Optical Waveguide Biosensors for Proteins and Cells

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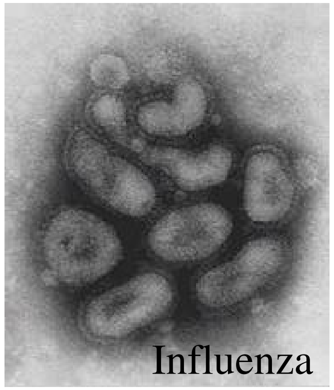
The objects of detection and their sizes in biosensing

Proteins and small molecules

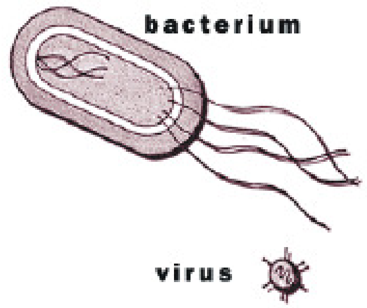
5-20 nm

Viruses



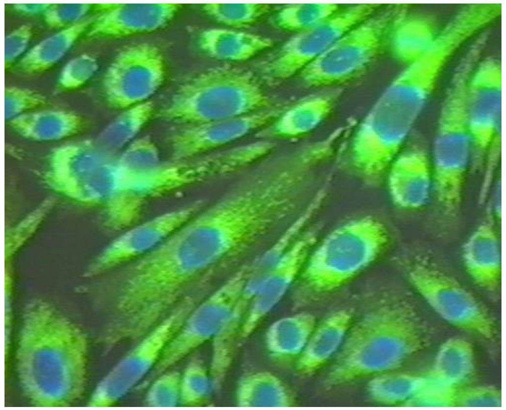
100 nm

Bacterial cells

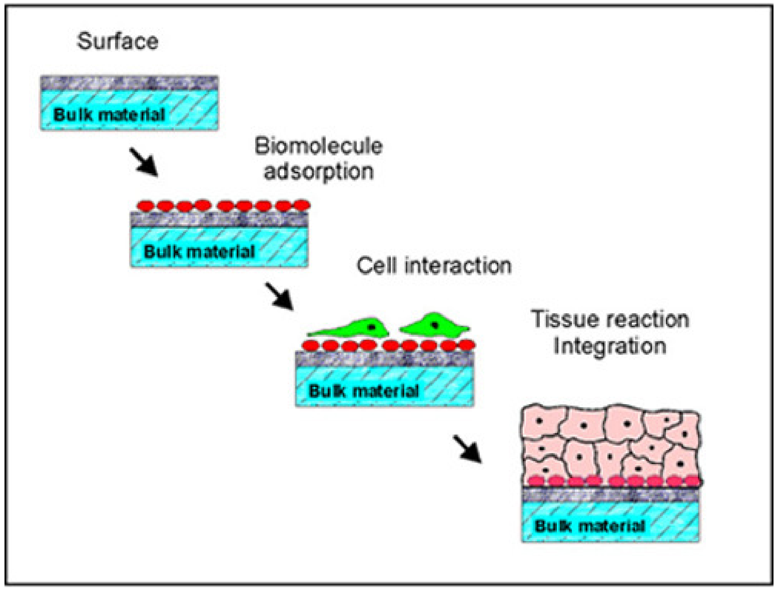


1-5 µm

Living cells



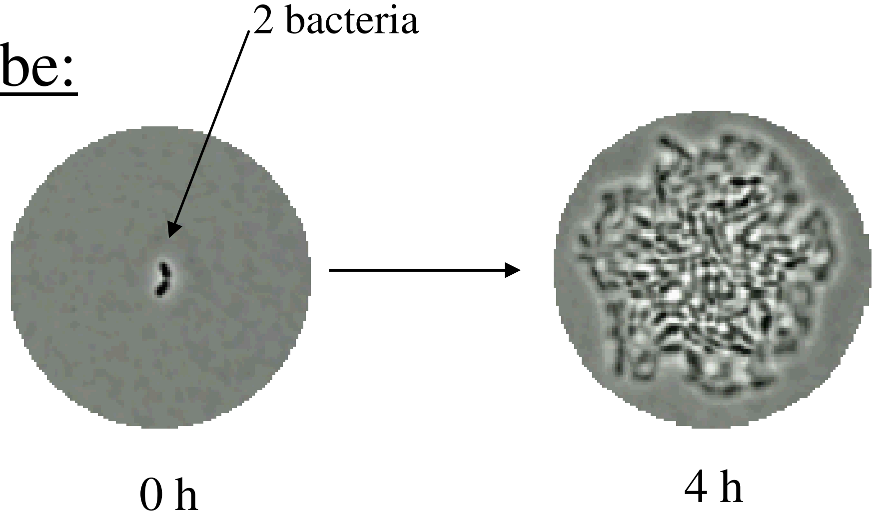
5-100 µm



The ideal sensor

The ideal sensor would be:

- Highly sensitive

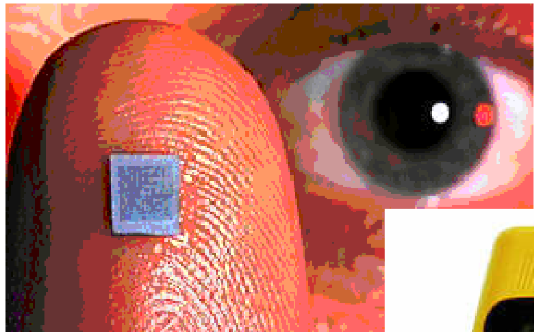


Protein adsorption: few ng/cm^2 is interesting

- Fast, on line: Measurement in real time

- Cheap, mass producible sensor elements

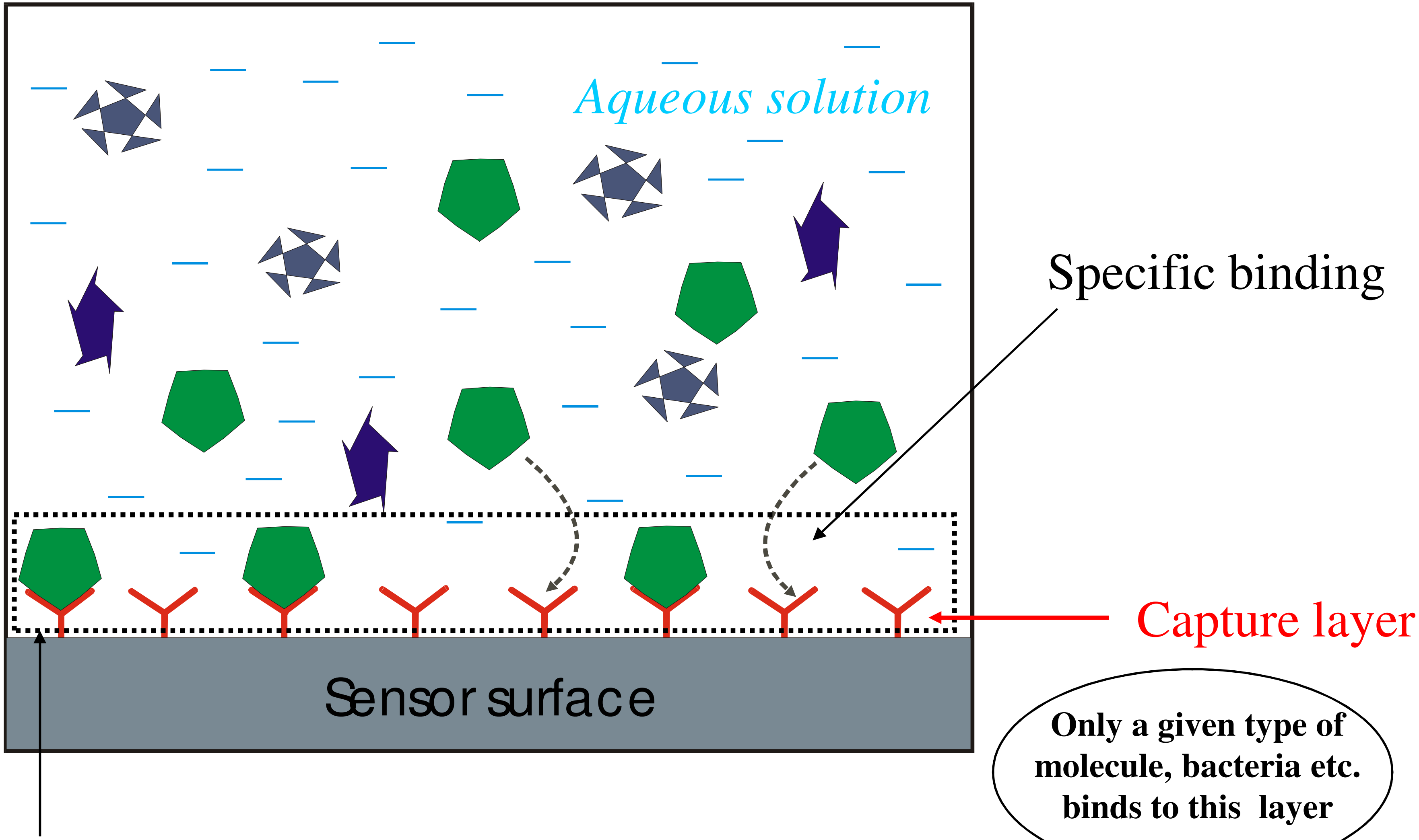
- Small, at least potential to miniaturization



- Selective!

for example: detect only one type of bacteria in a complex solution

The sensor selectivity – multidisciplinary approach



Only a given type of molecule, bacteria etc. binds to this layer

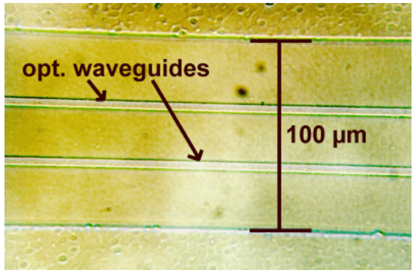
The sensor has to be selective in space too. Changes only close to the surface have to be detected!
(Physicists)

(Biologists and Chemists)

Optical devices in biosensing – advantages of optics

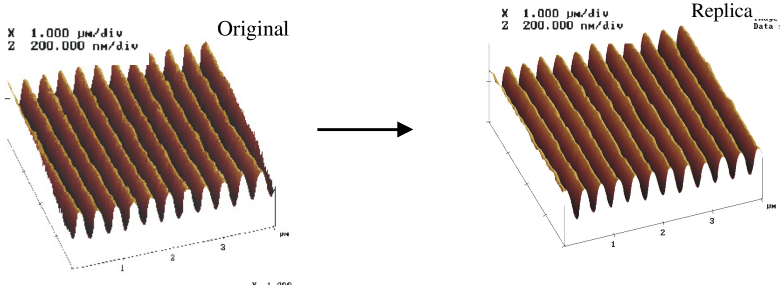
<u>What does optics offer?</u>

- An electromagnetic radiation what can interact, but does not damage the detected biological object



- Potential to miniaturization, integrated optics (1970-, "optical IC –s") micrometer scale devices

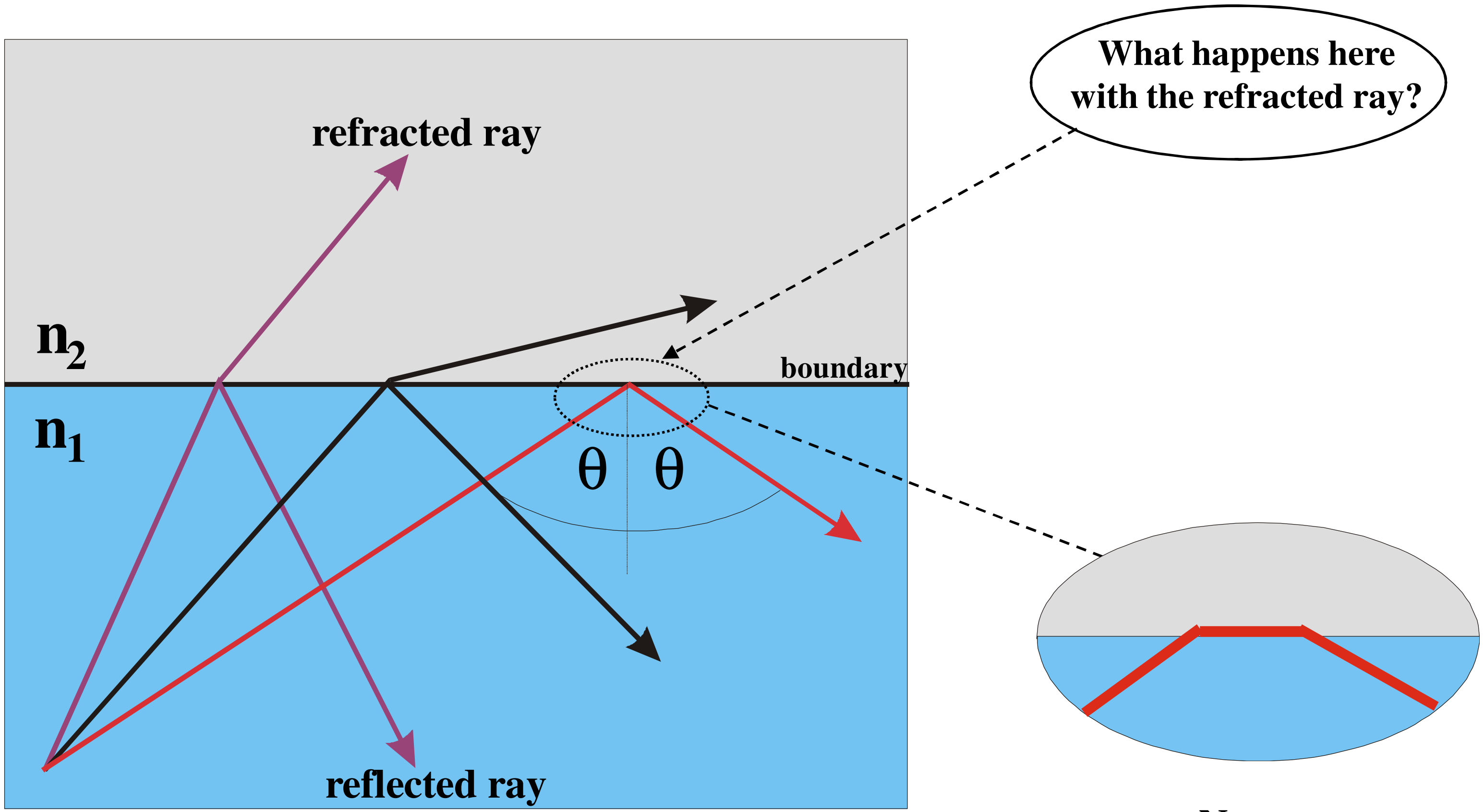
- Low prices, application of polymers and novel replication technologies



Surface selective detection and high sensitivity ?

We need to understand the behavior of light at surfaces…

Ray optics



Newton:
The light penetrates into the lower refractive index layer !

$n_1 > n_2$

$\sin(\Theta) > (n_2/n_1)$

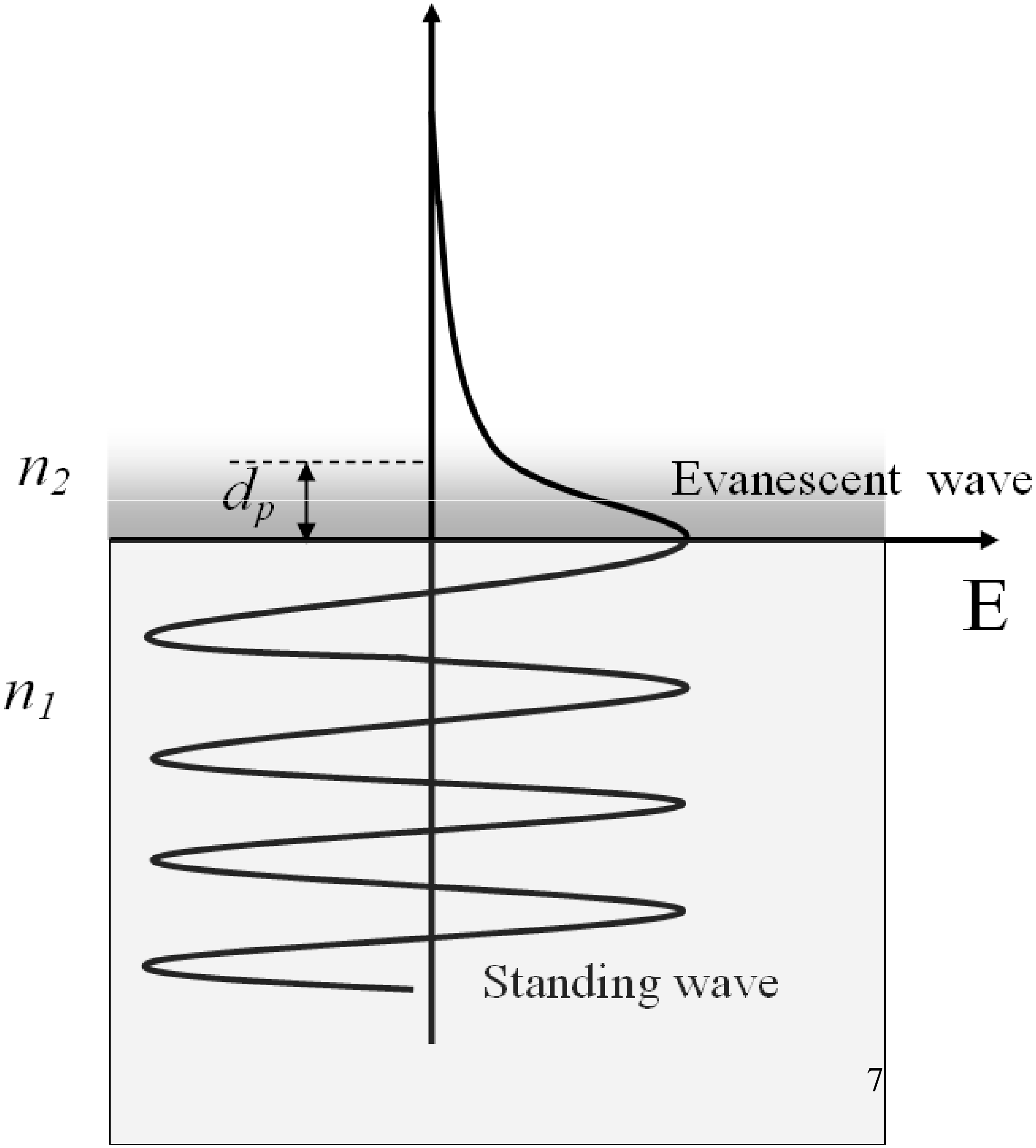
→ Total internal reflexion

Wave optics: the penetrating wave is the **evanescent wave**

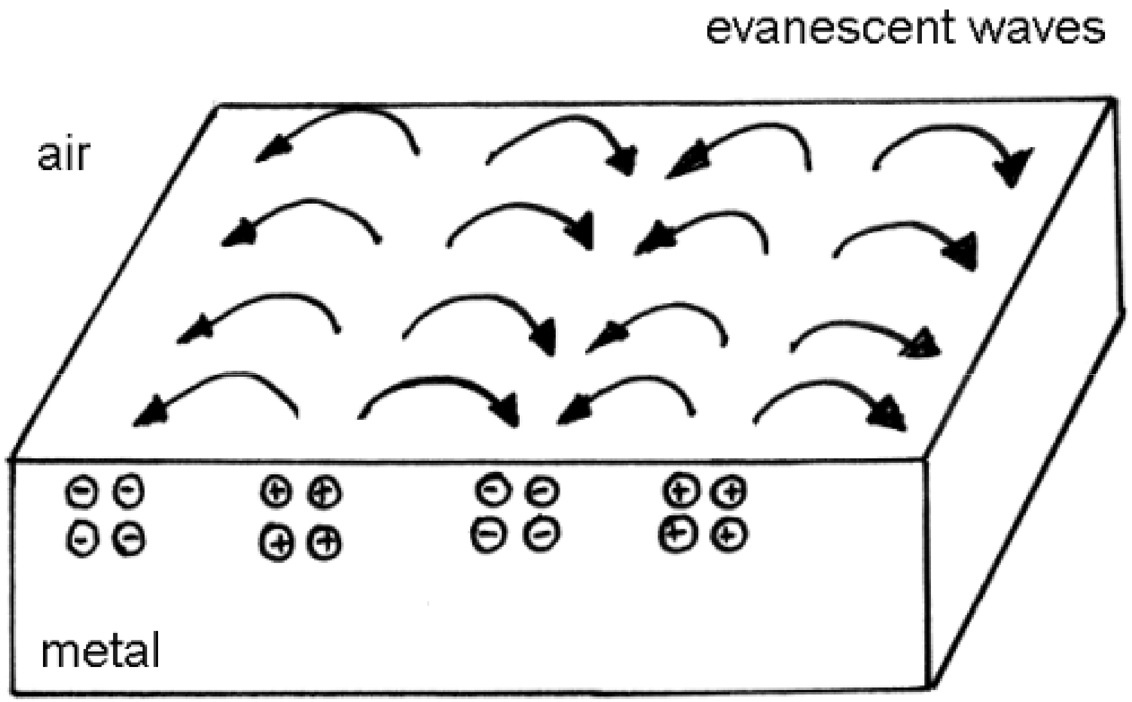
Exponential decay
penetration depth 100 nm

Highly surface sensitive...

How to generate and controll
its propagation?



Surface Plasmon Resonance



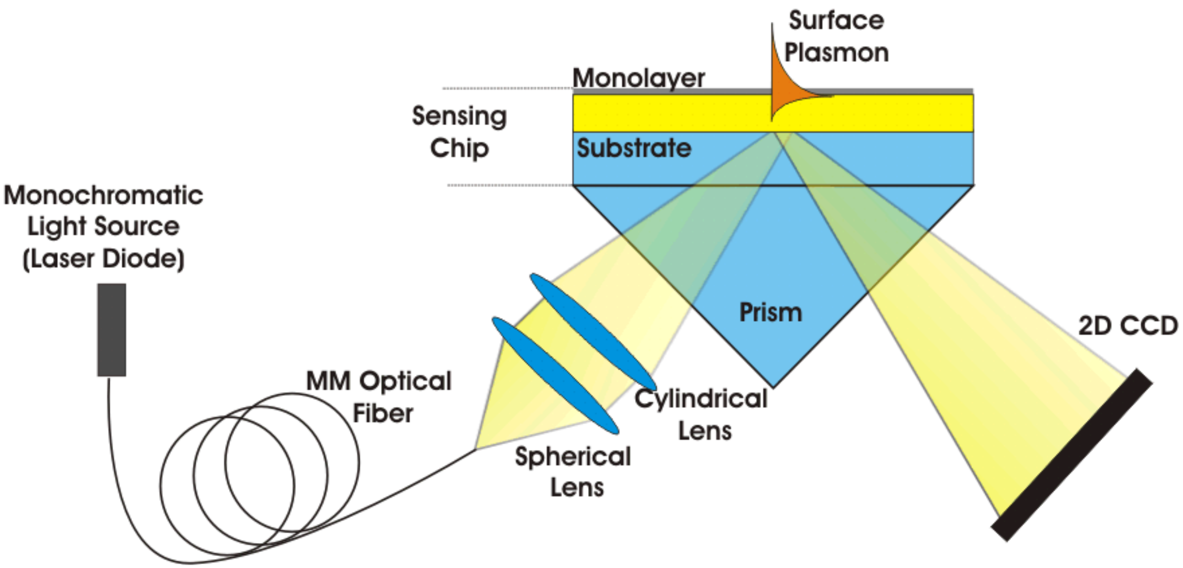
Using a metal surface the evanescent wave can be excited…

SPR (Surface Plasmon Resonance)

1990: Pharmacia Biosensor AB (Uppsala, Sweden) launched BIAcore "off the self" Instrument (Biomolecular Interaction Analysis):

Surface Plasmon Resonance (SPR)

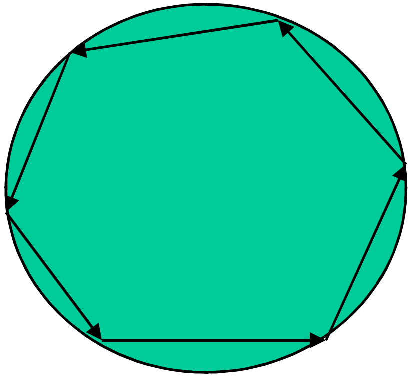
www.biacore.com

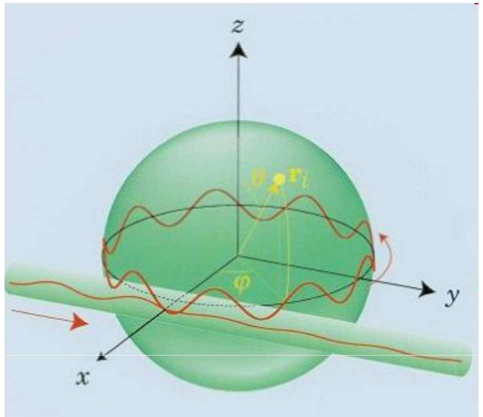


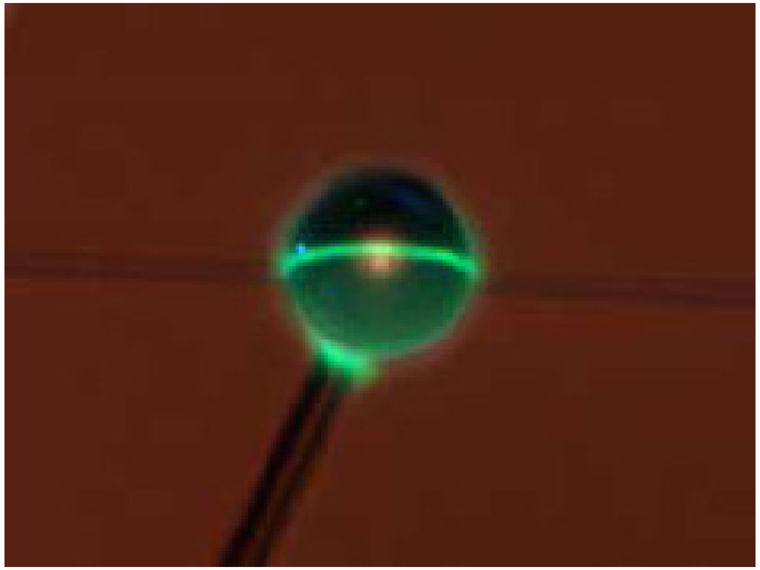
Unfortunately quantum tunneling does not work for cars!

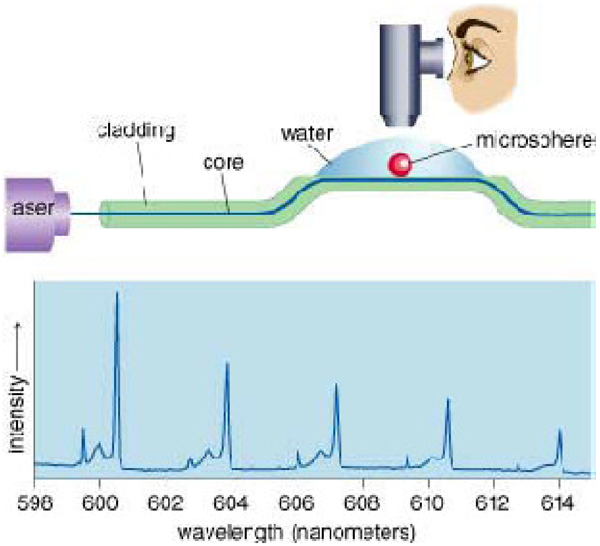
<u>2. Excitation type</u> The light can be trapped in a microsphere:

Whispering galery modes





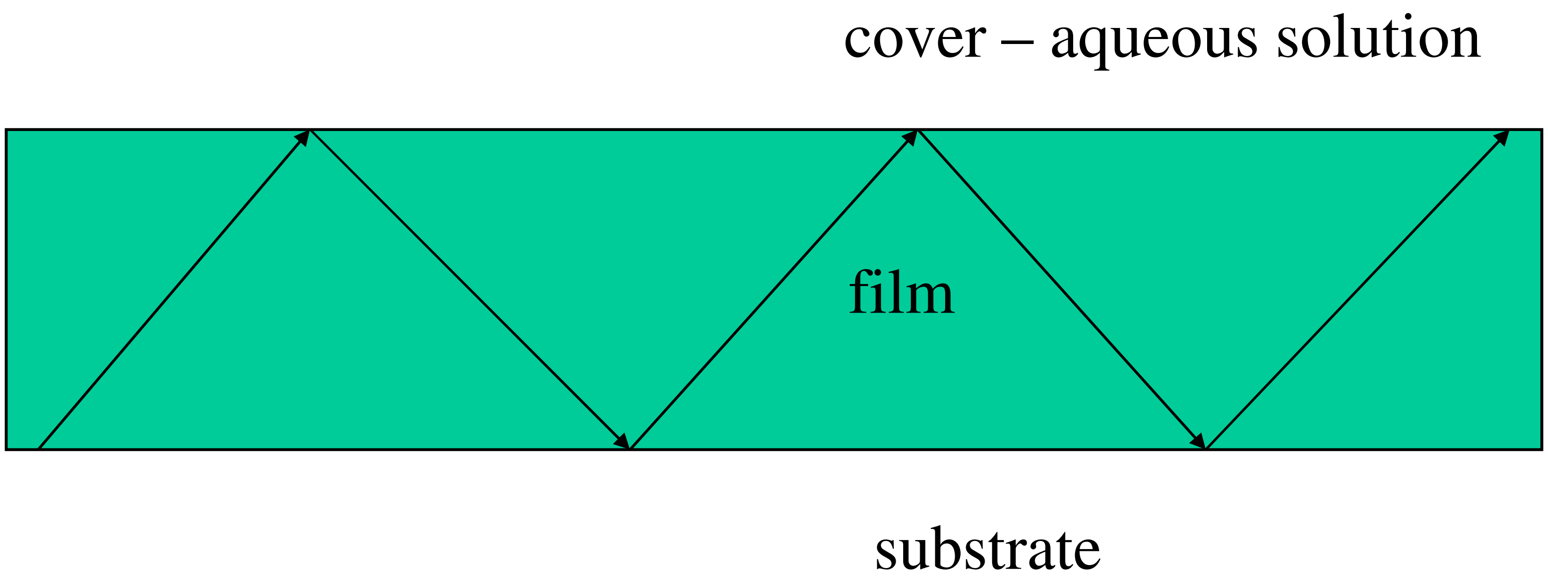




Single virus detection is possible!
(Harvard, 2008)

3. Excitation possibility of the evanescent waves

Light can be trapped in a thin dielectric film with high refractive index...

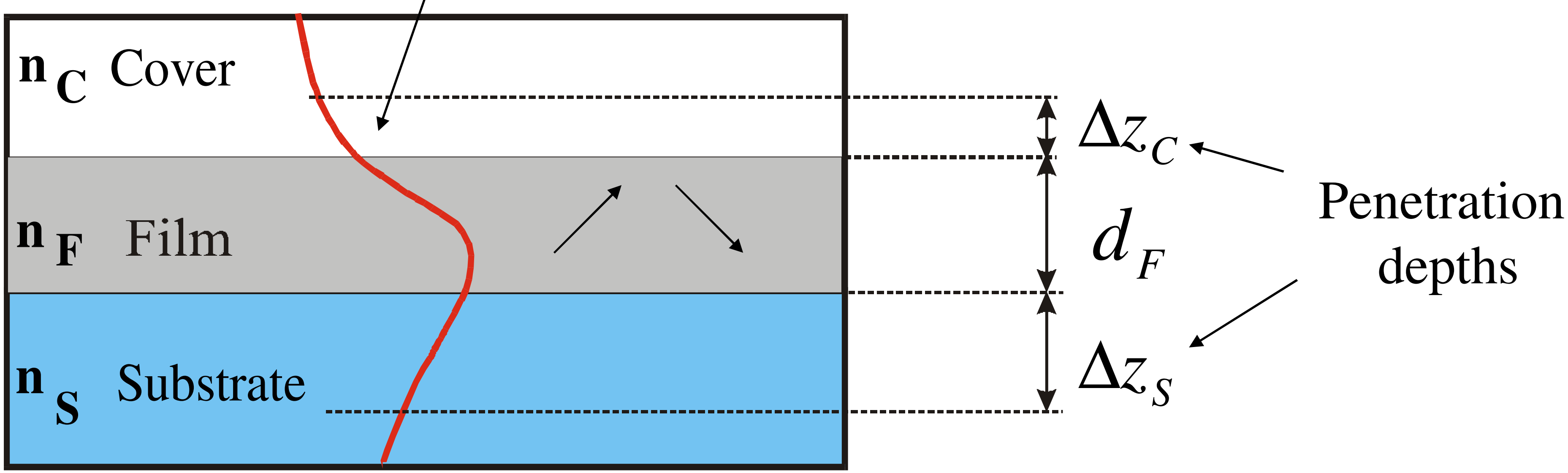


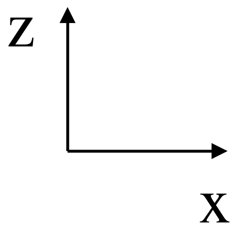
Modes of the waveguide – evanescent waves

Amplitude profile: $A(z)$

Evanescent wave:

$$A = A_0 \exp\left(-\frac{z}{\Delta z_C}\right)$$

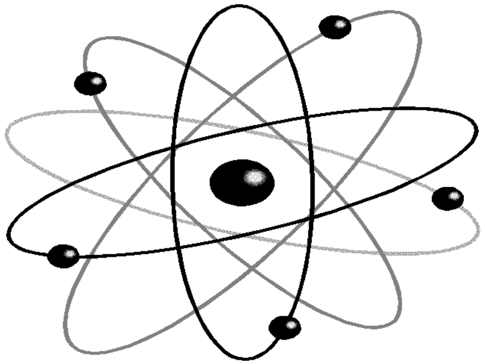


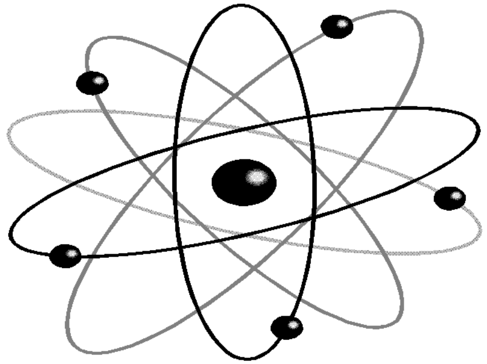


Phase velocity: $\frac{c}{N}$

Effective refractive index of the waveguide mode. It has discrete values.

Similar systems?





These modes are very similar to the modes (Eigenfunctions) observed in **quantum systems**.

(Film thickness in the order of wavelength!: we can expect new and unusual effects compared to bulk optics…)

Quantum mechanical analogy

Schrödinger eq. for a particle in square-wave potential:

$$\frac{d^2\Psi_J}{dz^2}+\frac{8\pi^2 m}{h^2}(E-V_J)\Psi_J=0 \qquad J=S,F,C$$

$$V_S>V_C>V_F$$

Wave eq.:

$$\frac{n_J^2}{c^2}\partial_t^2\Psi_J=\partial_z^2\Psi_J+\partial_x^2\Psi_J \qquad \Psi=a\exp[i(kNx+k_z z-\omega t)]$$

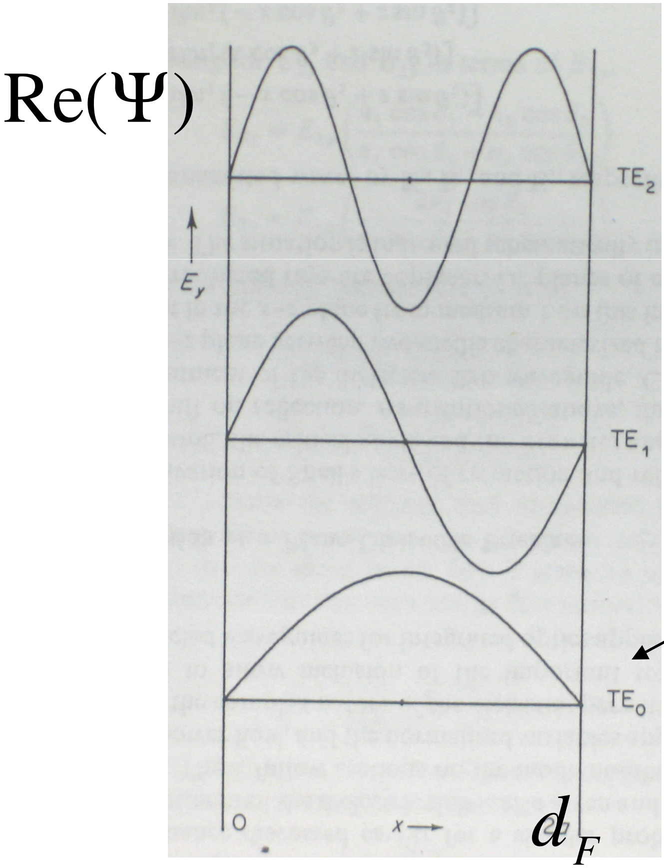
We get the same form as above:

$$\partial_z^2\Psi_J+k^2[(n_F^2-N^2)-(n_F^2-n_J^2)]\Psi_J=0$$

$$E\rightarrow\frac{h^2k^2}{8\pi^2 m}(n_F^2-N^2) \qquad V_J\rightarrow\frac{h^2k^2}{8\pi^2 m}(n_F^2-n_J^2)$$

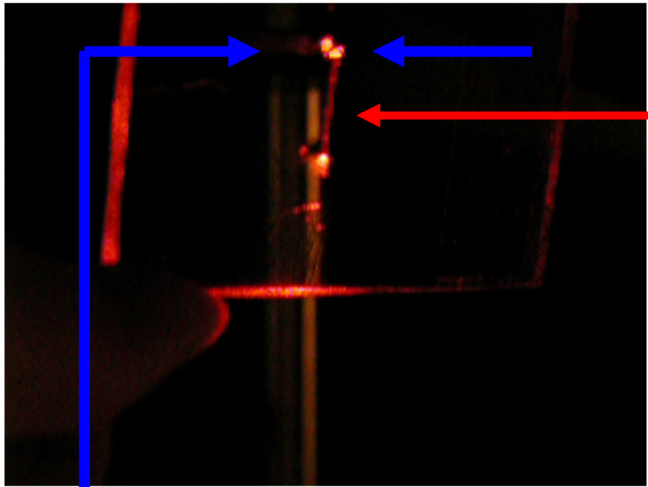
„Photons tend to be in high RI media”

- Example: Two parallel perfect mirrors; infinitely deep potential well

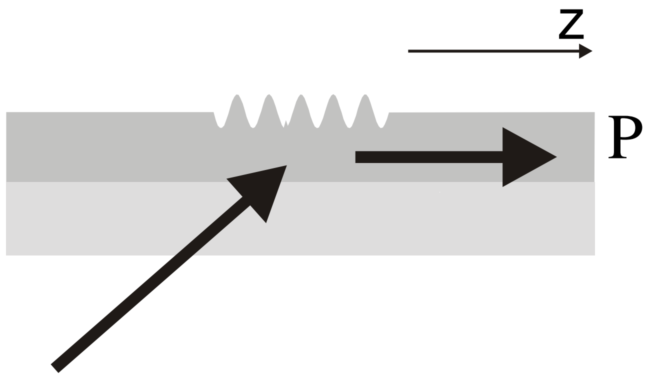


Lowest energy, highest N

How can we excite these modes? - Grating coupling

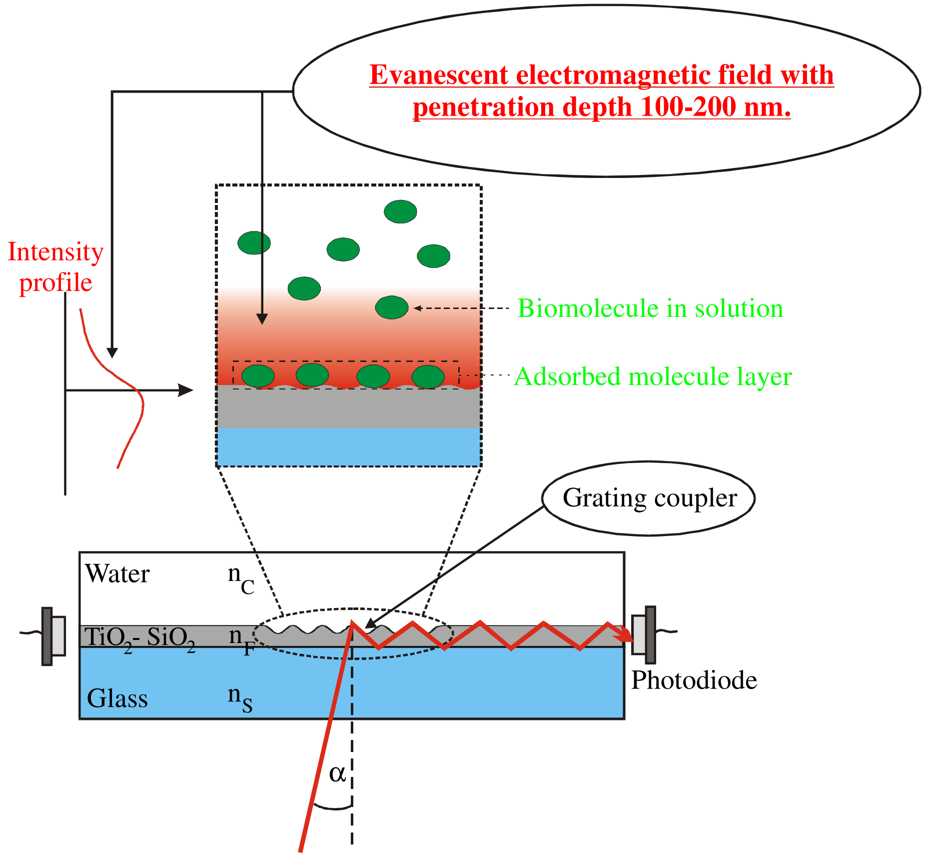


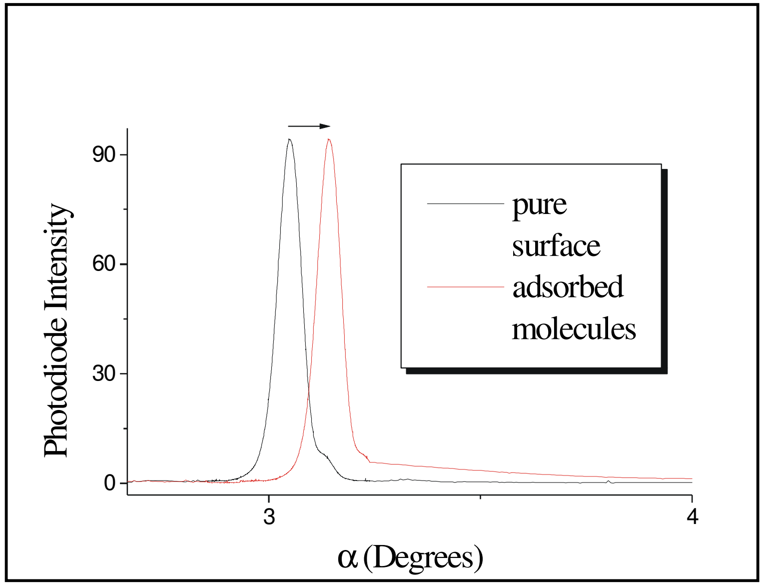
Propagating mode

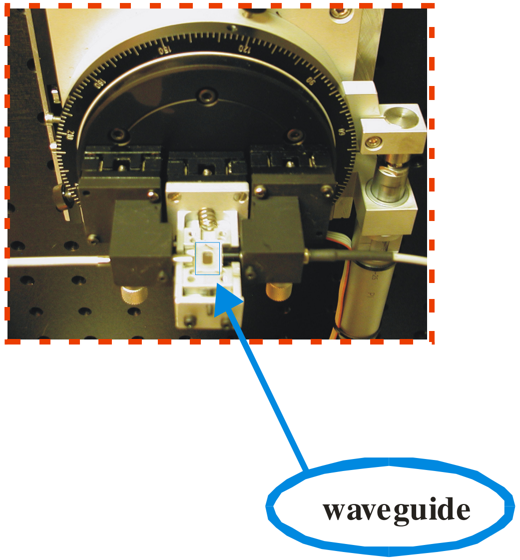


2 mm wide surface relief grating

Conventional sensors with grating coupler (ASI; Microvacuum)







Kurt Tiefenthaler and Walter Lukosz (Switzerland)

Planar Waveguide Biosensing

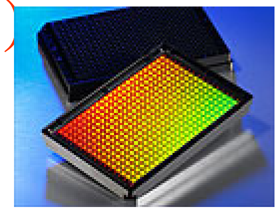
Label free detection!

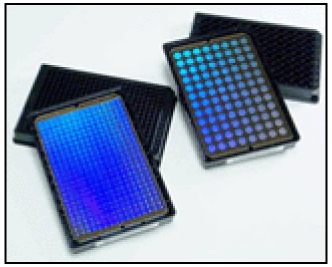
On-line. Measurement in real time

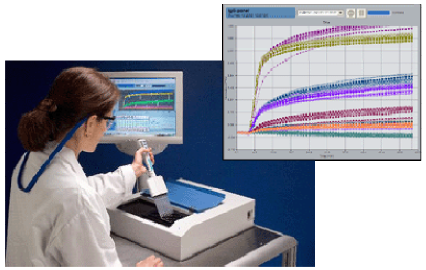
Multipoint screening is solved; HTS

Very well established quantitative technique:
Countless number of case studied are published
(proteins, lipids, DNA, living cells)
Highly sensitive! (1pg/mm^2)

Microvacuum (HU; ASI (CH))
Corning (USA)
SRU Biosystems (USA)







Farfield (UK)

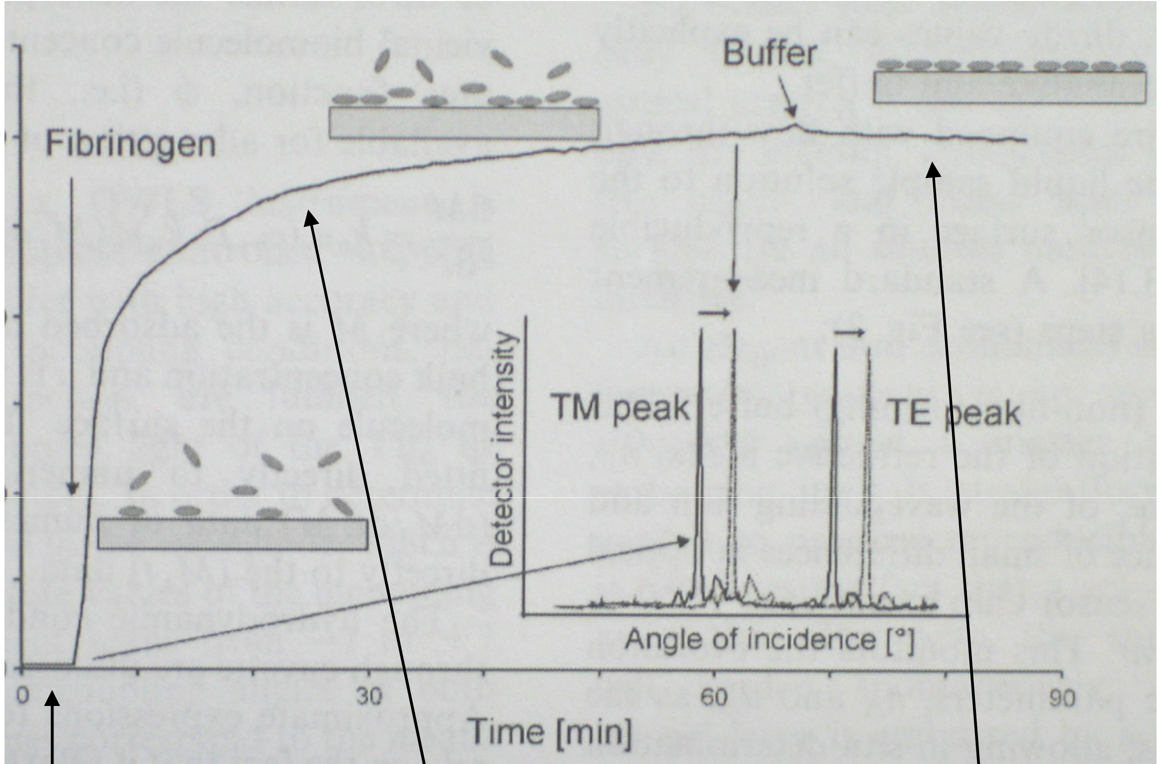
Zeptosense (CH; BAYER GMBH)
Luminescence (DK)

APPLICATION fields of these sensors

hot topics?

Application 1. on-line monitoring of protein adsorption

Adsorbed proteins shift the resonant angle



Testing medical implants

baseline

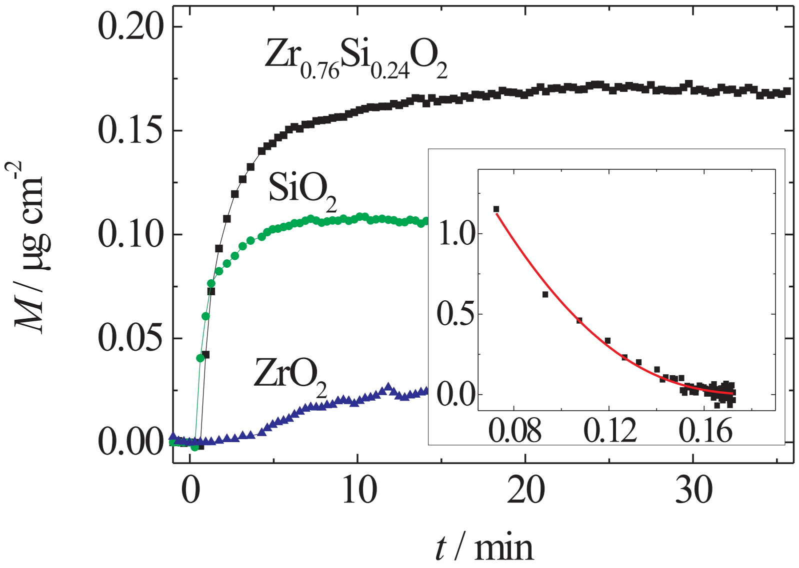
Adsorption

Desorption phase

Voros et al. Biomaterials 2002

Application 2. protein adsorption - KINETICS

Protein adsorption (Bovine Serum Albumin) on thin films



Random Sequential Adsorption Theory.

$$dM/dt = k_a c_b \phi(M,a),$$

Substrate	a (nm^2)
SiO_2	44
ZrO_2	122
$Zr_{0.76}Si_{0.24}O_2$	31

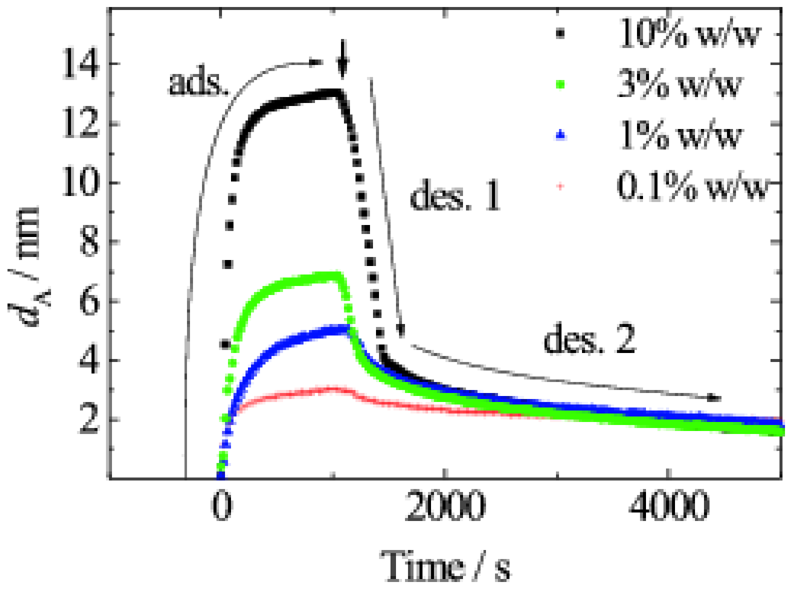
FROM KINETCS >>>>>>>>>> **STRUCTURE**

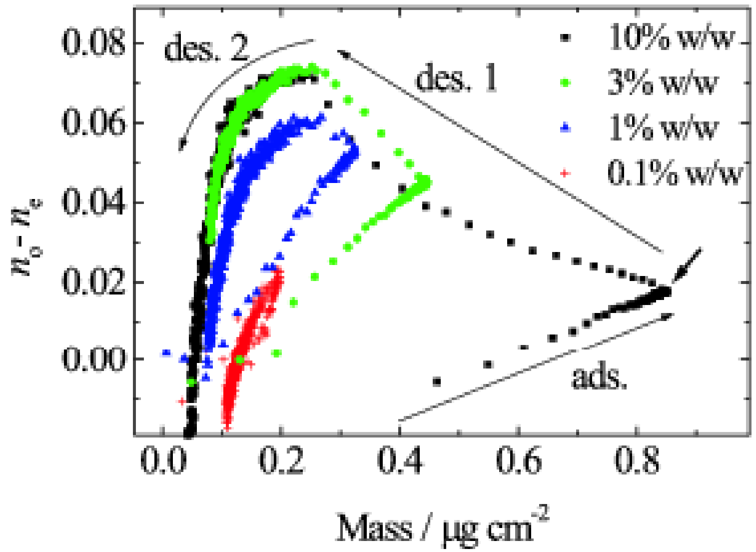
Nitesh Aggarwal, Ken Lawson, Matthew Kershaw, Robert Horvath, and Jeremy Ramsden
”Protein adsorption on heterogeneous surfaces”
Applied Physics Letters 94, 083110 (2009).
(Spotted in the March 1, 2009 issue of Virtual Journal of Biological Physics Research.)

Application 3. anisotropy of protein layers

More modes with different polarizations:

Measurement of optical anisotropy in glycoprotein (mucin) films



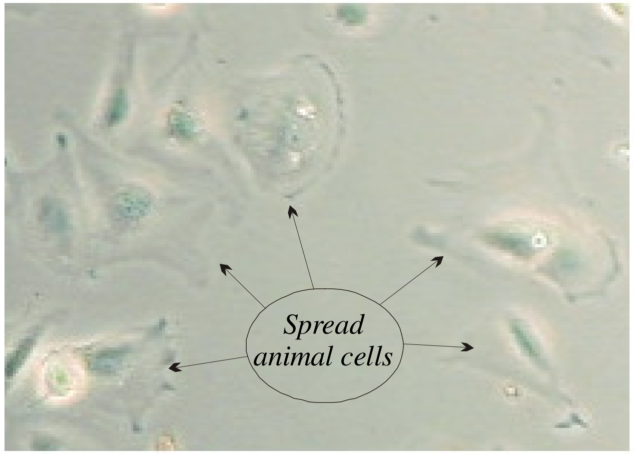


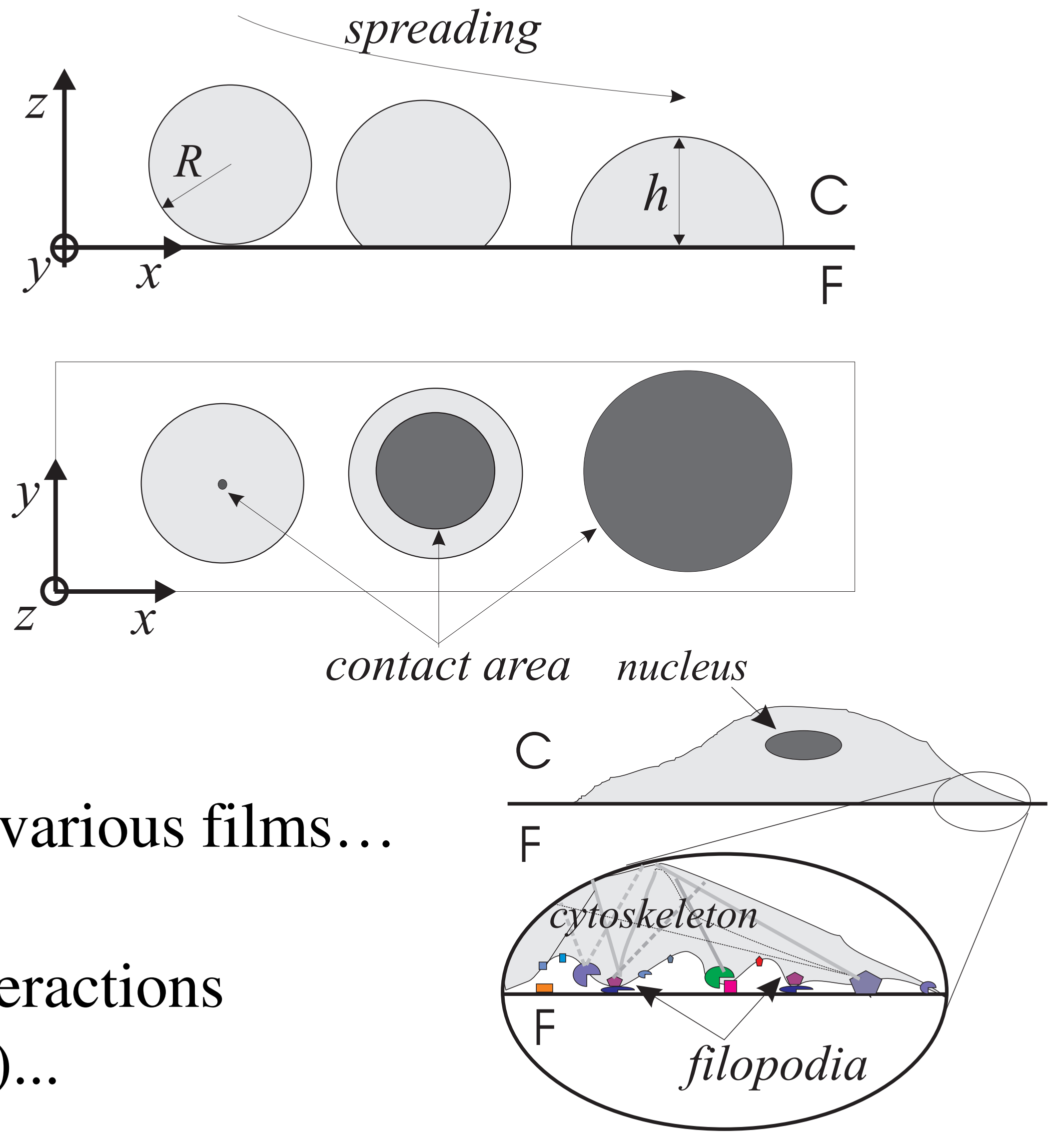
Structural information at the nanometer scale!

Two stage desorption – two forms of adsorbed molecules.

R. Horvath, J. McColl, G.E. Yakubov, J.J. Ramsden "Structural hysteresis and hierarchy in adsorbed glycoproteins" THE JOURNAL OF CHEMICAL PHYSICS 129 (7), art. no. 071102 (2008).

Application 4. On-line monitoring of living cell spreading



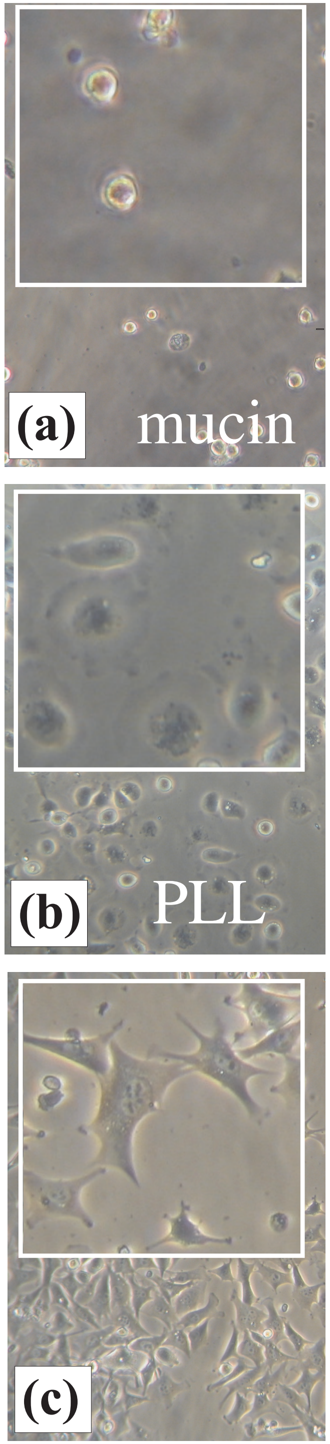


Waveguide can be coated with various films…

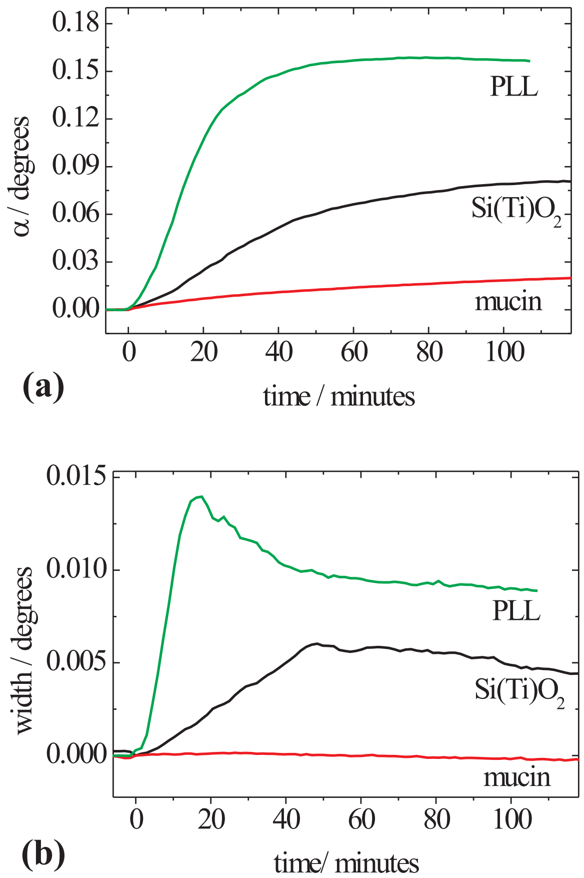
Testing implants, cell-cell interactions
(membrane fragments)...

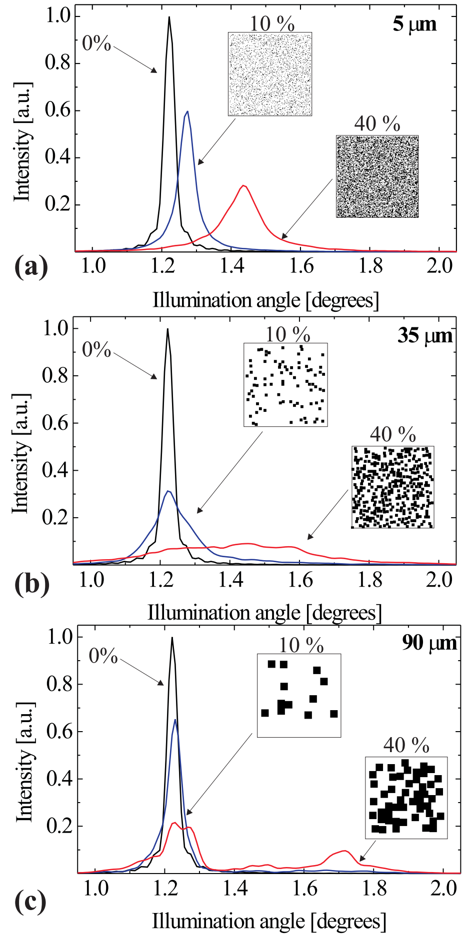
J.J. Ramsden and R. Horvath "Optical biosensors for cell adhesion" Journal of Receptors and Signal Transduction (Invited Review) (2009)

Monitoring cell adhesion on various surfaces



Experimental





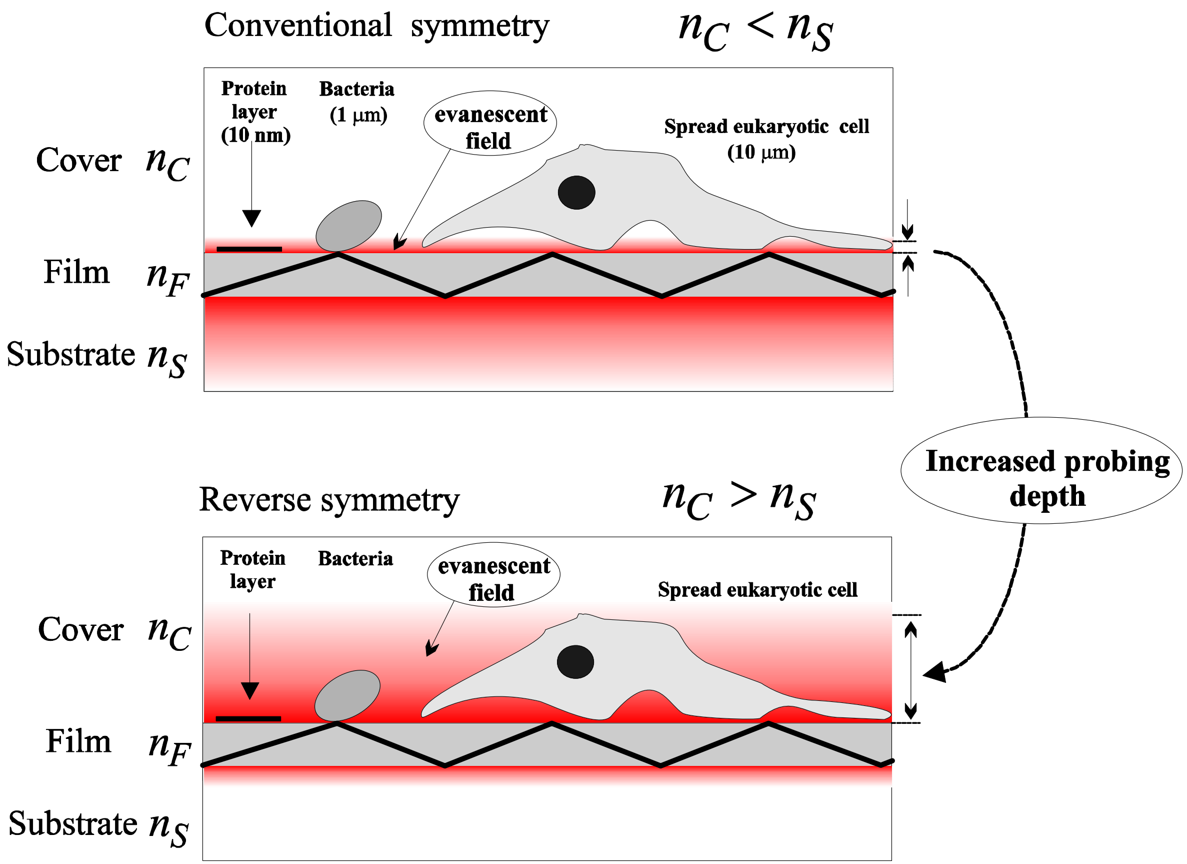
Numerical simulations

The strength of adhesion can be measured.

A.Aref, R. Horvath, J. McColl, J.J. Ramsden
“Optical monitoring of stem-cell substratum interactions”
Journal of Biomedical Optics Letters (2009).

Limited probing depth - reverse waveguide geometry

Problem with waveguide and SPR: Short and limited penetration depth into the cover media



Increased penetration depth and relative mode power in the cover.

→ Higher sensitivity

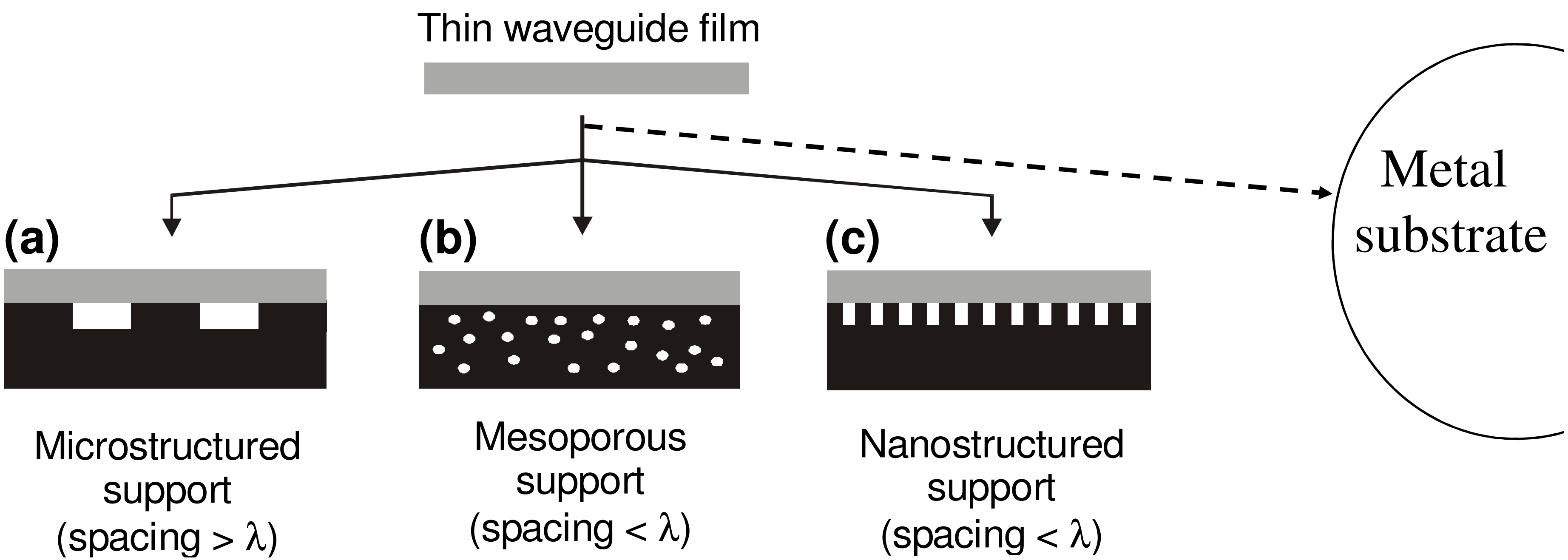
R. Horváth, L.R. Lindvold, and N.B. Larsen, "Reverse symmetry waveguides: Theory and fabrication", *Appl. Phys. B* **74**, 383 (2002).

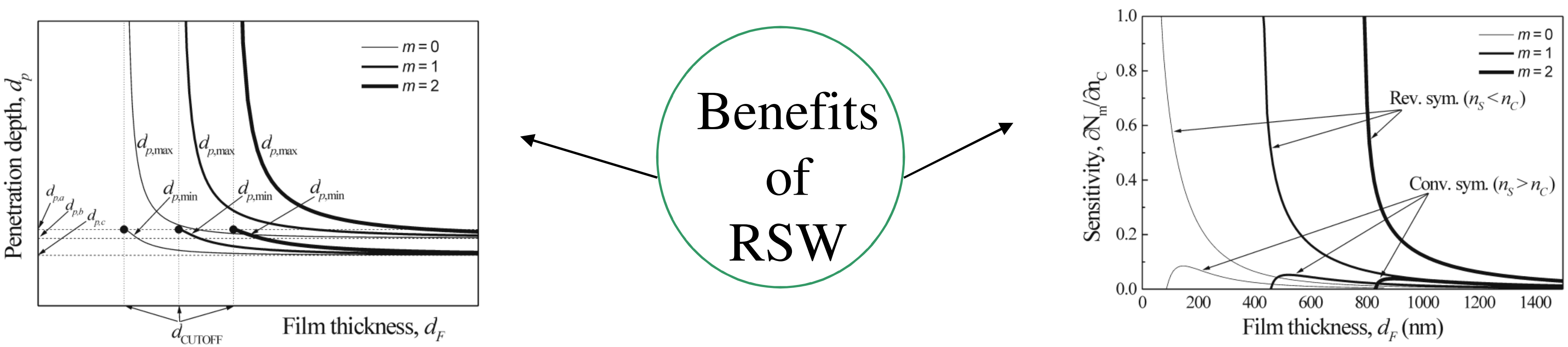
Substrate refractive index less than 1.33?!

Nano- and microtechnology can help us!

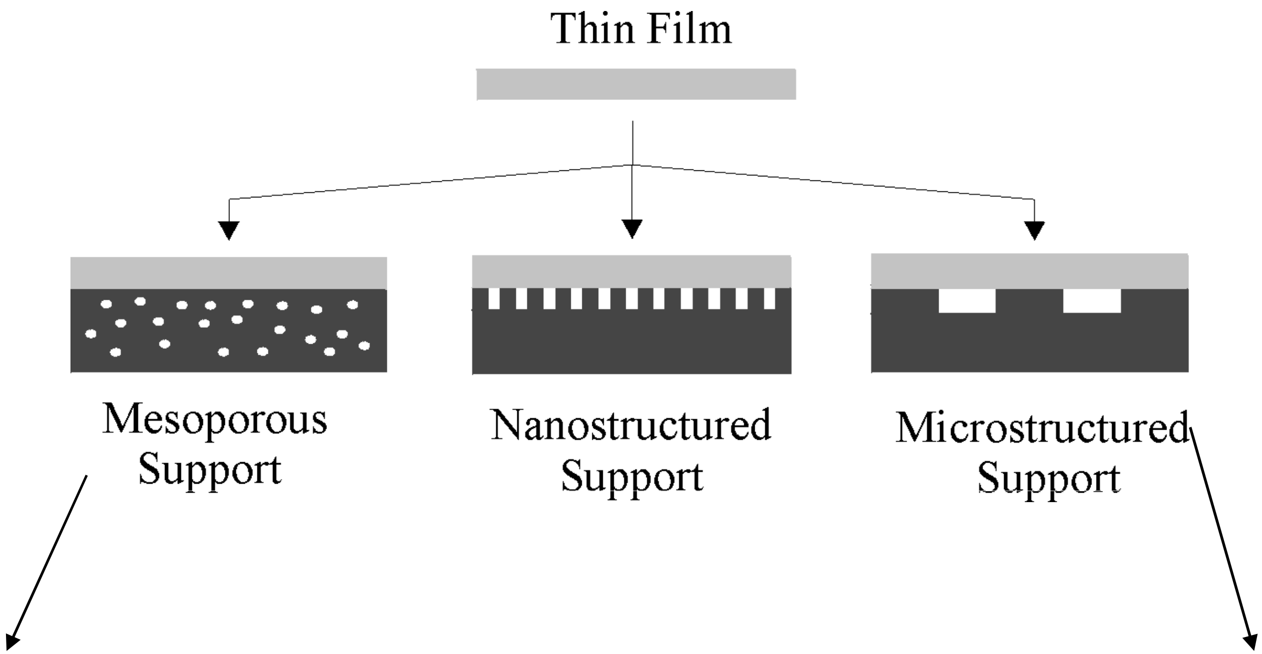
Possible reverse waveguide designs

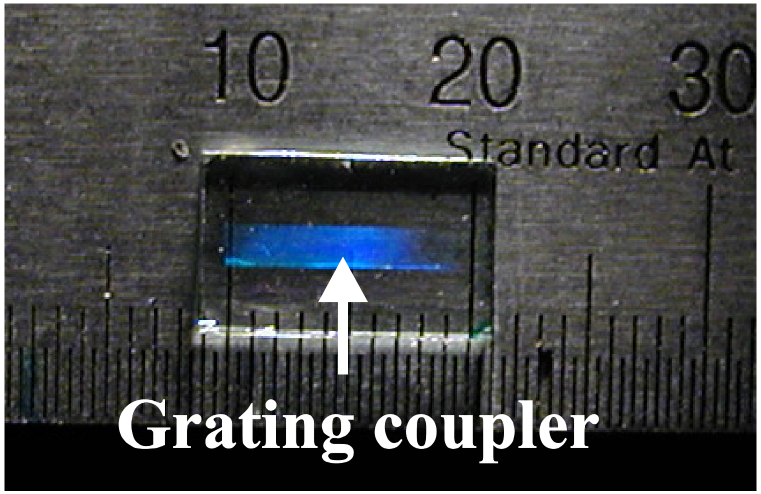
How to realize the reverse symmetry?:

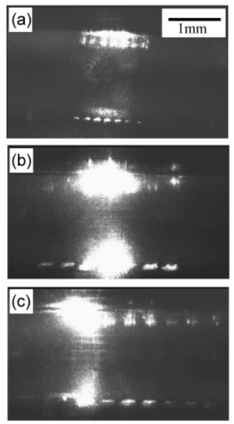


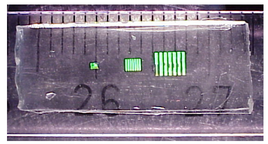


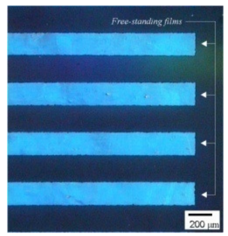
Fabricated structures



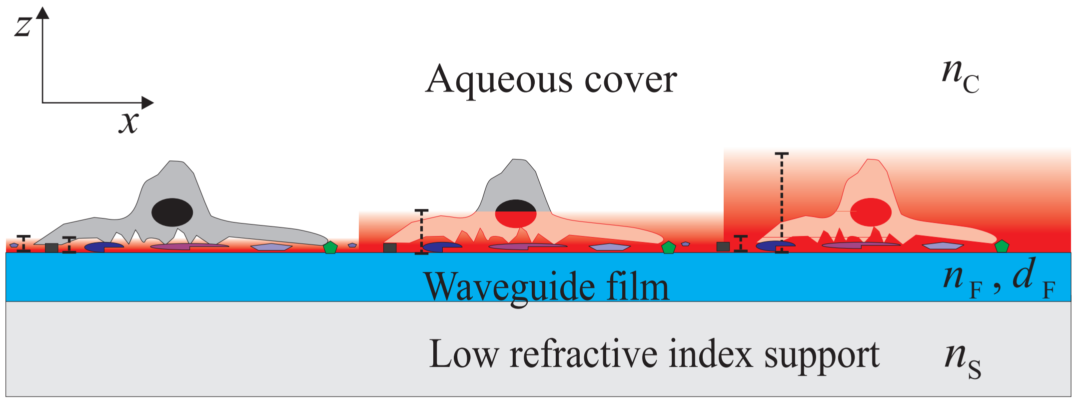




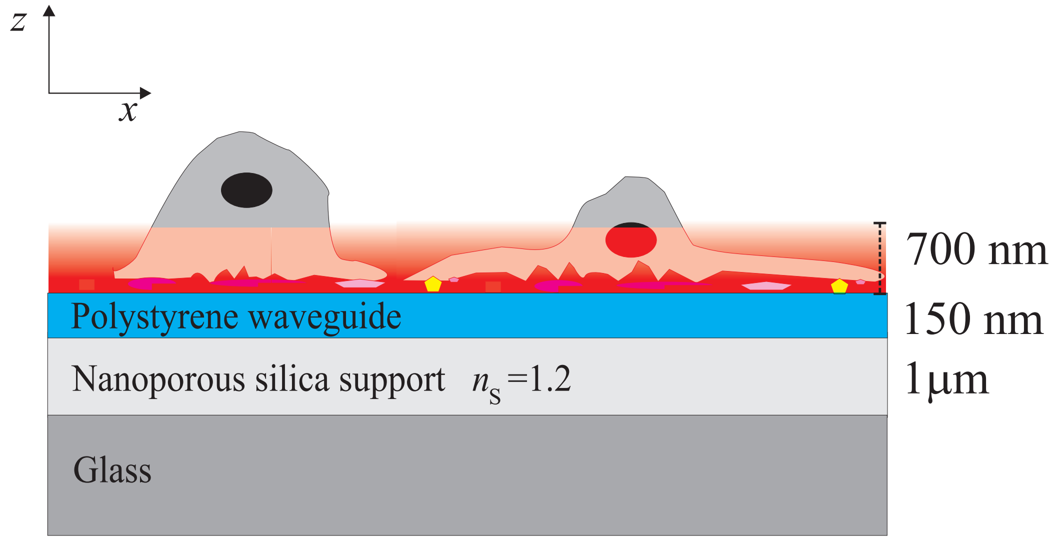




Multidepth screening of living cells using reverse waveguides

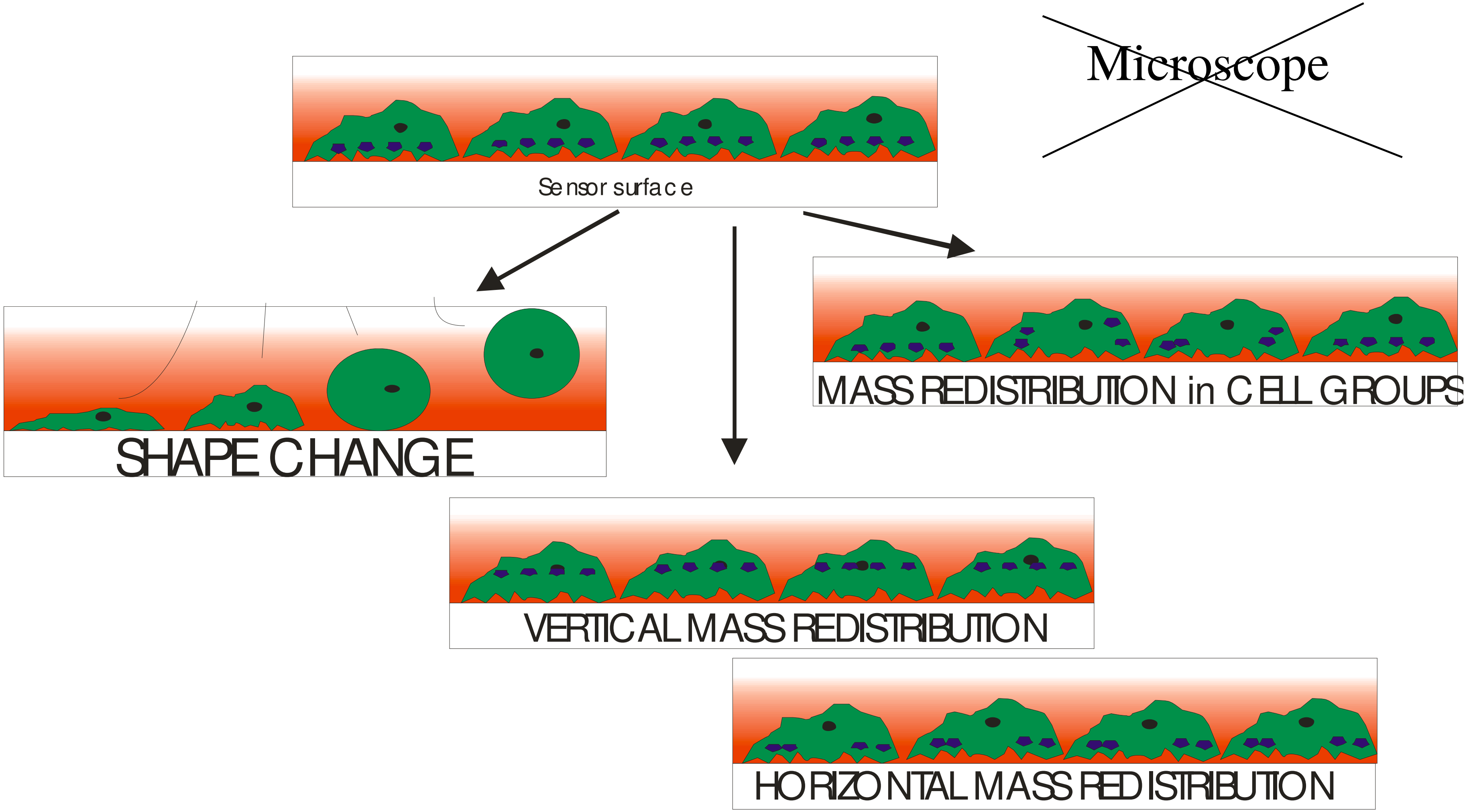


Optical slicing of the cell layer.



Horvath R, Cottier K, Pedersen HC, Ramsden JJ "Multidepth screening of living cells using optical waveguides" BIOSENSORS AND BIOELECTRONICS Volume 24, 1 December 2008, Pages 799-804 (2008).

Summary of cell refractive index variations in cell layers

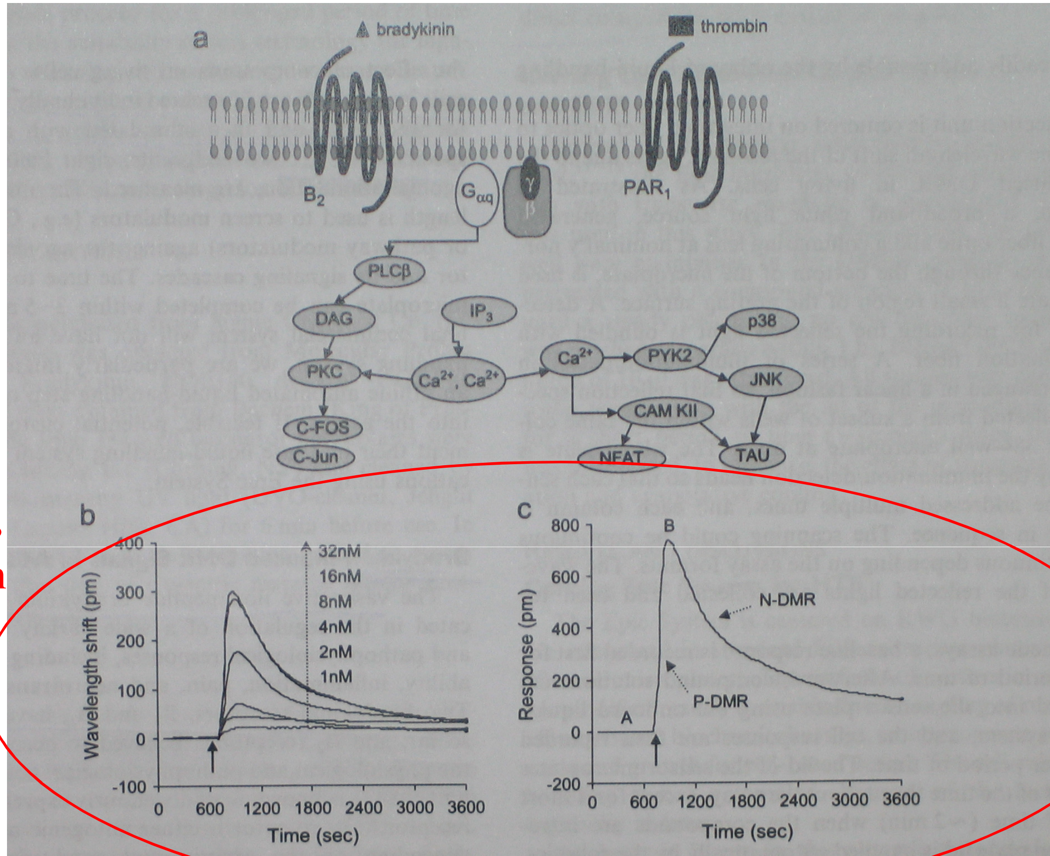


Cell based assays are more and more important in **drug development**.

EXAMPLE: study of GPCR signaling pathways

G Protein-Coupled Receptors

(Proven to be the most productive area for small drug discovery)



Activation of GPCR
Causes translocation of signaling molecules
Or in many cases cytoskelatal reorganization

Optical signature of RI variations measured by Corning EPIC System

(penetration depth of 100nm)

From Li et al. JALA 2006

Main projects in our laboratory

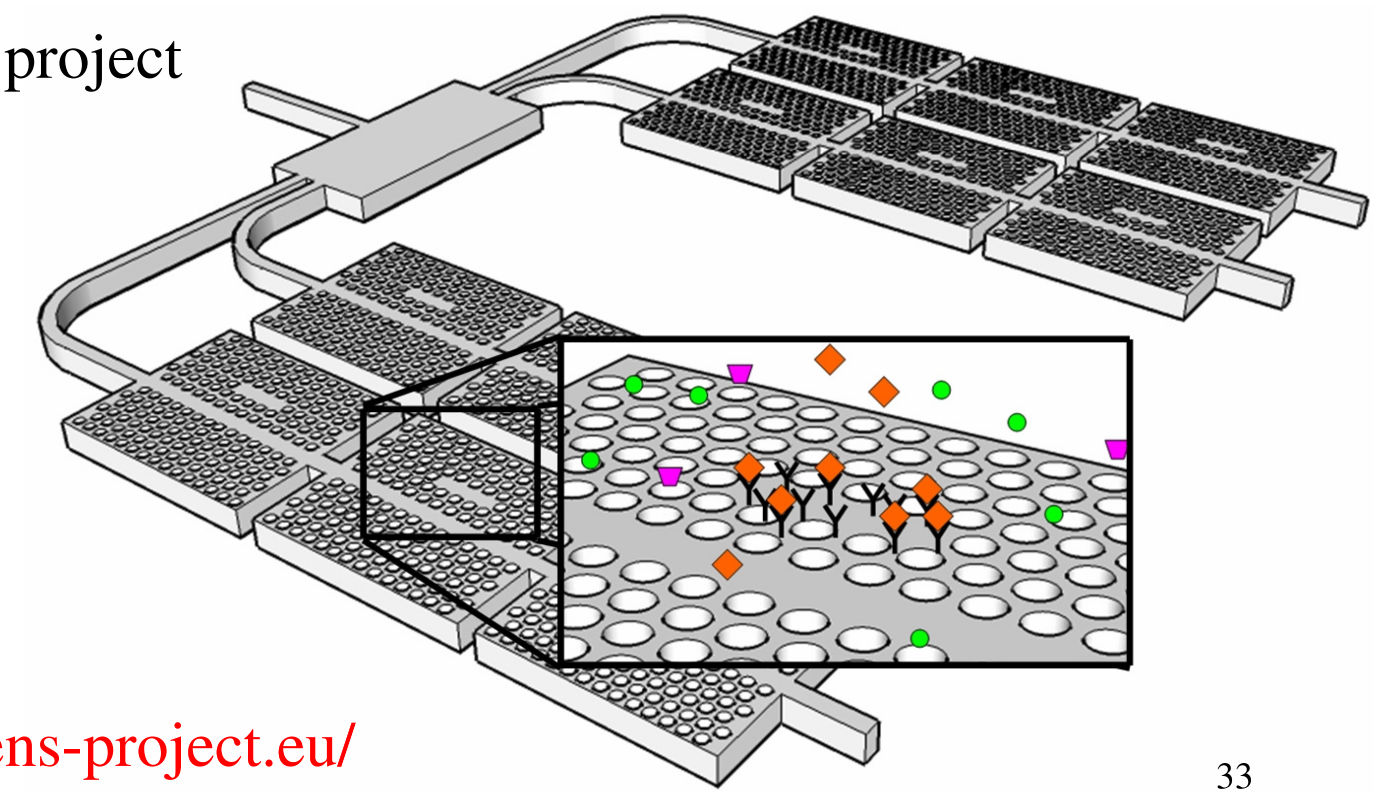
You are wellcome to visit us…

Detection of disease biomarkers in BLOOD

Can we tell the presence of cancer from a **drop of blood**?

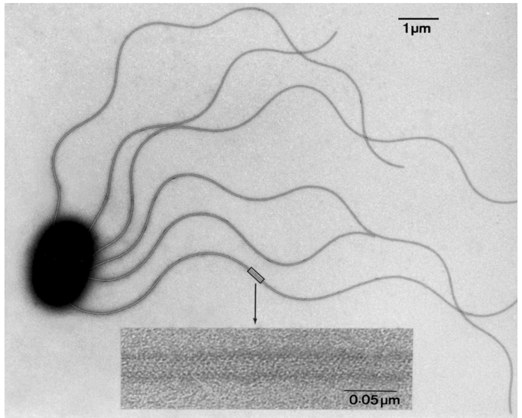
Significant challanges:
Glucose versus cancer detection in human blood

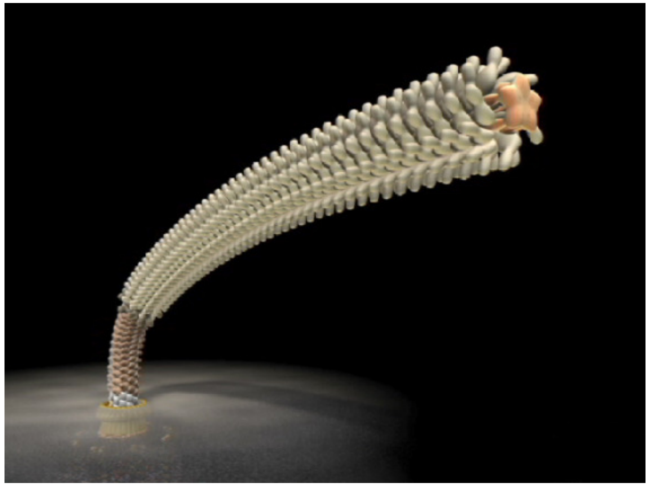
P3SENS FP7 project

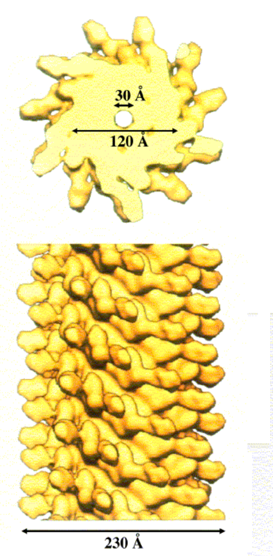


http://www.p3sens-project.eu/

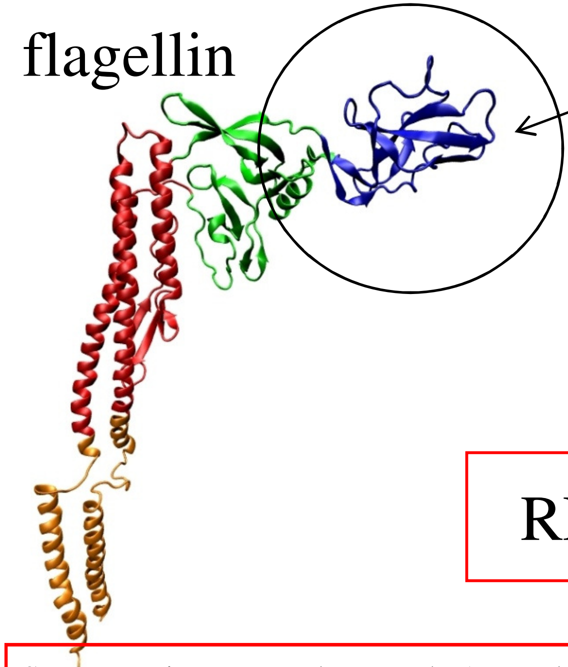
Novel sensor matrix based on modified flagellins – 3D receptors





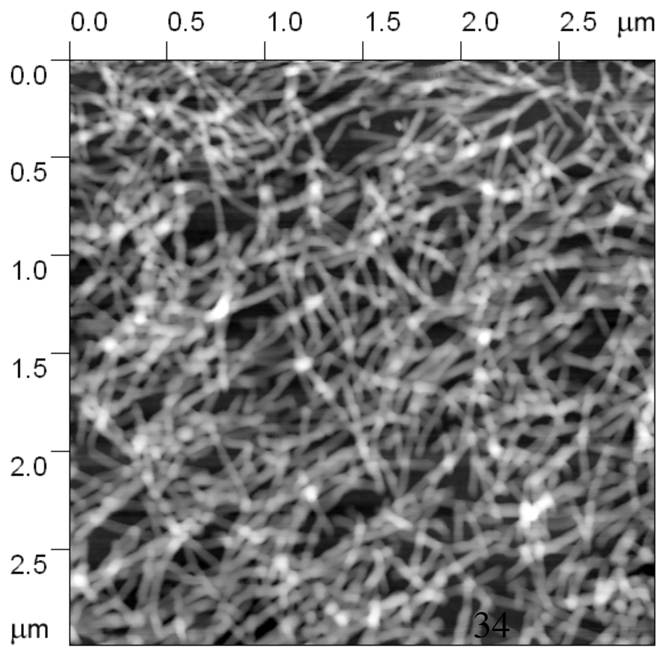


flagellin



Modify the D3 domain

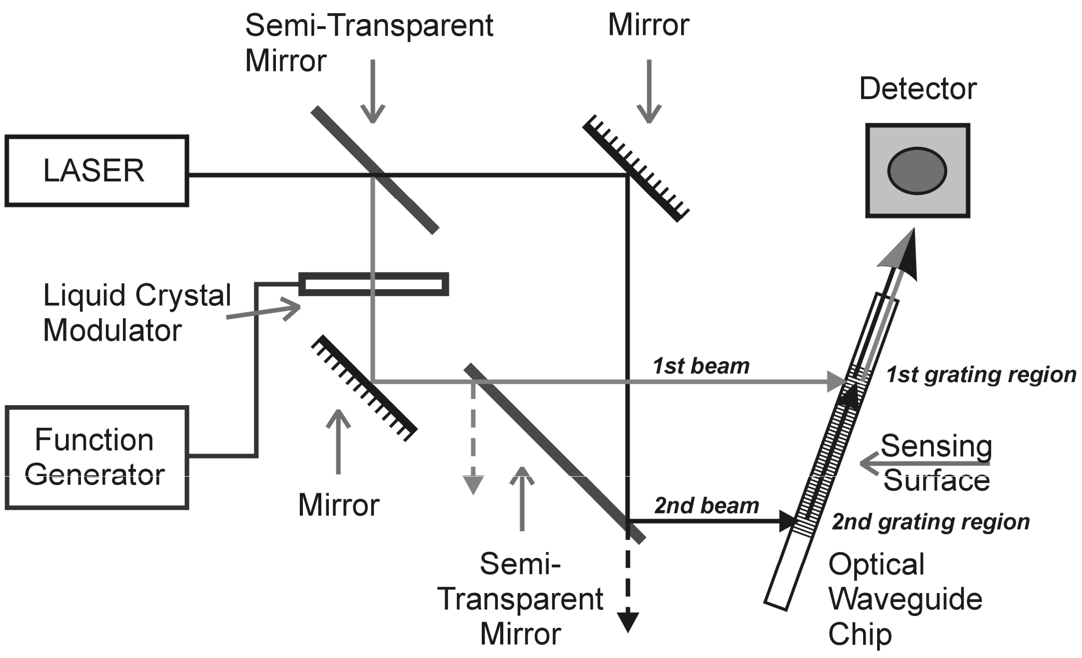
RECEPTOR functionality

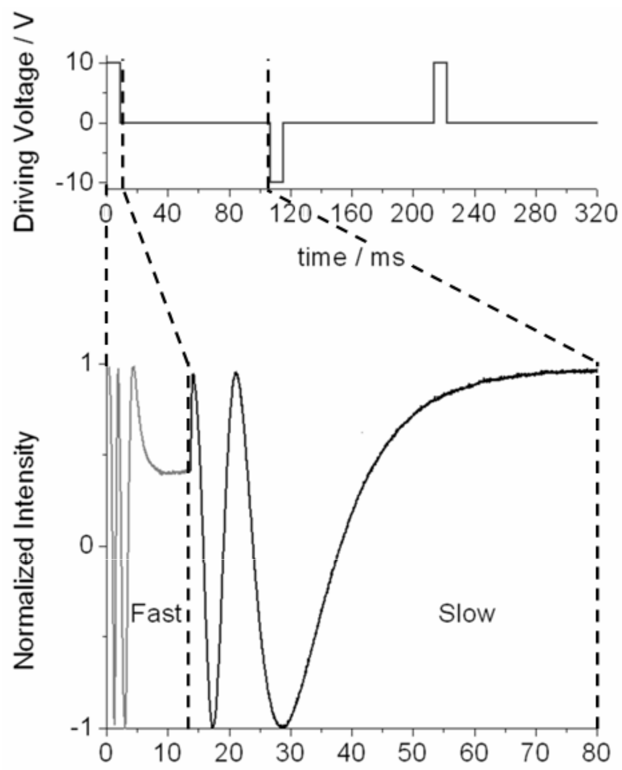


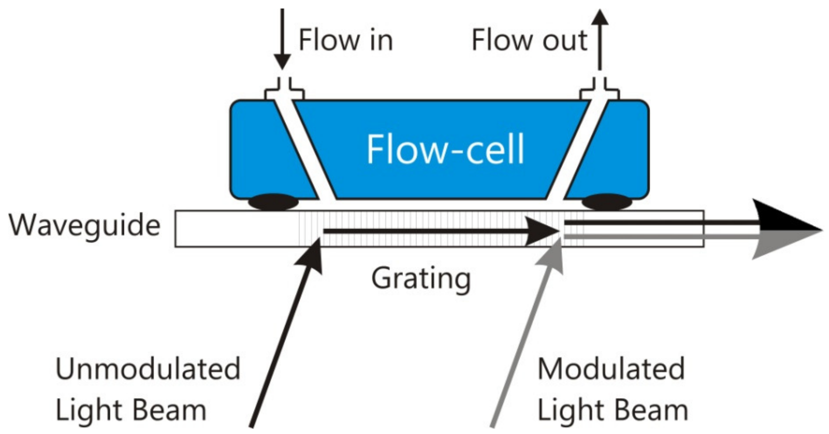
S. Kurunczi, R. Horvath, YP Yeh, A. Muskotál, A. Sebestyén, F. Vonderviszt, JJ Ramsden,
"Self assembly of rodlike receptors from bulk solutions"
The Journal of Chemical Physics, Volume 130, Issue 1, pp. 011101-011101-4 (2009).
(Spotted in the Virtual Journal of Nanoscale Science & Technology)

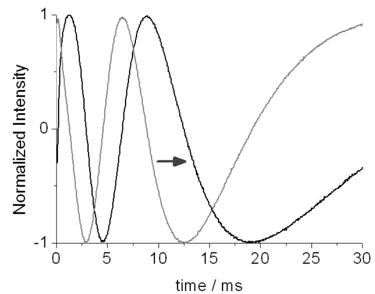
Interferometric hybrid biosensor development

Phase shifting with liquid cristal cell.





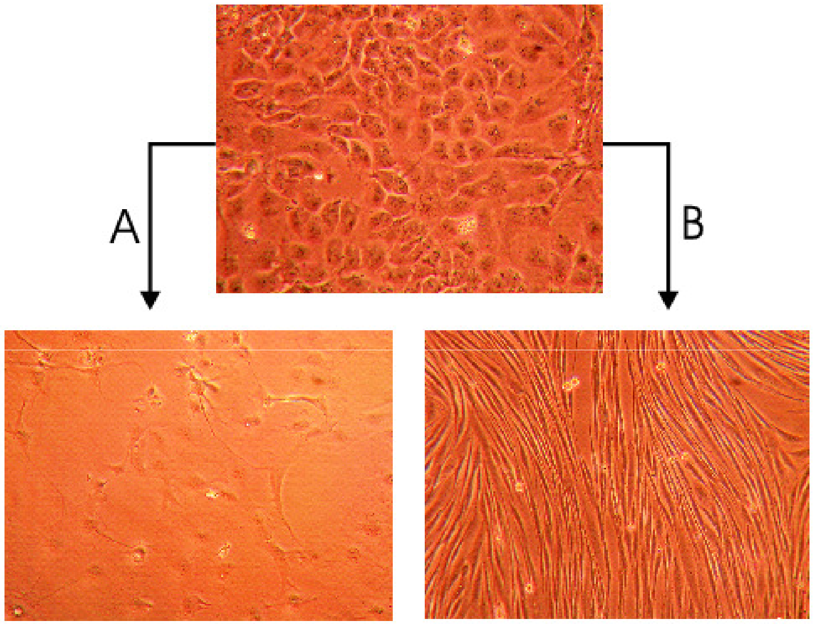


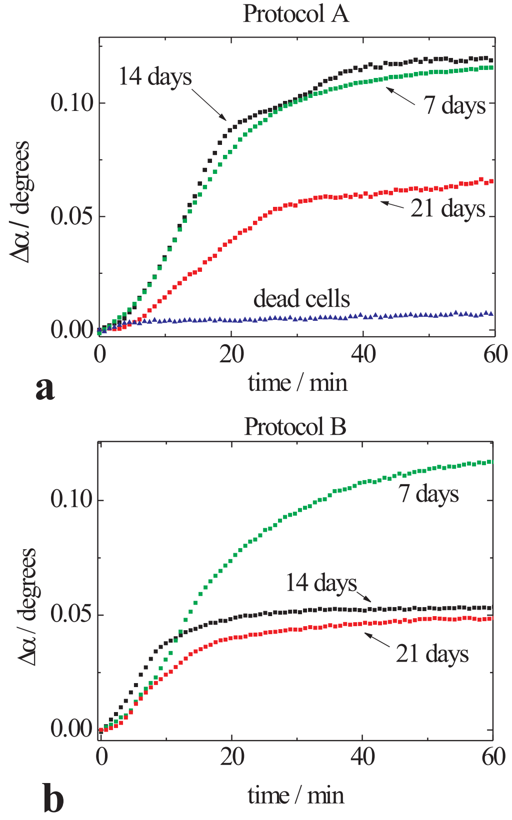


P. Kozma, A. Hamori, K. Cottier, S. Kurunczi, R. Horvath
"Grating coupled interferometry for optical sensing"
Applied Physics B – Lasers and Optics (2009).

Stem cell sensing

Cell adhesion kinetics during stem cell differentiation:





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