Biosensors

- Biosensing principles and figure of merits
- Optical biosensors
  1. DNA/protein microarrays
  2. Surface plasmon resonance (SPR)
  3. Surface-enhanced Raman scattering (SERS)
  4. Zero-mode waveguide
- Electrical biosensors
  1. Electrochemical (ECM) sensors
  2. Field-effect transistor (FET) sensors
  3. Nanopore sensors
- Mechanical biosensors
  1. Cantilever sensors

Fundamental Design and Operational Considerations for Affinity-Based Biosensors

Structural and design considerations	Operational considerations
Bioaffinity element properties	Sensitivity, selectivity, kinetic parameters, stability
Assay format	Homogeneous vs heterogeneous reversible, regenerable, disposable continuous, remote, *in situ* operation assay time
Sensor material	Immobilization method
Transducer type	Mechanism of signal transduction

Bioaffinity Elements for Affinity-Based Biosensors

Bioaffinity element	Types of analyte	Examples
Antibodies	Low mol-wt compounds	Drugs, hormones, environmental pollutants (pesticides, explosives, and so forth)
	Proteins	Antipathogen antibodies Toxins, insulin, serum proteins
	Microorganisms	*Candida albicans, Escherichia coil, Salmonella typhimurium, Salmonella dysenteria, Yersinia pestis*
Biological receptors -Interleukin-6 receptor -Acetylcholine receptor	Physiological ligands	
	Pharmacological ligands	Nicotine, carbamyl choline
	Toxicological ligands	Bungarotoxin
Nucleic acids	Identification of specific sequences	*Legionella pneumophila*
	Detection of intercalators	Ethidium, PAHs

Signal Transducers for Affinity-Based Biosensors

Transducer type	Assay format
Optical	
Fluorescence energy transfer	Direct
Bioluminescence	Indirect
TIRF[a]	Direct
SPR[b]	Direct
Grating coupler	Direct
Electrochemical	
Potentiometric	Indirect, direct
Amperometric	Indirect
Conductimetric	Indirect
Thermal	Indirect
Acoustic	Direct
QCM[c]	

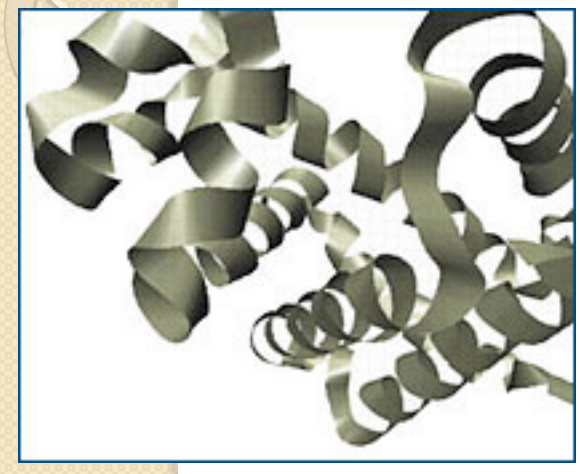
[a]Total internal reflectance fluorescence
[b]Surface plasmon resonance
[c]Quartz crystal microbalance

Types of Applications

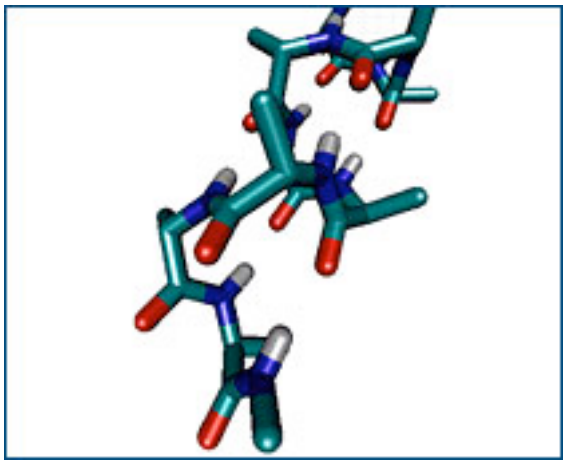
- Medical diagnostics (pathogens, diseases)
- Drug target discovery
- Forensics
- Food and environmental
- Genomic/Proteomic research
  - DNA analysis
  - mRNA analysis
  - Protein analysis
  - Disease-Gene association
  - Pharmacogenomics /pharmacogenetics

Monitor molecular interactions

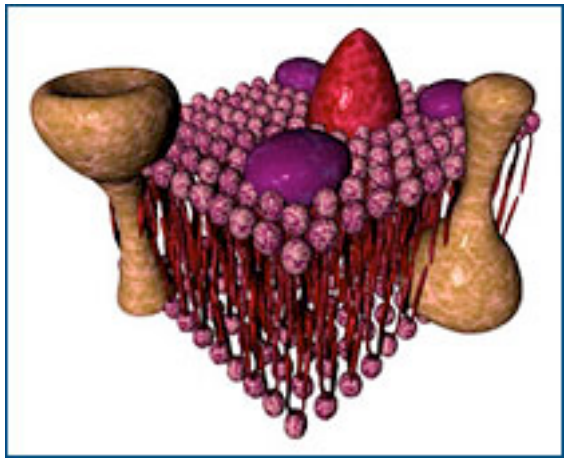
Protein interactions



Small molecules



Membrane proteins

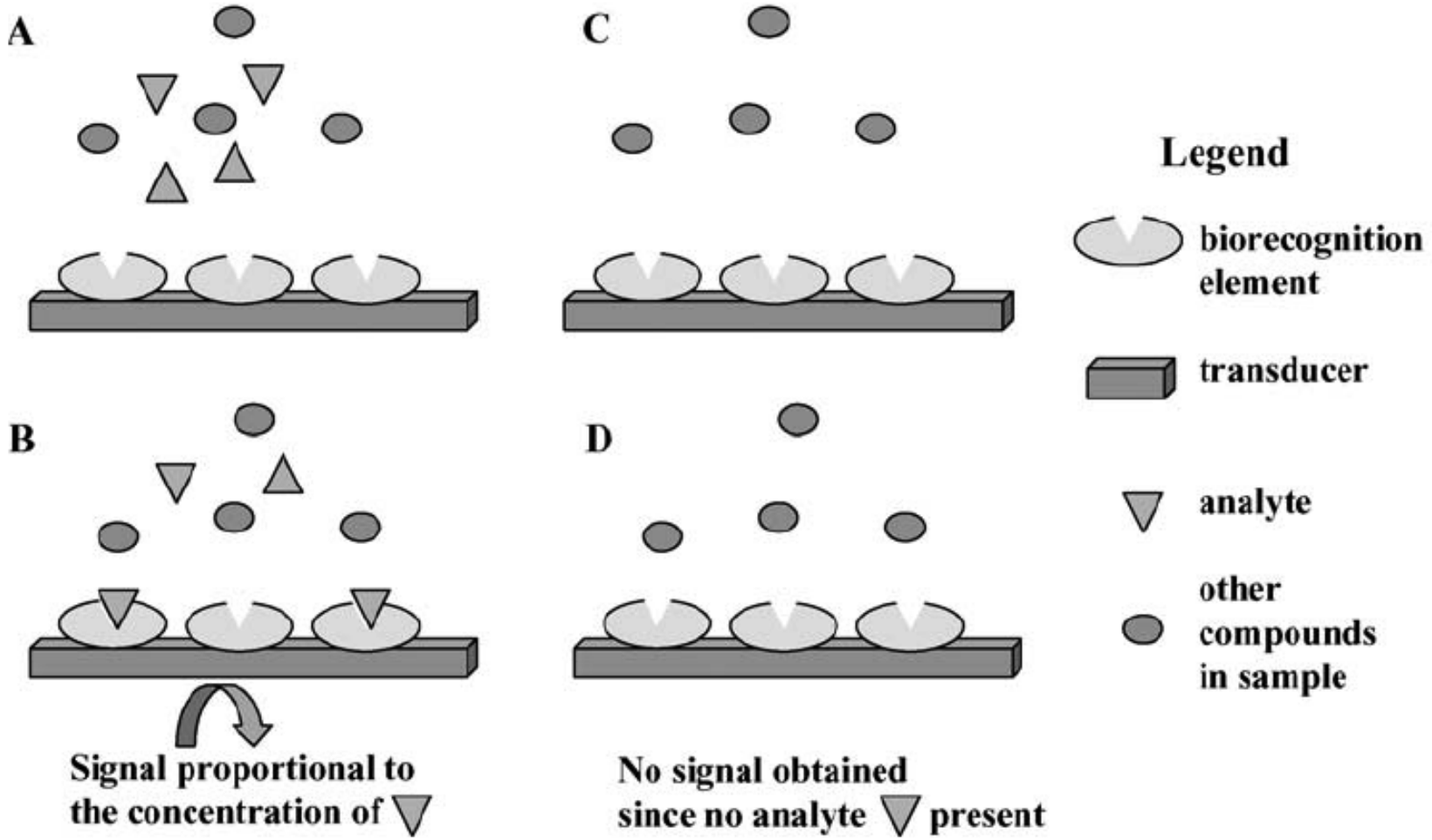


Nucleic acids

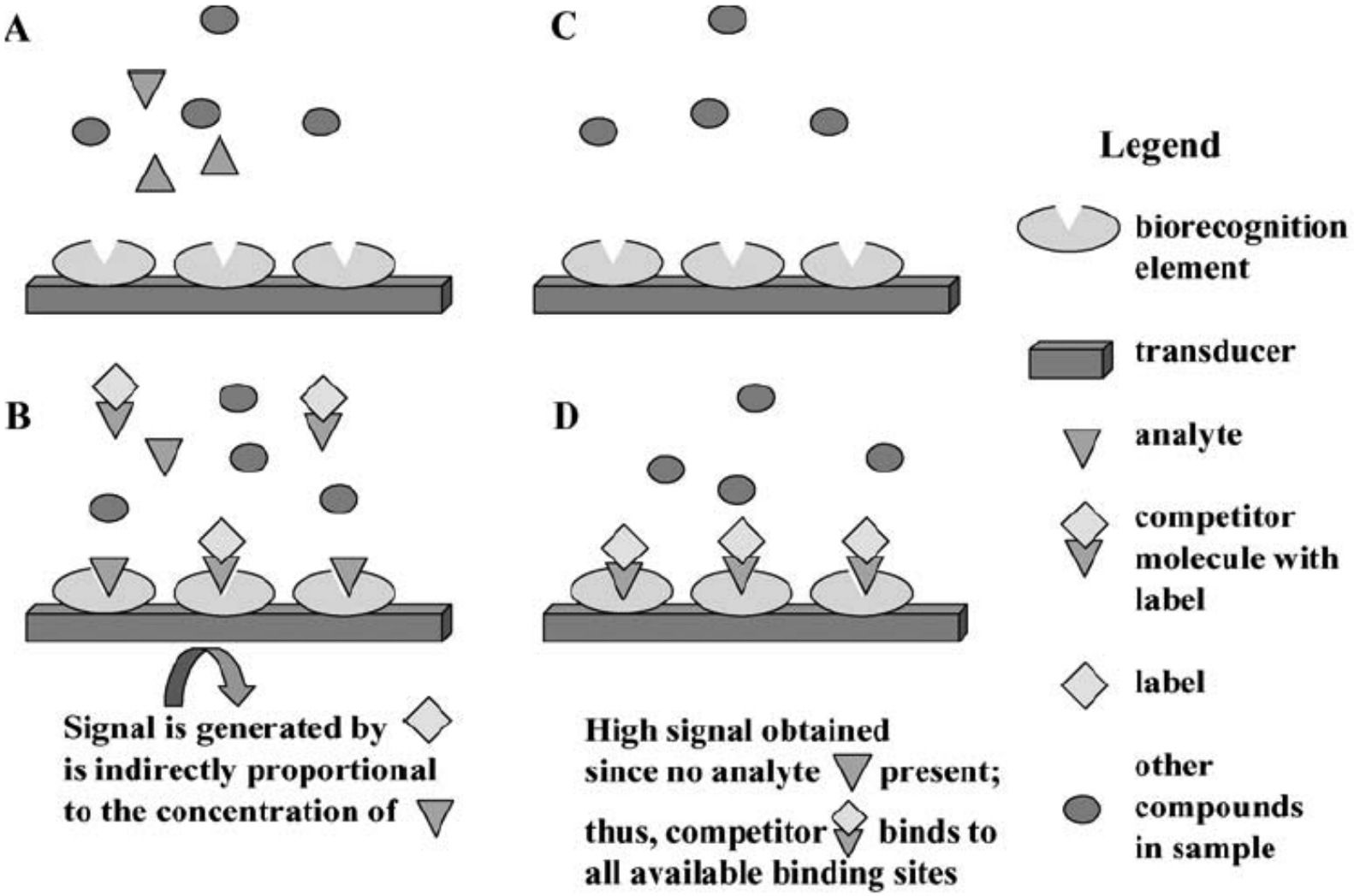
Cell and viruses

Carbohydrates

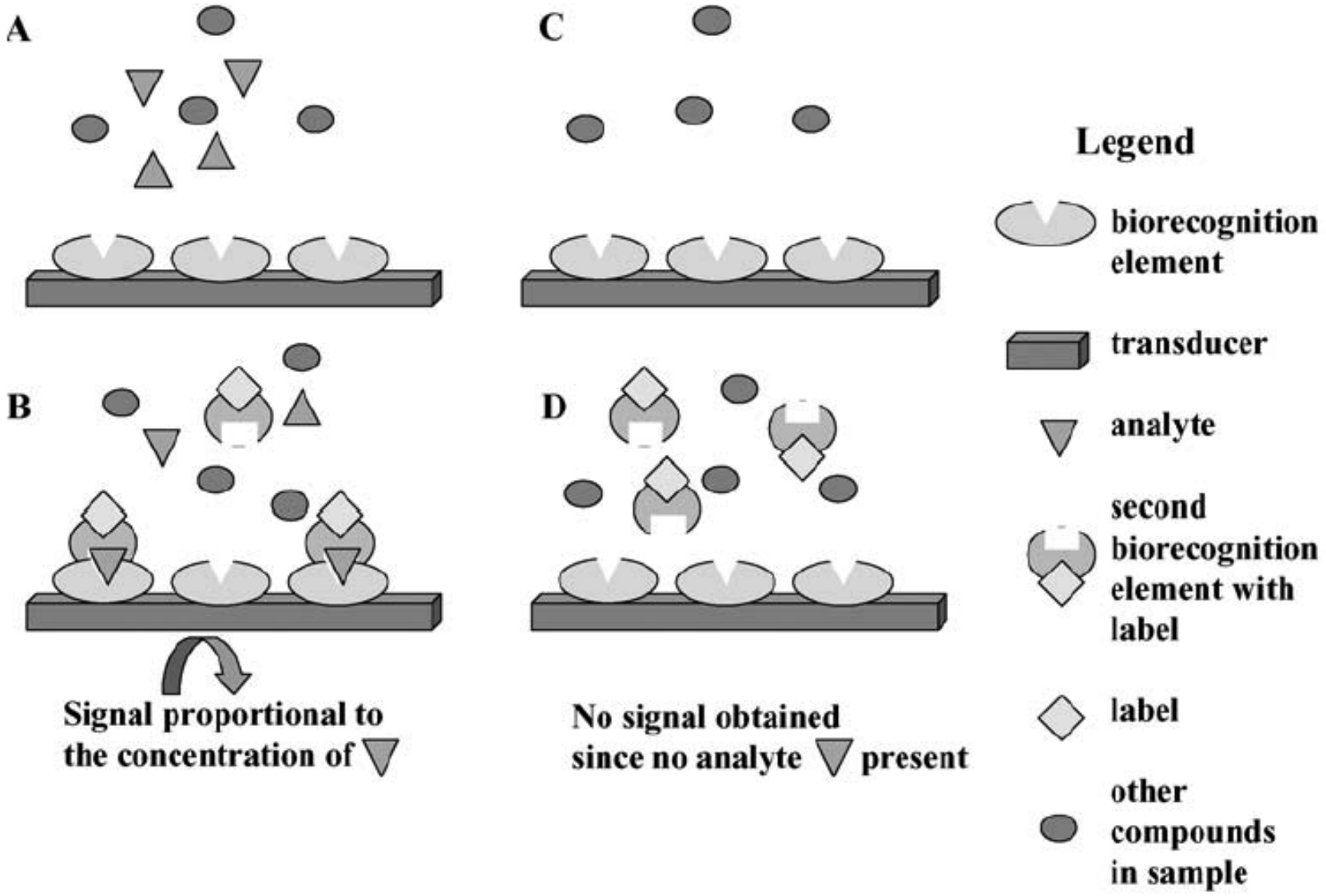
Direct assay format of biosensors



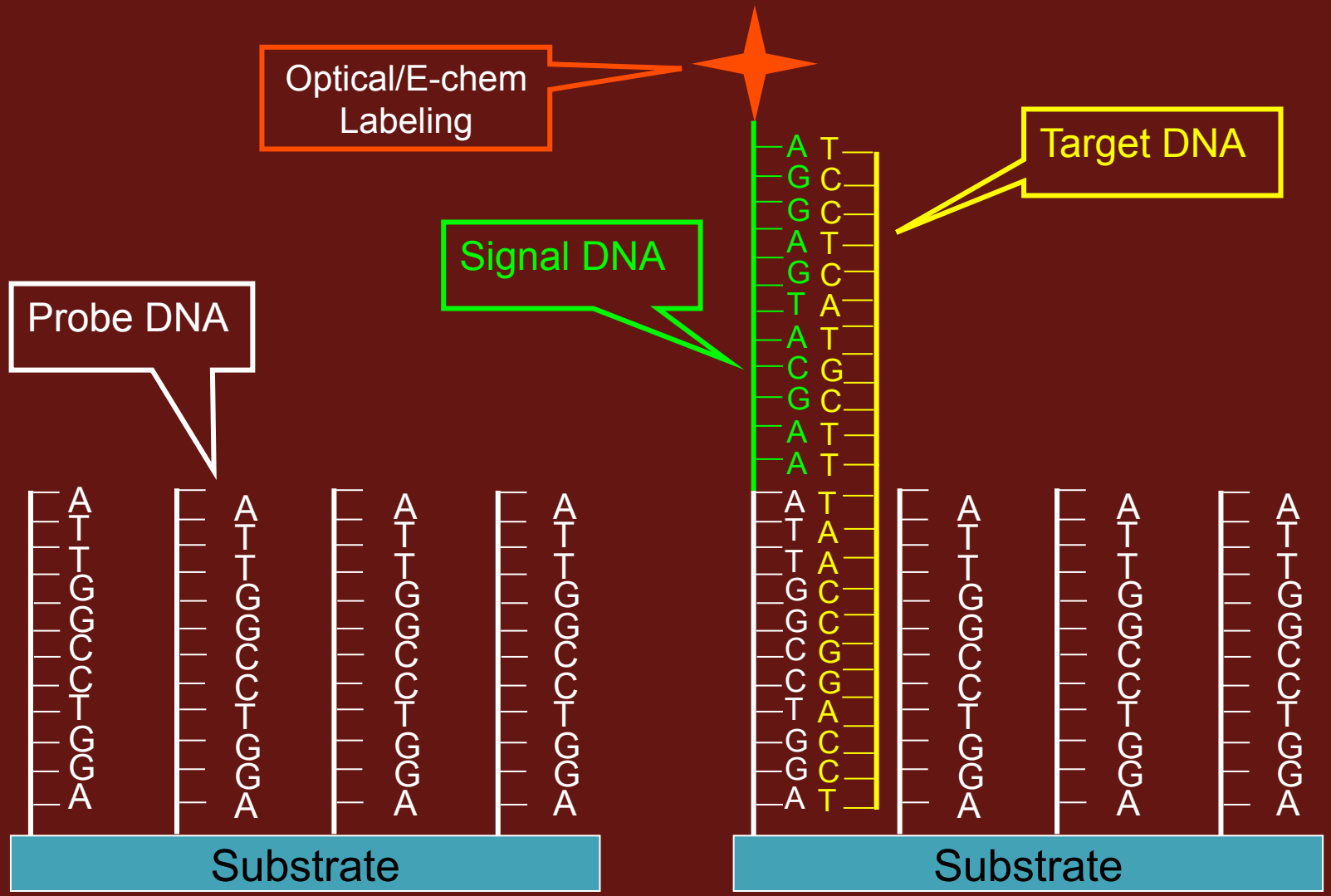
Indirect assay format with competitive binding



Indirect assay format with non-competitive binding



Principles of DNA Hybridization & Sensing



Different DNA hybridization array formats

	Probe generations method	Array size	Labeling and detection method	Hybridization method	Commercial suppliers
Microarrays [5]	Robotic printing or piezoelectric inkjet printing of PCR products	2.5 cm by 7.5 cm slide with approximately 10 000 genes	Fluorescent tag labeling prior to hybridization; fluorophore added after hybridization and washing	Passive	Agilent Technologies, Genometrix, Operon Technologies, Stratagene
Oligonucleotide arrays [6]	*In-situ* on the surface of the matrix	1 cm by 1 cm slide with approximately 40 000 genes; Affymetrix's GeneChip can contain up to 400 000 different oligonucleotides and is the densest array	Fluorescent tag labeling; fluorophore detector is added after hybridization	Passive	Affymetrix
Macroarrays [7]	Probes are spotted onto nylon, plastic or nitrocellulose solid matrix	8 cm by 12 cm with approximately 200 to 5000 genes	Radioactivity tag labeling; phosphorimager detector	Passive	Clontech Laboratories, Research Genetics
Microelectronics arrays [8]	Probes are drawn by electric current to chip surface	Number of genes is dependent on the number of electrodes that can be made onto the surface of the array	Fluorescent tag labeling and fluorescent detection	Active	Nanogen

Different array generation approaches

	Spatial resolution	Cost	Probe length	Ease of use
Robotic microprinting	Poorest	Most cost effective	Not restricted	Requires cloning and PCR steps
Photolithography	Highest	Highest as expensive equipments and particular expertise are required	Limited to 25-mers or less	Photolithography method is protected by patent and currently only Affymetrix has the rights to use this method
Inkjet printing	In between robotic printing and photolithography	In between robotic printing and photolithography	5–75-mers	Equipments need strict maintenance and experiment must be performed in a clean and uncontaminated environment

C M Li et al., in ELECTROCHEMICAL SENSORS, BIOSENSORS AND THEIR BIOMEDICAL APPLICATIONS (2008 by Academic Press, Inc.): Ch. 11

The first GeneChip (late 1980's)

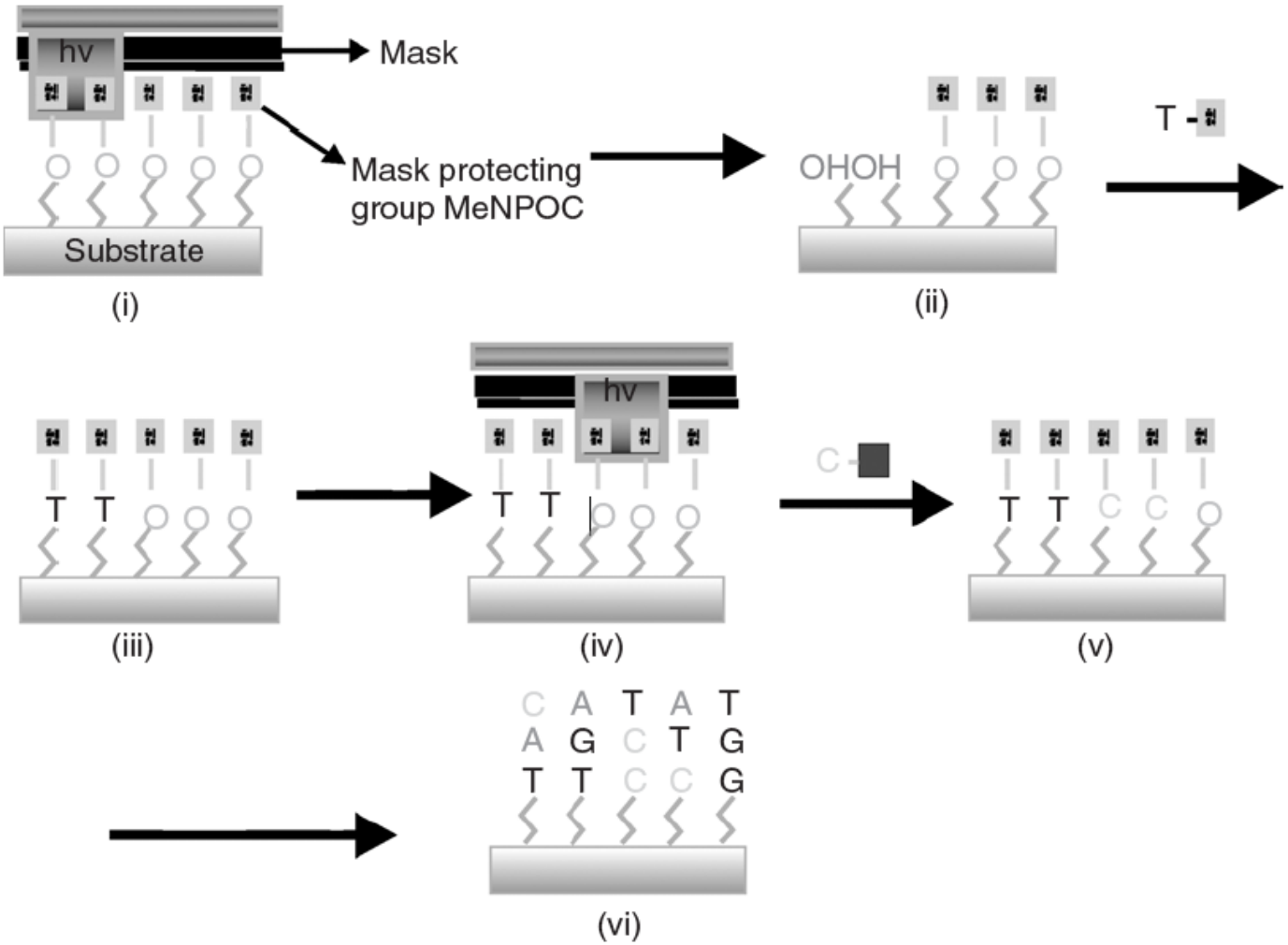
Revolutionary idea:
Semiconductor manufacturing techniques could be united with advances in combinatorial chemistry to build vast amounts of biological data on a small glass chip.

By Stephen P.A. Fodor, Ph.D., Affymetrix Founder, Chairman and CEO

Source: www.affymetrix.com

The first GeneChip (late 1980's)

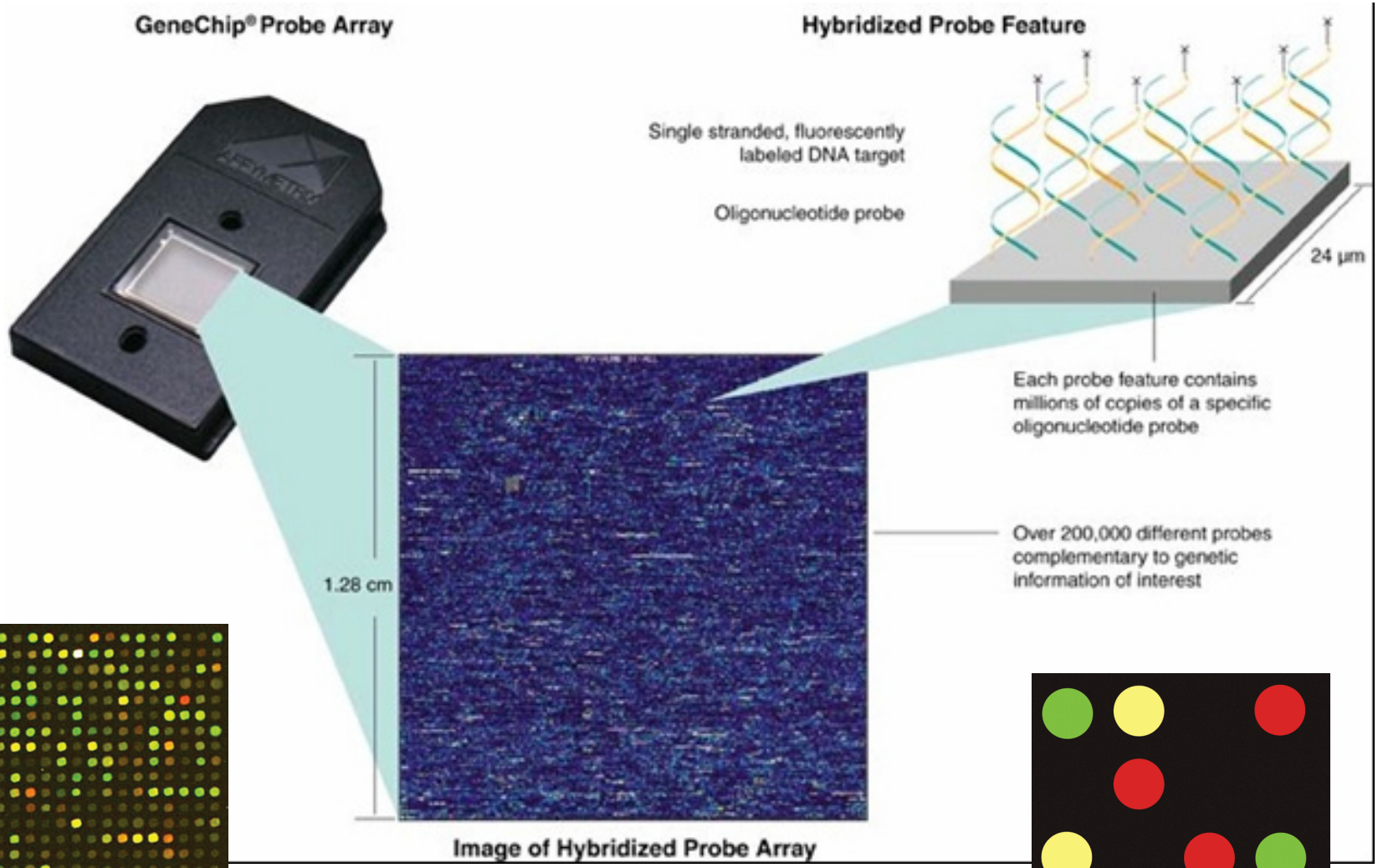
Photolithographic synthesis of oligonucleotide probe arrays

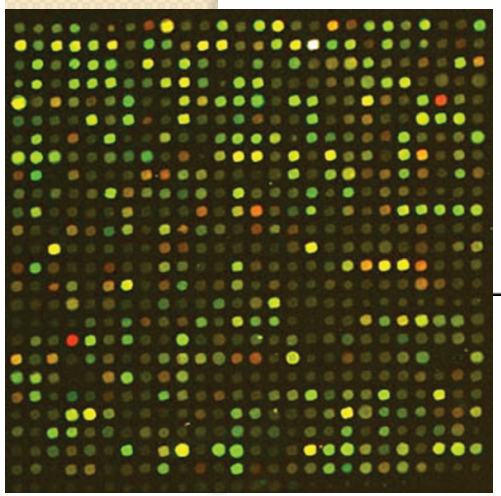


Source: www.affymetrix.com

Source: www.affymetrix.com

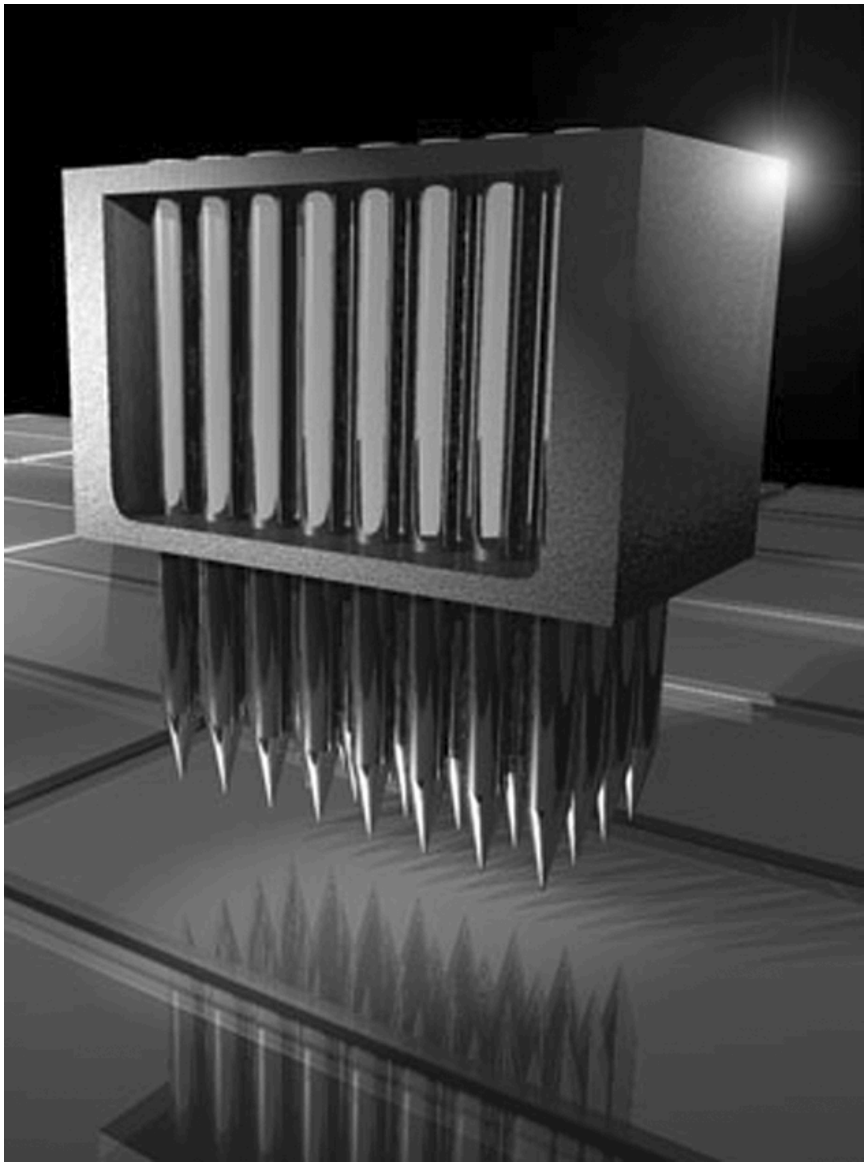
Affymatrix GeneChip





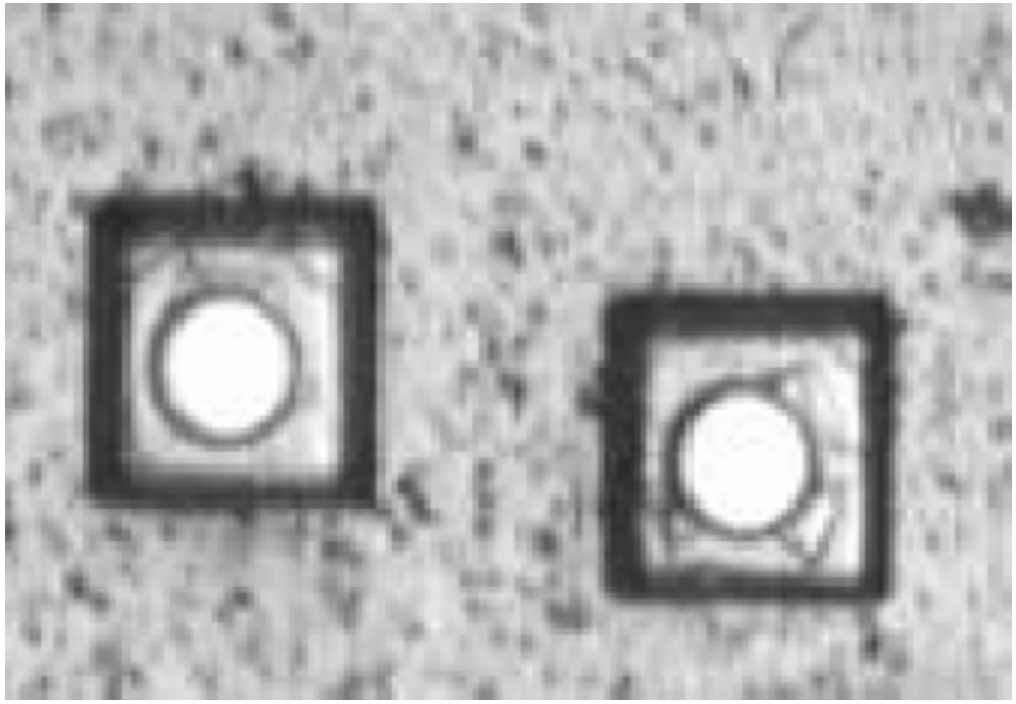
Ideal microarray spots

Printhead with a series of pins



Source: http://cmgm.stanford.edu/pbrown/

Biochip Fabrication by inkjet/piezoelectric methods



Orifice plate with 40 µm diameter orifices

Data from inkjet printing method

Dispense volume	Spot sizes	Spot densities	Delivery speed
50 pL	125–175 µm	500–2500 spots/cm^2	100–500 spots/s

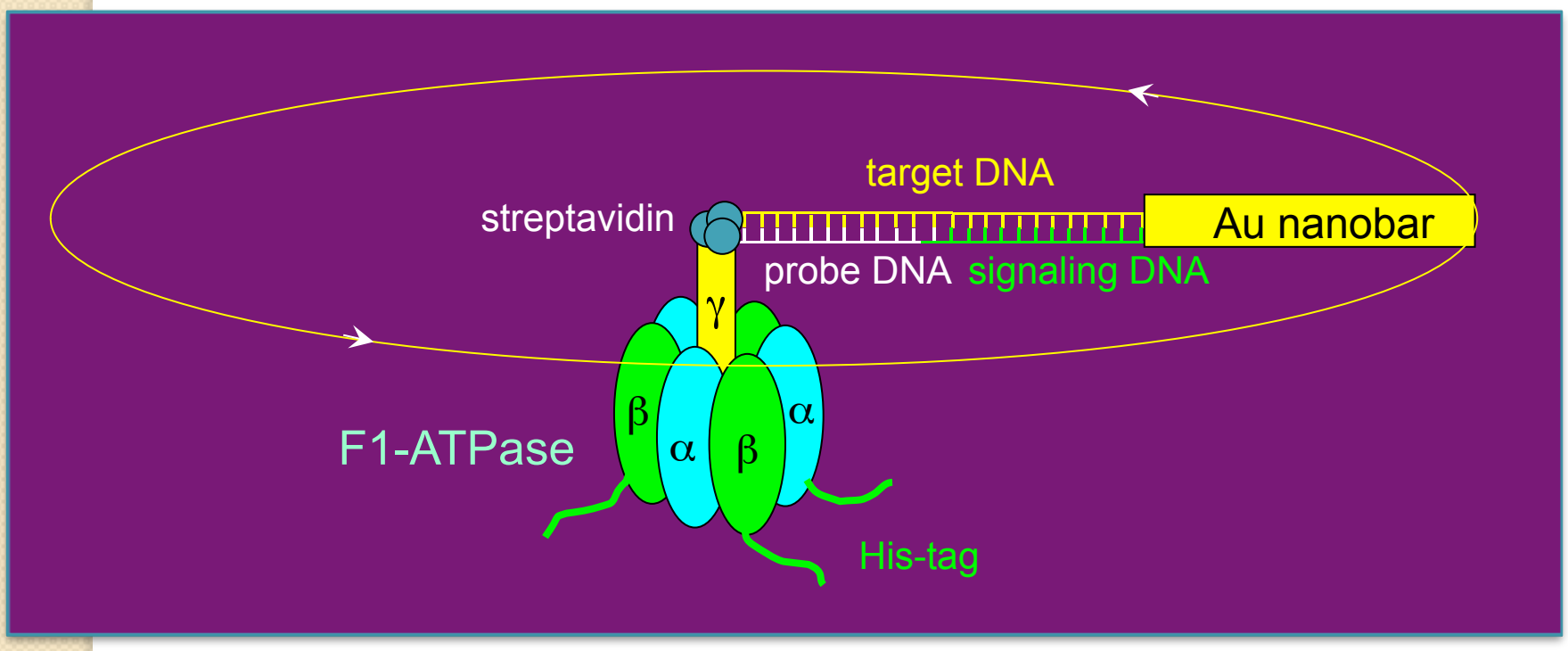
Detection limits of various techniques for DNA hybridization

Detection method	Detection limit (concentration of target molecules)	Sample volume	Detection limit (no. of hybridized target molecules)	Refs
Flourescence	5 pM	10–50 μl	10^3 per 100 x 100 μm spot	Taton et al., 2000; Duggan et al., 1999
"Scanometric" (nanoparticle-based)	50 fM			Taton et al., 2000
Surface plasmon resonance (label-free)	10 nM		6×10^8 per 500 x 500 μm spot	Nelson et al., 2001
Surface plasmon resonance (Au-amplified)	10 pM			He et al., 2000
Dye-containing liposomes	220 pM		6×10^8	Rule et al., 1996
BARC sensor (magnetic beads)	100 fM (using optical detection)			Edelstein et al., 2000
Microcantilever deflection	400 nM		10^{10}	Fritz et al., 2000
Molecular beacons	100 pM	10 μl		Steemers et al., 2000
electrochemical	100pM-100 fM	500 μl	10^8 per 100 μm pad	Umek et al., 2001 Motorola Life sciences data
Optical interference	10 fM	10–25 μl		Jenison et al., 2001

Biomotor-based single molecular sensors

Detecting Specific DNA Sequences

"Molecular Semaphore"



C.F. Chou *et al.*, US patent 6,989,235 (2006)

Optical Detection

- Optical detection is less interfered by impurities than other detection methods such as electrochemical sensors, and allows for higher specificity.
- Nano optical biosensors employ the interaction between biomolecules and light confined in or emitted from nanometer scale structures to report the bio-recognition events.

Why Nano Optical Sensors

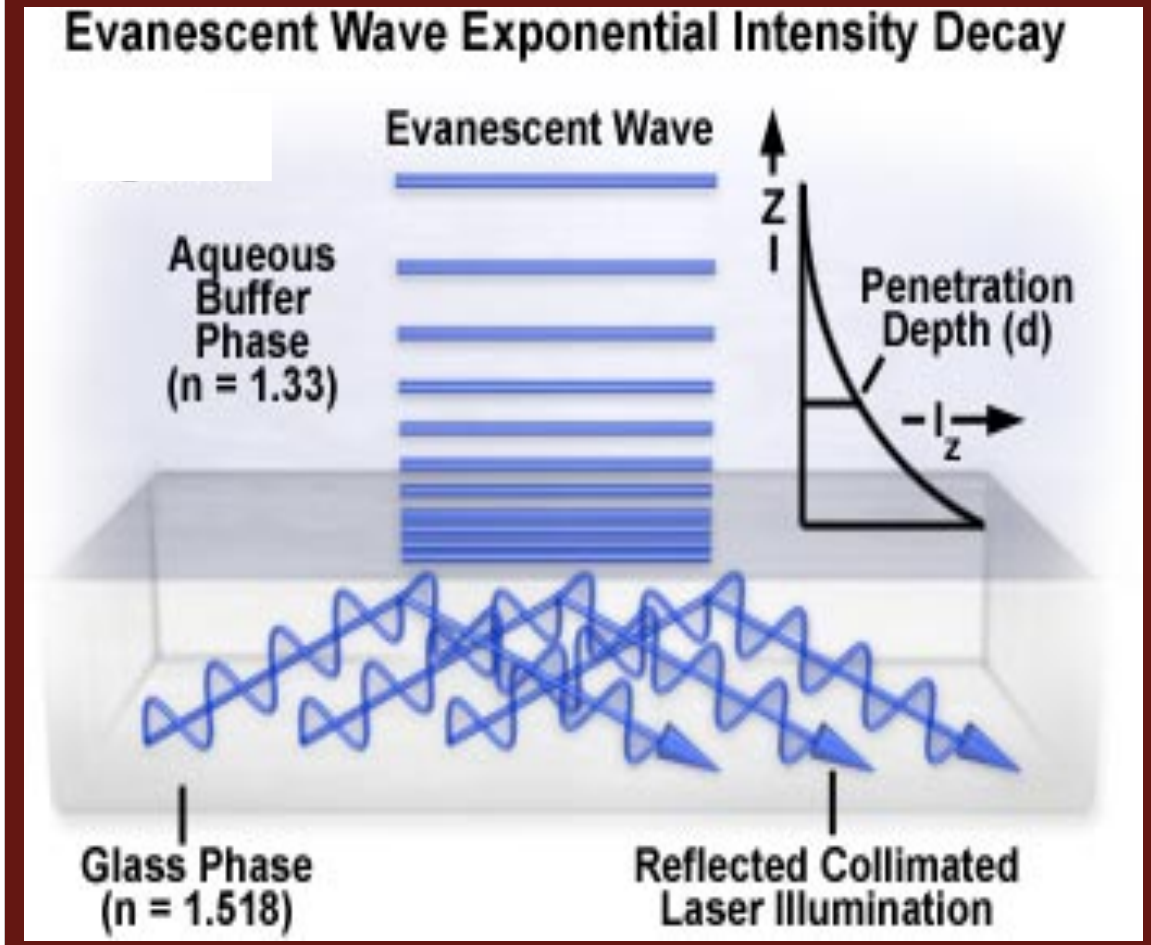
- Reduced excitation volume → minimized background noise, and therefore increased S/N ratio
- Enhanced local field → enhanced optical signals, and thus superior sensitivity up to single molecule level

Nano Optical Biosensors

- Manipulation and confining of light at sub-wavelength length scale by engineering surface plasmons
- Biomolecular signal amplifications
- Unique opportunities for interfacing with biomolecules especially proteins and drug molecules at extremely small spatial scales, for practical applications such as biosensing, single molecule kinetics, and drug safety and efficacy studies

a. NanoPair – DNA sensor
b. NanoBurger— Engineered Hotspots for Protein SERS
c. Nanopore sensors

Total Internal Reflection Fluorescence Microscopy (TIRFM)



$I(z) = I_o \exp(-z/d)$

$d = \lambda_o / 4\pi \cdot (n_1^2 \sin^2\theta_1 - n_2^2)^{-1/2}$

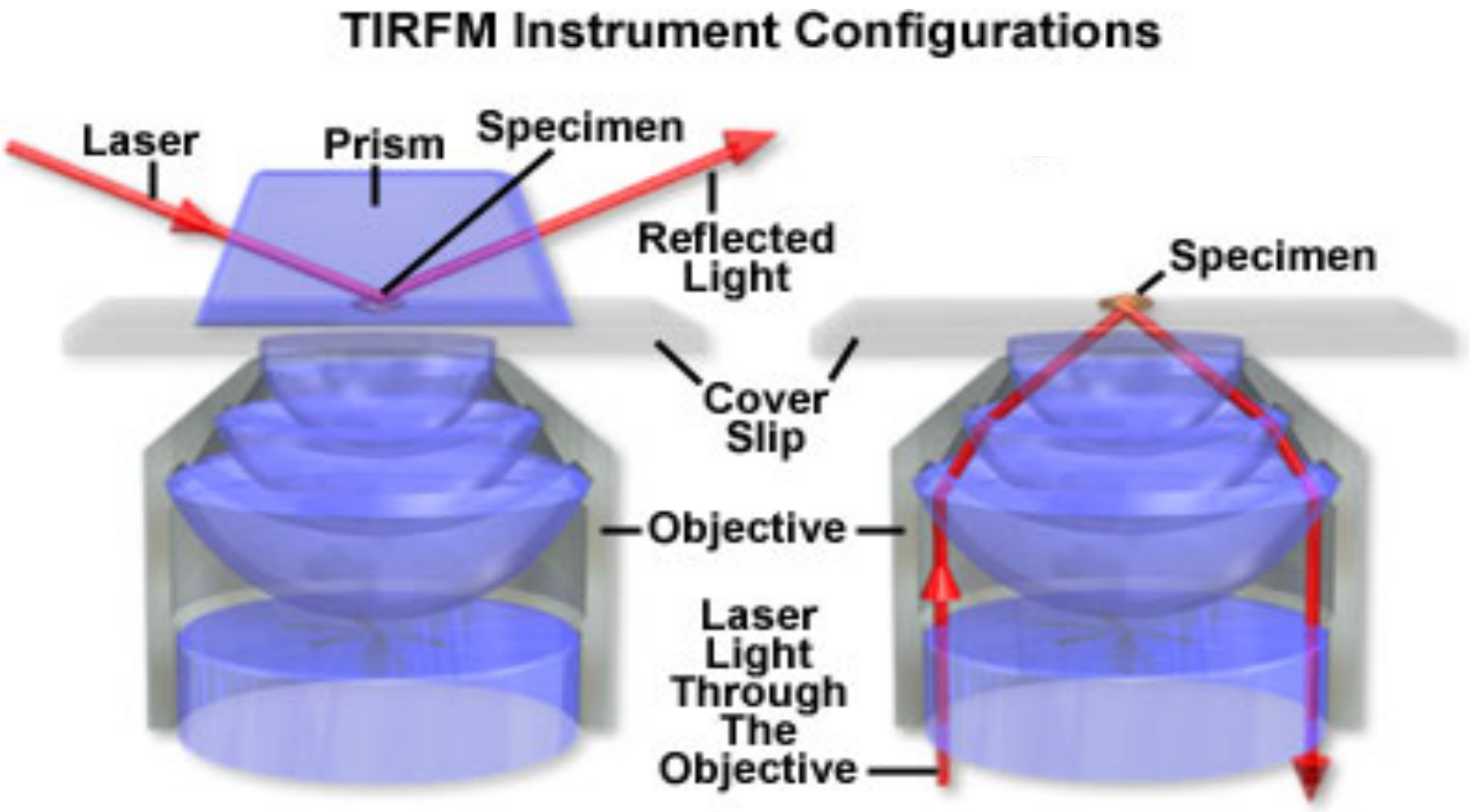
I_o: intensity at the interface

λ_o: the wavelength of incident light in a vacuum

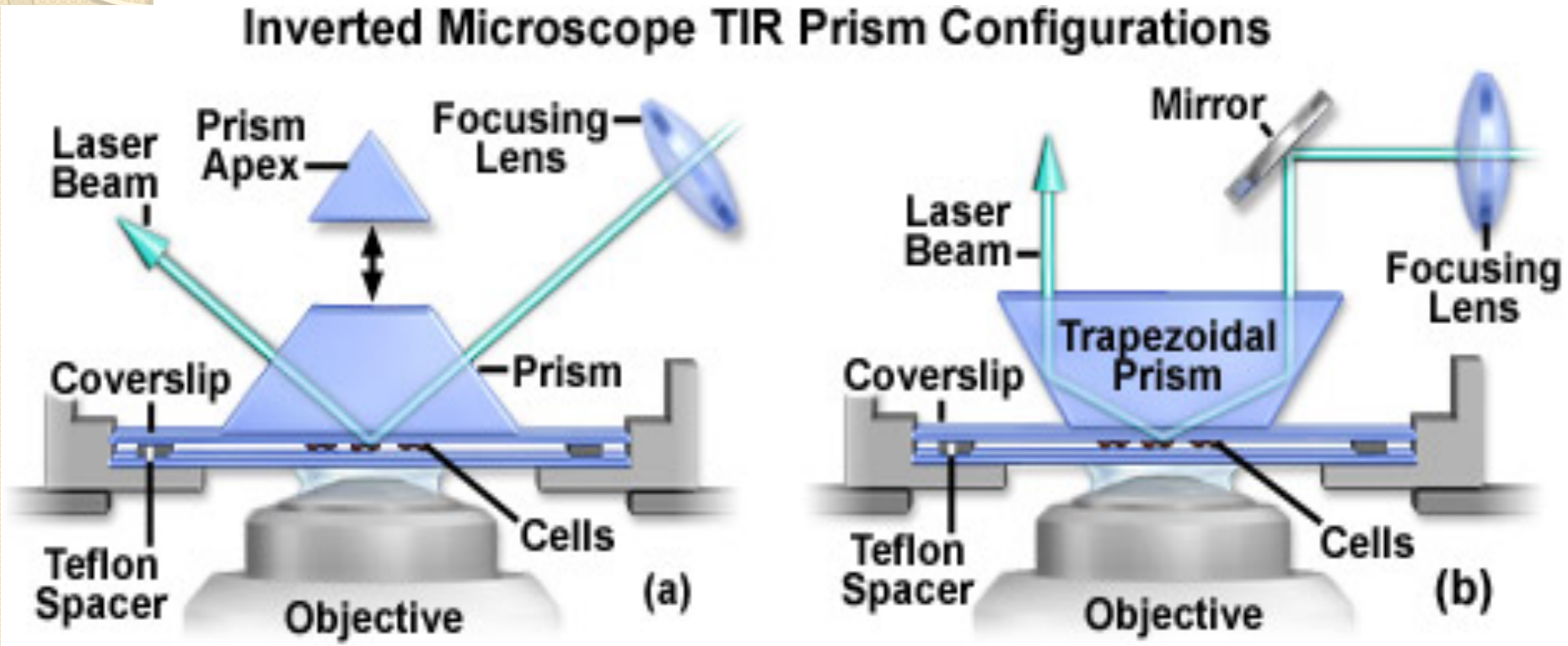
http://micro.magnet.fsu.edu

Fluorescence Intensity versus Penetration Depth

Distance (Nanometers)	Relative Intensity
1	0.99
10	0.92
100	0.43
1000	0.0002


TIRFM Instrument Configurations
Laser
Prism
Specimen
Reflected Light
Specimen
Cover Slip
Objective
Laser Light Through The Objective

Prism-based TIRFM



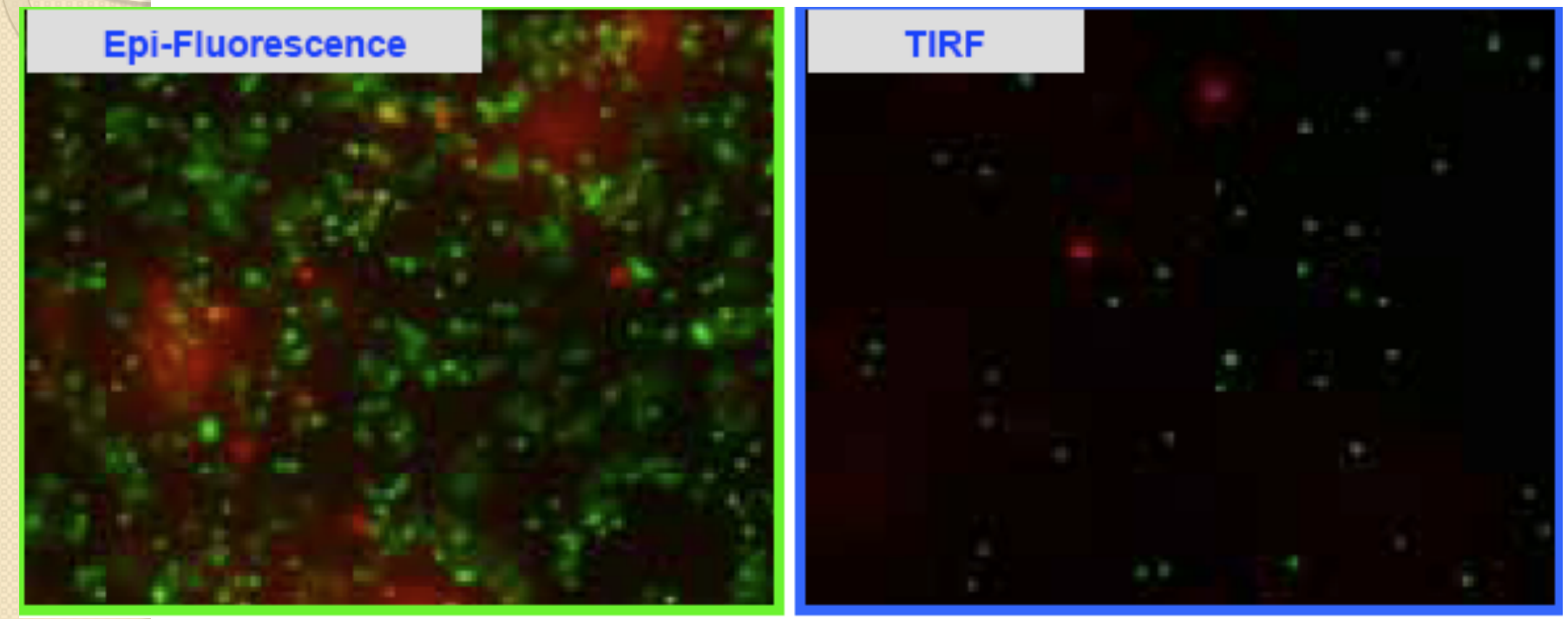
http://micro.magnet.fsu.edu

TIRFM tutorials

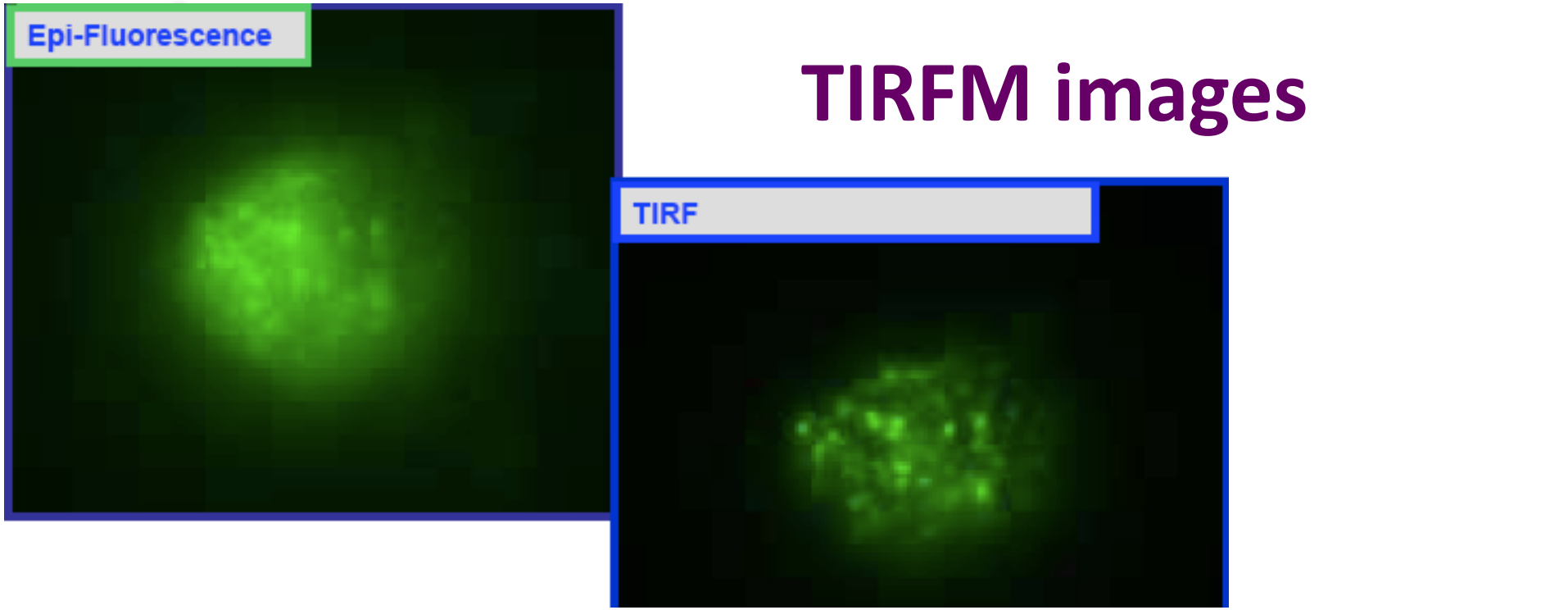
TIRFM Penetration Depth

TIRF excitation

TIRFM images



TIRFM images



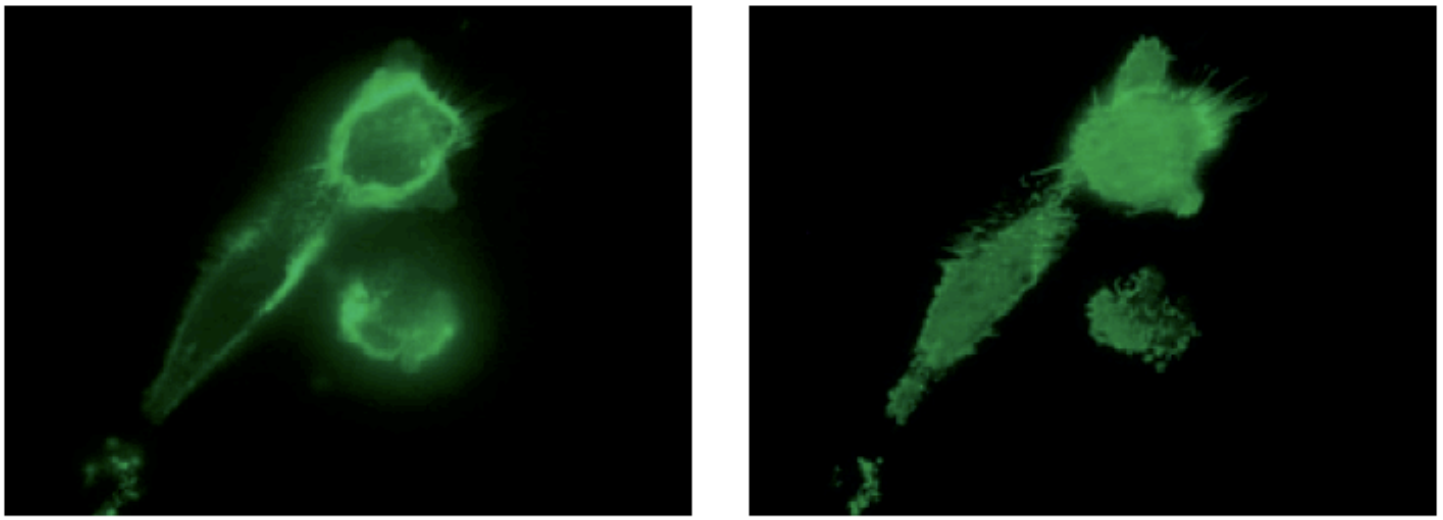
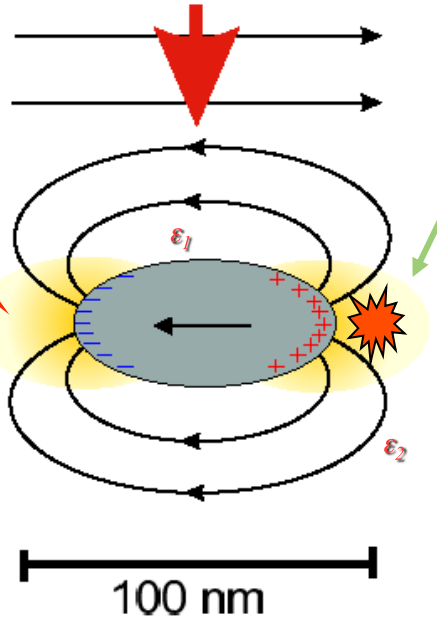


***Figure 2**: Breast cancer cell with GFP-marked CD44 on the cell membrane; left: photograph with wide-field fluorescence, right: with TIRF (image courtesy of Dr. M.C. Montoya, CNIO, Spanish National Cancer Center, Madrid)*

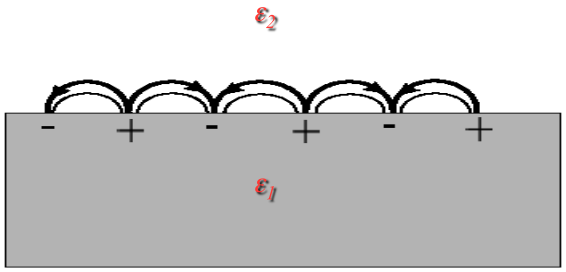
Surface Plasmon Resonance (SPR)

Enhanced local-field



$$P \sim \frac{\varepsilon_1 - \varepsilon_2}{\varepsilon_2 - d\left[\varepsilon_1 - \varepsilon_2\right]}$$

Enhanced Raman Signals (SERS)



$$k = \frac{\omega}{c}\sqrt{\frac{\varepsilon_1 \varepsilon_2}{\varepsilon_1 + \varepsilon_2}}$$

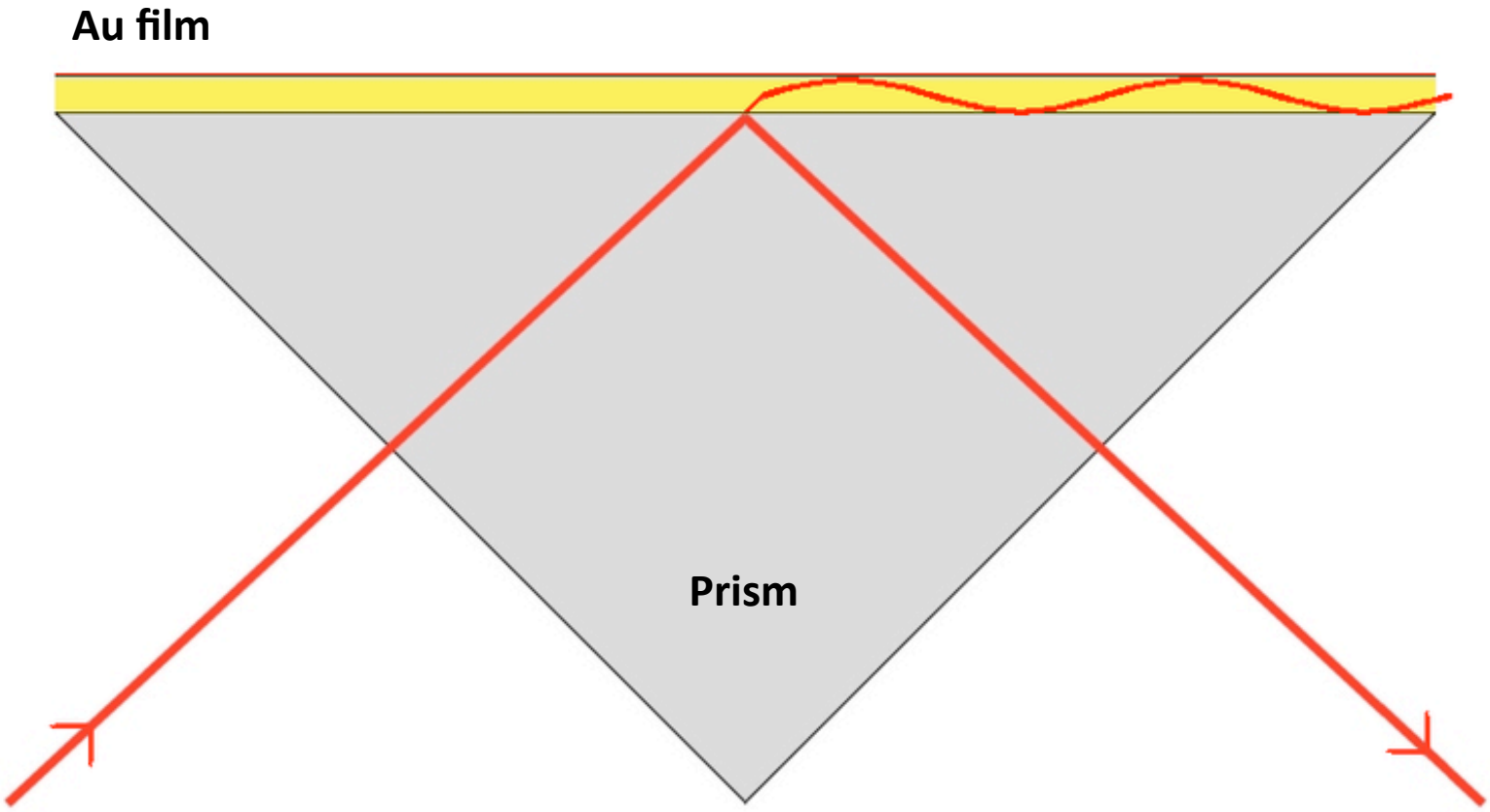
Advantages of SERS:

1. Direct molecular fingerprints
2. Multiplexed detection
3. Single-molecule sensitivity
4. No photobleaching

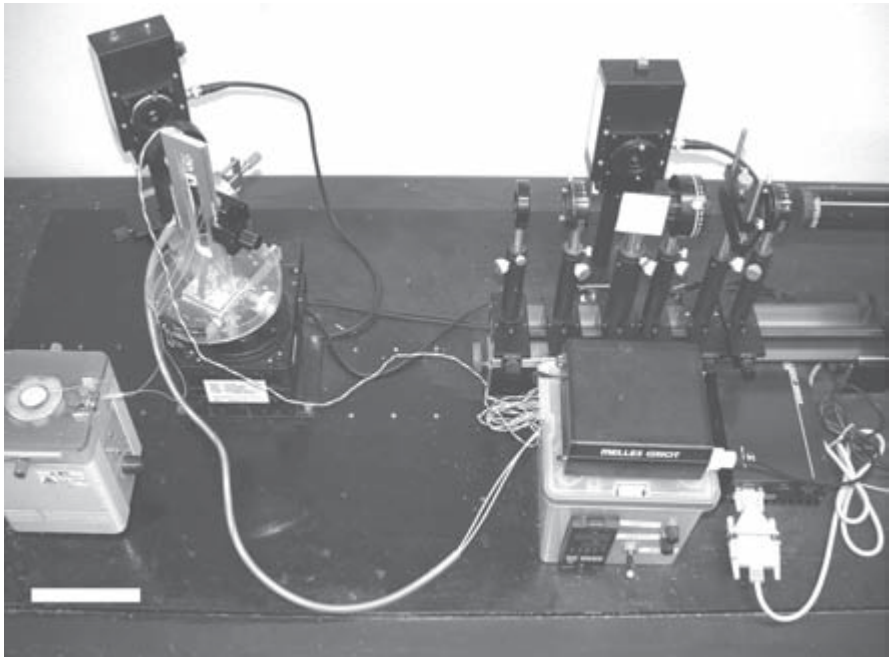
State-of-the-art of SERS substrates:

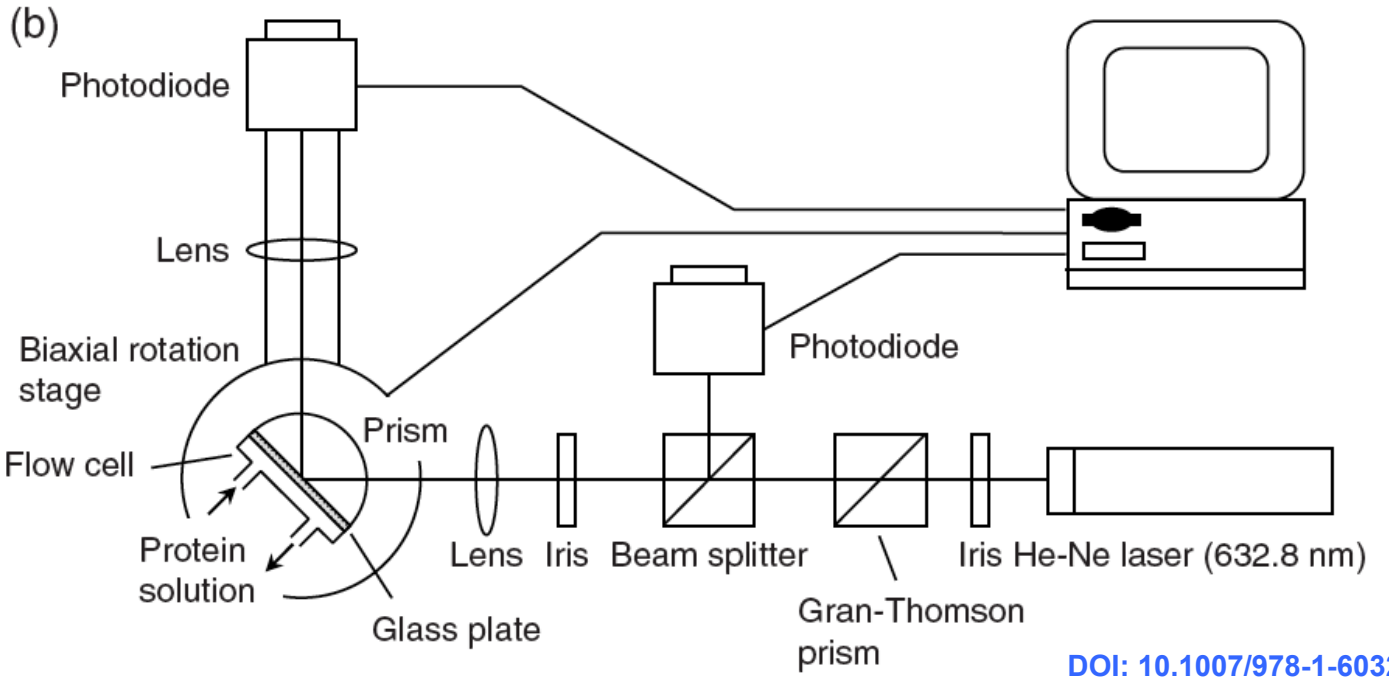
1. Colloid particles, not compatible with microfabrication processes
2. Unrepeatable enhancement factor

SPR–Kretschmann configuration



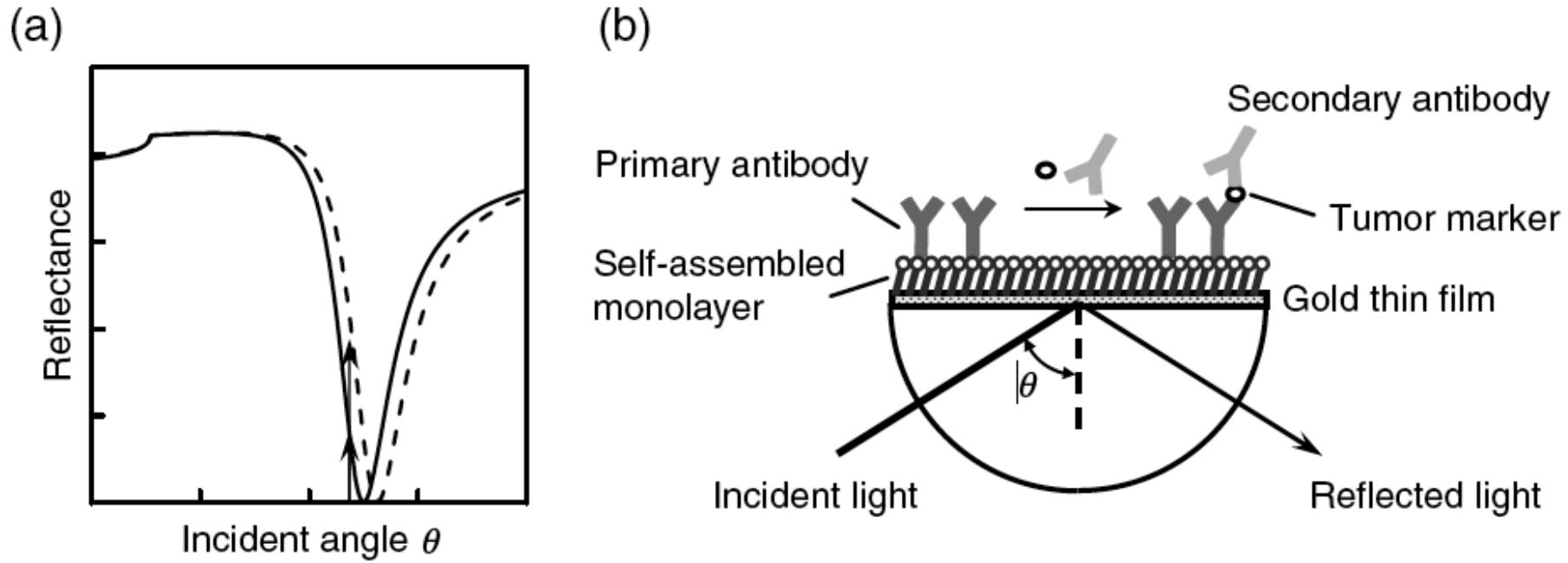
An SPR apparatus and its schematic representation





DOI: 10.1007/978-1-60327-567-5_1

Surface Plasmon Resonance (SPR)

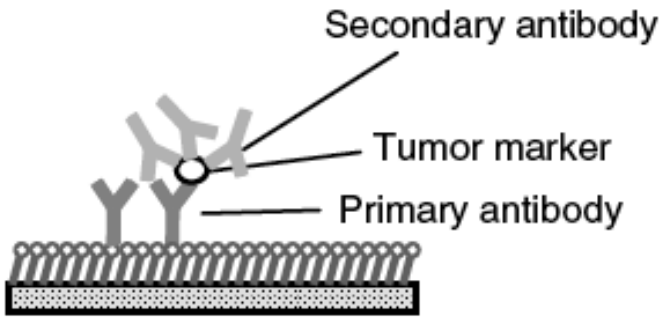


(a) Relationship between incident angle θ and intensity of reflected light before (solid line) and after (dashed line) protein adsorption. For real-time monitoring, the intensity of reflected light is monitored at a fixed angle throughout the measurement (arrow).

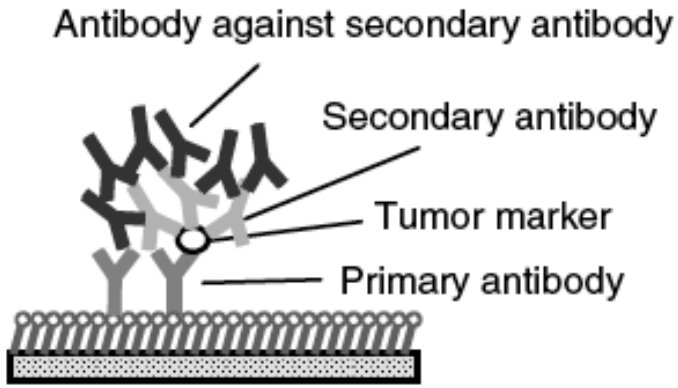
(b) Schematic representation of SPR-based sandwich-type immunoassay.

Signal amplification methods for detection of a minute amount of tumor marker

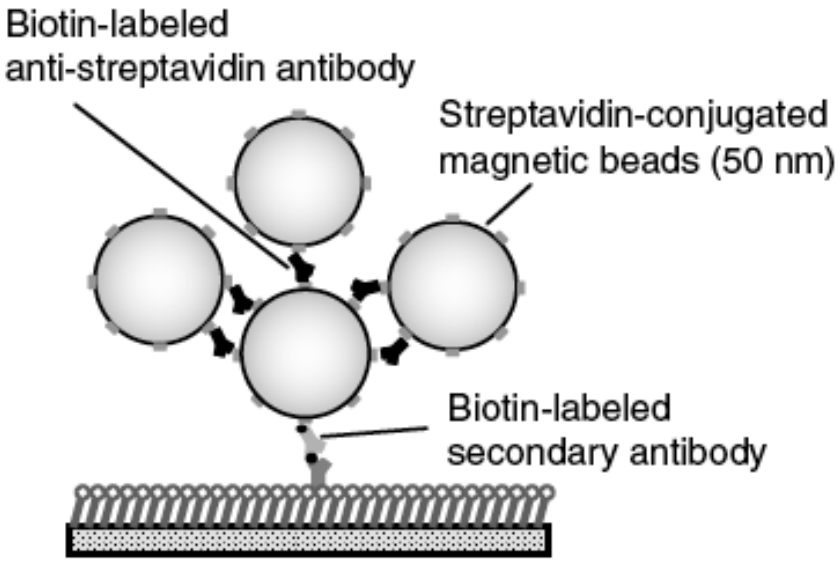
(a)



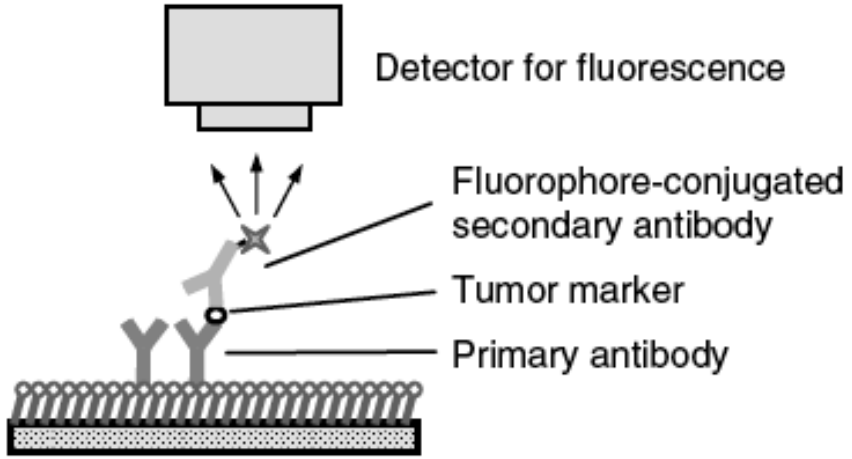
(b)



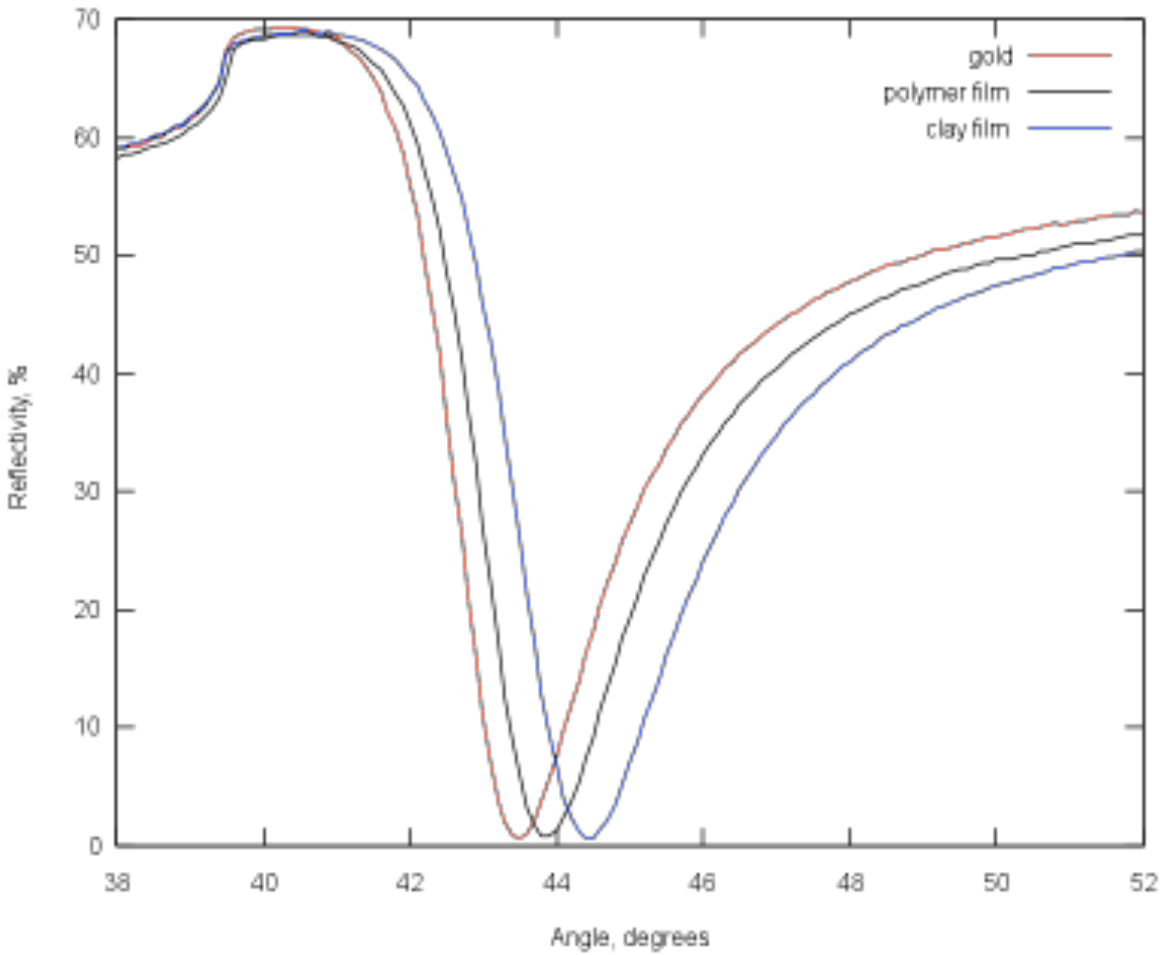
(c)



(d)

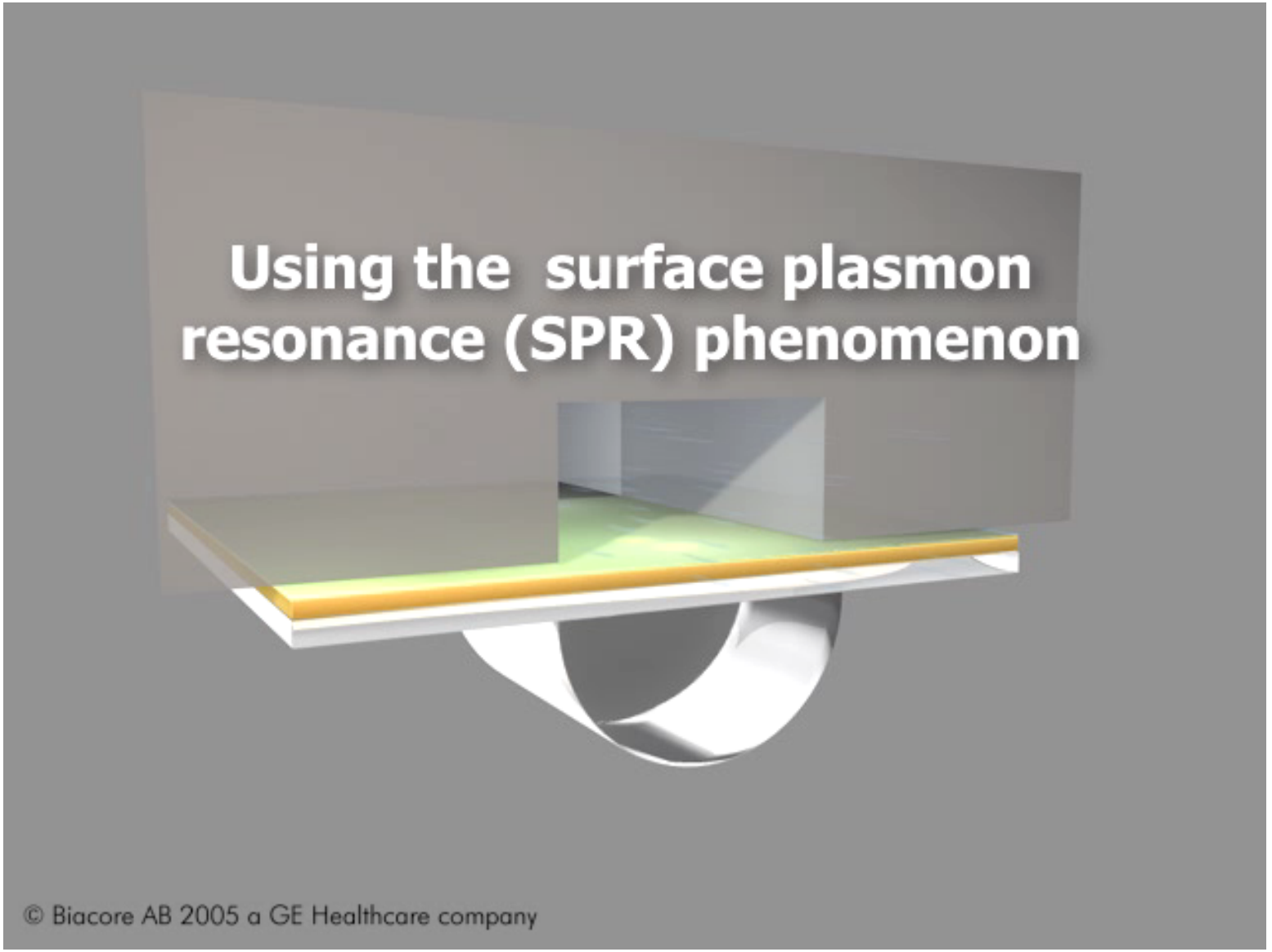


SPR-adsorption-data

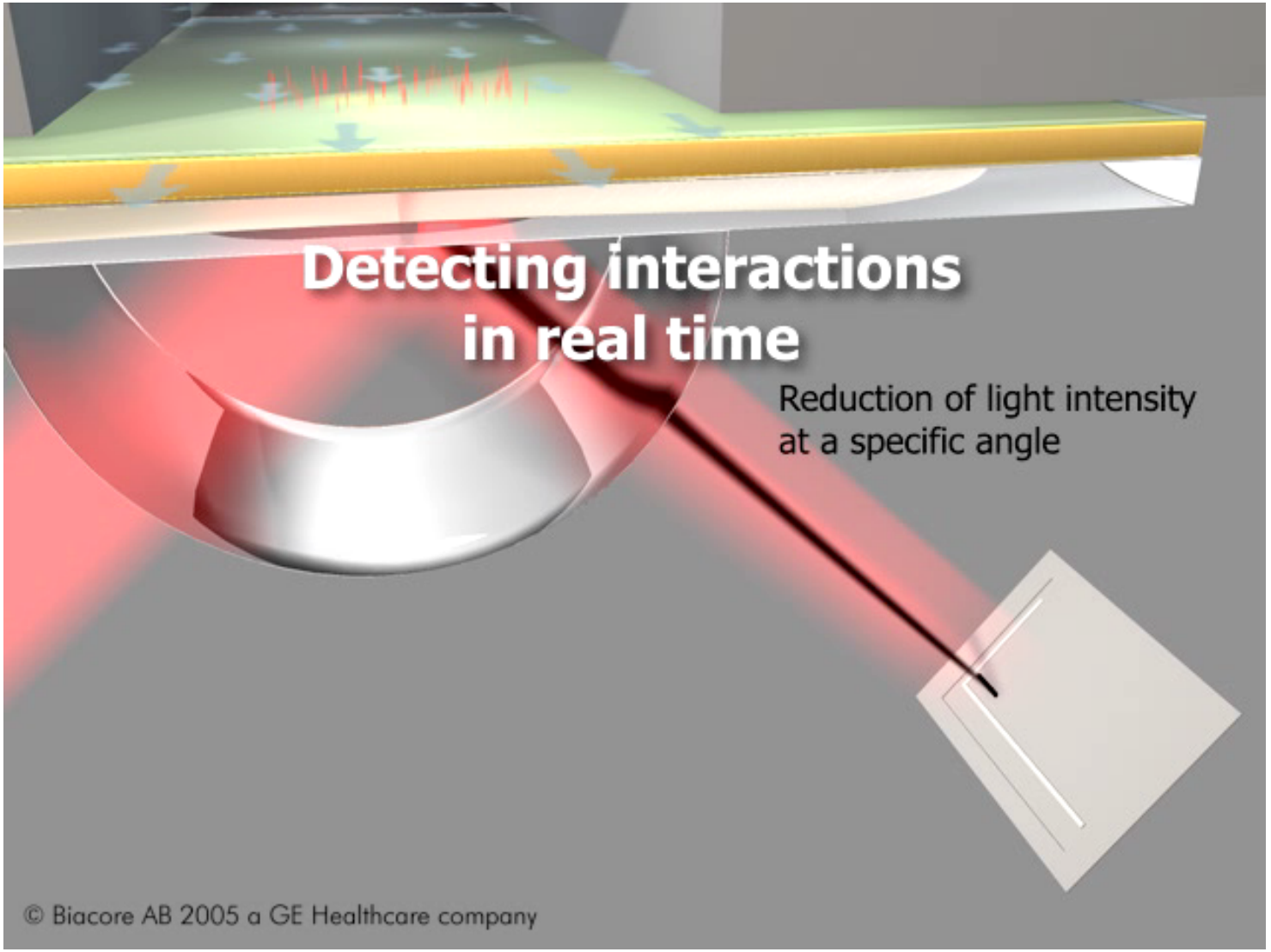


SPR data measured during layer-by-layer self-assembly of PDDACl and Na-montmorillonite clay on gold nanofilm (ca. 38 nm thick). Measurement data from Tamas Haraszti (at that time Department of Colloid Chemistry, University of Szeged, 1997)

Biocore SPR sensor platform



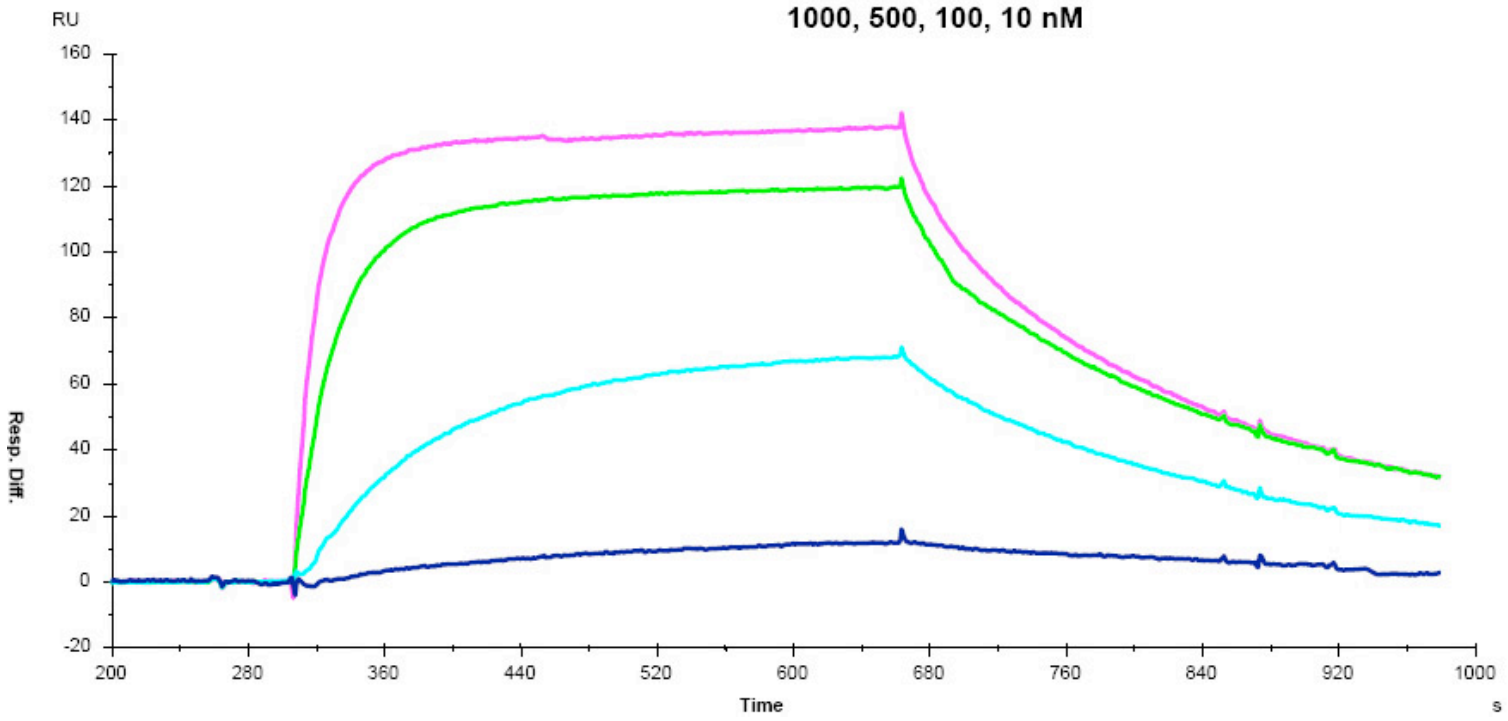
Biocore SPR sensor platform



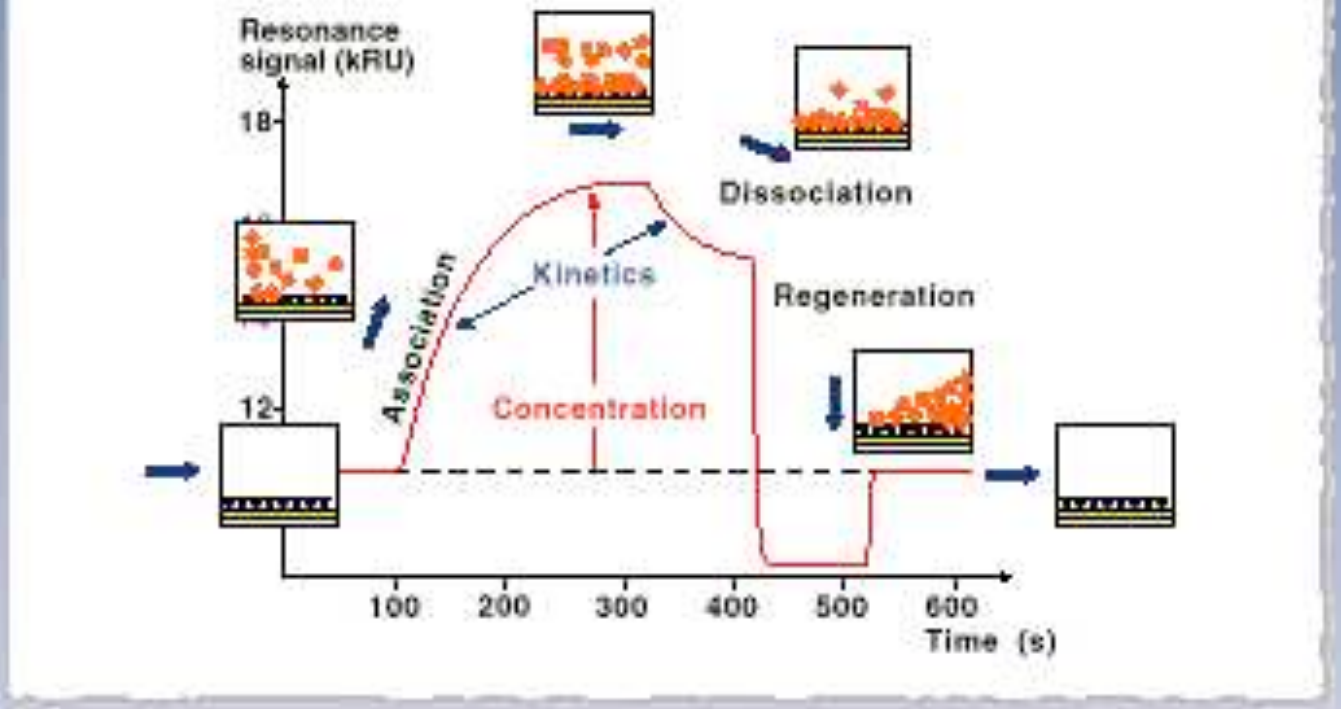
Biocore SPR sensor platform

Affinity:
Strong interaction

Biocore SPR sensor platform-Kinetics



The Sensorgram



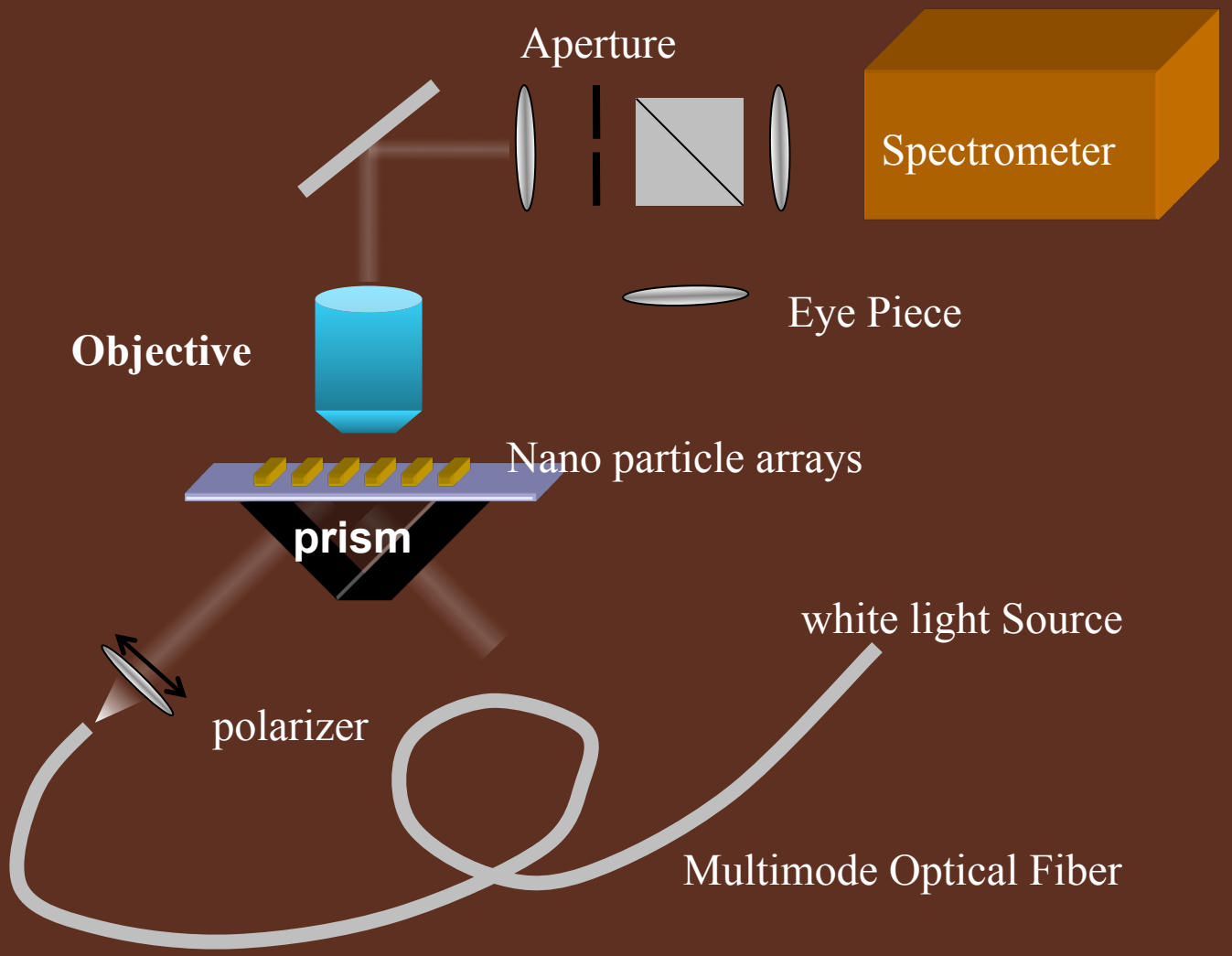
Biocore SPR sensor platform-Kinetics

Kinetics:
Rapid association &
dissociation

Biocore SPR sensor platform

Specificity:
Response

Far-field Nano-Optical Measurements



Raman Spectroscopy (SERS)

Why SERS

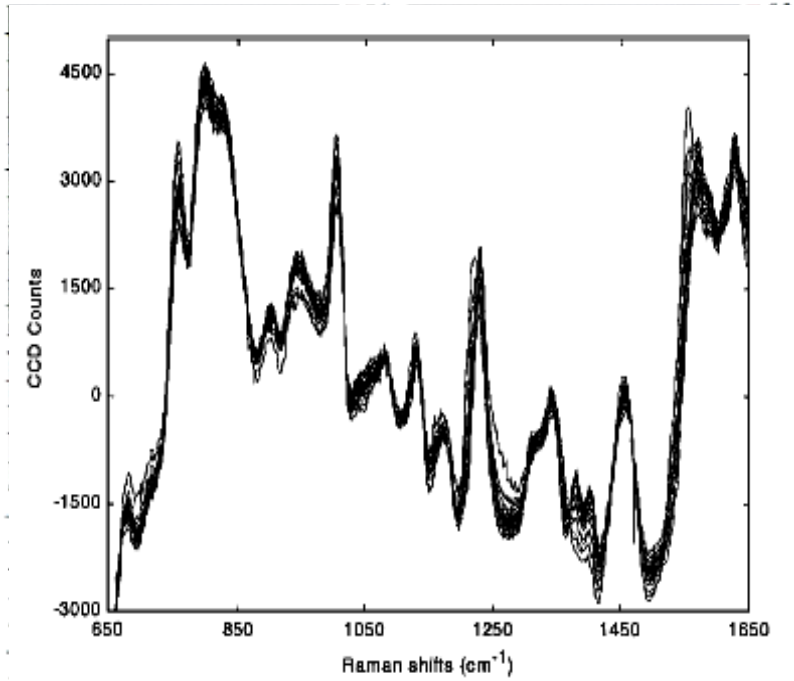
Sensitive, signals as molecular fingerprints, no photo-bleaching

Current method

Ag/Au colloidal particles, core-shell colloids, roughened Ag films (SERGen)

Open Questions

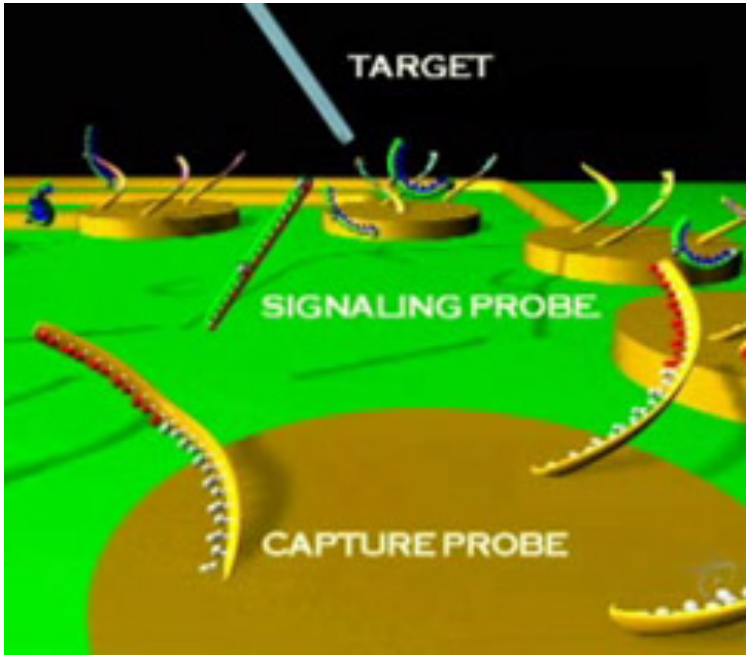
No compatibility with microfabrication, No integration with microfluidic Devices, un-repeatable enhancement factor



Raman Spectroscopy

ECM SNP Detection

Electrochemical detection



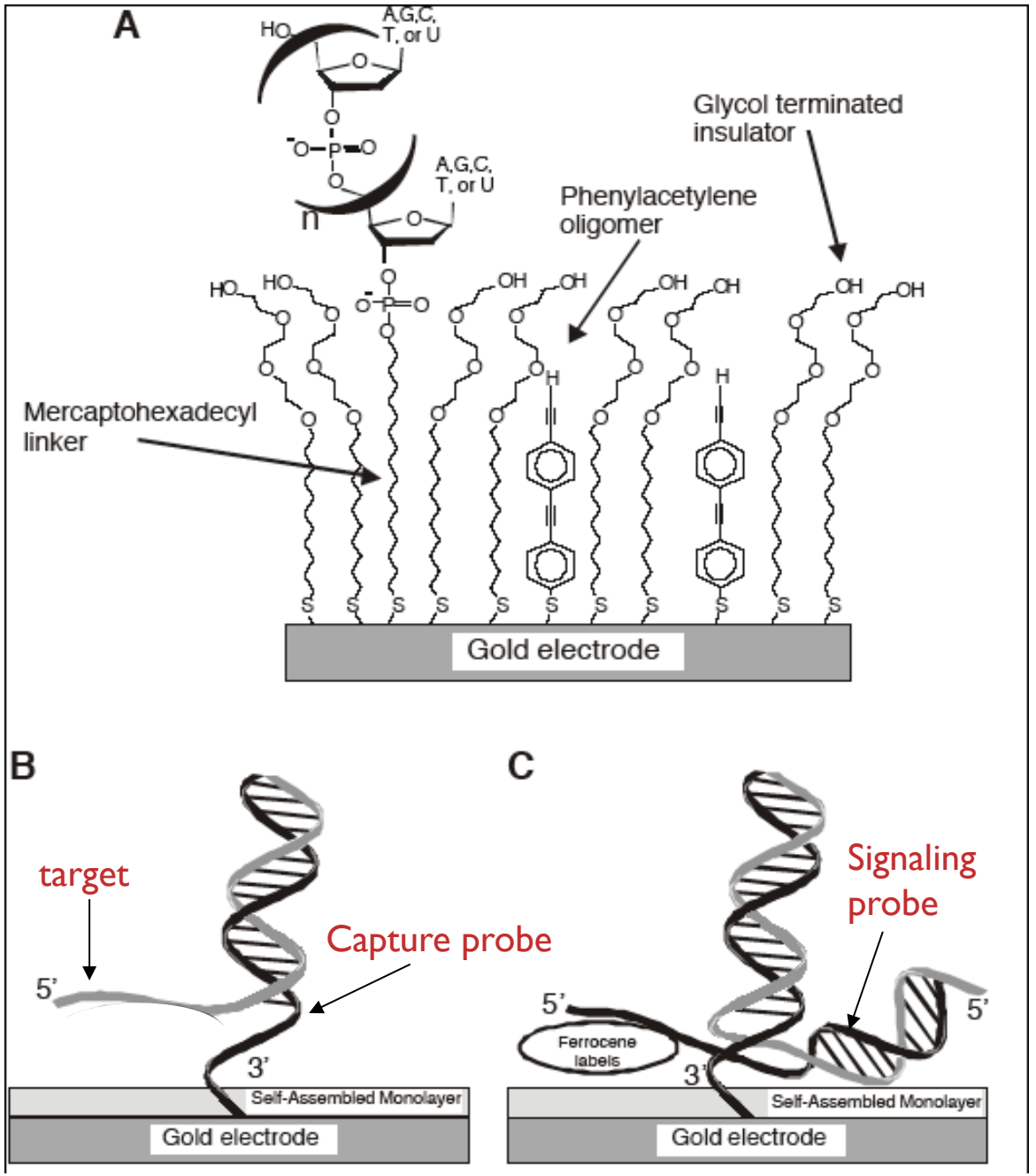
Use of 2 probes = double specificity

T

…ATC GTA…

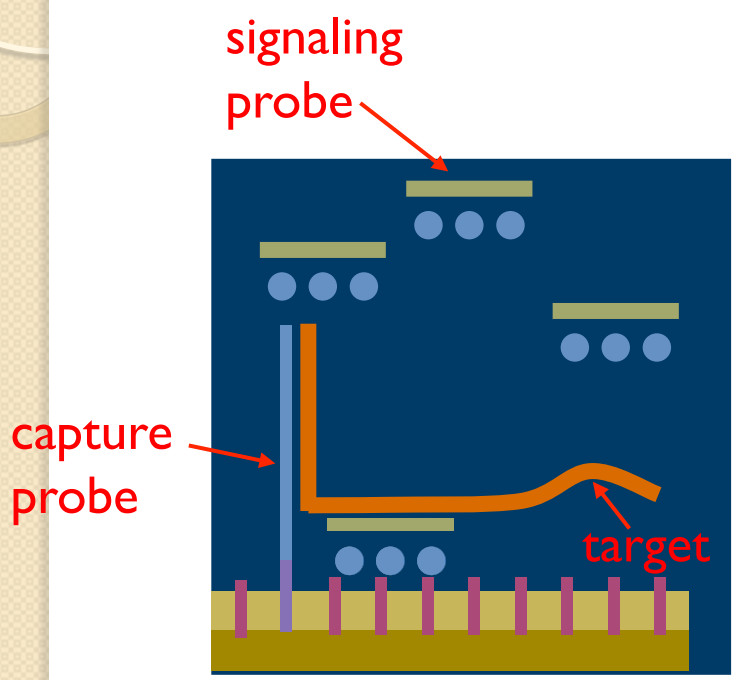
Single Nucleotide Polymorphism

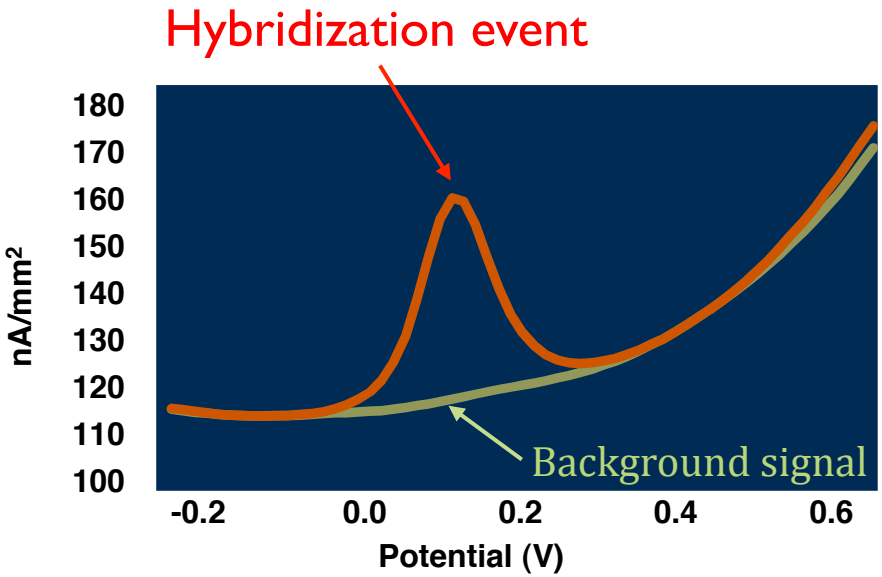
ECM SNP Detection



Umek, R. M.; et al. Electronic Detection of Nucleic Acids: A Versatile Platform for Molecular Diagnostics. *J. Mol. Diagn. 2001, 3,* 74.

ACV detection of DNA hybridization events





Umek, R. M.; et al. Electronic Detection of Nucleic Acids: A Versatile Platform for Molecular Diagnostics. *J. Mol. Diagn. 2001, 3,* 74.

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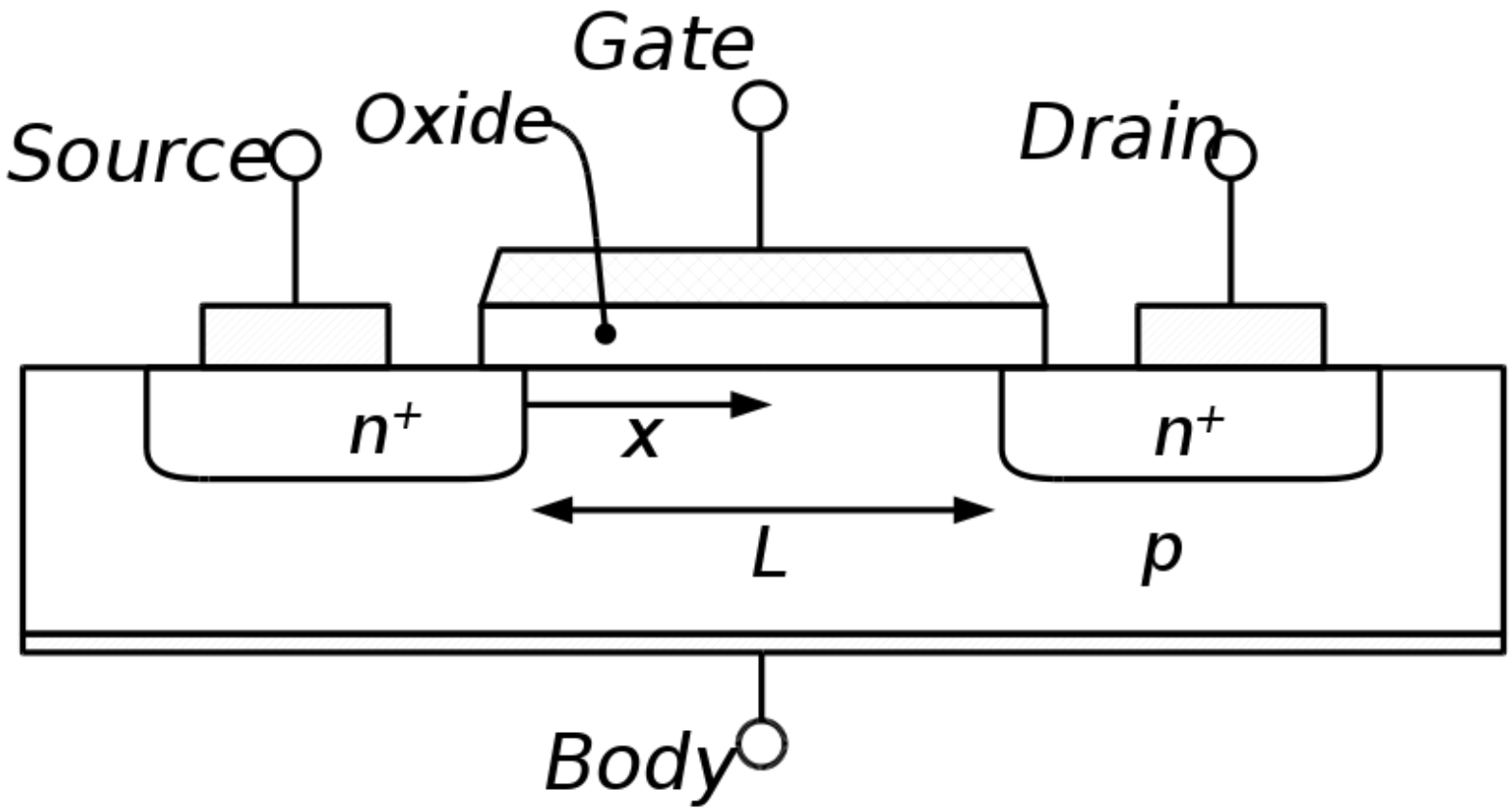
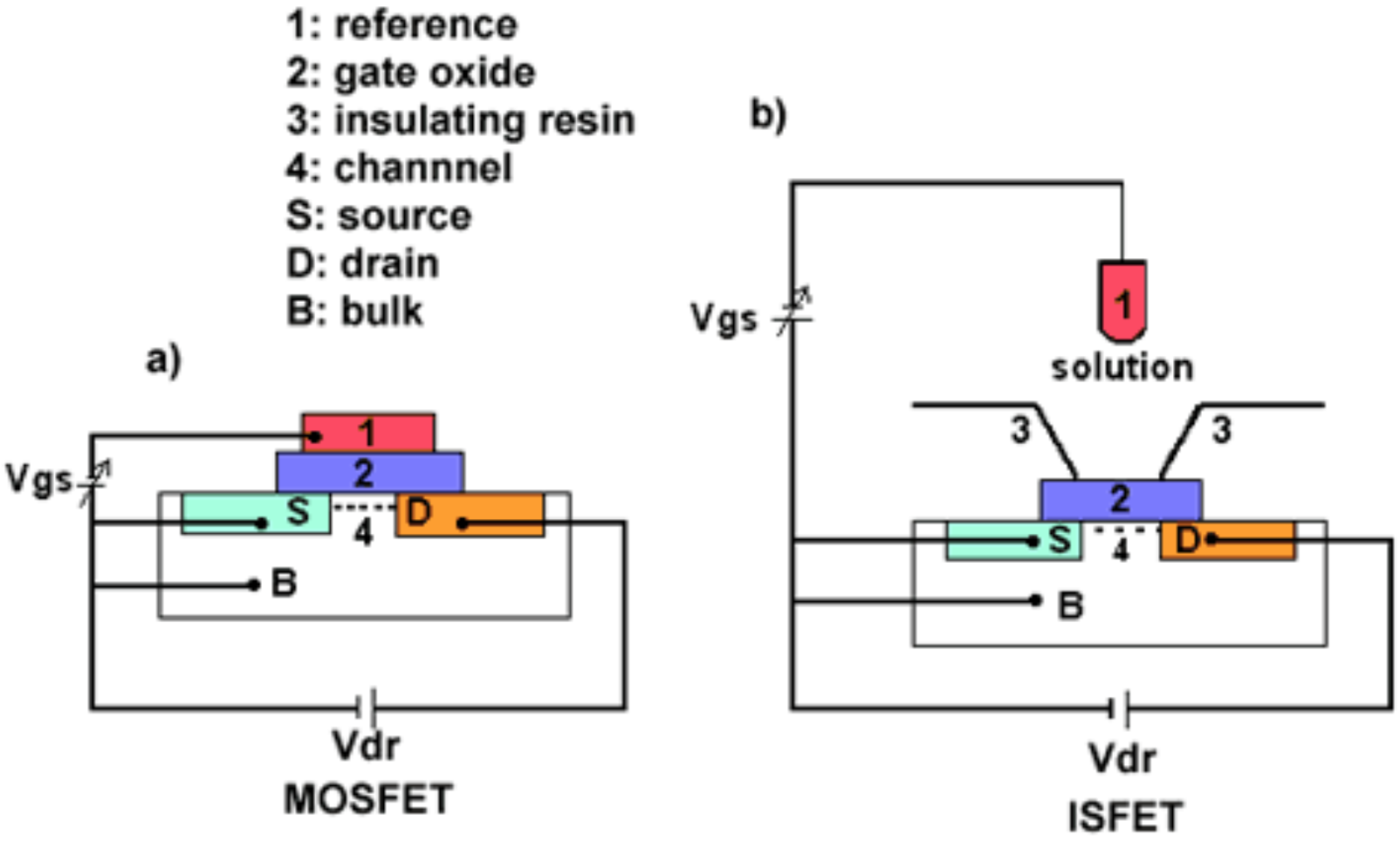
Cross section of an n-type MOSFET

(metal oxide semiconductor field effect transistor)

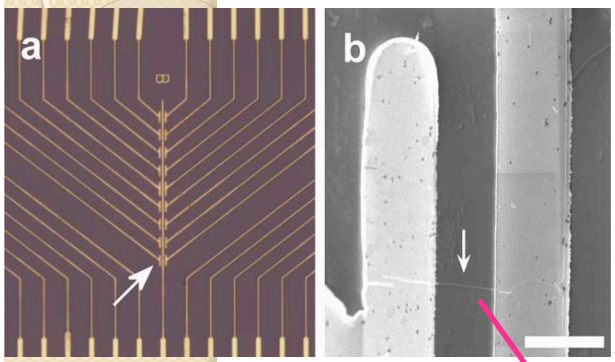


Field effect transistors (FETs) as transducers in electrochemical sensors

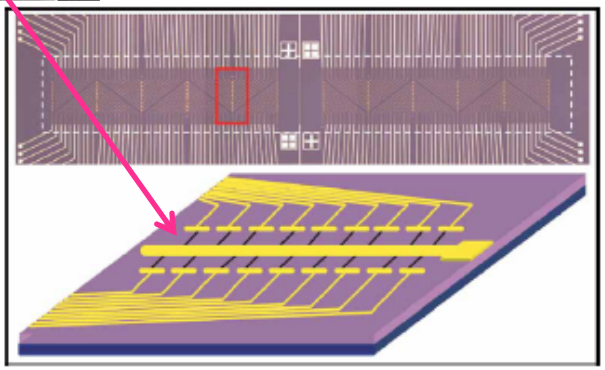


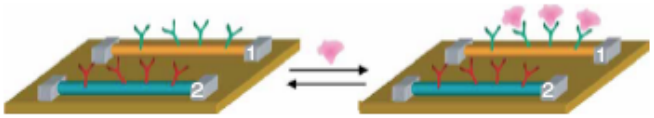
http://csrg.ch.pw.edu.pl/tutorials/isfet/

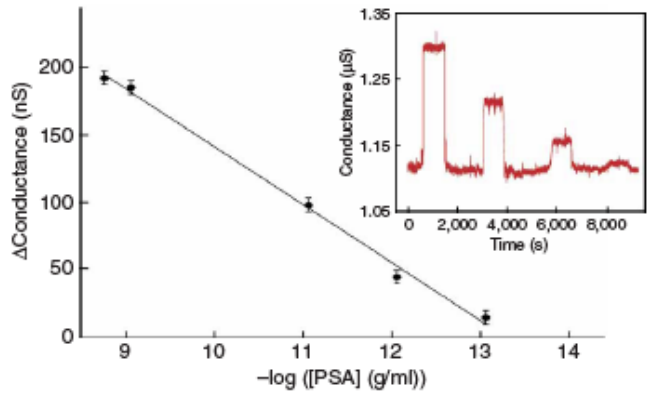
Nanowire sensor array

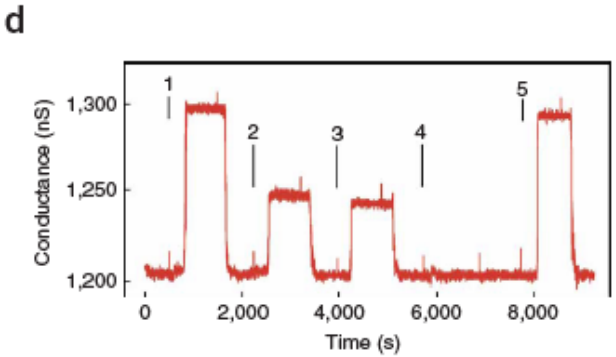


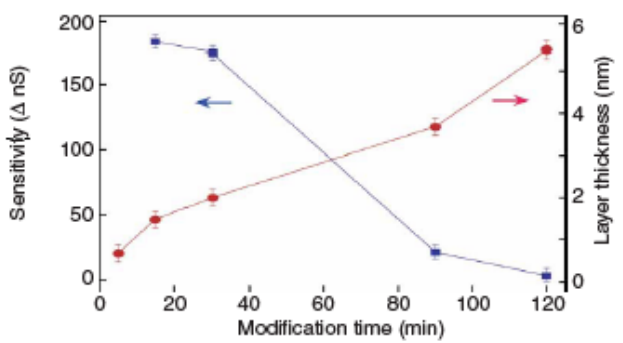
Zheng GF, Patolsky F, Cui Y, et al. Multiplexed electrical detection of cancer markers with nanowire sensor arrays. *NAT BIOTECH 23, 1294-1301 (2005).*



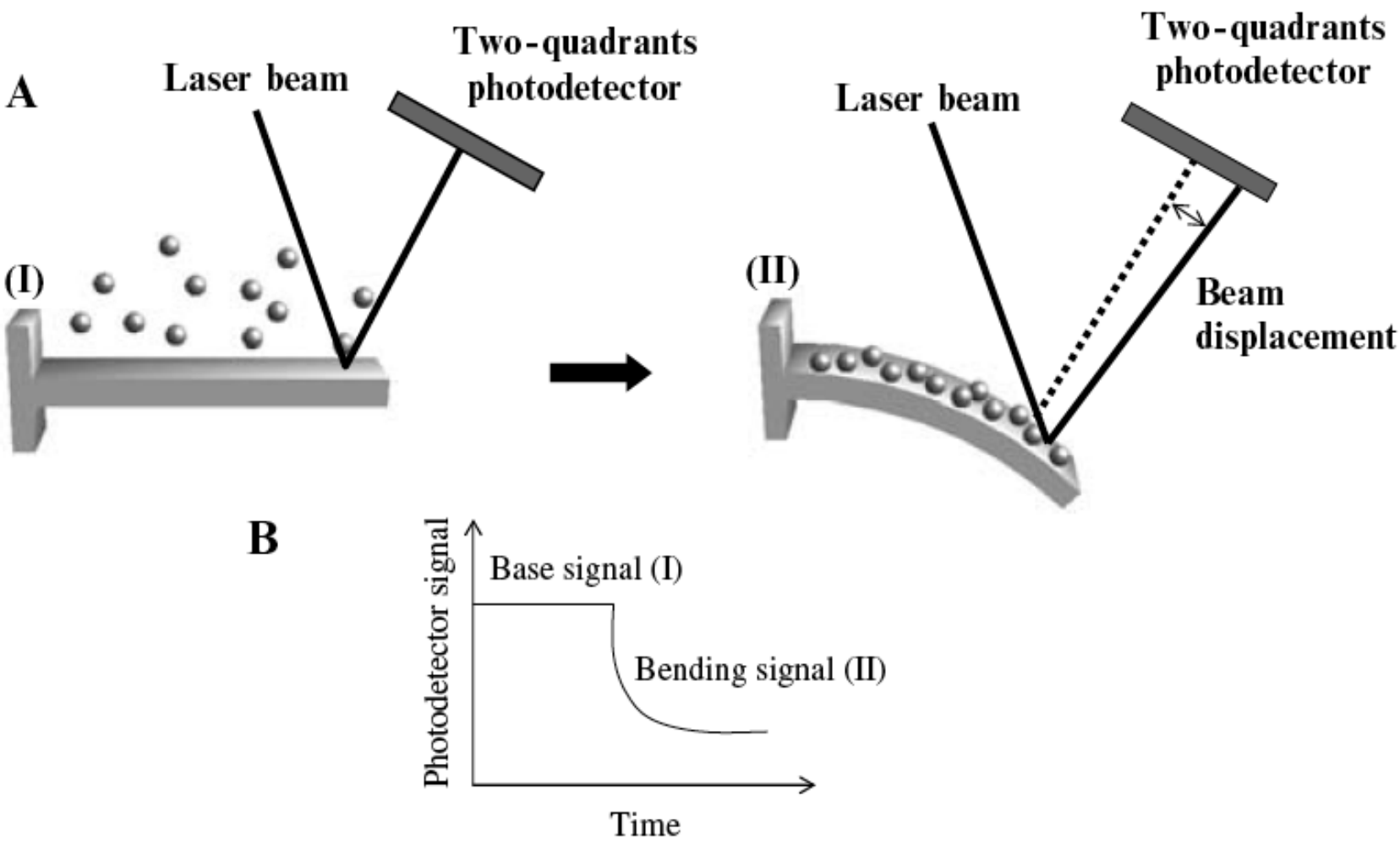




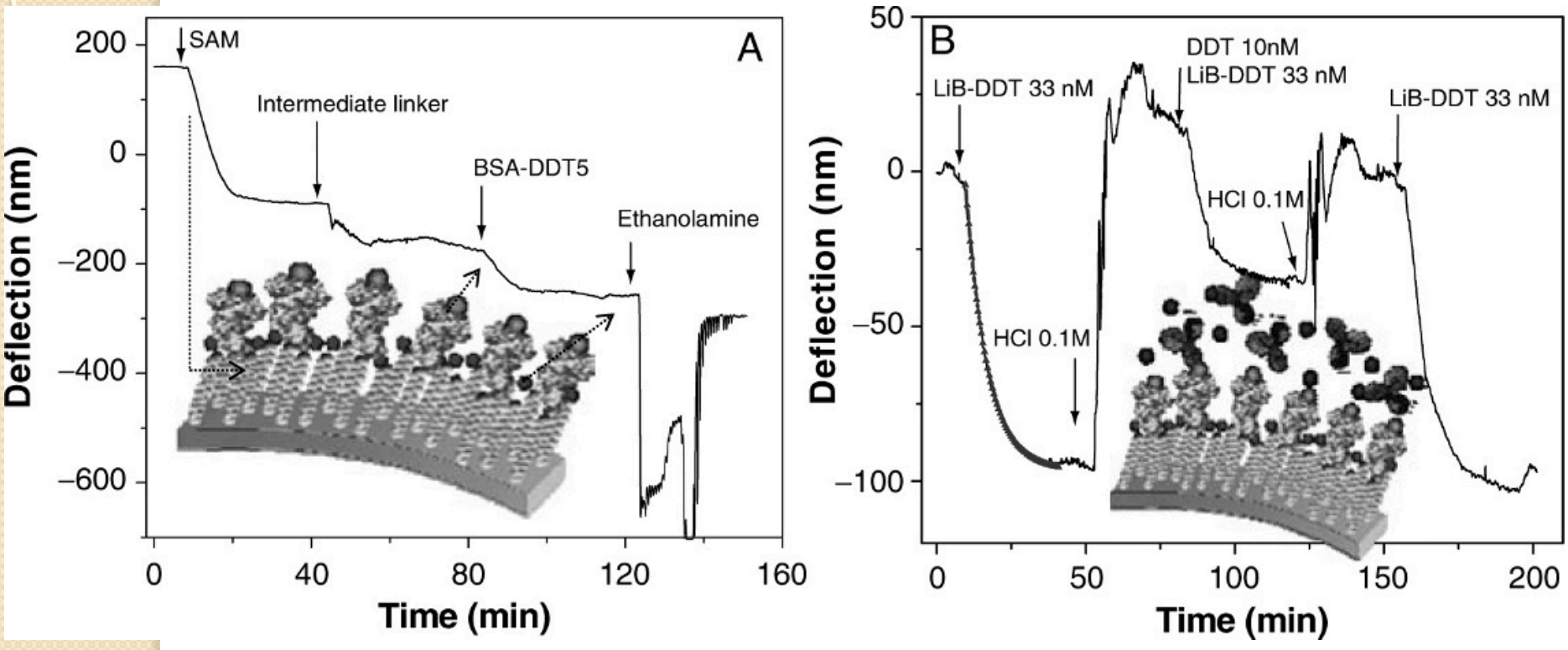




Biosensors Based on Cantilevers

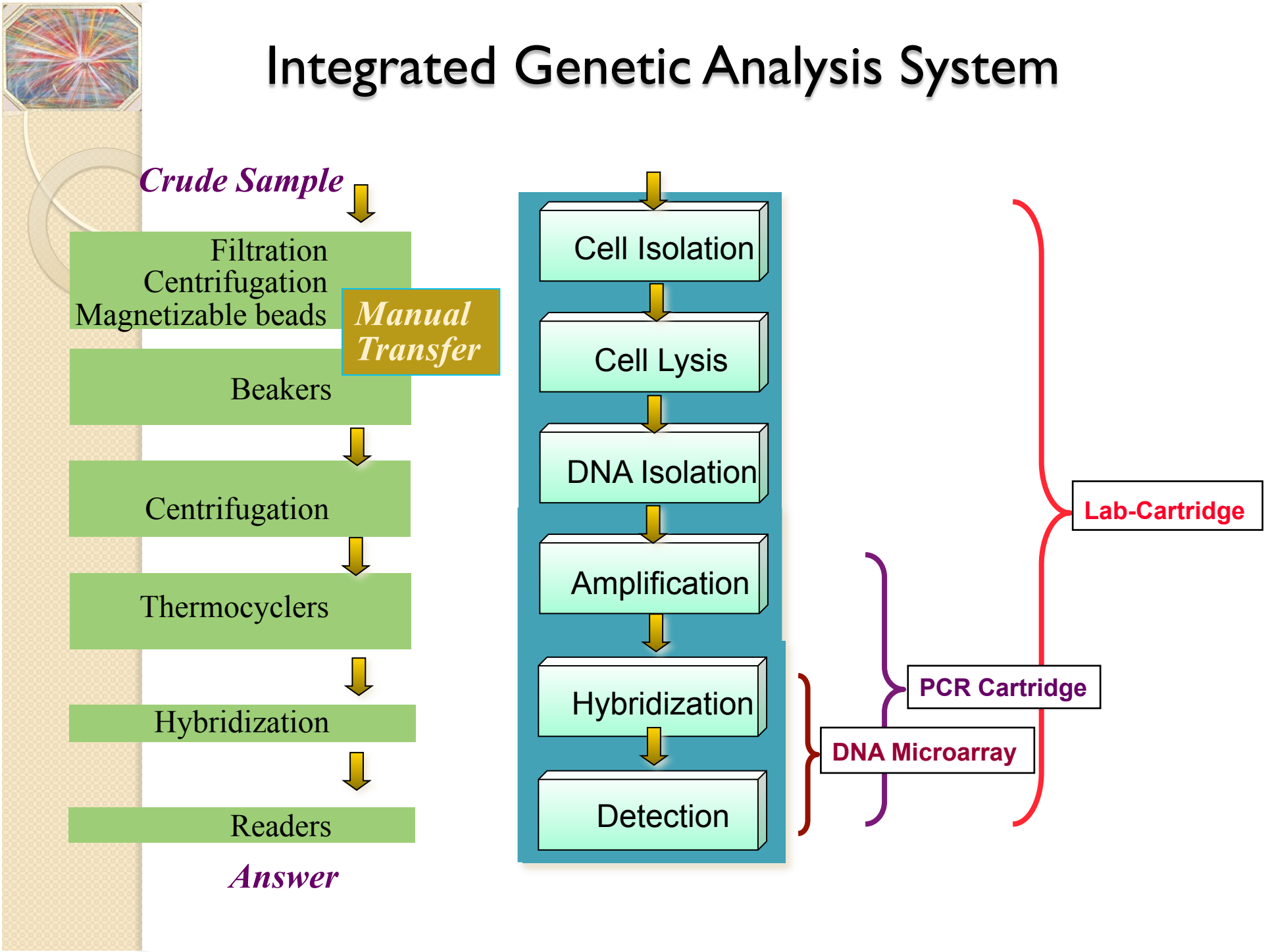


Cantilever surface functionalization in real time

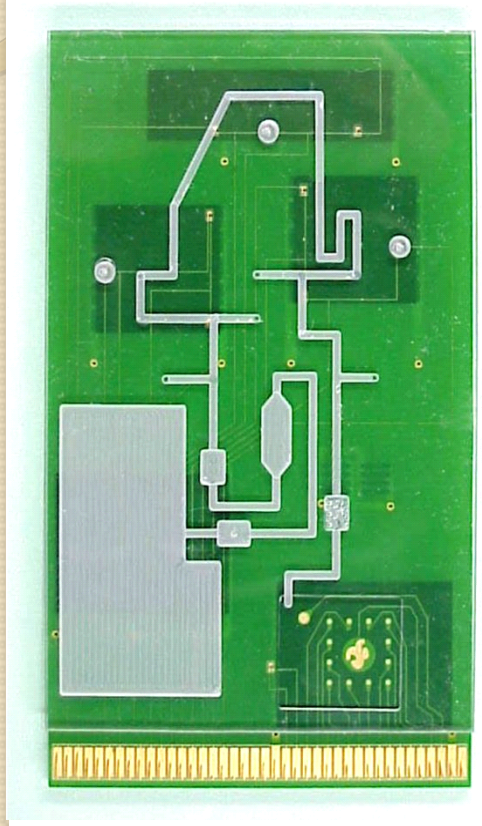


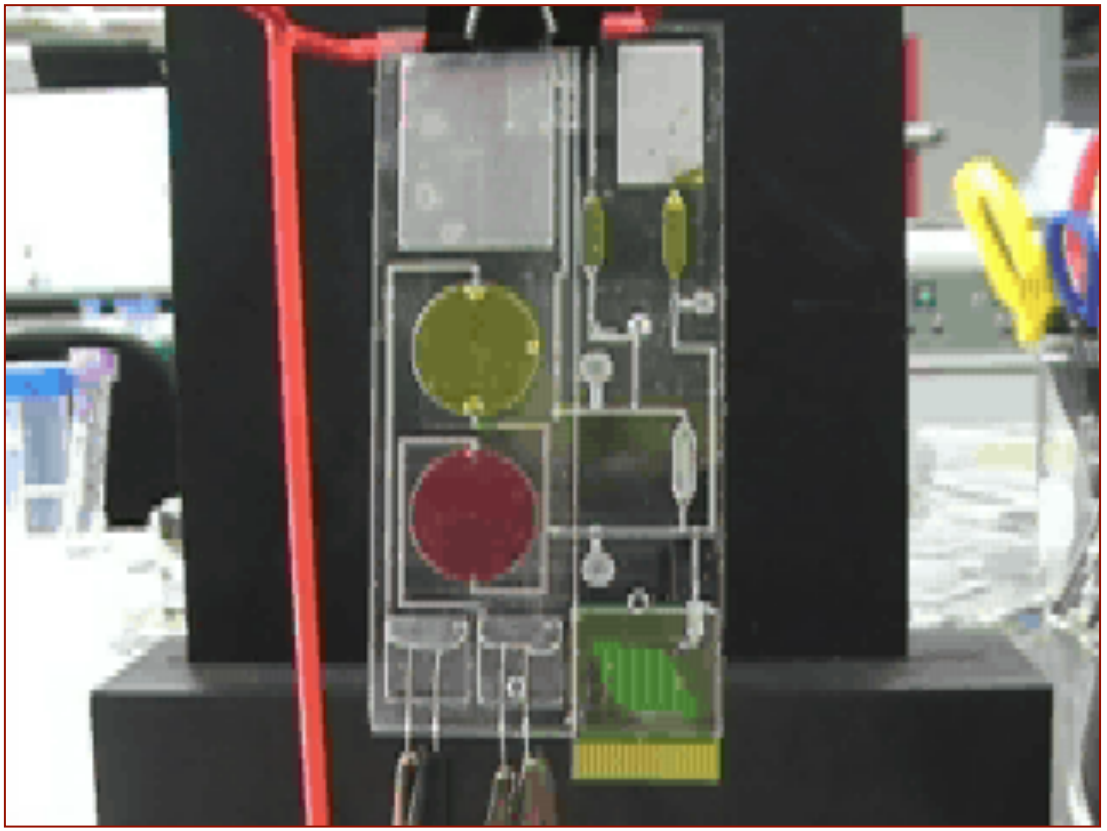
(A) Example of a cantilever surface functionalization in real time. Each one of the layers formed over the surface produce a cantilever bending. (B) Real-time monitoring of an antibody direct detection and a competitive immunoassay. The number of antibodies free in solution able to binding the cantilever surface is reduced due to the binding with the DDT free in solution. The cantilever surface was regenerated with 100 mM HCl (100 ml) to break the hapten/antibody complex; (M. Alvarez et al. (2003) Development of nanomechanical biosensors for detection of the pesticide DDT. *Biosen. Bioelectron.* 18, 649–653).

Integrated Genetic Analysis System



Integrated Genetic Analysis System





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Real-time Single Molecule Sequencing

Helicos platform

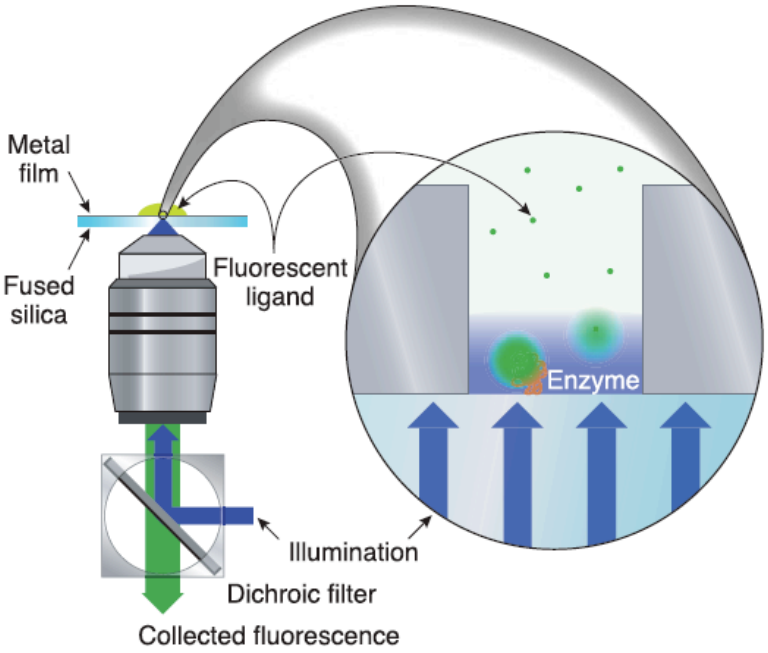
http://www.helicosbio.com/

Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations

M. J. Levene,[1] J. Korlach,[1,2] S. W. Turner,[1*] M. Foquet,[1]
H. G. Craighead,[1] W. W. Webb[1†]

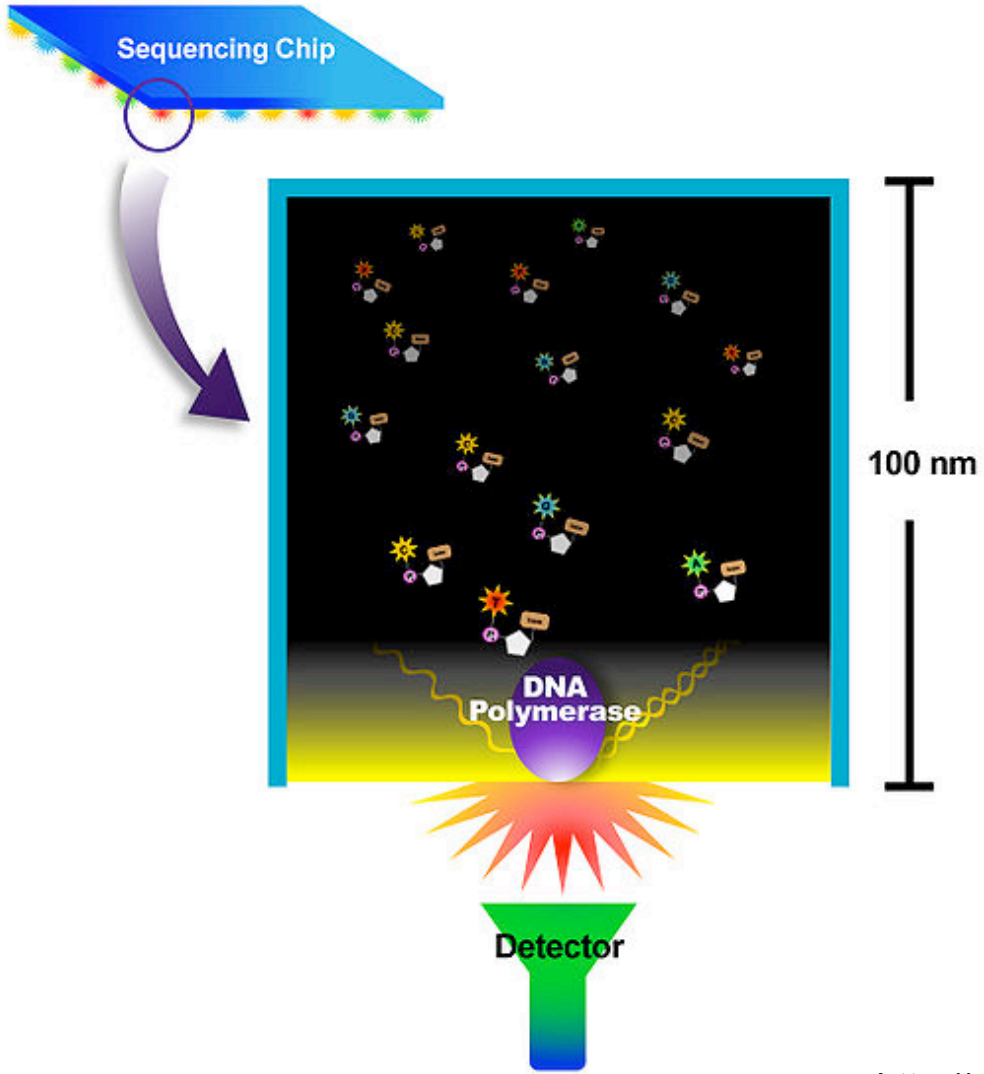
Science 299, 682-686, 2003

Optical approaches for observing the dynamics of single molecules have required pico- to nanomolar concentrations of fluorophore in order to isolate individual molecules. However, many biologically relevant processes occur at micromolar ligand concentrations, necessitating a reduction in the conventional observation volume by three orders of magnitude. We show that arrays of zero-mode waveguides consisting of subwavelength holes in a metal film provide a simple and highly parallel means for studying single-molecule dynamics at micromolar concentrations with microsecond temporal resolution. We present observations of DNA polymerase activity as an example of the effectiveness of zero-mode waveguides for performing single-molecule experiments at high concentrations.



An apparatus for single-molecule enzymology using zero-mode waveguides.

Real-time Single Molecule Sequencing



http://www.pacificbiosciences.com/

Real-time Single Molecule Sequencing

http://www.pacificbiosciences.com/

Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 13770–13773, November 1996
Biophysics

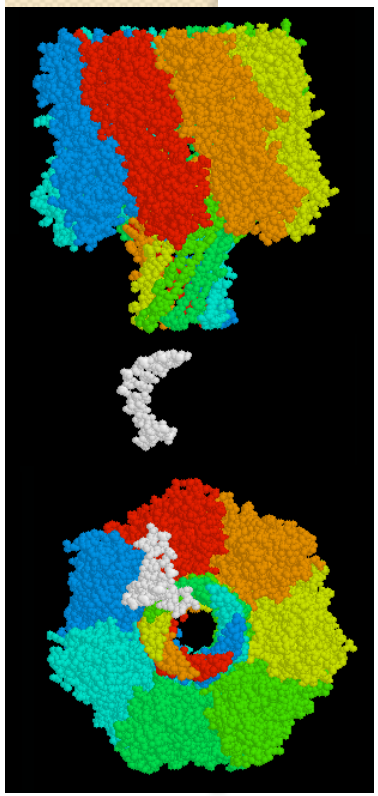
Nanopore DNA Sequencing

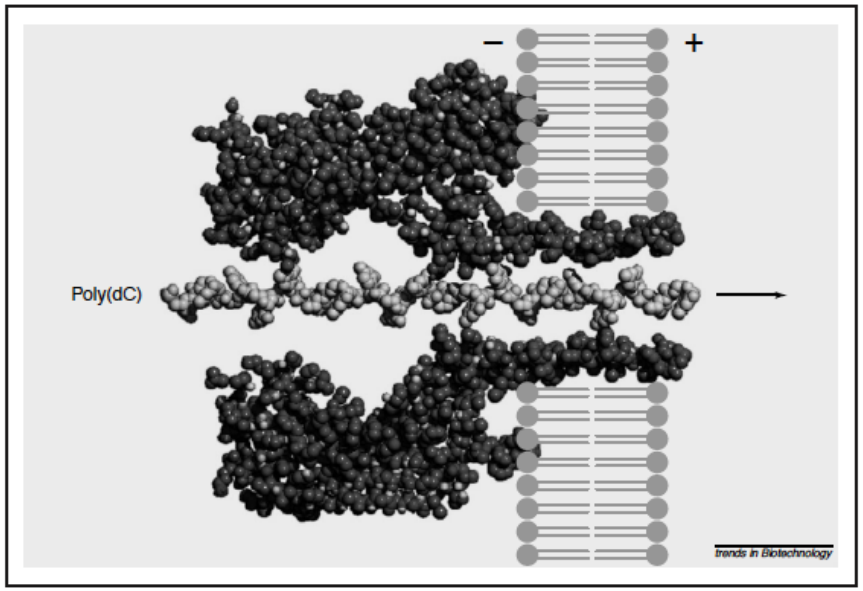
Characterization of individual polynucleotide molecules using a membrane channel

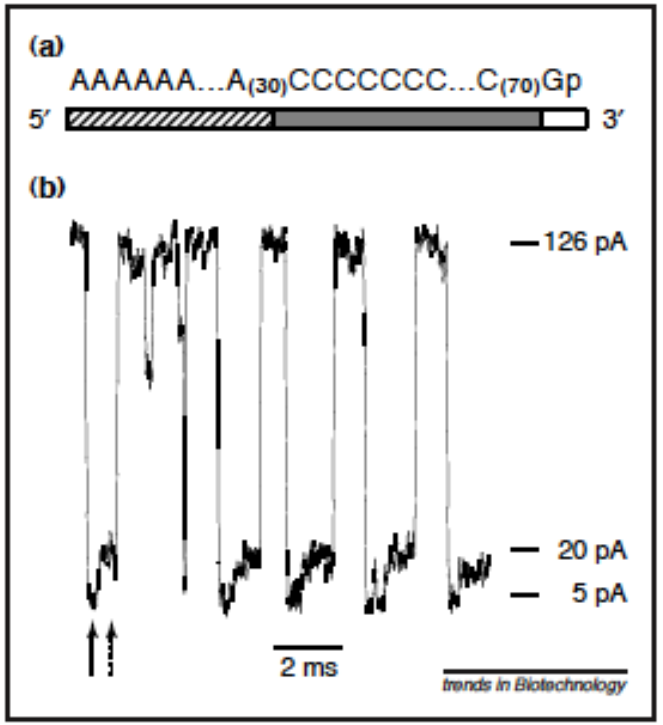
JOHN J. KASIANOWICZ*, ERIC BRANDIN†, DANIEL BRANTON†‡, AND DAVID W. DEAMER§

*Biotechnology Division, National Institute of Science and Technology, 222/A353, Gaithersburg, MD 20899; †Department of Molecular and Cellular Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138; and §Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064

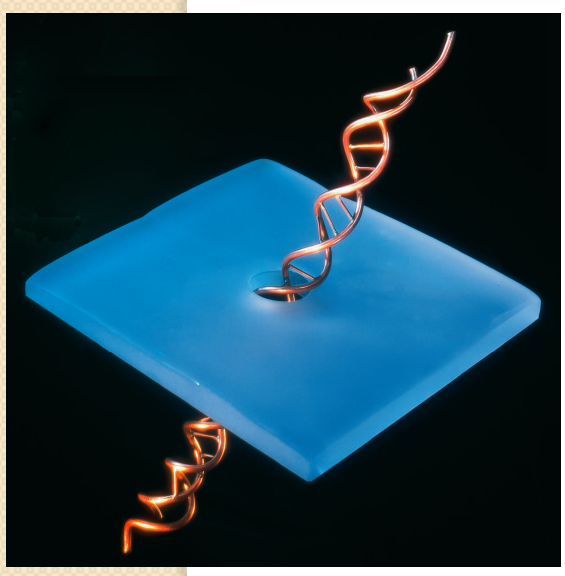
A single α-hemolysin channel (Ø =1.5 nm) embedded in a lipid bilayer



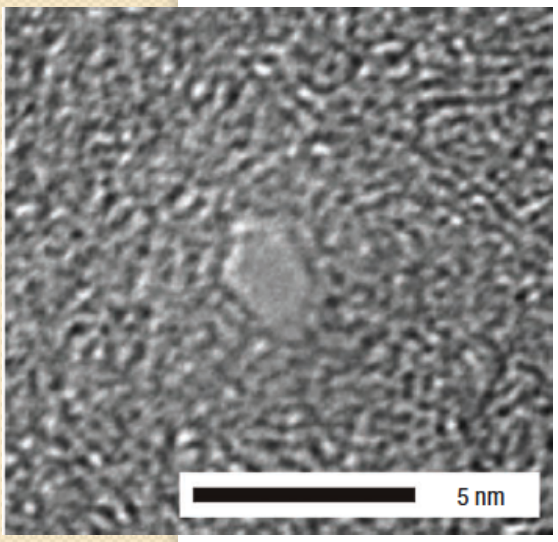


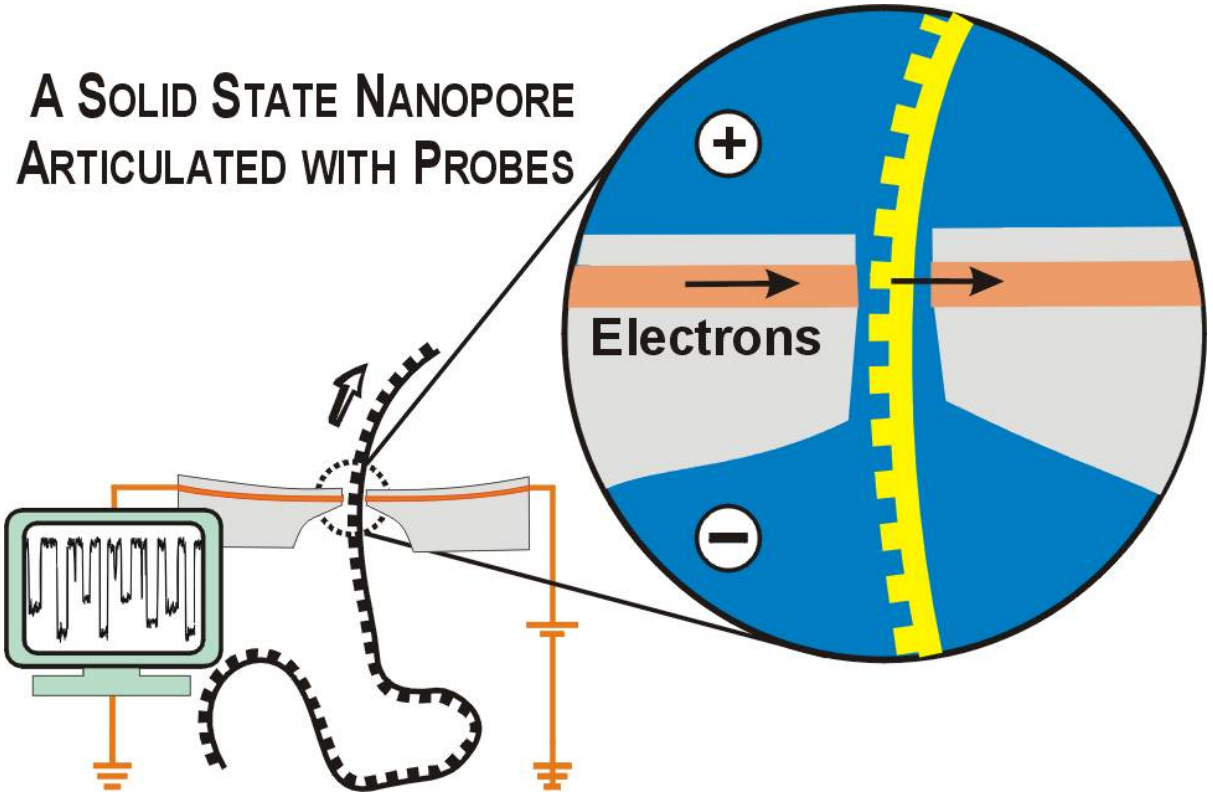


Solid State Nanopore



Dekker group, Nat. Mater. 2003





D. Branton, J. Golovchenko, Harvard

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4. Single-Molecule Detection in Solution Methods and Applications / edited by Christoph Zander, Jörg Enderlein, and Richard A. Keller: Wiley-VCH, 2002.