolution of the porous layer. For some applications, the degradation of PSi in aqueous solutions is however unwanted and solutions to improve the stability have been investigated. The solutions are numerous for PSi layers and include among others thermal oxidation [202], hydrosilylation [203], atomic layer deposition [204] and thermal hydrocarbonisation [205].

Some of these techniques cannot be transferred to PSiMs, like thermal stabilisation for example: thermal oxidation causes the expansion of the porous structure, and in mesoporous PSiMs, this causes the physical deformation and sometimes even degradation of the membranes [140]. Baraket *et al.* [140] studied alternatives for thermal stabilisation and found that the chemical etching using standard cleaning procedures RCA1 and RCA2, combined with silanisation, offered the best stability in physiological media while preserving the integrity and the biocompatibility of the PSiMs.

Oxidation is not always the preferred method of stabilisation, especially for long term applications. In such cases, more adequate methods, such as thermal hydrocarbonisation [206], silanisation [207] or hydrosilylation [122] are easily applied to PSiMs. Hydrocarbonisation however compromises the biocompatibility of the membrane, and remedies were investigated: by simply pre-incubating thermally hydrocarbonised PSiMs in physiological media, the biocompatibility was recovered [206].

3.2.3 Applications

The main applications of PSi membranes can be divided into five domains: microfluidics, medical applications, sensing, energy conversion and electronics. Table 3.3 categorises the applications of PSiM-based devices covered by this review. All these applications use PSiMs instead of other substrates because of one or several of their unique properties. It is however important to note that some other properties of PSiMs may form obstacles to the introduction of those devices to the market. For instance, the lack of mechanical stability is a major hindrance for

many applications, which encourages the thorough study of all parameters affecting the mechanical properties of PSiMs.

Microfluidics take advantage of the structural properties of PSiMs. The most common application is filtration, which makes use of the open-ended pores. As pore size can easily be controlled during the fabrication process, filtration in PSiMs is most often size based [129, 156, 157, 208, 209]. For these applications, mesoporous membranes are used, meaning that only small nanometre-sized molecules can pass through the filter. Charge-based filtration is also possible, since the membrane can exhibit a certain permselectivity in the presence of a charged buffer and an electric field [209].

PSiMs are used in the field of biomedical science because of their biocompatibility. PSiMs are mostly used as scaffolds for cell culture and tissue engineering. Other biomedical applications include drug delivery patches [121], hemodialysis membranes [210], DNA purification devices [137, 138] or supports for the investigation of pore-spanning lipid membranes [211–217].

Sensing and diagnostics are the most common application fields of PSiMs, especially of mesoporous PSiMs. This can be explained by the physical, optical, mass transport and biochemical properties of the membranes: analyte solutions can flow through the membrane; target molecules can be retained, based either on their size or on interactions with the pore walls, and both flow and immobilisation can be detected by a change in the optical properties of the membranes. PSiM sensors can be divided into two categories: gas sensors and biosensors. Gas sensors often detect the presence of solvent vapours [194, 218, 219]. PSiM-based biosensors have been fabricated for many target molecules, which will be detailed in Section 3.3.2.

The electrical properties and permselectivity of PSi encouraged its use in the area of energetics. A recurrent use for PSiMs is therefore as base material for microfuel cells [220]. PSiMs are also used as anodes for lithium-ion batteries, since they have many advantages: they form an alloy of $\text{Li}_{22}\text{Si}_5$, resulting in a very high capacity; they have a very low discharge potential; by tuning the physical properties, they can withstand many charge/discharge cycles; and finally, they are cost-effective [108, 221]. An exhaustive list of PSiM applications for Li-ion battery anodes is detailed by Luaïs *et al.* [222].

The last area of applications considered in this section is electronics. The low thermal conductivity of PSiMs renders them ideal substrates for thermal devices such as micro-heaters [133,151,223]. PSi is also used for RF isolation, in the form of layers or membranes [106]. The interest of PSi for RF applications originates from its lower effective resistivity and permittivity compared to bulk silicon which translate to a silicon-based substrate which is characterised by lower electromagnetic losses, reduced crosstalk and much weaker signal harmonics distortion. The low thermal conductivity is however a hindrance for RF application, as it causes the integrated circuit to self-heat. Other microelectronic-related applications include optoelectronic devices, like wavelength modulators [200] or light emitting capacitors [117].

Table 3.3: Recent advances in the applications fields of porous silicon membranes (*Note: C, F, FT, L, M, S stand for, respectively, Commercial, Freestanding, Full Thickness, Lateral, Microfabricated and Self-supported.*)

Application field	Membrane characteristics	Membrane thickness	Detailed application	Ref.
Microfluidics	M, mesoporous	15 nm	Size and charge selective filtration	[224]
	S, mesoporous	5 μm	Filtration of small biological molecules from mixtures	[129]
	L, mesoporous	10-20 μm	Size and charge selective filtration	[156, 157, 208, 209, 219]
Medical	S, macroporous	215 μm	Electro-osmotic pump	[142]
	F, macroporous	4.29 μm	Drug delivery patch and optical monitoring of drug release	[121]
	M, macroporous	50-60 nm	Culture of human umbilical vein vascular endothelial cells	[176]
	M, macroporous	500 nm	Reconstruction of the intestinal barrier of a trout via cell culture	[172]
	M, mesoporous	75 nm	Small-format hemodialysis with high toxin elimination capabilities	[210]
	M, macroporous	30 μm	Transmigration assay for cancer cells	[159]
	F, mesoporous	276 μm	Scaffold for culture of oral mucosal epithelial cells	[207]
	C, macroporous	1-10 μm	Support for the investigation of pore-spanning lipid membranes	[211-217]
	M, mesoporous	30 nm	Cell culture of endothelial cells	[171]
	F, macroporous	12-15 μm	Tissue scaffold integrated with cell-laden hydrogel biomaterials	[122]
	M, macroporous	5 μm	Lung-on-chip microfluidic device	[225, 226]

Table 3.3: Recent advances in the applications fields of porous silicon membranes (*Note: C, F, FT, L, M, S stand for, respectively, Commercial, Freestanding, Full Thickness, Lateral, Microfabricated and Self-supported.*)

Application field	Membrane characteristics	Membrane thickness	Detailed application	Ref.
Sensing	M, macroporous	10 μm	Culture of intestinal epithelial cells	[160]
	S, macroporous	130-150 μm	Mechanical cell lysis and DNA isolation	[137, 138]
	F, mesoporous	4-5 μm	Implantable scaffold for cell culture	[206]
	S, macroporous	~50 μm	Electrophysical NO_2 -gas detector	[134]
	F, mesoporous	1.7 μm	Silver-modified sensor for SERS-based detection of miRNA	[104]
	L, mesoporous	10 μm	Interferometric transducer for solvent detection	[219]
	F, mesoporous	1.6 μm	Optical detection of ethanol vapour	[194]
	S, mesoporous	2.5 μm	Impedance spectroscopy of the formation of a lipid membrane	[147, 148]
	F, mesoporous	29 μm	Electrostatic isopropanol vapour sensor	[218]
	F, meso- / macro-porous	5.5 μm	Optical detection of Bovine Serum Albumin	[189]
	F, mesoporous	17-21 μm	Optical detection of dissolved gas concentrations in liquids	[193]
	M, mesoporous	50 nm	Nanopore-based sensing of DNA translocation	[169]
	S, mesoporous	20 μm	Multi-assay solvent vapour optical detection	[227]
	F, mesoporous	870 nm	Multianalyte detection using silver-decorated sensors	[112, 113]
	M, mesoporous	15 nm	Optical detection of Bovine Serum Albumin permeation	[228]
	F, meso- / macro-porous	4.5 μm	Electrochemical detection of MS2 bacteriophage	[116]
	F, meso- / macro-porous	4.5 μm	Label-free electrochemical detection of bacterial toxin	[229]

Table 3.3: Recent advances in the applications fields of porous silicon membranes (Note: *C, F, FT, L, M, S* stand for, respectively, *Commercial, Freestanding, Full Thickness, Lateral, Microfabricated and Self-supported.*)

Application field	Membrane characteristics	Membrane thickness	Detailed application	Ref.
Energy conversion	M, meso-/macroporous	50 nm	Electrochemical detection of ion-transfer across the PSiM	[174]
	C, macroporous	475 μm	3D liquid core sensor array	[230]
	S, mesoporous	340 nm	DNA translocation detection	[149, 150]
	M, macroporous	50 μm	Immunoassay for specific leukocyte subsets	[162]
	S, macroporous	1-3 μm	Transmembranes proteins sensing	[136]
	C, meso-/macroporous	10-15 nm	DNA translocation detection	[231-233]
	F, mesoporous	19.9 μm	Optical aptasensors with multiple target-binding sites	[234]
	S, mesoporous	4-15 μm	Optical detection of enzyme adsorption and streptavidin binding	[10, 126, 235]
	F, micro/macroporous	10-30 μm	Wide-gap absorber for solar cells	[109, 111]
	S, mesoporous	$\sim 70 \mu\text{m}$	Implantable glucose biofuel cell	[140]
	S, mesoporous	5-20 μm	Ion-exchange membrane for micro fuel cells	[236]
	S, mesoporous	50 μm	Anion exchange membrane for Glucose/ O_2 micro fuel cell	[127]
	S, macroporous	125 μm	Membrane-electrode assembly for H_2 /air-fed micro fuel cells	[141]
	S, macroporous	230 μm	Microfluidic electric generator	[143]
	S, mesoporous	13 μm	Monolithic Si electrode for micro fuel cells	[153, 154]
	F, macroporous	50 μm	Anode for lithium-ion batteries	[105, 108, 110]

Table 3.3: Recent advances in the applications fields of porous silicon membranes (Note: C, F, FT, L, M, S stand for, respectively, Commercial, Freestanding, Full Thickness, Lateral, Microfabricated and Self-supported.)

Application field	Membrane characteristics	Membrane thickness	Detailed application	Ref.
Electronics	F, mesoporous	200 μm	Anode for lithium-ion batteries	[237]
	M, macroporous	5 μm	Electrode grids for micro fuel cells	[155]
	S, micro/macroporous	210 μm	High-conductivity electrodes for micro fuel cells	[144]
	M, macroporous	280 μm	Ion-exchange membrane for photoelectrochemical cell	[238]
	S, macroporous	50 μm	Electrode for methanol micro fuel cell	[152]
	S, macroporous	60 μm	Cathode for self-breathing microfuel cells	[139]
	FT, meso/-macroporous	500 μm	Membrane-electrode assembly for hydrogen/oxygen micro fuel cells	[101, 102]
	S, mesoporous	100 μm	Proton exchange membrane for methanol micro fuel cells	[131, 132]
	S, meso/macroporous	80-100 μm	Pervaporation membrane for methanol vapour-fed micro fuel cells	[128]
	F, mesoporous	46 μm	RF insulating and supporting substrate for microfabricated inductors	[106]
	S, mesoporous	10 μm	MEMS-based superheated loop heat pipes	[151]
	M, macroporous	600 nm	Thermal management device	[223]
	S, mesoporous	300 μm	Thermal isolation layer for high temperature micro-hotplates	[133]
	F, macroporous	2.46 μm	Flexible optoelectronics device	[117]
	F, mesoporous	1.92 μm	Modulator for photodetector	[119, 120]
	FT, mesoporous	380 μm	CMOS compatible RF insulating substrate	[103]

3.3 Porous silicon biosensors

Biosensing is a very widespread use of PSi. This is linked to the large surface area and many physical and chemical properties of the porous matrix [9].

3.3.1 PSi biosensing read-out methods

3.3.1.1 Optical read-out methods

Most PSi biosensors rely on changes in the optical features as means of detection, and can mostly be divided into two categories: sensors relying on a shift in the reflection spectrum and sensors exploiting photoluminescence (PL) [98, 239, 240].

Photoluminescence-based methods Room temperature photoluminescence can arise in porous silicon, under certain fabrication conditions, because of a quantum confinement effect inside the pore walls [81, 82]. This property is sensitive to the changes in state of the PSi internal surface, and is therefore quite useful for sensing applications. The detection can be based either on the quenching of the PL, or on the shift of the PL spectrum. PL quenching can be due to the adsorption of some molecules or due to irreversible reactions at the pore surface [241]. For biosensing, the former mechanism is most often used. The PL quenching is then induced by the biomolecular interaction between the bioelement that is fixed on the PSi internal surface and the biological target [242].

Reflection-based methods For biosensors relying on shifts in the optical spectrum of PSi, two subcategories can be distinguished: the ones that rely on the measurement of the reflectance spectrum of PSi photonic crystals or microcavities; and the ones utilising interferometric reflectance spectroscopy.

A biorecognition-event will induce a local change in refractive index (RI) in the porous structure, which results in a blue-shift or red-shift in the reflectance spectra. For photonic crystals, these spectra are characterised by typical spectral features, whose position can therefore be monitored quite easily, as reviewed by Arsahsky-Graham *et al.* [98]. The structural colour of the photonic crystal can also be used to monitor biological binding events, as demonstrated by Cao *et al.* who measure the colour shift in a microcavity upon biomolecule binding using a smartphone [243].

Interferometric reflectance spectroscopy, on the other hand, most often relies on single layer interferometers, whose reflectance spectrum is characterised by Fabry-Pérot fringes, due to the constructive and destructive interferences between the reflections at the top and bottom of the porous layer. As previously described in Section 3.1.2.2, the effective optical thickness (EOT) can be calculated from this fringe pattern by applying a Fourier transform. This method was described as the Reflective Interferometric Fourier Transform Spectroscopy (RIFTS) and is illustrated in Figure 3.17, a. Although it requires some post-processing of the recorded optical spectra, this method enables the real-time monitoring of the EOT, which is affected by local changes in the RI upon biorecognition events, as EOT is linked to the average refractive index (n) and the PSi thickness (L) by the following equation: $EOT = 2nL$ [97].

Recently, two alternative post-processing methods have been proposed to increase the sensitivity of PSi interferometers: the interferogram average over wavelength (IAW) technique developed by the *Barilaro* group [244] and the Morlet wavelet phase method, developed by both the *Weiss* group and the *Segal* group [245].

The IAW method is based on the difference between a *reference* reflectance spectrum before exposure of the target biomolecule and an *analyte* spectrum after biomolecule exposure. This difference is termed *interferogram* (Figure 3.17, b). This interferogram is zeroed by subtracting the mean for each value. Then, the absolute value of each point in the interferogram is integrated over the chosen spectral range [244]. While very sensitive, this method is however strongly dependent on the amplitude variations of the Fabry-Pérot fringes, and therefore more susceptible to noise contributions [245].

The Morlet wavelet phase method, on the other hand, relies on complex Morlet wavelet convolution, otherwise used to filter electrical signal, to reduce the noise contributions in the reflectance spectra. Similarly to the RIFTS method, a Fourier transform is applied to the optical spectra, and the full width at half-maximum (FWHM) of the obtained peak is used to define a complex Morlet wavelet. This wavelet is convolved with the reflectance data to obtain a complex-filtered output, from which the phase and amplitude can be extracted (Figure 3.17, c). Without going into details, the amplitude can be used to normalise the Fabry-Pérot fringes, and the phase can be used (after some post-processing) to monitor bio-recognition events (Figure 3.17, d) [245]. This method was

shown to improve the sensitivity of PSi sensors on theoretical optical spectra, as well as on experimental assays [245, 246].

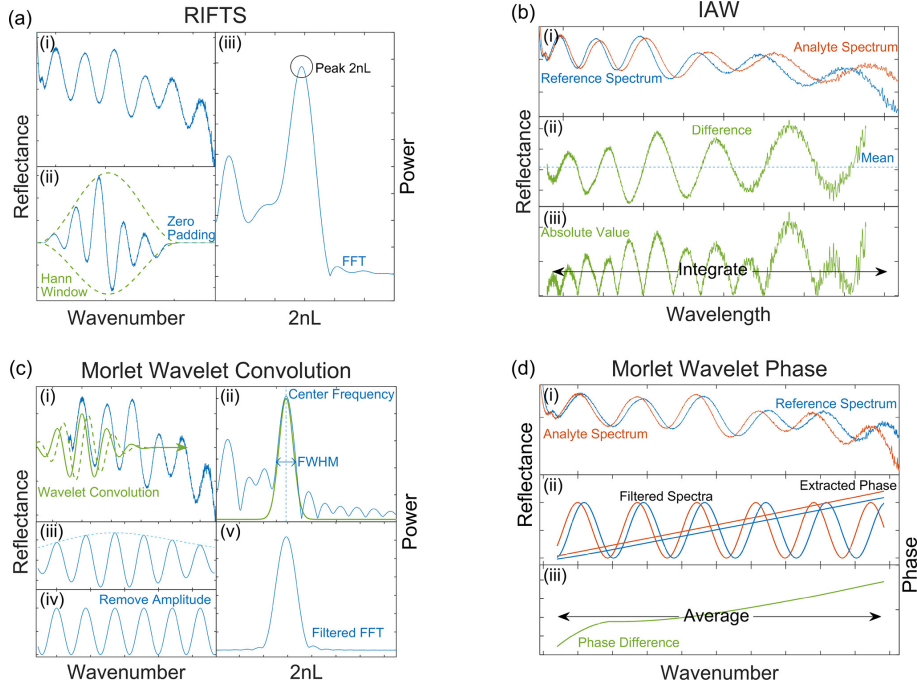


Figure 3.17: Illustration of different signal processing techniques for the reflectance spectra of a PSi single layer interferometer: (a) RIFTS, (b) IAW, (c) complex Morlet wavelet convolution, and (d) Morlet wavelet phase. Reprinted from [245].

Hybrid methods Other novel optical techniques have been studied as well, on PSi structures hybridised with plasmonic or fluorescent nanomaterials. The inclusion of quantum dots, fluorescent probes or noble metal nanoparticles into porous silicon enables their utilisation for new transducing techniques, such as LSPR, SERS or fluorescent microscopy [247]. The hybrid-PSi matrices offer superior sensing performances: the signal is enhanced, the sensitivity is higher and/or the stability of the signal is improved. They may also enable a dual-mode detection, which often yields a wider range of response [98].

3.3.1.2 Electrochemical read-out methods

Finally, PSi can also be used as an electrochemical transducer for biosensing applications. They offer the advantage of a higher exposed surface and hence a higher sensitivity compared to bulk silicon electrochemical sensors. The electrochemical transducing scheme can be based, among others, on conductance, resonant frequency, impedance or amperometry, as reviewed by RoyChaudhuri *et al.* [248].

3.3.2 Membrane-based biosensors

As per definition, biosensors are not only composed of a detection element or transducer; they also include a recognition element to capture the analyte. This element can take the form of antibodies, antigens, enzymes, complementary DNA, and so on. For PSi layers, the capture of analyte is however often limited, which is why PSiMs are more alluring for biosensing application. As detailed in Section 3.2.2.2, multiple comparative studies between PSi layer and PSi membranes have shown the advantages of the latter in terms of biosensing: the sensitivity is increased and the response time is shortened [10, 114, 126, 234].

Most PSiM-based biosensors rely on optical detection schemes. Kumar *et al.* [189] fabricated freestanding PSi microcavities for the detection of Bovine Serum Albumin (BSA). The recognition element of the biosensor was introduced via functionalisation: (3-Aminopropyl)triethoxysilane (APTES) was first used to provide amine terminations to the oxidised PSi, onto which glutaraldehydes were grafted. When BSA is captured inside the porous matrix by the glutaraldehydes, the refractive index of PSi increases and the optical signal shifts to higher wavelengths. The detection therefore consists in monitoring the shift of the microcavity resonance wavelength. Zhao *et al.* [10] used a similar photonic structure for their biosensor, but made the membrane self-supported in order to reduce the risk of surface inhomogeneity and mechanical failure. The sensor was APTES- and biotin-modified. The detection relied on the red-shift of the microcavity resonance wavelength upon streptavidin adsorption. The total detection time amounted to 20 minutes, which was 6 times faster than for a PSi layer-based biosensor, thus demonstrating the improved mass transport and the increased analyte capture of open-ended PSi films described in Section 3.2.2.2. The monitoring of photonic optical features is used in biomedical applications as well, where sensing is not the only role of the PSiM. Optical biosensing is used to track drug

delivery [121], cellular secretion and biomolecular release [122] as well as to report *in vivo* cell and tissue responses [206].

Not all optical biosensors rely on photonic structures for the detection of biomolecules: the monitoring of the EOT is also possible, using the RIFTS method described previously. This method enables the real-time monitoring of the EOT, relative to an EOT value acquired before the biosensing experiment. Yu *et al.* [234] used the relative EOT to track the binding of thrombin to aptamer. The biosensor consisted of a mesoporous freestanding PSiM, functionalised with thrombin-binding aptamers. The detection performance and selectivity of the sensor can be observed in Figure 3.18,a.

SERS is another optical method that can be used to detect biomolecules on PSiMs. The membranes were found to be promising supports for such biosensors [104]. PDMS was used as mechanical reinforcement, and silver nanoparticles were deposited onto the porous surface by inkjet printing or by immersion plating. A DNA probe was attached to the nanoparticles as recognition element. The thus-fabricated biosensor was able to detect the target microRNA with a sensitivity comparable to commercial techniques. The potential of the chip as multiplexing biosensing platform was studied in further works [112,113]. Polyadenine (polyA), polyguanine (polyG), polythymine (polyT) and polycytosine (polyC) solutions were injected in the four chambers of the biosensing platform, respectively. The SERS spectra measured for each chamber can be seen in Figure 3.18, b. The fingerprint of each DNA nucleobase can be easily recognised.

Besides optical detection methods, biosensors can also rely on electrical characterisations as means of diagnosis. Reta *et al.* [116] used differential pulse voltammetry (DPV) to probe the binding of either bacteriophages or of bacterial lipopolysaccharides on the pore walls. As illustrated in Figure 3.18,c, the biosensor consisted of an antibody-functionalised freestanding PSiM, deposited onto a gold slide. When the target biomolecules bound to the antibody, the pores became partially blocked. The blockage hindered the diffusion of electroactive species present in the solution to the gold slide, which resulted in a decrease in the current intensity. The thus-constructed platform formed a simple, direct and sensitive biosensor, and allowed for example the indirect detection of gram-negative bacteria, down to 1 CFU/mL [229].

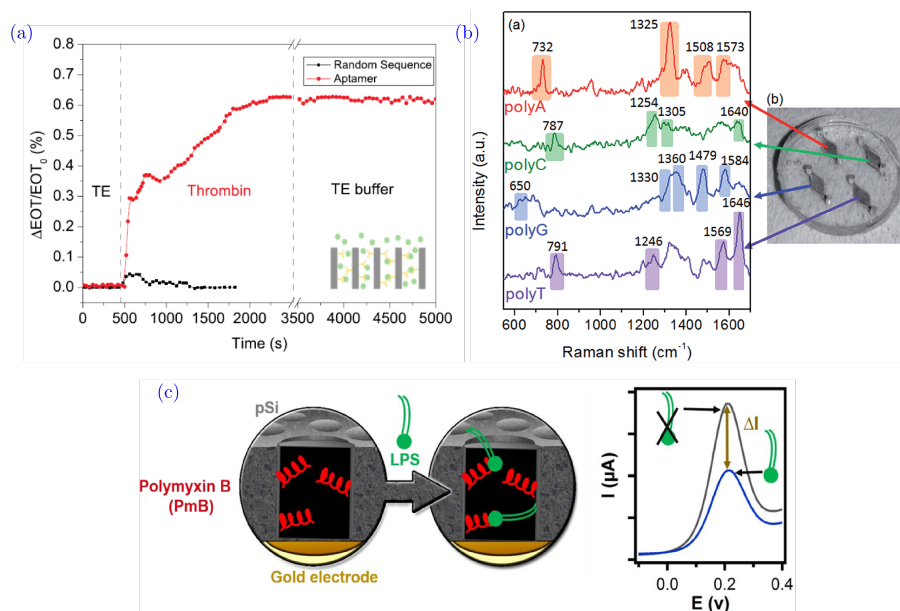


Figure 3.18: Examples of PSiM-based biosensors: (a) detection performance of aptamer-modified optical biosensors, relying on the increase of relative effective optical thickness upon thrombin binding, reprinted from [234]; (b) SERS spectra for multianalyte detection on four different silver nanoparticles-decorated PSiMs, reprinted from [112]; (c) sensing principle of a voltametric PSiM-on-gold biosensor, and corresponding DPV traces before and after the binding of lipopolysaccharides (LPS) bacterial toxins, reprinted from [229].

3.3.3 PSi biosensors for bacterial detection

PSi-based sensors for bacterial detection, be it membranes or single layers, have been widely studied over the last couple of decades, as reviewed in 2014 by Massad-Ivanir *et al.* [9]. This section will therefore only consider PSi-based bacterial detectors studied from late 2013 and onward, as summarised in Table 3.4.

PSi-based bacteria detectors can be categorised into direct and indirect sensors. The indirect detection relies on cell wall fragments, inner bacterial components (i.e. DNA, proteins, ...) or bacterial by-products (i.e. biomarkers, secreted proteins or enzymes, ...). The direct detection of bacteria, on the other hand, is based on the capture and detection of whole bacteria cells in or on the porous surface.

An example of indirect detection of bacteria has already been described

	Method	Matrix	Target	LoD	Time	Ref
Optical	RIFTS	Culture medium	<i>E. coli</i> & <i>S. aureus</i>	10^3 CFU/mL	1 h	[249]
	RIFTS	PBS	S-Layer protein from <i>L. acidophilus</i>	10^{-5} µg/mL	240 s	[250]
	SERS	SB	<i>E. coli</i>	1 CFU/mL	10 s	[68]
	RIFTS	Milk	NAGase Biomarker, linked to <i>S. dysgalactiae</i> or <i>E. coli</i>	31 µmol	1h30	[251]
	Bragg mirror	PBS	Ammonia-oxidising bacteria DNA	27.1 nmol	2h	[252]
	RIFTS	Water	<i>E. coli</i>	10^4 CFU/mL	30 min	[96]
	RIFTS	Food industry water	<i>E. coli</i>	10^3 CFU/mL	45 min	[253]
	RIFTS	Culture medium	<i>E. coli</i>	10^3 CFU/mL	1h	[254]
	RIFTS	SB	<i>L. acidophilus</i>	10^7 CFU/mL	40 min	[255]
	RIFTS	SB	<i>E. coli</i> cell wall fragments	10^3 cells/mL	3h	[256]
	RIFTS	Culture media	<i>E. coli</i>	10^6 CFU/mL	45 min	[257]
	RIFTS	SB	<i>E. coli</i>	10^5 CFU/mL	1 h	[258]
Electrochemical	DPV	SB	<i>E. coli</i> & <i>P. aeruginosa</i> via their lipopolysaccharides	1 CFU/mL	1 h	[229]
	EIS	Blood	<i>E. coli</i> & <i>Salmonella</i> Typhimurium	10^2 CFU/mL	10 min	[259]

Table 3.4: Porous silicon-based biosensors for the detection of bacteria, studied from 2013 onward. They are categorised based on their transducing principle. Both direct and indirect methods of bacterial detection are presented.

above, when discussing the biosensor developed by Reta *et al.* [116] for the detection *E. coli* & *P. aeruginosa* via their lipopolysaccharides. Other indirect bacterial sensors rely on optical detection schemes, such as RIFTS or photonic crystals. Zhang *et al.* [252], for example, developed a microcavity able to detect the DNA of ammonia-oxidising bacteria, which are essential for the ecosystem. The capture of the target DNA inside the APTES and a probe-functionalised cavity resulted in a red-shift of the reflectance resonant peak. Kumar *et al.* [251] concentrated on the biomarker of bovine mastitis, which is a recurrent inflammatory disease that can be caused by pathogenic agents such as *S. dysgalactiae* or *E. coli*. The lysosomal activity of the biomarker, N-acetyl- β -D-glucosaminidase or NAGase, was evaluated using the RIFTS technique and correlated to the number of infecting pathogens inside milk samples: the sensor could differentiate between different stages of the inflammation, which corresponds to bacterial concentration of $\sim 10^5$ CFU/mL to $> 10^6$ CFU/mL.

Another example of indirect bacterial detection on PSi sensors is given by Zhuo *et al.* [250]: they selected a bacterial surface protein, named S-layer protein (SPL), extracted from *Lactobacillus acidophilus*, as target. A TiO₂-coated PSi single layer was developed as transducer and coated with the antibody of SPL, and a response time of 5 min was observed. Tenenbaum *et al.* [256] also demonstrated the indirect detection of bacteria via the capture of cell wall fragments. Their oxidised PSi sensor was functionalised through silanisation, to bound a synthetic peptide probe. The *E. coli* suspension in saline buffer (SB) was lysed under ultrasounds and incubated on the PSi sensor for 1h, before being washed off. A detection limit of 10^3 CFU/mL could be reached by monitoring the intensity of the EOT peak via RIFTS. The experiment was repeated with intact bacteria, but no significant change in peak intensity was observed as illustrated in Figure 3.19, suggesting that no bacteria remained bound to the PSi surface after the final wash. This illustrates one of the advantages of indirect detection: the large size of the bacteria cell limits their access to the capture probes inside the porous layer, and therefore prevents their binding to the surface. Bacterial debris or secreted bacterial proteins are much smaller in size, and can hence penetrate the porous structure more easily.

The indirect detection of whole bacteria cells is however also possible, as demonstrated by Tang *et al.* [254]. They proposed an antibody-grafted PSi film for the optical detection of *E. coli* via pore blockage: a smaller

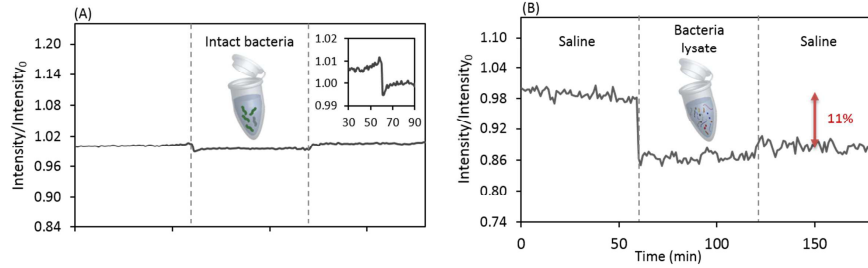


Figure 3.19: Relative intensity shift of peptide-modified PSi-based sensor upon exposure to *E. coli* bacterial suspension (A) and *E. coli* bacterial lysate (B), both with an initial concentration of 10^5 CFU/mL. Reproduced from [256].

probe molecules (BSA in this study) was used to fill the pores; when a bacteria cell is captured on top of the PSi surface, the pores are blocked and the diffusion of the probe molecules is hindered. RIFTS was used to monitor the EOT; the EOT shift was the highest during the control test in the absence of bacteria, but lower upon bacteria binding as fewer pores became accessible to the probe molecules. The LoD was measured as 10^3 CFU/mL, but could be decreased for longer sensing experiments or by adapting the flow rate of the analyte volume on top of the sensor.

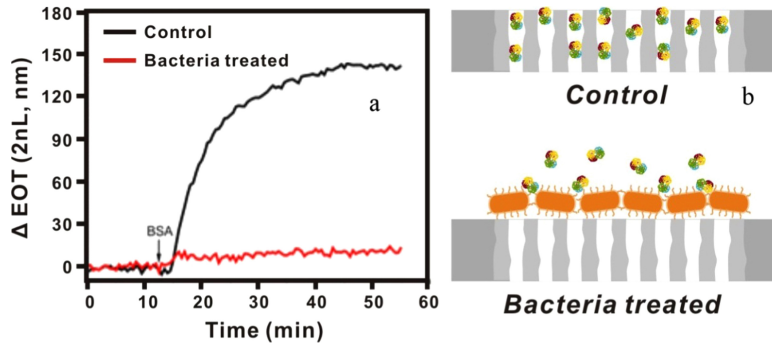


Figure 3.20: Indirect detection of *E. coli* via pore blockage: (a) EOT shift before and after bacteria binding on the PSi film surface; (b) Schematic illustration of the sensing principle, which is based on the blockage of pores by bacteria that prevent the diffusion of probe molecules inside the pores. Reproduced from [254].

The challenge of the large size of whole bacteria cells can be overcome in different ways in order to enable direct detection. One technique consists in making the pores big enough for the bacteria to penetrate, as

demonstrated by the *Segal* group [257,258]. A macroporous PSi photonic crystal, also called a 2D PSi lamellar grating and composed of a periodic array of pores that can fit *E. coli* cells (3.21,a), was fabricated using a combination of standard microfabrication techniques and electrochemical etching. The zero-order diffraction of the macroporous matrix (see Figure 3.21, b) exhibits a fringe pattern similar to the ones obtained for single layer PSi interferometers, which can be used for RIFTS. Mirsky *et al.* [258] conducted preliminary biosensing experiments for *E. coli* concentrations ranging from 10^5 CFU/mL to 10^7 CFU/mL and observed an increase in EOT and a decrease in the intensity of the EOT peak. Massad-Ivanir *et al.* pursued this research by functionalising the pore surface in order to promote the spontaneous capture of the bacteria cells. Exponential phase *E. coli* cells (10^6 CFU/mL) in their culture media were incubated onto the porous sensor, resulting in a direct EOT increase and intensity decrease as illustrated in Figure 3.21, c and d. The EOT increased further overtime, due to the bacterial growth and multiplication, as can be observed on the inset in Figure 3.21, c.

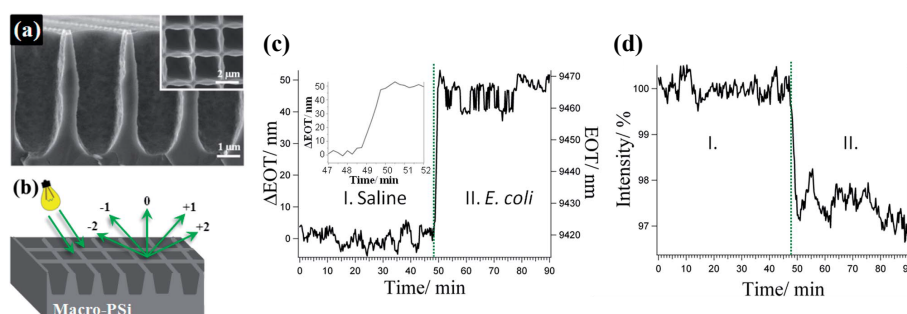


Figure 3.21: 2D PSi photonic crystal with a microporous array: (a) SEM image of the cross-section and the top view; (b) Schematic illustration of the RIFTS lamellar grating and the diffraction orders, the sensor only using the zero-order diffraction; (c) Real-time detection of *E. coli* with the macroporous sensor based on the EOT (in the inset: zoom of the EOT shift) and (d) detection based on the intensity of the EOT peak. Adapted from [9].

Another approach developed by *Segal's* group is the "direct cell capture" method that monitors the intensity of the EOT of the reflectivity spectrum. Urmann *et al.* [255], for example, functionalised their PSi-based sensor with aptamers, after silanisation, for the detection of *L. acidophilus*. The changes in optical reflectivity were quantified by applying a Fourier transform to the fringe patterned-optical spectra and by recording the maximum intensity of the thus-obtained peak. The rel-

ative change of intensity upon bacterial detection is illustrated in Figure 3.22. The main advantage of the presented sensor is its ability to distinguish between live and dead cells. This research was pursued by Massad-Ivanir *et al.* [253], who changed the surface chemistry and capture probe of the sensor: *E. coli* antibodies (IgG) were grafted onto the oxidised PSi matrix using silanisation chemistry and biotin-streptavidin coupling. The sensor was able to selectively detect the target bacteria in real food industry process water, over a range of 10^3 CFU/mL to 10^5 CFU/mL, in only 45 min. Recently, another research group used the same principle to detection for different bacterial strains. The probes were selective lectins, targeting *E. coli* and *S. aureus*. Different response patterns were observed for different bacterial species and lectin types, enabling the selective detection of both studied strains. The limit of detection was however not decreased compared to previous studies [249].

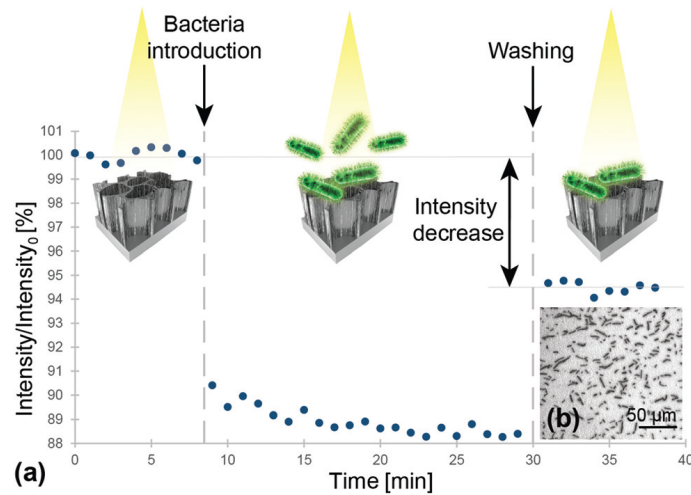


Figure 3.22: Relative intensity change upon exposure to *L. acidophilus* (10^7 CFU/mL) cells suspended in SB and after an extensive wash. The inset (b) is a microscope image of the biosensing experiment, depicting the bacteria cells captures on the aptamers-modified sensor. Reproduced from [255].

A variation to the "direct cell capture" method was introduced by Gongalsky *et al.* [96]: a double-layered PSi film was created by performing a MACE on an electrochemically etched mesoporous layer. The top layer is therefore made out of porous nanowires, which results in an increased roughness that favours the entrapment of bacteria. Infrared RIFTS was used to monitor the binding of bacteria, which led to an increased EOT

of the porous layer. The LoD was estimated as 10^4 CFU/mL.

While most sensors described above rely on RIFTS for the detection of bacteria, other transducing methods have also shown promising results. Samanta *et al.* pursued the research from Das *et al.* [260, 261] on impedance PSi-based biosensors. The sensor was composed of an oxidised macroporous layer, onto which electrodes were screen-printed. The antibody-modified porous matrix was able to capture the target bacteria, and by monitoring the impedance, the sensor could detect either *E. coli* or *S. Typhimurium* in spiked blood samples, with concentrations as low as 10^2 CFU/mL.

The lowest LoD was however obtained by Jabbar *et al.* [68], who relied on SERS for the monitoring and detection of *E. coli* and *S. aureus* cells. A gradient porosity PSi layer was etched by varying the current density during the electrochemical etch. Plasmonic silver and palladium bimetallic alloy nanoparticles ((Ag@Pd)NPs) were synthesised *in situ* using an ion reduction process. With this substrate, it was possible to identify the characteristic peaks identifying the *E-coli* bacteria molecules in the SERS-acquired intensity profile for ultra-low concentration of about 1 CFU/ml.

Part II

Results

CHAPTER 4

DESIGN OF A POROUS SILICON BIOSENSOR

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Abstract

The motives behind the design choices for our porous silicon biosensor will be presented in this chapter. First, the bacterial lysate will be examined using microscopy techniques. Next, the morphology of PSi obtained with varying fabrication conditions will be studied. All findings will then be used to model the biosensor and simulate its performance, both in terms of analyte diffusion and of optical response.

4.1 Motivation

When designing a biosensor, two crucial elements for the sensor's performance are the choice of the bioelement and the choice of the transducer. The former will dictate the specificity of the biosensor, and the latter will affect its overall sensitivity.

In this research, bacteriophage by-products, namely **endolysins**, were selected as bioelements. While most endolysin-based detection tools rely on their ability to bind a specific target bacterium, the current research will take advantage of their lytic activity as well. Indeed, this research will focus on the indirect detection of bacteria via their lysate. The endolysins will therefore be added to the sample volume, before the analyte is brought into contact with the transducer. The addition of the bioelement into the sample volume removes the need for a bio-functionalisation at the sensor's surface. This eliminates the constraints the functionalisation puts on the fabrication of the sensors (e.g. low temperature encapsulation), its storage conditions (e.g. controlled temperature and humidity) as well as its usage conditions (e.g. controlled temperature).

Another advantage of adding the bioelement to the sample volume is the versatility of the transducer itself: sensors fabricated within the same batch can be used to detect different target bacteria. The target bacteria can also be easily adapted by selecting and/or combining different endolysins: either one bacterial strain is targeted by the use of very specific endolysins, or multiple strains are targeted by a broader spectrum endolysins or by the combination of different endolysins. A final advantage of the use of endolysins is the ability to distinguish between live and dead bacteria: by comparing the transducer signal before and after lysis, one is able to highlight the contribution of the live bacteria that were originally in the analysed sample.

Porous silicon was then selected as transducer, because of its many advantages. First of all, it is characterised by a large internal surface area, which means it is very sensitive to minute changes in its environment [242,262]. Its fabrication process is relatively low cost and straightforward when building simple structures, and can be easily adapted to obtain a wide range of morphologies and properties [90,262]. Some of the most studied properties of PSi when considering biosensing applications are its optical properties. As detailed in the previous chapter, PSi

can exhibit either photoluminescence or specific features in its optical reflection spectrum. Both of these unique optical properties enable an easy and fast read-out of bio-recognition events that happen in the PSi-environment. In this research, the optical reflection spectra of PSi will be studied, and the fabrication of the PSi will be optimised for the best sensitivity. Furthermore, the fabrication process of PSi is also compatible with standard microfabrication techniques, meaning microelectronics can be integrated or complex structures can be built. Examples of complex structures are PSi membranes, whose geometry helps the diffusion of species to the porous surface and enhances the sensitivity [10].

The protocol of bacterial detection studied in this research, illustrated in 4.1, is thus based on the selective lysis of the target bacteria and the capture of the bacterial lysate inside a porous silicon matrix. Once bacterial fragments are captured, the optical properties of the PSi will be affected. To improve the diffusion of the lysate through the pores and encourage the capture, a porous membrane will be fabricated. This chapter will focus on the design of the PSi membranes. First, the bacterial lysate is studied using *B. cereus* as model. The endolysin selected for this example is PlyB221, which is encoded by the Deep-Blue phage targeting *B. cereus* [263]. The size distribution of bacterial fragments determines the aimed pore size of the PSi membrane, as it must be ensured that the target lysate is able to penetrate into the porous layer. To achieve the desired pore size, the morphology of PSi obtained with varying fabrication conditions is also studied.

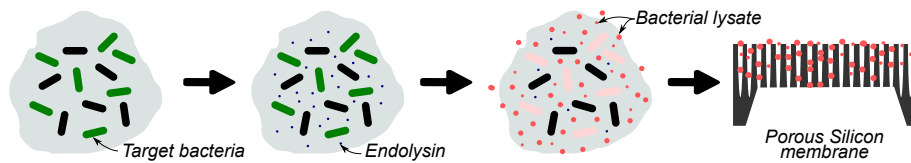


Figure 4.1: Conceptual protocol for the bacteria detection: a selective endolysin is added to the analyte; the endolysin specifically targets one bacteria strain; the resulting bacterial lysate is then captured inside a PSi membrane.

When designing a platform for the detection of analytes, one has to optimise the sensitivity of the platform. While this optimisation can be done experimentally, modelling and simulations enable a much faster way to study an even wider range of parameters. The optimisation of the biosensor studied in this research will focus on two aspects: the diffusion and capture of bacterial lysate inside the porous membrane

and the optical response of the porous structure.

First, the best PSi nanostructures must be selected; since the sensor must retain the bacterial lysate but let the endolysins permeate, a multi-layered structure is needed. Two options were compared: a double layer, relying on RIFTS for the optical read-out as detailed in section 3.3 and a microcavity, relying on the monitoring of its spectral features and/or on its structural colour. For the double layer, the optical read-out will mostly be affected by the analyte present in the top layer. For the microcavity, the analyte has to reach the defect layer for optimal results. The diffusion of analyte, modelled as particles, was therefore analysed using the Comsol Multiphysics simulation tool.

A second model focused on the optical response of the sensor, which can be modelled via transfer matrix simulation. The effects of the fabrication parameters on the optical response were studied; two main questions were answered: (1) which current densities give the best optical signal and (2) how thick the multi-layers should be. When combining this model with the data collected from the particles' diffusion simulation, a theoretical limit of detection (LoD) can be calculated.

4.2 Materials and methods

4.2.1 Bacterial lysis study and characterisation

Bacterial suspension of *B. cereus* ATCC 10987 were obtained by first spreading the bacteria from a stock solution onto a Lysogeny Broth (LB) plate using a loop, then incubating overnight (O/N) at 30 °C to allow the growth of the colonies. One colony was then taken from the LB plate and re-suspended in 5 mL of LB and incubated overnight at 30 °C at 120 RPM in order to obtain a stationary phase liquid culture. Finally, in order to obtain a liquid culture in the exponential growth phase, 20 mL of LB were inoculated with 200 μ L of the first liquid culture and incubated for 3 h at 30 °C, at 120 RPM. The culture was then centrifuged at 10000x g for 5 min at room temperature and the supernatants were re-suspended in 20 mL of PBS. This washing step was repeated once over and the optical density (OD_{600}) was adjusted to $OD_{600} = 0.2$ ($\sim 10^6$ CFU/mL). The concentration of bacterial suspensions for this OD_{600} was confirmed by plate count technique. This is done by performing ten-fold serial dilutions of the bacterial suspension and by plating 100 μ L of each dilution on LB petri dishes. These dishes are then incubated

overnight at 30 °C, before counting the colonies of at least two plates and averaging the results as a function of the dilution factor.

A detailed description of the expression and purification of PlyB221 endolysin can be found elsewhere [263]. The protein concentration was adjusted to 1 mg/mL. This concentration was selected based on previous characterisations of the bacteriolytic activity of PlyB221 [263]. The *B. cereus* lysate was prepared by adding 600 µL of PlyB221 endolysin to 5.4 mL of bacterial suspension and by incubating this mixture at 30 °C for 30 min. The success of the lysis could be observed visually by checking the obvious reduction of the opacity of the bacterial suspension, or by measuring the OD₆₀₀ before and after lysis.

B. cereus suspension and lysate were first observed with an inverted optical microscope (Leica DMI6000 B). For the observations, 15 µL of each suspension/solution were pipetted and transferred to a glass slide and then covered with a plastic coverslip. The glass slides were then observed using a 100x oil objective and a numerical aperture of 1.4. Several observations on the glass slide were recorded using the Leica LAS AF software. The images were then analysed with ImageJ to obtain information about the number and size of the bacteria and bacteria lysate. In short, the images were converted to black and white images and the "Analyse Particles" feature of the software was used to extract the average Feret diameter¹ of the measured fragments.

B. cereus suspension and lysate were also captured on silicon surfaces to enable their observation using SEM. Silicon dies of dimension 1 cm x 1 cm were placed inside a 12-well plate. The wells were filled with 2 mL of one of three samples: a control solution consisting of PBS, the *B. cereus* suspension or the *B. cereus* lysate. The 12-well plate was then incubated at 30 °C for 1 h, after which each die was rinsed 5 times in PBS by removing and adding 1 mL of PBS. After the last PBS wash, 1 mL of solutions remained in each well. A glutaraldehyde solution was added to each well in order to reach a final concentration of 2.5 %. The 12-well plate was left at room temperature for 1 h, enabling the crosslinking of the bacterial cell walls. The samples were then washed three times with PBS using the same technique as explained before, making sure that the dies were never exposed to air. For the dehydration, the samples were then immersed in DI water solutions of increasing ethanol content

¹The Feret diameter measures an object size along a specified direction. It is derived by computing the distance between two tangents on the contour of the object.

(25%, 50%, 75% and finally 99.9 %). Each immersion lasted 10 min. The silicon dies were then dried overnight at 58 °C. Directly before SEM observation, the samples were covered with a ~10 nm-thick layer of gold to prevent charging effects. The images were then analysed with ImageJ to obtain information about the number and size of the bacteria and the bacterial lysate. In brief, the images were converted to black and white images and the "Analyse Particles" feature of the software was used to extract the average Feret diameter of the measured particles.

4.2.2 Porous silicon study and characterisation

4.2.2.1 Porous silicon samples fabrication

The PSi layer samples were prepared by the electrochemical etch of a double-side polished, boron-doped silicon wafers ($\langle 100 \rangle$, 0.8-0.9 m Ω cm, 380-400 μ m) (Sil'tronix Silicon Technologies). The etching was carried out in a custom-made Teflon single bath etch-cell, with a platinum coil as the counter-electrode and a potentiostat/galvanostat (PGSTAT302N from Metrohm Belgium) as the current source. The porosification was performed in HF:ethanol (3:1, in volume) electrolyte. Aqueous hydrofluoric acid (HF, 49%) was acquired from Chem-Lab NV, and absolute ethanol was obtained from VWR Chemicals. The first step of the anodisation consisted in etching a sacrificial layer at 200 mA/cm² for 30 s and removing it with a 2 M aqueous solution of KOH until no more reaction was visible. By etching the sacrificial layer (or transitional layer) a larger and more homogeneous pore size is obtained. The sample was then rinsed once in deionised water and twice in 2-propanol before being etched again at current densities going from 50 to 250 mA/cm², with steps of 25 mA/cm². Most samples were etched for 60 s, but other samples were also etched for shorter or longer times. Once anodised, the PSi samples were rinsed three times in 2-propanol and gently dried with nitrogen.

4.2.2.2 PSi characterisation

PSi samples were first characterised optically using the SLIM method, as described in 3.1.2.2. This enables to approximate the porosity and thickness of the PSi layers. The characterisation was carried out using a fibre-coupled Ocean Optics JAZ spectrometer and a 10-mW halogen light source. The optical spectra, characterised by Fabry-Pérot fringes,

were analysed using a Fourier Transform over the 450 to 750 nm wavelength range.

The PSi samples were also characterised using a Carl Zeiss Ultra 55 SEM, both in cross-section and in top view. The thickness of the PSi layers was verified on the cross-section views. Based on the top views, the pore size distribution could be analysed using the ImageJ software: first the scale of the image was set, and the image was cropped; then the SEM image was converted into a purely black and white image, using fine-tuned threshold values that enable to best reflect the original image; finally, the "Analyse Particles" feature of the software was used in order to extract the average Feret diameter of the pores and approximate the pore size of the PSi samples.

The sensitivity of the PSi samples was characterised using a variation of the SLIM method. In brief, the reflection spectra of each PSi sample were measured in different media, namely air, ethanol and methanol. The EOT for each media was recorded and plotted with respect to the refractive index of the used medium. The slope of the linear regression fitted for each sample characterises the sensitivity of the samples. While for most optical sensors the sensitivity is expressed in $\text{nm} \cdot \text{RIU}^{-1}$, it will be expressed here in relative EOT changes, which is expressed in terms of the EOT_0 , the EOT of the layer in air:

$$\Delta EOT_{rel} = \frac{EOT - EOT_0}{EOT_0} * 100[\%] \quad (4.1)$$

4.2.3 Modelling of the optical biosensor response

4.2.3.1 Simulation of membrane filtration

The filtration capability of different porous silicon membrane nanostructures was assessed using the Comsol Multiphysics software, more specifically using the Computational fluid dynamics module. The porous silicon membrane was simplified into a 2D problem, with straight pores instead of branched ones. Only the first few micrometers of the PSi membranes are modelled. Two models were constructed, with different geometries: a 2D microcavity and a 2D double layer. For both models, the filtration of bacterial lysate was simulated using the *Creeping Flow* interface and the *Particle tracing in fluid flow* interface. The theory used for each interface is explained in Appendix A.

For both models, the following conditions were chosen:

1. Creeping flow
 - (a) Pressure driven inflow and outflow: this condition was selected because the experimental setup is pressure driven as well;
 - (b) No slip boundary conditions: the velocity of the flow is zero at the walls;
 - (c) One-way coupled flow: the flow is not impacted by the motion of the particles;
2. Particle tracing
 - (a) Only one set of particles is released at time $t=0$;
 - (b) When a particle hits the pore walls, it freezes;
 - (c) Particles do not interact with each other;
 - (d) The Stokes drag force and gravity force are the only forces taken into account. The Brownian motion of the particles is neglected to gain computational speed; preliminary studies illustrated that the contribution of the Brownian force was not significant.

In the particle tracing interface, two considerations are important: (1) particles do not displace the fluid they occupy and (2) their finite size is not taken into account when modelling particle-wall interactions. Indeed, the particles are treated as point masses. To add the contribution of the particle size to the filtration simulation, two workarounds were added. First, the "wall distance" interface was added to the model in order to calculate the distance from the pore walls; an expression-based boundary condition was then added to all the pore walls, stating that when the distance from the wall is equal or lower than the particle radius, the particle freezes. Secondly, a "fictitious" boundary was added at the top of each porous layer, with an expression-based boundary condition: if the particle diameter is larger than the defined pore size, the particle freezes; if not, the particle can pass through the boundary.

Microcavity 2D model The geometry of the 2D model of a porous microcavity is illustrated in Figure 4.2. It is composed of a porous microcavity membrane, encased in between two fluidic channels. To

simplify the morphology of the microcavity, the pores size of each layer remained constant over the entire layer. The first porous layer has larger pores, while the second has smaller ones. The thickness of each layer was fixed arbitrarily, not taking into account the requirements for the desired optical signal of a microcavity. All parameters are detailed in Table 4.1. The Bragg stacks surrounding the microcavity are each composed of two pairs, and the cavity itself is double as deep as the first porous layer.

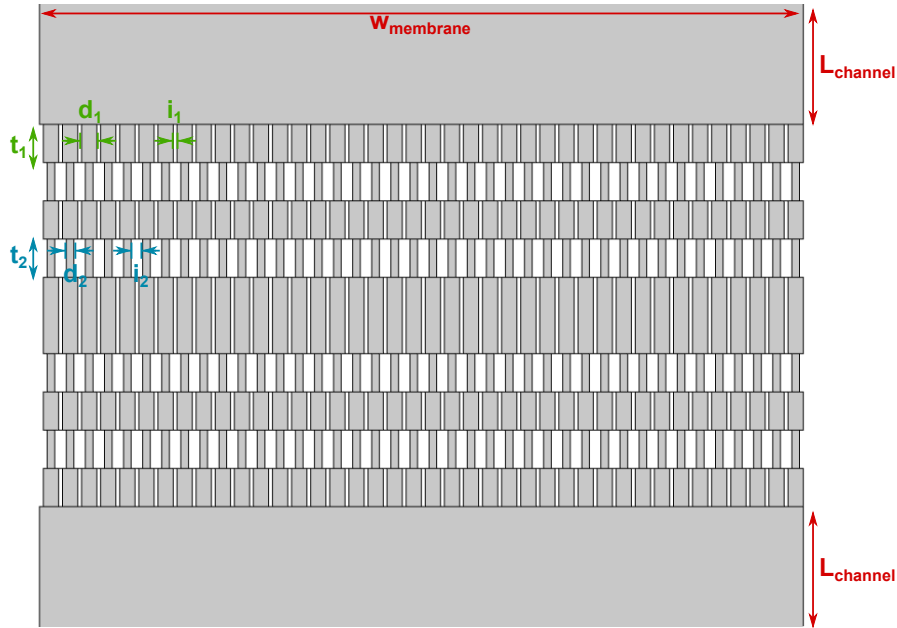


Figure 4.2: Schematic cross-section of a microcavity, which was used as geometry for the modelling of membrane filtration. The membrane-based microcavity is flanked by two fluidic channels.

The flow inlet is at the top of the upper fluidic channel, from which the particles are also released. The outlet was fixed at the bottom of the lower channel. The flow was normal to the inlet, and computed via a steady-state study. This flow was then used as the initial value for the time-dependent calculation of the motion of particles through the porous membrane.

Double layer 2D model The geometry of the double layer 2D model is depicted in Figure 4.3. It is composed of an upper fluidic channel and a first layer of pores, which counts a total of 20 pores. The diameter of each pore is selected using a random Gaussian function, with mean μ

Name	Value	Description
ρ_{PBS}	1060 [kg/m ³]	Density of PBS
μ_{PBS}	9.04e ⁻⁴ [Pa·s]	Dynamic viscosity of PBS
p ₁	40 [nm]	Pore diameter of the first layer
p ₂	20 [nm]	Pore diameter of the second layer
t ₁	100 [nm]	Layer thickness of the first layer
t ₂	100 [nm]	Layer thickness of the second layer
i ₁	10 [nm]	Inter-pore distance of the first layer
i ₂	30 [nm]	Inter-pore distance of the second layer
w _{membrane}	2 [μm]	Width of the membrane
L _{channel}	1 [μm]	Length of the channel
p _{in}	2 [bar]	Inlet pressure
p _{out}	1 [atm]	Outlet pressure
d _p	30 ± 20 [nm]	Particle diameter
ρ _p	2000 [kg/m ³]	Density of the particles

Table 4.1: Simulations parameters for the membrane filtration inside a 2D microcavity model.

and standard deviation σ . The second layer of the double PSi layer is not simulated, only the interface between top and bottom layer is represented. At this interface, segments are added, with length equal to the diameter of the selected pore minus *diff*, a parameter that represents the reduction of pore size between layers. These segments illustrate the top of the second layer of pores. The geometric parameters used for the simulation are listed in Table 4.2.

The flow inlet is at the top of the upper fluidic channel, from which the particles are also released. For the outlet, the segments representing the second porous layer were selected, as illustrated in Figure 4.3. Similarly to the 2D microcavity model, the flow rate was calculated in a stationary study, and then used as the initial condition for the time-dependent study of the motion of particles.

4.2.3.2 Optical responses modelling

Transfer matrix method The optical response of the multi-layered porous silicon membrane was modelled with the transfer matrix method (TMM) using scattering matrices. In this method, the device is presented as a stack of layers of infinite dimensions in the transverse plane. Typically, the x and y directions represent the transverse plane, while the z axis is the longitudinal direction, in which the waves propagate.

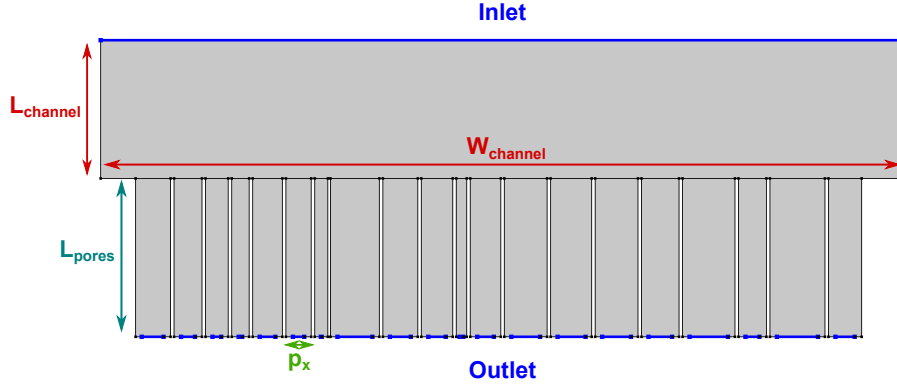


Figure 4.3: Schematic cross-section of a double-layer, which was used as geometry for the modelling of membrane filtration.

Name	Value	Description
ρ_{PBS}	1060 [kg/m ³]	Density of PBS
μ_{PBS}	9.04e ⁻⁴ [Pa·s]	Dynamic viscosity of PBS
p_x	50 ± 25 [nm]	Pore diameter of the first layer
diff	10-40 [nm]	Difference in pore size between layers
i	5 [nm]	Inter-pore distance of the first layer
L_{pores}	2.3 [μm]	Length of the pores
$w_{channel}$	1.1 [μm]	Width of the channel
$L_{channel}$	200 [nm]	Length of the channel
p_{in}	2 [bar]	Inlet pressure
p_{out}	1 [atm]	Outlet pressure
d_p	30 ± 20 [nm]	Particle diameter
ρ_p	2000 [kg/m ³]	Density of the particles

Table 4.2: Simulations parameters for the membrane filtration inside a 2D microcavity model.

The theory behind this method is presented in Appendix B.

Some assumptions and simplifications are made inside this model:

- The modelled materials are lossless, they are characterised by a real refractive index; there is no wavelength dispersion inside the materials; and finally, the materials are optically isotropic.
- Quantum and thermal effects are neglected.
- The modelled layers are treated as coherent layers, even for thicker layers.

A MATLAB (MathWorks) routine was developed in-house to perform the transfer matrix modelling (see Appendix C.1). First, the reflection of a multi-layered structure is calculated using the TMM with scattering matrices. Then this reflection spectrum is fed to a RIFTS method algorithm, which calculates the effective optical thicknesses of the layers using a Fast Fourier Transform. The input parameters of the routine are the following:

- Source parameters: the inputs needed for the source of the wave are the free space wavelength we are studying, as well as the elevation and azimuthal angles. In this study case, these angles are fixed at 0 since the light is perpendicular to the layers.
- External materials: the reflection and transmission media must be specified using their relative permeability and permittivity. In the two external regions, the media is air and its relative permeability and permittivity were both fixed to 1.
- Device parameters: the thickness and refractive index of each layer must be specified. These are obtained from the PSi characterisation presented in Section 4.3.2. Since the refractive index of a porous layer is dependent on its porosity and filling media, the porosity will be used as device parameters instead.

The simulation was divided into three stages, during which the device parameters were adapted: first a single layered membrane is simulated (Figure 4.4, ①); then a double-layered membrane (Figure 4.4, ②) and finally a triple-layered membrane (Figure 4.4, ③). For all three stages, the first layer is called the sensing layer. For the multi-layer simulations, the second layer is called the contrast layer. The third layer added for the last simulation step is called the support layer.

The main parameters that are studied during this simulation are the porous layers' porosities and thicknesses. These properties are respectively dictated by the current density used during the electrochemical etching and the etch duration. For each simulation step, these are the design questions that need to be answered:

1. Which porosity and thickness of the sensing layer gives the highest sensitivity ?
2. Which porosity and thickness of the contrast layer gives the biggest contrast with the sensing layer ?

3. Which porosity of the support layer impacts the overall optical signal the least ?

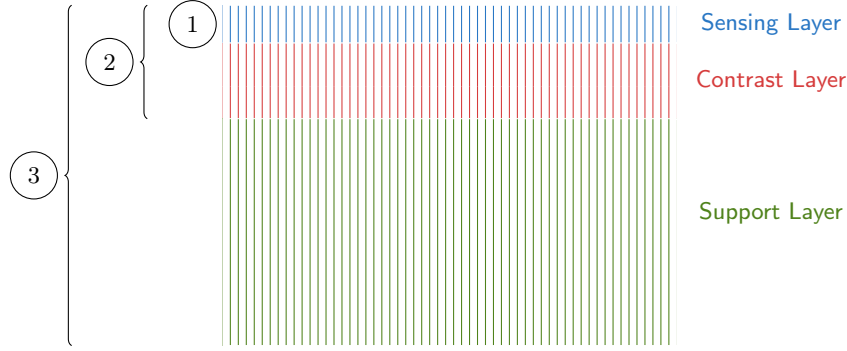


Figure 4.4: Schematic illustration of the geometry used at the different stages of the TMM simulations. The circled numbers illustrate the simulation steps.

The notion of sensitivity, which needs to be optimised in the first simulation step, was defined in Section 4.3.2 and relies on the notion of relative EOT shift (as defined in Equation 4.1). To compute the sensitivity, one must therefore first compute the relative EOT shifts for different media surrounding the porous layer. These different surrounding media will impact the overall refractive index of the layer. To compute this overall layer refractive index, the Looyenga effective media approximation [264] is used; this approximation is preferred over the Bruggeman one for high porosity layers. The Looyenga approximation expresses the layer refractive index n_{layer} , in terms of the porosity P , the refractive index of the skeleton of the porous material (e.g. Si or SiO₂) n_{skel} and the refractive index of the filling material n_{fill} :

$$n_{\text{layer}}^{2/3} = (1 - P) \cdot n_{\text{skel}}^{2/3} + P \cdot n_{\text{fill}}^{2/3} \quad (4.2)$$

This value of n_{layer} enables the calculation of the relative EOT shifts for different filling media. These EOT values can be plotted with respect to the filling media refractive index, fitted by a linear regression, and the sensitivity is given by the slope of this regression.

For simulation steps 2 and 3, the porosity and thickness of the top layers remains fixed, based on the observations made in the previous steps. The simulation parameters used at each step are detailed in Table 4.3.

Name	Value/Range			Description
	Step ①	Step ②	Step ③	
por1	50-80 [%]	75 [%]	75 [%]	Porosity of the sensing layer
por2	/	50-65 [%]	50 [%]	Porosity of the contrast layer
por3	/	/	50-75 [%]	Porosity of the support layer
L1	2-10 [μm]	5 [μm]	5[μm]	Thickness of the sensing layer
L2	/	5-30 [μm]	15 [μm]	Thickness of the contrast layer
L3	/	/	80 [μm]	Thickness of the support layer
nfill		1-1.4 [RIU]		Refractive index of filling media
nskel		3.1 [RIU]		Refractive index of Si skeleton

Table 4.3: Simulations parameters for TMM simulation of the optical response.

Theoretical limit of detection For the theoretical limit of detection computation, another MATLAB routine was encoded (see Appendix C.2). This routine only takes into consideration the top layer of the porous membrane and is based on the definition of the effective optical thickness $EOT=2nL$. Indeed, upon the penetration of bacterial lysate, the refractive index n in this expression will be affected. The impact on n was calculated in terms of the volumetric fraction of bacteria that accumulated inside the porous membrane, following the Gladstone-Dale equation [265]:

$$n - 1 = \sum_i \phi'_i (n_i - 1) \quad (4.3)$$

In this expression, $\phi'_i = V_i/V$ is the volumetric fraction term of each component of a mixture. The total volume of both components is calculated in terms of the porosity and size of the porous layer.

The bacterial lysate is modelled by spherical particles, whose diameters follow a gaussian distribution around 30 ± 20 nm. The total volume of particles is multiplied by a "*frac*" term, which denotes the fraction of lysate that remains in the porous layer after filtration and is calculated in Section 4.3.3. The theoretical response is first calculated with respect to the number of particles.

How the number of particles relates to the concentration of bacteria is dependent on the bacterial strains. The bacterial lysate can be modelled by the ribosomes and proteins present in a bacterial cell, as well as DNA, RNA and cell wall fragments. While the quantity of fragments

remains difficult to measure, the average number of ribosomes and proteins inside specific bacterial strains has been previously investigated in the literature [266,267]. For bacterial strains such as *E. coli*, the average number of ribosomes per cell is 15 000 [266], while the number of proteins per cell is approximated as $\sim 2 \cdot 10^6$ [267]. Smaller bacteria, such as *S. aureus*, contain less proteins: only $\sim 3 \cdot 10^5$ proteins per cell [267]. However, a large part of these proteins are smaller than 5 nm in diameter, and therefore flow through the porous membrane. Based on data gathered by Vendeville *et al.* [266] and Ando *et al.* [268], the number of particles larger than 10 nm of diameter inside a bacterial cell can be approximated as 20 000. The parameters used for the limit of detection calculation of detailed in Table 4.4.

Name	Value	Description	Ref.
por	80 [%]	Porosity of the sensing layer	/
t	5 [μm]	Thickness of the sensing layer	/
n_s	1.7 [RIU]	Refractive index of porous skeleton	/
L	1 [mm]	Size of the membrane	/
n_{lys}	1.3900 [RIU]	Refractive index of a bacterial cytoplasm	[269]
n_{PBS}	1.3348 [RIU]	Refractive index of PBS	[270]
frac	0.5	Fraction of lysate inside the layer	/
num	20000	Number of particles per bacterial cell	[266,267]
CFU	1 - 10^6 [CFU]	Number of bacteria in the analyte	/

Table 4.4: Simulations parameters for the membrane filtration inside a 2D microcavity model.

4.3 Results

As mentioned earlier, the detection protocol for the biosensor studied here relies on the capture of bacterial lysate inside a porous silicon membrane. To help the design of the biosensor by establishing the constraints on the porous matrix morphology, bacterial lysis is studied and characterised. To fulfil those constraints, the effect of the fabrication process on the porous silicon morphology is also studied in terms of pore size, etch rate, porosity and sensitivity.

To optimise the performance of the sensor, the filtration capability of different PSi nanostructures has been studied via Comsol Multiphysics simulations. The optical response was also optimised with respect to the pore morphology, using Transfer Matrix simulations. Finally, a theoretical limit of detection was established.

4.3.1 Bacterial lysis study and characterisation

A full study of the lytic activity of the PlyB221 endolysins on *B. cereus*, which was performed and published by Leprince *et al.* [263], can be found in Appendix D. The present section will focus on the effect of the lysis on the morphology of the bacteria.

A first visual inspection of the bacterial lysate was made with an inverted microscope. Examples of the observations for bacterial suspensions before and after lysis are illustrated in Figure 4.5. Figure 4.5, a) depicts living *B. cereus* cells; they are $\sim 6 \mu\text{m}$ long and $\sim 1 \mu\text{m}$ wide. Figure 4.5, b) shows the bacterial fragments that compose the bacterial lysate. Large fragments can be observed, surrounded by smaller ones.

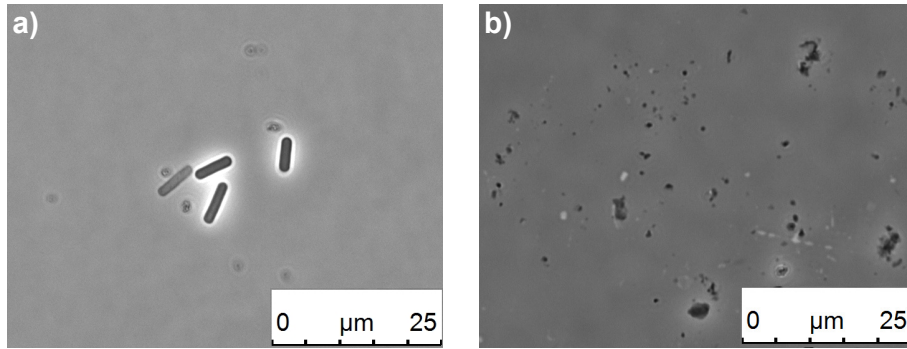


Figure 4.5: Microscopy images of bacterial suspension of *B. cereus*: a) before lysis, and b) after lysis, which corresponds to a 30 min of incubation with PlyB221 at 30 °C.

A set of ten images was analysed with the ImageJ software in order to extract data regarding the size distribution of these fragments, based on their Feret diameter. The histogram compiling this data is given in Figure 4.6. It can be observed that numerous bacterial fragments are smaller than $1 \mu\text{m}$. The precision of these measurements is however limited by the magnification of the microscope images: fragments smaller than 100 nm could not be measured.

SEM observations were therefore carried out in order to obtain higher magnification images. Examples of these observations are illustrated in Figure 4.7. *B. cereus* cells can be observed in Figure 4.7, a); their size is similar to the one observed on the microscopy images. It has to be noted that some PBS crystals remain in between bacteria cells, which might be linked to an incomplete removal of the PBS during the dehydration steps

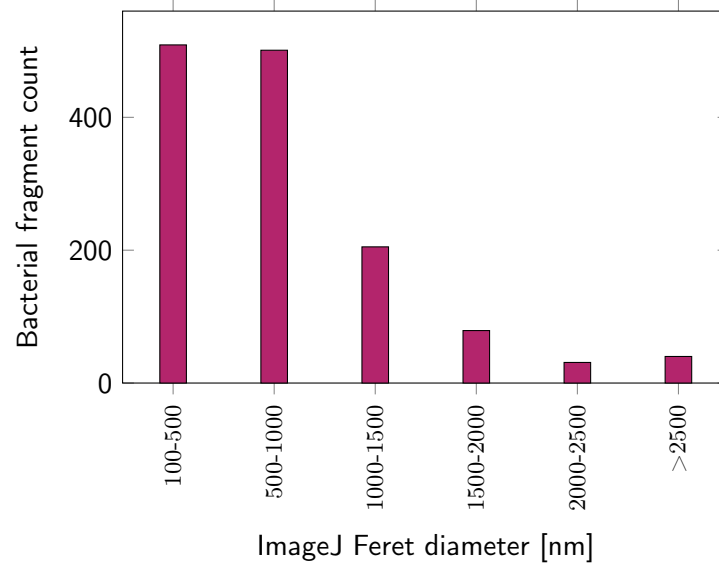


Figure 4.6: Histogram of the number of *B. cereus* bacterial fragments as a function of their measured Feret diameter, which can be approximated as their size.

of the sample preparation. Figure 4.7, b) depicts the bacterial lysate: as for the microscopy images, large fragments are observed, surrounded by much smaller fragments.

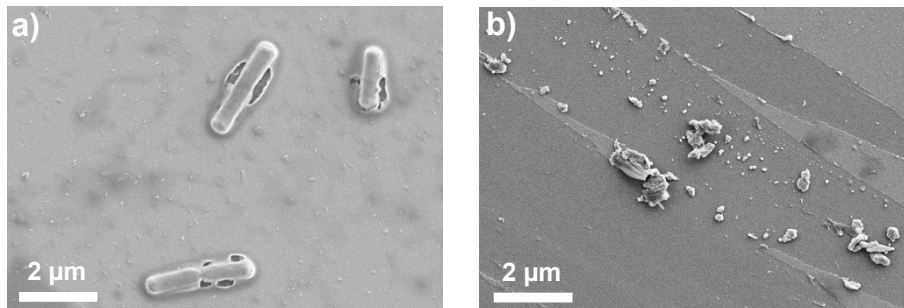


Figure 4.7: SEM observations of bacterial suspension of *B. cereus*: a) before lysis, and b) after lysis, which corresponds to a 30 min of incubation with PlyB221 at 30 °C.

Once again, a series of ten images was analysed for all three samples types, namely the bacterial suspension, the bacterial lysate and the PBS control, using the "Analyse Particles" feature on ImageJ. The resulting

data, from which the gold coating thickness is subtracted, is represented in Figure 4.8.

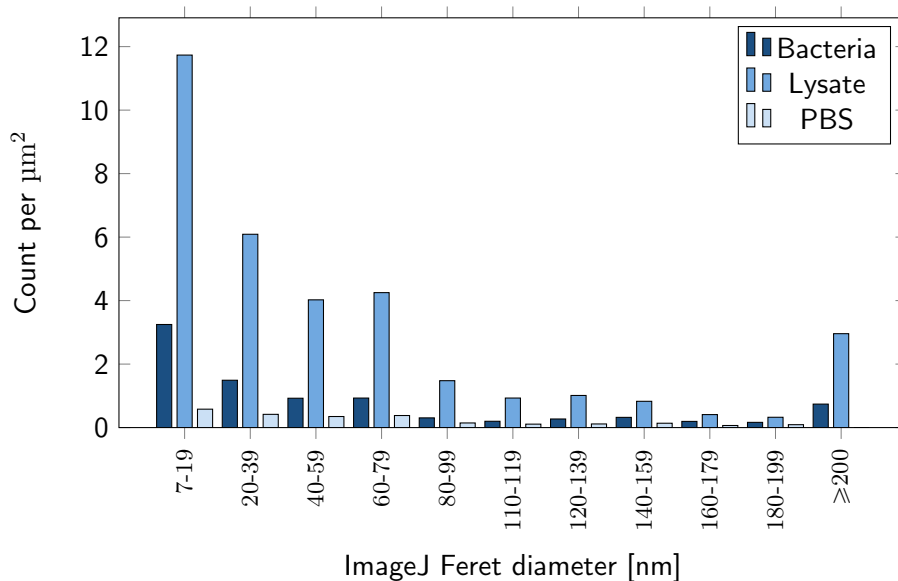


Figure 4.8: Histogram of the ImageJ Feret diameter distribution, measured on the bacterial suspension, the bacterial lysate and the PBS control SEM observations.

The cut-off value for the maximum particle size was fixed at 200 nm; while very large fragments or cells were present in all observed samples, their number was significantly lower than the number of smaller fragments. We can observe that the bacterial lysate presents the highest quantity of small fragments, compared to both control tests. Most of these bacterial fragments are smaller than 80 nm. Some smaller fragments can also be observed on the bacterial suspensions samples: these can be attributed to spontaneous cell death of bacteria cells as well as to the proteins they produce.

Higher magnification SEM images are depicted in Figure 4.9. In the bacterial lysate image, small particles are clearly visible. ImageJ analysis of a series of 10 images approximated their size to a couple of nanometers, after the subtraction of the gold coating thickness. These particles were not observed on the PBS control samples, as can be observed in Figure 4.9 as well.

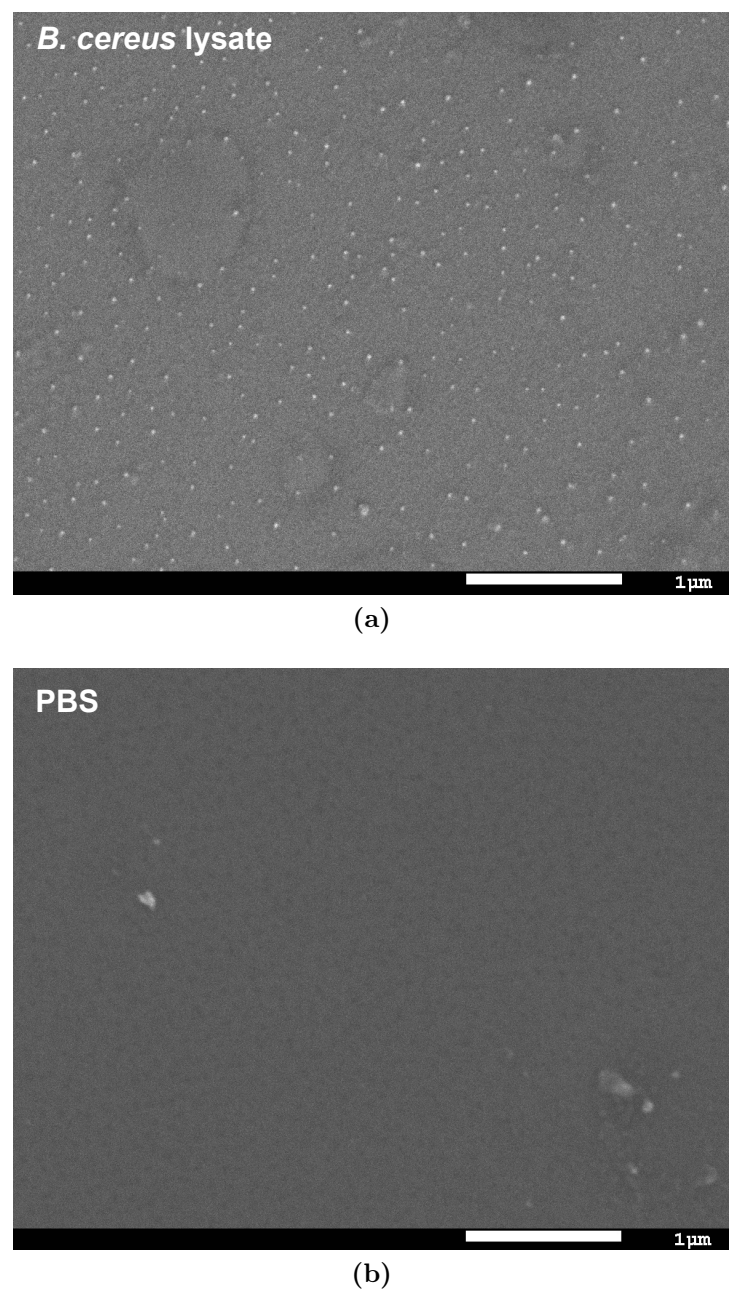


Figure 4.9: SEM observations of (a) *B. cereus* lysate and (b) the PBS control sample at very high magnification.

4.3.2 Porous silicon properties

Now that the lysate size distribution has been established, the porous silicon fabrication can be studied. As mentioned in Section 3.1.2, the range of possible pore size is large. Specifically, macroporous layers with micrometer pore size and mesoporous layers with pore size of 50 nm or smaller have been widely studied [6]. Pore sizes between 50 and 200 nm however remain to be extensively studied despite their wide range of applications; this might be linked to the very small parameter window that allows the formation of such pores [271]. For this research, it was decided to use highly doped *p*-type silicon substrates with the aim of reaching pores of between 10 and 100 nm; with this range of pore size, it would be possible to fabricate a multi-layered porous membrane, that would capture as much lysate as possible while still enabling the endolysins to flow through the membrane. Another important feature is porosity: the higher the porosity, the more bacterial lysate captured inside the PSi membrane.

Several PSi samples were thus fabricated using different fabrication parameters. The main parameter studied here is the current density, as it has been shown that pore dimension increases with current density [272]. The maximum current density was fixed at 250 mA/cm², as higher values led to highly porous and very fragile layers. It has to be noted that for all samples, the transitional layer was removed via wet etching before the electrochemical etch [87, 90].

The PSi samples were first optically characterised using SLIM in order to obtain the thickness and porosity of the porous layer. The values of the layer thickness were then verified via cross-section SEM measurements. An example of a SEM cross-section observation for a sample etched at 200 mA/cm² is given in Figure 4.10. The layer is 4.3 µm thick and the pore morphology is straight but branched. To compare all samples, the collected data is however expressed in etch rate instead of thickness. As can be observed in Figure 4.11, the higher the current density, the faster the etch. This variation appears to be linear with the current density.

The variation of porosity with current density is illustrated in Figure 4.12: there is also a linear increase of porosity with current density. The maximum average value of porosity is ~81 %, but samples with up to 90 % of porosity were measured. The increasing trend of both porosity and etch rate with current density matches the trends observed in the literature [192].

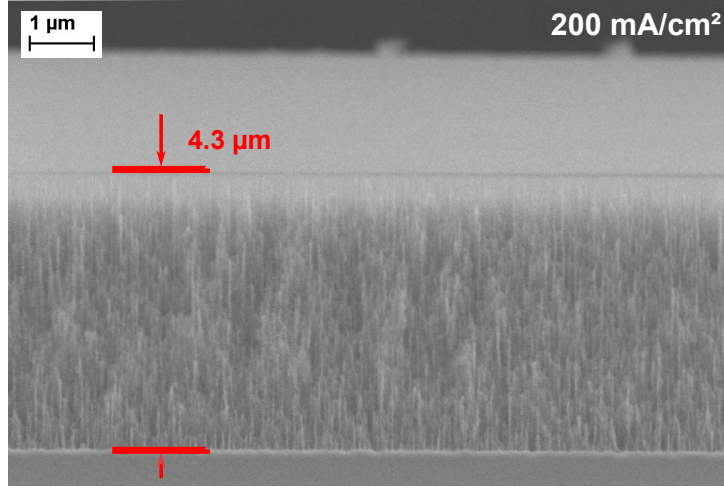


Figure 4.10: Cross-section SEM images PSi layer etched on a p++ wafer (0.8-0.9 mΩ cm) at 200 mA/cm² for 60 s in HF:etOH 3:1 in volume.

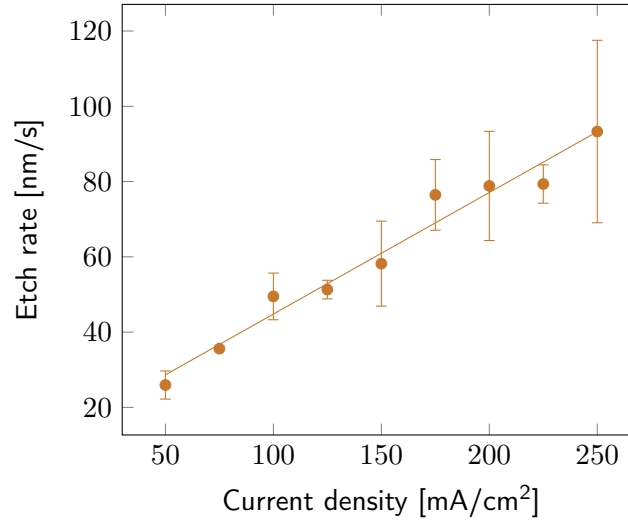


Figure 4.11: Calculated etch with varying current densities, on a p++ Si wafer (0.8-0.9 mΩ cm) in HF:etOH 3:1 in volume, based on SLIM measurements.

The pore sizes of all PSi samples were characterised based on SEM observations. Examples of these observations at different current densities are illustrated in Figure 4.13. While an increase in pore size is not very noticeable from 50 to 100 mA/cm², it is much more visible for higher

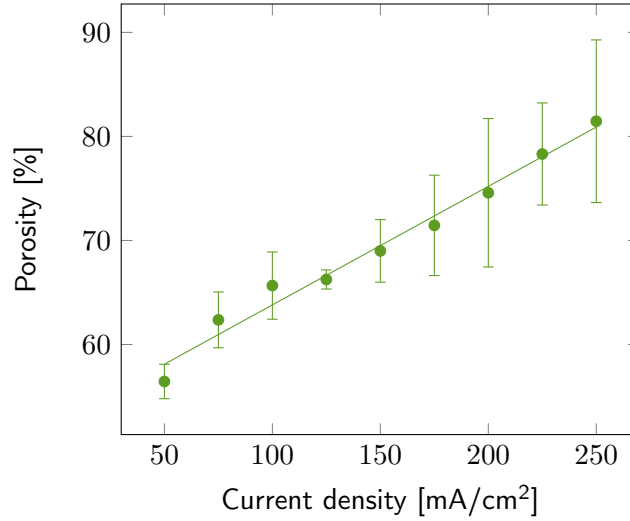


Figure 4.12: Calculated porosity with varying current densities, on a p++ Si wafer (0.8-0.9 mΩ cm) in HF:etOH 3:1 in volume, based on SLIM measurements.

current density values. It can also be observed that the pore wall does not seem affected by the current density; this has also been reported in the literature, as the width of the pore wall only seems to be affected by the doping concentration of the substrate and the HF content of the electrolyte [192].

Image analysis provided data about the average pore size for all current density values, as illustrated in Figure 4.14. Like porosity and etch rate, the average pore diameter increases with current density, but the trend is more quadratic than linear. Similar trends have been observed in the literature as well [192]. It can also be noted that the higher the current density, the higher the pore size range: while the average pore size for 250 mA/cm² is still less than 50 nm and therefore mesoporous, larger pores can be observed, up to a 100 nm for some samples.

Finally, the optical sensitivity of the layers was studied. The sensitivity is especially important when considering an optical sensor. The values for the sensitivity are gathered in Table 4.5, which summarised all the properties discussed above. It was observed that the sensitivity increases with current density. This might be linked to the increase in porosity, as the average refractive index of the porous layer is not only impacted

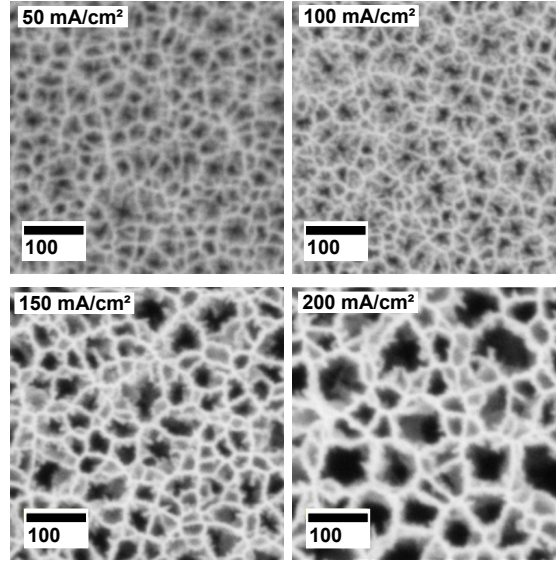


Figure 4.13: Top view SEM observations of porous silicon produced with different current densities: 50, 100, 150 and 200 mA/cm². The PSi was etched on a p++ Si wafer (0.8-0.9 mΩ cm) in HF:etOH 3:1 in volume. The scale bar represents 100 nm.

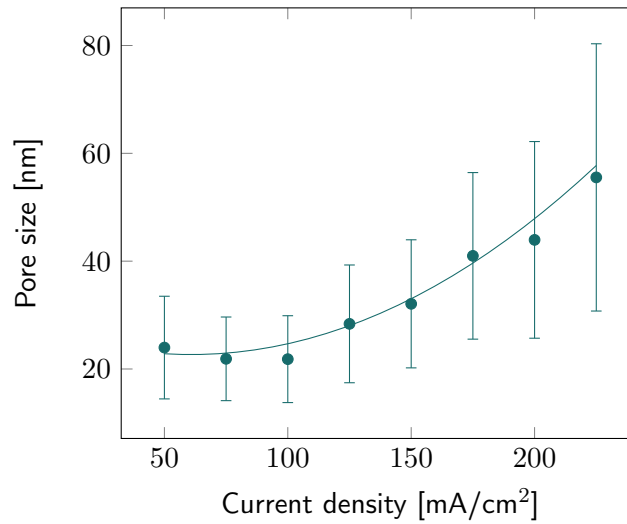


Figure 4.14: Calculated pore size with varying current densities for PSi layers etched on a p++ Si wafer (0.8-0.9 mΩ cm) in HF:etOH 3:1 in volume.

by the refractive index of the filling media, but also on the porosity. Indeed, the higher the porosity, the more filling material will be able to penetrate the layer, and the more it will affect the EOT.

Current density [mA/cm ²]	Etch rate [nm/s]	Pore size [nm]	Porosity [%]	Sensitivity [% _{EOT} /RIU]
50	25.9 ± 3.73	23.9 ± 9.52	56.4 ± 1.65	36.6 ± 0.03
75	35.6 ± 0.43	21.8 ± 7.75	62.4 ± 2.68	42.6 ± 1.43
100	49.5 ± 6.20	21.8 ± 8.05	65.7 ± 3.23	46.4 ± 1.74
125	51.3 ± 2.47	28.4 ± 10.91	66.2 ± 0.92	46.9 ± 0.18
150	58.2 ± 11.29	32.1 ± 11.88	69 ± 3.01	50.6 ± 2.07
175	76.5 ± 9.39	40.9 ± 15.44	71.4 ± 4.82	54.8 ± 4.96
200	78.8 ± 14.51	43.9 ± 18.24	74.6 ± 7.13	60.5 ± 7.15
225	93.3 ± 24.24	55.5 ± 24.78	78.3 ± 4.91	64.5 ± 7.85

Table 4.5: Properties of PSi samples etched at different current densities, on a p++ Si wafer (0.8-0.9 mΩ cm) in HF:etOH 3:1 in volume; these values were extracted from SLIM measurements and SEM observations.

It should be noted that none of these properties should vary for longer etching time using the same current density. This was verified by testing different etching times. The variation of most properties, such as sensitivity, exhibited some minor variations with etching times. More significant variations were observed for the porosity and etch rate. These can be attributed to the depletion of fluorine atoms with longer etching times, and can be avoided using the etch-stop etching method [273].

4.3.3 Simulation of membrane filtration

The filtration performance of porous membranes was studied using the Comsol Multiphysics software. Two different porous geometries were analysed: a microcavity and a double layer.

Microcavity 2D model The microcavity 2D model is composed of two Bragg stacks, surrounding a defect cavity. The Bragg stacks are a succession of larger pores and smaller pores. The simulated pore size is selected based on the observations made in Section 4.3.2: the large pore diameter is fixed at 40 nm (which corresponds to a current density of ~ 200 mA/cm²) and the small pore diameter is fixed at 20 nm (which corresponds to a current density of ~ 50 mA/cm²). The thickness of each layer is fixed arbitrarily.

Because of the small feature size, the Reynolds number is very low and

the flow inside the porous membrane can be modelled by a creeping flow. The flow rate is fixed by the pressure drop across the membrane. The lysate is modelled by particles of 30 ± 20 nm, which are released all at once at time $t=0$. A time-dependent simulation is ran in order to track the particles' displacement and stopped once all particles have either passed through the membrane or remain frozen inside. An illustration of the particles' trajectories just after the release is illustrated in Figure 4.15. Figures 4.16 and 4.17 illustrate the particles' distribution at and intermediate stage and after the last simulation step, respectively; in the latter, most particles are either stuck on top of the membrane, unable to enter the porous membrane, or frozen inside the first layer. No particles remain inside the cavity once all particles have flown through.

Figure 4.18 depicts the percentage of particles that remain both in the first layer and in the full membrane overtime. It can be observed that the vast majority of the particles present in the membrane are situated in the first layer.

Double layer 2D model The double layer model is composed of an upper fluidic channel and a porous membrane. Only the first layer is fully represented in the simulated geometry; the second layer is only depicted by the outlet of the first layer, which is reduced to the pore size of the second layer. The pore diameter of the first layer is fixed by a Gaussian distribution, based on a 50 nm mean and a 25 nm standard deviation (which corresponds to a current density of ~ 225 mA/cm²). The particles representing the bacterial lysate have a diameter fixed at 30 ± 20 nm.

Like for the microcavity model, the flow is modelled by a creeping flow, whose rate is fixed by the pressure drop across the geometry. The particles are released at time $t=0$, as illustrated in Figure 4.19 and the displacement across the membrane is analysed overtime, as depicted in Figure 4.20. The particles' position inside the geometry at the end of the simulation, meaning once all particles have flown through the membrane, is illustrated in Figure 4.21: the particles are either stuck on top of the membrane, or at the bottom of the first layer. No particles are stuck to the pore walls. It can also be observed in this figure that the flow rate is impacted by the pore diameter: larger pores experience a higher flow rate.

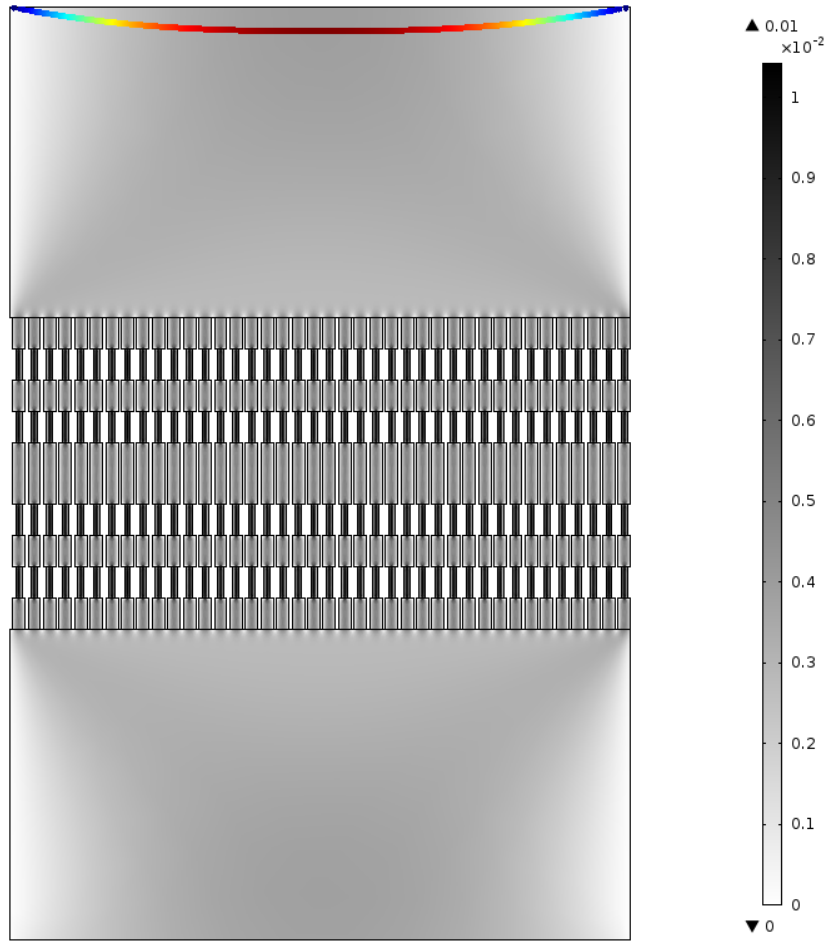


Figure 4.15: Illustration of the particle tracing inside the 2D microcavity model, just after the release of the particles from the top fluidic channel. The grey gradient depicts the flow rate inside the geometry.

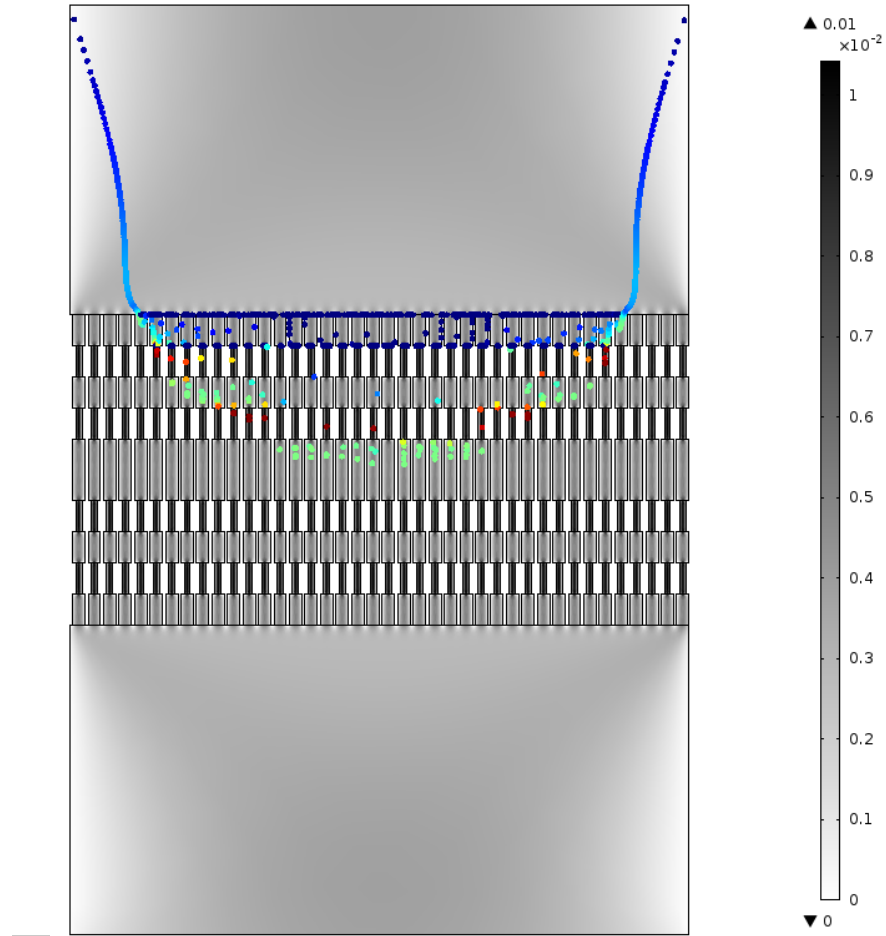


Figure 4.16: Illustration of the particle tracing inside the 2D microcavity model, at an intermediate time, when particles are still flowing through the membrane. The grey gradient depicts the flow rate inside the geometry.

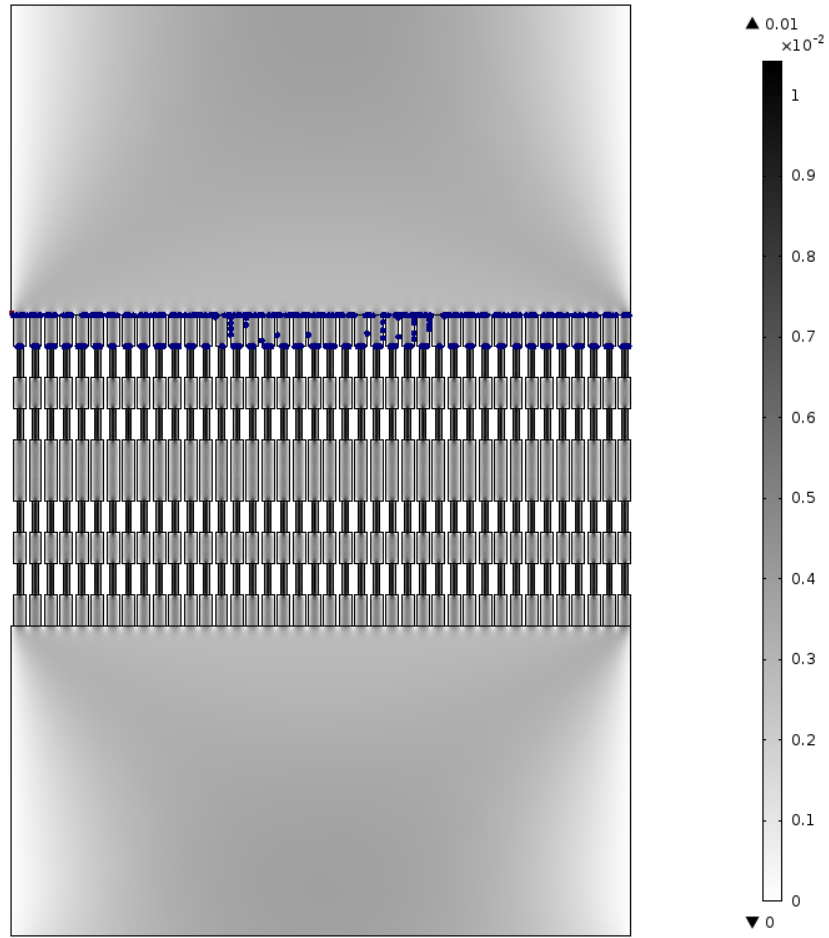


Figure 4.17: Illustration of the particle tracing inside the 2D microcavity model, once all particles have flown through the membrane. The grey gradient depicts the flow rate inside the geometry.

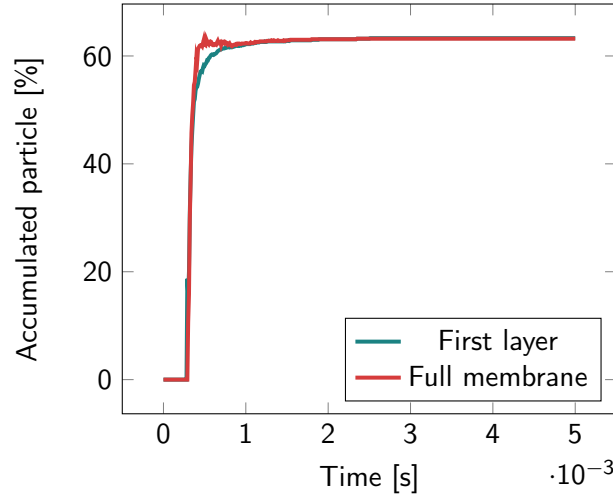


Figure 4.18: Percentage of the accumulated particles inside the full membrane and inside the first layer of the 2D microcavity overtime. The particles are released at the top of the upper fluidic channel at $t=0$.

Figure 4.22 counts the number of particles stuck inside the first layer of the porous membrane overtime, relative to the total number of released particles. This count initially increases up to 80 %, but then drops to ~ 47 % once all particles get dragged through the membrane by the flow.

The count of accumulated particles does not seem affected by the height of the fluidic channel or the thickness of the first porous layer, as the simulated value does not vary significantly (less than 1 %). It is however significantly affected when reducing the pore diameter of the second layer, which was achieved by adapting the *diff* parameter that characterises the difference in pore diameter between the first and second layer. Figure 4.23 illustrates the impact of the *diff* parameter on the percentage of accumulated particles: the smaller the pores of the second layer, the more particles are retained inside the first layer.

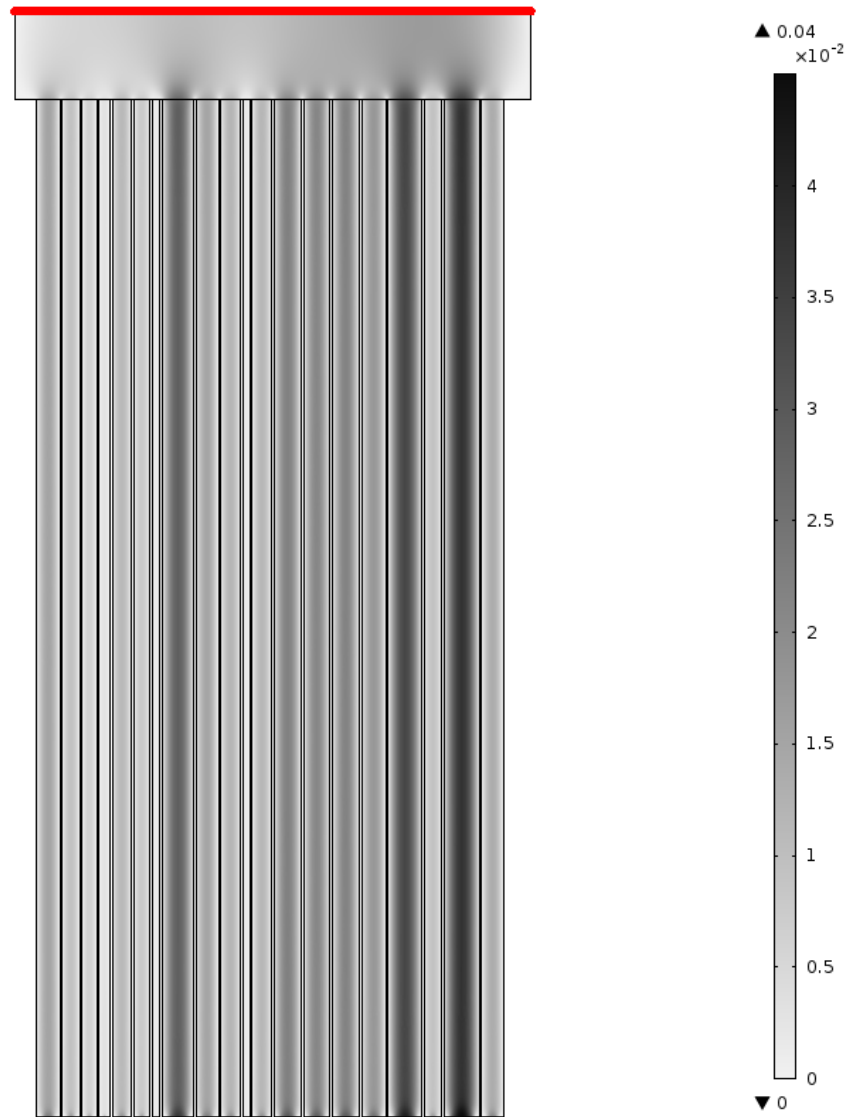


Figure 4.19: Illustration of the particle tracing inside the 2D double layer model, with parameter $diff=20$ nm, upon the release of the particles from the top fluidic channel. The grey gradient depicts the flow rate inside the geometry.

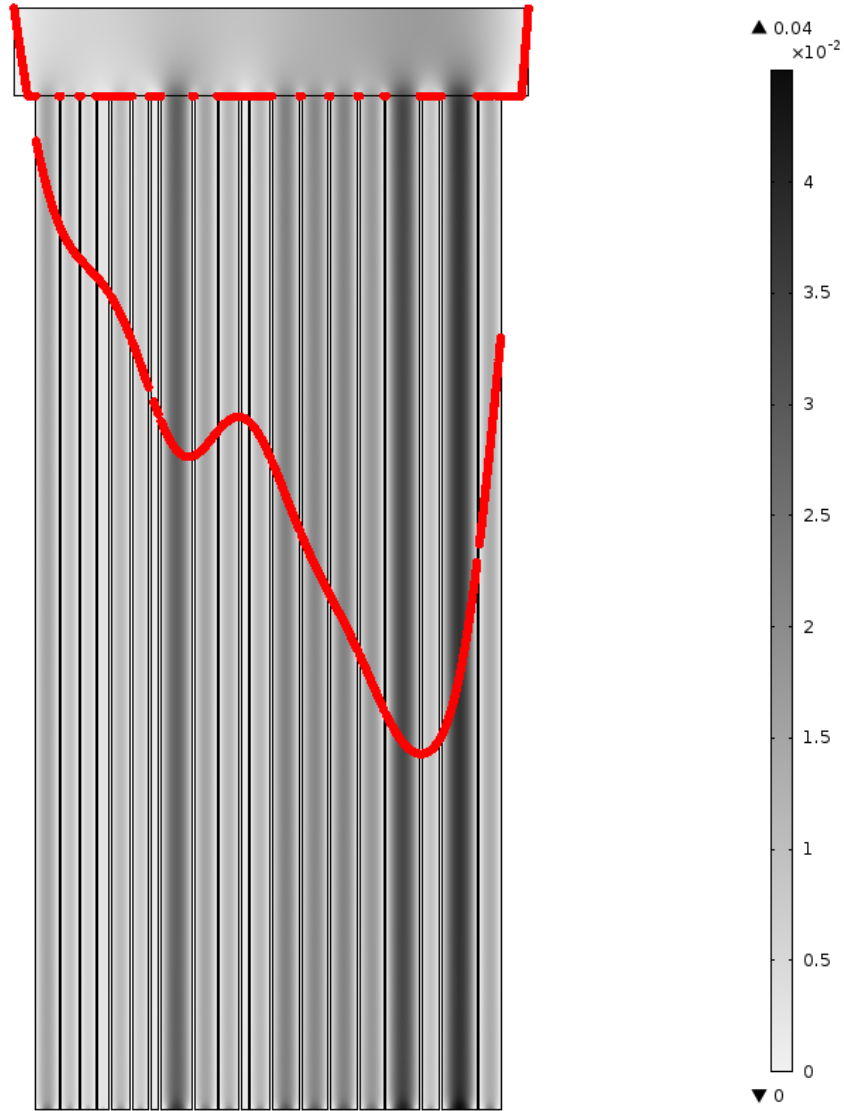


Figure 4.20: Illustration of the particle tracing inside the 2D double layer model, with parameter $diff=20$ nm, at an intermediate time, when particles are still flowing through the membrane. The grey gradient depicts the flow rate inside the geometry.

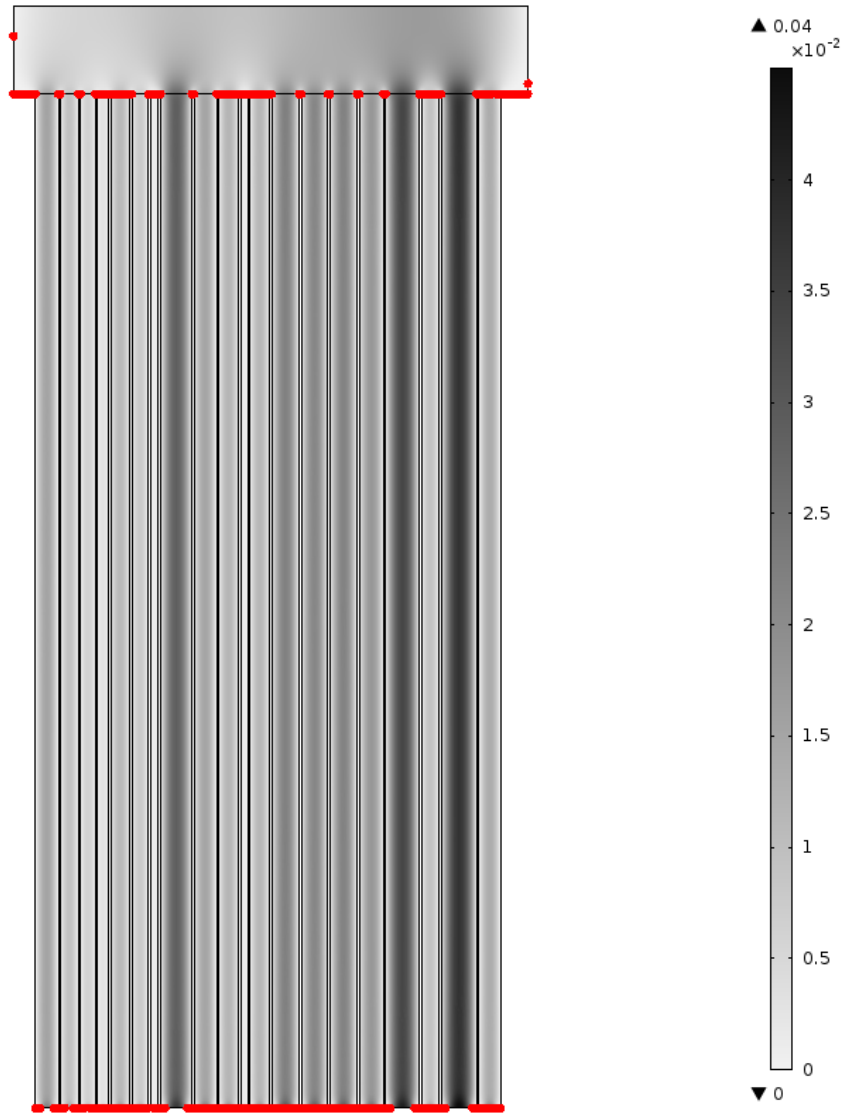


Figure 4.21: Illustration of the particle tracing inside the 2D double layer model, with parameter $diff=20$ nm, once all particles have flown through the membrane. The grey gradient depicts the flow rate inside the geometry.

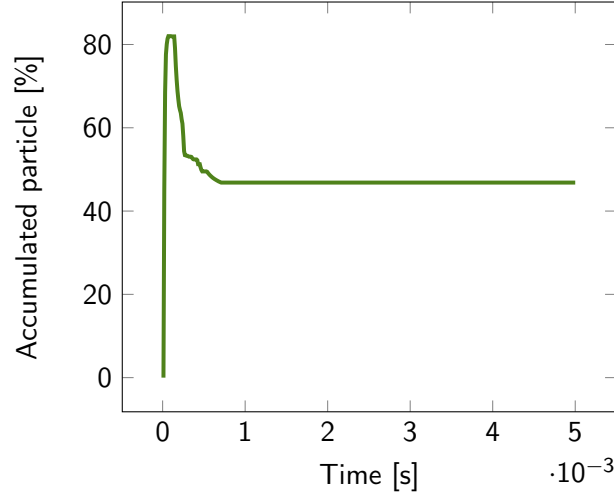


Figure 4.22: Percentage of the accumulated particles inside the first layer of the 2D double layer overtime, with parameter $diff=20$ nm. The particles are released at the top of the upper fluidic channel at $t=0$.

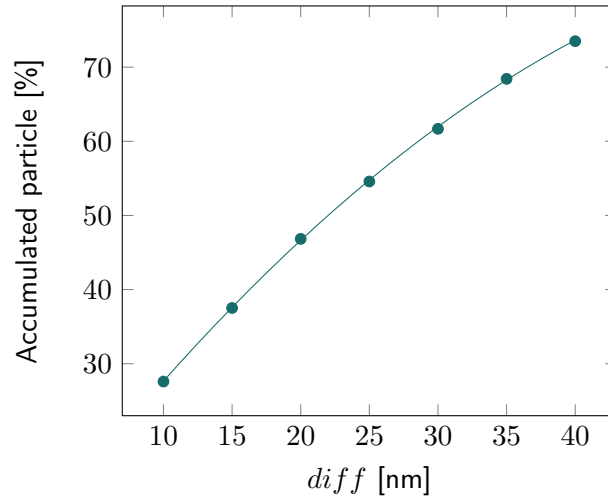


Figure 4.23: Percentage of the accumulated particles inside the first layer of the 2D double layer with varying $diff$, meaning with increasing difference in pore diameter between the first and second layers.

4.3.4 Optical response modelling

4.3.4.1 Transfer matrix simulation

The optical response of the porous sensors is modelled using transfer matrix method (TMM) simulations. The sensor is simulated as a multi-

layered structure, and the impact of the morphology of each layer on the overall optical response is studied. Based on the results of the membrane filtration simulation, microcavities and Bragg Stacks are excluded from the optical response study, since particles are not able to reach the porous layers of interest for these nanostructures. Only interferometric multi-layers are therefore considered here, and the optical response is based on RIFTS. The study is divided into three stages; the first stage only considers a single layer, and each subsequent stage adds one more porous layer beneath it.

Model validation Before diving into the optical response simulation, the model is validated by comparing the simulated theoretical optical output to an experimentally measured one. The experimental sample is a single porous layer etched at 200 mA/cm^2 for 30s. The thickness and porosity of the sample are measured using the SLIM method: the thickness is $3.3 \text{ }\mu\text{m}$ and the porosity is 75%. These parameters are used in the TMM model in order to calculate the optical signal. Both experimental and simulation optical spectra are depicted in Figure 4.24: a fringe pattern can be observed in both spectra, but it remains difficult to establish if the frequency of the fringes is similar.

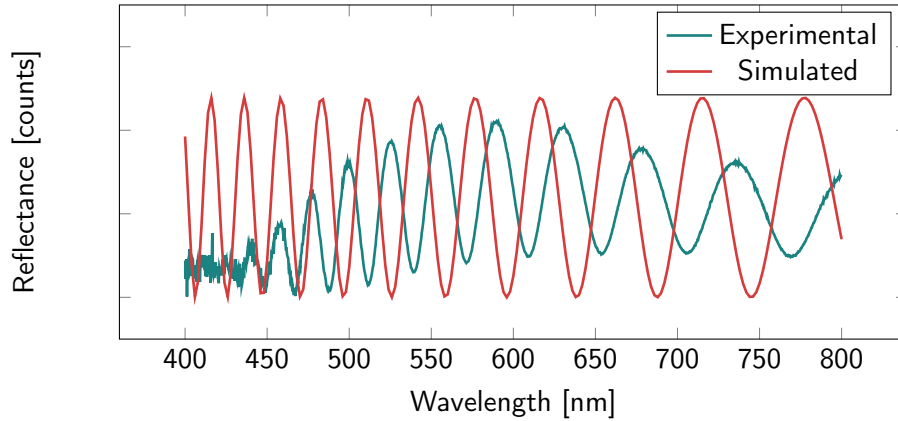


Figure 4.24: Interferometric spectra obtained experimentally and theoretically for a single porous layer etched at 200 mA/cm^2 for 30s. The values of thickness and porosity used for the simulated spectra were calculated via SLIM on experimentally acquired spectra.

A RIFTS routine is applied to both optical spectra in order to compare the fringe frequency, which is reflected by the EOT value. The resulting

FFT is illustrated in Figure 4.25, and one can see that both spectra are centred around the same $2nL$ values, namely ~ 9000 nm. More precisely, the EOT value of the experimental spectra is 9017 nm, and the simulated one is 8965 nm. The difference between these two values remains minor and can be attributed to the fact that the porosity and thickness used for the simulation are fitted with SLIM and not exact measurements. Moreover, the transfer matrix model considers an ideal situation, with no losses, absorption or wavelength dispersion, which also may explain the slight deviation between theory and experiment. It can however be concluded that the TMM simulation is able to model porous silicon layers.

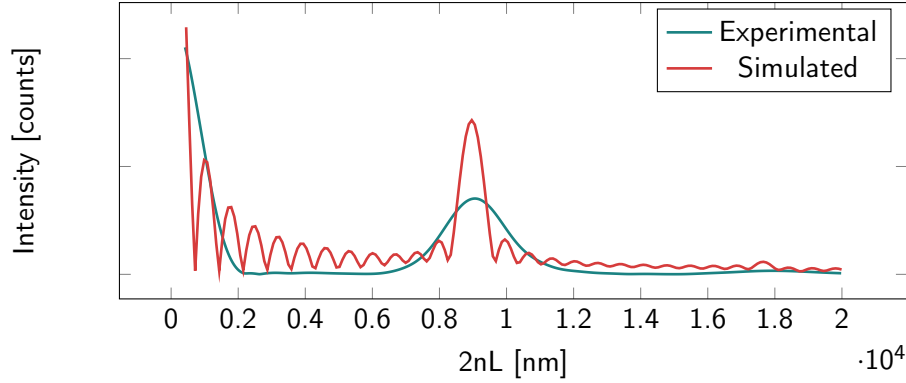


Figure 4.25: FFT of the interferometric spectra obtained experimentally and theoretically for a single porous layer etched at 200 mA/cm^2 for 30s. The values of thickness and porosity used for the simulated spectra were calculated via SLIM on experimentally acquired spectra.

Step 1: the single layer The first step of the TMM simulation focuses on the sensing layer, which is the top layer of our sensor and the most important one, as most of the bacterial lysate accumulates in this layer. The sensing layer is first studied as a single layer, surrounded by air, and both its thickness and porosity are optimised based on its optical response.

Porosity The porosity is dictated by the current density used during the electrochemical etching of the porous layer. The porosity enables the calculation of the refractive index of the layer, based on the surrounding environment, via the Looyenga approximation (see Equation 4.2) and therefore impacts the sensitivity of a PSi optical sensor. To analyse the

effect of porosity on sensitivity, the thickness of the layer is arbitrarily fixed at 5 μm and the porosity varies between 50% to 80%. Figure 4.26 depicts the observed variation: sensitivity increases with porosity. This trend can be explained by the higher volume of filling media present inside the porous layer for higher porosities, which increases the overall layer refractive index. The linear variation is similar for other layer thicknesses.

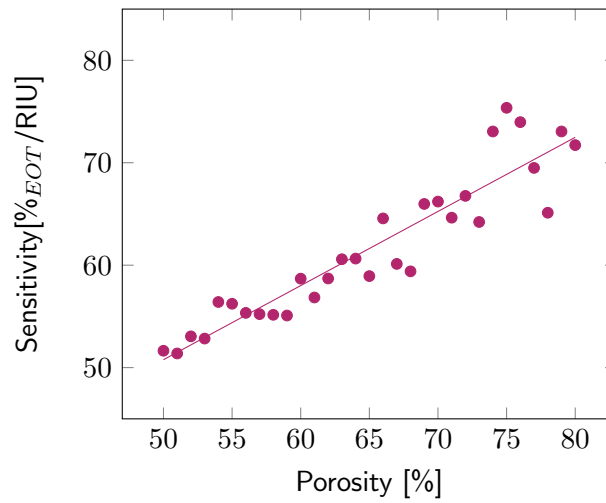


Figure 4.26: Variations of the sensitivity with the porosity of the first layer, with an arbitrary thickness of 5 μm .

Thickness The thickness of a PSi layer is dictated by the duration of the electrochemical etch. The impact of thickness on sensitivity is studied by varying the layer thickness from 2 to 10 μm , while keeping the porosity fixed at 80%. Figure 4.27 illustrates the variation of sensitivity with layer thickness: some variation can be observed, but the overall trend seems constant. The same trend is observed for other porosity values.

Step 2: the double layer The second step of the TMM simulation focuses on the contrast layer. The studied geometry is therefore a double layer, with the sensing layer on top and the contrast layer at the bottom. Light will reflect at the interface between the two layers, but only if there is enough contrast between layers. Indeed, should the refractive index be similar for both layers, then the two layers will be considered as one

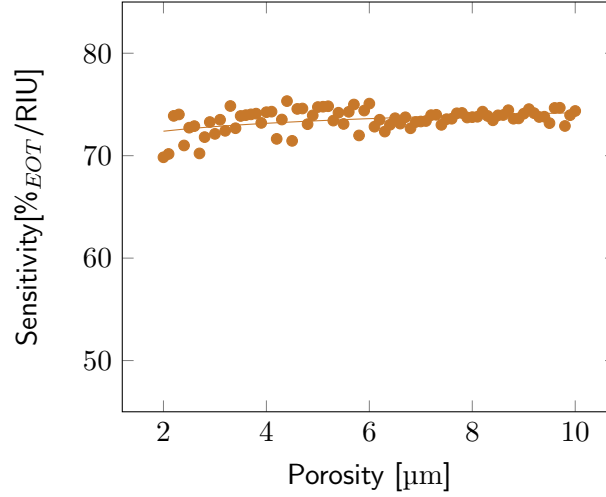


Figure 4.27: Variations of the sensitivity with the thickness of the first layer, with an arbitrary porosity of 80 %.

and there will be no EOT peak for the sensing layer. The morphology of the second layer and its impact on the optical response of the first layer is therefore studied. For this step, the porosity and thickness of the first layer are fixed at 75 % and 5 μm respectively.

Porosity The impact of the porosity of the contrast layer, which translates the impact of current density on the sensitivity of the overall structure, is studied over a range from 50 % to 70 %. The layer thickness is arbitrarily fixed at 10 μm . Figure 4.28 outlines the sensitivity values measured at different contrast layer porosities. The sensitivity remains roughly constant, and this trend is also observed for other layer thickness values.

To study the contrast between the first and second layers, the intensity of the EOT peak is compared to the surrounding noise, which is approximated using the standard deviation of the signal. The higher the EOT peak, the easier it is picked out and fitted by the RIFTS routine. The peak to noise ratio was therefore calculated by dividing the peak intensity by the standard deviation of the surrounding signal. The peak to noise ratio for a filling media of refractive index $n=1.3$ (which is close to the refractive index of PBS), for different contrast layer porosities, is detailed in Figure 4.29. It can be observed that the higher the contrast

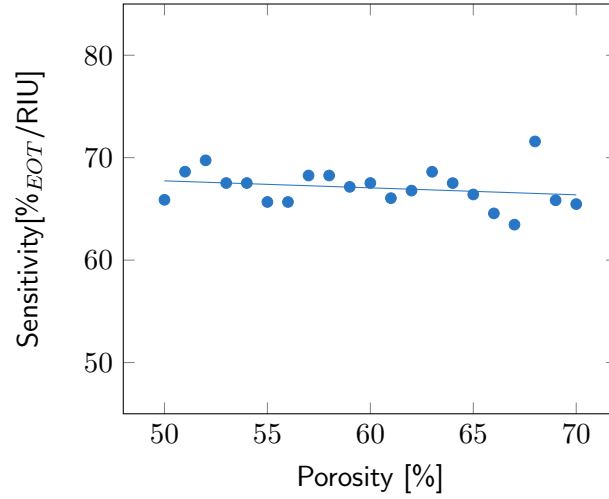


Figure 4.28: Variations of the sensitivity with the porosity of the contrast layer, with an arbitrary thickness of 10 μm .

layer porosity, the higher the peak to noise ratio.

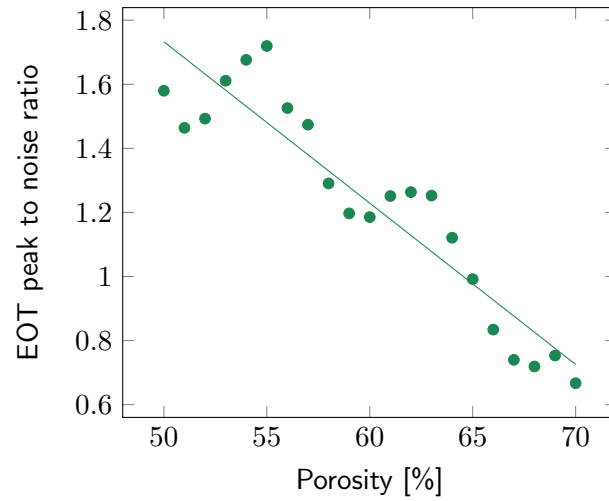


Figure 4.29: Variations of the peak-to-noise ratio of the EOT peak with the contrast layer porosity. The filling media refractive index was fixed at $n=1.3$ and the layer thickness at 10 μm .

Thickness To analyse the effect of the contrast layer thickness, its porosity is arbitrarily fixed at 50 %. The impact on sensitivity is illustrated in Figure 4.30. For thinner layers, the thickness does not seem to have a significant effect; for thicker layers, larger variations are observed, but the overall trend remains nearly constant.

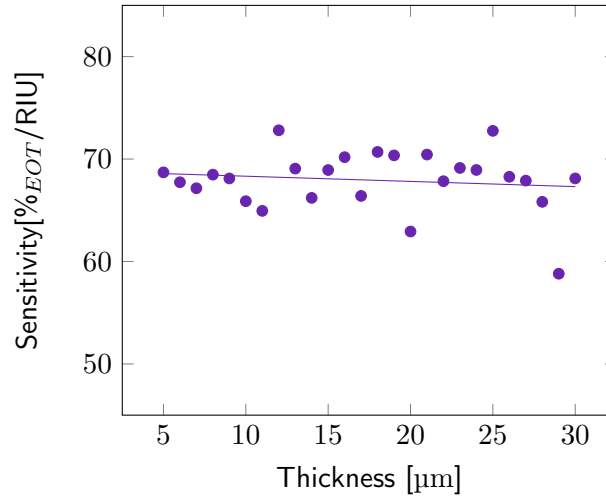


Figure 4.30: Variations of the sensitivity with the contrast layer thickness, with an arbitrary porosity of 50 %.

Step 3 : the triple layer The addition of a support layer to the porous structure has two purposes: (1) mechanically support the double layer during the pressure driven flow-through operation and (2) speed up the electrochemical etch by increasing the current density of this layer. The thickness of the layer will not be studied, but only the impact of the porosity of the support layer. Figure 4.31 illustrates the effect of porosity for an 80 μm layer. While some variations can be observed, a higher porosity support layer seems to slightly enhance the sensitivity.

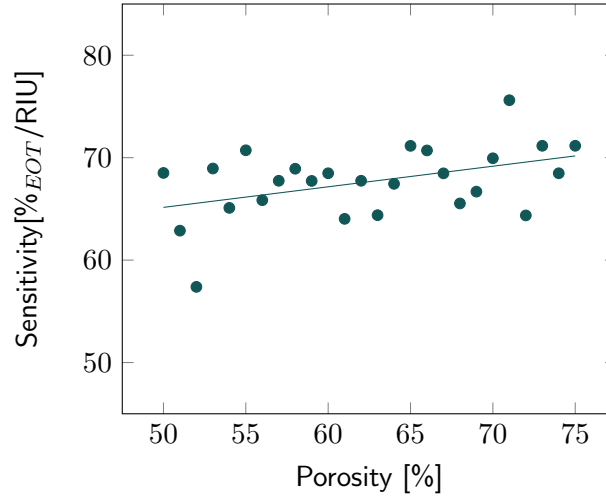


Figure 4.31: Variations of the sensitivity with the support layer porosity, with an arbitrary porosity of 80 μm .

4.3.4.2 Theoretical limit of detection

The theoretical limit of detection was approximated by considering only the top layer of the membrane, and calculating the change in EOT based on the volumetric fraction of particles accumulated inside the layer. This choice was motivated by the filtration simulations, which related that most of the lysate gets trapped in the sensing layer. The relative EOT shift with increasing quantities of particles, with 30 ± 20 nm diameters, is detailed in Figure 4.32. It can be observed that large quantities of particles are required to obtain noticeable relative EOT shifts.

To relate the number of particles to bacterial concentrations, typical values for the quantities of ribosomes and large proteins were extracted from the literature [266, 267]. The resulting relative EOT shift with increasing bacterial concentration is plotted in Figure 4.33. Both axes are expressed in a logarithmic scale, and the increasing trend is linear. The response was calculated based on 1 mL of analyte.

To verify these results, a volumetric based computation was also performed. Water composes 70% of a bacterial cell [266]; based on the remaining 30% of the typical volume of $0.9 \mu\text{m}^3$ for a rod-shaped bacterium [274, 275] and on a 0.5 fraction of lysate inside the top layer, we were able to approximate the response with increasing bacterial concen-

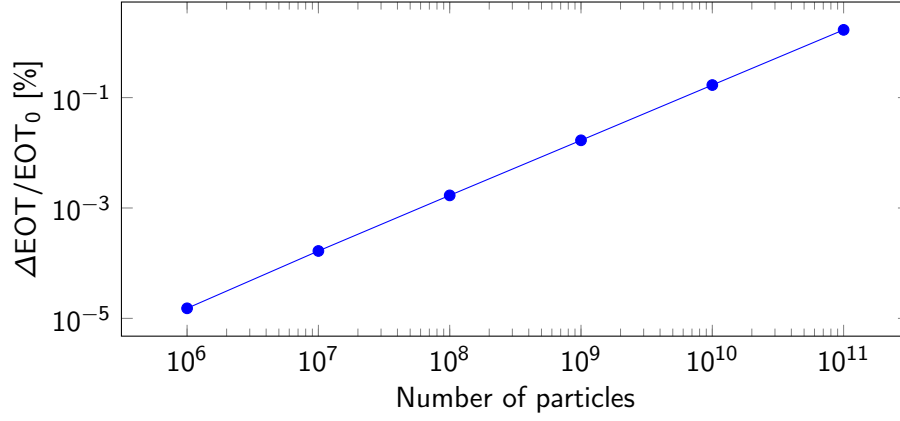


Figure 4.32: Theoretical response for increasing quantities of bacterial lysate particles, expressed in relative EOT shift.

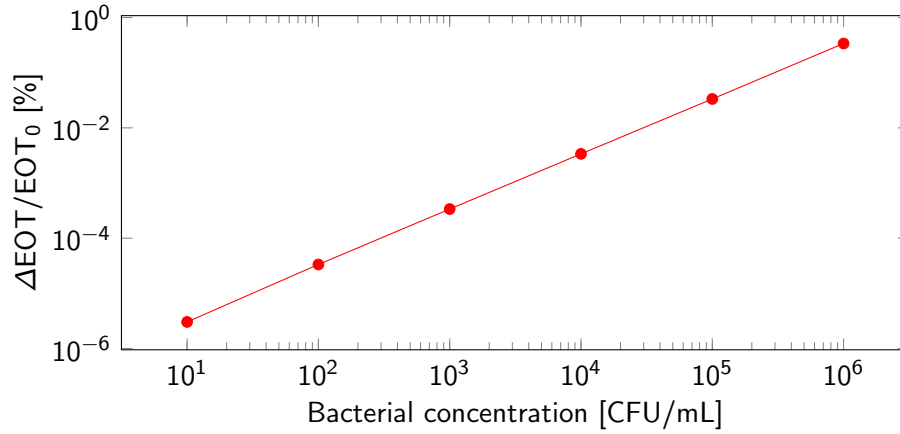


Figure 4.33: Theoretical response for increasing concentration of bacterial lysate, expressed in relative EOT shift.

trations. The obtained values were in the same order of magnitude as the values presented in 4.33.

While a small shift in EOT is observed even for concentrations below 10^3 CFU/mL, it seems unlikely that these will be distinguished from the baseline signal of the sensor. Indeed, the average error on the relative EOT value computation of the RIFTS routine, based on the measurements performed in Section 4.3.2 is in the 10^{-3} [%] range, which means that the theoretical limit of detection is computed as 10^4 CFU/mL.

The effect of several parameters on the magnitude of the detected shift is also studied. It was observed that the smaller the PSi layer, either in size or in thickness, the higher the EOT shift; indeed, the volume fraction of bacterial lysate is increased and hence the impact on the layer refractive index as well. The porosity of the layer and the fraction of lysate inside the layer have similar impacts, but to a lesser extent. By carefully selecting the biosensor design, meaning preferring a small membrane, with a thin sensing layer and a significant reduction in pore size for the contrast layer, the theoretical limit of detection can be decreased to 10^3 CFU/mL.

4.4 Discussion

In this chapter, the design of the PSi-based sensor was studied. This is a step that is not often documented in the porous silicon scientific community, but which is of the utmost importance. First, the target analyte was analysed, namely the bacterial lysate. The size distribution of the lysate was established: while some larger pieces are observed, most fragments are less than 200 nm wide, with a higher concentration of particles in the 10 to 80 nm range. These are the fragments that need to be targeted by the sensor. Their nature remains to be investigated, but it can be hypothesised that these are cell wall debris, ribosome complexes that have diameters from 25 to 40 nm [276], as well as protein-protein or DNA-protein complexes. The hypothesis that these are the PlyB221 endolysins can be excluded, as PlyB221 endolysins are much smaller: their molecular weight is 39 kDa [277], which can be approximated to a diameter of ~ 5 nm [278]. It can be assumed that the PlyB221 endolysins are represented by the particles observed in Figure 4.9 but further investigations are needed.

Next, the morphology of PSi layers was studied, in order to determine what is possible and which fabrication conditions can be used to achieve the needed morphology. The PSi sensor must be designed as a filter, with large pores on top and smaller pores beneath it to retain as many bacterial fragments as possible. It is observed that the morphology of the porous filter is limited by the fabrication conditions: pores with diameters larger than 200 nm cannot be fabricated on the same substrate as pores with diameters of 20 nm or less. On a $p++$ silicon substrate, the highest average pore size that can be achieved without risking the mechanical integrity of the sensor is ~ 55 nm, with a standard deviation

of ~ 25 nm. With this pore size range, a large portion of the bacterial fragments should already be retained. Moreover, the sensitivity to changes in the surrounding refractive index is also the highest with this pore morphology.

To study the scope of the bacterial lysate portion inside the PSi sensor, the membrane filtration was explored via fluid flow simulations. Two membrane geometries were compared: a microcavity and a double layer. While the presented 2D models offer only simplified versions of the reality of lysate filtration, the double layer was observed to be the best membrane geometry for the indirect detection of bacterial lysate, as most fragments are retained in the top porous layer. It was also observed that in order to optimise the retention of bacterial fragments inside the top layer of the membrane, the pore size of the second layer should be as small as possible. It should however be large enough to let the endolysins pass through, and also to allow the analyte to be pushed through the membrane at a reasonable pressure, in order to avoid any mechanical damage to the sensor. Indeed, the smaller the pores, the higher the pressure needed to push the analyte through at a given flow rate, which will be limited by the fragility of the porous membrane.

While the theoretical fraction of bacterial debris accumulated in the sensor could be quantified, how this value fits the reality remains to be investigated through more complex models or experimental work. Indeed, the nature of the interactions between the lysate fragments and the pore walls is still unknown; electrostatic or other effects might come into play, for example. These effects were not considered in the presented models. The effect of the particles on the fluid or the impact of pore blockage were also not investigated. Moreover, the interaction between bacterial debris was neglected; fragments may for example agglomerate, impacting the filtration. In addition, the geometry of PSi sensors is also more complex than the presented 2D model: large pores in the top layer may split into two smaller pores for the next layer, and some pores branch out to other pores. A 3D model, based on SEM images of the PSi structure to create the model geometry, may give more accurate results, but would require more computation time and power. Further investigations, studying the full membrane, may also enable the study of the fluidic resistance, which affects the flow rate through the membrane and also may impact the response.

The optical response of the sensor was also optimised using simulations.

These simulations were based on transfer matrices, which model the reflection of multi-layered structure. It was observed that the top layer, which is qualified as the sensing layer, should be as porous as possible. This parameter goes hand in hand with pore size. The thickness of the sensing layer should be as thin as possible, in order to concentrate the bacterial fragments in the smallest volume possible and hence increase the response. For the second layer, an optical contrast is needed, which is achieved by reducing the porosity. The thickness of this layer does not have a significant impact on the optical signal, but should be as thin as possible in order to limit the needed pressure to flow the analyte through it. A support layer is therefore needed, in order to make the porous membrane more robust. The porosity of this layer has a very limited impact on the optical signal. The ideal etching parameter ranges for each layer of the membrane are detailed in Table 4.6.

Layer	Current density [mA/cm ²]	Thickness [μm]	Porosity [%]	Justification
Sensing	200-250	2-5	75-80	Higher sensitivity and higher portion of bacterial fragments in the sensing layer
Contrast	50	5-15	~55	Higher optical contrast, higher portion of bacterial fragments in sensing layer and limitation of the increase in needed pressure of the inflow
Support	100-150	50-80	65-70	Mechanical support and reduction of the needed pressure of the inflow

Table 4.6: Ideal range of etching parameters for each layer of the PSi membrane

The transfer matrix model is however unable to give sufficient information about the signal intensity and signal-to-noise ratio, as it does not take into account the noise that is present during experimental measurements. This noise can be attributed to, among others, the dark noise, the baseline noise and to thermal effects of the optical read-out equipment. The noise level is also very important for the determination of the theoretical limit of detection. While it is possible to find

specifications of the noise contribution of each component of the optical read-out setup, it remains difficult to translate these in terms of EOT. As an approximation of the noise level, the average error on the EOT fitted by the RIFTS routine was calculated for multiple measurements and averaged. With this approximation, it was possible to calculate the theoretical limit of detection, which is situated in the 10^3 - 10^4 CFU/mL range depending on the PSi membrane design. This LoD is quite high, but one must keep in mind that it is based on simplified models of the sensor and the analyte. The reality of the described biosensing experiment is much more complex, with a lot of factors and effects that still need to be investigated.

4.5 Conclusion

In this chapter, we have studied both components of the biosensor presented in this research, which is based on the indirect detection of bacteria via the lysate inside porous silicon membranes. These components are the analyte and the sensor. The size distribution of the bacterial lysate was established, and the fabrication parameters of the porous silicon substrate were then adapted and investigated accordingly. Based on the membrane filtration simulation, the type of porous nanostructure best fitted to our biosensing experiment was selected, namely a double-layer porous membrane. The fabrication parameters of the multi-layered porous membrane were further fine-tuned via the simulation of the optical response of the sensor. Finally, using a simplified model of our sensor and sensing experiment, a theoretical response could be calculated. Concentrations of 10^5 and 10^6 CFU/mL induce a significant response, but for lower concentrations, the significance of the response will be dependent on the base-line signal.

CHAPTER 5

THE ADDED VALUE OF POROUS SILICON MEMBRANES FOR BACTERIAL DETECTION

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Abstract

This chapter reviews the fabrication and characterisation of our porous silicon-based biosensor and is adapted from [RV2]. First the benefits of using a membrane are demonstrated, followed by the study of the performance of the sensor, in terms of sensitivity and selectivity.

5.1 Introduction and motivation

The previous chapter focused on the design of our biosensor and on a theoretical study of its properties and performance. This chapter will focus on the experimental validation of the observations made previously. We demonstrate the concept of indirect optical detection of bacteria on porous silicon membranes with the selective detection of *B. cereus* lysate in PBS using the recently characterised PlyB221 endolysins, encoded by the Deep-Blue phage targeting *B. cereus* [263].

The detection principle of our optical sensor relies on RIFTS, as explained Section 3.3. While RIFTS-based biosensors mostly target small biomolecules, such as proteins [250, 279] or biomarkers [251, 280], they can also be applied to the detection of bacteria cells, as illustrated in Section 3.3.3. These bacterial detectors however lack sensitivity, with detection limits unable to go below 10^3 CFU/mL. Current bacteria detection techniques, such as PCR or ELISA, while time-consuming, can easily reach detection limits of 1 CFU/mL [228]. The insufficient sensitivity of PSi sensors is attributed to the hindered diffusion of bacteria to and into the porous matrix. Solutions, such as the electrokinetic transport for the preconcentration of analyte, have been proposed but remain to be tested on bacteria [281]. Another approach to increase the sensitivity of PSi biosensor is the fabrication of PSi membranes (PSiMs). Instead of a close-ended PSi layer over which the analyte must flow, an open-ended PSi membrane allows the analyte to flow through the porous matrix. PSi membranes have been fabricated as early as the 1990s [282] but the interest in PSiMs for sensing applications only arose recently, as discussed in Section 3.3.2). For biosensing applications, PSiMs have been found to increase both the time and amplitude of the response [10, 126, 283].

This chapter will therefore focus on the added value of PSiMs for the indirect detection of bacteria via their lysate. Their performance will thus be compared to PSi layers, with a similar porous morphology, using the same detection protocol. Next, the limit of detection will be studied, by decreasing the concentration of *B. cereus* before the lysis. The selectivity and versatility will also be analysed. Finally, the results will be analysed with the novel Morlet Wavelet method described in Section 3.3 and the observations will be compared to the ones made with RIFTS.

5.2 Materials and methods

5.2.1 Materials

Double-side polished, boron-doped silicon wafers ($\langle 100 \rangle$, 0.8-0.9 m Ω cm, 380-400 μ m) were purchased from Sil'tronix Silicon Technologies (France). Aqueous hydrofluoric acid (HF, 49%) was acquired from Chem-Lab, NV (Belgium), and absolute ethanol was obtained from VWR Chemicals (France). Phosphate buffered saline (PBS, 0.01 M phosphate, pH 7.4) and lysostaphin were purchased from Sigma-Aldrich (USA).

5.2.2 Fabrication of PSi-biosensors

5.2.2.1 Fabrication of Porous Silicon layers

The PSi layer samples were prepared by the electrochemical etch of a heavily doped *p*-type silicon substrate. The etching was carried out in a custom-made Teflon[®] single bath etch-cell, with a platinum coil as the counter-electrode and a potentiostat/galvanostat (PGSTAT302N from Metrohm Belgium) as the current source. The porosification was performed in HF:ethanol (3:1, in volume) electrolyte. The first step of the anodisation consisted in etching a sacrificial layer at 200 mA/cm² for 30 s and removing it with a 2 M solution of KOH until no more reaction was visible. By etching the sacrificial layer (or transitional layer) a more homogeneous pore size is obtained. The sample was then rinsed once in deionised water and twice in 2-propanol before being etched again at 200 mA/cm² for 50 s. The porous samples were thermally oxidised in an oven for 30 min at 350 °C under an oxygen flow (1.2 L/min).

5.2.2.2 Fabrication and Characterisation of Porous Silicon Membranes

Figure 5.1 sketches the process flow, which was inspired by the work from Zhao *et al.* [10]. First, 3-in. highly doped *p*-type silicon wafers were cleaned in a freshly prepared piranha solution (H₂O₂:H₂SO₄, 2:5), followed by two immersions in continuously flowing deionised (DI) water during 20 min. The native oxide is removed in a HF 2% solution, until the surface is hydrophobic. Afterwards, 500 nm of silicon nitride (Si₃N₄) was deposited using Plasma Enhanced Chemical Vapour Deposition (PECVD). To improve the chemical resistance of the nitride layer to HF, the wafers were annealed at 900 °C in ambient air for 3

h. A first i-line optical lithography with positive resist (AZ[®] MiR[™] 701, MicroChemicals GmbH) provided masking for the subsequent Reactive Ion Etching (RIE) of the silicon nitride layer. The patterned nitride layer itself served as a mask during the electrochemical etch of the silicon. The porosification followed the same protocol as explained in Section 5.2.2.1, but used three different current densities in order to obtain different porosities: the sensing layer was etched at 200 mA/cm² for 50 s; this was followed by a 1500 s-etch at 50 mA/cm², making a thick optical contrast layer characterised by lower porosity, enabling the reflection of the light required for the RIFTS method; finally, a thick mechanical support layer was etched at 100 mA/cm². The current densities chosen for each layer have been optimised such as to guarantee a good mechanical stability and optical signal, while retaining pores large enough for the flow-through operation. The porous multi-layers were passivated by thermal oxidation, for 30 min at 350 °C under an oxygen flow of 1.2 L/min. A second optical lithography was then performed on the backside of the wafers, where a thick positive resist (AZ[®] 9260, MicroChemicals GmbH) was patterned in alignment with the frontside. The thick resist served as a mask during the final step of the process, the Deep Reactive Ion Etching (DRIE) of the backside of the wafer, until the porous silicon was visible and the membranes were open. The entire fabrication process was performed in less than a week, and could be repeated several times. Slight variations in the porous structures can happen, which can be linked to the manual positioning of the platinum electrode.

The fabricated membranes have two shapes: either 4 mm² squares, or 3.14 mm² circles. This size was selected based on the beam diameter of the optical set-up. Each membrane is situated on a 1 cm² die, and a 3-in. wafer contains 32 dies. The total thickness of the membrane ranged from 75 to 150 µm, depending on the membrane area, position on the wafer and different batches.

The membranes were characterised using SEM, both in cross-section and in top view. Based on the top views, the pore size distribution could be analysed using the ImageJ software. The porosity of each layer was determined using SLIM.

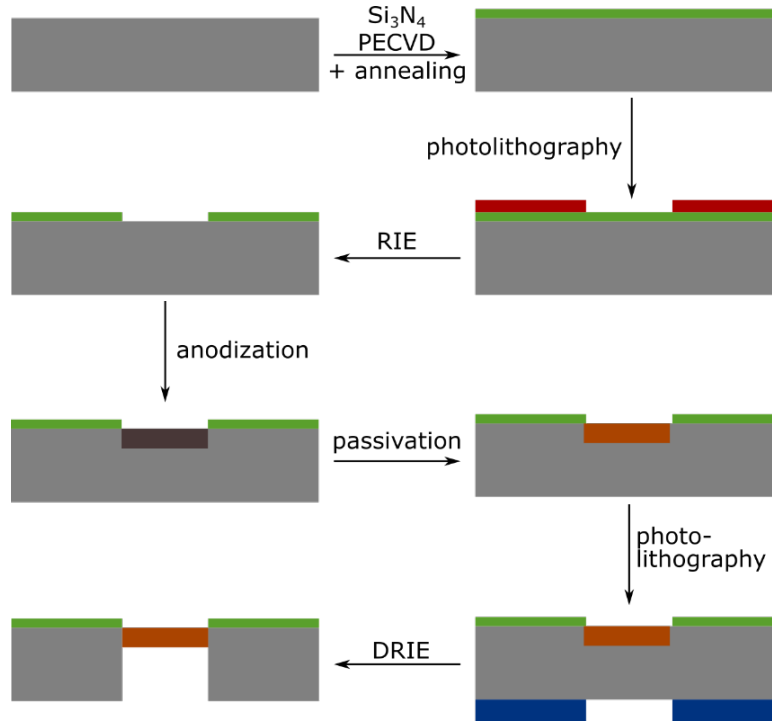


Figure 5.1: Schematic illustration of the process flow for the fabrication of a porous silicon membrane. Starting from a cleaned 3 in. highly doped silicon wafer, the process goes through the following steps: deposition of Si_3N_4 layer using Plasma Enhanced Chemical Vapor Deposition (PECVD) and annealing; positive photolithography on the frontside; opening of the nitride layer using Reactive Ion Etching (RIE); formation of the porous silicon layer by anodization; passivation by thermal oxidation; positive photolithography on the backside; and finally opening of the membrane using Deep Reactive Ion Etching (DRIE).

5.2.3 Bacterial detection on PSi-based biosensors

5.2.3.1 PlyB221 Endolysin Expression and Purification

A detailed description of the expression and purification of PlyB221 endolysins can be found elsewhere [263]. The protein concentration was adjusted to 1 mg/mL.

5.2.3.2 Bacterial Strains, Growth Conditions

B. cereus ATCC 10987 was used as reference strain and *S. epidermidis* ATCC 35984 as negative control in the PlyB221 endolysin experiments. *S. epidermidis* ATCC 35984 was also used as target when lysostaphin was applied as selective lytic agent. Bacteria were grown overnight (O/N) in Lysogeny Broth (LB) or LB-agar plates at 30 °C for *B. cereus* and in Tryptic Soy Broth (TSB) or Tryptic Soy Agar (TSA) plates at 37 °C for *S. epidermidis*. In brief, 20 mL of LB or TSB were inoculated with 200 μ L of each culture and incubated for 3 h at 30 °C (*B. cereus*) or 37 °C (*S. epidermidis*). The cultures were then centrifuged at 10000x g for 5 min at room temperature and the supernatants were resuspended in 20 ml of PBS. This washing step was repeated once over and the optical density (OD₆₀₀) was adjusted to OD₆₀₀ = 0.2 ($\sim 10^6$ CFU/ml) for *B. cereus* and OD₆₀₀ = 0.02 ($\sim 10^6$ CFU/ml) for *S. epidermidis*. For the determination of the limit of detection, the *B. cereus* suspension was diluted 10 times once in order to obtain concentrations of $\sim 10^5$ CFU/ml and then a second time to obtain concentrations of $\sim 10^4$ CFU/ml.

5.2.3.3 Experimental Setup and Optical Reflectivity Measurements

PSiM samples were integrated in a custom-built polycarbonate fluidic cell, as illustrated in Figure 5.2. A fibre-coupled Ocean Optics JAZ spectrometer and a 10-mW halogen light source were used to record reflectivity spectra. Data points were recorded every 10 s, with a spectral acquisition time of 1 s over a wavelength range of 500-800 nm. Analytes were injected at flow speed of 15 to 20 μ L/min using a Fluigent LINEUPTM fluidic setup. The obtained optical data were analysed using the RIFTS method in order to obtain the effective optical thickness, $EOT = 2nL$, with n being the refractive index and L the porous layer thickness. The relative change in EOT overtime was computed as a percentage, as indicated in Equation 4.1, with EOT_0 as the reference EOT value in PBS. The significance of the relative EOT shift was then established using a Student's t-test with a 5% confidence level, with a negative control test in PBS as reference.

A Morlet Wavelet routine, developed by the Weiss group [245]¹ was also used to process all the optical spectra. The resulting Morlet wavelet

¹The routine is available at <https://my.vanderbilt.edu/vuphotonics/resources/>

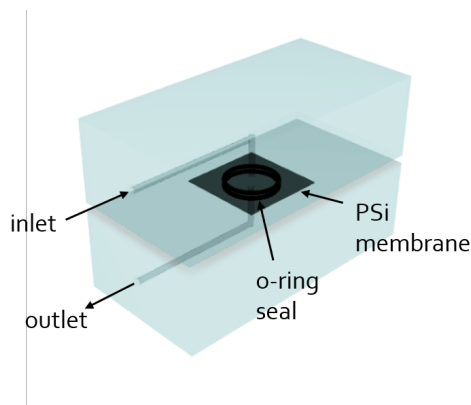


Figure 5.2: Schematic illustration of the custom-built polycarbonate fluidic cell.

phase signal is the average of the difference between the unwrapped phase of the reference spectrum and that of the spectrum collected at each time point. The significance of the phase shift was probed using a Student's t-test, similarly to the EOT shift.

5.2.3.4 Real-Time Detection of *B. cereus* in PBS on PSi layer and PSi membranes

The protocol for bacterial detection on a PSi sensor is illustrated in Figure 5.3. First 500 μL of purified PlyB221 endolysin were added to 4.5 mL of exponential phase *B. cereus* resuspended in PBS, so as to reach a final protein concentration of 100 $\mu\text{g/mL}$. The 5 mL final volume was sufficient for at least 4 detections. The suspension was then incubated for 30 min at 30 $^{\circ}\text{C}$. Before flowing the bacterial lysate, PBS solution was injected at 15 to 20 $\mu\text{L/min}$ for 60 min, flowing through the PSiM and over the PSi layer, and reference measurements were performed. *B. cereus* lysate suspensions were injected at the same flow speed. Optical measurements were carried out every 10 s for 60 min. The relative EOT was then extracted from these measurements using the method described above. A control test, with only the PlyB221 endolysins at the same concentration was also performed on both types of sensors, following the same protocol described previously. For all tests, measurements were performed at least 3 times.

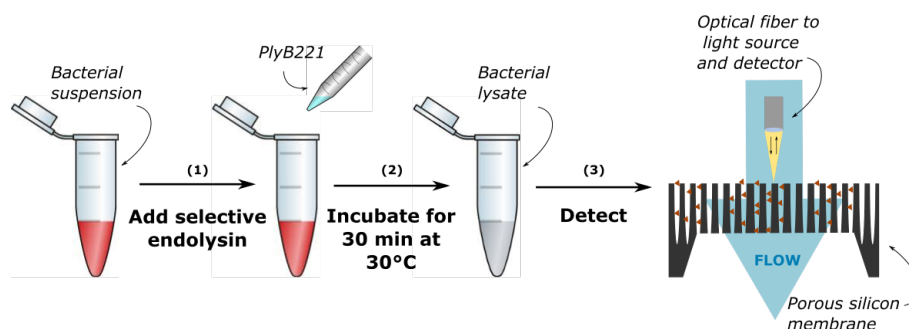


Figure 5.3: Protocol of bacteria detection through their lysate: (1) lysis of the bacteria using a selective endolysin, (2) incubation for 30 min and (3) optical detection on a porous silicon membrane.

5.2.3.5 Specificity Testing: Detection of *S. epidermidis* in PBS with the PlyB221 endolysin on PSi membranes

For this experiment, two tests were performed: a negative control using a *S. epidermidis* suspension and a positive control using a complex sample containing both *S. epidermidis* and *B. cereus*. For each test, 4.5 mL of bacterial suspension were incubated for 30 min at 30 °C with the 500 μ L of PlyB221 endolysin (1 mg/ml). This final volume was sufficient for at least 4 detections. Reference measurements in PBS were performed for 5 to 15 min, until liquid was observed at the other side of the PSi membranes. The bacterial suspension was then injected at a flow speed of 15 to 20 μ L/min and optical measurements were performed every 10 s for 60 min. The relative EOT shift was computed as described previously.

5.2.3.6 Versatility of the Platform: Detection of *S. epidermidis* in PBS with lysostaphin on PSi membranes

In this experiment, a different lytic enzyme-bacteria pair was tested. *S. epidermidis* was selected as the targeted strain, and lysostaphin was chosen as the selective agent. This glycyl-glycine endopeptidase is specific to the pentaglycine bridges present in the cell wall of certain Staphylococci. For the detection experiments, 1 mg of commercialised lysostaphin was diluted in 1 mL of PBS supplemented with 30 % of glycerol to reach a concentration of 20 μ M. For the lysis, 150 μ L of this 20 μ M lysostaphin was added to 2.850 mL of bacterial suspension, yielding a final endolysin concentration of 1 μ M [27]. The suspension was then incubated at 37

°C for 30 min. Reference measurements in PBS and in lysostaphin suspensions were performed for 1 h each. The bacterial suspension was injected at a flow speed of 15 to 20 $\mu\text{L}/\text{min}$ and optical measurements were performed every minute for 60 min. The relative EOT shift was computed as described previously.

5.3 Results

5.3.1 PSi-Based Biosensor Characterisation

The effective optical thickness of PSi is strongly dependent on its refractive index. When an analyte penetrates the pores, the refractive index of the porous layer increases, therefore inducing a shift in the EOT. In the case of a membrane, to make sure that the analyte remains inside the porous matrix, two approaches are possible: size exclusion or binding to the pore wall. For this project, size exclusion was chosen by accordingly selecting different pore size for each layer of the membrane. The first layer, also called the sensing layer, has an average pore size 41.05 nm, as described in Table 5.1 and illustrated in Figure 5.4. Larger pores could be etched, but the resulting layer was too easily damaged, as an increase in pore size induced an increase in porosity, rendering the layer more fragile. The selected pore size is a trade-off between pore opening and mechanical integrity. The bacterial lysate is composed, among others, of cell wall fragments, DNA and RNA molecules, cytoplasmic liquid and ribosomes, which are assumed to penetrate the sensing layer.

In order to keep the bulkier particles in the top layer, the contrast layer was designed to have a smaller pore size, as indicated in Table 5.1. As mentioned in the previous chapter, these choices in pore size also have two other motivations: (1) they allow the PlyB221 endolysin to flow through the membrane and not be retained in the sensing layer and (2) they prevent the non-lysed bacteria from penetrating the membrane, therefore enabling a selective detection. Figure 5.5 depicts a PSi membrane, with an up-close view of the transition between the top sensing layer and the contrast layer.

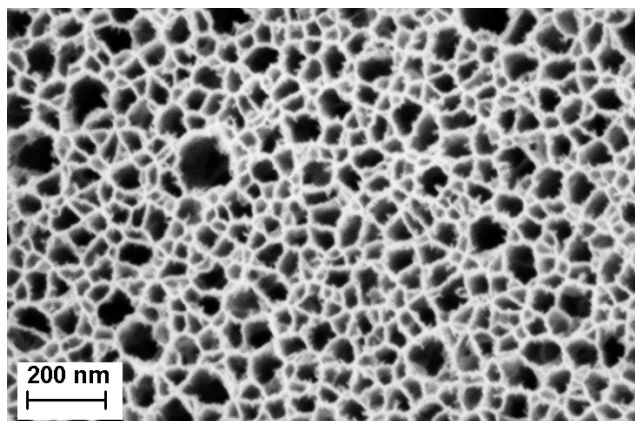


Figure 5.4: Scanning electron microscopy (SEM) image of the top layer porous silicon membrane. The average pore size is ~ 41 nm, with a standard deviation of ~ 20 nm.

Layer	Current density [mA/cm ²]	Time [s]	Pore size [nm]	Thickness [μ m]	Porosity [%]
Sensing layer	200	50	41.05 ± 20.4	4.09 ± 0.7	75.4
Contrast layer	50	1500	14.6 ± 7.8	22.8 ± 6.8	48.5
Support layer	100	2000	25.5 ± 10.4	_*	_*

Table 5.1: Properties of PSi samples etched at different current densities; these values were extracted from SLIM measurements and SEM observations.
* The thickness and porosity could not be accurately measured, as part of the layer was etched away during the DRIE step.

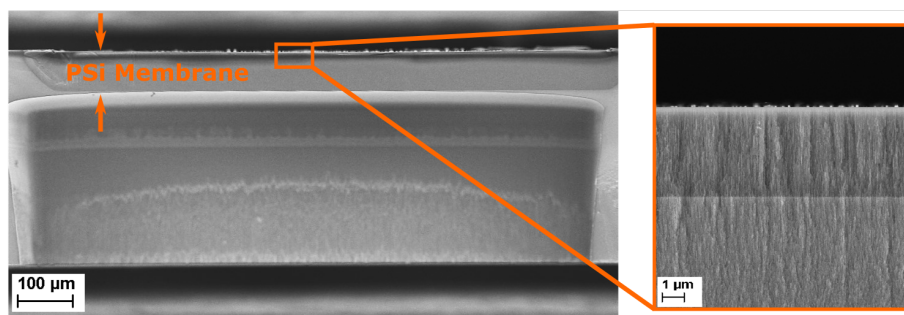


Figure 5.5: Scanning electron microscopy (SEM) cross-section image of a multi-layered porous silicon membrane. The inset shows a close-up of the top of the porous membrane, in which the sensing and the contrast layers can be identified.

5.3.2 PSi layers versus PSi membranes

In order to establish the added value of a flow-through approach to the optical detection with respect to the traditional flow-over approach, the performances of PSi membranes were compared to those measured on PSi layers. The average relative EOT shift measured on PSi layers, in a flow-over approach, is presented in Figure 5.6a. No distinction could be made between the bacterial lysate detection and the control tests using either the buffer or the endolysin suspension. Both control tests induced minute decreases of the relative EOT: on average -0.24% and -0.01% after 1 h for PBS and the PlyB221 endolysin, respectively. A small increase of relative EOT was measured in the presence of bacteria lysate, namely 0.05%, but this increase was not significant when compared to the noise level. This noise level was calculated based on the overall standard deviation of the signal in PBS and was expressed as $3\sigma = 1.08\%$.

The performances of PSi membranes for the same three tests in flow-through operation are illustrated in Figure 5.6b. The buffer control test induced a decrease in relative EOT of -0.32% after 1 h. The control test with only the PlyB221 endolysins gave rise to a 0.28% increase of relative EOT. This increase was however not significant and remained below the noise level, which was calculated in the same manner described previously and was equal to 0.84% in the case of PSi membranes. Upon the penetration of bacterial lysate inside the membrane, a significant increase of relative EOT was measured, exceeding the noise level after 6 min and reaching an average +2.43% after 1 h.

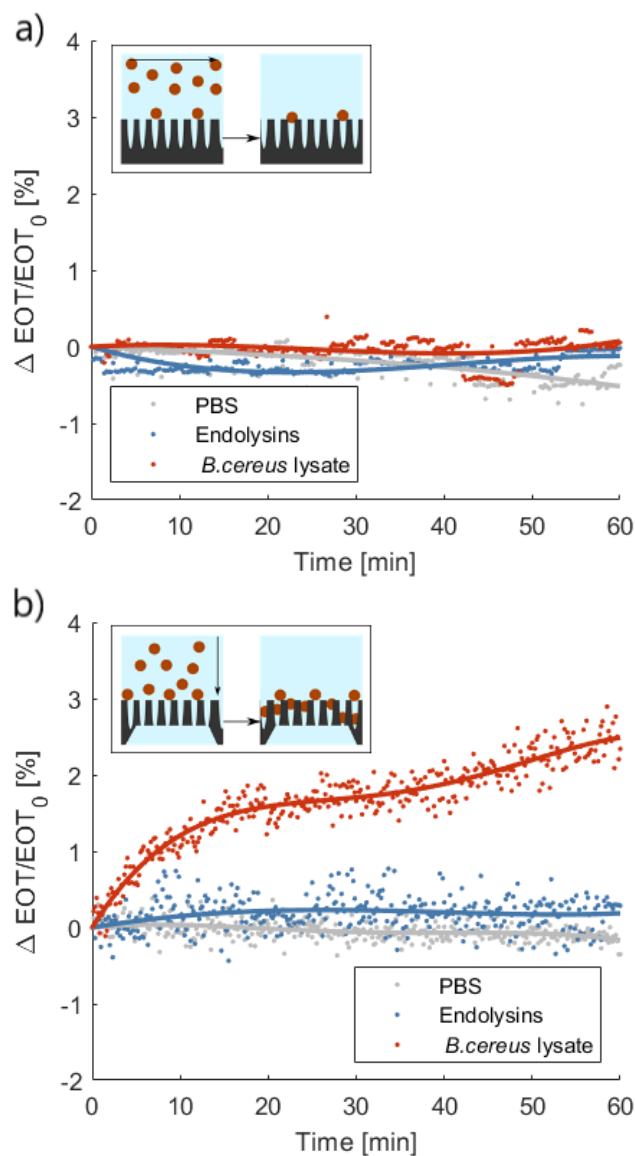


Figure 5.6: Characteristic relative effective optical thickness (EOT) shift measured on (a) a porous silicon (PSi) layer or (b) a PSi membrane for 1 h in phosphate buffered saline (PBS), in a PlyB221 endolysin suspension and in a *B. cereus* lysate ($n \geq 3$). In a), the inset illustrates the flow-over approach; there was no significant shift visible during the detection of either the PlyB221 endolysin or bacterial lysate. In (b), the inset illustrates the flow-through approach; there was a significant shift in the relative EOT during the detection of bacterial lysate.

5.3.3 Determination of the Limit of Detection

The limit of detection was determined using decreasing concentrations of *B. cereus*. Results are displayed in Figure 5.7. As presented above, the detection of 10^6 CFU/ml is significant compared to the negative control tests in PBS and in endolysin only. For a concentration of 10^5 CFU/ml, the relative EOT shift amounted 0.96%, which is just above the noise level but still significantly different to both control tests. For 10^4 CFU/ml, the relative EOT shift decreased below the noise level to 0.64%. While this difference remains significant with respect to the control test in PBS, it is not with respect to the endolysin control test.

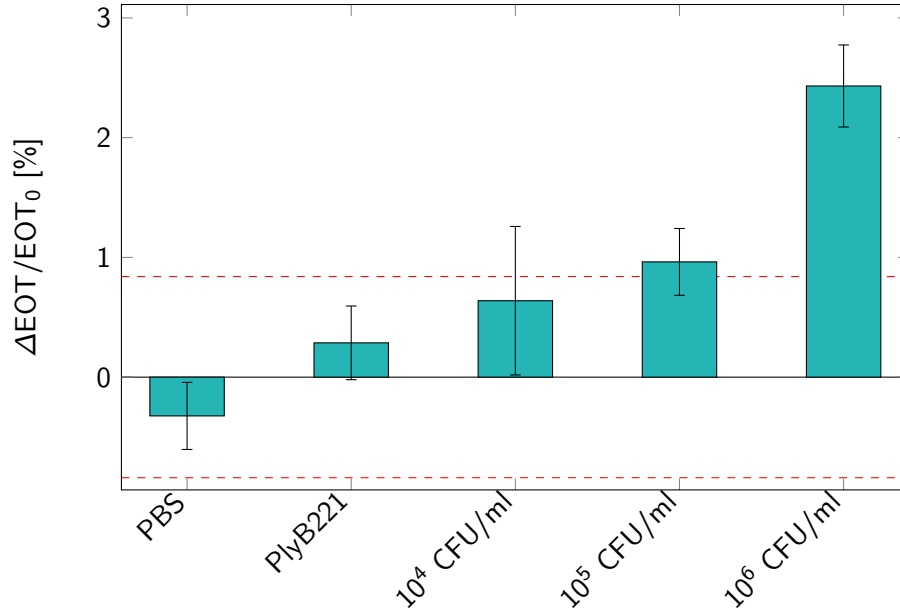


Figure 5.7: Characteristic relative effective optical thickness (EOT) shift measured on a PSi membrane after 1 h in PBS, in a PlyB221 endolysins suspension and in increasing concentrations of *B. cereus* lysate ($n \geq 3$). The dashed red line represents the noise level, fixed as 3σ of the signal measured in PBS. The detection limit is 10^5 CFU/ml of *B. cereus*.

5.3.4 Specificity testing with *S. epidermidis*

To illustrate the specificity of the sensing platform, a negative control test was performed using a *S. epidermidis* and a positive control test was carried out using a mixture of *B. cereus* and *S. epidermidis*. The relative

EOT shifts observed for both tests are depicted in Figure 5.8, where it is compared to controls in PBS and with the PlyB221 endolysins only, as well as to the detection of *B. cereus* lysate only. The detection using *S. epidermidis* induced no significant shift in relative EOT. After 1 h, the relative EOT was increased by 0.29%, which is comparable to the control test with only the PlyB221 endolysins. The positive control test showed a significant relative EOT increase of 1.59%.

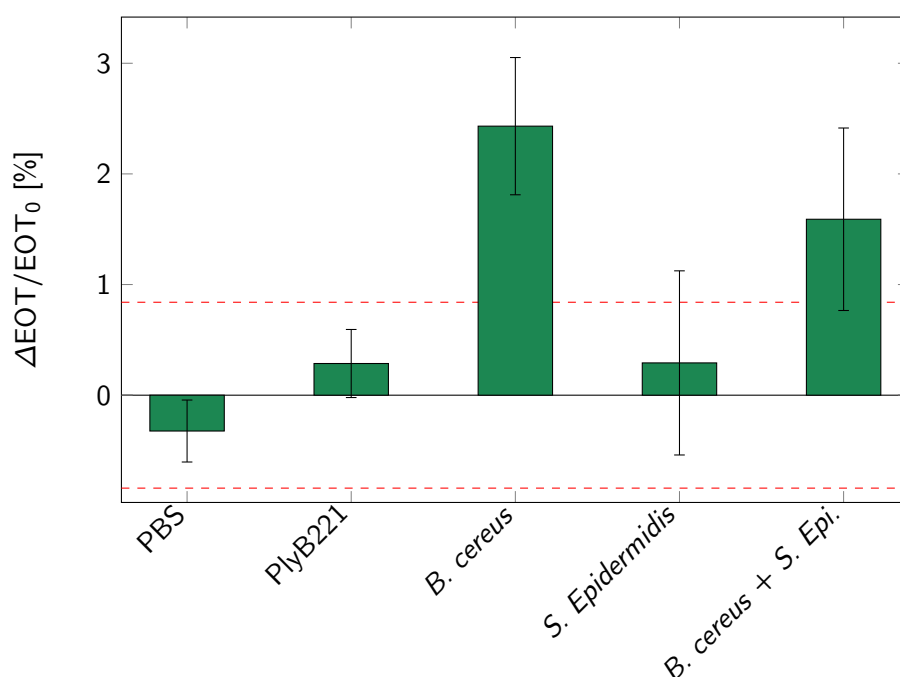


Figure 5.8: Characteristic relative EOT shift measured on a PSi membrane after 1 h in PBS, in a PlyB221 endolysin suspension, in a *S. epidermidis* suspension, in a mixture of *B. cereus* lysate and *S. epidermidis* and in *B. cereus* lysate only ($n \geq 3$). The dashed red line represents the noise level, fixed as 3σ of the signal measured in PBS. Only the detection of mixture of *B. cereus* lysate and *S. epidermidis* induced a significant shift

5.3.5 Versatility of the Platform

To illustrate that the biosensor can be used for the detection of other bacteria/lytic enzyme pairs, the same detection protocol was repeated using *S. epidermidis* as targeted strain and lysostaphin as selective lytic agent. The relative EOT shift was measured overtime and compared to

control tests in PBS and lysostaphin only. As depicted on Figure 5.9, the relative EOT shift induced after 1 h by the penetration of lysostaphin into the porous membrane is comparable to the one measured for the PlyB221 endolysin and amounts 0.26%. The penetration of *S. epidermidis* lysate causes a significant shift in relative EOT after 30 min, reaching a value of 1.83% after 1 h.

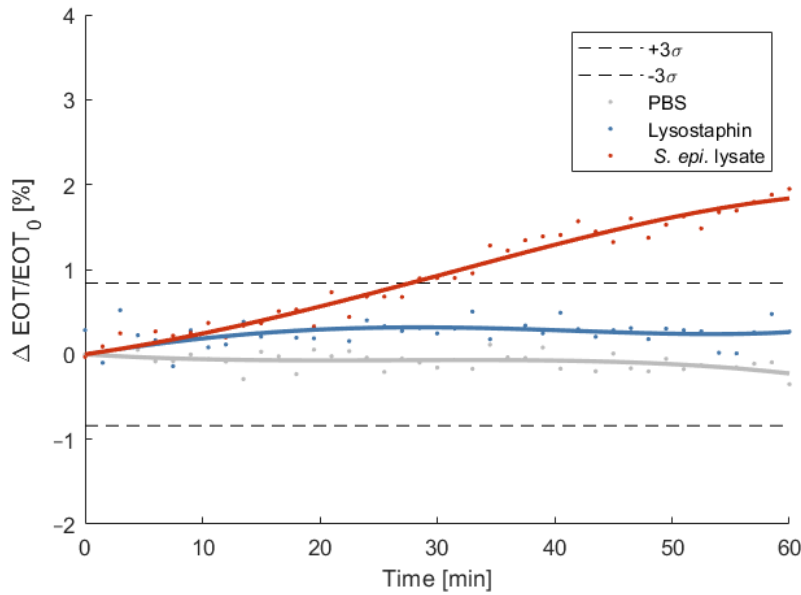


Figure 5.9: Relative effective optical thickness (EOT) shift measured on a PSi membrane for 1 h in PBS (grey), in a lysostaphin suspension (blue) and in a *S. epidermidis* lysate (red). The bacterial lysate is detected after 30 min.

5.3.6 Morlet Wavelet data processing

The data presented above was also analysed with a Morlet Wavelet routine. The results are summarised in Figure 5.10. This method was created in order to reduce the noise signal of the optical spectra. For the presented data set, the Morlet Wavelet routine does not seem to affect the limit of detection: according to the Student's t-test, the lowest concentration with a significant difference from the PBS baseline remains 10^5 CFU/mL. In terms of selectivity, the observations are the same as for RIFTS: our sensor is selective with respect to bacteria that are not targeted by the endolysin.

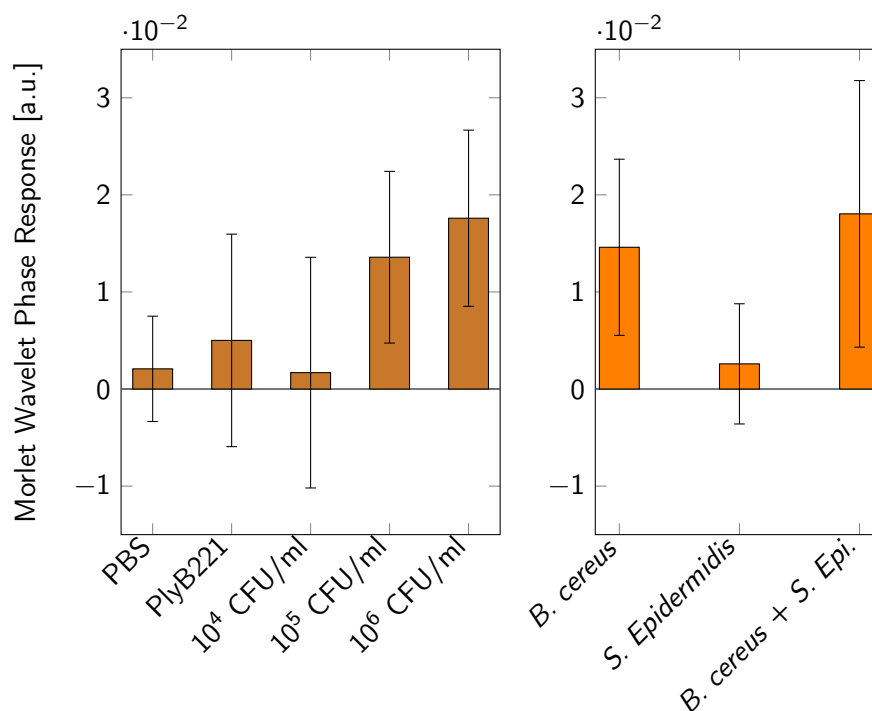


Figure 5.10: Characteristic Morlet Wavelet Phase Response measured on a PSi membrane after 1 h (right) in PBS, in a PlyB221 endolysin suspension and increasing concentration of a *B. cereus* lysate; and (left) in 10^6 CFU/mL of *B. cereus* lysate, in a *S. epidermidis* suspension and in a mixture of *B. cereus* lysate and *S. epidermidis* ($n \geq 3$).

5.4 Discussion

Porous silicon membranes are promising biosensors: with their flow-through operation, they overcome the lack of sensitivity of flow-over PSi-based biosensors and are characterised by short response times [10, 114, 189, 234]. Moreover, they do not require any stratagems to transport and concentrate the analyte on the transducer. In this work, we demonstrate once again the potential of these detection platforms and focus on their use for the detection of bacteria. PSiMs were fabricated using standard microfabrication techniques and electrochemical etching. The full fabrication process took less than a week, and dozens of samples could be produced in one attempt. No functionalisation was applied to the sensors, as the specificity was based on the use of endolysins.

Endolysins are phage-encoded enzymes that induce bacterial lysis for certain targeted strains. In this work, the PlyB221 endolysin was used, targeting *B. cereus*, whose efficiency is demonstrated in [263].

Combining PSiMs and the use of endolysins produced an innovative biosensing platform that was able to detect *B. cereus* lysate, with an initial concentration of 10^6 CFU/ml, in less than 10 min. In flow-over PSi-based biosensors, similar concentrations could not be detected. Two negative control tests were carried out: one in PBS and one with the PlyB221 endolysin only. The PBS control induced a slight decrease of relative EOT. This can be explained by the slow oxidation and dissolution that PSi undergoes in aqueous media [284]. This effect can be minimised by chemically modifying the pore surface using either hydrosilylation [203], thermal hydrocarbonisation [205] or atomic layer deposition (ALD) of oxides [204]. Adequate passivation may also help to reduce the noise level of the optical sensing, as demonstrated by Rason *et al.* with the use of ALD [204]. The second control test, which consisted in flowing a PlyB221 endolysin suspension through the sensor for 1 h, resulted in a slight increase of the relative EOT. While it was expected for the endolysins to pass through the membrane, this increase indicates that they were partly retained. We believe two hypotheses might explain this effect: (1) a minor size exclusion effect, which is not surprising since there is a large pore size distribution visible in Figure 3 and (2) the binding of proteins to the pore walls.

In order to determine the detection limit, the initial concentration of bacteria was reduced. The sensor was able to detect concentrations as low as 10^5 CFU/ml after 1 h. While concentrations of 10^4 CFU/ml induce a significant increase in signal compared to the PBS control test, this increase was however not significant with respect to the endolysin control test. Once again, adequate passivation may help to reduce the deviation between measurements and enable the lowering of the detection limit to 10^4 CFU/ml or lower. While the specificity of the PlyB221 endolysin has already been demonstrated [263], the specificity of the sensor was still exemplified by adding *S. epidermidis* to the PlyB221 endolysin suspension. The observed shift in relative EOT is similar to the one observed with the PlyB221 endolysins only. This confirms that bacteria, when not lysed, are not able to penetrate the membrane, thus demonstrating the specificity of the sensing platform. A second test was performed by adding both *B. cereus* and *S. epidermidis* to the endolysins: the results showed a significant increase in relative EOT, but

lower than the one measured for *B. cereus* lysate only. We believe this might be explained by the accumulation of *S. epidermidis* on top of the sensor, blocking part of the pores and preventing the penetration of *B. cereus* lysate. This accumulation of intact bacteria may also explain the larger deviation between measurements when in the presence of *S. epidermidis*: part of the light is scattered by the bacteria, therefore decreasing the optical signal and adding deviations during the fit of the EOT.

An alternative data processing method was also considered. The IAW method was excluded because of the large variations of the signal intensity, which can be attributed to the slow degradation of the PSi matrix. The application of a Morlet Wavelet routine, in order to reduce the effect of the noise on the optical signal, was however possible but did not result in any further reduction of the limit of detection. Similar observations have been made by Arshavsky-Graham *et al.* [246]. Further improvements could however be achieved by optimising the Morlet Wavelet parameters, in order to better filter the optical spectra. The selection of the wavelength range, for example, seems to have a significant effect on the results, and should therefore be carefully analysed.

While most PSiM-based sensors rely on functionalisation to capture the analyte [10,126,234], the biosensor presented in this work bases the capture on a size exclusion effect. This novelty has several benefits: the lack of functionalisation considerably shortens the production time of the sensors and puts no requirements on the storage and detection conditions. This choice of design was made with future industrial specifications in mind: without the constraints of functionalisation, a silicon-based device such as the one presented, which is compatible with all standard fabrication techniques used in microelectronic cleanrooms today, could be easily mass-produced and packaged, for a very low cost. The lack of functionalisation also enables the use of sensors from the same batch for the detection of different strains of bacteria, as long as a selective endolysin is available. This versatility was illustrated by the detection of *S. epidermidis* using lysostaphin as the selective lytic agent.

Besides specificity, the performance of our optical biosensor can be discussed in other terms: response time, sensitivity and limit of detection. The response time for this study is comparable to functionalised PSiMs [10,126,234] and since no rinsing is required to remove unbound species, the total detection time (which includes both lysis and optical

monitoring) can be reduced to less than 2 h. Another major advantage is the reduced volume of analyte that is needed: only 1 mL is required for the detection. In terms of bacterial concentration, a limit of detection of 10^5 CFU/ml remains high compared to other PSi bacteria sensors [249, 253, 254, 256], yet there is much room for improvement. Further studies should therefore aim to lower the detection limit to competitive levels ($< 10^3$ CFU/mL) by improving the stability of the sensor and reducing the noise level of the optical signal.

5.5 Conclusion

In this chapter, we demonstrated the fabrication of an innovative biosensor for the detection of bacteria through their lysate, by combining the use of both optically monitored porous silicon membranes and selective endolysins. We showed promising results in terms of sensitivity, selectivity and speed of response by demonstrating the targeted detection of 10^6 CFU/mL of *B. cereus* lysate in less than 10 min. These results, added to the easy fabrication and low design cost, pave the way for the development of a widespread multistrain bacteria sensor. There is however still room for improvement, especially when considering the stabilisation of the porous matrix in order to avoid the observed zero drift.

CHAPTER 6

STABLE POROUS SILICON MEMBRANES FOR BACTERIAL DETECTION

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Abstract

This chapter is partly adapted from [RV1] and relates to the optimisation of our biosensor prototype. Atomic layer deposition is used to passivate the pore walls with ultra-thin metal oxide layers. Several metal oxides are tested, and TiO₂ is selected as the most promising one for bacteria detection. The sensitivity, selectivity and versatility of the sensor are verified.

6.1 Concepts and motivation

The reactivity of porous silicon is governed by silicon-silicon (Si–Si) and silicon-hydrogen (Si–H) bonds. Both bonds can reduce in water to hydrogen. The reduction of Si–Si bonds in particular generates new Si–H bonds in addition to silicon oxides. These silicon oxides are then able to dissolve in aqueous media. The rate of this dissolution is dependent on the surrounding pH and the pore morphology [285]. The oxidative hydrolysis reaction was already discussed and described in Section 3.2.2.4. In the context of a biosensor, this reduction with water causes a drift of the sensor’s baseline signal in aqueous media, meaning that the device is unstable. Indeed, the cycles of oxidation and dissolution of the pore walls are a big disadvantage of PSi, which limits the sensitivity of the biosensors [90].

Section 3.2.2.4 listed some of the solutions that can be used to modify the chemistry of porous silicon and minimise or suppress the degradation of PSi; they will be discussed in the present section with more details. Atomic Layer Deposition (ALD) will be explored specifically, as this is the technique we selected to stabilise our PSi-based biosensor. In this work, we therefore demonstrate the conformal ALD coating of several metal oxides inside the porous membrane. The ALD-coated PSiMs exhibit an improved stability and enhanced optical properties, from which our biosensor can profit. Finally, the performance of our PSi-based biosensor, coated with ALD-TiO₂, is fully characterised, in terms of sensitivity, selectivity and versatility.

6.1.1 Overview of passivation techniques

There are two approaches to surface modification when it comes to porous silicon: (1) modifying the surface chemistry by covalent bonding or (2) the deposition inside the porous matrix, such as the electroless deposition of metals, the chemisorption of metal oxides or the layer-by-layer coating of electrolytes.

Most covalent chemistry modifications involve Si–O or Si–C bonds. These bonds are stronger than Si–Si and Si–H bonds, and therefore able to passivate a porous surface. Techniques used to generate Si–O or Si–C bonds will be discussed below. Oxidised porous silicon can further be modified by grafting other chemistries, such as silanol-based molecules, or even biomolecules. Only the former will be overviewed

here.

6.1.1.1 Formation of Si–O bonds

Porous silicon can be oxidised via several routes, which are listed below. Different surface oxide species can be generated with these techniques, such as Si–O–Si and Si–OH bonds. The nature of the oxide will affect the stability of the porous matrix, as the kinetics of the oxide dissolution in aqueous media is dependent on the oxide chemistry. More information about the oxide nature generated with each technique is given below and is inspired by [90, 285, 286].

- *Thermal oxidation:* this is the simplest technique, especially used in the microelectronics industry, in order to create a high-quality oxide layer. Thermal oxidation can be carried out in air or under O₂ flow. Different species are generated, depending on the temperature and the ambient humidity. Even at room temperature, a thin oxide layer is produced within a few hours, but both oxide species and hydroxy species are observed, as well as absorbed water when exposed to humid air. At higher temperatures, these surface hydroxy species start to disappear and, at >700 °C, the full porous skeleton is oxidised. High temperature oxides are more stable because they are dehydrated.
- *Water oxidation:* as mentioned above, water is a competent oxidant for Si–Si and Si–H bonds. The oxide created in aqueous solutions is however dissolved, via a nucleophilic attack (usually by OH[−] that cleaves the Si–Si bonds). In acidic solutions, the rate of dissolution is very slow, creating a stable oxide. Likewise, the use of high-pressure water vapour as an alternative to liquid water solutions, renders a very stable oxide layer.
- *Chemical oxidation:* chemical oxidants can also be used on porous silicon. For example, ozone oxidation generates a hydrated oxide; dimethyl sulfoxide or pyridine, can also be used, but created a very thin oxide layer. The advantage of chemical oxidation is the reduction of the strain in the porous layer, but it is not the most stable chemical modification.
- *Electrochemical oxidation:* the electrochemical anodisation of PSi using a mineral acid such as aqueous H₂SO₄ generates a fairly stable oxide layer. This oxide layer is very hydrophilic, which is an

interesting property for flow-through operation or for the loading of molecules inside the pores.

6.1.1.2 Modification of oxidised PSi

One further modification technique is silanisation, meaning porous silicon oxides are further modified by silanol groups. The most well-known silanol chemistry is the coupling of 3-aminopropyltriethoxysilane (APTES), which is able to link biomolecules on its $-NH_2$ group. However, APTES chains tend to couple to each other rather than to the PSi oxidised surface, causing the pores to clog. On the other hand, monoalkoxydimethylsilanes, such as 3-aminopropyldimethylethoxysilane (APDMES), are less likely to couple and hence penetrate better into the porous matrix [90,285].

Another stabilisation technique of oxidised PSi is “ring opening click chemistry”. The reagent for this type of modification is a 5-membered heterocyclic compound containing Si–S or Si–N bonds within the ring; via tandem coupling reactions, new functional chemistries, such as $-COOH$ or $-OH$ groups, were generated at the pore surface [286].

6.1.1.3 Formation of Si–C bonds

The formation of Si – C bonds leads to a very stable surface, which is highly desirable for applications that require a long-term exposure to aqueous media. Indeed, Si–C bonds are less electronegative than Si–O bonds, which renders them more kinetically stable despite their lower bond strength.

- *Thermal carbonisation:* this reaction was developed by Salonen *et al.* [205] and involves the decomposition and partial pyrolysis of acetylene gas on PSi, at very high temperature. The temperature of the reaction will dictate the hydrophobicity of the surface: at temperatures of $<500\text{ }^{\circ}\text{C}$, the porous silicon is more hydrophobic than at higher temperatures. The nature of the surface chemistry is also different; at lower temperatures, the surface also presents C–H, C=C and C–OH bonds; at higher temperature, the residual hydrogens are removed and most of the surface is carbonised [90,285].
- *Hydrosilylation:* in this stabilisation method, the Si–H surface species react with the alkene or alkyne terminals of hydrocarbon

chains. The other end of the chain can include a wide variety of organic functional groups. The reaction is helped by heat, light or catalysts and should be carried out in a Schlenk line, as the reacting compounds are often air- or water-sensitive. The coverage of PSi via hydrosilylation is however limited, both because the reagents are often too big to easily diffuse through the pores and because the spontaneous oxidation of the pore wall blocks the reaction. Microwave irradiation can be used to limit the latter side effects, increasing the surface coverage [90, 285].

- *Electrochemical grafting*: the coupling of Grignard, alkyl- or aryl-lithium reagents on freshly etched PSi (meaning on Si–H bonds) requires electrochemical assistance, in order to generate reactive carbon-based radical or carbanions. The reagents are often air- and water-sensitive as well, and for most too big to quickly diffuse through the pores. The surface coverage is therefore limited [285].

6.1.2 Atomic Layer Deposition (ALD)

Atomic layer deposition is a technique used in the microelectronics industry in order to deposit ultra-thin layer of materials. This technique, also known as atomic chemical vapour deposition, deposits films with an atomic control. Most ALD processes rely on binary reagent sequences, during which two surface reactions take place sequentially, resulting in the deposition of a binary compound film. Each surface reaction is self-limiting and only one monolayer of compounds is deposited, as illustrated in Figure 6.1. The self-limiting aspect ensures a good step coverage and a conformal deposition [287].

ALD is able to passivate the large internal surface areas of porous silicon with a precise control on the deposited thickness. It has been demonstrated that a conformal coating can be achieved in both macro- and mesoporous layers [204, 288, 289].

Stability tests performed on PSi layers coated with ALD-aluminium oxide (Al_2O_3) or ALD-hafnium(IV) oxide (HfO_2) under a buffer flow during 2 h indicated that the ALD passivation significantly reduces the signal drift characterising the porous matrix degradation [204]. Furthermore, the theoretical limit of detection of ALD-passivated biosensors was found to improve by a factor 13 and 16 for Al_2O_3 and HfO_2 passivated PSi layers respectively [94].

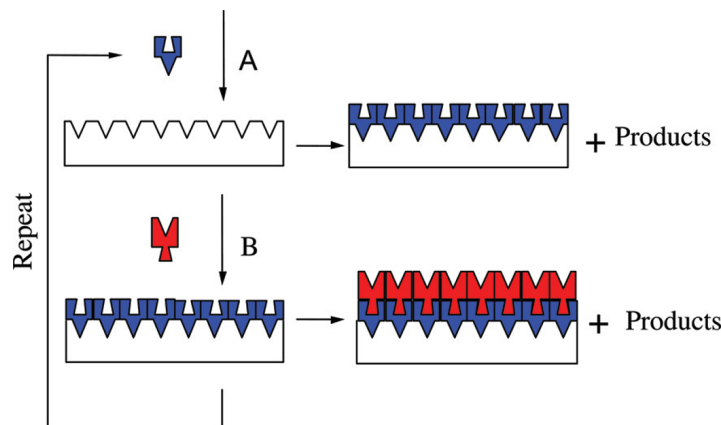


Figure 6.1: Schematic illustration of the working principle of ALD, using self-limiting surface chemistry and a binary reaction sequence, depicted by reagents A and B. Reprinted from [287].

One limitation of ALD is the hindered diffusion of the reagents for deep porous layer; even with an adapted recipe, in which long waiting times are added to enable a better diffusion, it remains difficult to have an even coating from the top to the bottom of deep mesoporous layer [94]. This limitation may be solved by PSi membranes, in which the porous matrix is accessible from both sides. This chapter therefore focuses on the ALD coating of PSi membranes, using three different metal oxides: aluminium oxide (Al_2O_3), hafnium(IV) oxide (HfO_2) and titanium oxide (TiO_2). The chemistry and conformity of the coating is characterised using several techniques, such as Energy Dispersive X-ray Spectroscopy (EDX). The performance of all three types of passivation is studied in terms of stability and of biosensing. Finally, TiO_2 -coated PSiMs are used for the indirect detection of bacterial lysate.

6.2 Materials and method

6.2.1 Study of ALD passivated PSiMs

6.2.1.1 Fabrication of the passivated PSiMs

Materials Double-side polished, 3-inch diameter boron-doped silicon wafers ($\langle 100 \rangle$, 0.8-0.9 $\text{m}\Omega\text{ cm}$, 380-400 μmm) were acquired from Sil'tronix Silicon Technologies (France). Aqueous hydrofluoric acid (HF, 49%) was obtained from Chem-Lab, NV (Belgium), and absolute ethanol

was purchased from VWR Chemicals (France). Phosphate buffered saline (PBS, 0.01 M phosphate, pH 7.4) was purchased from Sigma-Aldrich (USA). The atomic layer deposition precursors, trimethylaluminum (TMA), tetrakis(dimethylamido)hafnium (TDMAHf) and tetrakis(dimethylamido)titanium (TDMAT) for Al_2O_3 , HfO_2 and TiO_2 respectively, were purchased from SAFC Hitech (UK). Human serum (from human male AB plasma, USA origin, sterile-filtered) was purchased from Sigma-Aldrich (USA).

Fabrication and ALD passivation Figure 6.2 illustrates the process flow used for the manufacturing of PSi membranes. To start, the silicon wafers were dipped in a freshly prepared piranha solution ($\text{H}_2\text{O}_2:\text{H}_2\text{SO}_4$, 2:5), followed by two immersions in continuously flowing deionized (DI) water during 20 min. The native oxide is removed in an HF 2% solution, until the surface is hydrophobic. Next, a 90 nm-layer SiO_2 was grown using wet thermal oxidation at 1000 °C and a 500 nm-layer of polycrystalline silicon (PolySi) was deposited at 620 °C using Low Pressure Chemical Vapour Deposition (LPCVD). An i-line optical lithography with positive resist (AZ[®] MiRTM 701, MicroChemicals GmbH) provided masking for a RIE step, which was used to open the PolySi layer and remove it from the rear face. The SiO_2 at the bottom of these patterns was removed using diluted HF (5% v/v). The patterned double layer of SiO_2 and Poly Si then acts as a mask during the electrochemical etching of the silicon [11]. A negative resist (AZ[®] nLOFTM 5510, MicroChemicals GmbH) was coated and patterned on the backside of the wafer, forming a lift-off layer for the evaporation of aluminium. The metallisation step took place in an e-gun VST and resulted in a 300 nm thick aluminium layer. This layer has a double purpose: it serves as a hard mask during the backside etching, but also enables a better contact during the porosification step. This porosification was performed by anodisation in a custom-built Teflon[®] single bath etch cell, with a platinum coil as counter electrode, an aluminium working electrode and a potentiostat/galvanostat (PGSTAT302N from Metrohm, Belgium) as current source. The etching was carried out in HF(49%):ethanol (3:1, in volume) electrolyte and consists of a four-step process: (i) a sacrificial layer was etched at 200 mA/cm² for 30 s and removed using a 1 M solution of KOH until no more reaction is visible; (ii) then a first layer, forming the sensing layer, was etched at 225 mA/cm² for 30 s; (iii) a second layer, serving both as the contrast layer and the support layer, was etched at 100 mA/cm² for 4090 s, to ensure a mechanically stable

membrane. After the anodisation step, the silicon wafer was oxidised in a Heraeus heating oven under 1.5 L/min O₂ and 1.2 L/min N₂ flow at 250 °C for 30 min. The lower temperature was selected to any major thermal stresses inside the porous matrix. The thus-formed thin oxide layer protects the porous silicon during the last step of the fabrication process, namely the DRIE of the backside of the wafer. This DRIE etching is carried out until the porous silicon was visible from the backside. The fabricated membranes have two shapes: either 4 mm² squares, or 3.14 mm² circles. This size was selected based on the beam diameter of the optical set-up. Each membrane is situated on a 1 cm² die, and a 3-in. wafer contains 32 dies. The total thickness of the membrane ranged from 65 to 150 µm, depending on the membrane area, position on the wafer and different batches. Thinner membranes were avoided as they were easily mechanically deformed and/or cracked during the further processing steps.

Once fabricated, the membranes were divided in four batches: the first batch remained as is, while the three other batches were passivated using Atomic Layer Deposition (ALD) of three different metal oxides, namely HfO₂, Al₂O₃ and TiO₂. The oxide growths were carried out in a Fiji F200 ALD reactor (Veeco/CNT, MA, USA) in exposure mode, meaning that the deposition chamber is isolated from the pump during the precursor pulses to allow a deep penetration of the precursors and a homogeneous coating throughout the porous matrix. The samples were lifted onto Si wafer scraps, in order to enable the access from both sides of the membrane. All depositions were performed in thermal mode, at 200 °C. The first precursors are TMA, TDMAHf and TDMAT for Al₂O₃, HfO₂ and TiO₂ respectively. The second precursor is water vapour for all three types of deposition. The ALD parameters for the three metal oxides are detailed in Table 1. The numbers of cycles for each type of ALD was adjusted as to obtain layers with similar thicknesses, namely ~3 nm as measured per ellipsometry on flat silicon substrates submitted to the same deposition process.

Characterisation of ALD-passivated PSiMs The same techniques as described in Section 4.2.2.2 were used to characterise the ALD-passivated PSiMs. These techniques include SLIM and SEM observations. The SEM was also coupled with an Energy Dispersive X-ray Spectroscopy (EDX) detector, combined with the Esprit Software. These tools were used to analyse cross-sectional views of the membranes, and characterise

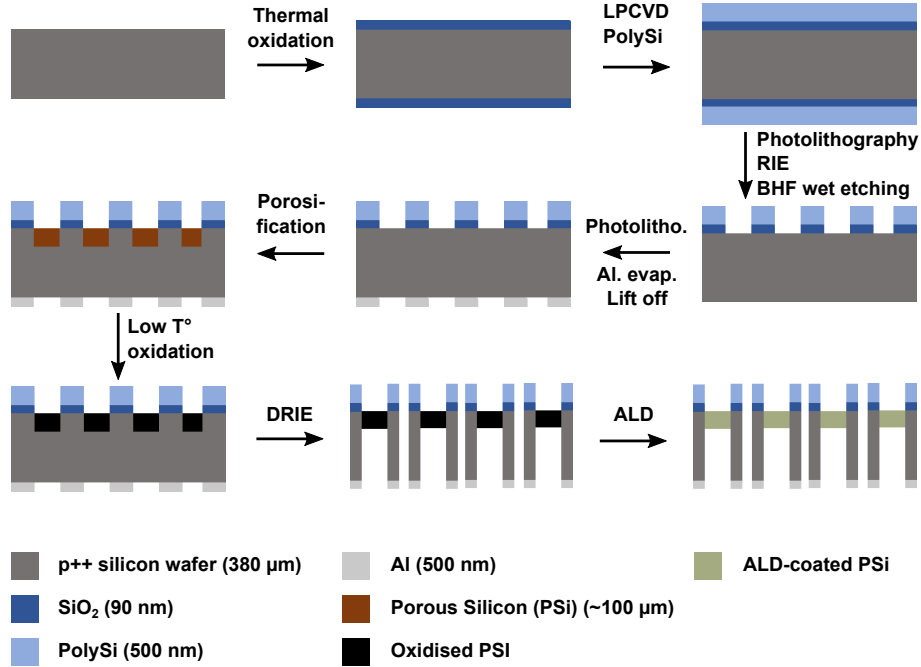


Figure 6.2: Schematic illustration of the manufacturing process of PSi membranes. Starting from a cleaned 3-in diameter highly doped silicon wafer, the process goes through the following steps: (1) thermal oxidation, (2) deposition of a polysilicon layer (PolySi) using Low Pressure Chemical Vapour Deposition (LPCVD); (3) positive photolithography on the frontside and opening of the PolySi and SiO₂ layers using Reactive Ion Etching (RIE) and diluted HF respectively; (4) negative photolithography on the backside, evaporation of an aluminium mask and lift off of the resist; (5) formation of the porous silicon layer by electrochemical etching; (6) low temperature thermal oxidation; and finally (7) opening of the membrane using Deep Reactive Ion Etching (DRIE).

conformity of the oxide deposition throughout the porous matrix depth. X-Ray Photoelectron Spectroscopy (XPS) was also performed to verify the chemistry of the deposited layer (see E.1.1).

6.2.1.2 Stability testing

The stability measurements were carried out in a custom-made polycarbonate fluidic cell. The cell was connected to a Fluigent LINEUP™ fluidic setup, which injected PBS at a flow speed of $\sim 1 \mu\text{L}/\text{min}$. Reflectivity spectra were recorded with the same equipment as detailed in Section 4.2.2.2. The spectra were acquired every 10 s for 4 hours, with

	HfO ₂	Al ₂ O ₃	TiO ₂
Precursor Pulse 1	0.3 s	0.5 s	0.2 s
Wait	120 s	120	120 s
Purge	360 s	360 s	360 s
Precursor Pulse 2	0.1 s	0.1 s	0.2 s
Wait	120 s	120 s	120 s
Purge	360 s	360 s	360 s
Total number of cycles	25	40	50
Deposition temperature	200 °C	200 °C	200 °C

Table 6.1: Parameters for ALD of different metal oxides in a Fiji F200 ALD reactor.

an integration time of 2000 ms and an averaging of five scans to reduce the noise level. The PSi samples were pre-wetted with PBS before their inclusion to the fluidic cell. The first 30 min of data were discarded to ensure that both the fluidic and the thermal transient are over.

The optical data was then analysed with a RIFTS routine in order to compute the relative EOT change overtime. The noise level of each measurement was also evaluated, following the RIFTS data processing. This was achieved by using the `wdenoise` routine on MATLAB (MathWorks). This function denoises the signal using a Symlets 4 wavelet. The mean and standard deviation of this calculated noise was then computed. A more detailed noise analysis can be found in Appendix E.2.

6.2.2 Bacterial lysate detection on ALD passivated PSiMs

The preparation of the endolysin mix and bacterial suspensions has been described in Section 5.2.3. In this chapter, *Bacillus subtilis* (Bs168) was selected to test the specificity of the sensor. In brief, *B. subtilis* bacteria were grown overnight (O/N) in Lysogeny Broth (LB) or LB-agar plates at 30 °C. The cultures were then centrifuged at 10000x g for 5 min at room temperature and the supernatants were resuspended in 20 ml of PBS. This washing step was repeated once over and the resulting suspension was diluted with by a factor 1/100, reaching an optical density $OD_{600} = 0.2$.

The experimental setup for optical measurement was also described in said section, with two differences: (1) the flow speed was reduced to 1 to 2 $\mu\text{L}/\text{min}$ and (2) the bacterial detection is preceded by a 30-min transitional period in PBS, during which the PSiMs are fully wetted.

Moreover, the concentration of endolysins in the bacterial suspension was halved to 50 $\mu\text{g/mL}$, as this concentration showed a similar lytic activity [263].

For all performed detections, at least three measurements were carried out. The collected optical data was then processed using a RIFTS routine, similarly to the first prototype. Some data was also analysed with the Morlet Wavelet routine [245]¹.

Finally, a complex sample was tested out: *B. cereus* bacteria grown as described above were suspended in human serum (pH 8). The concentration was adjusted to 10^6 CFU/mL. Part of the suspension was then incubated with PlyB221 endolysins, achieving a final concentration of 100 $\mu\text{g/mL}$, for 30 min at 30 °C and 120 RPM. Measurements were carried out for 1 h, after 30 min of pre-wetting in blank human serum. Controls test with endolysins only and intact bacteria were also carried out.

6.3 Results

6.3.1 Study of ALD passivated PSi membranes

6.3.1.1 Design of the new PSiM-based sensor

As ALD will deposit a thin layer of material inside the porous structure, the design of the PSi membranes must be adapted. The main adaptation relates to the increase of the pore size. In the previous chapter, the PSi membrane was composed of three layers; for the second prototype, the sensing layer was etched at a higher current density, and the contrast layer as well, merging with the support layer. A thinner sensing layer was also preferred in this new design, as it was observed in Section 4.3.4.2 that this improves the limit of detection, and because the EOT peak of a thinner layer was found to be higher in intensity as well. The fabrication parameters and morphology of both prototypes are listed in Table 6.2.

¹The routine is available at <https://my.vanderbilt.edu/vuphotonics/resources/>

Prototype 1			
	Pore size [nm]	Thickness [μm]	Parameters
Sensing layer	43.9 ± 18.24	4.09 ± 0.7	200 mA/cm^2 , 50 s
Contrast layer	23.9 ± 9.52	22.8 ± 6.8	50 mA/cm^2 , 1500 s
Support layer	21.8 ± 8.05	60 to 100*	100 mA/cm^2 , 2000 s

Prototype 2			
	Pore size [nm]	Thickness [μm]	Parameters
Sensing layer	55.5 ± 25.42	2.64 ± 0.13	225 mA/cm^2 , 30s
Contrast layer	21.8 ± 8.05	80 to 140*	100 mA/cm^2 , 4900 s

Table 6.2: Comparison of the fabrication parameters and properties of both fabricated prototypes (*=depends on the DRIE step at the end of the fabrication process).

6.3.1.2 Morphology of the ALD-coated porous silicon

As in the previous chapter, the morphology of the porous matrix was analysed using both SLIM characterisation and SEM observations. Figure 6.3 illustrates the decrease in pore size for all types of ALD coatings. It can be observed that the images for ALD-covered PSi layers are less sharp, which can be linked to a charging effect. For all types of metal oxides, a layer of $\sim 3 \text{ nm}$ was deposited. This expected thickness was derived from the growth per cycle rate measured on flat silicon sample using a Sentech SE850 ellipsometer (0.1 nm resolution): growth rates of $1.1 \text{ \AA/cycle}$, $0.9 \text{ \AA/cycle}$ and $0.7 \text{ \AA/cycle}$ were observed for HfO_2 , Al_2O_3 and TiO_2 layers respectively. With these growth rate, the expected layer thicknesses are 2.75 nm, 3.6 nm and 3.5 nm for HfO_2 , Al_2O_3 and TiO_2 respectively. However, based on SEM observations, the ALD layers coating the top layer of the porous membranes are much thicker than the expected $\sim 3 \text{ nm}$. The HfO_2 -coated sensors seem to have experienced an even higher decrease in pore size, which was verified by running an ImageJ routine on a set of 15 images to approximate the average pore size. Results are gathered in Table 6.3.

In this table, the porosity and sensitivity of each type of ALD coatings are also reported. It can be observed that the decrease in porosity does not always follow the decrease in pore size. This might be an artefact of the SLIM technique, which relies on the penetration of a solvent to measure porosity: however, the ALD tends to close up the top part of smaller pores, creating a closed porosity inaccessible to solvents, or the reduction of pore size just creates too much surface tension for the

solvent to infiltrate the pores easily [290]. For all types of sensors, a decrease in sensitivity is also observed.

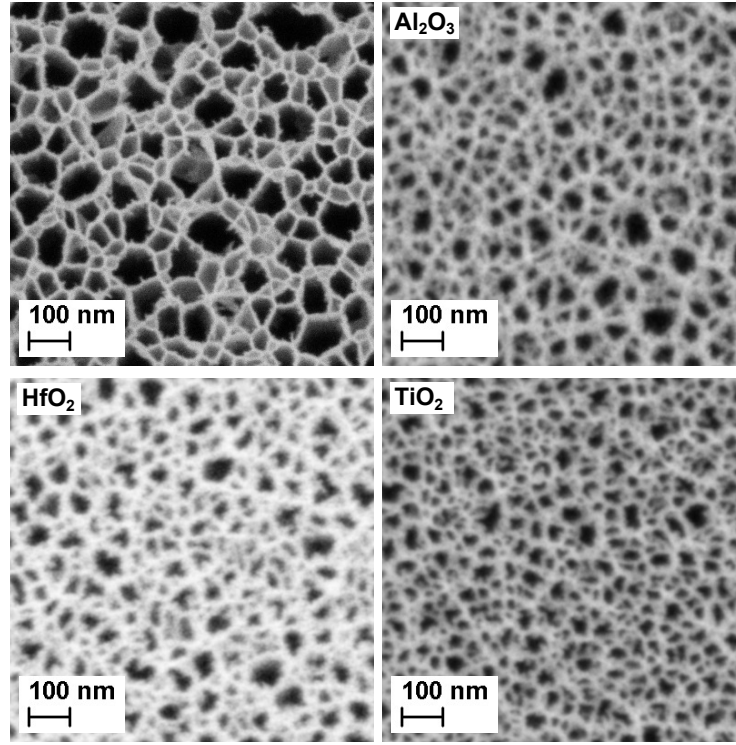


Figure 6.3: Top view SEM images of PSi membranes; from left to right and top to bottom: Blank PSiM, Al_2O_3 -coated PSiM, HfO_2 -coated PSiM and TiO_2 -coated PSiM.

Sample	Pore size [nm]	Porosity [%]	Sensitivity [% $_{EOT}$ /RIU]
Blank	55.53 ± 25.42	81.33 ± 4.38	64.7 ± 3.68
Al_2O_3 -coated	32.70 ± 17.68	48.68 ± 6.62	37.88 ± 0.68
HfO_2 -coated	27.71 ± 12.97	52.06 ± 12.89	44.33 ± 2.29
TiO_2 -coated	33.55 ± 15.14	55.01 ± 14.67	42.82 ± 3.64

Table 6.3: Properties of PSi samples coated with different ALD metal oxide layers; these values were extracted from SLIM measurements and SEM observations.

The optical signal intensity of the interferometric spectra was measured before and after ALD. The comparisons of the intensity of the Fabry-Pérot fringes over a wavelength range of 450 to 700 nm are illustrated

in Figure 6.4. For all metal-oxide coatings, there is an increase in the optical signal intensity, as well as a shift in the spectra.

6.3.1.3 Chemical composition of ALD layers

In order to ensure that no porous silicon or silicon oxide comes into contact with the buffer solution during the bacterial detection, and therefore prevent the degradation of the porous matrix, the ALD coatings must be uniform over the entire thickness of the membrane. The coverage of metal oxides through the depth of the membrane was evaluated using cross-section SEM, coupled with EDX in order to analyse the chemical composition. Three zones were considered in order to evaluate the uniformity of the deposition: the top of the membrane, the middle and the bottom. The EDX spectra for each type of metal oxide are depicted in Figure 6.5, 6.6 and 6.7. It must be noted that all three spectra show traces of aluminium, which can be explained by the Al layer at the back of the dies, that served as etching mask during the DRIE.

The $K\alpha$ Al peak at 1.486 keV is however most distinguishable on the PSi/ Al_2O_3 spectra in Figure 6.5. The other peaks that can be observed are the carbon, oxygen and silicon $K\alpha$ peaks at 0.277 keV, 0.525 keV and 1.739 keV respectively. The presence of carbon is related to the C_4F_8 used during the DRIE, which was not fully removed by O_2 plasma etching. In terms of peak intensity, it can be noted that the Al peak is higher at the top of the layer than at the middle and bottom. This means that more material accumulated at the top of the membrane during the ALD, but that metal oxide is still present through the entire membrane depth.

The HfO_2 -coated PSiM spectrum is represented in Figure 6.6. Once again, the carbon, oxygen, aluminium and silicon $K\alpha$ peaks are situated at 0.277 keV, 0.525 keV, 1.4486 keV and 1.739 keV respectively. The M band of hafnium at 1.644 keV is also present, as well as the $L\alpha$ Hf band at 7.898 keV, but which was not represented on the figure. The Hf M band overlaps partly with the Si $K\alpha$ band. At the top of the layer, the peak is more dislocated to the Hf M band. At the bottom, of the layer, the opposite is observed: the peak is more dislocated to the Si $K\alpha$ band. This observation suggests that while the deposition covers the membrane depth, more metal oxide accumulates at the top of the membrane. The small peak that can be observed at 1.27 keV can be attributed to an unknown contaminant, that remains to be identified.

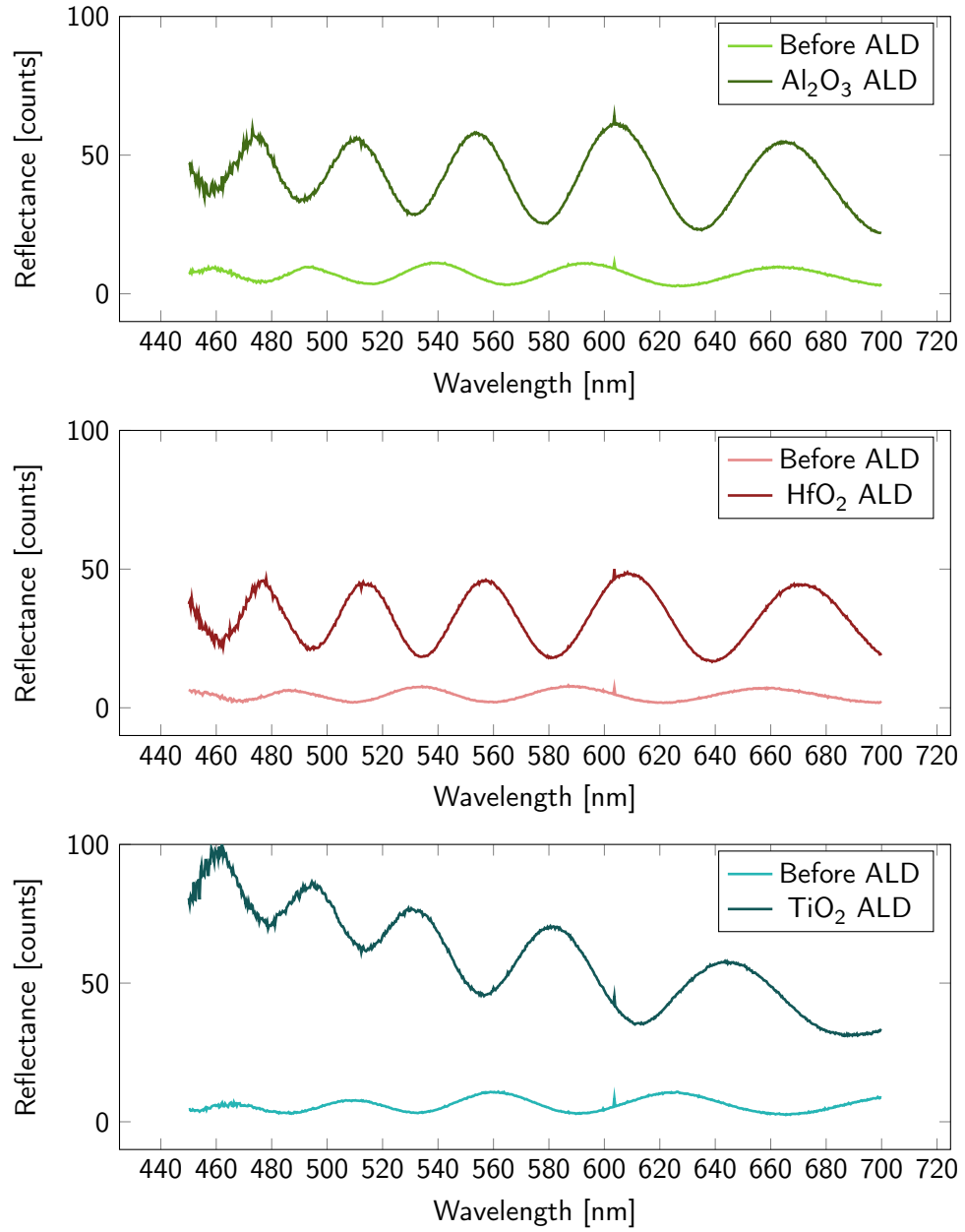


Figure 6.4: Interferometric spectra obtained before and after ALD for all three types of metal oxide layers, at the same integration time ($\Delta t=0.5s$).

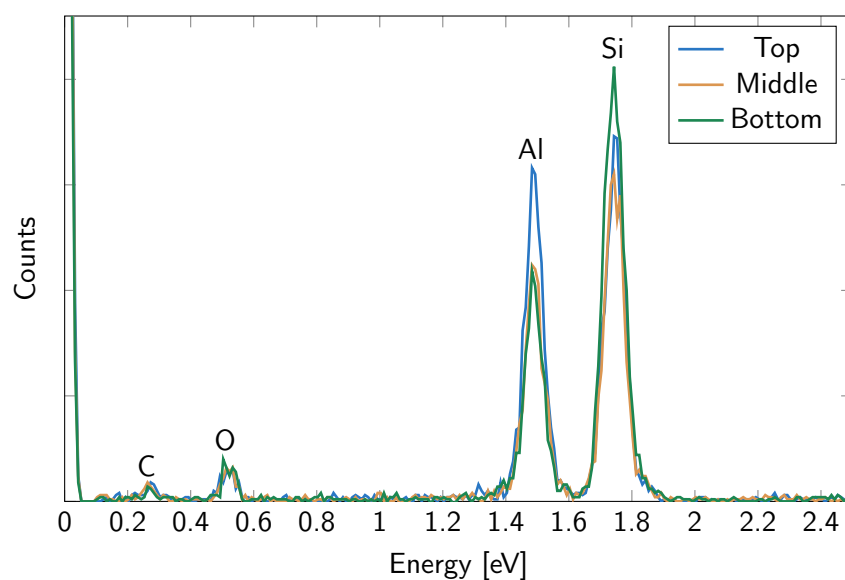


Figure 6.5: EDX spectra of Al₂O₃ coated PSiMs; the three traces correspond to the top, middle and bottom of the porous membrane.

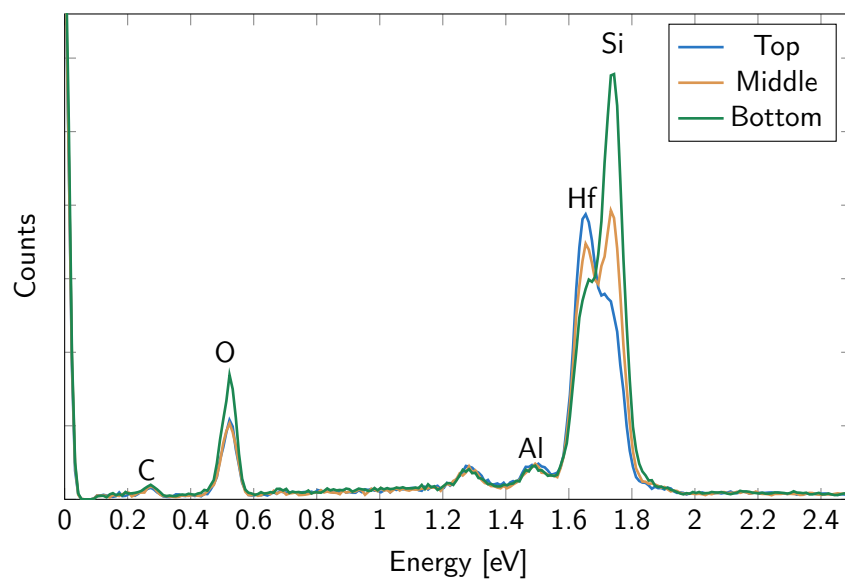


Figure 6.6: EDX spectra of HfO₂ coated PSiMs; the three traces correspond to the top, middle and bottom of the porous membrane.

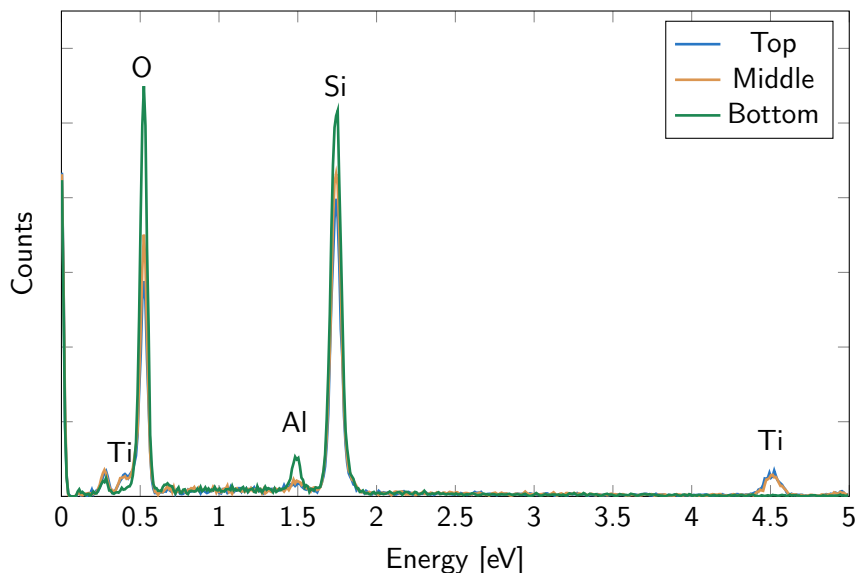


Figure 6.7: EDX spectra of TiO_2 coated PSiMs; the three traces correspond to the top, middle and bottom of the porous membrane.

Figure 6.7 represents the EDX spectra of a TiO_2 -coated PSiM. Once again, the oxygen, aluminium and silicon $K\alpha$ peaks are visible at 0.525 keV, 1.4486 keV and 1.739 keV respectively. Titanium is also represented by two peaks: one at 0.452 keV ($L\alpha$ band) and one at 4.508 keV ($K\alpha$ band). For both peaks, the signal intensity is similar at the top and middle of the membrane, but no signal comes from the bottom of the membrane. To further investigate this observation, a line scan was performed, which is depicted in Figure 6.8.

In the Al_2O_3 -coated PSiM line scan, it can be observed that more aluminium is present at the top of the membrane, which was also reported previously. In the line scan performed on HfO_2 -coated PSiMs, this effect is not observed: the presence of hafnium seems constant throughout the membrane depth. This contradicts the observations made from Figure 6.6. A possible explanation might be linked to the cutting of the samples: as the cutting is not perfect, the cross-section fully flat, and the difference in distance might affect the signal intensity. This explanation may also be applicable to the TiO_2 line scan, in which a decrease of Ti is reported after 50 μm .

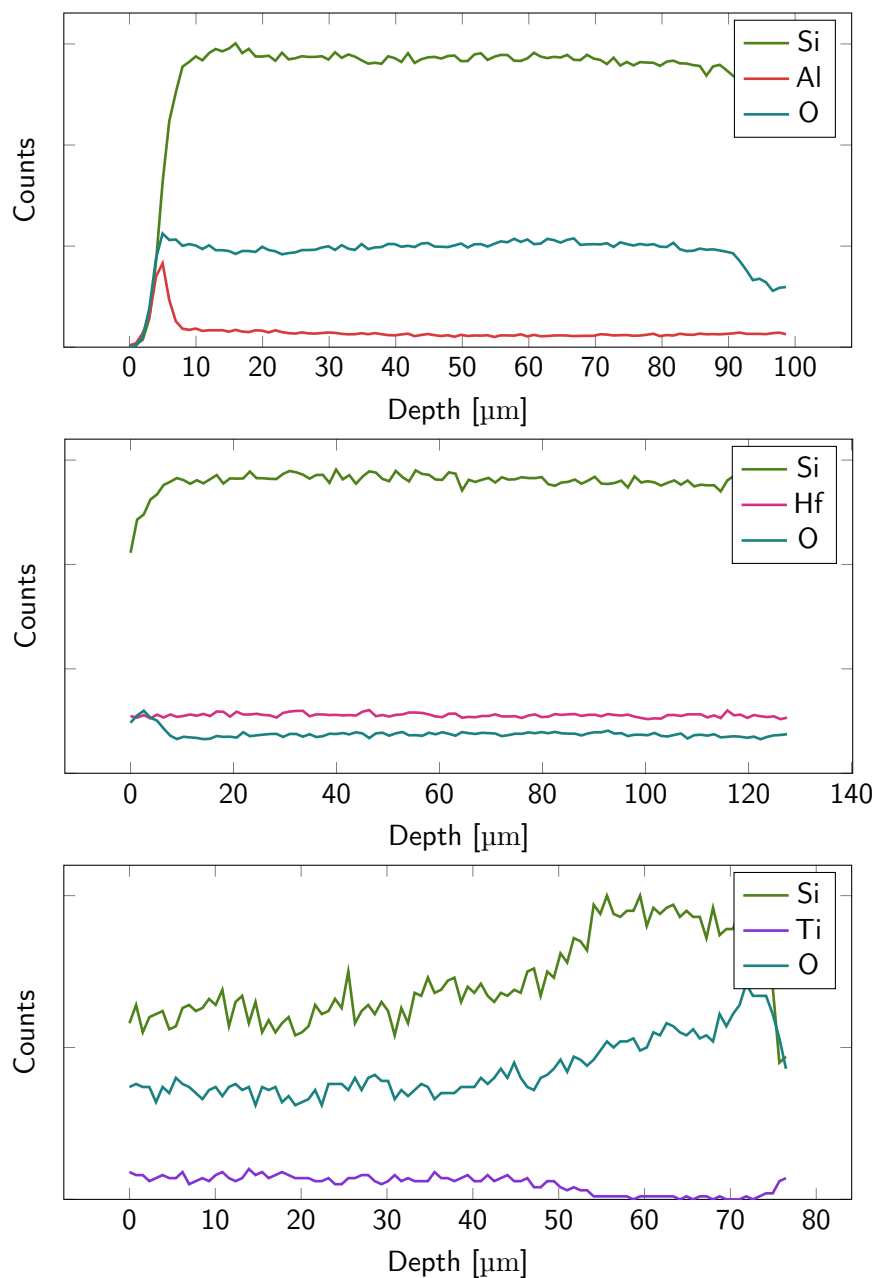


Figure 6.8: EDX line scan showing deposition on the full depth of the membrane for, from top to bottom, Al₂O₃-coated PSiMs, HfO₂-coated PSiMs and TiO₂-coated PSiMs.

To verify this, X-ray Photoelectron Spectroscopy (XPS) measurements were also carried both on the top and the bottom of the membranes. XPS is more surface-sensitive than EDX (1-10 nm for XPS compared to 0.5-10 μm), but does not enable the cross-section analysis of the PSi samples. Results are presented in Appendix E.1. Briefly, this technique is able to verify the valence state of elements, which indicates that the deposited materials are indeed the desired oxides. Moreover, the metal oxides were present both on the top and at the bottom of the membranes, indicating that the lack of Ti at the bottom of the TiO_2 -coated membranes observed on the EDX measurement is indeed an artefact linked to the sample cutting.

6.3.1.4 Stability of passivated PSiM in aqueous media

The optical stability of the ALD-coated membranes was established by flowing PBS through the membranes for a period of 4 hours, while monitoring their optical spectra. The first 30 min of data were discarded, as this period is considered as a transitional period, during which the PSiM gets fully wet. The duration of this period was fixed arbitrarily, but was observed to be sufficient to remove transient effects for all samples. The resulting relative EOT shift is presented in Figure 6.9.

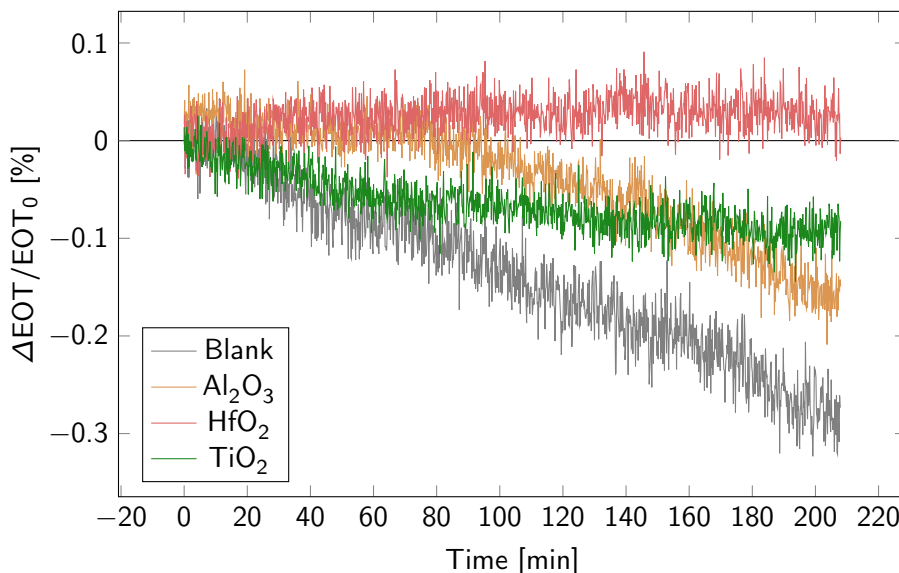


Figure 6.9: Average relative EOT shift of ALD-passivated membranes as a function of time, while exposed to a PBS flow ($n \geq 5$).

The blank samples exhibit a linear decrease in relative EOT, of ~ 0.8 % per hour. This is lower than the drift-rate observed on the previous prototype, which might be linked to the change in design and the reduced flow rate. For the HfO_2 -coated PSiMs, a minute increase in relative EOT is observed, which remains roughly constant overtime. The reason behind this increase remains to be investigated, but it might suggest either a slight pressure effect, or a minor swelling of the ALD coating when in contact with the buffer.

Both TiO_2 -coated and Al_2O_3 -coated PSiMs exhibit a decrease in relative EOT overtime, but not to the same extent as blank PSiMs. For PSi/ TiO_2 samples, a steeper decrease is first observed, but the relative EOT seems to stabilise after 2h. For PSi/ Al_2O_3 samples, the opposite is reported: there is first plateau for approximately 1 h, followed by a steep decrease.

The relative EOT shift after 3 h is detailed in Table 6.4, as well as the noise level (which is here approximated by the standard deviation of the overall noise), the deviation between measurements and a theoretical optical detection limit DL, which is calculated as $\text{DL} = 3 * \text{Noise level} / \text{Sensitivity}$ [291, 292]. HfO_2 -coated PSiMs are overall the most stable ones. It can also be noted that the ALD coating reduced both the noise level of the EOT shift and the deviation between samples, with TiO_2 -coated PSiMs exhibiting the lowest noise level and HfO_2 -coated PSiMs the lowest deviation. The impact of the ALD coating on the theoretical limit of detection was limited, with the lowest DL computed for PSi/ TiO_2 samples. This might be linked to the loss of sensitivity observed previously.

Sample	$\Delta\text{EOT}_{180}/\text{EOT}_0$ [%]	Noise level [%]	Deviation [%]	Theoretical DL [RIU]
Blank	-0.237 ± 0.003	$6.7 \cdot 10^{-4}$	0.0214	$3.1 \cdot 10^{-5}$
Al_2O_3 -coated	-0.114 ± 0.002	$3.9 \cdot 10^{-4}$	0.0014	$3.1 \cdot 10^{-5}$
HfO_2 -coated	$+0.006 \pm 0.001$	$3.9 \cdot 10^{-4}$	0.0008	$2.7 \cdot 10^{-5}$
TiO_2 -coated	-0.099 ± 0.002	$3.5 \cdot 10^{-4}$	0.0012	$2.5 \cdot 10^{-5}$

Table 6.4: Stability and performance of the ALD coated PSiM sensors: the relative EOT shift after 180 min, the noise level of the signal, the average response deviation between samples and the theoretical detection limit.

6.3.2 Bacterial detection

To check which type of ALD is best suited for bacterial detection, since the surface properties of each metal oxide is different (e.g. different zeta potentials), their performance as biosensor is tested via the detection of 10^6 CFU/ml of *B. cereus* lysate. The control test with only PlyB221 endolysins is also performed. Results are reported in Figure 6.10.

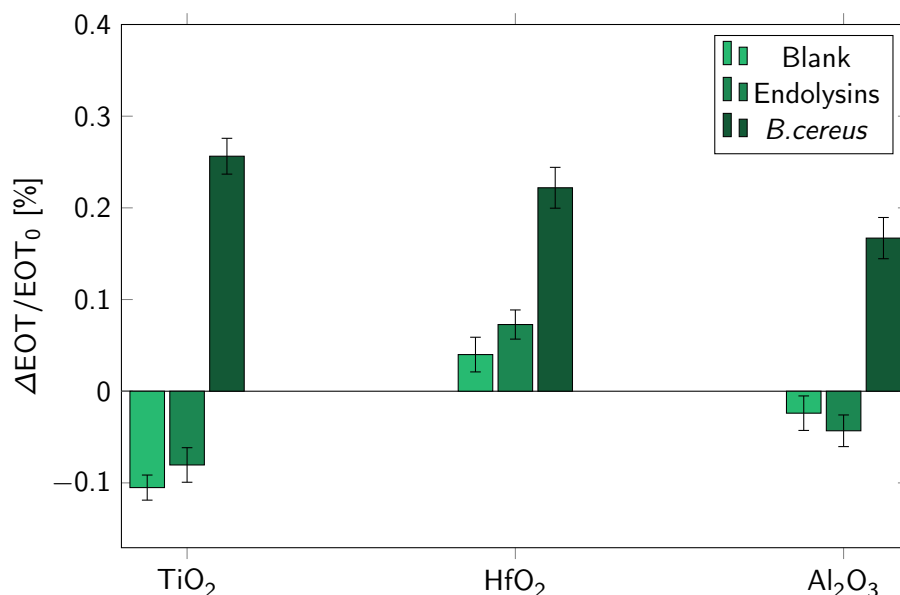


Figure 6.10: Detection of *B. cereus* lysate on PSiM-based sensors with different ALD coatings ($n \geq 5$), compared to the two negative controls tests (Blank and with endolysins only). The relative EOT shift for each experiment was measured after 1h.

For all ALD-coatings, the detection of *B. cereus* lysate induces a significant increase in relative EOT compared to the PBS baseline signal and the endolysins only control. It can be observed that while TiO₂-coated PSiMs are not the most stable, they exhibit the highest relative EOT shift upon penetration of bacterial lysate. In addition, the difference between the baseline signal in PBS and the bacterial lysate signal is the highest. This type of ALD passivation is therefore selected for further characterisation.

6.3.2.1 Sensitivity

The sensitivity of TiO_2 -coated sensors is established by decreasing the initial concentration of *B. cereus* bacteria, before the lysis. The relative EOT shift, from which the PBS baseline signal is subtracted, with increasing *B. cereus* concentration is plotted in Figure 6.11. For each concentration, the EOT shift is compared to the $3\sigma_{\text{PBS}}$ noise limit, which is depicted by the grey area on Figure 6.11. This $3\sigma_{\text{PBS}}$ limit was based on the IUPAC definition of the LoD as described in Equation 2.1. It can be observed that although 10^2 CFU/mL induces an average EOT shift that is above the noise limit, the detection is not always reliable, as some detected EOT shifts were below the limit.

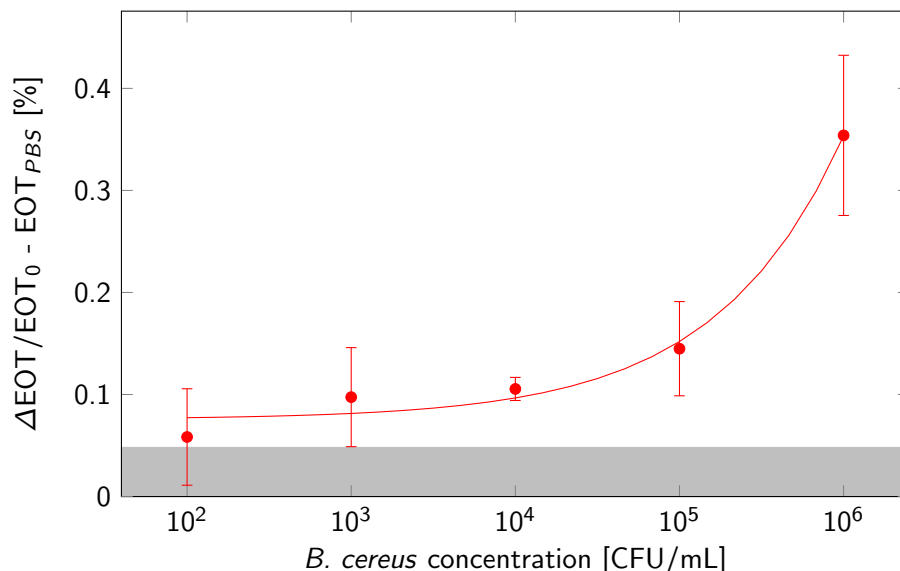


Figure 6.11: Detection of *B. cereus* lysate with increasing concentrations, on PSiM-based sensors with coated with ALD TiO_2 ($n \geq 5$). The relative EOT shift for each experiment was measured after 1h, and the average blank response was subtracted. The grey area depicts the $3\sigma_{\text{PBS}}$, as defined by the IUPAC's LoD definition.

The significance with both the PBS baseline and the endolysin control is also verified using a Student's t-test, with a p-value of 5 %. Concentrations in the 10^2 CFU/mL range do not induce a significant EOT shift, but higher concentrations do. The detection time can even be reduced, as even for concentrations of 10^3 CFU/mL, a significant relative EOT increase was observed after 15 min.

When considering how our biosensor will perform bacterial detection of more complex samples, with an unknown composition, the need for a final control test arises: the detection using only the analyte, before lysis. If there is a significant difference between the relative EOT shift before and after lysis, bacteria are detected. Results, for a bacterial concentration range of 10^3 to 10^6 CFU/mL, are presented in Figure 6.12. For all concentrations, the difference of EOT shift before and after lysis was significant, according to a Student's t-test (p-value < 0.05).

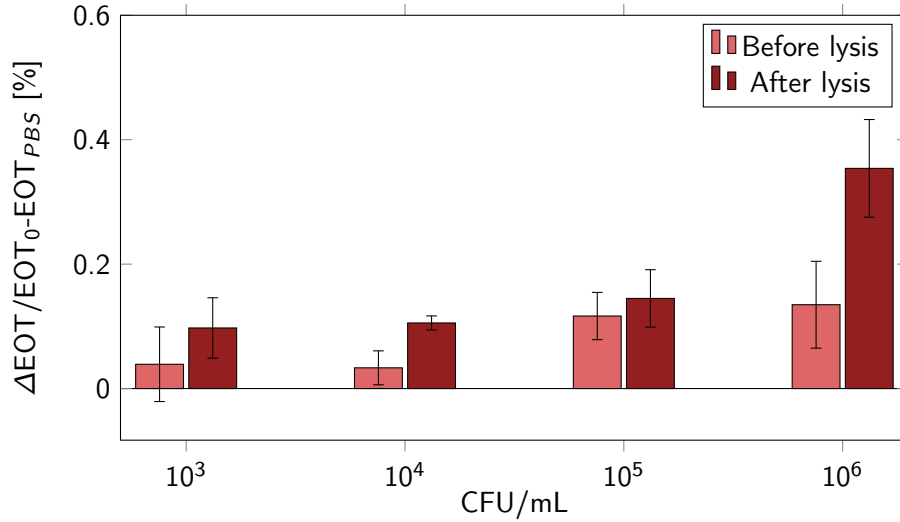


Figure 6.12: Detection of both *B. cereus* live bacteria and *B. cereus* lysate with increasing concentrations, on PSiM-based sensors with coated with ALD TiO_2 ($n \geq 3$). The relative EOT shift for each experiment was measured after 1h, and the average blank response was subtracted.

The collected data was also analysed with the Morlet Wavelet filtering routine. The results for the sensitivity are gathered in Figure 6.13. The significance of each bacterial concentration range was established using a Student's t-test with a 0.05 p-value: the lowest concentration range that induces a statistically significant shift is 10^3 CFU/mL. This means that the Morlet Wavelet processing does not reduce the detection limit in the case of our biosensor.

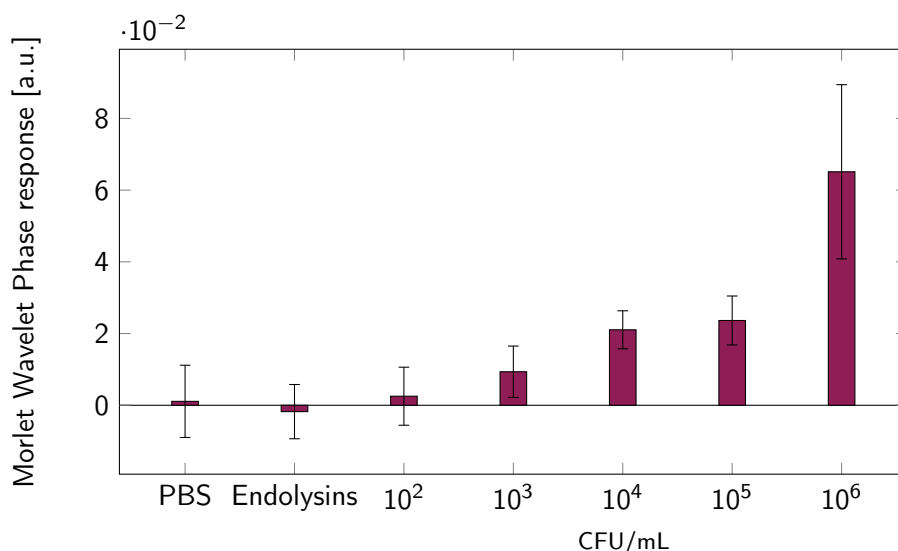


Figure 6.13: Morlet Wavelet response of the detection of *B. cereus* lysate with increasing concentrations, on PSiM-based sensors with coated with ALD TiO_2 .

6.3.2.2 Selectivity

In Section 5.3.4, we demonstrated the selectivity of our first prototype, using *S. epidermidis* as a control strain. With our second prototype, another strain is tested in order to validate its selectivity, namely *B. subtilis*. *B. subtilis* is also a Gram-positive bacterial strain, from the Bacillus genus, similarly to *B. cereus*, but is not targeted by the PlyB221 endolysin. Selectivity results are depicted in Figure 6.14.

It can be noted that the mixture of *B. subtilis* and PlyB221 endolysins does not create a significant relative EOT shift, meaning that there is no lysis induced by the PlyB221. When mixing both *B. subtilis* and *B. cereus* with PlyB221 endolysins, a significant relative EOT shift is observed. The extent of this shift is however reduced (only +0.26%) compared to the *B. cereus* lysate on its own (+0.35%). This may be attributed to the fact that *B. subtilis* bacteria accumulate on top of the porous membrane, preventing bacterial lysate to penetrate part of the pores.

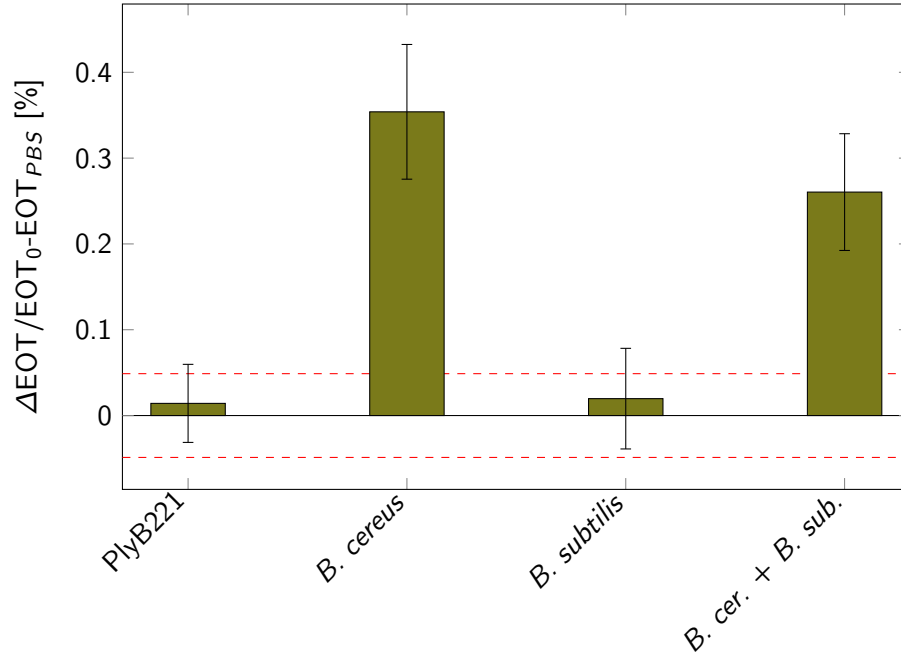


Figure 6.14: Characteristic relative EOT shifts measured on PSi membranes after 1 h in PBS, in a PlyB221 endolysin suspension, in a *B. subtilis* suspension, in a mixture of *B. cereus* lysate and *B. subtilis* and in *B. cereus* lysate only ($n \geq 3$). The dashed red line represents the 3σ level of the signal measured in PBS. The detection of *B. cereus* lysate, both with or without *B. subtilis* induced a significant shift

6.3.2.3 Versatility

Similarly to the selectivity, the versatility of our first prototype was validated in Section 5.3.5. The versatility of the optimised platform is also demonstrated using *S. epidermidis* as target strain and lysostaphin as lytic enzyme. In Figure 6.15, the detection of 10^6 CFU/mL of *S. epidermidis* lysate is compared to two control tests: one with only lysostaphin, and one with intact bacteria. The relative EOT shift of *S. epidermidis* lysate is significant to both control tests.

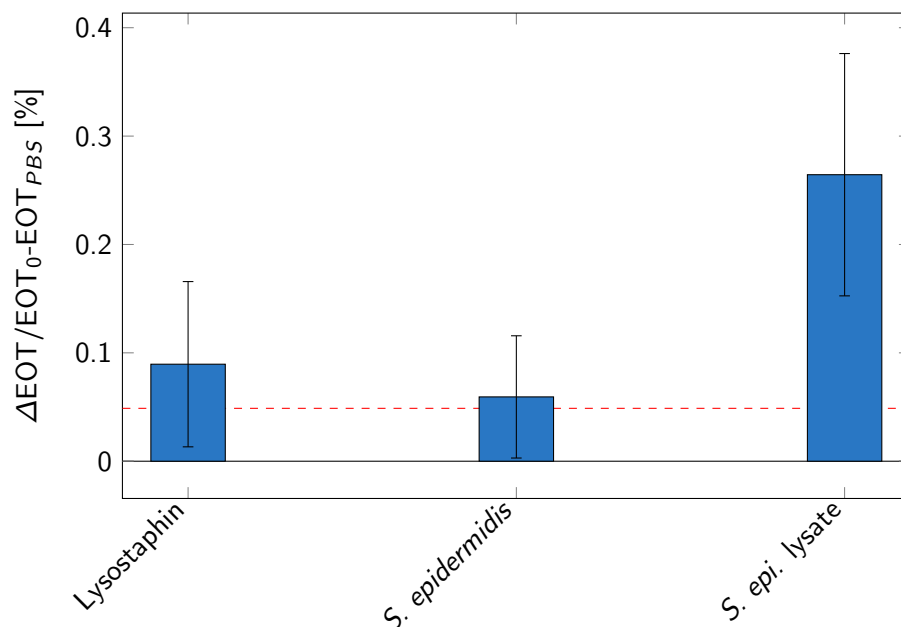


Figure 6.15: Characteristic relative EOT shifts measured on PSi membranes after 1 h in a Lysostaphin suspension, in a 10^6 CFU/mL *S. epidermidis* suspension, in 10^6 CFU/mL *S. epidermidis* lysate ($n \geq 3$). The dashed red line represents the 3σ level of the signal measured in PBS. Only the detection of *S. epidermidis* lysate induced a significant shift

6.3.2.4 Complex sample: detection of bacteria in human serum

To check the performance of our sensing platform with complex samples, human serum was spiked with *B. cereus*. Using a similar detection protocol as for PBS, the bacterial lysate was flown through the PSi membranes for 1 h; the results, compared to the control test in blank human serum, in a human serum and PlyB221 mix and in a *B. cereus* suspension in human serum, are illustrated in Figure 6.16.

The signification of the relative EOT shift of the detection of bacteria lysate with respect to the control tests was verified using a student t-test: the bacterial lysate induced a significant shift compared to all controls (p-value < 0.05).

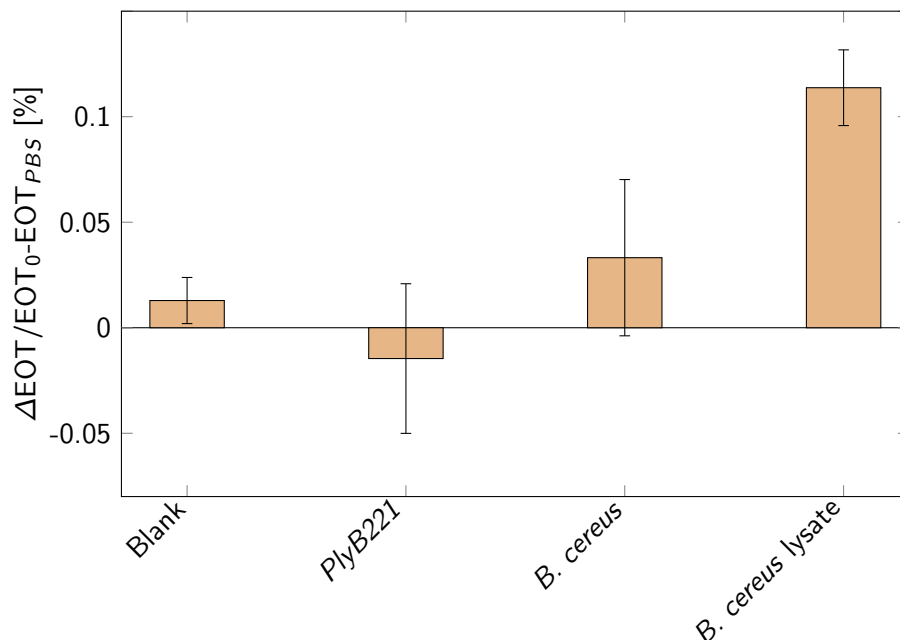


Figure 6.16: Characteristic relative EOT shifts measured on PSi membranes after 1 h in a blank human serum sample, in an endolysin-spiked human sample, in a 10^6 CFU/mL *B. cereus* suspension in human serum and in a 10^6 CFU/mL *B. cereus* lysate, suspended in human serum ($n \geq 3$).

6.4 Discussion

The passivation of porous silicon, especially for biosensing applications, has been a tremendous research question for a long time [285]. Indeed, the cycles of oxidation and dissolution of the pore walls limit the sensitivity of biosensors. Most passivation techniques rely on the creation of either Si–O or Si–C bonds [90, 285, 286], which can then be further modified by other chemistries. The most effective stabilisation methods are high temperature oxidation and thermal carbonisation. Both methods require a high temperature budget, which is not always ideal on fragile structures like PSi membranes. As an alternative to these techniques, atomic layer deposition can be used to deposit a thin layer of metal oxide inside the porous matrix, which then prevents the aqueous media to come in direct contact with the unstable porous silicon walls [204, 288, 289].

In this chapter, we demonstrated the successful deposition of several metal-oxide layers inside PSi membranes using ALD. The metal oxides were deposited on the entire membrane depth, and the surface chemical composition of each material was verified, both at the top and bottom of the membrane. Measurements, both of the pore morphology and chemical composition, however suggest that more metal oxide is deposited at the top of the membrane. Further investigations are needed to understand how the multilayered structure of the porous membranes affects the ALD process. We can however claim that the adaptation of the ALD recipe, including longer waiting times to enable the precursor's diffusion, and the lifting of the samples in the reaction chamber to allow the access from both sides of the membranes, was effective to coat the entire porous matrix. The ALD deposition naturally led to a decrease in pore size and porosity, but the design of our biosensor was adapted in order to take this into account. Nonetheless, a decrease in flow-through speed was observed (of one order of magnitude compared to the first prototype), which suggests a partial clogging of the porous membrane. It should also be noted that the fabrication process of the PSi membranes was also adapted with respect to the process developed in Chapter 5. First, for the front-side mask, a stack composed of a thin silicon oxide layer and a 500 nm polysilicon layer was used instead of a silicon nitride layer; this mask presented a better resistance to HF during the electrochemical etch. The backside mask was also adapted: an aluminium layer replaced the thick resist mask. This enabled the processing of multiple wafers in one step, exhibited a higher resistant to long reactive ion etches, and improved the electrical contact during the electrochemical etch as well.

After the ALD, the stability of the PSiMs was improved, to different extents for each metal oxides. HfO_2 exhibited the least drift when exposed to a buffer flow. This was already observed previously on PSi layers [204]. The addition of metal oxides also led to an enhancement of the optical signal: the interferometry spectra increased in intensity and the noise level was reduced. The signal enhancement is attributed to the increased relative permittivities (or dielectric constants) of the ALD coatings, compared to the blank samples. Indeed, the approximate relative permittivities of the metal oxides are 9, 80 and 25 for Al_2O_3 , HfO_2 and TiO_2 respectively, while the blank samples are covered with a thin layer of SiO_2 , which is characterised by a relative permittivity of 3.9 [293].

The biosensing performance of each type of ALD-coated PSiM was next

established, as the surface properties of each metal oxide are different and might affect the bacterial detection. This was indeed observed on PSi layers as well, for a similar principle of detection: PSi/HfO₂ layers exhibited the best stability in aqueous media and are characterised by the highest relative permittivity, but they did not perform well as biosensors [94]. In this work, it was observed that while TiO₂-coated PSiMs are not the most stable, they experienced the highest relative EOT shift upon bacterial lysate penetration. Some morphological properties may explain this observation: TiO₂-coated PSiMs were observed to have both the highest pore size and porosity, parameters which affect the LoD according to our simulations in Section 4.3.4.2. Moreover, the noise level of PSi/TiO₂ membranes in PBS was the lowest amongst all samples, similarly to the calculated theoretical LoD.

Other properties, such as the zeta potential or the hydrophilicity, may also affect the biosensing performance of the ALD-coated PSiMs, but remain to be further investigated. In terms of zeta potential and possible electrostatic effects, it can be assumed that the bacterial lysate is mostly negatively charged, as ribosomes, DNA-protein complexes and cell wall fragments (containing teichoic and lipoteichoic acids) are mostly negatively charged [268, 294–296]. At physiological pH (~ 7.4 , which is the pH of PBS), HfO₂ is often characterised by a negative zeta potential while Al₂O₃ and TiO₂ ALD coatings are characterised by positive zeta potentials [297, 298]. Indeed, for ALD coatings on flat Si samples, Xia *et al.* measured that Al₂O₃ and TiO₂ exhibit isoelectric points of 7.4 and 7.9 respectively [298]. This may explain why PSi/TiO₂ biosensors perform better than PSi/HfO₂ ones, as charge effects may enable the retention of more lysate in the porous matrix. Hydrophilicity may also be part of the explanation of the better performance of TiO₂-coated sensors: ALD-TiO₂ was observed to be more hydrophilic than ALD-Al₂O₃ [298]; analyte penetrates more easily in PSi/TiO₂ biosensors, which may justify why they perform the best for bacterial detection.

It must however be noted that the relative EOT shift for bacterial concentration of 10⁶ CFU/mL was much lower for the passivated prototype, compared to the first prototype presented in Chapter 5: for the first prototype, a shift of +2.43% was measured, while the optimised prototype only showed a shift of +0.35%. This may be linked once again to the decreased flow speed, which went from ~ 15 μ L/min to ~ 1 μ L/min for the second prototype. This hypothesis was verified using of theoretical LoD simulation presented in Section 4.3.4.2: higher flow speeds lead to

more accumulation of bacterial lysate inside the porous membrane, and therefore to higher EOT shifts.

The full performance of PSi/TiO₂ membranes for the indirect detection of bacteria via their lysate was characterised in terms of sensitivity and specificity. The LoD of our device was found to be 10³ CFU/mL; more precisely, the lowest bacterial concentration detected reliably was 9·10² CFU/mL. Lower concentrations could be detected, but not without a risk of false negatives. The significance of the detection was compared to three negative control tests: the baseline control test in PBS, the control with only endolysin and the control with intact bacteria. It was observed that while the addition of endolysins causes a minor increase in relative EOT, the control test with intact bacteria caused a more notable EOT shift. This increase was even statistically significant for concentrations of 10⁵ CFU/mL and 10⁶ CFU/mL. It can possibly be attributed to the spontaneous cell death of bacteria, as well as the production of by-products, such as proteins complexes.

We also demonstrated the versatility of our detection platform by switching the target bacteria and the lytic agent to *S. epidermidis* and lyso-staphin, respectively. Once again, the detection of bacterial lysate caused a significant increase in relative EOT. This experiment also illustrates the fact that commercial lytic enzymes can be used with our device. It can however be noted that relative EOT shift induced by the *S. epidermidis* lysate is lower than the shift induced by *B. cereus* lysate for the same concentration range. This might be explained by the smaller size of the *S. epidermidis* bacterial cells. The volumetric fraction of the bacterial lysate inside the PSiM is therefore lower, and the effect on the relative EOT is diminished.

Next, we performed a bacterial detection assay on a complex sample, namely human serum. *B. cereus* is one of the pathogens causing nosocomial bloodstream infections and its detection in blood samples is therefore crucial [299]. In the present work, we opted for human serum instead of blood as the optical detection method and our current fluidic cell does not enable the detection of opaque matrices. Human serum, while still turbid, allowed the transmission of enough light to enable optical detection. The blank human serum sample induced a slight increase in relative EOT of +0.01%, which is to be expected as the proteins, peptides, nutrients and organic waste present in the serum can agglomerate inside the porous matrix. The assay with human serum containing live

B. cereus bacteria induced an even larger relative EOT shift, namely +0.03%. This increase in EOT was also observed in PBS samples, and can also be attributed to bacterial debris, by-products and proteins complexes. Finally, the detection of bacterial lysate induced a relative EOT shift of +0.11 %. While this shift was significant compared to all control tests, it is quite lower than the shift induced in PBS. A possible explanation for this decrease might be the impact of the human serum composition on the lytic activity of the PlyB221 endolysins: while the NaCl content (100-200 mM) and pH (8) of the serum are both within the ideal conditions for the optimal lytic activity of the endolysins (see Appendix D), the presence lipids and other proteins may still impact the lysis. This impact needs to be further investigated. To extrapolate the LoD in human serum, we scaled the preliminary trend measured in PBS to the data obtained in serum: the LoD is hence estimated at 10^6 CFU/mL. This estimation remains to be verified experimentally.

When comparing our device to traditional methods for bacterial detection, such as PCR and ELISA, we can say that where we lack in sensitivity, we make up for it in assay time: the total assay time amounts to 1h30, lysis included. Indeed, the PBS baseline signal can be established at the same time as the lysis occurs. Our device however still requires a lab environment and some additional equipments, but these could be easily miniaturised to a small bench top instrument. In terms of the comparison to other P*Si*-based bacteria sensors, our detection platform shows a similar LoD and response time to the devices that rely on RIFTS as detection method [96, 249, 253, 254, 256]. Other methods, such as SERS, are faster and more sensitive, but require more bulky equipments if a high precision is required [68]. Electrochemical methods are also faster and more sensitive, but they are easily impacted by the nature of the analyte matrix [259]. However, when considering a similar detection protocol, relying on lytic agents instead of functionalisation, the use of P*Si* membranes instead of P*Si* layers tremendously improved the LoD, which was estimated at 10^7 CFU/mL for P*Si* layers [94].

The strength and the novelty of our device lies not only the optical gain achieved by ALD, but also in the lack of functionalisation, which requires specific fabrication, storage and usage conditions. Any drift in temperature, for example, may cause the functionalisation to lose its binding power, and impact the sensitivity of the sensor. In the present detection platform, the lack of functionalisation not only means that there are limited constraints on the storage and usage conditions, but it

also makes our device versatile. Any lytic enzyme/bacteria pair could be detected using our sensor; a cocktail of lytic enzymes could even be used to target multiple bacteria strains. This opens up a lot of possibilities in terms of fields of application.

Our device however still faces some challenges. Improvements are yet possible in terms of stability, for example. This might be achieved by using different ALD parameters or different layer thicknesses; multilayer of ALD coatings might also be investigated, combining for example the passivation capabilities of HfO_2 and the interesting properties of TiO_2 by depositing a $\text{HfO}_2/\text{TiO}_2$ bilayer in the PSi membranes. One important constraint seems to be the limited flow speed, which affects the limit of detection. Either by increasing the pore size, or by improving the mechanical stability of the membranes, a higher pressure may be used, leading to an increased flow speed. Chemical modification is also worthy of further investigations, to either improve the stability (e.g. via the addition of APTES or APDMES), or to modify the surface charges and the hydrophilicity. Finally, the impact of complex media on the lysis and sensing of bacteria still needs to be further investigated.

6.5 Conclusion

In this chapter, we demonstrated the successful stabilisation of PSiM using ALD. Several metal oxides were deposited, and the homogeneity of the coating was verified. The addition of metal oxides did not only improve the stability of the sensor, but also enhanced its optical properties. TiO_2 -coated PSiMs were found to exhibit the best biosensing performance, and were therefore used for the indirect detection of bacteria via their lysate. The achieved LoD is in the 10^3 CFU/mL range and the total assay time amounts to 1h30. The platform is both specific and versatile. The detection of bacteria in a complex medium, namely human serum, is also illustrated.

Part III

Discussion & Conclusion

CHAPTER 7

GENERAL DISCUSSION

Contents

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Abstract

This chapter takes a step back and analyses the added value and the limitations of the bacterial detection platform presented in this thesis. It also concentrates on the performance of porous silicon as a biosensor in general: it is a promising material, or should other materials be preferred ? The arguments raised to answer this question are partly adapted from [RV3].

7.1 The added value and limitations of PSiMs for the detection of bacterial lysate

The aim of this thesis was to combine the known benefits of PSi-based optical sensors [8,9,242] and the use of lytic proteins for the indirect detection of bacteria via their lysis or via their lysate [27,52]. The former was improved by establishing a membrane-based flow thorough operation, while the latter enabled the development of a versatile biodetection scheme.

Porous silicon biosensors have been researched for more than two decades [300]. They have a high surface area (up to $800 \text{ m}^2/\text{g}$ [301]) leading to a high surface reactivity, a tunable morphology, and are biocompatible as well as biodegradable, unless their surface is chemically modified by one of the many techniques available [285]. Most importantly, porous silicon can exhibit specific optical properties, which are impacted by changes in the environment surrounding the pores. The PSi optical sensing mechanism is based on changes in the average layer refractive index, which occurs when any substance penetrates into the porous matrix. The extent of the refractive index change depends on the refractive index of the substance and level of pore-filling that is achieved. The refractive index then impacts the optical properties, be it the frequency of the Fabry-Pérot fringes for porous interferometers, or the shift in special optical features for photonic layers. One advantage of PSi optical sensors is therefore the fast response time [8,239,242,262,302].

The amplitude of the response is however limited by the diffusion of substances through the porous matrix. The hindrance of diffusion can be attributed to the structural properties of PSi (pore size, porosity, layer thickness, etc.) and to steric or hydrodynamic interactions between the diffusing analyte, the pores and molecules already adsorbed on the pore wall [303]. An illustration of the diffusion hindrance levels is depicted in Figure 7.1.

One way to enable a better diffusion is to fabricate porous silicon membranes that enable flow-through operation. This can be achieved using several techniques, some of which involve standard microfabrication techniques, all which we detailed in Chapter 3. PSi membranes show both an increased response time and an improved sensitivity [10,126,283]. This was therefore the chosen path for our biosensor: we built a multilayered Fabry-Pérot interferometer, which we then opened from

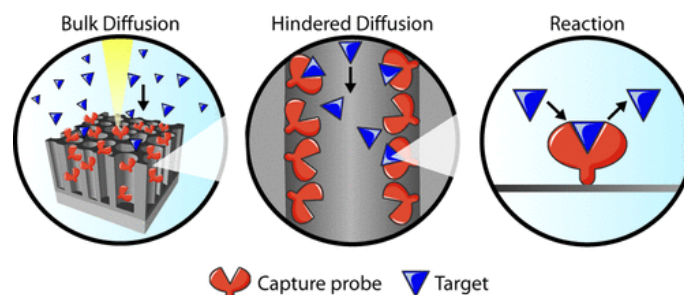


Figure 7.1: Schematic illustration of the different diffusion hindrances when considering PSi layer-based biosensors. Reproduced from [303].

the back using a dry etching technique from the microelectronics industry. We demonstrated that, with our bacterial detection protocol, the use of PSi membranes improved the sensitivity of the biosensor: compared to PSi layers [94], the limit of detection decreased by 4 orders of magnitude, going from 10^7 CFU/mL to 10^3 CFU/mL.

Another particular feature of PSi is its surface chemistry: PSi spontaneously degrades in aqueous media, and therefore needs to be chemically modified for biosensing application. This is both a weakness and a strength of PSi biosensors, as many functionalisation methods have hence been researched, making the PSi surface highly customisable [262, 286, 302]. For our biosensor, the surface chemistry was modified via atomic layer deposition, but only to render the sensor stable when in contact with aqueous media.

By using lytic enzymes to selectively lyse our target bacteria, we eliminated the need for complex and time-consuming surface chemical functionalisation, that binds biorecognition elements to the sensor surface. The lack of functionalisation limits the necessity for specific fabrication, storage and usage conditions. Lytic proteins were selected in this research because they can have different targets as well as various origins: they can be endolysins, coded by bacteriophages [52], or enzymes produced by bacteria (such a lysostaphin for example [304]). Both were used with our biosensor, in order to demonstrate the versatility of our detection platform. Indeed, sensors from the same batch can be used to detect different targets, simply by changing the lytic protein. Lytic proteins can also be combined, in order to create a multi-target biosensor. Furthermore, by using lytic enzymes, the sensor is able to distinguish between live and dead bacteria. At this stage, one hindrance is the cost

of commercial lytic proteins: for example, 1 mg of lysostaphin currently sells for ~ 140 €¹.

Via the combination of lytic enzymes and PSiM-based optical sensors, we were able to detect bacterial concentrations as low as 10^3 CFU/mL in less than 2 h. Therefore, our biosensor fits most optimisation criteria for bacterial detection methods, as defined in Chapter 2:

- Assay time: the total assay time is 1 h 30 min.
- Sensitivity: the limit of detection is 10^3 CFU/mL, which is sufficient for many food-borne infections [305] or for some common medical infections, like urinary tract infections for example [18].
- Selectivity: the selectivity is guaranteed by the endolysins and can be extended by either adapting the endolysins or using several endolysins at once.
- Sample volume: the assay uses less than 100 μ L of analyte.
- Cost: the current fabrication cost per sensor is just under 4 €; this is still quite high, but one must keep in mind that these sensors are fabricated on 3-inch wafers, which can only hold 32 sensors. Should the fabrication process be upscaled to industry-size wafers, namely 200 mm or 300 mm, the cost per sensor would be much reduced. It must however be noted that the price of the endolysins and eventually an on-chip fluidic integration still has to be added to the total cost as well.
- Sample preparation: there is only one step of sample preparation, namely the lysis, which only takes 30 min of the total assay time.

The only criteria that is not fulfilled is portability: our biosensor still needs external equipments for the optical measurement and fluidic operation. Next steps could include the integration of the biosensor into a miniaturised signal acquisition platform to ensure its portability.

The biosensor presented in this work is also aimed as a single-use device, going with the Point-of-Care testing trend. The re-use of the sensor, by performing a cleaning, has not been explored, but is worthy of investigation.

¹according to <https://www.sigmaaldrich.com/BE/en/product/sigma/17386>

7.1.1 Performance as biosensors for bacterial detection

Even if the sensitivity achieved by our biosensor is not comparable to methods such as PCR and bacterial culture that can both detect down to one bacterium per mL, we have reached a noteworthy performance in terms of assay time. Much research and development efforts have been dedicated to PCR, contributing to its miniaturisation, improved response time and portability [306, 307]. However, the biosensor presented in this work is still a viable alternative to traditional detection methods, especially for applications in which the distinction between live and dead bacteria is crucial.

Our detection limit of 10^3 CFU/mL is indeed sufficient for many applications, as can be verified in Table 1.1. Other biosensors have however reached lower limits of detection, even as low as a single bacteria cell per mL [64]. These ultra-sensitive sensors often rely on electrochemical detection methods, such as EIS or DPV [308]. Optical biosensors often read LoDs in the 10 CFU/mL range, but have a response time that is expressed in tens of minutes [309]. Moreover, optical biosensors can now rely on smartphones for the optical read-out, which renders them portable and accessible to all users [309]. It however remains difficult to compare our device to each and every biosensor for bacterial detection published in the literature, as there are more than 400 publications on bacterial biosensors each year.

Nevertheless, we can compare our biosensor to other PSi-based bacterial sensors, as the number of publications on this topic is much more reduced. In Table 3.4, we listed the PSi-based sensors for bacterial detection, published from 2013 onward. Our biosensor has a similar LoD and response time as the most sensitive optical biosensor relying on RIFTS as detection method [249, 253, 254]. These sensors are constituted of porous silicon layers and are based on the capture of bacteria on top of the porous matrix, instead of inside the pores. Bacteria are indeed too big to penetrate the pores. In the works of both Yaghoubi *et al.* [249] and Massad-Ivanir *et al.* [253], bioelements bind the bacteria on top of the layer, which causes a reduction of the EOT peak intensity; this intensity change is established after a rinsing step that removes all unbound cells. On the other hand, Tang *et al.* developed an indirect detection method based on an EOT shift: as bacteria bind on top of the porous layer, they prevent the penetration of a probe molecule inside the pores and hence inhibit the EOT shift that occurs in the absence of bacteria [254]. These

three biosensors have thus a different working principle than ours, but have one common feature: they are all functionalised by capture probes.

Other PSi-based bacterial sensors, not relying on RIFTS, were able to achieve lower LoDs. By using SERS, Jabbar *et al.* were able to detect the presence of a single bacterium [68]. However, SERS often requires a large measurement equipment; some miniaturised versions are now available on the market, but remain either lack precision or are relatively expensive. A LoD of 1 CFU/mL was also achieved by Reta *et al.*, using DPV to measure the blockage of pores by bacteria [229]. While electrochemical transducers can easily be integrated in point-of-care devices, the fabrication of the presented PSiM-based devices seems difficult to upscale to an industrial level, as freestanding PSiMs, which are very fragile, must be detached from their substrate and transferred to gold electrodes.

So, while our sensor still bears some limitations in terms of sensitivity and response time, it has three interesting features: (1) its compatibility with microfabrication techniques makes it possible for the fabrication process to be easily upscaled, (2) the improvement of the optical properties via the addition of ALD metal oxides and (3) the use of lytic enzymes instead of functionalisation opens the possibility to versatile or multi-target detectors.

To discuss potential improvements for our sensing platform, one must however first discuss the limitations of the sensor, but also the limitations of the measurement equipments. The limitation in terms of the sensitivity is linked to high noise levels. On the sensor's scale, this noise can be attributed to scattering effects due to the surface roughness (both at the PSi-air interface and at the interface between PSi layers), to thickness fluctuations that induce incoherent effects or to absorption losses [192]. These effects can be minimised, but not fully eliminated. During the detection, the accumulation of large bacterial debris on the top of the PSi membrane can also induce light scattering or cause the blockage of part of the pores, which impacts the optical response and creates noise.

Not all the noise is however attributed to the sensor itself; some of the error level is linked to the measurement set-up. The optical set-up is for example required to be exactly perpendicular to the sensor's surface, at the optimal height; small deviations in angle or height induce errors and sensitivity losses. The fluidic cell also has its limitations: polycarbonate is characterised by a light absorption peak around 700

nm, which has to be eliminated by performing a reference measurement; this reference measurement can be a source of error. Scratches and spots on the fluidic cell also cause scattering and losses of the optical signal. When considering complex samples, losses can also be attributed to the matrix itself, as the light has to pass through the complex sample before reaching the PSi membrane. With the current fluidic set-up, turbid matrices cannot be used for the detection of bacteria, as the height of the fluidic channel above of the PSi membrane is about 3 to 4 mm; a microfluidic on-chip integration, with micrometric channels, may reduce the losses induced by the sample matrix and enable the detection of turbid samples.

Other potential improvements could certainly be envisaged, from different points of view:

- Mechanical instability: the flow-through operation of the membrane is pressure driven, but limited by its mechanical stability. The PSiMs in their current design tend to break when exposed to pressures higher than 2 bar. The flow speed is inhibited, which limits the volume of bacterial lysate that can accumulate inside the porous membrane and therefore impacts the sensitivity, as discussed in Section 4.3.4.2. Improving the mechanical properties of the PSiMs is however only a question of technological optimisation, and it seems unlikely that it will improve the sensitivity by more than one order of magnitude.
- Filtration: while we aim for the bacterial lysate to accumulate inside the sensing layer, this has yet to be investigated experimentally. The presence of any binding effect, be it electrostatic or otherwise, of the bacterial lysate and the pore walls also remains unknown. The limited knowledge of the actual filtration mechanism of the bacterial lysate may conceal some optimisation routes.
- Optical response: while the optical properties of the bacteria lysate remain to be investigated, some information can be found in the literature [310,311]. Most debris of bacterial cell are characterised by refractive indices that are quite close to the refractive index of buffer solutions. The nature of the analyte therefore already challenges the performance of the detection platform.

7.1.2 Performance as optical sensor

Regardless of the application of bacterial detection, our biosensor has an optical detection limit of $2.5 \cdot 10^{-5}$ RIU. By expressing the optical detection limit, we can compare our sensor's performance to other optical sensors, no matter the application. It must however be noted that the LoD can be calculated in multiple manners, as presented in Section 2.5.1. In our case, we expressed the detection limit based on the average noise level of our blank measurements and on the sensitivity [291].

Most ultra-sensitive optical sensors have a detection limit in the 10^{-7} - 10^{-8} RIU range [312, 313]. Optical sensors based on surface-plasmon resonance are often the most sensitive, with detection limits as low as 10^{-8} RIU [312]. Interferometers and ring resonators, for example, often have a slightly higher LoD, in the 10^{-6} - 10^{-7} RIU range [312]. For porous silicon-based optical sensors, the LoD is not often expressed in RIU, which limits the number of comparisons. However, some publications, as discussed hereafter, report the optical detection limit. It can be observed that the detection limit is influenced by the nanostructures of the PSi sensors: PSi photonic crystals are able to reach detection limits in the 10^{-7} - 10^{-8} RIU range [292, 314], while for PSi interferometers, the LoD is limited to 10^{-4} RIU [227, 292, 315]. This poorer performance is often attributed to a higher noise level [292].

The detection limit of our PSiM-based interferometric sensor is therefore slightly lower than the expected range. Two publications also relate to optical sensors which based on porous silicon membranes: Martín-Sánchez *et al.* reached a resolution of $2 \cdot 10^{-4}$ RIU [227], while He *et al.* built a lateral PSi membrane with a detection limit of $2 \cdot 10^{-3}$ RIU, which could be lowered to $6 \cdot 10^{-4}$ RIU with a higher resolution optical setup [316]. Compared to both these PSiMs, our sensor is thus more sensitive to changes in the refractive index, at least by one order of magnitude.

Similarly to He *et al.*, the sensitivity of our sensor is limited by our optical setup and fluidic set-up. For example, the resolution of the spectrometer used in this work is reported as 0.3 to 10 nm; studies have however demonstrated that by improving the set-up resolution to 0.1 nm, the LoD is nearly decreased by one order of magnitude [316, 317].

7.2 PSi optical biosensor: is it worth it?

The advantages and drawbacks of porous silicon as a material for the optical transduction of biomolecules were recently listed by De Stefano [262], and are gathered in Table 7.1.

Pros	Cons
Low costs and simple (but not trivial) fabrication equipment and procedures	Intrinsic limitation of the etching process
Easy and fast read-out	Spontaneous ageing and chemical instability
Integrated microelectronics compatibility	Single device calibration
Tunable morphology, dielectric properties and surface chemistry	
Huge specific surface area	
Biocompatibility	
Extreme flexibility in different application fields	

Table 7.1: The advantages and drawbacks of porous silicon biosensors, replicated from [262]

Based on this table, it would seem that the advantages of PSi largely outweigh the disadvantages. Indeed, the many benefits of PSi, gathered in one single material, explain why PSi biosensors have been researched for more than two decades, in laboratories spread all over the world. Many of these good features were already discussed above. But to verify if these merits overcome the main drawbacks of PSi, we must look into the passivation and integration challenges that PSi biosensors face.

7.2.1 Passivation challenges for aqueous applications

The chemical instability and spontaneous oxidation/dissolution of porous silicon was already discussed at length in Chapter 6. One thing that was however not discussed was the impact of the passivation techniques on the surface contact angle. This impact is however not negligible when considering biosensing applications, as hydrophobic surface properties may limit the penetration of analyte inside the porous matrix. Table 7.2 gathers some contact angle values for some of the passivation techniques presented in Chapter 6 [90,318].

Process	Surface Species	Contact Angle [°]
Native surface	Si–H	102 ± 3
Ozone oxidation	Si–O–Si, Si–OH	11 ± 1
Thermal oxidation (600 °C, 90 min)	Si–O–Si	20 ± 1
Hydrosilylation with 1-dodecene	Si–(CH ₂) ₁₁ CH ₃	119 ± 2
Electrochemical methylation	Si–CH ₃	102 ± 3
Thermal acetylation (300 °C, 30 min)	Si–C	53 ± 3
Thermal acetylation (485 °C, 30 min)	Si–C	80 ± 3
Thermal acetylation (500 °C, 30 min)	Si–C	76 ± 7

Table 7.2: Surface chemistries prepared on PSi samples, adapted from [318].

It can be noted, with the exception of hydrosilylation and electrochemical methylation, most surface modification techniques reduce the hydrophobicity of the porous layer. The most effective passivation methods, such as high temperature thermal oxidation and thermal acetylation, render the native porous silicon surface hydrophilic, but to different extents.

High temperature thermal oxidation offers the best compromise between chemical stability and hydrophilicity. However, as silicon oxide is transparent, thermal oxidation tends to deteriorate the sensing performance of PSi, mainly by increasing the noise level [204]. Moreover, for more fragile structures, such as PSi membranes, the high temperature budget of thermal oxidation puts at risk the physical integrity of the porous layer, often resulting in cracks or bending.

With regard to ALD as passivation method for PSi, not much information can be found in the literature regarding the wettability. For our biosensor, the contact angle of ALD-coated PSiMs could not be measured, as the membrane area was too small for the measuring tool. It has however been observed that ALD-deposited metal oxides, such as the ones tested in Chapter 6, have a hydrophilic nature [298, 319]. How each ALD coating compares to thermally oxidised PSi remains to be investigated.

7.2.2 Integration challenges

While thermal oxidation is still a very common technique in the microelectronics industry, the other passivation techniques are less common, and often difficult to upscale. This introduces the second challenge of PSi biosensors: their integration into industrial devices.

Because PSi is compatible with microfabrication techniques and clean-room environment, it has the potential to become integrated in microelectronics devices. This potential is currently hampered by a series of obstacles, among which the lack of reliable processes and equipment, the low reproducibility, the need for high-quality silicon substrates and the disparate properties. There are still too many unknowns in the field of PSi and PSiMs that block the transition from research to industry. The absence of standards in the PSi-research community makes it difficult to fill the present gaps of knowledge.

To fully benefit from the properties of PSi and introduce innovative PSi-based products to the market, PSiMs must be combined with other microelectronics devices, such as integrated light sources, communications systems or electronic interfaces. This is only possible if the access to cleanrooms and microfabrication techniques is guaranteed. Yet, when the access to CMOS technologies is assured, the introduction of PSi hinders further microfabrication process steps. The mechanical stability of the substrate is indeed compromised and some process steps such as high temperature annealing are incompatible with PSi since they cause cracking and bending. This makes the integration of PSiMs at a wafer level into a full CMOS process difficult, but not impossible. Indeed, PSi was already integrated in a CMOS process over a decade ago, namely in Bosch's Advanced Porous Silicon Membrane process, which is used in the fabrication of pressure sensors for automotive applications [320–323]. More recent advances and challenges in the integration of PSi at the industrial level are presented by Gautier *et al.* [324]. Despite their limited reproducibility and homogeneity, the various attempts at industrialisation discussed by the authors show great promise. Two examples of prototypes worth mentioning are Barillaro *et al.*'s gas sensor [325, 326] and Capelle *et al.*'s RF devices [327, 328], both produced in Si processing environments and integrated with CMOS electronics. Moreover, novel work from Scheen *et al.* [103] presented a step towards large-scale industrial production. They demonstrated the integration of porous silicon in a post-process anodisation: porosification occurs as the last step of the process and only involves the backside of the wafer, leaving the front-side circuitry intact. This progress in the integration of PSi and PSiMs paves the way for the development of new applications and for future industrialisation attempts.

7.2.3 The future of porous silicon

Recently, a European Cooperation in Science and Technology (COST) initiative was launched with the aim of creating a network for research, innovation and product development on porous semiconductors and oxides. This action, named Netpore, seeks to bridge the gap between fundamental developments and practical applications of technologies, focusing on the fields of health, energy and environment. PSi sensors and biosensors will be part of both the health and the environment work group. The goal is to bring PSi (bio)sensors to higher levels of sensitivity, while developing affordable, low maintenance portable systems [329].

Despite this action, the most interesting prospect in the field of porous silicon application is not that of PSi devices but that of porous silicon nanoparticles. These silicon nanostructured powders show the greatest marketing possibilities and do not face the same challenges as the PSi devices. For example, they take advantage of the lack of chemical stability of PSi by making self-degrading drug delivery carriers. Moreover, PSi nanoparticles are able to combine diagnostics and therapeutics, extending their prospects even further [262].

In conclusion, PSi biosensors are promising from a scientific point of view, despite the challenges they face. With more effort focused on their development and their integration, they could become successful industrial devices in the near future [242, 262]. Whether future efforts in PSi technology will focus on biosensors or on more promising fields remains to be seen, as economic and financial factors will determine the future industrial initiatives.

CHAPTER 8

CONCLUSIONS AND PERSPECTIVES

Thesis conclusions

In this thesis, we investigated the design and the fabrication of a single-use biosensor based on porous silicon membranes, for the indirect detection of bacteria via their lysate. Four key aspects have been investigated:

1. The bacterial lysate morphology and its impact on the porous silicon transducer's design and performance (Chapter 4).
2. The added value of PSi membranes compared to PSi layers for the indirect detection of bacteria via their lysate (Chapter 5).
3. The chemical stabilisation of PSi membranes with atomic layer deposition for their use in aqueous media and its impact on the biosensors' sensitivity (Chapter 6).
4. The versatility of the bioassay, via the detection of both *B. cereus*, targeted by the phage-coded PlyB221 endolysins, and *S. epidermidis*, lysed by the commercial lysostaphin (Chapters 5 and 6).

Each studied aspect led to innovations, answering known challenges in the field of biosensing and porous silicon technologies. The main contributions are listed below:

- Avoiding functionalisation and complex sample pre-treatments by

using lytic enzymes for the selectivity of the biosensor; this method has already proven its worth on capacitive CMOS sensors [27].

- Understanding and optimising porous silicon optical transducers by studying the effect of the fabrication parameters on the PSi properties, modelling the filtration of analyte through the porous membranes and reproducing the optical response of PSi sensors via simulations; a simple analytical model of the theoretical limit of detection qualifies the impact of key system parameters on the sensitivity of the detection platform.
- Fabricating PSi membranes via a robust and repeatable full wafer process, combining electrochemical etching and standard micro-fabrication techniques; the fabrication process is developed such that the addition of porous silicon does not hinder later fabrication steps.
- Passivating PSi membranes using atomic layer deposition to enable their use in aqueous media; the developed deposition technique enables a homogeneous deposition of metal oxides throughout the full membrane depth and the resulting porous structure not only exhibits an improved chemical stability, but also enhanced optical properties.
- Detecting bacteria via our novel detection scheme, reaching a limit of detection of 10^3 CFU/mL for a total assay time of 1 h 30 min; our indirect detection method relies on a rapid and simple pre-treatment of the analyte with selective lytic enzymes, during which target bacteria are destroyed. The target of the detection can easily be adapted by changing the lytic enzyme, as is demonstrated by the detection of both *B. cereus* using the PlyB221 endolysins and *S. epidermidis* using lysostaphin. The bacterial detection is also possible in complex media, as illustrated by the detection of *B. cereus* in human serum.

These findings illustrate some of the steps that need to be taken when developing a sensing platform, here with the specific application of bacterial detection. Biosensing being a multidisciplinary subject, the work involved in this research went from material characterisation, to fluid mechanics simulations, optical modeling, standard microfabrication and biological samples preparation. The outcome of this research is a stable and permanent, versatile platform that can still deliver more promising

achievements. We hope that our small contribution to the field of porous silicon biosensors will drive the field to new possibilities and challenges.

The sensitivity of the detection platform can still reach better performances. The first challenge for our biosensor intrinsically lies within its application: the lack of optical contrast between bacterial lysate and buffer solutions. Especially for low bacterial concentrations, the change in refractive index is very small. This means only ultra-sensitive optical transducers can detect a single bacterial cell. To further improve the sensitivity of our sensor, the intensity of the optical response needs to be addressed. Indeed, the incident light tends to get transmitted rather than reflected. The ALD coatings have contributed to the reflectivity and increased the signal intensity, but further research efforts are needed to improve the reduction in noise levels even further. The measurement set-up must also be optimised, opting for a higher resolution optical instrumentation, as well as a fluidic integration that induces less optical losses.

A second aspect that is raising more and more interest in the porous silicon community is the mechanical stability of these layers. Porous silicon membranes could greatly benefit from such studies because of their sensitivity to pressure variations, which can impact the optical output. Moreover, the tendency to crack nucleation and propagation in these substrates needs to be further investigated to ensure the robustness and reliability of PSiMs based biosensors. In this research, the mechanical stability challenge has been the target of many reflections and some suggestions based on design optimisations are formulated afterwards.

Finally, our current detection protocol requires comparing our detection to a control test, using the analyte without pre-treatment. An alternative detection protocol would be to flow the analyte through the PSi membrane, hence accumulating the bacteria on top of it, then adding the lytic enzymes and measuring the optical response. The effect of this adapted protocol on the response time and the sensitivity has been quickly investigated in Appendix E.3. The response time was increased by 1 h 30 min, totalling to 3 h including the PBS pre-wetting. The sensitivity was also decreased, which might be linked to the unideal conditions for the lysis on chip. Another protocol would consist in carrying both detections out in parallel. By using two sensors, two detections can be carried in parallel and a control group can be easily established, but the deviation between sensors should be taken into account. An alterna-

tive protocol that might also be investigated is the sequential detection of the control and lysate test on a single sensor, with a PBS rinse in between both assays: the response time would be increased, but both tests would be performed on the same sensor.

In conclusion, we have demonstrated the added value of porous silicon membranes for biosensing. We have chosen bacterial detection as our target application, but this sensor is not only limited to this field and might present an even better performance when applied to other types of analytes. We also illustrated the use of lytic enzymes as bioelement in biosensors, which enables the fabrication of versatile and multi-target detection platforms.

Perspectives

Based on the improvement suggestions discussed above, some paths can be followed to enhance the presented bacterial detection platform. With these developments, it might be possible to decrease the limit of detection by one or two orders of magnitude.

- *Design optimisation:* as mentioned earlier, the mechanical aspect of the porous membrane impacts the sensitivity. By adapting the membrane design and, for example, adding supporting structures beneath the membrane, more robust sensors could be built. Thinner membranes could hence be fabricated, allowing increased flow speeds. Lateral PSi membranes [208, 209, 219, 316] may also prove to be a viable solution, facilitating any possible further on-chip integration.
- *Porosification:* while most publications use ethanol as wetting agent for the electrolyte used during porosification, it has been observed that by switching to 1-butanol instead, larger pores could be obtained without reaching the electropolishing range. These pores are more stable as well [330, 331]. By adapting the electrolyte for the porosification, the pore size of the sensing layer could be increased, without impacting the mechanical stability of the PSi membrane. This increase in pore size would enable more bacterial lysate to penetrate the sensing layer and hence increase the sensitivity.
- *Fluidic integration:* Arshavsky-Graham *et al.* recently demon-

strated that the integration of a PSi biosensor into a microchannel setup, instead of using a re-usable fluidic cell similar to the one used in this work, increases the sensitivity of the biosensor [246]. While a PDMS microchannel fluidic integration was briefly tested out in the course of this research, the high pressure needed to push the analyte through the membrane requires more robust fluidic integration techniques, such as anodic glass bonding, for example. The advantage of microelectronic bonding techniques is that they can once again easily be upscaled to industrial levels. A microfluidic integration would also enable the detection of turbid matrices. Moreover, with a microfluidic integration enabling both a flow-through and a flow-over operation, it may be possible to quickly rinse away the debris accumulated on the top of the PSi membrane and hence permit the use of the same sensor for both the control and lysate detection assay.

- *Capture probe*: the inner surface of the sensor could be modified with simple non-specific capture probes, in order to increase the amount of bound bacterial lysate. A possible candidate is dopamine, which was reported to efficiently bind biomolecules [332]. Dopamine can be deposited by immersing the transducer in dopamine hydrochloride [27, 333].
- *Lytic agent blending*: by combining different lytic agents, the assay time and/or the detection limit may be reduced. Several studies have indeed observed that endolysins that target different bonds in the peptidoglycan often cooperate against the same target bacteria when combined [334–336].
- *Multiplexing*: by combining multiple PSi membranes on a single chip, the multiplexing of measurements could be made possible. Either to perform the control test and the detection at the same time, or to test for different targets, the multiplexing either requires multiple optical setups, or a programmable moving stage. Indeed, as the optical spectra of the PSi sensors does not need to be recorded every second, a movable stage could be used to move each PSi membrane underneath the light spot for the recording of its optical spectra, at given intervals of time. Such a setup could be implemented with the aid of a precise movement interface and an adapted data collection software.

Other outlooks

PSi membranes have proven their added value compared to PSi layers for the indirect detection of bacteria via their lysate. When compared with the literature, the obtained limit of detection is similar to PSi biosensors that use complex analyte-binding routines. Here, by improving design features and using lytic enzymes, we have developed a scalable, clean-room compatible and versatile device. The current method can however still be improved to decrease the limit of detection and response time.

This does not mean that the research on PSiM-based bacterial biosensors must stop here. While we listed many routes for the optimisation of the presented sensor, we also believe that PSiMs have many other areas of applications, as we briefly illustrated in Section 3.2.3. The added value of PSiMs may, for example, be more highlighted in the detection of smaller biomolecules [10, 228, 234] or even in gas sensing applications like the detection of air contaminants [134, 194].

The detection protocol, which is based on the selective lysis of target bacteria, is also a scheme that is worth researching to a further extent. The lysis might be induced by lytic enzymes, endolysins or even bacteriophages. The combination of the detection protocol with another transducer has already shown its promise [27]. Most biosensors in the literature focus on the detection of a very specific target, but this is not always the desired application by the industry. Some industries would benefit from a rapid sterility test, that would screen for the presence of any type of pathogens in just one test, regardless of the bacterial strain. The use of lytic enzyme mixes enables this type of applications.

In short, the combination of selective lytic agents and optical sensors based on PSi-membranes shows encouraging results. Both technologies, either combined or separated, are worthy of further research efforts and promise to deliver more achievements and challenges.

APPENDIX A

MEMBRANE FILTRATION: CONCEPTS

In the membrane filtration modelling, two Comsol Multiphysics interfaces are combined: a single face creeping flow and the particles tracing interface.

A.1 Theoretical concepts

A.1.1 The creeping flow

To study the flow of bacterial lysate through the membrane, we have modelled it as a single phase flow of PBS containing dispersed particles. The following properties were used in the model

The PBS flow follows the Naviers-Stokes equations, which is a set of three equations that respectively represent the conservation of mass, of momentum and of energy:

Property	PBS	Particles
Density ρ [kg/m ³]	1060	2000
Viscosity μ [Pa · s]	9.04E ⁻⁴	/

Table A.1: Properties of the PBS flow and the particles.

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \mathbf{u}) = 0 \quad (\text{A.1})$$

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho (\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot [-p \mathbf{I} + \boldsymbol{\tau}] + \mathbf{F} \quad (\text{A.2})$$

$$\rho C_p \left(\frac{\partial T}{\partial t} + (\mathbf{u} \cdot \nabla) T \right) = -(\nabla \cdot \mathbf{q}) + \boldsymbol{\tau} : \mathbf{S} - \frac{T}{\rho} \frac{\partial \rho}{\partial T} \bigg|_p \left(\frac{\partial p}{\partial t} + (\mathbf{u} \cdot \nabla) p \right) + Q \quad (\text{A.3})$$

where

- ρ is the density (SI unit: kg/m^3)
- $\mathbf{u}$ is the velocity vector (SI unit: m/s)
- p is the pressure (SI unit: Pa)
- $\boldsymbol{\tau}$ is the viscous stress tensor (SI unit: Pa)
- $\mathbf{F}$ is the volume force vector (SI unit: N/m^3)
- C_p is the specific heat capacity at constant pressure (SI unit: $\text{J}/(\text{kg} \cdot \text{K})$)
- T is the absolute temperature (SI unit: K)
- $\mathbf{q}$ is the heat flux vector (SI unit: W/m^2)
- Q contains the heat sources (SI unit: W/m^3)
- $\mathbf{S}$ is the strain-rate tensor: $\mathbf{S} = \frac{1}{2} (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)$

We assumed a Newtonian and incompressible flow. For a Newtonian flow, there is a linear relationship between stress and strain, dependent on the viscosity μ (SI units: $\text{Pa} \cdot \text{s}$):

$$\boldsymbol{\tau} = 2\mu \mathbf{S} - \frac{2}{3}\mu (\nabla \cdot \mathbf{u}) \mathbf{I} \quad (\text{A.4})$$

For an incompressible flow, ρ is constant or nearly constant, which reduces Equations A.1 and A.2 to

$$\rho \nabla \cdot \mathbf{u} = 0 \quad (\text{A.5})$$

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho(\mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot [-p\mathbf{I} + (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)\mathbf{I}] + \mathbf{F} \quad (\text{A.6})$$

A fundamental number in fluid mechanics is the Reynolds number, which is expressed in terms of the velocity scale U and the representative length L :

$$\text{Re} = \frac{\rho U L}{\mu} \quad (\text{A.7})$$

In the case of this simulation, U is in the $\mu\text{m/s}$ range and L is expressed in nm, the Reynolds number is very low. This means that we can simulate the flow using a creeping flow, also called Stokes flow, for which the inertial terms are neglected and Equation A.1.1 becomes.

$$\rho \frac{\partial \mathbf{u}}{\partial t} = \nabla \cdot [-p\mathbf{I} + (\nabla \mathbf{u} + (\nabla \mathbf{u})^T)\mathbf{I}] + \mathbf{F} \quad (\text{A.8})$$

Inlet conditions An inlet of fluid requires velocity components. An alternative to prescribing the complete velocity field is to define the pressure and all but one velocity component. In this simulation, the flow was set as normal to the inlet boundary. The pressure boundary condition is described as:

$$\begin{aligned} \mathbf{n}^\top [-p\mathbf{I} + \mu (\nabla \mathbf{u} + (\nabla \mathbf{u})^\top)] \mathbf{n} &= -\hat{p}_0 \\ \hat{p}_0 &\geq p_0 \end{aligned} \quad (\text{A.9})$$

in which p_0 is the prescribed pressure. The $\hat{p}_0 \geq p_0$ condition translates the "Suppress Backflow" option. This condition means that the prescribed pressure is p_0 if $\mathbf{u} \cdot \mathbf{n} \geq 0$, but bigger where $\mathbf{u} \cdot \mathbf{n} < 0$.

A no-tangential-stress condition must also be defined:

$$\mathbf{u} \cdot \mathbf{t} = 0 \quad (\text{A.10})$$

Outlet conditions The outlet condition is also pressure driven. Similarly to the inlet conditions, the pressure p_0 is prescribed using the following equations:

$$\begin{aligned} [-p\mathbf{I} + \mu (\nabla \mathbf{u} + (\nabla \mathbf{u})^\top)] \mathbf{n} &= -\hat{p}_0 \mathbf{n} \\ \hat{p}_0 &\leq p_0 \end{aligned} \quad (\text{A.11})$$

Boundary conditions When the "No Slip" condition is selected for a boundary, it is assumed that the fluid velocity tends to zero at the wall interface:

$$\mathbf{u} = 0 \quad (\text{A.12})$$

A.1.2 Particle tracing in fluid flow

In order to trace the motion of a particle in a fluid flow, Newton's second law is applied:

$$\frac{d(m_p \mathbf{v})}{dt} = F_t \quad (\text{A.13})$$

in which m_p is the particle's mass, $\mathbf{v}$ is its velocity and F_t is the sum of all forces acting on the particle. In a fluid, these forces can be drag forces, buoyancy forces or gravity forces. To model this motion, several assumptions were made:

- The flow is one-way coupled, meaning that the flow impacts the particles, but there is no reverse effect.
- The flow is undisturbed, meaning that the pressure and shear stress in the flow is undisturbed by the presence of particles.

Drag force

$$\mathbf{F}_D = \left(\frac{1}{\tau_p} \right) m_p (\mathbf{u} - \mathbf{v}) \quad (\text{A.14})$$

where

- m_p is the particle mass (SI unit: kg)
- τ_p is the particle velocity response time (SI unit: s)
- v is the velocity of the particle (SI unit: m/s)
- $\mathbf{u}$ is the fluid velocity (SI unit: m/s)
- $\mathbf{F}_g$ is the gravitational force vector (SI unit: N)
- $\mathbf{F}_{\text{ext}}$ is any other external force (SI unit: N).

For creeping flow, the Stokes drag is applied, which defines the response time of spherical particles such as:

$$\tau_p = \frac{\rho_p d_p^2}{18\mu} \quad (\text{A.15})$$

where μ is the fluid viscosity (SI unit: $\text{Pa} \cdot \text{s}$), ρ_p is the particle density (SI unit: kg/m^3), and d_p is the particle diameter (SI unit: m). This is frequently known as Stokes drag law. The Stokes drag law is applicable for creeping flows, $\text{Re}_r \geq 1$.

Gravity force The gravity force is given by:

$$\mathbf{F}_g = m_p \mathbf{g} \frac{(\rho_p - \rho)}{\rho_p} \quad (\text{A.16})$$

where ρ is the density of the surrounding fluid and $\mathbf{g}$ is the gravity vector.

APPENDIX B

TRANSFER MATRIX

In this section, we present the mathematical study and design of the multi-layered porous structure that composes our biosensor. Several methods enable the simulation of this type of structures, the most used ones being the transfer matrix and scattering matrix methods [?, 337–340]. In these methods, the device is presented as a stack of layers of infinite dimensions in the transverse plane. Typically, the x and y directions represent the transverse plane, while the z axis is the longitudinal direction, in which the waves propagate. While the transfer matrix method is faster, the scattering matrix method is not only more memory efficient, it is also the most direct and clear way to describe wave propagation in a multi-layered structure as the parameters gave physical meaning [341].

Indeed, the wave propagation in each layer can be characterised by a *scattering matrix* $\mathbf{S}$, whose parameters as well as their physical interpretation are illustrated in Figure B.1. The $\mathbf{S}_{11}$ and $\mathbf{S}_{22}$ parameters correspond to the reflection of the wave from Port 1 and Port 2 respectively. The $\mathbf{S}_{12}$ and $\mathbf{S}_{21}$ represent the transmission from one port to the other. The global scattering matrix for a device can be expressed in terms of the mode coefficients of the incident, reflected and transmitted

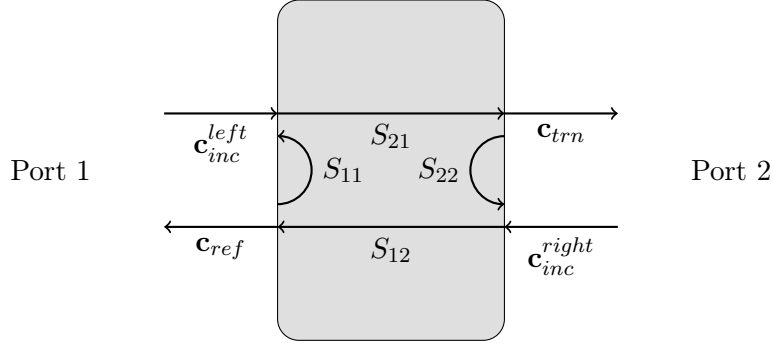


Figure B.1: Conventional definition of the scattering parameters.

waves, such that

$$\begin{bmatrix} \mathbf{c}_{ref} \\ \mathbf{c}_{trn} \end{bmatrix} = \begin{bmatrix} \mathbf{S}_{11}^{(global)} & \mathbf{S}_{12}^{(global)} \\ \mathbf{S}_{21}^{(global)} & \mathbf{S}_{22}^{(global)} \end{bmatrix} \begin{bmatrix} \mathbf{c}_{inc}^{left} \\ \mathbf{c}_{inc}^{right} \end{bmatrix}. \quad (\text{B.1})$$

Assuming that there is no wave incident from the transmission region, $\mathbf{c}_{inc}^{right} = 0$, and the expression can be rewritten as

$$\mathbf{c}_{ref} = \mathbf{S}_{11}^{(global)} \mathbf{c}_{inc} \quad (\text{B.2})$$

$$\mathbf{c}_{trn} = \mathbf{S}_{21}^{(global)} \mathbf{c}_{inc} \quad (\text{B.3})$$

To correlate the scattering matrix method to the computation of the transmitted and reflected fields, one must start with the normalised Maxwell's equation, in which we eliminate the longitudinal field components E_z and $\tilde{H}_z$ by substitution. The four remaining equations are discretised in the x - y plane and take the following $\mathbf{PQ}$ matrix form:

$$\frac{d}{dz'} \begin{bmatrix} E_x \\ E_y \end{bmatrix} = \mathbf{P} \begin{bmatrix} \tilde{H}_x \\ \tilde{H}_y \end{bmatrix} \quad (\text{B.4})$$

$$\frac{d}{dz'} \begin{bmatrix} \tilde{H}_x \\ \tilde{H}_y \end{bmatrix} = \mathbf{Q} \begin{bmatrix} E_x \\ E_y \end{bmatrix} \quad (\text{B.5})$$

in which $\mathbf{P}$ and $\mathbf{Q}$ depend on the effective permeability μ_r and effective permittivity ϵ_r , both derived from the effective refractive index, as well

as on the normalised wave vectors which are characterised by the $\tilde{k}$ terms, such that

$$\mathbf{P} = \frac{1}{\epsilon_r} \begin{bmatrix} \tilde{k}_x \tilde{k}_y & \mu_r \epsilon_r - \tilde{k}_x^2 \\ \tilde{k}_y^2 - \mu_r \epsilon_r & -k_x \tilde{k}_y \end{bmatrix} \quad (\text{B.6})$$

$$\mathbf{Q} = \frac{1}{\mu_r} \begin{bmatrix} \tilde{k}_x \tilde{k}_y & \mu_r \epsilon_r - \tilde{k}_x^2 \\ \tilde{k}_y^2 - \mu_r \epsilon_r & -k_x \tilde{k}_y \end{bmatrix} \quad (\text{B.7})$$

The solutions for the magnetic and electric fields from $\mathbf{P}$ and $\mathbf{Q}$ become

$$\begin{bmatrix} E_x(z') \\ E_y(z') \end{bmatrix} = \mathbf{W} e^{-\lambda z'} \mathbf{c}^+ + \mathbf{W} e^{\lambda z'} \mathbf{c}^- \quad (\text{B.8})$$

$$\begin{bmatrix} \tilde{H}_x(z') \\ \tilde{H}_y(z') \end{bmatrix} = \mathbf{V} e^{\lambda z'} \mathbf{c}^+ - \mathbf{V} e^{-\lambda z'} \mathbf{c}^- \quad (\text{B.9})$$

where $\mathbf{c}^+$ and $\mathbf{c}^-$ are the amplitude coefficients of the forward and backward wave respectively, $\mathbf{W} = \mathbf{I}$ is the eigen-vector matrix, which is equal to the identity matrix, λ is the diagonal eigen-value matrix and $\mathbf{V} = \mathbf{QW}\lambda^{-1}$.

The combined solution describes the total field inside one layer of the device and is written as

$$\Psi(z') = \begin{bmatrix} E_x(z') \\ E_y(z') \\ \tilde{H}_x(z') \\ \tilde{H}_y(z') \end{bmatrix} = \begin{bmatrix} \mathbf{W} & \mathbf{W} \\ \mathbf{V} & -\mathbf{V} \end{bmatrix} \begin{bmatrix} e^{\lambda z'} & 0 \\ 0 & e^{-\lambda z'} \end{bmatrix} \begin{bmatrix} \mathbf{c}^+ \\ \mathbf{c}^- \end{bmatrix} \quad (\text{B.10})$$

Based on this equation, the scattering parameters for a layer i of thickness L_i such as illustrated in Figure B.1 can be formulated by defining the boundary condition at each interface, which are

$$\begin{bmatrix} \mathbf{W}_1 & \mathbf{W}_1 \\ \mathbf{V}_1 & -\mathbf{V}_1 \end{bmatrix} \begin{bmatrix} \mathbf{c}_1^+ \\ \mathbf{c}_1^- \end{bmatrix} = \begin{bmatrix} \mathbf{W}_i & \mathbf{W}_i \\ \mathbf{V}_i & -\mathbf{V}_i \end{bmatrix} \begin{bmatrix} \mathbf{c}_i^+ \\ \mathbf{c}_i^- \end{bmatrix} \quad (\text{B.11})$$

$$\begin{bmatrix} \mathbf{W}_i & \mathbf{W}_i \\ \mathbf{V}_i & -\mathbf{V}_i \end{bmatrix} \begin{bmatrix} e^{\lambda k_0 L_i} & 0 \\ 0 & e^{-\lambda k_0 L_i} \end{bmatrix} \begin{bmatrix} \mathbf{c}_i^+ \\ \mathbf{c}_i^- \end{bmatrix} = \begin{bmatrix} \mathbf{W}_2 & \mathbf{W}_2 \\ \mathbf{V}_2 & -\mathbf{V}_2 \end{bmatrix} \begin{bmatrix} \mathbf{c}_2^+ \\ \mathbf{c}_2^- \end{bmatrix} \quad (\text{B.12})$$

For computational reasons, Port 1 and Port 2 are considered as gaps composed of free space, of zero thickness. This enables to separate the

effect of the materials on either side of the layer, with no effect on the performance of the device. The scattering matrices of each layer can therefore be arbitrarily interchanged. We can therefore rewrite the eigen-modes $\mathbf{W}_1 = \mathbf{W}_2 = \mathbf{W}_0$ and $\mathbf{V}_1 = \mathbf{V}_2 = \mathbf{V}_0$. The parameters of the scattering matrix for layer i are expressed as

$$\mathbf{S}_{11}^{(i)} = \mathbf{S}_{22}^{(i)} = (\mathbf{A}_i - \mathbf{X}_i \mathbf{B}_i \mathbf{A}_i^{-1} \mathbf{X}_i \mathbf{B}_i)^{-1} (\mathbf{X}_i \mathbf{B}_i \mathbf{A}_i^{-1} \mathbf{X}_i \mathbf{A}_i - \mathbf{B}_i) \quad (\text{B.13})$$

$$\mathbf{S}_{12}^{(i)} = \mathbf{S}_{21}^{(i)} = (\mathbf{A}_i - \mathbf{X}_i \mathbf{B}_i \mathbf{A}_i^{-1} \mathbf{X}_i \mathbf{B}_i)^{-1} \mathbf{X}_i (\mathbf{A}_i - \mathbf{B}_i \mathbf{A}_i^{-1} \mathbf{B}_i) \quad (\text{B.14})$$

$$\mathbf{A}_i = \mathbf{W}_i^{-1} \mathbf{W}_0 + \mathbf{V}_i^{-1} \mathbf{V}_0 \quad (\text{B.15})$$

$$\mathbf{B}_i = \mathbf{W}_i^{-1} \mathbf{W}_0 - \mathbf{V}_i^{-1} \mathbf{V}_0 \quad (\text{B.16})$$

$$\mathbf{X}_i = e^{-\lambda_i k_0 L - i} \quad (\text{B.17})$$

In case of a multi-layered device, the combined scattering matrix can be computed using the Redheffer star product for each layer [341]. This combined scattering matrix can then be connected to the reflection region and transmission region, via scattering matrices $\mathbf{S}^{(ref)}$ and $\mathbf{S}^{(trn)}$ respectively, resulting in the global scattering matrix of the device

$$\mathbf{S}^{(global)} = \mathbf{S}^{(ref)} \otimes [\mathbf{S}^{(1)} \otimes \mathbf{S}^{(2)} \otimes \dots \otimes \mathbf{S}^{(N)}] \otimes \mathbf{S}^{(trn)} \quad (\text{B.18})$$

The scattering parameters for $\mathbf{S}^{(ref)}$ can be derived by fixing $L = 0$, $\mathbf{W}_1 = \mathbf{W}_i = \mathbf{W}_{ref}$, $\mathbf{V}_1 = \mathbf{V}_i = \mathbf{V}_{ref}$, $\mathbf{W}_2 = \mathbf{W}_0$ and $\mathbf{V}_2 = \mathbf{V}_0$. Likewise, the parameters for $\mathbf{S}^{(trn)}$ can be derived by letting $L = 0$, $\mathbf{W}_2 = \mathbf{W}_i = \mathbf{W}_{ref}$, $\mathbf{V}_2 = \mathbf{V}_i = \mathbf{V}_{ref}$, $\mathbf{W}_1 = \mathbf{W}_0$ and $\mathbf{V}_1 = \mathbf{V}_0$.

Now that we have expressed the global scattering matrix, we take expressions B.1 and B.2 again and derive the reflected and transmitted fields.

$$\begin{bmatrix} E_x^{ref} \\ E_y^{ref} \end{bmatrix} = \mathbf{W}_{ref} \mathbf{c}_{ref} = \mathbf{W}_{ref} \mathbf{S}_{11}^{(global)} \mathbf{c}_{inc} \quad (\text{B.19})$$

$$\begin{bmatrix} E_x^{trn} \\ E_y^{trn} \end{bmatrix} = \mathbf{W}_{trn} \mathbf{c}_{trn} = \mathbf{W}_{trn} \mathbf{S}_{21}^{(global)} \mathbf{c}_{inc} \quad (\text{B.20})$$

$$(\text{B.21})$$

In linear, homogeneous and isotropic media, the mode coefficient can be

written as

$$\mathbf{c}_{inc} = \begin{bmatrix} E_x^{inc} \\ E_y^{inc} \end{bmatrix} \quad (\text{B.22})$$

We then solve the longitudinal field components E_z^{ref} and E_z^{trn} in order to calculate the total reflectance of the wave

$$E_z^{ref} = -\frac{\tilde{k}_x E_x^{ref} \tilde{k}_y E_y^{ref}}{\tilde{k}_z} \quad (\text{B.23})$$

$$E_z^{trn} = -\frac{\tilde{k}_x E_x^{trn} \tilde{k}_y E_y^{trn}}{\tilde{k}_z} \quad (\text{B.24})$$

$$R = \left| \vec{E}_{ref} \right|^2 \quad (\text{B.25})$$

APPENDIX C

MATLAB CODES

C.1 MATLAB image processing for pore morphology analysis

The following MATLAB code corresponds to the routine used for the Transfer Matrix Simulation of the optical response of a PSi membrane. This simulation was performed to select the fabrication parameters that result in the best optical signal. The principal code, `tmm_double_layer` was adapted in order to simulate the response using different input porosity or refractive indices for a double layer. It was also adapted to simulate a single layer or a triple layer, but as those codes are very similar, they will not be added here. The `tmm_double_layer` script calls to two other functions: `tmm1D` which performs the TMM calculation ¹ and `FFT_REF`, which applies a Fourier transform to the optical spectrum, similarly to the RIFTS method.

C.1.1 `tmm_double_layer.m`

```
1 %% TRANSFER MATRIX METHODS using SCATTERING MATRICES %%  
2 % by R. Vercauteren  
3
```

¹based on the code found here <https://github.com/cm403/cem/blob/master/tmm1d.m>

```

4 close all;
5 clear all;
6
7
8 % UNITS
9 degrees = pi/180;
10
11 %-----
12 %% DEFINE SIMULATION PARAMETERS
13 %-----
14
15 % SOURCE PARAMETERS
16 lam=(400:2:900)*1e-9;%free space wavelength
17 k0=(2*pi)./lam;
18 SRC.theta = 0 * degrees; %elevation angle
19 SRC.phi = 0 * degrees; %azimuthal angle
20 SRC.pte = 1/sqrt(2); %amplitude of TE polarization
21 SRC.ptm = 1i*SRC.pte; %amplitude of TM polarization
22
23 % EXTERNAL MATERIALS
24 DEV.ur1 = 1.0; % permeability in the reflection region
25 DEV.er1 = 1.0; % permittivity in the reflection region
26 DEV.ur2 = 1.0; % permeability in the transmission region
27 DEV.er2 = 1.0; % permittivity in the transmission region
28
29
30 %% LOOP THROUGH THE POROSITY (OR LENGTH)
31
32 L1=5; %Thickness of first layer
33 L2=100; % Thickness of second layer
34 p=[20, 40, 60 ,80]; % porosity range
35 Lp = length(p);
36 sensitivity = zeros(1,Lp);
37 int=zeros(5, Lp);
38 noise=zeros(5, Lp);
39
40 for i=1:Lp
41 %% LOOP THROUGH refractive index
42 por=p(i); %porosity layer 1
43 nfill=1:0.1:1.4; %refractive index of filling media
44 n1=zeros(1,length(nfill));
45 n_s=3.1; % refractive index of skeleton
46 por2=0.656; %porosity layer 2
47 n2=zeros(1,length(nfill));
48 %Bruggeman effective meadium approximation
49 % for r=1:length(nfill)
50 %     n1(r)=1/2*sqrt(2*n_s^2-nfill(r)^2-3*por*n_s^2
51 % +3*por*nfill(r)^2+sqrt(8*n_s^2*nfill(r)^2+(nfill(r)^2
52 % -2*n_s^2+3*por*n_s^2-3*por*nfill(r)^2)^2));

```

```

53 %      n2(r)=1/2*sqrt(2*n_s^2-nfill(r)^2-3*por2*n_s^2
54 %+3*por2*nfill(r)^2+sqrt(8*n_s^2*nfill(r)^2+(nfill(r)^2
55 %-2*n_s^2+3*por2*n_s^2-3*por2*nfill(r)^2)^2));
56 % end
57
58 %Looyenga effective meadium approximation
59 for r=1:length(nfill)
60     n1(r)=((1-por)*n_s^(2/3)+por*nfill(r)^(2/3))^(3/2);
61     n2(r)=((1-por2)*n_s^(2/3)+por2*nfill(r)^(2/3))^(3/2);
62 end
63
64 Ln1 = length(n1);
65 peakInt = zeros(1,Ln1);
66 peakPos = zeros(1,Ln1);
67
68 for m=1:Ln1
69
70 %% DEFINE LAYERS
71 DEV.n = [n1(m) n2(m)];% array of the refractive indices
72 % array of the thickness of each layer
73 DEV.L = [L1 L2];
74
75 %% LOOP THROUGH THE WAVELENGTH
76
77 for q=1:length(lam)
78     SRC.lam0 = lam(q);
79     DAT = tmm1d(DEV, SRC);
80     REF(q) = DAT.REF;
81     TRN(q)= DAT.TRN;
82 end
83
84 EOT=2*n1(m)*5e-6
85 % figure(1)
86 % plot(lam*1e9,REF,'r','linewidth',2)
87 % %axis([100e-9 900e-9 -0.05 1.05])
88 % xlabel('\lambda','fontsize',14)
89 % ylabel('T (in blue) or R (in red)','fontsize',14)
90 % title('Transmittance (T) and Reflectance(R)','fontsize',14)
91 % fh = figure(1);
92 % set(fh, 'color', 'white');
93
94 %% RIFTS
95 [Lv, FFT]=FFT_REF(lam, REF, 3);
96 %[pks,idx] = findpeaks(abs(FFTref(Iv))*2);
97
98 EOTcalc=2*DEV.n(1)*DEV.L(1);
99 insiderange=find(Lv > 0.95*EOTcalc & Lv < 1.05*EOTcalc);
100 Lvrage=Lv(insiderange);
101 [maxpeaks, idmax]=max(FFT(insiderange));

```

```

102 % peakInt(m) = maxpeaks;
103 % peakPos(m)= Lvrangle(idmax);
104 % noise(m,i)=std(FFT);
105 %
106 figure, hold on;
107 plot(Lv, abs(FFT/length(FFT))*2,'r','linewidth',2)
108 %xlim([0 120]);
109 xlabel('2nL','fontsize',14)
110 ylabel('Intensity','fontsize',14)
111 title(' ','fontsize',14)
112 end
113
114
115 peakPosrel=(peakPos-peakPos(1))/peakPos(1)*100;
116 [P S]=polyfit(n1, peakPosrel,1);
117 sensitivity(i)= P(1);
118 int(:,i)=peakInt;
119
120
121 end

```

C.1.2 tmm1D.m

```

1 function DAT = tmm1d(DEV, SRC)
2     % TMM1D One-Dimensional Transfer Matrix Method
3     %
4     % DAT = tmm1d(DEV,SRC);
5     %
6     % INPUT ARGUMENTS
7     % =====
8     % DEV Device Parameters
9     % .er1 relative permittivity in reflection region
10    % .ur1 relative permeability in reflection region
11    % .er2 relative permittivity in transmission region
12    % .ur2 relative permeability in transmission region
13    % .n array containing refractive indices of each layer
14    % .L array containing thickness of each layer
15    %
16    % SRC Source Parameters
17    % .lam0 free space wavelength
18    % .theta elevation angle of incidence (degrees)
19    % .phi azimuthal angle of incidence (degrees)
20    % .ate amplitude of TE polarisation
21    % .atm amplitude of TM polarisation
22    %
23    % OUTPUT ARGUMENTS
24    % =====
25    % DAT Output Data

```



```

26      % .REF Reflectance
27      % .TRN Transmittance
28      % .S11 GLOBAL (DEVICE)
29      % .S12 GLOBAL (DEVICE)
30      % .S21 GLOBAL (DEVICE)
31      % .S22 GLOBAL (DEVICE)
32      % This MATLAB program implements the transfer matrix ←
        method.

33
34      % UNITS
35      degrees = pi/180;
36      %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
37      %% DEFINE SIMULATION PARAMETERS
38      %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
39      % SOURCE PARAMETERS
40      lam0 = SRC.lam0; %free space wavelength
41      theta = SRC.theta * degrees; %elevation angle; convert ←
        from deg to rad
42      phi = SRC.phi * degrees; %azimuthal angle; convert from ←
        deg to rad
43      pte = SRC.pte; %amplitude of TE polarisation
44      ptm = SRC.ptm; %amplitude of TM polarisation
45      % EXTERNAL MATERIALS
46      url = DEV.url; %permeability in the reflection region
47      erl = DEV.erl; %permittivity in the reflection region
48      ur2 = DEV.ur2; %permeability in the transmission region
49      er2 = DEV.er2; %permittivity in the transmission region
50      % DEFINE LAYERS
51      n = DEV.n; %array of refractive indices in each layer
52      L = DEV.L; %array of the thickness of each layer
53
54      %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
55      %% IMPLEMENT TRANSFER MATRIX METHOD
56      %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
57      % DEFINE I AND O FOR MODES
58      I = eye(2);
59      O = zeros(2);
60      % LHI SIMPLE CASE
61      nincl = sqrt(erl * url);
62      kxt = sqrt(url*erl) * sin(theta) * cos(phi);
63      kyt = sqrt(url*erl) * sin(theta) * sin(phi);
64      QG = [ kxt * kyt    1 + kyt^2;
65            -(1+kxt^2)  -kxt * kyt;];
66      VG = -1i * QG;
67      k0 = 2 * pi / lam0;
68      % INITIALIZE S GLOBAL
69      S11G = O; S12G = I; S22G = O; S21G = I;
70      % CALCULATE # OF LAYERS FROM ER INPUT
71      N_layer = length(n);

```

```

72
73 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
74 %% LOOP THROUGH LAYERS
75 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
76
77 kzti = nan(size(n));
78 i = 1;
79 for i = 1:N_layer
80     % CALCULATE PARAMETERS FOR LAYER I
81     kzti(i) = sqrt(n(i)^2 - kxt^2 - kyt^2);
82     Qi = [kxt * kyt      n(i)^2 - kxt^2;
83           kyt^2 - n(i)^2  -kxt * kyt]; % ←
84                                     only valid for non magnetic ←
85                                     materials
86     OMEGAi = li * kzti(i) * eye(size(Qi));
87     Vi = Qi * inv(OMEGAi);
88     % CALCULATE SCATTERING MATRIX FOR LAYER I
89     Ai = eye(size(Vi)) + inv(Vi) * VG;
90     Bi = eye(size(Vi)) - inv(Vi) * VG;
91     Xi = expm(OMEGAi * k0 * L(i) ) ;
92     D = Ai - Xi * Bi * inv(Ai) * Xi * Bi;
93
94     S11 = inv(D) * (Xi * Bi * inv(Ai) * Xi * Ai - Bi);
95     S12 = inv(D) * Xi * (Ai - Bi * inv(Ai) * Bi);
96     S21 = S12;
97     S22 = S11;
98
99     % UPDATE GLOBAL SCATTERING MATRIX
100     DG = S12G * inv(I - S11 * S22G);
101     FG = S21 * inv(I - S22G * S11);
102
103     S11G = S11G + DG * S11 * S21G;
104     S12G = DG * S12;
105     S21G = FG * S21G;
106     S22G = S22 + FG * S22G * S12;
107
108 end
109
110 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
111 %% EXTERNAL REGIONS
112 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
113
114 % CALCULATE SCATTERING MATRIX FOR REFLECTION REGION
115 kztr = sqrt(erl * url - kxt^2 - kyt^2);
116 QR = 1 / url * [kxt * kyt,      url * erl - kxt^2;
117                 kyt^2 - url * erl,  -kxt * kyt];
118 OMEGAR = li * kztr * eye(size(QR));
119 VR = QR * inv(OMEGAR);
120 AR = eye(size(VR)) + inv(VG) * VR;

```

```

119     BR = eye(size(VR)) - inv(VG) * VR;
120
121     S11R = - inv(AR) * BR;
122     S12R = 2 * inv(AR);
123     S21R = 0.5 * ( AR - BR * inv(AR) * BR);
124     S22R = BR * inv(AR);
125     %%
126     % CALCULATE SCATTERING MATRIX FOR TRANSMISSION REGION
127     kztT = sqrt(er2 * ur2 - kxt^2 - kyt^2);
128     QT = 1 / ur2 * [kxt * kyt    ur2 * er2 - kxt^2;
129                    kyt^2 - ur2 * er2  -kxt * kyt];
130     OMEGAT = 1i * kztT * eye(size(QT));
131     VT = QT * inv(OMEGAT);
132     AT = eye(size(VT)) + inv(VG) * VT;
133     BT = eye(size(VT)) - inv(VG) * VT;
134
135     S11T = BT * inv(AT);
136     S12T = 0.5 * ( AT - BT * inv(AT) * BT);
137     S21T = 2 * inv(AT);
138     S22T = -inv(AT) * BT;
139
140     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
141     % CONNECT TO EXTERNAL REGIONS
142     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
143     % SG = SR (X) SG
144     DR = S12R * inv(I - S11G * S22R);
145     FR = S21G * inv(I - S22R * S11G);
146
147     S22G = S22G + FR * S22R * S12G;
148     S21G = FR * S21R;
149     S12G = DR * S12G;
150     S11G = S11R + DR * S11G * S21R;
151
152     % SG = SG (X) ST
153     DG = S12G * inv(I - S11T * S22G);
154     FG = S21T * inv(I - S22G * S11T);
155
156     S11G = S11G + DG * S11T * S21G;
157     S12G = DG * S12T;
158     S21G = FG * S21G;
159     S22G = S22T + FG * S22G * S12T;
160
161     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
162     % SOLVE SCATTERING PROBLEM
163     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
164
165     n = [0; 0; 1;];
166     kinc = k0 * ninc1 * ...
167         [sin(theta) * cos(phi); sin(theta)*sin(phi); ←

```

```

        cos(theta)];
168
169     % Check if theta = 0
170     if theta == 0
171         ate = [0 1 0];
172     else
173         ate = cross(n, kinc) ./ norm(cross(n, kinc));
174     end
175     atm = cross(ate, kinc) ./ norm(cross(ate, kinc));
176
177     P = pte * ate + ptm * atm;
178
179     Esrc = [P(1); P(2)];
180     Eref = [S11G * Esrc; nan];
181     Etrn = [S21G * Esrc; nan];
182
183     Eref(3) = -(kxt * Eref(1) + kyt * Eref(2)) / kztR;
184     Etrn(3) = -(kxt * Etrn(1) + kyt * Etrn(2)) / kztT;
185
186     DAT.REF = norm(Eref).^2;
187     DAT.TRN = norm(Etrn).^2 * real(kztT * k0 / ur2) / ←
        real(kinc(3) / ur1);
188     DAT.Eref = Eref;
189     DAT.Etrn = Etrn * real(kztT * k0 / ur2) / real(kinc(3) ←
        / ur1);
190     DAT.S11 = S11G;
191     DAT.S12 = S12G;
192     DAT.S21 = S21G;
193     DAT.S22 = S22G;
194 end

```

C.1.3 FFT_REF.m

```

1 function [Lv, FFT]=FFT_REF(lam, REF, res)
2
3 freq=fliplr(1./lam);
4 REFflip=fliplr(REF);
5 L=length(REF);
6 freqsort=linspace(freq(1), freq(end), L);
7 Fs=1/(mean(diff(freqsort))); %sampling interval
8
9 REFfit=spline(freq, REFflip, freqsort);
10
11 lpad=L*2^res; % lenght of the padding with 0 (to increase ←
    the resolution of the FFT)
12 FFTref=fft(REFfit, lpad)/L;%/((2^res)*L); %normalized fft
13
14 Lv=0:Fs/lpad:FsWith2;

```

```

15 FFT=abs(FFTref(1:lpad/2+1));
16
17 end

```

C.2 MATLAB theoretical limit of detection

This code relates to the calculation of the theoretical limit of detection. It computes the shift in EOT by first determining the refractive index shift caused by the penetration of bacteria lysate.

C.2.1 TheoreticalLOD.m

```

1  % by R. Vercauteren
2
3  close all;
4  clear all;
5
6
7
8  %% Define the porous membrane
9  por=0.8; %porosity of the 1st layer
10 t1= 5e-6; %2.655e-6; %microns thickness of 1st layer
11 n_s=1.7; % refractive index of skeleton
12 L=1e-3; %1.3348e-3; %size of the membrane
13
14 voltot=pi*(L/2)^2*t1*por;%round
15 %voltot=L*L*t1*por; %square
16
17 %% Define the lysate
18 bact= 0.9e-18; %volume of B. subtilus according to
19 %https://bionumbers.hms.harvard.edu/bionumber.aspx?s=n&v=1&id=115203
20 %bact= 1.1e-18; %volume of E Coli
21 %https://bionumbers.hms.harvard.edu/bionumber.aspx?id=100004&ver=19
22
23 N=100000; %number of particle per cell
24 CFU=[1e0 1e1 1e2 1e3 1e4 1e5 1e6]; %CFU/ml
25
26 nlys=1.390;% Refractive index bacterial cytoplasm with ↔
    %proteins from doi: 10.1128/jb.160.3.1151-1157.1984
27 nPBS=1.3348;% Refractive index PBS from 1145; ↔
    %doi:10.3390/app9061145
28
29 fraction=0.5; % fraction of the particles inside the 1st layer
30 n=zeros(1,length(CFU));
31
32

```

```

33 %% Calculate EOT shift based on number of particles
34 for i=1:length(CFU)
35     R=normrnd(15,10,N,1);
36     volume=sum(4/3*pi*(abs(R)*1e-9).^3)*fraction*CFU(i);
37     vollys=volume/voltot;
38     volPBS=1-vollys;
39
40     nfill=(vollys*(nlys-1)+volPBS*(nPBS-1))+1; % ←
41     %Gladstone-Dale equation
42     %a=(vollys*(nlys^2-1)/(nlys^2+2)+volPBS*(nPBS^2-1)/(nPBS^2+2));
43     %nfill=sqrt((2*a+1)/(1-a)); %Lorentz-Lorenz equation
44
45     n(i)=((1-por)*n_s^(2/3)+por*nfill^(2/3))^(3/2); ←
46     %Looyenga effective meadium approximation
47 end
48 C=categorical({'10^4','10^5','10^6','10^7','10^8','10^9', ←
49     '10^{10}'});
50 EOT=2*n*t1;
51 EOTshift=(EOT-EOT(1))/EOT(1)*100
52 figure, hold on;
53 bar(C, EOTshift)
54 xlabel('Number of particles','fontsize',14)
55 ylabel('EOT shift [%]','fontsize',14)
56 %set(gca,'XScale','log')
57 set(gca,'YScale','log')
58
59 %% Calculate EOT shift based on volumetric fraction
60 volume=0.3*bact*fraction*CFU;
61 vollys=volume/voltot;
62 volPBS=1-vollys;
63
64 nfill=(vollys*(nlys-1)+volPBS*(nPBS-1))+1;
65 n=((1-por)*n_s^(2/3)+por*nfill^(2/3))^(3/2);
66
67 C=categorical({'1','10','10^2','10^3','10^4','10^5', ←
68     '10^6'});
69 EOT2=2*n*t1;
70 EOTshift2=(EOT2-EOT2(1))/EOT2(1)*100
71 figure, hold on;
72 bar(C, EOTshift)
73 xlabel('Number of particles','fontsize',14)
74 ylabel('EOT shift [%]','fontsize',14)
75 %set(gca,'XScale','log')
76 set(gca,'YScale','log')

```

APPENDIX D

CHARACTERISATION OF PLYB221

This section relates to results published by *Leprince et al.* in [263].

Endolysins are enzymes coded by bacteriophages that mediate bacterial lysis via the degradation of peptidoglycan. They accumulate inside the cytoplasm during bacteriophage replication and cooperate with another protein, the holin, in order to induce the cell lysis.

PlyB221 is an endolysin encoded by Deep-Blue, a phage targeting the *Bacillus cereus* group. This group is a cluster of closely related species, with the most commonly recognised members being *B. cereus sensu stricto* (s.s), *Bacillus thuringiensis*, *Bacillus anthracis*, *Bacillus weihenstephanensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, and *Bacillus cytotoxicus*.

The activity of PlyB221 was tested against several members of the *B. cereus* group, as well as some *Bacillus* spp. A broad range of pH, salt tolerance and temperature were also tested in order to establish if PlyB221 is suitable for food industry applications.

Lytic activity assessment The lytic activity was studied in a turbidity reduction experiment, using *B. cereus* ATTC 10987 as model strain. The PlyB221 was diluted from 100 to 3 µg/mL and incubated with bacteria resuspended in Tris-HCl buffer with pH fixed at 8. The assay was performed at 30 °C and the OD₅₉₅ was monitored for 30 min. Figure

D.1 shows that at the highest PlyB221 concentration, only 4 min of incubation are needed for a 50 % reduction of the OD₅₉₅, which reaches a plateau after 12 min. Reducing the concentration to 50 µg/mL does not overly affect the observed lytic activity. For 6 µg/mL of PlyB221, the 50 % reduction mark is reached after 50 min.

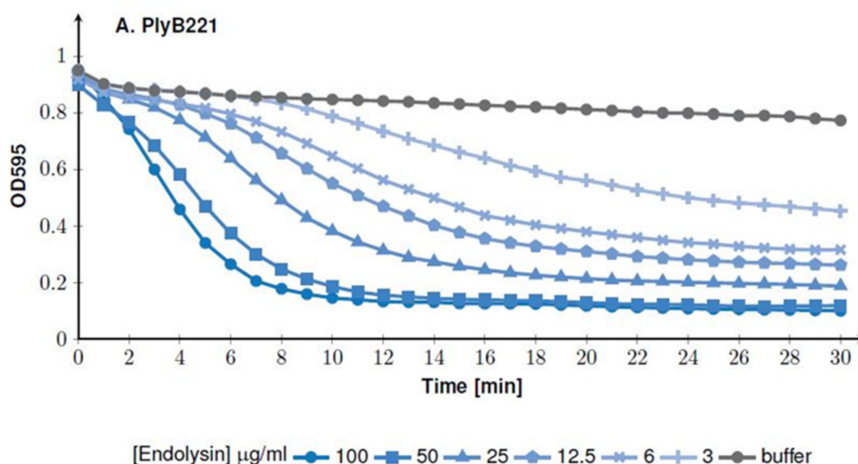


Figure D.1: Bacteriolytic activity of PlyB221 assessed by OD monitoring. The activity test was performed on exponentially growing *B. cereus* ATCC 10,987 at 30 °C in the optimal buffer and using enzyme concentrations ranging from 3 to 100 µg/mL. Reproduced from [263].

The lytic activity range was then tested using strains from the *B. cereus* group and other Gram-positive bacteria, via a turbidity reduction assay. The PlyB221 concentration was fixed at 100 µg/mL. In parallel to this test, the susceptibility of each strain to the Deep-Blue phage was also assessed via spot-on-plate. This assay resulted in 3 different bacterial responses: sensitive strains (i.e., formation of phage plaques), insensitive strains and those affected by the process of ‘lysis from without’ [342]. Results are gathered in Table D.1. All the strains tested within the *B. cereus* group are sensitive to PlyB221, but to different extents: the turbidity reduction ranges from 47 % to 76 %. Surprisingly, the Deep-Blue phage host spectrum was narrower, as three strains were insensitive to the phage. For other Gram-positive bacteria, PlyB221 is able to lyse *Bacillus licheniformis* and *Bacillus pumilus* in a reduced way, and *Bacillus megaterium* in a highly active way.

PlyB221 has an intermediate activity spectrum, as they are active against

Strain	Species	Φ	PlyB221	CBD
Strains of the <i>B. cereus</i> group				
VD021	<i>B. cereus</i>	S	74.9 ± 3.9	+
ATCC 10987	<i>B. cereus</i>	I	72.6 ± 2.3	+
F4810/72	<i>B. cereus</i> *	L	67.8 ± 10.7	+
H3081.97	<i>B. cereus</i> *	L	57.3 ± 5.9	+
WSBC10202	<i>B. weihenstephanensis</i>	S	61.1 ± 3.4	+
WSBC10204	<i>B. weihenstephanensis</i>	L	76.4 ± 9.1	+
Si0239	<i>B. weihenstephanensis</i> *	S	66.6 ± 4	+
LH002	<i>B. weihenstephanensis</i> *	I	69.3 ± 10.5	+
GBJ001	<i>B. thuringiensis</i>	L	76.2 ± 3	+
HD4	<i>B. thuringiensis</i>	L	38.1 ± 0.9	+
NVH 391-98	<i>B. cytotoxicus</i>	L	74.2 ± 6	+
BcA20 086	<i>B. cytotoxicus</i>	L	46.6 ± 11	+
KBS 2-12	<i>B. mycoides</i>	L	62.1 ± 2.7	+
NC2-18	<i>B. mycoides</i>	I	66 ± 23.3	+
Other Gram-positive bacteria				
Bs168	<i>B. subtilis</i>	I	0.2 ± 0.4	-
ATCC 10716	<i>B. licheniformis</i>	I	23.2 ± 1	-
SI0279	<i>B. pumilus</i>	I	6.8 ± 0.9	-
10A-B5	<i>B. pumilus</i>	I	28.3 ± 1.5	-
SI0003	<i>B. megaterium</i>	L	73.9 ± 2.7	+
10A-B3	<i>B. megaterium</i>	I	79.9 ± 0.5	+
Liv001	<i>L. ivanovii</i>	I	0.5 ± 0.9	-
ATCC 12228	<i>S. epidermidis</i>	I	0.1 ± 0.2	-

Table D.1: Activity and binding spectra of PlyB221 endolysins and their cell wall binding domain (CBD) compared to Deep-Blue phage host spectra. The phage host spectra were established via spot-on-plate. The lytic activity was assessed in triplicate by a turbidity reduction assay on exponentially growing cells using 100 $\mu\text{g}/\text{mL}$ of endolysin. The binding spectra were established in a cell wall binding assay using exponentially growing cells and the GFP fused CBD. Stars indicate emetic strains. Reproduced from [263].

Φ : phage host spectrum; S: Sensitive/plaque forming strain; I: Insensitive strain; L: Strain affected by “lysis from without”

members of the *B. cereus* group but also a select number of other Gram-positive bacteria. It could be assumed that PlyB221 is active against most, if not all, pathogenic strains of the *B. cereus* group, including *B. anthracis*; indeed, although this strain could not be tested, PlyB221 has close relationships to PlyP56 and PlyB, which have demonstrated their

activity against this strain [343,344].

Biochemical characterisation The variation of lytic activity with different pH values and NaCl concentrations was assessed via a turbidity reduction assay using *B. cereus* ATCC 10987 as model strain. PlyB221 was active in a wide pH range (5 to 9) but the highest activity was observed at pH 7 and 8 (Figure D.2, A). This is consistent with the predicted p.I, namely 7.67. In terms of salt concentration, the lytic activity decreased for concentration higher than 300 mM (Figure D.2, C). The thermal stability of PlyB221 was assessed in Figure D.2, E and it can be observed that the endolysins retain their activity up to 45 °C, with a 25 % decrease in activity at 50 °C, but a major loss in activity after 60 °C. Overall, PlyB221 remains active in a wide range of pH and NaCl conditions and exhibits a thermal stability that is consistent with other endolysins encoded by the phages targeting the *B. cereus* group [345,346].

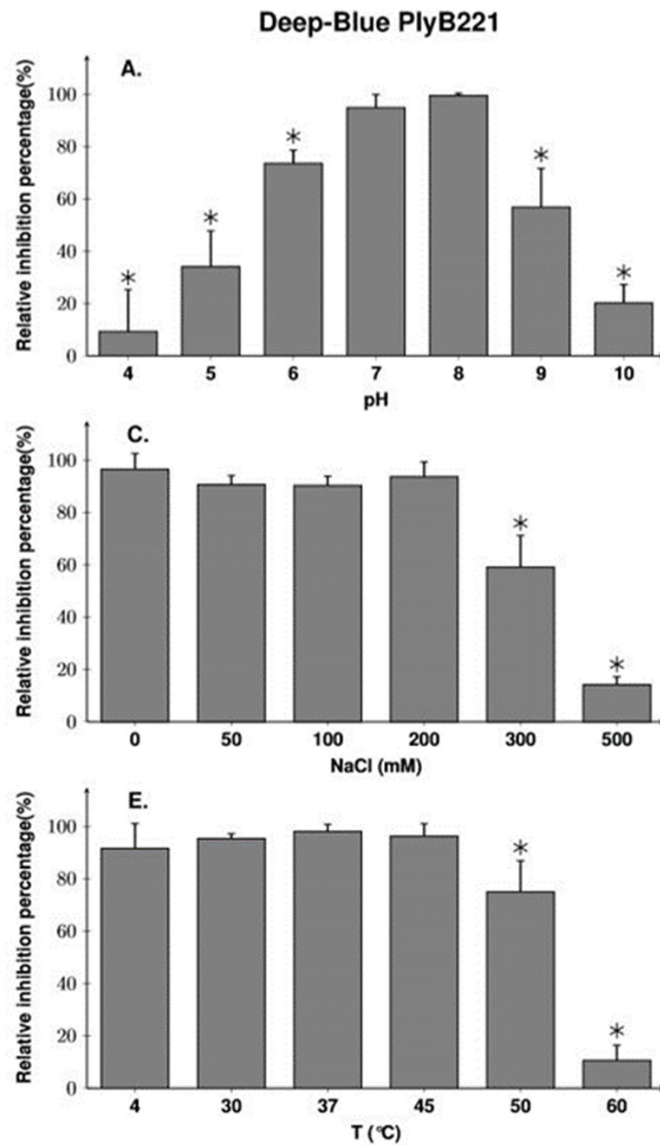


Figure D.2: Biochemical characterization of PlyB221. Exponentially growing *B. cereus* ATCC 10,987 cells were resuspended in buffer of various pH (A) and NaCl concentration (C) and challenged with 50 µg/mL of protein during 30 min to assess the endolysins optimal activity. PlyB221 stability to temperature (E) was tested by incubating the proteins (50 µg/mL) at given temperatures during 30 min and transferred for 5 min on ice. The experiments were done in triplicate. Stars indicate significant differences (Student's t-test, $p < 0.05$). Reproduced from [263].

APPENDIX E

STUDY OF ALD COATING INSIDE PSIMS

E.1 Chemical characterisation: XPS

This section is issued from [RV1].

In order to determine the chemical composition of the coatings inside the membrane, XPS measurements were performed. Both the front- and the backside of the membranes were analysed. It must however be pointed out that these ALD deposition were carried out at lower temperatures (100 °C for HfO₂ and Al₂O₃, and 150 °C for TiO₂).

E.1.1 Methods

X-Ray Photoelectron Spectroscopy (XPS) analyses were performed to determine the chemical composition of the ALD films deposited on the pore surface. Therefore, XPS measurements were carried out on the top and back sides of the PSi membranes with an SSX 100/206 photoelectron spectrometer from Surface Science Instruments (USA) equipped with a monochromatised micro focused Al X-ray source (powered at 20 mA and 10 kV). The chamber pressure was fixed at 10⁻⁶ Pa and the data was collected at a 55 ° angle. The spot size was approximately 300 µm and the pass energy was set at 150 eV. In these conditions, the full width measured at half maximum (FWHM) of the Au 4f_{7/2} peak for a clean gold standard sample was about 1.6 eV. A flood gun set at

8 eV and a Ni grid placed 3 mm above the sample surface were used for charge stabilisation. The C–(C_H) component of the C1s peak has been fixed to 284.8 eV to set the binding energy scale. Data treatment was performed with CasaXPS program (Casa Software Ltd, UK), some spectra were decomposed with the least squares fitting routine provided by the software with a Gaussian/Lorentzian (85/15) product function and after subtraction of a non-linear baseline. Molar fractions were calculated using peak areas normalised based on acquisition parameters and sensitivity factors provided by the manufacturer.

E.1.2 Results

The full spectra for each type of ALD are available in Figures E.1, E.2 and E.3.

The XPS survey spectra PSiO₂/HfO₂ presented mainly Hf, O and C but also Si, F and P. The presence of fluorine, especially at the backside, can be explained by contamination due to the SF₆ gas used during the DRIE step, but the origin of the phosphorous signal remains unknown. Figure E.4 a,b show the Hf 4f spectra for the front and the back of the membrane. After curve fitting, two peaks are observed at 17 eV and 19 eV on both the front- and back side of the membrane, attributed to the binding energies of Hf 4f7/2 and Hf 4f5/2, respectively. These peaks indicate a valence state of +4 for hafnium and validate that the product of the ALD process is indeed HfO₂.

For the PSiO₂/TiO₂, peaks for Ti, O and C but also Si, and F can be observed in the XPS survey spectra. The presence of fluorine can once again be attributed to the DRIE step. Figure 7c,d reveal two peaks at 459 eV and 464 eV, both on front and back, which correspond to Ti 2p3/2 and Ti 2p1/2, respectively. These peaks also indicating a valence state of +4 for titanium and corroborate the deposition of TiO₂.

In the XPS survey spectra of PSiO₂/Al₂O₃, the presence of mainly Al, O and C but also F contamination can be observed. In Figure 7e,f, a peak at 74.5 eV can be observed on both front and back. This peak corresponds to the Al 2p binding energy and indicated a +3 valence, validated that Al₂O₃ is indeed the deposited product.

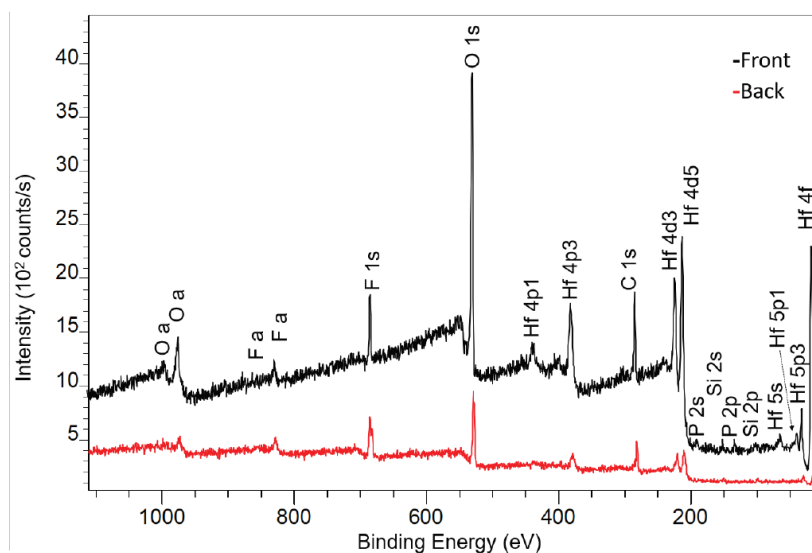


Figure E.1: XPS survey spectra of the $\text{PSiO}_2/\text{HfO}_2$ membrane front (black) and back (red). The main core levels are labelled. The data are normalised to each C-(C,H) component of the C 1s peak and separated vertically.

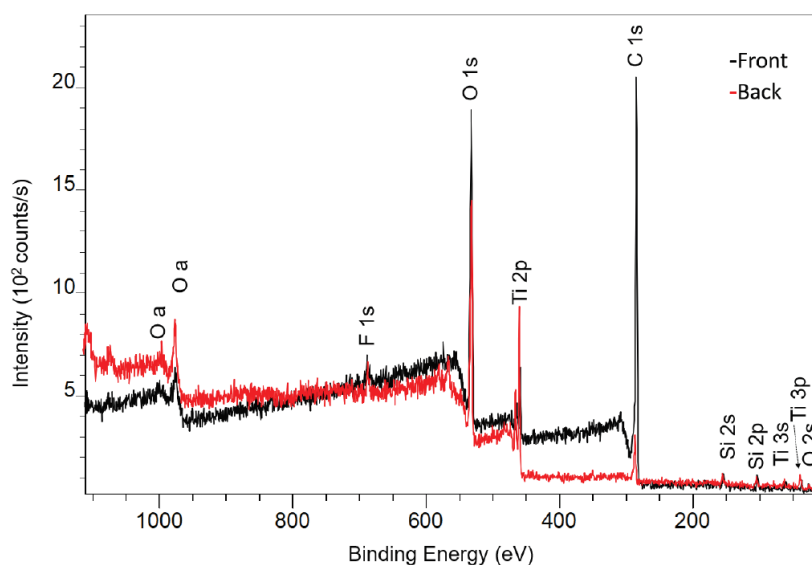


Figure E.2: XPS survey spectra of the $\text{PSiO}_2/\text{TiO}_2$ membrane front (black) and back (red). The main core levels are labelled. The data are normalised to each C-(C,H) component of the C 1s peak and separated vertically.

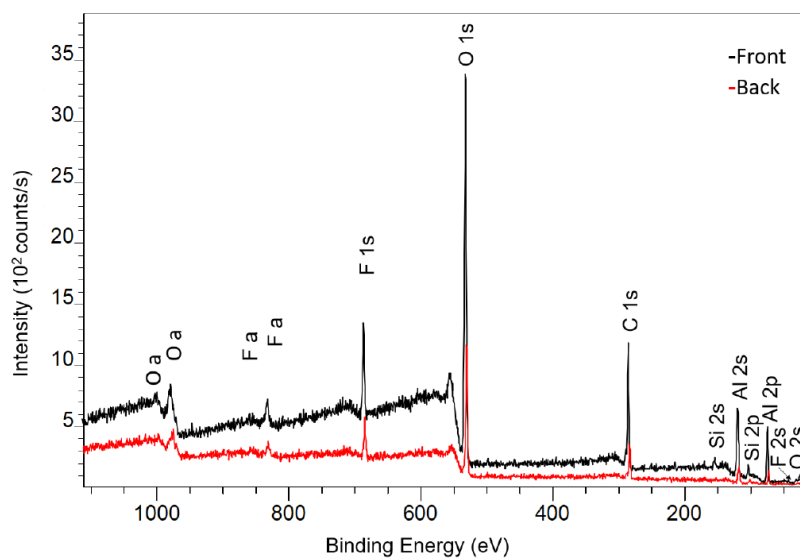


Figure E.3: XPS survey spectra of the $\text{PSiO}_2/\text{Al}_2\text{O}_3$ membrane front (black) and back (red). The main core levels are labelled. The data are normalised to each C-(C,H) component of the C 1s peak and separated vertically.

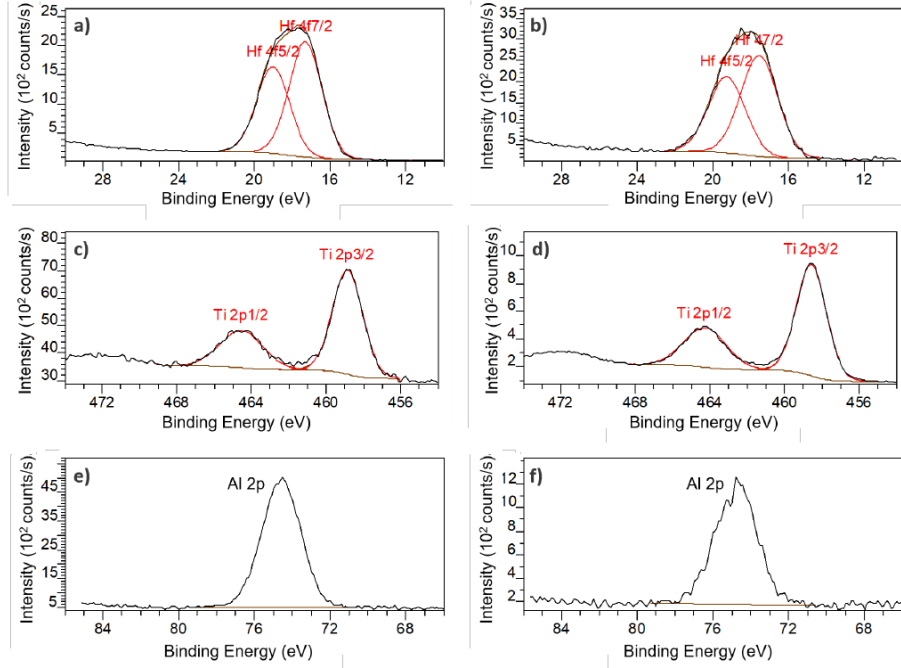


Figure E.4: High resolution XPS core level spectra: Hf 4f on $\text{PSiO}_2/\text{HfO}_2$ membrane (a) front, and (b) back; Ti 2p on $\text{PSiO}_2/\text{TiO}_2$ membrane (c) front, and (d) back; Al 2p on $\text{PSiO}_2/\text{Al}_2\text{O}_3$ membrane (e) front, and (f) back.

E.2 Noise analysis

E.2.1 Methods

The noise level of each measurement was also evaluated, both before and after the RIFTS data processing. This was achieved by using the `wdenoise` on MATLAB (MathWorks). This function denoises the signal using a Symlets 4 wavelet. The mean and standard deviation of this calculated noise was then computed. The signal-to-noise ratio was also computed using the `snr` function on Matlab and expressed in dB.

E.2.2 Results

Noise is unavoidable when taking optical measurements. They are linked to, among others, the dark noise, the baseline noise and the thermal noise of the read-out equipment. While noise can be minimised by taking a background reference and subtracting it from the optical signal, some

contributions are still present. In the case of PSiMs, the signal intensity is reduced because of the slight transparency of the porous matrix, reducing the reflected light. The noise level and the signal-to-noise ratio were therefore analysed to study the impact of the ALD coatings on the optical spectra. Values averaged for all spectra and samples are listed in Table E.1

Sample	SNR [dB]	Noise level [Relative intensity]
Blank	34.9	-0.0026 ± 0.0007
Al ₂ O ₃ -coated	37.9	-0.0013 ± 0.0004
HfO ₂ -coated	38.1	-0.0015 ± 0.0004
TiO ₂ -coated	39.3	-0.0008 ± 0.0005

Table E.1: signal-to-noise ratio and noise level of the optical signal of ALD coated PSiM sensors.

The addition of ALD inside the porous matrix increases the SNR of the optical signal, which can be explained by the increased optical signal presented in 6.4. The noise level was also decreased after ALD coating. A possible explanation might be linked to the decrease in pore size, which reduced the overall roughness of the layer surface and affect the scattering of the light.

The same routine was applied to the signal after the application of a RIFTS routine to the optical spectra. Values averaged for all samples are listed in Table E.2

Sample	SNR [dB]	Noise level [%]
Blank	12.3	$-8.6\text{E-}07 \pm 0.0007$
Al ₂ O ₃ -coated	13.5	$1.9\text{E-}06 \pm 0.0004$
HfO ₂ -coated	12.5	$1.3\text{E-}06 \pm 0.0004$
TiO ₂ -coated	15.1	$1.1\text{E-}07 \pm 0.0003$

Table E.2: signal-to-noise ratio and noise level of the RIFTS processed optical signal of ALD coated PSiM sensors.

After RIFTS data processing, the effect of ALD is less noticeable. SNR is similar for most samples, except for TiO₂ coated sensors, which exhibit a higher ratio. This increase may be explained by the minute reduction in noise level for TiO₂ coated sensors.

E.3 Lysis on chip

E.3.1 Methods

As part of the biosensing performance characterisation, the detection protocol was adapted by performing the lysis on the porous silicon membrane, instead of as a pre-treatment. This enables to perform both the control test and the detection at once. In short, after a pre-wetting of the PSi membrane in PBS for 30 min, a bacterial suspension (as prepared in Section 5.2.3) was flown through the membrane for 1 h. Next, a PlyB221 endolysin suspension in PBS (100 mg/mL) was added to the fluidic set-up for 30 min. The detection was then finished completed by rinsing the PSi membrane with PBS for 30 min. The RIFTS routine was applied to the measured optical spectra, as described previously. The relative EOT value was then averaged before the introduction of lytic agents, over the 40 to 60 min range (control relative EOT value) as well as after the rinsing step, namely over the 100 to 120 min range. The difference between these average EOT values expressed the relative EOT shift.

E.3.2 Results

When considering more complex matrices, the need for a control test of the sample before lysis arises, as mentioned at the end of Section 6.3.2.1. Previously, we performed this control test on a second sensor, in parallel to the bacterial lysate detection. An alternative protocol is investigated here, which performs the lysis of the target bacteria on the PSi membranes themselves. In short, the bacterial suspension is flown through the porous membrane for 1 h, after which the lytic agents are introduced to the fluidic setup for 30 min. A final rinsing step is performed, as indicated in Figure E.5. The relative EOT shift is measured by averaging the relative EOT before the lysis and after the rinsing step, as illustrated again in Figure E.5.

To verify if this relative EOT shift is significant, it must be compared to the noise level and a control test with endolysins only. This comparison is illustrated in Figure E.6: the average relative EOT shift for an initial concentration of 10^6 CFU/mL of *B. cereus* amounts to 0.12 ± 0.01 %, which is significant compared to both noise level and the endolysin control test.

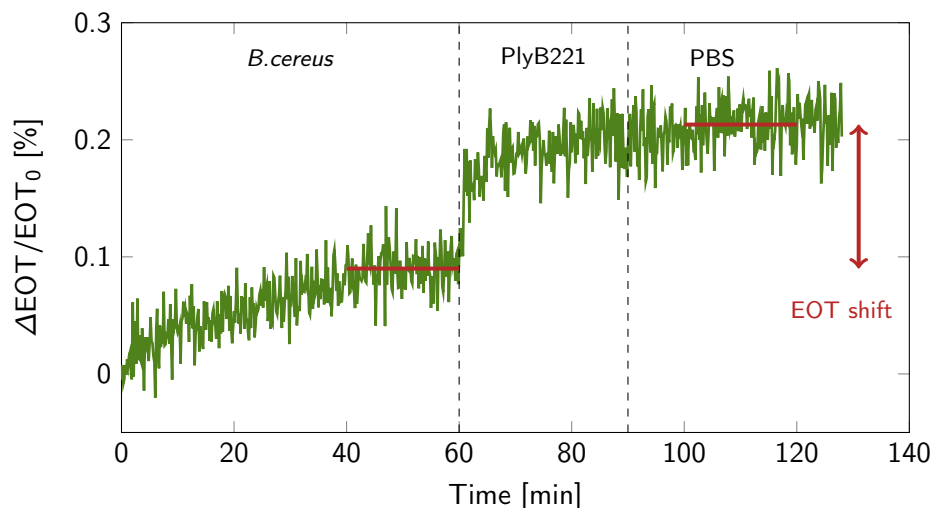


Figure E.5: Characteristic relative EOT shift overtime when performing the lysis on the PSi membrane chip, for 1h in a 10^6 CFU/mL *B. cereus* suspension, followed by 30 min during which the PlyB221 endolysins are added and finally a 30 min PBS rinse.

By performing the lysis on the PSi membranes, we eliminated the need for additional control tests. The relative EOT shift of +0.12 % before and after lysis on chip is however lower than the relative EOT shift between the bacterial suspension control and the bacterial lysate detection performed on two separate sensors (which amounts to +0.22 %). This decrease suggests that the LoD of this alternative protocol is higher than the LoD of the original detection scheme. While the reasons behind this decreased EOT shift remains to be investigated, some possible explanations may be linked to the conditions in which the lysis on chip occurs. Indeed, the lysis as pre-treatment occurs at a controlled temperature, under motion to favour the interactions between bacteria and lytic agents; on chip, the lysis occurs at room temperature, in a static fluidic cell. It must also be noted that the lysis-on-chip protocol is also longer than the original protocol: the total duration of the detection amounts to nearly 3 h, including the pre-wetting of the PSi membranes in PBS.

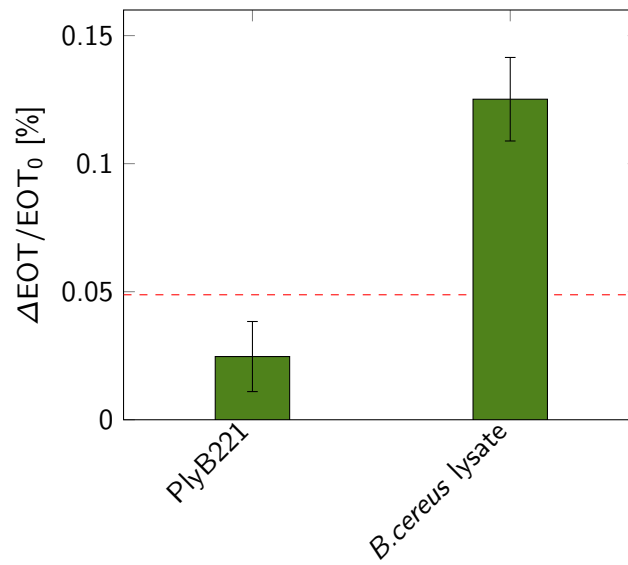


Figure E.6: Relative EOT shifts measured on PSi membranes when performing the lysis on chip: control test with only PlyB221 endolysins and shift measured when lysing a 10^6 CFU/mL *B. cereus* suspension on chip ($n \geq 3$). The dashed red line represents the 3σ level of the signal measured in PBS.

BIBLIOGRAPHY

- [1] M. L. Cohen, "Changing patterns of infectious disease," *Nature*, vol. 406, pp. 762–767, Aug. 2000. Number: 6797 Publisher: Nature Publishing Group.
- [2] J. O'Neill, "Tackling drug-resistant infections globally: final report and recommendations," report, Government of the United Kingdom, May 2016.
- [3] Centers for Disease Control and Prevention (U.S.), "Antibiotic resistance threats in the United States, 2019," tech. rep., Centers for Disease Control and Prevention (U.S.), Nov. 2019.
- [4] M. Zourob, S. Elwary, and A. Turner, eds., *Principles of Bacterial Detection: Biosensors, Recognition Receptors and Microsystems*. New York, NY: Springer New York, 2008.
- [5] A. Gopal, L. Yan, S. Kashif, T. Munshi, V. A. L. Roy, N. H. Voelcker, and X. Chen, "Biosensors and Point-of-Care Devices for Bacterial Detection: Rapid Diagnostics Informing Antibiotic Therapy," *Advanced Healthcare Materials*, vol. 11, no. 3, p. 2101546, 2022. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adhm.202101546>.
- [6] L. Canham, *Handbook of porous silicon*. New York: Springer, 2014.
- [7] T. Tieu, M. Alba, R. Elnathan, A. Cifuentes, and N. H. Voelcker, "Advances in Porous Silicon-Based Nanomaterials for Diagnostic and Therapeutic Applications," *Advanced Therapeutics*, vol. 2, no. 1, p. 1800095, 2019. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adtp.201800095>.
- [8] A. A. Leonardi, M. J. Lo Faro, and A. Irrera, "Biosensing platforms based on silicon nanostructures: a critical review," *Analytica Chimica Acta*, p. 338393, Mar. 2021.

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- [9] N. Massad-Ivanir and E. Segal, "Porous silicon for bacteria detection," in *Porous Silicon for Biomedical Applications*, pp. 286–303, Elsevier, 2014.
- [10] Y. Zhao, G. Gaur, S. T. Retterer, P. E. Laibinis, and S. M. Weiss, "Flow-Through Porous Silicon Membranes for Real-Time Label-Free Biosensing," *Analytical Chemistry*, vol. 88, pp. 10940–10948, Nov. 2016.
- [11] J. C. Pommerville, *Alcamo's Fundamentals of Microbiology*. Jones & Bartlett Publishers, Aug. 2010. Google-Books-ID: 2o79wdtgJDMC.
- [12] T. E. Love and B. Jones, "Introduction to Pathogenic Bacteria," in *Principles of Bacterial Detection: Biosensors, Recognition Receptors and Microsystems* (M. Zourob, S. Elwary, and A. Turner, eds.), pp. 3–13, New York, NY: Springer New York, 2008.
- [13] O. Lazcka, F. J. D. Campo, and F. X. Muñoz, "Pathogen detection: A perspective of traditional methods and biosensors," *Biosensors and Bioelectronics*, vol. 22, pp. 1205–1217, Feb. 2007.
- [14] M. Ferone, A. Gowen, S. Fanning, and A. G. M. Scannell, "Microbial detection and identification methods: Bench top assays to omics approaches," *Comprehensive Reviews in Food Science and Food Safety*, vol. 19, no. 6, pp. 3106–3129, 2020. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/1541-4337.12618>.
- [15] H. Bergis, L. Bonanno, A. Asséré, and B. Lombard, "On challenge tests and durability studies for assessing shelf-life of ready-to-eat foods related to *Listeria monocytogenes*," tech. rep., European Union Reference Laboratory for *Listeria monocytogenes*, 2021.
- [16] P. Herman, M. Fauville-Dufaux, D. Breyer, B. V. Vaerenbergh, K. Pauwels, C. D. D. Thi, M. Sneyers, M. Wanlin, R. Snacken, and W. Moens, "Biosafety Recommendations for the Contained Use of *Mycobacterium tuberculosis* Complex Isolates in Industrialized Countries.," *Royal Library of Belgium*, no. D/2006/2505/22, p. 17, 2006.
- [17] O. Opota, A. Croxatto, G. Prod'hom, and G. Greub, "Blood culture-based diagnosis of bacteraemia: state of the art," *Clinical Microbiology and Infection*, vol. 21, pp. 313–322, Apr. 2015.
- [18] R. Colodner, T. Eliasberg, B. Chazan, and R. Raz, "Clinical significance of bacteriuria with low colony counts of *Enterococcus* species," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 25, pp. 238–241, Apr. 2006.
- [19] L. Váradi, J. L. Luo, D. E. Hibbs, J. D. Perry, R. J. Anderson, S. Orenge, and P. W. Groundwater, "Methods for the detection and identification of pathogenic bacteria: past, present, and future," *Chemical Society Reviews*, vol. 46, pp. 4818–4832, Aug. 2017. Publisher: The Royal Society of Chemistry.

- [20] J. M. Willey, L. Sherwood, C. J. Woolverton, and L. M. Prescott, *Prescott, Harley, and Klein's microbiology*. New York: McGraw-Hill Higher Education, 7th ed ed., 2008. OCLC: ocm71044581.
- [21] T. Tominaga and M. Ishii, "Chapter 11 - Detection of microorganisms with lateral flow test strips," in *Methods in Microbiology* (C. S. Pavia and V. Gurtler, eds.), vol. 47 of *Immunological Methods in Microbiology*, pp. 351–394, Academic Press, Jan. 2020.
- [22] E. E. Antunez, M. A. Martin, and N. H. Voelcker, "Chapter 11 - Porous silicon-based sensors for protein detection," in *Porous Silicon for Biomedical Applications (Second Edition)* (H. A. Santos, ed.), Woodhead Publishing Series in Biomaterials, pp. 359–395, Woodhead Publishing, Jan. 2021.
- [23] N. Bhalla, P. Jolly, N. Formisano, and P. Estrela, "Introduction to biosensors," *Essays in Biochemistry*, vol. 60, pp. 1–8, June 2016.
- [24] A. Ahmed, J. V. Rushworth, N. A. Hirst, and P. A. Millner, "Biosensors for Whole-Cell Bacterial Detection," *Clinical Microbiology Reviews*, vol. 27, pp. 631–646, July 2014.
- [25] Z. Aliakbar Ahovan, A. Hashemi, L. M. De Plano, M. Gholipourmalek-abadi, and A. Seifalian, "Bacteriophage Based Biosensors: Trends, Outcomes and Challenges," *Nanomaterials*, vol. 10, p. 501, Mar. 2020. Number: 3 Publisher: Multidisciplinary Digital Publishing Institute.
- [26] T. M. Squires, R. J. Messinger, and S. R. Manalis, "Making it stick: convection, reaction and diffusion in surface-based biosensors," *Nature Biotechnology*, vol. 26, pp. 417–426, Apr. 2008. Number: 4 Publisher: Nature Publishing Group.
- [27] N. Couniot, T. Vanzieleghem, J. Rasson, N. Van Overstraeten-Schlögel, O. Poncelet, J. Mahillon, L. A. Francis, and D. Flandre, "Lytic enzymes as selectivity means for label-free, microfluidic and impedimetric detection of whole-cell bacteria using ALD-Al₂O₃ passivated microelectrodes," *Biosensors and Bioelectronics*, vol. 67, pp. 154–161, May 2015.
- [28] Ł. Richter, M. Janczuk-Richter, J. Niedziółka-Jönsson, J. Paczesny, and R. Hołyst, "Recent advances in bacteriophage-based methods for bacteria detection," *Drug Discovery Today*, vol. 23, pp. 448–455, Feb. 2018.
- [29] U. Farooq, Q. Yang, M. W. Ullah, and S. Wang, "Bacterial biosensing: Recent advances in phage-based bioassays and biosensors," *Biosensors and Bioelectronics*, vol. 118, pp. 204–216, Oct. 2018.
- [30] J. Paczesny, Ł. Richter, and R. Hołyst, "Recent Progress in the Detection of Bacteria Using Bacteriophages: A Review," *Viruses*, vol. 12, p. 845, Aug. 2020.
- [31] V. Ongenae, A. Briegel, and D. Claessen, "Cell wall deficiency as an escape mechanism from phage infection," *Open Biology*, vol. 11, no. 9, p. 210199, 2021. Publisher: Royal Society.

- [32] J. Onsea, J. Wagemans, J. Pirnay, M. Di Luca, M. Gonzalez-Moreno, R. Lavigne, A. Trampuz, T. Moriarty, and W.-J. Metsemakers, "Bacteriophage therapy as a treatment strategy for orthopaedic-device-related infections: where do we stand?," *European Cells and Materials*, vol. 39, pp. 193–210, May 2020.
- [33] L. Tilton, G. Das, X. Yang, N. Wisuthiphaet, I. M. Kennedy, and N. Nitin, "Nanophotonic Device in Combination with Bacteriophages for Enhancing Detection Sensitivity of Escherichia coli in Simulated Wash Water," *Analytical Letters*, vol. 52, pp. 2203–2213, Sept. 2019. Publisher: Taylor & Francis _eprint: <https://doi.org/10.1080/00032719.2019.1604726>.
- [34] A. Mortari, A. Adami, and L. Lorenzelli, "An unconventional approach to impedance microbiology: Detection of culture media conductivity variations due to bacteriophage generated lyses of host bacteria," *Biosensors and Bioelectronics*, vol. 67, pp. 615–620, May 2015.
- [35] H. Anany, L. Brovko, N. K. El Dougdoug, J. Sohar, H. Fenn, N. Alasiri, T. Jabrane, P. Mangin, M. Monsur Ali, B. Kannan, C. D. M. Filipe, and M. W. Griffiths, "Print to detect: a rapid and ultrasensitive phage-based dipstick assay for foodborne pathogens," *Analytical and Bioanalytical Chemistry*, vol. 410, pp. 1217–1230, Feb. 2018.
- [36] K. V. Sergueev, A. A. Filippov, and M. P. Nikolich, "Highly Sensitive Bacteriophage-Based Detection of Brucella abortus in Mixed Culture and Spiked Blood," *Viruses*, vol. 9, p. 144, June 2017. Number: 6 Publisher: Multidisciplinary Digital Publishing Institute.
- [37] M. Vinay, N. Franche, G. Grégori, J.-R. Fantino, F. Pouillot, and M. Ansaldi, "Phage-Based Fluorescent Biosensor Prototypes to Specifically Detect Enteric Bacteria Such as E. coli and Salmonella enterica Typhimurium," *PLOS ONE*, vol. 10, p. e0131466, July 2015. Publisher: Public Library of Science.
- [38] N. Wisuthiphaet, X. Yang, G. M. Young, and N. Nitin, "Rapid detection of Escherichia coli in beverages using genetically engineered bacteriophage T7," *AMB Express*, vol. 9, p. 55, Apr. 2019.
- [39] S. Srivastava, H. Hamo, A. Kushmaro, R. Marks, C. Grüner, B. Rauschenbach, and I. Abdulhalim, "Highly sensitive and specific detection of E. coli by a SERS nanobiosensor chip utilizing metallic nanosculptured thin films," *Analyst*, vol. 140, no. 9, pp. 3201–3209, 2015.
- [40] A. Lai, S. Almaviva, V. Spizzichino, D. Luciani, A. Palucci, S. Mengali, C. Marquette, O. Berthuy, B. Jankiewicz, and L. Pierno, "Bacillus spp. cells captured selectively by phages and identified by surface enhanced raman spectroscopy technique," *Proceedings*, vol. 1, no. 4, 2017.
- [41] F. Moghtader, G. Congur, H. M. Zareie, A. Erdem, and E. Piskin, "Impedimetric detection of pathogenic bacteria with bacteriophages using gold

- nanorod deposited graphite electrodes,” *RSC Advances*, vol. 6, pp. 97832–97839, Oct. 2016. Publisher: The Royal Society of Chemistry.
- [42] N. Bhardwaj, S. K. Bhardwaj, J. Mehta, G. C. Mohanta, and A. Deep, “Bacteriophage immobilized graphene electrodes for impedimetric sensing of bacteria (*Staphylococcus arlettae*),” *Analytical Biochemistry*, vol. 505, pp. 18–25, July 2016.
- [43] N. Hiremath, R. Guntupalli, V. Vodyanoy, B. A. Chin, and M.-K. Park, “Detection of methicillin-resistant *Staphylococcus aureus* using novel lytic phage-based magnetoelastic biosensors,” *Sensors and Actuators B: Chemical*, vol. 210, pp. 129–136, Apr. 2015.
- [44] J. D. Mack, T. Yehualaeshet, M.-K. Park, B. Tameru, T. Samuel, and B. A. Chin, “Phage-Based Biosensor and Optimization of Surface Blocking Agents to Detect *Salmonella Typhimurium* on Romaine Lettuce,” *Journal of Food Safety*, vol. 37, no. 2, p. e12299, 2017. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1111/jfs.12299>.
- [45] I.-H. Chen, S. Horikawa, K. Bryant, R. Riggs, B. A. Chin, and J. M. Barba-ree, “Bacterial assessment of phage magnetoelastic sensors for *Salmonella enterica Typhimurium* detection in chicken meat,” *Food Control*, vol. 71, pp. 273–278, Jan. 2017.
- [46] H. Peng and I. A. Chen, “Rapid Colorimetric Detection of Bacterial Species through the Capture of Gold Nanoparticles by Chimeric Phages,” *ACS Nano*, vol. 13, pp. 1244–1252, Feb. 2019. Publisher: American Chemical Society.
- [47] Z. Wang, D. Wang, A. J. Kinchla, D. A. Sela, and S. R. Nugen, “Rapid screening of waterborne pathogens using phage-mediated separation coupled with real-time PCR detection,” *Analytical and Bioanalytical Chemistry*, vol. 408, pp. 4169–4178, June 2016.
- [48] S. Liébana, D. A. Spricigo, M. P. Cortés, J. Barbé, M. Llagostera, S. Alegret, and M. I. Pividori, “Phagomagnetic Separation and Electrochemical Magneto-Genosensing of Pathogenic Bacteria,” *Analytical Chemistry*, vol. 85, pp. 3079–3086, Mar. 2013. Publisher: American Chemical Society.
- [49] S. Poshtiban, M. A. Javed, D. Arutyunov, A. Singh, G. Banting, C. M. Szymanski, and S. Evoy, “Phage receptor binding protein-based magnetic enrichment method as an aid for real time PCR detection of foodborne bacteria,” *Analyst*, vol. 138, pp. 5619–5626, Aug. 2013. Publisher: The Royal Society of Chemistry.
- [50] M. Dunne and M. J. Loessner, “Modified Bacteriophage Tail Fiber Proteins for Labeling, Immobilization, Capture, and Detection of Bacteria,” in *Foodborne Bacterial Pathogens: Methods and Protocols* (A. Bridier, ed.), Methods in Molecular Biology, pp. 67–86, New York, NY: Springer, 2019.

- [51] Y. He, Y. Shi, M. Liu, Y. Wang, L. Wang, S. Lu, and Z. Fu, "Nonlytic Recombinant Phage Tail Fiber Protein for Specific Recognition of *Pseudomonas aeruginosa*," *Analytical Chemistry*, vol. 90, pp. 14462–14468, Dec. 2018. Publisher: American Chemical Society.
- [52] V. A. Fischetti, "Bacteriophage lytic enzymes: novel anti-infectives," *Trends in Microbiology*, vol. 13, pp. 491–496, Oct. 2005.
- [53] M. Tolba, M. U. Ahmed, C. Tlili, F. Eichenseher, M. J. Loessner, and M. Zourob, "A bacteriophage endolysin-based electrochemical impedance biosensor for the rapid detection of *Listeria* cells," *Analyst*, vol. 137, pp. 5749–5756, Nov. 2012. Publisher: The Royal Society of Chemistry.
- [54] Y. Wang, Y. He, S. Bhattacharyya, S. Lu, and Z. Fu, "Recombinant Bacteriophage Cell-Binding Domain Proteins for Broad-Spectrum Recognition of Methicillin-Resistant *Staphylococcus aureus* Strains," *Analytical Chemistry*, vol. 92, pp. 3340–3345, Feb. 2020. Publisher: American Chemical Society.
- [55] P. Liu, Y. Wang, L. Han, Y. Cai, H. Ren, T. Ma, X. Li, V. A. Petrenko, and A. Liu, "Colorimetric Assay of Bacterial Pathogens Based on Co3O4 Magnetic Nanozymes Conjugated with Specific Fusion Phage Proteins and Magnetophoretic Chromatography," *ACS Applied Materials & Interfaces*, vol. 12, pp. 9090–9097, Feb. 2020. Publisher: American Chemical Society.
- [56] M. Kong, J. Shin, S. Heu, J.-K. Park, and S. Ryu, "Lateral flow assay-based bacterial detection using engineered cell wall binding domains of a phage endolysin," *Biosensors and Bioelectronics*, vol. 96, pp. 173–177, 2017.
- [57] G. Le Brun, M. Hauwaert, A. Leprince, K. Glinel, J. Mahillon, and J.-P. Raskin, "Electrical Characterization of Cellulose-Based Membranes towards Pathogen Detection in Water," *Biosensors*, vol. 11, p. 57, Feb. 2021. Number: 2 Publisher: Multidisciplinary Digital Publishing Institute.
- [58] V. Naresh and N. Lee, "A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors," *Sensors*, vol. 21, p. 1109, Jan. 2021. Number: 4 Publisher: Multidisciplinary Digital Publishing Institute.
- [59] X. Guo, S. Sang, A. Jian, S. Gao, Q. Duan, J. Ji, Q. Zhang, and W. Zhang, "A bovine serum albumin-coated magnetoelastic biosensor for the wireless detection of heavy metal ions," *Sensors and Actuators B: Chemical*, vol. 256, pp. 318–324, Mar. 2018.
- [60] D. Sung and J. Koo, "A review of BioFET, Ås basic principles and materials for biomedical applications," *Biomedical Engineering Letters*, vol. 11, pp. 85–96, May 2021.
- [61] D. Grieshaber, R. MacKenzie, J. Voros, and E. Reimhult, "Electrochemical biosensors - sensor principles and architectures," *Sensors*, vol. 8, no. 3, pp. 1400–1458, 2008.

- [62] J. Hammond, N. Formisano, P. Estrela, S. Carrara, and J. Tkac, "Electrochemical biosensors and nanobiosensors," *Essays in Biochemistry*, vol. 60, pp. 69–80, June 2016.
- [63] Y. Chen, J. Liu, Z. Yang, J. S. Wilkinson, and X. Zhou, "Optical biosensors based on refractometric sensing schemes: A review," *Biosensors and Bioelectronics*, vol. 144, p. 111693, Nov. 2019.
- [64] Z. Kotsiri, J. Vidic, and A. Vantarakis, "Applications of biosensors for bacteria and virus detection in food and water, A systematic review," *Journal of Environmental Sciences*, vol. 111, pp. 367–379, Jan. 2022.
- [65] A. Roda, M. Mirasoli, E. Michelini, M. Di Fusco, M. Zangheri, L. Cevenini, B. Roda, and P. Simoni, "Progress in chemical luminescence-based biosensors: A critical review," *Biosensors and Bioelectronics*, vol. 76, pp. 164–179, Feb. 2016.
- [66] Y.-T. Chen, Y.-C. Lee, Y.-H. Lai, J.-C. Lim, N.-T. Huang, C.-T. Lin, and J.-J. Huang, "Review of Integrated Optical Biosensors for Point-of-Care Applications," *Biosensors*, vol. 10, p. 209, Dec. 2020.
- [67] J. Langer, D. Jimenez de Aberasturi, J. Aizpurua, R. A. Alvarez-Puebla, B. Augu  , J. J. Baumberg, G. C. Bazan, S. E. J. Bell, A. Boisen, A. G. Brolo, J. Choo, D. Cialla-May, V. Deckert, L. Fabris, K. Faulds, F. J. Garc  a de Abajo, R. Goodacre, D. Graham, A. J. Haes, C. L. Haynes, C. Huck, T. Itoh, M. K  ll, J. Kneipp, N. A. Kotov, H. Kuang, E. C. Le Ru, H. K. Lee, J.-F. Li, X. Y. Ling, S. A. Maier, T. Mayerh  fer, M. Moskovits, K. Murakoshi, J.-M. Nam, S. Nie, Y. Ozaki, I. Pastoriza-Santos, J. Perez-Juste, J. Popp, A. Pucci, S. Reich, B. Ren, G. C. Schatz, T. Shegai, S. Schl  cker, L.-L. Tay, K. G. Thomas, Z.-Q. Tian, R. P. Van Duyne, T. Vo-Dinh, Y. Wang, K. A. Willets, C. Xu, H. Xu, Y. Xu, Y. S. Yamamoto, B. Zhao, and L. M. Liz-Marz  n, "Present and Future of Surface-Enhanced Raman Scattering," *ACS Nano*, vol. 14, pp. 28–117, Jan. 2020. Publisher: American Chemical Society.
- [68] A. A. Jabbar, A. M. Alwan, M. Q. Zayer, and A. J. Bohan, "Efficient single cell monitoring of pathogenic bacteria using bimetallic nanostructures embedded in gradient porous silicon," *Materials Chemistry and Physics*, vol. 241, p. 122359, Feb. 2020.
- [69] D. Duval and L. M. Lechuga, "Optical Waveguide Biosensors," in *Photonics* (D. L. Andrews, ed.), pp. 323–365, Wiley, 1 ed., Feb. 2015.
- [70] H. Mukundan, A. S. Anderson, W. K. Grace, K. M. Grace, N. Hartman, J. S. Martinez, and B. I. Swanson, "Waveguide-Based Biosensors for Pathogen Detection," *Sensors (Basel, Switzerland)*, vol. 9, pp. 5783–5809, July 2009.
- [71] C. Chen and J. Wang, "Optical biosensors: an exhaustive and comprehensive review," *Analyst*, vol. 145, pp. 1605–1628, Mar. 2020. Publisher: The Royal Society of Chemistry.

- [72] D. Rho, C. Breaux, and S. Kim, "Label-Free Optical Resonator-Based Biosensors," *Sensors*, vol. 20, p. 5901, Jan. 2020. Number: 20 Publisher: Multidisciplinary Digital Publishing Institute.
- [73] S. Li, J. Huang, and L. Cai, "A porous silicon optical microcavity for sensitive bacteria detection," *Nanotechnology*, vol. 22, p. 425502, Oct. 2011.
- [74] D. Threm, Y. Nazirizadeh, and M. Gerken, "Photonic crystal biosensors towards on-chip integration," *Journal of Biophotonics*, vol. 5, no. 8-9, pp. 601–616, 2012. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/jbio.201200039>.
- [75] D. Gowdhami, V. R. Balaji, M. Murugan, S. Robinson, and G. Hegde, "Photonic crystal based biosensors: an overview," *ISSS Journal of Micro and Smart Systems*, Jan. 2022.
- [76] H. Inan, M. Poyraz, F. Inci, M. A. Lifson, M. Baday, B. T. Cunningham, and U. Demirci, "Photonic crystals: emerging biosensors and their promise for point-of-care applications," *Chemical Society Reviews*, vol. 46, pp. 366–388, Jan. 2017. Publisher: The Royal Society of Chemistry.
- [77] G. Scheen, *Metal electrode integration and Palladium nanoparticle functionalization on a miniaturized macroporous silicon chemiresistor*. PhD thesis, UCL - Université Catholique de Louvain, 2015.
- [78] A. Shrivastava and V. Gupta, "Methods for the determination of limit of detection and limit of quantitation of the analytical methods," *Chronicles of Young Scientists*, vol. 2, no. 1, p. 21, 2011.
- [79] S. Franssila, *Introduction to Microfabrication*. Wiley, 2004.
- [80] A. Uhler Jr., "Electrolytic shaping of germanium and silicon," *Bell System Technical Journal*, vol. 35, no. 2, pp. 333–347, 1956.
- [81] L. T. Canham, "Silicon quantum wire array fabrication by electrochemical and chemical dissolution of wafers," *Applied Physics Letters*, vol. 57, pp. 1046–1048, Sept. 1990.
- [82] V. Lehmann and U. Gösele, "Porous silicon formation: A quantum wire effect," *Applied Physics Letters*, vol. 58, no. 8, pp. 856–858, 1991.
- [83] D. L. Kendall, "A new theory for the anisotropic etching of silicon and some underdeveloped chemical micromachining concepts," *Journal of Vacuum Science & Technology A*, vol. 8, no. 4, pp. 3598–3605, 1990.
- [84] G. Kittilsland, G. Stemme, and B. Nordén, "A sub-micron particle filter in silicon," *Sensors and Actuators A: Physical*, vol. 23, no. 1, pp. 904 – 907, 1990.
- [85] K. W. Kolasinski, "Electron transfer during metal-assisted and stain etching of silicon," *Semiconductor Science and Technology*, vol. 31, p. 014002, Jan. 2016.

- [86] Z. C. Feng and R. Tsu, *Porous Silicon*. WORLD SCIENTIFIC, 1994.
- [87] X. G. Zhang, "Morphology and Formation Mechanisms of Porous Silicon," *Journal of The Electrochemical Society*, vol. 151, no. 1, p. C69, 2004.
- [88] P. Kumar, P. Lemmens, M. Ghosh, F. Ludwig, and M. Schilling, "Effect of HF Concentration on Physical and Electronic Properties of Electrochemically Formed Nanoporous Silicon," *Journal of Nanomaterials*, vol. 2009, pp. 1–7, 2009.
- [89] J. Carstensen, M. Christophersen, G. Hasse, and H. Föll, "Parameter Dependence of Pore Formation in Silicon within a Model of Local Current Bursts," *physica status solidi (a)*, vol. 182, no. 1, pp. 63–69, 2000.
- [90] M. Sailor, *Porous Silicon in Practice: Preparation, Characterization and Applications*. Wiley, 2012.
- [91] R. M. Sasaki, R. A. Douglas, M. U. Kleinke, and O. Teschke, "Structure imaging by atomic force microscopy and transmission electron microscopy of different light emitting species of porous silicon," *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures Processing, Measurement, and Phenomena*, vol. 14, pp. 2432–2437, July 1996. Publisher: American Institute of Physics.
- [92] S. Chuang, S. D. Collins, and R. L. Smith, "Porous silicon microstructure as studied by transmission electron microscopy," *Applied Physics Letters*, vol. 55, pp. 1540–1542, Oct. 1989. Publisher: American Institute of Physics.
- [93] O. Bisi, S. Ossicini, and L. Pavesi, "Porous silicon: a quantum sponge structure for silicon based optoelectronics," *Surface Science Reports*, vol. 38, pp. 1–126, Apr. 2000.
- [94] J. Rasson, *Stable porous silicon-based optical sensing platform towards detection of bacterial lysis*. PhD Thesis, Université Catholique de Louvain, Université Catholique de Louvain, 2018.
- [95] G. Korotcenkov, *Porous Silicon: From Formation to Application: Biomedical and Sensor Applications, Volume Two: Biomedical and Sensor Applications*. CRC Press, Jan. 2016. Google-Books-ID: czo0CwAAQBAJ.
- [96] M. B. Gongalsky, A. A. Koval, S. N. Schevchenko, K. P. Tamarov, and L. A. Osminkina, "Double Etched Porous Silicon Films for Improved Optical Sensing of Bacteria," *Journal of The Electrochemical Society*, vol. 164, no. 12, pp. B581–B584, 2017.
- [97] C. Pacholski, M. Sartor, M. J. Sailor, F. Cunin, and G. M. Miskelly, "Biosensing Using Porous Silicon Double-Layer Interferometers: Reflective Interferometric Fourier Transform Spectroscopy," *Journal of the American Chemical Society*, vol. 127, pp. 11636–11645, Aug. 2005. Publisher: American Chemical Society.

- [98] S. Arshavsky-Graham, N. Massad-Ivanir, E. Segal, and S. Weiss, "Porous Silicon-Based Photonic Biosensors: Current Status and Emerging Applications," *Analytical Chemistry*, vol. 91, pp. 441–467, Jan. 2019.
- [99] J. Zheng, M. Christophersen, and P. L. Bergstrom, "Thick macroporous membranes made of p-type silicon," *physica status solidi (a)*, vol. 202, no. 8, pp. 1402–1406, 2005.
- [100] F. Lucklum, A. Schwaiger, and B. Jakoby, "Development and Investigation of Thermal Devices on Fully Porous Silicon Substrates," *IEEE Sensors Journal*, vol. 14, pp. 992–997, Apr. 2014. Conference Name: IEEE Sensors Journal.
- [101] V. A. Yuzova, F. F. Merkushev, and O. V. Semenova, "A monolithic silicon-based membrane-electrode assembly for micro fuel cells," *Technical Physics Letters*, vol. 43, pp. 764–766, Aug. 2017.
- [102] V. A. Yuzova, F. F. Merkushev, and E. A. Lyaykom, "Formation of cross-cutting structures with different porosity on thick silicon wafers," *Modern Electronic Materials*, vol. 3, pp. 72–75, June 2017.
- [103] G. Scheen, R. Tuyaerts, M. Rack, L. Nyssens, J. Rasson, M. Nabet, and J.-P. Raskin, "Post-process porous silicon for 5G applications," *Solid-State Electronics*, vol. 168, p. 107719, June 2020.
- [104] A. Chiadò, C. Novara, A. Lamberti, F. Geobaldo, F. Giorgis, and P. Rivolo, "Immobilization of Oligonucleotides on Metal-Dielectric Nanostructures for miRNA Detection," *Analytical Chemistry*, vol. 88, pp. 9554–9563, Oct. 2016. Publisher: American Chemical Society.
- [105] N. E. Preobrazhenskiy, E. V. Astrova, S. I. Pavlov, V. B. Voronkov, A. M. Rumyantsev, and V. V. Zhdanov, "Anodes for Li-ion batteries based on p-Si with self-organized macropores," *Semiconductors*, vol. 51, pp. 78–87, Jan. 2017.
- [106] B. Bardet, S. Desplobain, J. Billoué, L. Ventura, and G. Gautier, "Integration of low-loss inductors on thin porous silicon membranes," *Microelectronic Engineering*, vol. 194, pp. 96–99, July 2018.
- [107] H. Ning, N. A. Krueger, X. Sheng, H. Keum, C. Zhang, K. D. Choquette, X. Li, S. Kim, J. A. Rogers, and P. V. Braun, "Transfer-Printing of Tunable Porous Silicon Microcavities with Embedded Emitters," *ACS Photonics*, vol. 1, pp. 1144–1150, Nov. 2014. Publisher: American Chemical Society.
- [108] G. V. Li, E. V. Astrova, A. M. Rumyantsev, V. B. Voronkov, A. V. Parfen, A. A. Tolmachev, T. L. Kulova, and A. M. Skundin, "Microstructured silicon anodes for lithium-ion batteries," *Russian Journal of Electrochemistry*, vol. 51, pp. 899–907, Oct. 2015.

- [109] R. Mentek, D. Hippo, B. Gelloz, and N. Koshida, "Photovoltaic effect with high open circuit voltage observed in electrochemically prepared nanocrystalline silicon membranes," *Materials Science and Engineering: B*, vol. 190, pp. 33–40, Dec. 2014.
- [110] G. V. Li, E. V. Astrova, N. E. Preobrazhenskii, A. M. Rumyantsev, S. I. Pavlov, and E. V. Beregulin, "Negative Electrodes for Lithium-Ion Batteries Obtained by Photoanodization of Solar-Grade Silicon," *Technical Physics*, vol. 64, pp. 660–665, May 2019.
- [111] M. Balucani, K. Kholostov, V. Varlamava, F. Palma, M. Izzi, L. Serenelli, and M. Tucci, "Porous silicon solar cells," in *2015 IEEE 15th International Conference on Nanotechnology (IEEE-NANO)*, pp. 724–727, July 2015. ISSN: null.
- [112] C. Novara, A. Lamberti, A. Chiadò, A. Virga, P. Rivolo, F. Geobaldo, and F. Giorgis, "Surface-enhanced Raman spectroscopy on porous silicon membranes decorated with Ag nanoparticles integrated in elastomeric microfluidic chips," *RSC Advances*, vol. 6, pp. 21865–21870, Feb. 2016.
- [113] C. Novara, A. Chiadò, N. Paccotti, S. Catuogno, C. L. Esposito, G. Condorelli, V. De Franciscis, F. Geobaldo, P. Rivolo, and F. Giorgis, "SERS-active metal-dielectric nanostructures integrated in microfluidic devices for label-free quantitative detection of miRNA," *Faraday Discussions*, vol. 205, pp. 271–289, 2017.
- [114] D. Martín-Sánchez, S. Ponce-Alcántara, and J. García-Rupérez, "Sensitivity Comparison of a Self-Standing Porous Silicon Membrane Under Flow-Through and Flow-Over Conditions," *IEEE Sensors Journal*, vol. 19, pp. 3276–3281, May 2019. Conference Name: IEEE Sensors Journal.
- [115] I. S. Kryukova, D. S. Dovzhenko, Y. P. Rakovich, and I. R. Nabiev, "Enhancement of the photoluminescence of semiconductor nanocrystals in transfer-printed microcavities based on freestanding porous silicon photonic crystals," *Journal of Physics: Conference Series*, vol. 1439, p. 012018, Jan. 2020.
- [116] N. Reta, A. Michelmore, C. Saint, B. Prieto-Simón, and N. H. Voelcker, "Porous silicon membrane-modified electrodes for label-free voltammetric detection of MS2 bacteriophage," *Biosensors and Bioelectronics*, vol. 80, pp. 47–53, June 2016.
- [117] F. Morales-Morales, L. Palacios-Huerta, S. A. Cabañas-Tay, A. Coyopol, and A. Morales-Sánchez, "Luminescent Si quantum dots in flexible and semitransparent membranes for photon down converting material," *Optical Materials*, vol. 90, pp. 220–226, Apr. 2019.
- [118] I.-T. Chen, D. A. Sessoms, Z. Sherman, E. Choi, O. Vincent, and A. D. Stroock, "Stability Limit of Water by Metastable Vapor, A Liquid Equilibrium with Nanoporous Silicon Membranes," *The Journal of Physical Chem-*

- istry B*, vol. 120, pp. 5209–5222, June 2016. Publisher: American Chemical Society.
- [119] M. R. Jimenéz-Vivanco, G. García, J. Carrillo, V. Agarwal, T. Díaz-Becerril, R. Doti, J. Faubert, and J. E. Lugo, “Porous Si-SiO₂ based UV Microcavities,” *Scientific Reports*, vol. 10, pp. 1–21, Feb. 2020. Number: 1 Publisher: Nature Publishing Group.
- [120] M. R. Jimenéz-Vivanco, G. García, J. Carrillo, F. Morales-Morales, A. Coyopol, M. Gracia, R. Doti, J. Faubert, and J. E. Lugo, “Porous Si-SiO₂ UV Microcavities to Modulate the Responsivity of a Broadband Photodetector,” *Nanomaterials*, vol. 10, p. 222, Feb. 2020. Number: 2 Publisher: Multidisciplinary Digital Publishing Institute.
- [121] P. Dardano, A. Calì, J. Politi, I. Rea, I. Rendina, and L. D. Stefano, “Optically monitored drug delivery patch based on porous silicon and polymer microneedles,” *Biomedical Optics Express*, vol. 7, pp. 1645–1655, May 2016. Publisher: Optical Society of America.
- [122] Y. Pei, S. H. Shahoei, Y. Li, P. J. Reece, E. R. Nelson, J. J. Gooding, and K. A. Kilian, “Vertical Integration of Cell-Loaded Hydrogels with Bioinspired Photonic Crystal Membranes,” *Advanced Materials Interfaces*, vol. 5, p. 1801233, Dec. 2018.
- [123] L. G. Cenchá, C. Antonio Hernández, L. Forzani, R. Urteaga, and R. R. Koropecski, “Optical performance of hybrid porous silicon-porous alumina multilayers,” *Journal of Applied Physics*, vol. 123, p. 183101, May 2018.
- [124] D. J. J. Fandio, S. Sauze, A. Boucherif, R. Arès, and D. Morris, “Structural, optical and terahertz properties of graphene-mesoporous silicon nanocomposites,” *Nanoscale Advances*, vol. 2, pp. 340–346, Jan. 2020. Publisher: RSC.
- [125] S. De La Luz-Merino, M. E. Calixto, and A. Méndez-Blas, “Nanostructured CuInSe₂ by electrodeposition with the assistance of porous silicon templates,” *Materials Chemistry and Physics*, vol. 163, pp. 362–368, Aug. 2015.
- [126] Y. Zhao, G. Gaur, R. L. Mernaugh, P. E. Laibinis, and S. M. Weiss, “Comparative Kinetic Analysis of Closed-Ended and Open-Ended Porous Sensors,” *Nanoscale Research Letters*, vol. 11, p. 1, Dec. 2016.
- [127] R. Haddad, J. Thery, B. Gauthier-Manuel, K. Elouarzaki, M. Holzinger, A. Le Goff, G. Gautier, J. El Mansouri, A. Martinet, and S. Cosnier, “High performance miniature glucose/O₂ fuel cell based on porous silicon anion exchange membrane,” *Electrochemistry Communications*, vol. 54, pp. 10–13, May 2015.
- [128] Z. Wu, X. Wang, and L. Liu, “A Passive Vapor-Feed Direct Methanol Fuel Cell Based on a Composite Pervaporation Membrane,” *Journal of Microelectromechanical Systems*, vol. 24, pp. 207–215, Feb. 2015.

- [129] N. Burham, A. A. Hamzah, J. Yunas, and B. Y. Majlis, "Electrochemically etched nanoporous silicon membrane for separation of biological molecules in mixture," *Journal of Micromechanics and Microengineering*, vol. 27, p. 075021, July 2017.
- [130] N. Burham, A. A. Hamzah, and B. Yeop Majlis, "Self-Adjusting Electrochemical Etching Technique for Producing Nanoporous Silicon Membrane," *New Research on Silicon - Structure, Properties, Technology*, May 2017. Publisher: IntechOpen.
- [131] M. Wang, L. Liu, and X. Wang, "A novel proton exchange membrane based on sulfo functionalized porous silicon for monolithic integrated micro direct methanol fuel cells," *Sensors and Actuators B: Chemical*, vol. 253, pp. 621–629, Dec. 2017.
- [132] M. Wang, L. Liu, and X. Wang, "Effects of grafting parameters on the properties of proton exchange membranes based on sulfo-functionalized porous silicon for micro direct methanol fuel cells," *Journal of Polymer Engineering*, vol. 39, pp. 620–627, July 2019.
- [133] F. Lucklum, A. Schwaiger, and B. Jakoby, "Highly insulating, fully porous silicon substrates for high temperature micro-hotplates," *Sensors and Actuators A: Physical*, vol. 213, pp. 35–42, July 2014.
- [134] V. V. Bolotov, K. E. Ivlev, E. V. Knyazev, I. V. Ponomareva, and V. E. Roslikov, "Formation of Multilayer Structures with Integrated Membranes Based on Porous Silicon," *Semiconductors*, vol. 54, pp. 609–613, May 2020.
- [135] T. Pichonat and B. Gauthier-Manuel, "A new process for the manufacturing of reproducible mesoporous silicon membranes," *Journal of Membrane Science*, vol. 280, pp. 494–500, Sept. 2006.
- [136] K. H. Tantawi, B. Berdiev, R. Cerro, and J. D. Williams, "Porous silicon membrane for investigation of transmembrane proteins," *Superlattices and Microstructures*, vol. 58, pp. 72–80, June 2013.
- [137] H. So, K. Lee, N. Murthy, and A. P. Pisano, "All-in-One Nanowire-Decorated Multifunctional Membrane for Rapid Cell Lysis and Direct DNA Isolation," *ACS Applied Materials & Interfaces*, vol. 6, pp. 20693–20699, Dec. 2014.
- [138] H. So, K. Lee, Y. H. Seo, N. Murthy, and A. P. Pisano, "Hierarchical Silicon Nanospikes Membrane for Rapid and High-Throughput Mechanical Cell Lysis," *ACS Applied Materials & Interfaces*, vol. 6, pp. 6993–6997, May 2014. Publisher: American Chemical Society.
- [139] Z. Yu, D. Zheng, K. Zhang, T. Yang, Y. Chen, and X. Li, "Optimally catalyzed porous-silicon electrode of self-breathing micro fuel cells," *Microsystem Technologies*, vol. 23, pp. 3257–3262, Aug. 2017.

- [140] A. Baraket, J.-P. Alcaraz, C. Gondran, G. Costa, G. Nonglaton, F. Gailard, P. Cinquin, M.-L. Cosnier, and D. K. Martin, "Long duration stabilization of porous silicon membranes in physiological media: Application for implantable reactors," *Materials Science and Engineering: C*, vol. 108, p. 110359, Mar. 2020.
- [141] G. Nagayama, A. Kuromaru, M. Kaneda, and T. Tsuruta, "Effects of nano/microstructures on performance of Si-based microfuel cells," *Applied Thermal Engineering*, vol. 72, pp. 298–303, Nov. 2014.
- [142] M. A. Parashchenko, N. S. Filippov, V. V. Kirienko, and S. I. Romanov, "Electroosmotic pump based on asymmetric silicon microchannel membranes," *Optoelectronics, Instrumentation and Data Processing*, vol. 50, pp. 315–322, May 2014.
- [143] M. A. Parashchenko, N. S. Filippov, and V. V. Kirienko, "Microfluidic electric generator based on a silicon microchannel membrane," *Optoelectronics, Instrumentation and Data Processing*, vol. 51, pp. 94–102, Jan. 2015.
- [144] V. V. Starkov, D. M. Sedlovets, M. A. Knyazev, and A. N. Red, "Composite electrodes for current sources based on graphene-like films in porous silicon," *Protection of Metals and Physical Chemistry of Surfaces*, vol. 53, pp. 85–87, Jan. 2017.
- [145] V. V. Bolotov, K. E. Ivlev, E. V. Knyazev, I. V. Ponomareva, and V. E. Roslikov, "The formation of membranes based on oxidized two-layer porous silicon," in *AIP Conference Proceedings 2141*, (Selangor, Malaysia), p. 040017, 2019.
- [146] M. S. Khan and J. D. Williams, "Fabrication of Solid State Nanopore in Thin Silicon Membrane Using Low Cost Multistep Chemical Etching," *Materials*, vol. 8, pp. 7389–7400, Nov. 2015. Number: 11 Publisher: Multidisciplinary Digital Publishing Institute.
- [147] M. S. Khan, N. S. Dosoky, B. K. Berdiev, and J. D. Williams, "Electrochemical impedance spectroscopy for black lipid membranes fused with channel protein supported on solid-state nanopore," *European Biophysics Journal*, vol. 45, pp. 843–852, Dec. 2016.
- [148] M. S. Khan, N. S. Dosoky, G. Mustafa, D. Patel, B. Berdiev, and J. D. Williams, "Electrophysiology of Epithelial Sodium Channel (ENaC) Embedded in Supported Lipid Bilayer Using a Single Nanopore Chip," *Langmuir*, vol. 33, pp. 13680–13688, Nov. 2017. Publisher: American Chemical Society.
- [149] T. Schmidt, M. Zhang, I. Sychugov, N. Roxhed, and J. Linnros, "Nanopore arrays in a silicon membrane for parallel single-molecule detection: fabrication," *Nanotechnology*, vol. 26, p. 314001, Aug. 2015.

- [150] M. Zhang, T. Schmidt, A. Jemt, P. Sahlén, I. Sychugov, J. Lundberg, and J. Linnros, "Nanopore arrays in a silicon membrane for parallel single-molecule detection: DNA translocation," *Nanotechnology*, vol. 26, p. 314002, Aug. 2015.
- [151] I.-T. Chen and A. D. Stroock, "A tree-on-a-chip: design and analysis of MEMS-based superheated loop heat pipes exploiting nanoporous silicon membranes," *Journal of Physics: Conference Series*, vol. 557, p. 012067, Nov. 2014.
- [152] C.-R. Yang, C.-W. Lu, P.-C. Fu, C. Cheng, Y.-C. Chiou, R.-T. Lee, and S.-F. Tseng, "Performance evaluation of μ DMFCs based on porous-silicon electrodes and methanol modification," *Energy*, vol. 192, p. 116666, Feb. 2020.
- [153] I. Morisawa, T. Suzuki, N. Katayama, K. Dowaki, and M. Hayase, "Miniature fuel cell with monolithically fabricated Si electrodes ,Ä Uniformity of Catalyst Layer Thickness -," *Journal of Physics: Conference Series*, vol. 557, p. 012108, Nov. 2014.
- [154] M. Kobayashi, T. Suzuki, and M. Hayase, "A miniature fuel cell with monolithically fabricated Si electrodes ,Ä Reduction of residual porous Si on catalyst layer," *Journal of Power Sources*, vol. 267, pp. 622–628, Dec. 2014.
- [155] N. Sabaté, J. P. Esquivel, J. Santander, J. G. Hauer, R. W. Verjullo, I. Gràcia, M. Salleras, C. Calaza, E. Figueras, C. Cané, and L. Fonseca, "New approach for batch microfabrication of silicon-based micro fuel cells," *Microsystem Technologies*, vol. 20, pp. 341–348, Feb. 2014.
- [156] T. Leïchl  and D. Bourrier, "Integration of lateral porous silicon membranes into planar microfluidics," *Lab on a Chip*, vol. 15, no. 3, pp. 833–838, 2015.
- [157] Y. He and T. Leïchl , "Fabrication of lateral porous silicon membranes for planar microfluidics by means of ion implantation," *Sensors and Actuators B: Chemical*, vol. 239, pp. 628–634, Feb. 2017.
- [158] H. Hagino, S. Tanaka, N. Tanimura, and K. Miyazaki, "Thermal and Electrical Conductivities of Porous Si Membranes," *International Journal of Thermophysics*, vol. 36, pp. 2548–2564, Nov. 2015.
- [159] Y. Hosseini, S. Soltanian-Zadeh, V. Srinivasaraghavan, and M. Agah, "A Silicon-Based Porous Thin Membrane as a Cancer Cell Transmigration Assay," *Journal of Microelectromechanical Systems*, vol. 26, pp. 308–316, Apr. 2017.
- [160] B. N. G. Sajay, C. S. Yin, and Q. Ramadan, "Optimization of micro-fabricated porous membranes for intestinal epithelial cell culture and *in vitro* modeling of the human intestinal barrier," *Journal of Micromechanics and Microengineering*, vol. 27, p. 124004, Dec. 2017.

- [161] M. Sledzinska, B. Graczykowski, F. Alzina, U. Melia, K. Termentzidis, D. Lacroix, and C. M. S. Torres, "Thermal conductivity in disordered porous nanomembranes," *Nanotechnology*, vol. 30, p. 265401, Apr. 2019. Publisher: IOP Publishing.
- [162] A. Stephens, R. Nidetz, N. Mesyngier, M. T. Chung, Y. Song, J. Fu, and K. Kurabayashi, "Mass-producible microporous silicon membranes for specific leukocyte subset isolation, immunophenotyping, and personalized immunomodulatory drug screening *in vitro*," *Lab on a Chip*, vol. 19, no. 18, pp. 3065–3076, 2019.
- [163] B. Graczykowski, A. E. Sachat, J. S. Reparaz, M. Sledzinska, M. R. Wagner, E. Chavez-Angel, Y. Wu, S. Volz, Y. Wu, F. Alzina, and C. M. S. Torres, "Thermal conductivity and air-mediated losses in periodic porous silicon membranes at high temperatures," *Nature Communications*, vol. 8, pp. 1–9, Sept. 2017. Number: 1 Publisher: Nature Publishing Group.
- [164] M. Verdier, R. Anufriev, A. Ramiere, K. Termentzidis, and D. Lacroix, "Thermal conductivity of phononic membranes with aligned and staggered lattices of holes at room and low temperatures," *Physical Review B*, vol. 95, p. 205438, May 2017.
- [165] M. Verdier, D. Lacroix, and K. Termentzidis, "Heat transport in phononic-like membranes: Modeling and comparison with modulated nanowires," *International Journal of Heat and Mass Transfer*, vol. 114, pp. 550–558, Nov. 2017.
- [166] T. Ghoshal, J. D. Holmes, and M. A. Morris, "Development of Ordered, Porous (Sub-25 nm Dimensions) Surface Membrane Structures Using a Block Copolymer Approach," *Scientific Reports*, vol. 8, pp. 1–11, May 2018. Number: 1 Publisher: Nature Publishing Group.
- [167] S. Greil, A. Rahman, M. Liu, and C. T. Black, "Gas Transport Selectivity of Ultrathin, Nanoporous, Inorganic Membranes Made from Block Copolymer Templates," *Chemistry of Materials*, vol. 29, pp. 9572–9578, Nov. 2017. Publisher: American Chemical Society.
- [168] S. R. Gillmer, D. Z. Fang, S. E. Wayson, J. D. Winans, N. Abdolrahim, J.-P. S. DesOrmeaux, J. Getpreecharsawas, J. D. Ellis, P. M. Fauchet, and J. L. McGrath, "Predicting the failure of ultrathin porous membranes in bulge tests," *Thin Solid Films*, vol. 631, pp. 152–160, June 2017.
- [169] G. R. Madejski, K. Briggs, J.-P. DesOrmeaux, J. J. Miller, J. A. Roussie, V. Tabard-Cossa, and J. L. McGrath, "Monolithic Fabrication of NPN/SiNx Dual Membrane Cavity for Nanopore-Based DNA Sensing," *Advanced Materials Interfaces*, vol. 6, no. 14, p. 1900684, 2019. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/admi.201900684>.
- [170] C. Qi, C. C. Striemer, T. R. Gaborski, J. L. McGrath, and P. M. Fauchet, "Highly Porous Silicon Membranes Fabricated from Silicon Nitride/Silicon Stacks," *Small*, vol. 10, pp. 2946–2953, July 2014.

- [171] B. J. Nehilla, N. Nataraj, T. R. Gaborski, and J. L. McGrath, "Endothelial vacuolization induced by highly permeable silicon membranes," *Acta Biomaterialia*, vol. 10, pp. 4670–4677, Nov. 2014.
- [172] C. Drieschner, S. Könemann, P. Renaud, and K. Schirmer, "Fish-gut-on-chip: development of a microfluidic bioreactor to study the role of the fish intestine in vitro," *Lab on a Chip*, vol. 19, pp. 3268–3276, Sept. 2019. Publisher: The Royal Society of Chemistry.
- [173] C. Zhou, N. Tambo, E. M. Ashley, Y. Liao, J. Shiomi, K. Takahashi, G. S. W. Craig, and P. F. Nealey, "Enhanced Reduction of Thermal Conductivity in Amorphous Silicon Nitride-Containing Phononic Crystals Fabricated Using Directed Self-Assembly of Block Copolymers," *ACS Nano*, May 2020. Publisher: American Chemical Society.
- [174] M. Sairi, N. Chen-Tan, G. Neusser, C. Kranz, and D. W. M. Arriagan, "Electrochemical Characterisation of Nanoscale Liquid|Liquid Interfaces Located at Focused Ion Beam-Milled Silicon Nitride Membranes," *ChemElectroChem*, vol. 2, pp. 98–105, Jan. 2015.
- [175] Y. Bdour, J. Gomez-Cruz, and C. Escobedo, "Structural Stability of Optofluidic Nanostructures in Flow-Through Operation," *Micromachines*, vol. 11, p. 373, Apr. 2020. Number: 4 Publisher: Multidisciplinary Digital Publishing Institute.
- [176] J. P. S. DesOrmeaux, J. D. Winans, S. E. Wayson, T. R. Gaborski, T. S. Khire, C. C. Striemer, and J. L. McGrath, "Nanoporous silicon nitride membranes fabricated from porous nanocrystalline silicon templates," *Nanoscale*, vol. 6, pp. 10798–10805, Aug. 2014. Publisher: The Royal Society of Chemistry.
- [177] R. Maboudian, "Micro Devices: Stiction and Adhesion," in *Encyclopedia of Materials: Science and Technology* (K. H. J. Buschow, R. W. Cahn, M. C. Flemings, B. Ilshner, E. J. Kramer, S. Mahajan, and P. Veyssière, eds.), pp. 5591–5593, Oxford: Elsevier, Jan. 2001.
- [178] T. Sumigawa, S. Ashida, S. Tanaka, K. Sanada, and T. Kitamura, "Fracture toughness of silicon in nanometer-scale singular stress field," *Engineering Fracture Mechanics*, vol. 150, pp. 161–167, Dec. 2015.
- [179] G. Y. Gor, L. Bertinetti, N. Bernstein, T. Hofmann, P. Fratzl, and P. Huber, "Elastic response of mesoporous silicon to capillary pressures in the pores," *Applied Physics Letters*, vol. 106, p. 261901, June 2015.
- [180] A. Grosman, J. Puibasset, and E. Rolley, "Adsorption-induced strain of a nanoscale silicon honeycomb," *EPL (Europhysics Letters)*, vol. 109, p. 56002, Mar. 2015.
- [181] E. Rolley, N. Garroum, and A. Grosman, "Using capillary forces to determine the elastic properties of mesoporous materials," *Physical Review B*, vol. 95, p. 064106, Feb. 2017.

- [182] T. Hofmann, D. Wallacher, R. Toft-Petersen, B. Ryll, M. Reehuis, and K. Habicht, "Phonons in mesoporous silicon: The influence of nanostructuring on the dispersion in the Debye regime," *Microporous and Mesoporous Materials*, vol. 243, pp. 263–270, May 2017.
- [183] X. Gong, J. Bustillo, L. Blanc, and G. Gautier, "FEM simulation on elastic parameters of porous silicon with different pore shapes," *International Journal of Solids and Structures*, vol. 190, pp. 238–243, May 2020.
- [184] C. Populaire, B. Remaki, V. Lysenko, D. Barbier, H. Artmann, and T. Pannek, "On mechanical properties of nanostructured meso-porous silicon," *Applied Physics Letters*, vol. 83, pp. 1370–1372, Aug. 2003.
- [185] R. S. Dariani and M. Nazari, "Comparison of stress, strain, and elastic properties for porous silicon layers supported by substrate and corresponding membranes," *Journal of Molecular Structure*, vol. 1119, pp. 308–313, Sept. 2016.
- [186] R. Guider, C. Traversa, and P. Bettotti, "Mechanical stress relief in porous silicon free standing membranes," *Optical Materials Express*, vol. 5, p. 2128, Oct. 2015.
- [187] N. Burham, A. A. Hamzah, and B. Y. Majlis, "Mechanical characteristics of porous silicon membrane for filtration in artificial kidney," in *2014 IEEE International Conference on Semiconductor Electronics (ICSE2014)*, pp. 119–122, Aug. 2014. ISSN: null.
- [188] M. Fahmi bin Jaafar, R. Latif, and B. Y. Majlis, "Simulation on mechanical properties of porous nanocrystalline silicon membrane for artificial kidney," in *2017 IEEE Regional Symposium on Micro and Nanoelectronics (RSM)*, pp. 26–29, Aug. 2017. ISSN: null.
- [189] N. Kumar, E. Froner, R. Guider, M. Scarpa, and P. Bettotti, "Investigation of non-specific signals in nanoporous flow-through and flow-over based sensors," *The Analyst*, vol. 139, no. 6, p. 1345, 2014.
- [190] R. W. Baker, *Membrane Technology and Applications*. John Wiley & Sons, July 2012. Google-Books-ID: FhtBKUq4rL8C.
- [191] R. M. A. Roque-Malherbe, *Adsorption and Diffusion in Nanoporous Materials*. CRC Press, Feb. 2018. Google-Books-ID: p3FQDwAAQBAJ.
- [192] G. Korotcenkov, *Porous Silicon: From Formation to Application: Formation and Properties, Volume One: Formation and Properties*. CRC Press, Apr. 2016. Google-Books-ID: JWjdCgAAQBAJ.
- [193] Y. Lee and K. Lee, "Sensing of dissolved-gas concentration in water using a rugate-structured porous silicon membrane," *Sensors and Actuators B: Chemical*, vol. 202, pp. 417–425, Oct. 2014.

- [194] M. D. R. Jiménez Vivanco, G. García, R. Doti, J. Faubert, and J. E. Lugo Arce, “Time-Resolved Spectroscopy of Ethanol Evaporation on Free-Standing Porous Silicon Photonic Microcavities,” *Materials*, vol. 11, p. 894, June 2018. Number: 6 Publisher: Multidisciplinary Digital Publishing Institute.
- [195] W. He, I. V. Yurkevich, L. T. Canham, A. Loni, and A. Kaplan, “Determination of excitation profile and dielectric function spatial nonuniformity in porous silicon by using WKB approach,” *Optics Express*, vol. 22, p. 27123, Nov. 2014.
- [196] J. R. Knab, X. Lu, F. A. Vallejo, G. Kumar, T. E. Murphy, and L. M. Hayden, “Ultrafast carrier dynamics and optical properties of nanoporous silicon at terahertz frequencies,” *Optical Materials Express*, vol. 4, pp. 300–307, Feb. 2014. Publisher: Optical Society of America.
- [197] A. Zakar, S. J. Park, V. Zerova, A. Kaplan, L. T. Canham, K. L. Lewis, and C. D. Burgess, “MWIR optical modulation using structured silicon membranes,” in *Proceedings Volume 9992, Emerging Imaging and Sensing Technologies* (K. L. Lewis and R. C. Hollins, eds.), (Edinburgh, United Kingdom), p. 999203, Oct. 2016.
- [198] A. Zakar, R. Wu, D. Chekulaev, V. Zerova, W. He, L. Canham, and A. Kaplan, “Carrier dynamics and surface vibration-assisted Auger recombination in porous silicon,” *Physical Review B*, vol. 97, p. 155203, Apr. 2018.
- [199] W. He, R. Wu, I. V. Yurkevich, L. T. Canham, and A. Kaplan, “Reconstructing charge-carrier dynamics in porous silicon membranes from time-resolved interferometric measurements,” *Scientific Reports*, vol. 8, pp. 1–7, Nov. 2018. Number: 1 Publisher: Nature Publishing Group.
- [200] S. J. Park, A. Zakar, V. L. Zerova, D. Chekulaev, L. T. Canham, and A. Kaplan, “All-optical modulation in Mid-Wavelength Infrared using porous Si membranes,” *Scientific Reports*, vol. 6, pp. 1–8, July 2016. Number: 1 Publisher: Nature Publishing Group.
- [201] S. Anderson, H. Elliott, D. Wallis, L. Canham, and J. Powell, “Dissolution of different forms of partially porous silicon wafers under simulated physiological conditions,” *Physica Status Solidi (A) Applied Research*, vol. 197, no. 2, pp. 331–335, 2003. cited By 186.
- [202] M. Hecini, A. Khelifa, B. Bouzid, N. Drouiche, S. Aoudj, and H. Hamitouche, “Study of formation, stabilization and properties of porous silicon and porous silica,” *Journal of Physics and Chemistry of Solids*, vol. 74, pp. 1227–1234, Sept. 2013.
- [203] M. Stewart, E. Robins, T. Geders, M. Allen, H. Choi, and J. Buriak, “Three methods for stabilization and functionalization of porous silicon surfaces via hydrosilylation and electrografting reactions,” *Physica Status*

- Solids (A) Applied Research*, vol. 182, no. 1, pp. 109–115, 2000. cited By 57.
- [204] J. Rasson and L. A. Francis, “Improved Stability of Porous Silicon in Aqueous Media via Atomic Layer Deposition of Oxides,” *The Journal of Physical Chemistry C*, vol. 122, pp. 331–338, Jan. 2018.
- [205] J. Salonen, M. Björkqvist, E. Laine, and L. Niinistö, “Stabilization of porous silicon surface by thermal decomposition of acetylene,” *Applied Surface Science*, vol. 225, pp. 389–394, Mar. 2004.
- [206] W. Y. Tong, M. J. Sweetman, E. R. Marzouk, C. Fraser, T. Kuchel, and N. H. Voelcker, “Towards a subcutaneous optical biosensor based on thermally hydrocarbonised porous silicon,” *Biomaterials*, vol. 74, pp. 217–230, Jan. 2016.
- [207] Y. D. Irani, S. Klebe, S. J. P. McInnes, M. Jasieniak, N. H. Voelcker, and K. A. Williams, “Oral Mucosal Epithelial Cells Grown on Porous Silicon Membrane for Transfer to the Rat Eye,” *Scientific Reports*, vol. 7, pp. 1–11, Aug. 2017. Number: 1 Publisher: Nature Publishing Group.
- [208] Y. He, D. Bourrier, E. Imbernon, A. Bhaswara, X. Dollat, F. Cristiano, and T. Leïchlé, “Lateral porous silicon membranes with tunable pore size for on-chip separation,” in *2016 IEEE 29th International Conference on Micro Electro Mechanical Systems (MEMS)*, pp. 497–500, Jan. 2016. ISSN: null.
- [209] Y. He, D. Bourrier, E. Imbernon, and T. Leïchlé, “Lateral porous silicon membranes with size and charge selectivity,” in *2017 IEEE 12th International Conference on Nano/Micro Engineered and Molecular Systems (NEMS)*, pp. 770–773, Apr. 2017. ISSN: 2474-3755.
- [210] K. Hill, S. N. Walker, A. Salminen, H. L. Chung, X. Li, B. Ezzat, J. J. Miller, J. S. DesOrmeaux, J. Zhang, A. Hayden, T. Burgin, L. Piraino, M. N. May, T. R. Gaborski, J. A. Roussie, J. Taylor, L. DiVincenti, A. A. Shestopalov, J. L. McGrath, and D. G. Johnson, “Second Generation Nanoporous Silicon Nitride Membranes for High Toxin Clearance and Small Format Hemodialysis,” *Advanced Healthcare Materials*, vol. 9, p. 1900750, Feb. 2020.
- [211] J. W. Kuhlmann, I. P. Mey, and C. Steinem, “Modulating the Lateral Tension of Solvent-Free Pore-Spanning Membranes,” *Langmuir*, vol. 30, pp. 8186–8192, July 2014. Publisher: American Chemical Society.
- [212] M. Gleisner, I. Mey, M. Barbot, C. Dreker, M. Meinecke, and C. Steinem, “Driving a planar model system into the 3rd dimension: generation and control of curved pore-spanning membrane arrays,” *Soft Matter*, vol. 10, no. 33, pp. 6228–6236, 2014. Publisher: Royal Society of Chemistry.

- [213] I. Kusters, A. M. van Oijen, and A. J. M. Driessen, "Membrane-on-a-Chip: Microstructured Silicon/Silicon-Dioxide Chips for High-Throughput Screening of Membrane Transport and Viral Membrane Fusion," *ACS Nano*, vol. 8, pp. 3380–3392, Apr. 2014. Publisher: American Chemical Society.
- [214] L. L. G. Schwenen, R. Hubrich, D. Milovanovic, B. Geil, J. Yang, A. Kros, R. Jahn, and C. Steinem, "Resolving single membrane fusion events on planar pore-spanning membranes," *Scientific Reports*, vol. 5, pp. 1–15, July 2015. Number: 1 Publisher: Nature Publishing Group.
- [215] J. W. Kuhlmann, M. Junius, U. Diederichsen, and C. Steinem, "SNARE-Mediated Single-Vesicle Fusion Events with Supported and Freestanding Lipid Membranes," *Biophysical Journal*, vol. 112, pp. 2348–2356, June 2017.
- [216] J. Sibold, V. E. Tewaag, T. Vagedes, I. Mey, and C. Steinem, "Phase separation in pore-spanning membranes induced by differences in surface adhesion," *Physical Chemistry Chemical Physics*, p. 10.1039.D0CP00335B, 2020.
- [217] Y. Sun, X. Zang, Y. Sun, L. Wang, and Z. Gao, "Lipid membranes supported by planar porous substrates," *Chemistry and Physics of Lipids*, vol. 228, p. 104893, May 2020.
- [218] H.-G. Kim and K.-W. Lee, "Electrostatic gas sensor with a porous silicon diaphragm," *Sensors and Actuators B: Chemical*, vol. 219, pp. 10–16, Nov. 2015.
- [219] Y. He, D. S. D. Vasconcellos, V. Bardinal, D. Bourrier, E. Imbernon, L. Salvagnac, A. Laborde, X. Dollat, and T. Leichlé, "Lateral Porous Silicon Interferometric Transducer for Sensing Applications," in *2018 IEEE SENSORS*, pp. 1–3, Oct. 2018.
- [220] G. Gautier and S. Kouassi, "Integration of porous silicon in microfuel cells: a review," *International Journal of Energy Research*, vol. 39, no. 1, pp. 1–25, 2015. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/er.3206>.
- [221] M. Ge, X. Fang, J. Rong, and C. Zhou, "Review of porous silicon preparation and its application for lithium-ion battery anodes," *Nanotechnology*, vol. 24, p. 422001, Oct. 2013.
- [222] E. Luais, F. Ghamouss, J. Sakai, T. Defforge, G. Gautier, and F. Tran-Van, "Improved cycling performances of binder-free macroporous silicon Li-ion negative electrodes using room temperature ionic liquid electrolyte," *Journal of Solid State Electrochemistry*, vol. 23, pp. 937–941, Mar. 2019.
- [223] D. Hanks, J. Sircar, Z. Lu, D. Antao, K. Bagnall, B. Barabadi, T. Salamon, and E. Wang, "High heat flux evaporative nanoporous silicon membrane device for advanced thermal management," in *2016 Solid-State, Actuators, and Microsystems Workshop Technical Digest*, (Hilton Head, South Carolina, USA), pp. 380–383, Transducer Research Foundation, May 2016.

- [224] P. Bhadra, S. Sengupta, N. P. Ratchagar, B. Achar, A. Chadha, and E. Bhattacharya, "Selective transportation of charged ZnO nanoparticles and microorganism dialysis through silicon nanoporous membranes," *Journal of Membrane Science*, vol. 503, pp. 16–24, Apr. 2016.
- [225] T. H. Punde, W.-H. Wu, P.-C. Lien, Y.-L. Chang, P.-H. Kuo, M. D.-T. Chang, K.-Y. Lee, C.-D. Huang, H.-P. Kuo, Y.-F. Chan, P.-C. Shih, and C.-H. Liu, "A biologically inspired lung-on-a-chip device for the study of protein-induced lung inflammation," *Integrative Biology*, vol. 7, pp. 162–169, Feb. 2015.
- [226] W.-H. Wu, T. H. Punde, P.-C. Shih, C.-Y. Fu, T.-P. Wang, L. Hsu, H.-Y. Chang, and C.-H. Liu, "A capillary-endothelium-mimetic microfluidic chip for the study of immune responses," *Sensors and Actuators B: Chemical*, vol. 209, pp. 470–477, Mar. 2015.
- [227] D. Martín-Sánchez, T. Angelova, and J. García-Rupérez, "Simultaneous refractive index sensing using an array of suspended porous silicon membranes," *IEEE Sensors Journal*, pp. 1–1, 2020. Conference Name: IEEE Sensors Journal.
- [228] N. P. Ratchagar, V. T. Fidal, P. Bhadra, A. Ghosh, A. Chadha, and E. Bhattacharya, "Sensor for Continuous and Real-Time Monitoring of Biomolecule Permeation Through Ultrathin Silicon Nanoporous Membranes," *IEEE Sensors Journal*, vol. 19, pp. 4419–4427, June 2019. Conference Name: IEEE Sensors Journal.
- [229] N. Reta, A. Michelmoro, C. P. Saint, B. Prieto-Simon, and N. H. Voelcker, "Label-Free Bacterial Toxin Detection in Water Supplies Using Porous Silicon Nanochannel Sensors," *ACS Sensors*, vol. 4, pp. 1515–1523, June 2019. Publisher: American Chemical Society.
- [230] S.-W. Seo, H. R. Azmand, and A. N. Enemu, "Hollow Core Waveguide Sensor Array Based on a Macroporous Silicon Membrane Structure," *Journal of Lightwave Technology*, vol. 37, pp. 2036–2041, May 2019. Conference Name: Journal of Lightwave Technology.
- [231] H. Yamazaki, S. Kimura, M. Tsukahara, K. Esashika, and T. Saiki, "Optical detection of DNA translocation through silicon nanopore by ultraviolet light," *Applied Physics A*, vol. 115, pp. 53–56, Apr. 2014.
- [232] H. Yamazaki, S. Ito, K. Esashika, Y. Taguchi, and T. Saiki, "Optical observation of DNA translocation through Al₂O₃ sputtered silicon nanopores in porous membrane," *Applied Physics A*, vol. 122, p. 216, Feb. 2016.
- [233] H. Yamazaki, K. Esashika, and T. Saiki, "A 150 nm ultraviolet excitation volume on a porous silicon membrane for direct optical observation of DNA coil relaxation during capture into nanopores," *Nano Futures*, vol. 1, p. 011001, Apr. 2017.

- [234] N. Yu and J. Wu, "Rapid and reagentless detection of thrombin in clinic samples via microfluidic aptasensors with multiple target-binding sites," *Biosensors and Bioelectronics*, vol. 146, p. 111726, Dec. 2019.
- [235] Y. Zhao, G. A. Rodriguez, Y. M. Graham, T. Cao, G. Gaur, and S. M. Weiss, "Resonant photonic structures in porous silicon for biosensing," in *Frontiers in Biological Detection: From Nanosensors to Systems IX*, vol. 10081, p. 100810D, International Society for Optics and Photonics, Feb. 2017.
- [236] M. G. Bölen and T. Karacali, "Crossover of ion through porous silicon based membrane," in *2018 5th International Conference on Electrical and Electronic Engineering (ICEEE)*, pp. 302–305, May 2018. ISSN: null.
- [237] A. Roland, A. Dupuy, D. Machon, F. Cunin, N. Louvain, B. Fraisse, A. Boucherif, and L. Monconduit, "In-depth study of annealed porous silicon: Understand the morphological properties effect on negative LiB electrode performance," *Electrochimica Acta*, vol. 323, p. 134758, Nov. 2019.
- [238] W. J. C. Vijselaar, P. Perez, Rodríguez, P. J. Westerik, R. M. Tiggelaar, A. H. M. Smets, H. Gardeniers, and J. Huskens, "A Stand-Alone Si-Based Porous Photoelectrochemical Cell," *Advanced Energy Materials*, vol. 9, no. 19, p. 1803548, 2019. [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/aenm.201803548](https://onlinelibrary.wiley.com/doi/pdf/10.1002/aenm.201803548).
- [239] F. A. Harraz, "Porous silicon chemical sensors and biosensors: A review," *Sensors and Actuators B: Chemical*, vol. 202, pp. 897 – 912, 2014.
- [240] S. N. A. Jenie, S. E. Plush, and N. H. Voelcker, "Recent Advances on Luminescent Enhancement-Based Porous Silicon Biosensors," *Pharmaceutical Research*, vol. 33, pp. 2314–2336, Oct. 2016.
- [241] M. J. Sailor and E. C. Wu, "Photoluminescence-Based Sensing With Porous Silicon Films, Microparticles, and Nanoparticles," *Advanced Functional Materials*, vol. 19, no. 20, pp. 3195–3208, 2009.
- [242] R. Moretta, L. De Stefano, M. Terracciano, and I. Rea, "Porous Silicon Optical Devices: Recent Advances in Biosensing Applications," *Sensors*, vol. 21, p. 1336, Jan. 2021. Number: 4 Publisher: Multidisciplinary Digital Publishing Institute.
- [243] T. Cao, Y. Zhao, C. A. Nattoo, R. Layouni, and S. M. Weiss, "A smart-phone biosensor based on analysing structural colour of porous silicon," *Analyst*, vol. 144, no. 13, pp. 3942–3948, 2019.
- [244] S. Mariani, L. M. Strambini, and G. Barillaro, "Femtomole Detection of Proteins Using a Label-Free Nanostructured Porous Silicon Interferometer for Perspective Ultrasensitive Biosensing," *Analytical Chemistry*, vol. 88, pp. 8502–8509, Sept. 2016.

- [245] S. J. Ward, R. Layouni, S. Arshavsky-Graham, E. Segal, and S. M. Weiss, "Morlet Wavelet Filtering and Phase Analysis to Reduce the Limit of Detection for Thin Film Optical Biosensors," *ACS Sensors*, p. acssensors.1c00787, Aug. 2021.
- [246] S. Arshavsky-Graham, S. J. Ward, N. Massad-Ivanir, T. Scheper, S. M. Weiss, and E. Segal, "Porous Silicon-Based Aptasensors: Toward Cancer Protein Biomarker Detection," *ACS Measurement Science Au*, vol. 1, pp. 82–94, Oct. 2021. Publisher: American Chemical Society.
- [247] N. Abu-Thabit and E. Ratemi, "Hybrid Porous Silicon Biosensors Using Plasmonic and Fluorescent Nanomaterials: A Mini Review," *Frontiers in Chemistry*, vol. 8, 2020. Publisher: Frontiers.
- [248] C. RoyChaudhuri, "A review on porous silicon based electrochemical biosensors: Beyond surface area enhancement factor," *Sensors and Actuators B: Chemical*, vol. 210, pp. 310–323, Apr. 2015.
- [249] M. Yaghoubi, F. Rahimi, B. Negahdari, A. H. Rezayan, and A. Shafiekhani, "A lectin-coupled porous silicon-based biosensor: label-free optical detection of bacteria in a real-time mode," *Scientific Reports*, vol. 10, p. 16017, Sept. 2020. Number: 1 Publisher: Nature Publishing Group.
- [250] S. Zhuo, M. Xun, M. Li, X. Kong, R. Shao, T. Zheng, D. Pan, J. Li, and Q. Li, "Rapid and Label-free Optical Assay of S-layer Protein with High Sensitivity Using TiO₂-coated Porous Silicon-based Microfluidic Biosensor," *Sensors and Actuators B: Chemical*, p. 128524, June 2020.
- [251] D. N. Kumar, N. Pinker, and G. Shtenberg, "Porous Silicon Fabry-Pérot Interferometer for *N*-Acetyl- β -d-Glucosaminidase Biomarker Monitoring," *ACS Sensors*, vol. 5, pp. 1969–1976, July 2020.
- [252] H. Zhang, J. Lv, and Z. Jia, "Detection of Ammonia-Oxidizing Bacteria (AOB) Using a Porous Silicon Optical Biosensor Based on a Multilayered Double Bragg Mirror Structure," *Sensors*, vol. 18, p. 105, Jan. 2018.
- [253] N. Massad-Ivanir, G. Shtenberg, N. Raz, C. Gazenbeek, D. Budding, M. P. Bos, and E. Segal, "Porous Silicon-Based Biosensors: Towards Real-Time Optical Detection of Target Bacteria in the Food Industry," *Scientific Reports*, vol. 6, Dec. 2016.
- [254] Y. Tang, Z. Li, Q. Luo, J. Liu, and J. Wu, "Bacteria detection based on its blockage effect on silicon nanopore array," *Biosensors and Bioelectronics*, vol. 79, pp. 715–720, May 2016.
- [255] K. Urmann, S. Arshavsky-Graham, J. G. Walter, T. Scheper, and E. Segal, "Whole-cell detection of live *Lactobacillus acidophilus* on aptamer-decorated porous silicon biosensors," *The Analyst*, vol. 141, no. 18, pp. 5432–5440, 2016.

- [256] E. Tenenbaum and E. Segal, "Optical biosensors for bacteria detection by a peptidomimetic antimicrobial compound," *The Analyst*, vol. 140, no. 22, pp. 7726–7733, 2015.
- [257] N. Massad-Ivanir, Y. Mirsky, A. Nahor, E. Edrei, L. M. Bonanno-Young, N. Ben Dov, A. Sa'ar, and E. Segal, "Trap and track: designing self-reporting porous Si photonic crystals for rapid bacteria detection," *The Analyst*, vol. 139, no. 16, pp. 3885–3894, 2014.
- [258] Y. Mirsky, A. Nahor, E. Edrei, N. Massad-Ivanir, L. M. Bonanno, E. Segal, and A. Sa'ar, "Optical biosensing of bacteria and cells using porous silicon based, photonic lamellar gratings," *Applied Physics Letters*, vol. 103, p. 033702, July 2013.
- [259] N. Samanta, O. Kundu, and C. R. Chaudhuri, "A Simple Low Power Electronic Readout for Rapid Bacteria Detection With Impedance Biosensor," *IEEE Sensors Journal*, vol. 13, pp. 4716–4724, Dec. 2013.
- [260] R. Dev Das, A. Dey, S. Das, and C. RoyChaudhuri, "Interdigitated Electrode-Less High-Performance Macroporous Silicon Structure as Impedance Biosensor for Bacteria Detection," *IEEE Sensors Journal*, vol. 11, pp. 1242–1252, May 2011.
- [261] R. D. Das, N. Mondal, S. Das, and C. RoyChaudhuri, "Optimized Electrode Geometry for an Improved Impedance Based Macroporous Silicon Bacteria Detector," *IEEE Sensors Journal*, vol. 12, pp. 1868–1877, June 2012.
- [262] L. De Stefano, "Porous Silicon Optical Biosensors: Still a Promise or a Failure?," *Sensors*, vol. 19, p. 4776, Jan. 2019. Number: 21 Publisher: Multidisciplinary Digital Publishing Institute.
- [263] A. Leprince, M. Nuytten, A. Gillis, and J. Mahillon, "Characterization of PlyB221 and PlyP32, Two Novel Endolysins Encoded by Phages Preying on the *Bacillus cereus* Group," *Viruses*, vol. 12, p. 1052, Sept. 2020. Number: 9 Publisher: Multidisciplinary Digital Publishing Institute.
- [264] H. Sohn, "Refractive Index of Porous Silicon," in *Handbook of Porous Silicon* (L. Canham, ed.), pp. 231–243, Cham: Springer International Publishing, 2014.
- [265] N. An, B. Zhuang, M. Li, Y. Lu, and Z.-G. Wang, "Combined Theoretical and Experimental Study of Refractive Indices of Water, Acetonitrile, Salt Systems," *The Journal of Physical Chemistry B*, vol. 119, pp. 10701–10709, Aug. 2015. Publisher: American Chemical Society.
- [266] A. Vendeville, D. Larivière, and E. Fourmentin, "An inventory of the bacterial macromolecular components and their spatial organization," *FEMS Microbiology Reviews*, vol. 35, pp. 395–414, Mar. 2011.

- [267] R. Milo, “What is the total number of protein molecules per cell volume? A call to rethink some published values,” *Bioessays*, vol. 35, pp. 1050–1055, Dec. 2013.
- [268] T. Ando and J. Skolnick, “Crowding and hydrodynamic interactions likely dominate in vivo macromolecular motion,” *Proceedings of the National Academy of Sciences*, vol. 107, pp. 18457–18462, Oct. 2010. Publisher: Proceedings of the National Academy of Sciences.
- [269] J. A. Valkenburg and C. L. Woldringh, “Phase separation between nucleoid and cytoplasm in *Escherichia coli* as defined by immersive refractometry,” *Journal of Bacteriology*, vol. 160, pp. 1151–1157, Dec. 1984.
- [270] V. T. Hoang, G. Stepniewski, K. H. Czarnecka, R. Kasztelanic, V. C. Long, K. D. Xuan, L. Shao, M. Śmietana, and R. Buczyński, “Optical Properties of Buffers and Cell Culture Media for Optofluidic and Sensing Applications,” *Applied Sciences*, vol. 9, p. 1145, Mar. 2019.
- [271] D. H. Ge, J. W. Jiao, S. Zhang, and Y. L. Wang, “Fast speed nano-sized macropore formation on highly-doped n-type silicon via strong oxidizers,” *Electrochemistry Communications*, vol. 12, pp. 603–606, Apr. 2010.
- [272] X. G. Zhang, *Electrochemistry of Silicon and Its Oxide*. Springer Science & Business Media, May 2007. Google-Books-ID: eIFCAAQBAJ.
- [273] S. Billat, M. Thönissen, R. Arens-Fischer, M. G. Berger, M. Krüger, and H. Lüth, “Influence of etch stops on the microstructure of porous silicon layers,” *Thin Solid Films*, vol. 297, pp. 22–25, Apr. 1997.
- [274] J. W. Jeong, J. Snay, and M. M. Ataai, “A mathematical model for examining growth and sporulation processes of *Bacillus subtilis*,” *Biotechnology and Bioengineering*, vol. 35, pp. 160–184, Jan. 1990.
- [275] H. E. Kubitschek and J. A. Friske, “Determination of bacterial cell volume with the Coulter Counter,” *Journal of Bacteriology*, vol. 168, pp. 1466–1467, Dec. 1986.
- [276] R. A. Cox and H. R. V. Arnstein, “Translation of RNA to Protein,” in *Encyclopedia of Physical Science and Technology (Third Edition)* (R. A. Meyers, ed.), pp. 31–51, New York: Academic Press, Jan. 2003.
- [277] A. Leprince, *Characterization of the adsorption and lysis processes of bacteriophages infecting the *Bacillus cereus* group*. PhD thesis, Université Catholique de Louvain, 2022.
- [278] M. J. Love, D. Coombes, S. Ismail, C. Billington, and R. C. J. Dobson, “The structure and function of modular *Escherichia coli* O157:H7 bacteriophage FTBEc1 endolysin, LysT84: defining a new endolysin catalytic subfamily,” *Biochemical Journal*, vol. 479, pp. 207–223, Jan. 2022.

- [279] N. H. Maniya and D. N. Srivastava, "Fabrication of porous silicon based label-free optical biosensor for heat shock protein 70 detection," *Materials Science in Semiconductor Processing*, vol. 115, p. 105126, Aug. 2020.
- [280] D. N. Kumar, N. Pinker, and G. Shtenberg, "Inflammatory biomarker detection in milk using label-free porous SiO₂ interferometer," *Talanta*, vol. 220, p. 121439, Dec. 2020.
- [281] S. Arshavsky-Graham, N. Massad-Ivanir, F. Paratore, T. Scheper, M. Bercovici, and E. Segal, "On Chip Protein Pre-Concentration for Enhancing the Sensitivity of Porous Silicon Biosensors," *ACS Sensors*, vol. 2, pp. 1767–1773, Dec. 2017. Publisher: American Chemical Society.
- [282] L. Canham, "Porous Silicon Membranes," in *Handbook of Porous Silicon* (L. Canham, ed.), pp. 163–170, Cham: Springer International Publishing, 2014.
- [283] D. Martín-Sánchez, S. Ponce-Alcántara, and J. García-Rupérez, "Flow-Through Self-Standing Porous Silicon Sensor," *Proceedings*, vol. 4, p. 17, Nov. 2018.
- [284] A. Janshoff, K.-P. S. Dancil, C. Steinem, D. P. Greiner, V. S.-Y. Lin, C. Gurtner, K. Motesharei, M. J. Sailor, and M. R. Ghadiri, "Macroporous p-Type Silicon Fabry-Perot Layers. Fabrication, Characterization, and Applications in Biosensing," *Journal of the American Chemical Society*, vol. 120, pp. 12108–12116, Nov. 1998.
- [285] M. J. Sailor, "Chemical Reactivity and Surface Chemistry of Porous Silicon," in *Handbook of Porous Silicon* (L. Canham, ed.), pp. 355–380, Cham: Springer International Publishing, 2014.
- [286] S. H. Lee, J. S. Kang, and D. Kim, "A Mini Review: Recent Advances in Surface Modification of Porous Silicon," *Materials*, vol. 11, p. 2557, Dec. 2018. Number: 12 Publisher: Multidisciplinary Digital Publishing Institute.
- [287] S. M. George, "Atomic Layer Deposition: An Overview," *Chemical Reviews*, vol. 110, pp. 111–131, Jan. 2010.
- [288] I. Iatsunskyi, M. Jancelewicz, G. Nowaczyk, M. Kempinski, B. Peplińska, M. Jarek, K. Zaleski, S. Jurga, and V. Smyntyna, "Atomic layer deposition TiO₂ coated porous silicon surface: Structural characterization and morphological features," *Thin Solid Films*, vol. 589, pp. 303–308, Aug. 2015.
- [289] I. Iatsunskyi, M. Kempinski, M. Jancelewicz, K. Zaleski, S. Jurga, and V. Smyntyna, "Structural and XPS characterization of ALD Al₂O₃ coated porous silicon," *Vacuum*, vol. 113, pp. 52–58, Mar. 2015.
- [290] E. Segal, L. A. Perelman, F. Cunin, F. Di Renzo, J.-M. Devoisselle, Y. Y. Li, and M. J. Sailor, "Confinement of Thermoresponsive Hydrogels in Nanostructured Porous Silicon Dioxide Templates," *Advanced Functional Materials*, vol. 17, no. 7, pp. 1153–1162, 2007. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adfm.200601077>.

- [291] D. MacDougall, W. B. Crummett, and . et al., "Guidelines for data acquisition and data quality evaluation in environmental chemistry," *Analytical Chemistry*, vol. 52, pp. 2242–2249, Dec. 1980. Publisher: American Chemical Society.
- [292] S. Mariani, L. M. Strambini, and G. Barillaro, "Electrical Double Layer-Induced Ion Surface Accumulation for Ultrasensitive Refractive Index Sensing with Nanostructured Porous Silicon Interferometers," *ACS Sensors*, vol. 3, pp. 595–605, Mar. 2018.
- [293] J. Robertson, "High dielectric constant oxides," *The European Physical Journal Applied Physics*, vol. 28, pp. 265–291, Dec. 2004. Number: 3 Publisher: EDP Sciences.
- [294] W. W. Wilson, M. M. Wade, S. C. Holman, and F. R. Champlin, "Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements," *Journal of Microbiological Methods*, vol. 43, pp. 153–164, Jan. 2001.
- [295] D. V. Fedyukina, T. S. Jennaro, and S. Cavagnero, "Charge Segregation and Low Hydrophobicity Are Key Features of Ribosomal Proteins from Different Organisms," *Journal of Biological Chemistry*, vol. 289, pp. 6740–6750, Mar. 2014.
- [296] A. R. Arnold, M. A. Grodick, and J. K. Barton, "DNA Charge Transport: From Chemical Principles to the Cell," *Cell chemical biology*, vol. 23, pp. 183–197, Jan. 2016.
- [297] D. Zamani, M. Keswani, O. Mahdavi, J. Yan, S. Raghavan, and F. Shadman, "Dynamics of Interactions Between HF and Hafnium Oxide During Surface Preparation of High-K Dielectrics," *IEEE Transactions on Semiconductor Manufacturing*, vol. 25, pp. 511–515, Aug. 2012. Conference Name: IEEE Transactions on Semiconductor Manufacturing.
- [298] Z. Xia, V. Rozyyev, A. U. Mane, J. W. Elam, and S. B. Darling, "Surface Zeta Potential of ALD-Grown Metal-Oxide Films," *Langmuir*, vol. 37, pp. 11618–11624, Oct. 2021. Publisher: American Chemical Society.
- [299] M. Ikeda, Y. Yagihara, K. Tatsuno, M. Okazaki, S. Okugawa, and K. Moriya, "Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* blood stream infections," *Annals of Clinical Microbiology and Antimicrobials*, vol. 14, p. 43, Sept. 2015.
- [300] V. S.-Y. Lin, K. Motesharei, K.-P. S. Dancil, M. J. Sailor, and M. R. Ghadiri, "A Porous Silicon-Based Optical Interferometric Biosensor," *Science*, vol. 278, pp. 840–843, Oct. 1997.
- [301] R. Herino, G. Bomchil, K. Barla, C. Bertrand, and J. L. Ginoux, "Porosity and Pore Size Distributions of Porous Silicon Layers," *Journal of The Electrochemical Society*, vol. 134, p. 1994, Aug. 1987. Publisher: IOP Publishing.

- [302] G. Shtenberg and E. Segal, "Porous Silicon Optical Biosensors," in *Handbook of Porous Silicon* (L. Canham, ed.), pp. 857–868, Cham: Springer International Publishing, 2014.
- [303] S. Arshavsky Graham, E. Boyko, R. Salama, and E. Segal, "Mass Transfer Limitations of Porous Silicon-Based Biosensors for Protein Detection," *ACS Sensors*, vol. 5, pp. 3058–3069, Oct. 2020.
- [304] M. d. C. d. F. Bastos, B. G. Coutinho, and M. L. V. Coelho, "Lysostaphin: A Staphylococcal Bacteriolysin with Potential Clinical Applications," *Pharmaceuticals*, vol. 3, pp. 1139–1161, Apr. 2010.
- [305] T. C. O. T. E. COMMUNITIES, "Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs (Text with EEA relevance)," Nov. 2005. Legislative Body: COM.
- [306] L. Powell, R. S. Wiederkehr, P. Damascus, M. Fauvart, F. Buja, T. Stakenborg, S. C. Ray, P. Fiorini, and W. O. Osburn, "Rapid and sensitive detection of viral nucleic acids using silicon microchips," *Analyst*, vol. 143, pp. 2596–2603, May 2018. Publisher: The Royal Society of Chemistry.
- [307] Q. Cai, M. Fauvart, R. S. Wiederkehr, B. Jones, P. Cools, P. Goos, M. Vaneechoutte, and T. Stakenborg, "Ultra-fast, sensitive and quantitative on-chip detection of group B streptococci in clinical samples," *Talanta*, vol. 192, pp. 220–225, Jan. 2019.
- [308] H. O. Kaya, A. E. Cetin, M. Azimzadeh, and S. N. Topkaya, "Pathogen detection with electrochemical biosensors: Advantages, challenges and future perspectives," *Journal of Electroanalytical Chemistry*, vol. 882, p. 114989, 2021.
- [309] G. Xing, W. Zhang, N. Li, Q. Pu, and J.-M. Lin, "Recent progress on microfluidic biosensors for rapid detection of pathogenic bacteria," *Chinese Chemical Letters*, vol. 33, no. 4, pp. 1743–1751, 2022.
- [310] Q. Zhang, L. Zhong, P. Tang, Y. Yuan, S. Liu, J. Tian, and X. Lu, "Quantitative refractive index distribution of single cell by combining phase-shifting interferometry and AFM imaging," *Scientific Reports*, vol. 7, pp. 1–10, May 2017.
- [311] Y. P. Liu, "Refractive Index Distribution of Single Cell and Bacterium Usingan Optical Diffraction Tomography System," 2016.
- [312] X. Fan, I. M. White, S. I. Shopova, H. Zhu, J. D. Suter, and Y. Sun, "Sensitive optical biosensors for unlabeled targets: A review," *Analytica Chimica Acta*, vol. 620, pp. 8–26, July 2008.
- [313] A. Fernández Gavela, D. Grajales García, J. C. Ramirez, and L. M. Lechuga, "Last Advances in Silicon-Based Optical Biosensors," *Sensors*, vol. 16, p. 285, Mar. 2016. Number: 3 Publisher: Multidisciplinary Digital Publishing Institute.

- [314] R. Caroselli, D. Martán Sánchez, S. Ponce Alcántara, F. Prats Quilez, L. Torrijos Morán, and J. García-Rupérez, “Real-Time and In-Flow Sensing Using a High Sensitivity Porous Silicon Microcavity-Based Sensor,” *Sensors*, vol. 17, p. 2813, Dec. 2017. Number: 12 Publisher: Multidisciplinary Digital Publishing Institute.
- [315] R. F. Balderas-Valadez, R. Schürmann, and C. Pacholski, “One Spot-Two Sensors: Porous Silicon Interferometers in Combination With Gold Nanostructures Showing Localized Surface Plasmon Resonance,” *Frontiers in Chemistry*, vol. 7, 2019. Publisher: Frontiers.
- [316] Y. He, D. S. de Vasconcellos, D. Bourrier, K. Hajdu, J.-O. Durand, F. Cunin, V. Bardinal, and T. Leichlé, “Lateral porous silicon interferometric transducer for on-chip flow-through sensing applications,” *Sensors and Actuators A: Physical*, vol. 332, p. 113089, Dec. 2021.
- [317] A. Keating, “Porous Silicon Diffraction Gratings,” in *Handbook of Porous Silicon* (L. Canham, ed.), pp. 823–833, Cham: Springer International Publishing, 2014.
- [318] A. M. Ruminski, B. H. King, J. Salonen, J. L. Snyder, and M. J. Sailor, “Porous Silicon-Based Optical Microsensors for Volatile Organic Analytes: Effect of Surface Chemistry on Stability and Specificity,” *Advanced Functional Materials*, vol. 20, no. 17, pp. 2874–2883, 2010. _eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/adfm.201000575>.
- [319] L. Ahmadi, M. Hiltunen, J. Hiltunen, S. Aikio, J. Saarinen, S. Honkanen, and M. Roussey, “Influence of an Al₂O₃ surface coating on the response of polymeric waveguide sensors,” *Optics Express*, vol. 25, p. 25102, Oct. 2017.
- [320] S. Armbruster, F. Schafer, G. Lammel, H. Artmann, C. Schelling, H. Benzel, S. Finkbeiner, F. Larmer, R. Ruther, and O. Paul, “A novel micromachining process for the fabrication of monocrystalline Si-membranes using porous silicon,” in *TRANSDUCERS '03. 12th International Conference on Solid-State Sensors, Actuators and Microsystems. Digest of Technical Papers (Cat. No.03TH8664)*, vol. 1, pp. 246–249 vol.1, June 2003.
- [321] H. Artmann, F. Schaefer, G. Lammel, S. Armbruster, H. Benzel, C. Schelling, H. Weber, H.-G. Vossenberger, R. Gampp, J. Muchow, F. Laermer, and S. Finkbeiner, “Monocrystalline Si membranes for pressure sensors fabricated by a novel surface-micromachining process using porous silicon,” in *MEMS Components and Applications for Industry, Automobiles, Aerospace, and Communication II*, vol. 4981, pp. 65–70, International Society for Optics and Photonics, Jan. 2003.
- [322] K. Knese, S. Armbruster, H. Weber, M. Fischer, H. Benzel, M. Metz, and H. Seidel, “Novel Technology for Capacitive Pressure Sensors with Monocrystalline Silicon Membranes,” in *2009 IEEE 22nd International*

- Conference on Micro Electro Mechanical Systems*, pp. 697–700, Jan. 2009. ISSN: 1084-6999.
- [323] A. C. Fischer, F. Forsberg, M. Lapisa, S. J. Bleiker, G. Stemme, N. Roxhed, and F. Niklaus, “Integrating MEMS and ICs,” *Microsystems & Nanoengineering*, vol. 1, pp. 1–16, May 2015. Number: 1 Publisher: Nature Publishing Group.
- [324] G. Gautier, T. Defforge, S. Desplobain, J. Billoue, M. Capelle, P. Poveda, K. Vanga, B. Lu, B. Bardet, J. Lascaud, C. Seck, A. Fevre, S. Menard, and L. Ventura, “Porous Silicon in Microelectronics: From Academic Studies to Industry,” *ECS Transactions*, vol. 69, pp. 123–134, Oct. 2015.
- [325] G. Barillaro and L. M. Strambini, “An integrated CMOS sensing chip for NO₂ detection,” *Sensors and Actuators B: Chemical*, vol. 134, pp. 585–590, Sept. 2008.
- [326] G. Barillaro, P. Bruschi, G. M. Lazzerini, and L. M. Strambini, “Validation of the Compatibility Between a Porous Silicon-Based Gas Sensor Technology and Standard Microelectronic Process,” *IEEE Sensors Journal*, vol. 10, pp. 893–899, Apr. 2010. Conference Name: IEEE Sensors Journal.
- [327] M. Capelle, J. Billoué, P. Poveda, and G. Gautier, “Porous Silicon/Silicon Hybrid Substrate Applied to the Monolithic Integration of Common-Mode and Bandpass RF Filters,” *IEEE Transactions on Electron Devices*, vol. 62, pp. 4169–4173, Dec. 2015. Conference Name: IEEE Transactions on Electron Devices.
- [328] M. Capelle, J. Billoue, J. Concord, P. Poveda, and G. Gautier, “Monolithic integration of low-pass filters with ESD protections on p+ silicon/porous silicon substrates,” *Solid-State Electronics*, vol. 116, pp. 12–14, Feb. 2016.
- [329] “NETPORE.”
- [330] T. Urata, K. Fukami, T. Sakka, and Y. H. Ogata, “Pore formation in p-type silicon in solutions containing different types of alcohol,” *Nanoscale Research Letters*, vol. 7, p. 329, June 2012.
- [331] D. S. de Vasconcellos, *Monolithic Integration of Multiple Porous Silicon Membranes for Lab-on-a-chip Applications*. Micro and nanotechnologies/Microelectronics, Université Paul Sabatier Toulouse III, 2020.
- [332] H. Lee, S. M. Dellatore, W. M. Miller, and P. B. Messersmith, “Mussel-Inspired Surface Chemistry for Multifunctional Coatings,” *Science*, vol. 318, pp. 426–430, Oct. 2007.
- [333] K. Kang, I. S. Choi, and Y. Nam, “A biofunctionalization scheme for neural interfaces using polydopamine polymer,” *Biomaterials*, vol. 32, pp. 6374–6380, Sept. 2011.

- [334] S. C. Becker, J. Foster-Frey, and D. M. Donovan, "The phage K lytic enzyme LysK and lysostaphin act synergistically to kill MRSA," *FEMS microbiology letters*, vol. 287, pp. 185–191, Oct. 2008.
- [335] M. Schmelcher, A. M. Powell, S. C. Becker, M. J. Camp, and D. M. Donovan, "Chimeric phage lysins act synergistically with lysostaphin to kill mastitis-causing *Staphylococcus aureus* in murine mammary glands," *Applied and Environmental Microbiology*, vol. 78, pp. 2297–2305, Apr. 2012.
- [336] M. Schmelcher, A. M. Powell, M. J. Camp, C. S. Pohl, and D. M. Donovan, "Synergistic streptococcal phage Φ^{a} SA2 and B30 endolysins kill streptococci in cow milk and in a mouse model of mastitis," *Applied Microbiology and Biotechnology*, vol. 99, pp. 8475–8486, Oct. 2015.
- [337] R. B. Balili, "Transfer matrix method in nanophotonics," *International Journal of Modern Physics: Conference Series*, vol. 17, pp. 159–168, Jan. 2012. Publisher: World Scientific Publishing Co.
- [338] M. C. Troparevsky, A. S. Sabau, A. R. Lupini, and Z. Zhang, "Transfer-matrix formalism for the calculation of optical response in multilayer systems: from coherent to incoherent interference," *Optics Express*, vol. 18, pp. 24715–24721, Nov. 2010. Publisher: Optical Society of America.
- [339] N. P. K. Cotter, T. W. Preist, and J. R. Sambles, "Scattering-matrix approach to multilayer diffraction," *Journal of the Optical Society of America A*, vol. 12, p. 1097, May 1995.
- [340] C. Kneale, J. Chandezon, and K. S. Booksh, "Mode Characterization for Planar and Corrugated Multilayer Structures via Scattering Matrix Analysis," *arXiv:1611.09896 [physics]*, Jan. 2017. arXiv: 1611.09896.
- [341] R. C. Rumpf, "Improved formulation of scattering matrices for semi-analytical methods that is consistent with convention," *Progress In Electromagnetics Research B*, vol. 35, p. 21, 2011.
- [342] V. A. Fischetti, "Development of phage lysins as novel therapeutics: A historical perspective," *Viruses*, vol. 10, no. 6, 2018.
- [343] R. Schuch, A. J. Pelzek, D. C. Nelson, and V. A. Fischetti, "The PlyB Endolysin of Bacteriophage ν B_{bans}_bcp1 Exhibits Broad-Spectrum Bactericidal Activity against *Bacillus cereus* Sensus Lato Isolates," *Applied and Environmental Microbiology*, vol. 85, pp. e00003–19, May 2019. Publisher: American Society for Microbiology.
- [344] I. Etobayeva, S. B. Linden, F. Alem, L. Harb, L. Rizkalla, P. D. Mosier, A. A. Johnson, L. Temple, R. M. Hakami, and D. C. Nelson, "Discovery and Biochemical Characterization of PlyP56, PlyN74, and PlyTB40-Bacillus Specific Endolysins," *Viruses*, vol. 10, p. 276, May 2018. Number: 5 Publisher: Multidisciplinary Digital Publishing Institute.

-
- [345] Y. Yuan, Q. Peng, and M. Gao, "Characteristics of a broad lytic spectrum endolysin from phage BtCS33 of *Bacillus thuringiensis*," *BMC Microbiology*, vol. 12, p. 297, Dec. 2012.
- [346] Q. Peng and Y. Yuan, "Characterization of a novel phage infecting the pathogenic multidrug-resistant *Bacillus cereus* and functional analysis of its endolysin," *Applied Microbiology and Biotechnology*, vol. 102, pp. 7901–7912, Sept. 2018.