

Investigation of the Electrical Detection of Hybridized DNA Concentrations lower than 1 nM, based on CMOS Compatible Al Capacitors Coated with Metal Oxides

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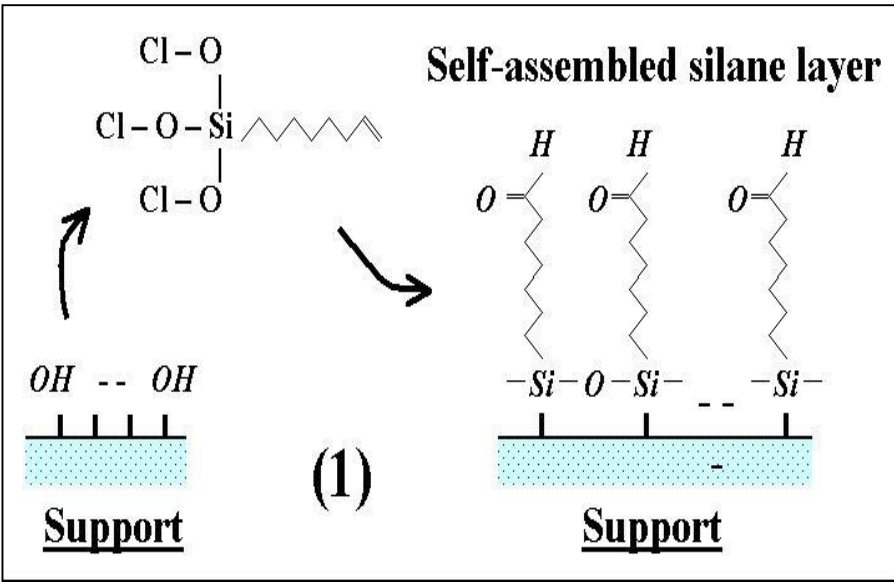
** B. Foutier, J. Remacle

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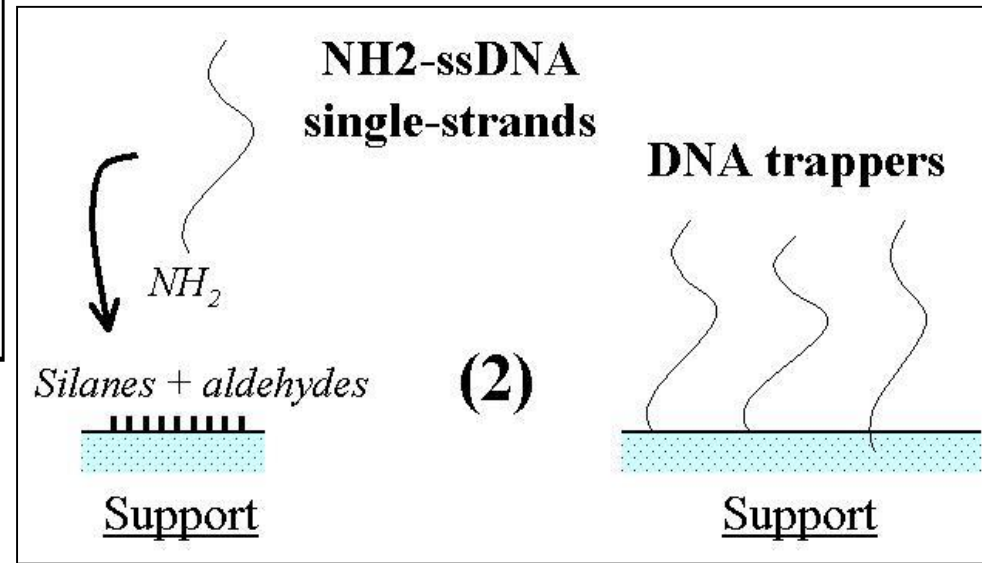
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- 1. Introduction**
 - **Biological Process**
 - **Materials and Fabrication**
 - **Previous Results**
- 2. Process Characterization**
- 3. Silver Quantification**
- 4. Interdigitated Capacitors**
 - **Design & Simulation**
 - **Electrical Results**
- 5. Conclusions**

Introduction: DNA binding

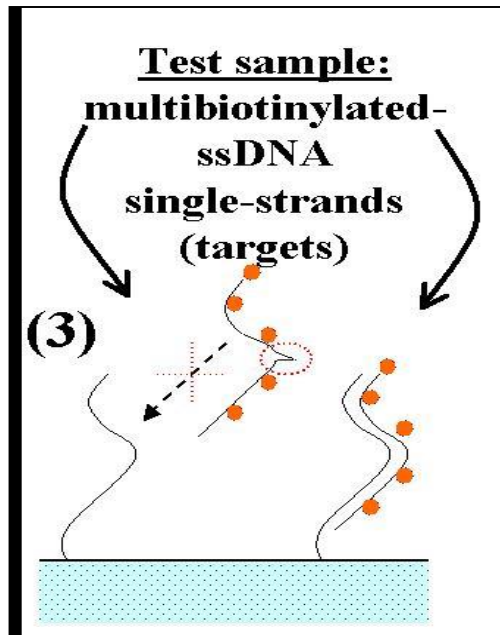


**Functionalization
(trichlorosilane)**

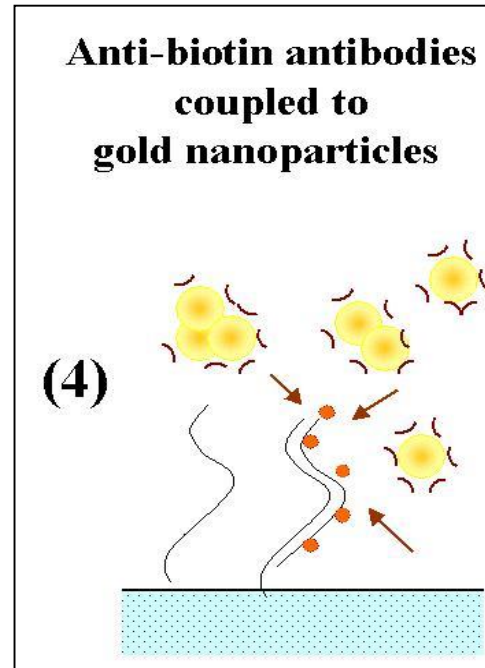


**DNA probes spotting
(covalent bonding)**

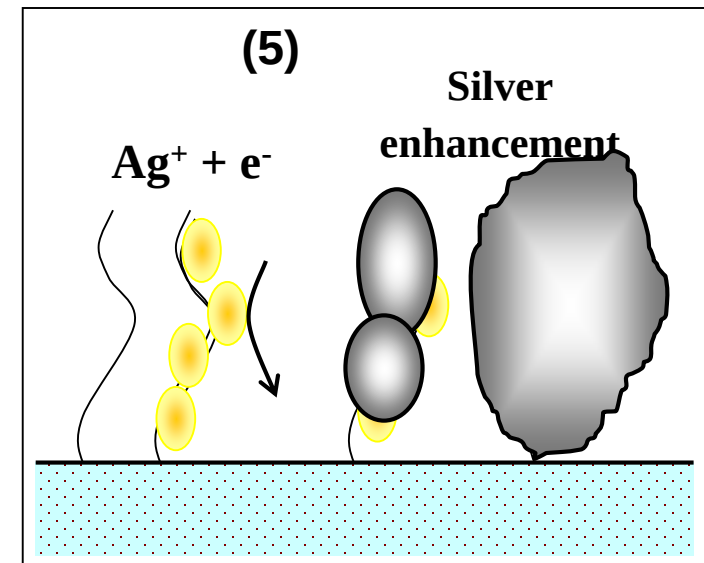
Introduction: Silver amplification



**Hybridization
(selective bonding)**



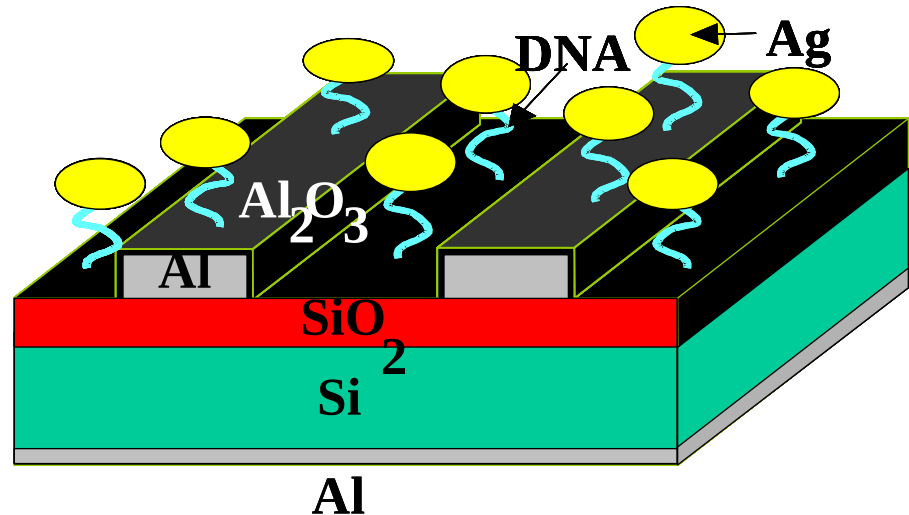
**Gold labeling
(20-100 nm balls)**



**Amplification 40x
(Ag grains $\approx 1 \mu\text{m}$)**

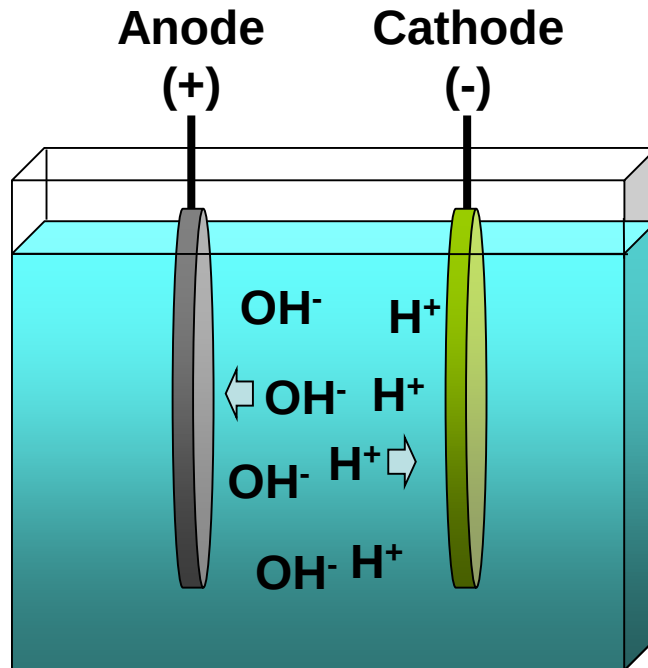
New concept

Metal oxide coating of Al structures



- Metal Oxide obtained by anodization.
 - Protection against chemicals during the biological process.
 - OHs terminals in the surface to allow the functionalization.
 - Reduction of non-specific Ag precipitation.
- Advantage of Capacitive detection: calibration of the sensor at a reference level before or in the absence of hybridization, on the contrary to resistive values which tend to infinity and are hence limited by the measurement set-up.

Anodization process



Chemical solution to form oxide:

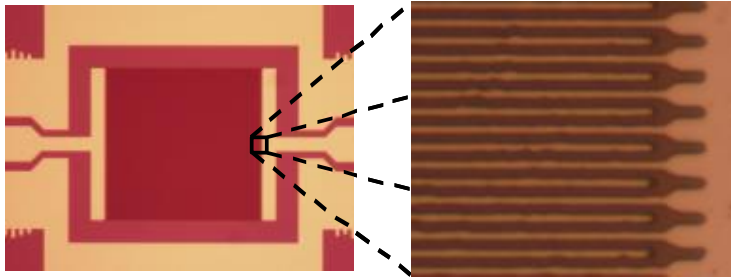
- Non-porous: Boric acid/ammonium hydroxide/ethylenglycol
- *Porous*: Sulfuric acid or Phosphoric acid in water

Anode: $2\text{Al}^{+3} + 3\text{OH}^- \rightarrow \text{Al}_2\text{O}_3 + 3\text{H}^+$ (CMOS wafer)

Cathode: $2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\uparrow$ (reference electrode)

Introduction: Previous Results

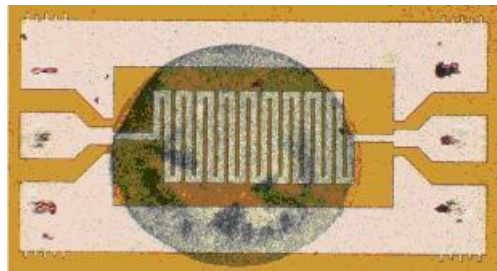
Structure



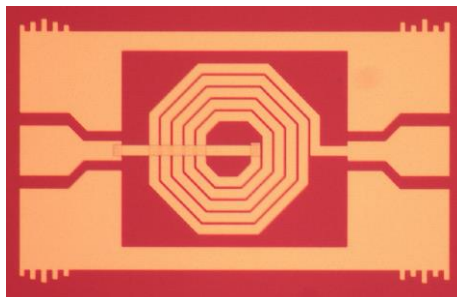
Variable Measured

Range Tested

Capacitance	0.5 – 20 nM dsDNA
Resonance Frequency	2.5 – 20 nM ssDNA



Resonance Frequency	5 – 20 nM ssDNA
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Resonance Frequency	5 – 10 nM ssDNA
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- Interdigitated Capacitors seems to be the best option to detect low complementary ssDNA conc.

Process Characterization

Definitions

Noise:

Two possibilities:

- a) Silver precipitated in an area non-spotted (no probes).
- b) Silver precipitated where the hybridization did not take place, 0 nM ssDNA target concentration.

Signal:

Silver precipitated on the area where the hybridization took place, concentration > 0 nM (target ssDNA).

Anodization characterization

Process Parameters:

Electrolyte (chemicals)

Current density

Ending voltage

Effect on oxide:

porosity, roughness

porosity, roughness, density

thickness

Test done:

Two electrolytes, aqueous sulfuric acid and boric acid/ammonium hydroxide/ethyleneglycol

Current density: 0.1, 1.0, 2.0, 3.0, 4.0 mA/cm²

Comparison:

Surface analysis by ToF-SIMs (OHs groups) and AFM (Roughness)

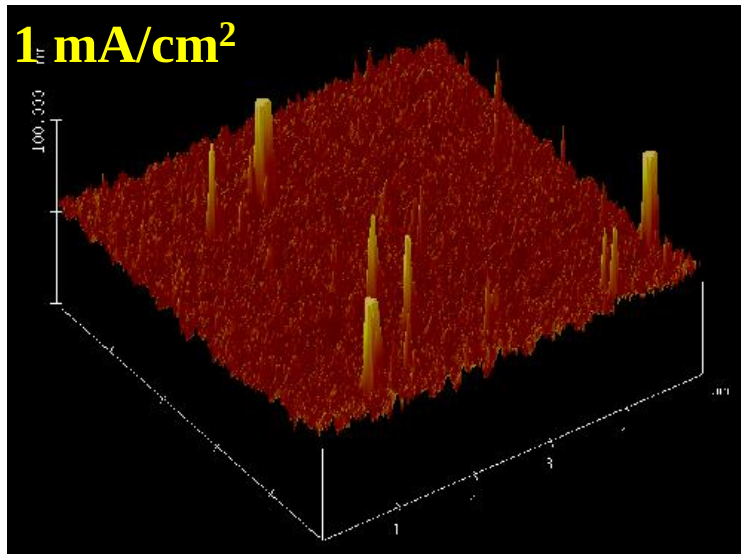
Anodization

ToF-SIMS Results

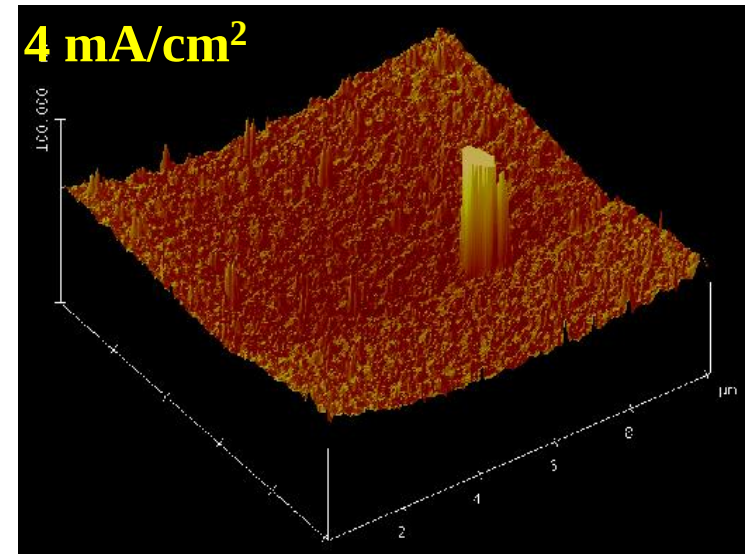
Anodizing Chemical	Anodizing speed (mA/cm ²)	AlOH/AlO negative	AlOH/AlO positive
Boric acid	0.1	0.1291	14.31
Boric acid	1	0.1532	19.84
Boric acid	2	0.1547	22.96
Boric acid	4	0.1534	20.30
Sulfuric acid	2	0.0850	10.12
Sulfuric acid	4	0.0932	09.84

- Non-porous anodic Al₂O₃ is better for DNA attachment, anodizing current density around 1 to 4 mA/cm² could be used.

Non-porous: Effect of anodizing speed (AFM)



Img Z range: 11.04 nm
Img rms (rq): 1.09 nm



Img Z range: 16.04 nm
Img rms (rq): 1.32 nm

- **Faster oxide forming means more roughness, but how does this affect functionalization?**

Functionalizing Solutions Effect

Test done:

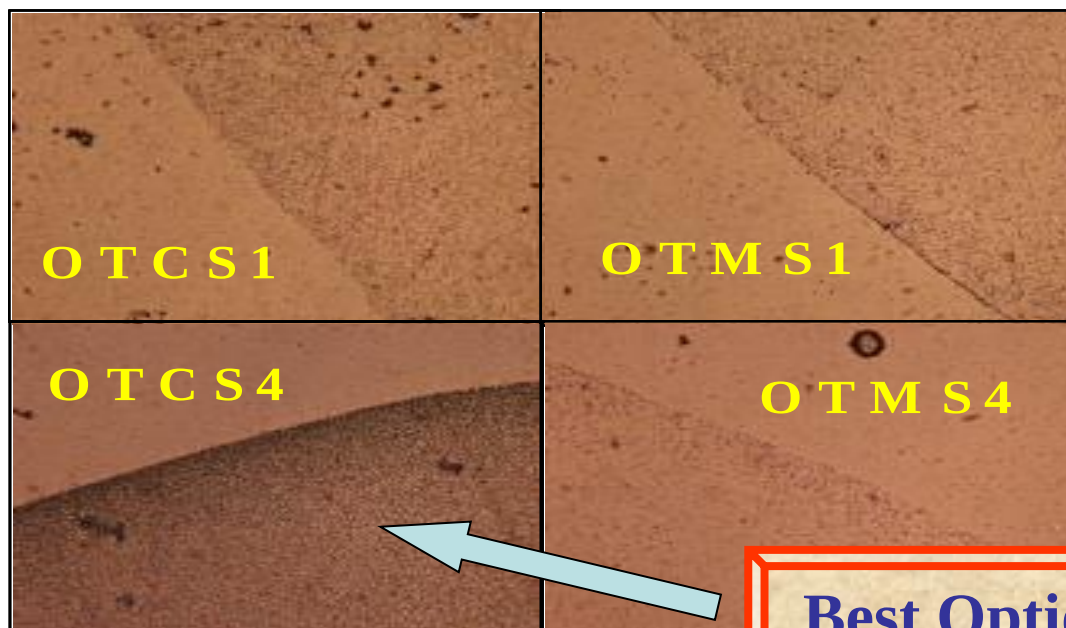
Chemical: 7-octényltrichlorosilane (OTCS), 7-octényltrimethoxysilane (OTMS)

Samples: wafers with Anodized Al_2O_3 (2 oxidation rates, 1 and 4 mA/cm^2 , 100 nm thick)

All samples followed the standard biological process.

Verification test: pictures after silver precipitation.

Signal on darker area
(spot), Noise on lighter
one



Best Option

Silver Quantification

Silver Quantification



Biological Process Parameters

20 nl of solution, 400 μm spots,

**Capture probe Cy2b2 a 120 nucleotides-long 5' amino modified single strand DNA (ssDNA): 5'-NH₂ATAAAAAAAGTGGGTCTTAGAAA
TAAATTTTCGAAGTGCAATAATTATTATTCACAACATTTTCGATT
TTTGCAACTACTTCAGTTCACTCCAAATTACACATCTCATGCT
GAGTCACTTCCC-3'**

**Target DNA, was prepared by polymerase chain reaction (PCR)
Cy2b2, 534 pb, concentration was adjusted at 50 nM by dilution in
MilliQ water.**

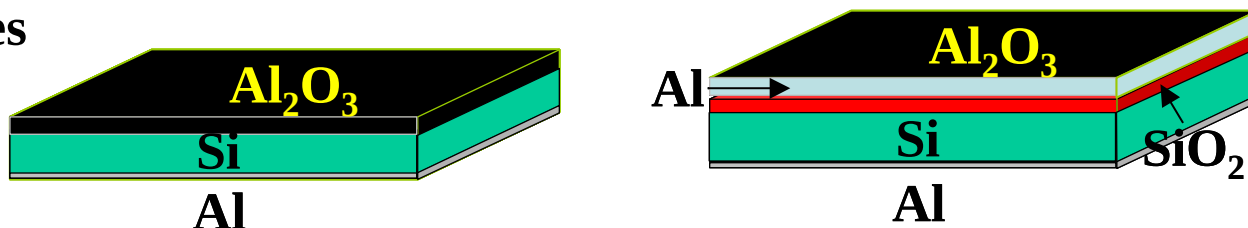
Hybridization Reaction Volume: 200 μl

Silver Quantification

Silver Quantification Procedure

Samples preparation:

2 types



2 anodizing current densities: 1 and 3 mA/cm²

Test done:

Biological process was followed as explained before using cited DNA targets and 20 nm Au particles onto the anti-biotin to make the silver precipitation.

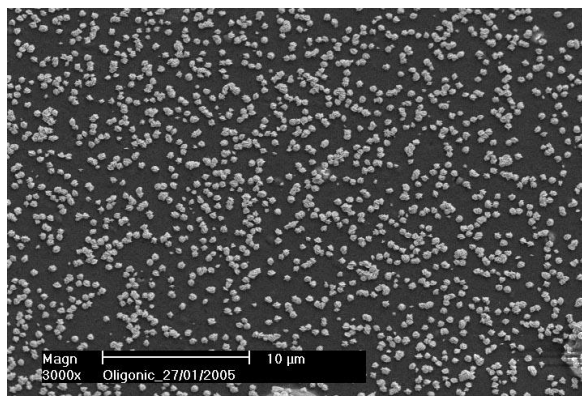
Comparison:

Silver coverage quantification from SEM pictures

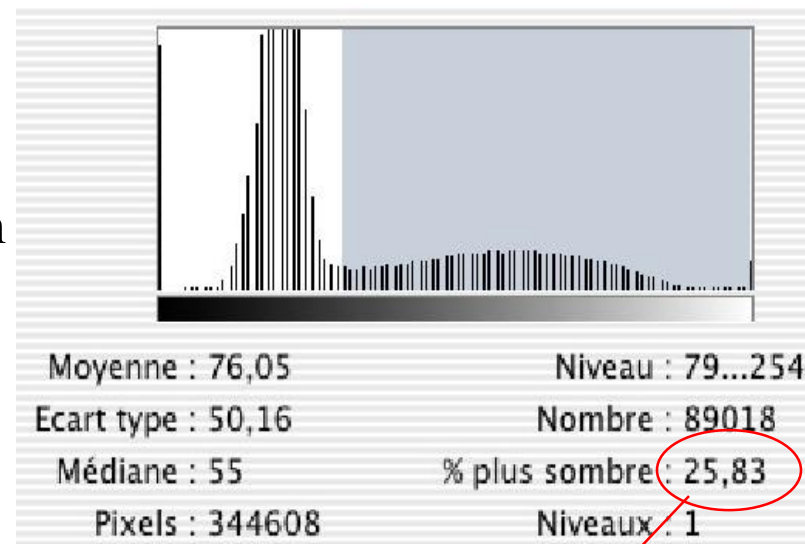
Silver Quantification

Principle

- > Obtain quantitative results, test reproducibility of the process
 - Compare experimental conditions
- > Establish silver coverage, target ssDNA concentration and electrical signal correlation



Signal
Integration
→



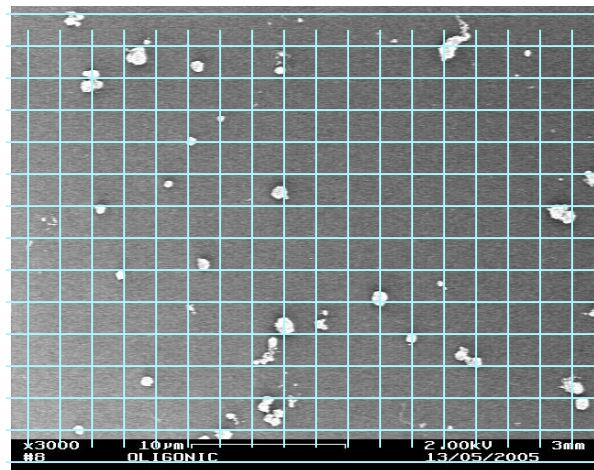
% Ag coverage

SEM Image x3000, Gray level, 8bits
Analysis Area: 37.5 μm x 30.2 μm

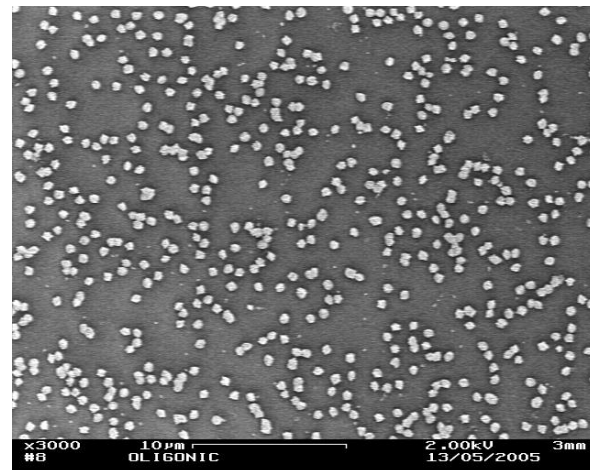
Silver Quantification

Principle

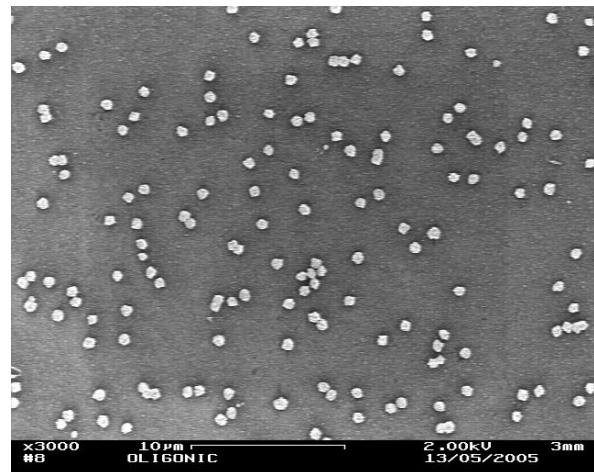
0 nM (Noise)



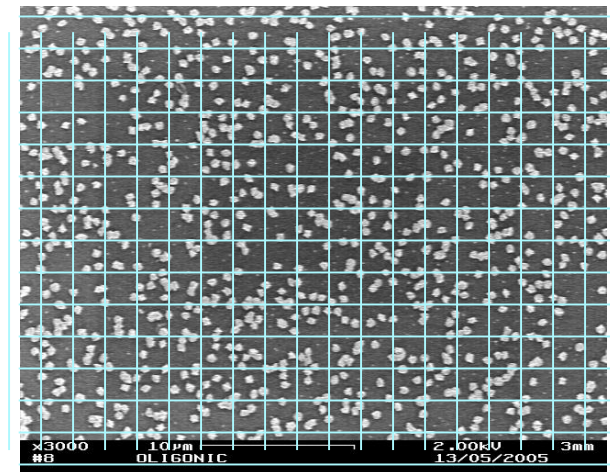
0.05 nM



0.01 nM



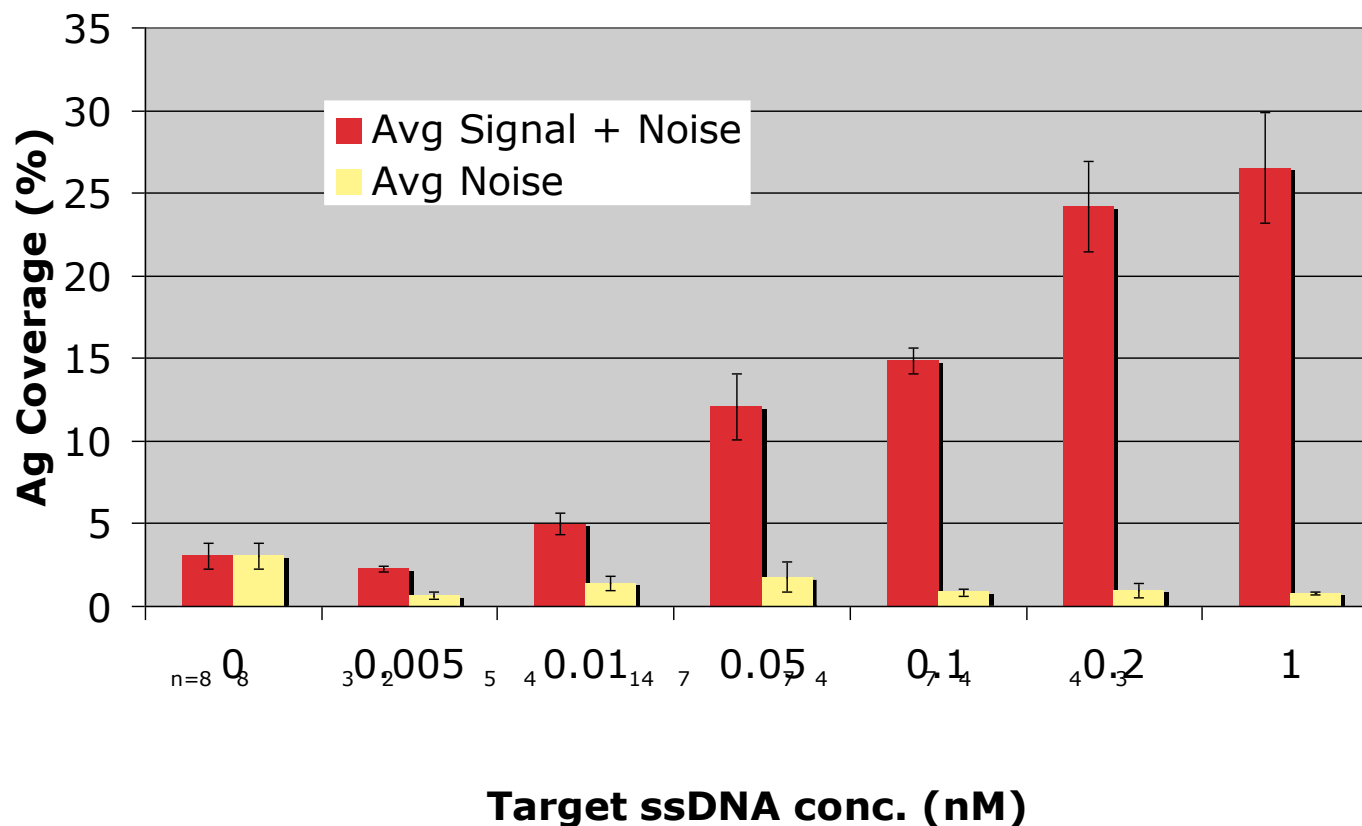
0.1 nM



Silver Quantification



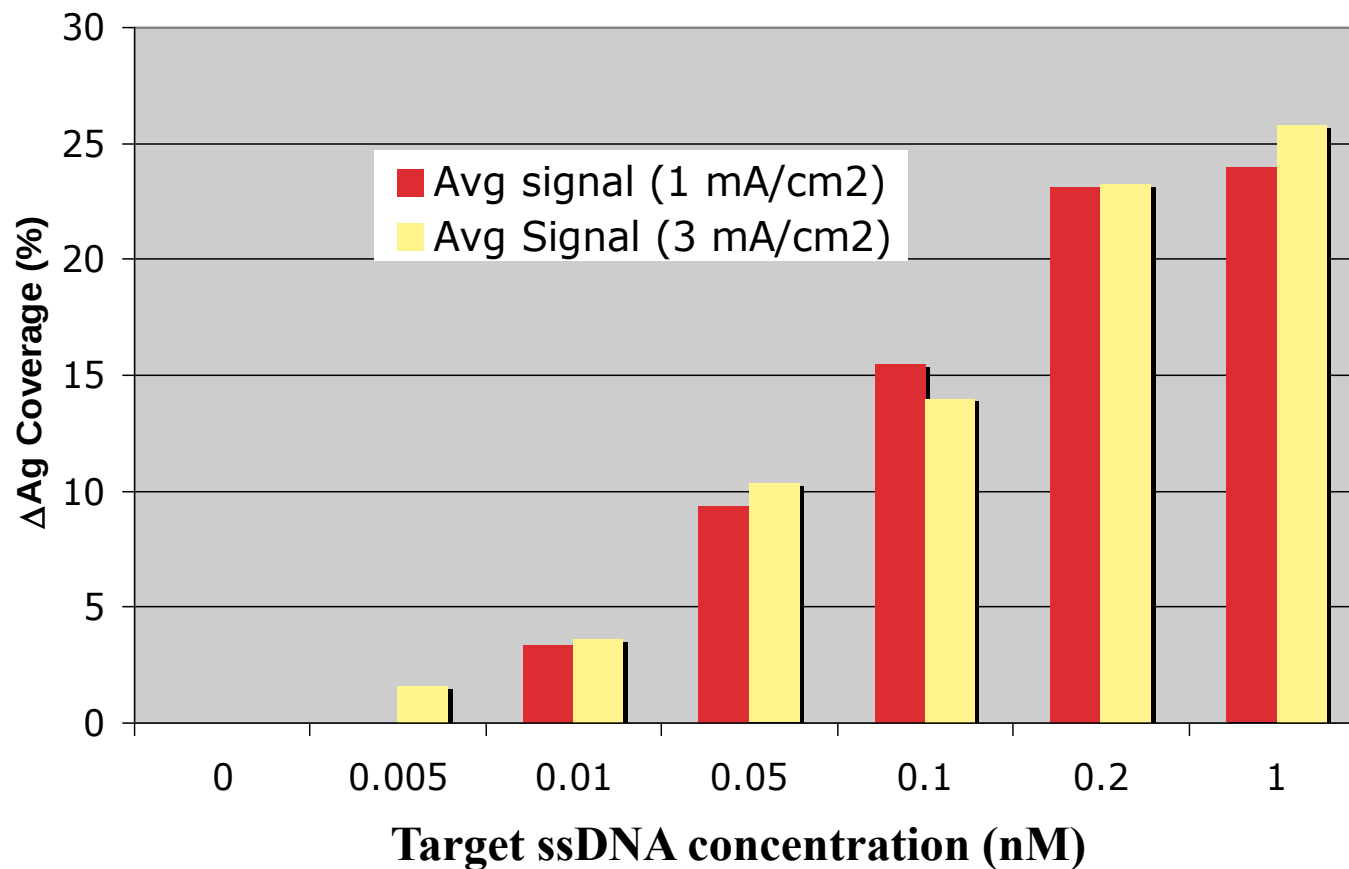
Signal & Noise over Si/Al₂O₃ (3 mA/cm²)



Silver Quantification



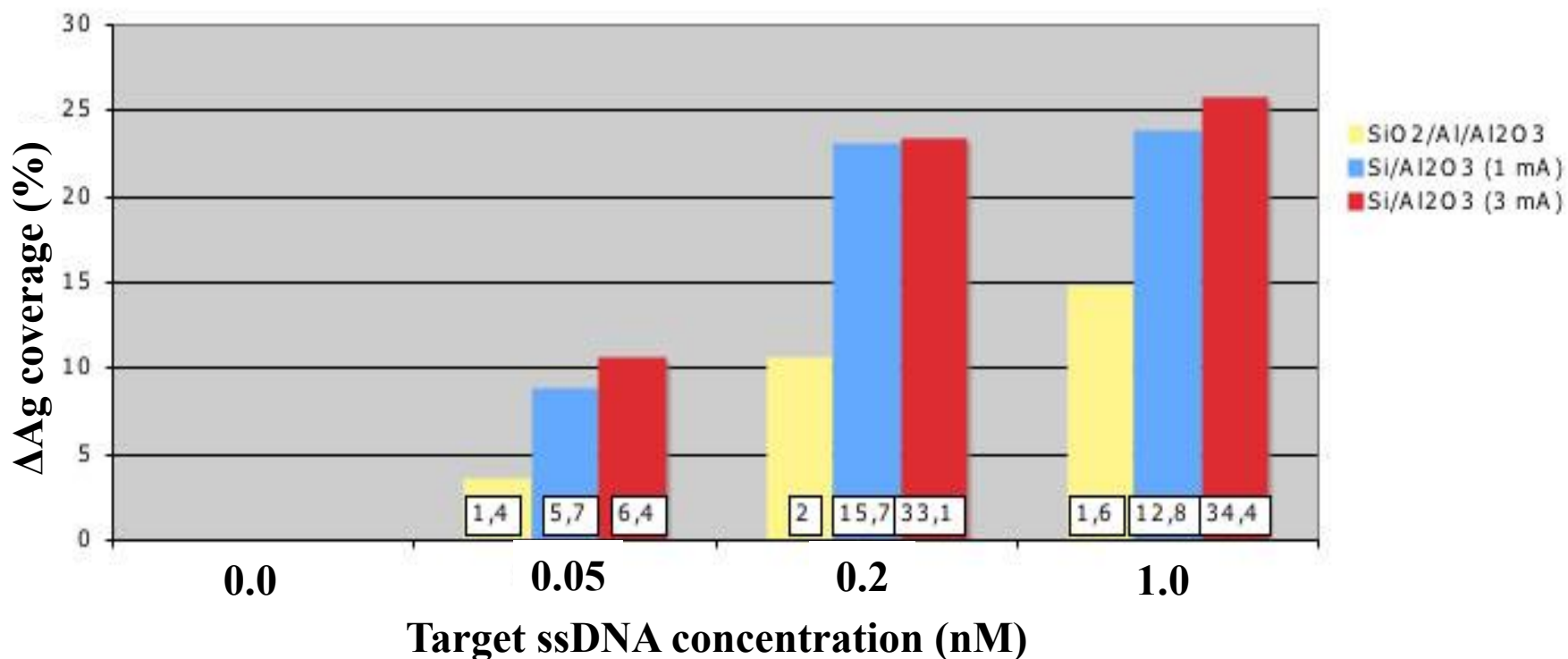
$\Delta\text{Ag coverage} = \text{Signal} - \text{Noise}$, for $\text{Al}_2\text{O}_3/\text{Si}$



Silver Quantification



ΔAg coverage = Signal – Noise, for Al_2O_3/Si and $Al_2O_3/Al/SiO_2$



Silver Quantification



Observations

- Good Reproducibility of Biochemical Process over Anodic Aluminum Oxide
- Signal more significant over $\text{Si}/\text{Al}_2\text{O}_3$ than $\text{SiO}_2/\text{Al}/\text{Al}_2\text{O}_3$
- Significant SEM image difference found at all concentrations level (down to 0.005 nM), but influence might not be sufficient below 0.01 nM for electrical detection using IDFs capacitors.

Interdigitated Capacitors

Interdigitated Capacitors

IDFs Design

Interdigitated fingers:

Fingers / space

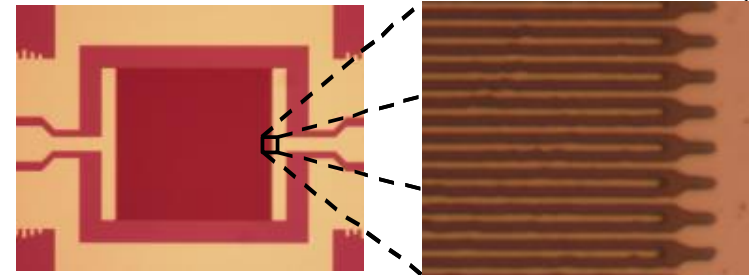
1.3 / 1.0

3.0 / 1.0

400 nm SiO_2

1000 nm thick Al Structures

100 nm Al_2O_3 formed



Two configurations

Fingers / space:

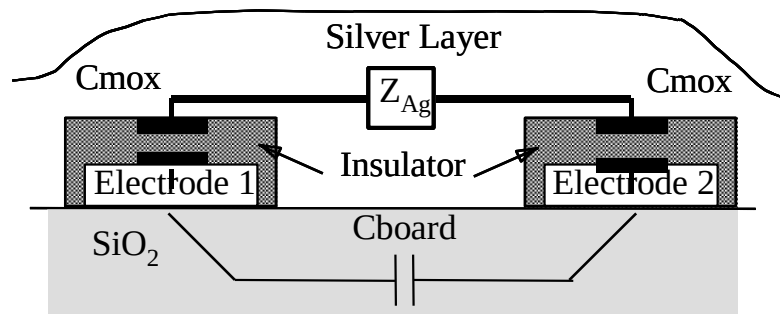
Al_2O_3 / SiO_2

Al_2O_3 / Al_2O_3

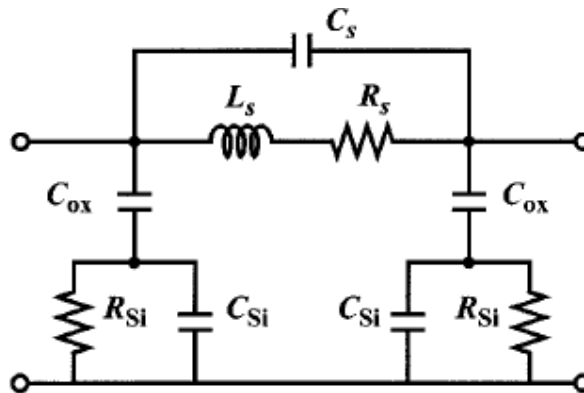
Interdigitated Capacitors

Basics of IDFs

Capacitance



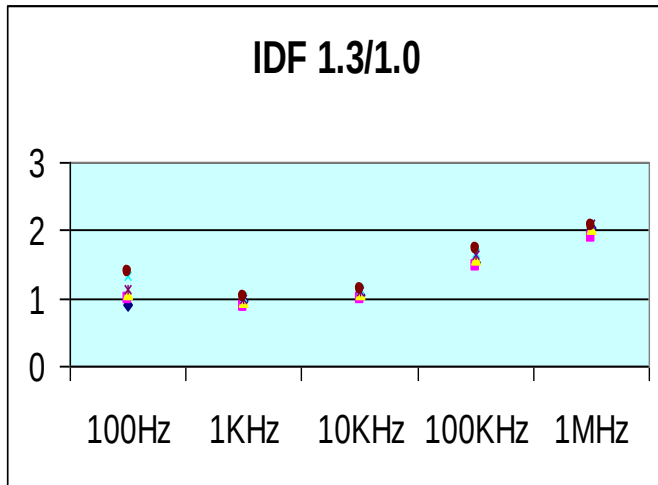
Resonance Frequency



$$f_{res} \approx \frac{1}{2\pi} \cdot \frac{1}{\sqrt{L_s \cdot C_s}}$$

Interdigitated Capacitors

As Fabricated, Capacitance in pF (Low Frequency)

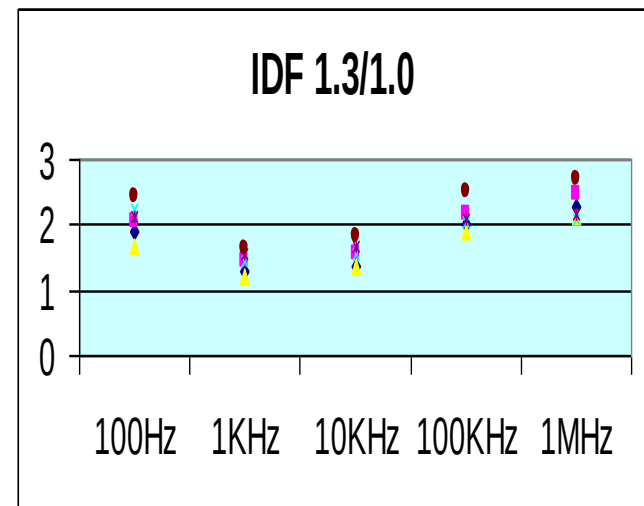


$\text{Al}_2\text{O}_3\text{-SiO}_2$

1,3/1,0	100Hz	1KHz	10KHz	100KHz	1MHz
avg	1.13	0.97	1.08	1.60	2.04
stdev	0.19	0.05	0.06	0.10	0.08
3,0/1,0	100Hz	1KHz	10KHz	100KHz	1MHz
avg	0.90	0.77	0.92	1.59	2.12
stdev	0.08	0.02	0.04	0.06	0.04

$\text{Al}_2\text{O}_3\text{-Al}_2\text{O}_3$

1,3/1,0	100Hz	1KHz	10KHz	100KHz	1MHz
avg	2.06	1.43	1.55	2.12	2.31
stdev	0.28	0.16	0.18	0.22	0.23
3,0/1,0	100Hz	1KHz	10KHz	100KHz	1MHz
avg	1.41	0.99	1.15	1.92	2.23
stdev	0.10	0.06	0.06	0.11	0.20



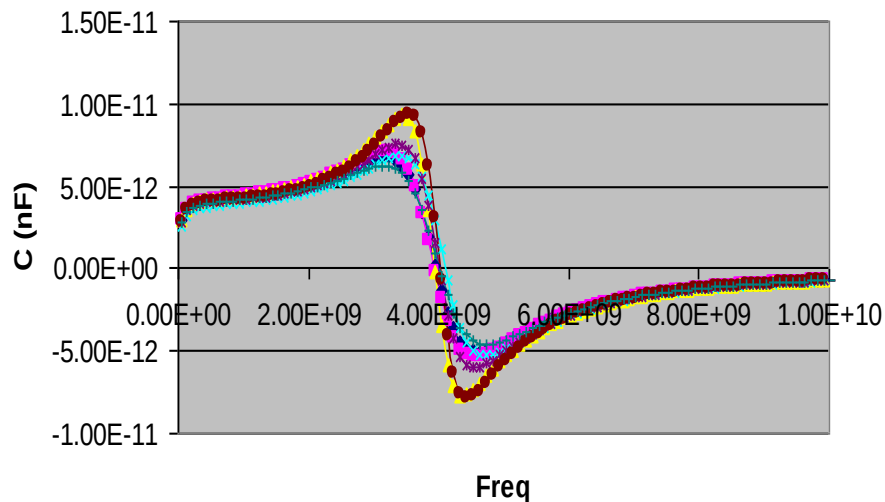
Interdigitated Capacitors



As Fabricated, Resonance Frequency

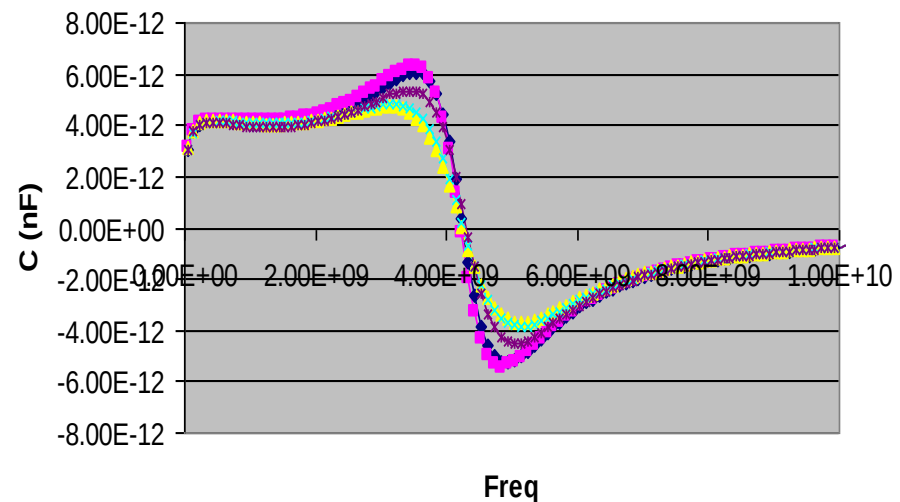
$\text{Al}_2\text{O}_3\text{-SiO}_2$

IDF 1.3/1.0



Res Freq = 3.94 – 4.04 GHz

IDF 3.0/1.0

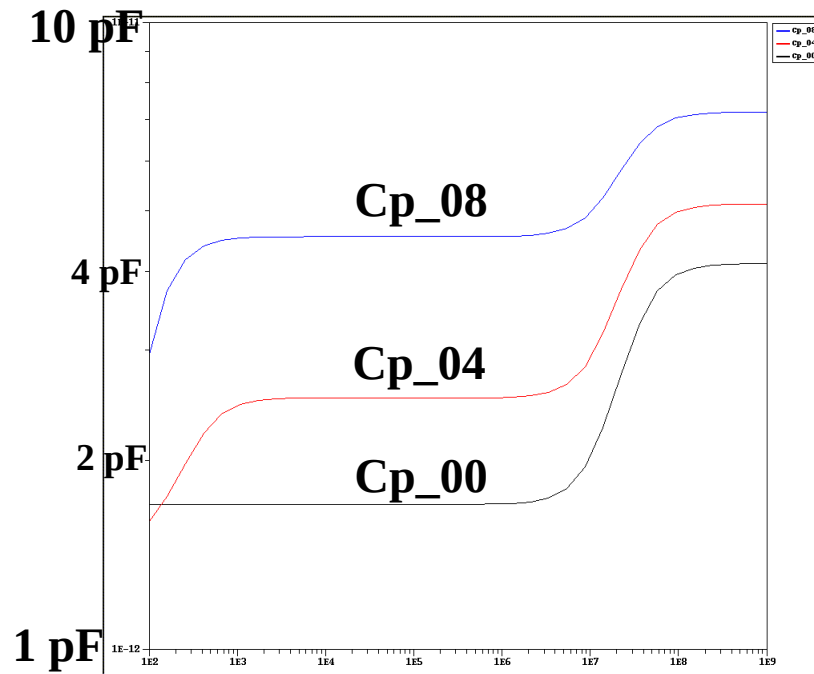
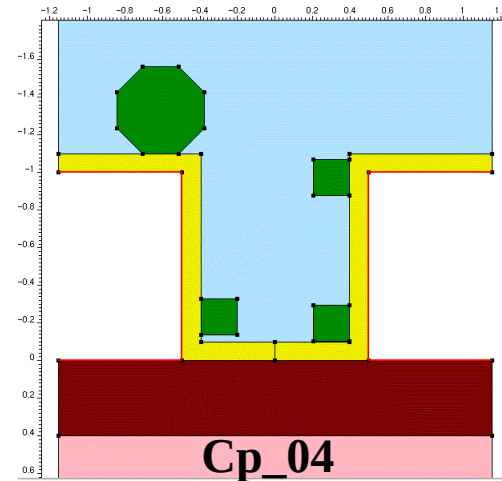
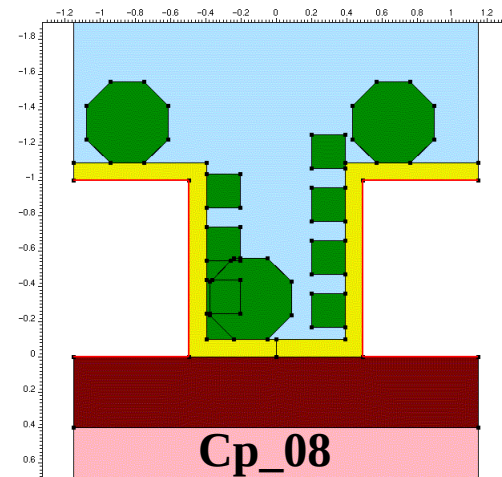


Res Freq = 4.28 - 4.38 GHz

Interdigitated Capacitors

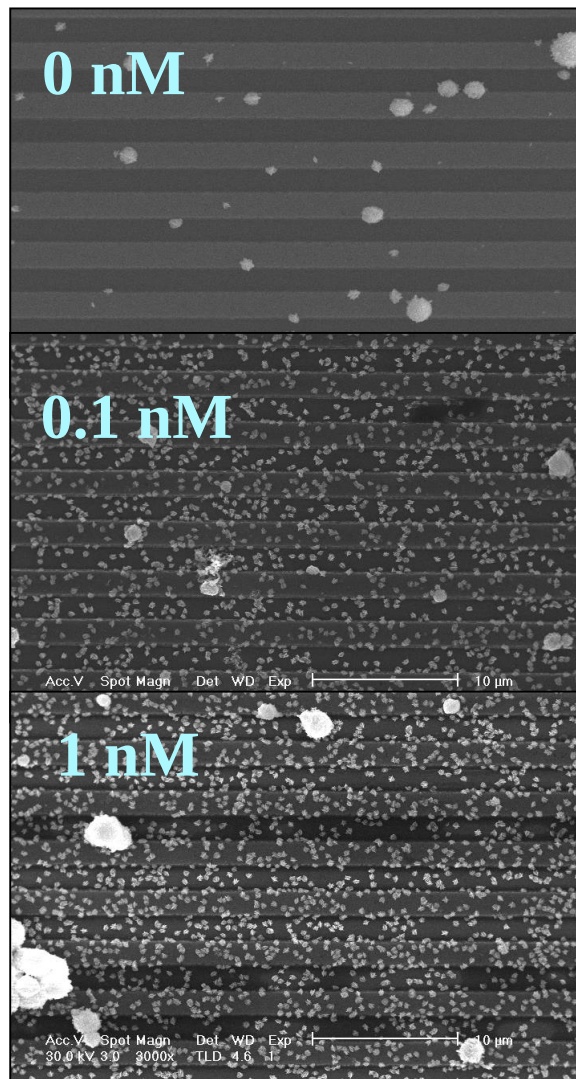
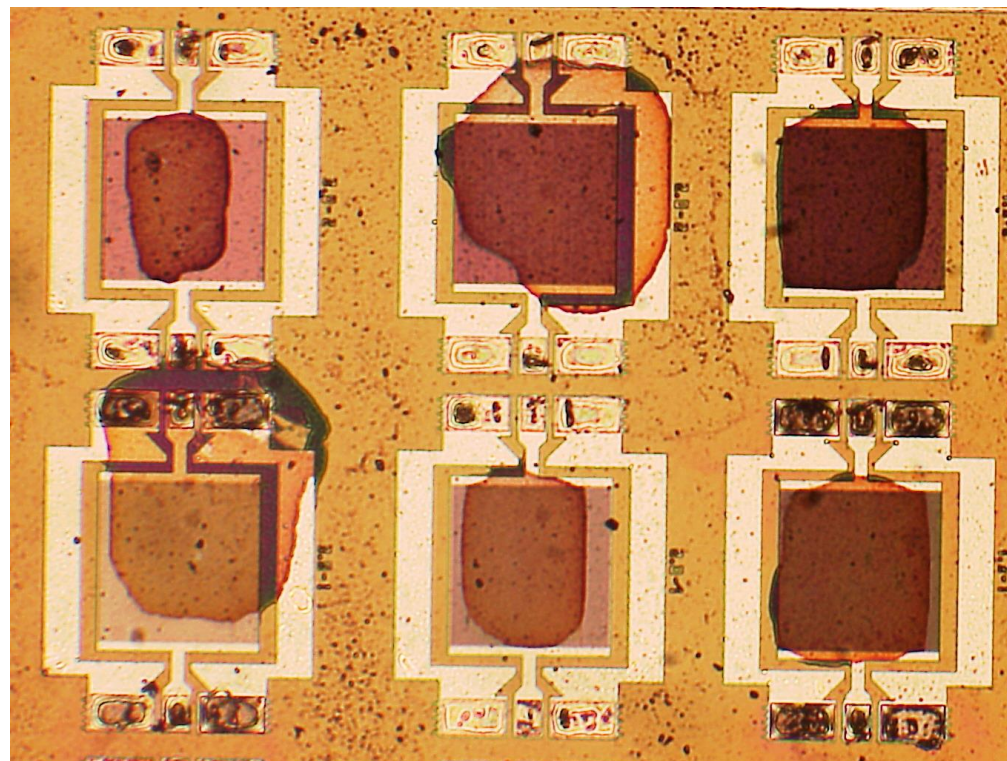
Interdigitated fingers (simulation):

Fingers / space
1.3 / 1.0



Interdigitated Capacitors

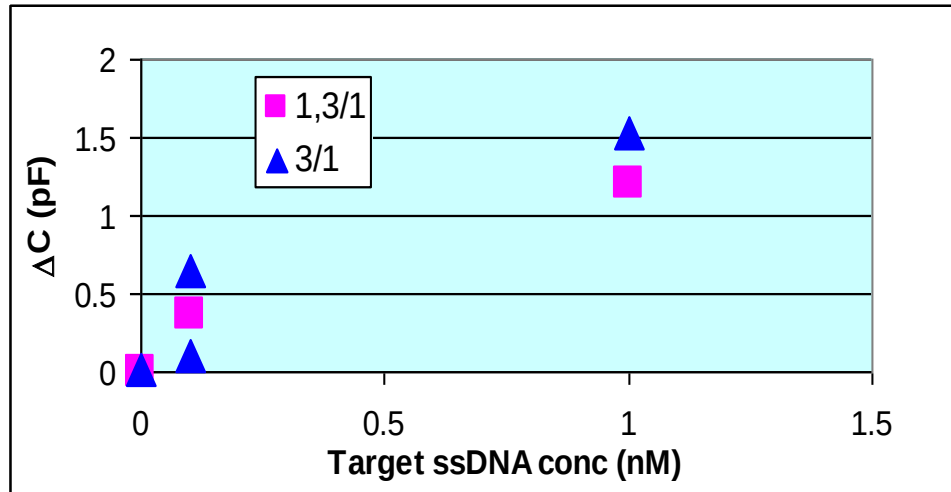
DNA Spots SEM Pictures



Interdigitated Capacitors

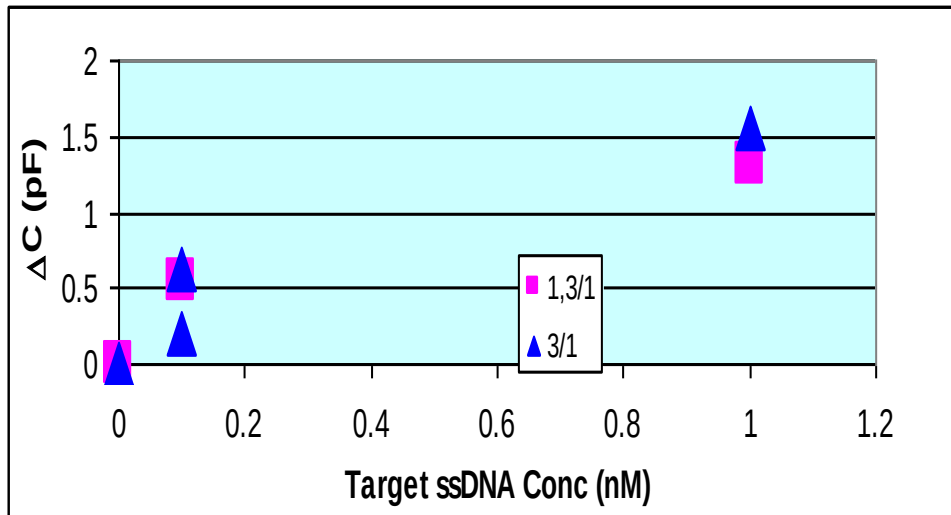
$\text{Al}_2\text{O}_3\text{-SiO}_2$ After Ag Precipitation

10KHz



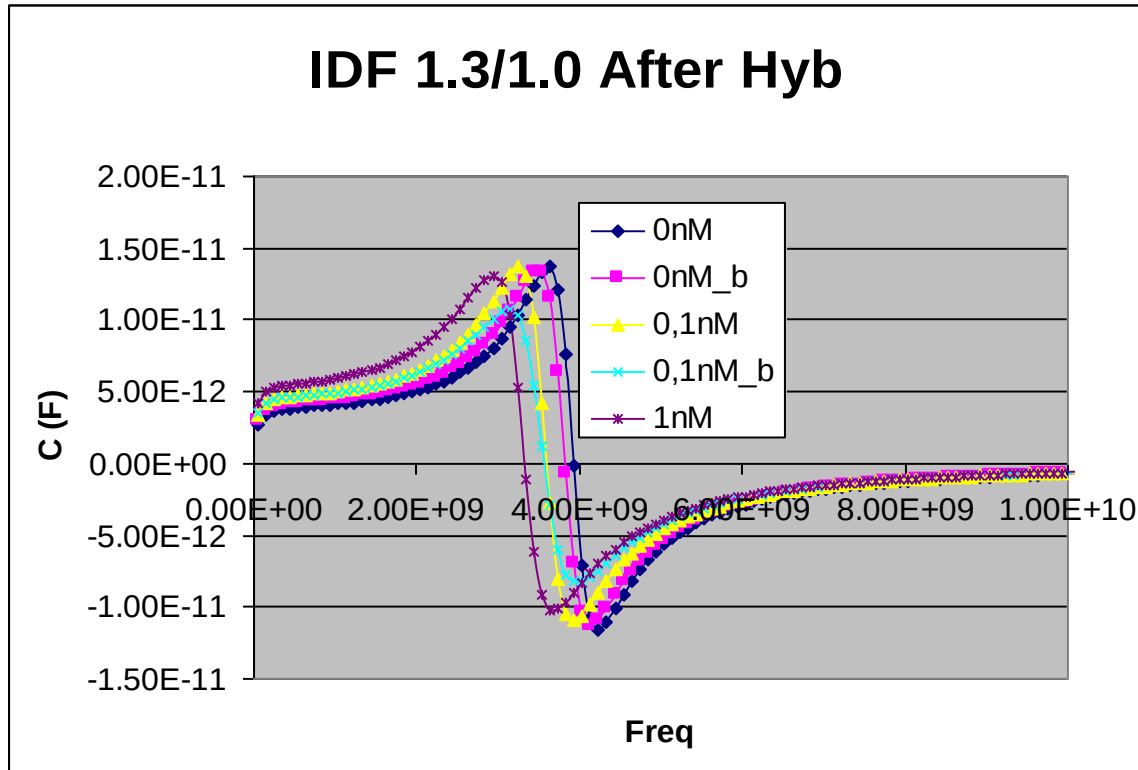
$$\Delta C = C_{Ci} - C_{C0}$$

100KHz



Interdigitated Capacitors

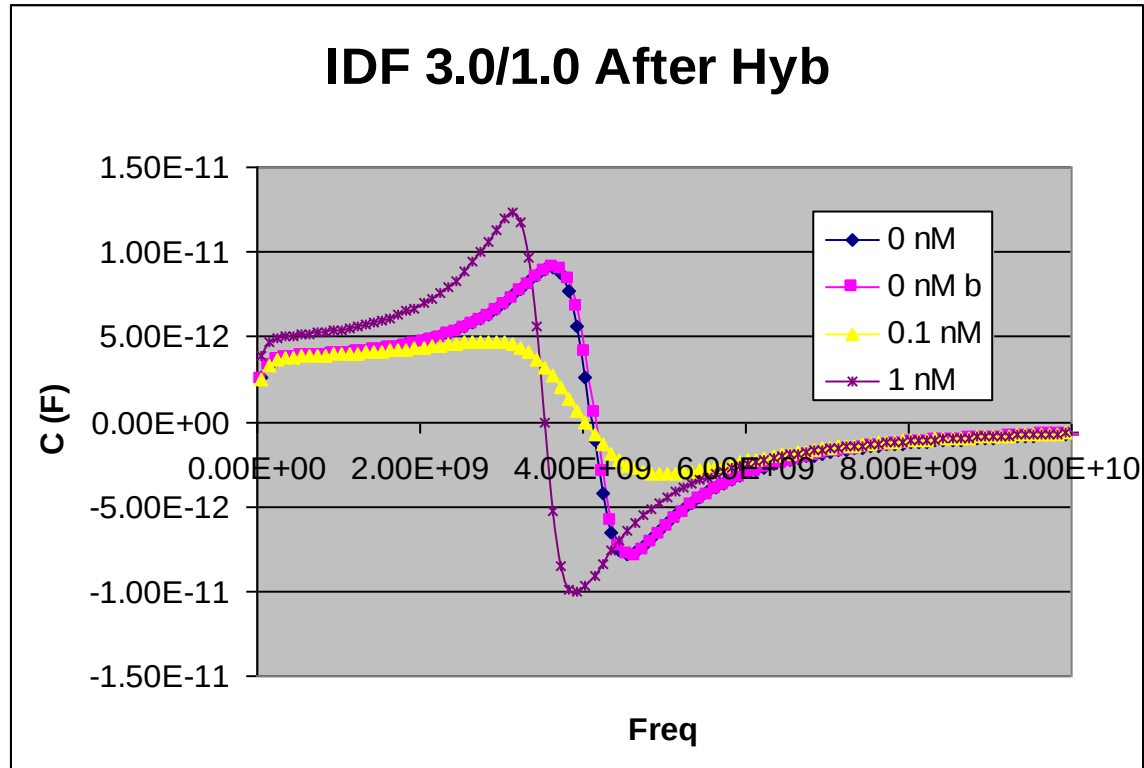
Resonance Frequency After Ag Precipitation



	Vide	0nM	0nM_b	0,1nM	0,1nM_b	1nM
F Res	3.99E+09	3.89E+09	3.79E+09	3.59E+09	3.59E+09	3.29E+09
Vs Vide		1.00E+08	2.00E+08	4.00E+08	4.00E+08	7.00E+08
Vs 0nM				2.50E+08	2.50E+08	5.50E+08

Interdigitated Capacitors

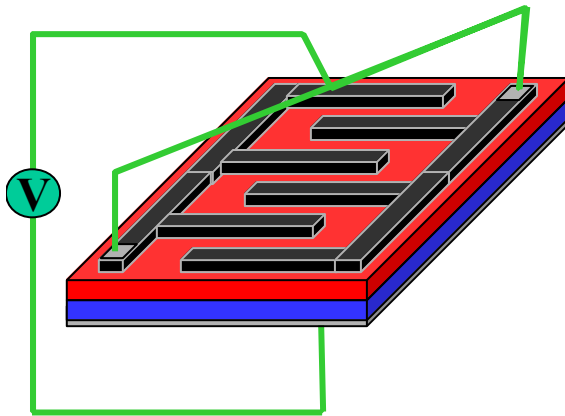
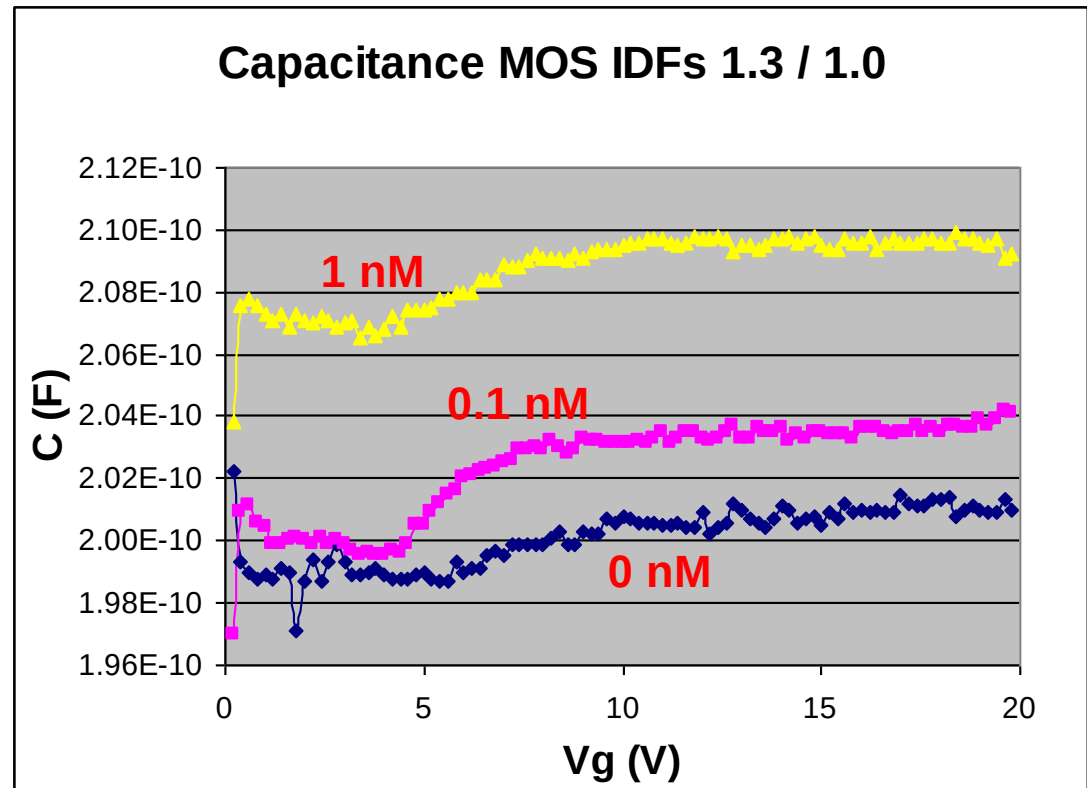
Resonance Frequency After Ag Precipitation



	Vide	0nM	0nM b	0,1nM	0,1nM b	1nM
F Res	4.14E+09	4.09E+09	4.19E+09	3.99E+09	2.69E+09	3.49E+09
Vs Vide		2.00E+08	1.00E+08	3.00E+08	1.60E+09	8.00E+08
Vs 0nM				1.50E+08	1.45E+09	6.50E+08

Interdigitated Capacitors

MOS Capacitance After Ag Precipitation



Interdigitated Capacitors



IDF 1.3 / 1.0 Electrical Results Comparison

<u>Variable Measured</u>	<u>Target ssDNA conc.</u>	<u>Delta Range</u>
Capacitance	0.1 nM	0.37 – 0.62 pF
	1.0 nM	1.21 – 1.31 pF
Resonance Frequency	0.1 nM	0.24 – 0.26 GHz
	1.0 nM	0.54 – 0.56 GHz
MOS Capacitance	0.1 nM	0.003 pF
	1.0 nM	0.009 pF

- Optimization has made possible the noise reduction after silver precipitation.
- Now concentration as low as 0.1 nM (100pM) Target ssDNA (hybridization) was successfully electrical detected using IDFs Capacitors.
- Silver coverage study suggests that detection of a concentration of Target ssDNA could be also possible down to the 0.01 nM (study still in progress).
- Same Structure, Three Different Measurement Methods, opens new data processing towards an increased overall sensitivity and precision.