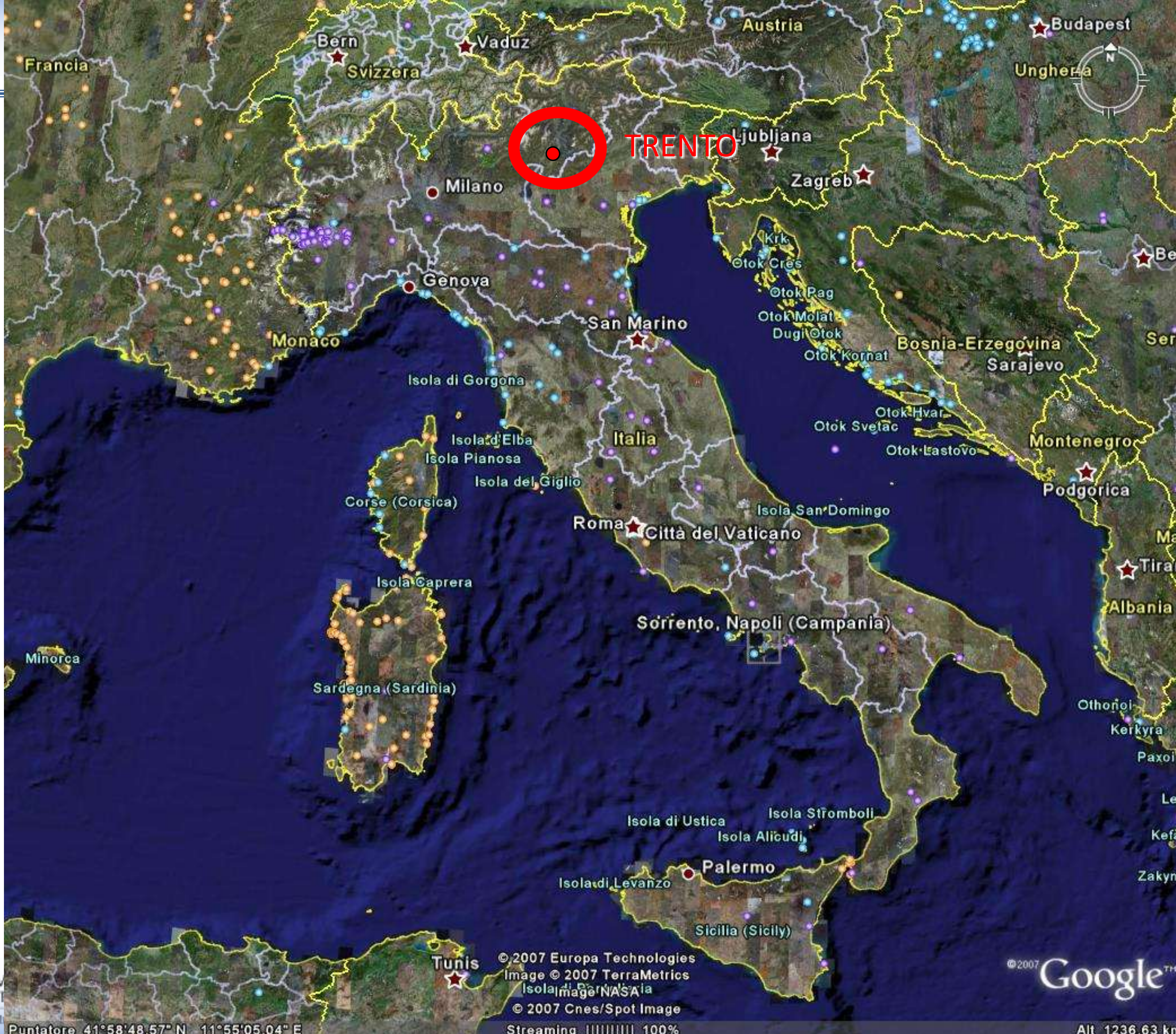
NanoSilicon nanoPhotonic for lab-on-chip applications

Lorenzo Pavesi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Isola di Gorgona
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Roma
Città del Vaticano
Tiran
Albania
Isola Caprera
Sorrento, Napoli (Campania)
Minorca
Sardegna (Sardinia)
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Kerkyra
Paxoi
Isola di Ustica
Isola Stromboli
Isola Alicudi
Kefal
Palermo
Isola di Levanzo
Zakynt
Sicilia (Sicily)
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Puntatore 41°58'48.57" N 11°55'05.04" E
Streaming 100%
Alt 1236.63 km

coworkers

- UNITN
  - P. Bettotti
  - M. Scarpa
  - E. Froner
  - F. J. Aparicio Rebollo
  - D. Gandolfi
  - N. Kumar
- FBK
  - G. Pucker
  - M. Ghulinyan

- FBK
  - Elisa Morganti (FBK-CMM)
  - Lucio Pancheri (FBK-CMM)
  - Laura Pasquardini (FBK-CMM)
  - Leandro Lorenzelli (FBK-CMM)
  - Cecilia Pederzolli (FBK-CMM)
  - David Stoppa (FBK-CMM)
- SSSA-CRIM
  - Elisa Buselli (SSSA-CRIM)
  - Arianna Menciassi (SSSA-CRIM)

MiNaSens workshop 2013

Chairman: Prof. Dimitris Tsoukalas

SENSING PLATFORMS II

14:15-14:45	*NanoSilicon nanoPhotonic for lab on chip applications* **Prof. Lorenzo Pavesi** Department of Physics, University of Trento, ITALY
14:45-15:05	*Acoustic devices as the sensing element in diagnostic platforms* **Prof. Electra Gizeli** Department of Biology, University of Crete & IMBB-FORTH Crete, GREECE
15:05-15:25	*Microfabricated voltammetric sensors for environmental and clinical monitoring* **Prof. Anastasios Economou** Analytical Chemistry Lab, Department of Chemistry, National & Kapodistrian University of Athens, GREECE
15:25-15:45	*Mammalian cell-based biosensors in food safety control* **Prof. Spyridon Kintzios** Faculty of Agricultural Biotechnology, Agricultural University of Athens, GREECE
15:45-16:05	*Laser technology for sensor applications* **Prof. Ioanna Zergioti** School of Applied Mathematical & Physical Sciences, National Technical University of Athens, GREECE

MiNaSens

Chairman: Prof. Dimitris Tsoukalas

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outline

- Nanosilicon nanoPhotonics
- Few examples
  - Silicon nanocrystals as chromophore
  - Naomi test vehicle: contact sensor
  - Polarimetric sensor based on porous silicon membranes
  - Integrated waveguide for marked protein detection
  - Wedge microdisk resonator for label free biosensors
- Conclusions

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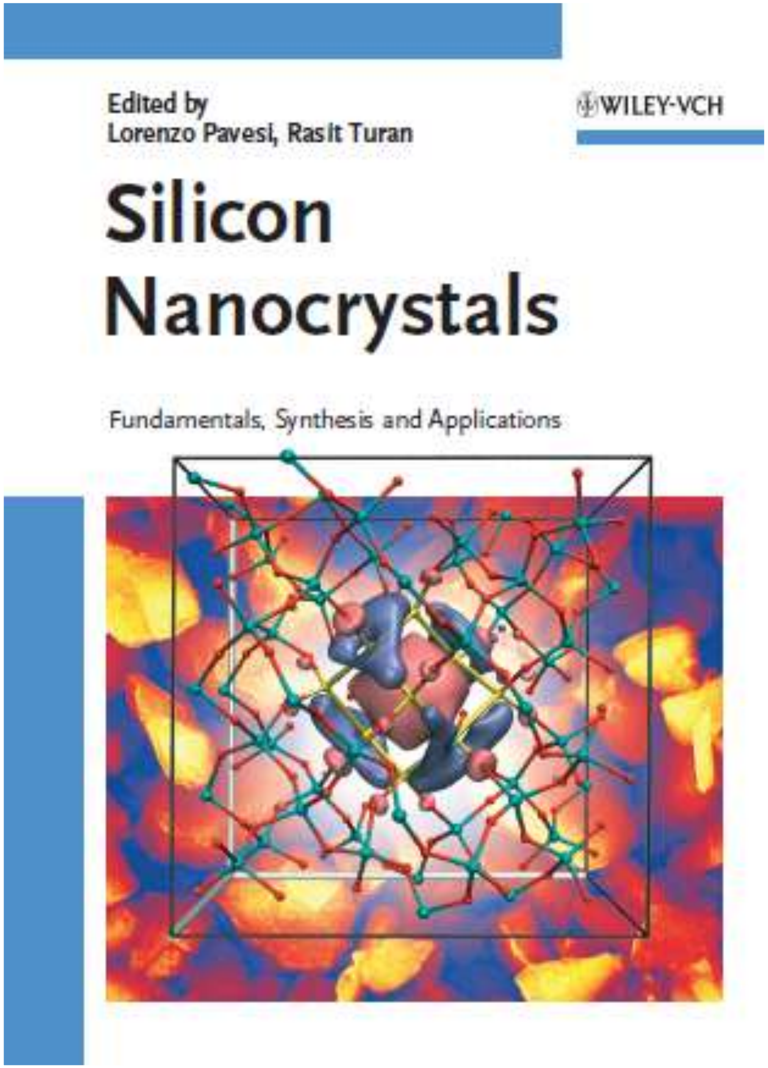
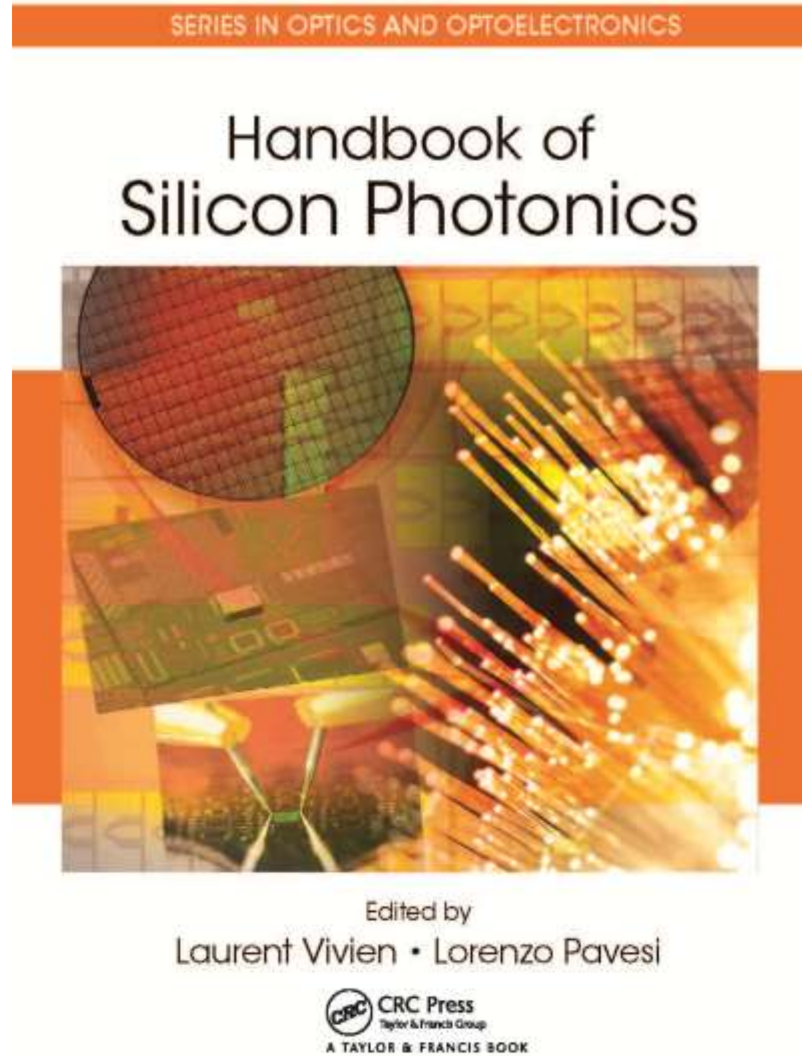
nanoSilicon nanoPhotonics



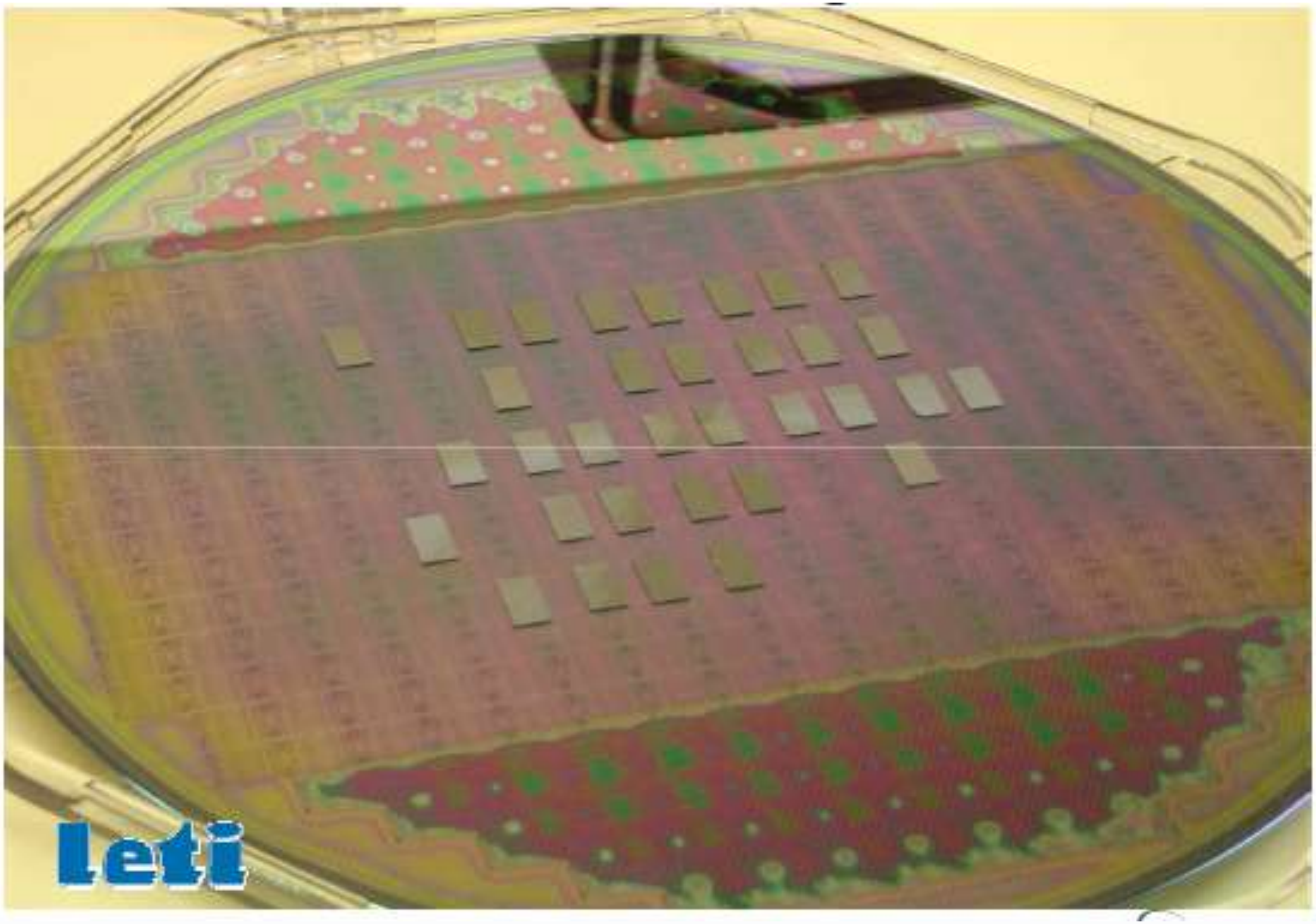


nanoSilicon nanoPhotonics

A platform where photon or electron confinement enables new functionalities in silicon photonics for bisoensing, i. e. lab-on-chip applications

Silicon photonics because of the mass manufacturability which means advantages in terms of cost and performances

Full wafers with thousands of photonics sensors



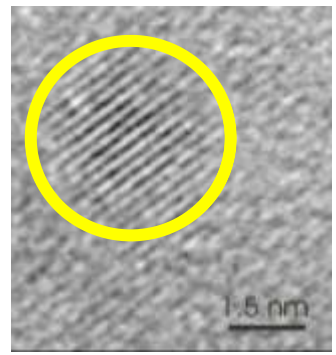
nanoSilicon nanoPhotonics

- Confine carriers on nanoscale dimensions
  - Length scale =
    electron DeBroglie wavelength

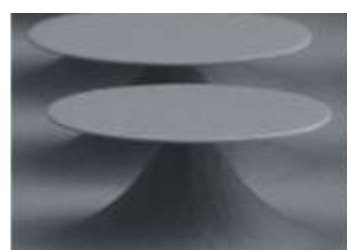
- Confine photons on nanoscale dimensions
  - Length scale =
    light wavelength

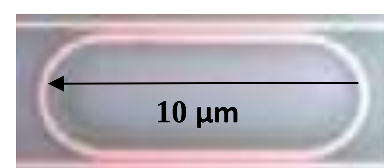
nanoSilicon nanoPhotonics

- Confine carriers on nanoscale dimensions



- Confine photons on nanoscale dimensions





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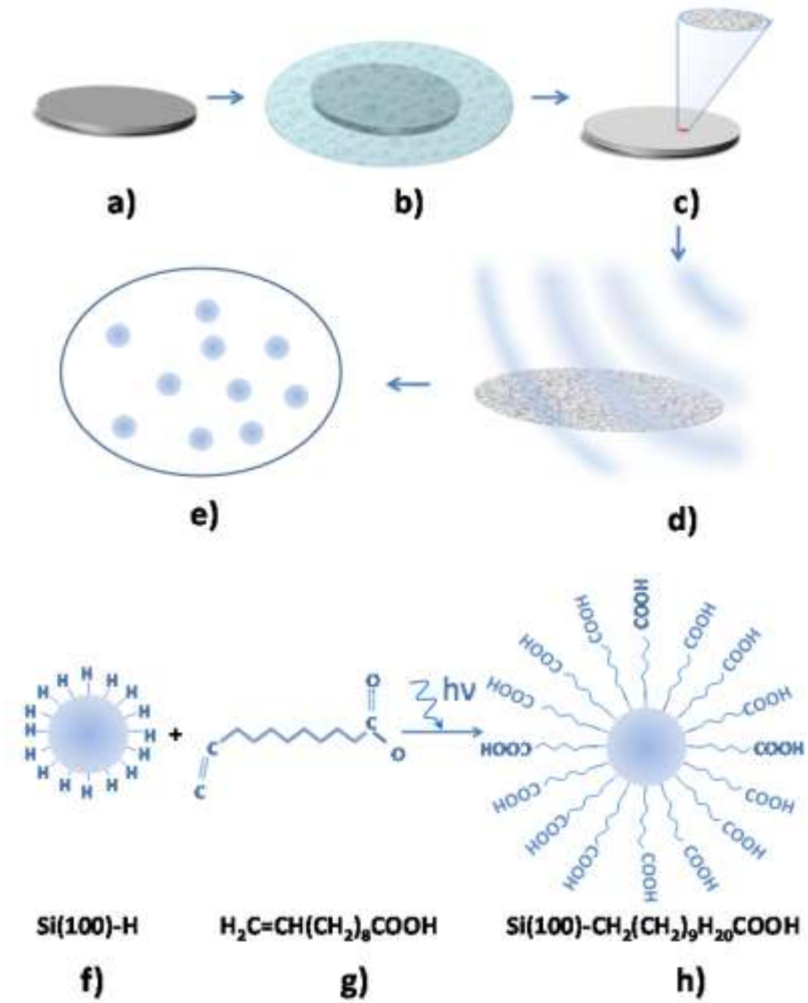
Si-nc as imaging agents

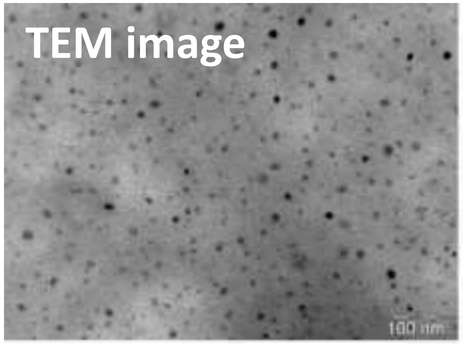


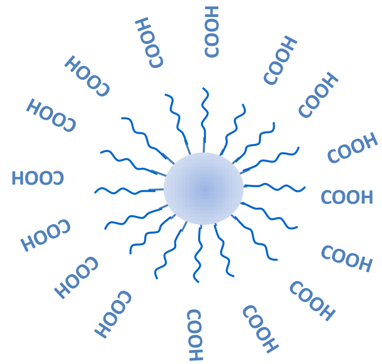
Preparation:

1. Sonication of porous silicon

2. Photoinduced hydrosilylation reaction between undecylenic acid and hydrogen passivated Si-nc surface

Si-nc as bioimaging agent



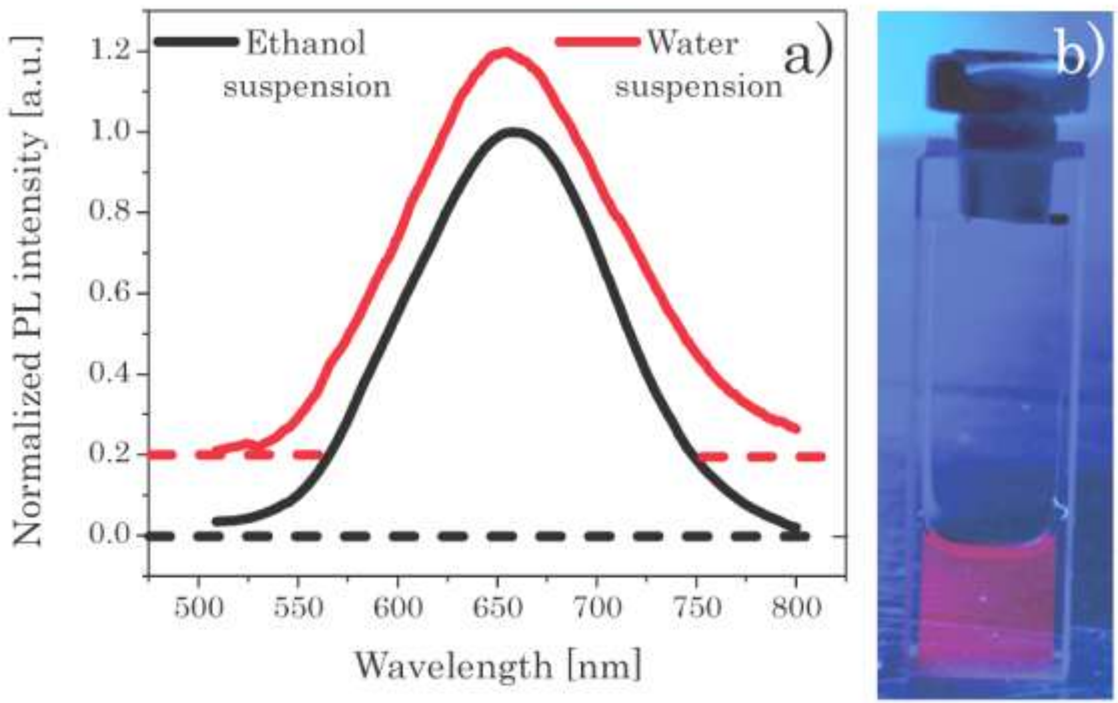


Hydrophilic alkyl-capped Si-nc

Luminescent clear suspension in different solvents (water, ethanol).

No change in PL lineshape in different solvents.

High quantum yield
QY ~ 30 %

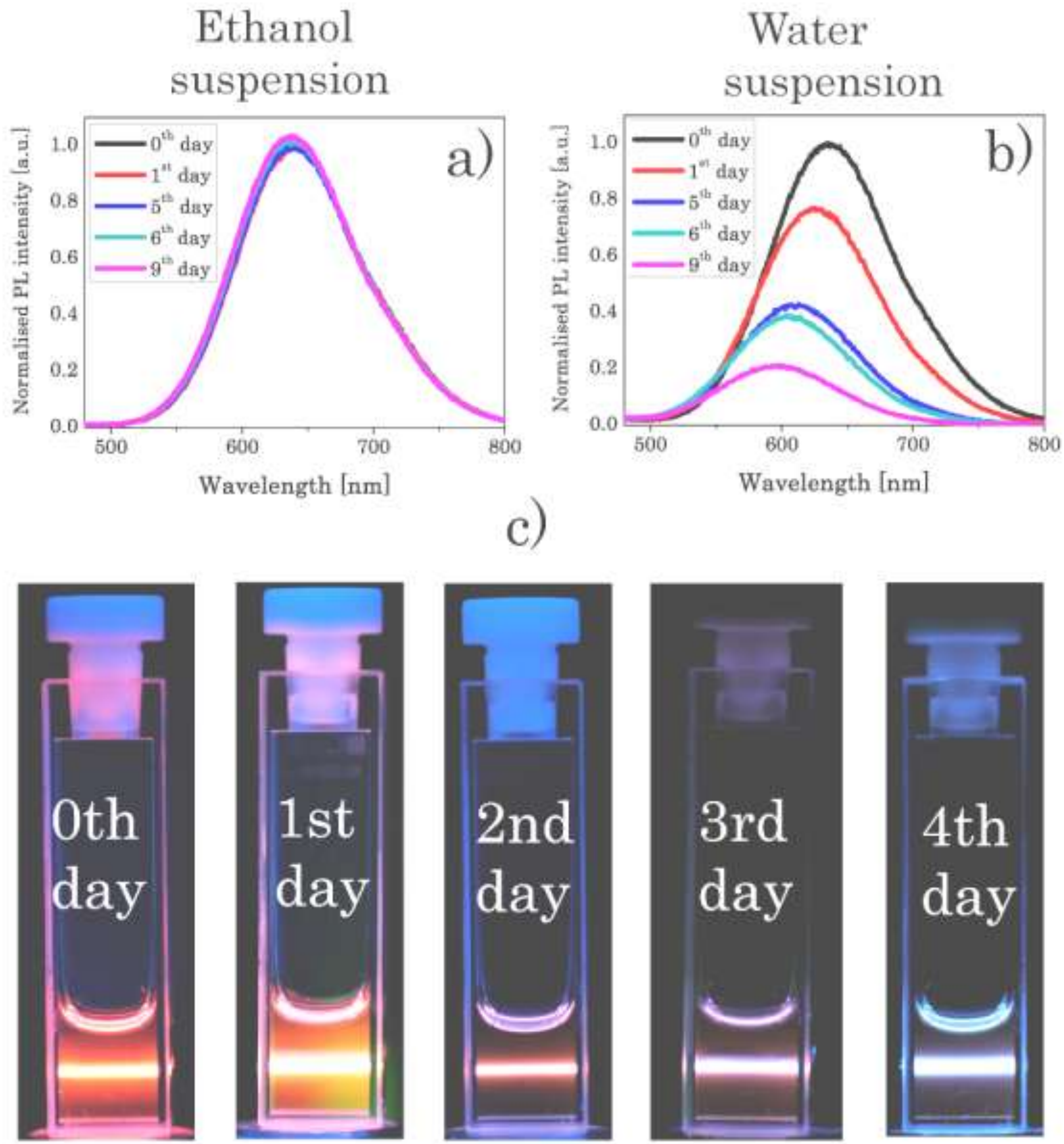


Si-nc as bioimaging agent

<u>Si-nc-COOH without physical coating:</u>

1. Si-nc-COOH can be stored in ethanol for long periods of time.
2. In water Si-nc-COOH slowly oxidize and dissolve.

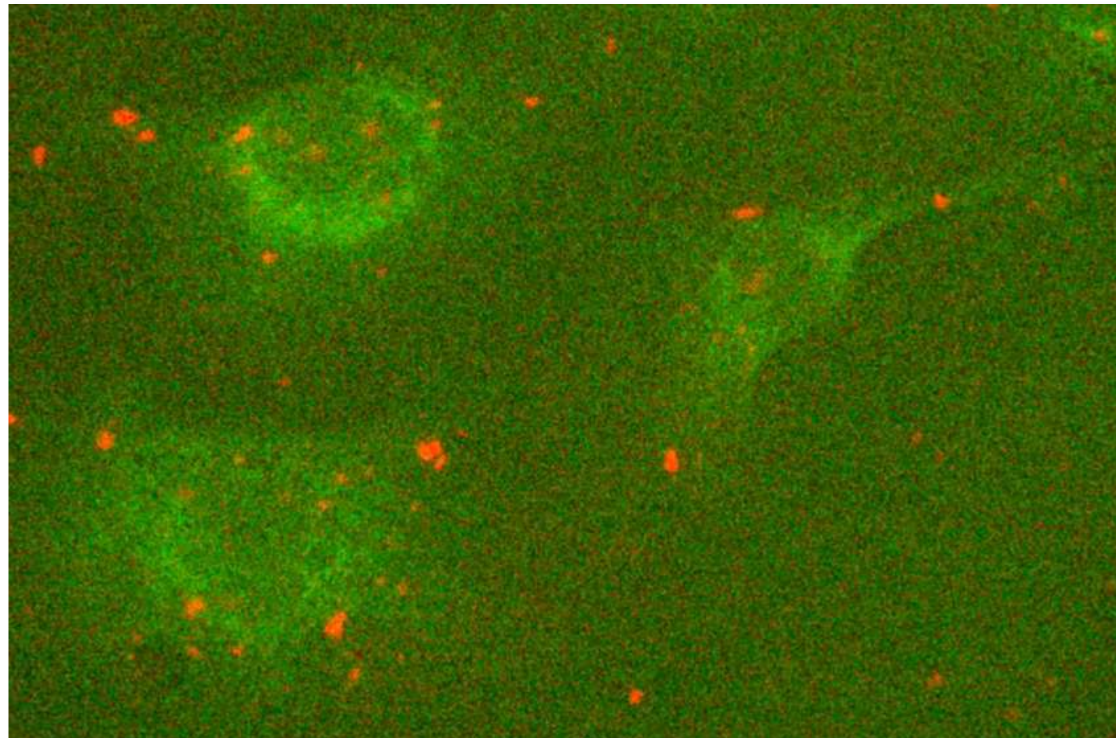
Biodegradability is achieved.



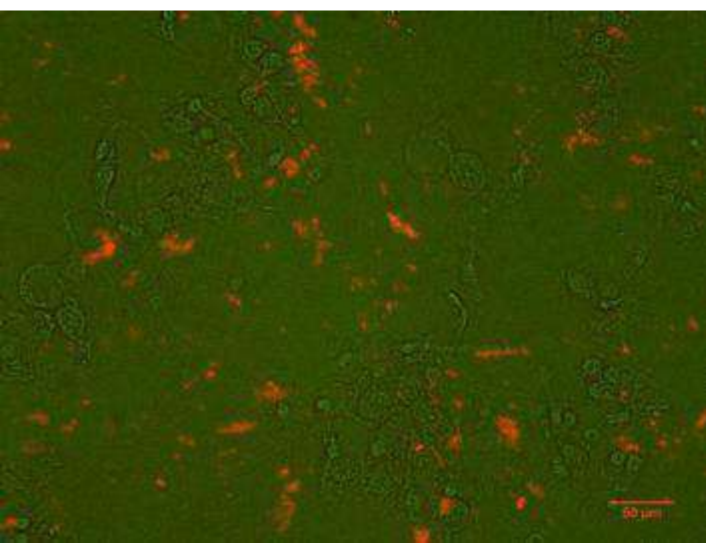
Bio imaging

- **DCA** (sodium deoxycholate monohydrate) shows similiar behaviour as **SDS**

- **DCA** less toxic than **SDS**



Fluorescence images of SKOV-3 cells incubated with Si-nc-COOH+DCA for 30 min.



DCA was not added

Advantages of silicon nanocrystals with respect to dyes

- Biocompatible
- No bleaching
- Long lifetimes (μs)
- Two photon absorption
- Broad absorption band
- Silicon surface chemestry

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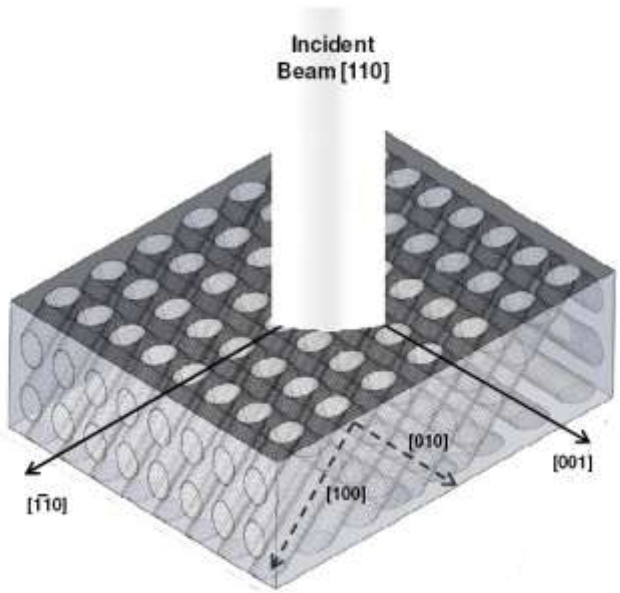
Polarimetric sensor



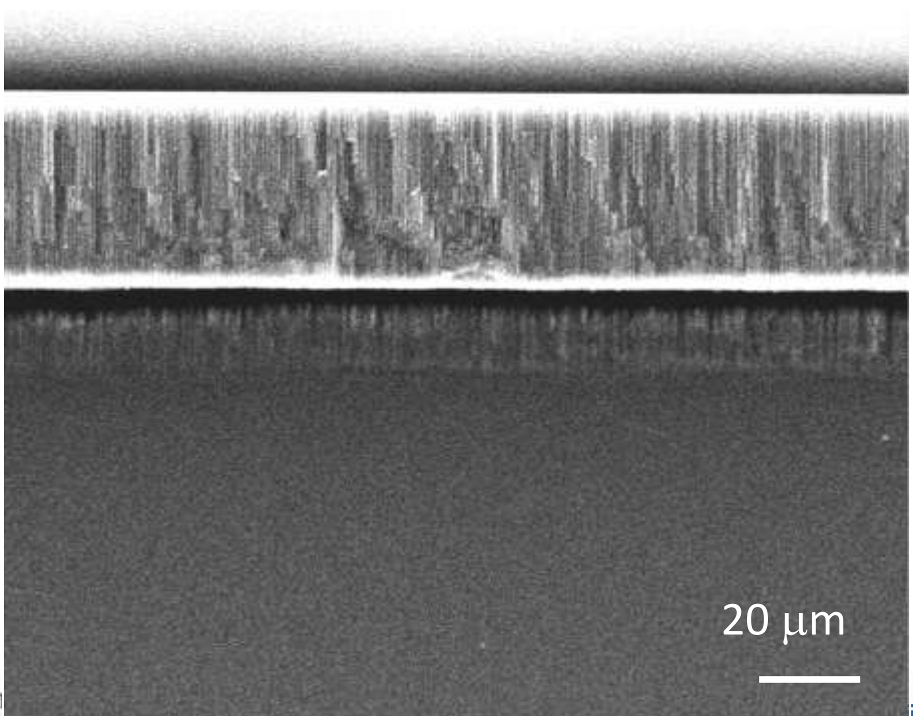
$$\Delta\phi(\lambda) = \frac{2\pi}{\lambda} \cdot d \cdot \Delta n(\lambda)$$

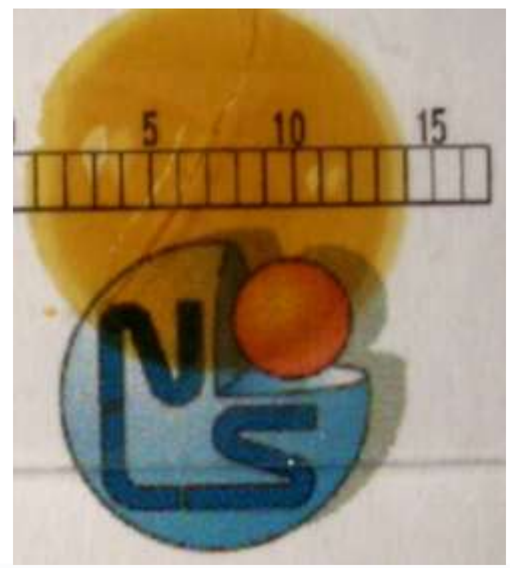
$$T_{\parallel}(\lambda) = \cos^2\left(\Delta\phi(\lambda)/2\right)$$

$$T_{\perp}(\lambda) = \sin^2\left(\Delta\phi(\lambda)/2\right)$$

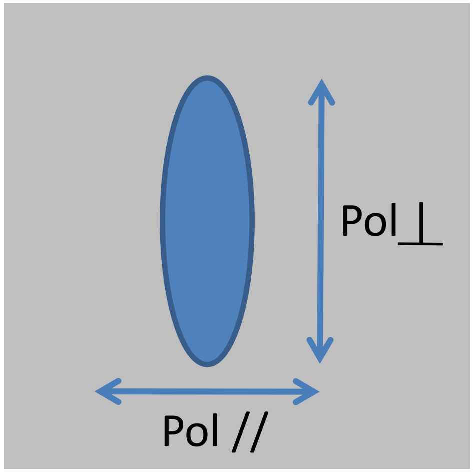
Porous Silicon

PorSi etching High current burst Membrane detachment

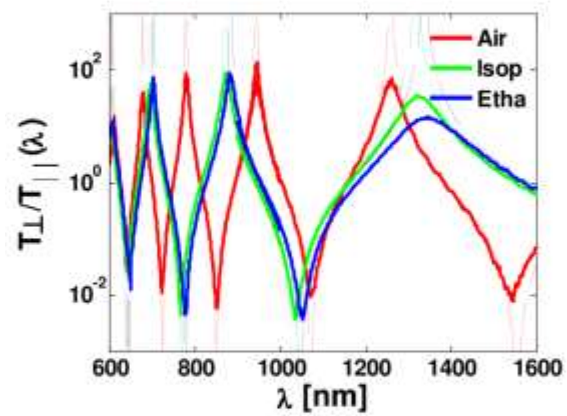


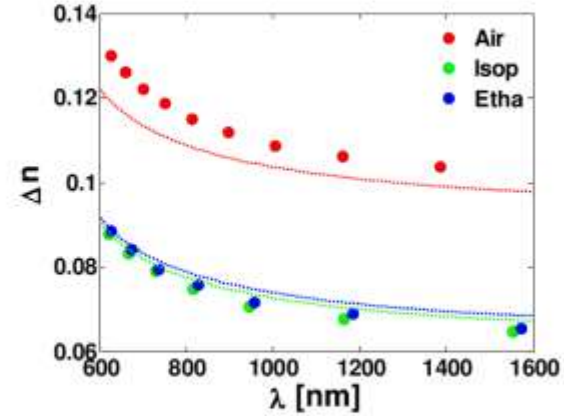


Polarimetry



$$\Delta\varphi(\lambda) = \frac{2\pi}{\lambda} \cdot d \cdot \Delta n(\lambda)$$





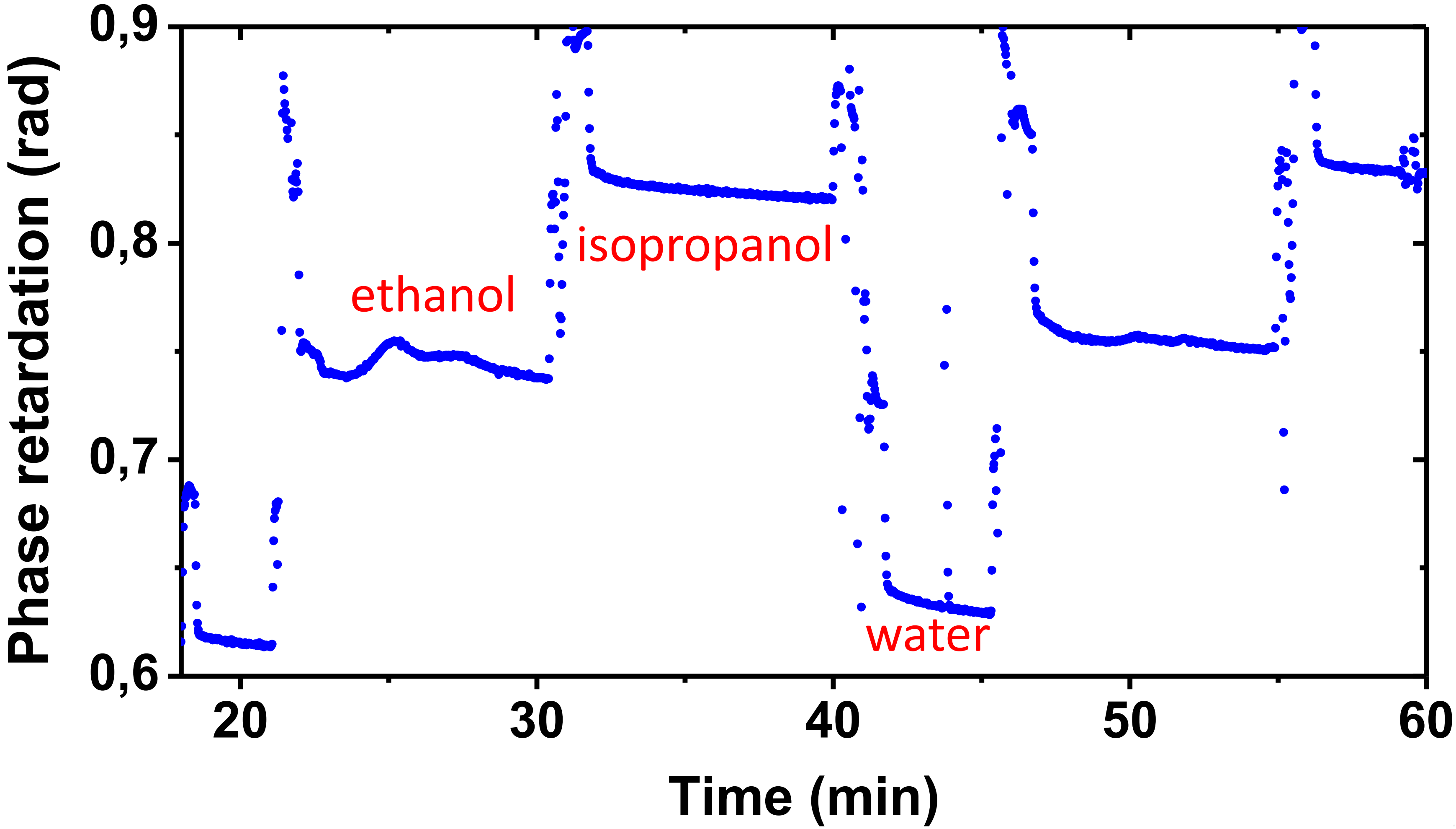
$$n_\perp \neq n_{//}$$

Porous Silicon is a (form) birefringent material

Wavelength (nm)	Sensitivity (nm/RIU)
810	626
1300	1135
1500	1247

In collaboration with University of Valencia

Flow through measurements



Food allergies & point of care diagnosis

POSITIVE

An FP7 project that aims to develop a food allergies point of care diagnostic tool.

Food allergens

Food allergies affect 1-2% of adult population and up to 8% of children (15 milions people in Europe). A serious public health problem.

Lab on a Chip

POSITIVE is developing a compact LoC with an integrated blood sample preparation technique.

Optical detection

Porous Silicon free standing membranes are used to quantitatively check the allergic reaction to specific foods. Through an integrated approach a multiplexed approach is developed.

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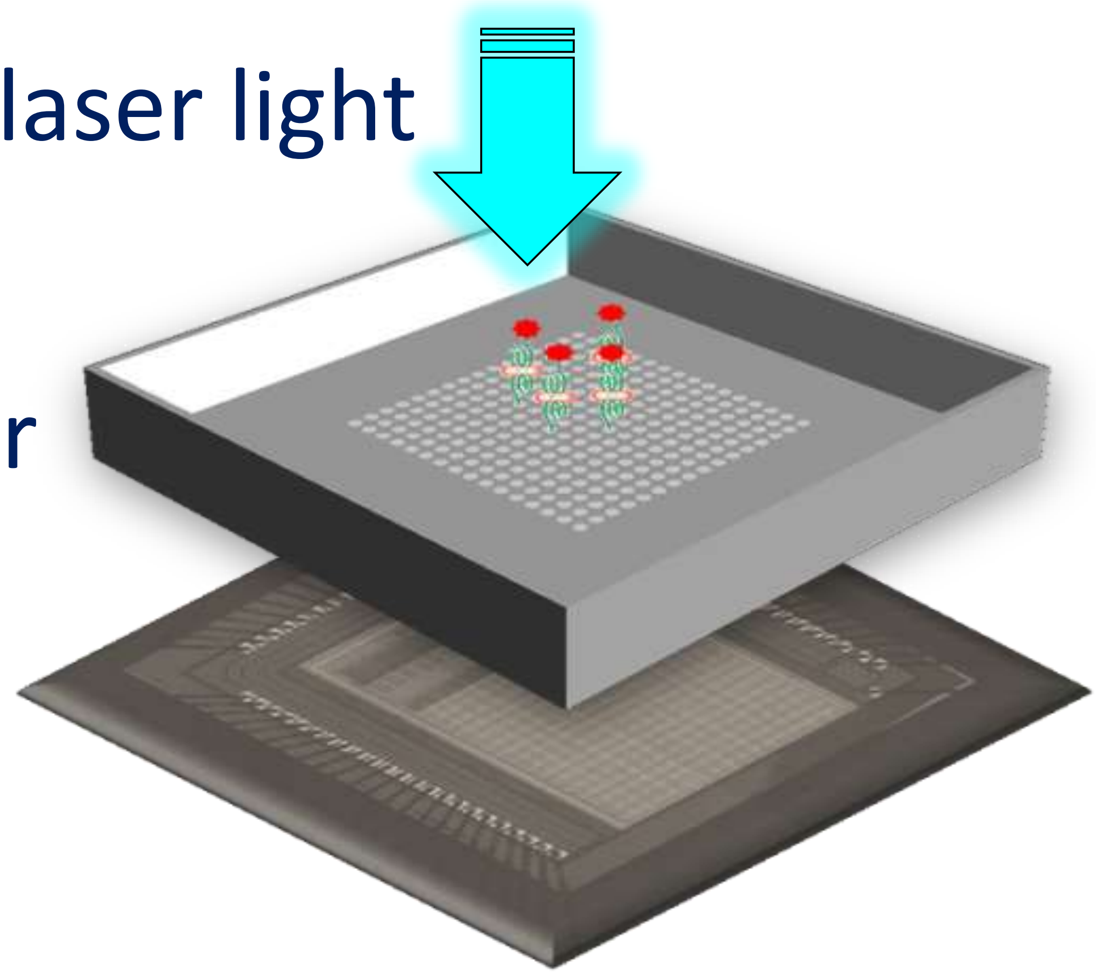
Affinity biosensor

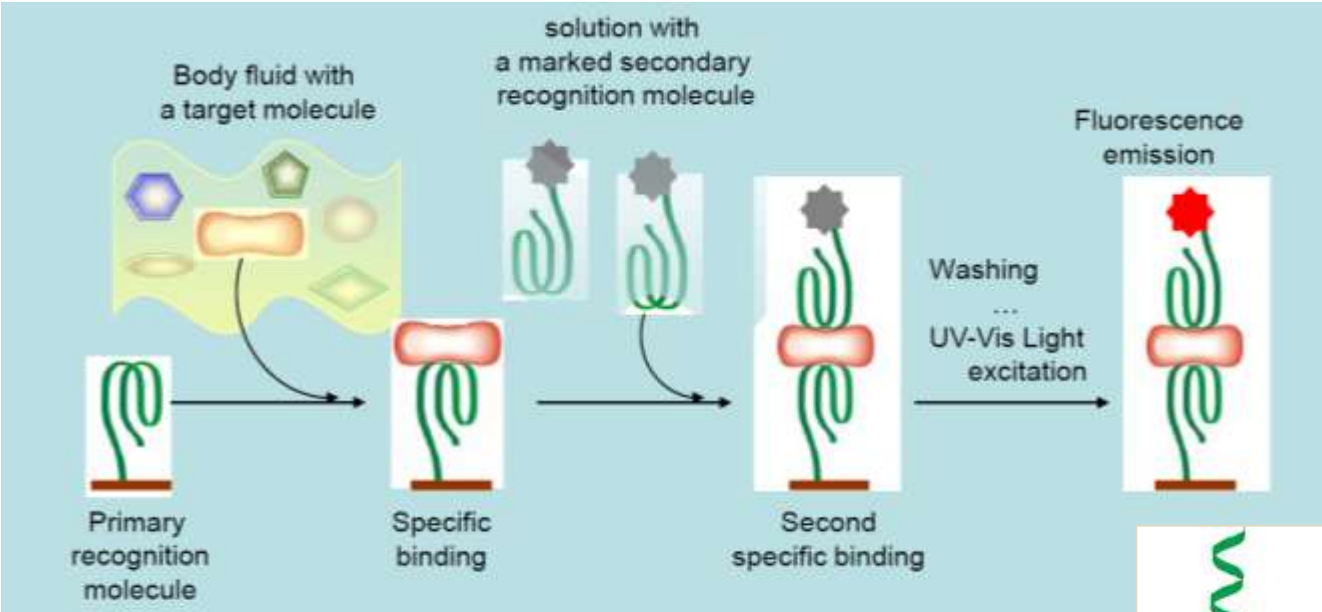
Pulsed laser light

Reactor

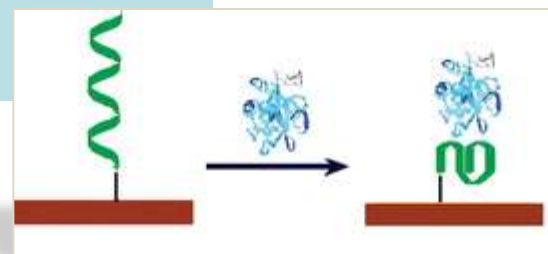
SPAD



An affinity biosensor constituted of nucleic acid based probes (**DNA-aptamers**) designed to bind specific proteins

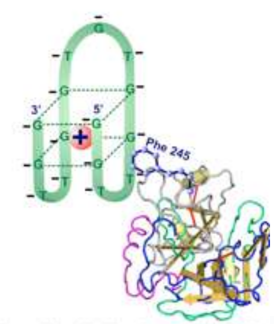


Detecting biomolecular interactions with high sensitivity and reliability

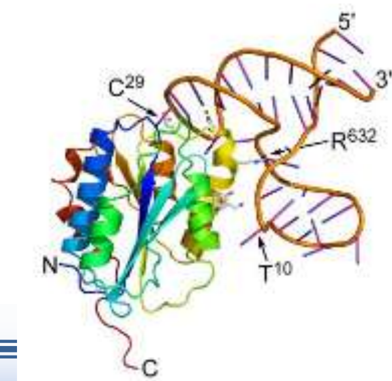


Aptamers

- in vitro selection (SELEX)
- high specificity and affinity
- high reproducibility and purity
- highly chemically stable
- great flexibility in design of novel biosensors



Thrombin-binding Aptamer (TBA)
5'-d(GGTTGGTGTGGTTGG)-3'



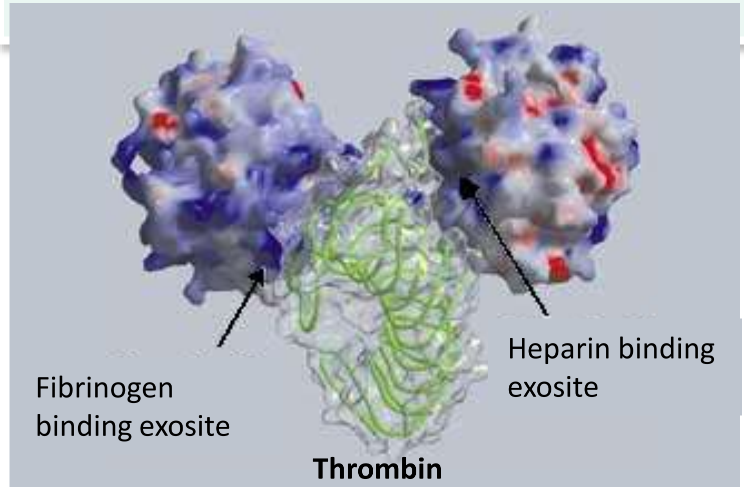
Biological target

1) Initial model: THR

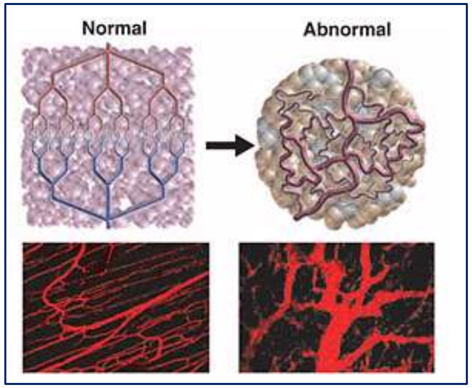
Thrombin: is the last enzyme protease involved in coagulation cascade

Central role in a number of cardiovascular diseases, in inflammation and tissue repair at the vessel wall

Thrombin concentration in blood:
- 0 (normal conditions) ÷ mM (coagulation process)
- low levels (~nM) of thrombin generated early in hemostasis are also important to the overall process



2) Validation system: VEGF



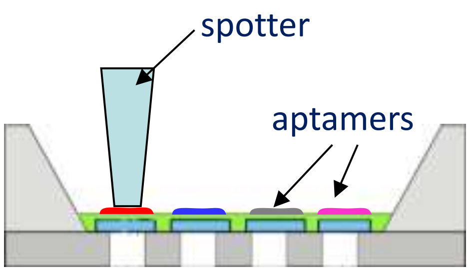
Normal human serum values 0.3-0.8 ng/ml

VEGF: **vascular endothelial growth factor** - stimulates the growth of new blood vessels.

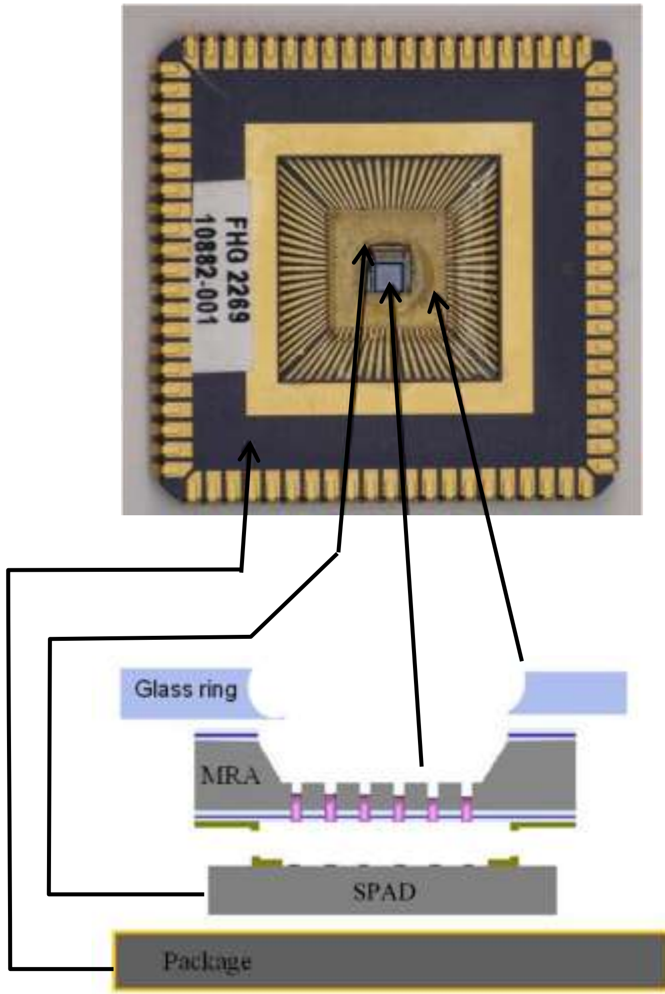
Central role in pathologies such as tumors, cronical ischemia, retinopathy

Transparent micro reactor array (MRA)

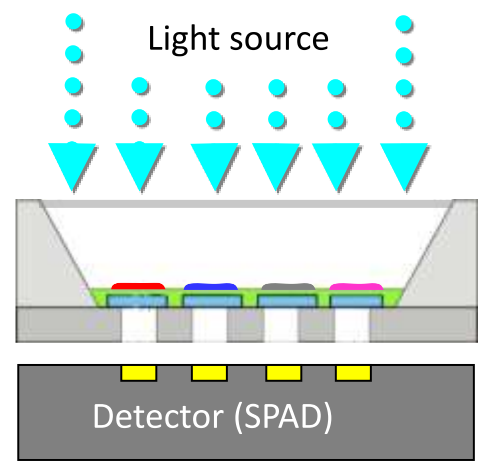
Total dimensions	2.2mm x 2.2mm
Wells area	1.6mm x 1.6mm
Number of wells	256
Wells diameters	50 µm
Centers Distance	100 µm
Chamber volume	0.9 µL



1: spotting



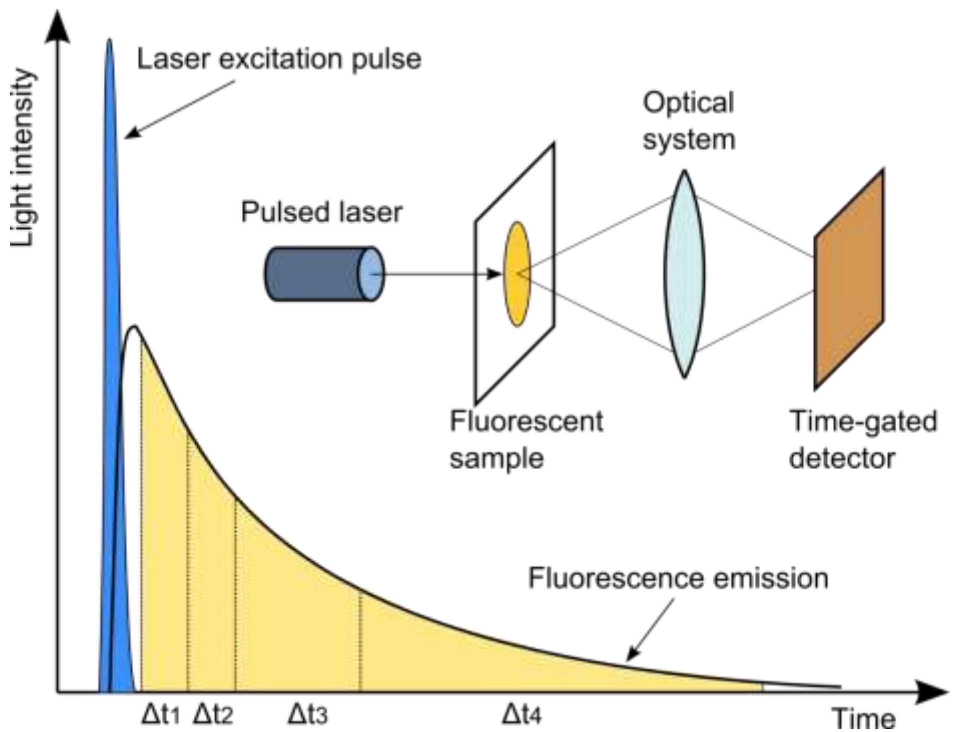
2: assembling, loading the sample

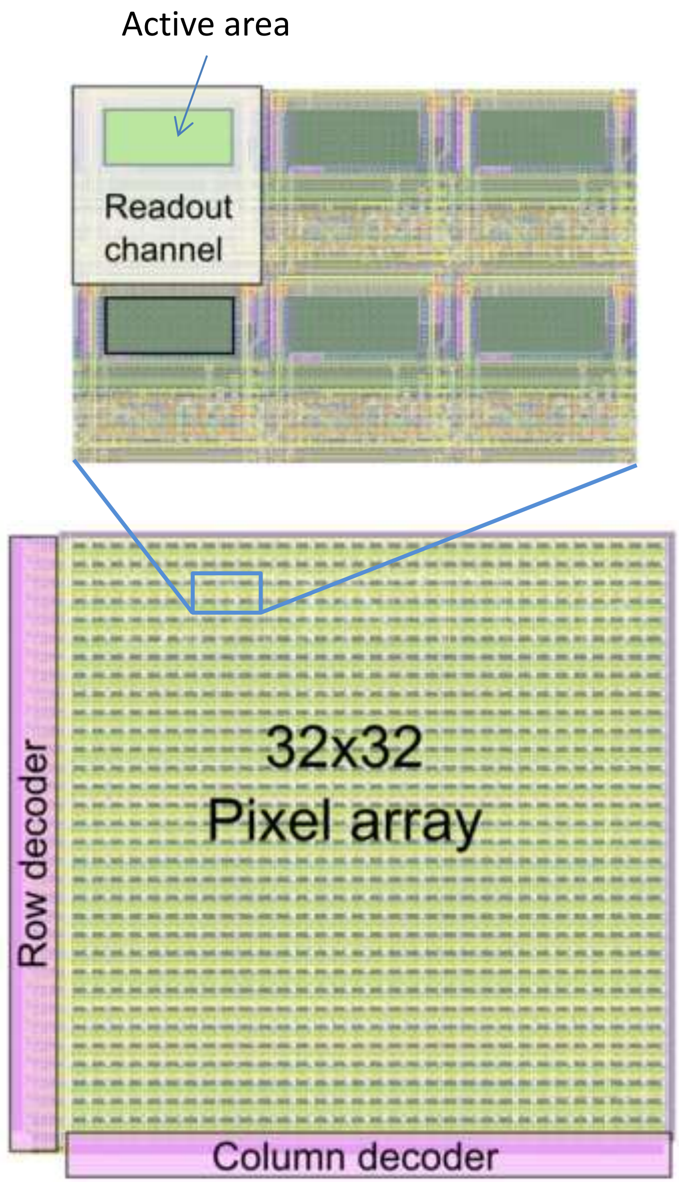


3: detection

SPAD sensor

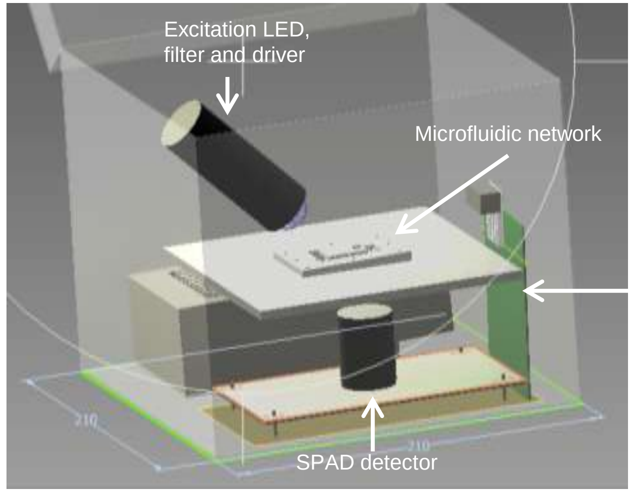
CMOS Visible Detectors: Time-gated Lifetime Measurement Technique





32x32 SPAD pixel array layout
Array size: 0.8 x 0.8 mm
Pixel pitch: 25um
Fill factor: 20.8%

Protein Detection using a fluorescence approach based on SPAD detector

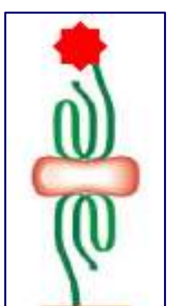


Integrated system

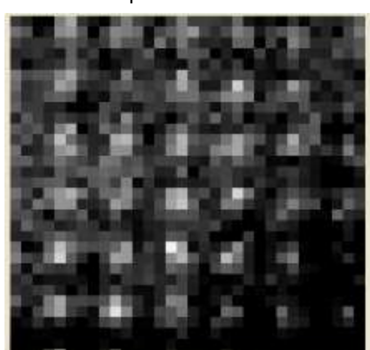
Two blood proteins are tested: Human Thrombin and Vascular Endothelial Growth Factor (VEGF)

THROMBIN

A secondary fluorescent-labelled aptamers is used to detect the protein



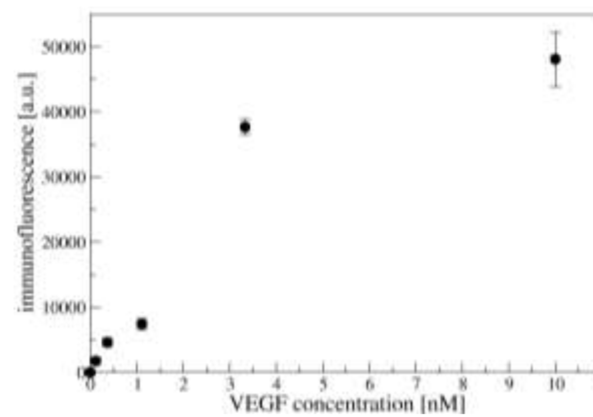
256 micro-reactor array
4 SPAD pixels/micro-reactor



Thrombin concentration: 300nM
Total measurement time: 2.5 min

VEGF

Using an amplification system based on the immunofluorescence technique we are able to detect a protein concentration up to 100pM concentration



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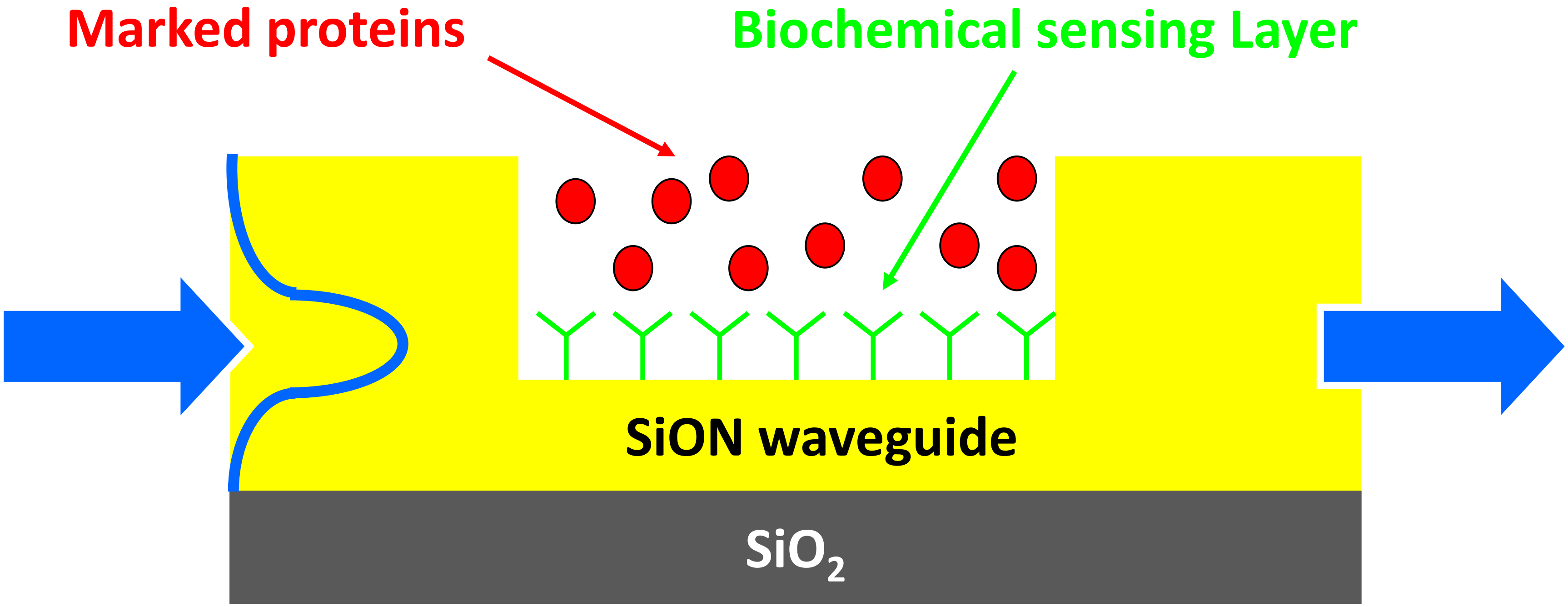
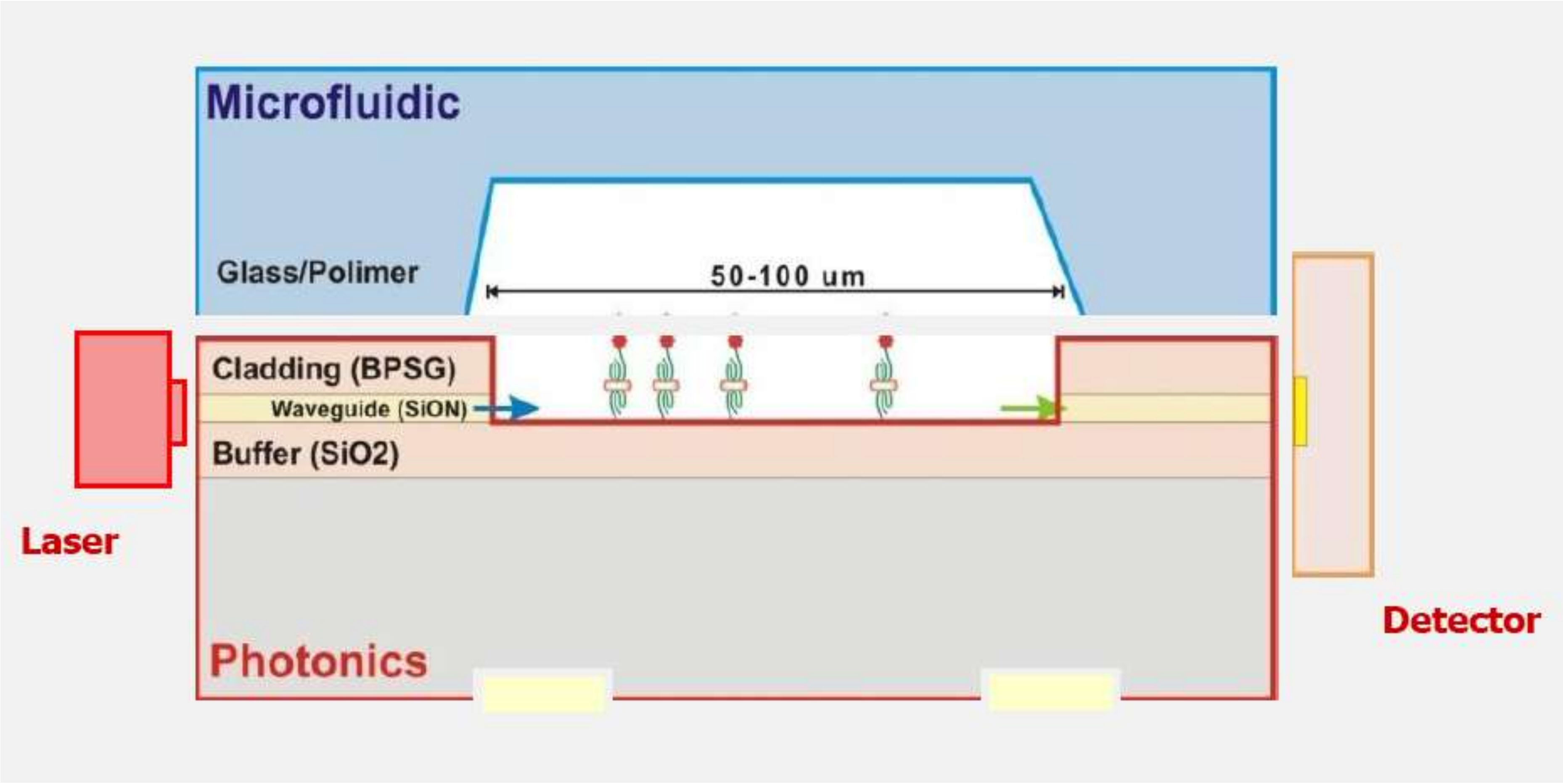
waveguide based system



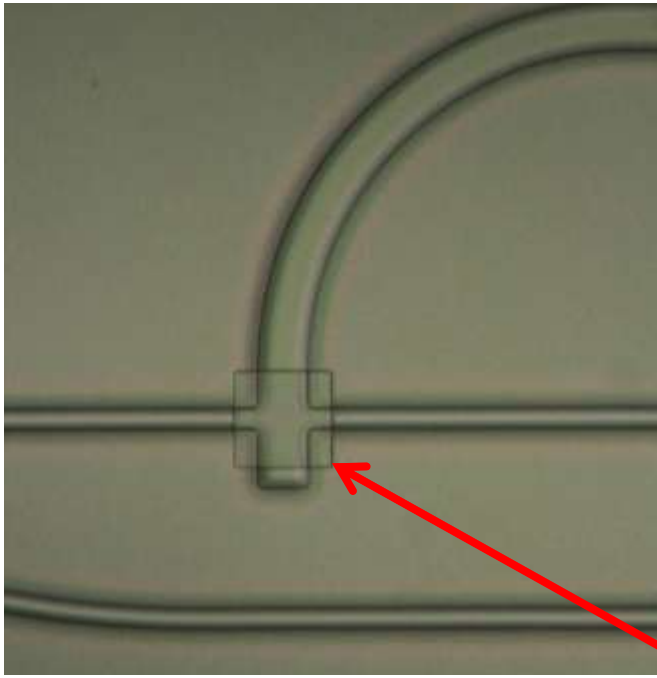
- high sensitivity
- localized and uniform illumination
- low background and scattering

The overall packaged system

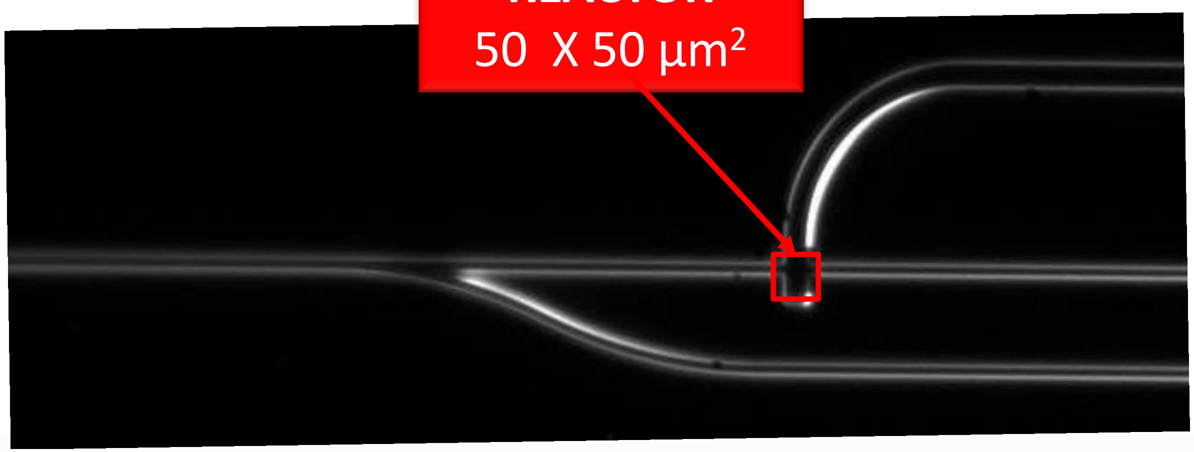


1st Test Vehicle

Densely Packed Arrays



REACTOR
50 X 50 μm²



Output WG
Width 15/25 μm

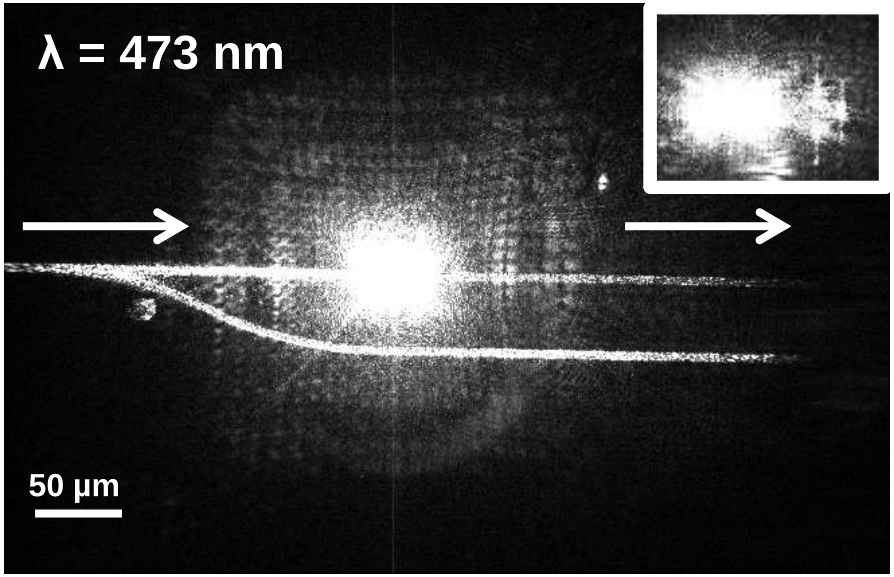
Transmission WG
Width 5/10 μm

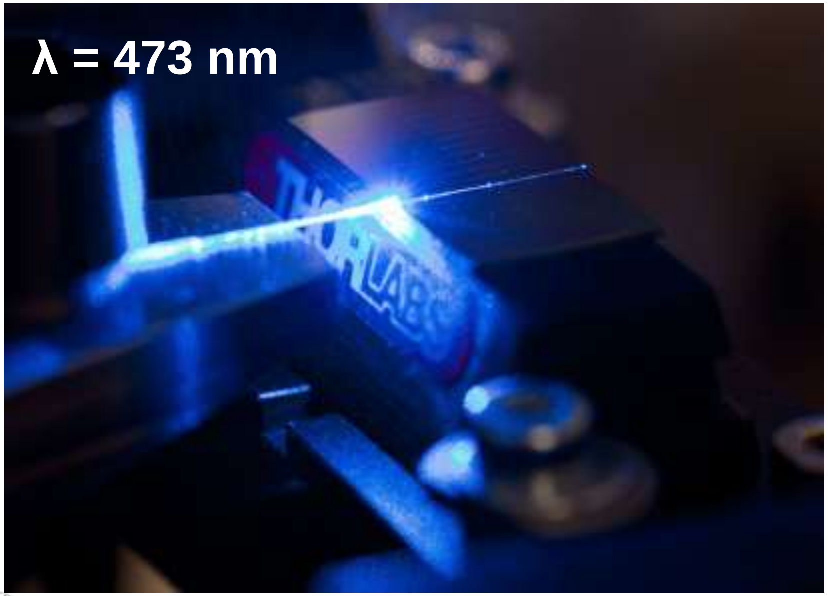
Reference WG
Width 5/10 μm

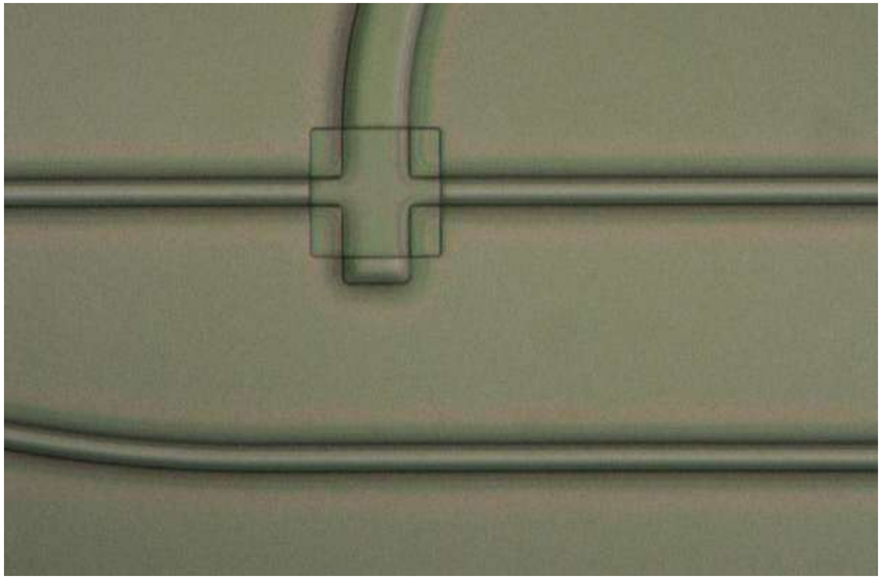
Input WG
Width 5/10 μm

Propagation losses below 3 dB/cm <u>@ 473 nm</u>

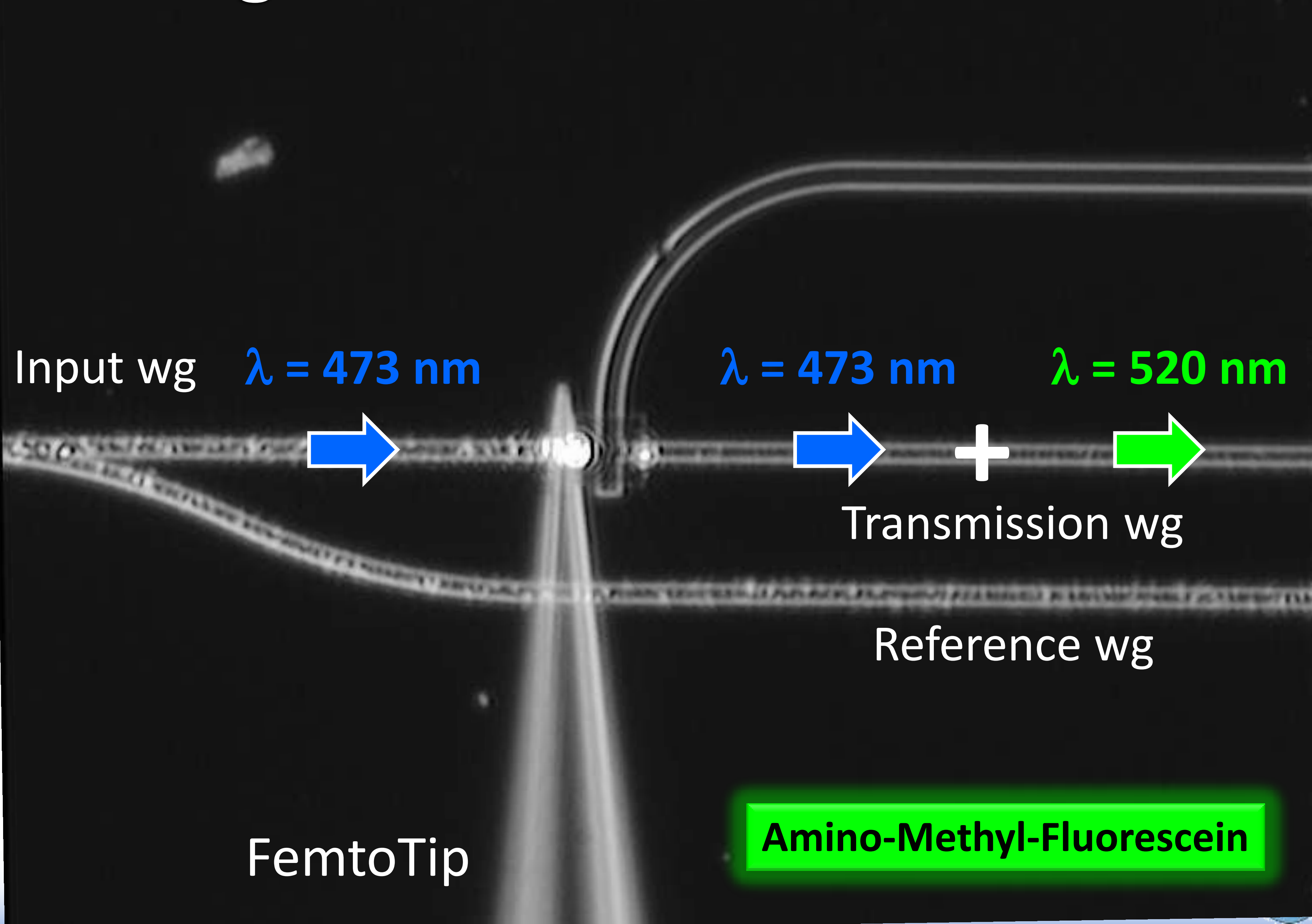
An intense excitation beam is transmitted by the waveguide up to the bioreactor







Sensing measurements: in transmission



The bioreactor depth

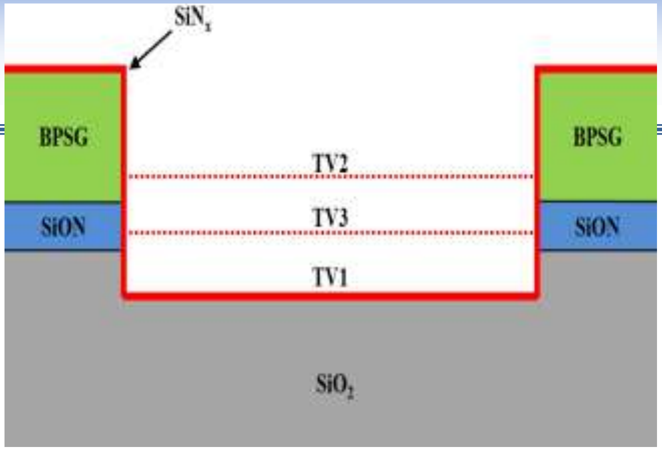
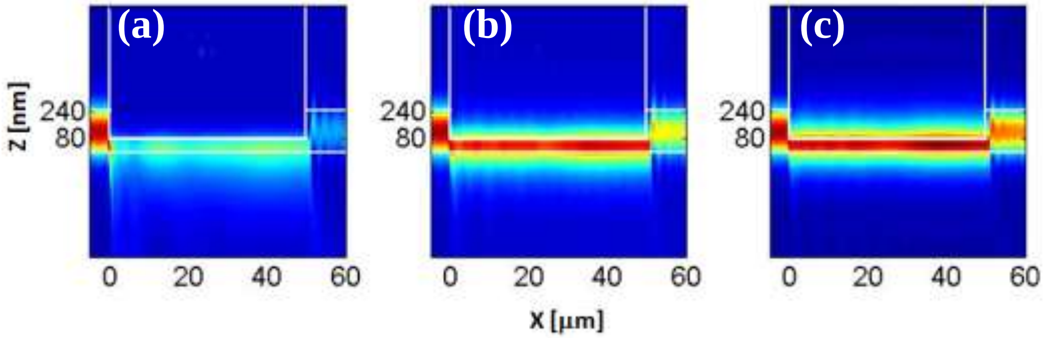


TABLE I

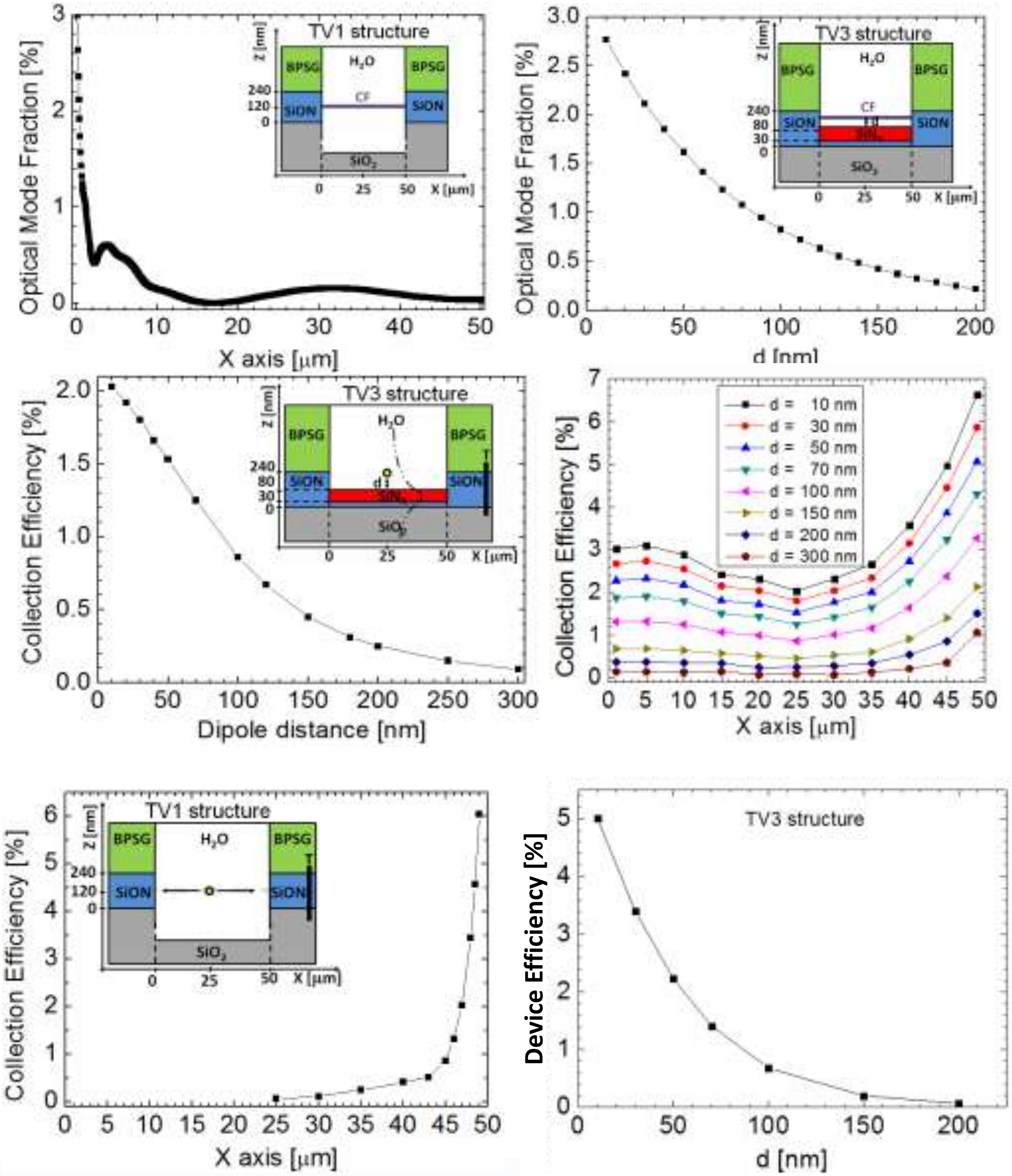
OPTICAL LOSSES DUE TO THE BIOREACTOR AT 670 NM, EXPERIMENTALLY AND THEORETICALLY DETERMINED FOR THREE FILLING SOLUTIONS AS INDICATED IN THE TABLE. THE SECOND COLUMN REFERS TO THE ETCH DEPTH MEASURED BY ATOMIC FORCE MICROSCOPY. THE ERROR BARS ON THE EXPERIMENTAL DATA RESULT FROM REPEATED EXPERIMENTS [18].

Sample	Etch depth (µm)	Bioreactor Insertion Losses (dB)					
		AIR (n = 1)		2 vol H_2O + 1 vol Glycerol (n = 1.37)		Glycerol (n = 1.47)	
		Measured	Simulated	Measured	Simulated	Measured	Simulated
TV1	1.4 ± 0.2	no signal	34.0	17.9 ± 5.3	20.4	13.4 ± 2.7	11.2
TV2	0.8 ± 0.2	0 ± 1	0.0	0 ± 1	0.0	0 ± 1	0.0
TV3	1.0 ± 0.2	6.9 ± 2.1	8.8	3.4 ± 2.0	4.0	2.5 ± 1.0	1.7

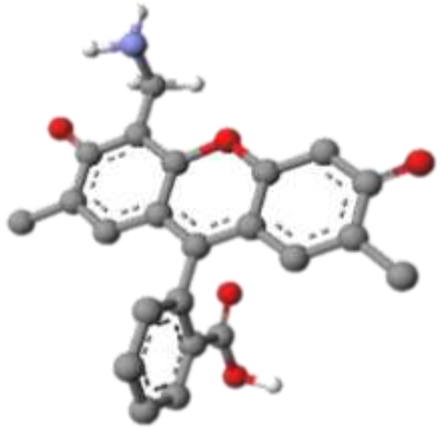


(a) air (n = 1), (b) dilute water/glycerol solution (estimated n = 1.37) and (c) glycerol (n = 1.47).

Modeling the bioreactors

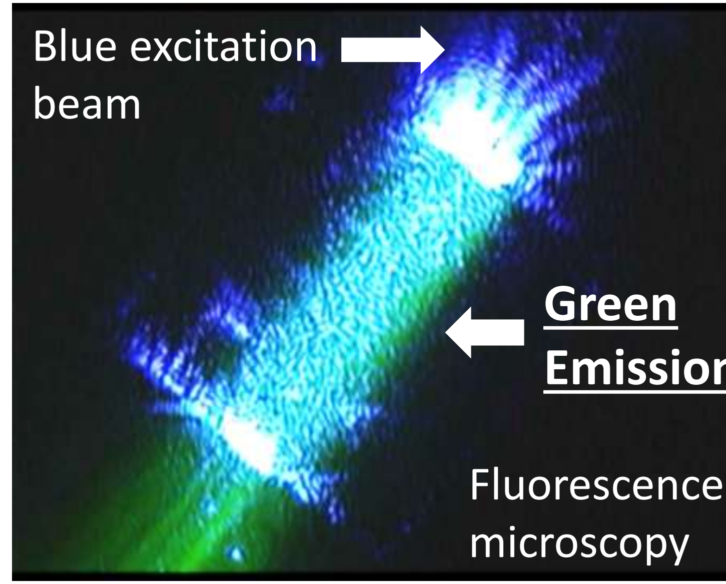


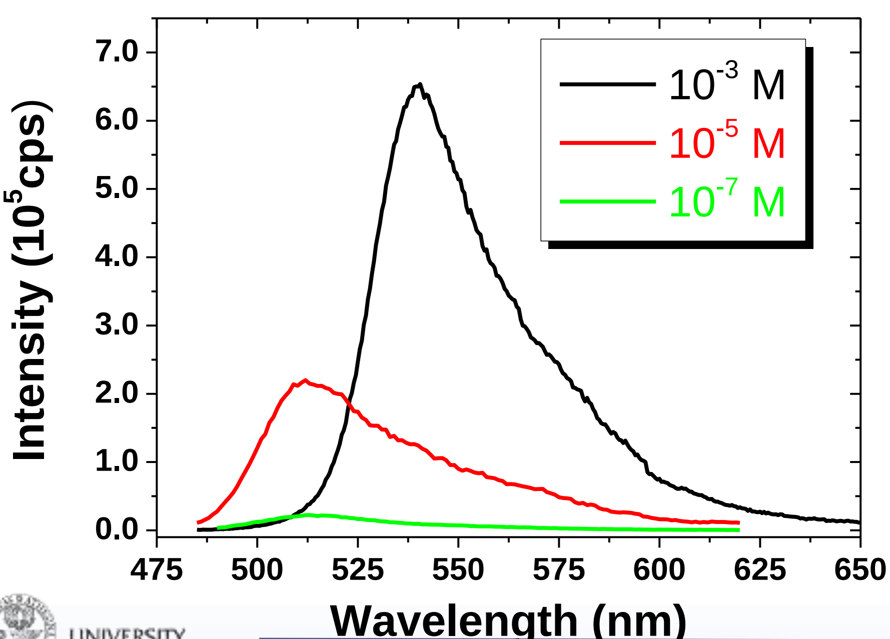
DETECTION OF A LUMINESCENT DYE IN SOLUTION

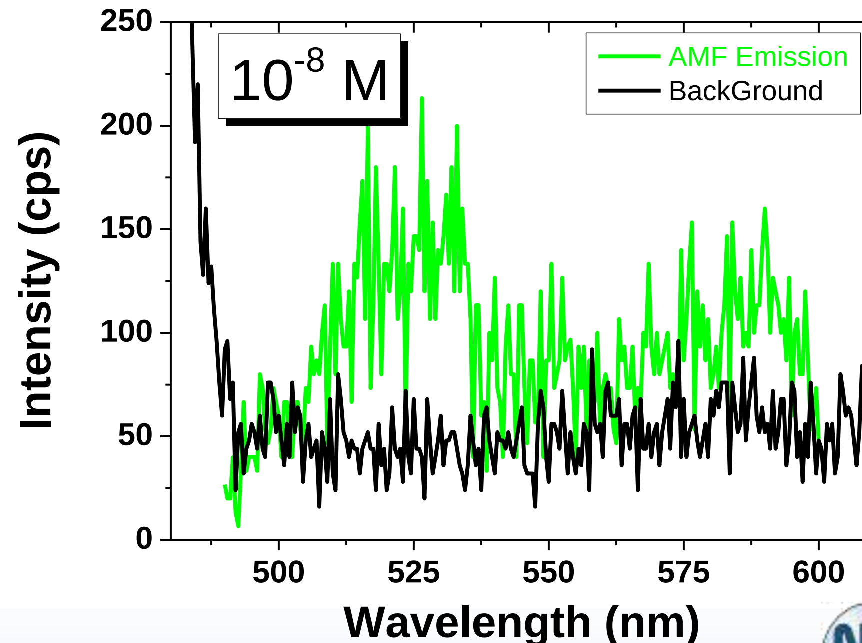


4'-(aminomethyl)fluorescein hydrochloride **(AMF)**

Bioreactor filled with a AMF drop







outline

- Nanosilicon nanoPhotonics
- Silicon nanocrystals as chromophore
- Naomi test vehicle: contact
- Polarimetric sensor based on porous silicon membranes
- Integrated waveguide for marked protein detection
- Wedge microdisk resonator for label free biosensors
- Conclusions

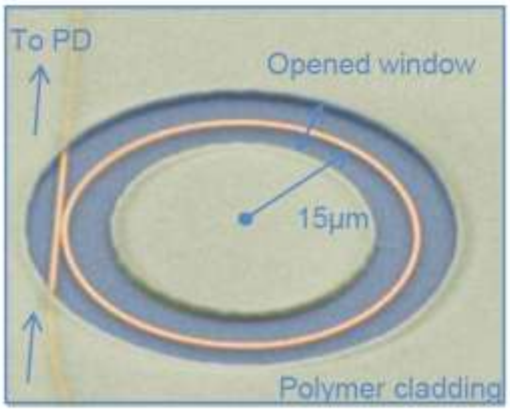
Alternative approach

LABEL FREE APPROACH

Principal of Operation

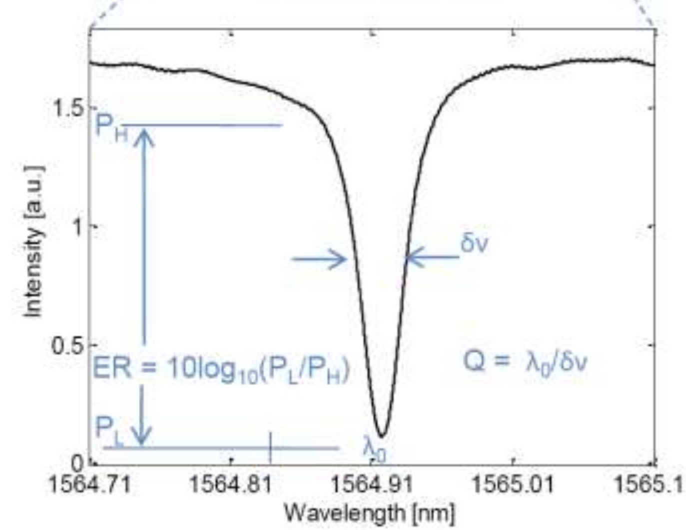
Swept wavelength light source

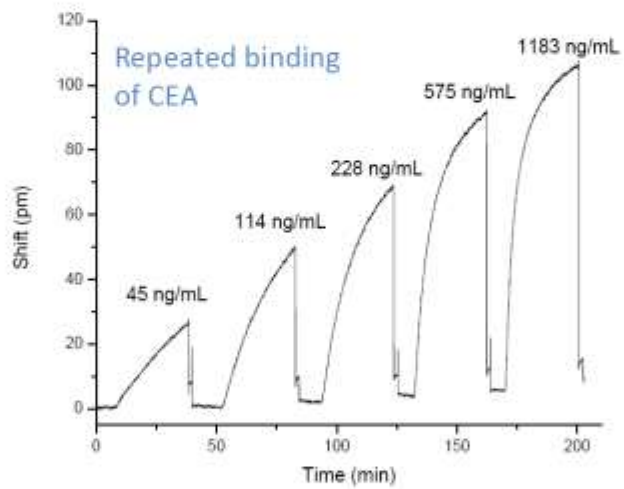
To detector



Light "resonates" in ring when the number of wavelengths around the circumference is EXACTLY an integer

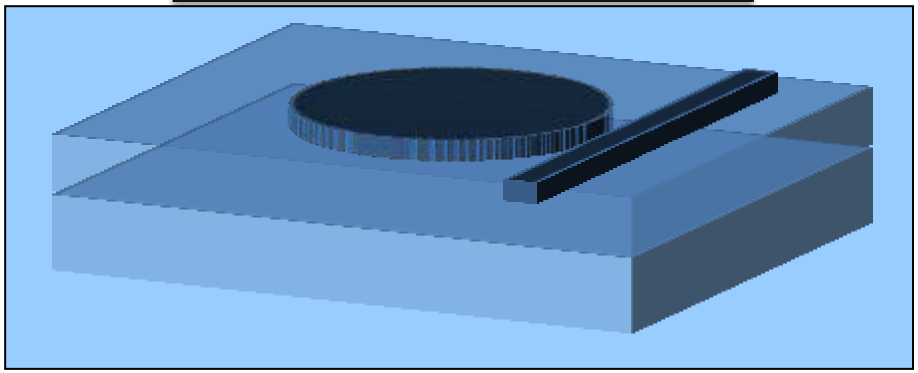
Resonant wavelength strongly attenuated at detector

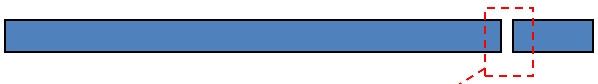




Resonator coupling configuration

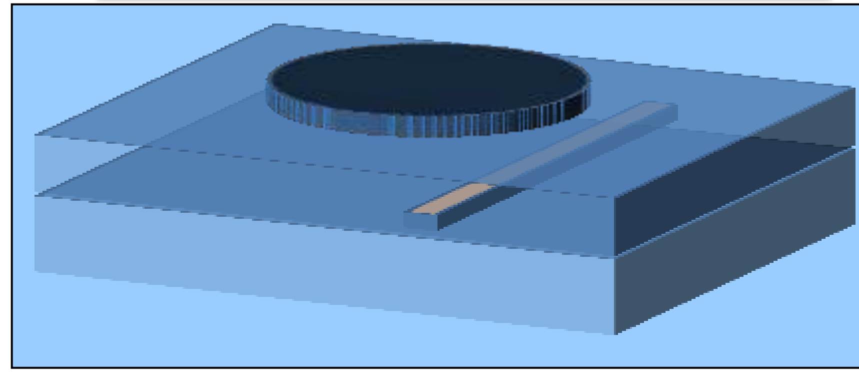
In-plane coupling





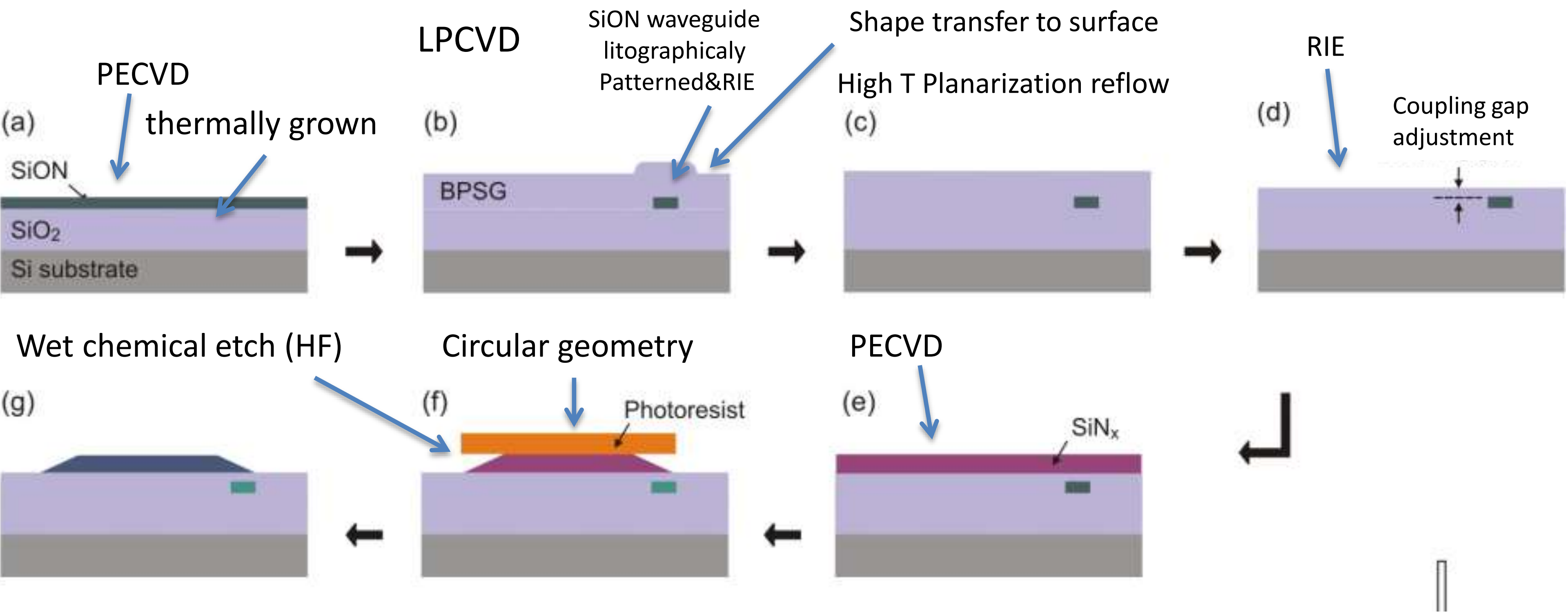
1. Requires reduced coupling-gap (~100nm)
2. Gap defined through E-beam or deep-UV Litho
3. A <u>1-mask process</u> imposes equal waveguide and resonator thicknesses, because of a single deposition
4. A <u>1-mask process</u> imposes the same material for both the waveguide and the resonator

Vertical (bus) coupling



1. <u>nm-controlled</u> gap defined through deposition, use of conventional optical Lithography
2. A <u>2-mask process</u> allows for independent waveguide and resonator thicknesses, multiple depositions
3. A 2-<u>mask process</u> allows for use of different materials for the waveguide and the resonator

Fabrication process



Depending on the gap (NIR or VIS)

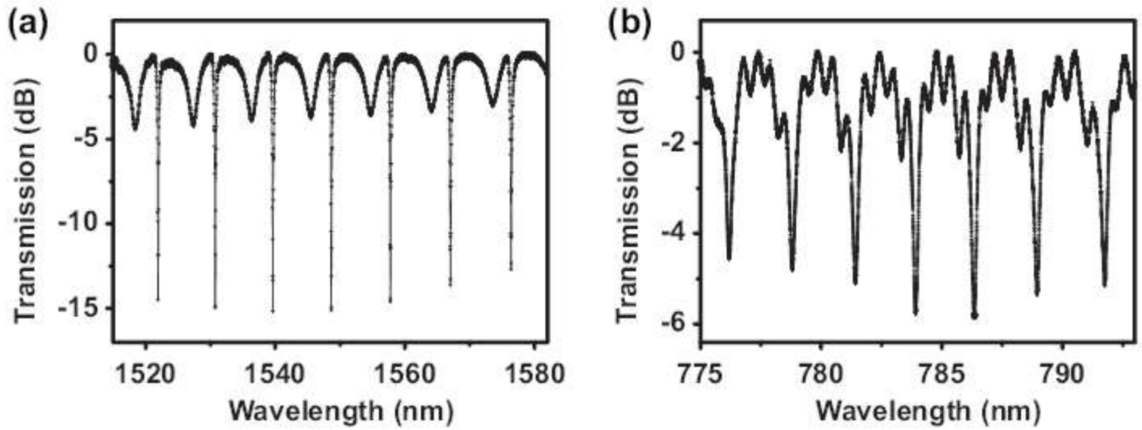
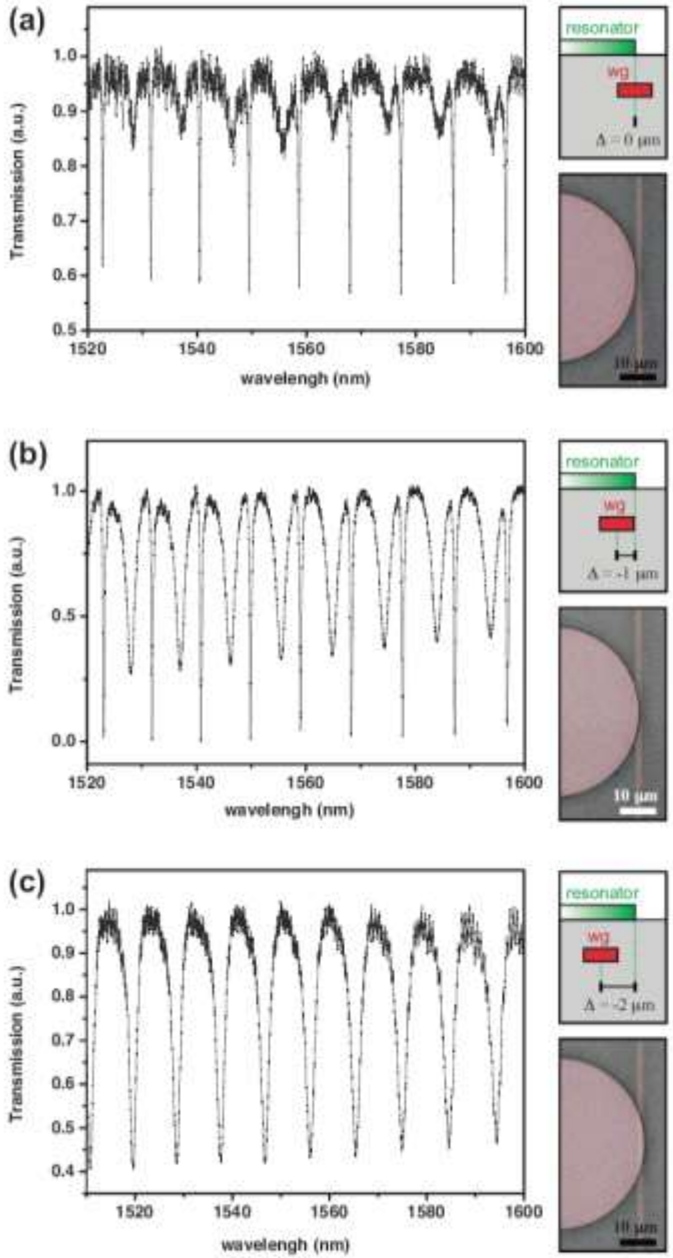
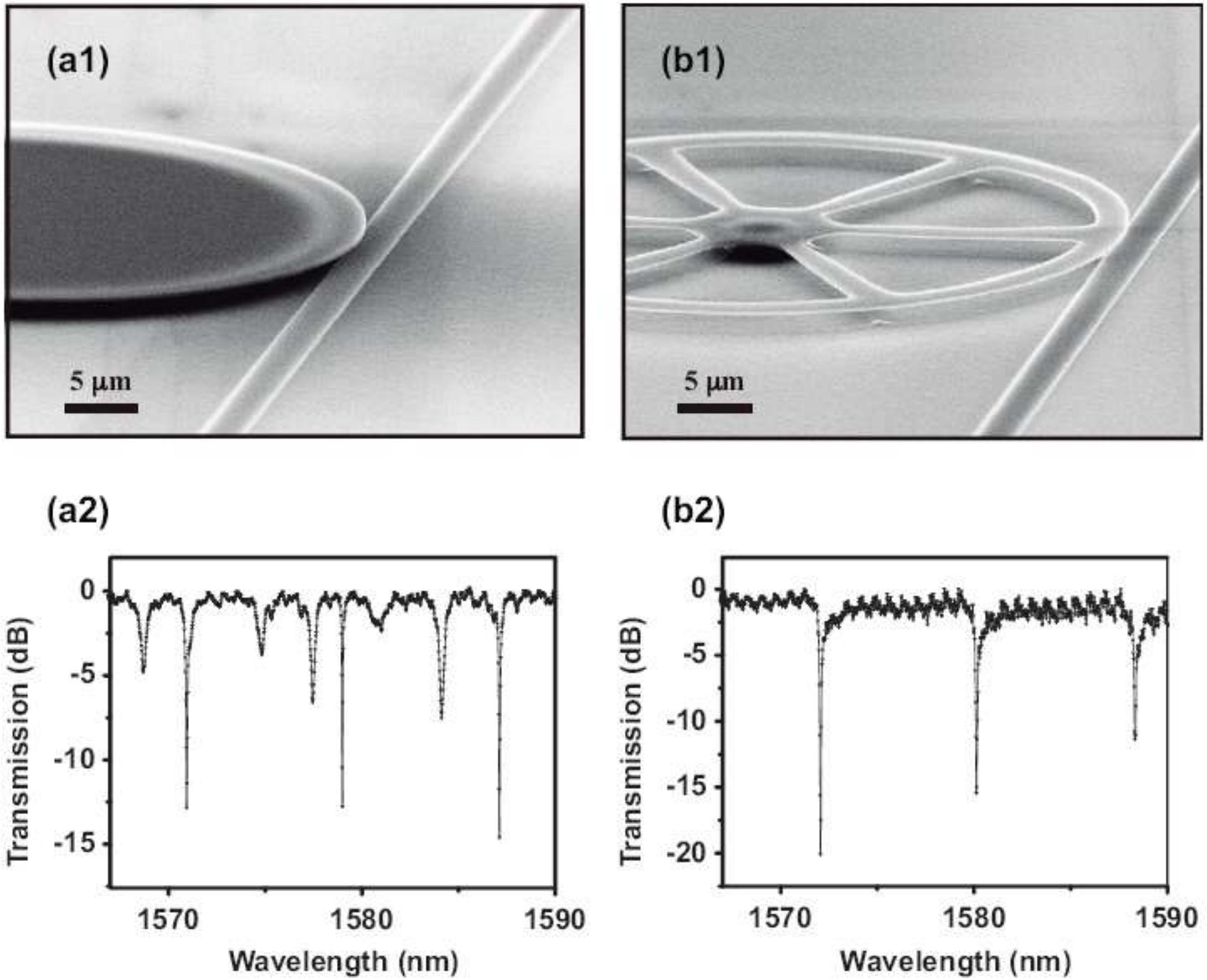


Figure 2. Normalized waveguide transmission spectra of vertically coupled SRO disk resonators at IR (a) and visible (b) wavelengths with 700 nm and 300 nm vertical gaps, respectively.

Mode selection



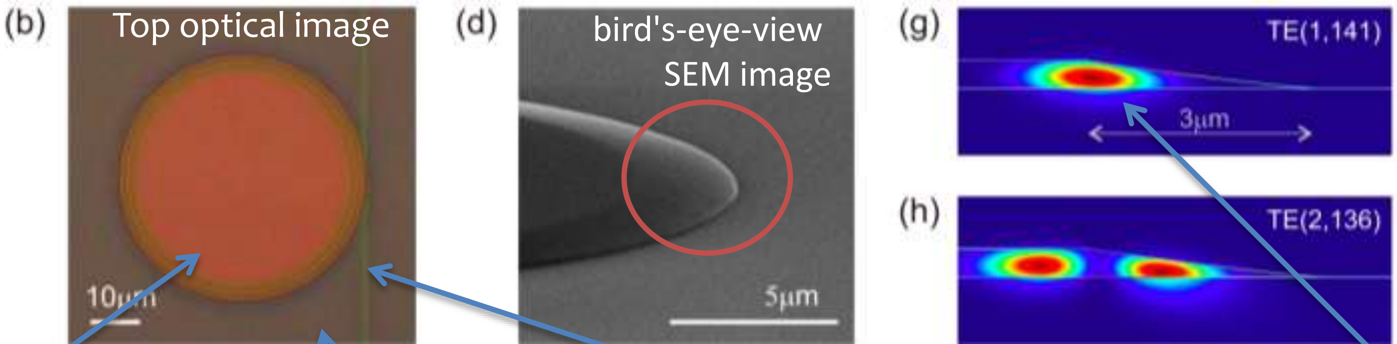
Free standing disk or ring



On-chip wedge WGM resonator

Vertically waveguide coupled Wedge resonator

Calculated Mode intensity profiles



1[st] radial family

2[nd] radial family

SiNx wedge resonator

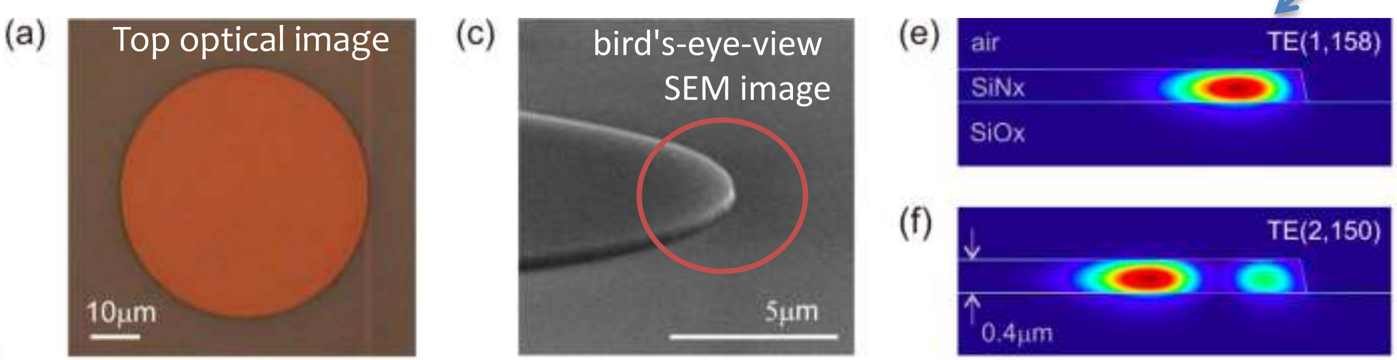
Buried waveguide

BPSG Cladding

Different shape of the borders

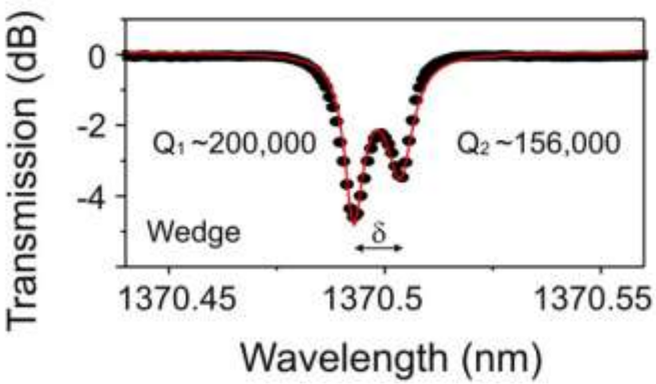
Different position of the mode profiles

Comparison with conventional disk resonators



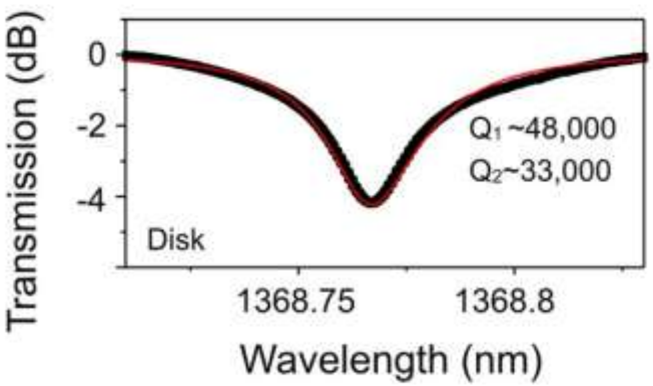
Q factor analysis

Q-factor could be extracted from the lorentzian fit of the transmission spectrum dips → $$Q \approx \frac{\lambda(n,m)}{FWHM}$$



FOR THE WEDGE RESONATOR

- The mode is split into a doublet because of the scattering-induced coupling between clockwise and counter-clockwise modes

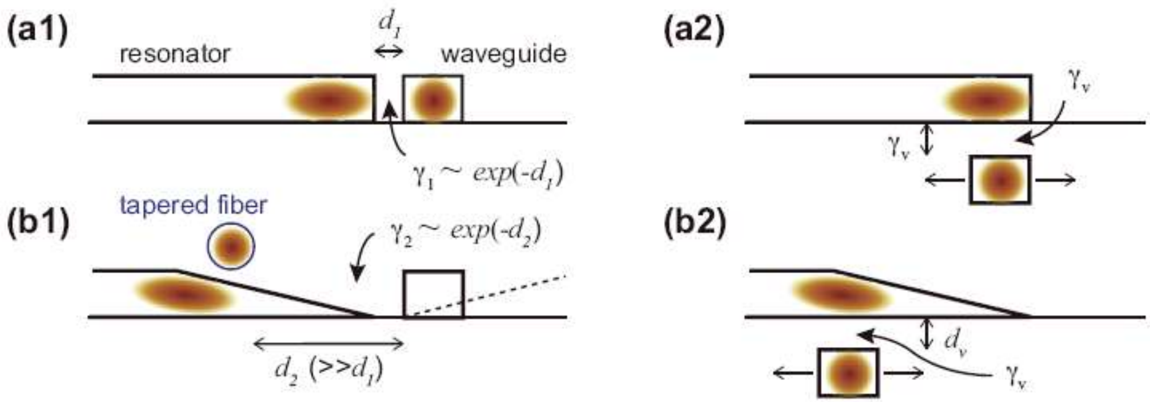


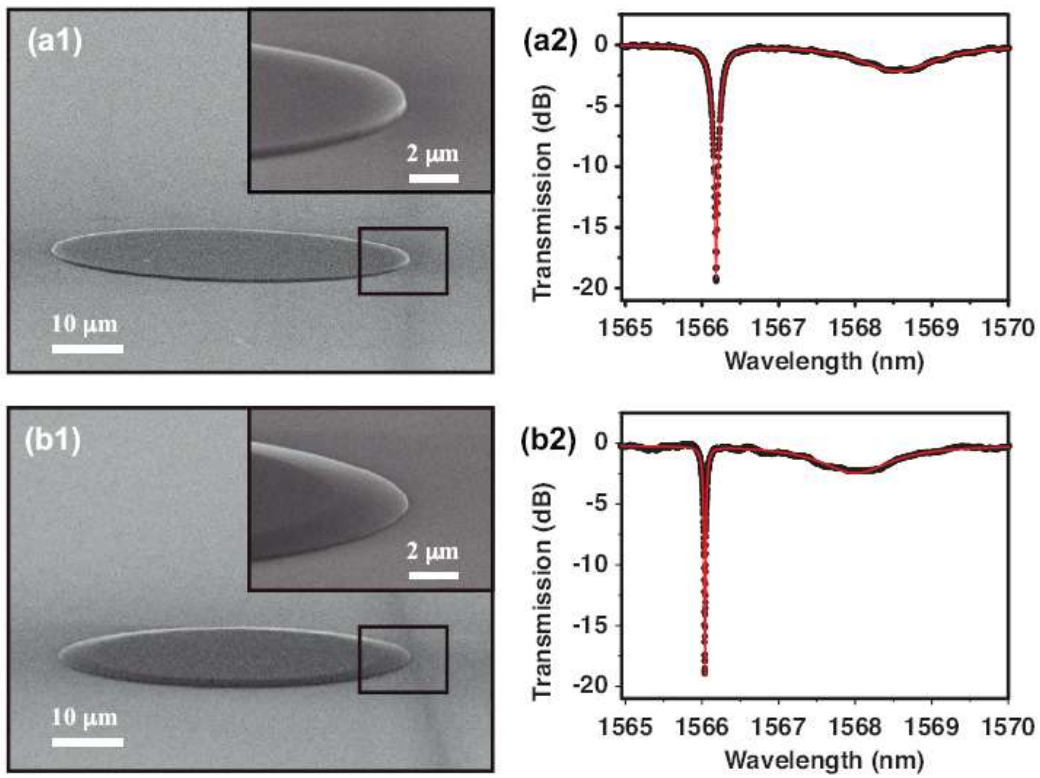
FOR THE DISK RESONATOR

- The coherent sum of lorentzians hides the splitting of the modes

wedge resonator shows 5 times larger Q-value due to the reduced scattering losses

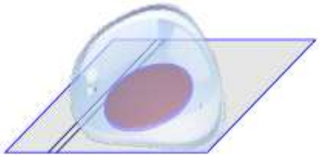
Wedge vs. Sharp edges

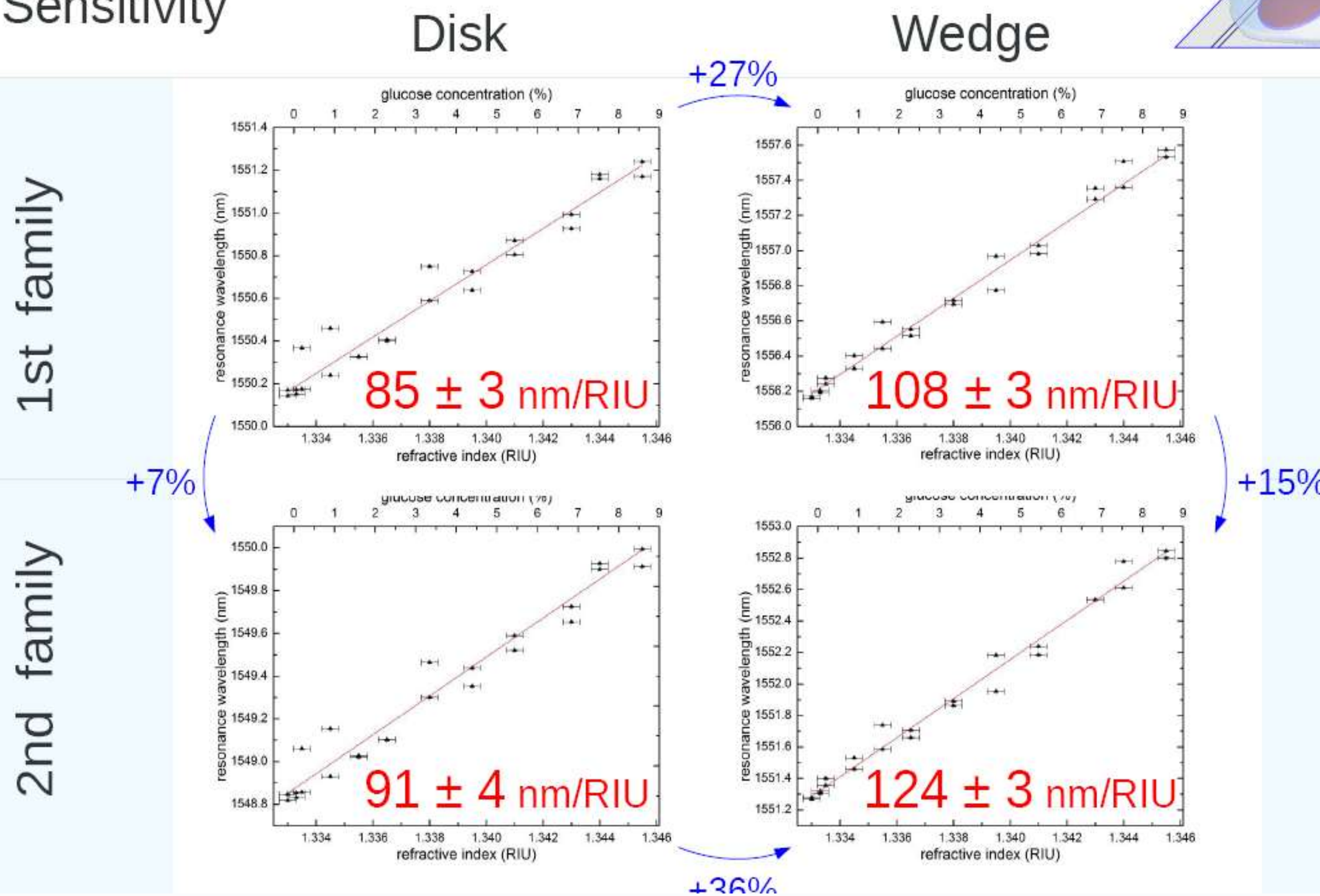




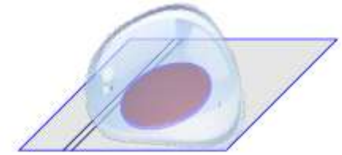
Enhancing the sensitivity

Sensitivity





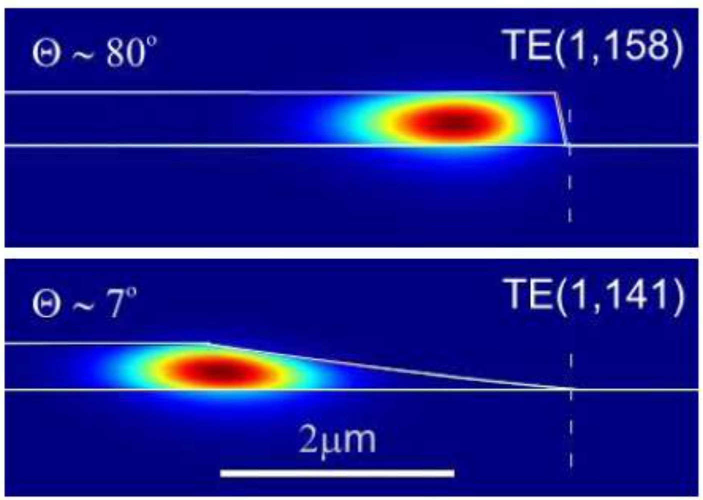
Enhancing the sensitivity

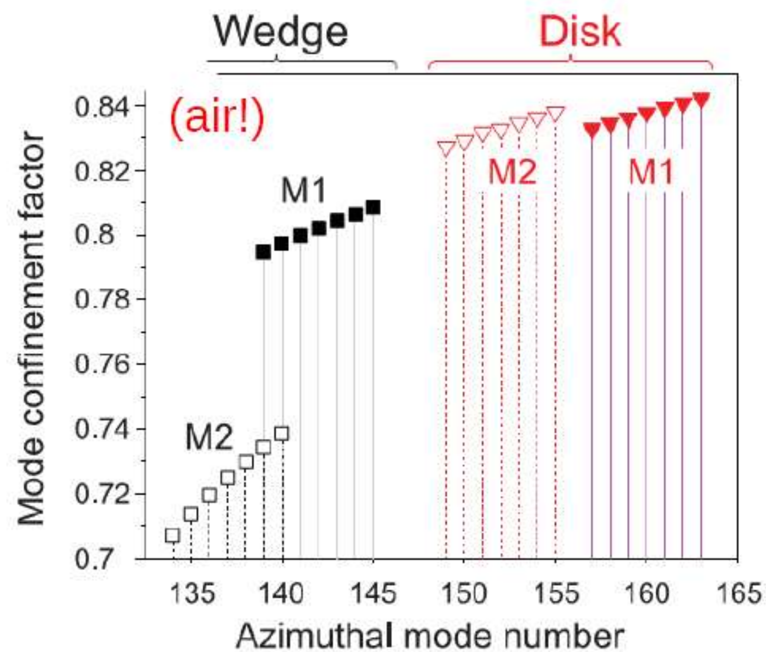


Sensitivity:

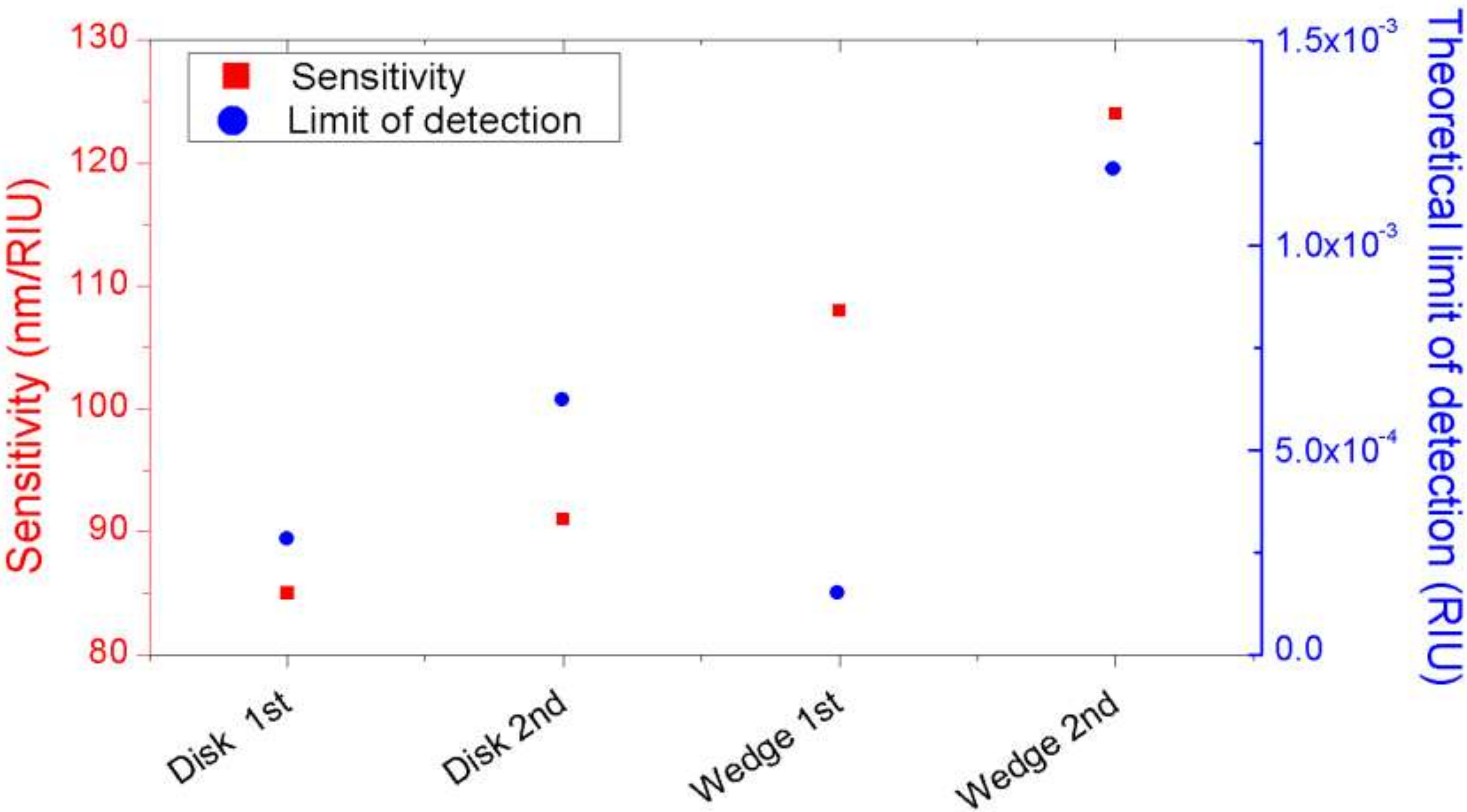
- Wedge generally better than disk
- Second family better mainly for wedge

  └→ Confinement factor





Preformance comparison

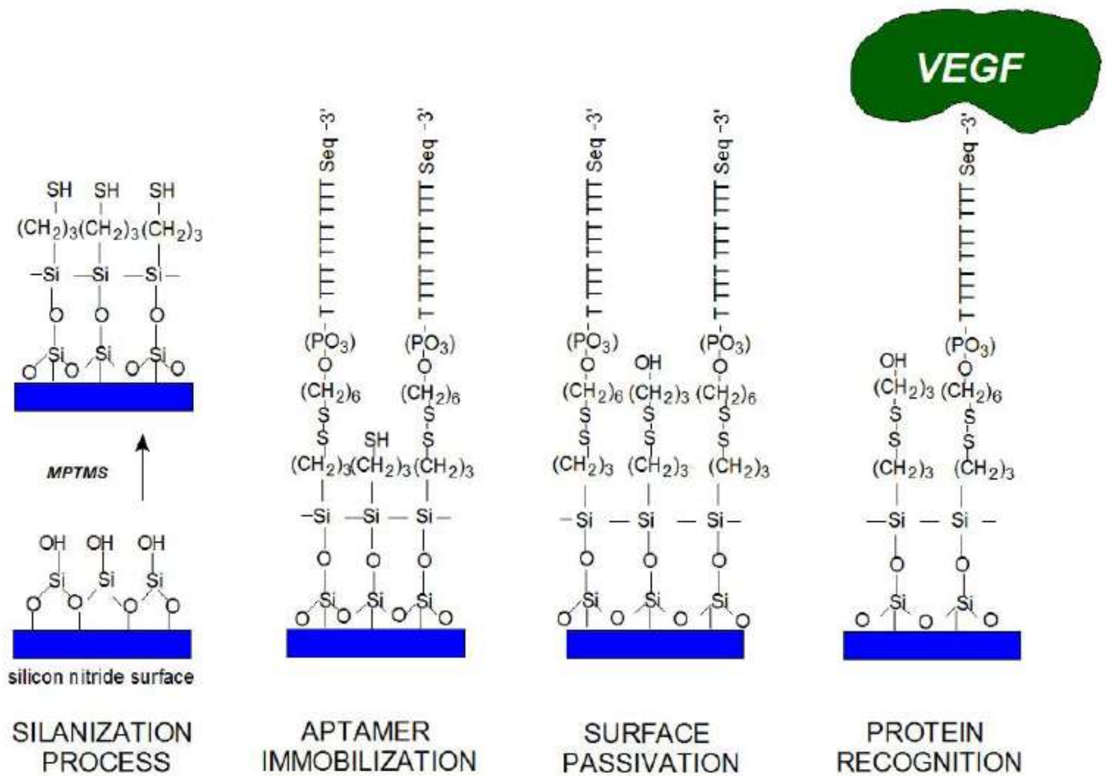


Protein recongnition test

Target: vascular endothelial growth factor (VEGF)

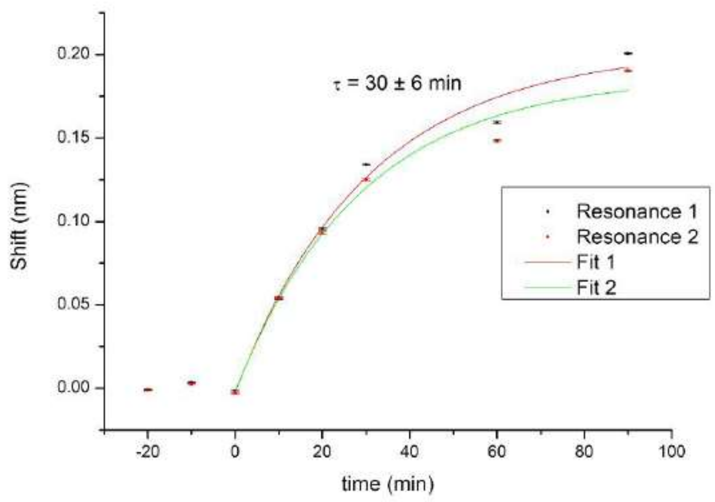
Specific detection with aptamers

└→ Surface functionalization step needed

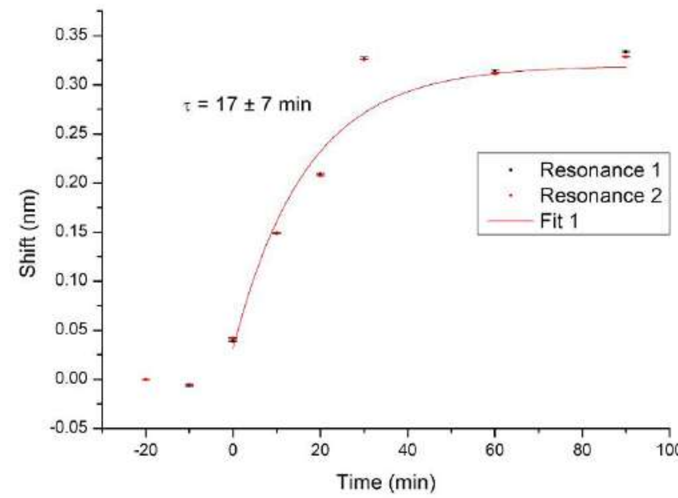


Protein recongnition test

Different kinetics for different concentrations



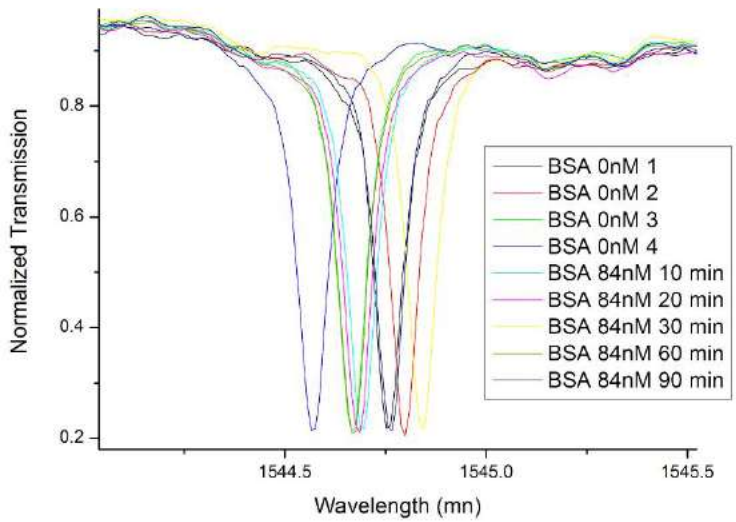
84 nM



211 nM

Protein recongnition test

Aspecific sensing using Bovine Serum Albumin (BSA)



Shifts are present but no dependance with incubation time

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Conclusions

- Silicon photonics for biosensing
  - Mass manufacturing
  - Low cost
  - High versatile
- Nanosilicon nanophotonics
  - Many different platforms
  - Many different sensing schemes
- Open issues is not the photonics
  - Biofunctionalization
  - microfluidics

Conclusions

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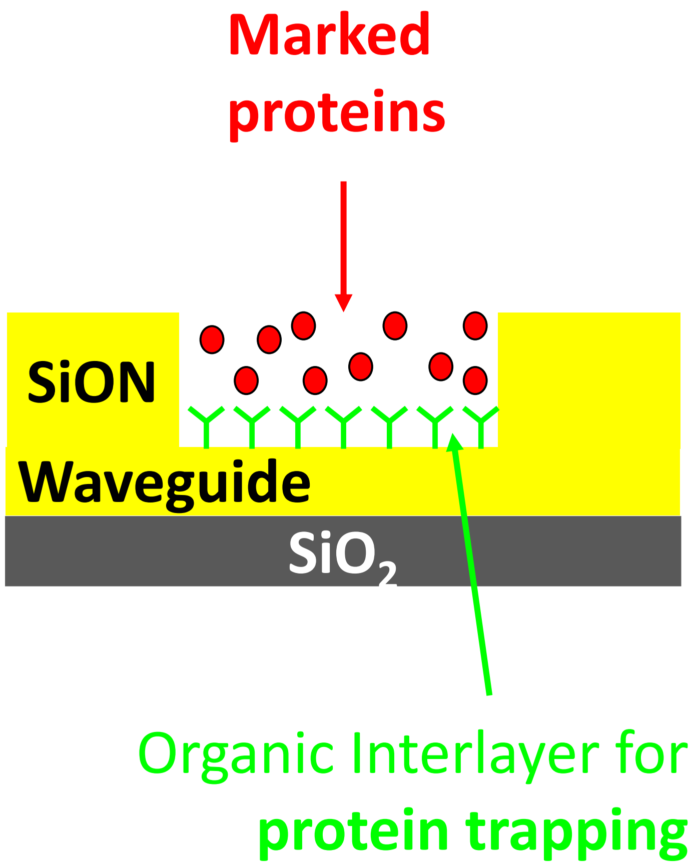
Acknowledgments

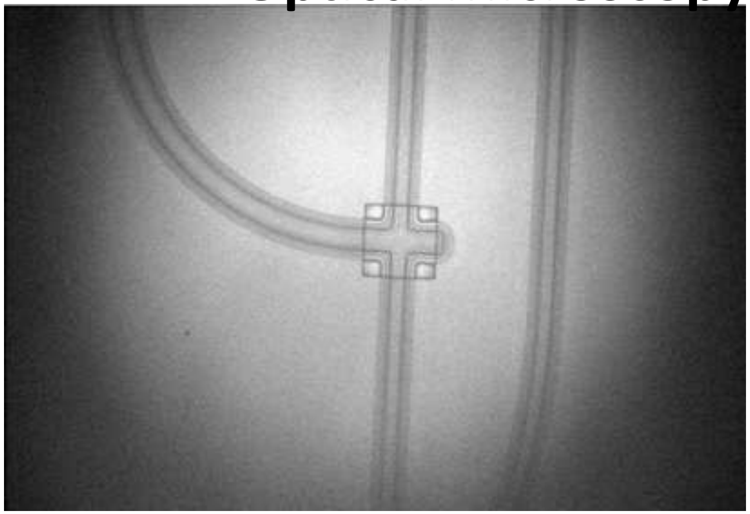
We are hiring on biosensing, nanophotonics and integrated quantum :

- Assistant professors
- Post docs
- Ph students
    - Email me your CV lorenzo.pavesi@unitn.it

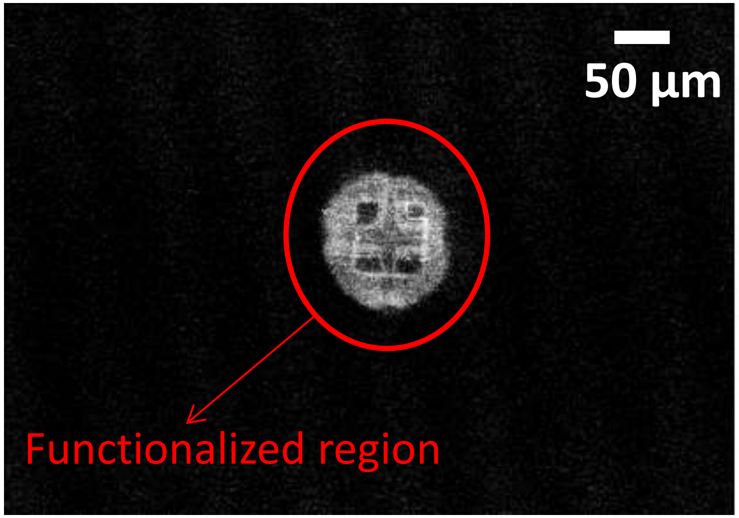
DYE IMMOBILIZATION



Optical Microscopy



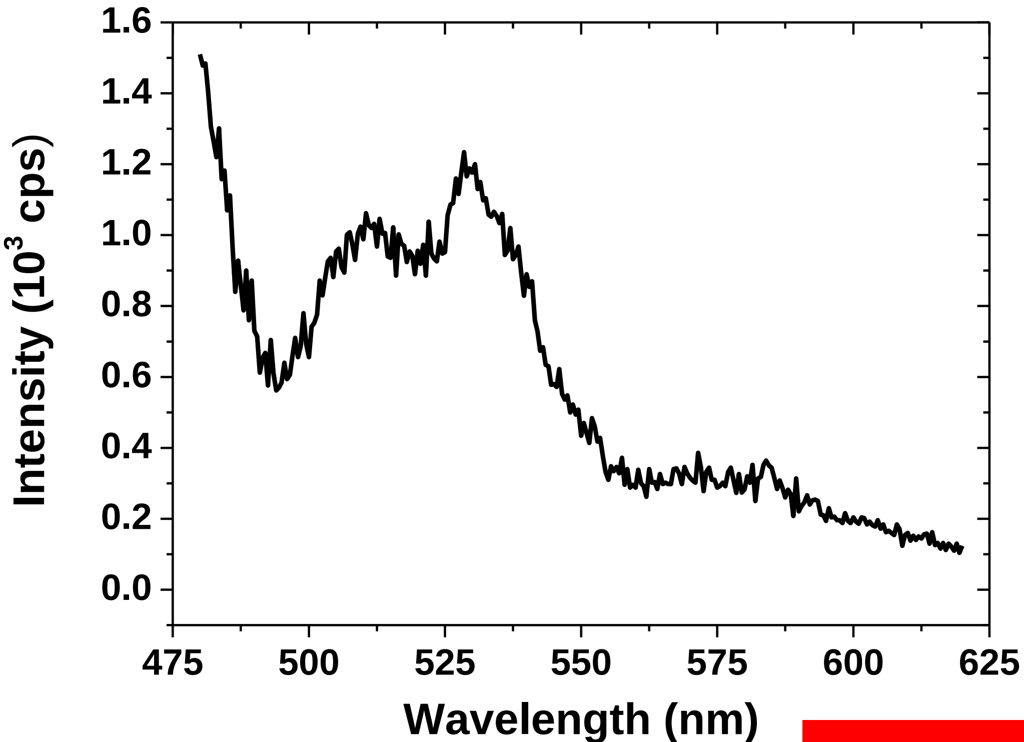
Fluorescence Microscopy.

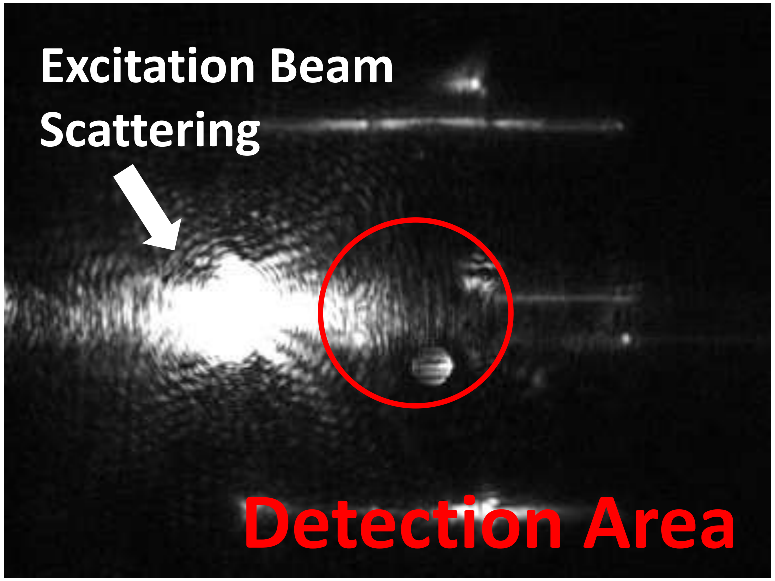


The luminescent markers remain just in the functionalized regions.

DETECTION OF IMMOBILIZED DYE MOLECULES

Surface concentration 10^{-12} Moles/cm^2

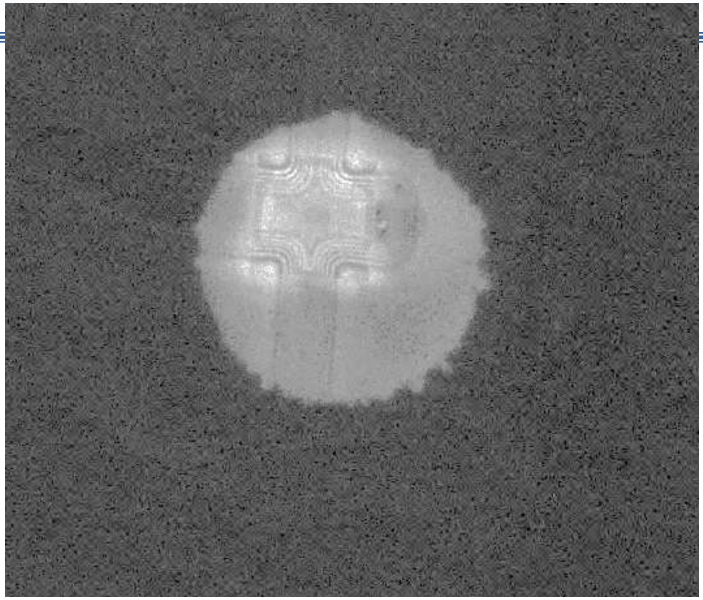




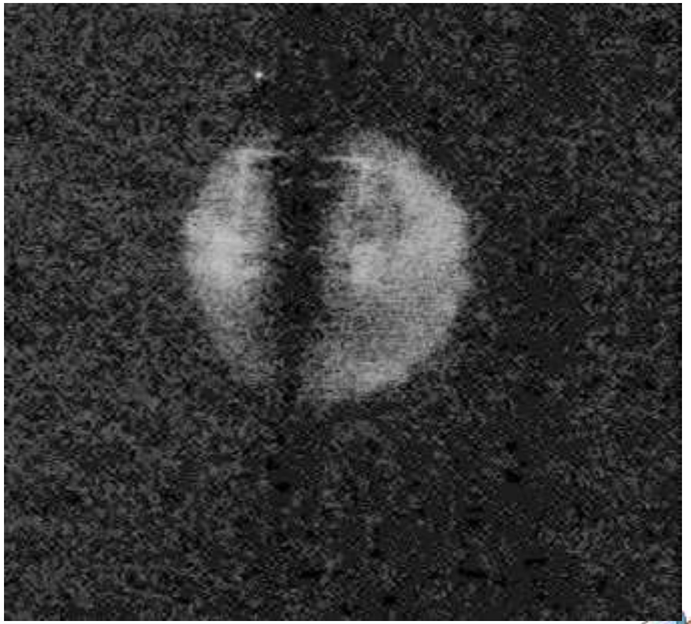
Limit of detection 10^{-17} Moles

Experiments conducted after bioreactor washing and in dry conditions

Photobleaching of the dye layer by the excitation beam transmitted along the waveguide



Before dye immobilization



After detection experiments

The photodegradation process damages much more efficiently the dye molecules near to the waveguide