PHOTONIC MATERIALS IN BIOLOGICAL APPLICATIONS

~ BIOPHOTONICS ~

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PhOREMOST

THANKS

To the members of the team:

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50% women !

To our collaborators:

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OUTLINE

Biophotonics – definition

Laser in health care

Bioimaging: functional and spectroscopic microscopy

Novel biosensing

Natural photonic materials

Towards hybrid photonic devices

Biophotonics – definition

Photonics for Life Science and Health → Biophotonics

« the study of the interaction of light with biological material »

“light” includes all forms of radiant energy whose quantum unit is the photon

Biophotonics utilizes light-based technologies to solve problems in medecine and the life sciences

- **Light measures contact-free**
- **Light measures fast**
- **Light measures precisely**

→ Photonic tools are capable to manipulate molecules and living cells

Surgery
use of lasers
combined with in-situ
imaging

Bioimaging
based on
novel microscopic and
spectroscopic techniques

Photonics for Nanomedecine

Diagnosis
novel biosensors for
preventive medecine

Therapy
targeted drug delivery
with follow-up monitoring

Lasers in health care

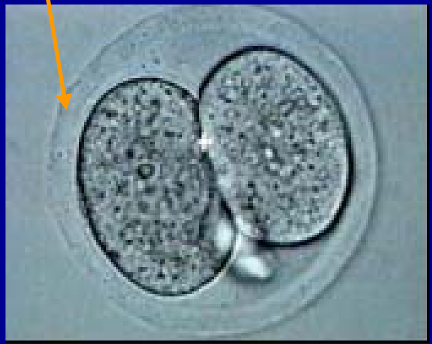
Laser Surgery: conventional and novel

A minimal invasive corneal ablation inside the cornea using a femto-second laser system

New: frequency tripled solid state lasers
- Microplasma in the focal spot ($1\mu m$)
- All material is fragmented in the focal point
- Duration 3 ns, thus no heat effect

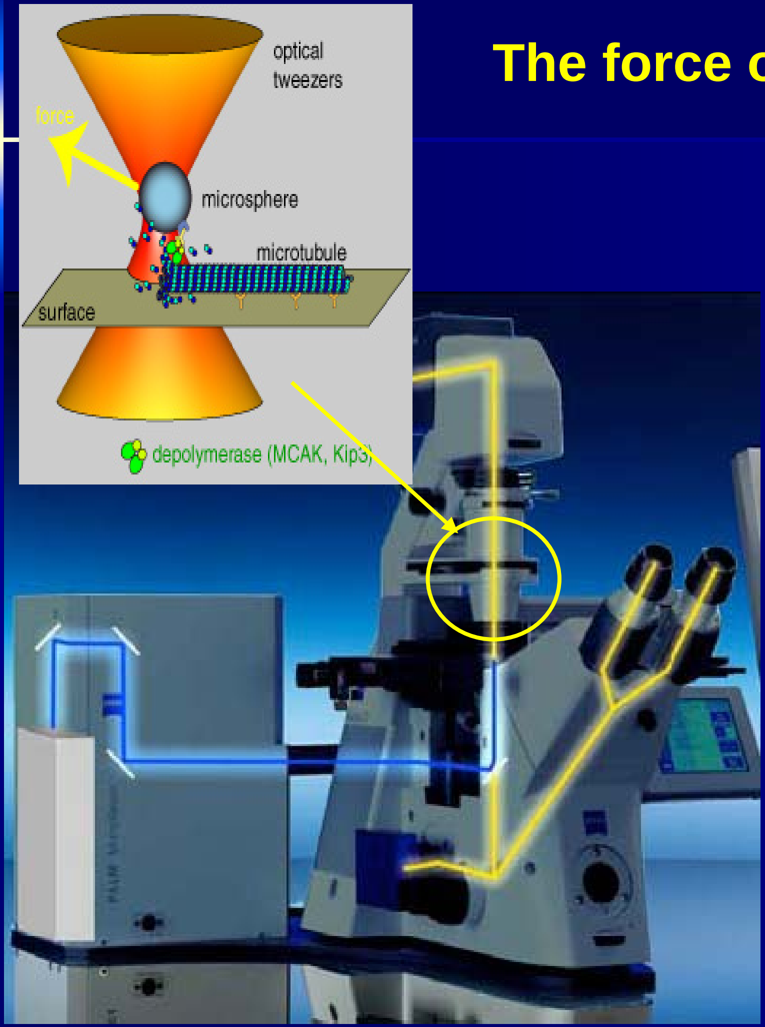
Laser Microsurgery

Microinjection



Laser fusion

The force of focused light – Optical Tweezers



Lasers coupled into microscopes
→ precise micromanipulation tools

- Catch, move viruses, bacteria or cells
- Force measurements: binding forces between molecules, organelles
- Cell fusion
- Laser microdissection of cells

Bioimaging:

Functional and spectroscopic microscopies

Photonics → novel functional and spectroscopic microscopies

Fluorescence 3D imaging combined with FRET

Fluorescence Lifetime Imaging Microscopy (FLIM)

Multiphoton Microscopy (MPM)

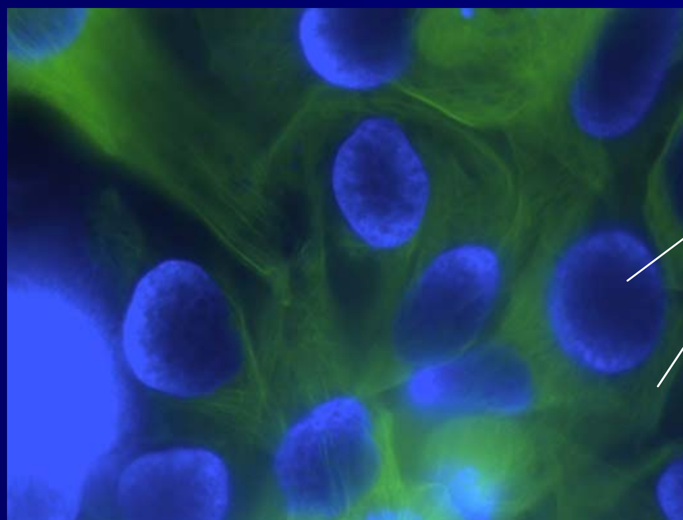
Optical Coherence Tomography (OCT)

Combined coherent anti-Stokes Raman spectroscopy (CARS) and two-photon confocal microscope

Scanning near-field microscopy combined with Raman micro-imaging

Near field microscopy

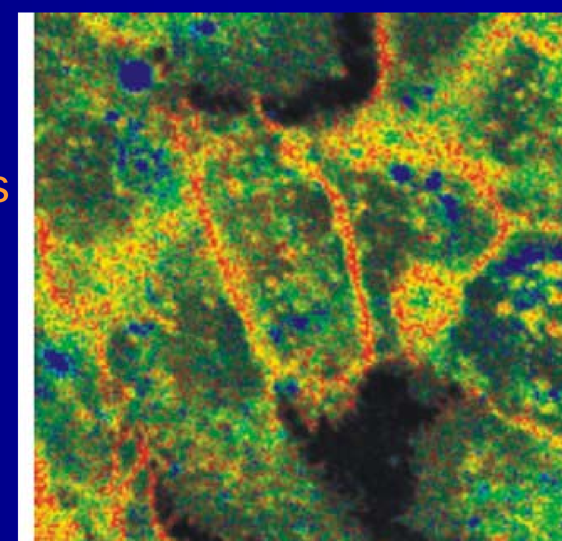
Fluorescence imaging combined with FRET, FLIM



Adenocarcinoma breast cancer cells (MCF7)

nuclei stained with Hoescht 33342
actin filaments stained with Alexa Fluor 488

Cancer cell line of liver cells

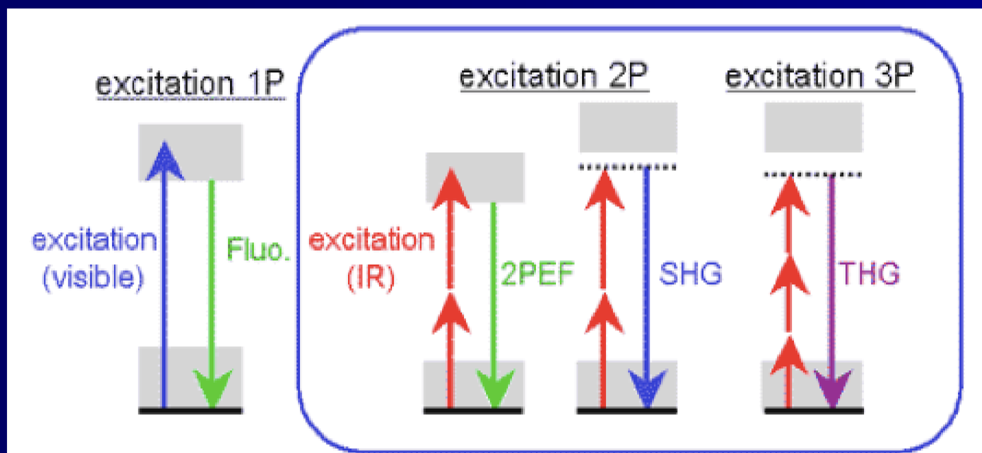


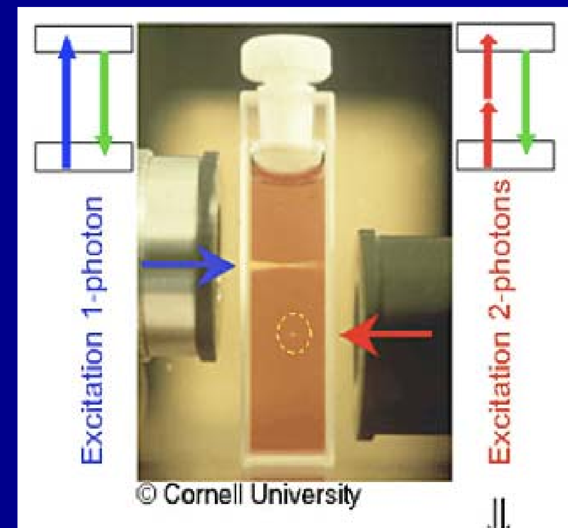
- stained with phospholipids labeled with NBD
- the lifetime is depending on the hydrophobicity

→ lifetime allows to gain information about the molecular structure of cellular compartments

A. Tannert, T. Korte, Humboldt Univ. Berlin, Germany

Multiphoton Microscopy (MPM)



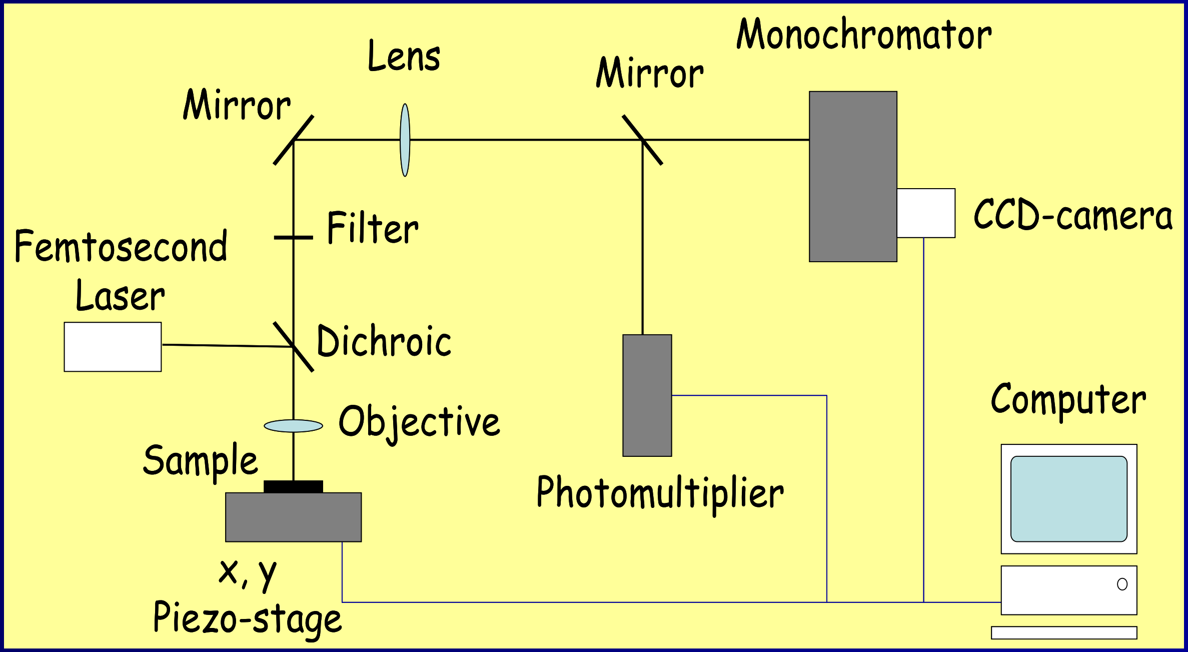


Confined excitation

- The excitation using near-infrared wavelengths allows excellent depth penetration → 400 μm

- Good light confinement in the focal point of the laser

Modular components of the MPM



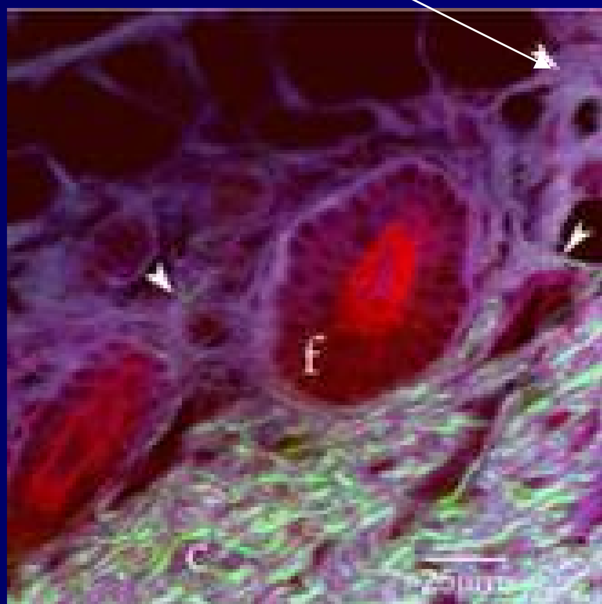
- Laser excitation → non-linear phenomena (2PEF, SHG, THG)

- SH, TH coherent → useful information on the structure and optical properties of a specimen

MPM interesting for health care:

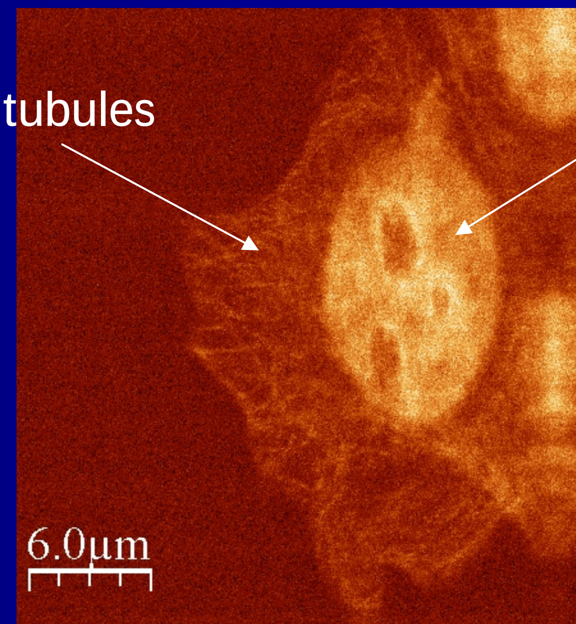
1. Cells, tissues are transparent for IR radiation
2. The non centro–symetric systems produce SH, TH → visible without labeling

SHG image of collagen in mouse dermis



microtubules

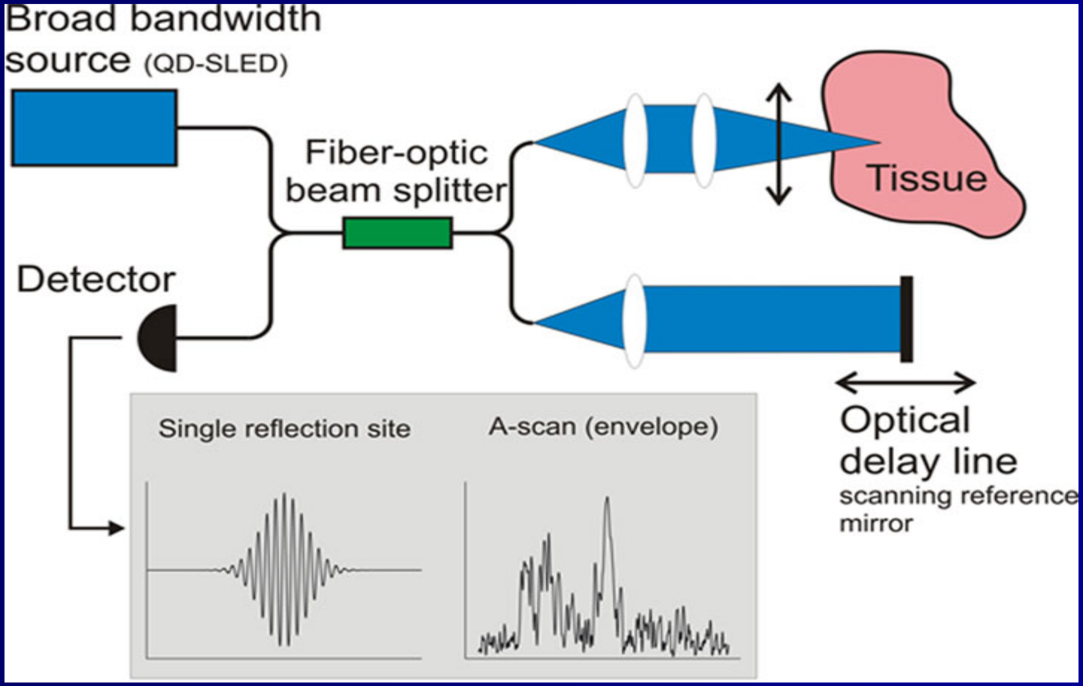
nucleus



MCF7 mammalian cells imaged by MPM
Excitation is at 880 nm

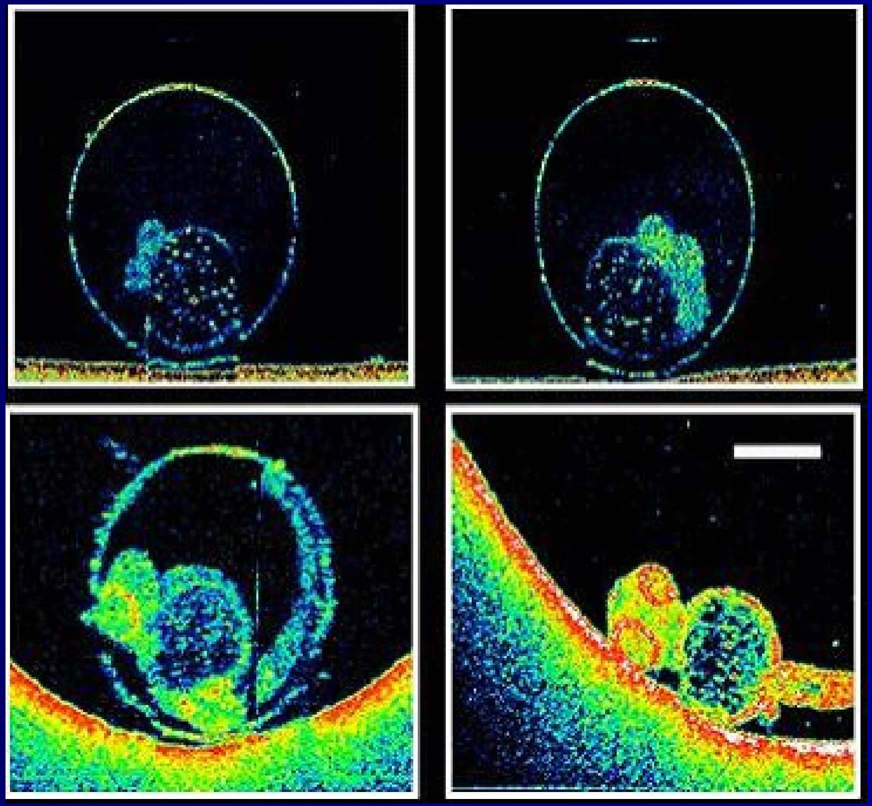
CJR Sheppard

Optical coherence tomography



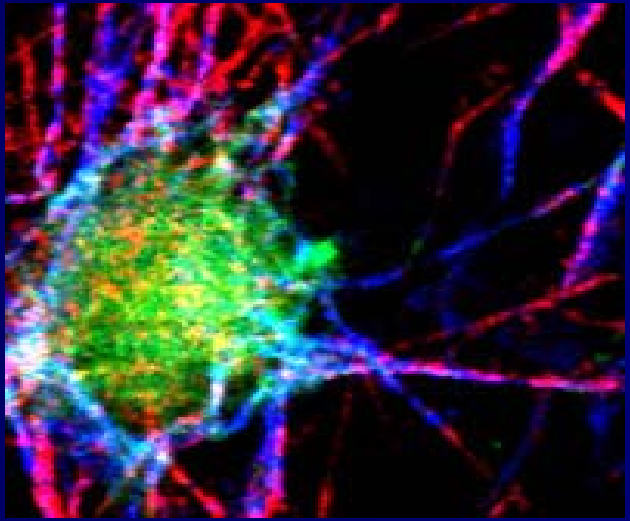
- A broadband source illuminates a fiber-optic **Michelson interferometer**
- **An interference pattern is detected** when the sample and reference path lengths match within the coherence length of the source
- **Images of tissue (2D, 3D,cross-sectional**) may be obtained non-invasively and in situ with appropriate scanning

Optical coherence tomography: developing zebrafish



Biophotonics Imaging Laboratory
University of Illinois Urbana Champagne

Integrating multiphoton microscopy (MPM) with optical coherence tomography (OCT)

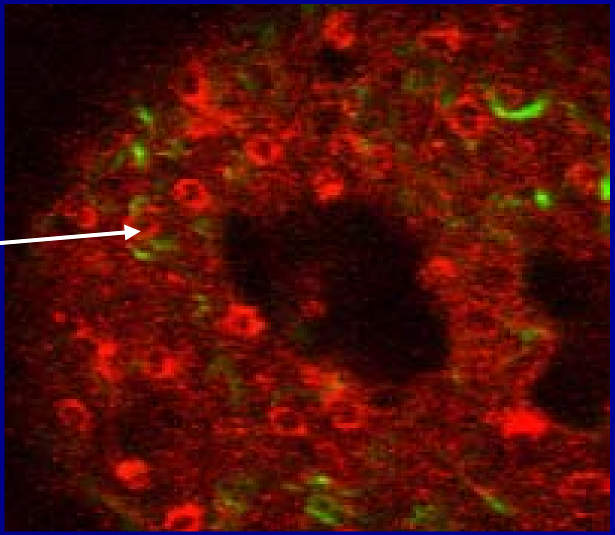


- MPM is sensitive to cells and extracellular matrix
- OCT to structural interfaces and tissue layers.

→ acquire structural and functional imaging of tissues simultaneously

Micro-endoscopes are applied to study lung and ovarian cancers

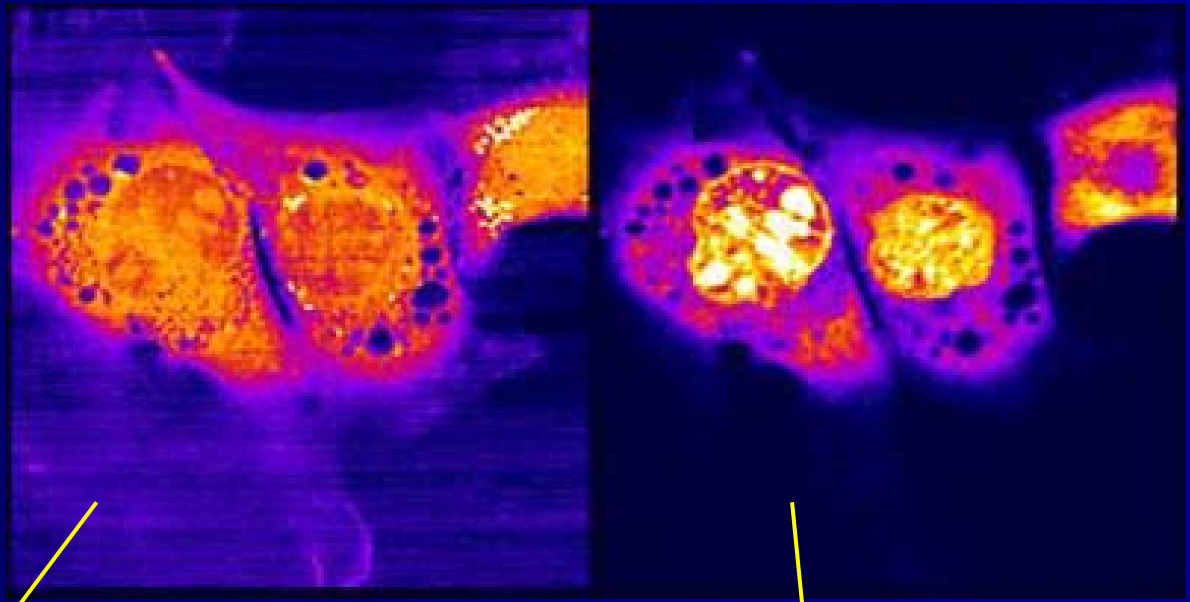
→In vivo optical imaging to detect cancer in its early stage



Mina, University of British Columbia

Combined coherent anti-Stokes Raman spectroscopy (CARS) and two-photon confocal microscope

→ visualize molecules based on their vibrational and fluorescent properties

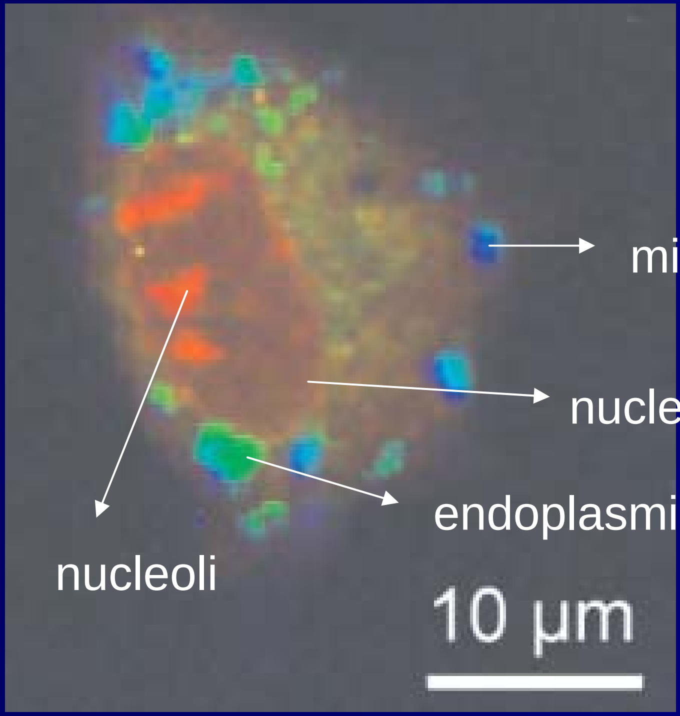


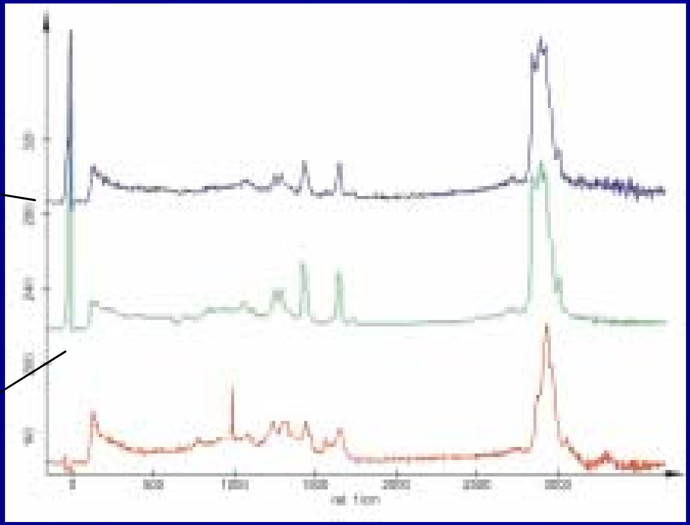
Concentrations of carbon-hydrogen bonds in live D2XRII murine bone marrow stromal cells
(white is the most intense)
- no stain was required.

Fluorescence of stained DNA and RNA obtained simultaneously as the CARS image
The nuclear region is shown very clearly and serves to identify known cell structures

Scanning near-field microscopy combined with Raman micro-imaging

Live cell Raman image



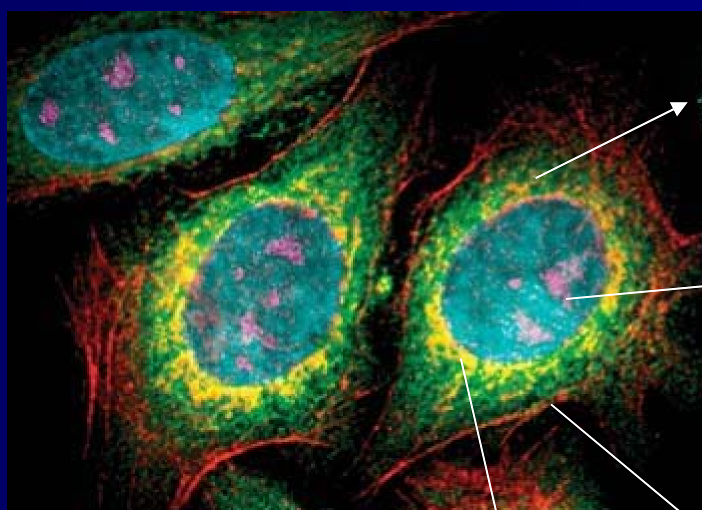


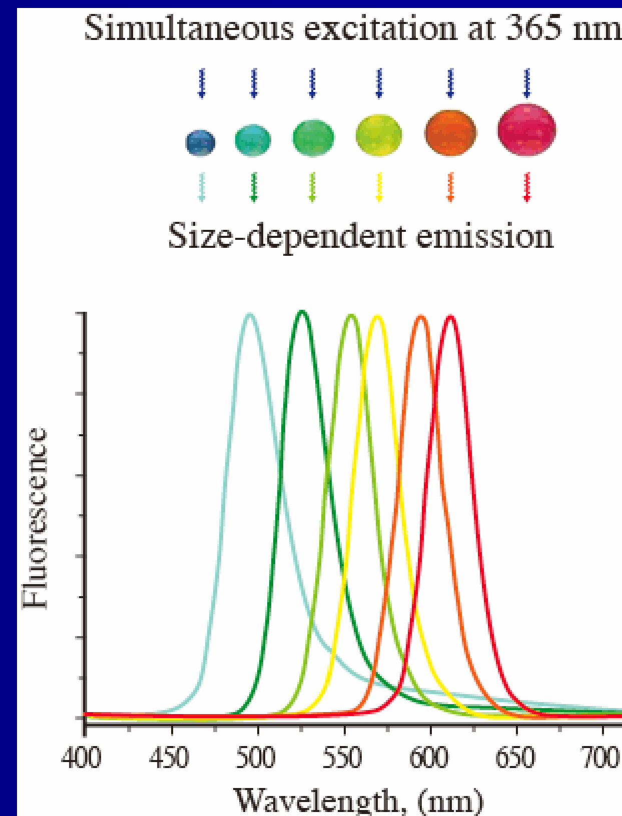
Raman spectra of the various cell components

Witec, Ulm, Germany

Nanoparticles: Quantum dots as biolabels

- they can simultaneously reveal the fine details of many cell structures



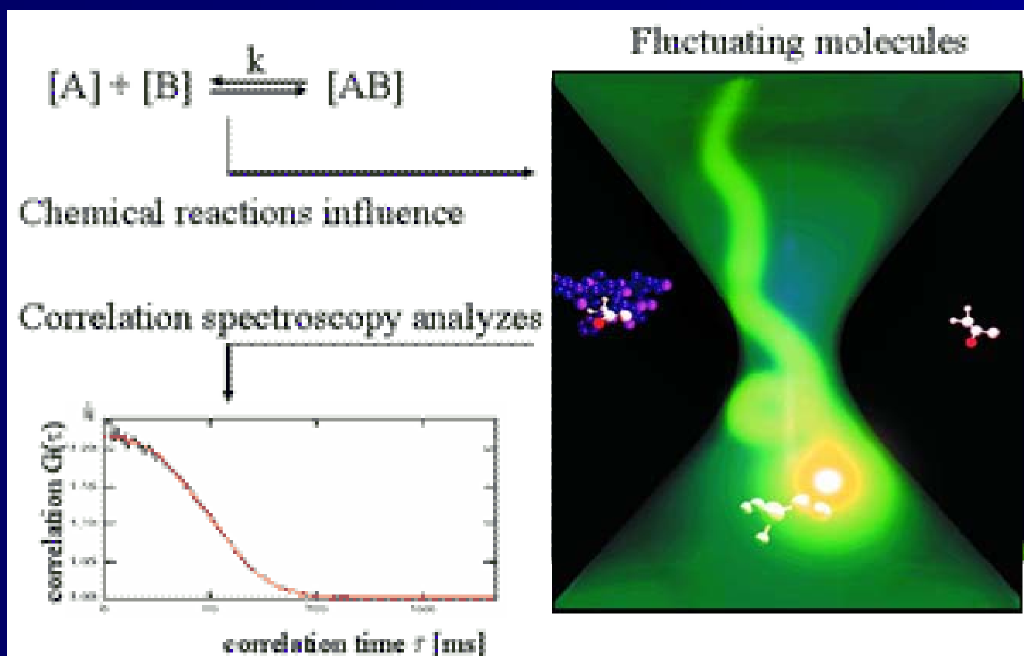


Novel Biosensing

Diagnosis: towards novel biosensing

Simple molecule detection using Fluorescence Correlation Spectroscopy (FCS)

FCS is based on the fluctuation of light emitted by dye molecules crossing a small laser spot and detected with confocal optics



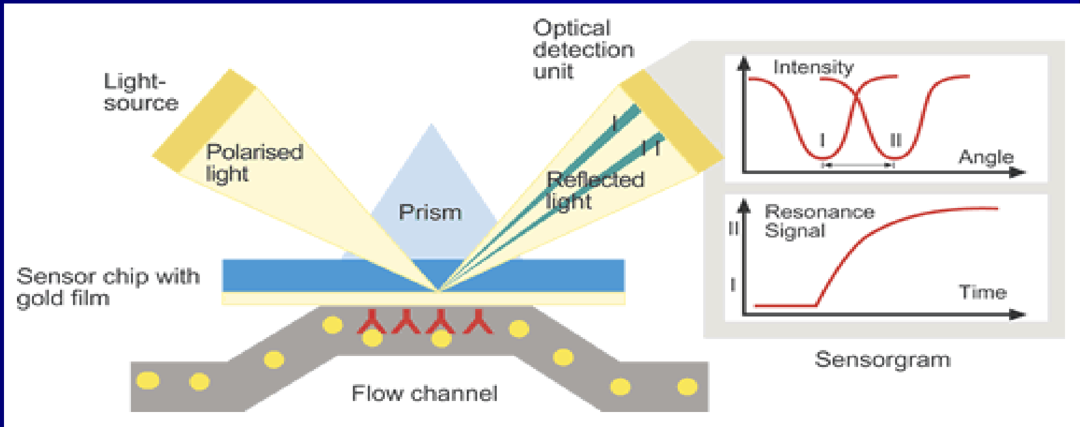
Detecting two different sizes of molecules by their different diffusion constants

Stowers Institute, USA

Affinity based optical biosensors

to detect selective binding between target molecules and capture agents: ligand-receptor, antibody-antigen, nucleotides pairing, etc.

Biacore:
optical biosensor that uses surface plasmon resonance for detection

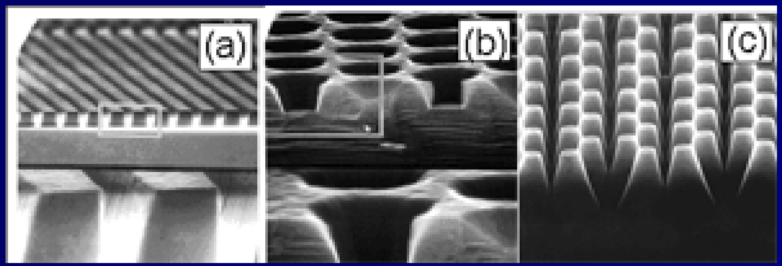


O. Chaloin, IBMC, Strasbourg

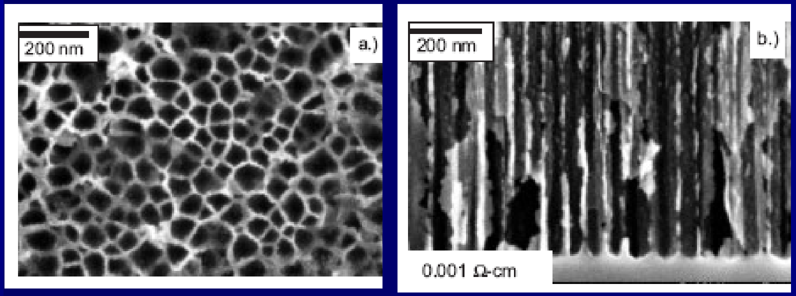
-large sensing area
-substrate dependent binding of molecules driven by unspecific interactions → giving rise to serious limitations in the detection accuracy

? Miniaturization + large sensing area + specificity needed at the same time

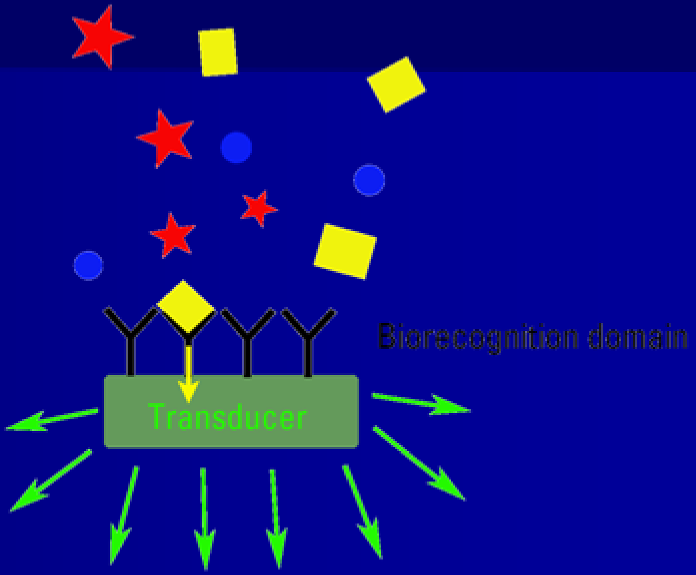
Photonic crystals → Biosensors



Y. Chen, LPN, Marcoussis



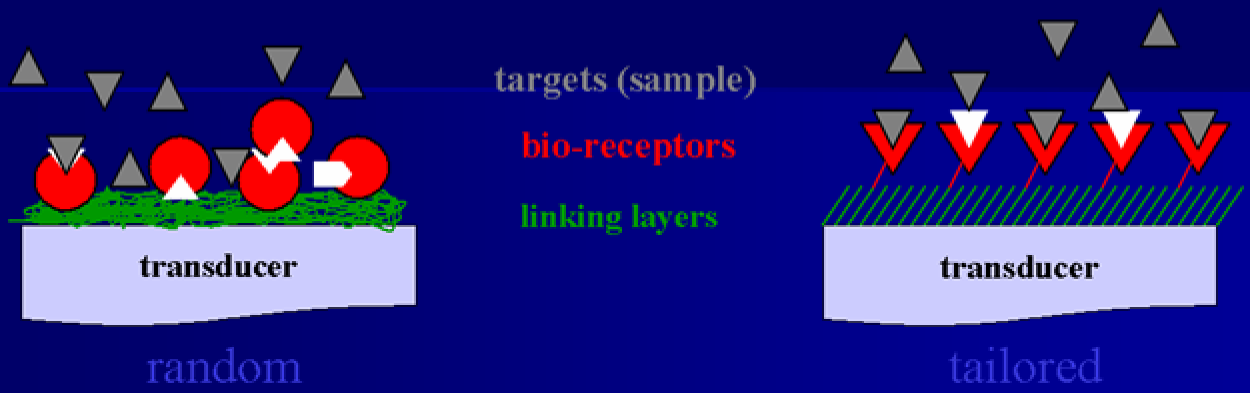
✓ Miniaturization
✓ Large sensing area



➢detection based on the presence of the topological defects (biomolecules) within the photonic crystals

➢specific recognition of the substrates by biological molecules

Key aspects in the development of biosensors



- the interaction between inorganic (the transducer) and organic material (the biological receptors)

- the increasing miniaturization of biosensor transducers (and thus of their active areas) + the demand for high sensitivity require a

<u>covalent and tailored coupling of bio-molecules to the transducer surface</u>

- certain semiconductors (GaAs, InAs) are toxic → for biocompatibility previous surface functionalization is needed

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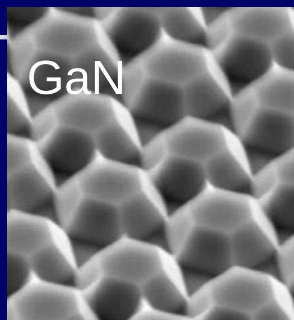
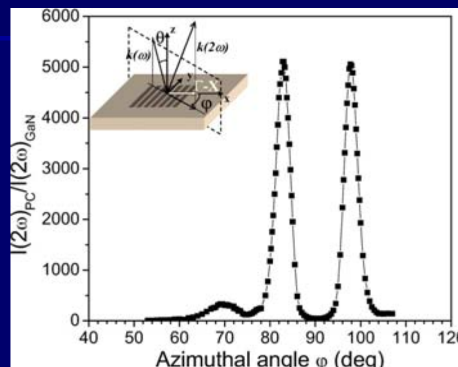
Photonic Crystals: Refractive Index Sensors



(I,Watson, Glasgow)



Advantages of GaN

Transparence: 365nm – 13µm

Non toxic

Chemical stability

Luminescent source

-confinement of light in very reduced zones

- exaltation of the nonlinear answer

-to reduce probed volumes and the quantity of fluorophores to reach a high sensitivity

PhC: - nanostructured substrate

- contrast in refractive indices

-specific detection based on refractive index modifications

-hybrid structures for light guiding

?

➢Selective localization of molecules to keep r.i. contrast

→ ordered array of a specific molecule

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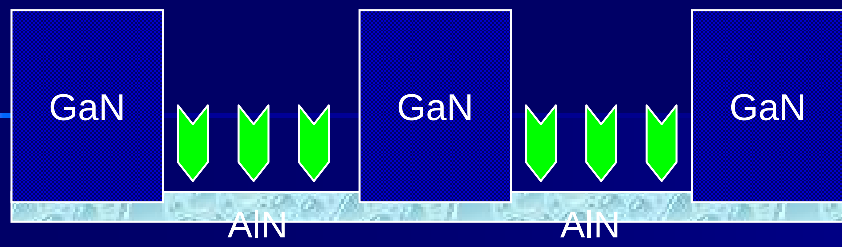
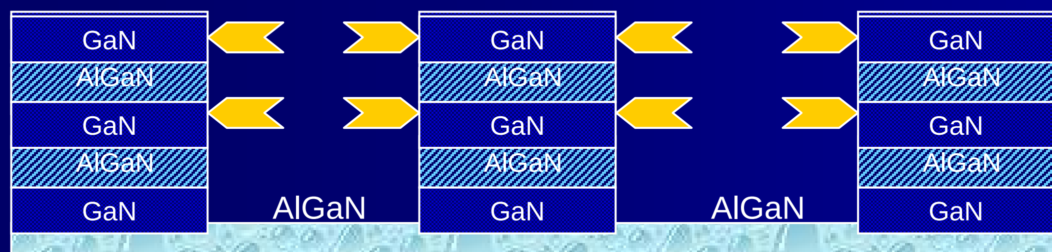
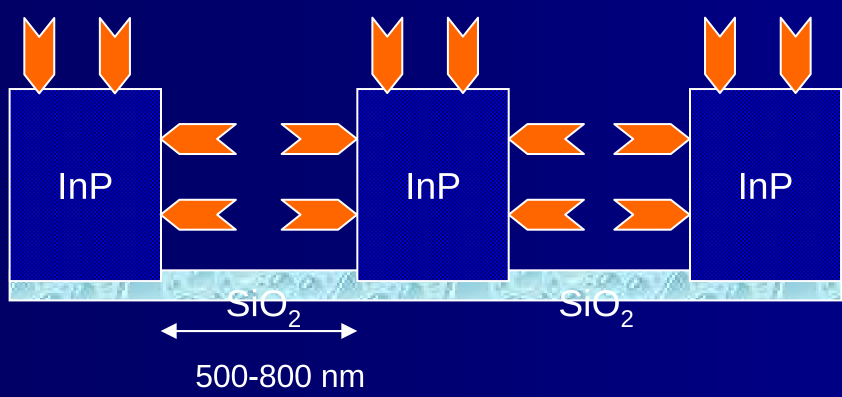
Examples of photonic structures







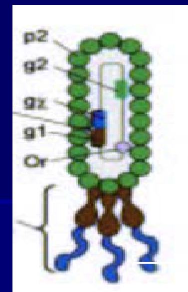
their functionalization with peptides which recognize the SC specifically

Optical detection based on refractive index changes monitored by :

-PhC resonances
-Evanescent field propagation within the waveguide structures

Functionalization by phage display technique

Phage



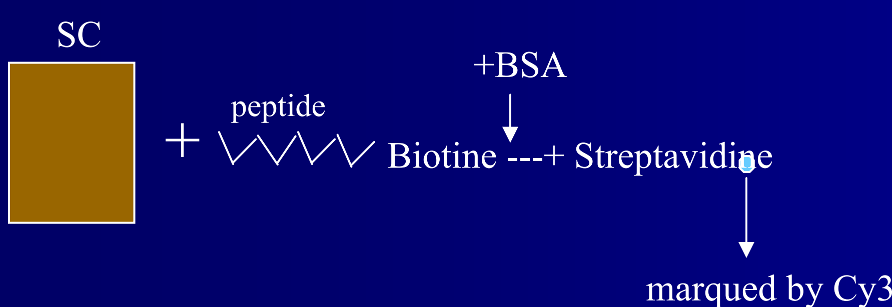
10^{10} combination → ~ some peptides with the best affinity

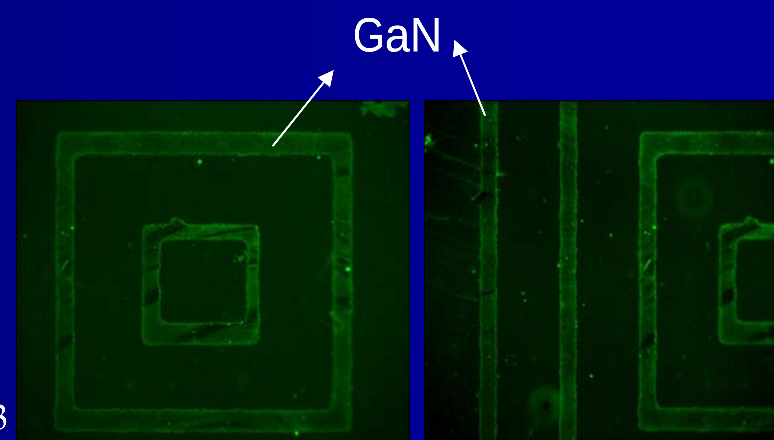
→ Adhesion peptides

Peptides with specific recognition for:

GaAs(100), InAs(100), GaN(0001), ZnSe(100), ZnTe(100), InP(001)

Selective functionalisation of GaN patterned structures

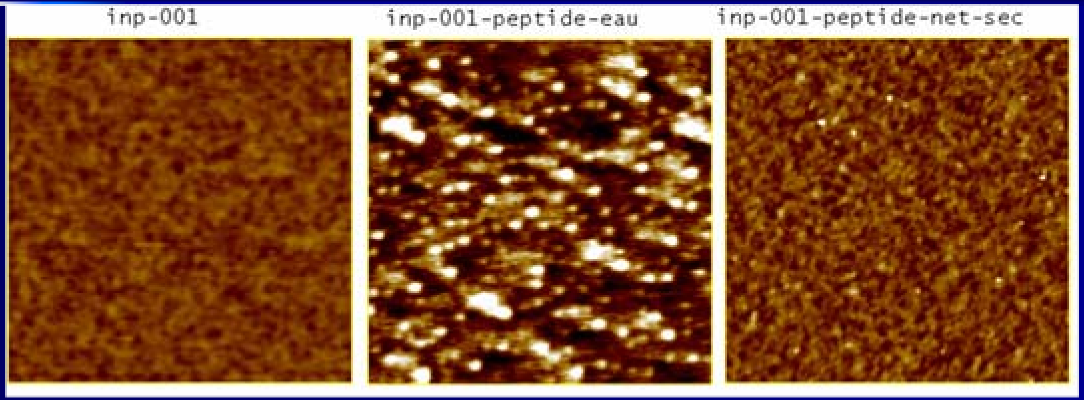




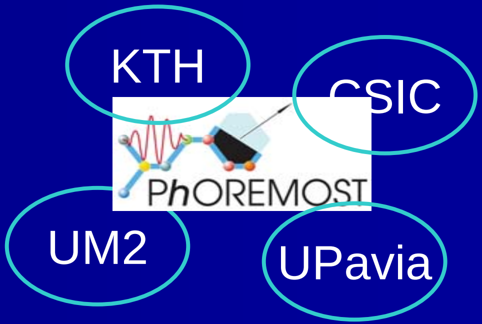
E. Estephan et al. (2007) *Proc. SPIE Vol. 6592, 65920Z1-9*

Selective functionalisation of InP patterned structures

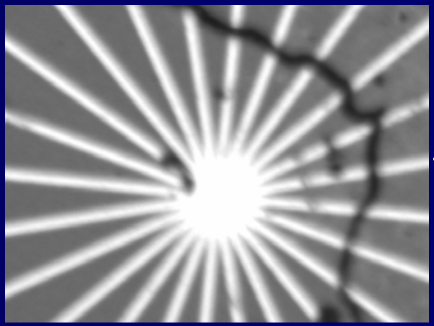
- ✓ bio activating the InP surface via the InP-specific peptide (phage display)
- ✓ reversibility of the functionalization process



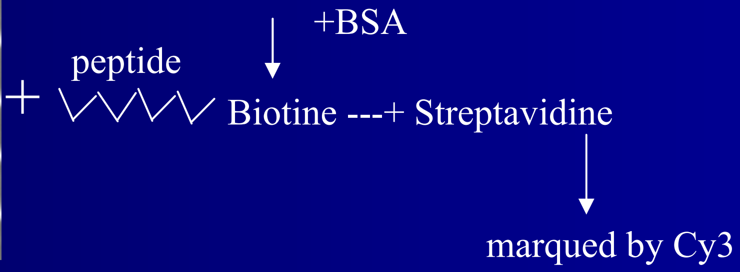
InP ⟶ + peptide-biotin ⟶ after cleaning



✓Specific localization of fluorescence has been demonstrated



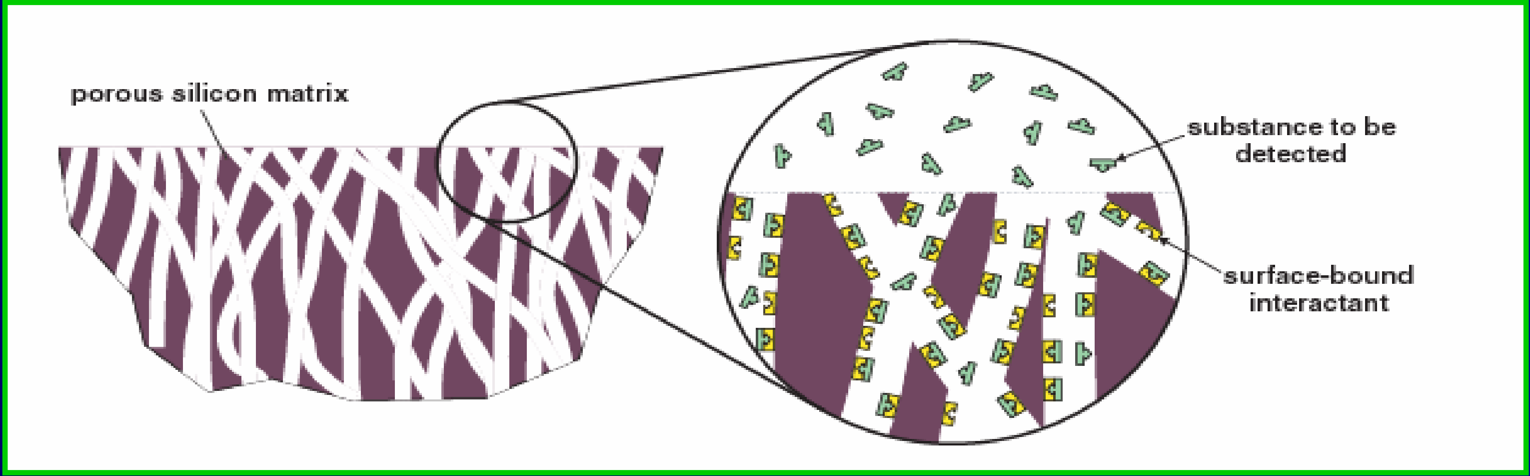
InP patterns on Si layers



Biosensors:

Simple and inexpensive analytical devices which converts a biological response into an optical or electrical signal.

Porous Silicon

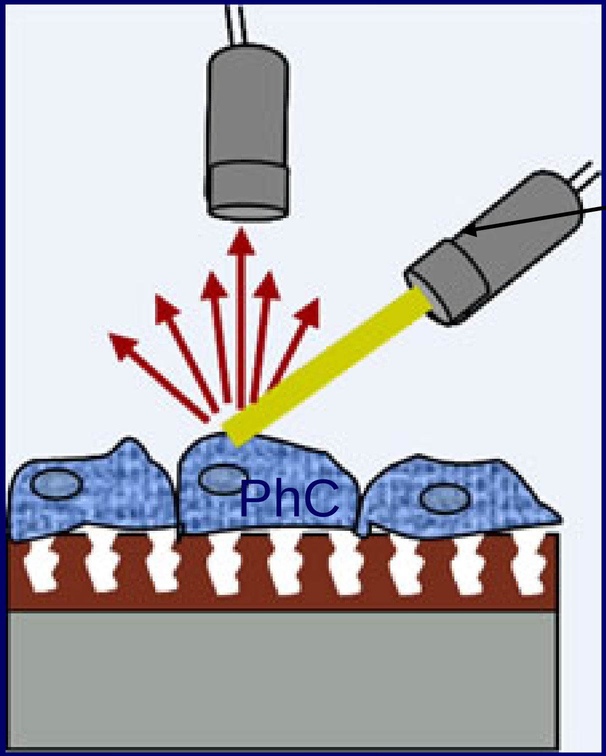


The internal surface is coated with biomolecule that selectively binds to a target substance to be detected.

When the target material is immobilized on the surface, the macroscopic optical properties of the porous silicon film change in a measurable way

Psi photonic crystal:

capable of controlling light within the structure analogous to the way that semiconductors transmit electricity through computer chips

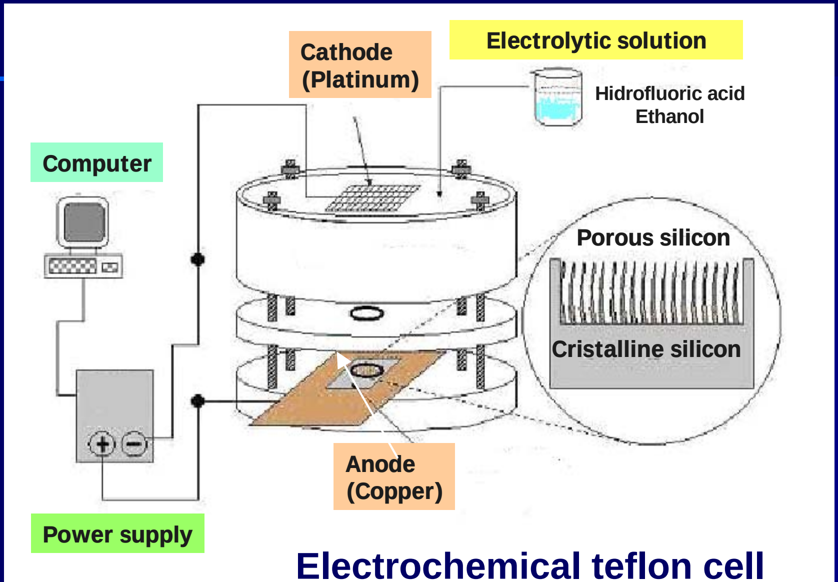


measuring the scattering of light with a sensitive spectrometer

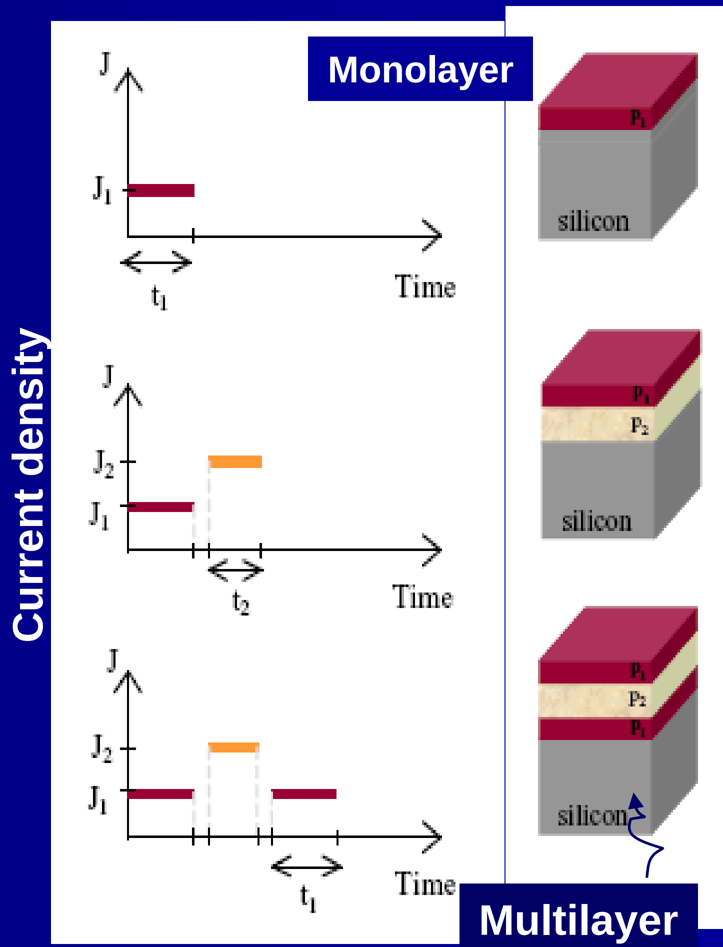
detect small changes in the shapes of liver cells as they reacted to toxic doses of cadmium

M. Sailor, USC San Diego

Psi fabrication: wet etching in an electrochemical anodization system



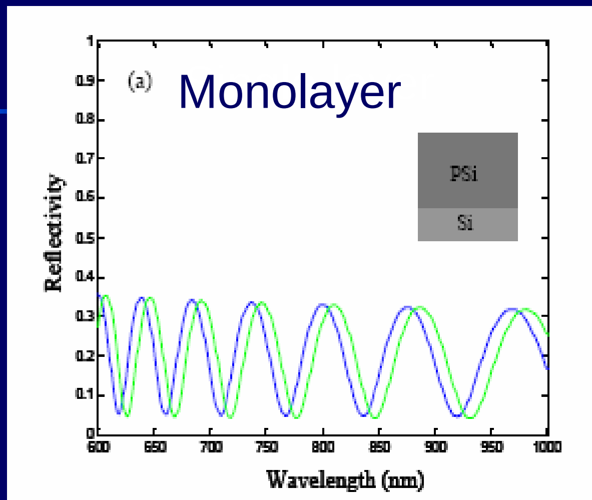
Electrochemical teflon cell

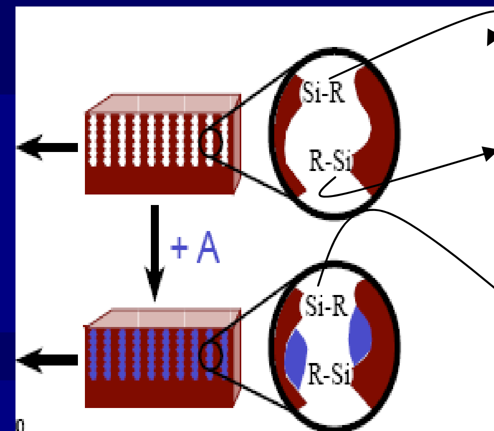


Periodic stack

Porosity and pore size can be easily tuned by adjusting the electrochemical conditions

Reflectance spectra of various pSi structures



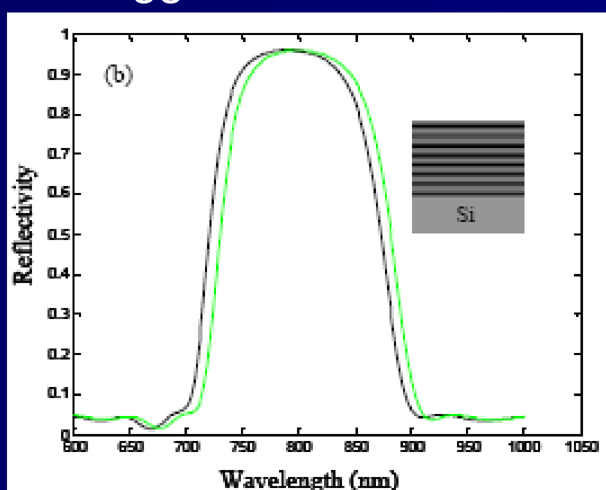


Hidride bond

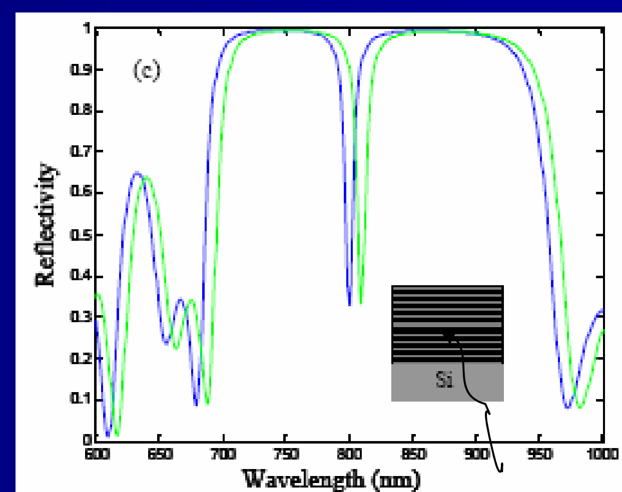
Oxide bond

Biological or chemical species

Bragg Mirror



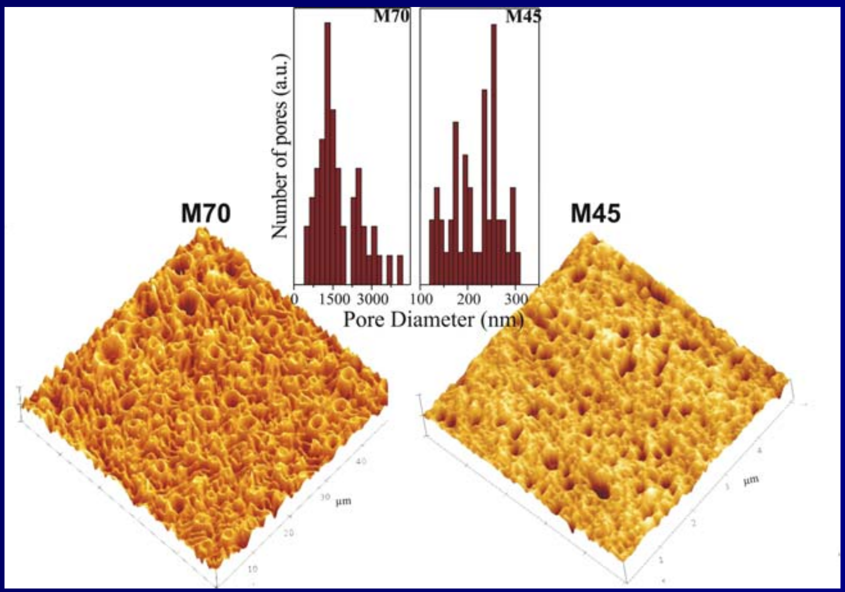
Microcavity

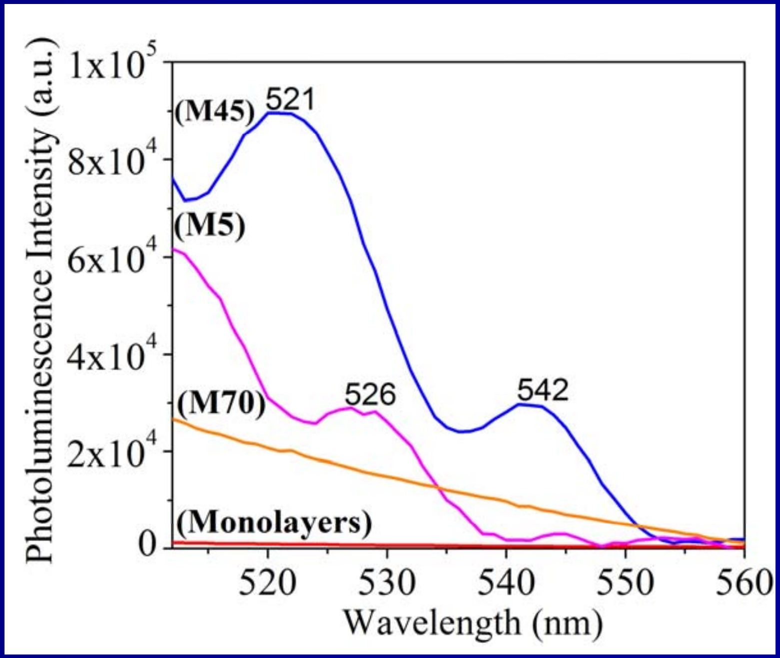


Defect layer
Breaks symmetry

Fluorescence Enhancement in pSi

Functionalised pSi Bragg Mirrors + confinement of fluorescein



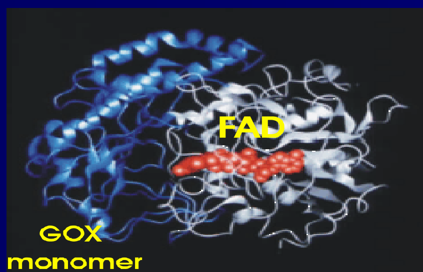


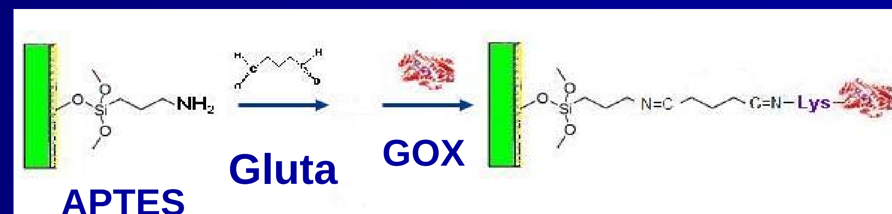
Fluorescent emission of the fluorescein molecules adsorbed on the M45 mirror cooperative effect of the pores dimensions + resonance conditions

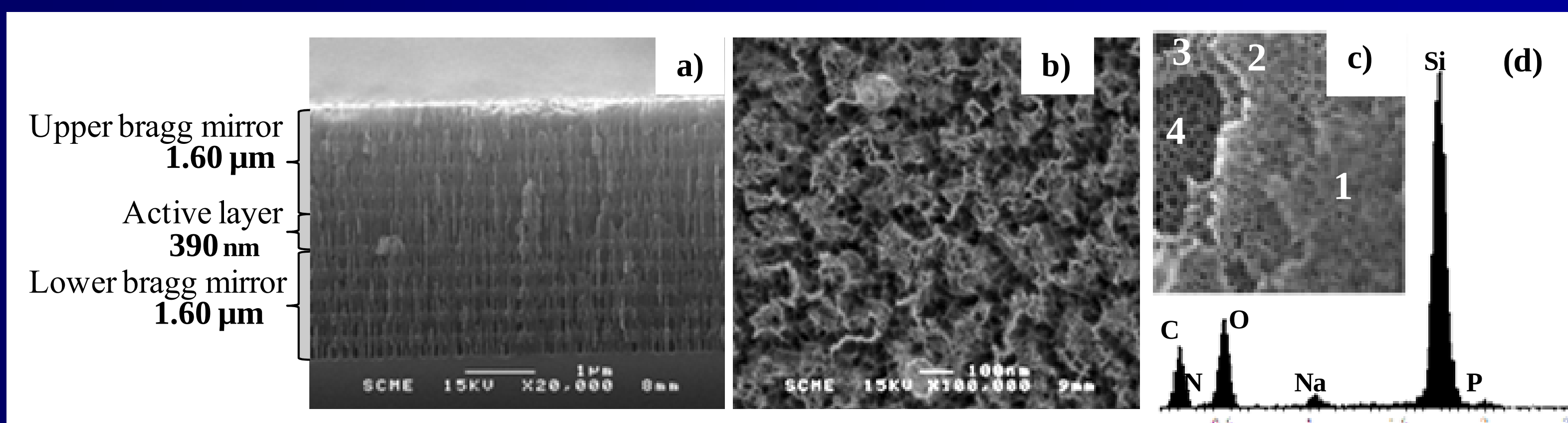
G. Palestino et al. APL, 91, 12 (2007) + Biophotonics Int/ by. Hogan, 2007

Biosensor: Glucose Oxydase (GOX) + porous silicon microcavity

GOX adsorbed on PSi after functionalisation





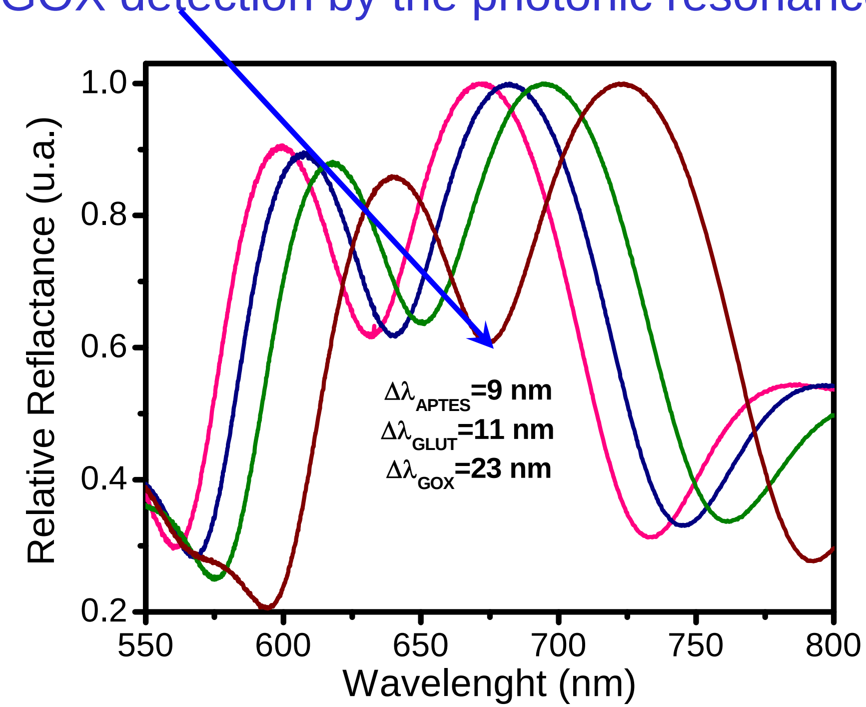


Organic molecules inflitrate the whole structure

G. Palestino et al. (2007) *Proc. SPIE Vol. 6592, 65920E1-9*

Molecular sensing by specular reflectance

GOX detection by the photonic resonance



PSi Microcavity

ext pore : 400-4000nm

-- pSi after themal oxidation
-- pSi + APTES (silanization)
-- pSi + APTES + Glu
-- pSi + APTES + Glu + GOX

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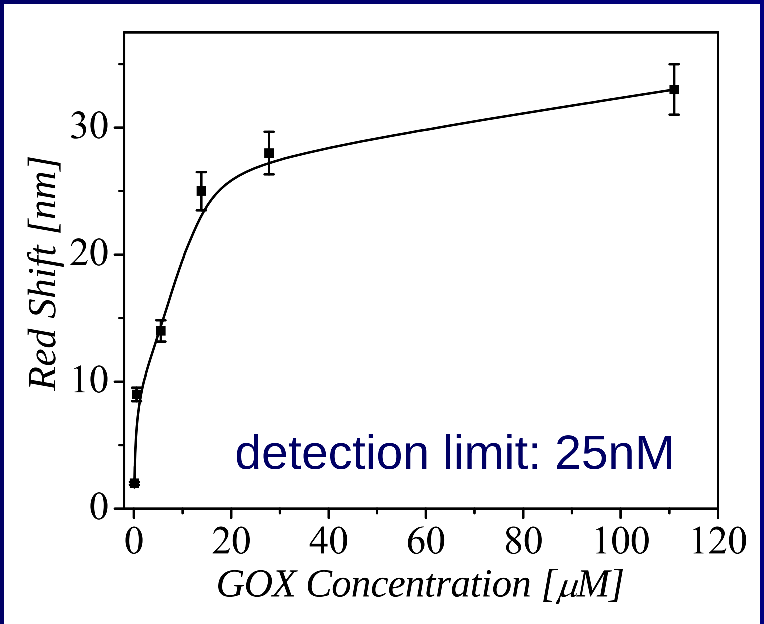
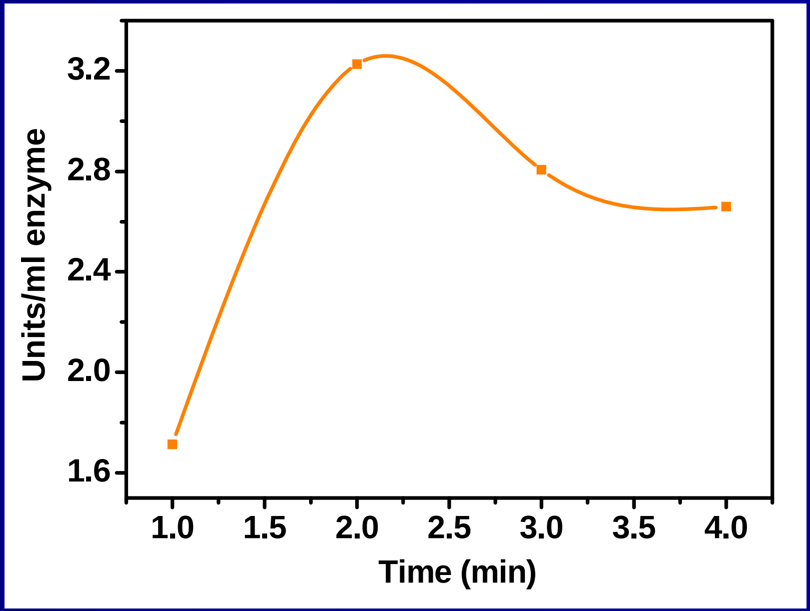
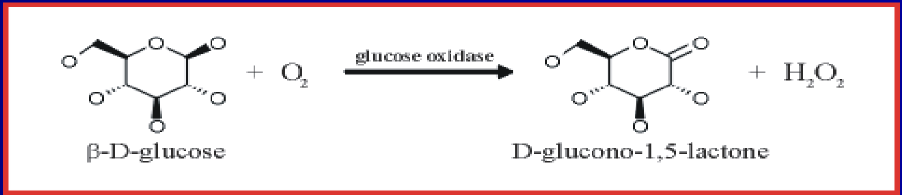
A functional GOX-Psi sensor has been obtained

Dose response curve



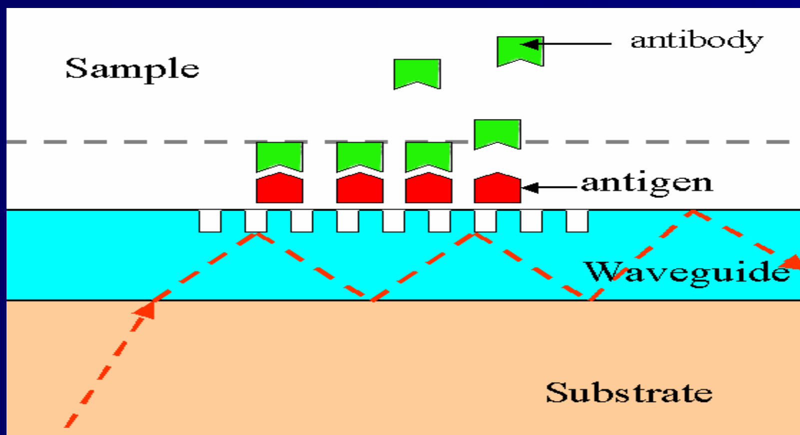
Enzymatic acitivity of the adsorbed GOX

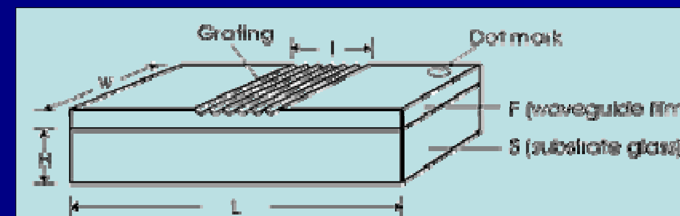




Evanescent wave sensing

Optical waveguide lightmode spectroscopy

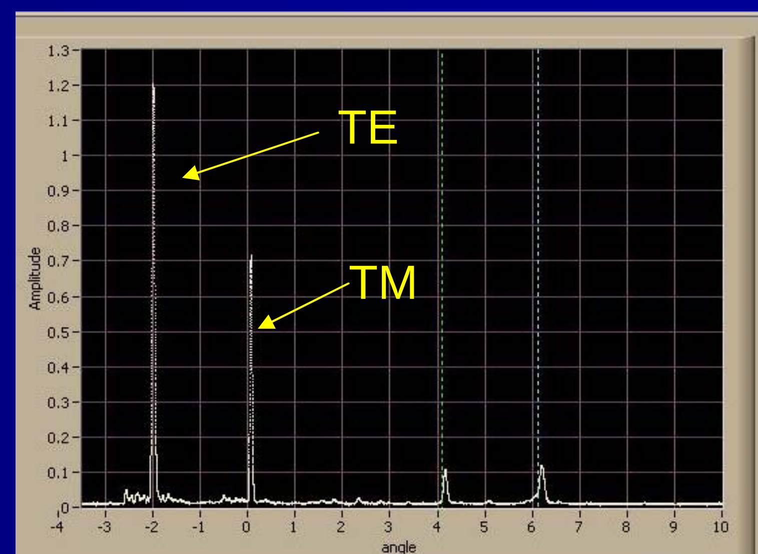




Input grating sensor: **waveguide** (SiO_2-TiO_2, n=1.8) Microvacuum Ltd

Coupling equation

$$N = n \sin \alpha + \frac{l\lambda}{\Lambda}$$



Tiefenthaler and Lukosz, 1982

4 layer mode equation:

$$2k_{z,F} + \Phi_{S,F} + \Phi_{F,A} = 2\pi m$$

The phase shifts at interfaces:

$$\Phi_{F,S} = -2\arctan\left(\frac{n_F^{2\rho} s}{n_S^{2\rho} f}\right)$$

$$\Phi_{F,A,C} = -2\arctan\left[\frac{n_F^{2\rho} a}{n_A^{2\rho} f}\,\frac{\frac{c}{n_C^{2\rho}} + \frac{a}{n_A^{2\rho}}\tanh(k_0 a d_A)}{\frac{a}{n_A^{2\rho}} + \frac{c}{n_C^{2\rho}}\tanh(k_0 a d_A)}\right]$$

Solutions:

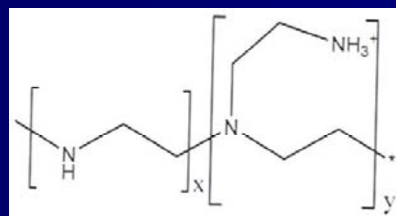
(for thin and thick layers)

n_A ; d_A - refractive index, thickness of the adlayers

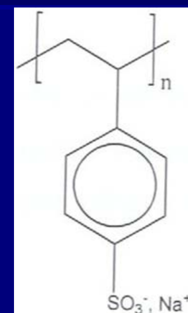
$\Gamma = (dn/dc)^{-1} (n_A - n_C) d_A$
– adsorbed quantity in $\mu g/cm^2$

C. Gergely et al. 2004 Langmuir, 20, 557

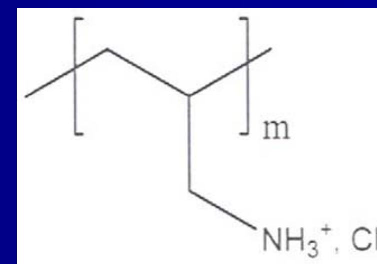
In situ monitoring adsorption of molecules on the waveguide chip



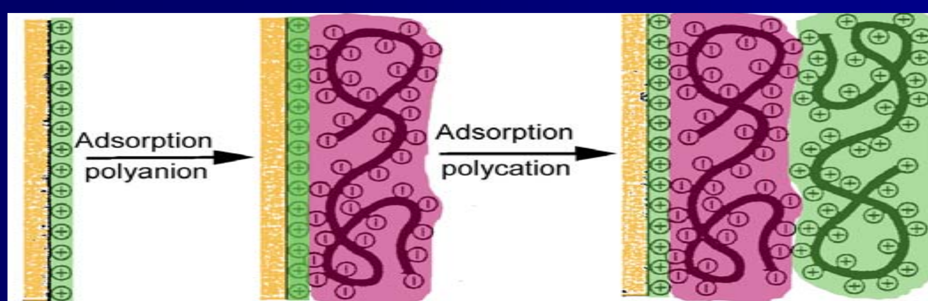
PEI^{+}



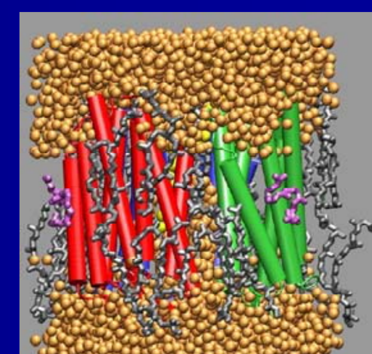
PSS^{-}



PAH^{+}



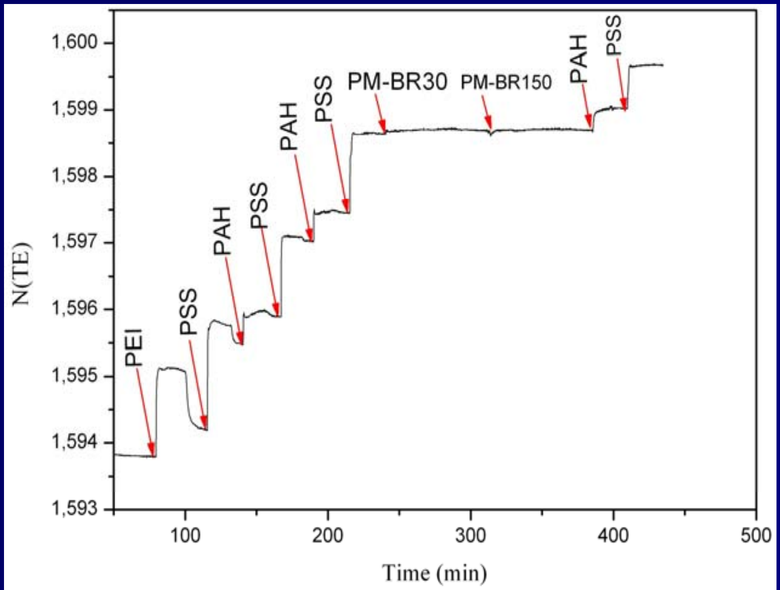
+

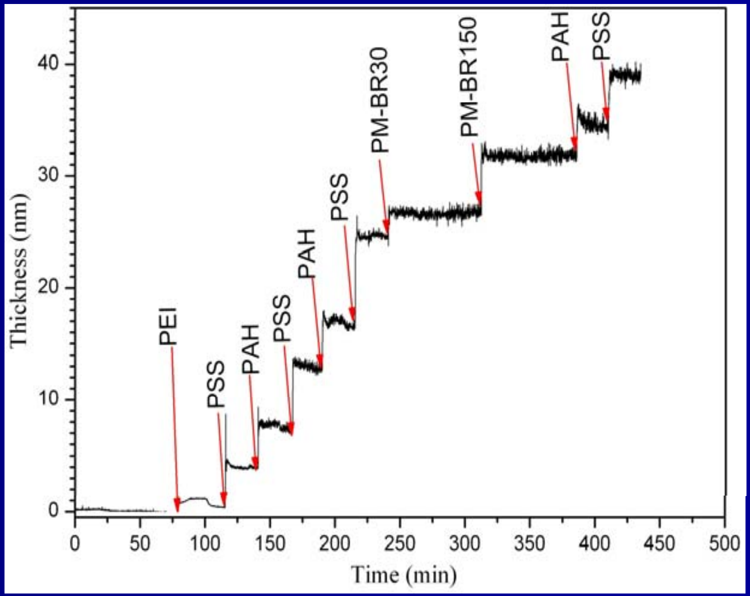


bR

Layer-by-layer buildup of polyelectrolyte multilayered film and adsorption of membrane bound bacteriorhodopsin protein

In situ monitoring adsorption of molecules on surfaces by OWLS



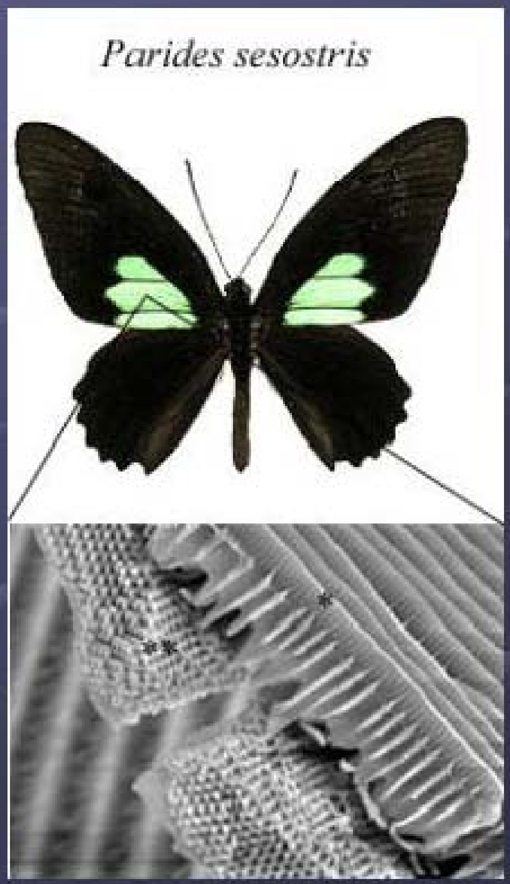


Changes of the effective refractive index of the transverse electric mode (N_{TE}) and the corresponding layer thicknesses upon buildup of PEI-(PSS-PAH)$_2$-PSS-PMBR$_{30}$-PMBR$_{150}$-PAH-PSS matrix

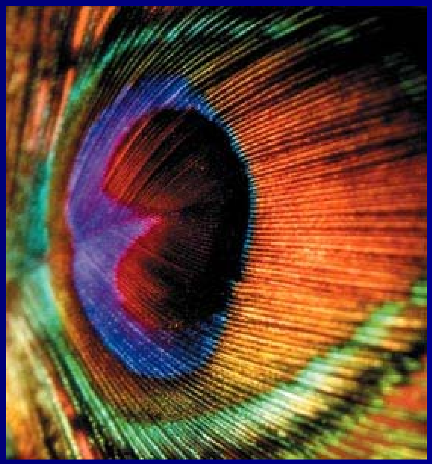
C. Gergely et al 2007 Biomacromolecules 8, 2228

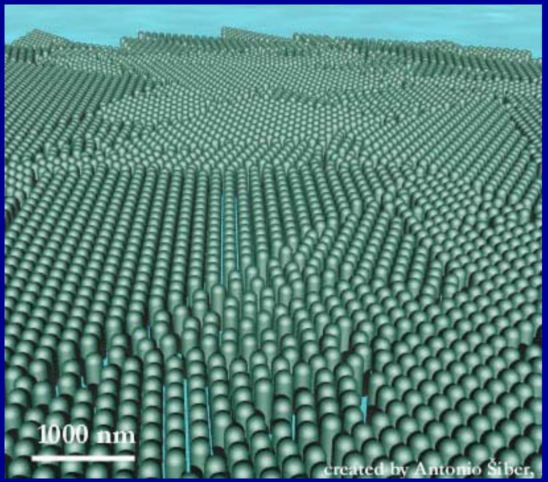
Natural photonic crystals
Towards hybrid photonic devices

Natural Photonic Crystals



Nanometric two-dimensional structures found in eyes of some insects

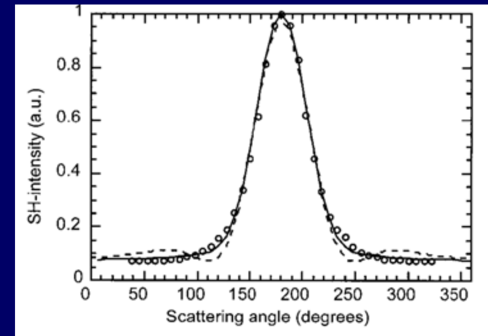




The brown in the feathers of male peacocks arise from natural photonic crystals.

Science at the shine Dome 2004
Michael Shake/Dreamstime.com; P. Vukusic Nature, 2003

Bacteriorhodopsin (bR): natural photonic crystals

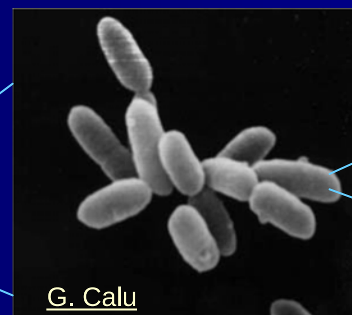


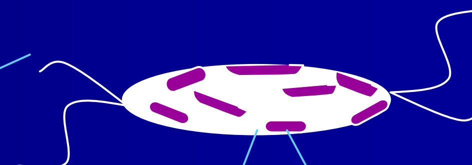
bR → SHG

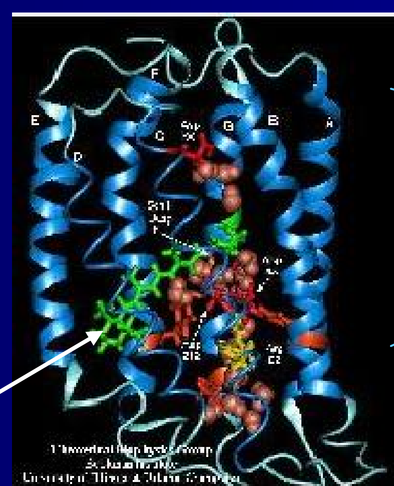
J. Rinuy et al; Biophys. J 1999

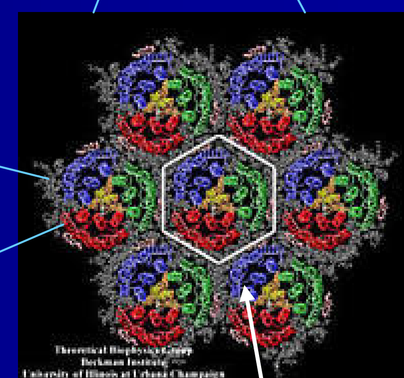
Halobacterium Salinarium

Salt lake







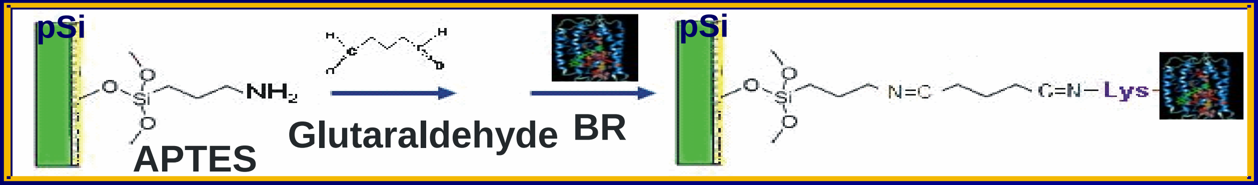


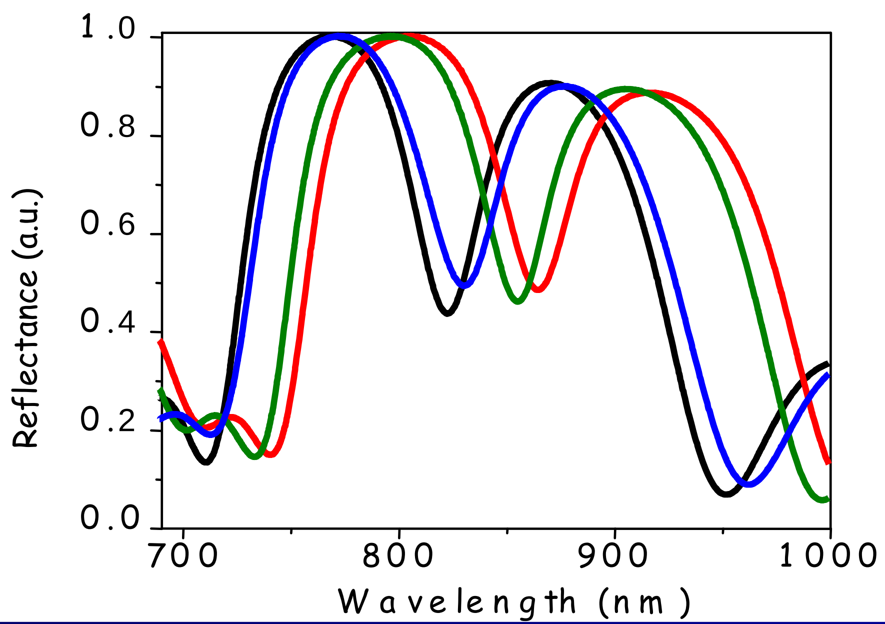
Cromophore retinal within the 7 helices of the protein

Hexagonal arrangement of bR trimers within the purple membrane

Combine organic/ inorganic photonic structures

PSiMc functionalisation and BR immobilization



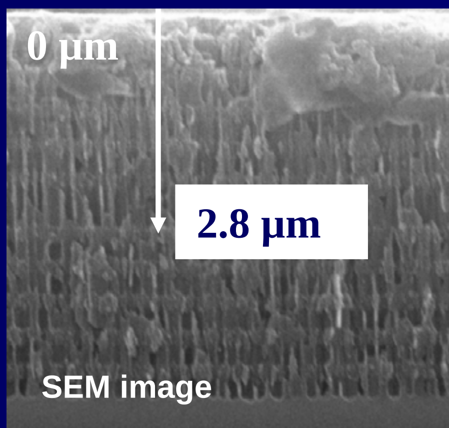


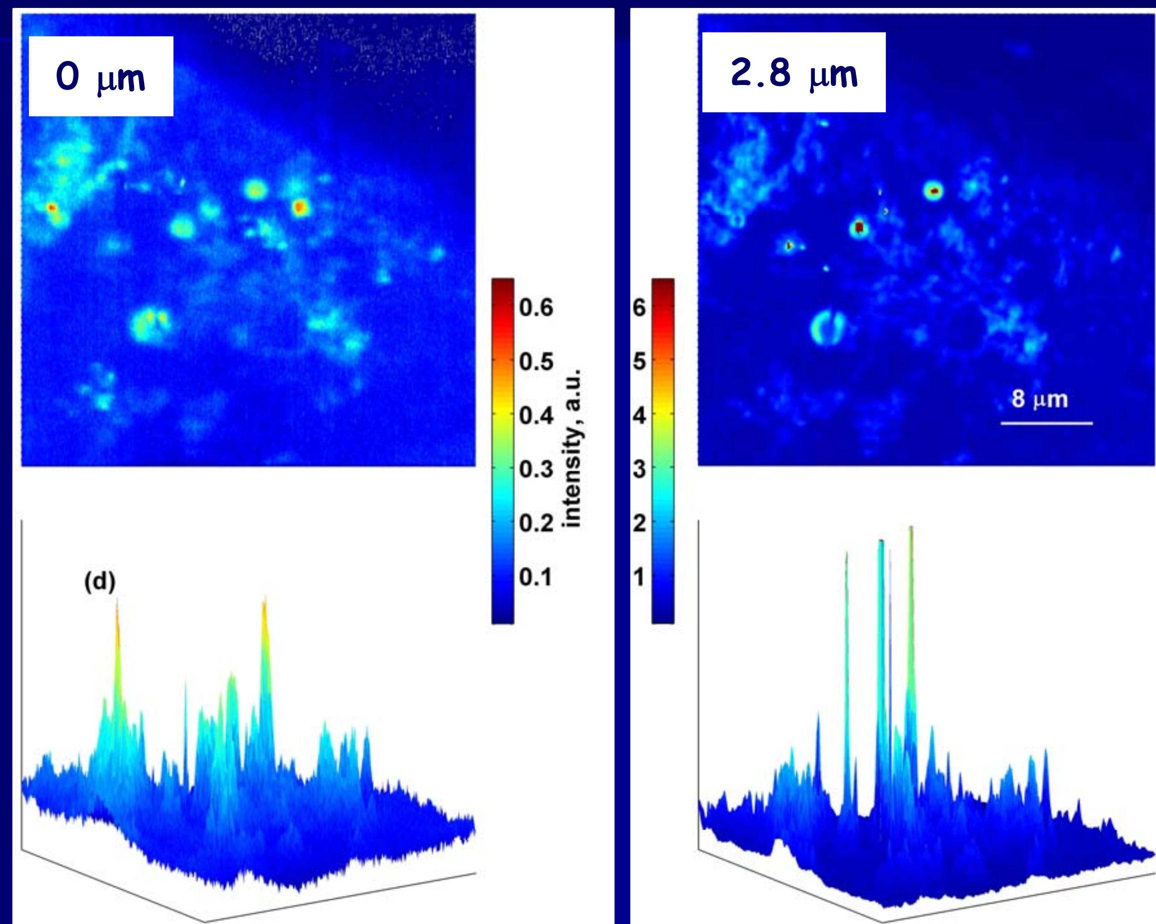
Cavity mode position:
PSi: 824 nm
APTES: 831 nm
GTA: 856 nm
BR: 866 nm

⇒ Covalent binding of solubilized BR on the surface and on the pore walls

G. Palestino *et al.* Proc. SPIE Vol 6592, 1 (2007).

PSi-BR light enhancement detection by Multiphoton Microscopy

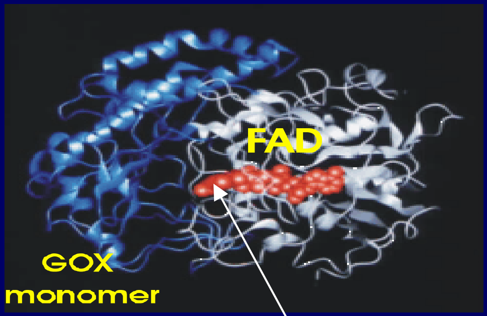




2PEF (visible emission from the dry retinal chromophore) and SHG (max. 440 nm)

2PEF and SHG enhancement occurs at the microcavity

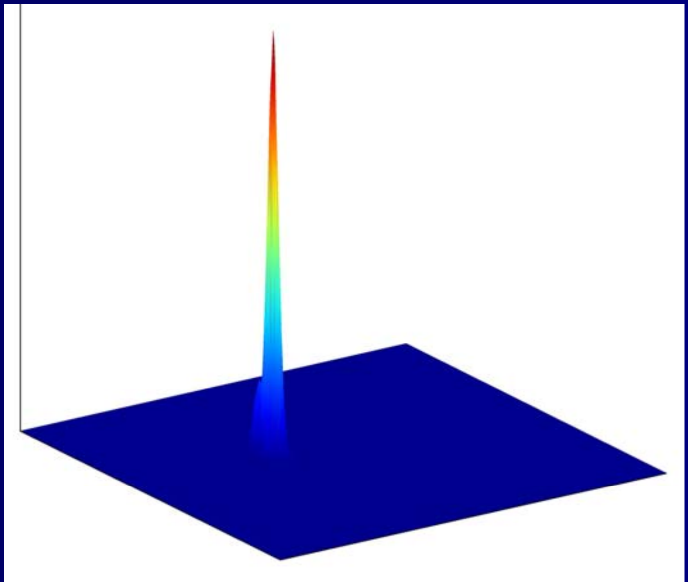
Hybrid organic/inorganic photonic devices



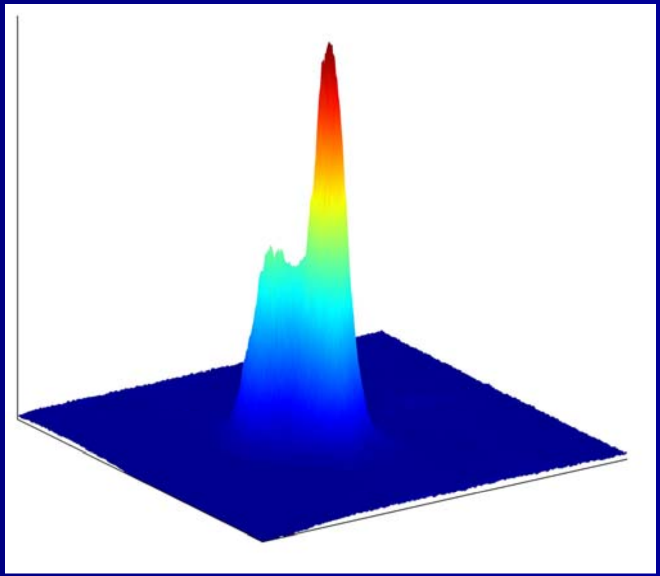
GOX → SHG

2PEF and SHG measured by multiphotonic microscopy

Psi microcavity



Psi microcavity + GOX



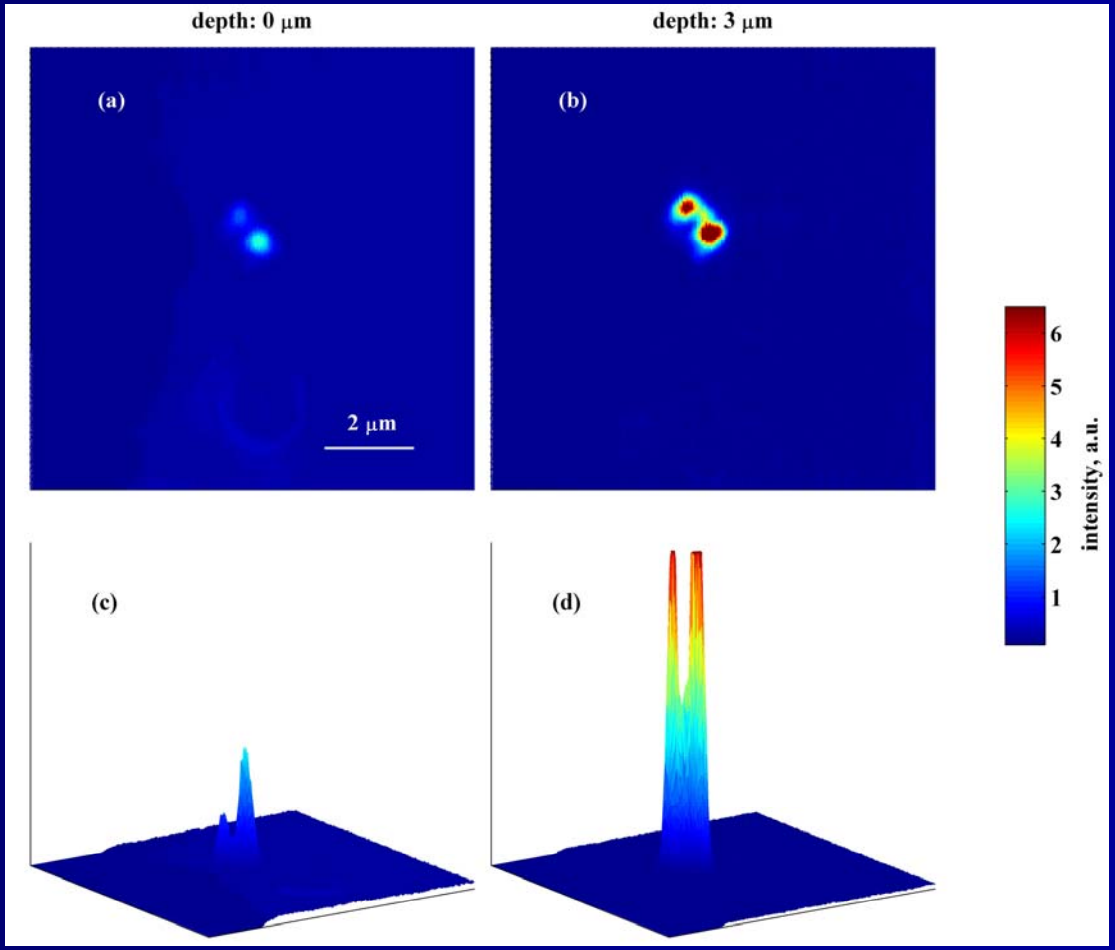
Enhanced 2PEF and SHG emission

Multiphotonic microscopy monitors light emission inside photonic structures

Psi microcavity
+ GOX

- Luminescente biosensing

- Individual pores, thus better detection limit



Smart photonic structures

are needed

to be smartly bio-functionalized

to support device functionality that includes
strongly confined and localized light emission and detection processes

go on towards 'lab-on-a-chip'
high selectivity-based optical biosensors

Go for biophotonics for a better life

Thanks for your attention