EPFL

Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #13

CMOS Circuits for DNA Detection

Lecture Outline

(Book Bio/CMOS: Chapter' paragraphs § 7.1-8)

- CMOS for DNA capacitance detection
- Charge-Based Capacitance Measurement (CBCM) Method
- Frequency-to-Capacitance Measurement (FTCM) Method
- CMOS for DNA Amperometric detection

CMOS architectures for VLSI

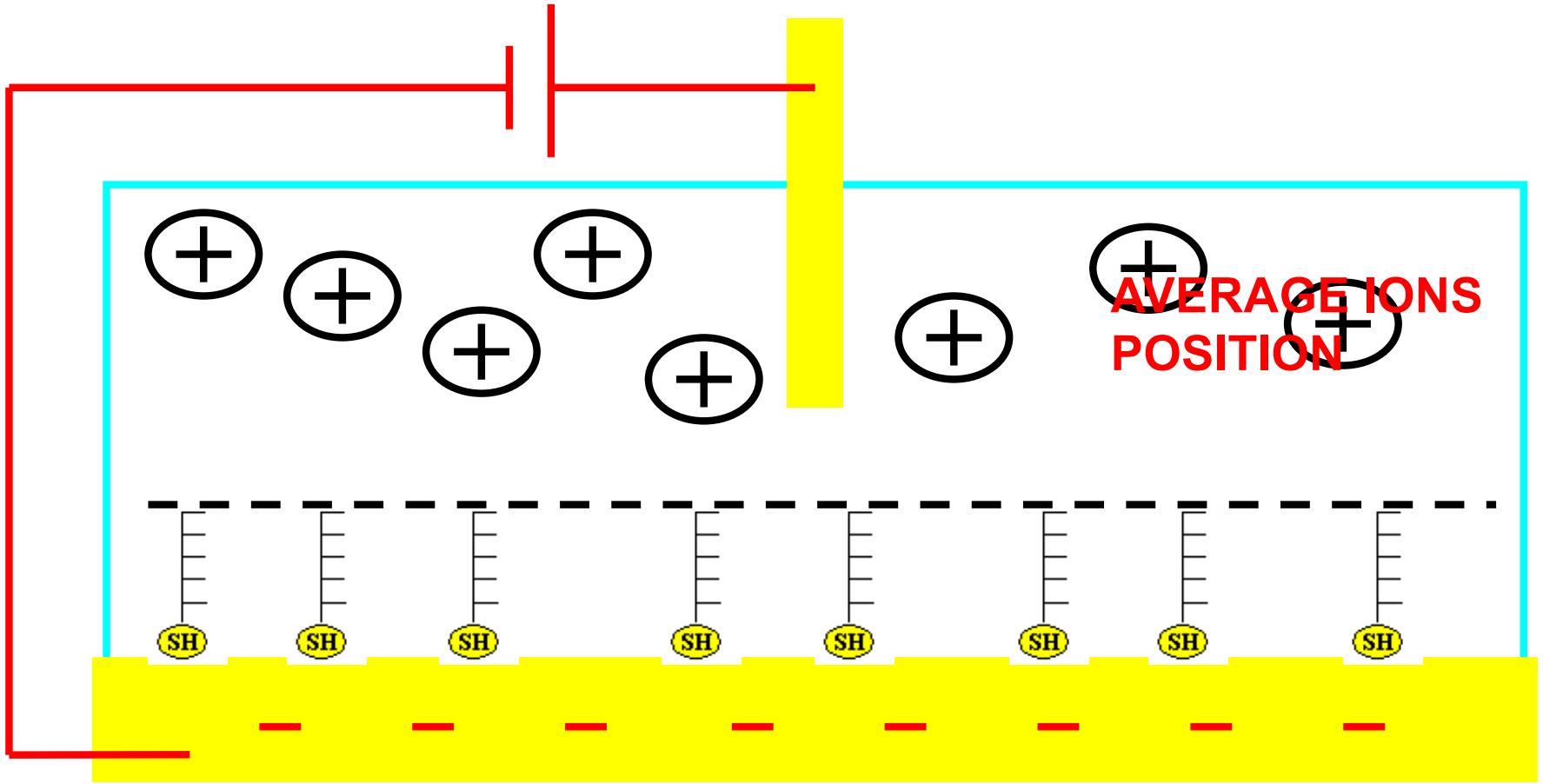
in DNA Detection

Q1

Do we really need point-of-care portable DNA detectors?

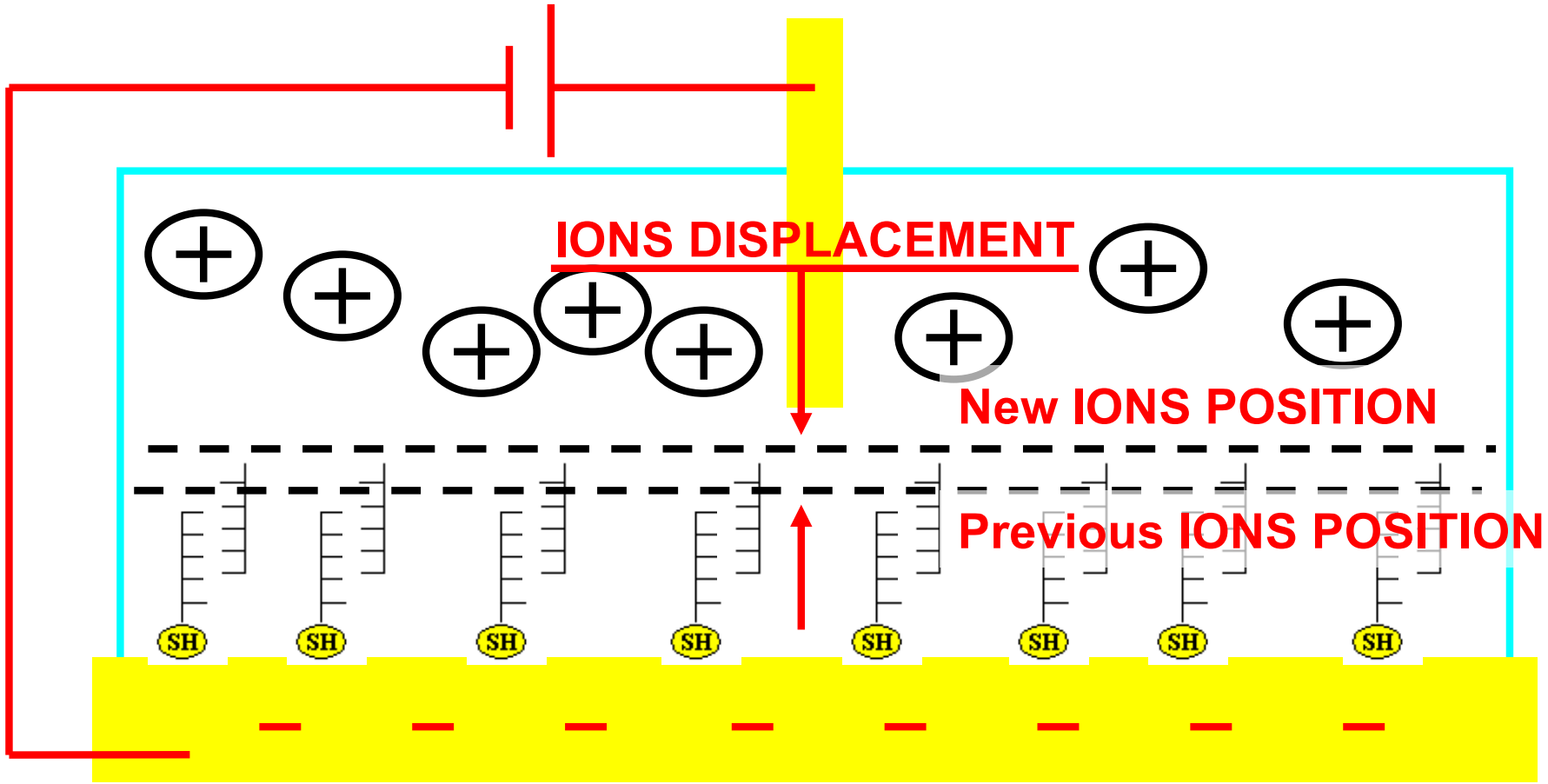
A. No, that's just an academic fashion!
B. Not really, even though it might be useful for precision medicine
C. Yes, since it is useful for precision medicine
D. Yes, since it might be useful to establish genetic predispositions
E. Yes, of course!

Electrochemical Interface



Ion planes are formed at the interface when electrodes immersed in solution are polarized

Electrochemical Interface



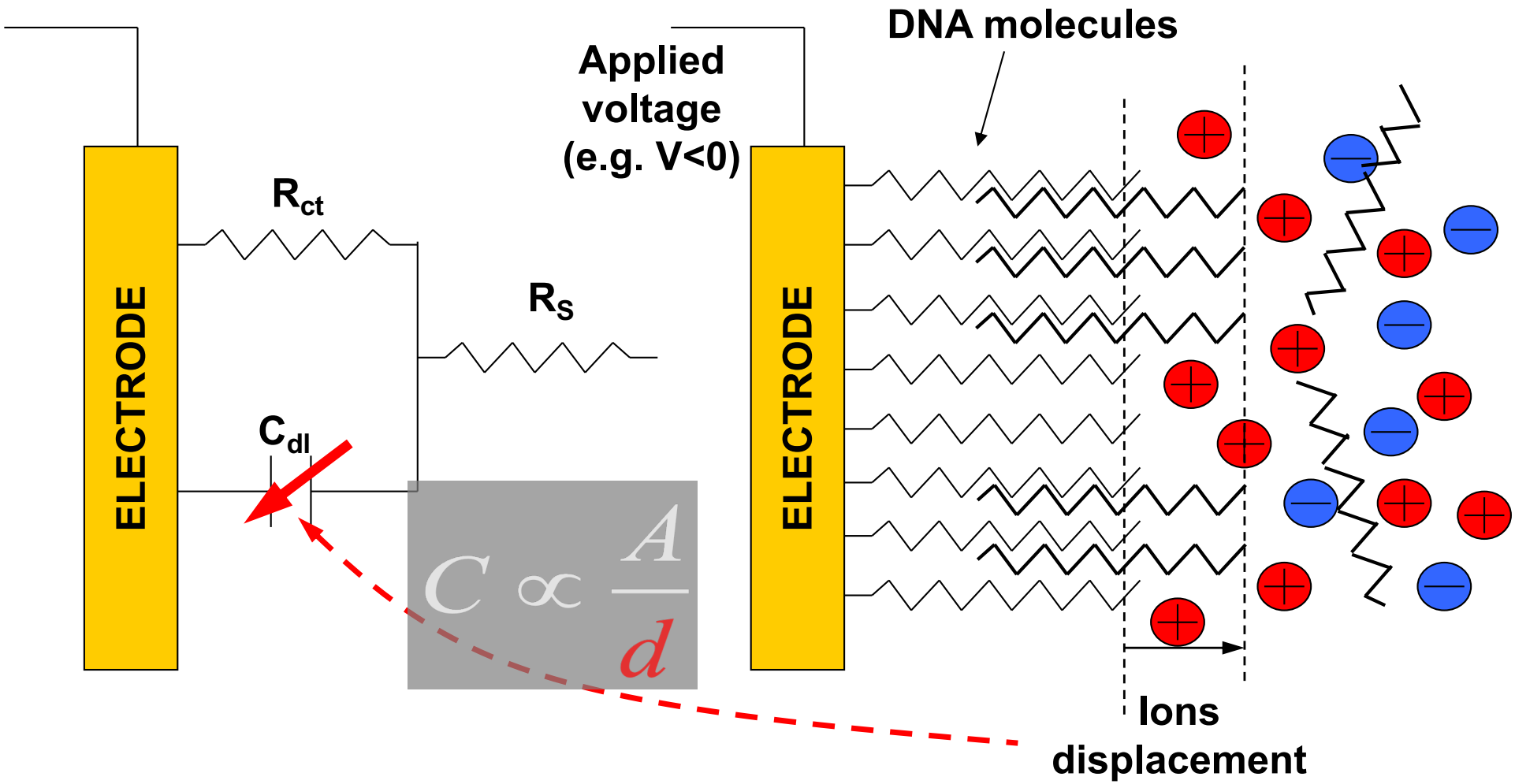
Ion planes are formed at the interface when electrodes immersed in solution are polarized

Q2

Do we usually get only the fully matching sequences?

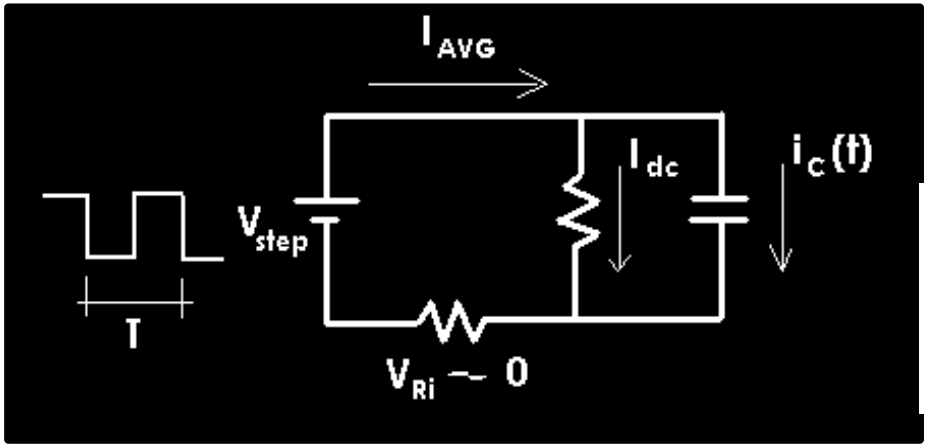
A. No, we always have mismatches
B. Yes, but we might get few-bases mismatch
C. May be, since we might get non-specific adsorption on the surface
D. On same probes, we may get a wrong sequence
E. Yes, of course!

The Capacitance DNA Detection



Unlabeled ssDNA may be detected with capacitance measurements as due to charge displacement

Charge-Based Capacitance Measurement (CBCM)



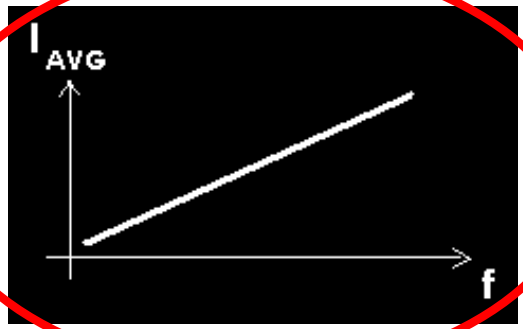
$$i(t) = I_{dc} + i_C(t)$$

Frequency!

$$I_{AVG} = \frac{I_{dc}}{2} + \frac{1}{T}\int_0^{T/2} i_C(t)\,dt$$

$$I_{AVG} = \frac{I_{dc}}{2} + CV_{step}f$$

THE CAPACITANCE !



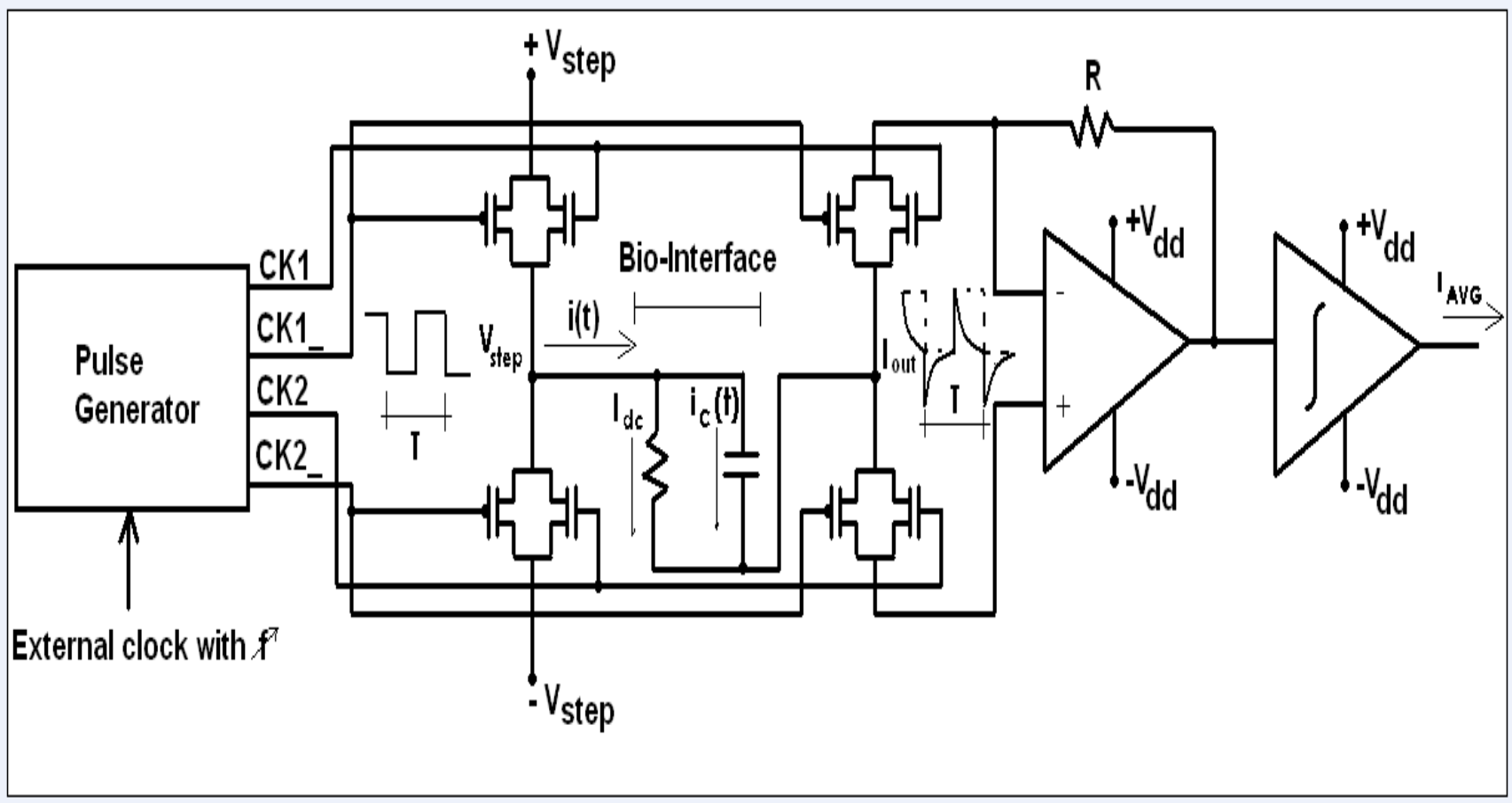
Method for a precise Capacitance measurement

Q3

Is that correct to estimate the C-value in frequency?

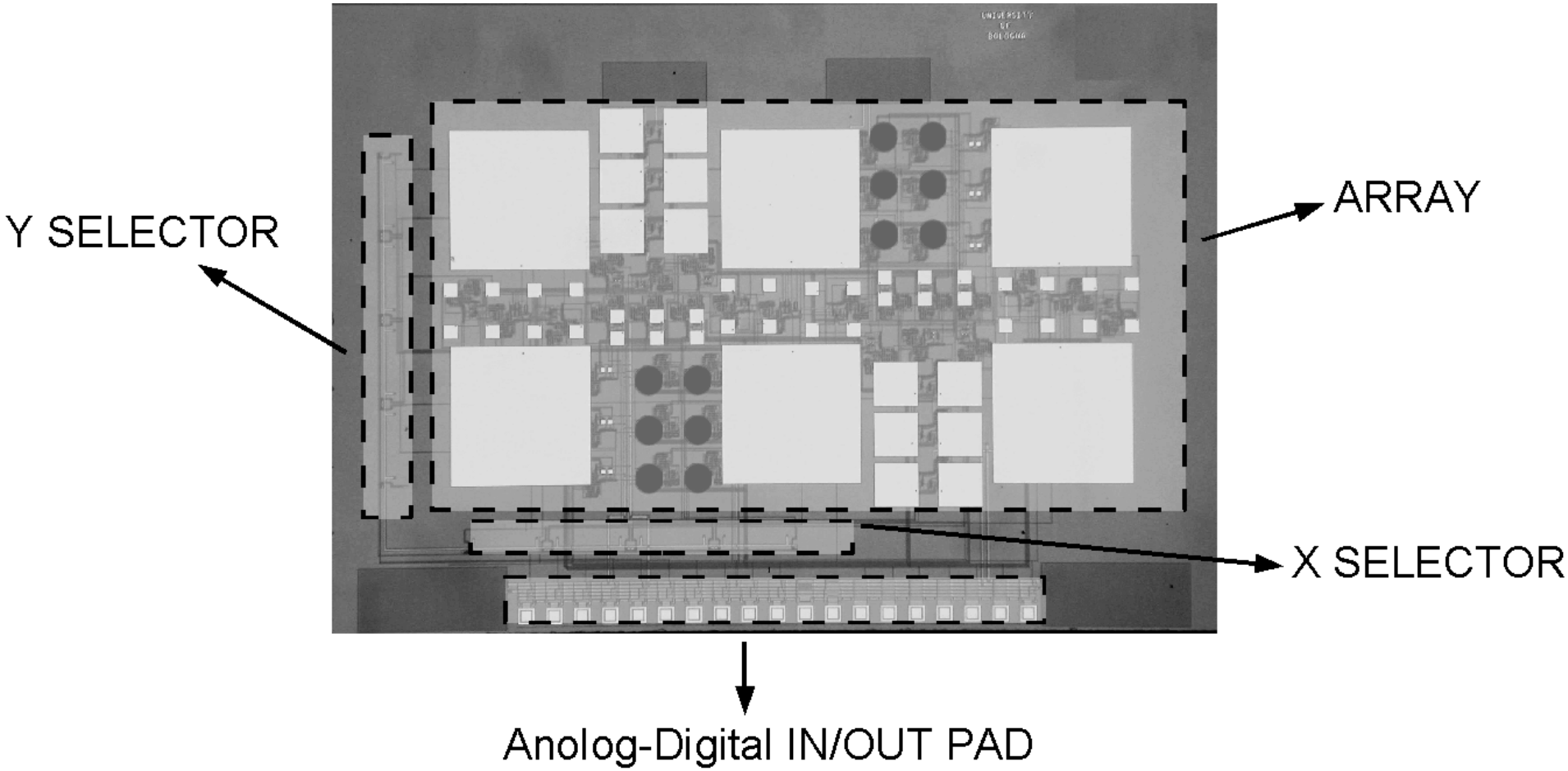
A. No, C always changes with the frequency
B. Not always, since C might change with the frequency
C. May be, in case of some good interfaces
D. Some times, we got a totally wrong estimation
E. Yes, of course!

CMOS for CBCM detection



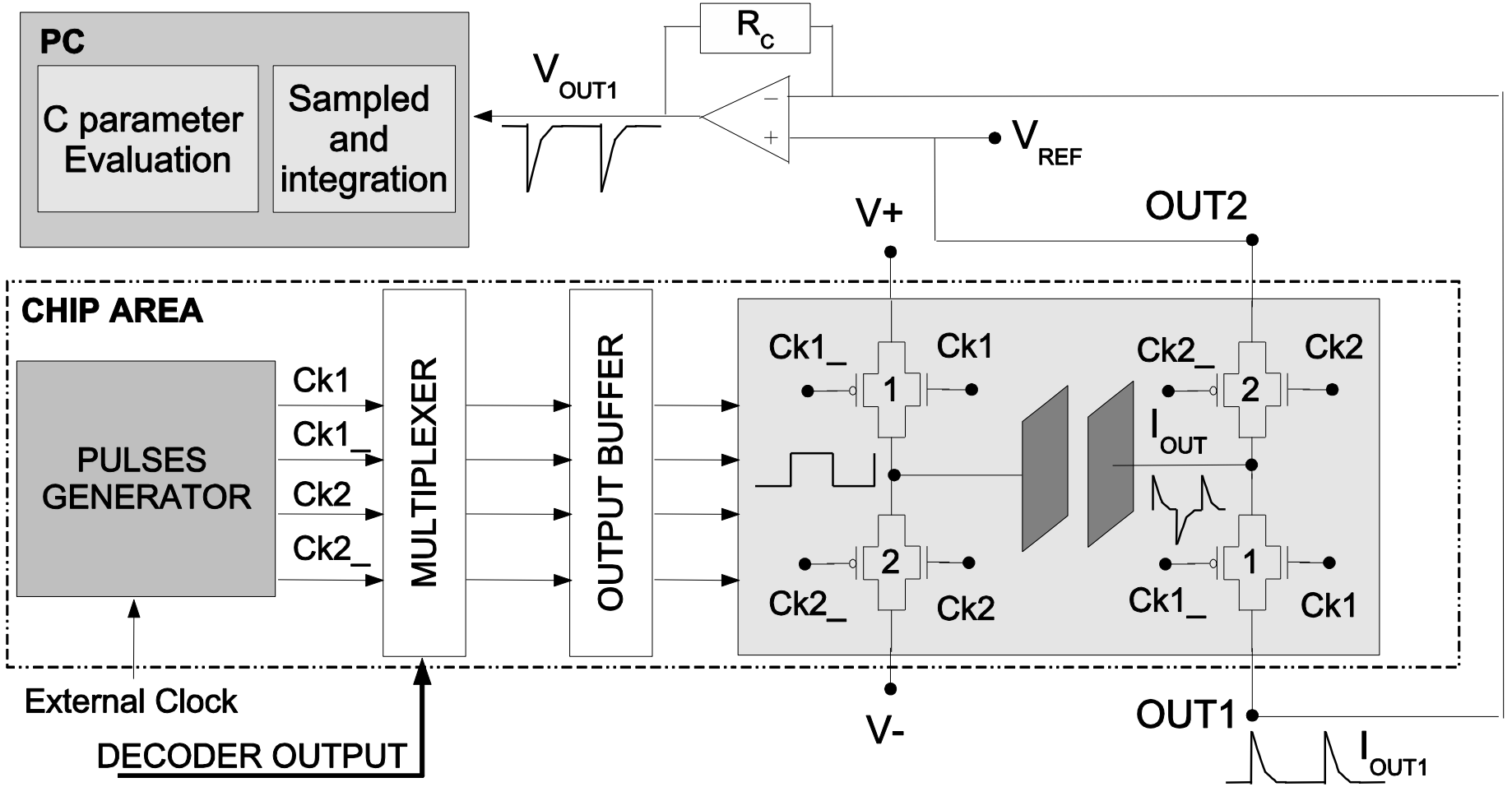
The circuit assures the square signal generator, an inverters, and an integrator to calculate the average current

The Chip Electrodes Layout



The VLSI Implementation of the Chip (CBCM method)

(CBCM = Charge Base Capacitance Mode)



The problem of overlapping signals

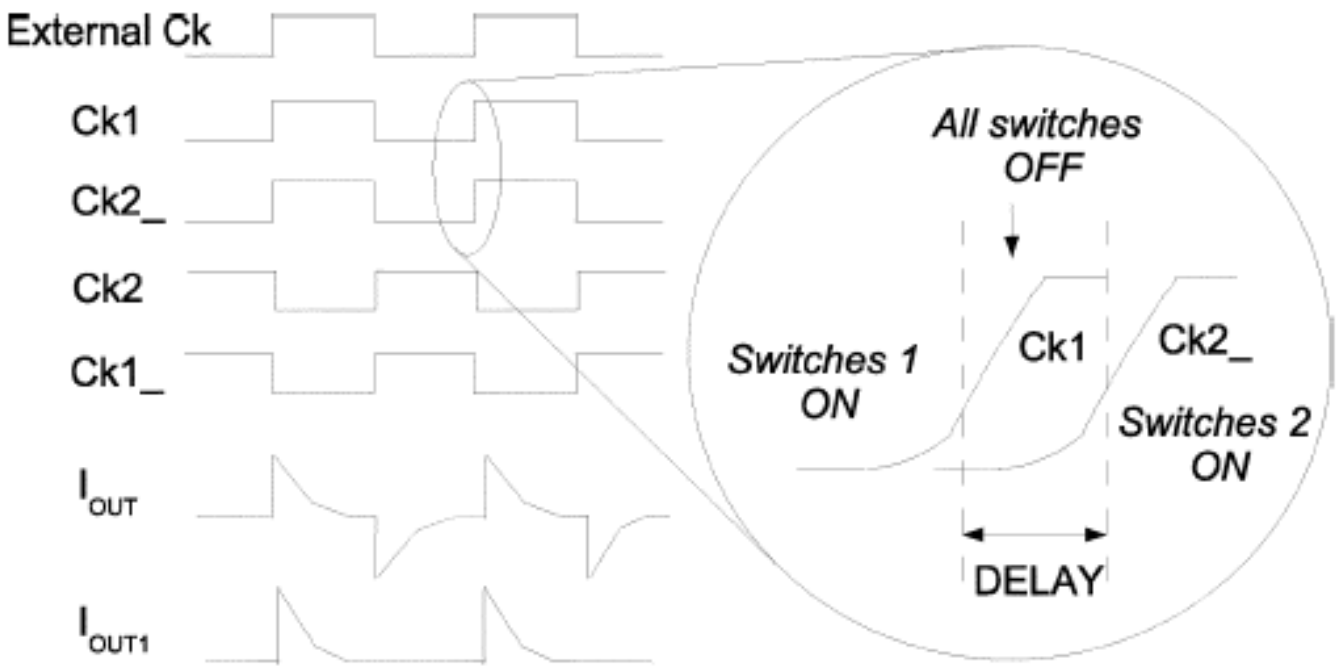


Fig. 4. Schematic representation of the signals flow used in the experiments.

Ck and Ck_ signals need to be not-overlapping in order to assure the correct square signal generation

The circuit solution

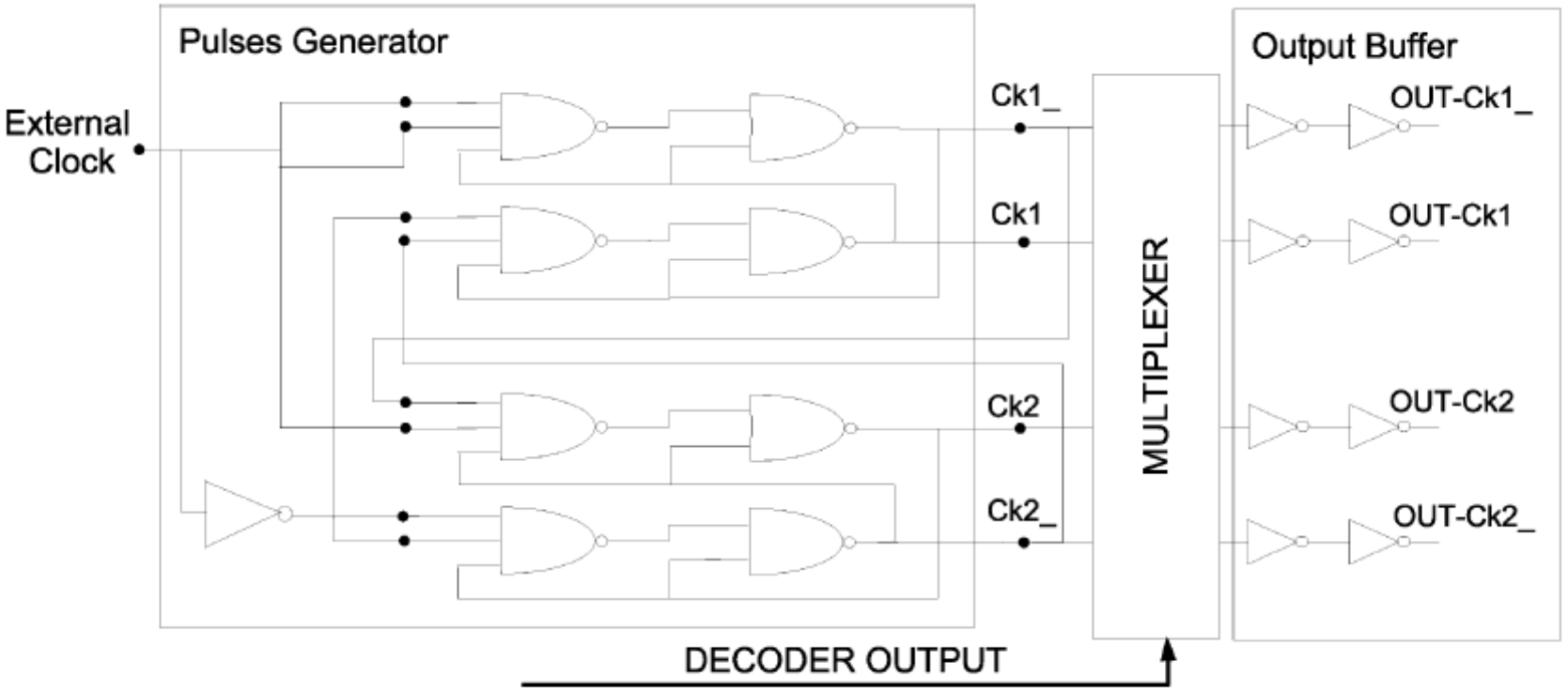


Fig. 5. Schematic plot of the block used to generate not overlapping clock signals.

A simple logical circuit and a digital multiplexer assures not-overlapping Ck and Ck_ signals

The Measurements Set-up

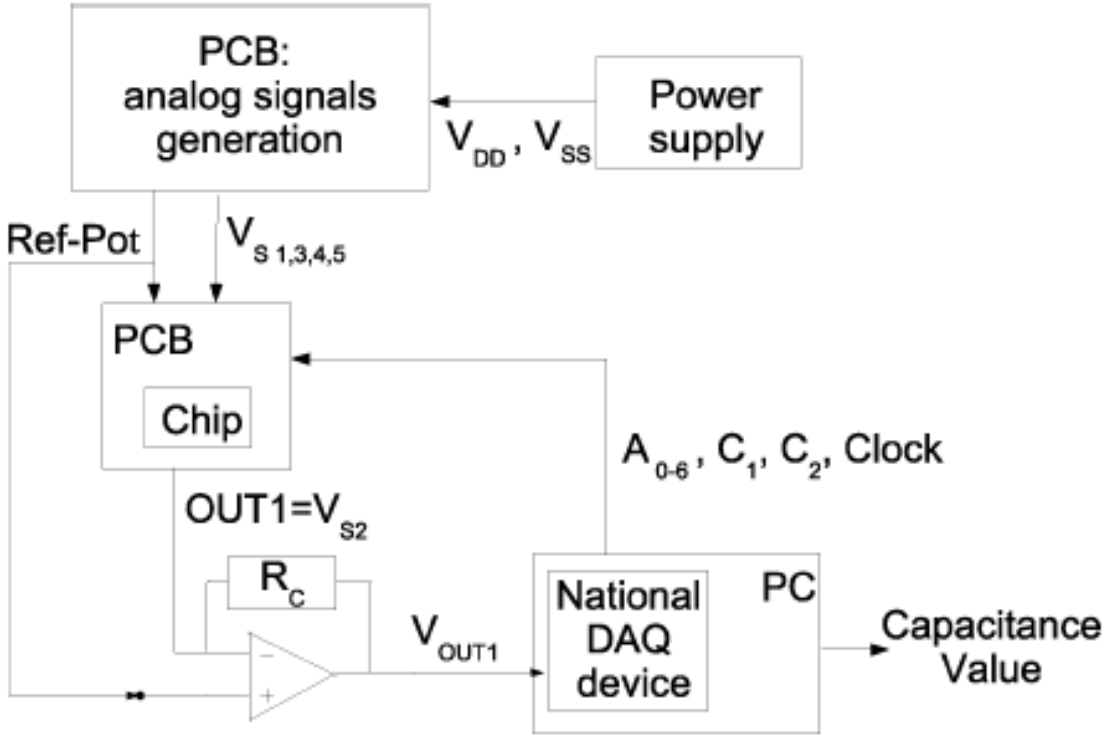


Fig. 8. Schematic representation of the measurement setup.

The Chip has been mounted onto a PCB for PC remote control and testing

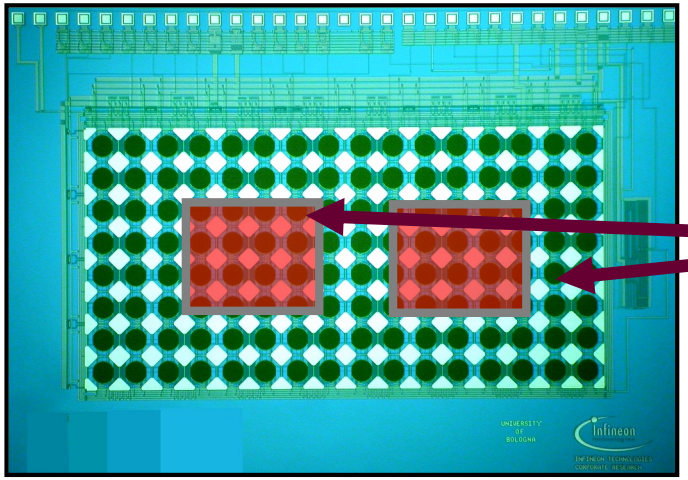
Liquid Measurement set-up

Chip is glued on a PCB

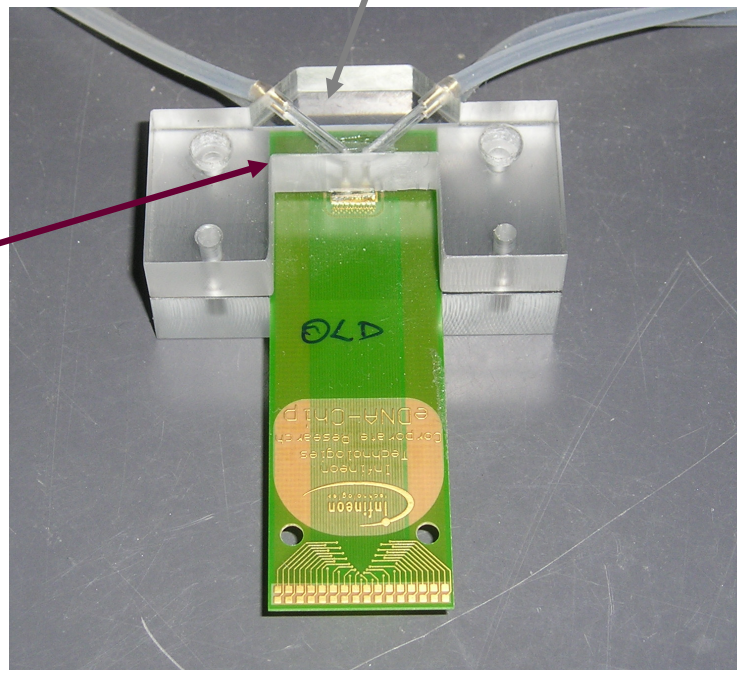
Output PCB pads

Bonding wires

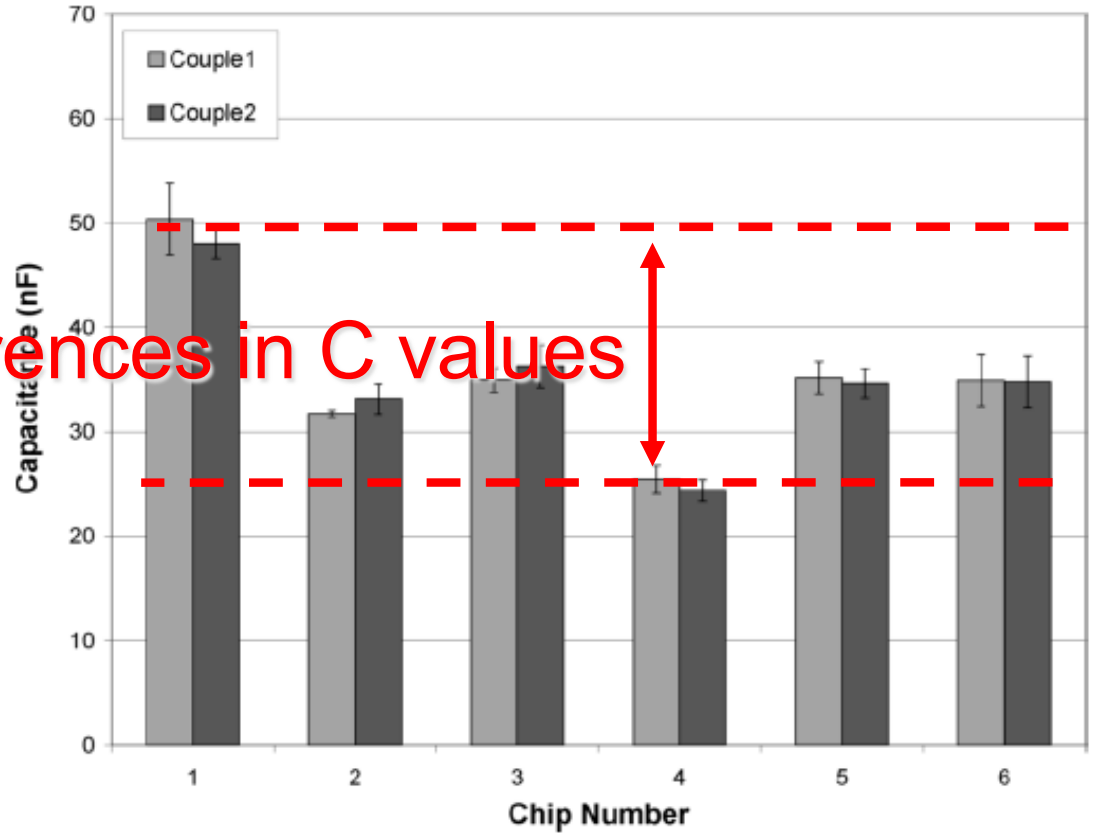
Fluidic cell



Two different Chambers 1mmX1mm



DNA detection in CBCM mode



Large differences in C values

Fig. 10. Capacitance measurements of electrode couples on different chips.

The chip-by-chip reproducibility has been not so high: the problem is on the chip electrodes cleaning

Q4

Is it correct to use CBCM for the C-value on bare electrodes?

A. No, C always changes with the frequency
B. Not always, since C might change with the frequency
C. May be, in case of some good interfaces
D. Some times, we got a totally wrong estimation
E. Yes, of course!

Capacitance vs Frequency

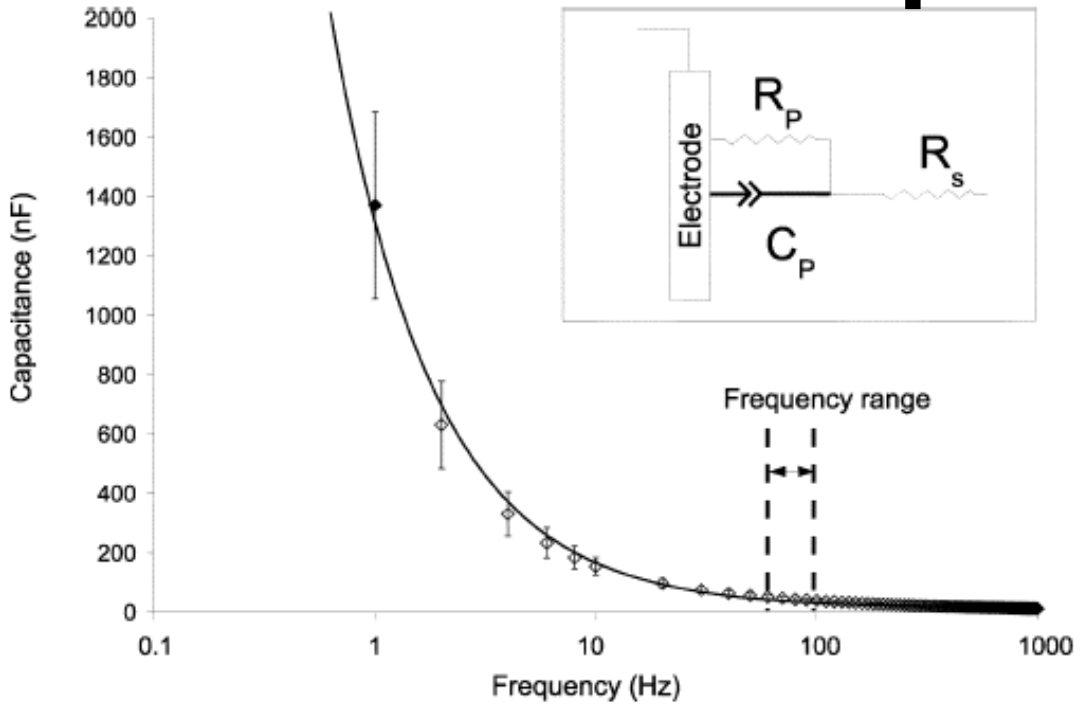


Fig. 9. Measured capacitance versus charge/discharge frequency on clean gold electrodes. The continuous line shows the fitting.

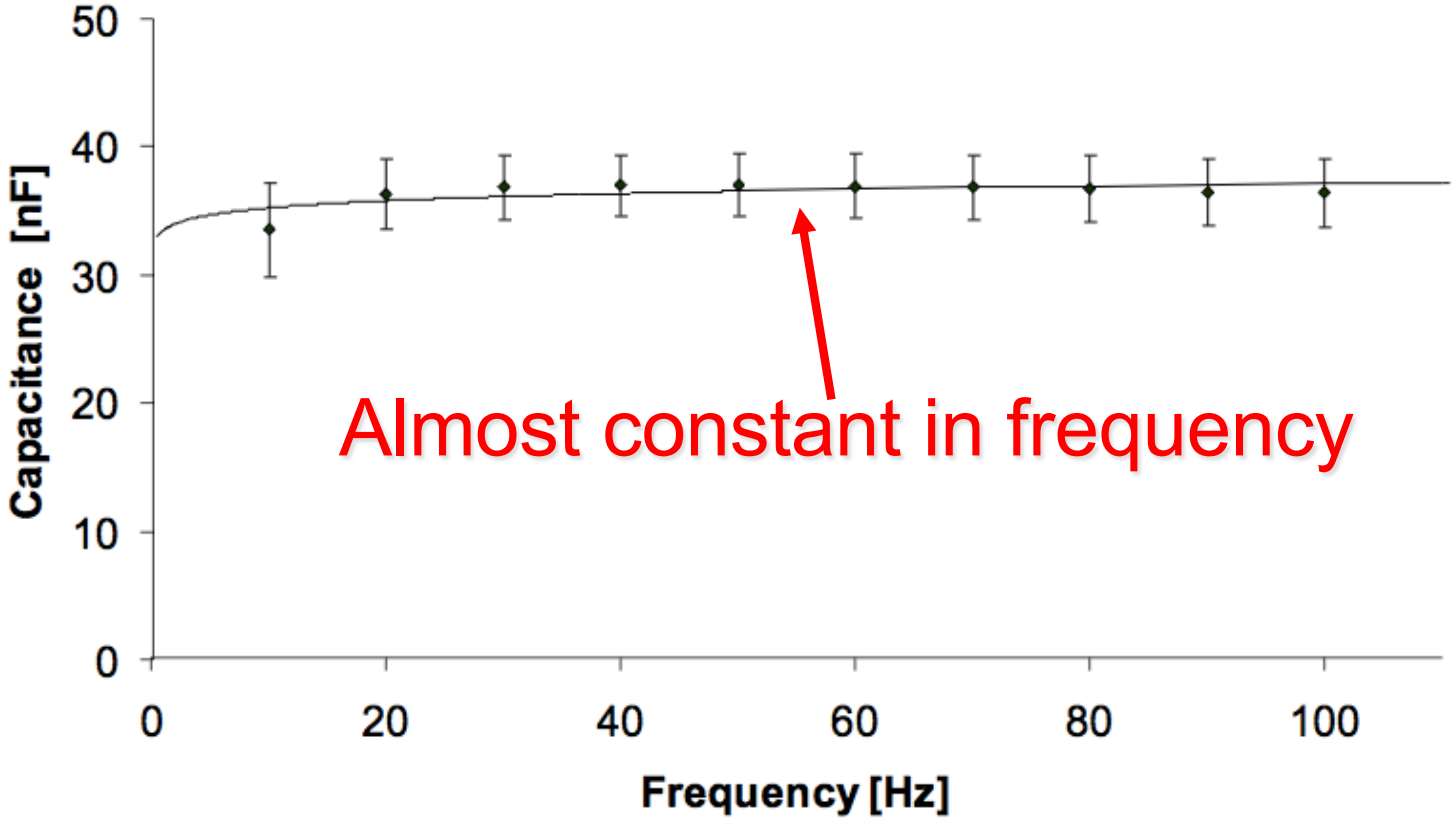
The trends of the measured capacitance vs frequency decrease the accuracy of the measurements in CBCM mode

Q5

May we improve the ideality of C-behaviour of gold electrodes?

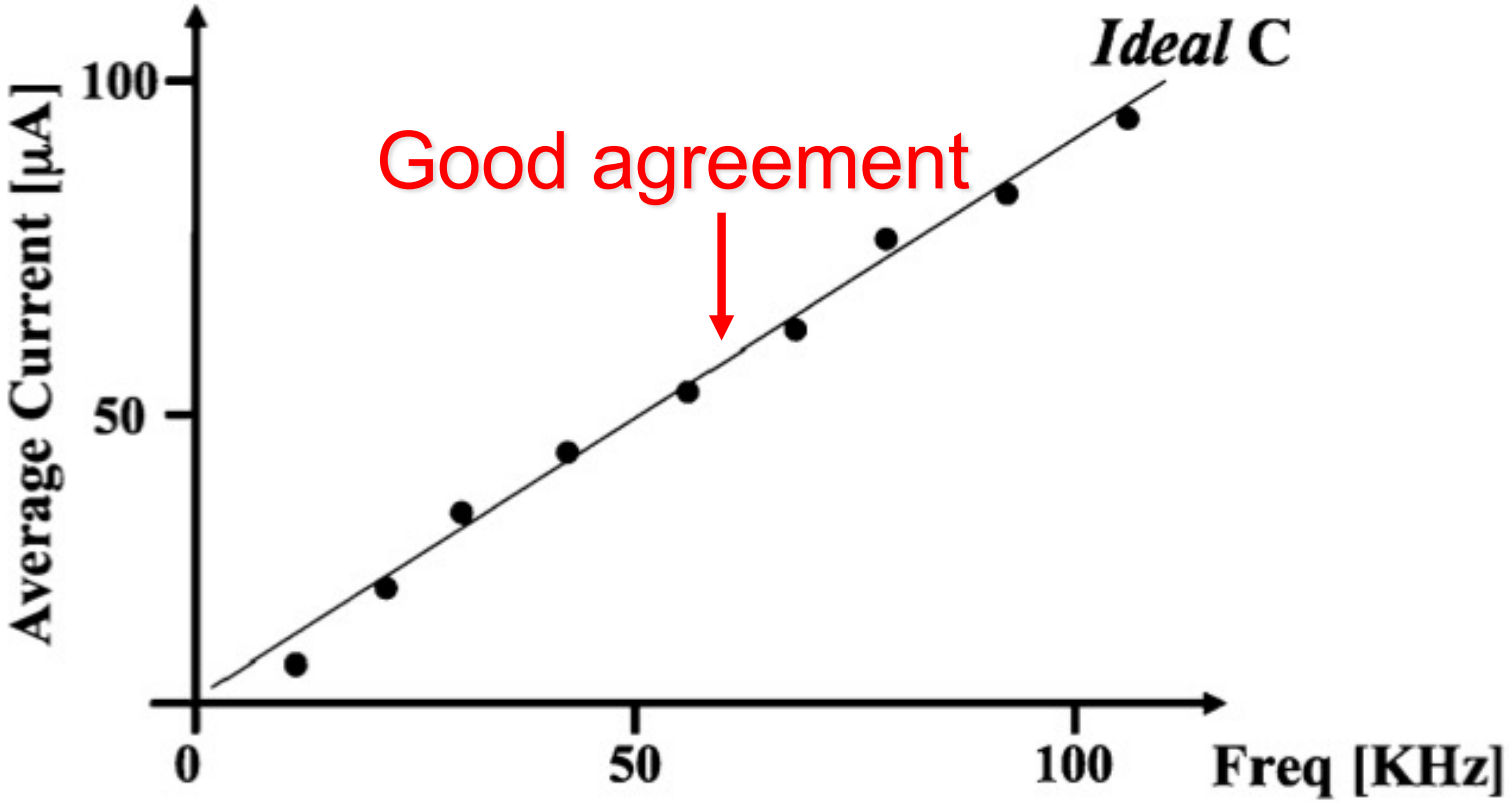
A. Impossible, C always changes with the frequency
B. Not easy, since C might change any way with the frequency
C. May be, in case of some good self-assembly
D. Some times, we get the right behavior
E. Yes, by measuring at high frequency!

Good DNA Layer



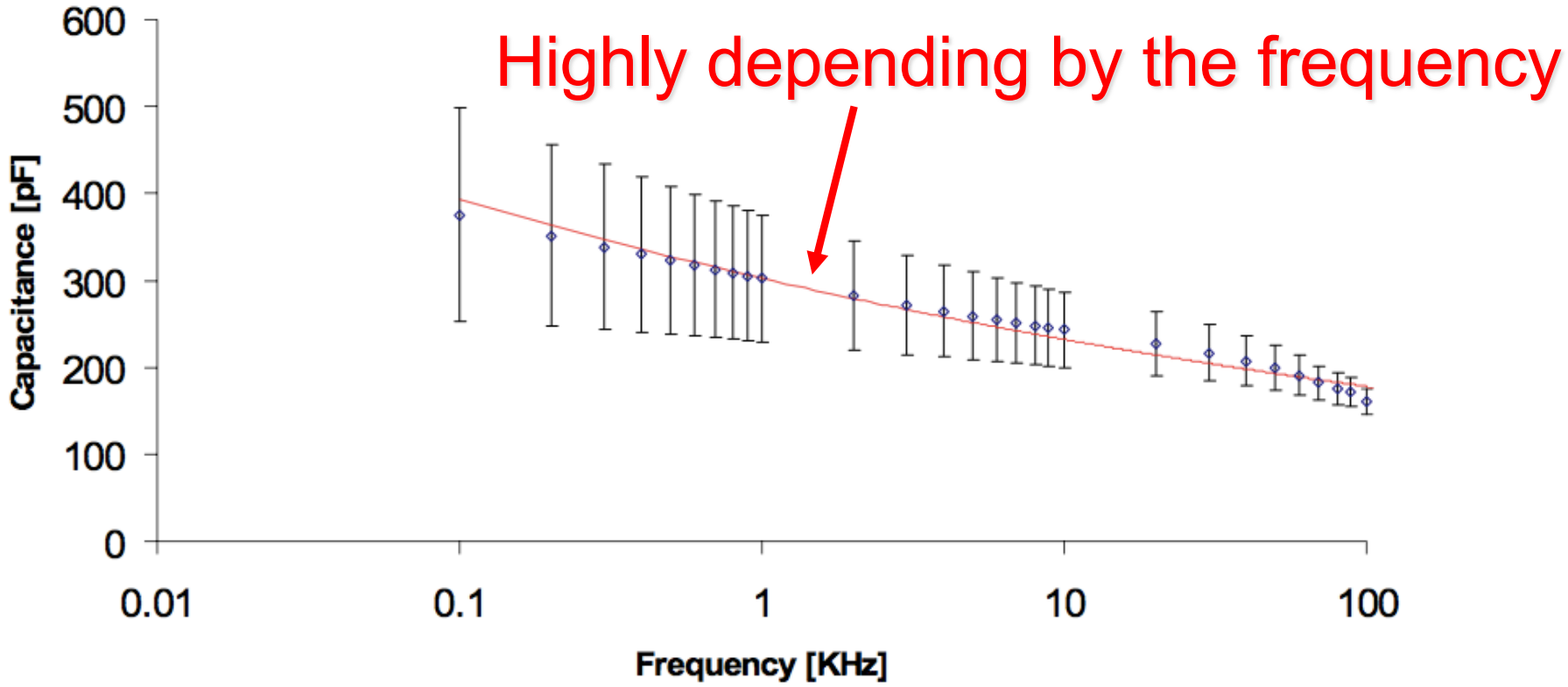
DNA Layer is independent by the frequency thanks to probes immobilized on Ethylene-Glycol Thiols

CBCM on good DNA Layer



CBCM method on a DNA Layer that is independents by the frequency

Bad DNA Layer



DNA Layer is dependent by the frequency since the monolayer is not extremely well formed

Q6

How much wrong may be the C-value estimation by CBCM?

A. Always really bad since C always changes in frequency

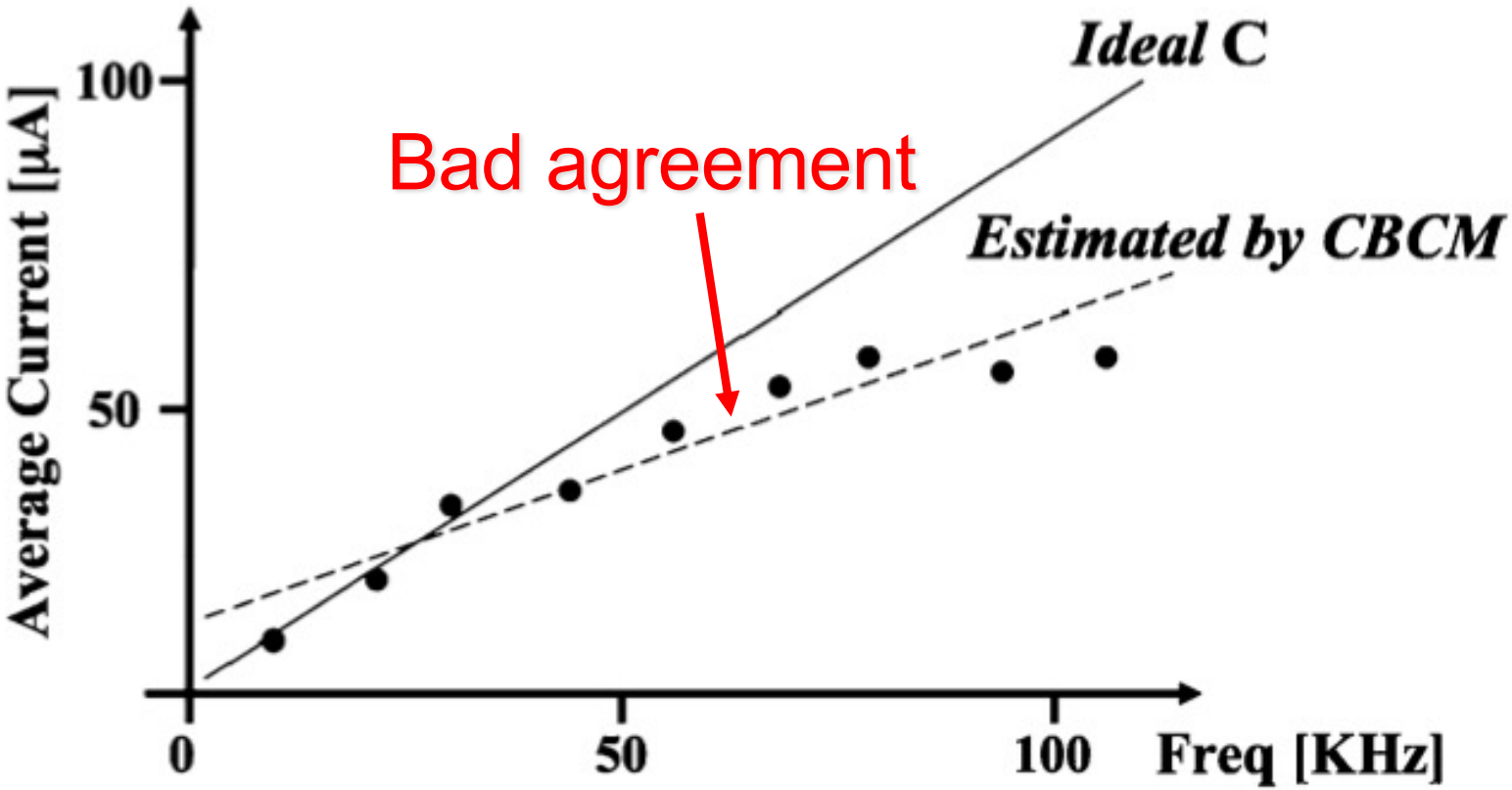
B. Some times really wrong, when C changes in frequency

C. Almost always good, in case of DNA interfaces

D. Always largely good

E. Always largely wrong

CBCM on bad DNA Layer



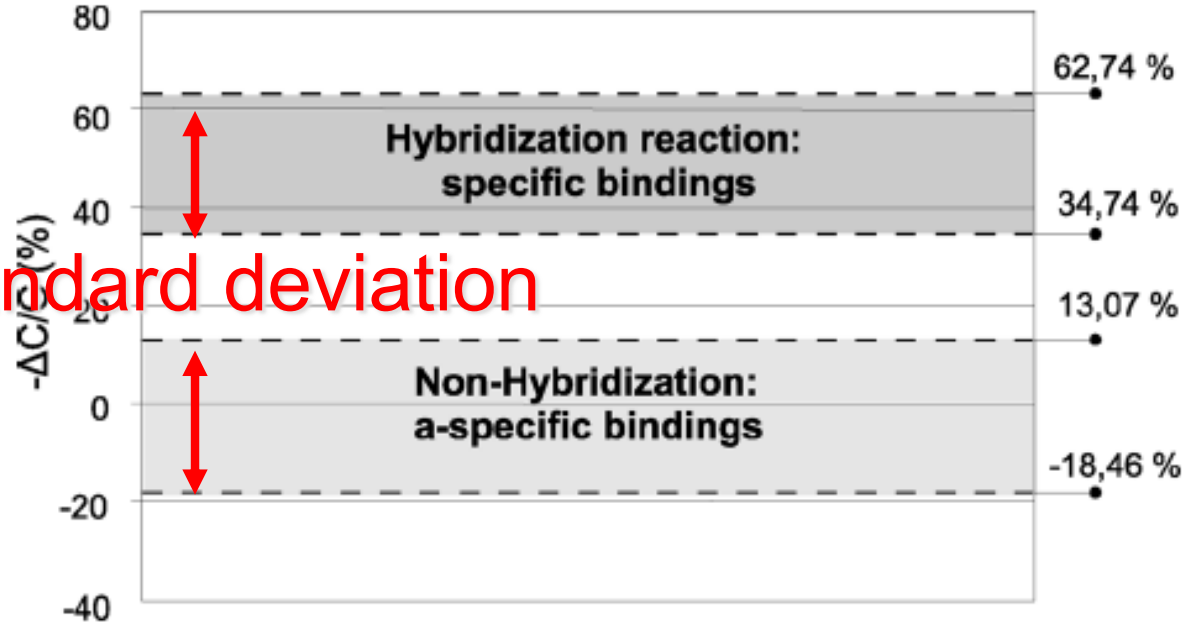
CBCM method on a DNA Layer that dependents by the frequency

Q7

Bad interfaces impact only on the wrong estimation of C-values?

A. Definitely yes: that's the only effect of bad interfaces
B. Some times that's the only effect of bad interfaces
C. Usually also impact the Sensitivity
D. Usually also impact the LoD
E. Usually also impact the reproducibility

DNA detection in CBCM mode

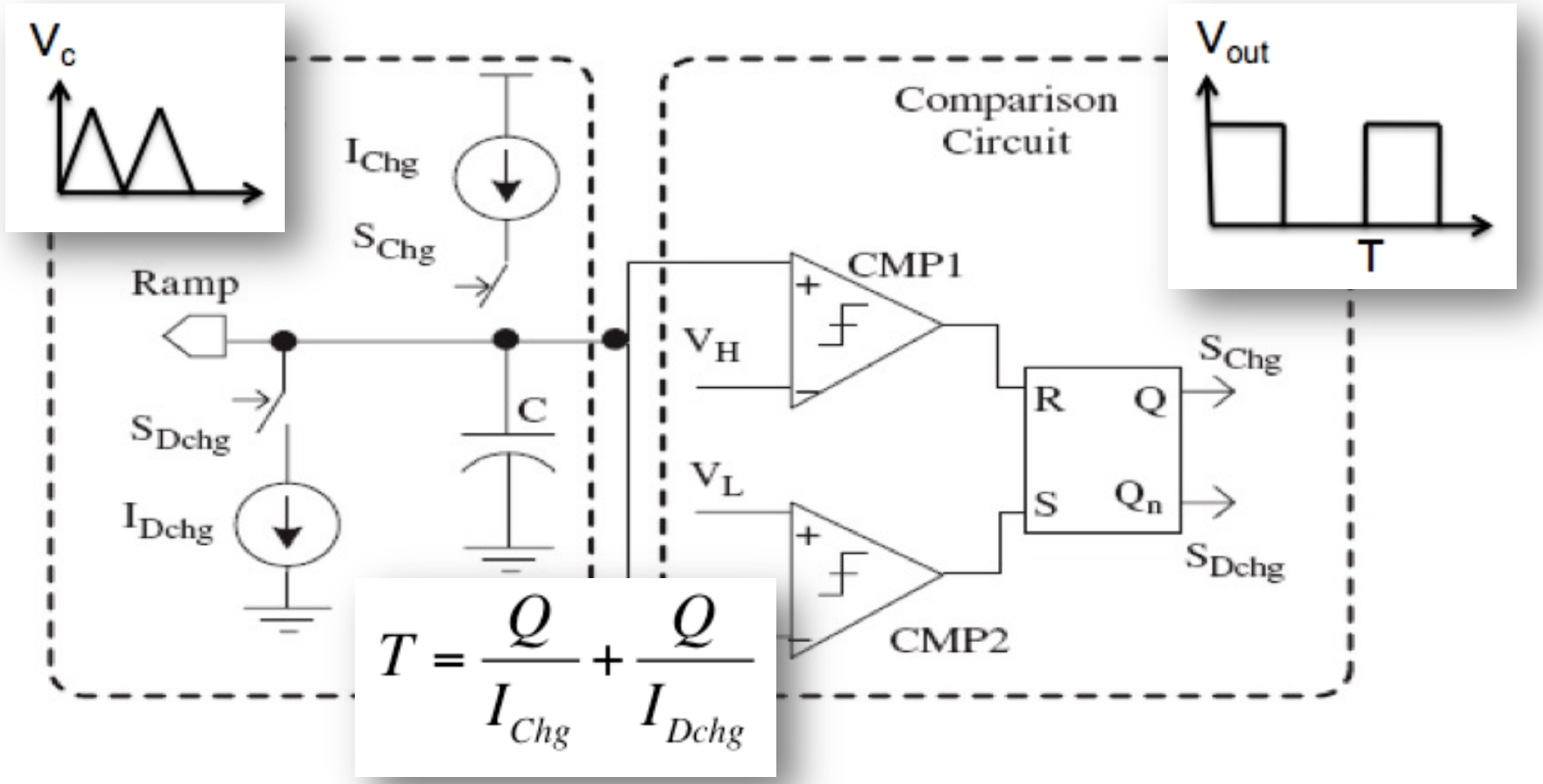


Large Standard deviation

Fig. 12. Capacitance variations due to specific and a-specific bindings (upper and lower bands of measured capacitances, respectively). Positive values indicate capacitance decrease.

The reproducibility on the same chip-spot is not so high: here the problem is on the nano-scale aperture in the probes surfaces

Current to frequency concept

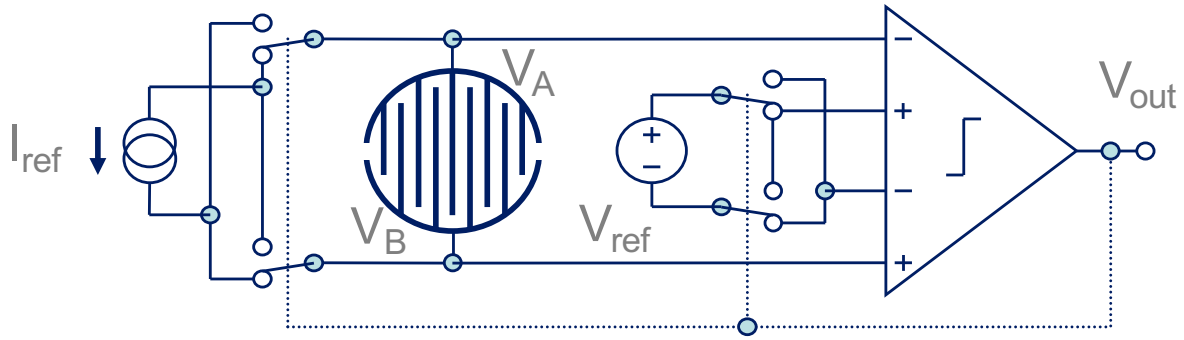


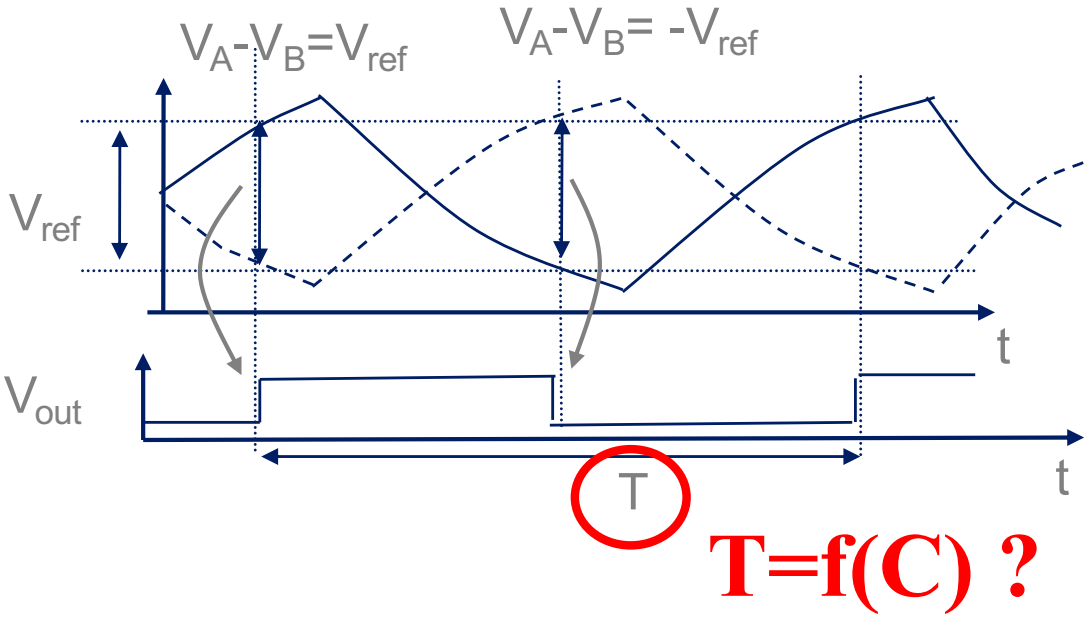
$$T = \frac{Q}{I_{Chg}} + \frac{Q}{I_{Dchg}}$$

- Two current sources are used to charge and discharge the capacitor:
- The output period depends on both currents

Frequency to Capacitance Measurement (FTCM)

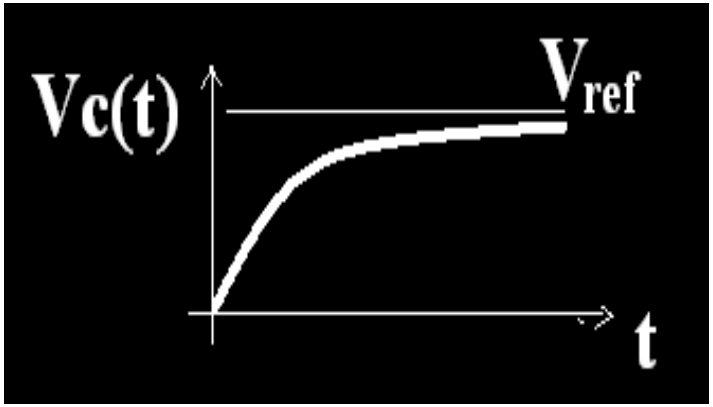
Principle: Frequency To Capacitance Mode





T=f(C) ?

Frequency to Capacitance Measurement (FTCM)



$$V_c(t) = V_{charge}\left[1 - e^{-\frac{t}{RC}}\right]$$

$$V_c\left(\frac{T}{2}\right) = V_{ref} = V_{charge}\left[1 - e^{-\frac{T}{2RC}}\right]$$

$$V_c\left(\frac{T}{2}\right) = V_{ref} = RI_{ref}\left[1 - e^{-\frac{T}{2RC}}\right]$$

$$\frac{T}{2RC} = -\ln\left[1 - \frac{V_{ref}}{RI_{ref}}\right] \qquad \rightarrow T = 2RC\ln\left[1 - \frac{V_{ref}}{RI_{ref}}\right]^{-1}$$

The Taylor Series

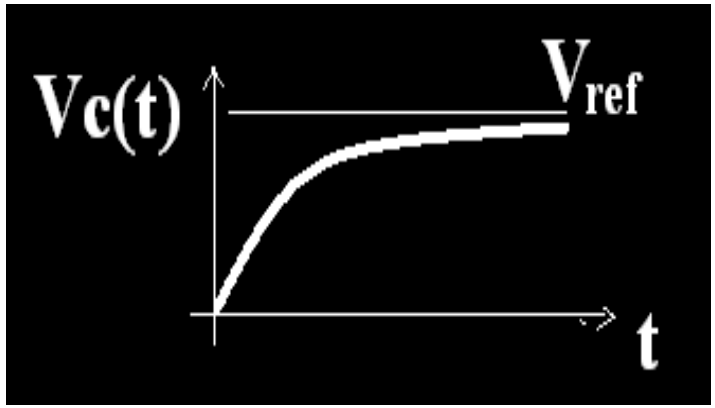
$$f(x) = f(0) + \left\{\frac{\partial f(x)}{\partial x}\right\}x + \left\{\frac{\partial^2 f(x)}{\partial x^2}\right\}x^2 + o(3)$$

$$\frac{1}{1-x} = 1 + \{-(-1)\}x + o(2) \cong 1 + x$$

$$\ln\left[\frac{1}{1-x}\right] = \ln[1 + x + o(2)] \cong \ln[1 + x] = 0 + x + o(2) \cong x$$

Linearity by approximation in the right range of values

Frequency to Capacitance Measurement (FTCM)



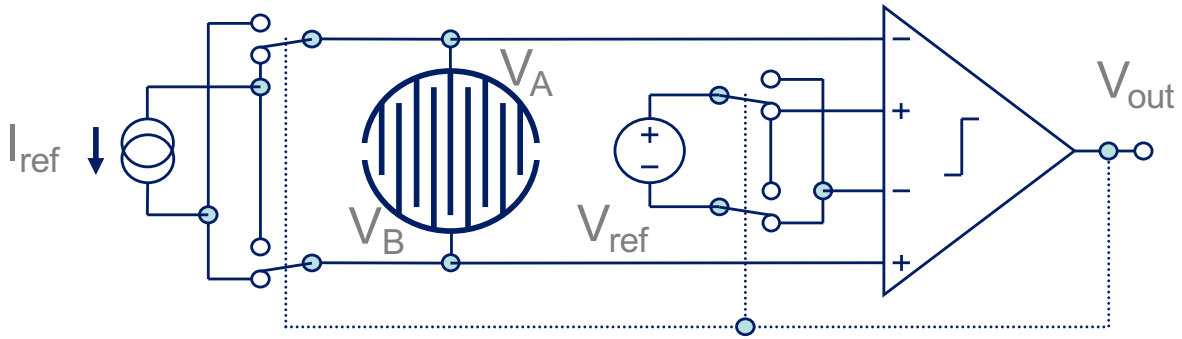
$$T = 2RC\ln\left[1-\frac{V_{ref}}{RI_{ref}}\right]^{-1}$$

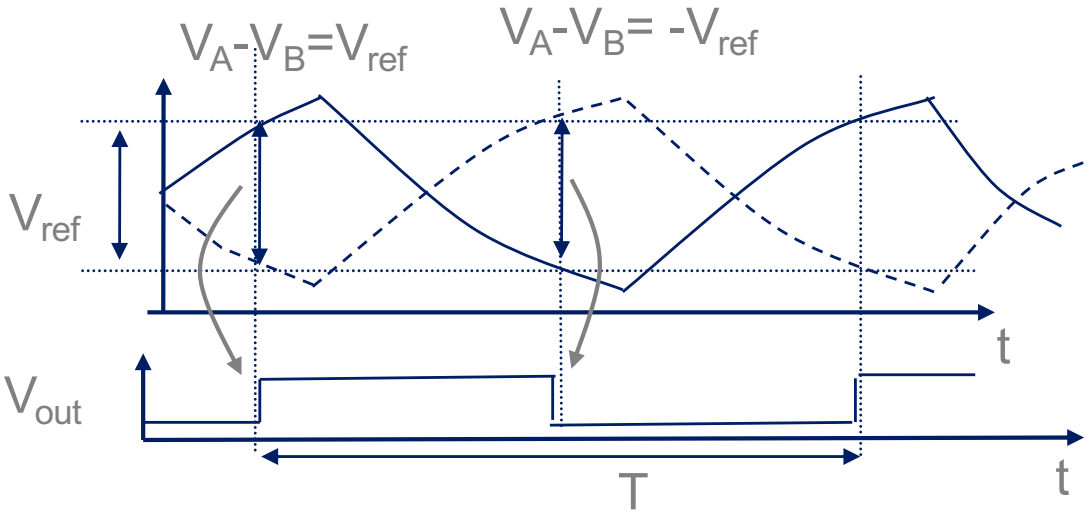
$$T = 2RC\ln\left[\frac{1}{1-\frac{V_{ref}}{RI_{ref}}}\right] \cong 2RC\ln\left[1+\frac{V_{ref}}{RI_{ref}}\right] \cong \frac{2CV_{ref}}{I_{ref}}$$

Method for the estimation of the Capacitance

Frequency to Capacitance Measurement (FTCM)

Principle: Frequency To Capacitance Mode



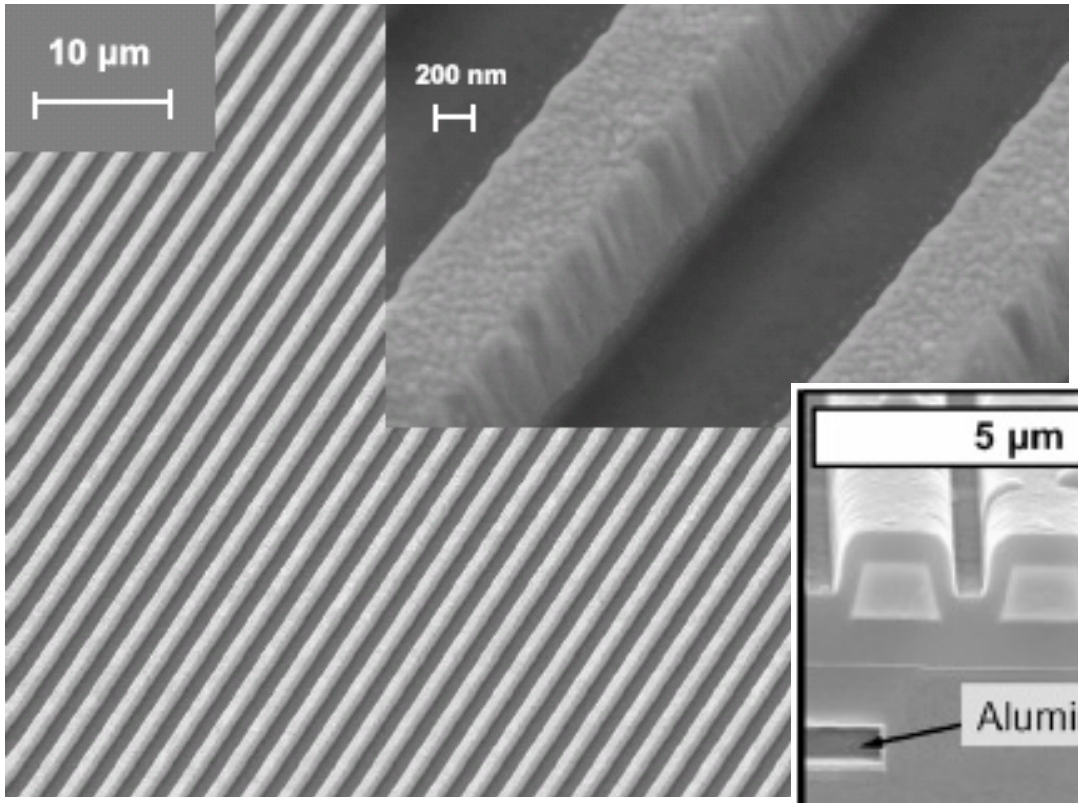


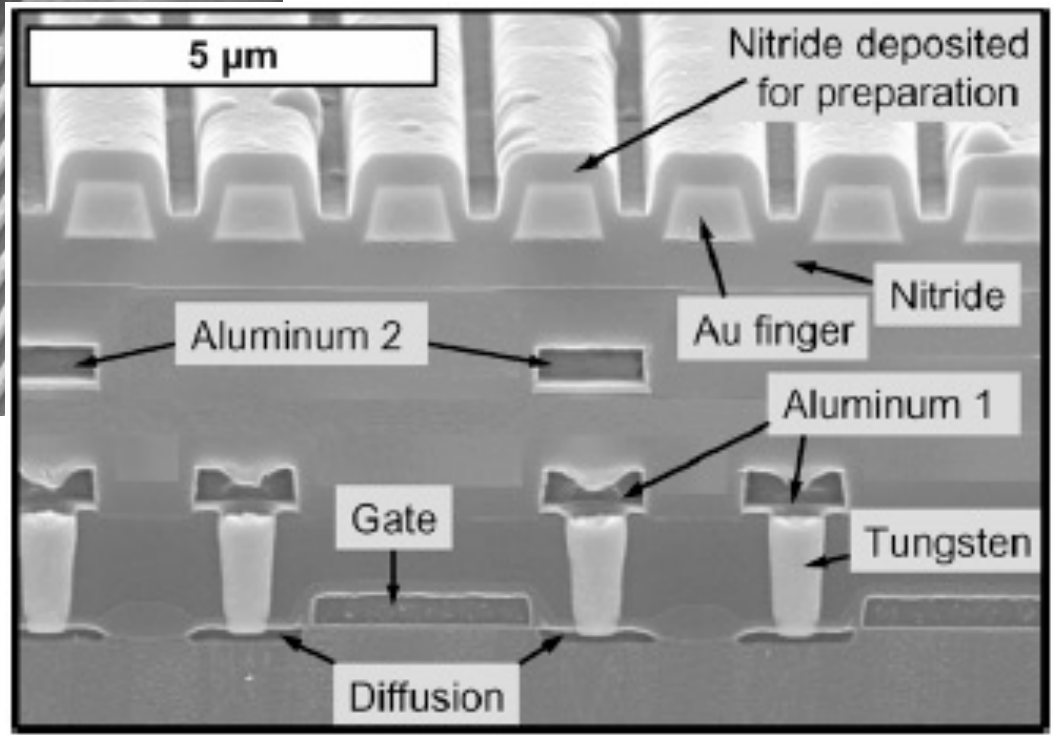
$$T = 2RC\ln\frac{1}{1-\frac{V_{REF}}{I_{REF}R}}$$

$V_{REF}/I_{REF}R \rightarrow 0$

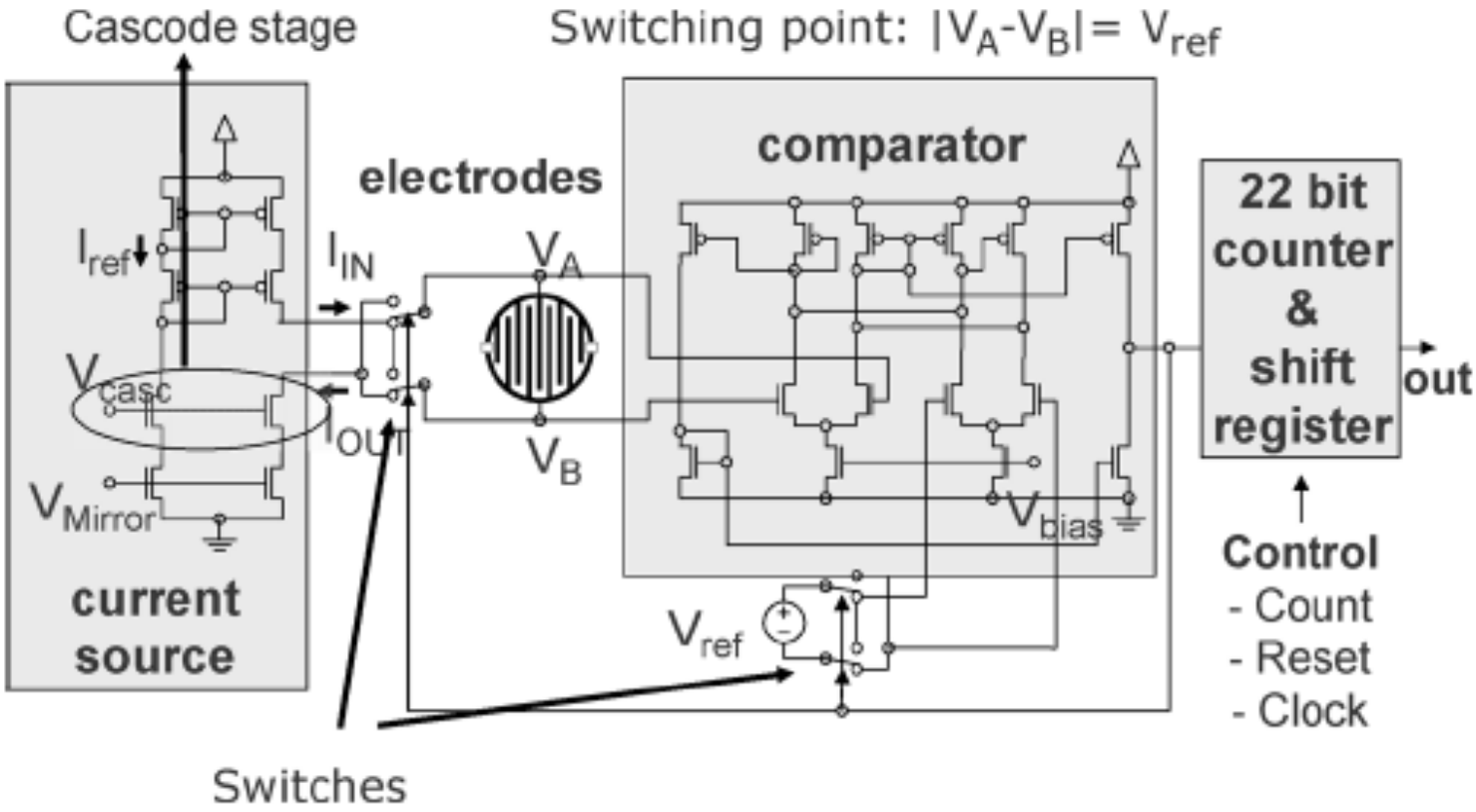
$$T = \frac{2 \cdot V_{REF} \cdot C}{I_{REF}}$$

Electrodes Layout





Chip Architecture (FTCM)



Measurements Set-up

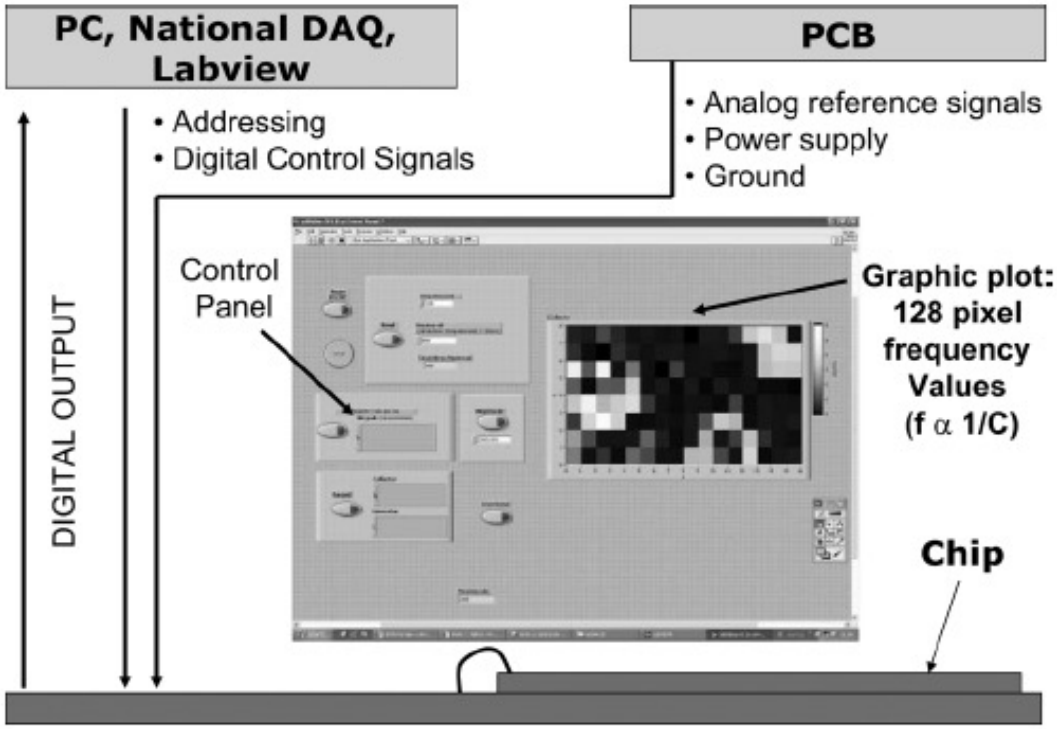
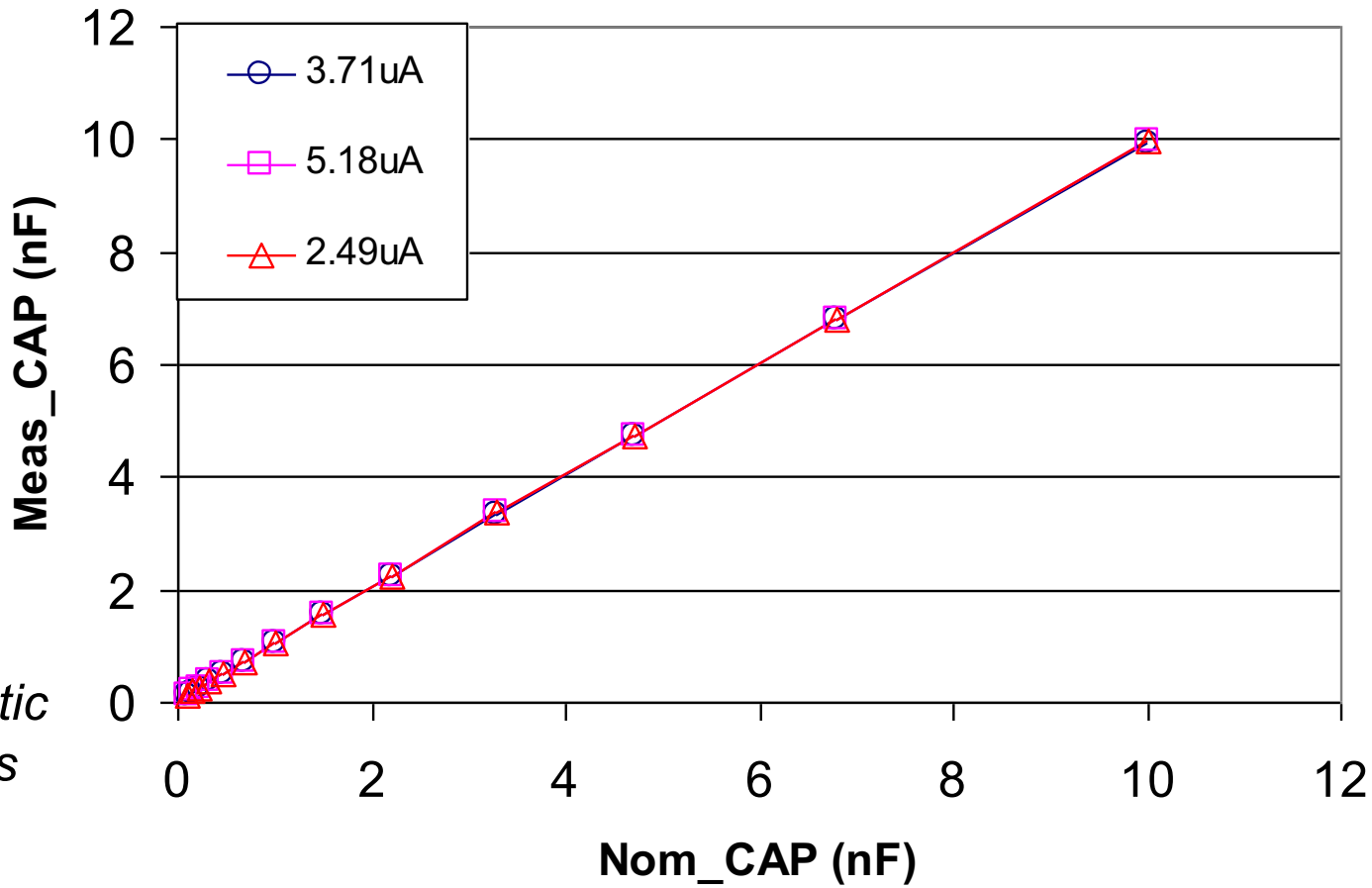


Fig. 9. Schematic representation of the measurement set-up. Voltage reference signals and power supply are generated by circuitry on the PCB. Digital control signals are provided by a PC. The LabView interface manages all the parameters involved in the measurements and shows directly on the screen the measurement results of the whole array.

Validation Test

Slope = 0.9837
Intercept = 62 pF
σ < 0,3 %

Offset is due to parasitic capacitances of cables



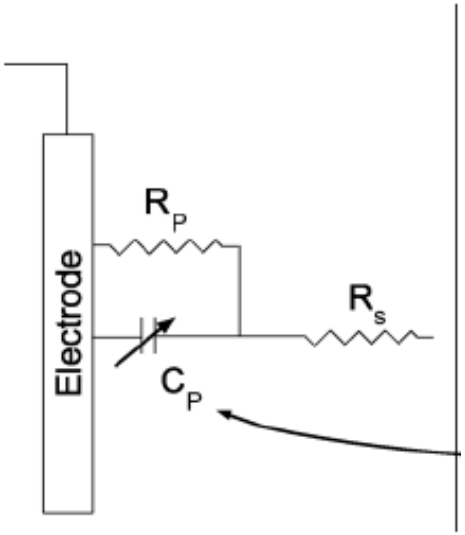
A test structure has been implemented on chip beside the array to characterize the measurement circuit with discrete test capacitances (10 pF -10 nF)

Q8

Does the FTCM returns good C-value estimations also on bad interfaces?

A. Definitely not, if C changes with the frequency
B. Not really, since C might change with the frequency
C. Not always, since R_P might be extremely small
D. Some times, since R_P is usually extremely large
E. Yes, of course!

Probes property on FTCM mode



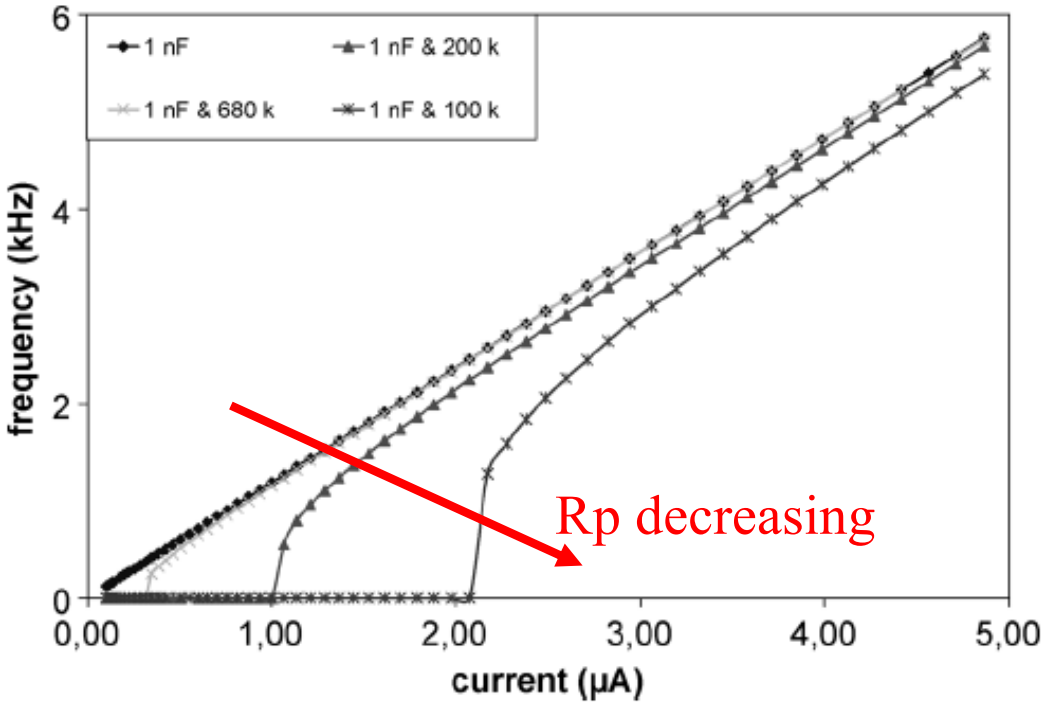


Fig. 12. Frequency versus reference current showing that a significant influence of the parallel resistance on the measurement result occurs only at low current values and at R_P values lower than 680 kΩ.

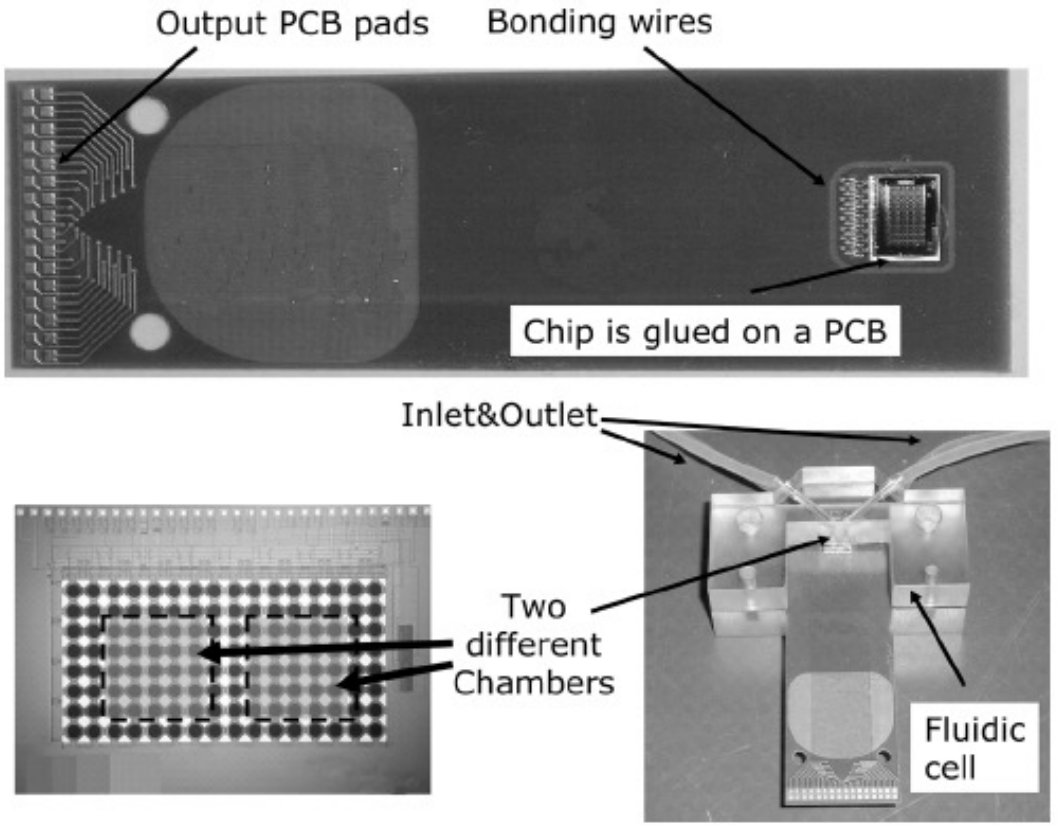
The linearity between the current and the measured frequency is lost at low current if the CMOS/Bio interface is not a perfect capacitor

Q9

May we obtain ideal interfaces for FTCM estimations?

A. Impossible, R_P always is too small
B. Not easy, since R_P might change in frequency
C. May be, in case of some good self-assembly
D. Some times, by assuring large R_P
E. Some times, by assuring small R_P

Liquid Measurement set-up



DNA detection in FTCM mode

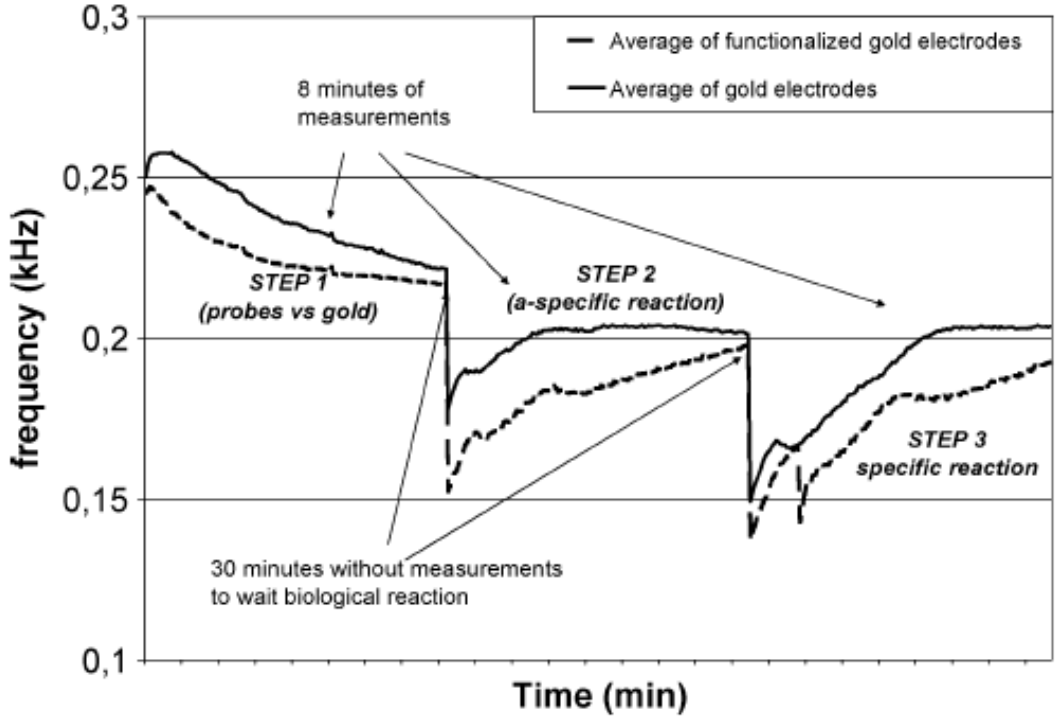


Fig. 13. Frequency changes of the average of reference electrodes (continuous line), and the average of functionalized electrodes (dashed line) show a larger gap after DNA hybridization step considering the stable value reached at the end of the transient.

Time stability on the single chip-spot is pour due to nano-scale aperture in the probes surfaces

DNA detection in FTCM mode

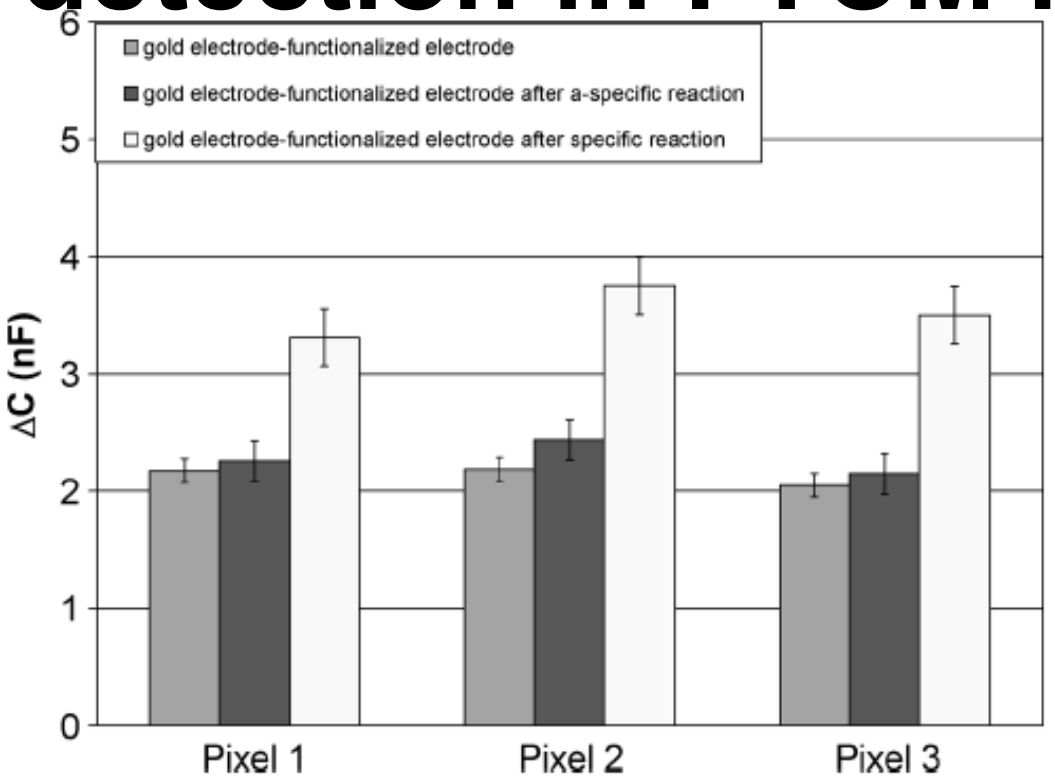


Fig. 14. Typical variations for several pixels among functionalized electrodes and the average value of reference gold electrodes. Capability to distinguish between specific and nonspecific binding is shown for each pixel.

In chip spot-by-spot reproducibility is improved also due to better cleaning of the spot gold electrodes

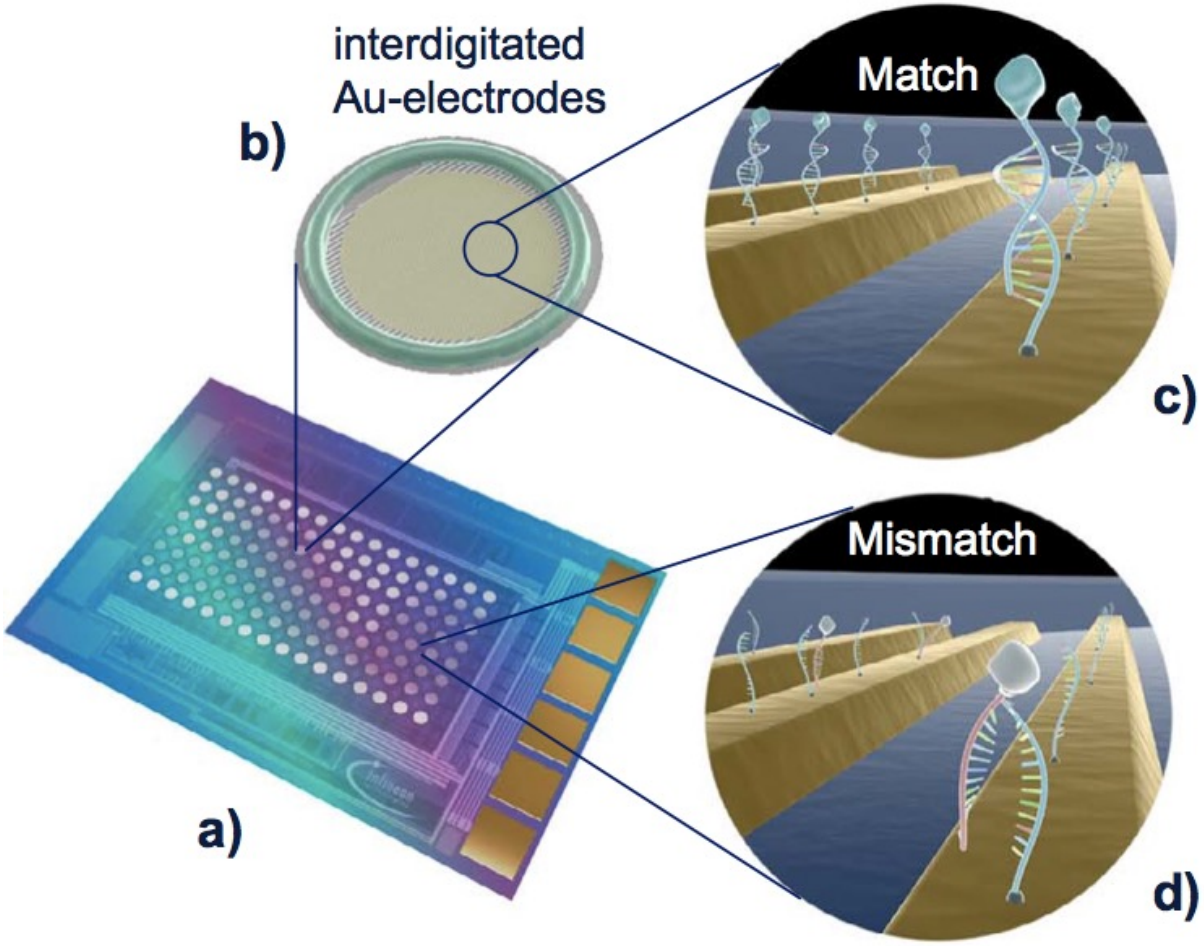
Q10

How we may detect DNA with Amperometric Methods?

A. Impossible, Amperometry works only with enzymes
B. Not easy, since Amperometry works mainly with enzymes
C. May be by using DNA-Polymerase
D. May be by using electrochemical labels
E. May be by using DNA-Helicase

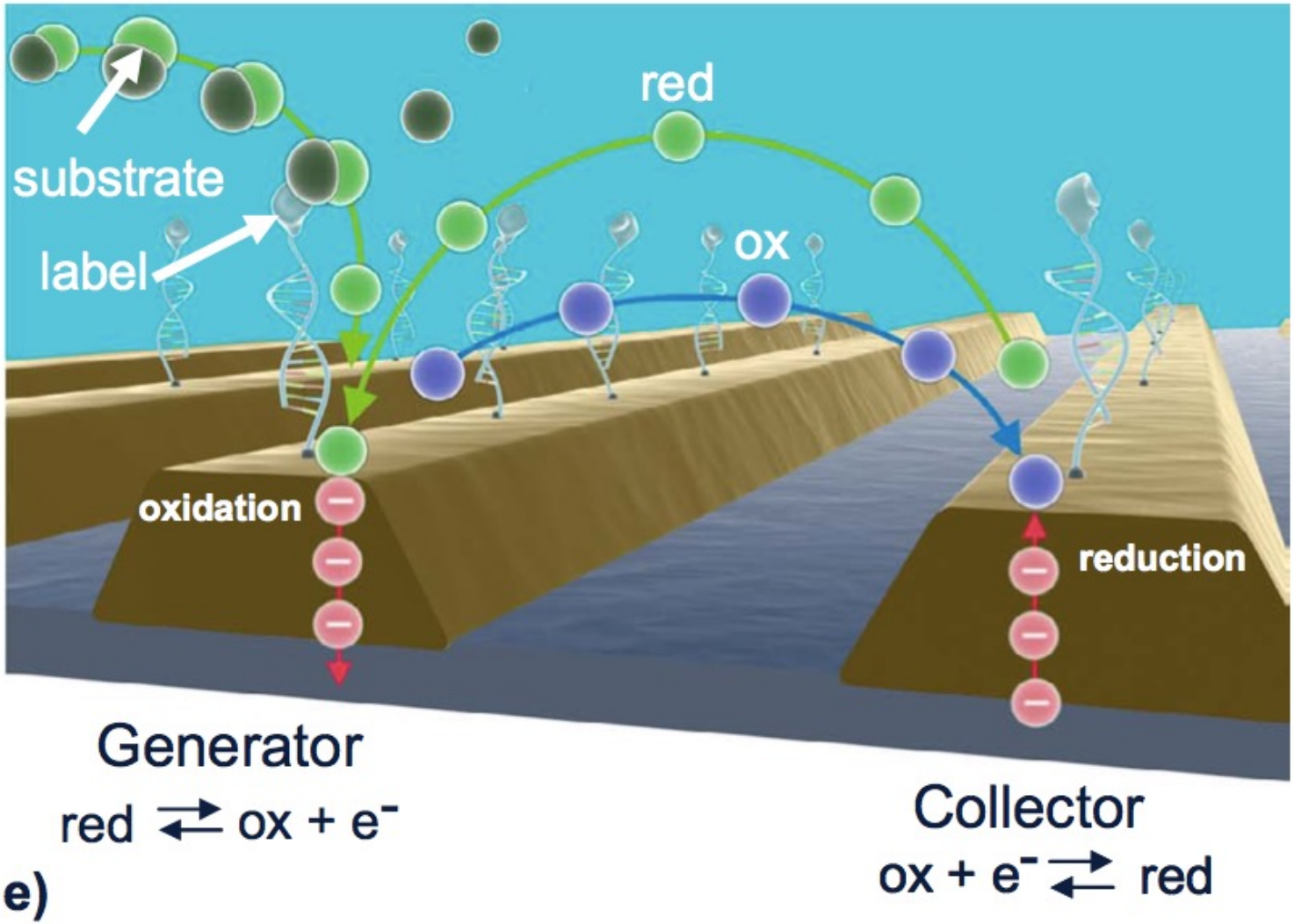
Amperometric Detection of DNA

Figure by *Frey et al, IEEE ISCAS 2015*



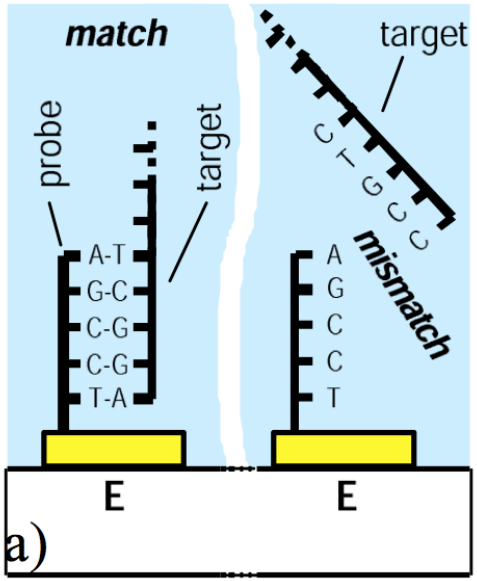
Electrochemical labels might be used to detect DNA

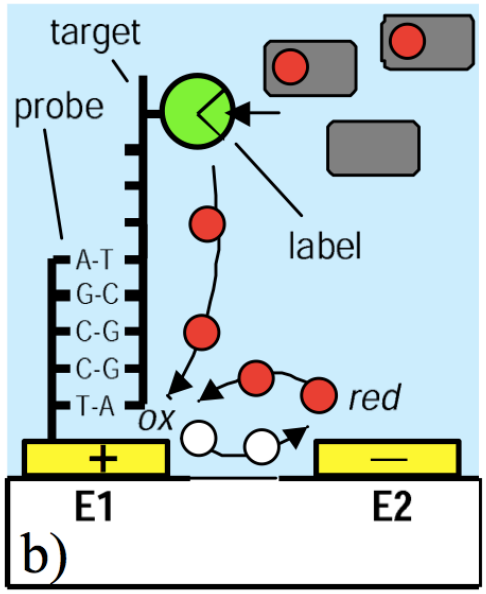
Amperometric Detection of DNA



Redox species can be then measured at the electrodes

Amperometric Detection Principle





How it works:

✓ First, single stranded DNA molecules (about 20 bases) are immobilized by using a spotting machine on top of the gold electrodes due to gold-thiol coupling.

✓ Then, the chip is flooded with an analyte containing labeled target DNA ss: hybridization takes place in case of matching.

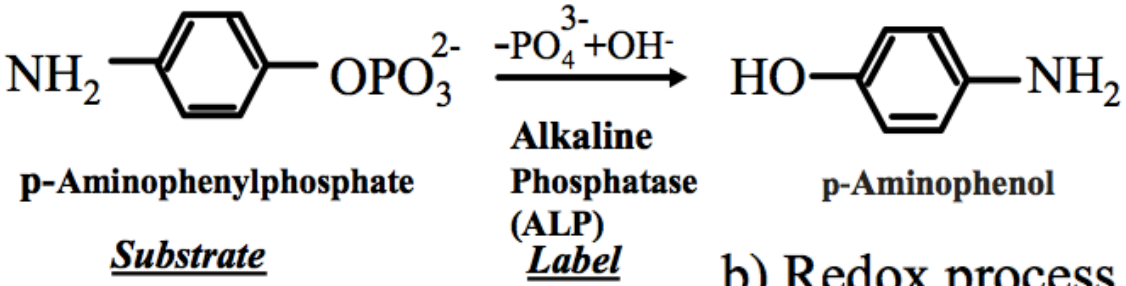
✓ A suitable substrate is applied to the buffer solution and it is enzymatically cleaved by the label.

✓Resulting species starts an electrochemical redox process at the electrodes.

✓Faradaic currents generated by the related redox process is detected and transduces DNA hybridization

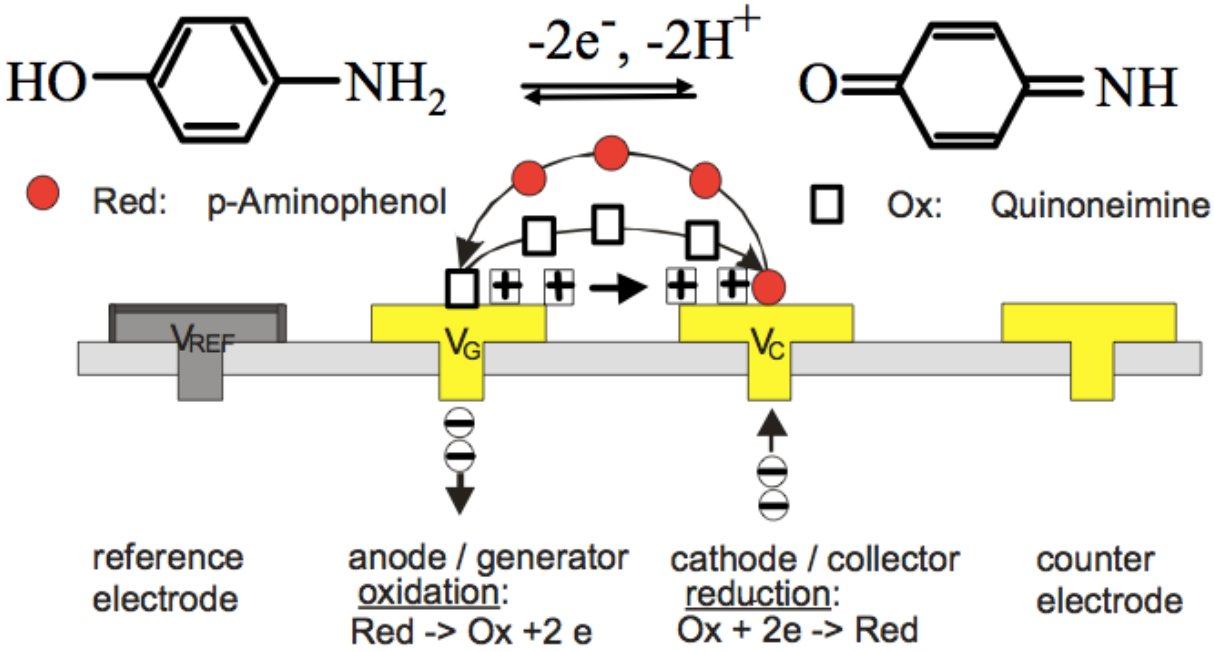
Enzymatically cleavage & redox process

a) Process at the label



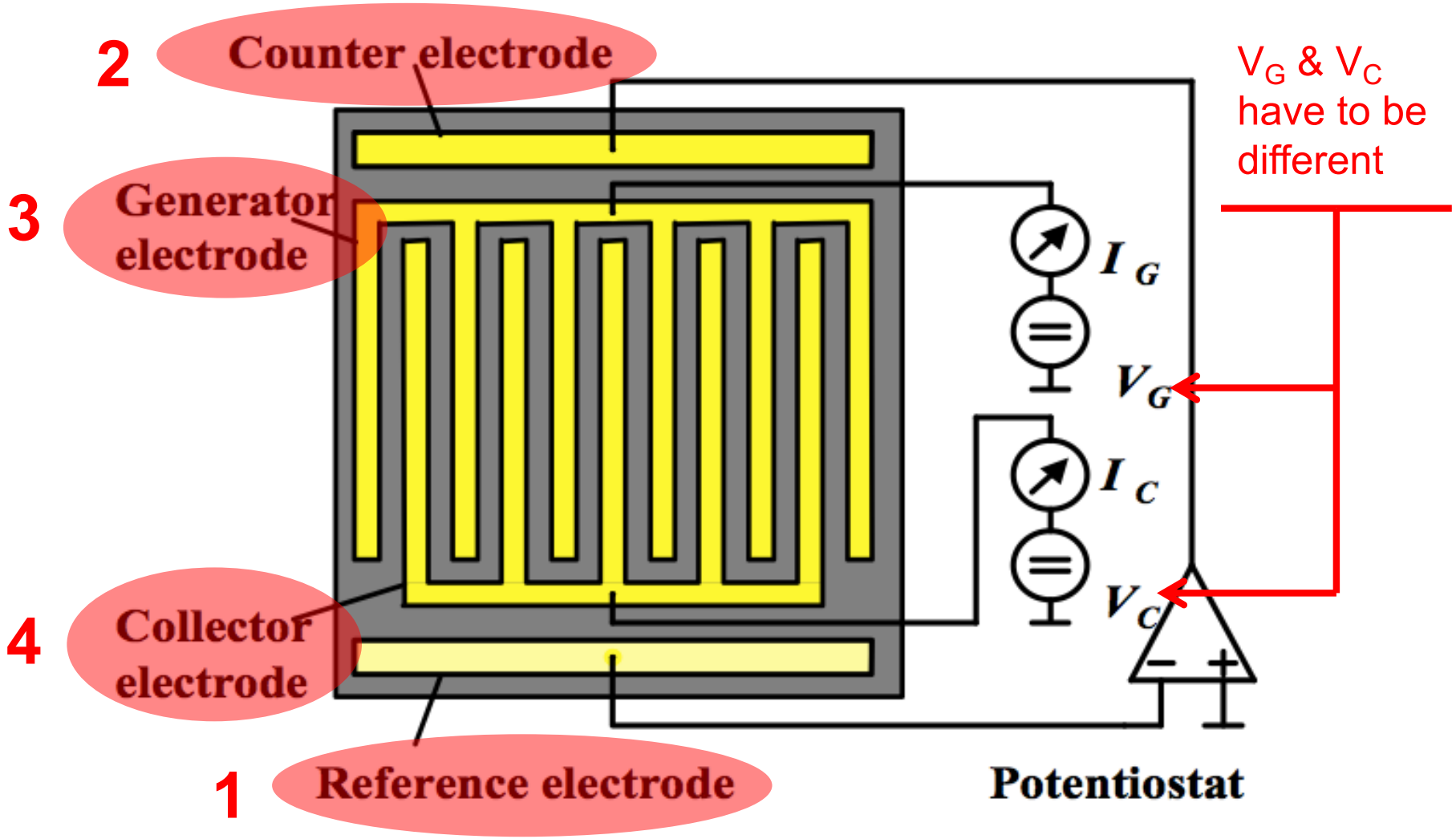
✓ The label cleaves the secondary probe

b) Redox process at the electrodes



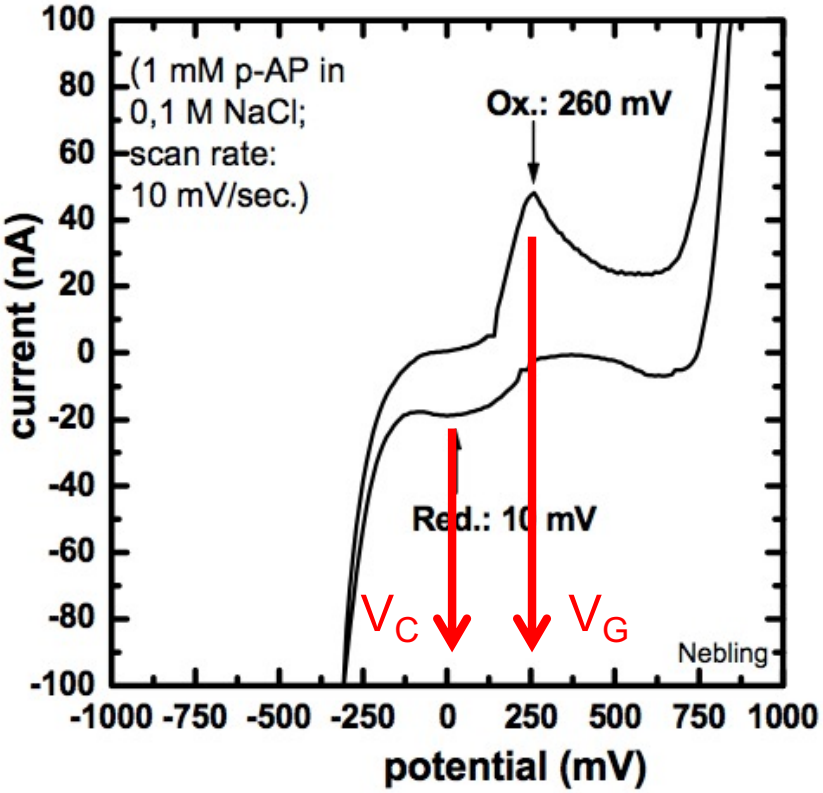
✓ The product of the cleavage is generating an oxidation process at the anode and, once oxidized, a reduction at the cathode

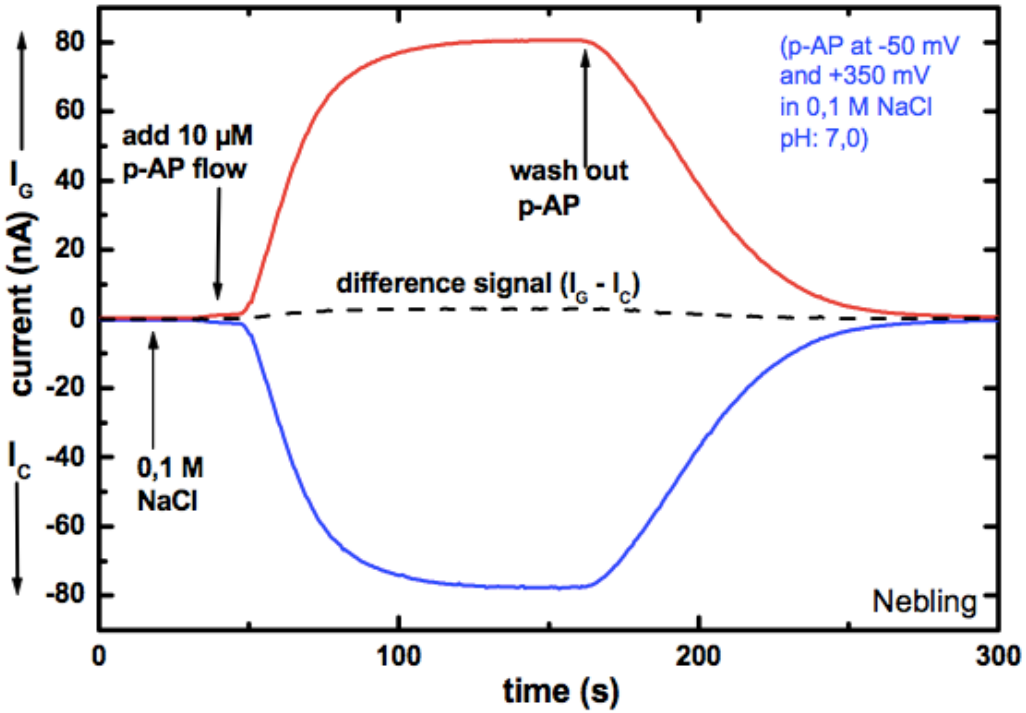
The Electrochemical Cell



A 4-electrode Electrochemical Cell is here required

Cyclic Voltammetry



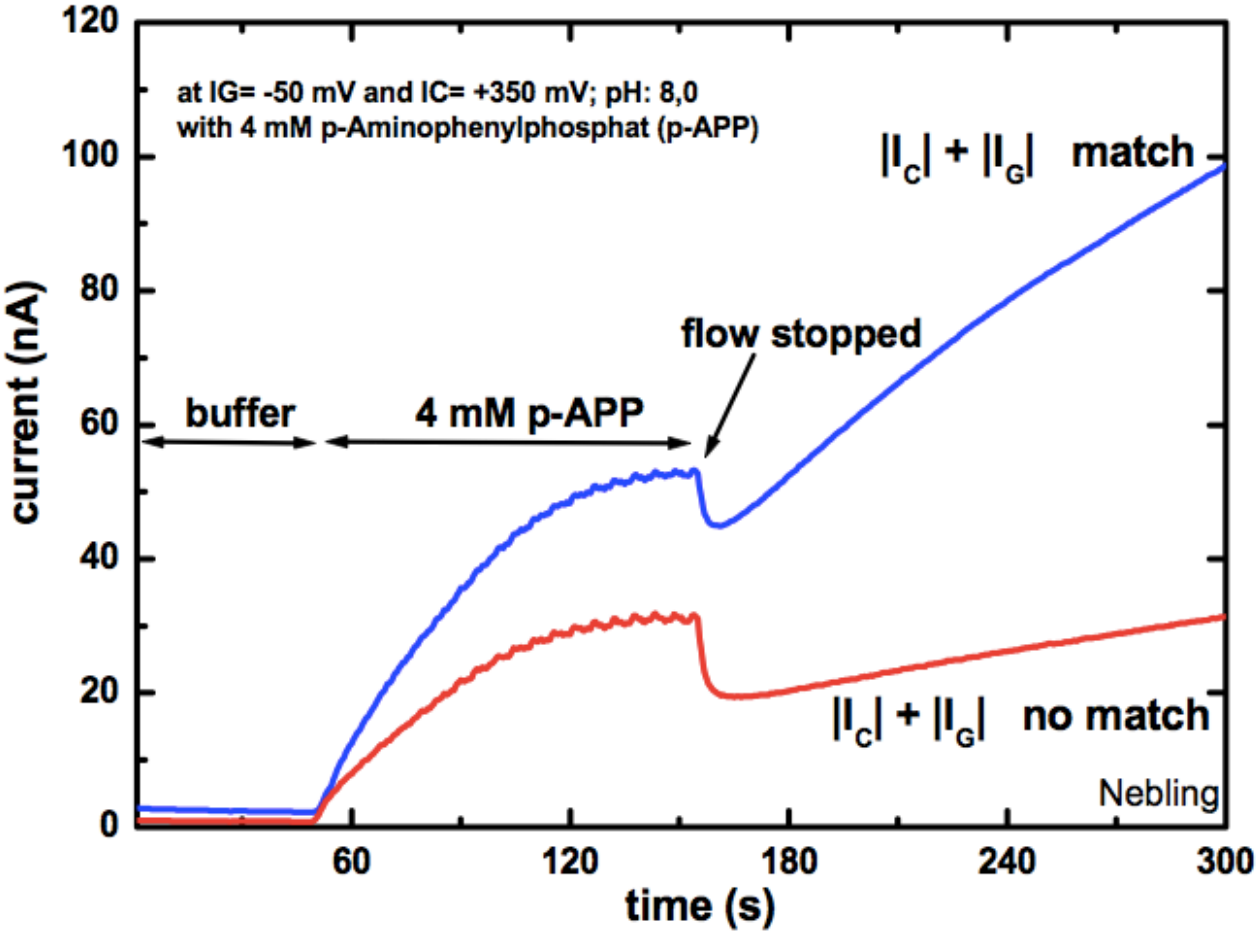


✓ Typical Cyclic Voltammetry acquired with 3-electrode cell

✓ Chronoamperometry acquired with 4-electrode cell

Simultaneous acquisition of Ox/Red current with 4-el

Match/Mismatch DNA Hybridization



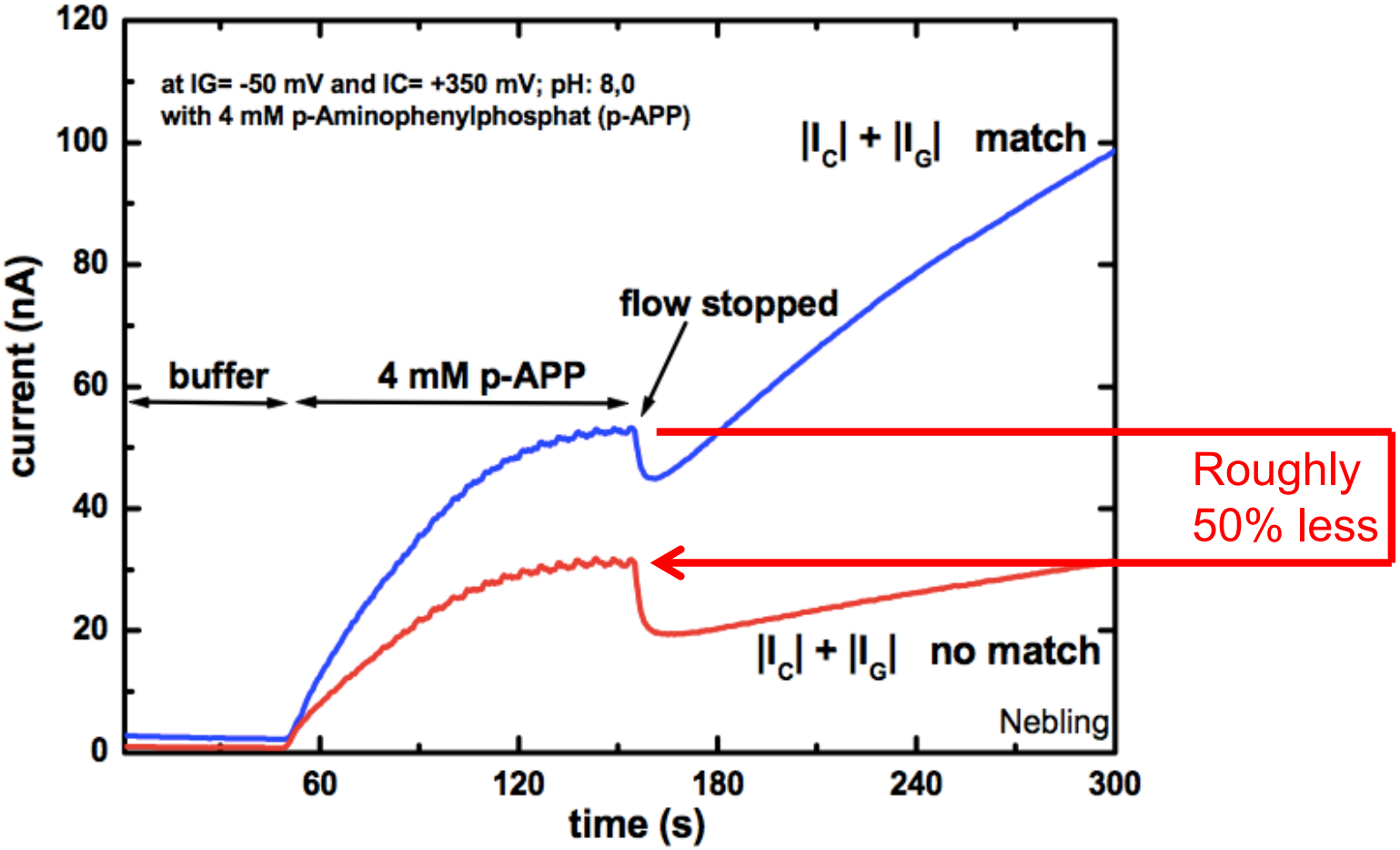
Successful detection of the matching sequence by significant signal by non-matching ones too

Gibbs free energy for Match/Mismatch

duplex	*Experimental* ΔG [kJ/mol]
GGTTATTGG CCAATAACC	-26.8
GGTTCTTGG CCAAGAACC	-31.4
GGTTTTTGG CCAAAAACC	-29.5
GGTTATTGG CCAAAAACC	-12.0
GGTTCTTGG CCAATAACC	-12.4
GGTTTTTGG CCAAGAACC	-17.5

Roughly 50% less

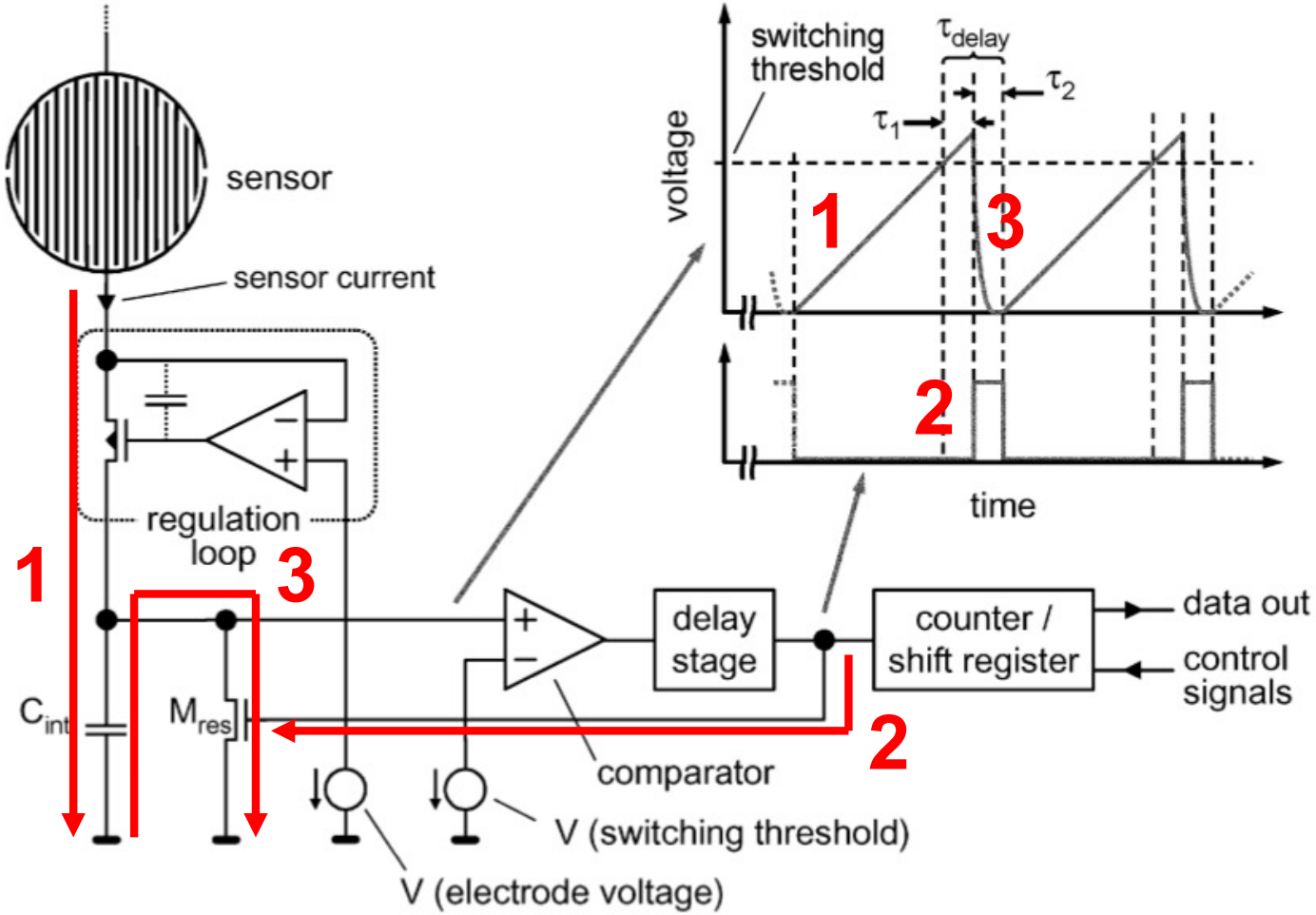
Match/Mismatch DNA Hybridization



Successful detection of the matching sequences

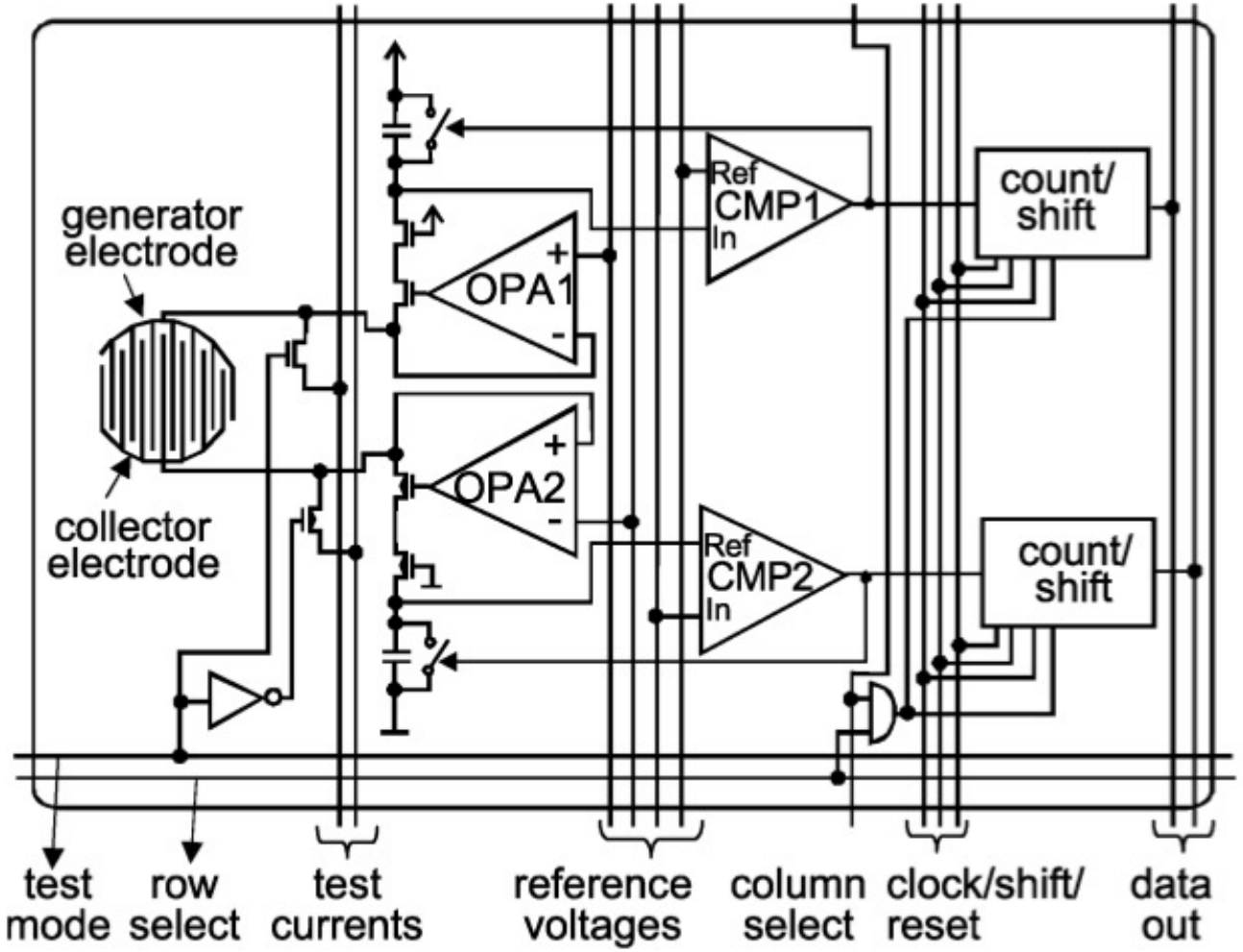
but significant signal by non-matching too

Current CMOS Readout



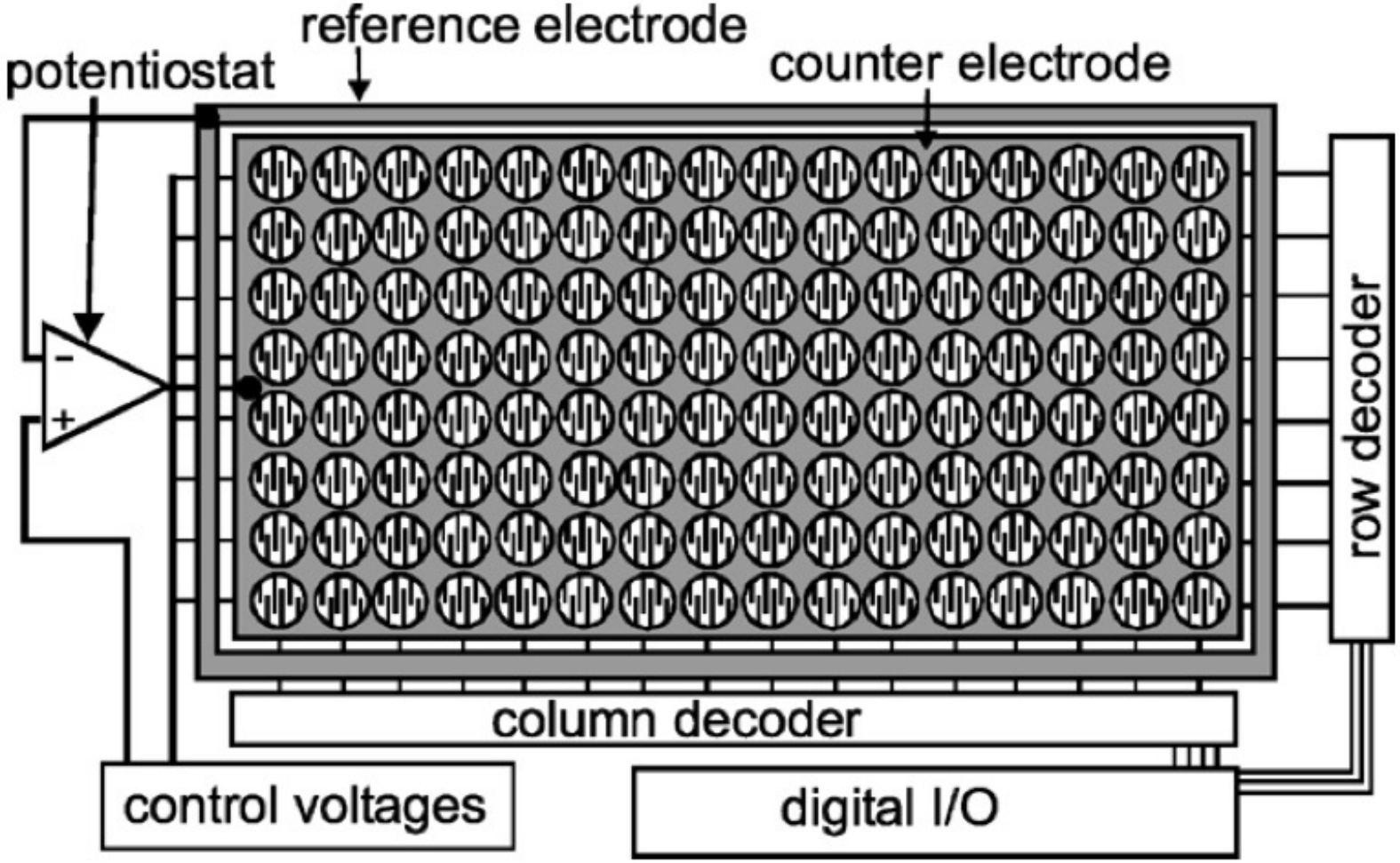
Frequency-To-Current Conversion (FTCC) method is used here too

Current CMOS Readout



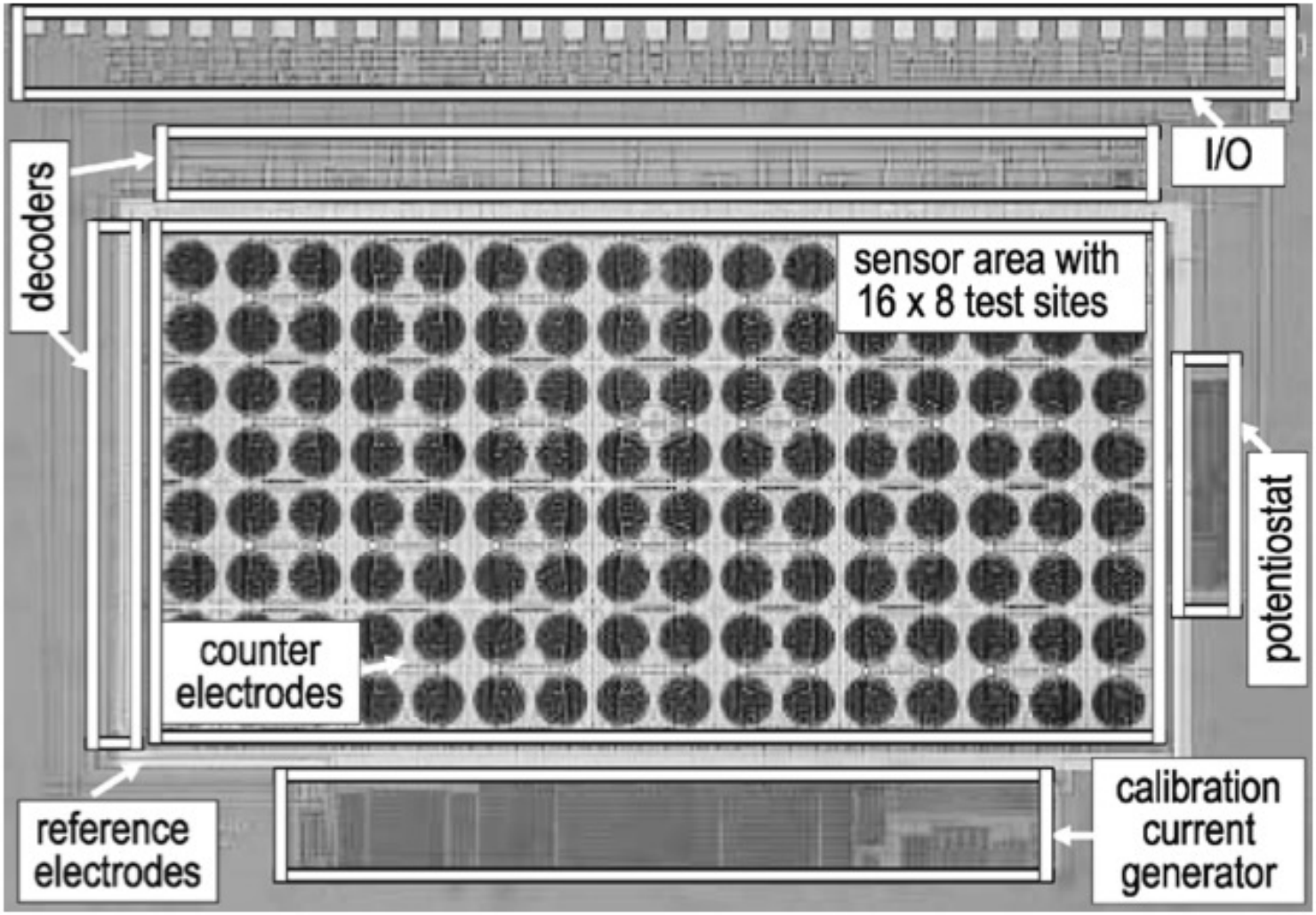
Sensor-site circuit architecture with digital output

Array Architecture



Whole Chip architecture including Row/Column decoders

The realized IC



Chip microphotograph. Total dimensions are 6.4 x 4.5 mm^2.

Exposed IC-Die Electrodes

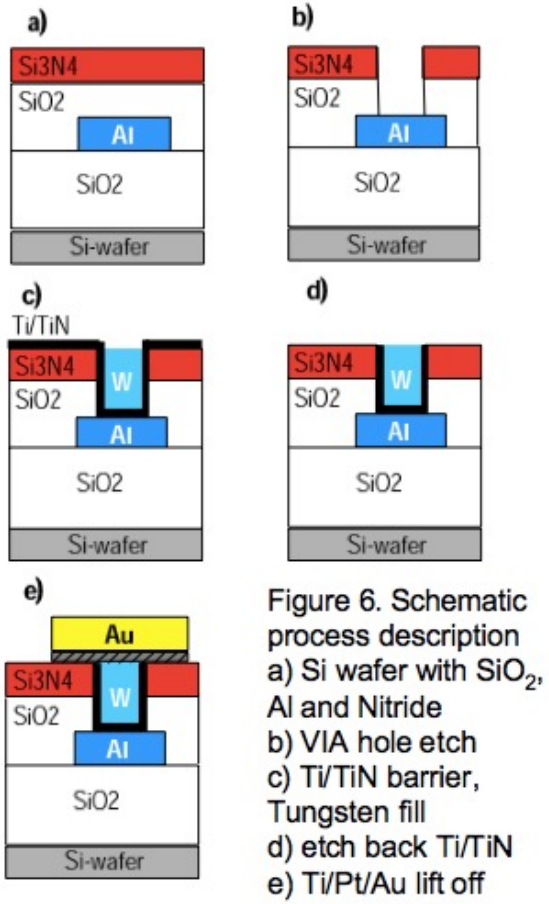
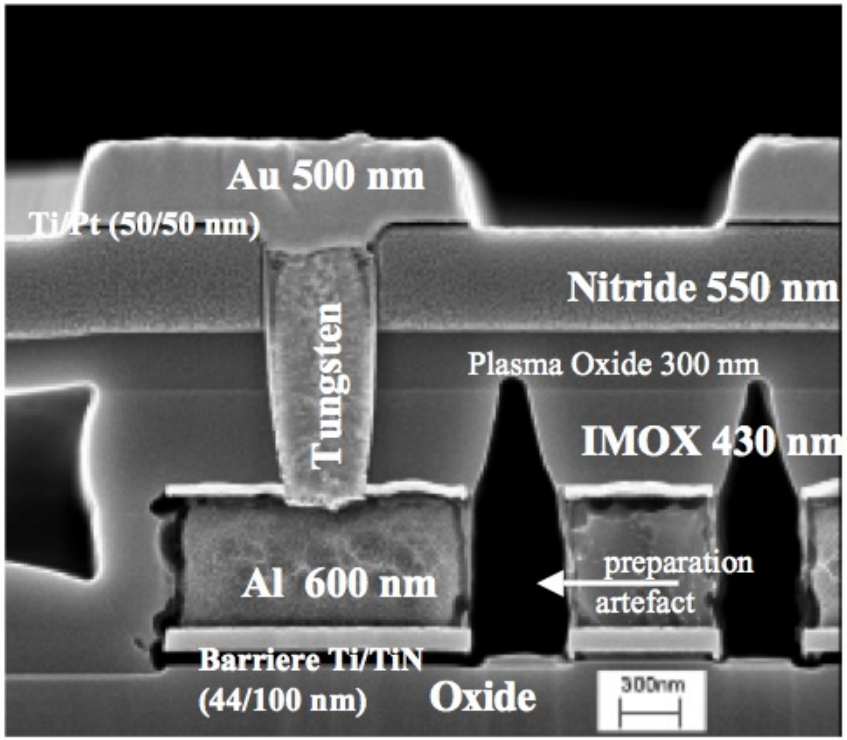
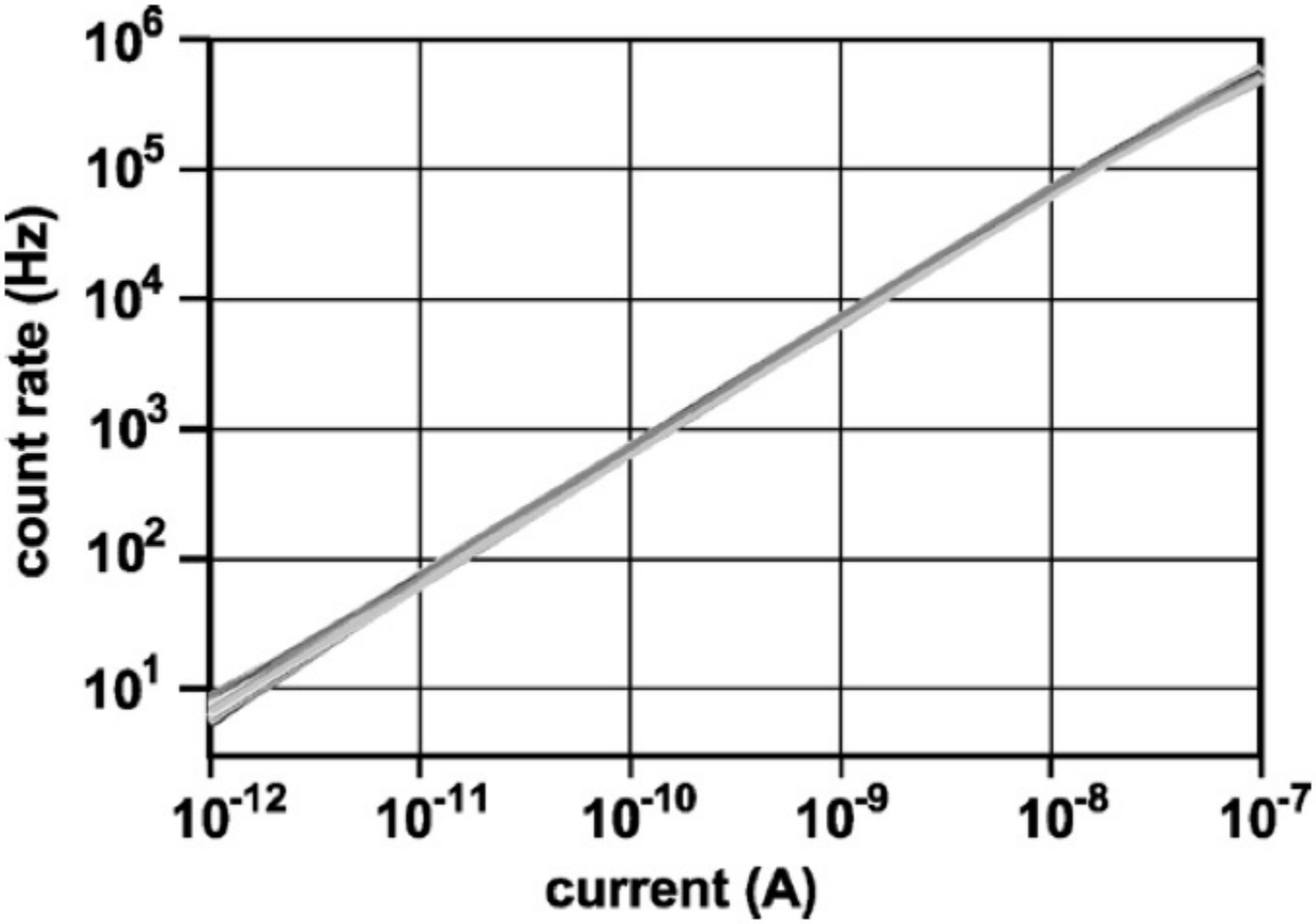


Figure 6. Schematic process description
a) Si wafer with SiO_2, Al and Nitride
b) VIA hole etch
c) Ti/TiN barrier, Tungsten fill
d) etch back Ti/TiN
e) Ti/Pt/Au lift off



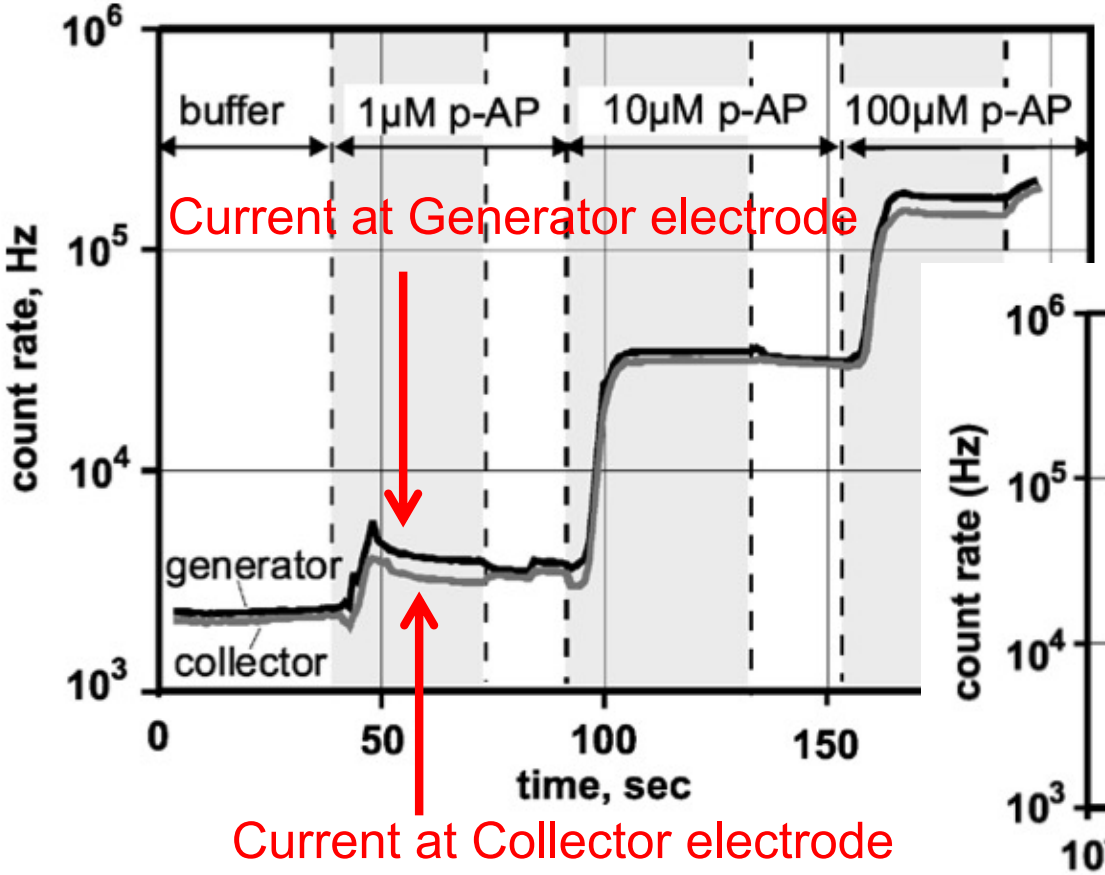
Electrochemical electrodes are created on top of the last CMOS metal Al layer

Frequency Readout

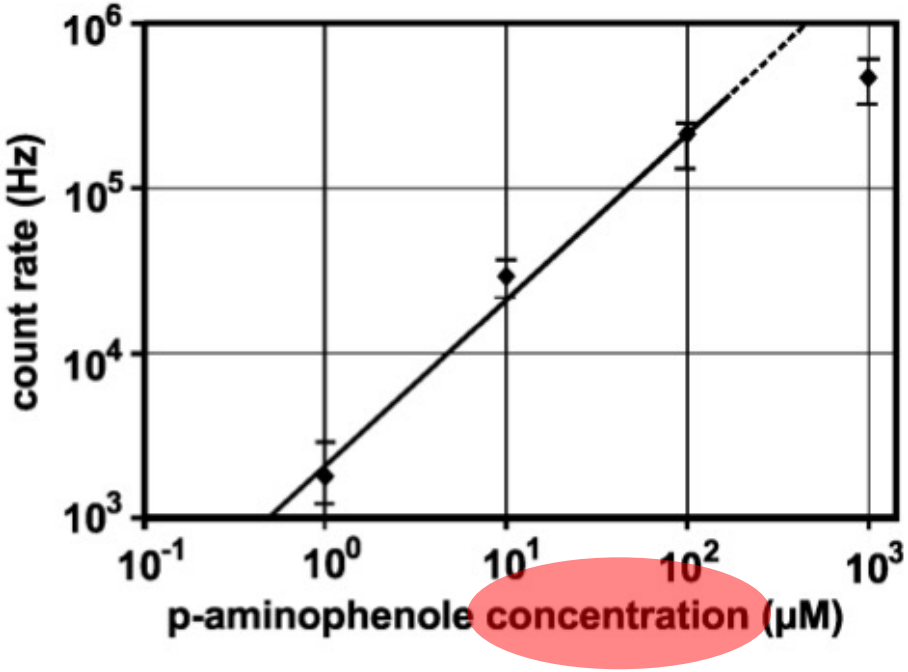


Measured count rate of all 128 DNA sensors

Test Measures



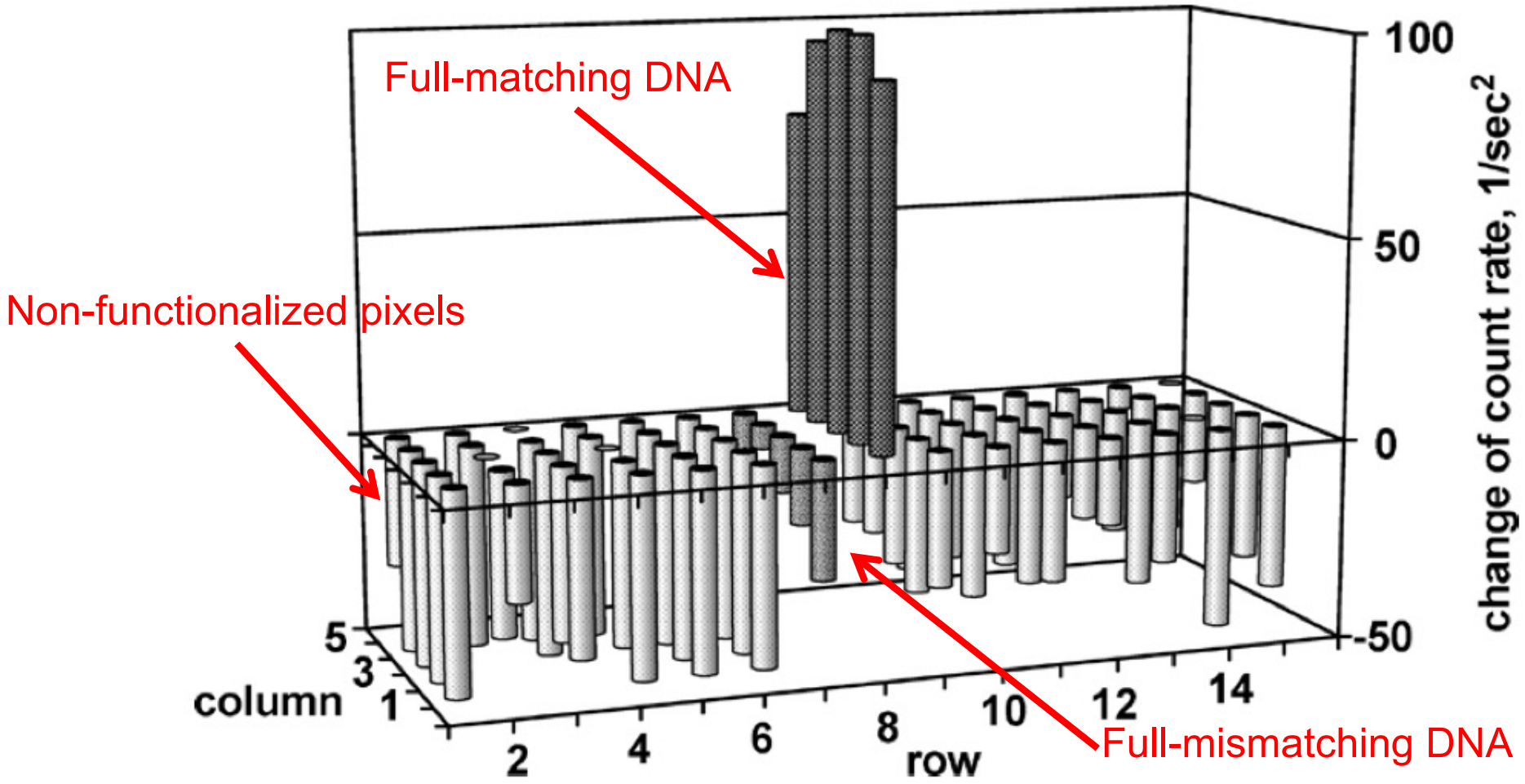
Response Calibration



Concentration of the secondary probe, not of the DNA

Response of the sensor versus secondary probe

DNA Detection



Row 8: full matching DNA, row 7: full mismatching DNA, all other positions not functionalized.