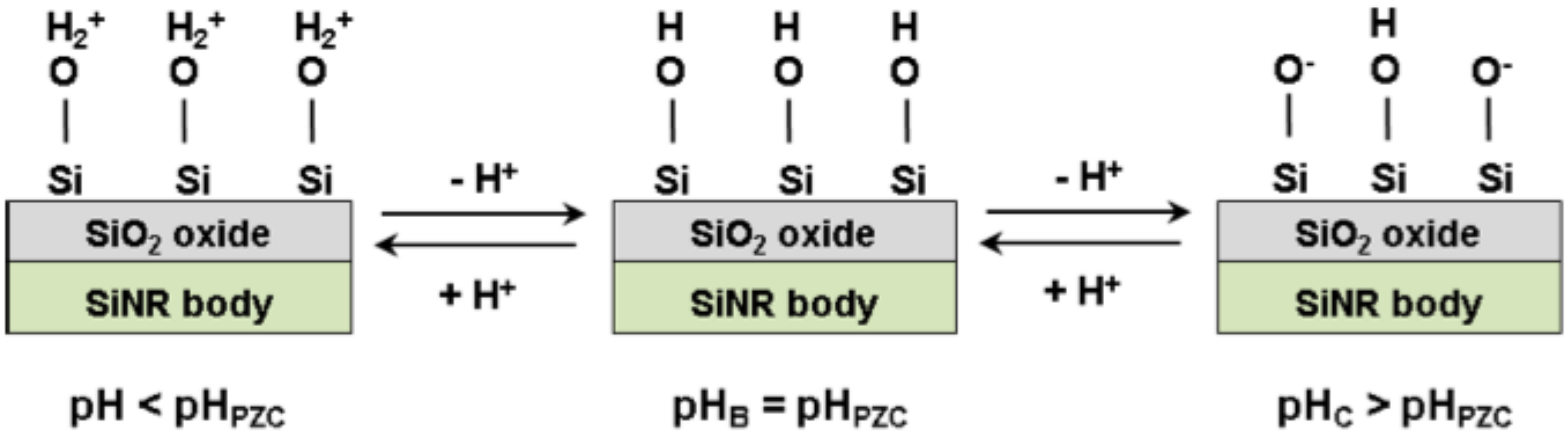
(7) OXIDES INTERFACES- FIELD-EFFECT-TRANSISTORS

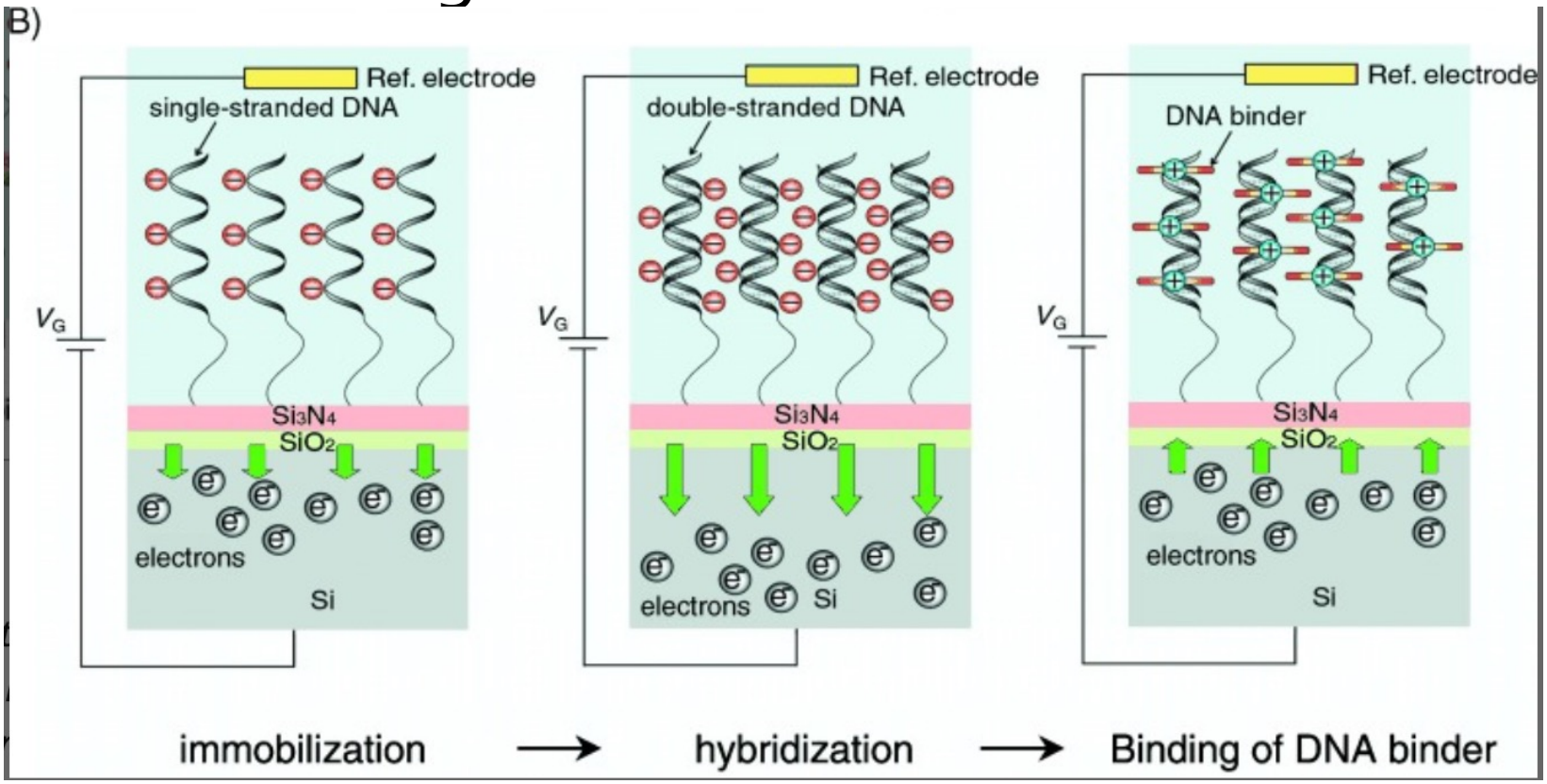
CHARGE SENSING VIA SEMICONDUCTOR DEVICES

Site-binding model



This model assumes the dielectric material constituting the gate oxide to be amphoteric, meaning that the surface hydroxyl groups can be neutral, protonized (thus positively charged) or deprotonized (thus negatively charged) depending on the pH of the solution. This idea was first applied to the electrolyte/insulator/silicon (EIS) structures, and in particular to devices featuring SiO_2 as gate oxide. In particular, the surface of SiO_2 becomes negatively charged when in contact with electrolyte solutions with pH values higher than its point of zero charge ($pH_{PZC} \approx 2$).

Effect of charged molecules

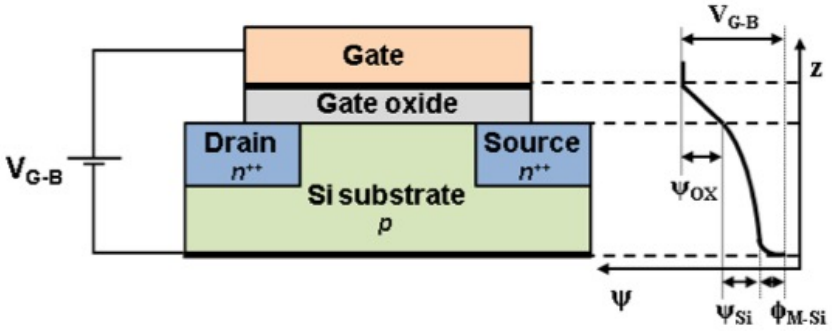


DNA molecules have negative charges in aqueous solution, while DNA binders have positive charges and bind specifically to double-stranded DNA.

The charged molecules on the gate surface interact electrostatically with electrons in the Silicon substrate through the thin gate insulator.

METAL-OXIDE-SEMICONDUCTOR STRUCTURE

MOS structure



The applied bias between the gate and the substrate (V_{G-B}) can be divided into three different components:

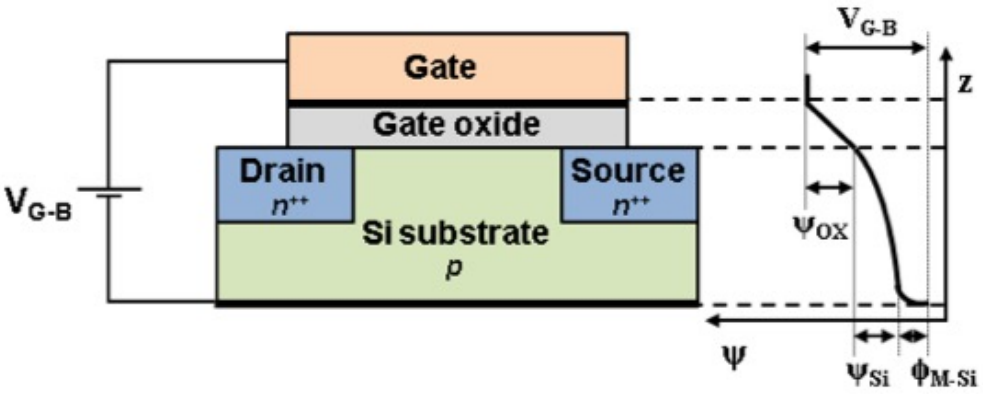
- A linear potential drop that occurs across the gate oxide (ψ_{OX}).
- An exponential potential drop that occurs between the silicon/oxide interface and the bulk semiconductor (ψ_{Si}) and that can be described by combining the Poisson equation with the Boltzmann statistic.
- A potential drop that compensates for the different work functions of the metal and semiconductor (Φ_{M-Si}).

The three components of the potential drop can be related to the applied potential V_{G-B} as follows:

$$V_{G-B} = \phi_{M-Si} + \psi_{OX} + \psi_{Si}$$

Responsible for the modulation of the channel conductivity

MOS structure

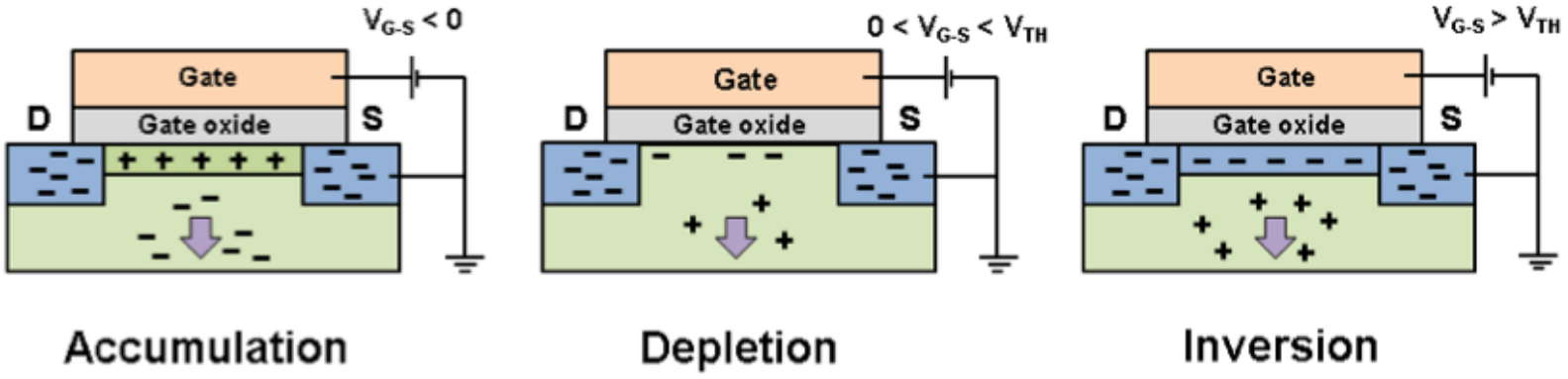


When the gate is made positive with respect to the source (source is short-circuited with the bulk), electrons (negatively charged) are attracted toward the silicon/gate oxide interface, while the holes (positively charged) are repelled from it.

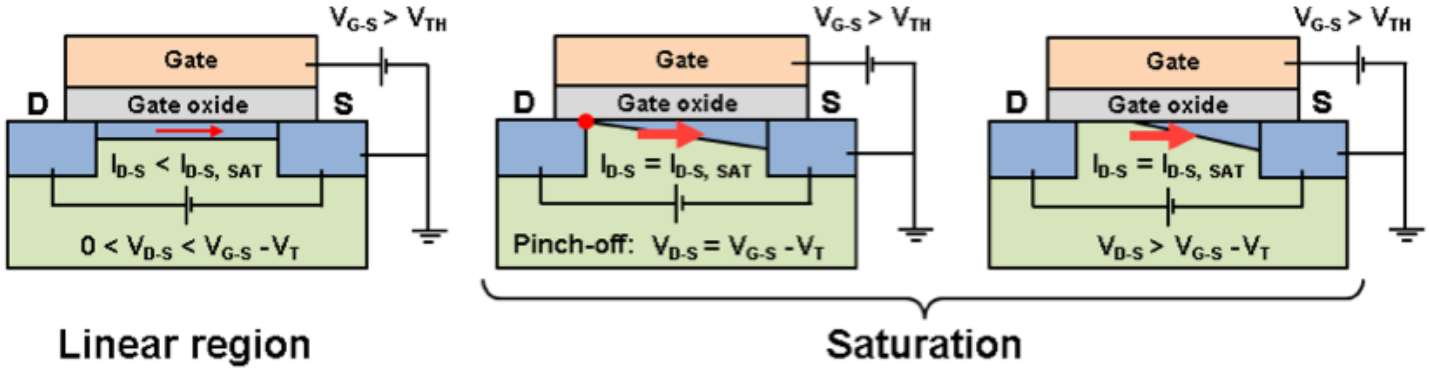
If the gate bias is further increased, more minority carriers (electrons) are attracted and eventually form a conducting layer, called channel, that allows for the passage of current, when a potential difference between the source and drain terminals is applied.

The key parameter that quantifies the magnitude of the gate voltage that needs to be applied in order to create the channel is the threshold voltage V_{TH}. The threshold voltage is defined as the value of the gate–source voltage ($V_{G\text{-}S}$) for which the conducting channel has just begun to connect the source and drain, allowing significant current to flow.

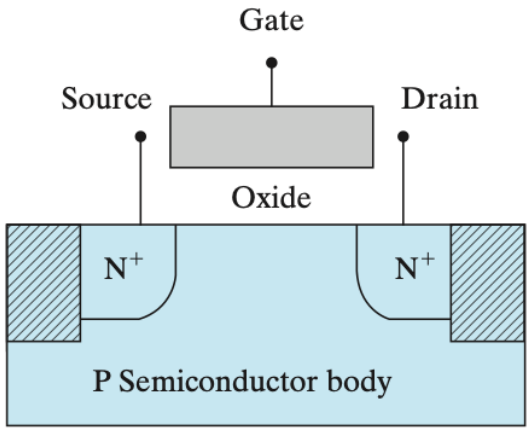
Polarization of MOSFET (n-type). V_{gs} and V_{ds}

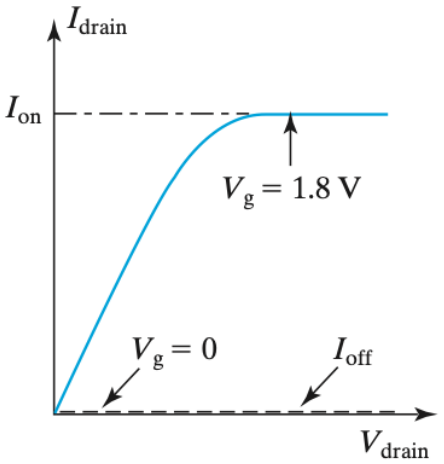


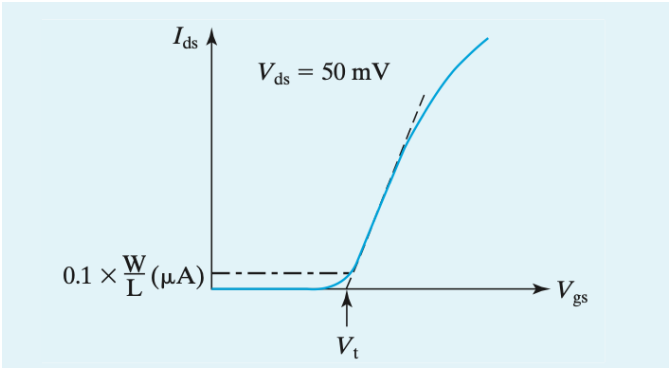
Provided that free carriers are present in the channel ($V_{GS} > V_{TH}$), a current can flow from the source to the drain terminal when a source-drain voltage $V_{D\text{-}S}$ is applied. Depending on the applied $V_{D\text{-}S}$, we can distinguish two working regions (linear and saturation):

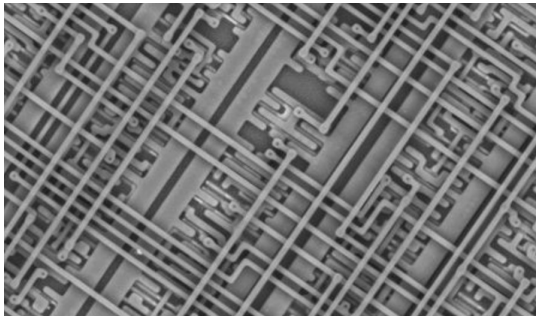


MOS-FET (Metal Oxide Semiconductor) Field-effect transistor



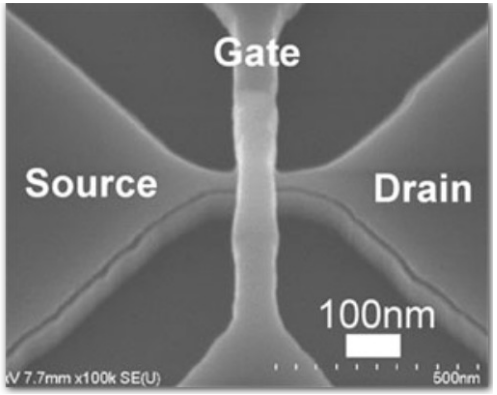


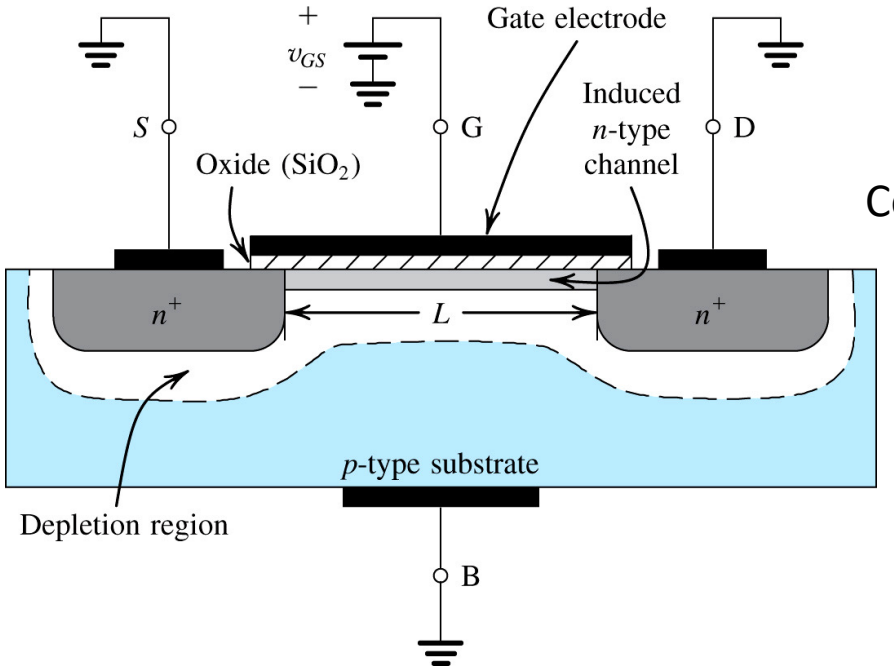




—

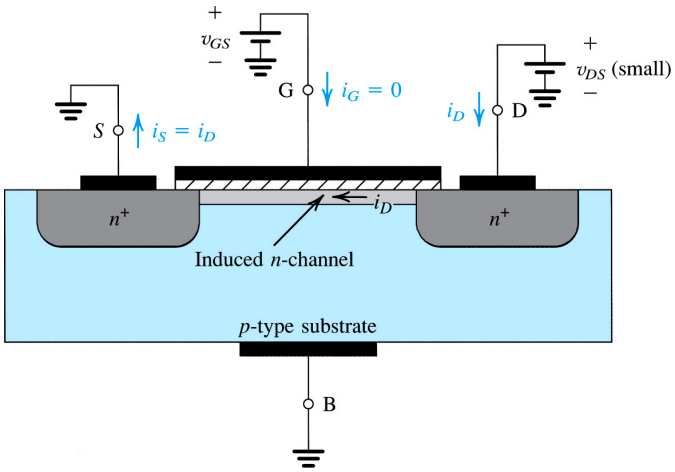
tens of microns



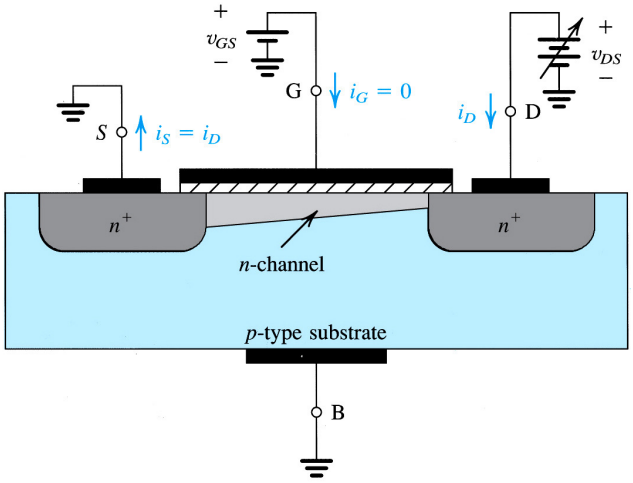


Conduction region (Vgs > Vth)

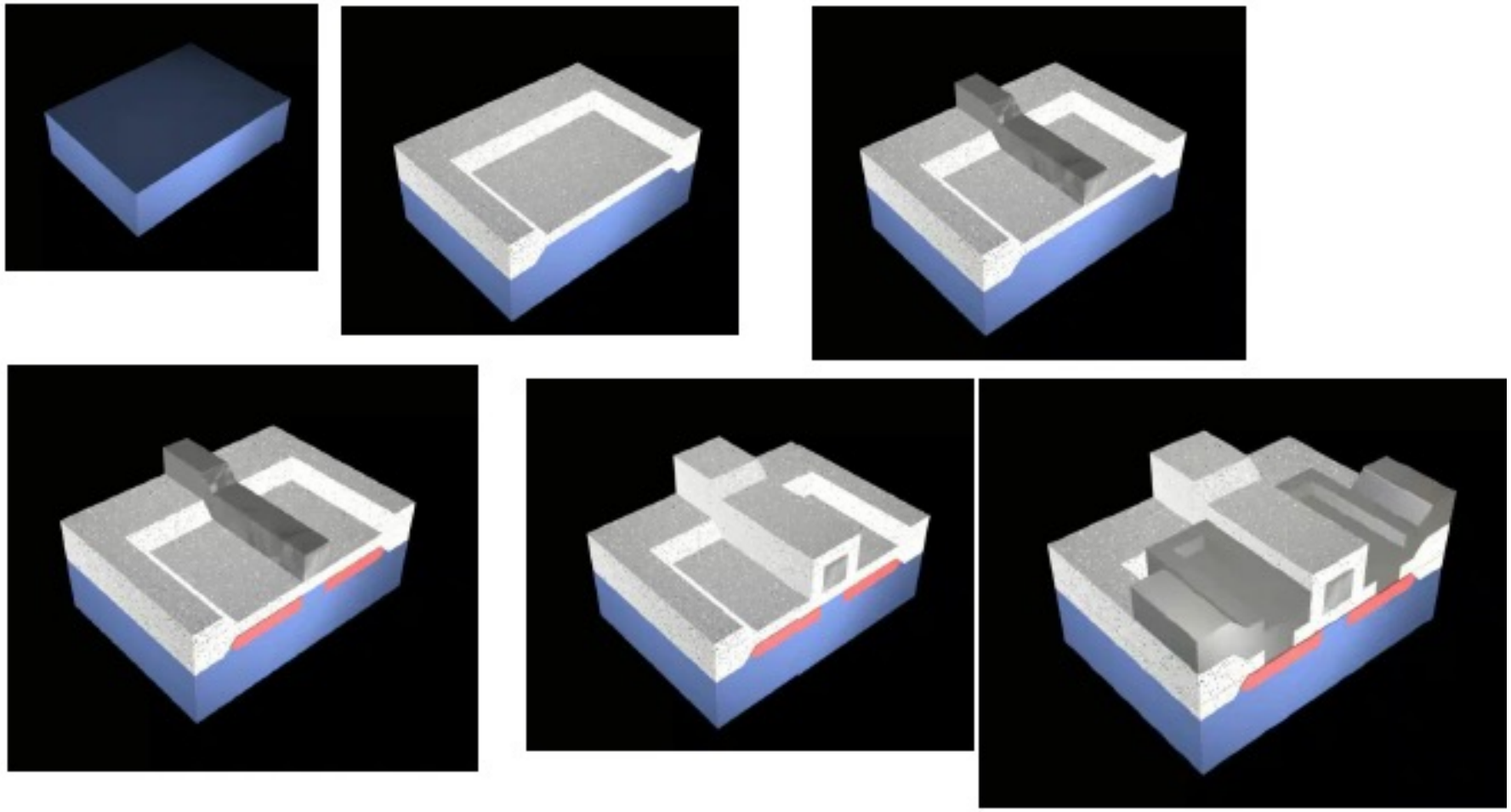
Linear region (conduction)



Saturation region (conduction)

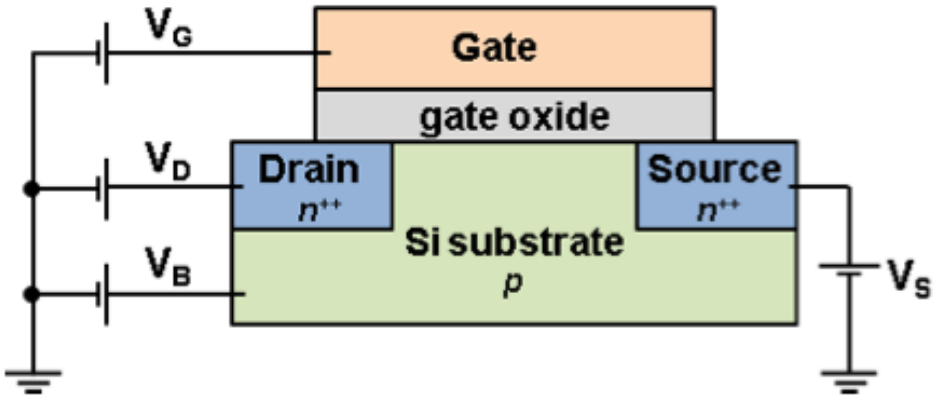


MOSFET transistor fabrication process

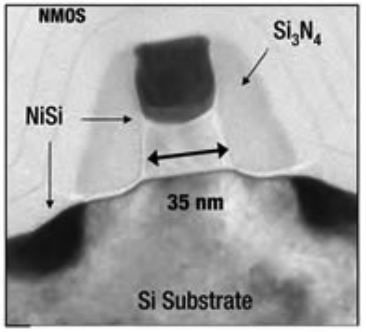


MOSFET

65 nm Transistor



Schematic representation of an *n*-type MOSFET



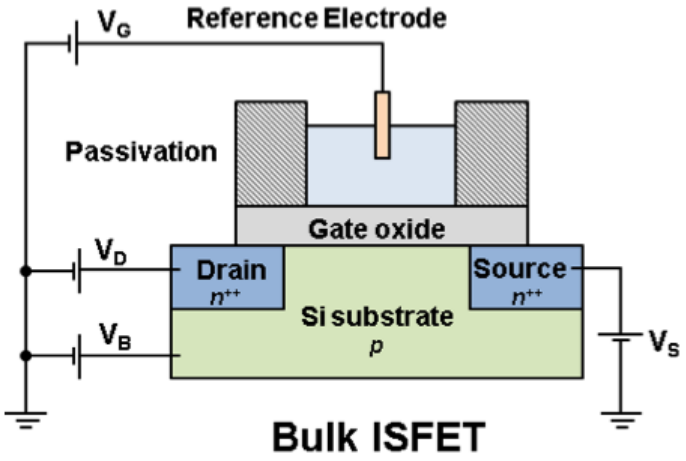
Transistor for 65 nm Process

Intel

The working principle of a MOSFET can be described by considering separately the vertical and horizontal components of the electric field that can be applied through the terminals. If we consider only the vertical component (gate and bulk terminals), we have the so called two-terminal MOS structure.

ION SENSITIVE FIELD EFFECT TRANSISTOR (ISFET) AND CHEMICAL FIELD EFFECT TRANSISTOR (CHEMFET)

Electrolyte-insulator-silicon structure (EISFET)



In the EISFET the gate metal is replaced by an aqueous solution contacted by a reference electrode. The gate oxide is, therefore, exposed to the liquid environment and hydrated. This way, the biochemical event of interest occurring on the gate oxide can directly modulate the channel conductivity.

As for the case of a MOSFET, the drain current ID-S is a unique function of the input voltage $V_{G\text{-}S}$, provided that $V_{D\text{-}S}$ and V_{TH} are constant. In the case of the ISFET, however, we need to consider an **extra component that takes into account the ion activity occurring in the electrolyte solution and the surface charge at the gate oxide surface**. The effect of this additional component can be described as a modification of $V_{G\text{-}S}$ or a modification of V_{TH}.

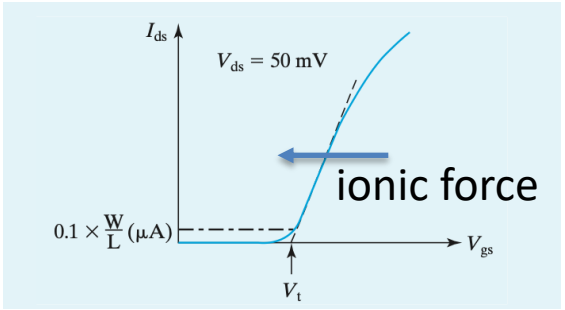
The Grahame equation

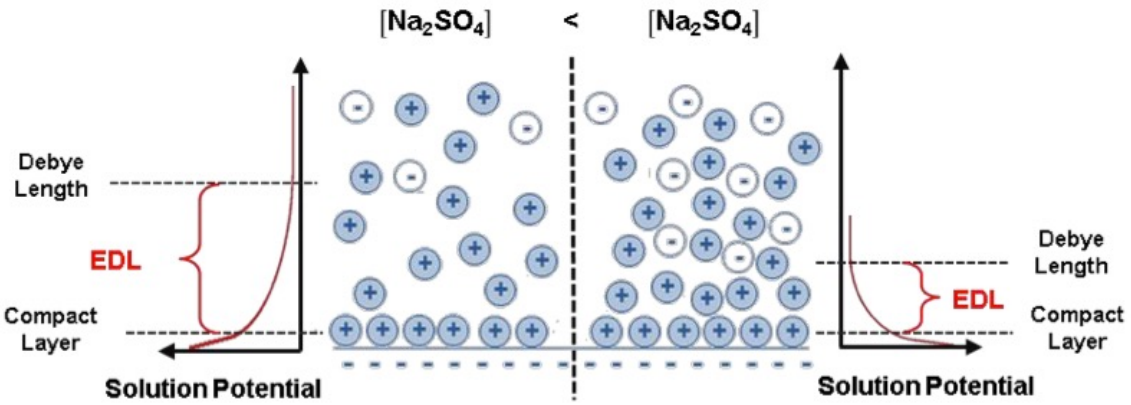
The relationship between the surface potential ψ_0 and the surface charge density σ_0 is given by the Grahame equation

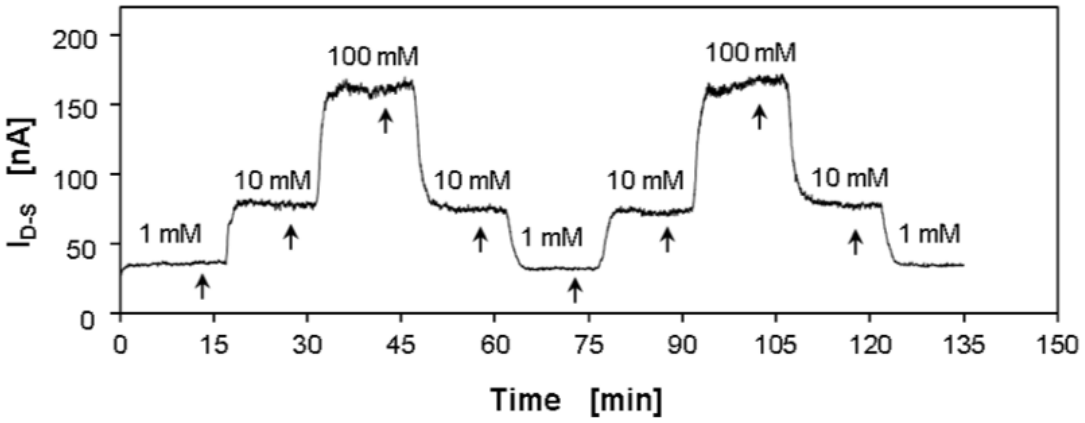
$$\sigma_0 = \sqrt{8\varepsilon_r\varepsilon_0\, k_B T\, c_0}\,\sinh\left(\frac{q\psi_0}{2\, k_B T}\right)$$

where k_B denotes the Boltzmann constant, T the absolute temperature, q the elementary charge, ε_0 the permittivity of free space, ε_r the dielectric constant of the aqueous solution, and c_0 the ionic strength of the solution.

Impact of ionic strength (concentration of ions in solution)

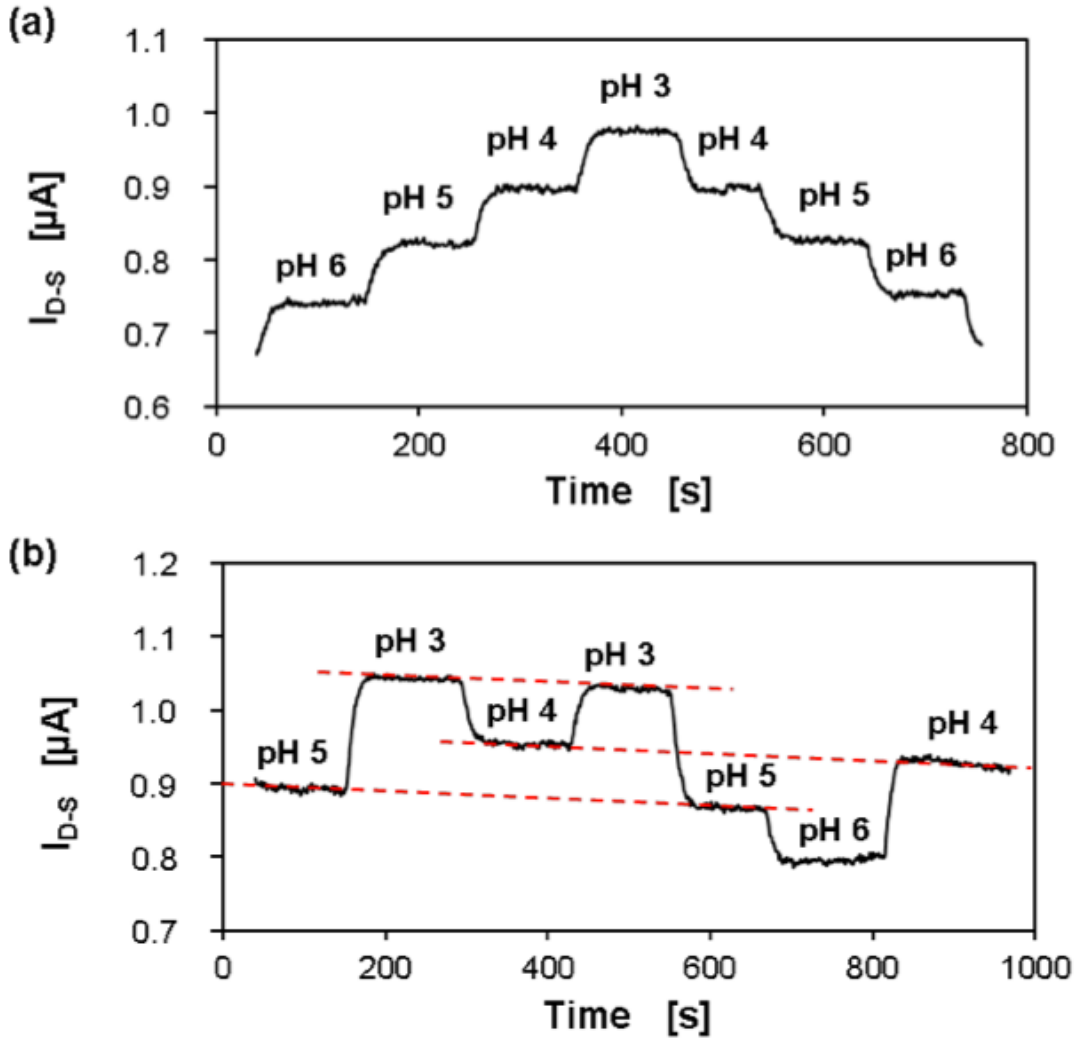






Higher ionic forces results in a larger concentration of positive ions in proximity of the interface, which translates in a higher number of negative carriers in the silicon channel, hence higher conductivity of the transistor

pH sensing



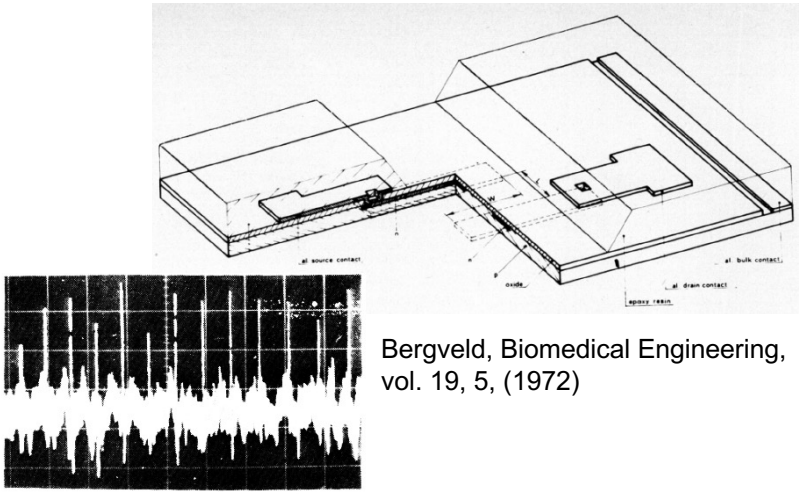
$V_{FG-S} - V_{TH} = 0.2$ V,
$V_{D-S} = 1.5$ V
The device features a width of 50 nm and a length of 2375 nm.
The flow-rate is 40 µl/min.

The protons in solution reduce the negative charge of the SIO_2 surface, thus attracting negative carriers in the Silicon channel by field effect.

ISFET-BASED ANALYTICAL SYSTEMS

Traditional applications for pH sensing semiconductor-based microdevices

Extracellular ion pulses



Bergveld, Biomedical Engineering, vol. 19, 5, (1972)

Physiological parameters

i-STAT Abbott

pH sensor

Wisc.edu

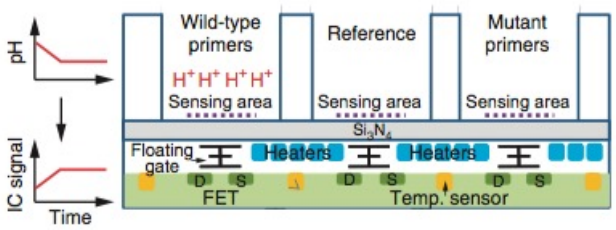
Temperature sensor

Inherent scalability

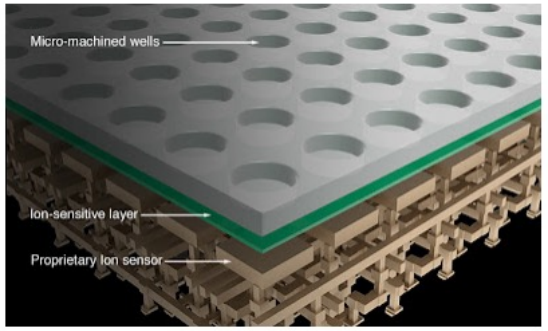
- Signal does not scale with the device size (W/L) (*but SNR still does*)
- Feature size scales down and complexity scales up at ever decreasing costs (IC integration)

⇨ **Ideal sensor for massively parallel system**

Semiconductor-based analytical systems

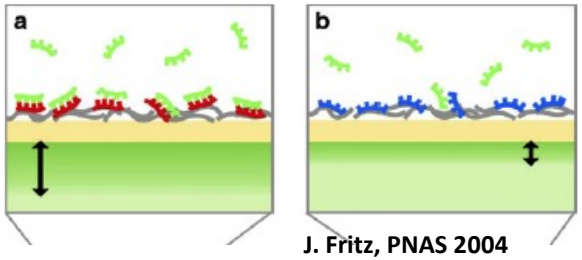


Quantitative PCR
DNA electronics



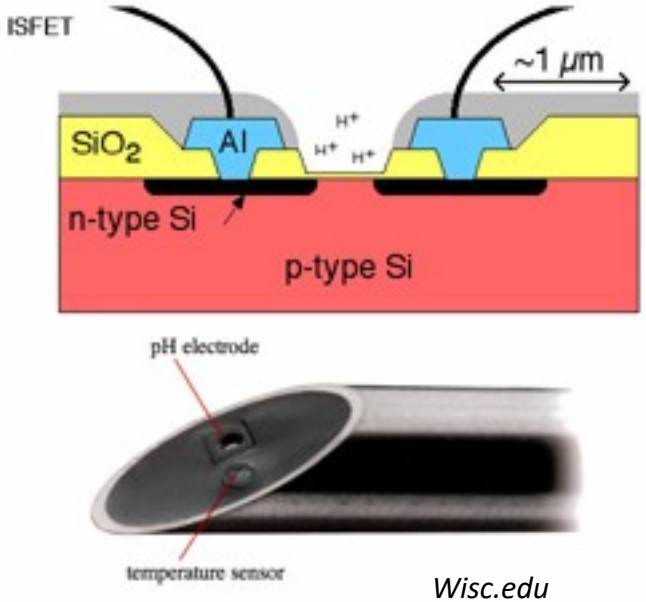
High-throughput sequencing
Ion Torrent

Blood analysis, physiological parameters
i-STAT Abbott

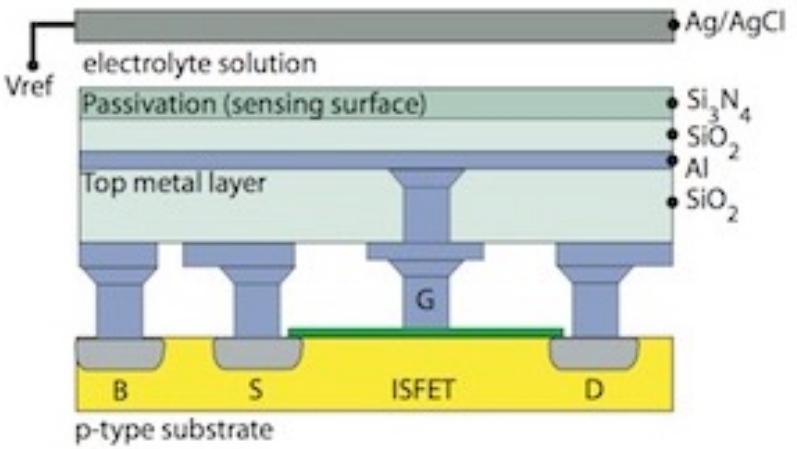


Molecular affinity sensor
No existing commercial products

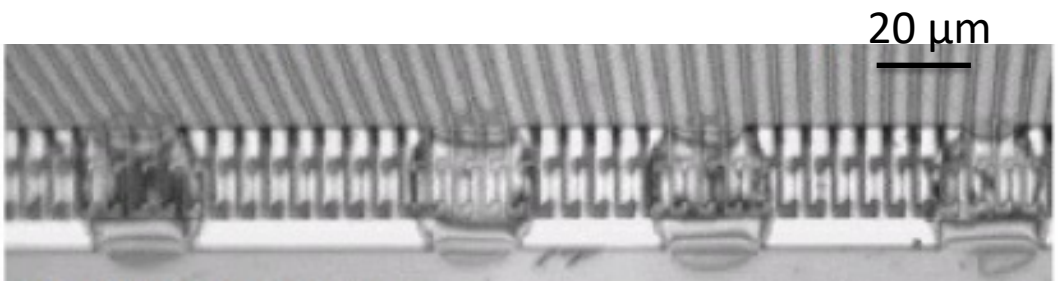
ISFETS structures



Wisc.edu

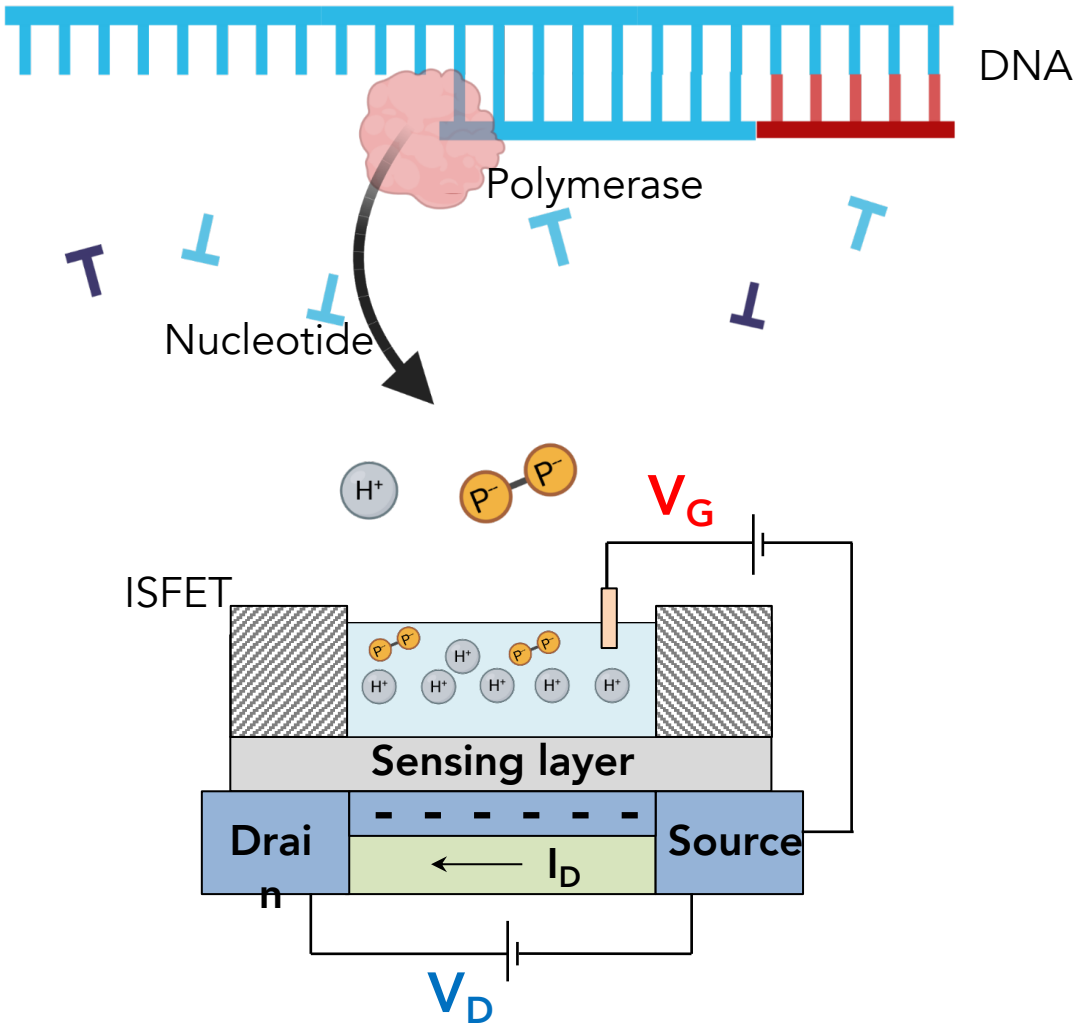


Imperial college UK

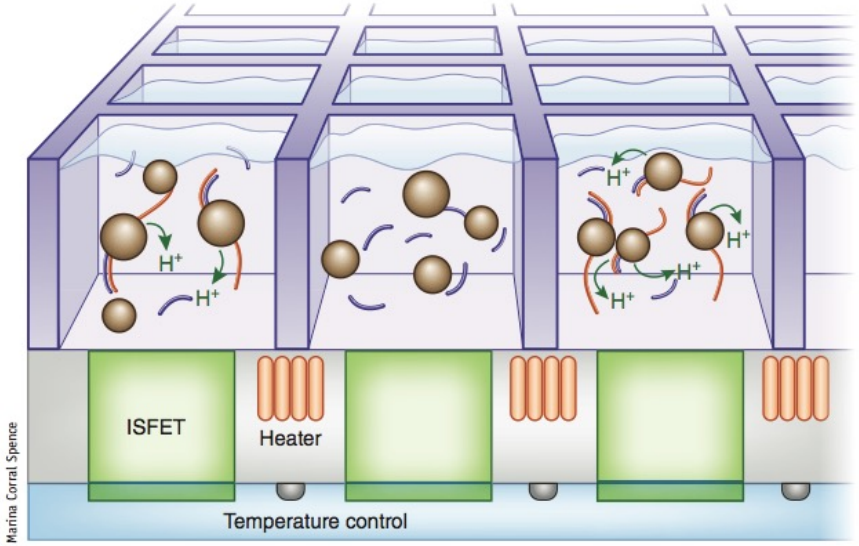


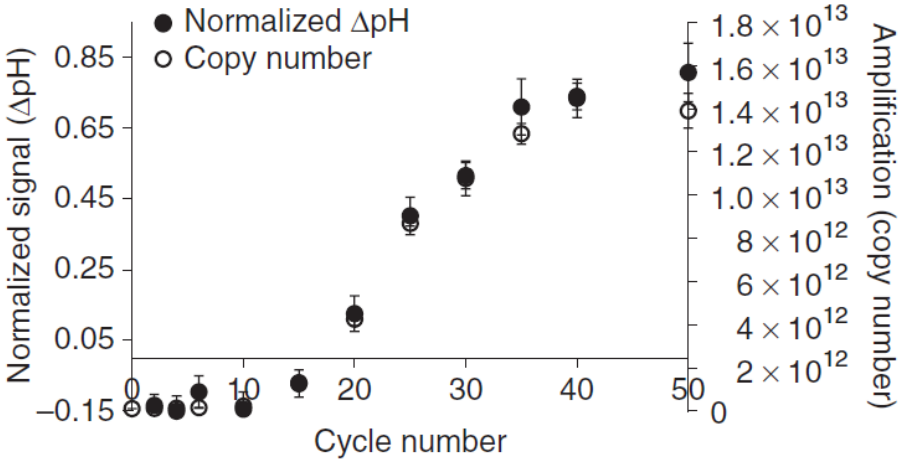
Quantitative readout of Nucleic acid amplification

Chemical by-products of nucleic acids elongation



Quantitative qPCR

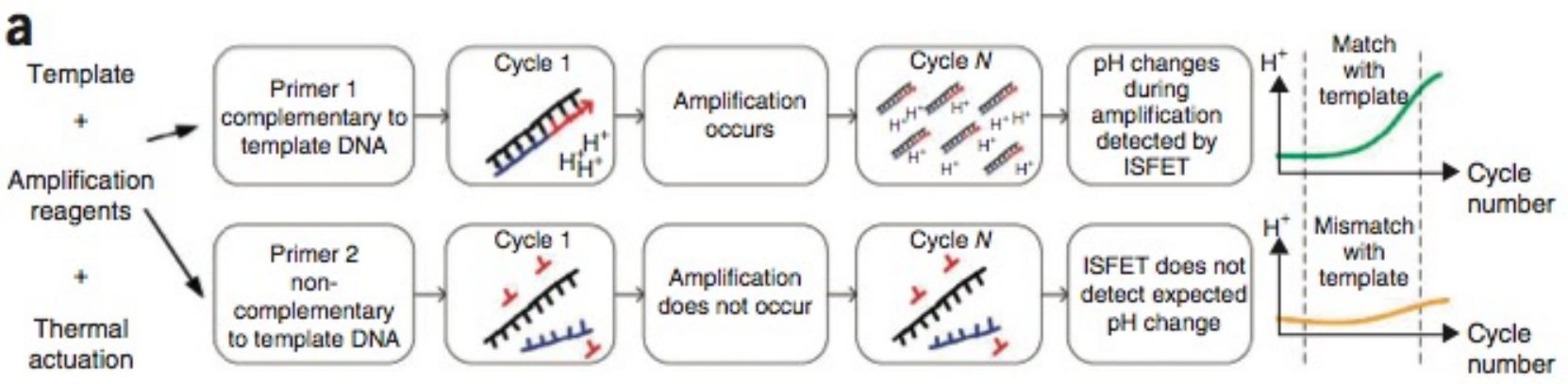


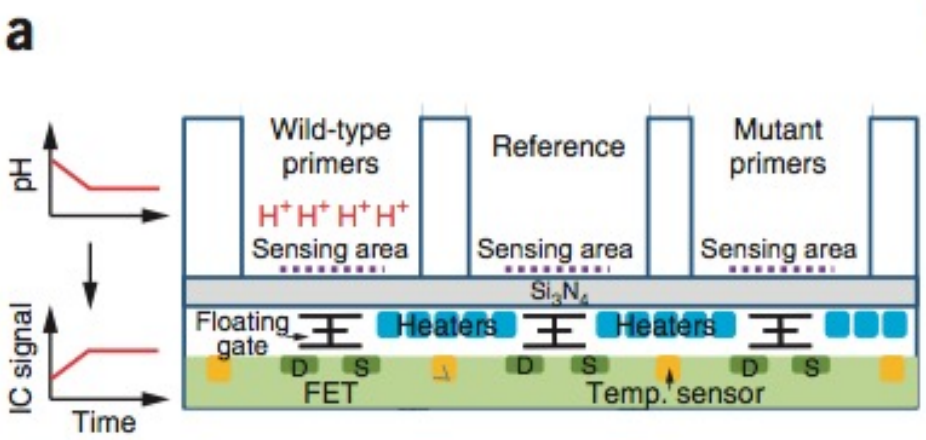


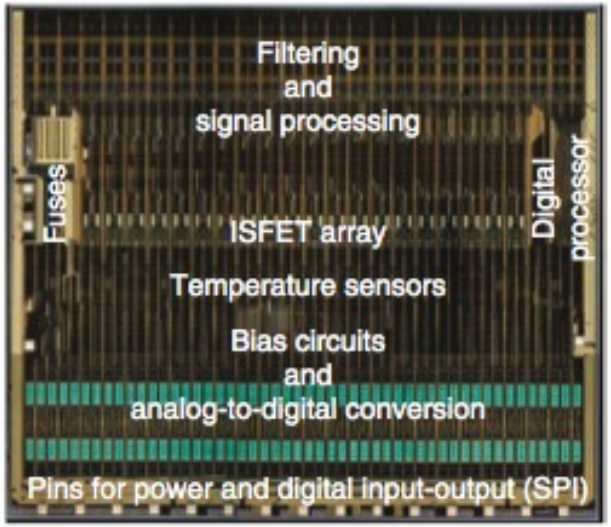
- Same sensitivity than light-based system (10 copies)
- Demonstrated on chambers with low parallelism
- Large transistors (lower noise)

Toumazou *et al.* Nature Methods 10, 641–646, 2013.
Guiducci, *et. al*, Nature Methods, News and Views, 2013

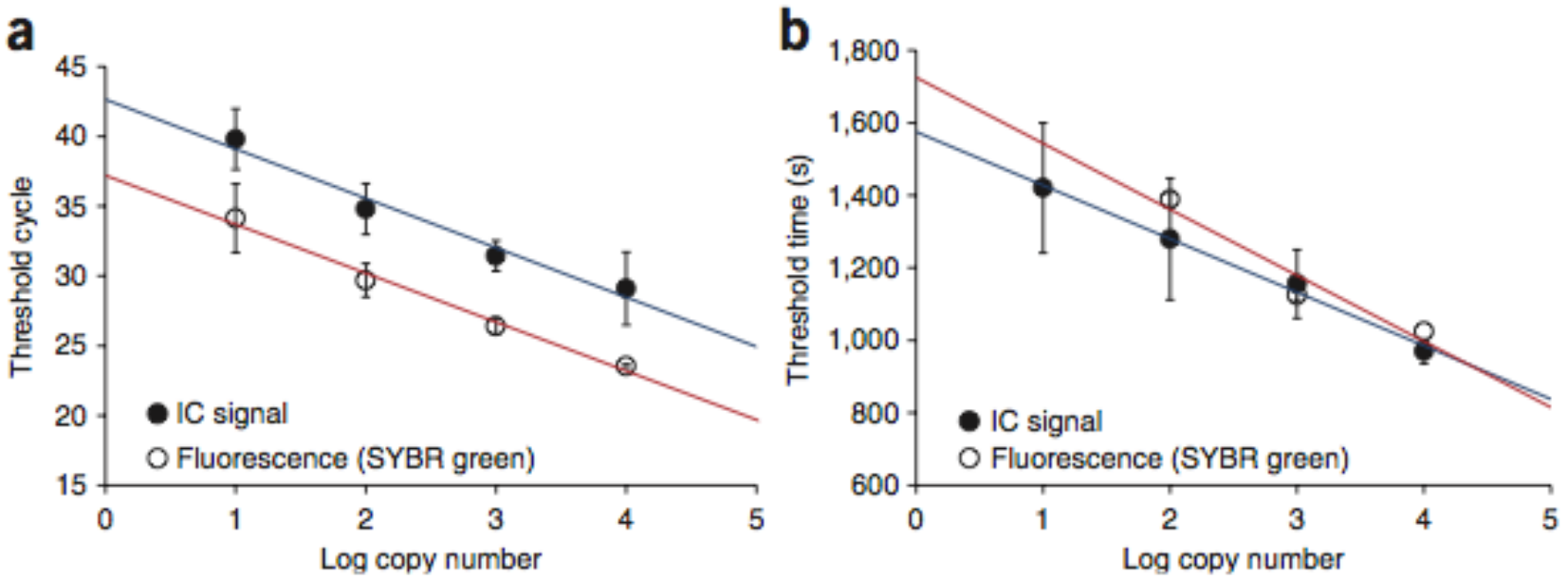
Quantitative PCR







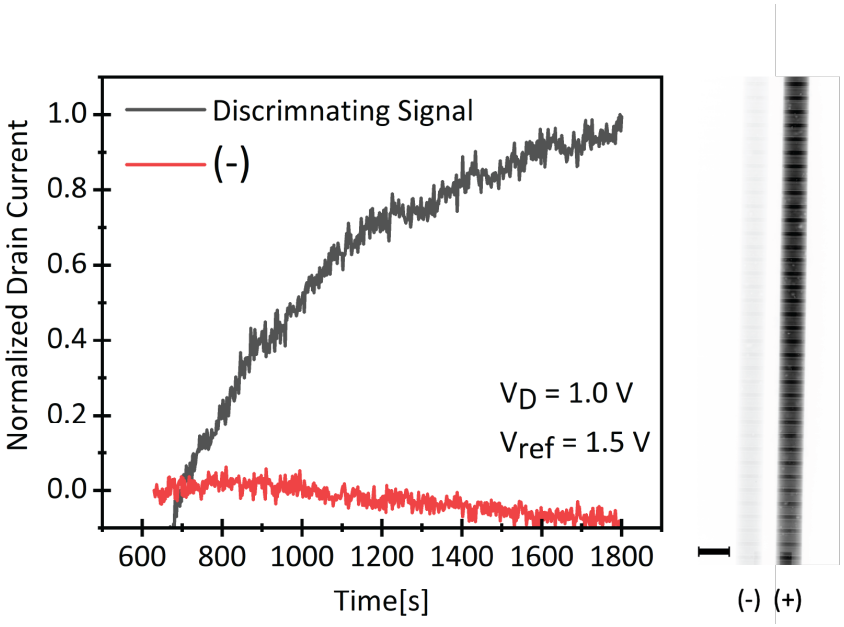
Nat methods July 2013

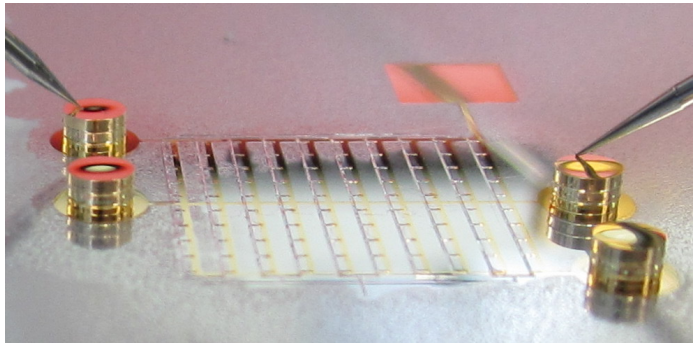


Evaluation of integrated circuit sensitivity.
(a) Standard curves of GH1 gene generated from the on-chip pH-PCR and the conventional fluorescence-based SYBR green PCR. Mean ± s.d. of the threshold cycle (Ct) for the on-chip pH-PCR (two independent experiments, total of 6–8 chips per dilution) and SYBR green–based, tube-based PCR were plotted against log of copy number of genomic DNA.
(b) Standard curves of *NAT2* gene generated from the on-chip pH-LAMP and the fluorescence-based SYBR green q-LAMP method. Mean ± s.d. of the threshold time for the on-chip pH-LAMP (5 independent experiments) and the fluorescence-based q-LAMP (2 independent experiments, six replicates per dilution) were plotted against log of copy number of genomic DNA.

Nat methods | VOL.10 NO.7 | JULY 2013 |

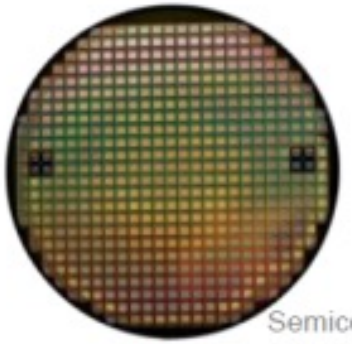
DNA amplification in nanoliter chambers





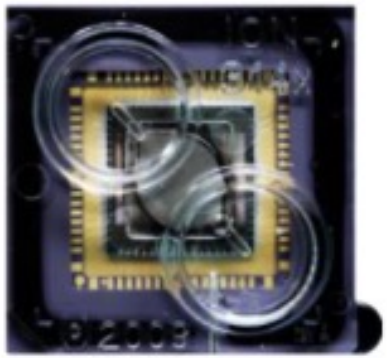
High-throughput sequencing and quantitative PCR

Sequencing. Leveraging upscaling of transistors arrays



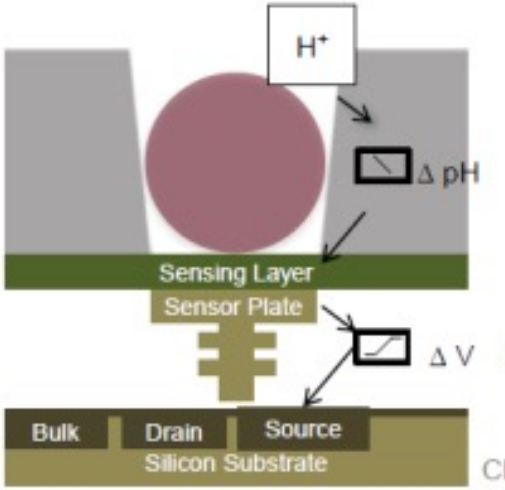
Wafer

Semiconductor Manufacturing



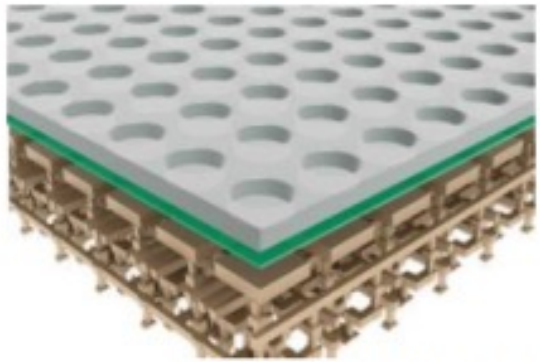
Chip

Semiconductor Packaging



Single Sensor

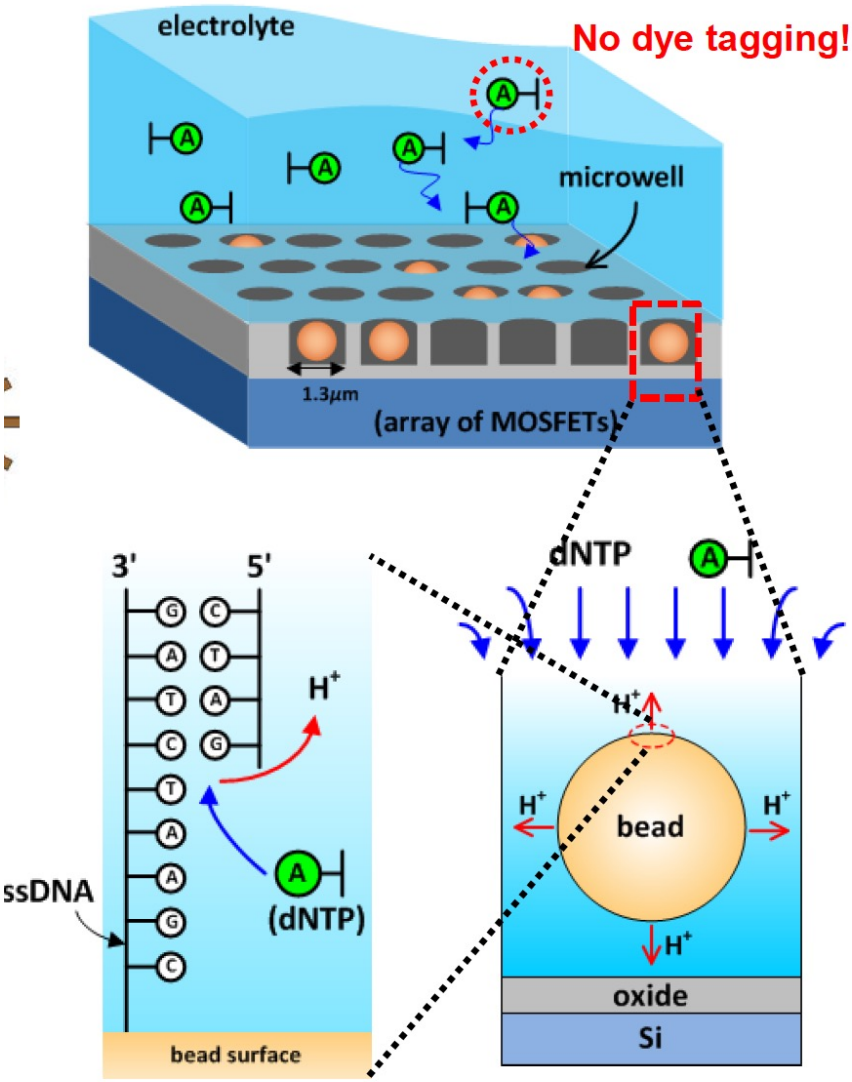
Chemical to Digital Sequence

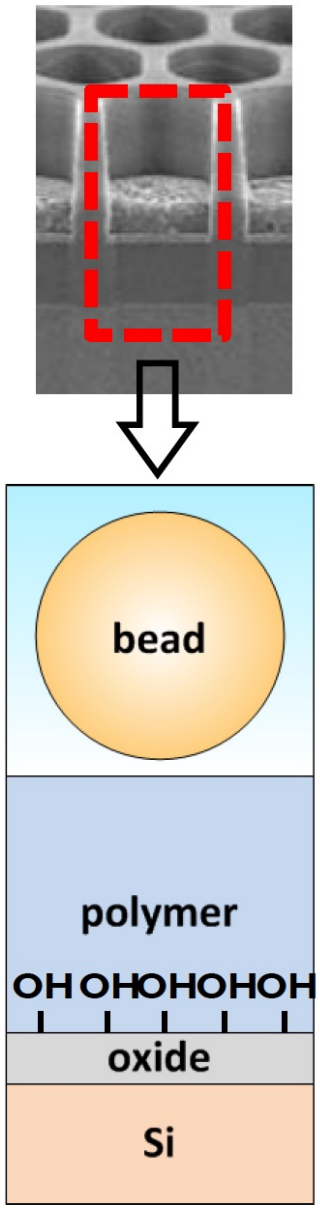


Millions of Sensors

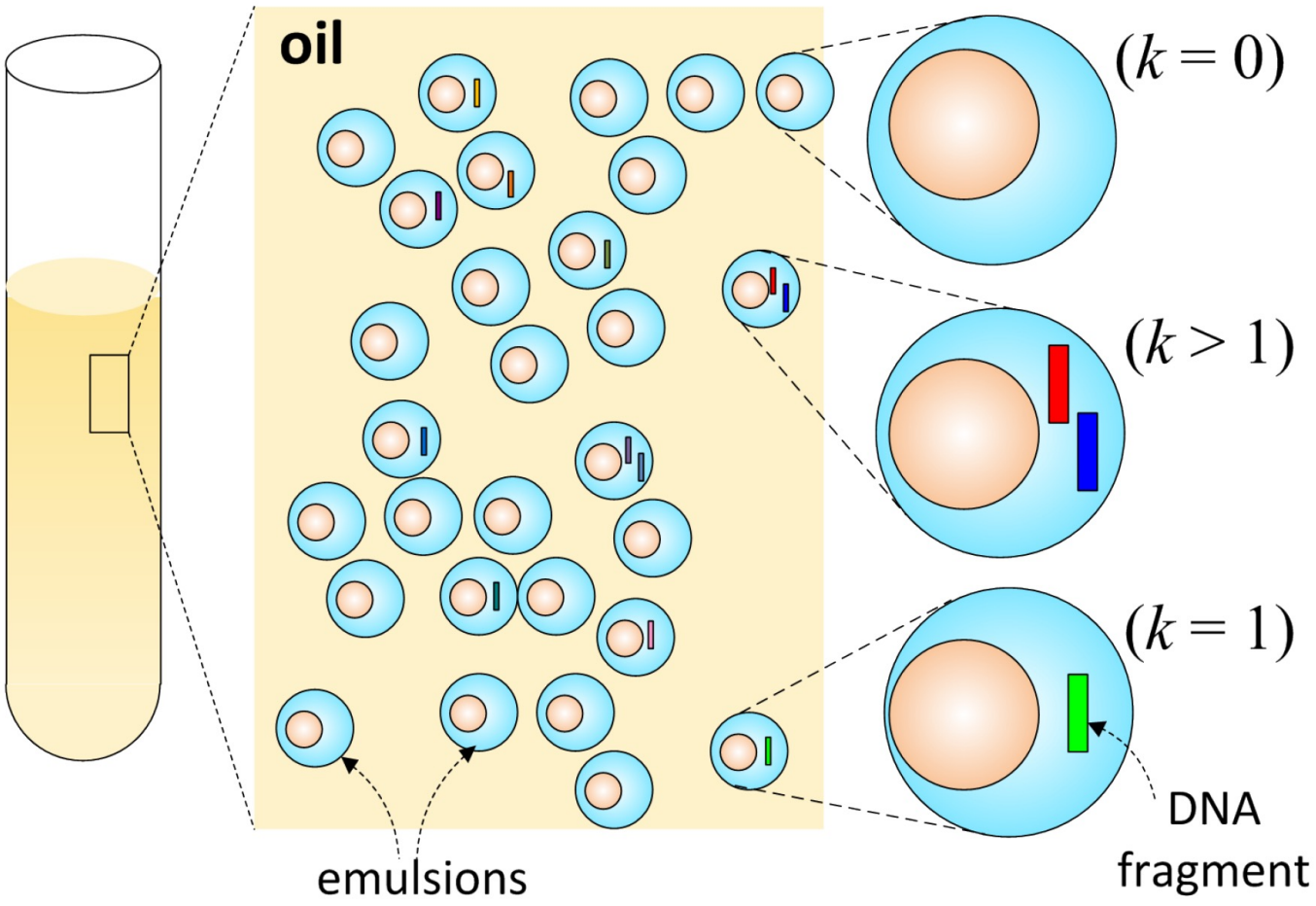
Semiconductor Design

Reference: Rothberg et al., Nature 2011





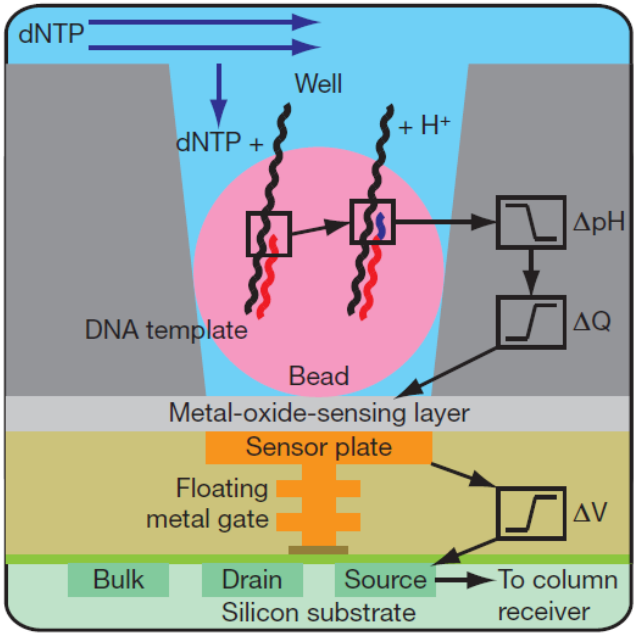
Emulsion PCR

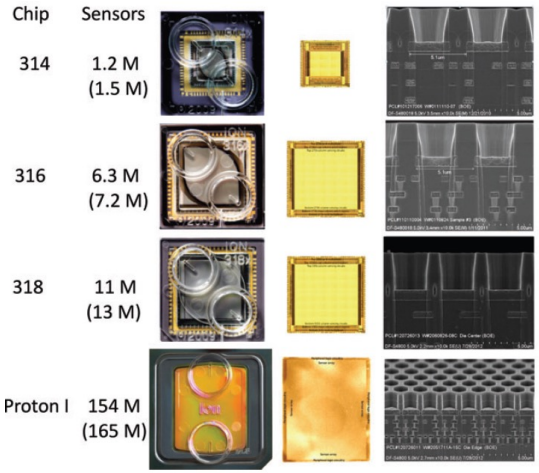


Alam, Principles of Nanobiosensors, 2013

Non-optical NGS

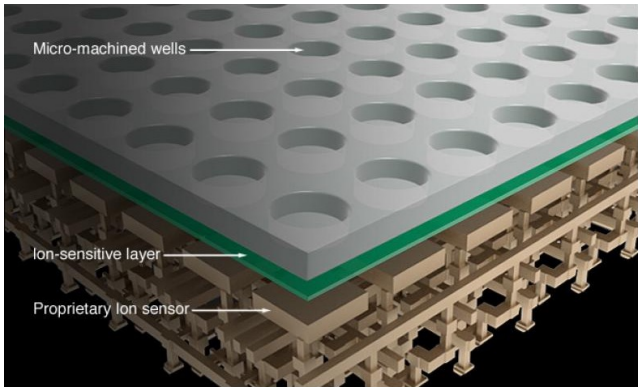
ion torrent
by life technologies™





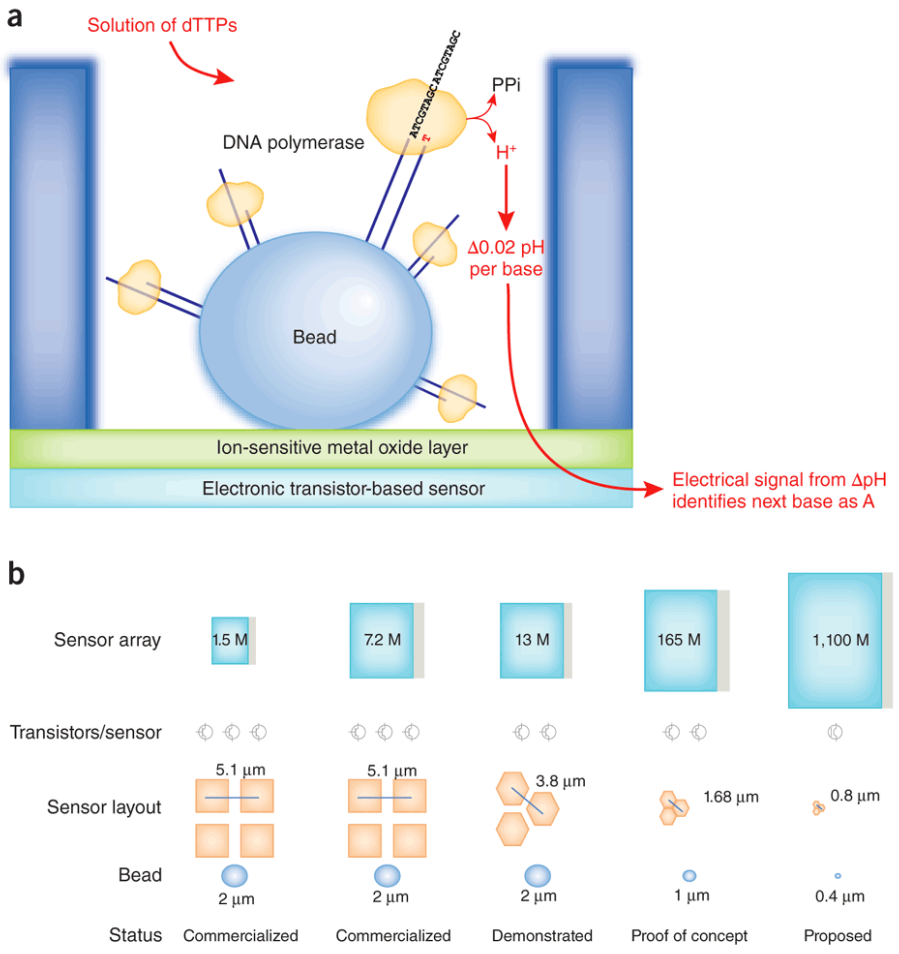
Proton II

660 M sensors



- Proves scalability of the technique
- Fast, “reagentless”
- Based on established pH sensing technologies

Rothberg *et al.*, Nature, Vol. 475, 348–352, July 2011

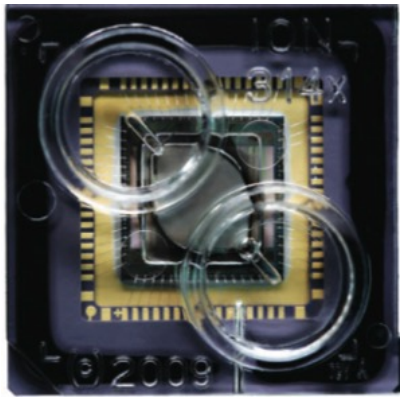


a) Schematic of a well on an ion sequencing chip. Clonal DNA immobilized on a bead is extended by polymerase in the presence of a pure solution of one nucleotide (here 'T'). Nucleotide incorporation releases a pyrophosphate (PPi) and a hydrogen ion. The change in pH caused by release of the hydrogen ion alters the surface potential of the ion-sensitive metal oxide layer. This is converted to a voltage signal by transistors. The wells are washed and exposed sequentially to pure solutions of other nucleotides. For comparison, in high-throughput pyrosequencing, the pyrophosphate is converted to chemiluminescence by an enzymatic cascade and optically imaged. The size of the well relative to the bead has been exaggerated, although each well contains a single bead.

b) Evolution of ion sequencing chips. Increases in sensors per chip can be achieved by (i) increasing the physical area of the sensor array, (ii) reducing the number of transistors per sensor, (iii) arranging the sensors in a hexagonal rather than rectilinear geometry and reducing the well and bead size; (iv) reducing the size of the single transistor.

Nature Biotechnology **29**, 805–807 (2011)

Semiconductor-based sequencing



- ☺ Overcome issue of dead fluorophores
- ☺ Highest throughput (80 Mb/h)
- ☹ Long homopolymers difficult to read

Table 1 Price comparison of benchtop instruments and sequencing runs

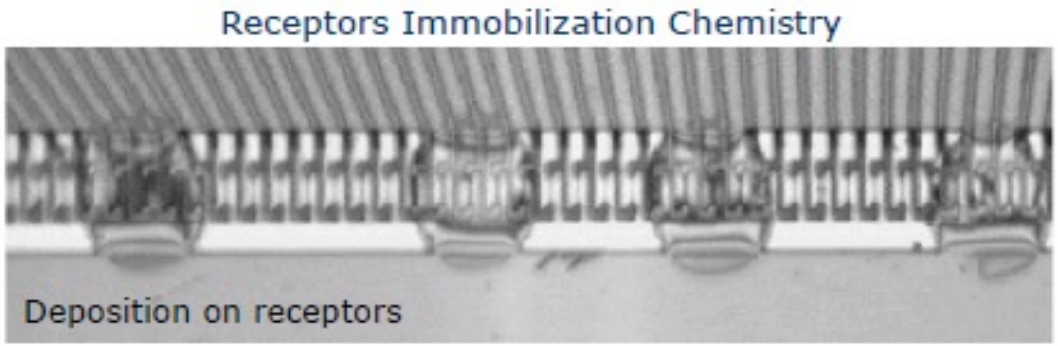
Platform	List price	Approximate cost per run	Minimum throughput (read length)	Run time	Cost/Mb	Mb/h
454 GS Junior	$108,000	$1,100	35 Mb (400 bases)	8 h	$31	4.4
Ion Torrent PGM						
(314 chip)	$80,490[a,b]	$225[c]	10 Mb (100 bases)	3 h	$22.5	3.3
(316 chip)		$425	100 Mb[d] (100 bases)	3 h	$4.25	33.3
(318 chip)		$625	1,000 Mb (100 bases)	3 h	$0.63	333.3
MiSeq	$125,000	$750	1,500 Mb (2 × 150 bases)	27 h	$0.5	55.5

Note pricing may vary between countries and/or sales territories. Instrument prices do not include service contracts. Sample prices do not include the cost of generating the initial fragmented genomic DNA library with adaptors (an additional cost of between $75–200 depending on method used). Cost per megabase assumes one sample and one sample sequencing kit per run. Unless stated, pricing information is from the online supplement of ref. 3.
[a]Ion Torrent PGM pricing from Invitrogen US territory website (http://www.invitrogen.com/, accessed 21 February 2012). [b]Price includes Ion Torrent PGM, server, OneTouch and OneTouch ES sample automation systems. [c]Ion Torrent PGM prices include chip and sample preparation kit. [d]Configuration used in this study.

N.J.Loman, Performance comparison of benchtop high-throughput sequencing platforms, Nat Biotech, 2012
http://iontorrent.com/lomanpaper?CID=fl-lomanpaper

Sensing of molecular binding with EISFET

Critical step: immobilization chemistry (functionalization)



Electrostatic

Immobilization of probes by adsorption (PLL).

Lower selectivity: **density of specific target/ density of non specific targets**

Covalent

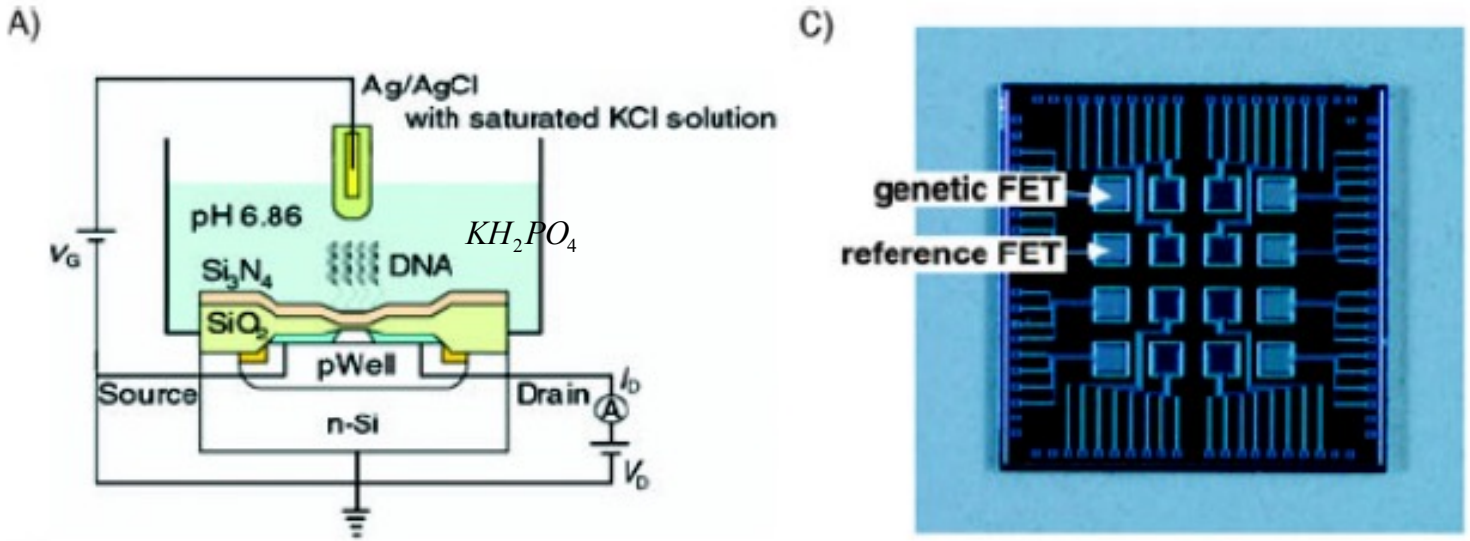
Immobilization of probes by covalent attachments (eg: MPTS-PEG_NH_2)

Higher selectivity : **density of specific target/ density of non specific targets**

Sometimes too aggressive for chips

The spacer (PEG) is undesirable

Sensing of DNA hybridization



The shift of the threshold voltage (VT) is determined from **the gate voltage (VG)- drain current (ID) characteristics** in a phosphate buffer solution

An Ag/AgCl electrode with saturated KCl solution is used as a reference electrode.

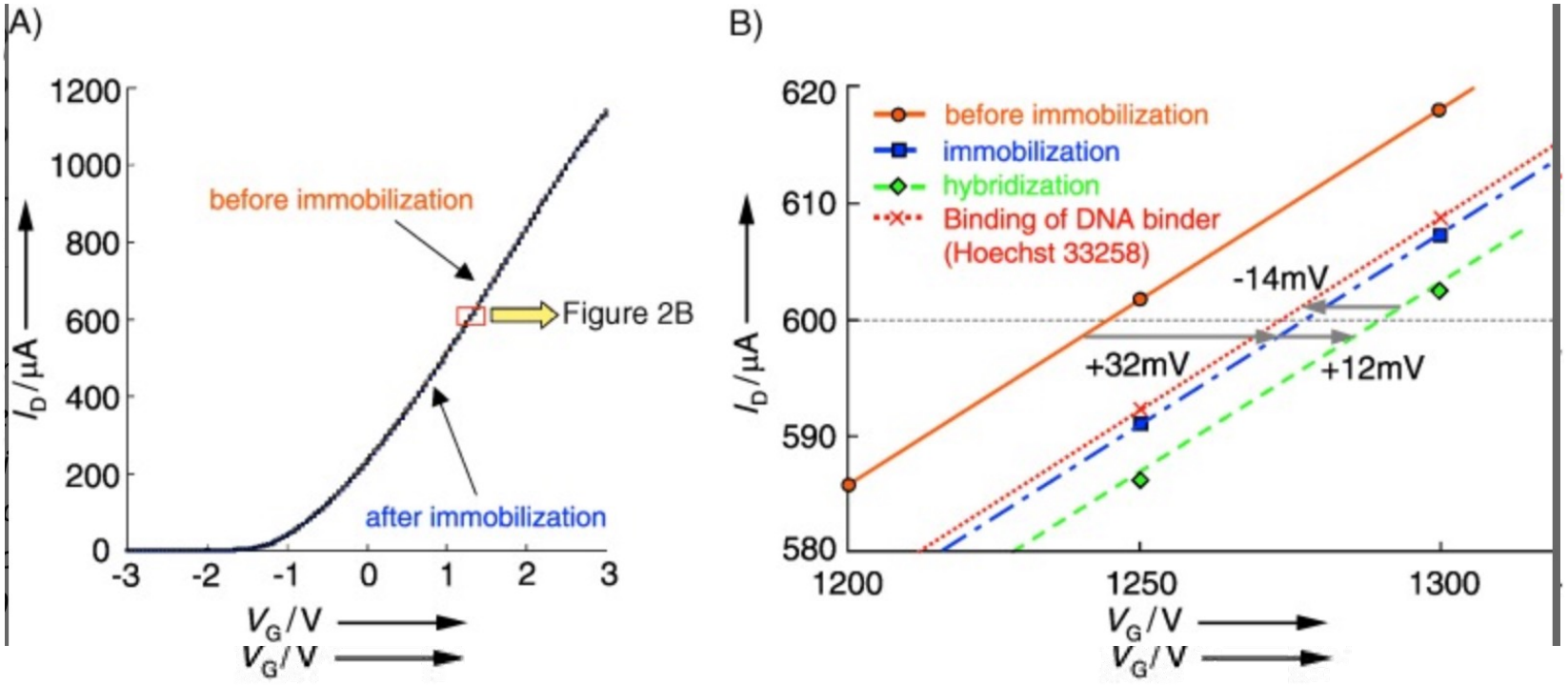


Table 1. *Base sequences for oligonucleotide probes at the R353Q locus of factor VII gene.*[21]

Locus	Function	Sequence	T_m [°C]
R353Q	R353Q-normal (N)		
	probe	5′-amino group-CCACTACC**G**GGGCACGT-3′ (17 mer)	60 (T_{m1})
	target	5′-ACGTGCCCCGGTAGTGG-3′ (17 mer)	
	R353Q-mutant (M)		
	probe	5′-amino group-CCACTACC**A**GGGCACGT-3′ (17 mer)	57 (T_{m2})
	target	5′-ACGTGCCCTGGTAGTGG-3′ (17 mer)	

N and M indicate normal (wild-type) and mutant allele-specific oligonucleotides, respectively. T_m shows the melting temperature.

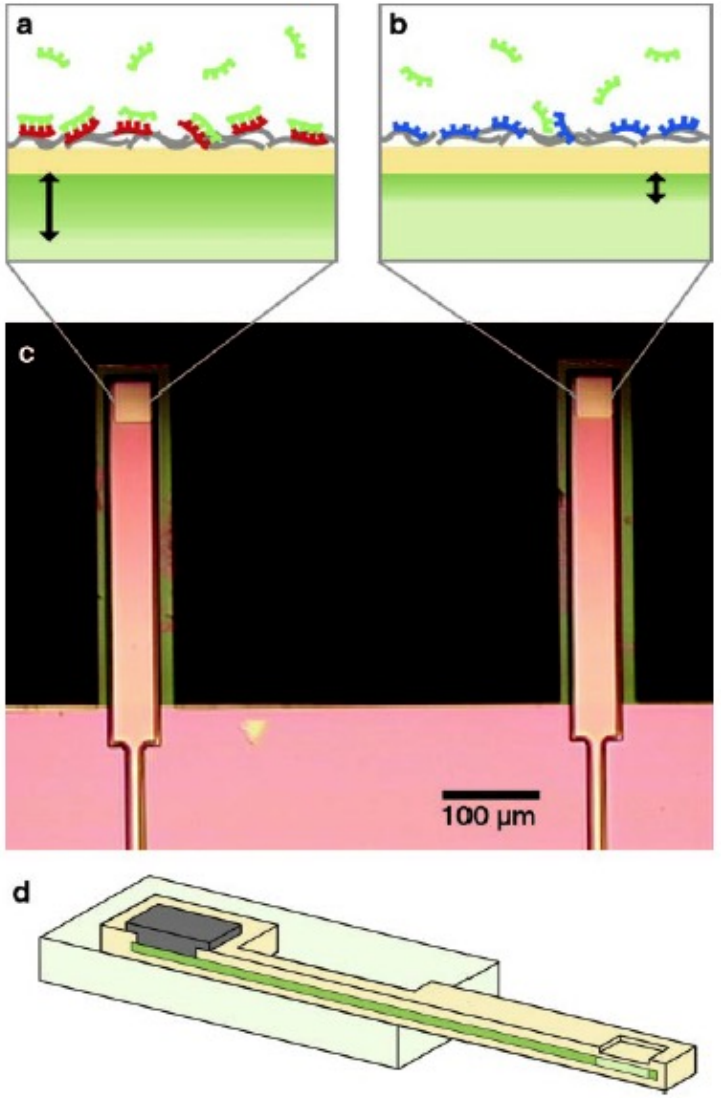
FYI

Two terminal MOS. Measurement of DNA hybridization

Nanomolar DNA concentrations can be detected within minutes, and a single base mismatch within 12-mer oligonucleotides can be distinguished by using a differential detection technique with two sensors in parallel.

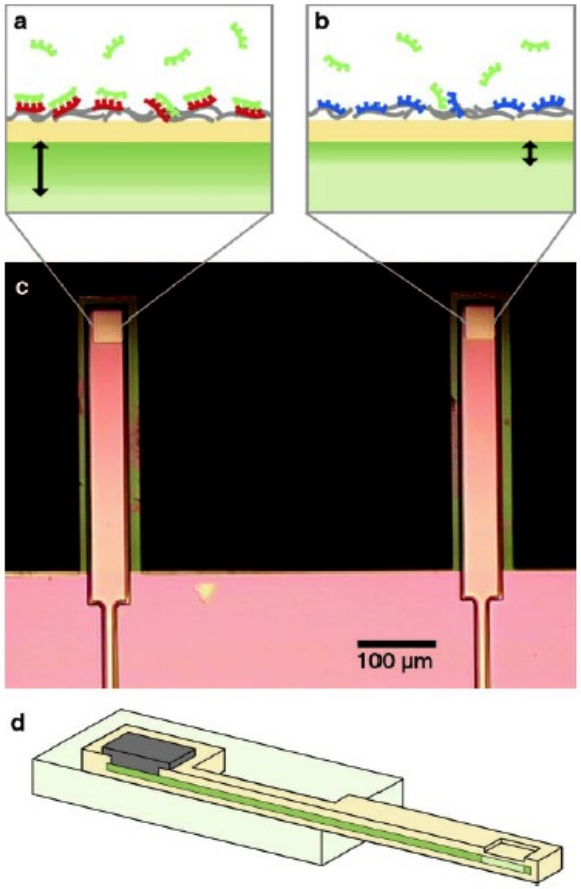
To explore the utility of this field-effect sensor for detecting DNA in solution, two sensors were first functionalized with a PLL layer.

Next, the sensing area of one sensor was functionalized with the 12-mer oligonucleotide *A* (sensor 1), and the adjacent sensor was functionalized with the unrelated 12-mer oligonucleotide *B* (sensor 2).

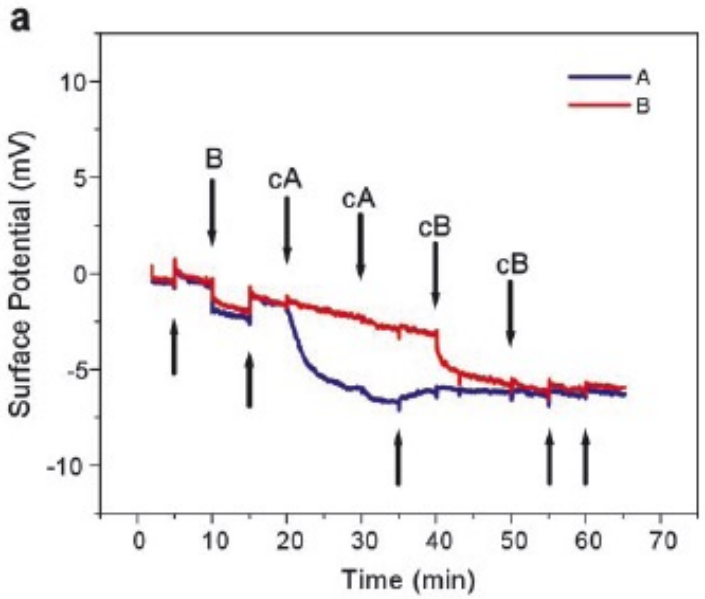


S. Manalis, MIT

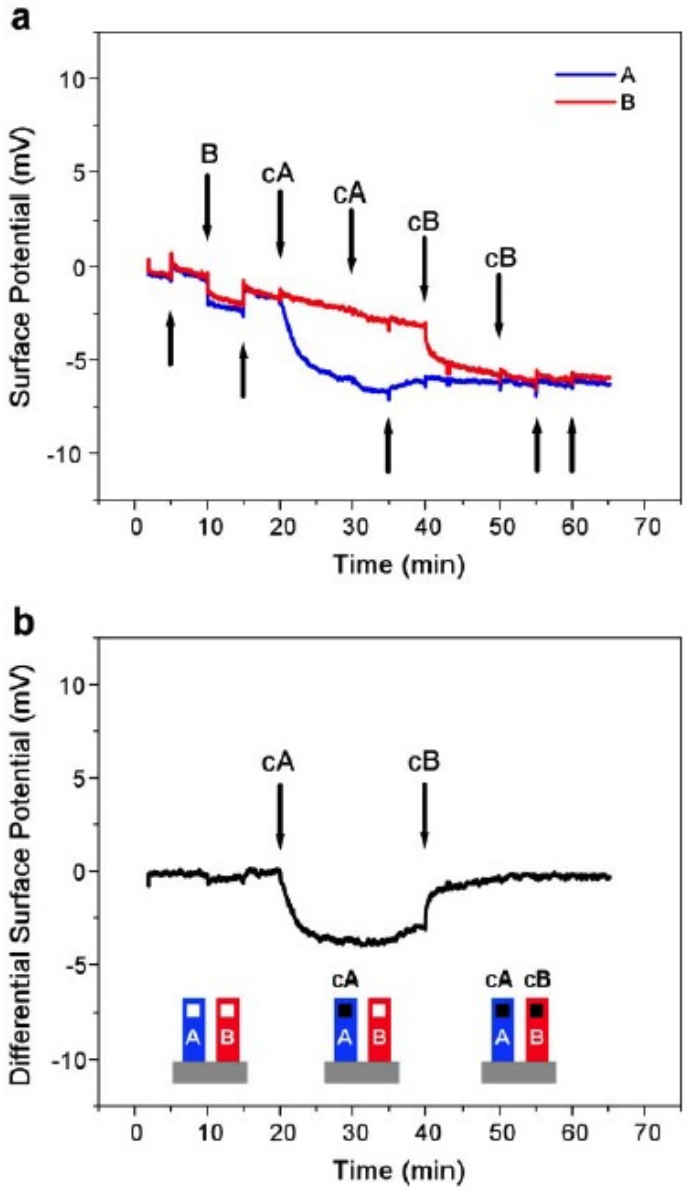
Two terminal MOS. Measurement of DNA hybridization



- EIS capacitors at the termini of cantilevers
- 2nm gate oxide
- Measurement of the surface potential by 1 kHz, 150 mVpp
- 80 nM 12 mer complementary oligonucleotides



J. Fritz, PNAS 2004



Unwanted signals arose because the surface potential is sensitive to thermal fluctuations, drifts, nonspecific binding, and changes in electrolyte composition.

When oligonucleotide cA, complementary to A, was injected, the surface potentials of sensor 1 and sensor 2 diverged. The sensors showed a differential response of 3 mV for oligonucleotide cA and of 3 mV for the subsequent addition of cB (Fig.b).

These observations demonstrate that a differential field-effect sensor configuration is able to measure the sequence-specific formation of A– cA and B–cB hybrids.

S. Manalis, MIT

J. Fritz, PNAS 2004

Differential analysis

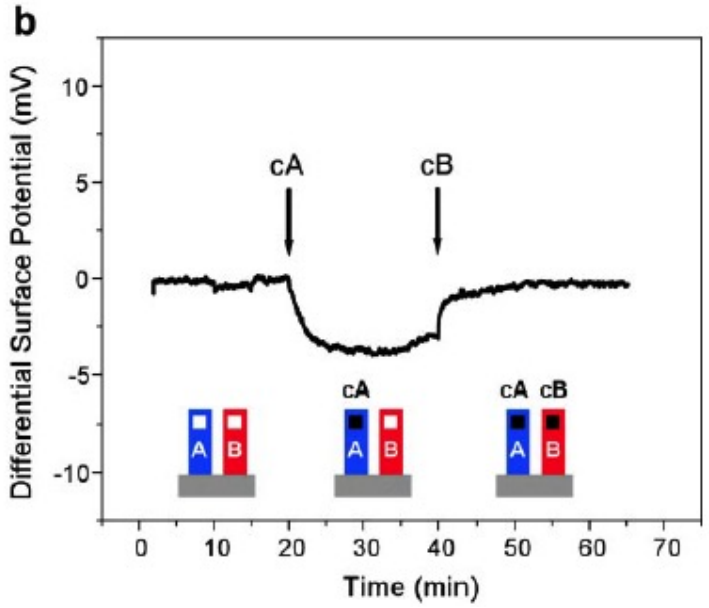
The sensors were then mounted in a fluid cell. Solutions containing various target DNA oligonucleotides were injected in succession, and the surface potential of the sensors was measured.

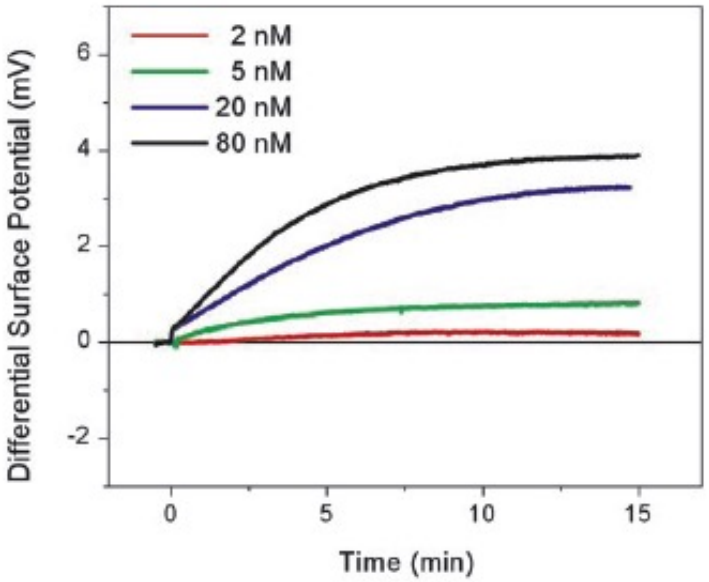
SELECTIVITY: The addition of control solutions such as buffer or oligonucleotide B generated similar signals from both sensors.

These signals arose because the surface potential is sensitive to thermal fluctuations, drugs, nonspecific binding, and changes in electrolyte composition.

DIFFERENTIAL ANALYSIS: However, because these unwanted signals are similar for both sensors, they can be eliminated by taking the differential response from the two sensors (sensor 1-sensor2).

EISCAP. Measurement of DNA hybridization





Experiments:

3 mV surface potential change

having on one cantilever 10^4 molecules of 12 mer specifically hybridized oligonucleotides (quantitative data obtained by comparative radiolabeling technique)

Simplified estimation:

Graham Eq.

Surface charge is a function of the surface potential

$$\sigma_0 = \sqrt{8\varepsilon_w\varepsilon_o kTc_o}\,\sinh\left(\frac{e\Psi_0}{2kT}\right)$$

where k is the Boltzmann constant, T is absolute temperature, e is elementary charge, ε_0 is the permittivity of free space, ε_w is the dielectric constant of water, and c_o is the buffer ionic strength.