2nd WINFAB DAY 2014

Impedance spectroscopy using lysin as selectivity means for detection of bacteria

N. Couniot[1], T. Vanzieleghem[2], J. Rasson[1], N. Van-Overstraeten[1], O. Poncelet[1], J. Mahillon[2], L.A. Francis[1], D. Flandre[1]

Based on ***"Lytic enzymes as selectivity means for impedimetric detection of bacteria using ALD-Al_2O_3 passivated microelectrodes"***
, In Press, Biosensors and Bioelectronics, 2014

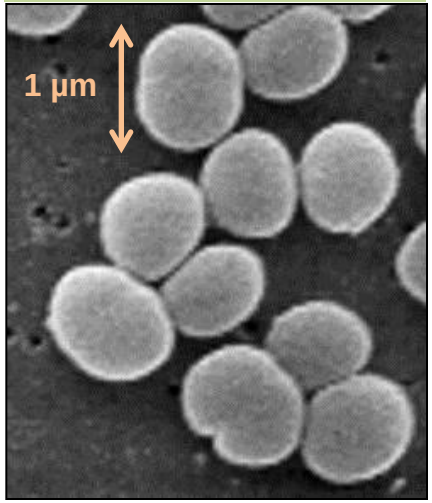
[1]: ICTEAM Institute, UCL
[2]: ELIM Institute, UCL

Slowest Part

Frame of this study

Multidisciplinary work

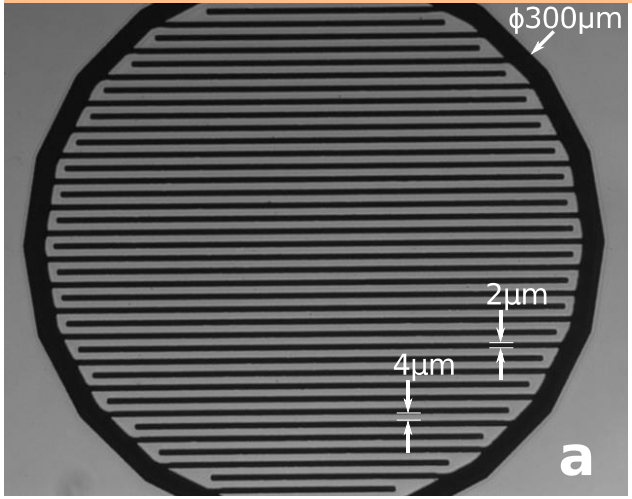
Bacterial cell



Staphylococcus epidermidis

- Similar to S. aureus
- Non-pathogenic

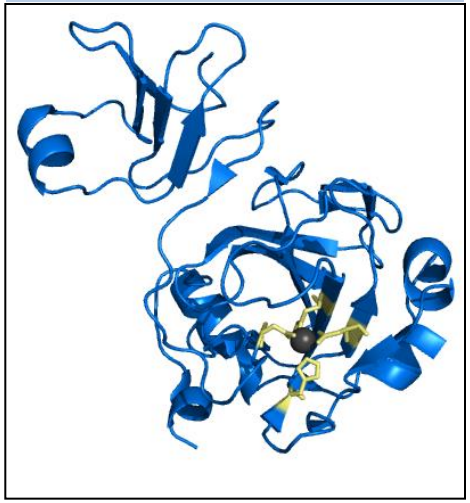
Transducer



Interdigitated microelectrodes (Al/Al_2O_3)

- High active area
- Similar dimensions as bacteria
- Electric field in surface
- Compact and CMOS-compatible

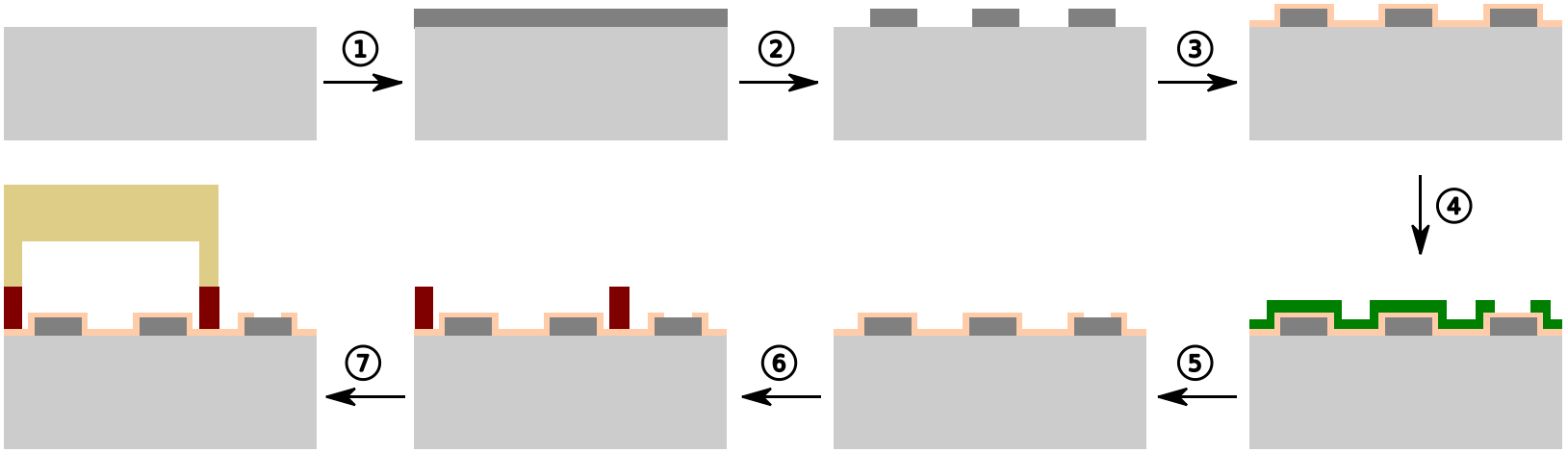
Selective agent



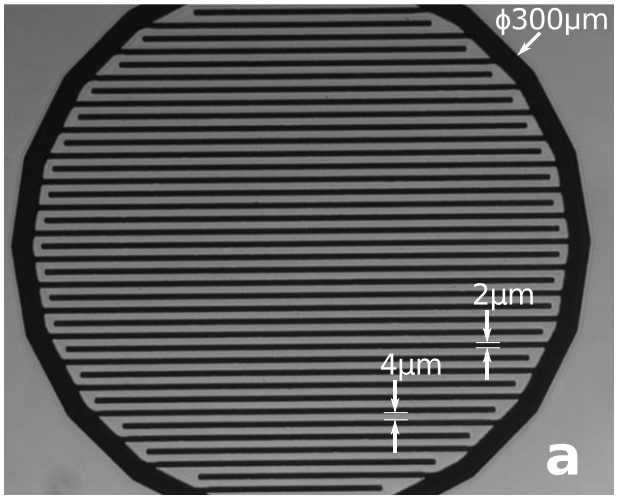
Lysostaphin

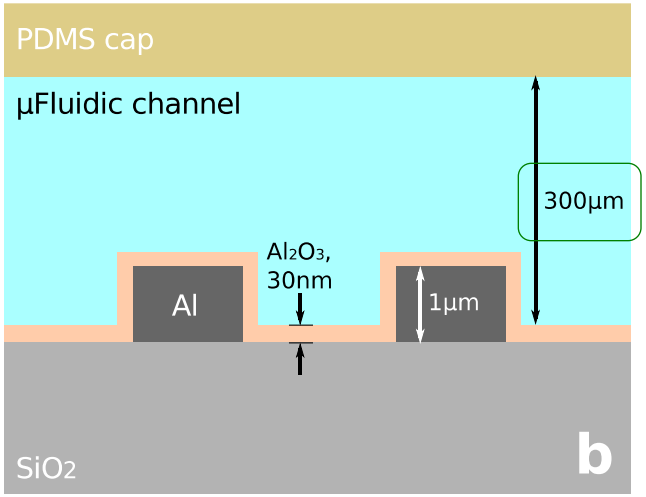
- Selectively destroy bacterial cell wall
- Low cost
- Can be extended to all Gram+ bacteria by using lysins

Transducer microfabrication



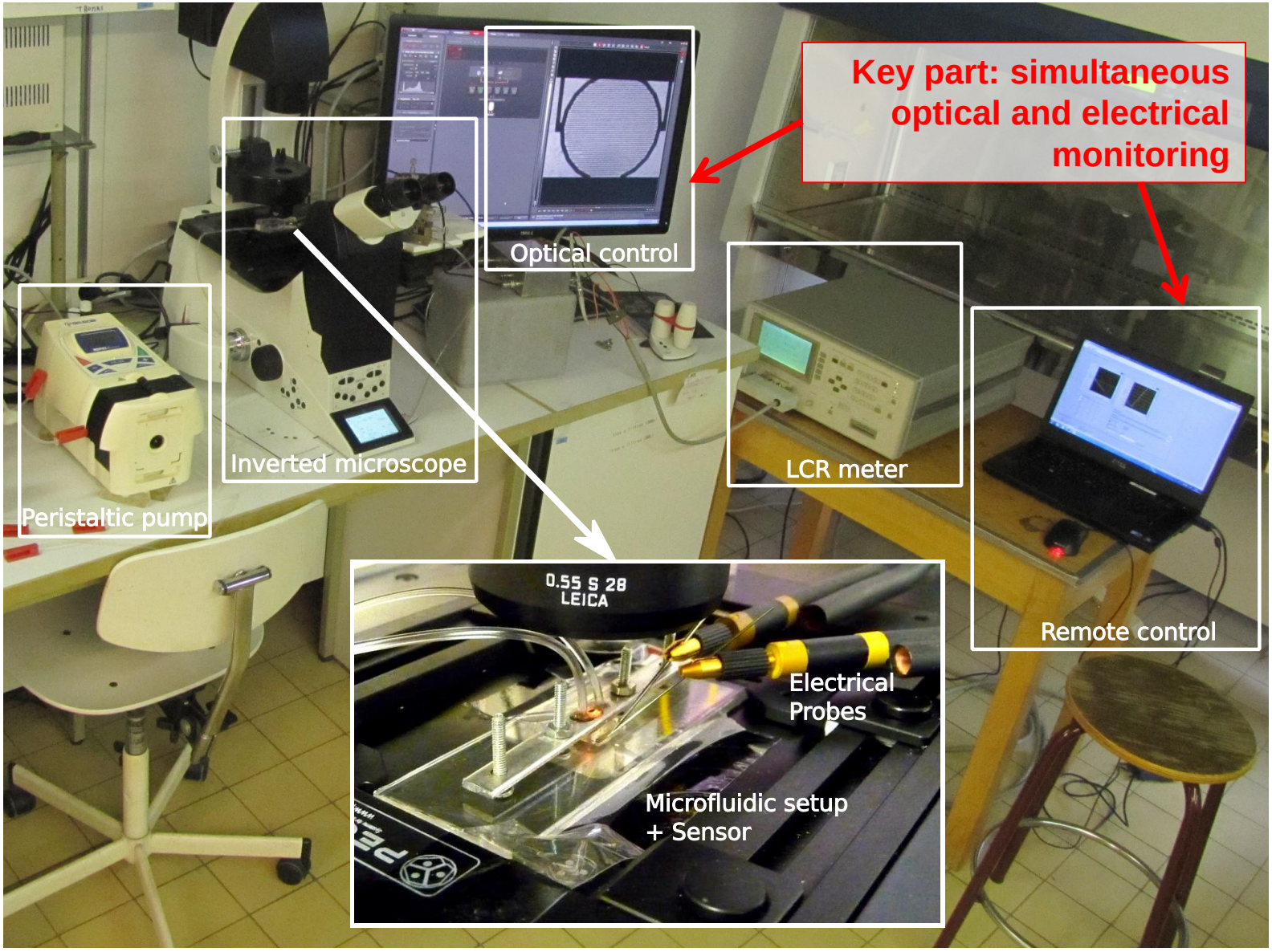
Transparency **CMOS-compatibility**



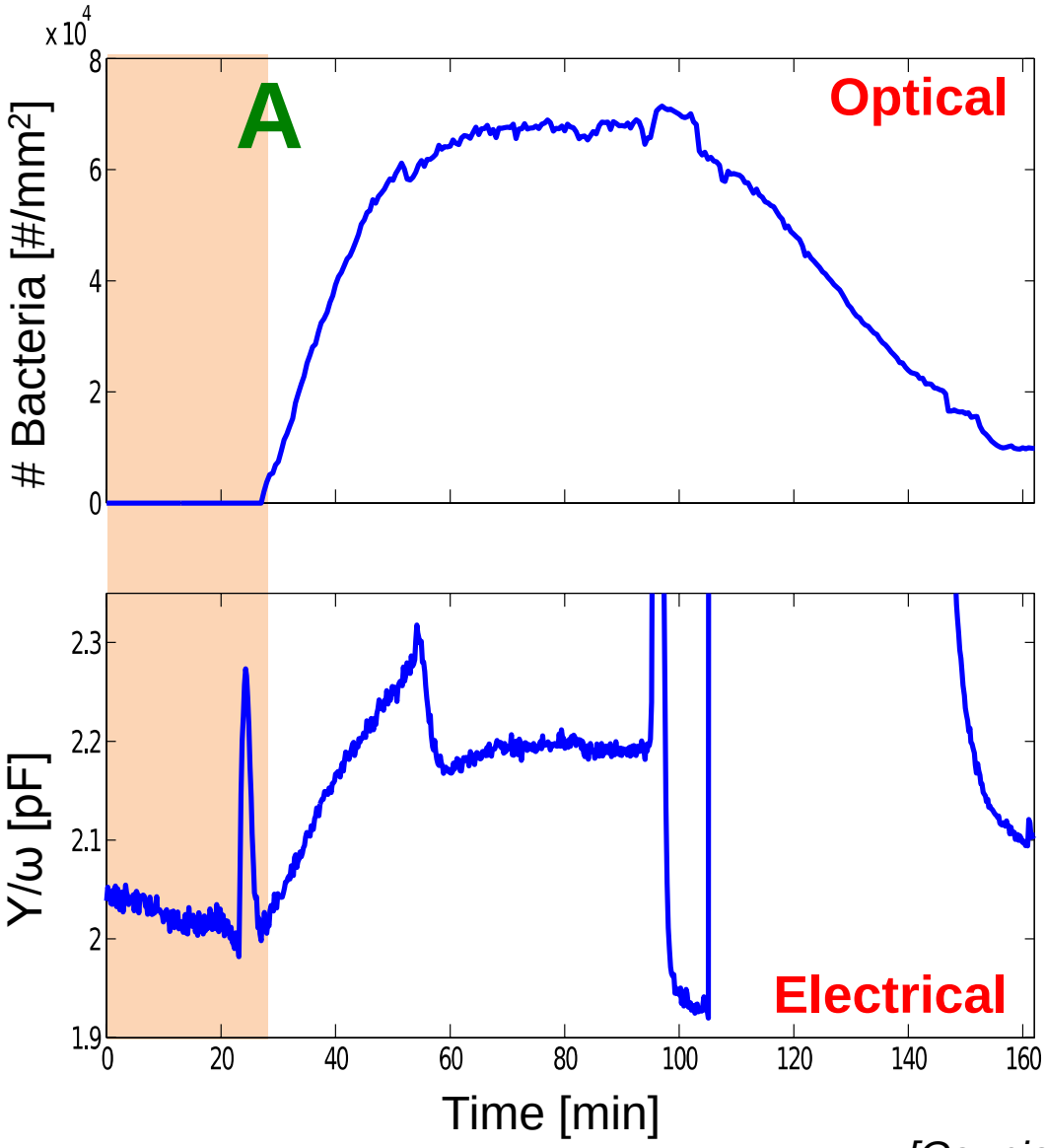


Good bacterial speed with regards to peristaltic pump

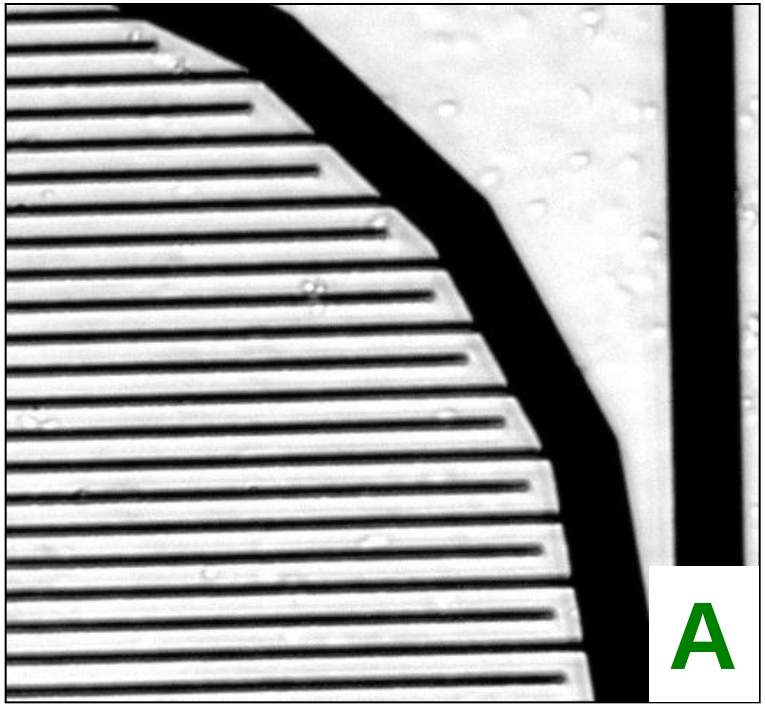
Measurement setup



Real-time monitoring in PBS 1:1000, @ 1 MHz

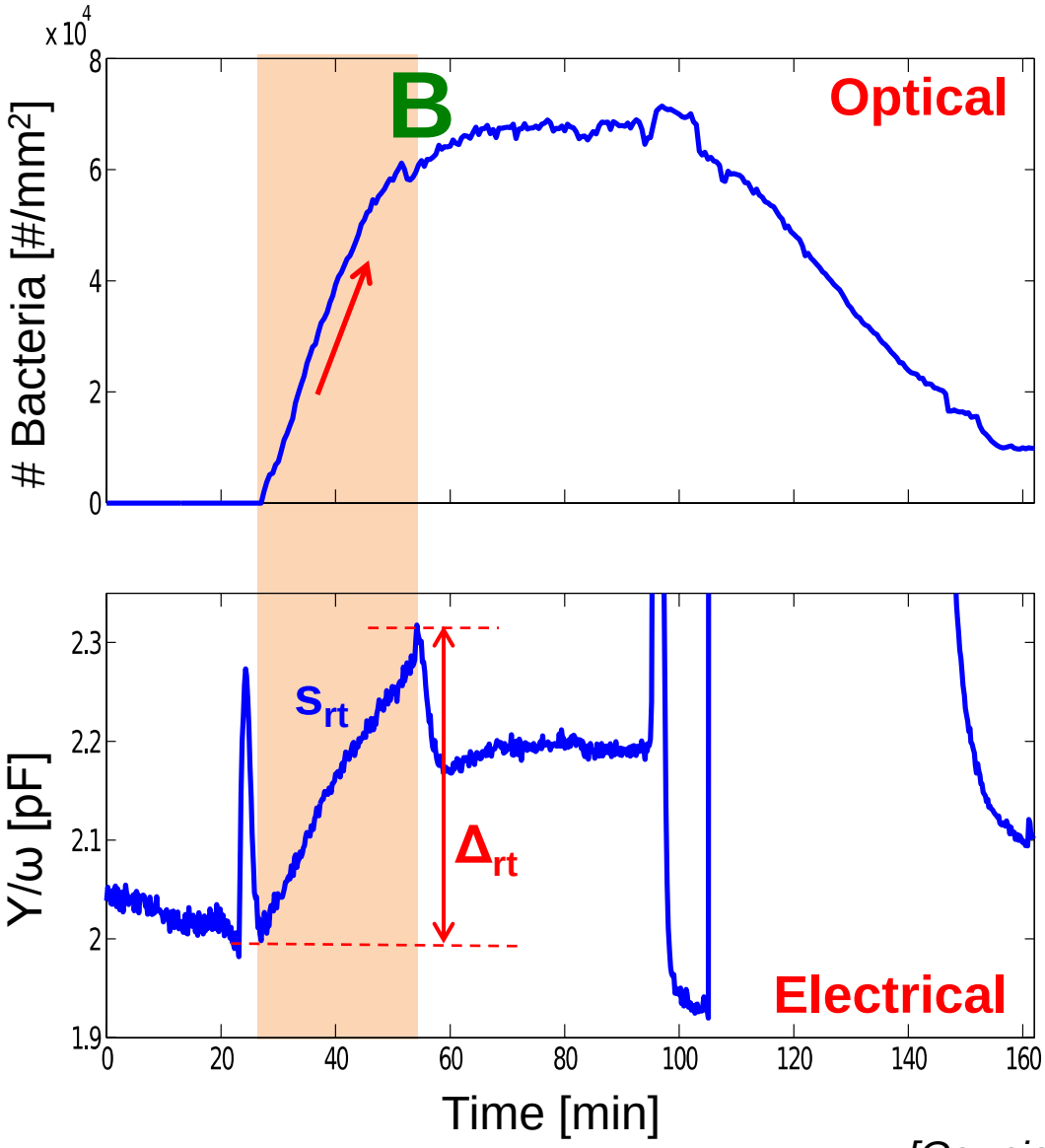


1: Reference buffer



[Couniot, Biosensors and Bioelectronics, 2014]

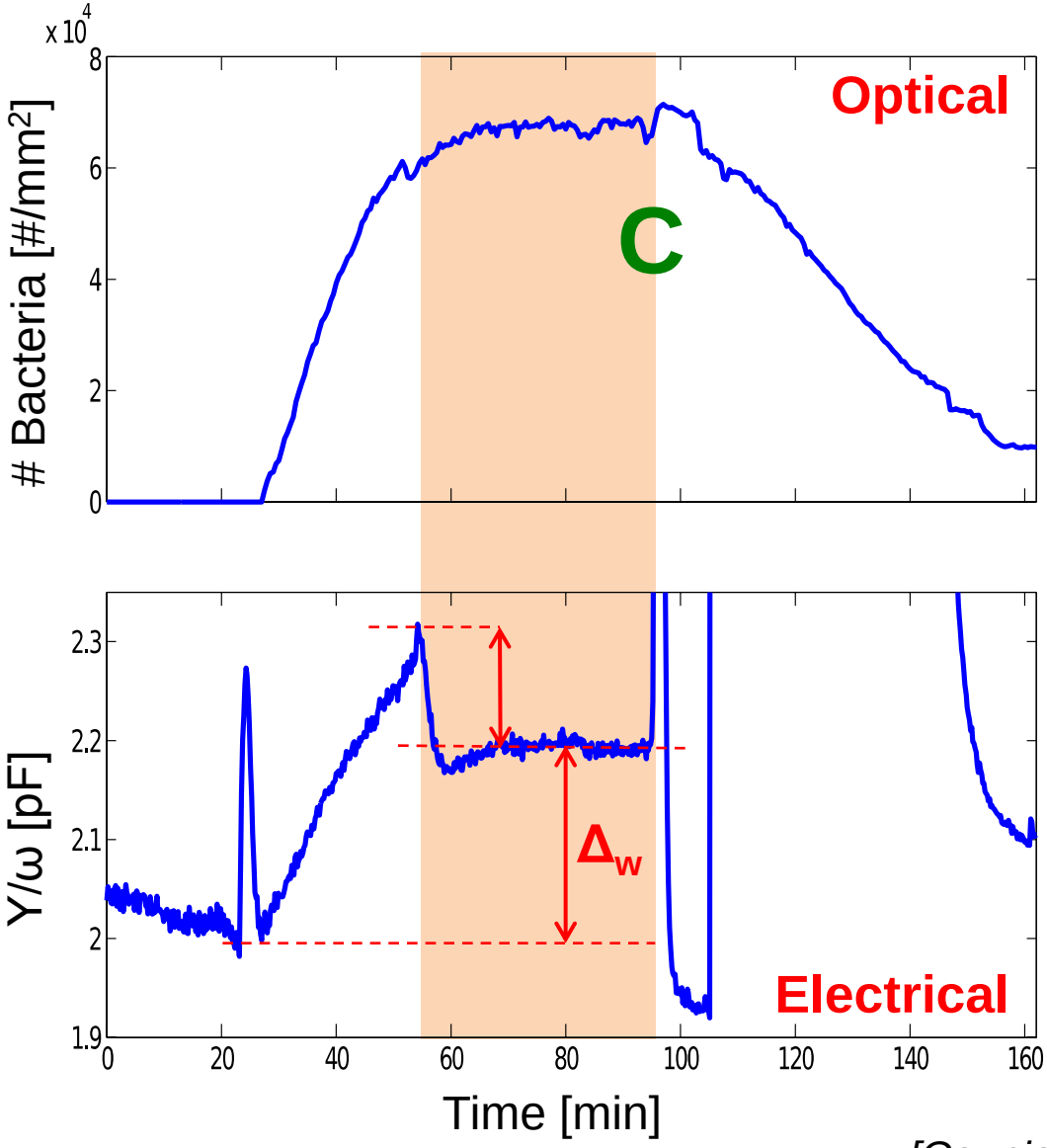
Real-time monitoring in PBS 1:1000, @ 1 MHz



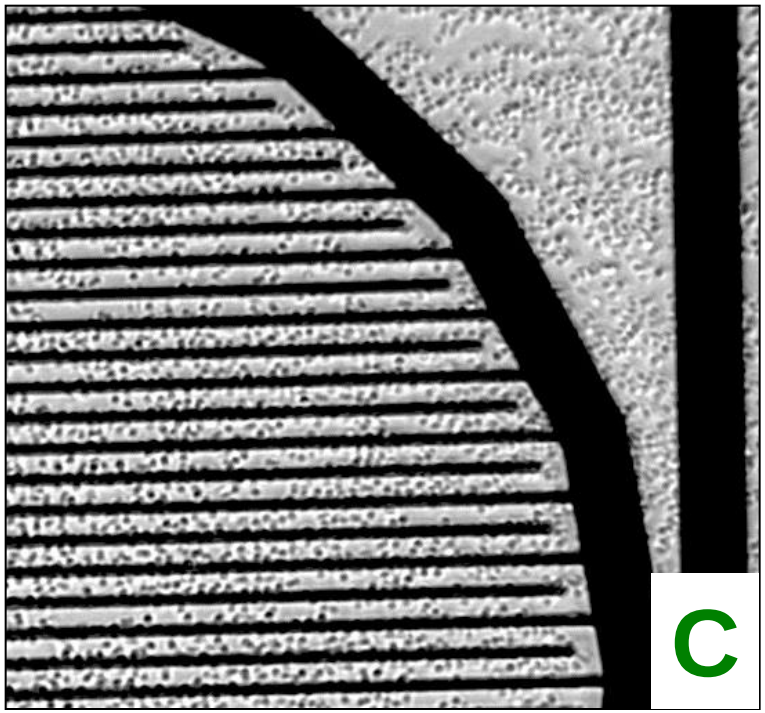
2: Bacterial incubation

[Couniot, Biosensors and Bioelectronics, 2014]

Real-time monitoring in PBS 1:1000, @ 1 MHz

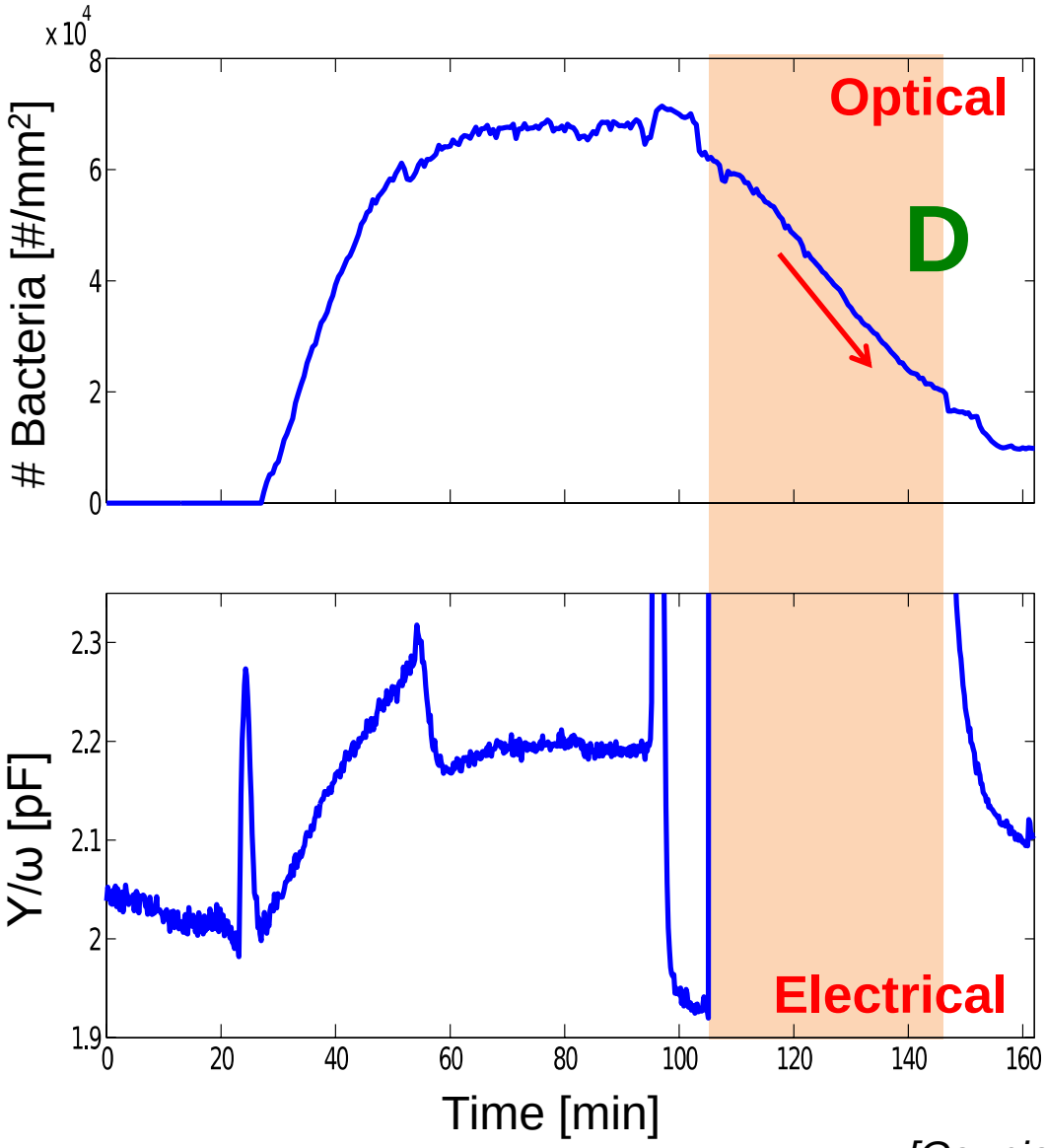


3: Wash with reference buffer

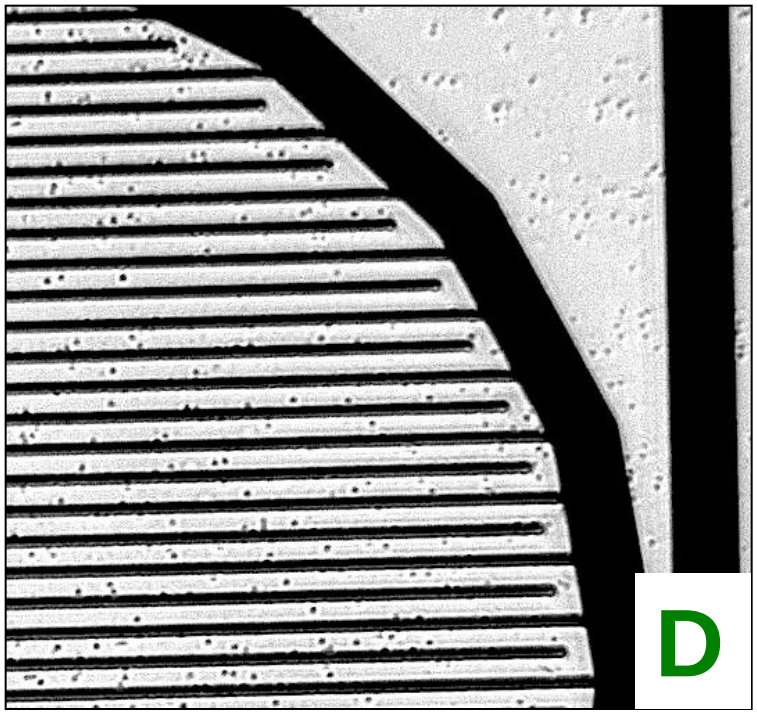


[Couniot, Biosensors and Bioelectronics, 2014]

Real-time monitoring in PBS 1:1000, @ 1 MHz

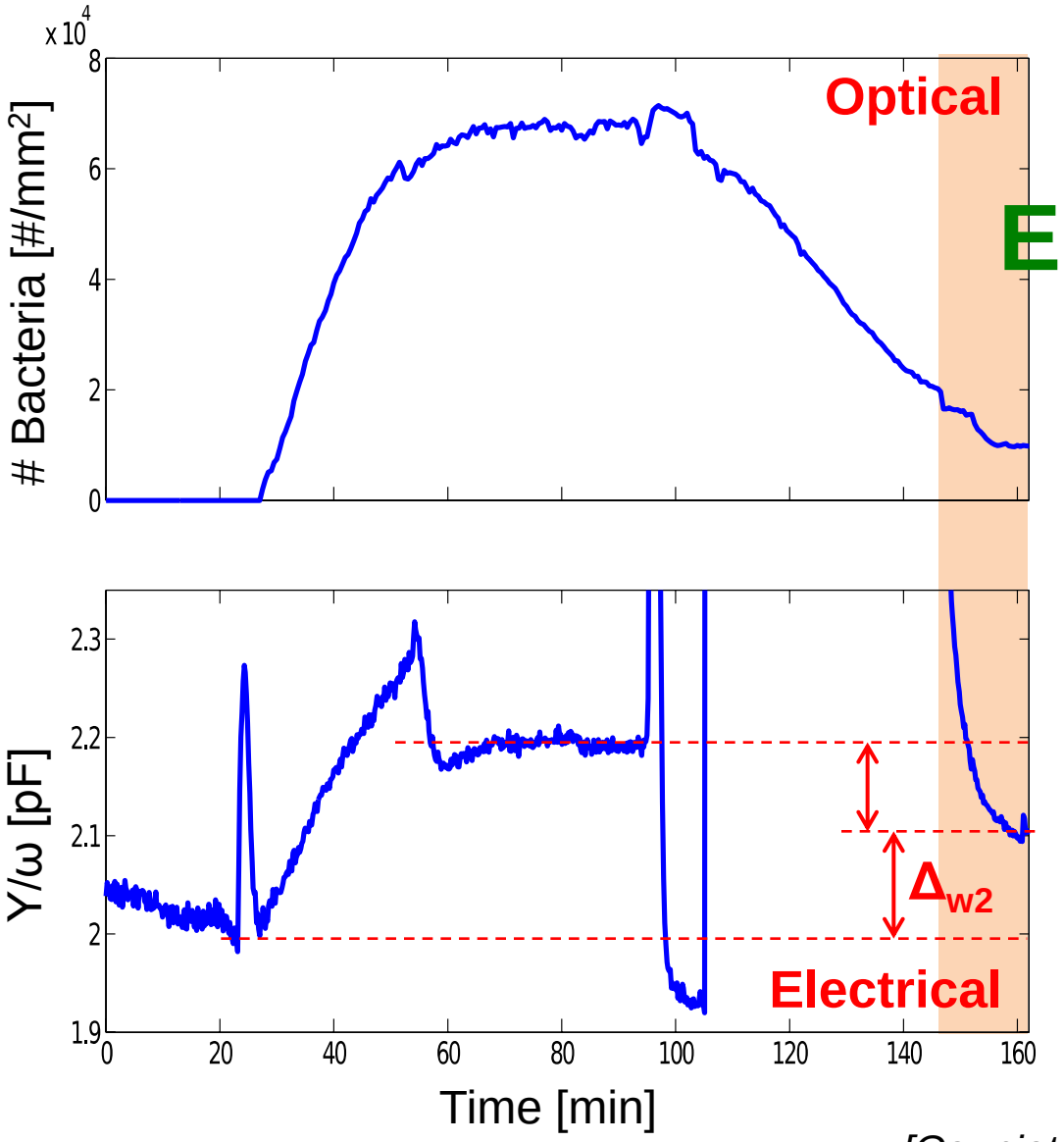


4: Lysostaphin incubation

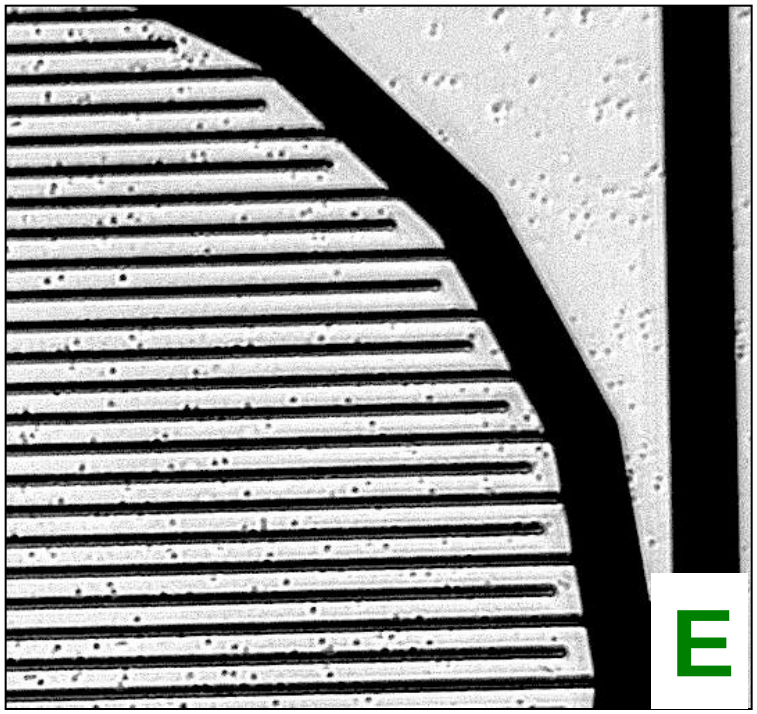


[Couniot, Biosensors and Bioelectronics, 2014]

Real-time monitoring in PBS 1:1000, @ 1 MHz



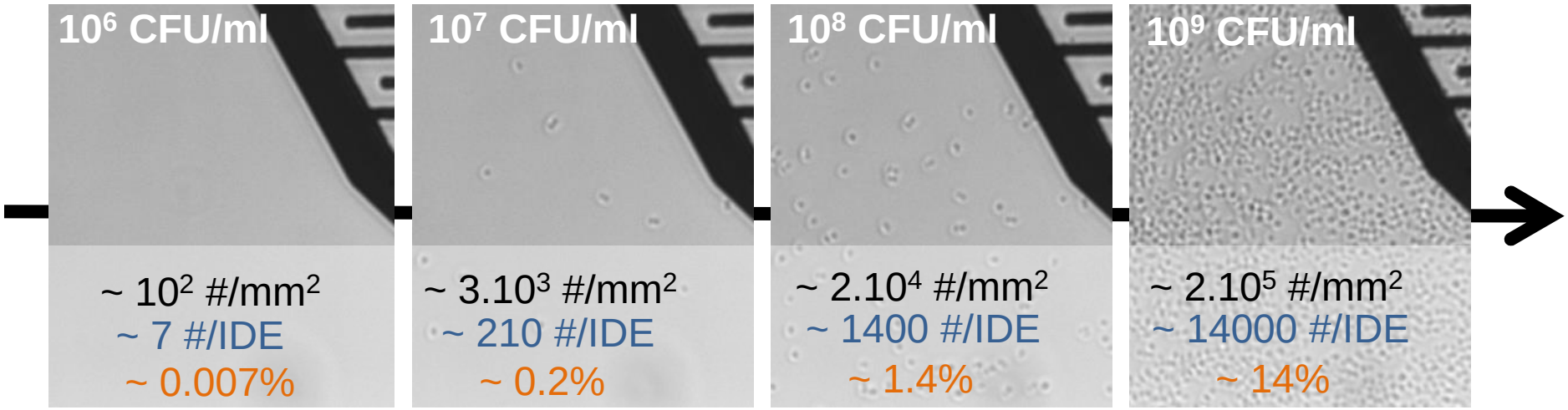
5: Wash with reference buffer

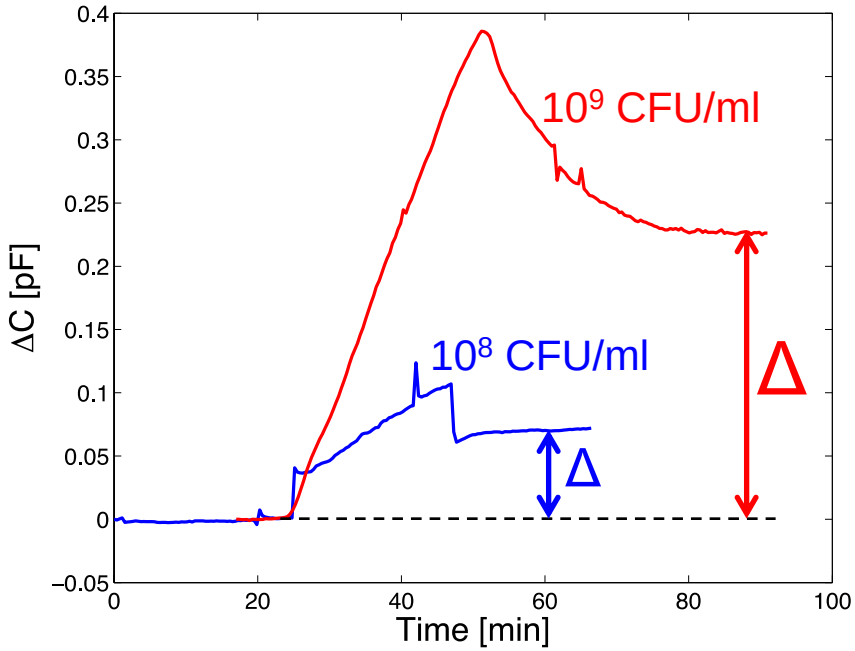


[Couniot, Biosensors and Bioelectronics, 2014]

Real-time monitoring in PBS 1:1000, @ 1 MHz

Impact of bacterial concentration after 30 min





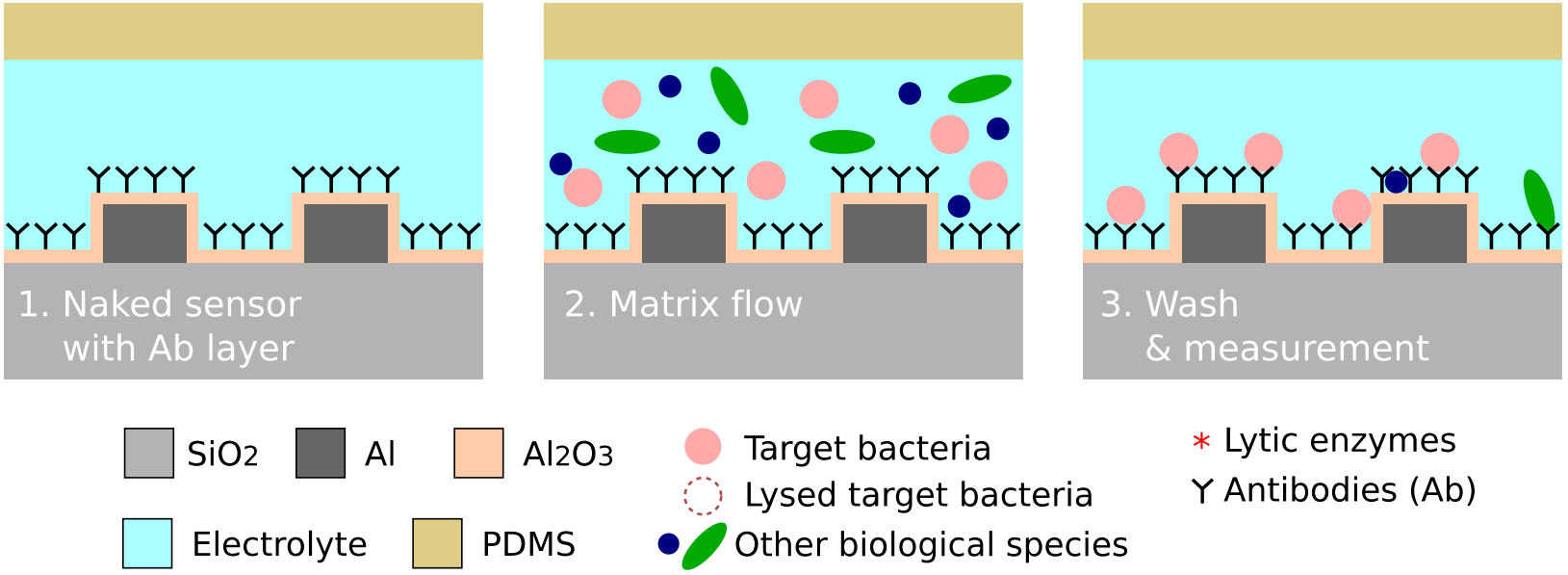
[Couniot, Biosensors and Bioelectronics, 2014]

Dealing with real situation:

Urine with *S. epidermidis* (target) *and E. faecium* (control -)

Conventional method with Ab coating:

- ✖ Expensive
- ✖ Complex chemistry
- ✖ Low reproducibility/uniformity
- ✖ Low temporal stability (during wash)
- ✖ Aspecificity/Background noise



[Van Overstraeten et al., Biofabrication, 6(3), 035007, 2014

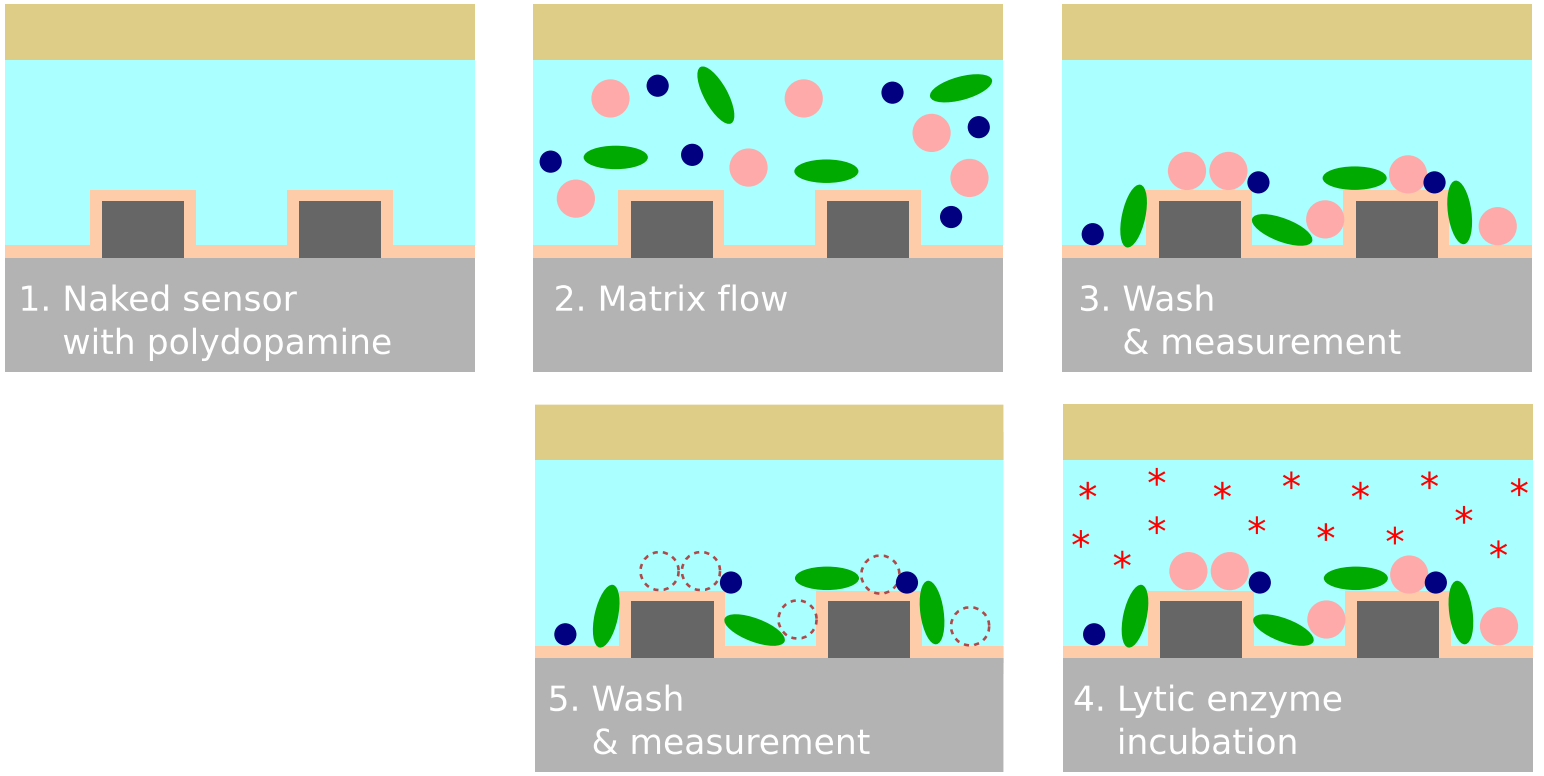
Dealing with real situation:

Urine with *S. epidermidis* (target) *and E. faecium* (control -)

Innovative method with lytic enzymes:

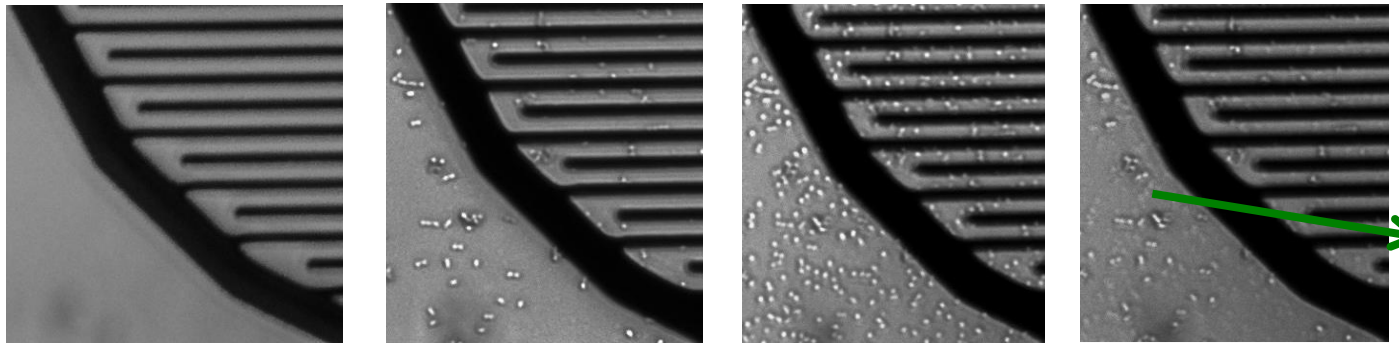
[Couniot, Biosensors and Bioelectronics, 2014]

- ✔ Low-cost and for all Gram + bacteria
- ✔ Robust to wash
- ✔ Almost no background noise

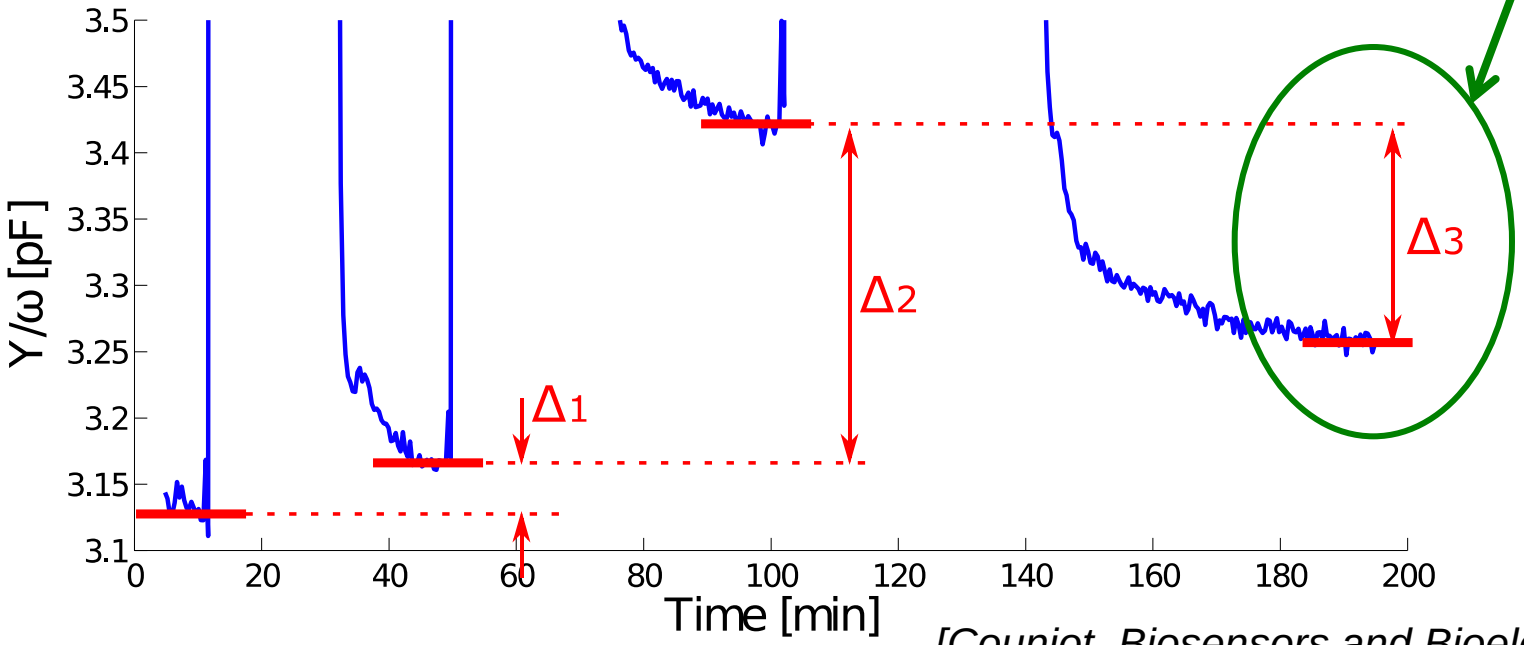


Dealing with real situation:

Urine with *S. epidermidis* (target) *and E. faecium* (control -)



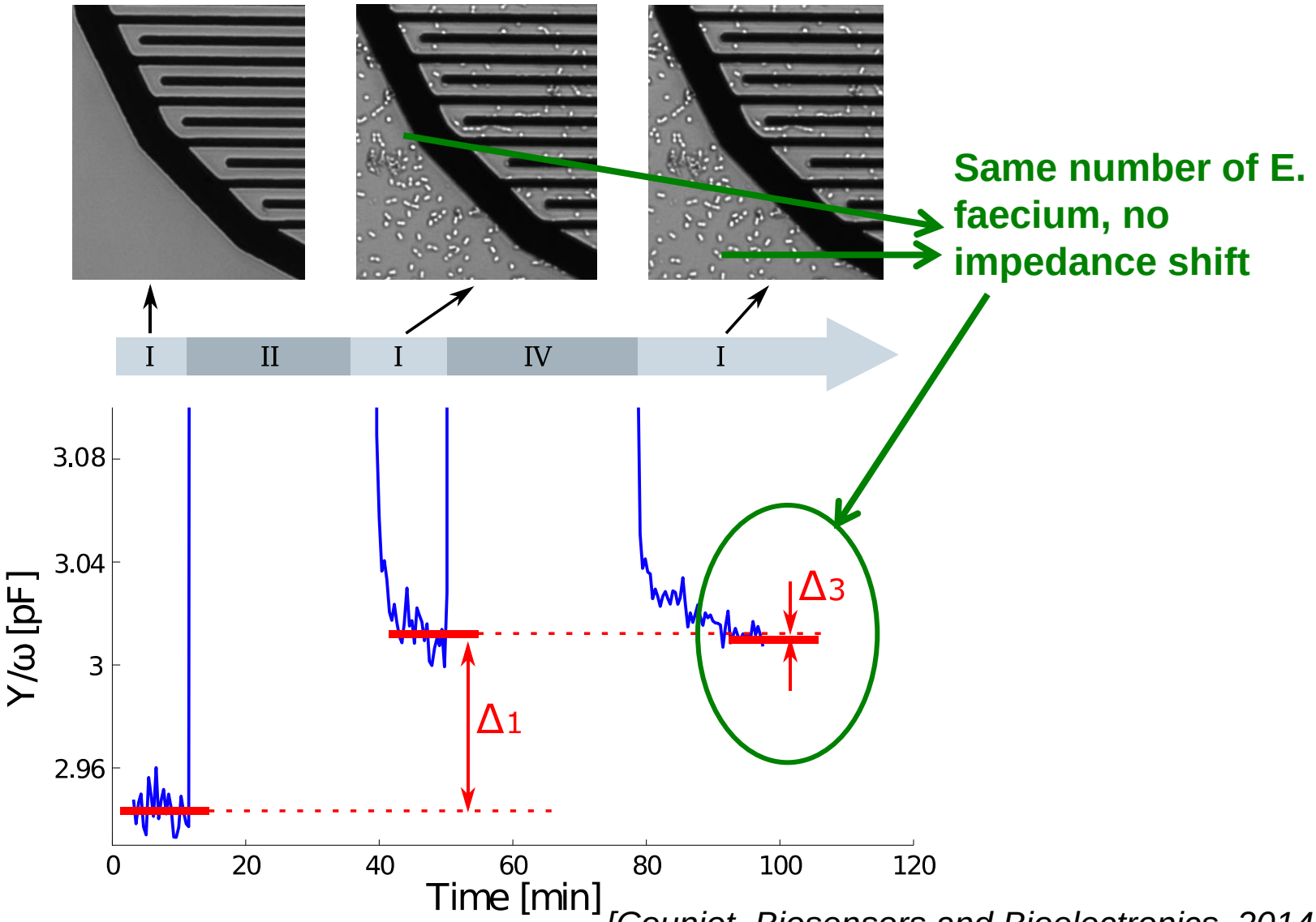
Destroyed *S. epidermidis* causes decrease in capacitance



[Couniot, Biosensors and Bioelectronics, 2014]

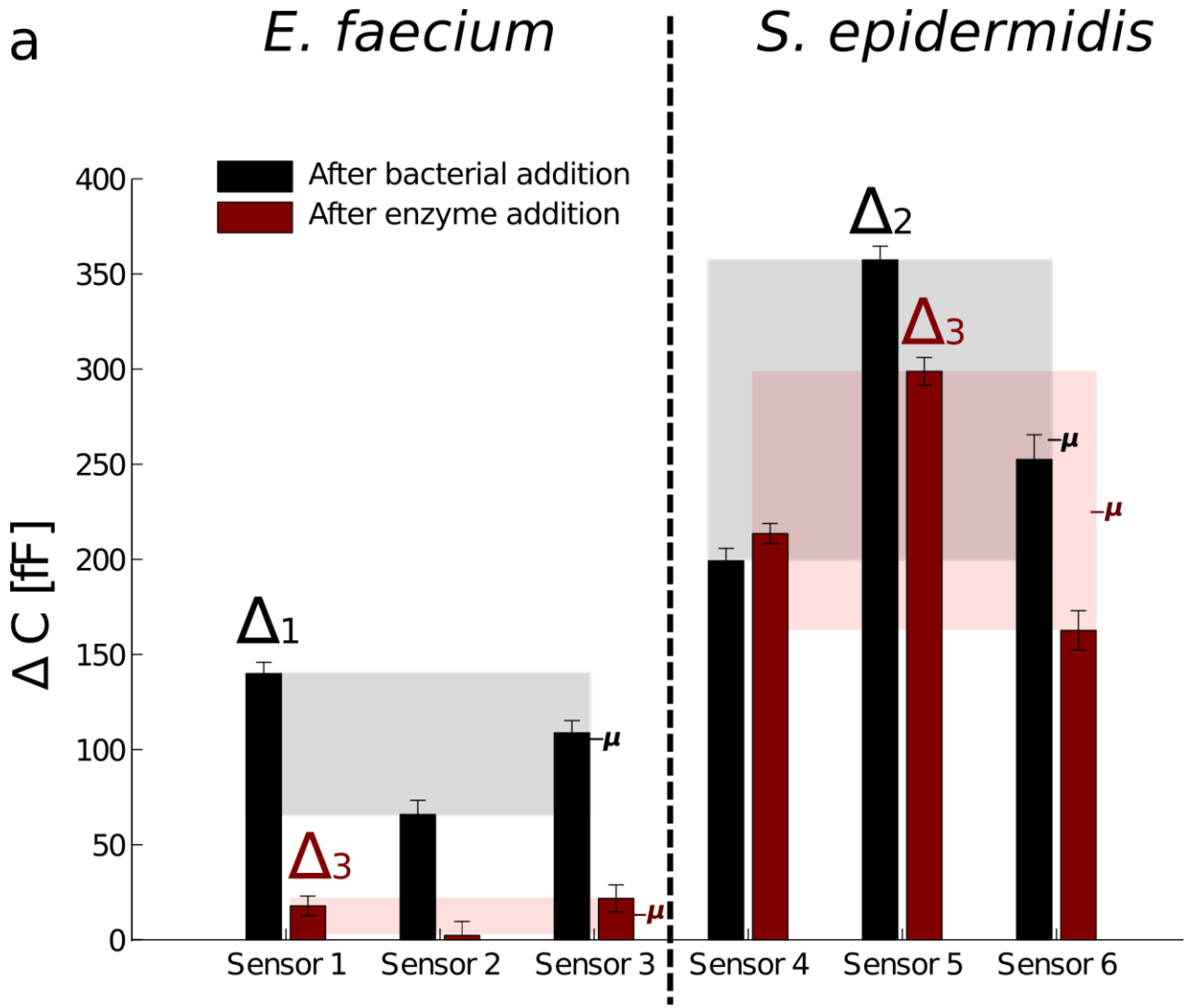
Dealing with real situation:

Urine with *E. faecium* only (control -)



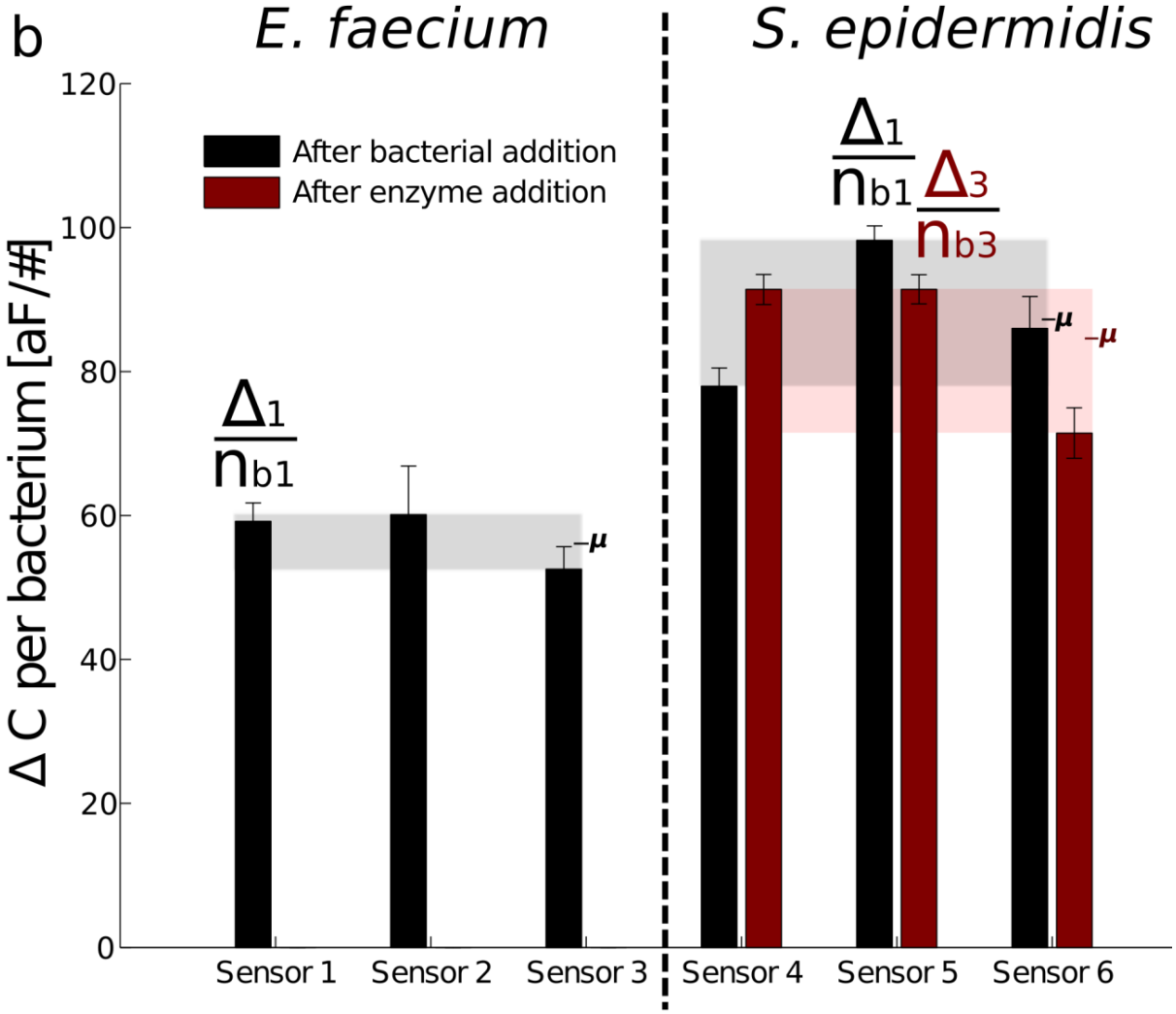
[Couniot, Biosensors and Bioelectronics, 2014]

Dealing with real situation:
Reproducibility of the shift



[Couniot, Biosensors and Bioelectronics, 2014]

Dealing with real situation:
Reproducibility of the normalized shift

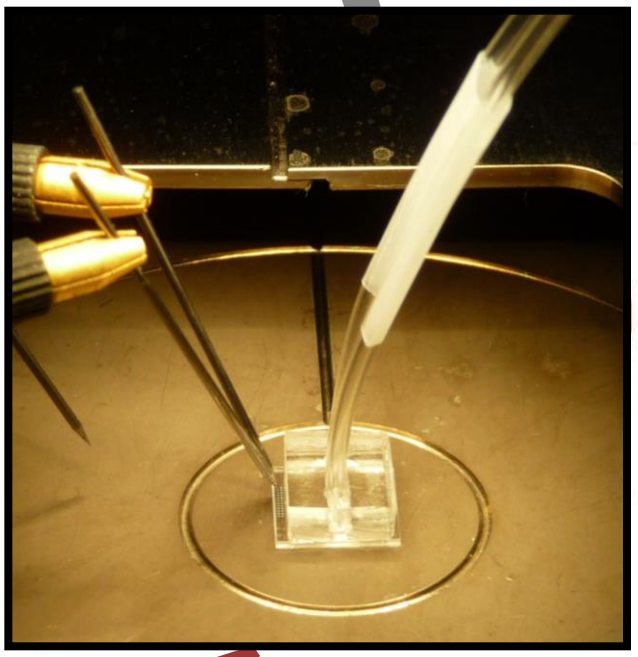


[Couniot, Biosensors and Bioelectronics, 2014]

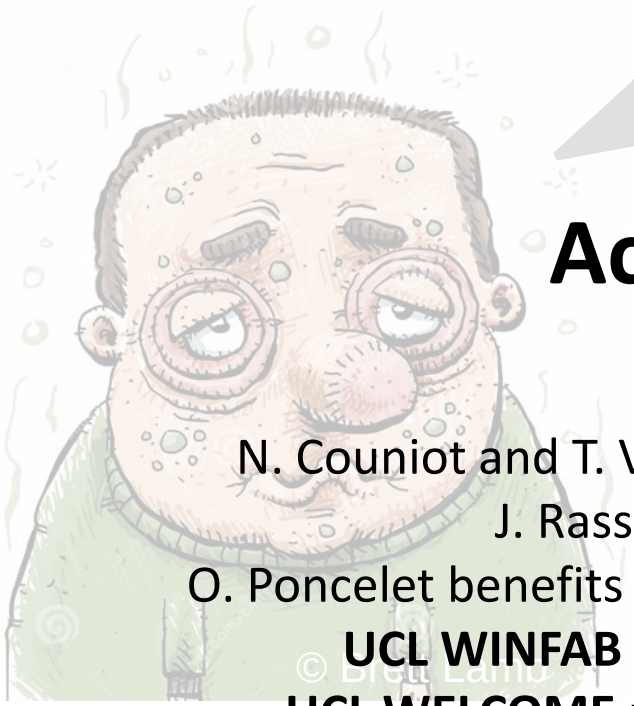
Conclusions

- New selective method based on lytic enzymes:
  - Works for all Gram + bacteria
  - Robustness
  - Low-cost
  - No background noise
- CMOS-compatible microelectrodes
  - Low-cost
  - Miniaturizable
  - Integrable with readout circuit
  - High-througput
- Performances:
  - 10^8 (CFU/mL)*min without pre-concentration steps

More details in
"Lytic enzymes as selectivity means for impedimetric detection of bacteria using ALD-Al_2O_3 passivated microelectrodes"
, Biosensors and Bioelectronics, In Press, 2014



Quicker part



Acknowledgements

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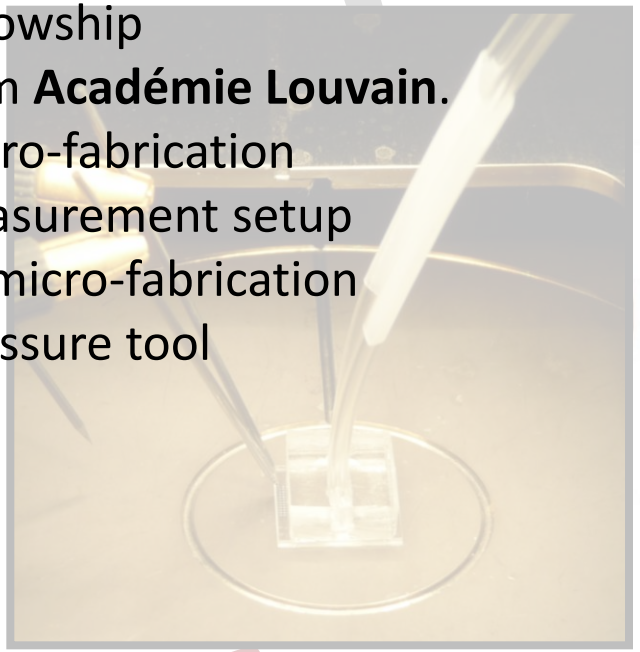
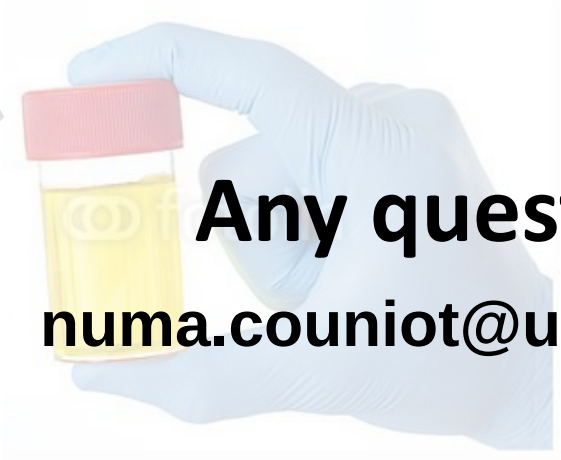
J. Rasson benefits from a **FRIA** Fellowship
O. Poncelet benefits from an ARC Fellowship from **Académie Louvain**.
UCL WINFAB platform for help during micro-fabrication
UCL WELCOME platform for help during measurement setup
C.A. Dutu for technical help with PDMS cap micro-fabrication
D. Spôte for the fabrication of the pressure tool





Any questions?

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Quicker part