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Label-free Electric Detection of Biomolecular Interactions using Semiconductor Integrated Circuit

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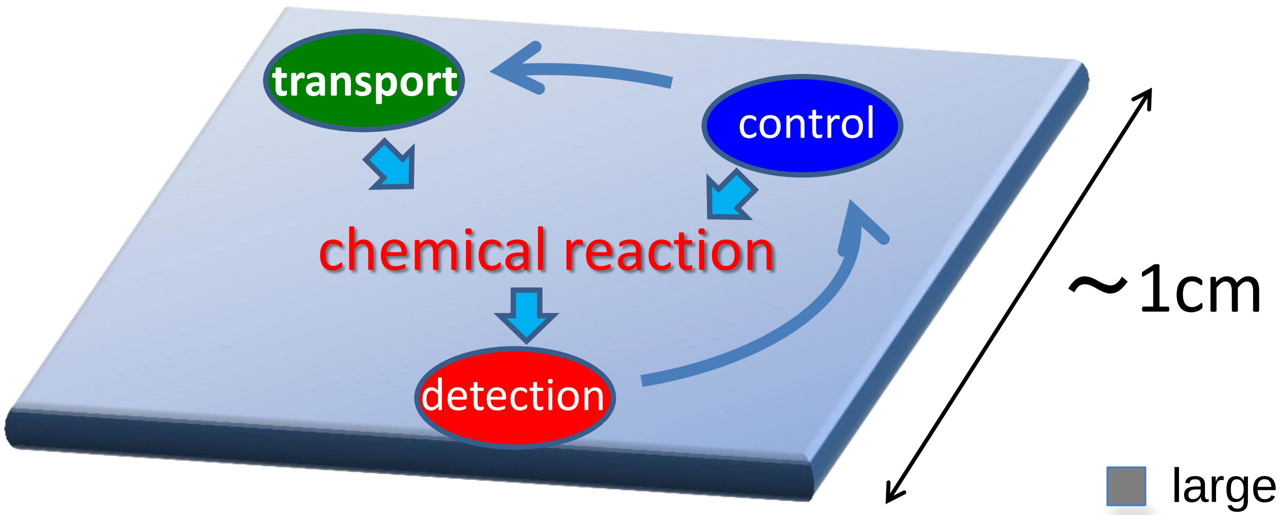
Integration of Chemistry on a Chip



smart lab-on-a-chip

- Detection of Chemical Reaction
  - Sensing of Specific Molecule
- Control of Chemical Reaction
  - Amplification of Specific Molecule
- Transport
  - Transportation and Selection of Molecule

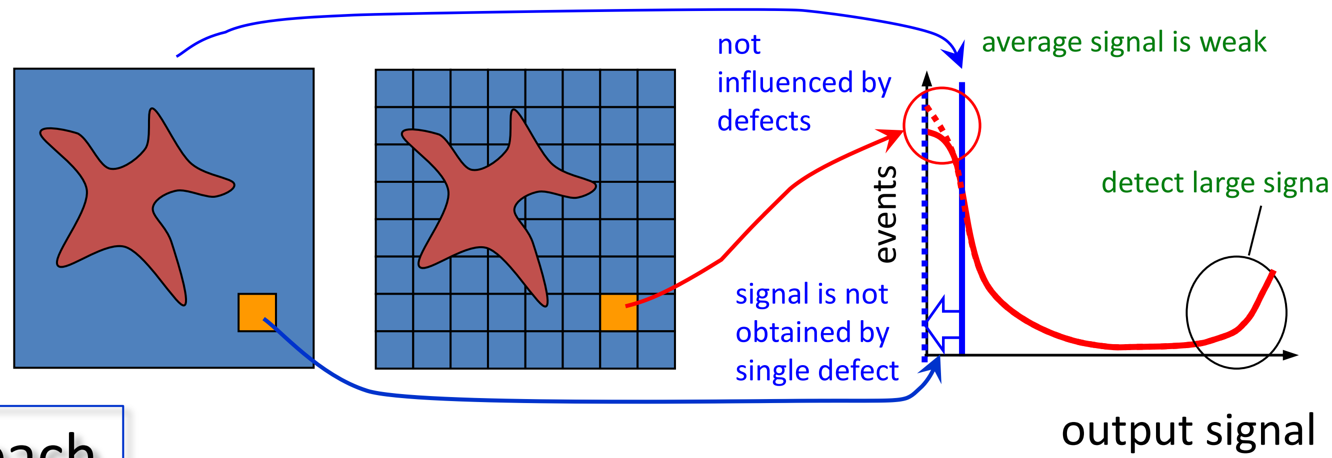
Advantages

- large Signal/Noise ratio enables the detection of subtle signals
- high speed (1μs) and local (1~100μm) detection
- parallel detection
  - real time imaging
  - massive parallel detection
- control of chemical reaction
  - temperature, electric field, magnetic field, fluid flow
- compact ($1cm^2$)

Sensor Array

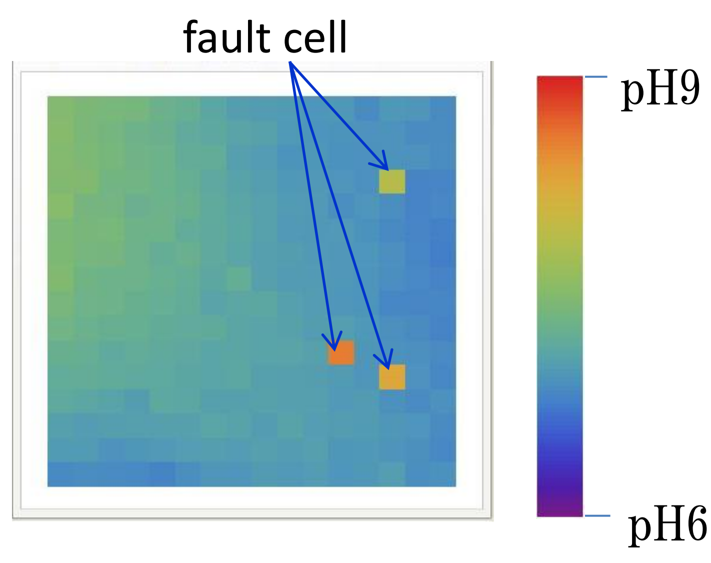
sensor array

- parallel detection
- 2-dimensional image
- redundancy



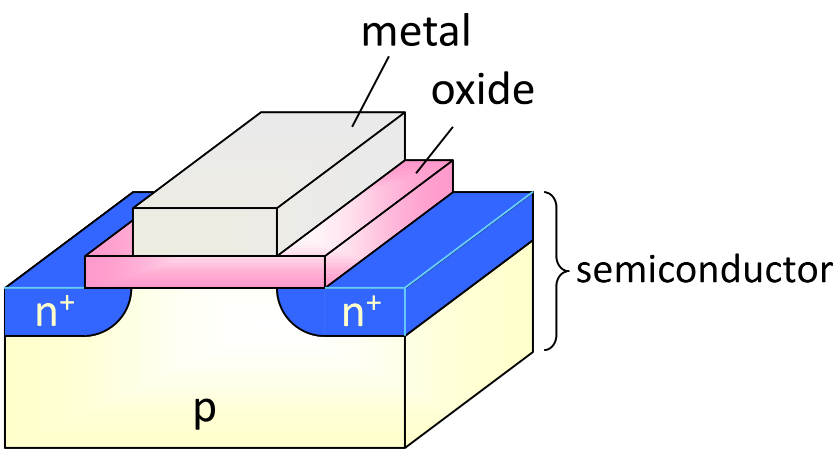
our research approach

- semiconductor chips are fabricated by standard CMOS process line
- a few post-CMOS processes
- focus on unit cell circuit
  - no influence to chemical reaction
  - high density
  - low power consumption
  - stable operation



MOSFET (Metal-Oxide-Semiconductor Field Effect Transistor)

MOSFET structure



- The present LSIs are constructed by MOSFETs
- More than one million transistors can be integrated on 1 square cm chip.
- Large amount of chips can be fabricated at once (~ ten thousand chips/lot)

Initial cost is high.

It is very difficult to add non standard process.

Mass production cuts the chip cost.

Technology	Initial Cost ($)	Chip Cost ($/chip)
0.6μm	16 k	2
0.25μm	100 k	2.5
0.18μm	250 k	3
0.13μm	600 k	5

Initial Cost : set of photomasks
Chip Cost : 1 square cm chip

Small production is not suitable.

Standardization is essential for semiconductor chips.

Portable Gene-based Diagnostic Inspection System

Food Security
Healthcare
Evidence-based Care
Infectious disease
Tailor-maid Medicine

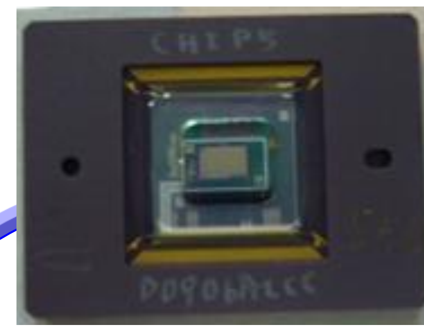
portable gene-based POCT

everybody, everywhere, immediately

conventional DNA chip

optical detection labeled with fluorophors

- technically trained operator
- expensive, not portable



new DNA chip

electrical } detection
label free }

system-on-a-chip + lab-on-a-chip

new area of semiconductor LSI

More than Moore

Electrochemical Sensing Methods

- potentiometric
- amperometric
- impedimetric

Direct Charge Detection Method

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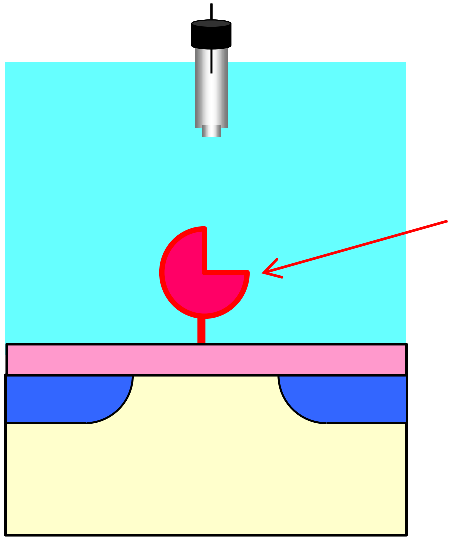
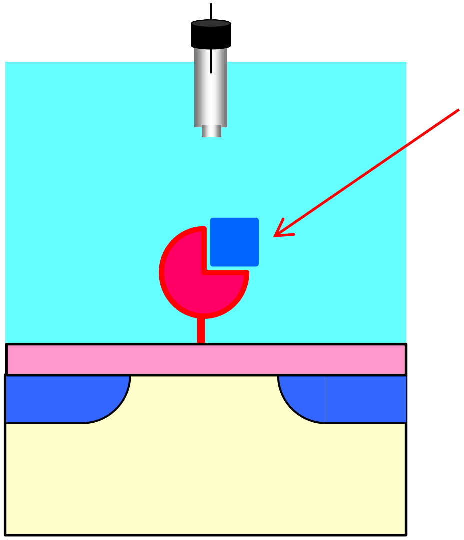
immobilization



prove is fixed
known molecule
interaction with specific molecule

hybridization



target is supplied
specific interaction with probe
target has specific charge

Noninteracting molecules are washed out.

If specific molecule is included in target, potential is changed which can be detected by transistor.

Potentiometric Sensor Circuit

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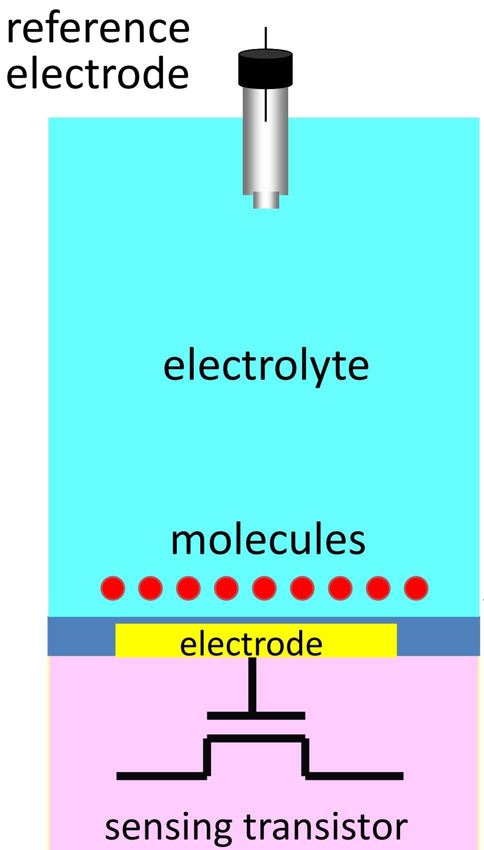


ideal detection

no influence on measured system

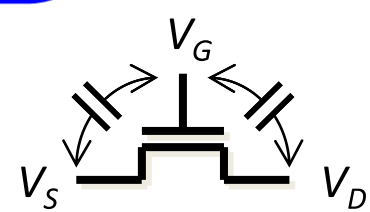
↓

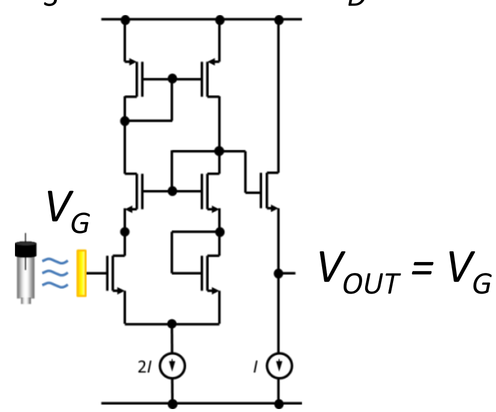
infinite input resistance
of sensor circuit

↓

CMOS source-drain follower

$$V_S = V_G + \text{const.}$$
$$V_D = V_G + \text{const.}$$





Compared to conventional circuit

area	1/30
power consumption	1/10,000
precision	x100

K.Nakazato, M.Ohura & S.Uno
IEICE Trans. Electron. **E91-C**
(2008) 1505

16 x 16 sensor array

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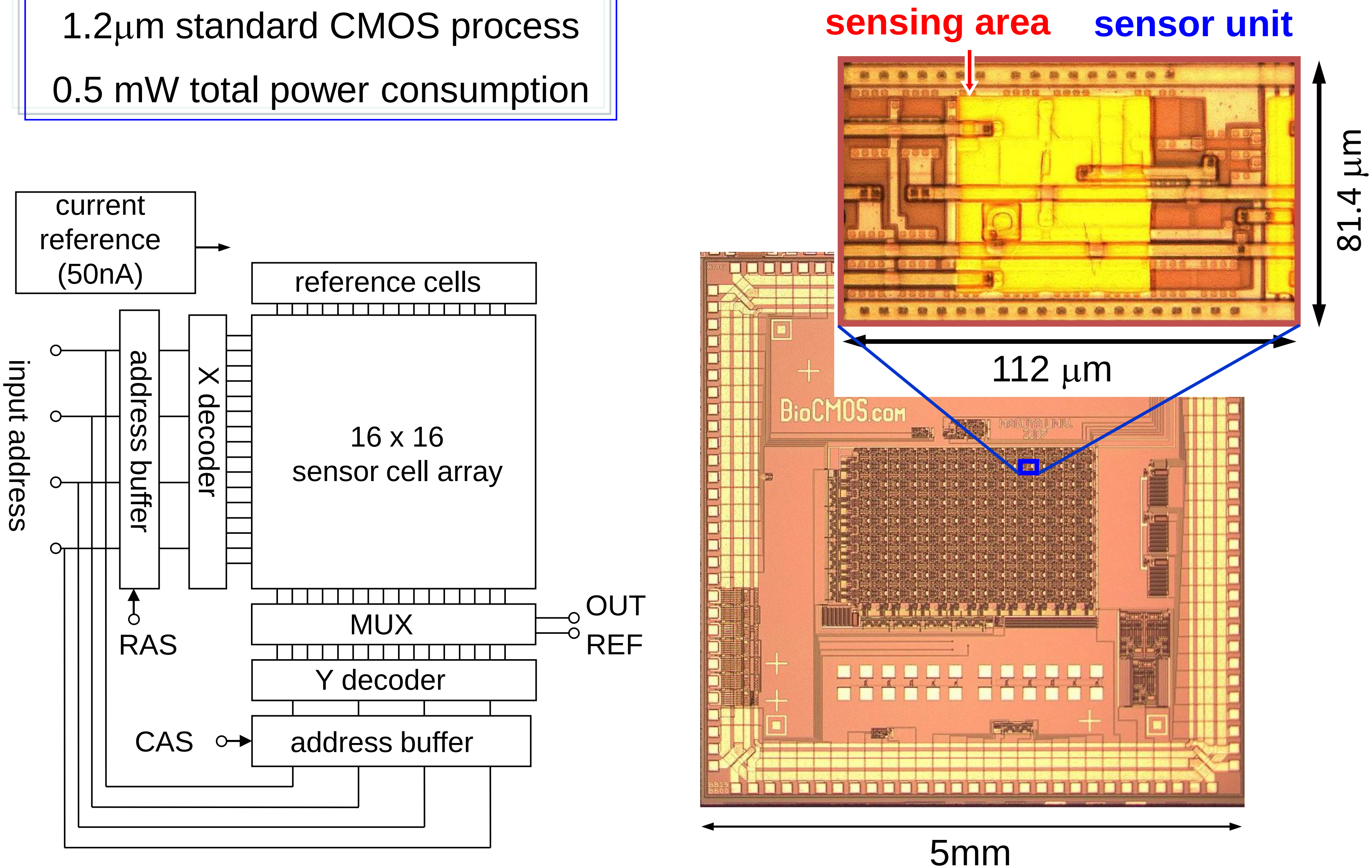
1.2μm standard CMOS process

0.5 mW total power consumption



pH Detection on Catalytic-CVD Si_3N_4 Layer

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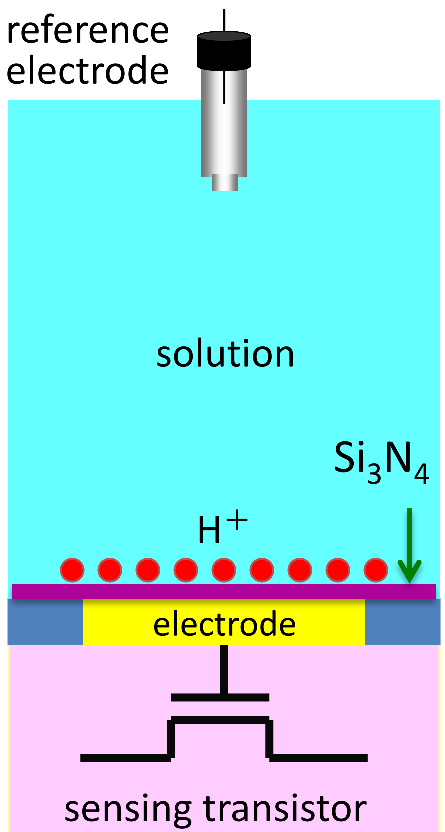
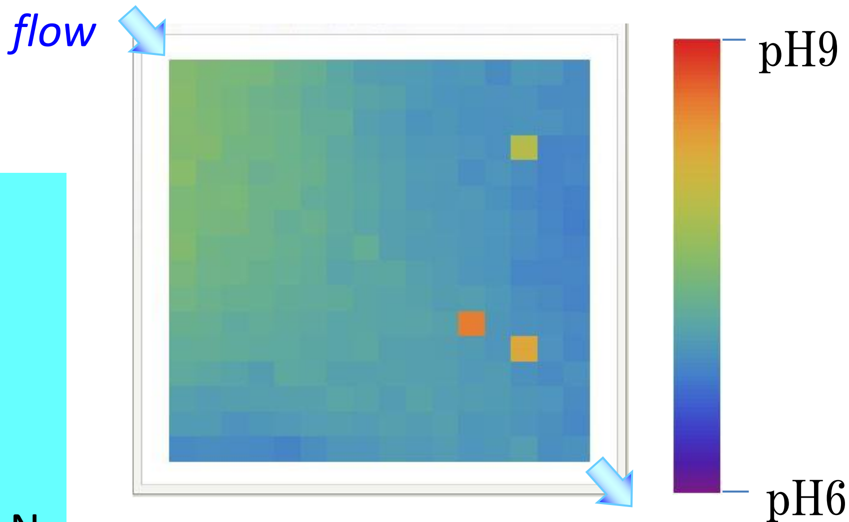
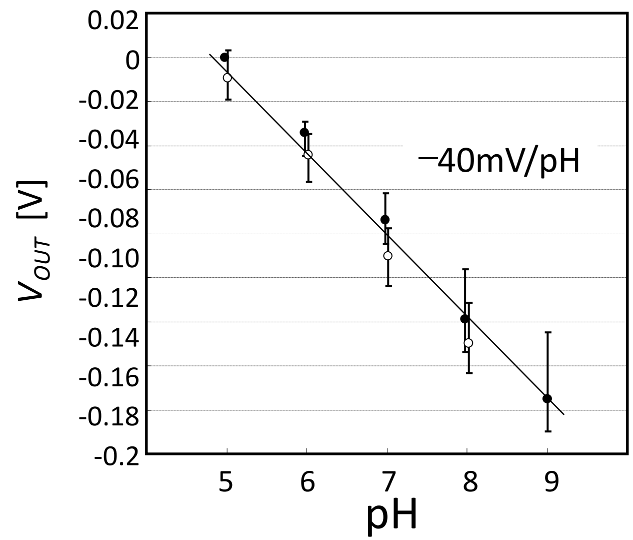
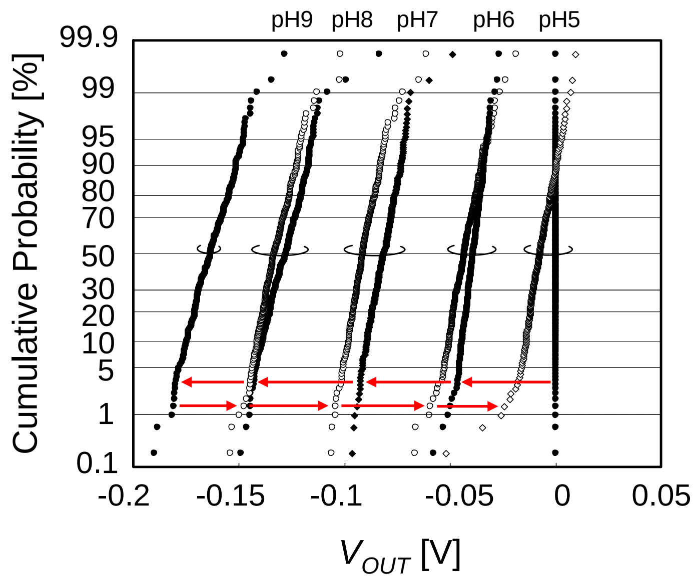
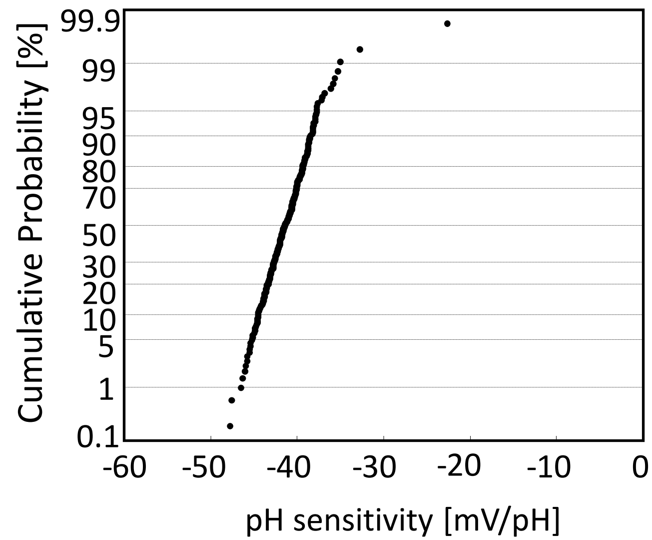


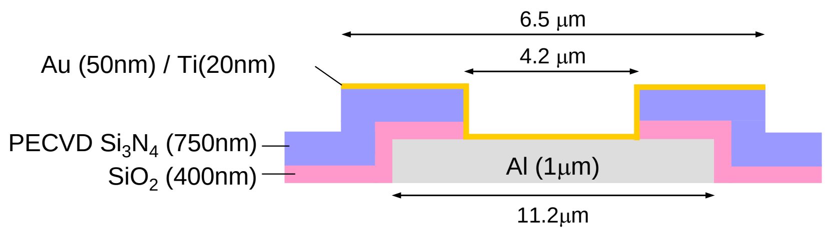




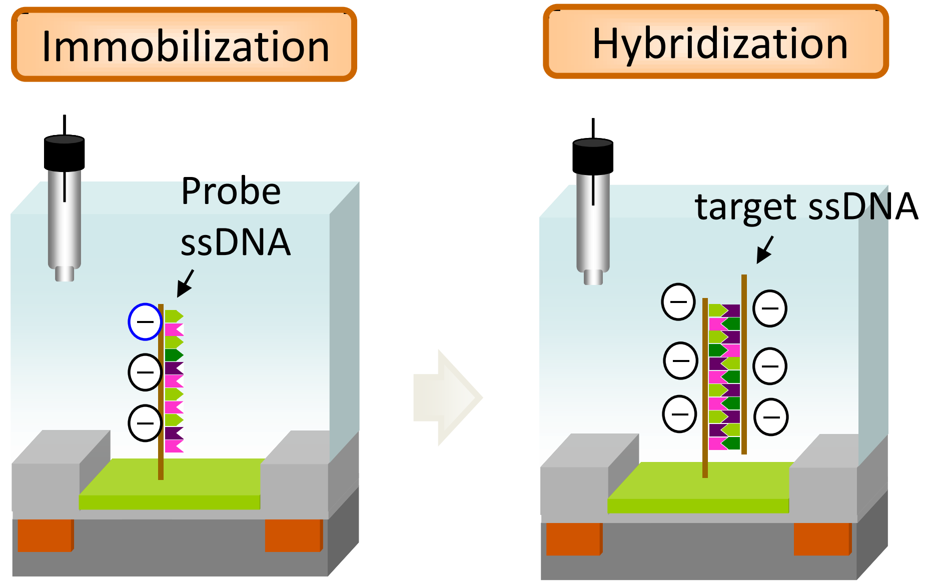


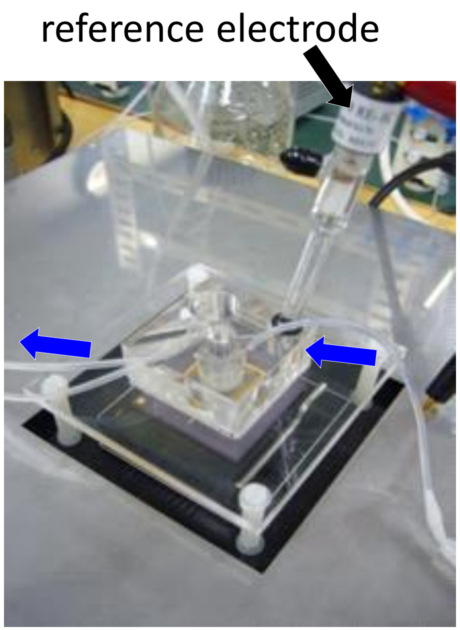


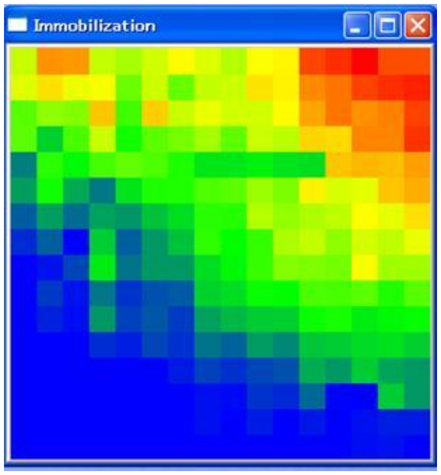
Detection of DNA Hybridization

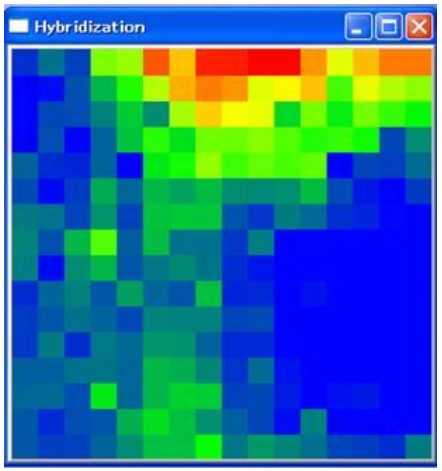


probe: 5' thiol modified GGGAAAAAAAAAAAAAAGGG
target: CCCTTTTTTTTTTTTTTCCC
buffer: 1mM NaCl & 1uM EDTA & 1mM KH_2PO_4 &
1mM K_2HPO_4 (pH7.0)









K.Nakazato
Sensors **9** (2009)
8831

Genetic Field Effect Transistor

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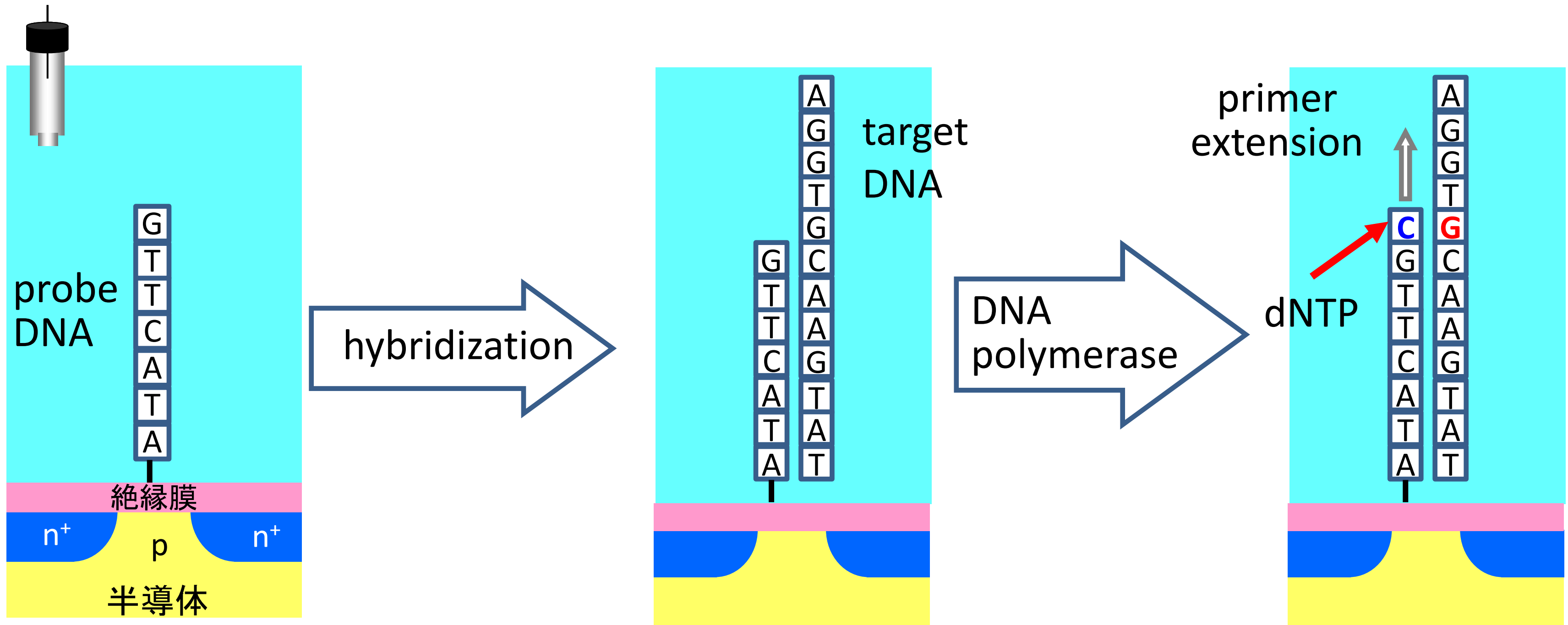
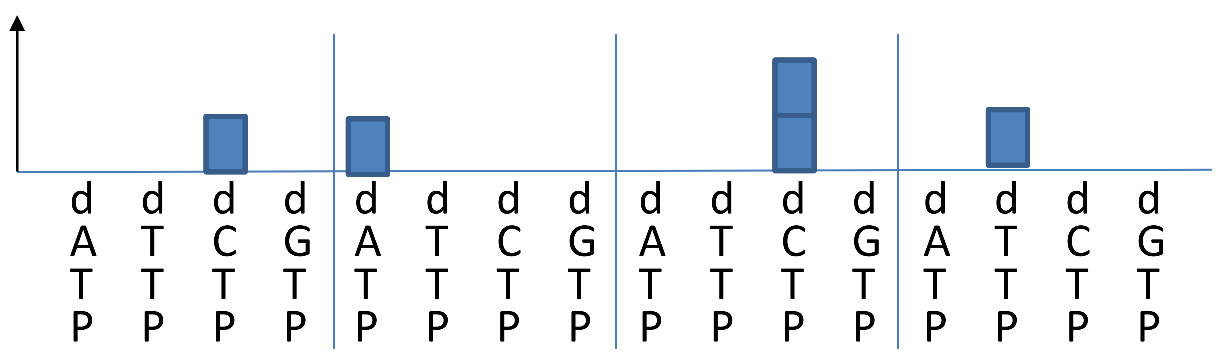


Change of Charge



T.Sakata & Y. Miyahara
Biosensors Bioelectron.
22 (2007) 1311

Problems of Direct Charge Detection Method

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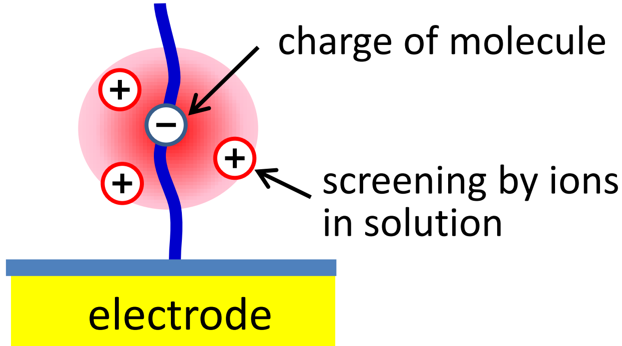
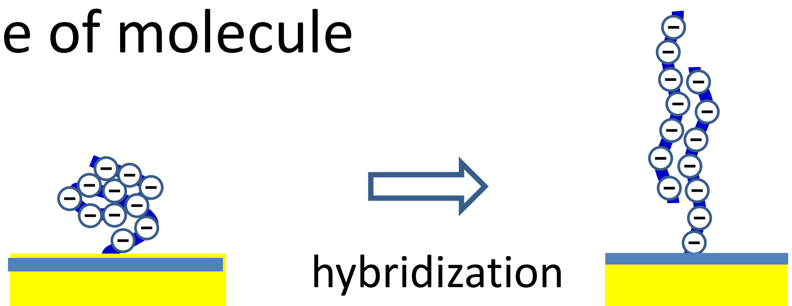
I. screening by ions



concentration	screening length
10 mM	3 nm
1 mM	10 nm

low ion concentration
→ high impedance environment
→ unstable of electric potential

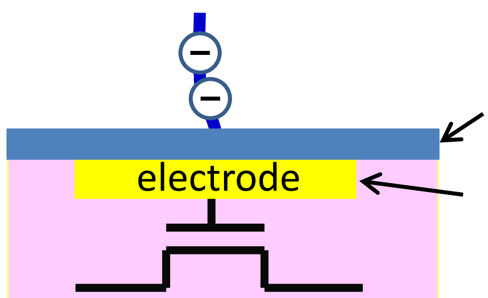
II. influence of shape of molecule



What detects
change of charge ?
change of structure ?

flow system : fluctuation of shape → unstable of electric potential

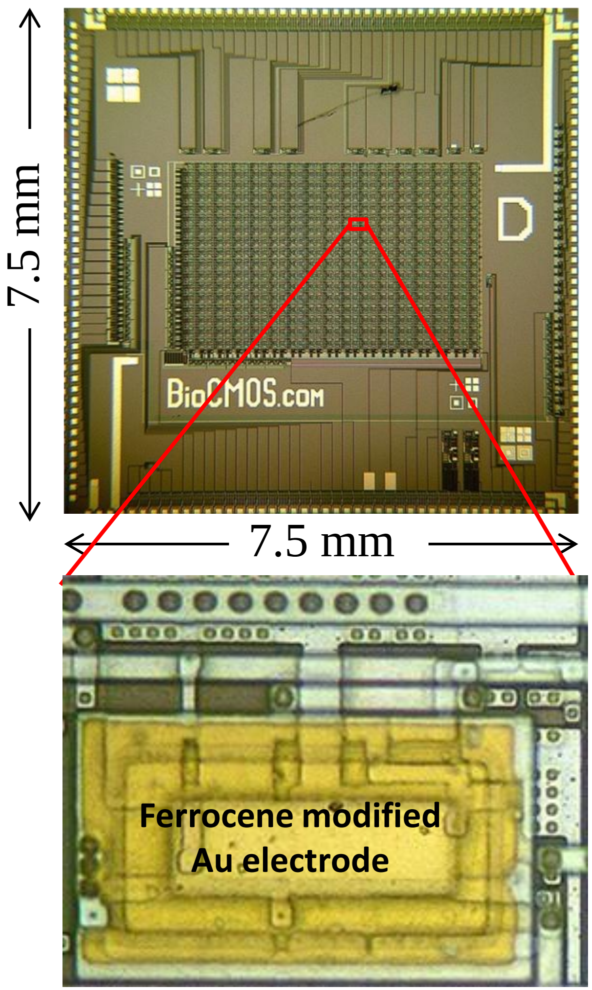
III. floating gate

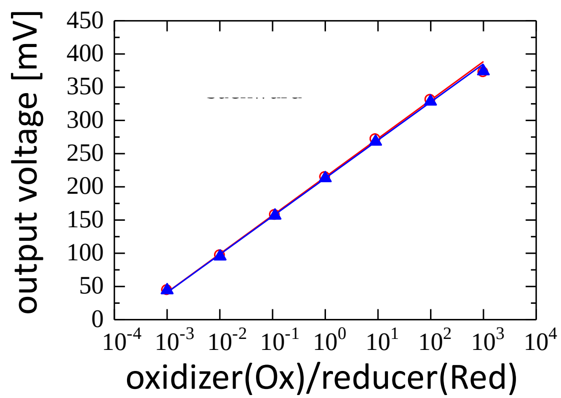


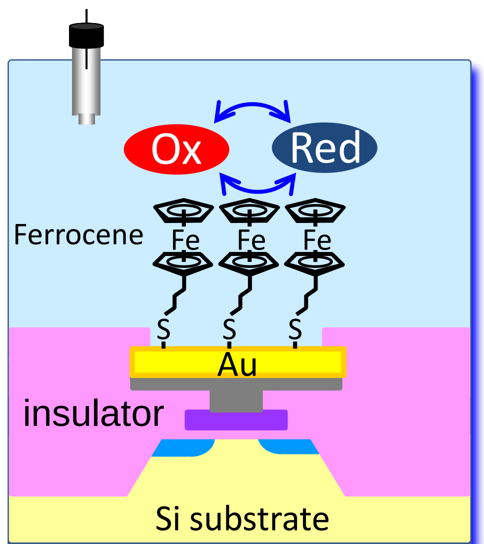
Insulator or SAM

embedded charge → large VTH variation

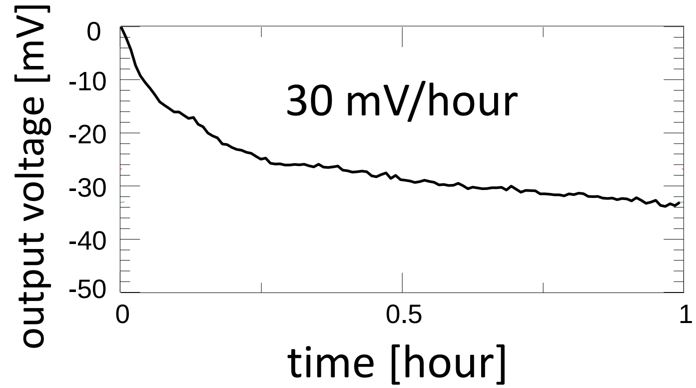
Redox Potential Sensor





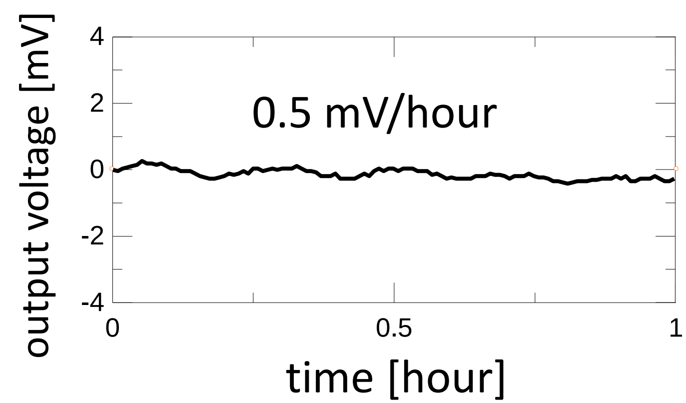


direct charge detection method



stability improvement
2 orders

redox potential detection method



FET Enzyme Sensor

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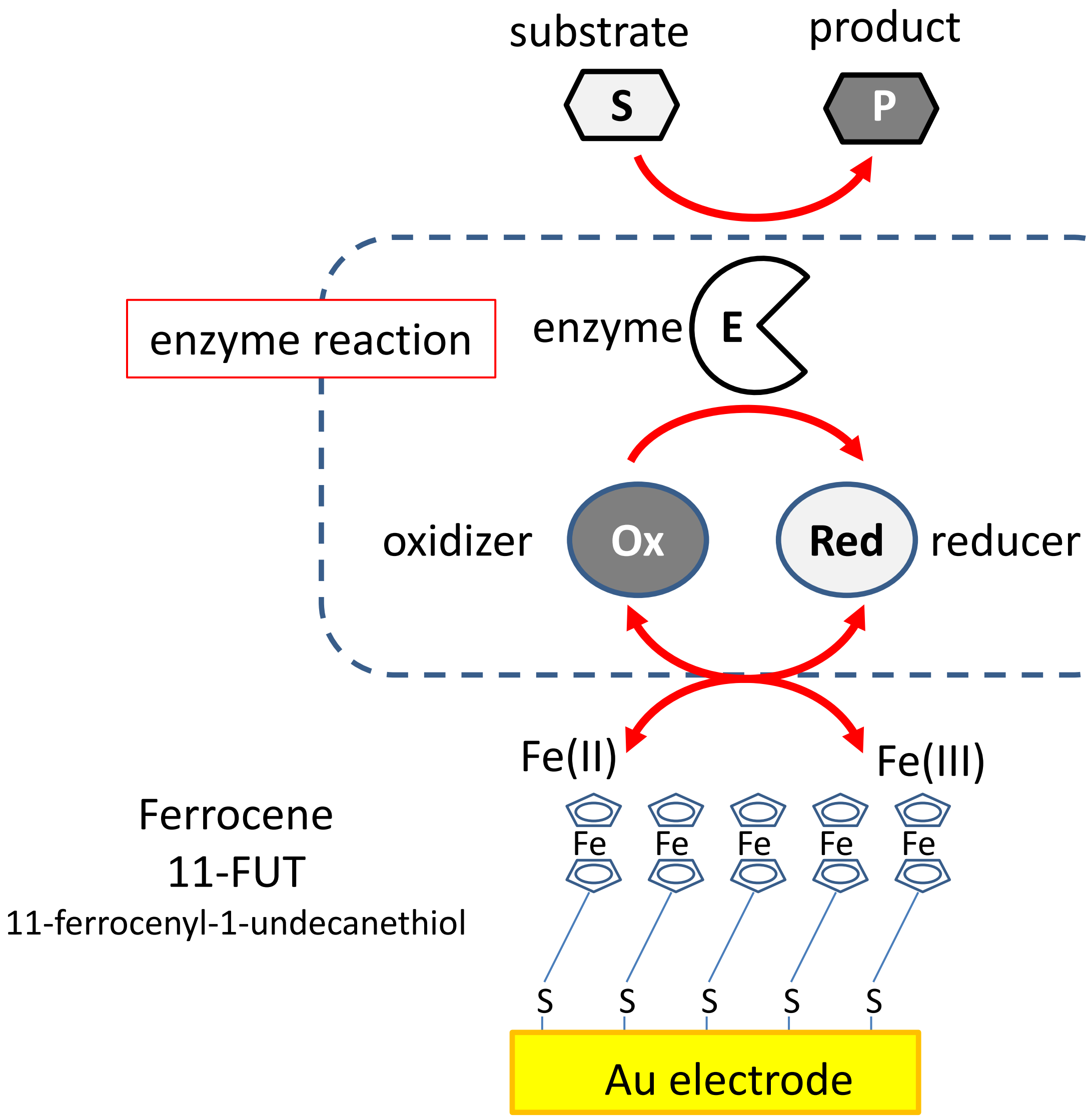

merits
- independent of pH
- sensitivity
- stable
- reusable
- general purpose

hexacyanoferrate

$Fe(CN)_6^{3-}$ $Fe(CN)_6^{4-}$

$$V_T = V_{T0} - \frac{k_B T}{nq} \log_e([S])$$

Examples of Detection

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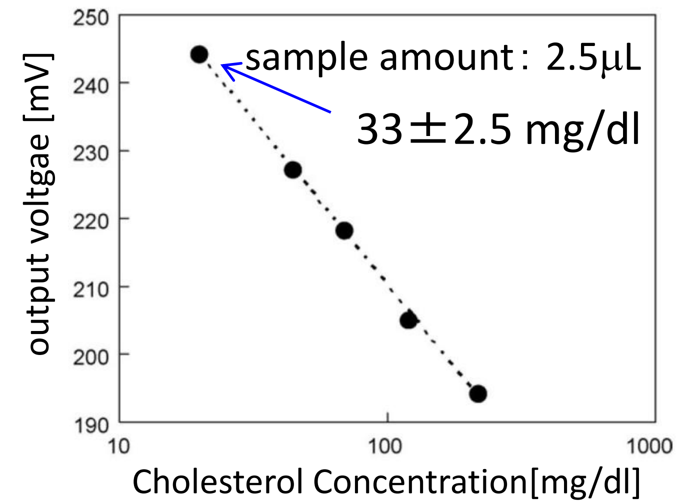
Cholesterol Detection

Cholesterol + NAD $\xrightarrow{\text{CDH}}$ Cholestenon +NADH

$2[Fe(CN)_6]^{3-}$ + NADH $\xrightarrow{\text{Diaphorase}}$ $2[Fe(CN)_6]^{4-}$ + NAD



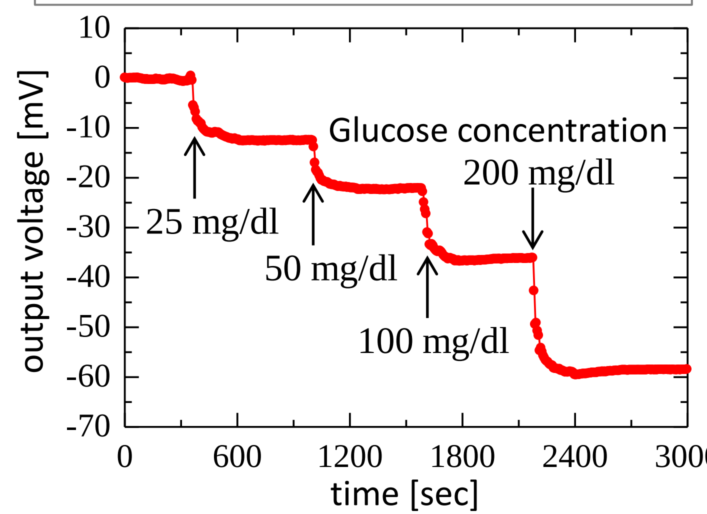
T.Ishige, M. Shimoda & M. Kamahori Biosensors Bioelectron. **24** (2009) 1096

Glucose Detection

Glucose + ATP $\xrightarrow{\text{HK}}$ Glucose-6-Phosphate +ADP

Glucose-6-Phosphate + NAD $\xrightarrow{\text{G6PDH}}$ Gluconolactone-6-phosphate +NADH

$2[Fe(CN)_6]^{3-}$ + NADH $\xrightarrow{\text{Diaphorase}}$ $2[Fe(CN)_6]^{4-}$ + NAD



H. Anan, T.Ishige, M. Kamahori, & K. Nakazato Sensors an Actuators B: Chemical (2013)

DNA Sequencer using Redox Potential Sensor

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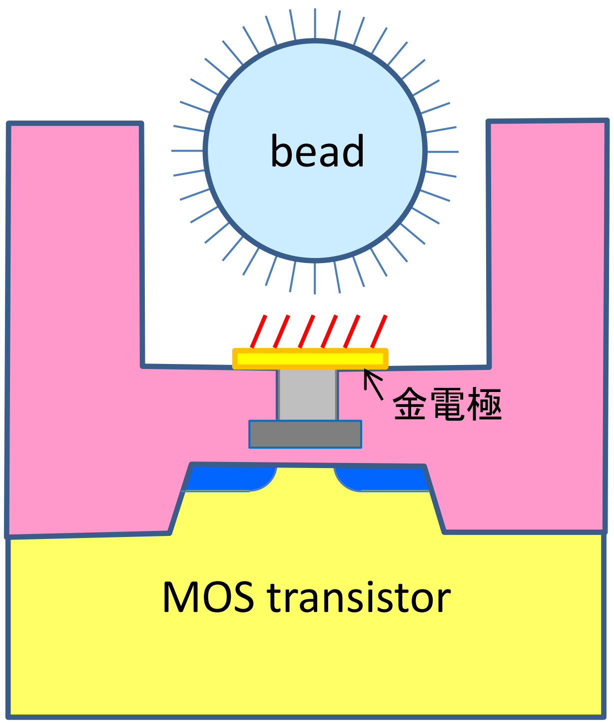

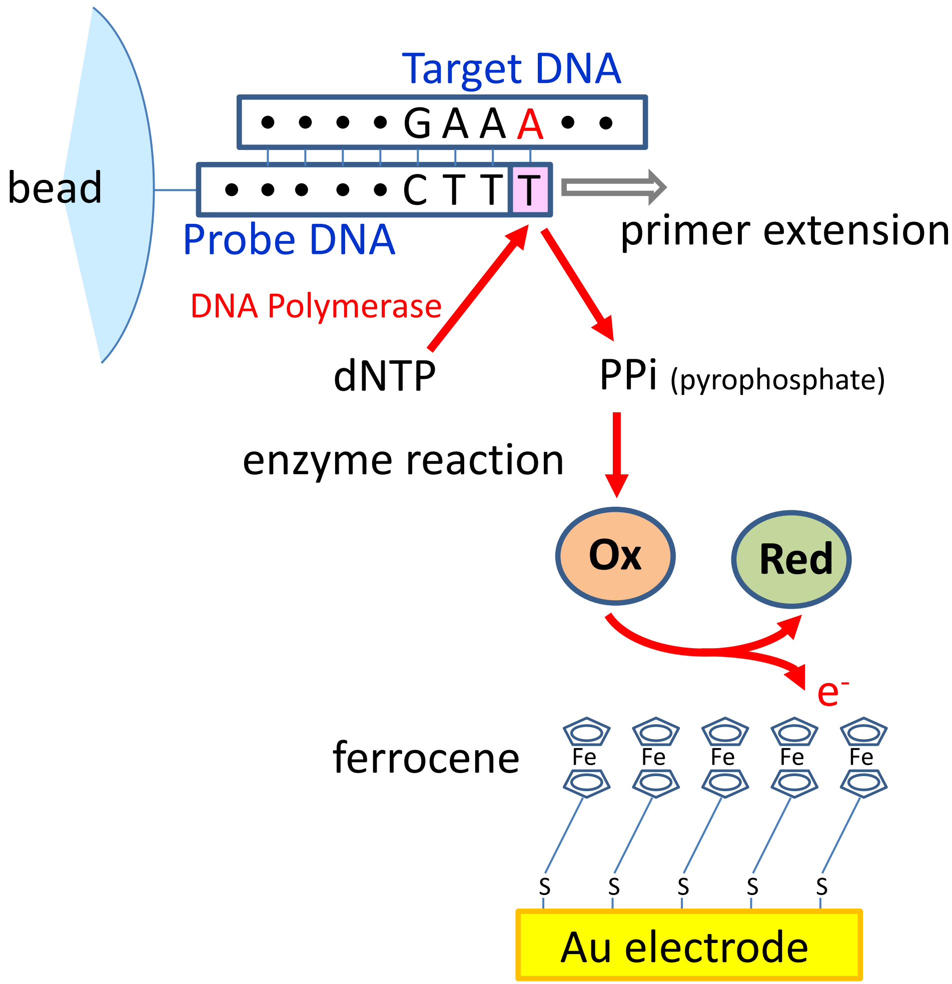

separated detection from reaction
large and stable signal
general purpose
reusable

Amperometric Sensor Array

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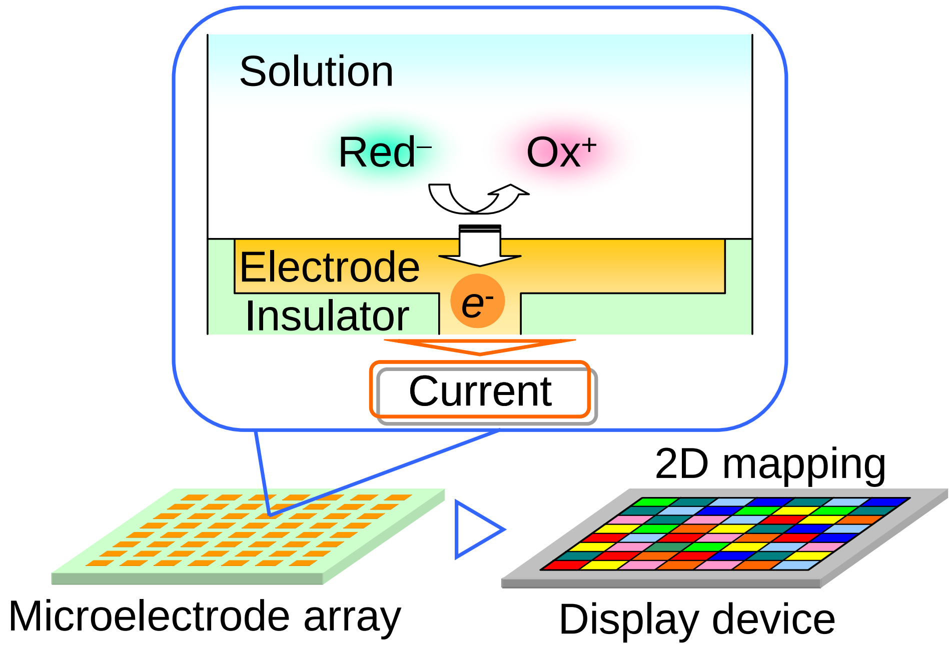
Measurement principle



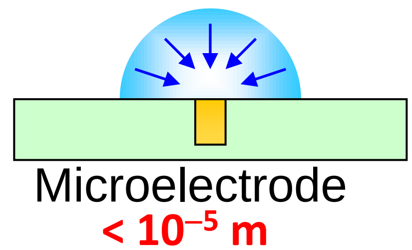
$$\mathrm{Red}^{-} \rightleftarrows \mathrm{Ox}^{+} + ne^{-}$$

Measuring the current induced by a redox reaction at the working electrode.

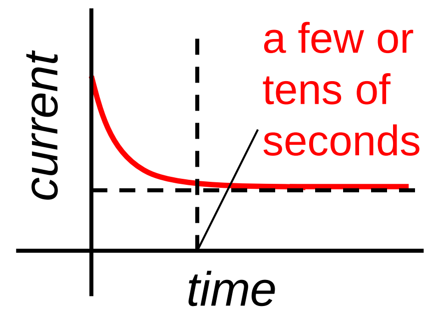
Microelectrode

- Diffusion layer

Hemisphere



- Current response

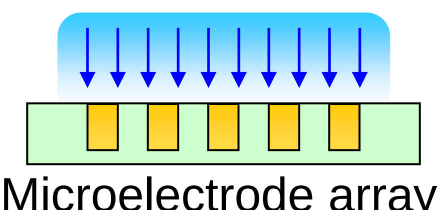


Steady-state current is observed.

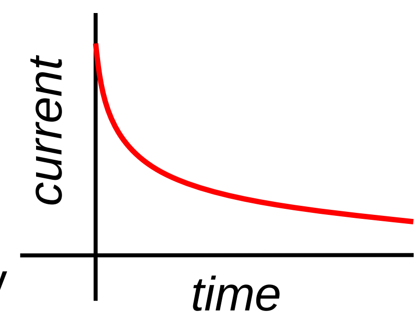
Microelectrode array

- Diffusion layer

Planar



- Current response



Current magnitude continuously decreases.

Microelectrode Array Structure

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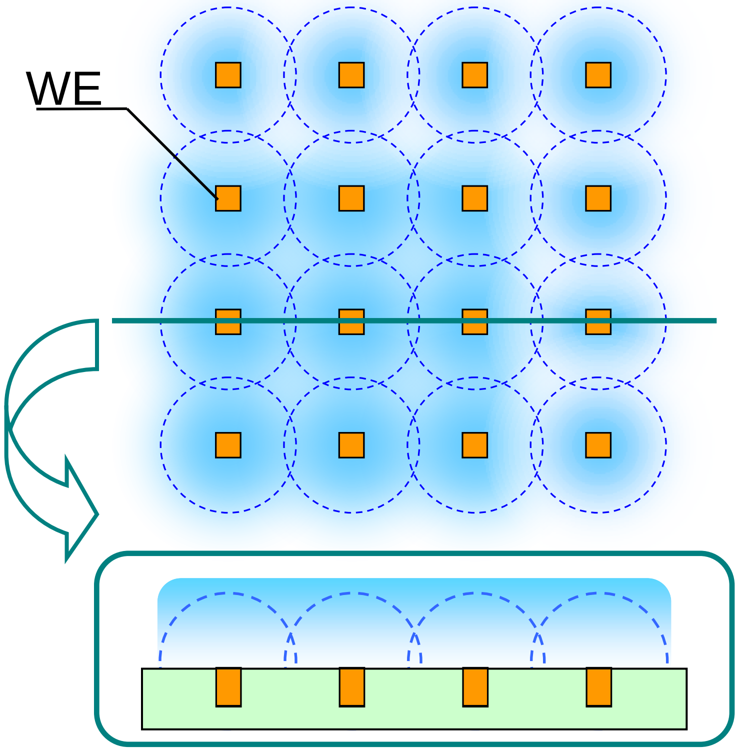
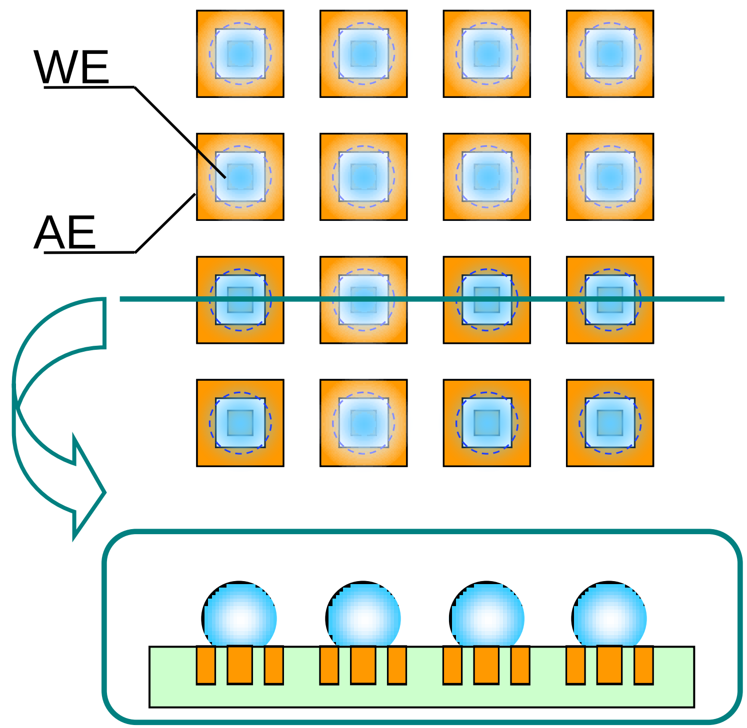
Conventional



WE : Working electrode

- The diffusion layers of the closely spaced microelectrodes overlap, and planar diffusion layer over the entire array is formed.

Proposed



AE: Auxiliary electrode

- The diffusion layer is confined near the working electrode,
and the overlapping is suppressed.

Time Dependence of the Current

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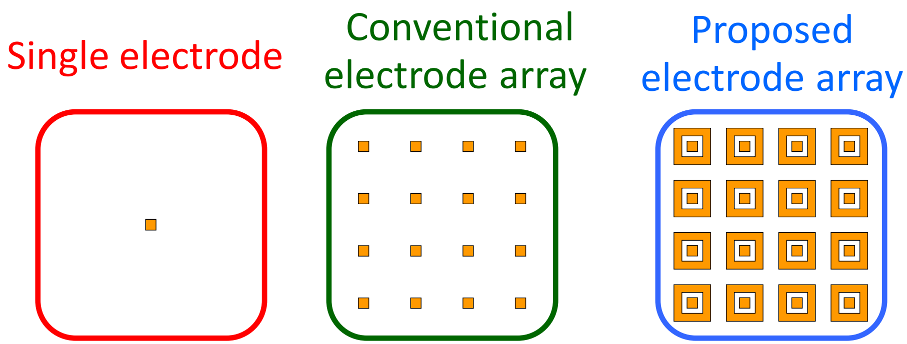
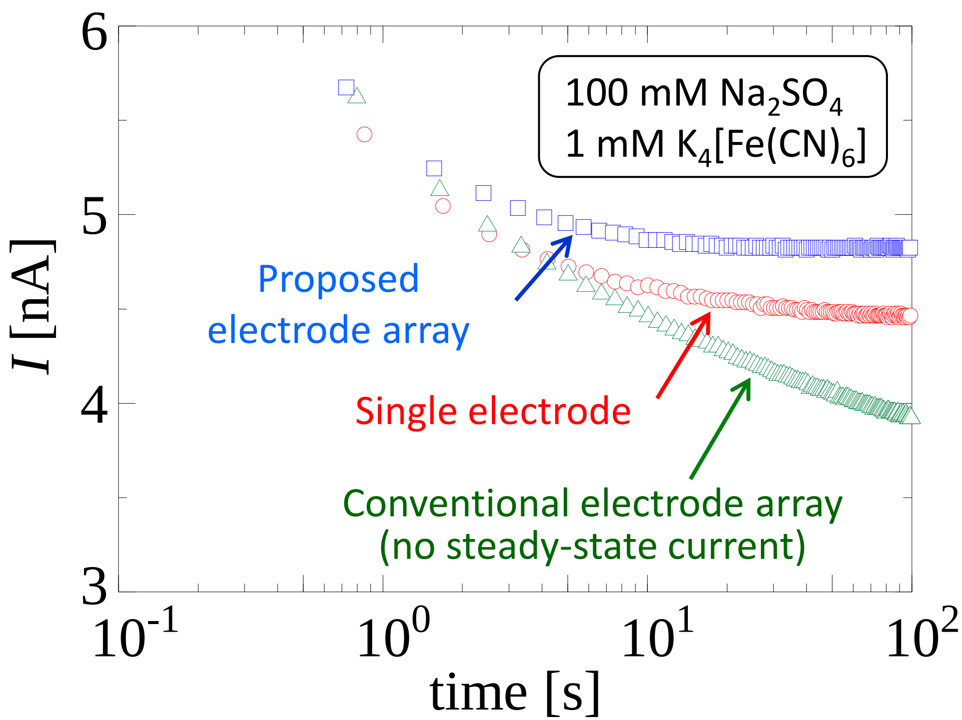
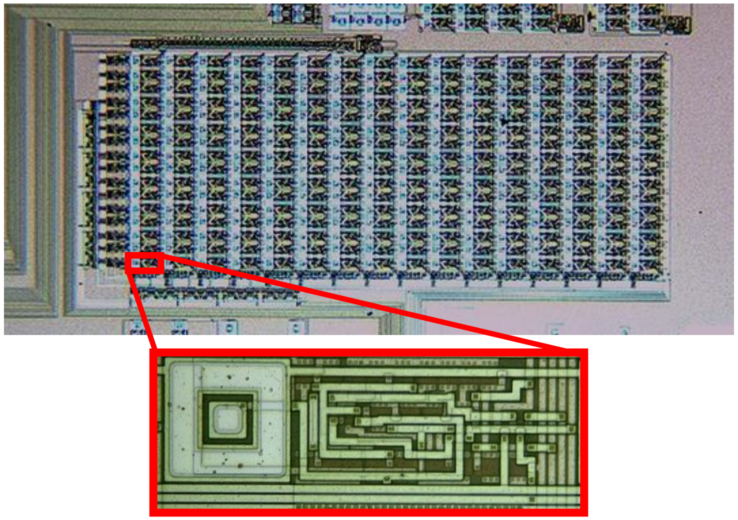
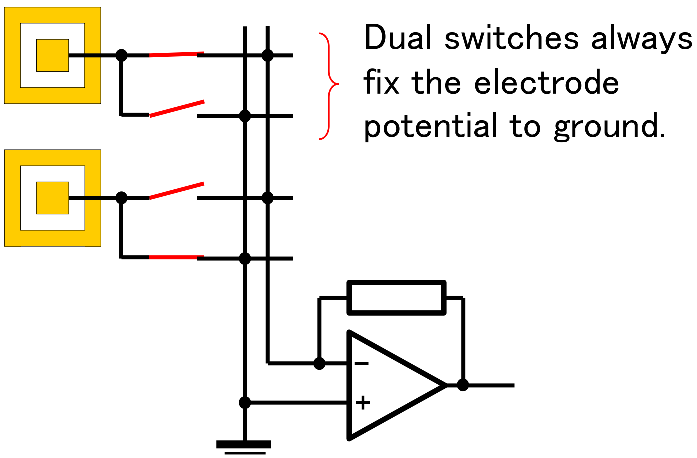




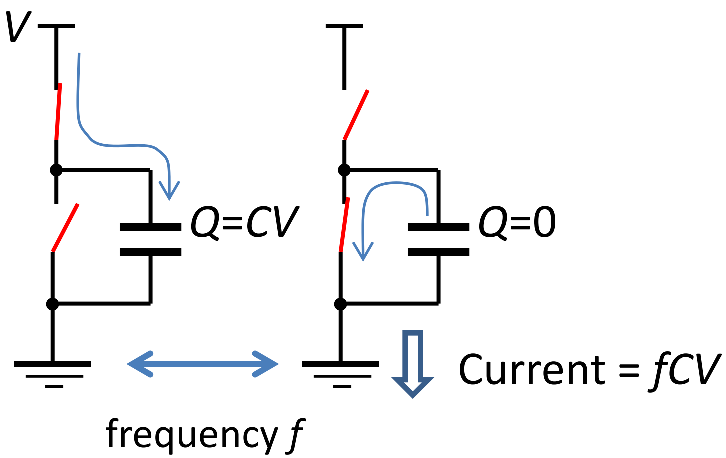


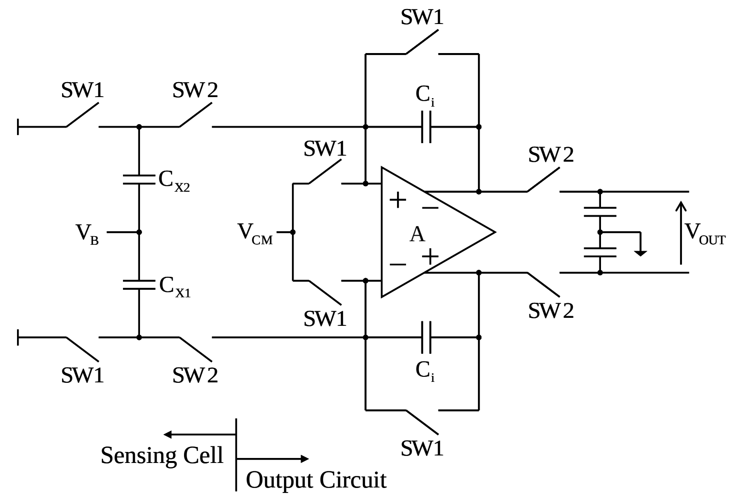


J.Hasegawa, S. Uno & K. Nakazato
Jpn. J. Appl. Phys. **50** (2011) 04DL03

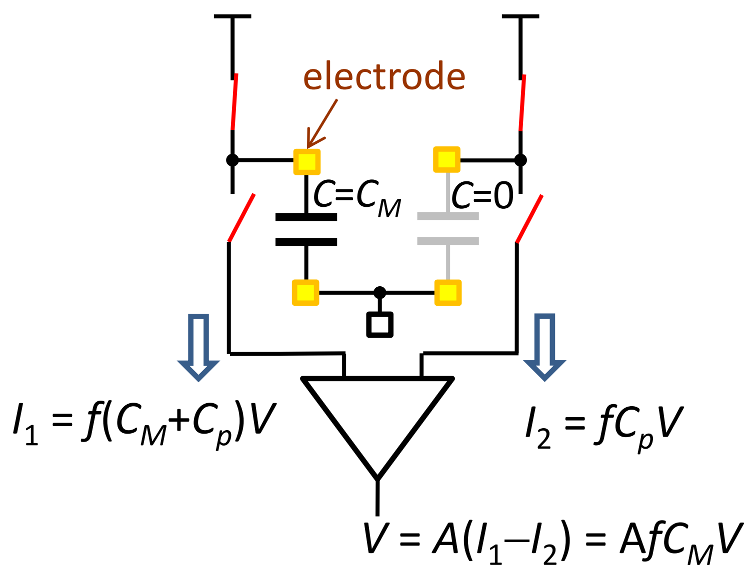
Detection of Capacitance

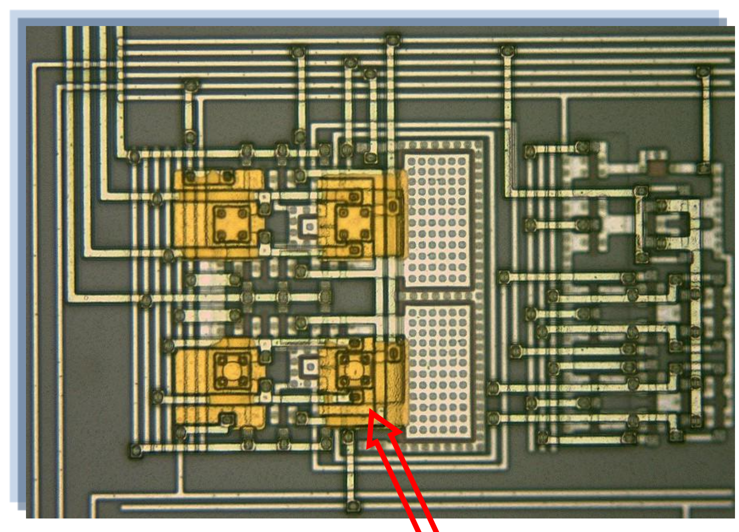
CBCM (Charge Based Capacitance Measurement)





differential method to improve the precision





electrode 4μmx4μm

Capacitive Detection of DNA

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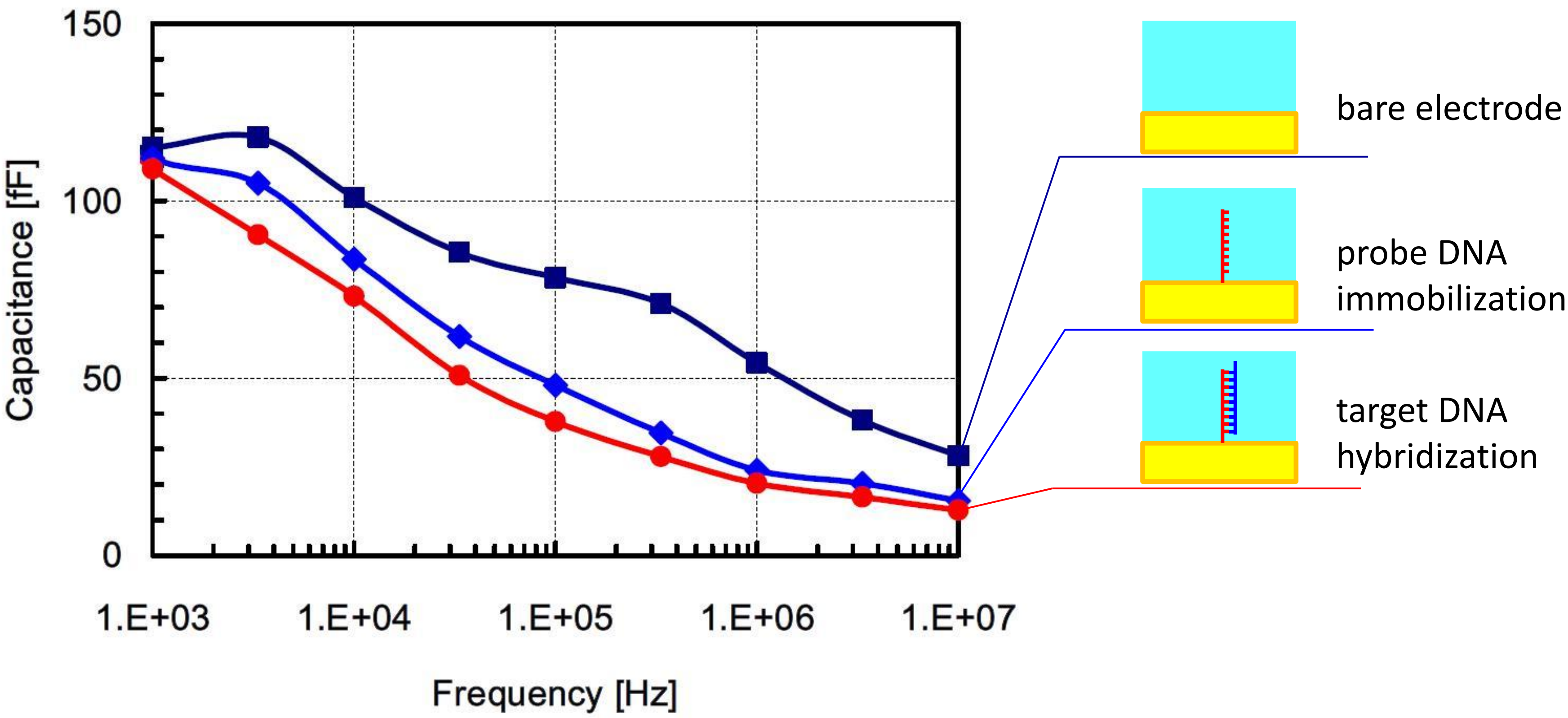

Y.B.Yusof, K.Sugimoto, H.Ozawa, S.Uno & K. Nakazato
Jpn. J. Appl. Phys. **49** (2010) 01AG05

Electrochemical Impedance Spectroscopy (EIS)

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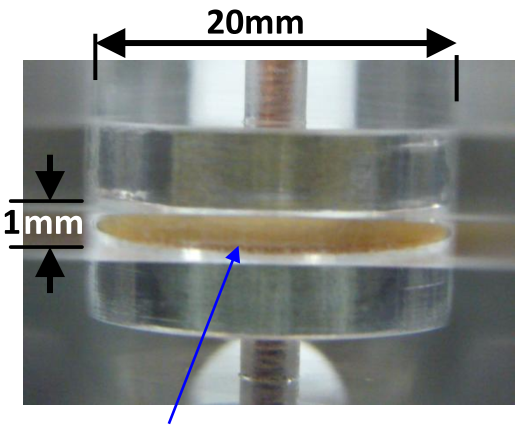
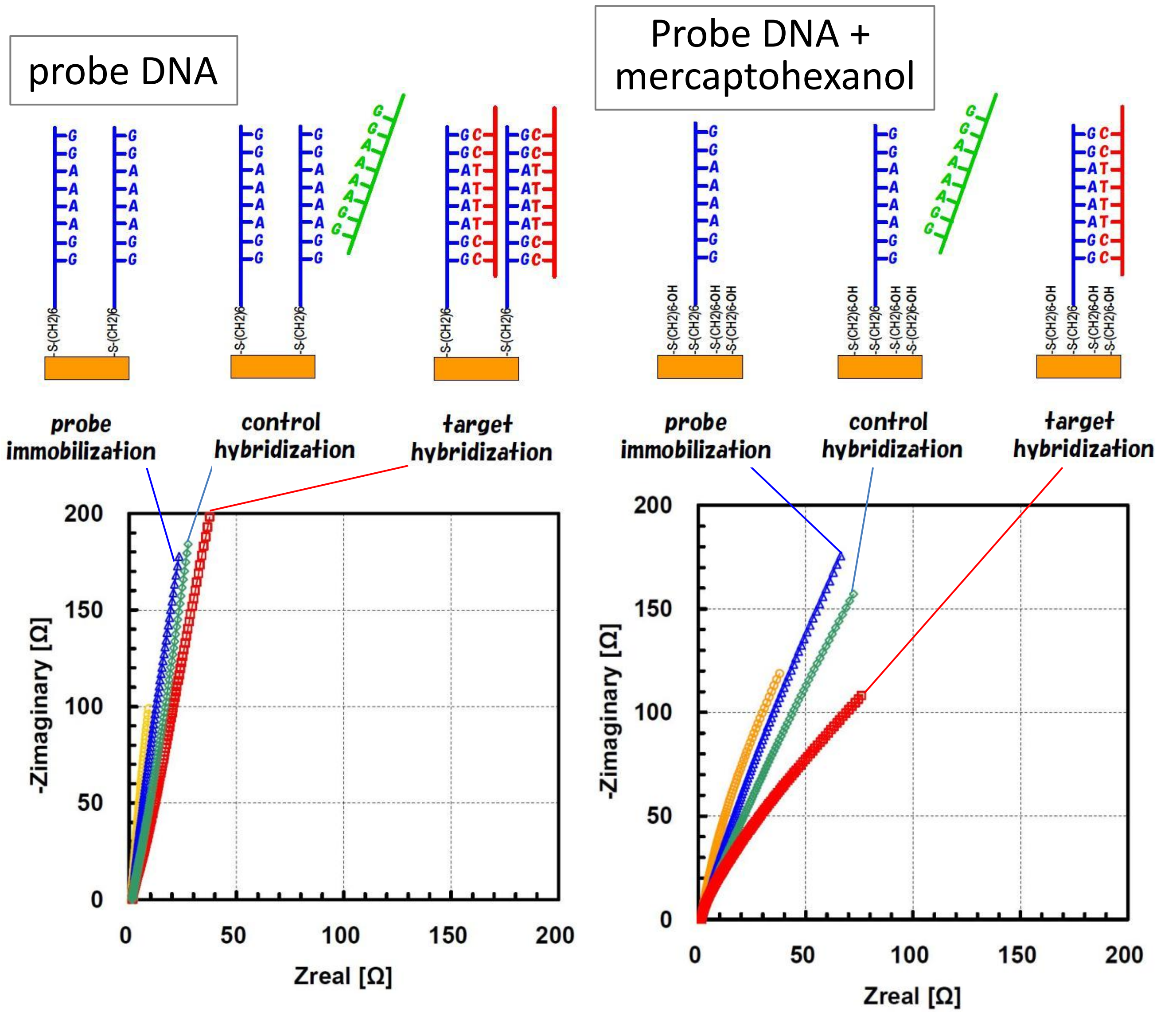


金電極



- sensitive to DNA shape
- not simple capacitance and resistance
- change of constant phase element factor α

$$Z = \frac{1}{Q(j\omega)^{\alpha}} \qquad j = \sqrt{-1}$$

$$\frac{-Z_{imaginary}}{Z_{real}} = \tan\left(\frac{\alpha\pi}{2}\right)$$

Constant Phase Element

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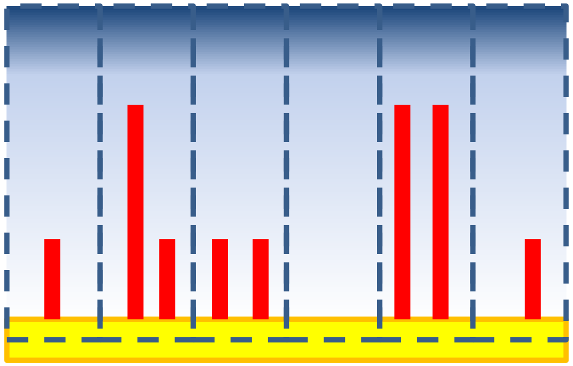
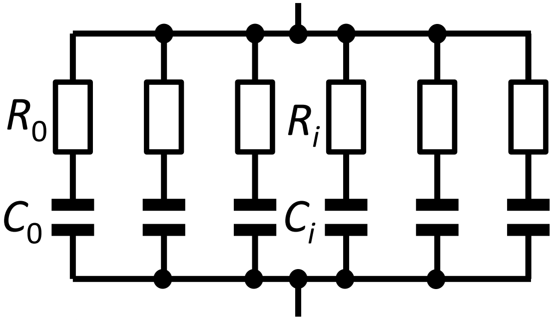
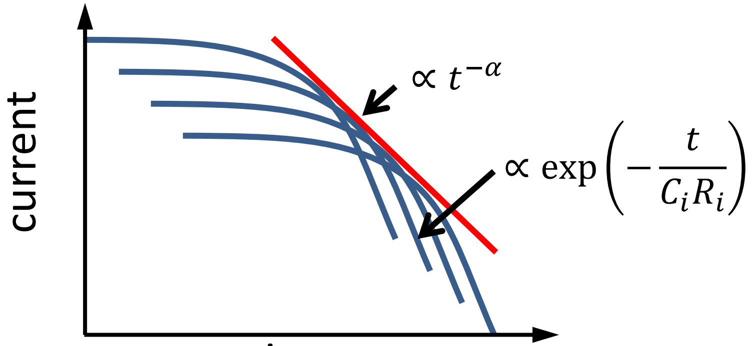
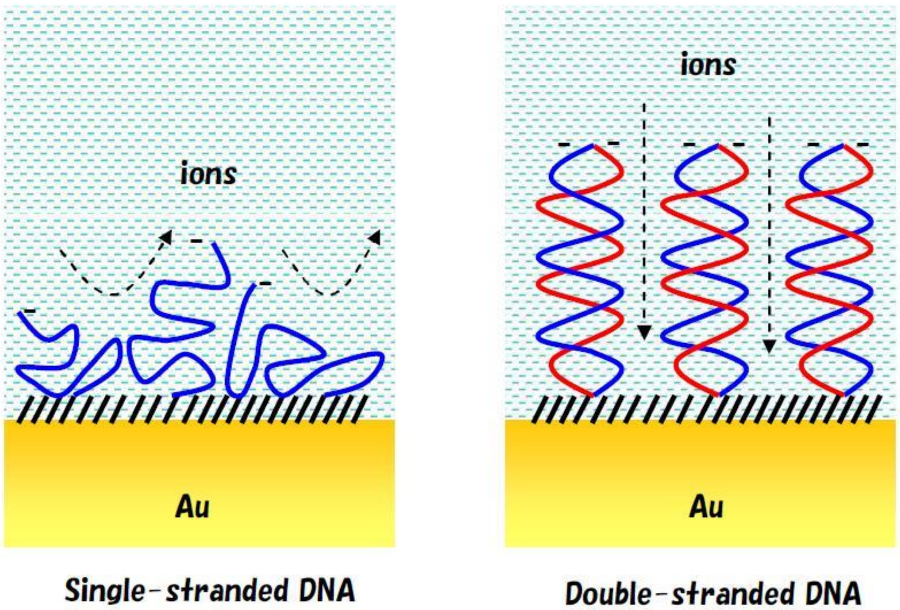
random distribution







structural change



Y.B.Yusof
ph. D thesis, Nagoya University
(October, 2011)

Impedimetric Single Molecule Detection

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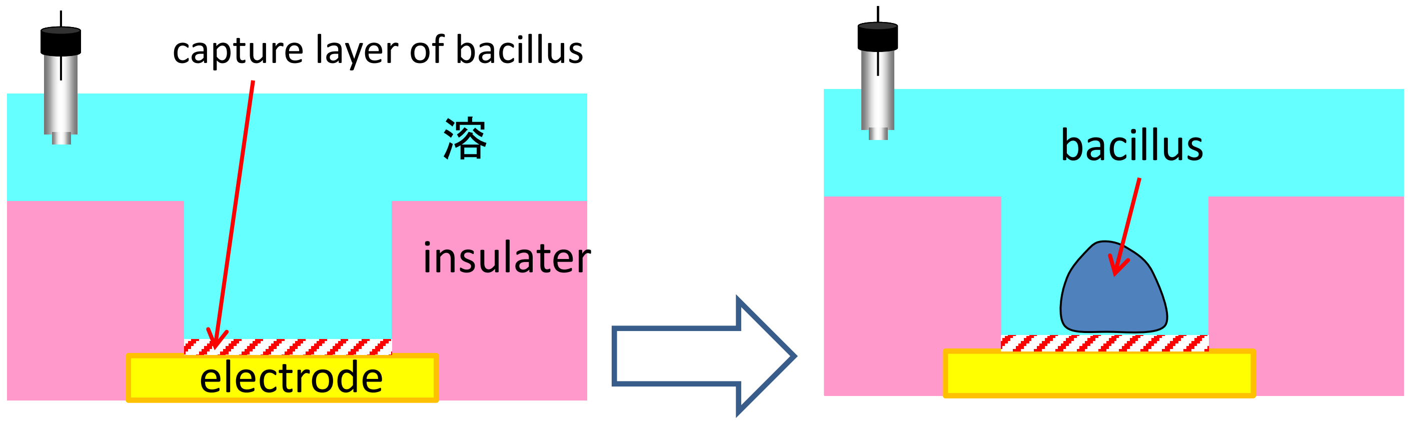
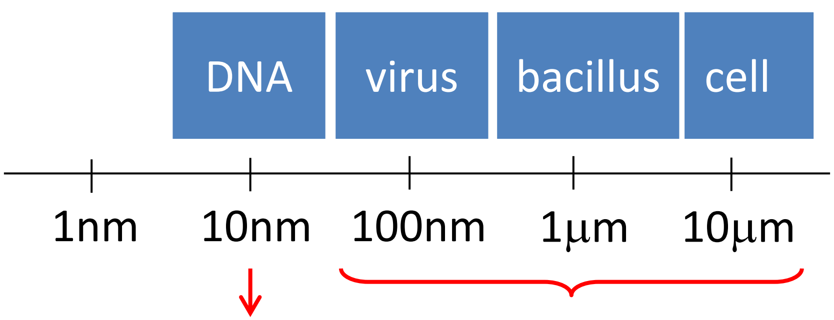


decrease of effective area

decrease of capacitance
increase of resistance

increase of impedance



nanopore

The present semiconductor technology enables electrode with molecular size.

Qualitative difference between average and single-molecule detections

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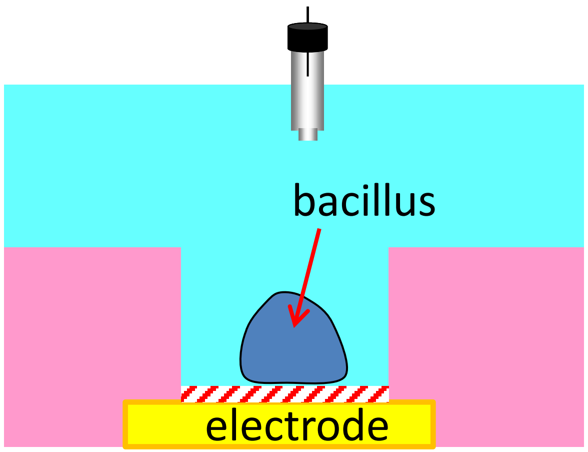
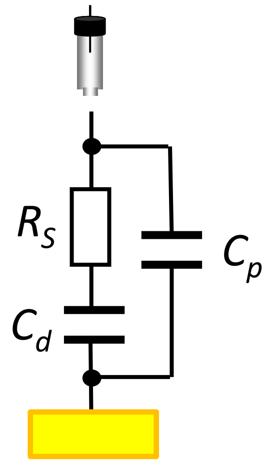
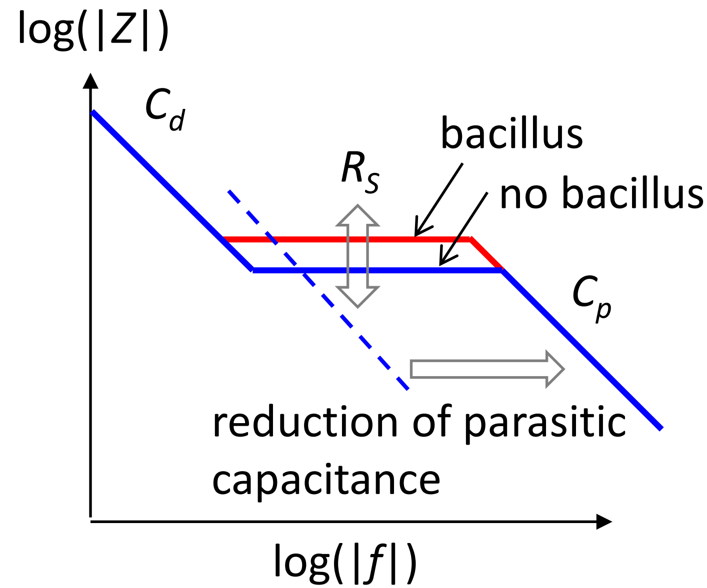


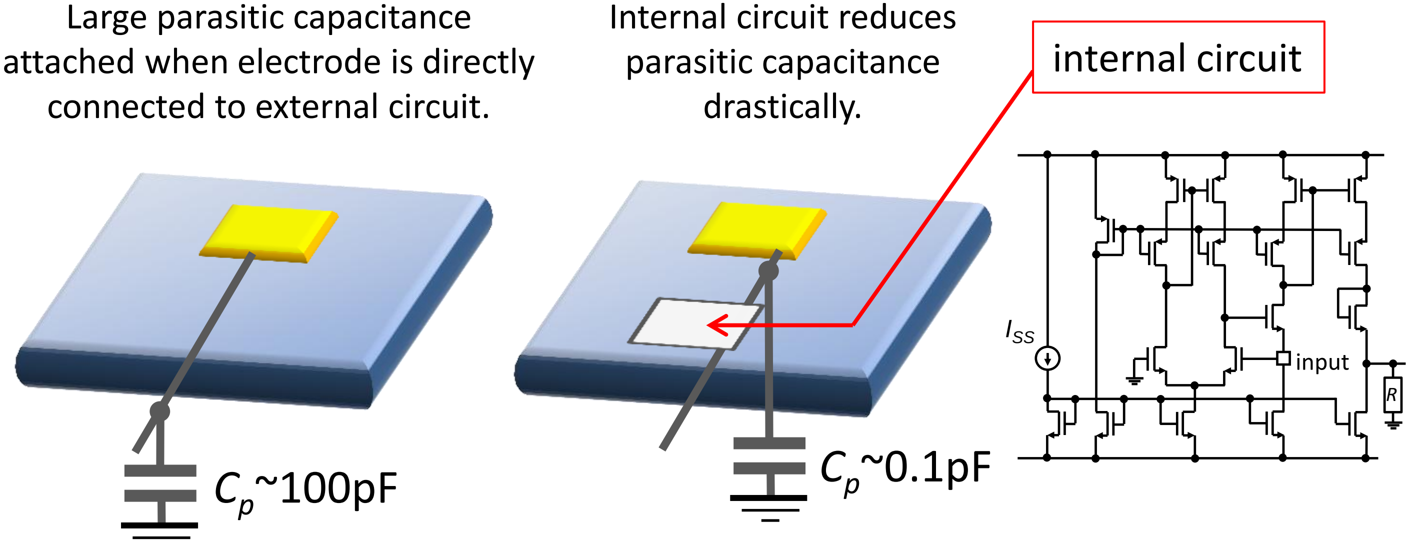


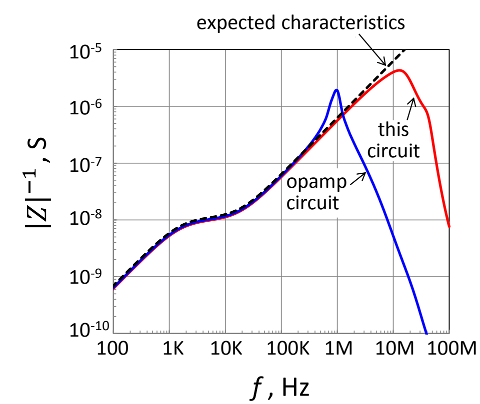
equivalent circuit



Large parasitic capacitance attached when electrode is directly connected to external circuit.

Internal circuit reduces parasitic capacitance drastically.





Bacillus Counting Chip

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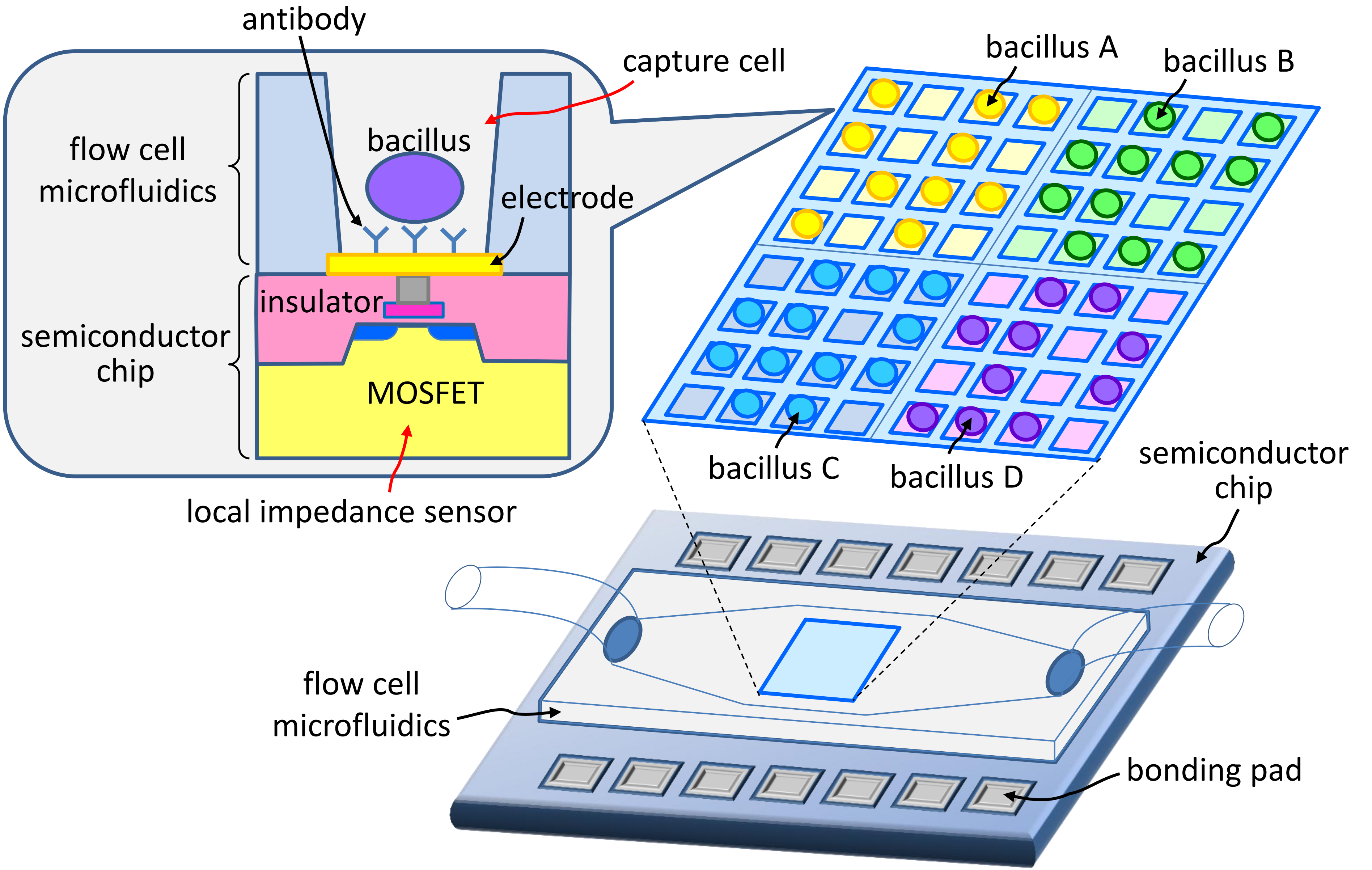
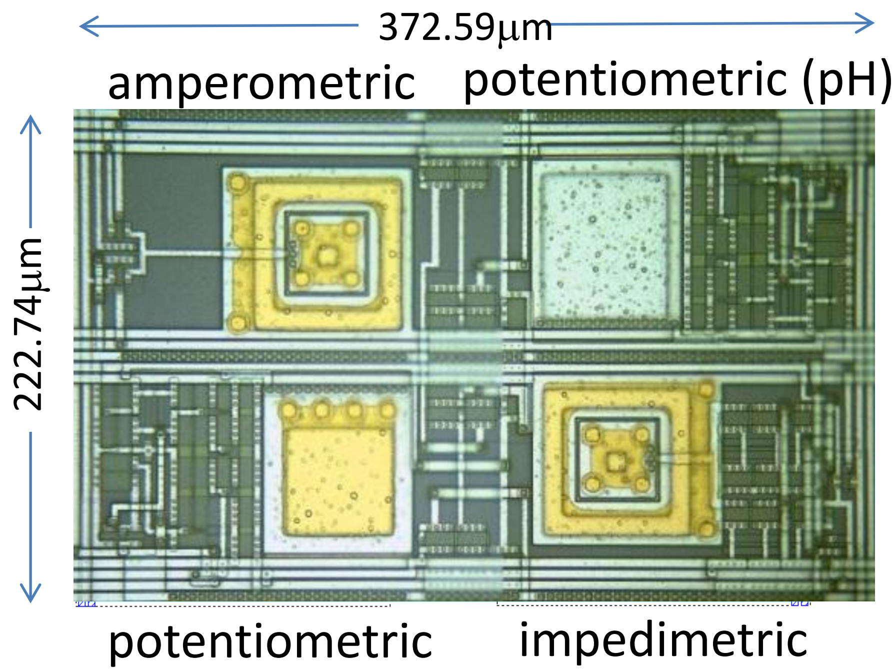


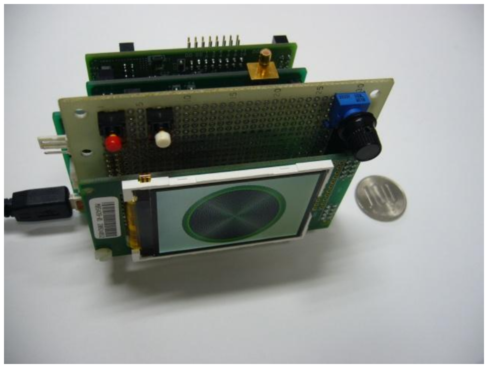
Synthetic Analysis

example of blood test

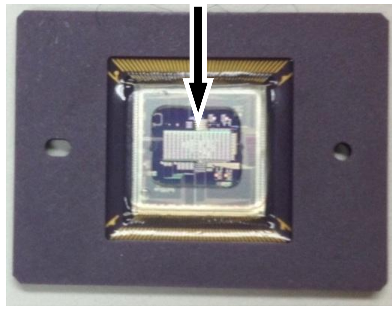
detection	symbol	sensing method
hematocrit	Hct	impedimetric
hemoglobin	Hb	amperometric
glycated hemoglobin	HbA1c	potentiometric
glucose	GLU	potentiometric



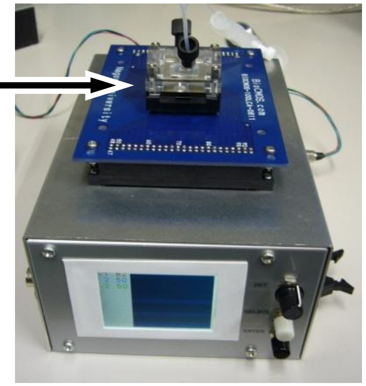
Prototype



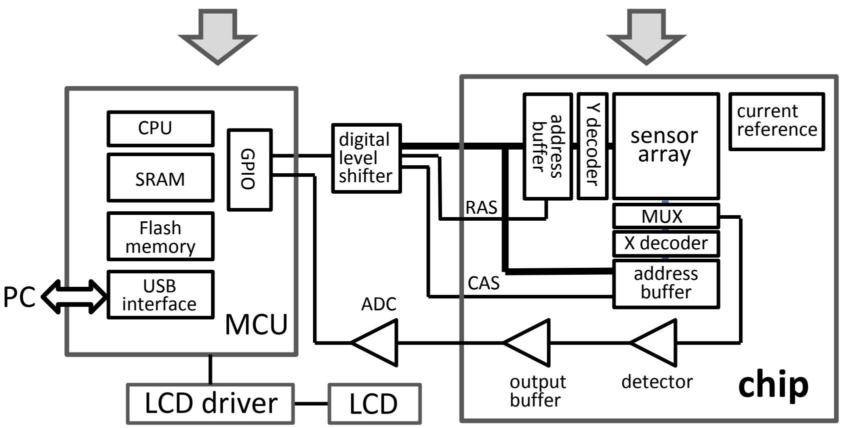
chip

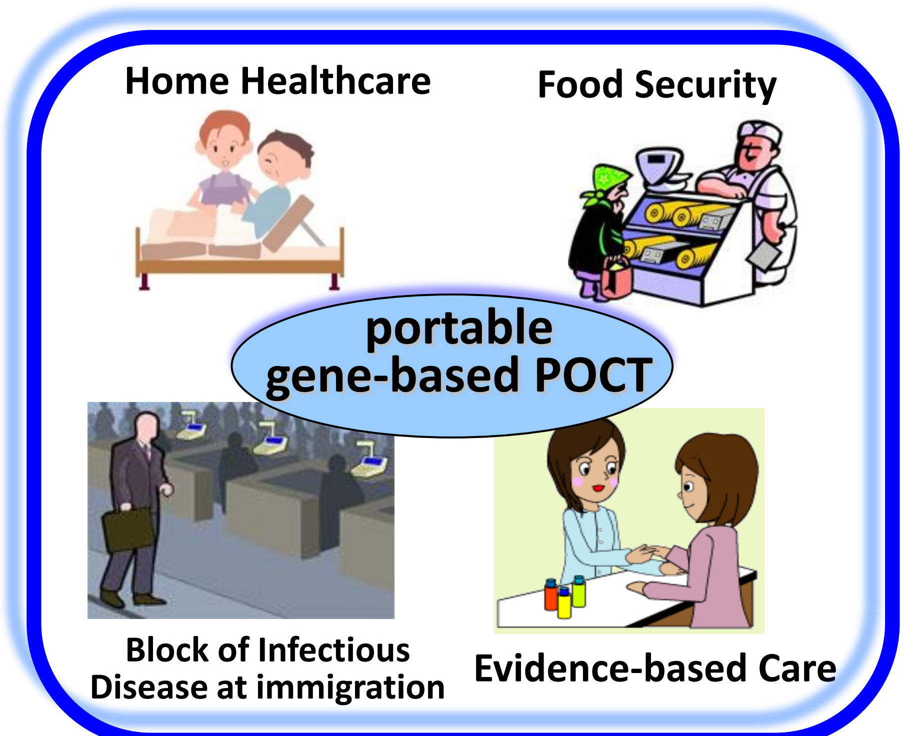


2cm



stand-alone prototype
12 cm x 18 cm x 14 cm





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