



Master in Electrical and Electronics Engineering

EE-517: Bio-Nano-Chip Design

Lecture #13

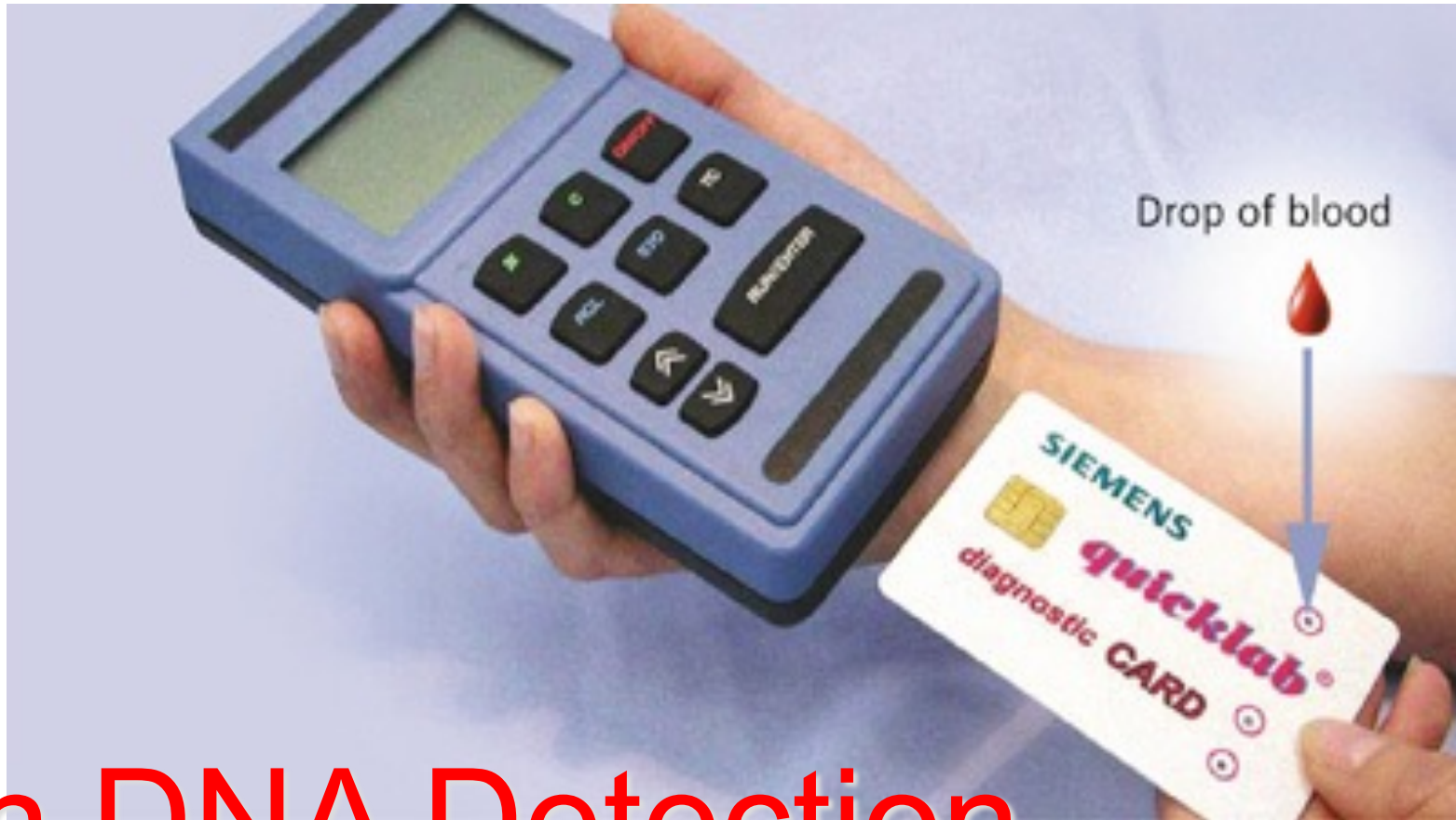
CMOS Circuits for DNA Detection

Lecture Outline

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

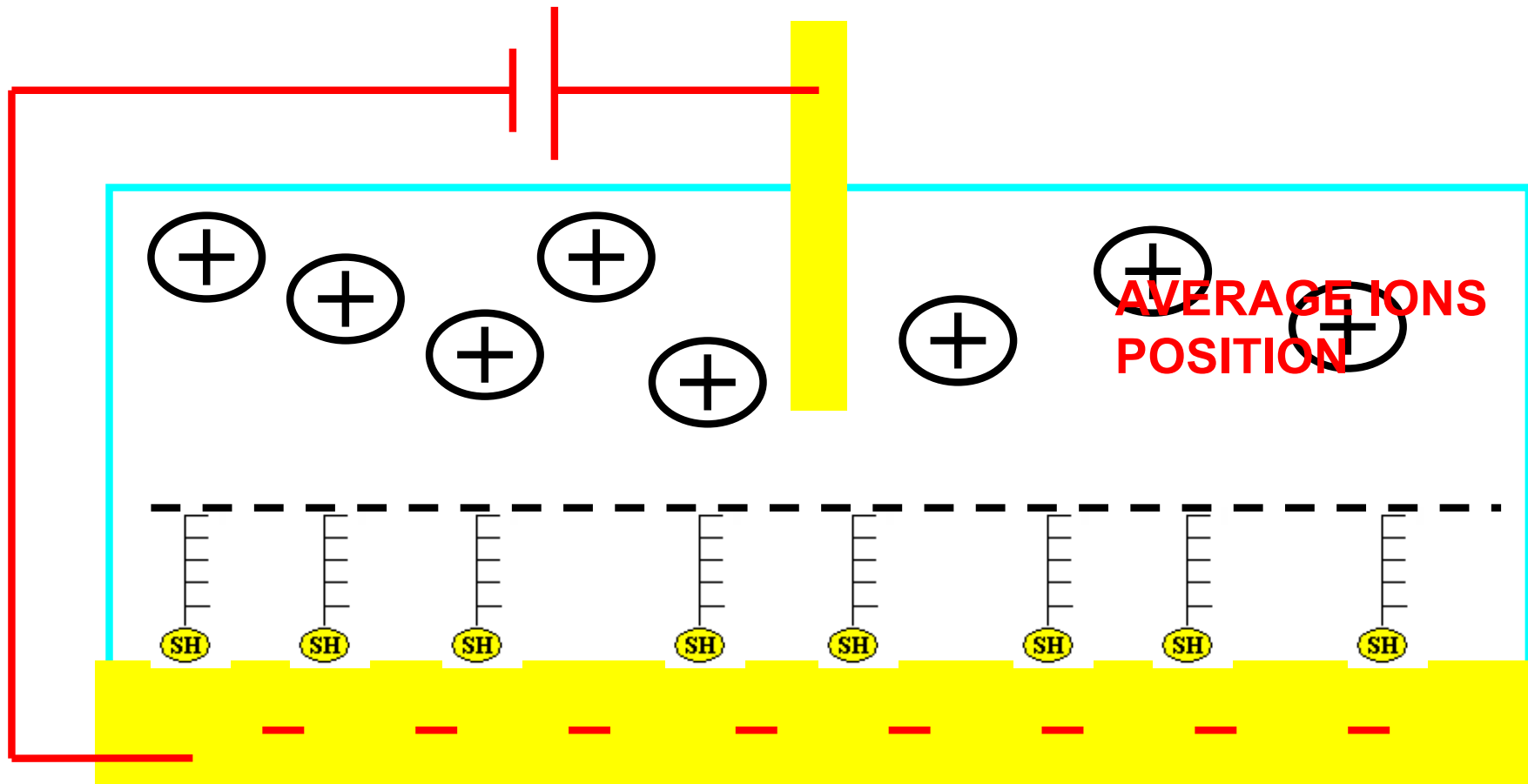
- CMOS for DNA capacitance detection
- Charge-Based Capacitance Measurement (CBCM) Method
- Frequency-to-Capacitance Measurement (FTCM) Method
- CMOS for DNA Amperometric detection

CMOS architectures for VLSI



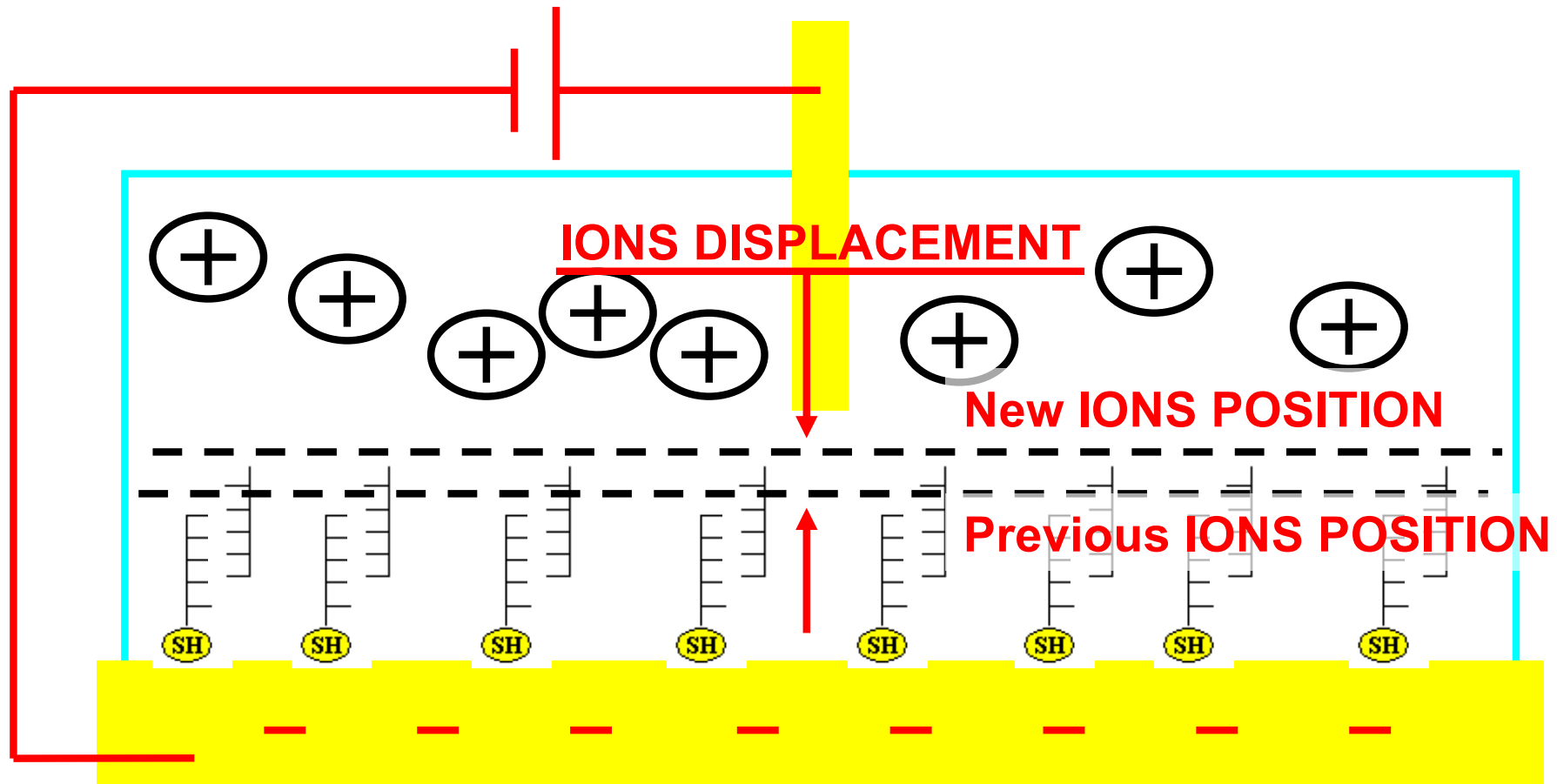
in DNA Detection

Electrochemical Interface



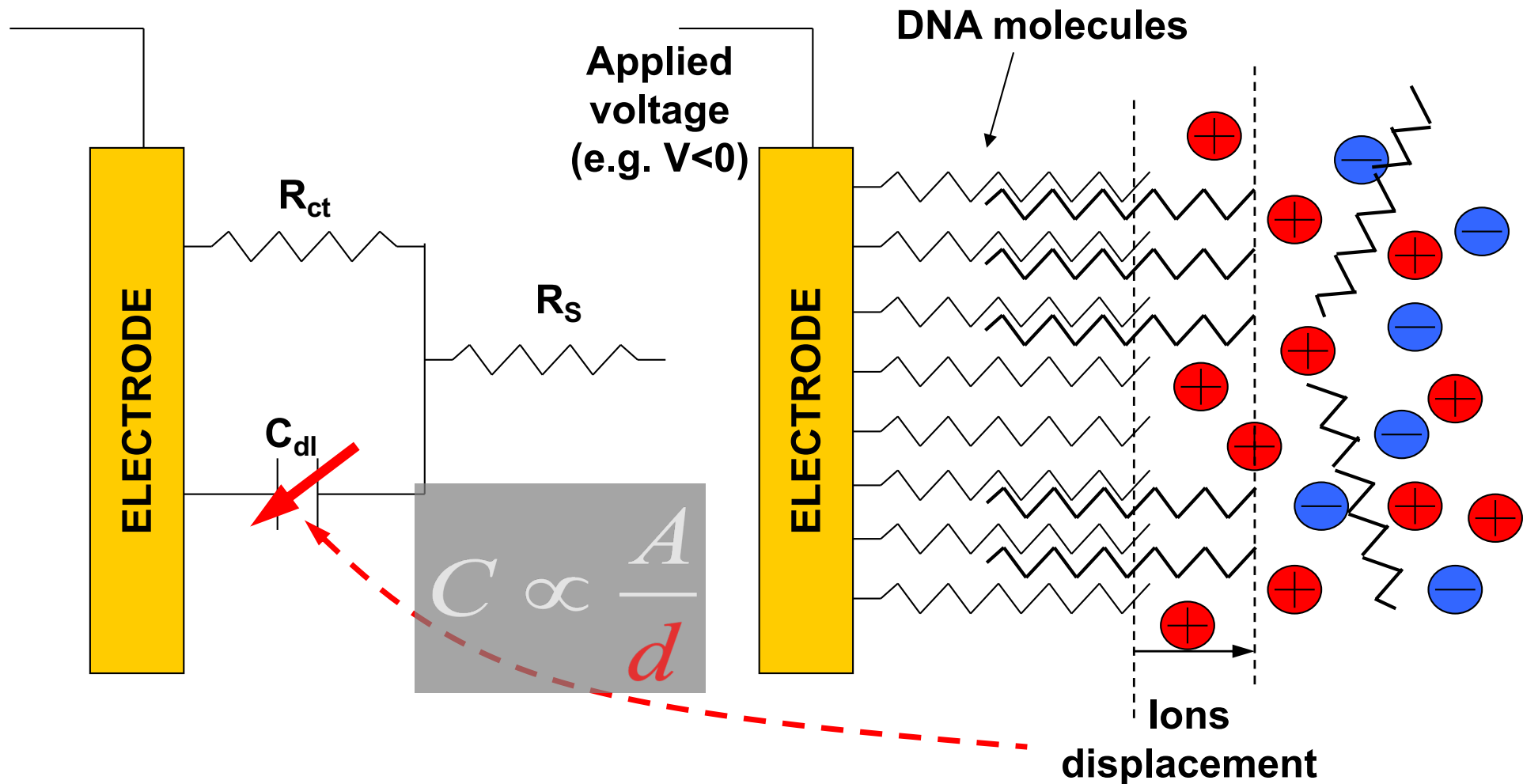
Ion planes are formed at the interface when electrodes immersed in solution are polarized

Electrochemical Interface



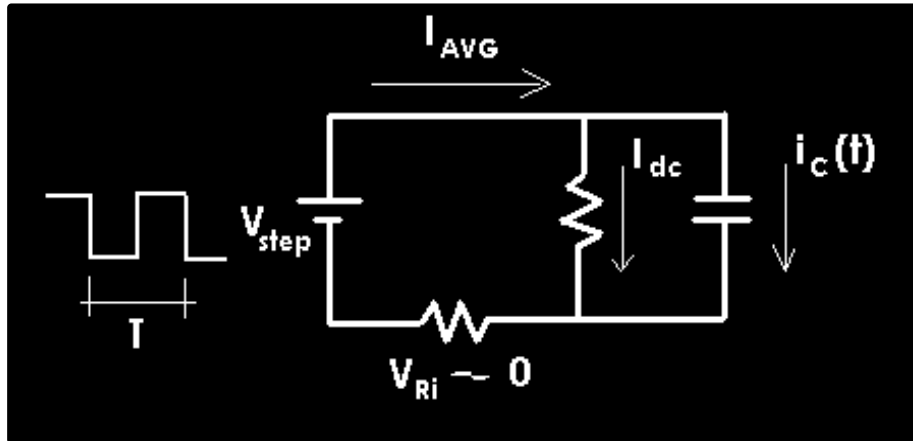
Ion planes are formed at the interface when electrodes immersed in solution are polarized

The Capacitance DNA Detection



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

Charge-Based Capacitance Measurement (CBCM)



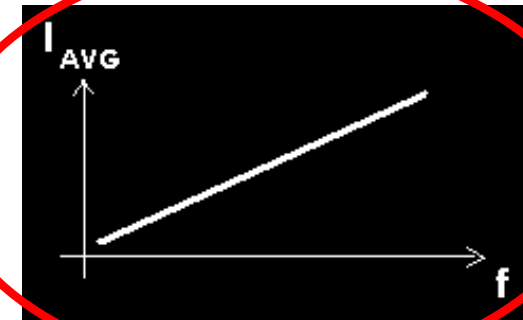
$$i(t) = I_{dc} + i_C(t)$$

Frequency!

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

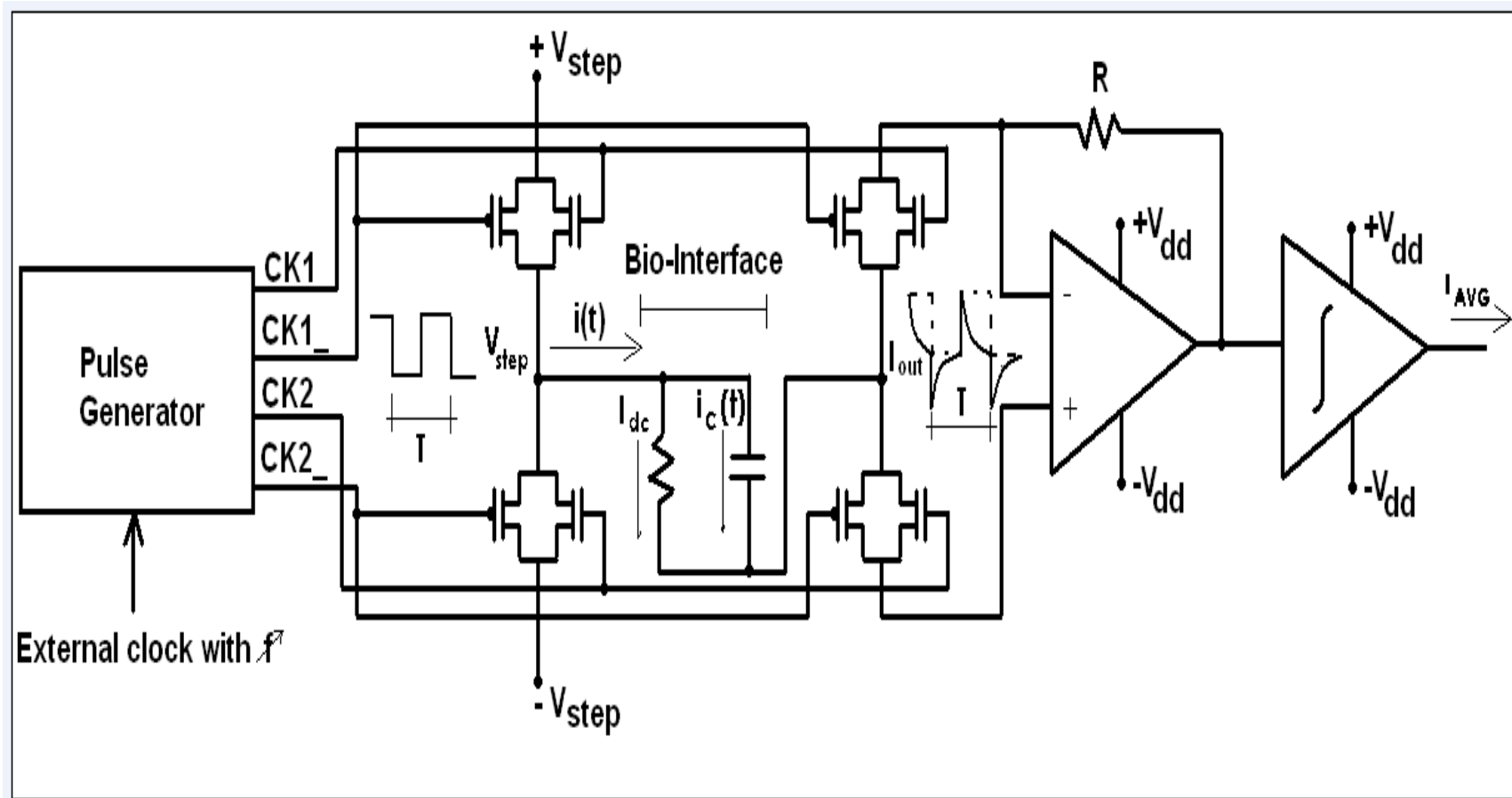
$$I_{AVG} = \frac{I_{dc}}{2} + CV_{step}f$$

THE CAPACITANCE !



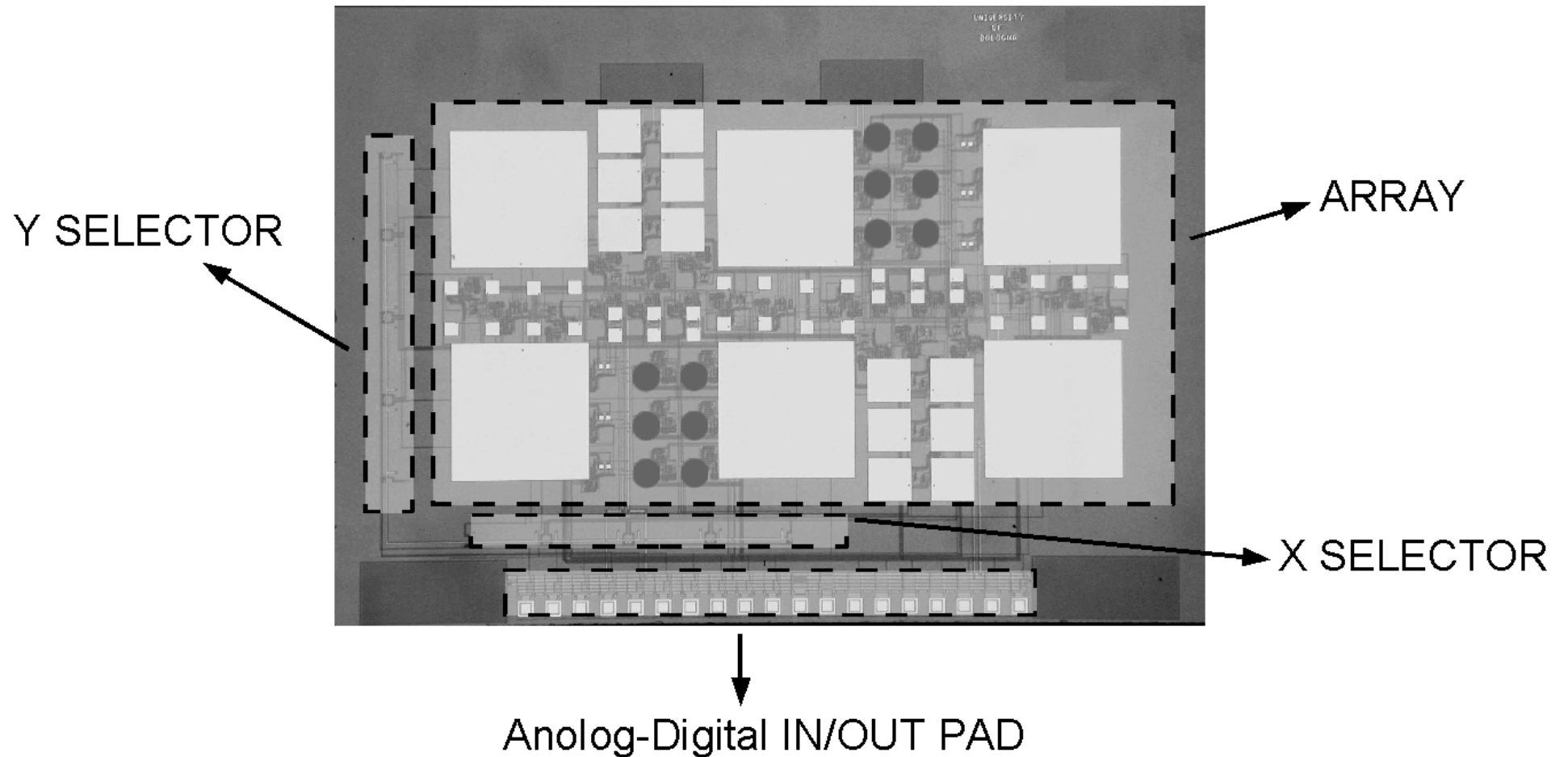
Method for a precise Capacitance measurement

CMOS for CBCM detection



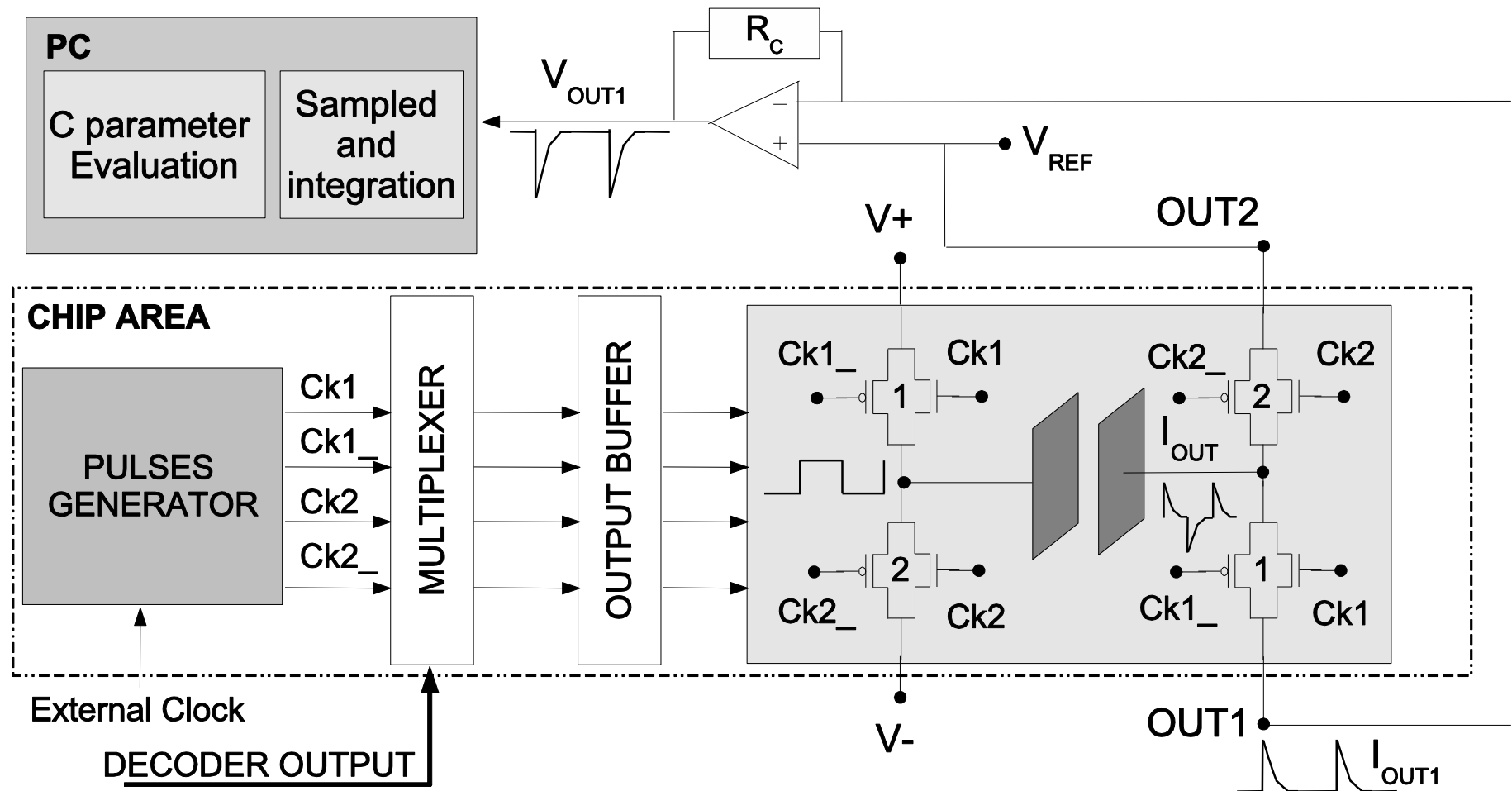
The circuit assures the square signal generator, an inverters, and an integrator to calculate the average current

The Chip Electrodes Layout



The VLSI Implementation of the Chip (CBCM method)

(CBCM = Charge Base Capacitance Mode)



The problem of overlapping signals

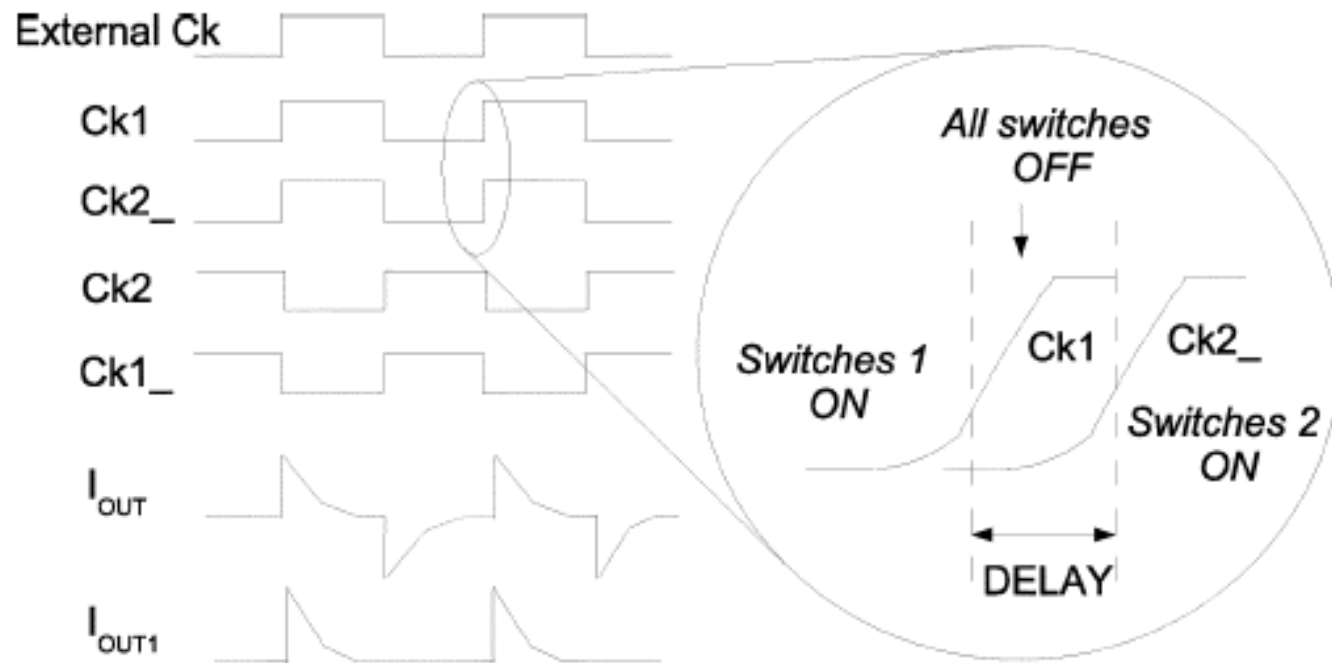


Fig. 4. Schematic representation of the signals flow used in the experiments.

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

The circuit solution

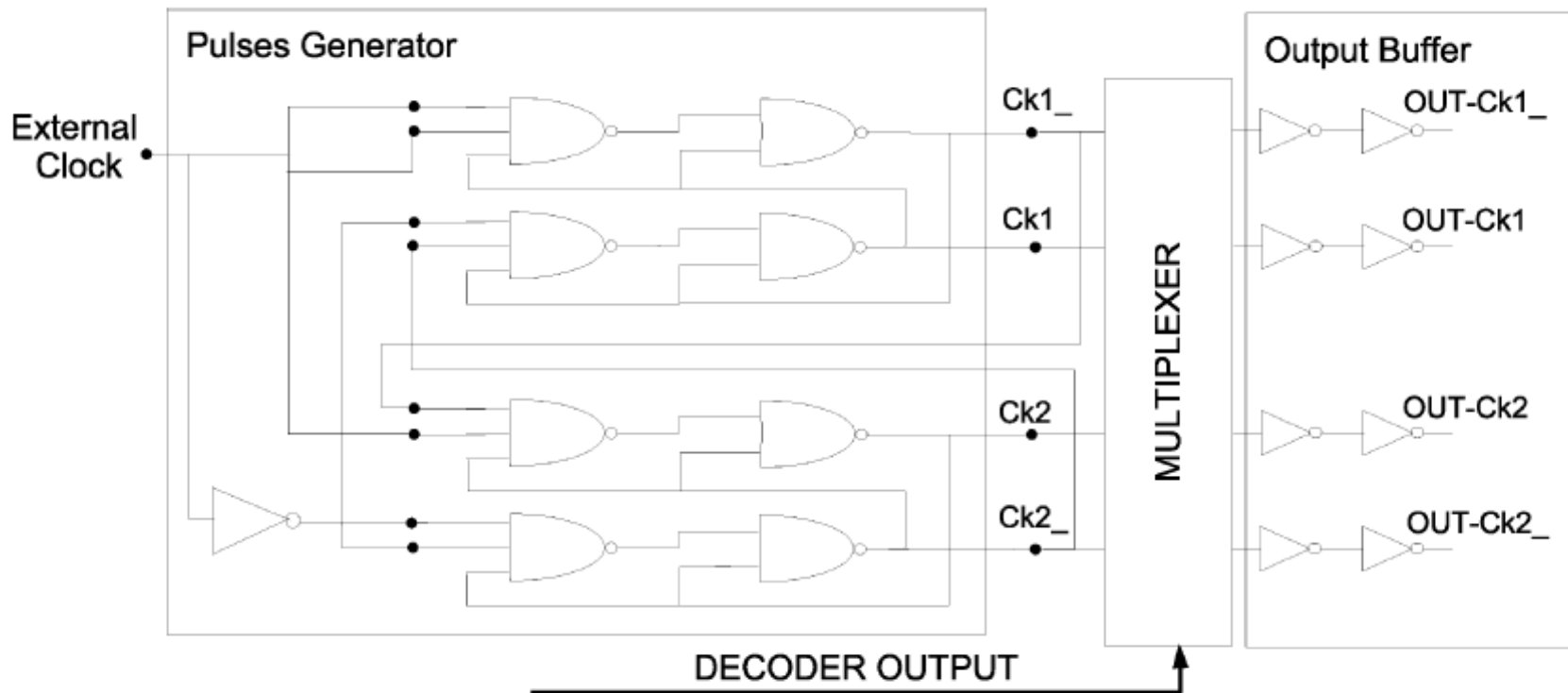


Fig. 5. Schematic plot of the block used to generate not overlapping clock signals.

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

The Measurements Set-up

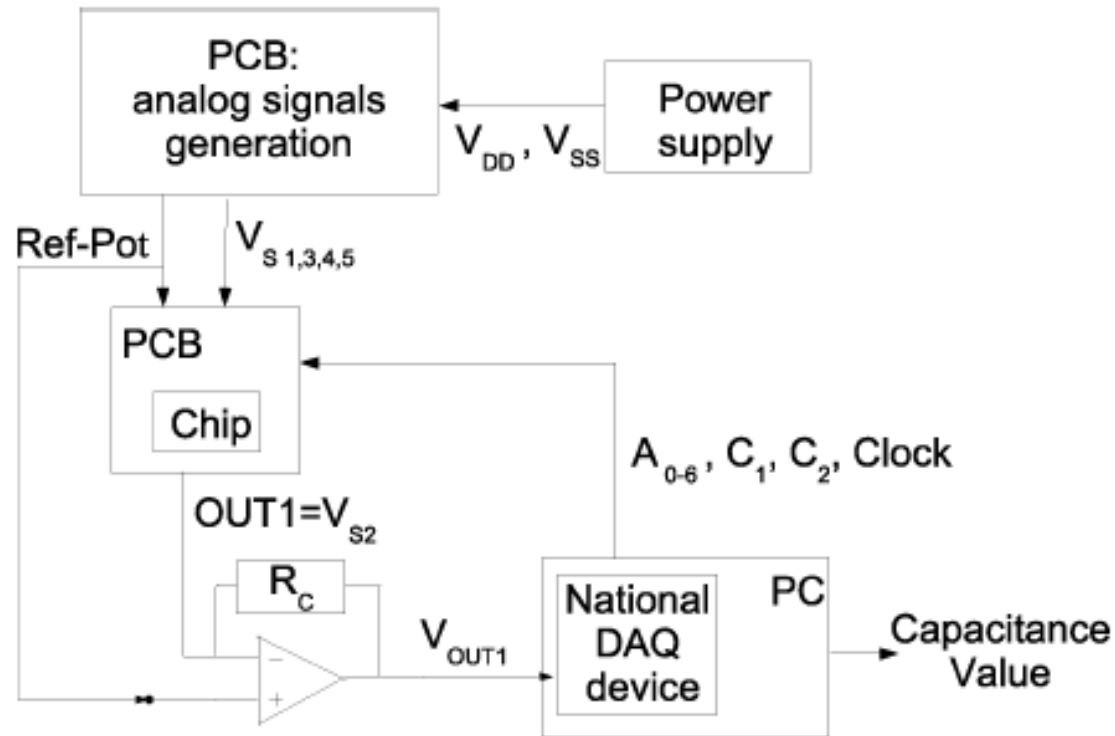


Fig. 8. Schematic representation of the measurement setup.

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

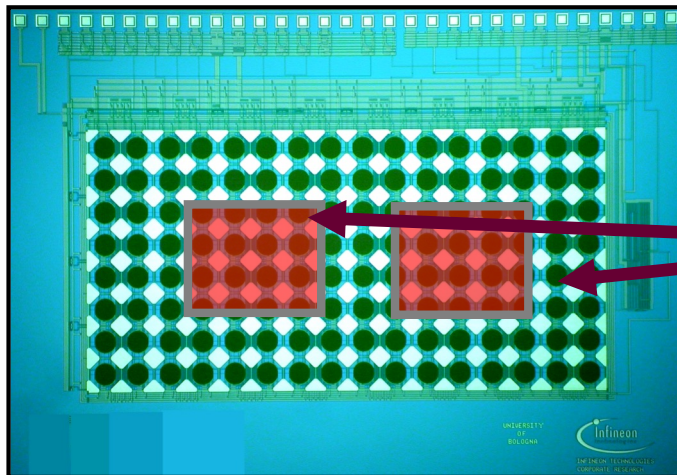
Liquid Measurement set-up



Chip is glued on a PCB

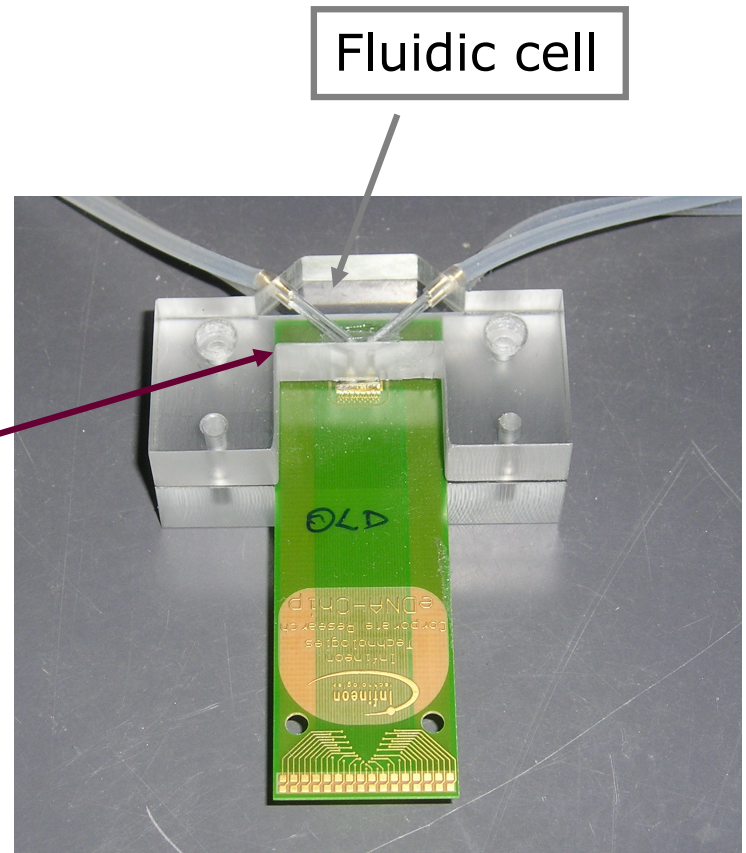
Output PCB pads

Bonding wires



Two different
Chambers
1mmX1mm

(c) S.Carrara



Fluidic cell

DNA detection in CBCM mode

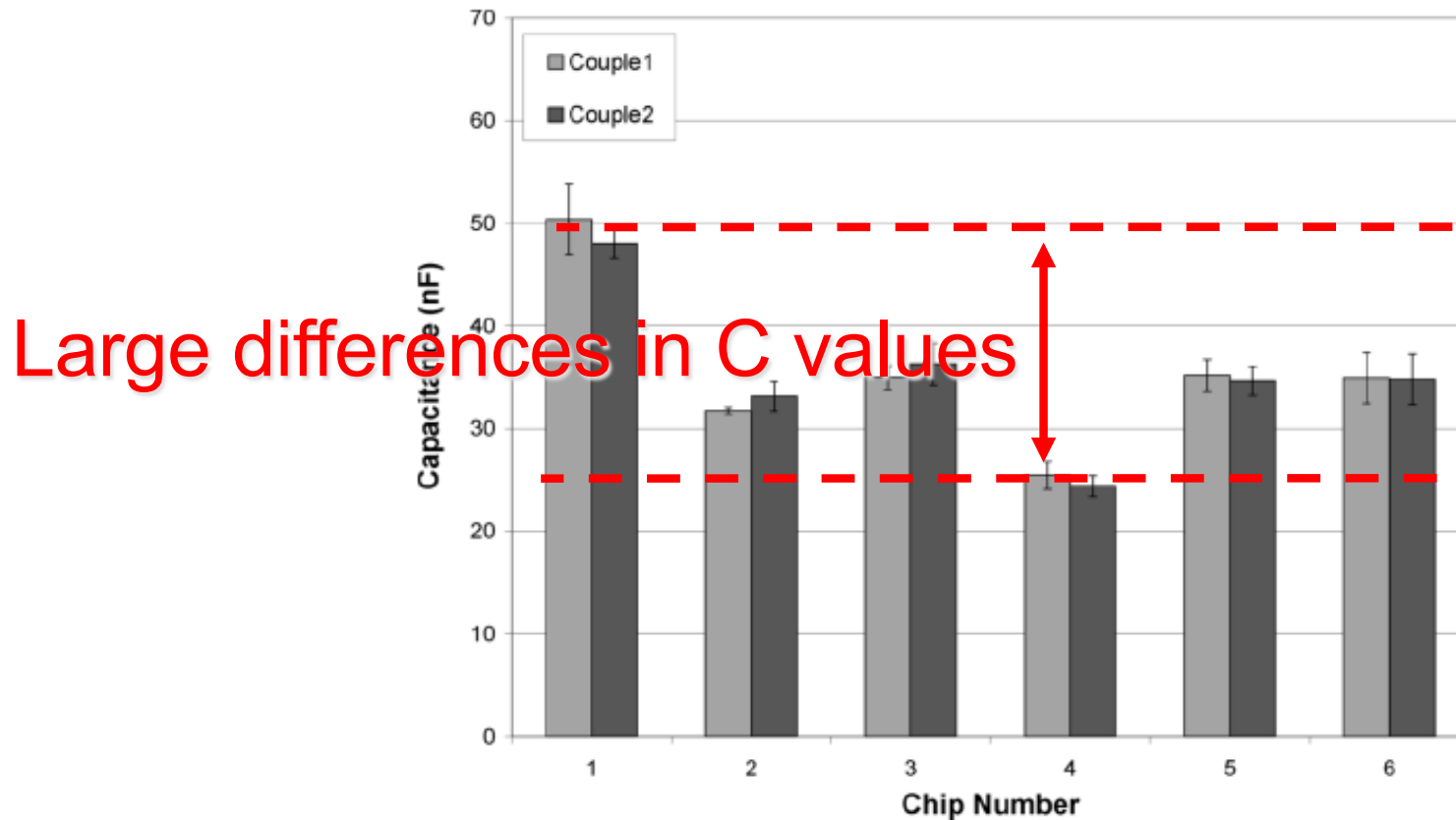


Fig. 10. Capacitance measurements of electrode couples on different chips.

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

Capacitance vs Frequency

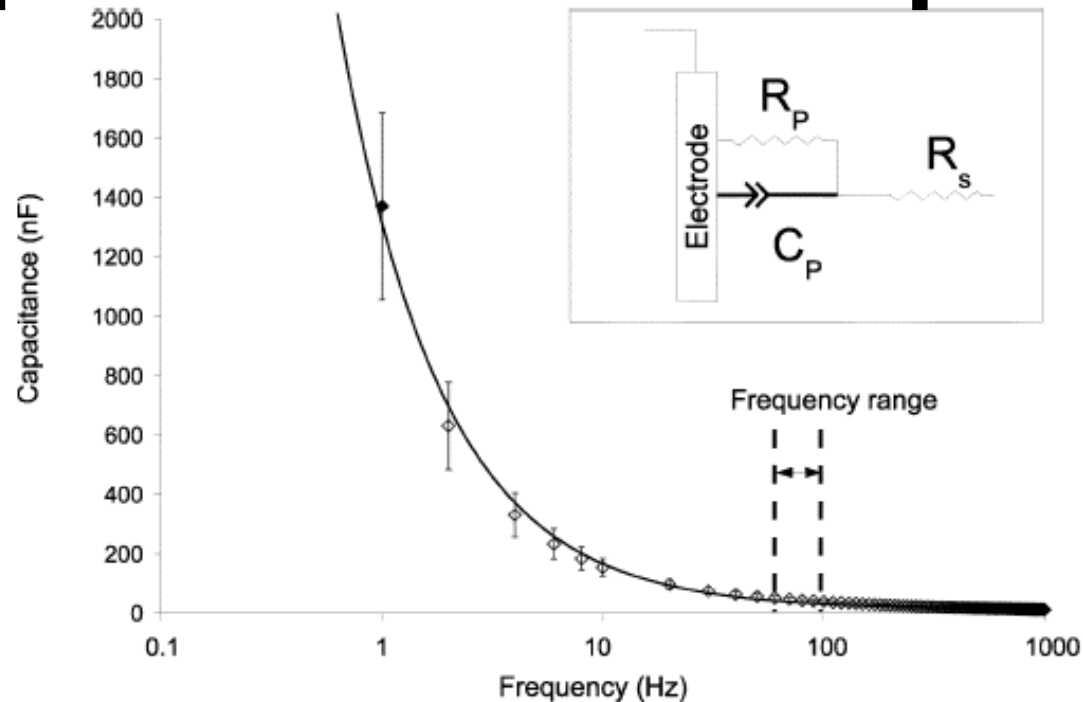
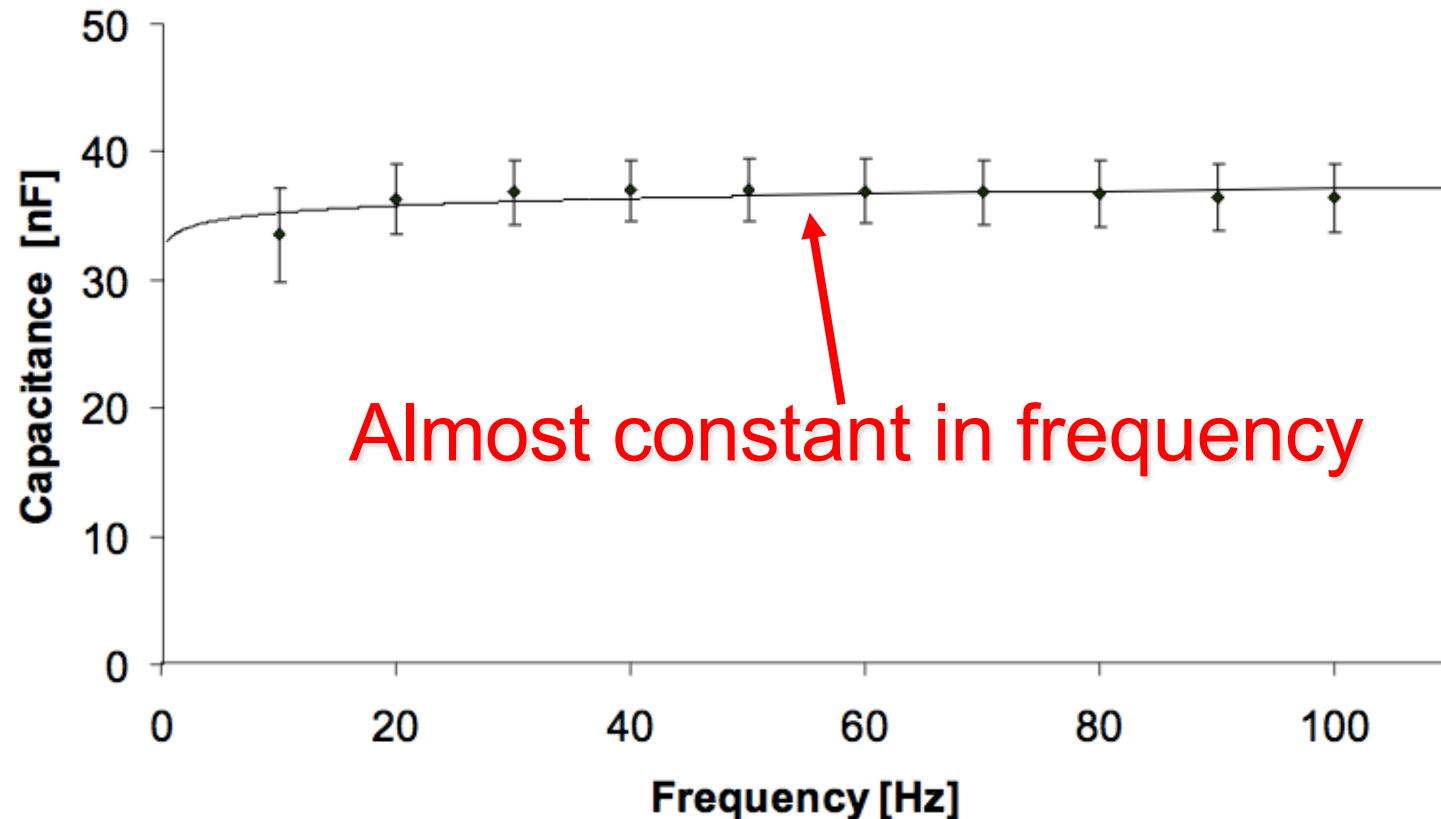


Fig. 9. Measured capacitance versus charge/discharge frequency on clean gold electrodes. The continuous line shows the fitting.

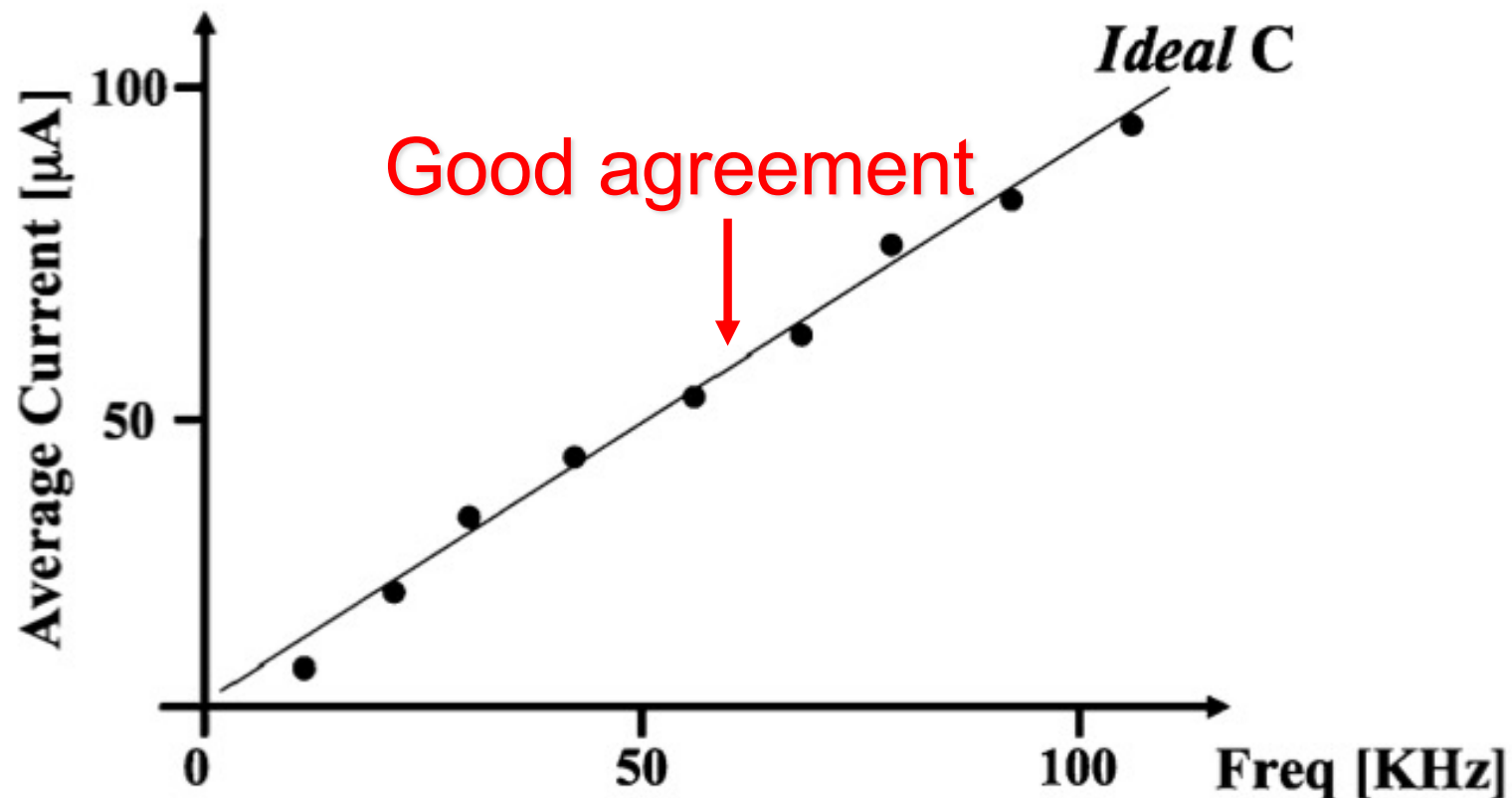
The trends of the measured capacitance vs frequency decrease the accuracy of the measurements in CBCM mode

Good DNA Layer



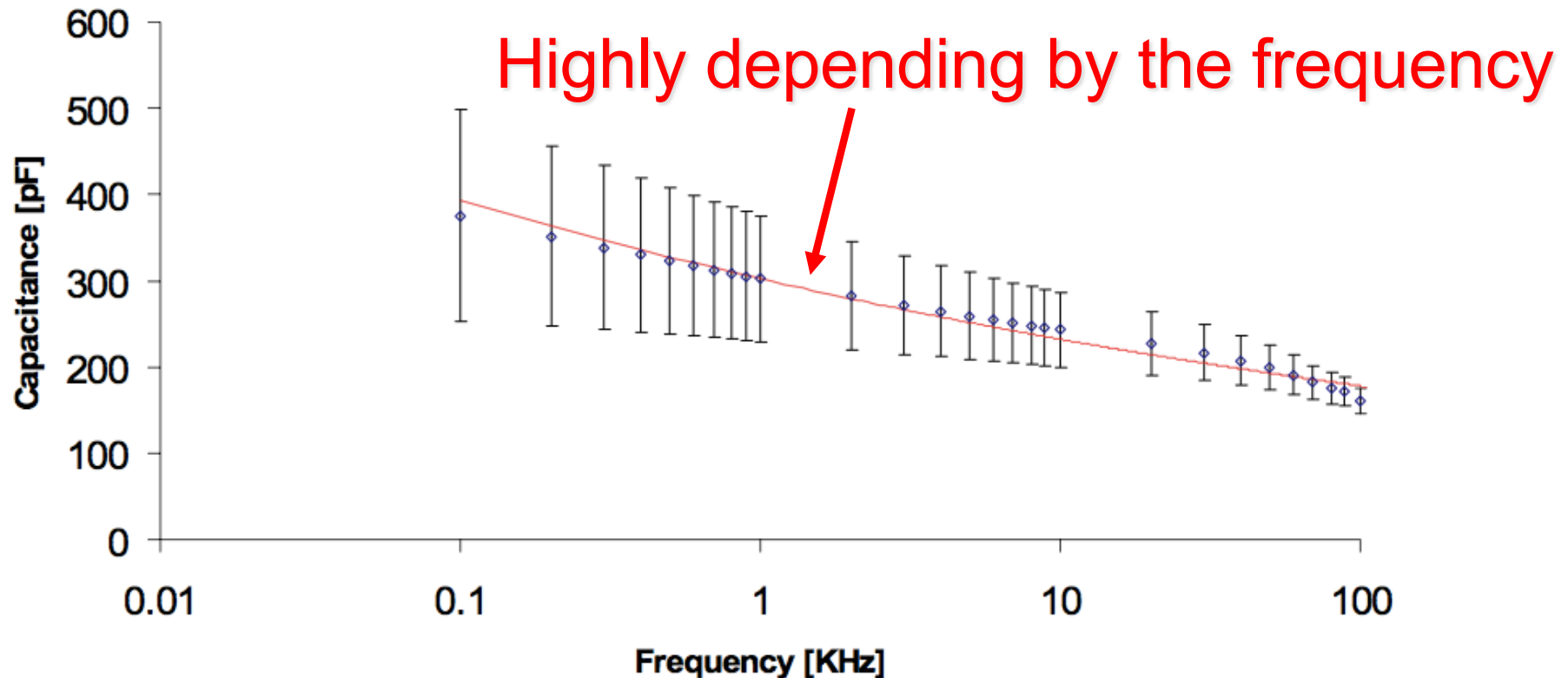
DNA Layer is independent by the frequency thanks to probes immobilized on Ethylene-Glycol Thiols

CBCM on good DNA Layer



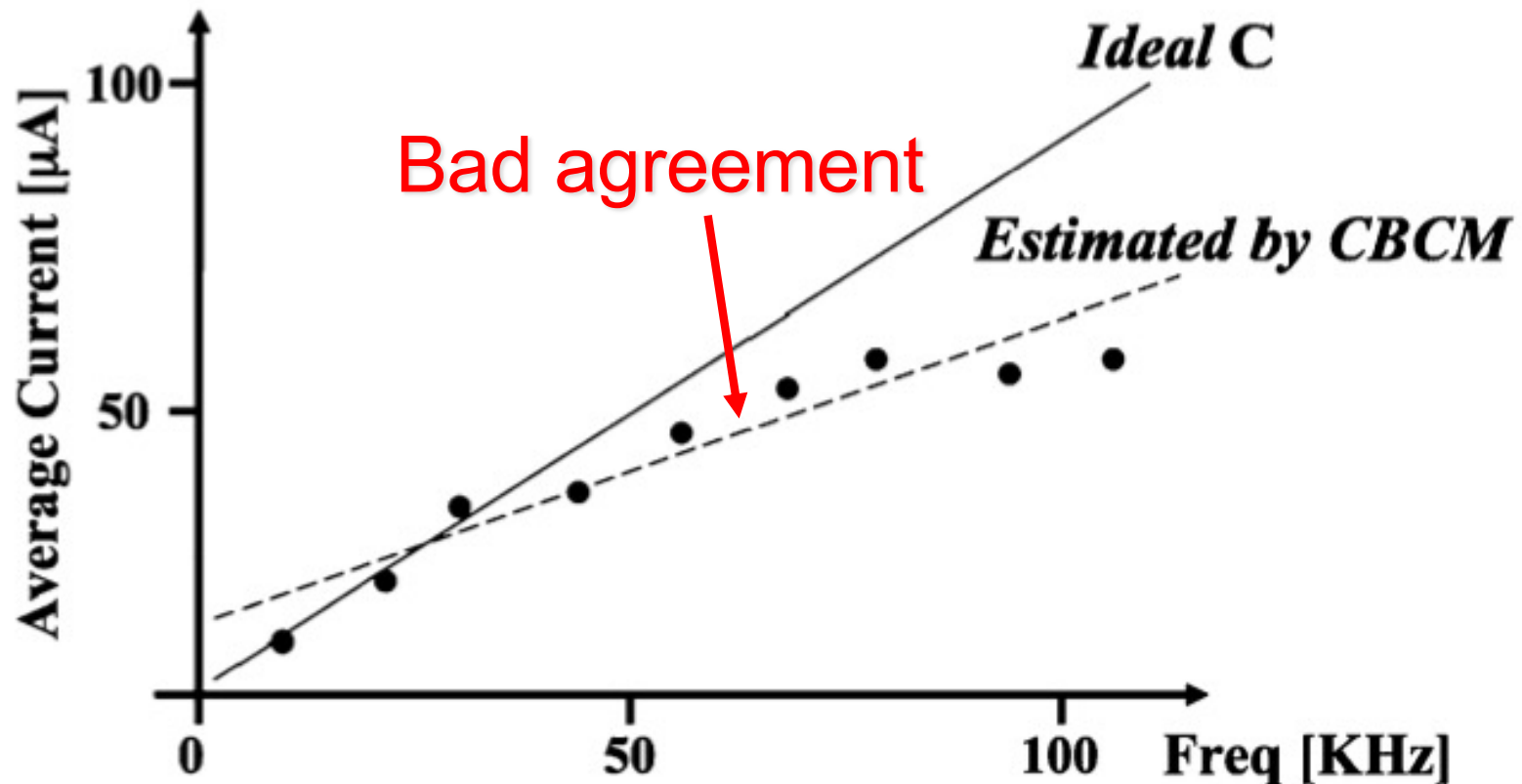
CBCM method on a DNA Layer that is independent by the frequency

Bad DNA Layer



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

CBCM on bad DNA Layer



CBCM method on a DNA Layer that depends by the frequency

DNA detection in CBCM mode

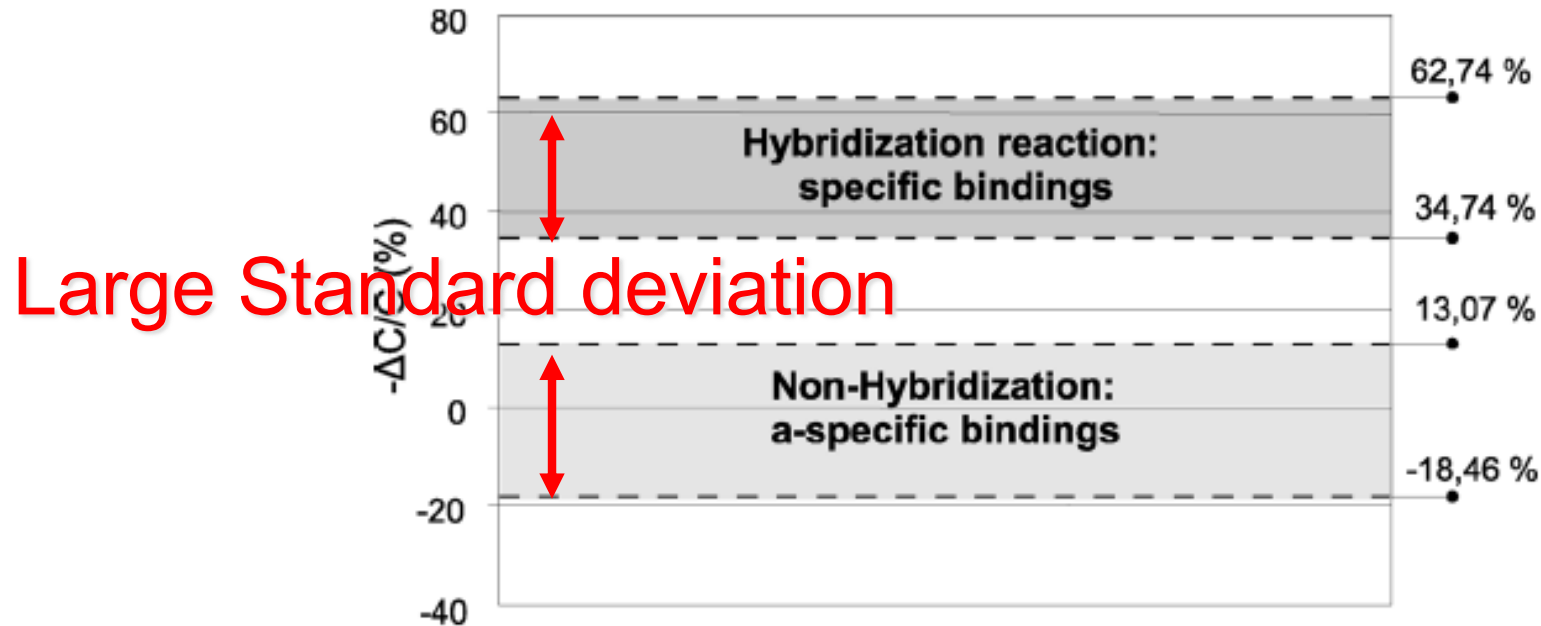
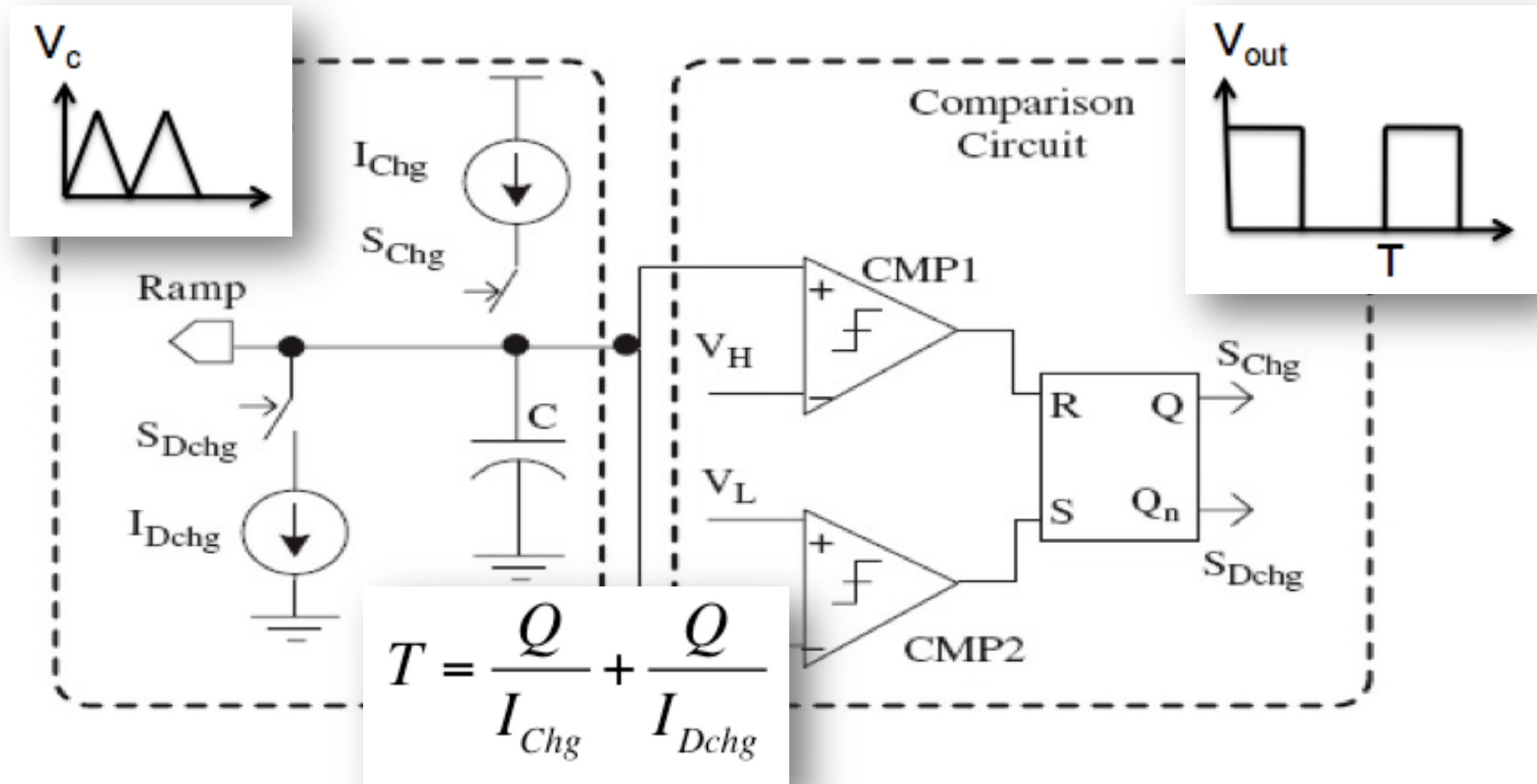


Fig. 12. Capacitance variations due to specific and a-specific bindings (upper and lower bands of measured capacitances, respectively). Positive values indicate capacitance decrease.

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

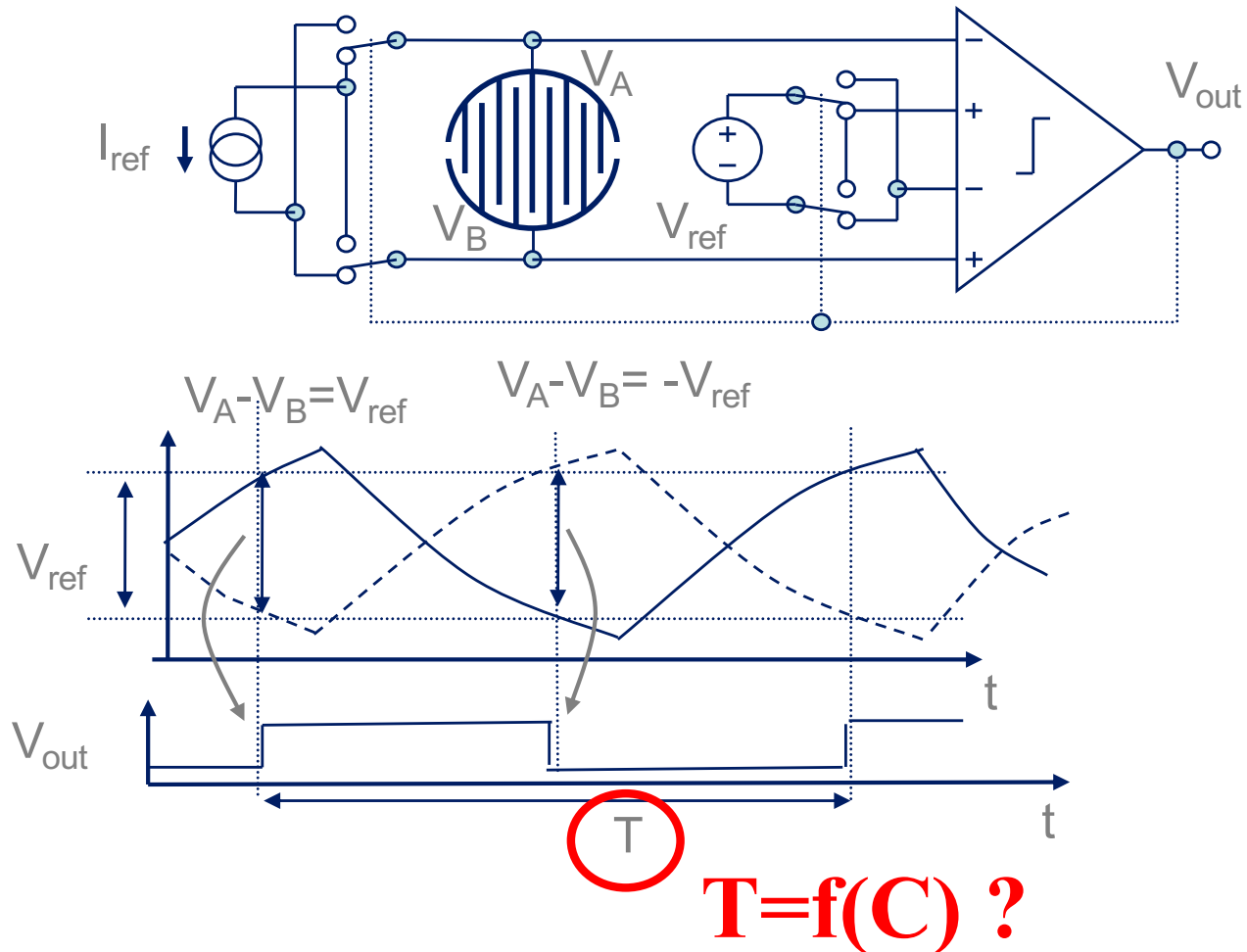
Current to frequency concept



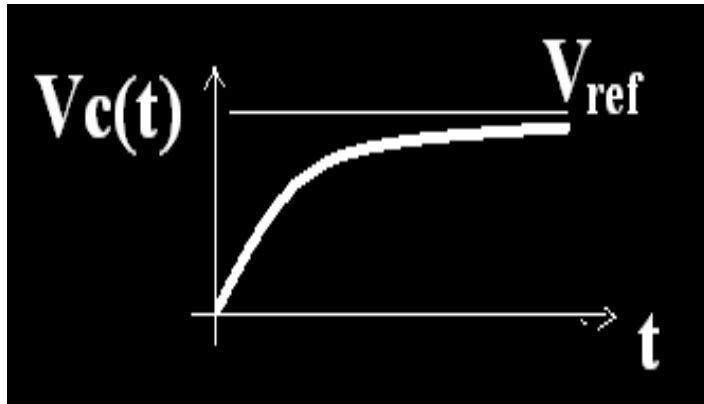
- Two current sources are used to charge and discharge the capacitor:
- The output period depends on both currents

Frequency to Capacitance Measurement (FTCM)

Principle: Frequency To Capacitance Mode



Frequency to Capacitance Measurement (FTCM)



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

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

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

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

The Taylor Series

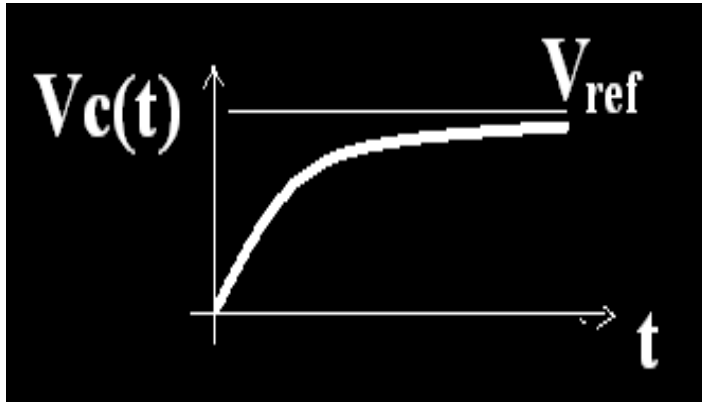
$$f(x) = f(0) + \left\{ \frac{\partial f(x)}{\partial x} \right\} x + \left\{ \frac{\partial^2 f(x)}{\partial x^2} \right\} x^2 + o(3)$$

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

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

Linearity by approximation in the right range of values

Frequency to Capacitance Measurement (FTCM)



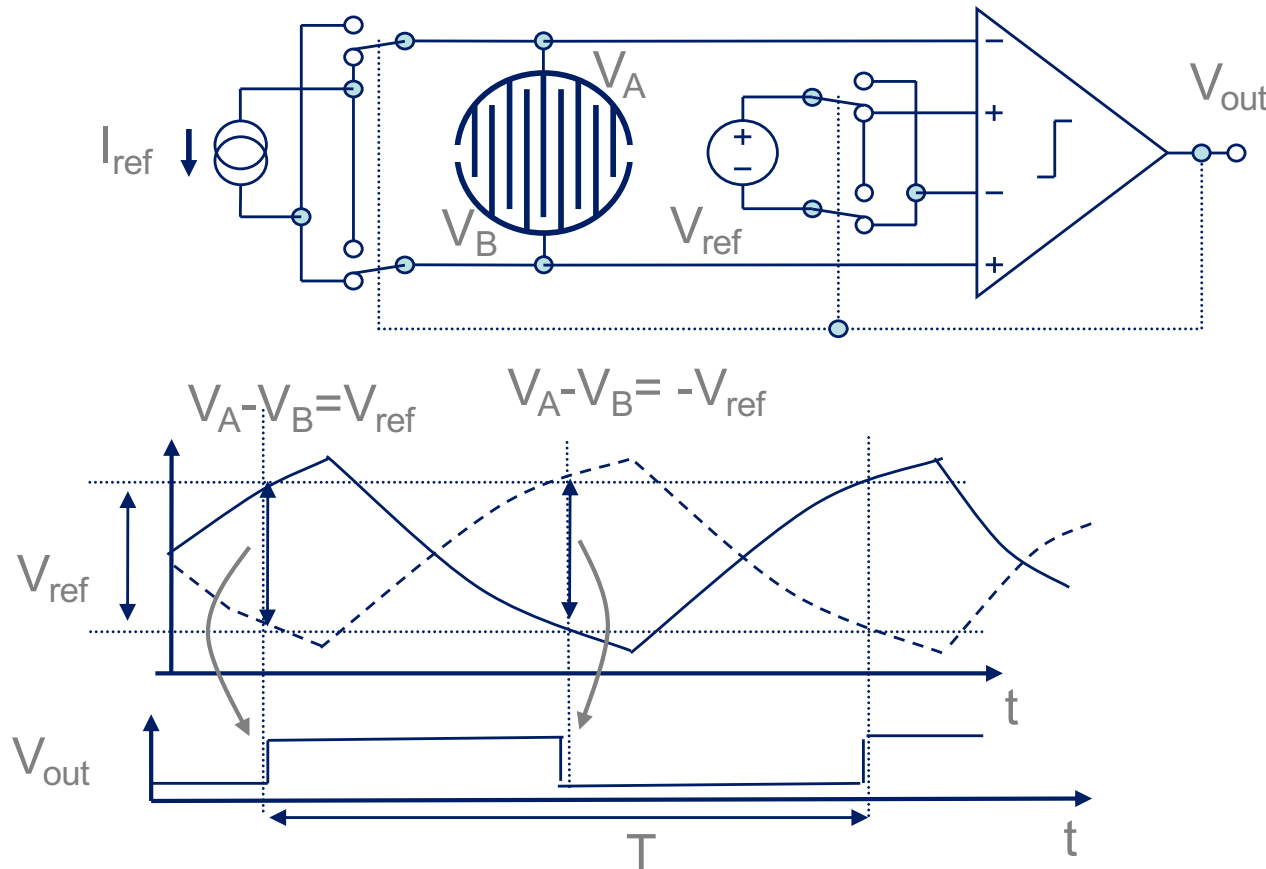
$$T = 2RC \ln \left[1 - \frac{V_{ref}}{RI_{ref}} \right]^{-1}$$

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

Method for the estimation of the Capacitance

Frequency to Capacitance Measurement (FTCM)

Principle: Frequency To Capacitance Mode



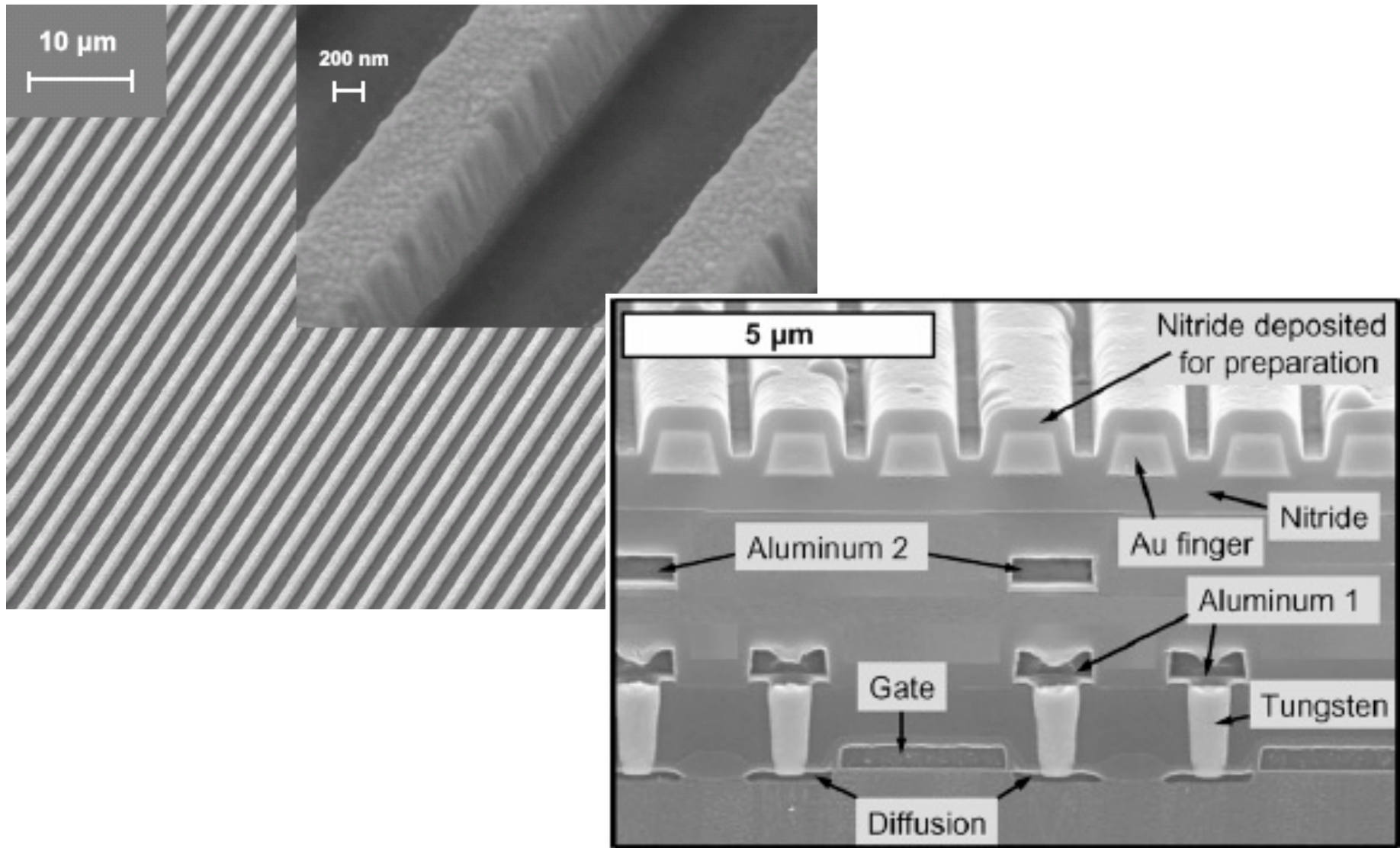
$$T = 2RC \ln \frac{1}{1 - \frac{V_{REF}}{I_{REF}R}}$$

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

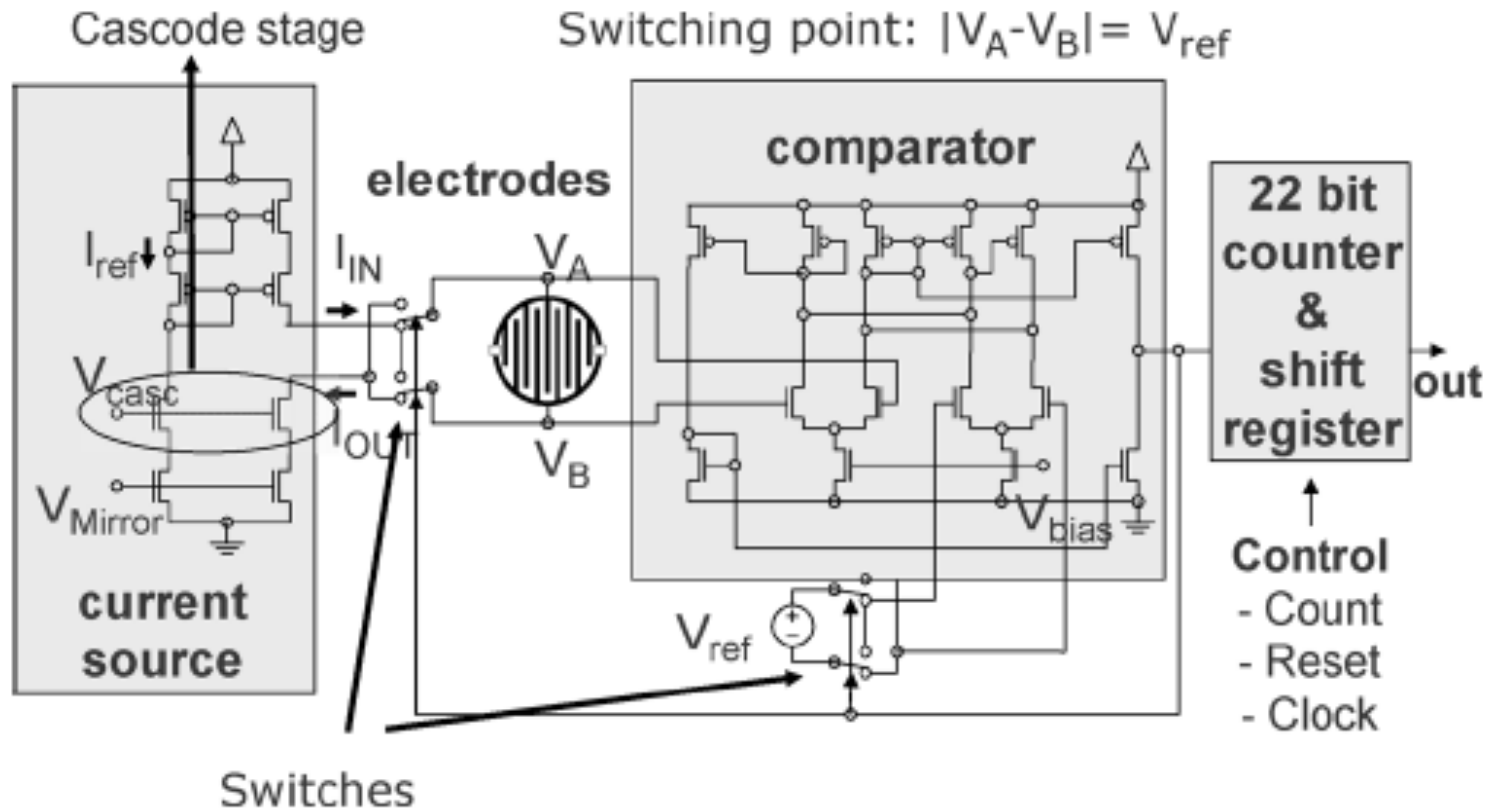


$$T = \frac{2 \cdot V_{REF} \cdot C}{I_{REF}}$$

Electrodes Layout



Chip Architecture (FTCM)



Measurements Set-up

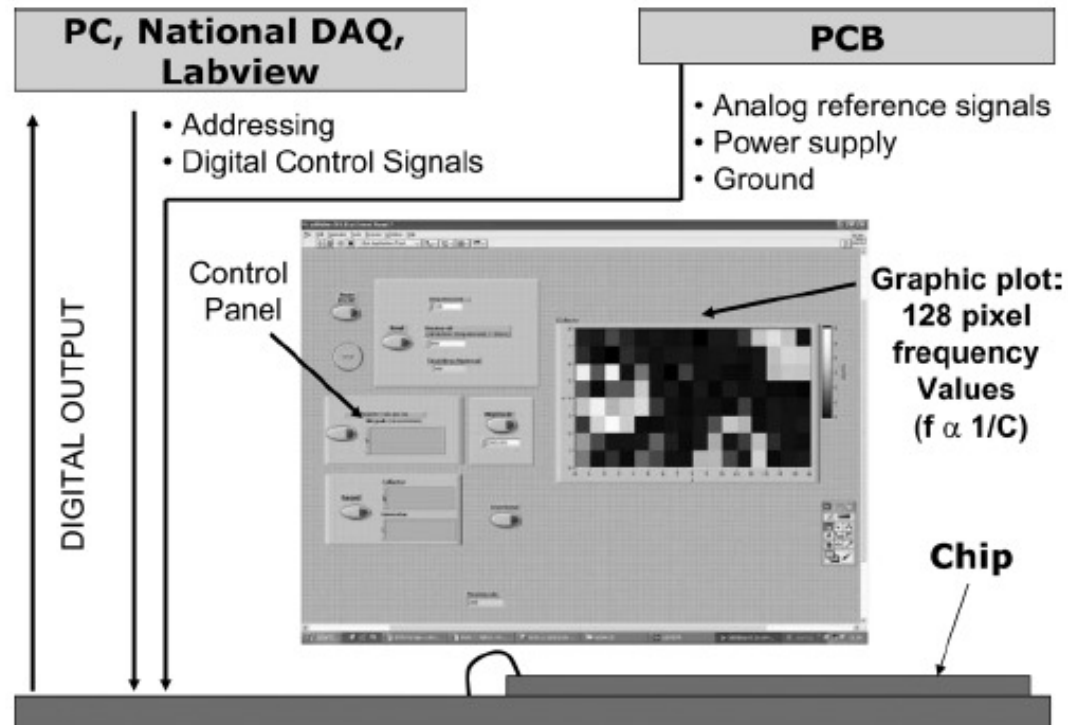
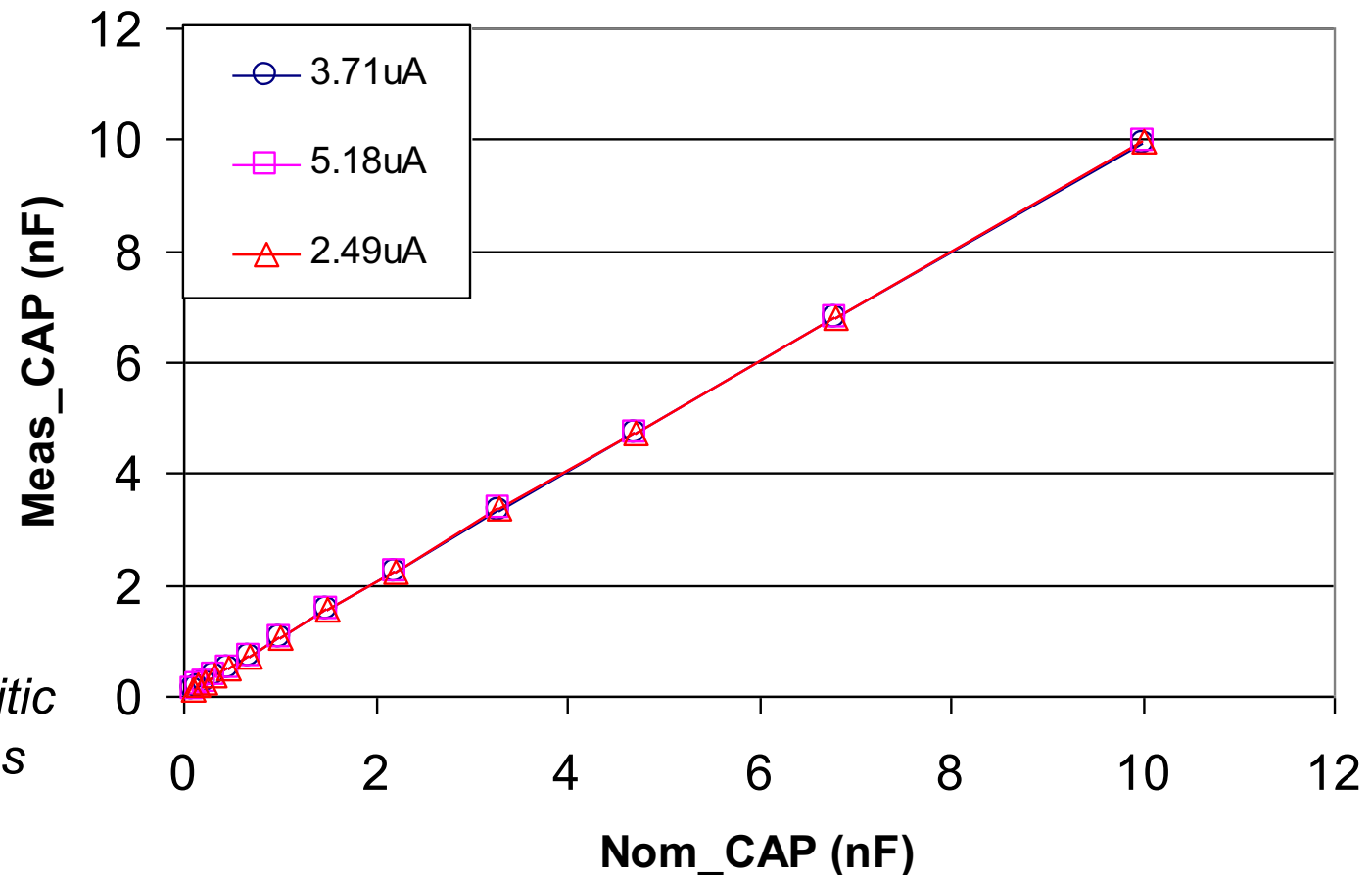


Fig. 9. Schematic representation of the measurement set-up. Voltage reference signals and power supply are generated by circuitry on the PCB. Digital control signals are provided by a PC. The LabView interface manages all the parameters involved in the measurements and shows directly on the screen the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

Validation Test

Slope = 0.9837
Intercept = 62 pF
 $\sigma < 0,3 \%$

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)

Probes property on FTCM mode

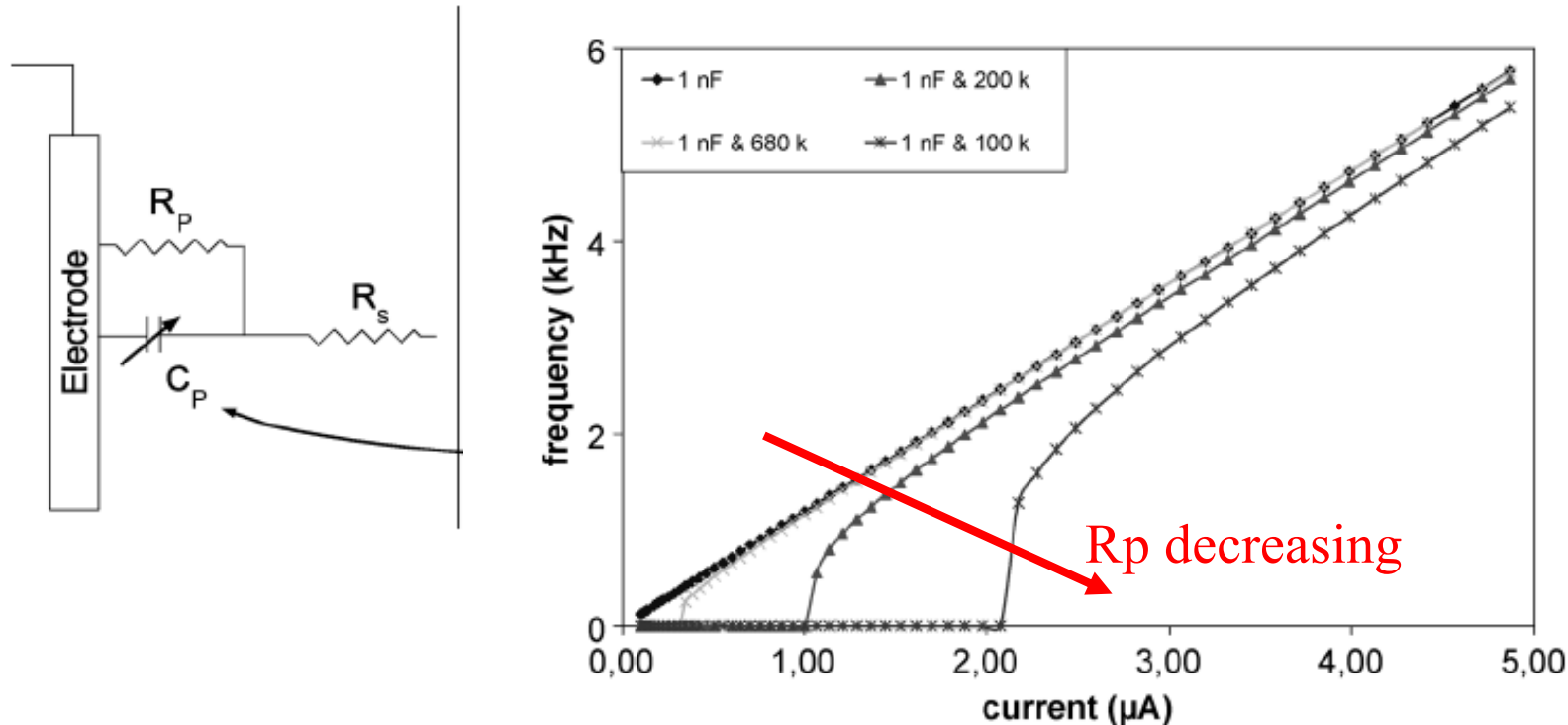
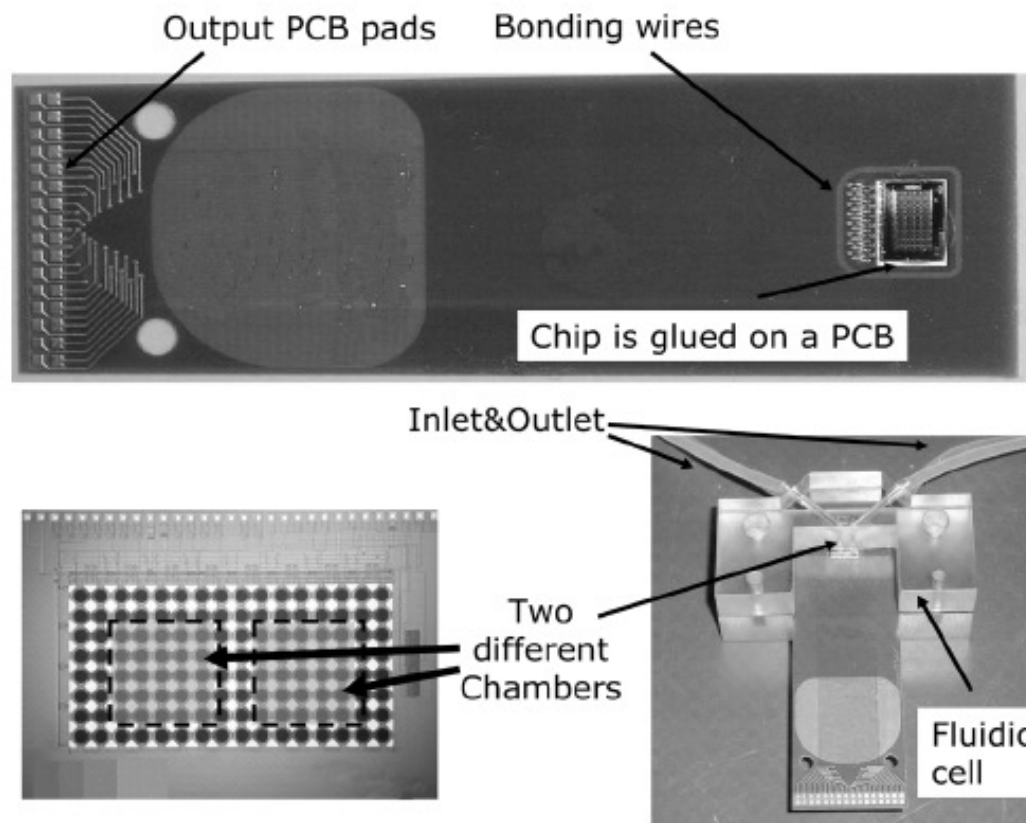


Fig. 12. Frequency versus reference current showing that a significant influence of the parallel resistance on the measurement result occurs only at low current values and at R_P values lower than 680 k Ω .

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

Liquid Measurement set-up



DNA detection in FTCM mode

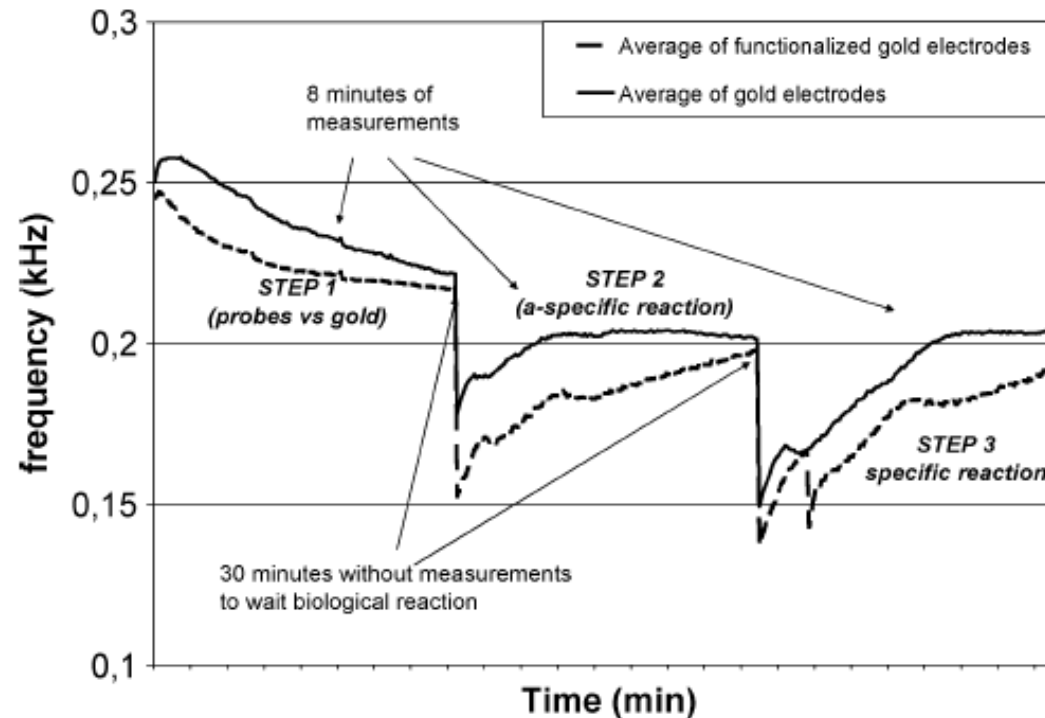


Fig. 13. Frequency changes of the average of reference electrodes (continuous line), and the average of functionalized electrodes (dashed line) show a larger gap after DNA hybridization step considering the stable value reached at the end of the transient.

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

DNA detection in FTCM mode

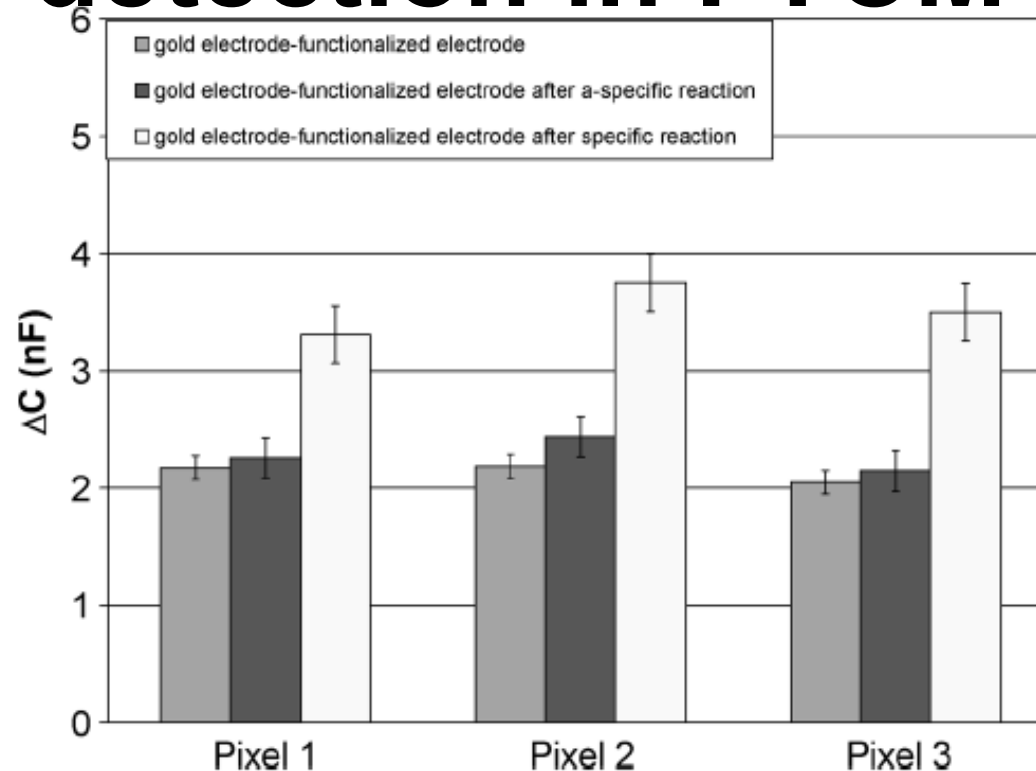
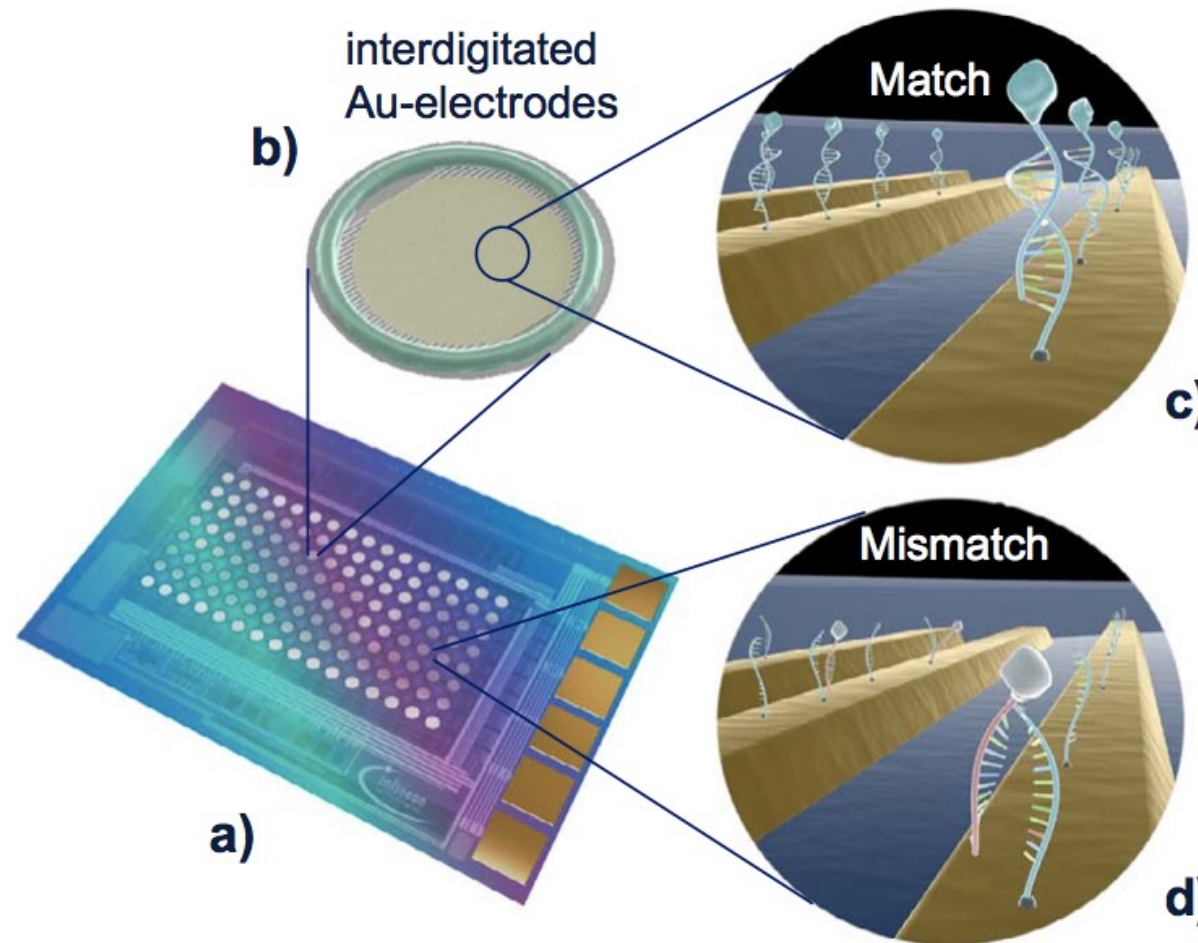


Fig. 14. Typical variations for several pixels among functionalized electrodes and the average value of reference gold electrodes. Capability to distinguish between specific and nonspecific binding is shown for each pixel.

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

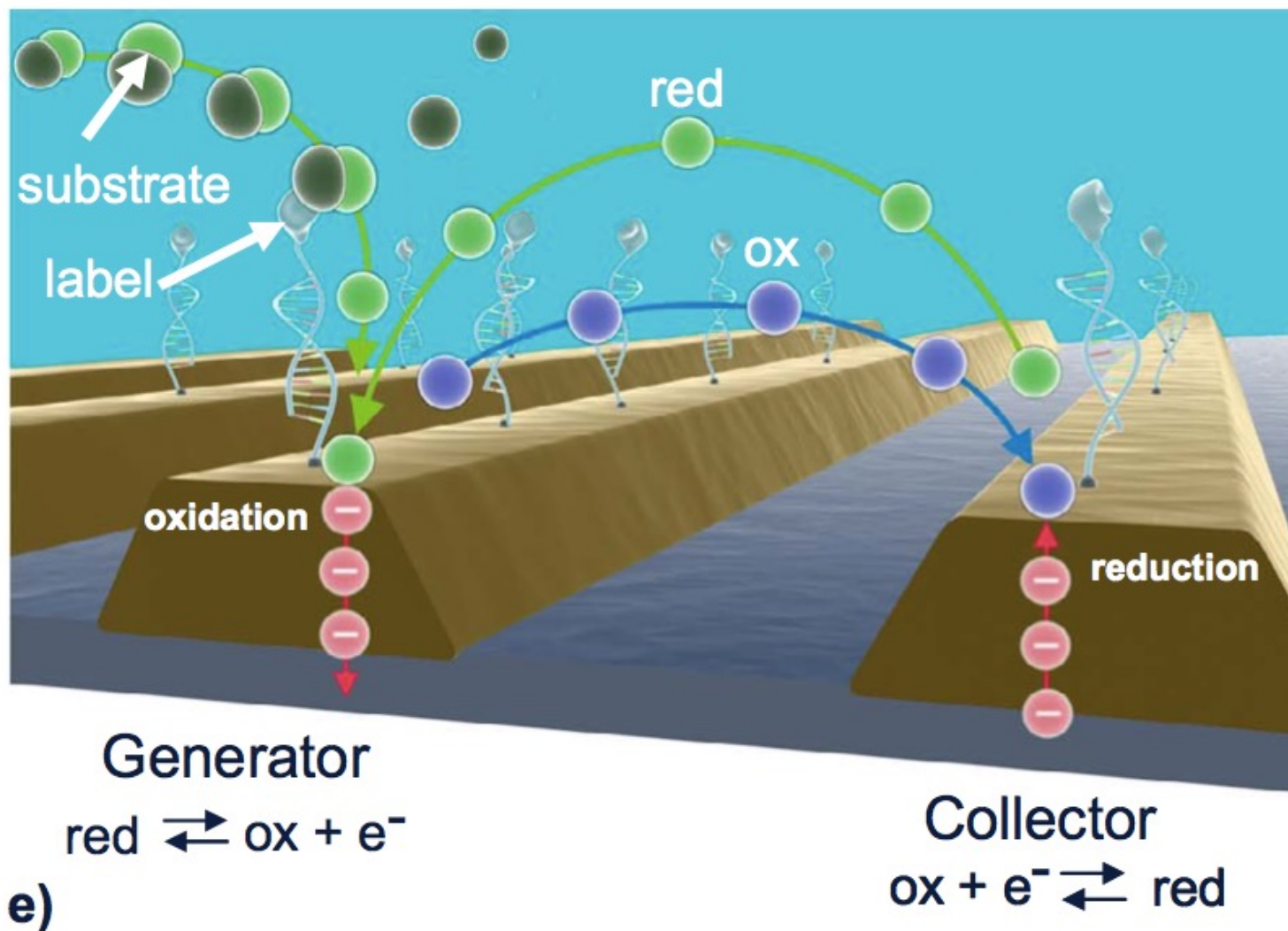
Amperometric Detection of DNA

Figure by Frey et al, IEEE ISCAS 2015



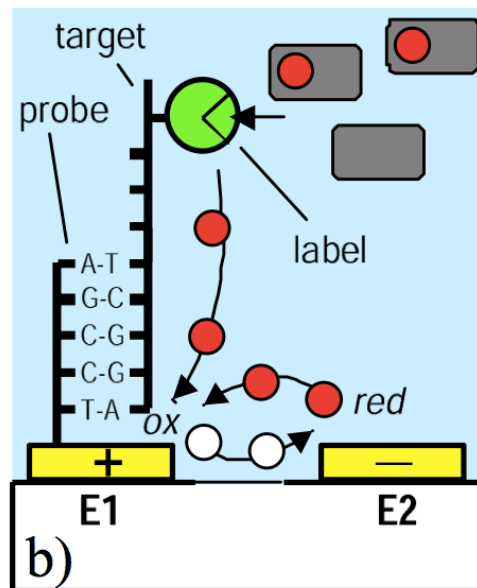
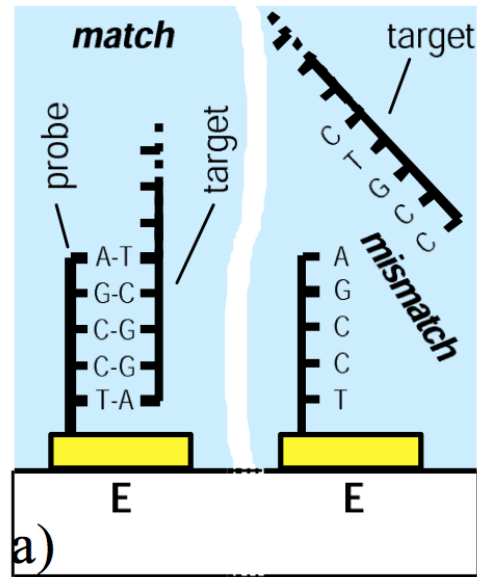
Electrochemical labels might be used to detect DNA

Amperometric Detection of DNA



Redox species can be then measured at the electrodes

Amperometric Detection Principle

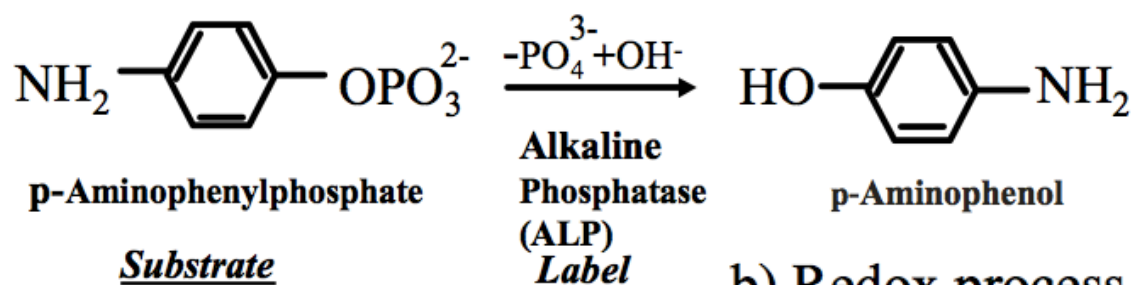


How it works:

- ✓ 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

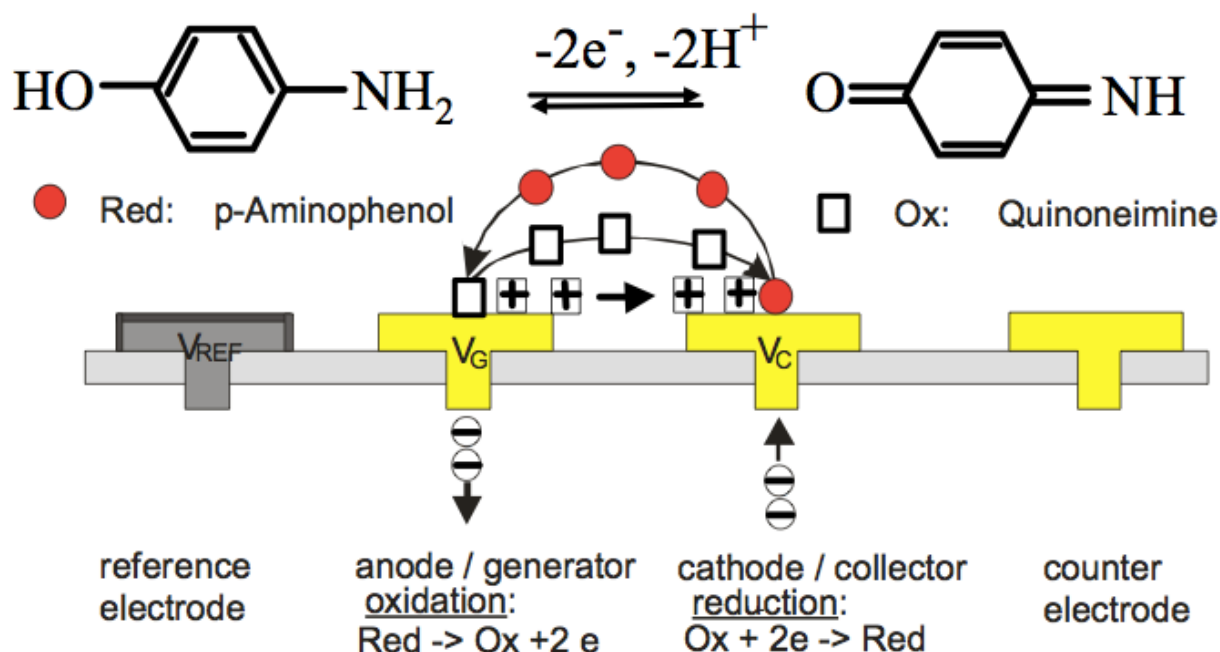
a) Process at the label



✓ The label cleaves the secondary probe

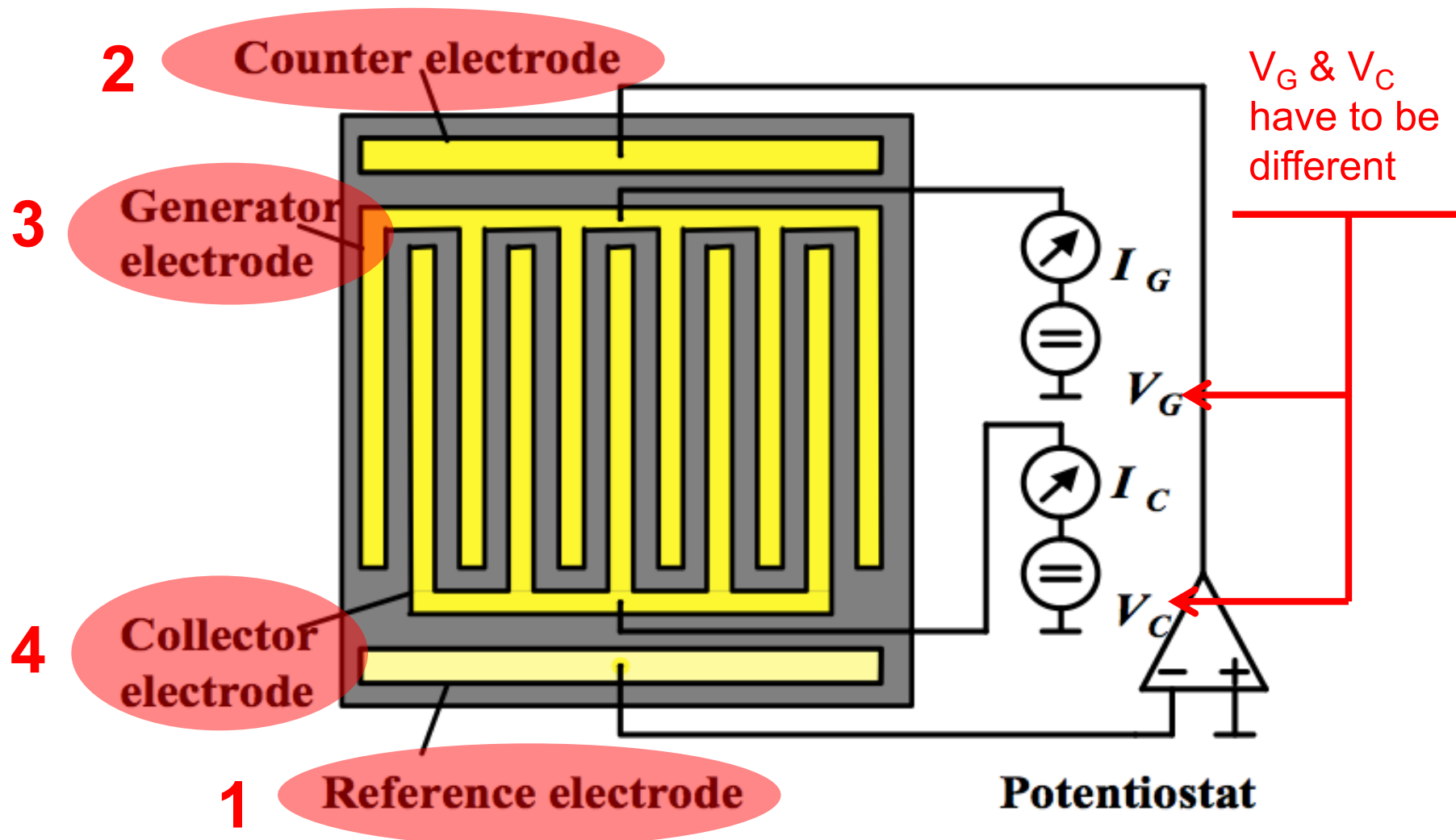
✓ The product of the cleavage is generating an oxidation process at the anode and, once oxidized, a reduction at the cathode

b) Redox process at the electrodes



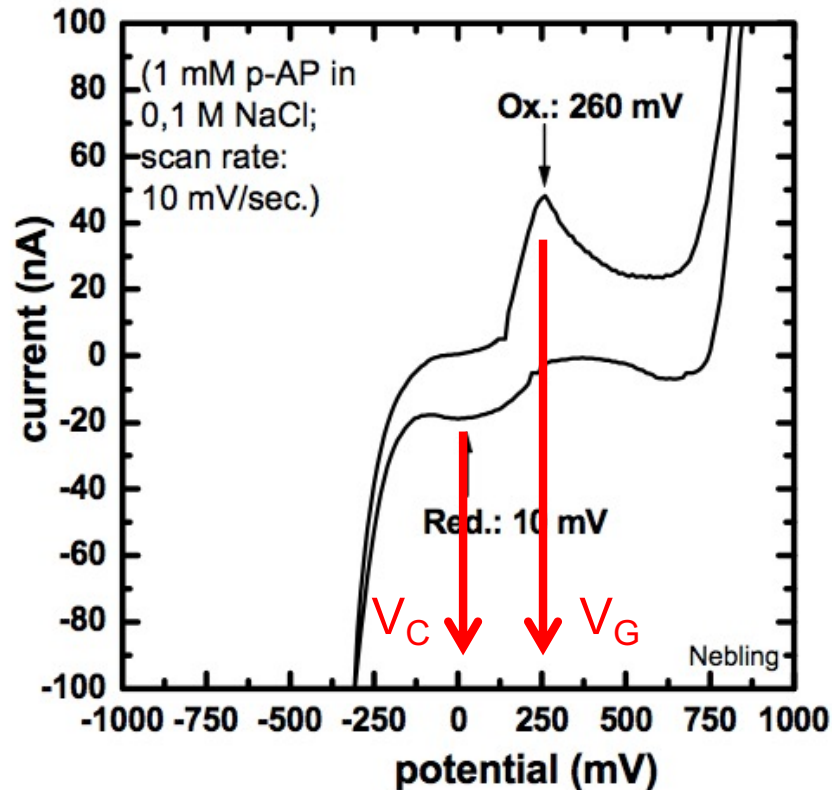
(c) S.Carrara

The Electrochemical Cell

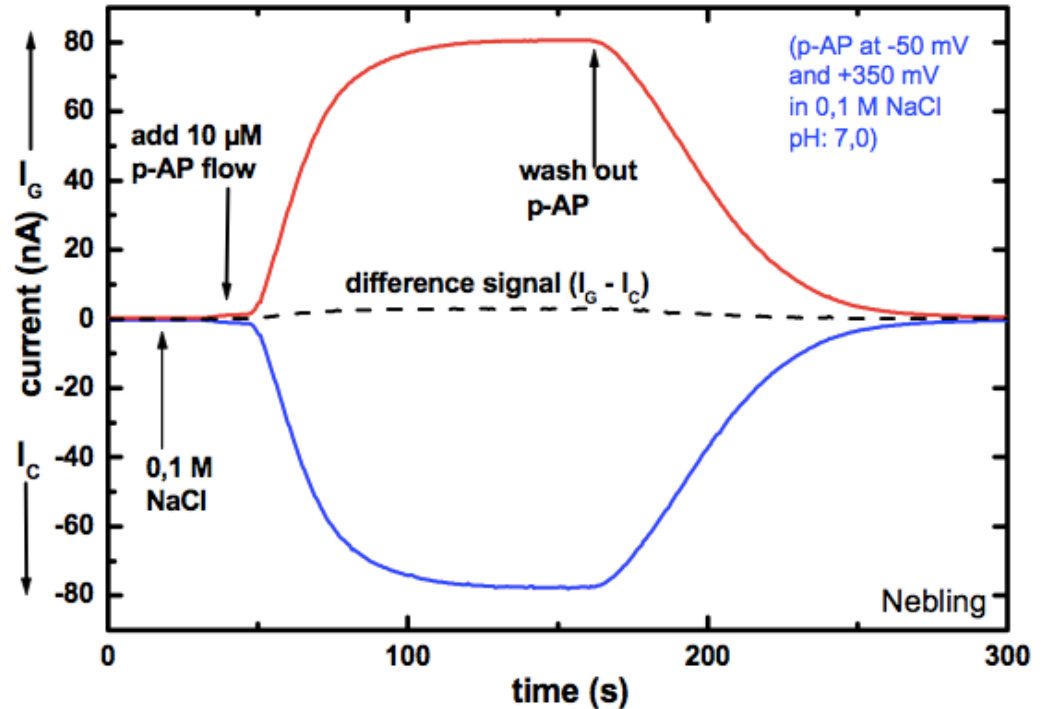


A 4-electrode Electrochemical Cell is here required

Cyclic Voltammetry



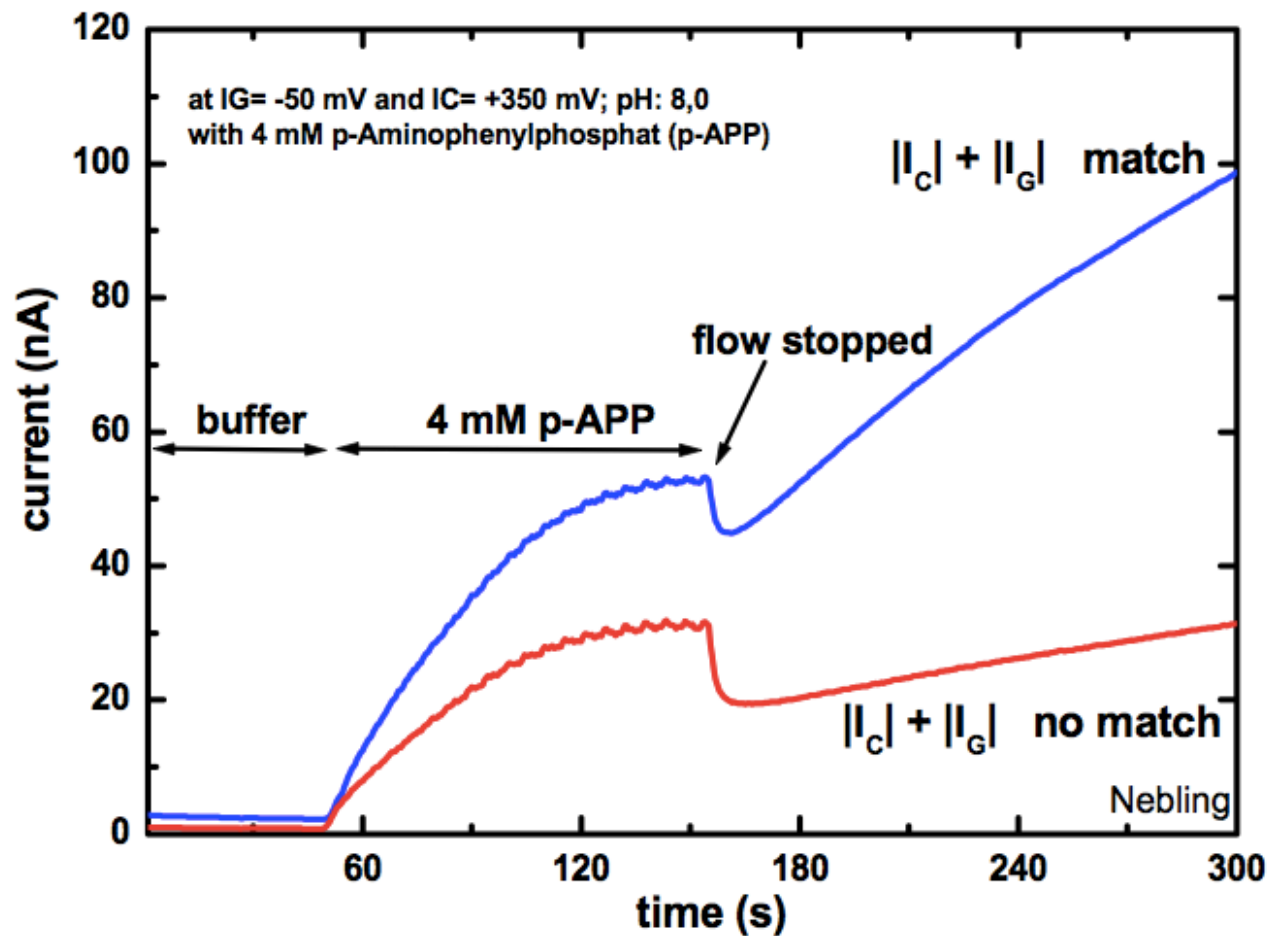
- ✓ Typical Cyclic Voltammetry
acquired with 3-electrode cell



- ✓ 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

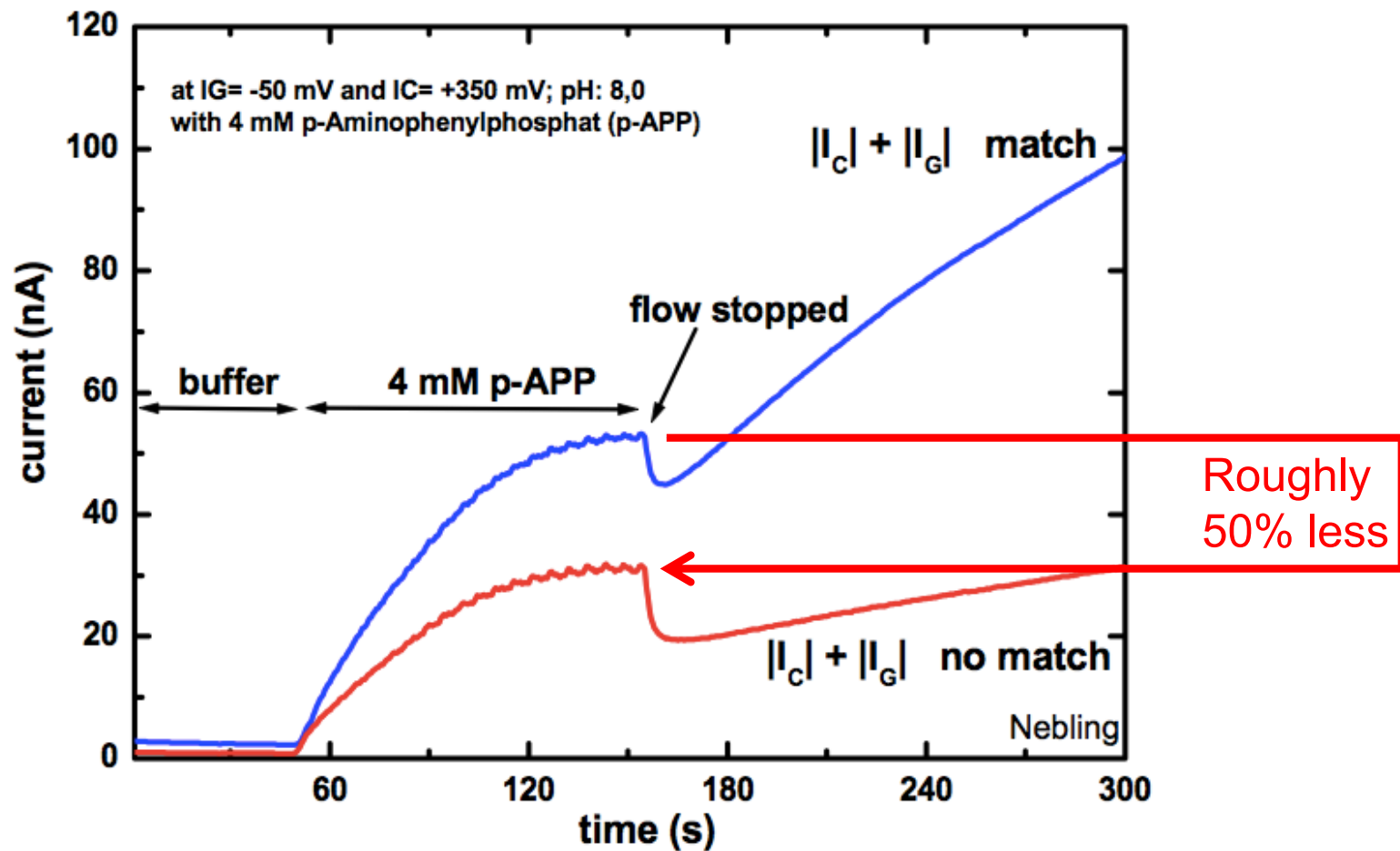
Gibbs free energy for Match/Mismatch

duplex	<i>Experimental</i> ΔG [kJ/mol]
GGTTATTGG CCAATAACC	-26.8
GGTTCCTGG CCAAGAACC	-31.4
GGTTTTTGG CCAAAAACC	-29.5
GGTTATTGG CCAAAACC	-12.0
GGTTCCTGG CCAATAACC	-12.4
GGTTTTTGG CCAAGAAACC	-17.5

Roughly
50% less

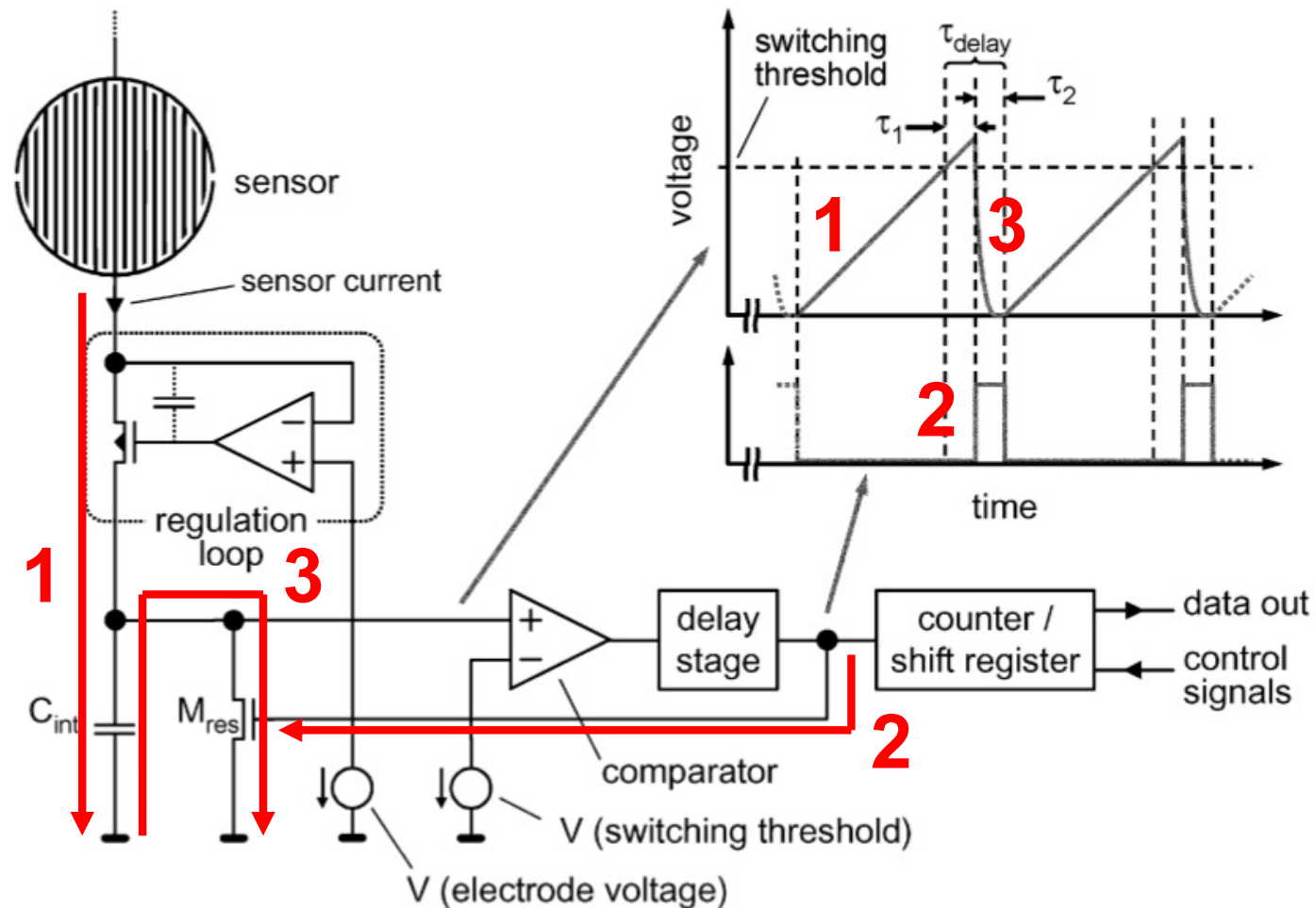
(c) S. Carrara

Match/Mismatch DNA Hybridization



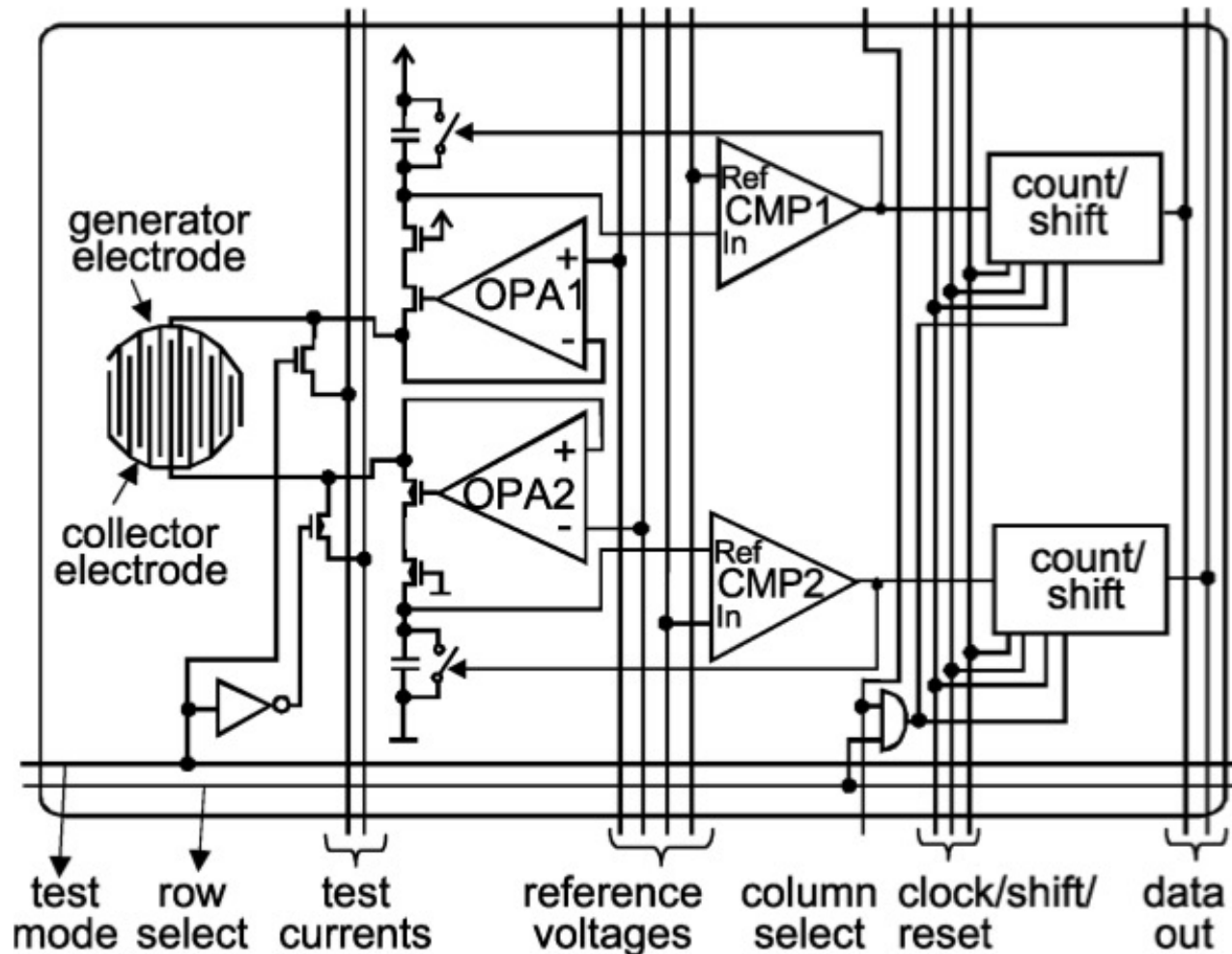
Successful detection of the matching sequences
but significant signal by non-matching too

Current CMOS Readout



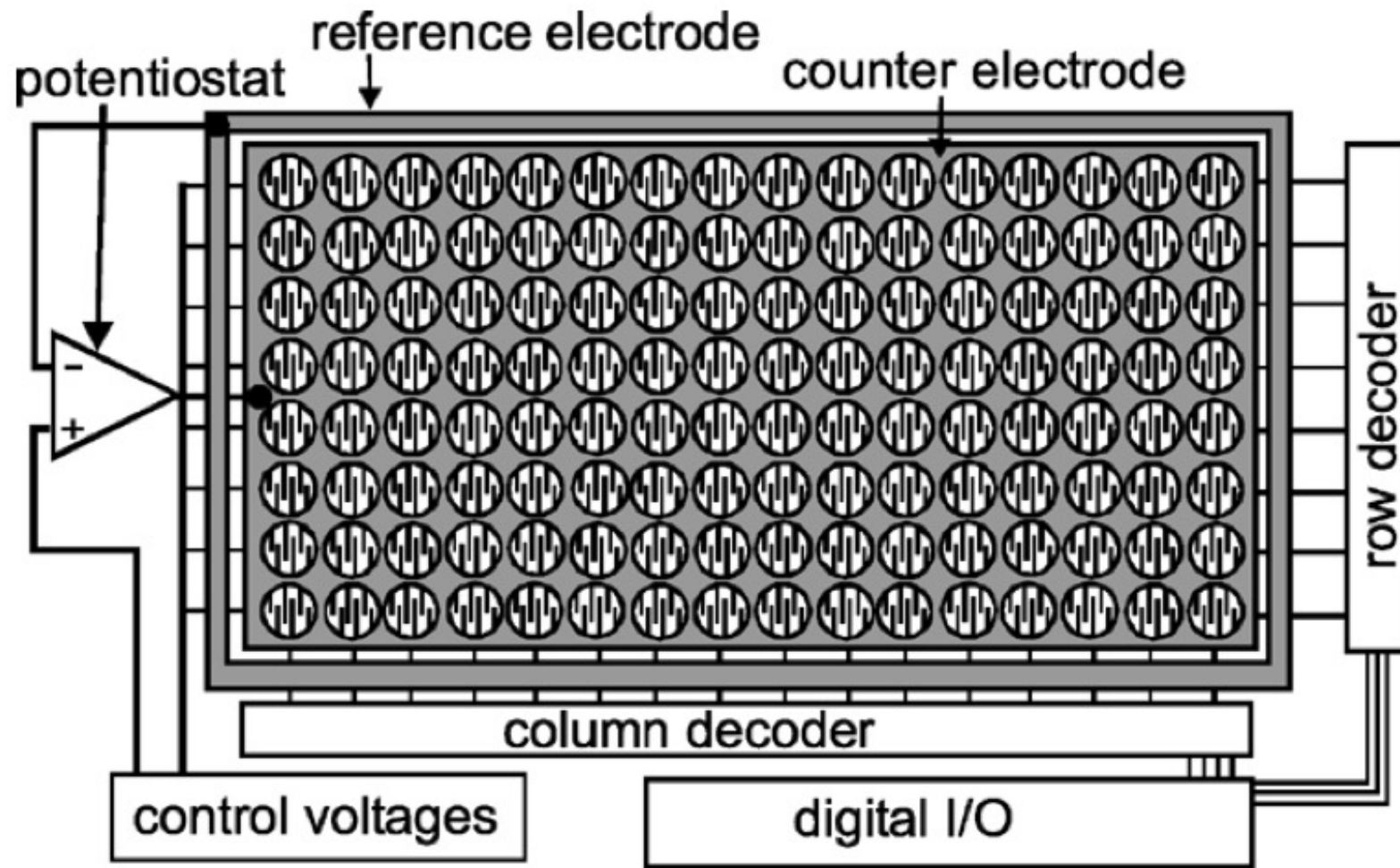
Frequency-To-Current Conversion (FTCC)
method is used here too

Current CMOS Readout



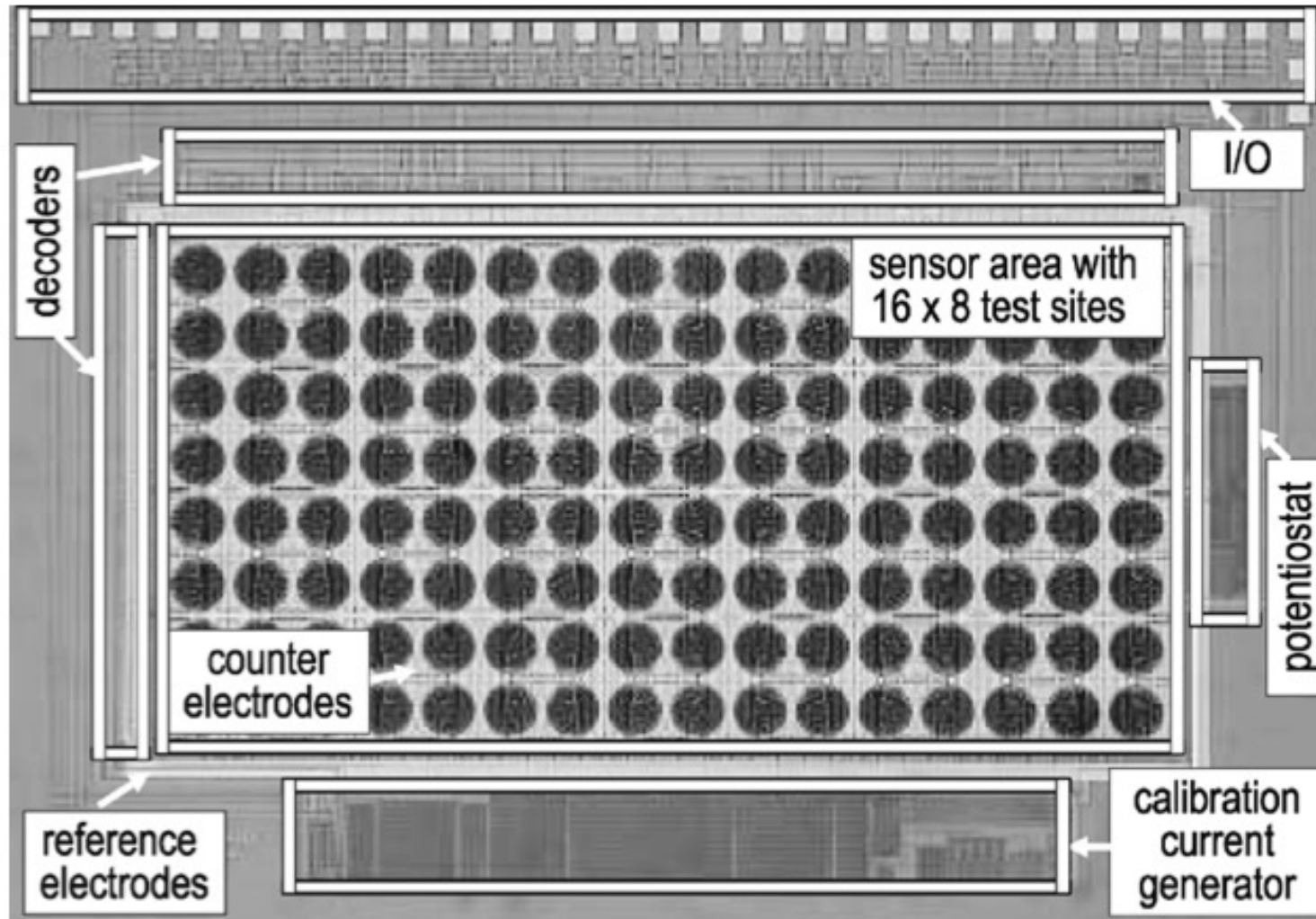
Sensor-site circuit architecture with digital output

Array Architecture



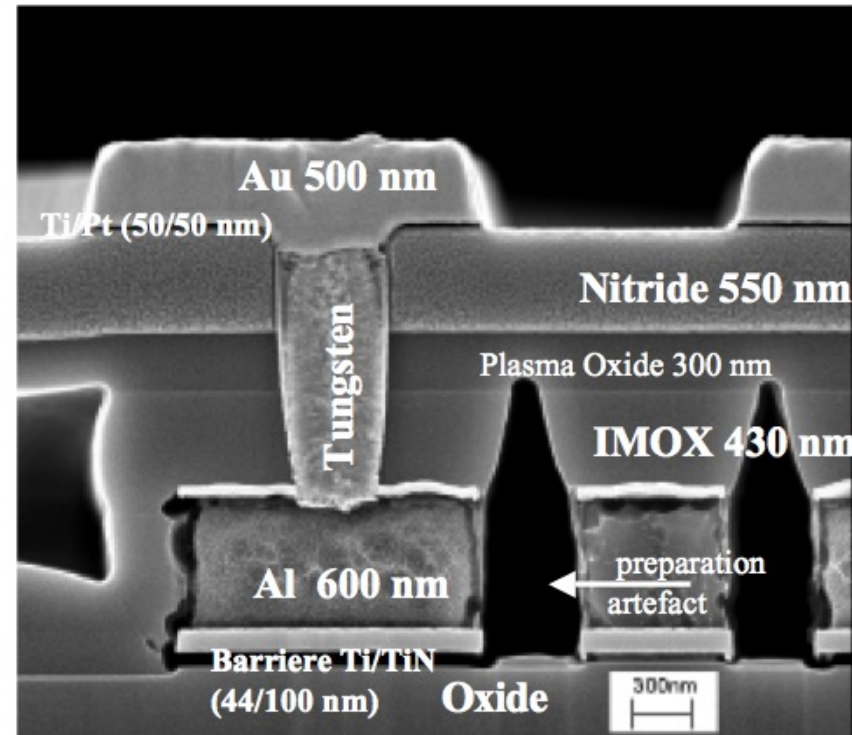
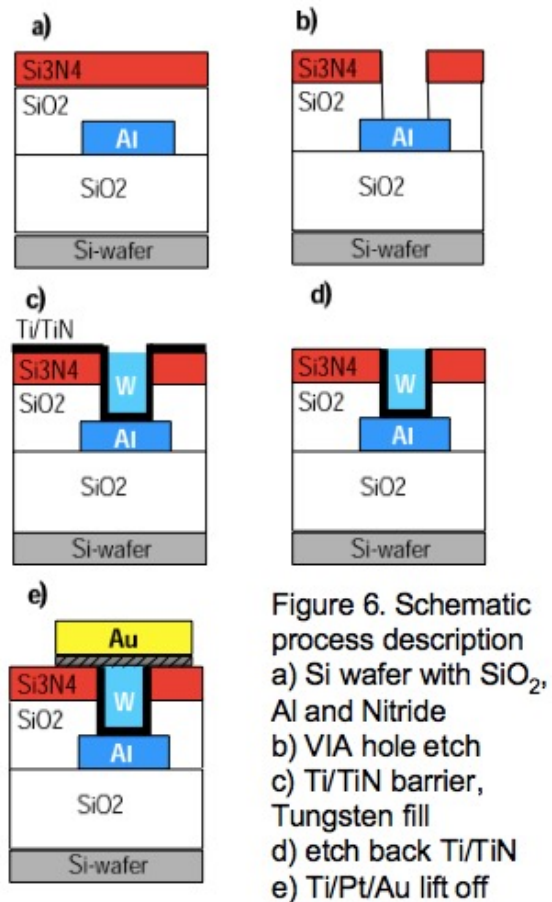
Whole Chip architecture including Row/Column decoders

The realized IC



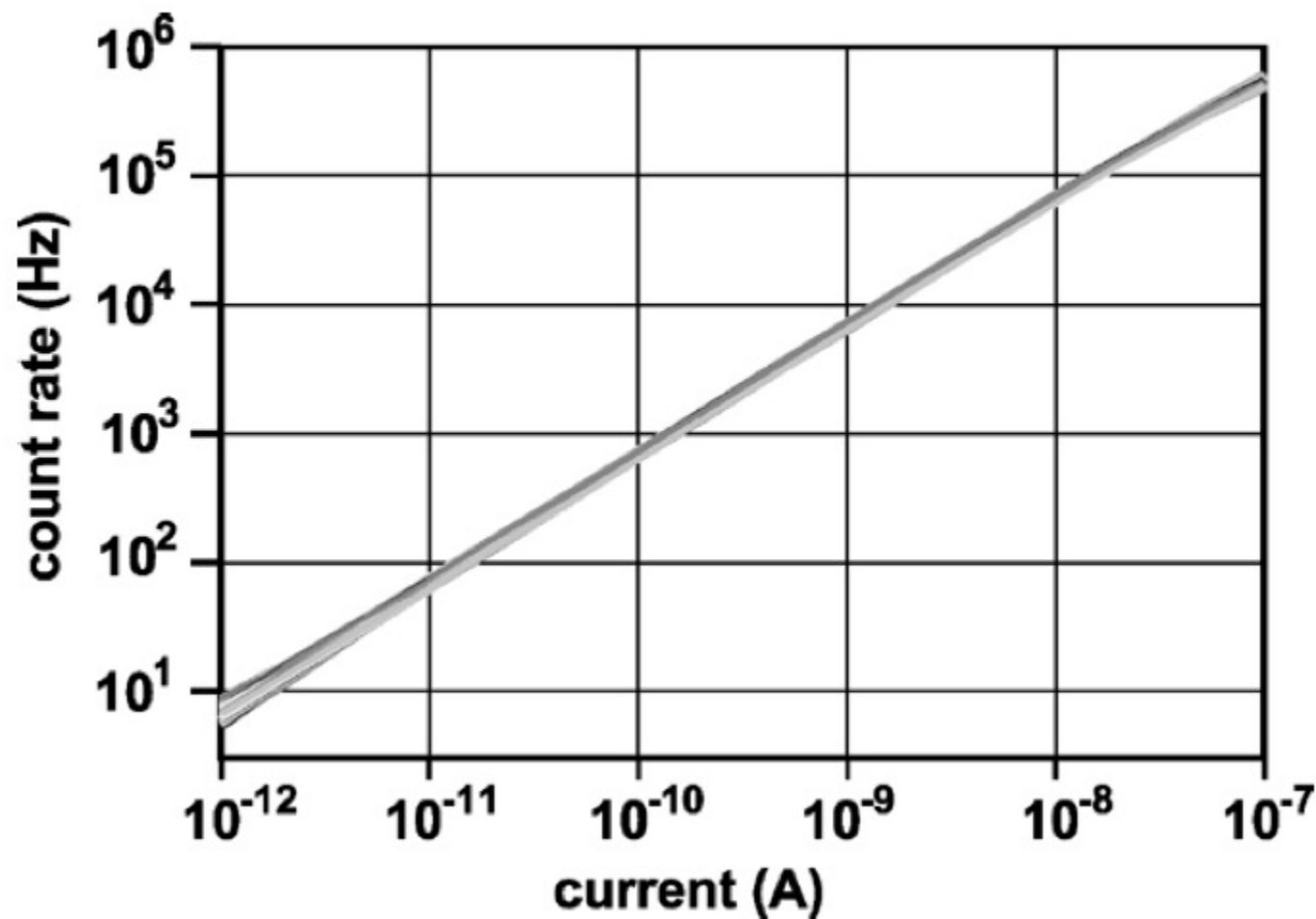
Chip microphotograph. Total dimensions are 6.4 x 4.5 mm².

Exposed IC-Die Electrodes



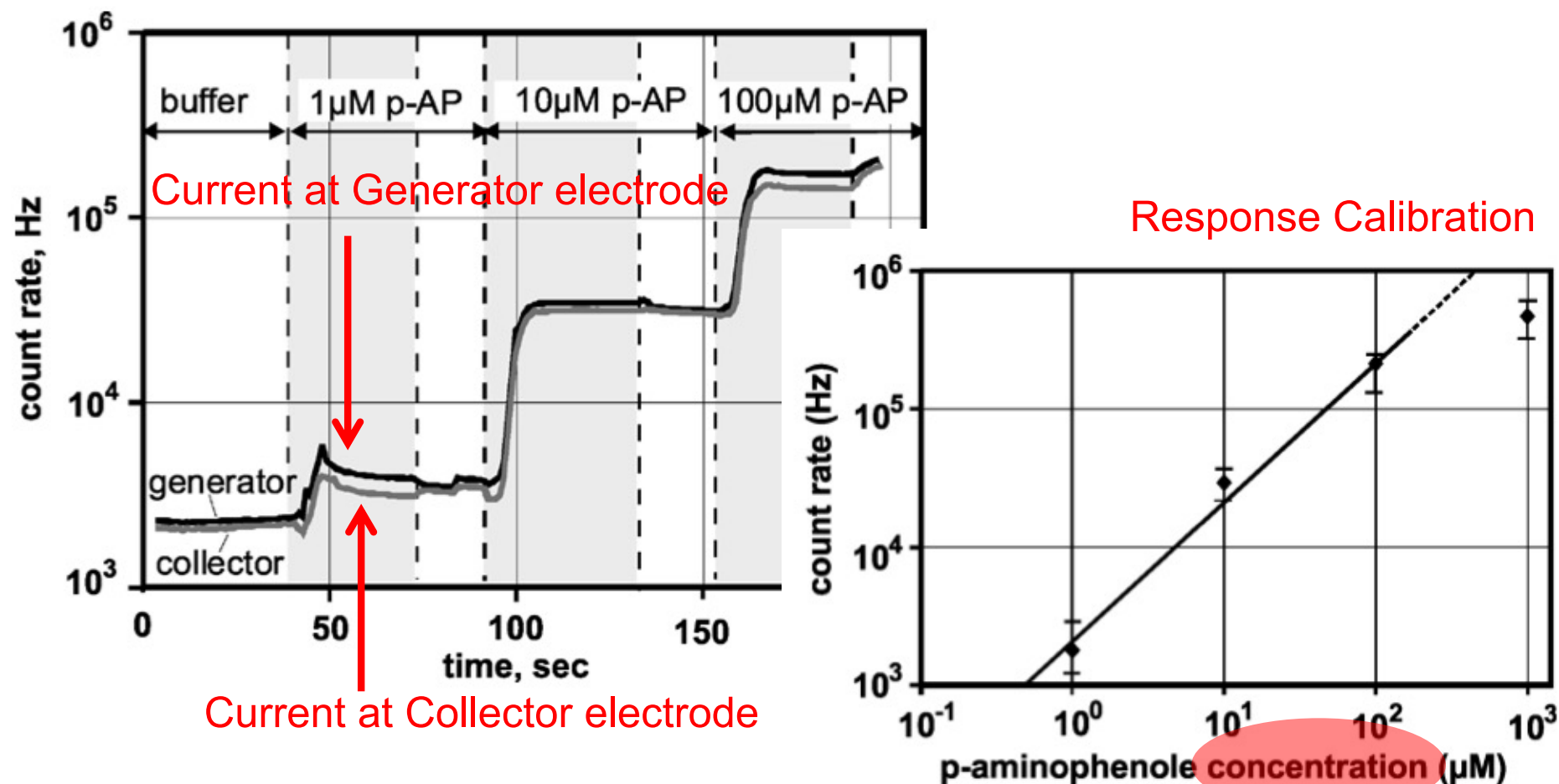
Electrochemical electrodes are created on top of the last CMOS metal Al layer

Frequency Readout



Measured count rate of all 128 DNA sensors

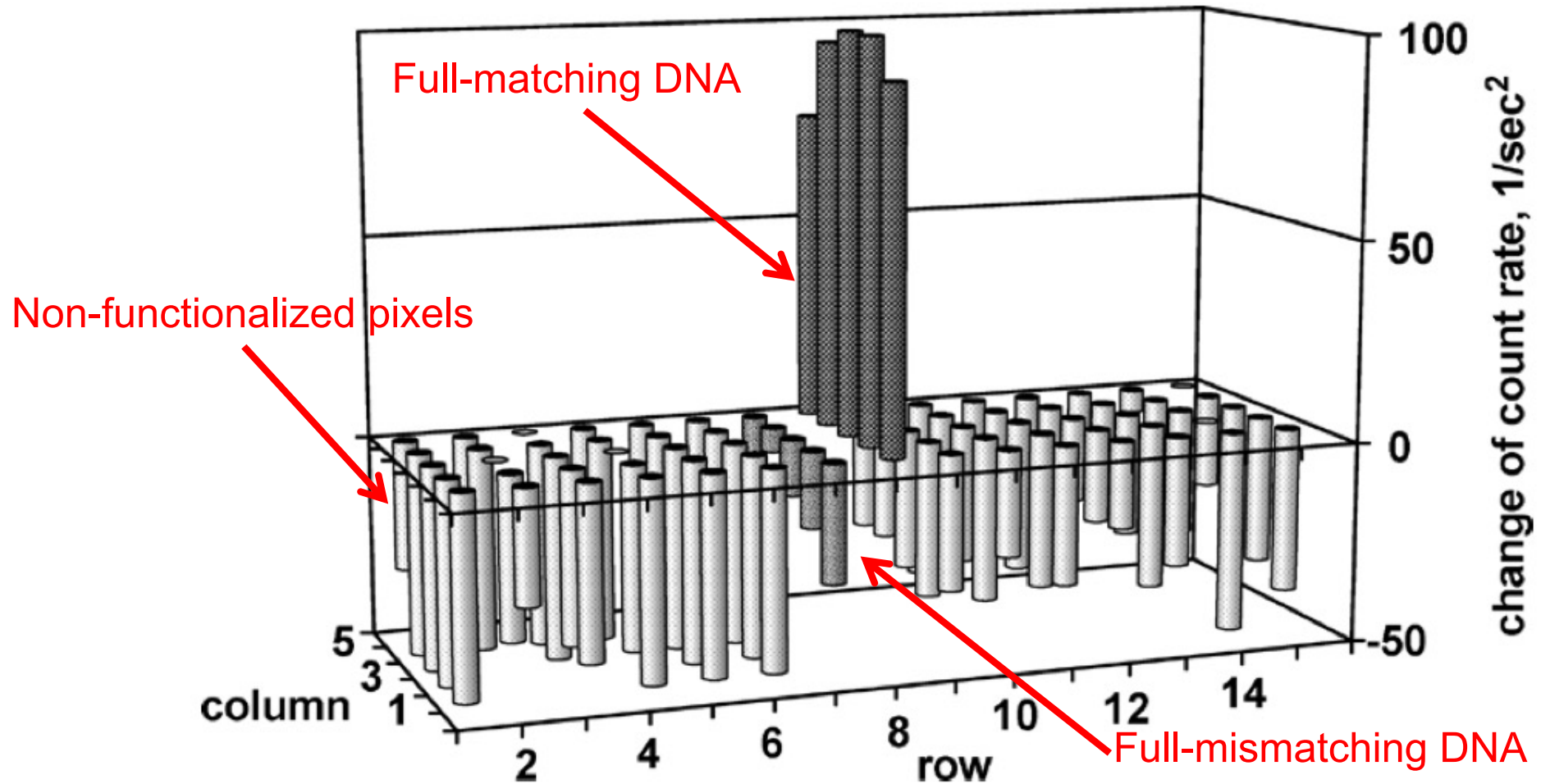
Test Measures



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.