



**The Abdus Salam
International Centre for Theoretical Physics**



2328-16

**Preparatory School to the Winter College on Optics and the Winter College on
Optics: Advances in Nano-Optics and Plasmonics**

30 January - 17 February, 2012

Applications to bio-sensing

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BLOCH SURFACE WAVES ON PHOTONIC CRYSTALS

APPLICATIONS TO GAS SENSING AND BIOPHOTONICS



SAPIENZA Università di Roma

***Department of Basic and Applied Sciences for Engineering
Molecular Photonics Laboratory***

Francesco Michelotti

International Centre for Theoretical Physics, Trieste, February 2010



Lecture 4

**Applications of BSW to biophotonics
(Experimental)**



Applications of BSW to biophotonics

We applied the BSW concept to several experimental configurations in the field of biophotonics. Here we shall discuss the following results:

- Experimental determination of fluorescence enhancement by BSW in far and near field experiments.
- Theoretical predictions on the resolution and fluorescence enhancement in Standing Wave – BSW Resonance Microscopy
- Label-Free Biosensing with BSW and its application to the detection of cancer biomarkers



Many other configurations used in biophotonics can take advantage of the properties of BSW but we have not yet investigated them (SERS, SEIRA, TERS, S-TPA, S-SHG, ...)



Biophotonics

Experimental determination of fluorescence enhancement by BSW in far and near field experiments



Examples – Fluorescence Imaging

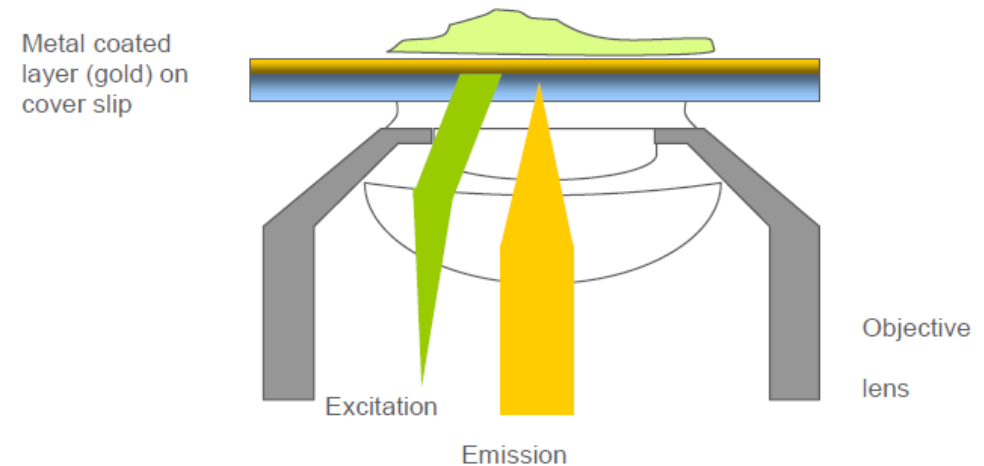
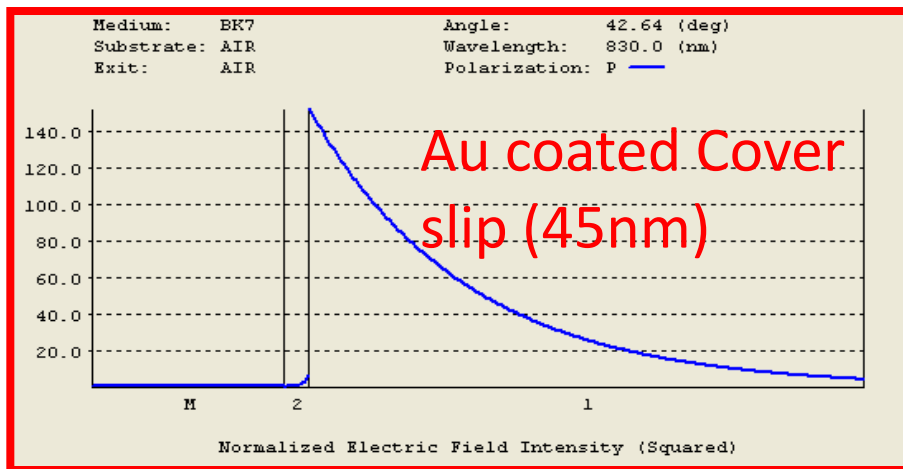
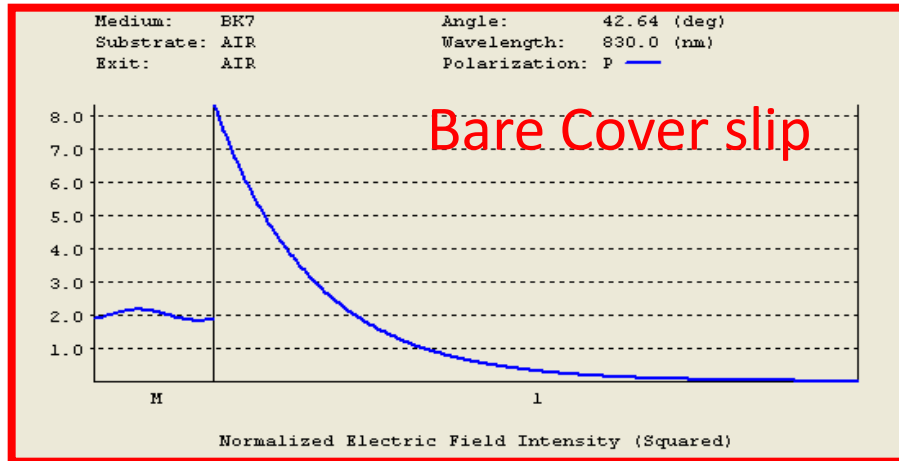
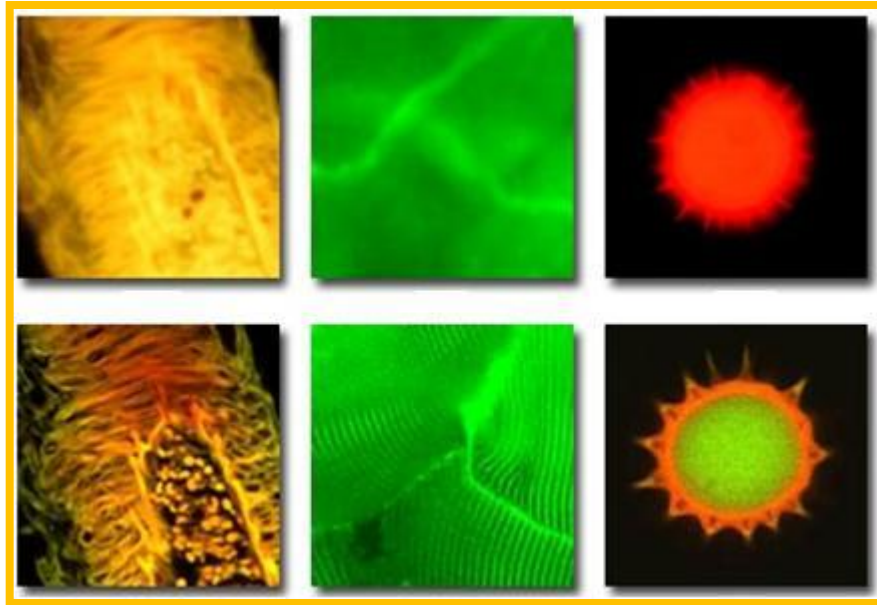


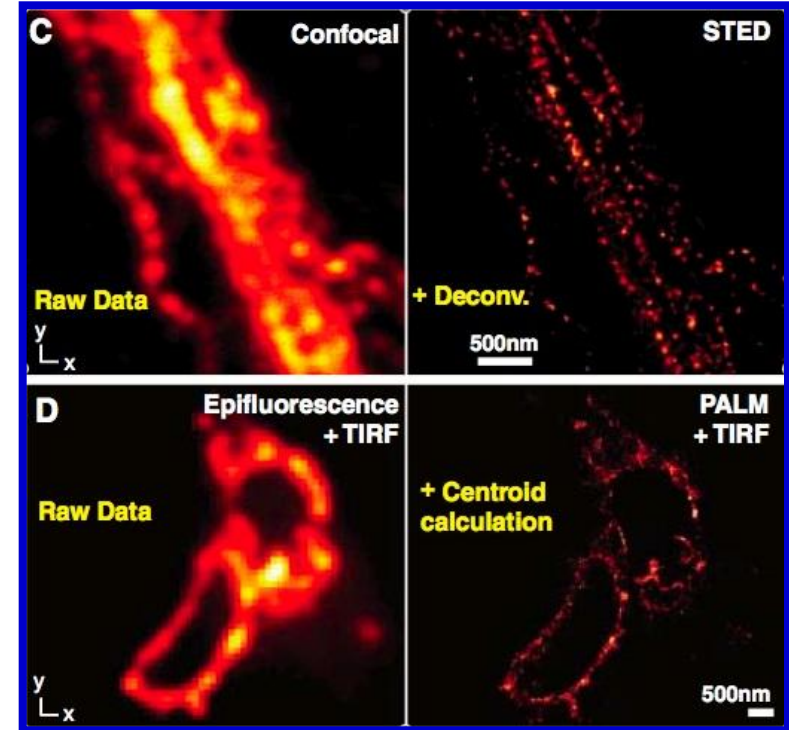
Figure 2-2. An objective-launched set-up for SPCE imaging.

Surface Plasmon Coupled Emission (SPCE) and Surface Plasmon Field-enhanced Fluorescence (SPFS)

Fluorescence microscopy



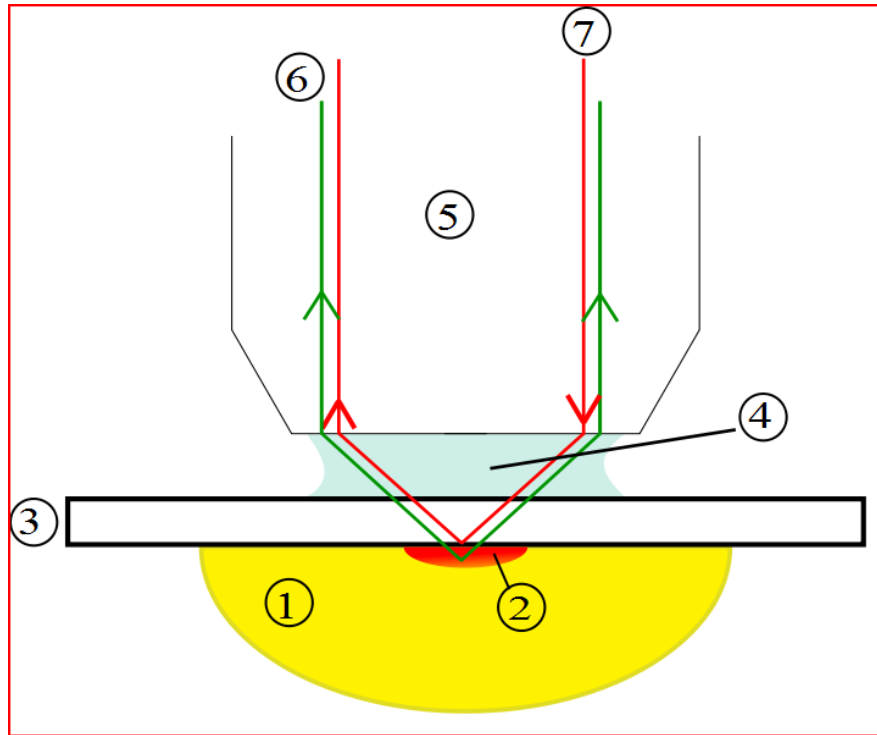
Wide Field Fluorescence Microscopy
VS
Confocal Fluorescence Microscopy



STED and PALM
VS
Confocal and TIRF Microscopies



Fluorescence microscopy



**Total internal reflection
fluorescence microscopy
(TIRFM)**

**J.R.Lakowicz
Principles of Fluorescence
Microscopy
3rd Edition
Chapter 26**

**Chapter 26 is the
LAST CHAPTER**

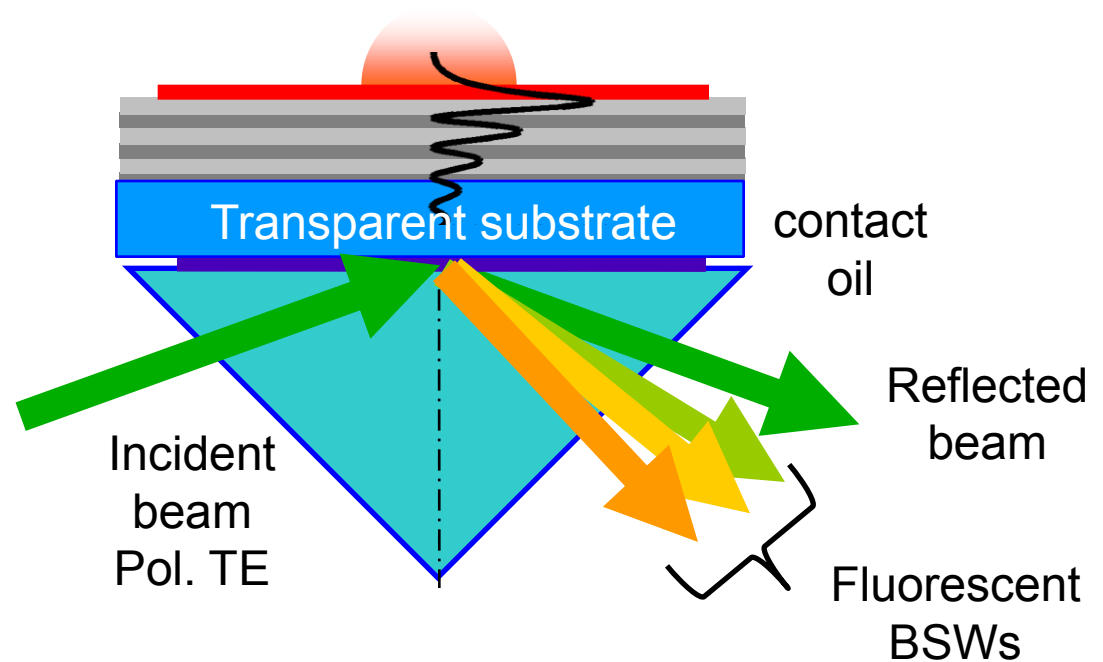
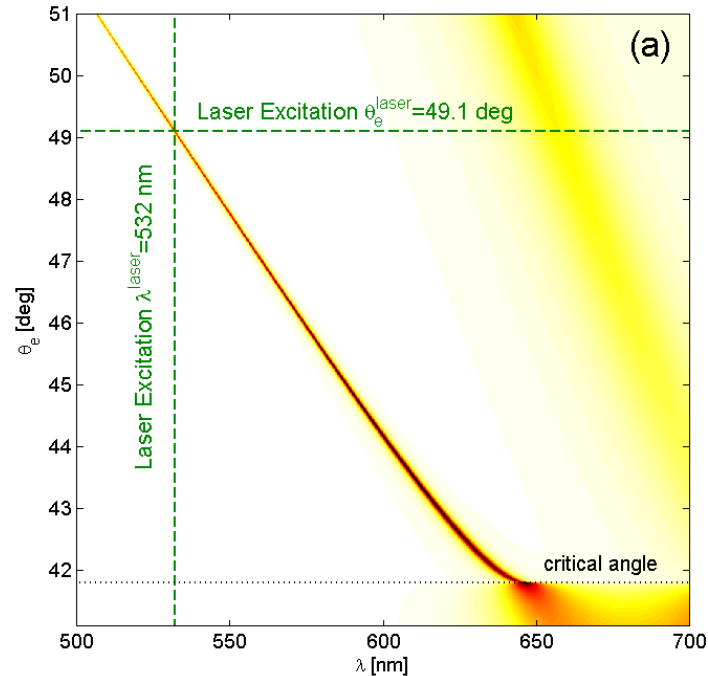


SPCE and SPCF Fluorescence with BSW (FF)

Far Field



SPCE and SPCF Fluorescence with BSW (FF)

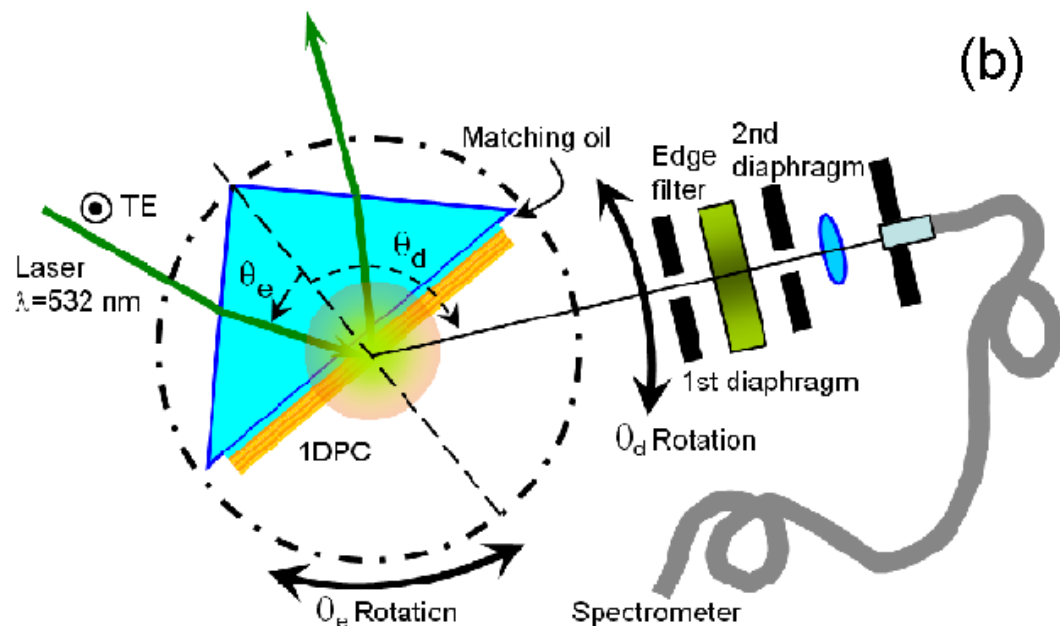
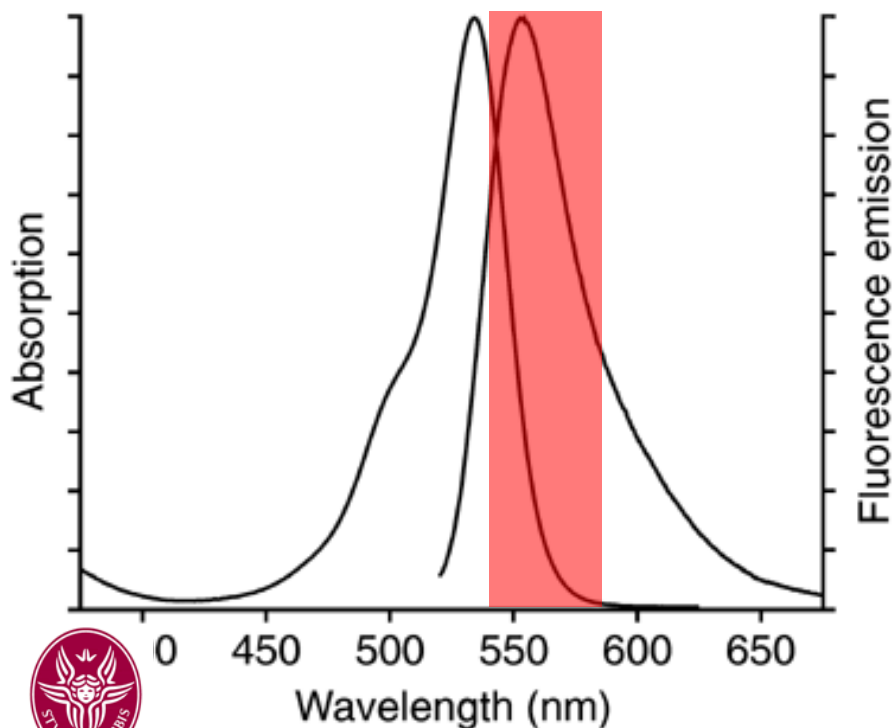


1DPC - Glass / (HL)⁷ L1 / P / Protein A(Alexa546) / air

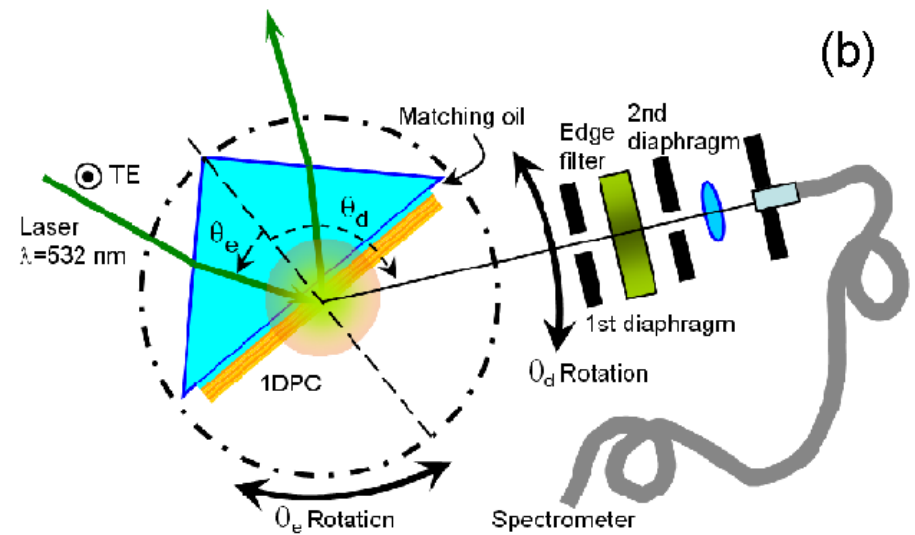
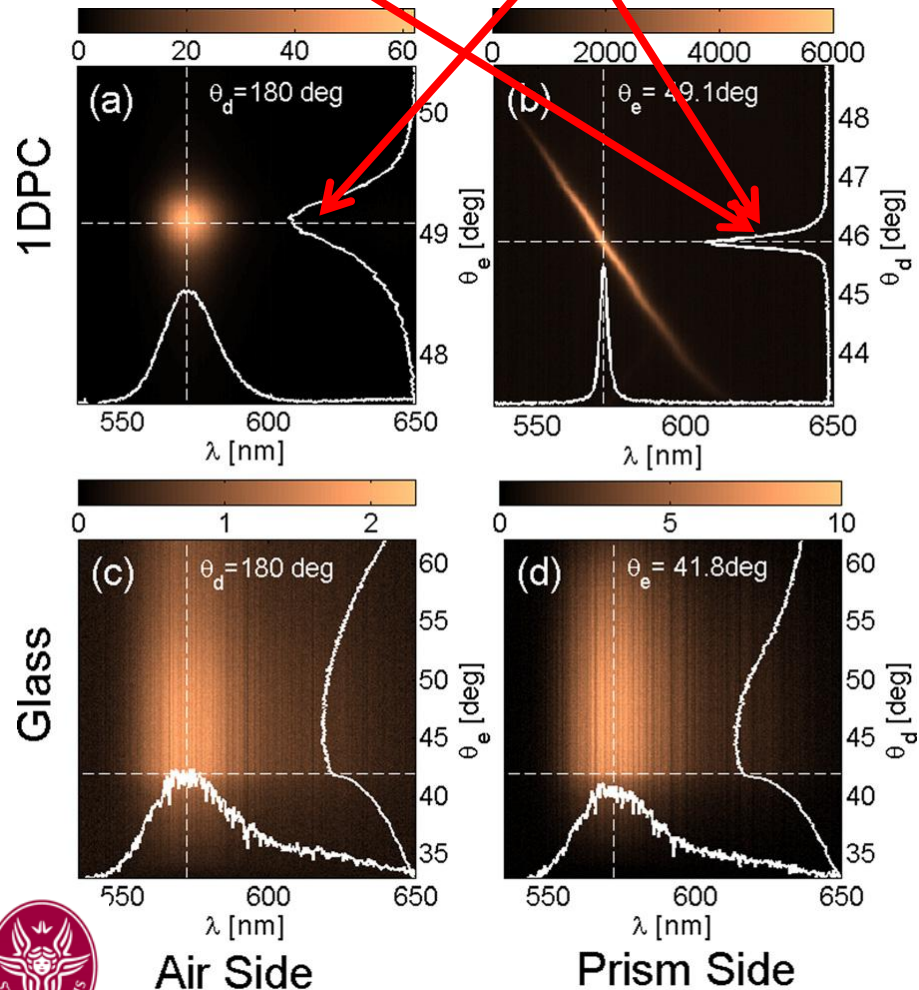
H	(silicon nitride)	$n_H = 1.99$	$t_H = 79 \text{ nm}$
L	(silicon dioxide)	$n_L = 1.48$	$t_L = 134 \text{ nm}$
L1	(silicon dioxide)	$n_{L1} = 1.48$	$t_{L1} = 28 \text{ nm}$
P	(PPAA polymer)	$n_P = 1.6$	$t_P = 30 \text{ nm}$

PPAA was decorated with Protein A labelled with Alexa Fluor 546

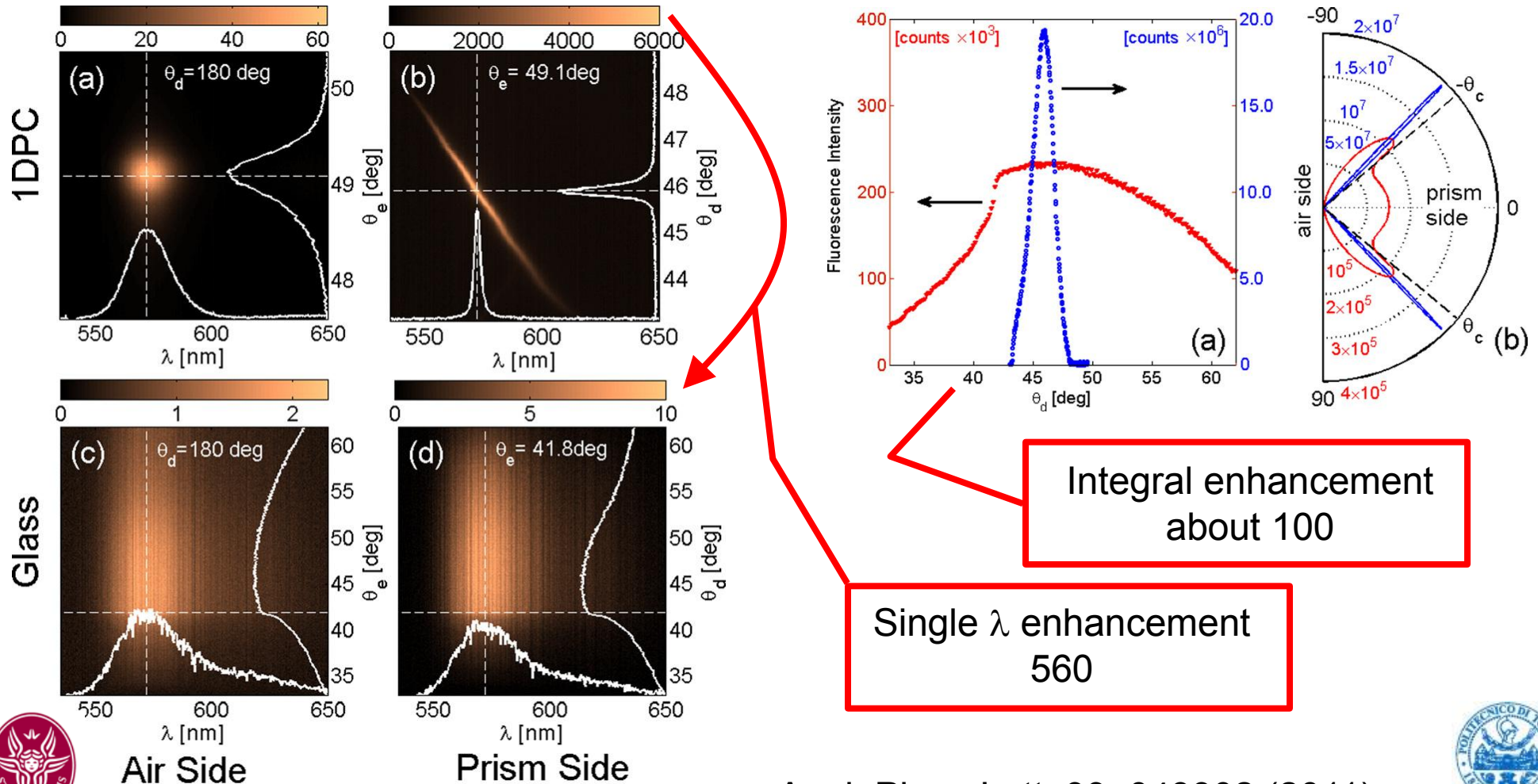
SPCE and SPCF Fluorescence with BSW (FF)



SPCE and SPCF Fluorescence with BSW (FF)



SPCE and SPCF Fluorescence with BSW (FF)



SPCE and SPCF Fluorescence with BSW (NF)

Near Field



SPCE and SPCF Fluorescence with BSW (NF)

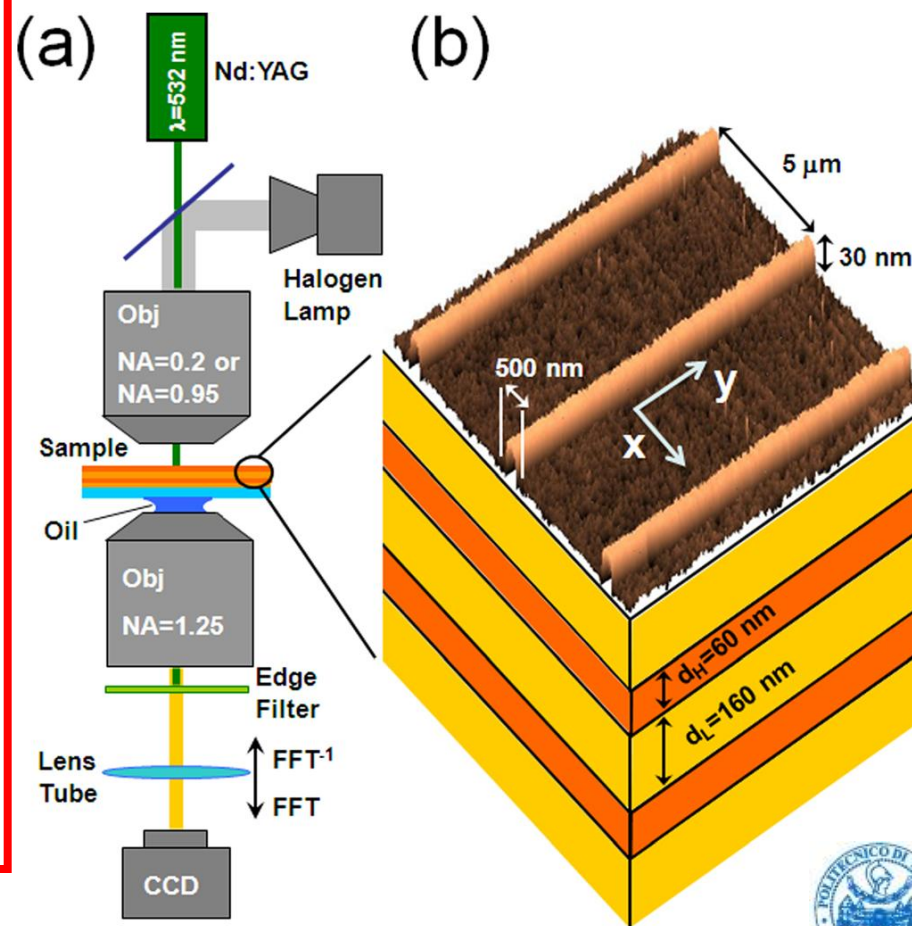
1DPC

170 μm cover slip / (HL)⁸ / P / PtA(AF546) / air

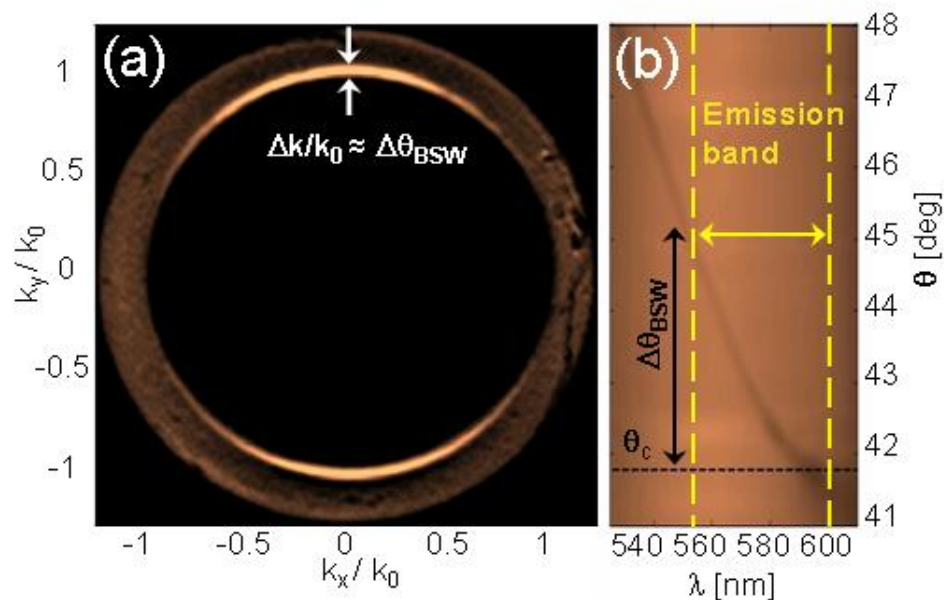
H	(silicon nitride)	$n_H=1.99$	$t_H=60\text{ nm}$
L	(silicon dioxide)	$n_L=1.48$	$t_L=160\text{ nm}$
P	(PPAA polymer)	$n_P=1.6$	$t_P=30\text{ nm}$

The PPAA polymer layer was patterned by means of standard photo-lithography to get a set of parallel ridges with:

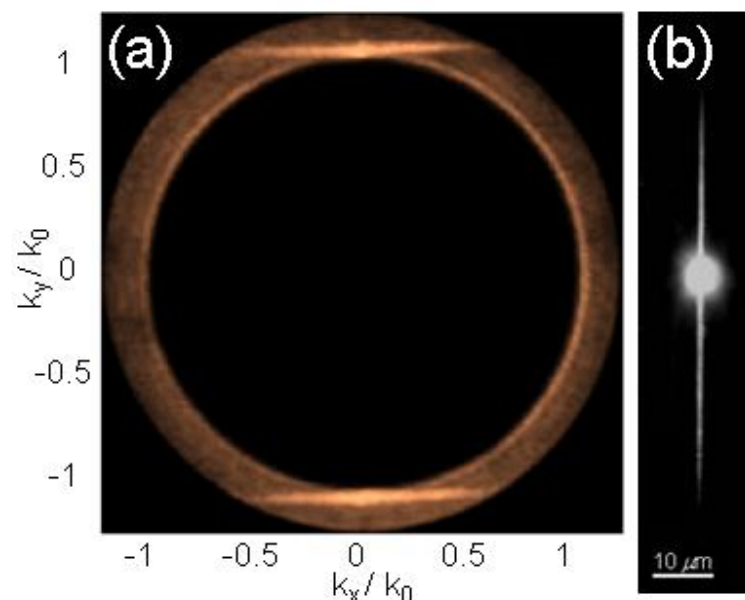
Thickness	30nm
Width	500nm
Separation	5 μm



SPCE and SPCF Fluorescence with BSW (NF)



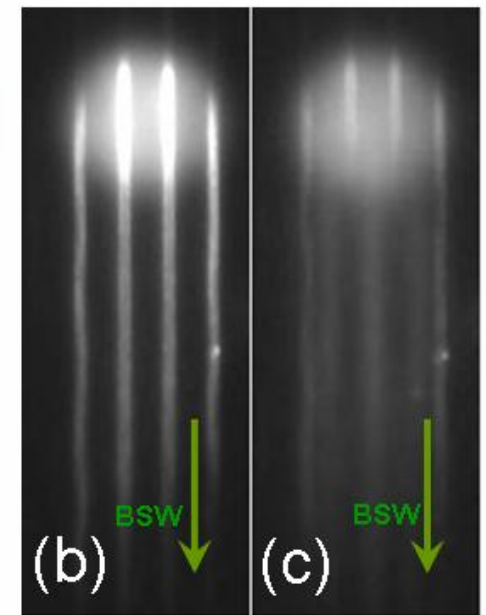
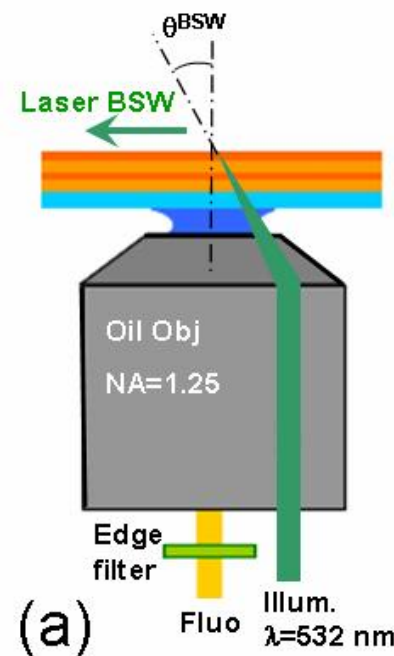
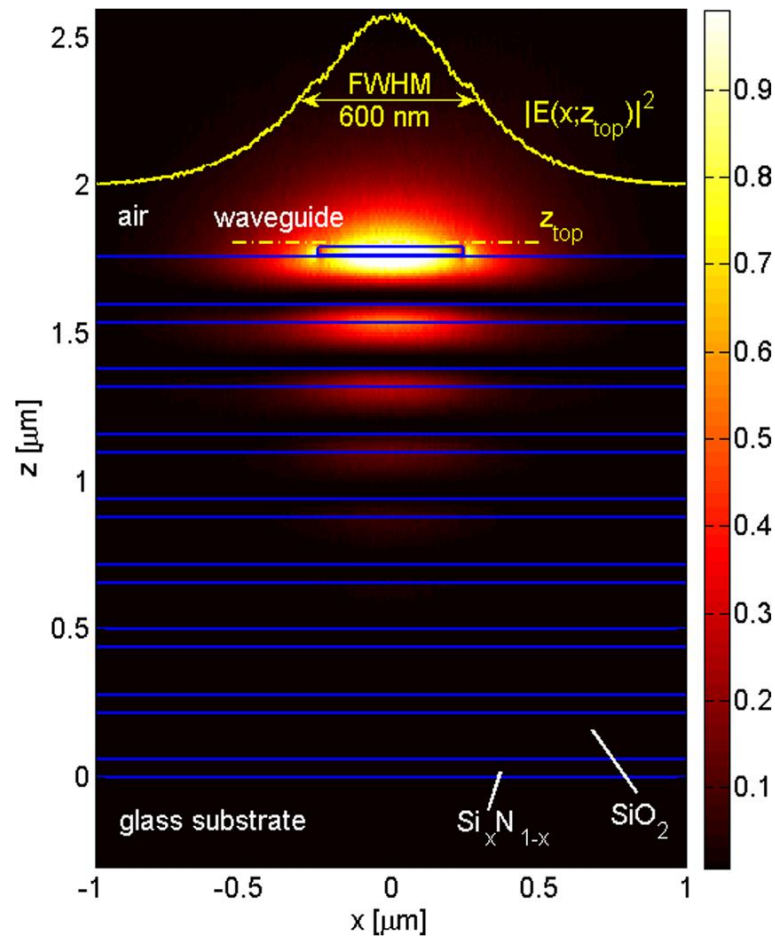
**Unpatterned
PPAA coated 1DPC**



**Patterned
PPAA coated 1DPC**



SPCE and SPCF Fluorescence with BSW (NF)



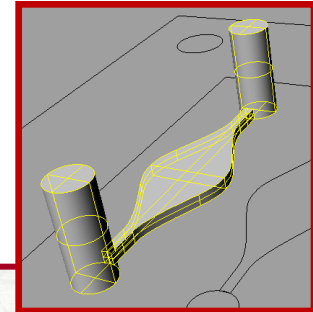
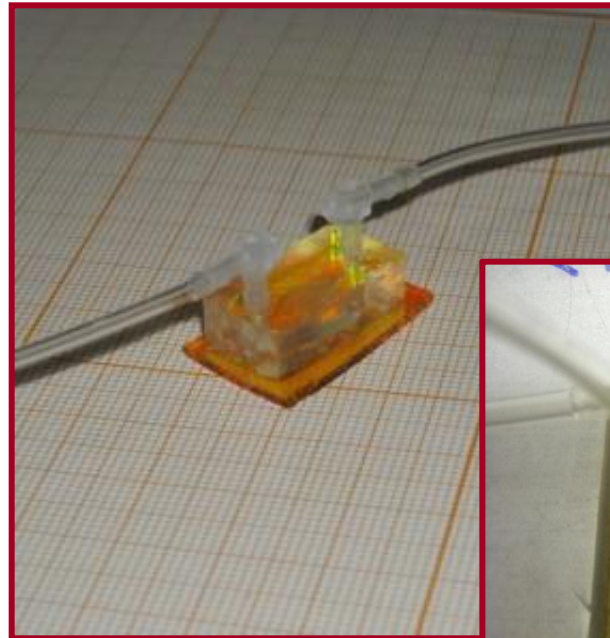
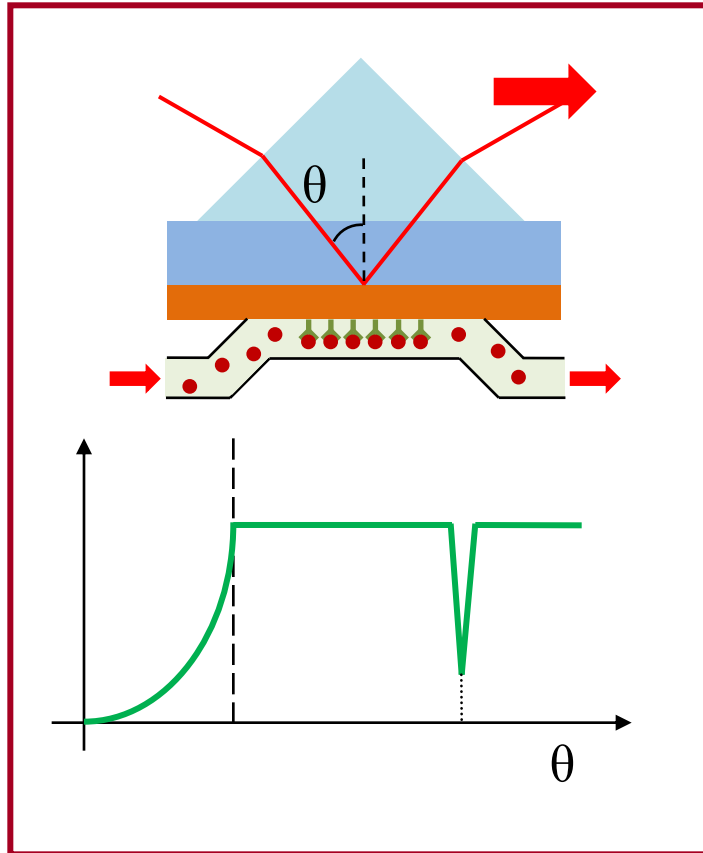


Biophotonics

Label-Free Biosensing with BSW and its application to the detection of cancer biomarkers



Label-Free Biosensing with BSW



PDMS Microfluidics



Label-Free Biosensing with BSW



Available online at www.sciencedirect.com



Sensors and Actuators B 105 (2005) 360–364



www.elsevier.com/locate/snb

Surface plasmon-like sensor based on surface electromagnetic waves in a photonic band-gap material

M. Shim, W.M. Robertson*

Department of Physics and Astronomy, Middle Tennessee State University, Murfreesboro, TN 37132 USA

Received 6 February 2004; accepted 16 June 2004

Available online 7 August 2004



Label-Free Biosensing with BSW

Anal. Chem. **2007**, *79*, 4729–4735

Photonic Crystal Surface Waves for Optical Biosensors

Valery N. Konopsky* and **Elena V. Alleva**

Institute of Spectroscopy, Russian Academy of Sciences, Troitsk, Moscow region, 142190, Russia

APPLIED PHYSICS LETTERS **90**, 241116 (2007)

Bragg surface wave device based on porous silicon and its application for sensing

E. Guillermain,^{a)} V. Lysenko, R. Orobchouk, and T. Benyattou^{b)}

Institut des Nanotechnologies de Lyon INL, CNRS-UMR5270, INSA-Lyon, Villeurbanne F-69621, France

S. Roux and A. Pillonnet

Laboratoire de Physico-Chimie des Matériaux Luminescents LPCML, CNRS-UMR5620, Université Lyon1, Villeurbanne F-69622, France

P. Perriat

Matériaux: Ingénierie et Science MATEIS, CNRS-UMR5510, INSA-Lyon, Villeurbanne F-69621, France



Label-Free Biosensing with BSW

APPLIED PHYSICS LETTERS 91, 253125 (2007)

Enhancement of diffraction for biosensing applications via Bloch surface waves

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2318 OPTICS LETTERS / Vol. 34, No. 15 / August 1, 2009

Strong modification of light emission from a dye monolayer via Bloch surface waves

Marco Liscidini,^{1,*} Matteo Galli,¹ Molu Shi,² Giacomo Dacarro,¹ Maddalena Patrini,¹
Daniele Bajoni,³ and J. E. Sipe^{1,2,3}

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*Corresponding author: marco.liscidini@gmail.com



Label-Free Biosensing with BSW

Protease detection using a porous silicon based Bloch surface wave optical biosensor

Hong Qiao,^{1,2} Bin Guan,^{1,2} J. Justin Gooding,² and Peter J Reece,^{1,*}

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²*School of Chemistry, The University of New South Wales, Sydney, New South Wales 2052, Australia*

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5 July 2010 / Vol. 18, No. 14 / OPTICS EXPRESS 15174

Anal. Chem. 2010, 82, 5211–5218

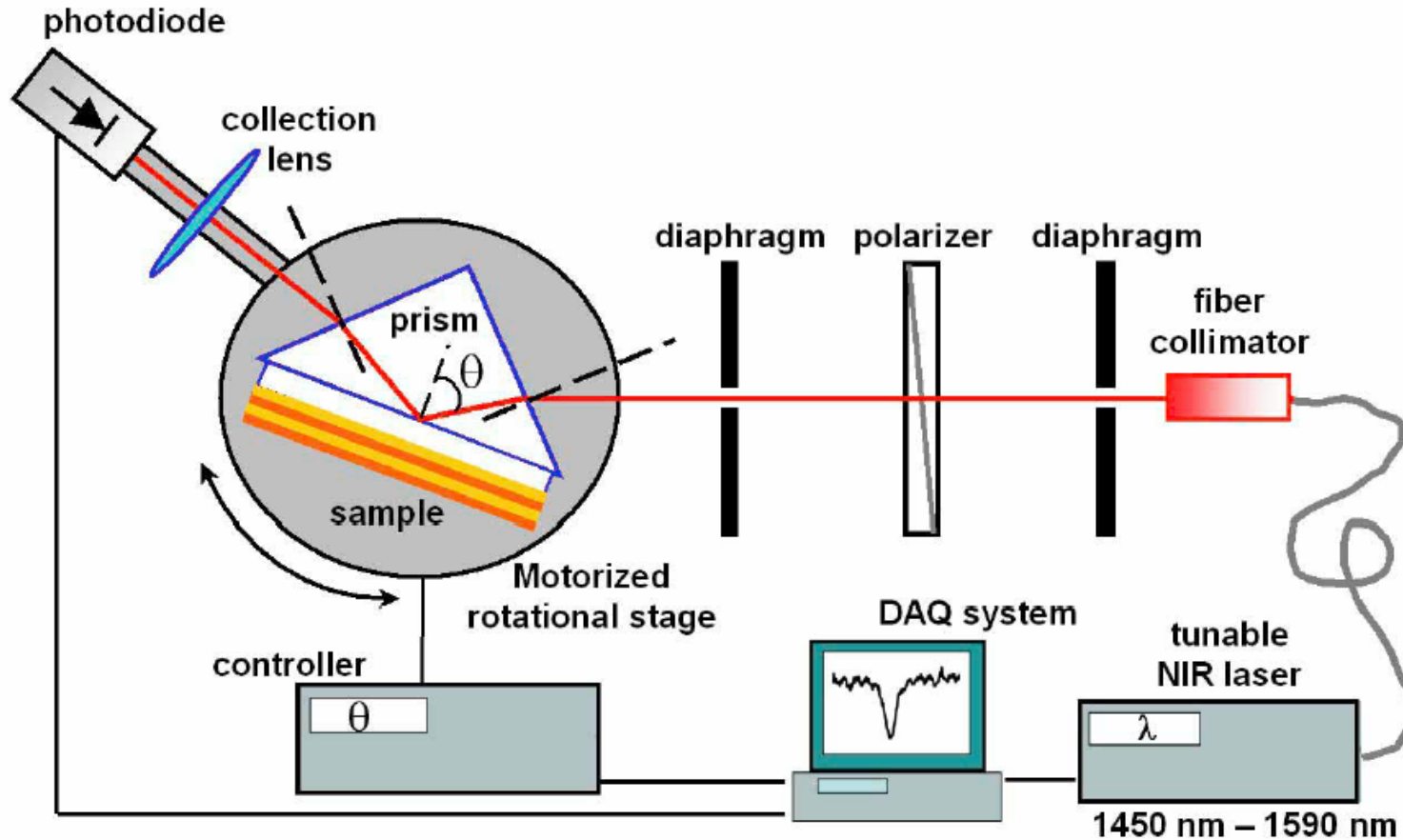
Real-Time Biomolecular Binding Detection Using a Sensitive Photonic Crystal Biosensor

Yunbo Guo,^{*,†,‡} Jing Yong Ye,^{†,‡,§} Charles Divin,[†] Baohua Huang,[‡] Thommey P. Thomas,[‡]
James R. Baker, Jr.,[‡] and Theodore B. Norris^{†,‡}

Center for Ultrafast Optical Science, Department of Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, Michigan 48109, and Michigan Nanotechnology Institute for Medicine and Biological Sciences, University of Michigan, Ann Arbor, Michigan 48109



$a\text{-Si}_{1-x}\text{N}_x\text{:H}$ based 1D Photonic Crystal

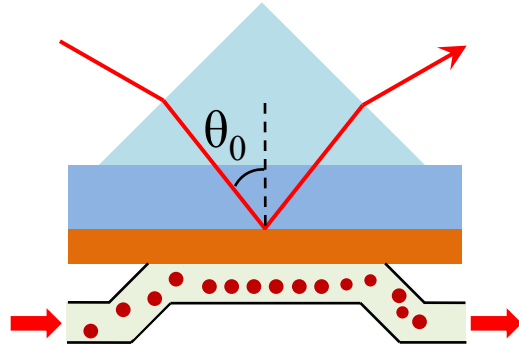


Bio Sensing - Demonstration of sensitivity – Glucose in H₂O

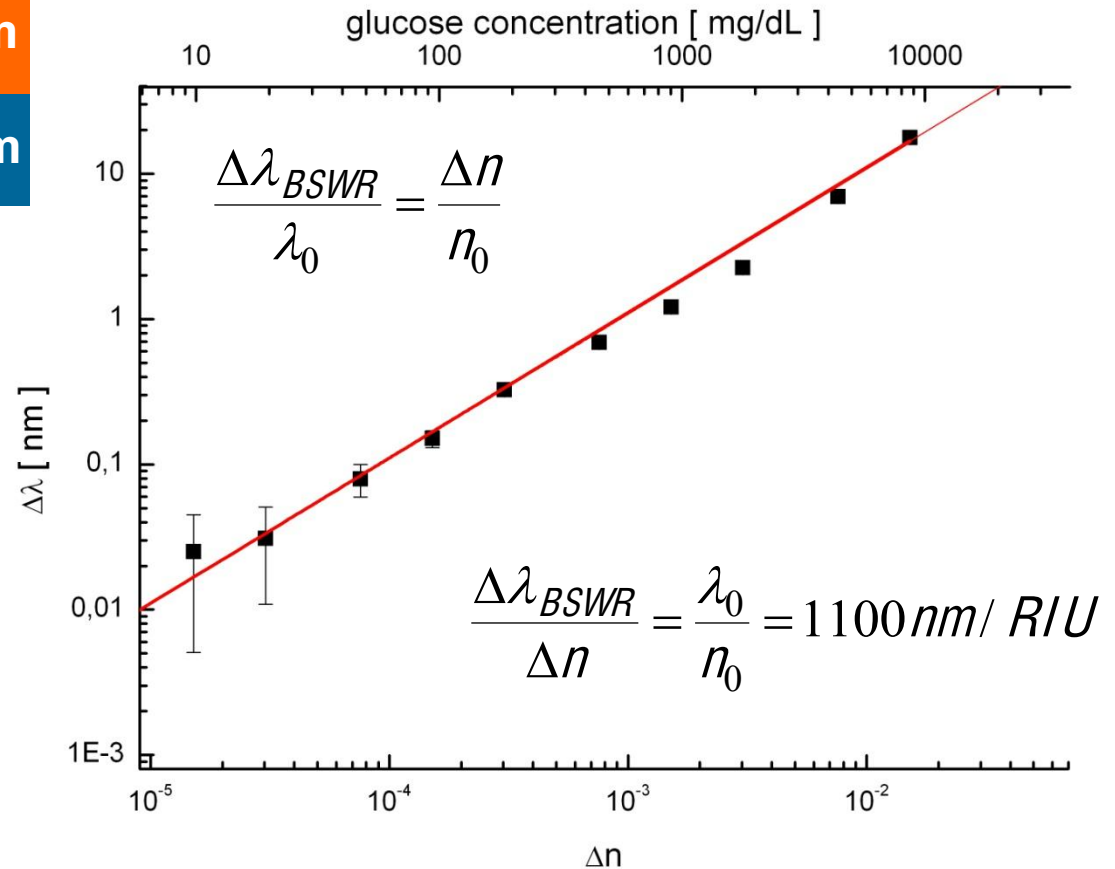
L t=318nm n=1.72 @ λ=1530nm

H t=265nm n=2.19 @ λ=1530nm

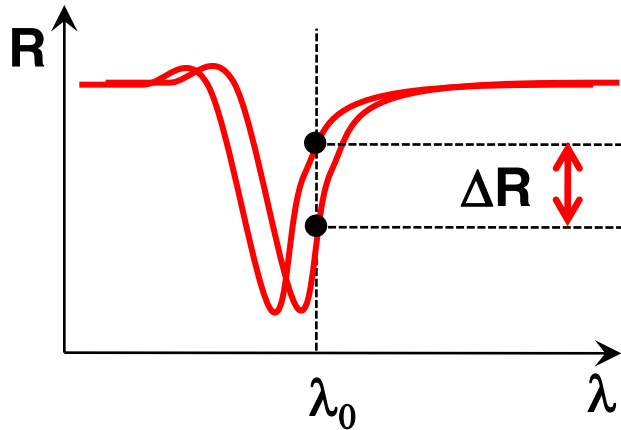
Si_xN_{1-x} - N=10



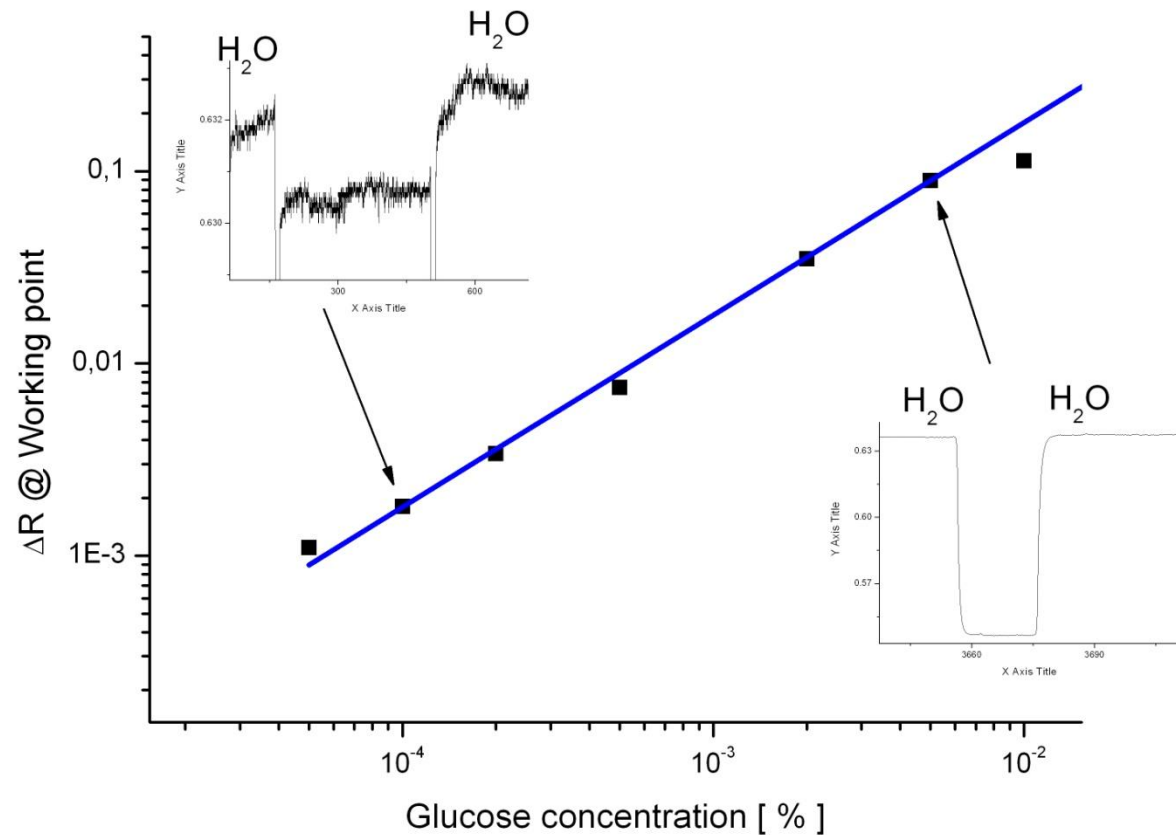
The reflectance spectrum is measured at fixed θ_0 for increasing stationary concentrations of glucose in water



Bio Sensing - Demonstration of sensitivity – Glucose in H₂O



The reflectance spectrum can be dynamically measured in a working point (fixed θ_0 and λ_0) upon injection of a solution of glucose in water



$$\Delta n_{\text{min,pub}} = 3.8 \cdot 10^{-6} \quad (\text{now } \Delta n_{\text{min}} = 1 \cdot 10^{-6})$$

Bio Sensing - Demonstration of selectivity – Antibody pairing

Primary / Secondary standard antibody system

Primary Antibody

Rabbit IgG (**AbI**)

Secondary Antibodies

Specific Goat Antirabbit IgG – HRP

(**POSITIVE - AbII**)

Non specific Anti-Mouse IgG – HRP

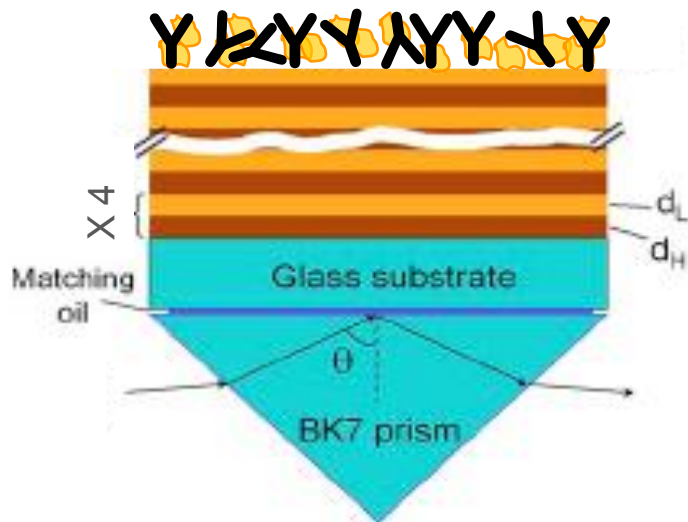
(**NEGATIVE**)



Both secondary antibodies are marked with horseradish peroxidase (HRP) in order to subsequently perform ELISA assays for quantitative analysis

Bio Sensing - Demonstration of selectivity – Antibody pairing

Surface functionalisation

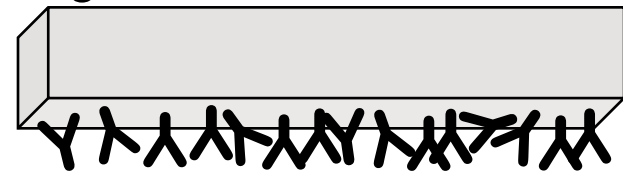


L $t=290\text{nm}$ $n=1.74$ @ $\lambda=1530\text{nm}$

H $t=149\text{nm}$ $n=3.40$ @ $\lambda=1530\text{nm}$

3) Addition of bovine serum albumin (BSA) solution in ddH₂O (250 $\mu\text{g}/\text{mL}$)

2) Antibodies transfer onto SiN surface by soft printing



1) PDMS incubation with:
Rabbit IgG solution (50 $\mu\text{g}/\text{mL}$)

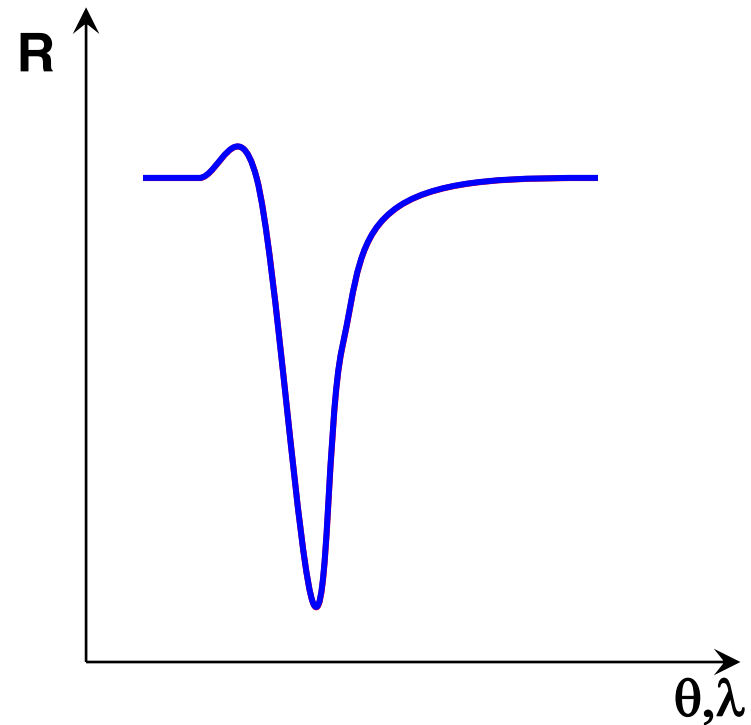
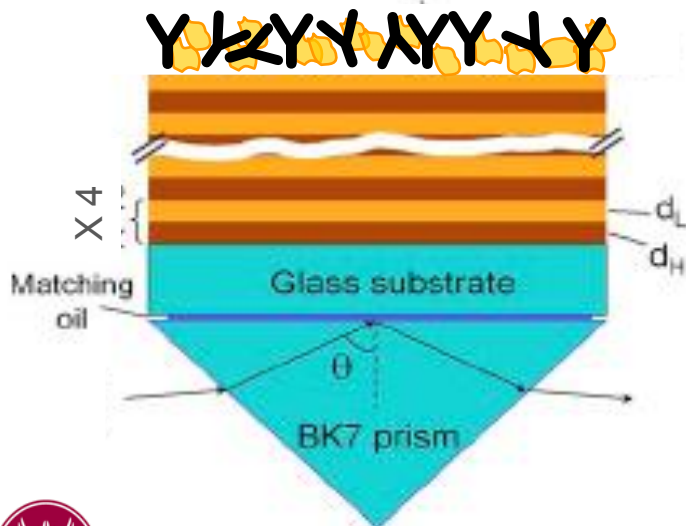


Bio Sensing - Demonstration of selectivity – Antibody pairing

Bio-Sensor operation

The flow cell is filled with Goat specific Anti-Rabbit IgG -HRP in ddH₂O

Binding takes place

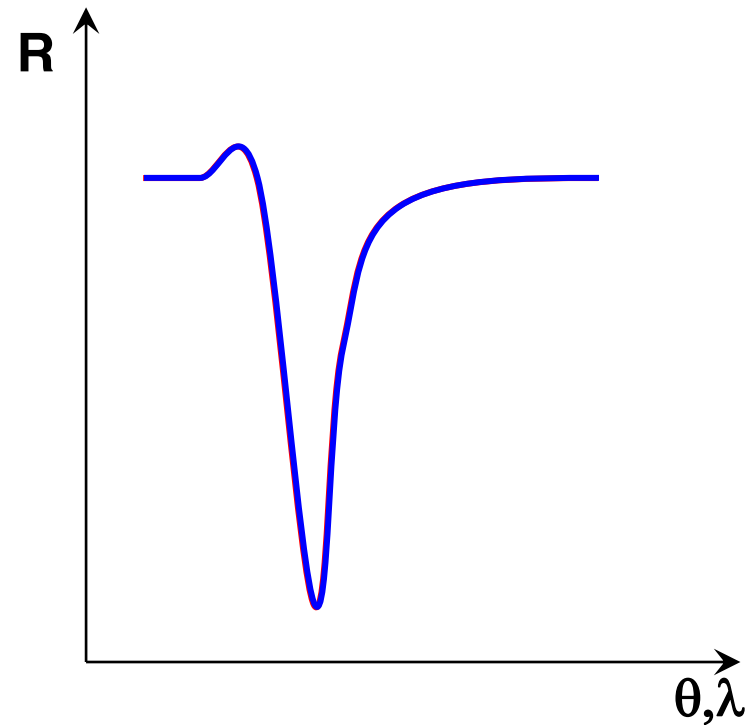
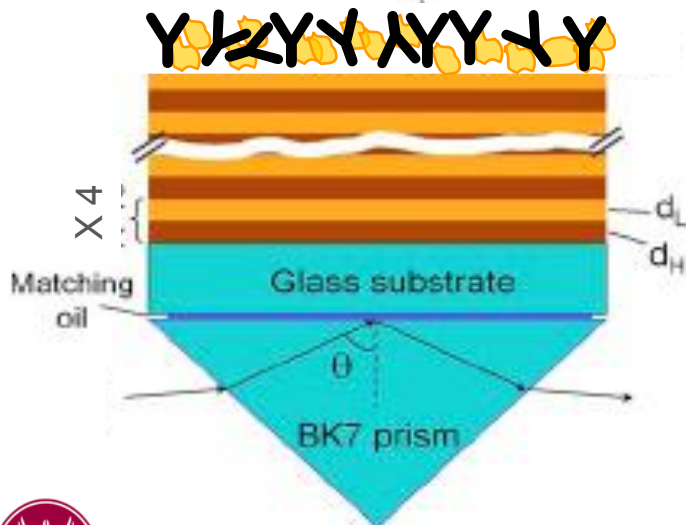


Bio Sensing - Demonstration of selectivity – Antibody pairing

Bio-Sensor operation

The flow cell is filled with Anti-Mouse IgG - HRP in ddH₂O

Binding doesn't take place

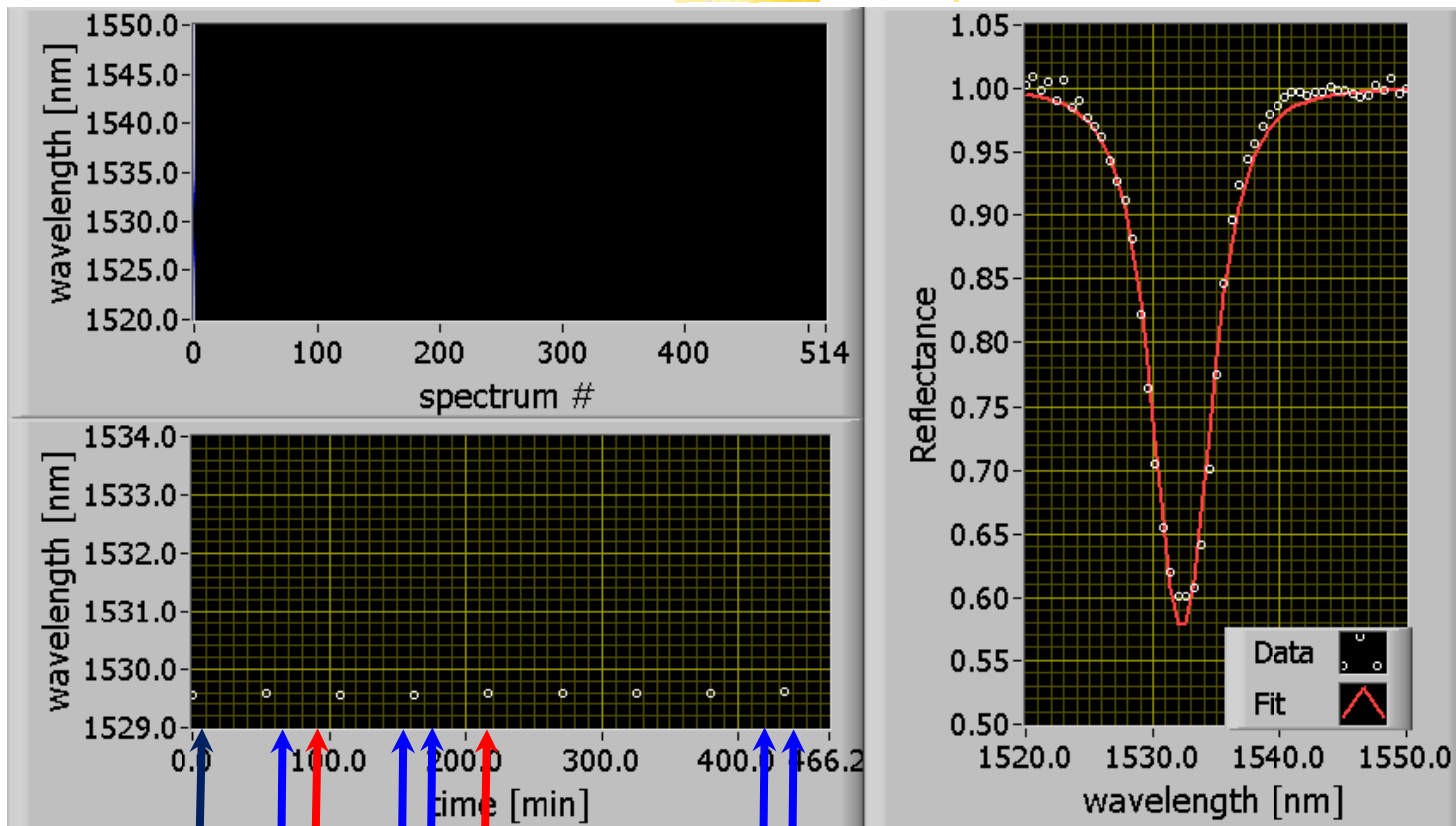


Bio Sensing - Demonstration of selectivity – Antibody pairing

A typical sensing experiment



Bio Sensing - Demonstration of selectivity – Antibody pairing



250 $\mu\text{g}/\text{mL}$ \leftarrow BSA

ddH₂O

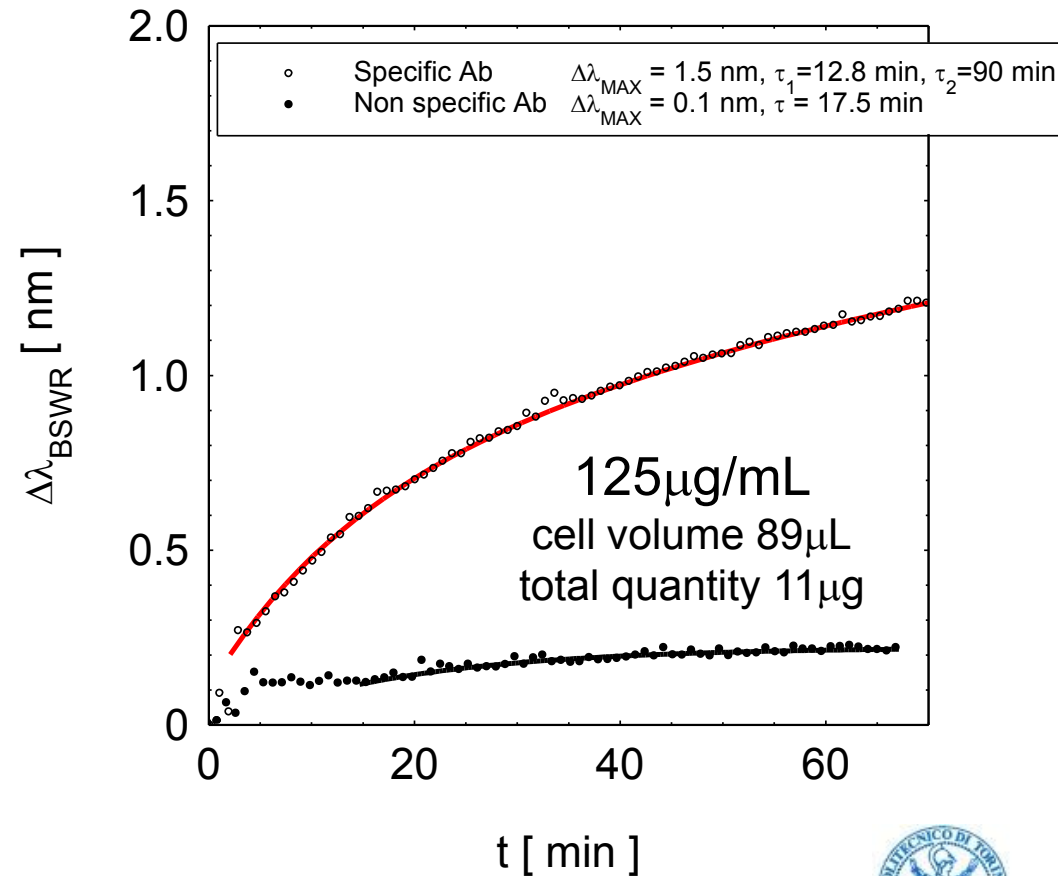
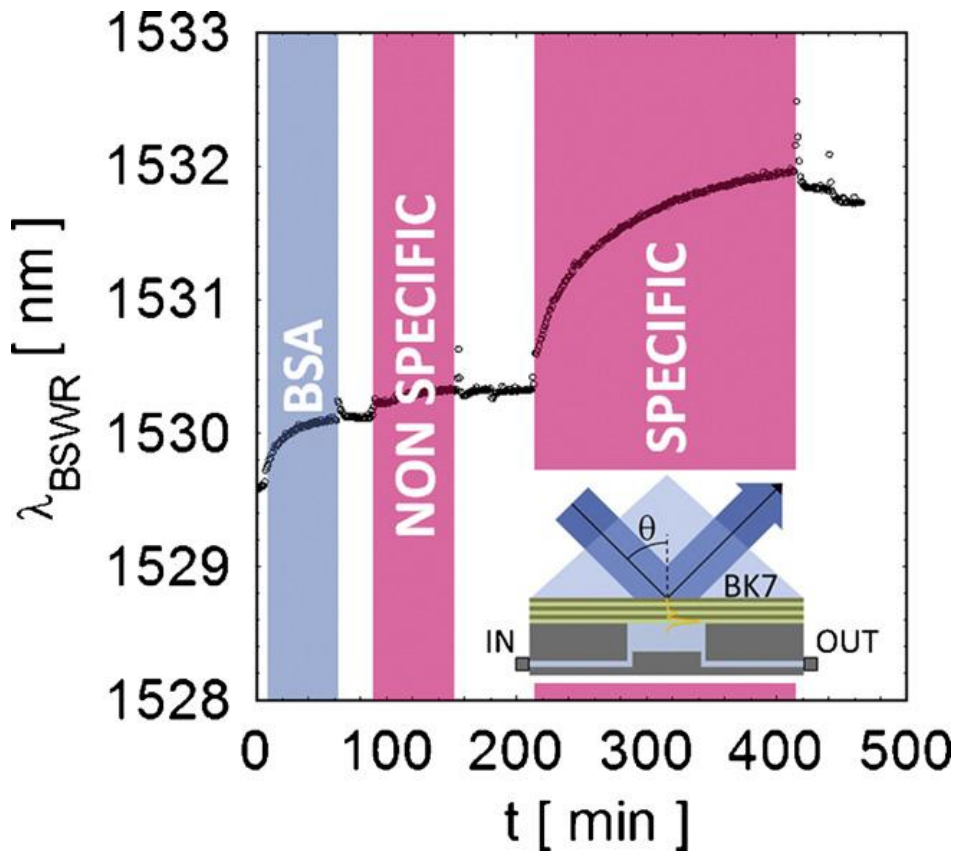
Anti-Mouse IgG (NEG)

Goat Anti-Rabbit IgG (POS)

\rightarrow 125 $\mu\text{g}/\text{mL}$

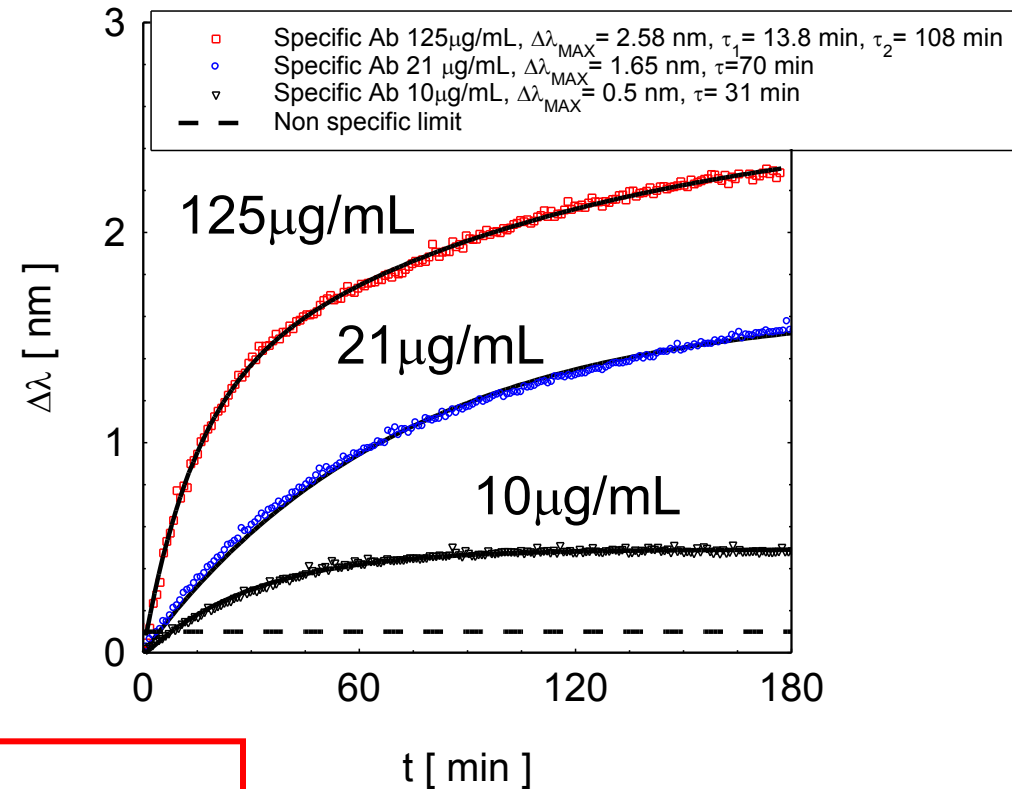


Bio Sensing - Demonstration of selectivity – Antibody pairing



Bio Sensing - Demonstration of selectivity – Antibody pairing

Specific AbII concentration [$\mu\text{g/mL}$]	Fit Parameters	
	τ_1 (τ_2) [min]	$\Delta\lambda_{\text{MAX},1}$ ($\Delta\lambda_{\text{MAX},2}$) [nm]
125	14.0 108.0	1.15 1.43
21	70.0	1.65
10	31.0	0.50



Resolution	with NEG	2 $\mu\text{g/mL}$
	without NEG	0.2 $\mu\text{g/mL}$ =1pM



Bio Sensing - Demonstration of selectivity – Antibody pairing

ELISA QUANTITATIVE ANALYSIS

Quantitative ELISA carried out on biosensors' surface after **Goat Anti-Rabbit IgG - HRP (125 $\mu\text{g}/\text{mL}$)** BSW sensing experiments indicate that:

- the number of counted AbII molecules is $5 \cdot 10^8$
- corresponding to an absolute quantity of 2.5 fg of AbII (~attomol)
- Further investigations are on the way.



Side product of the research – Thermal stability of BSW

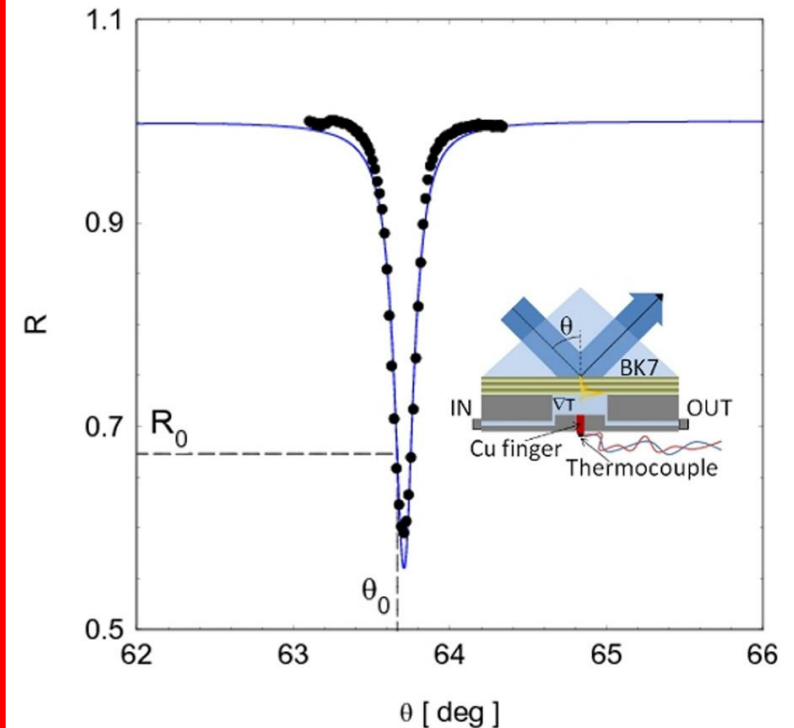
1DPC

Glass/ (HL)⁴ / P / air

H	(a-Si)	$n_H=3.486$	$t_H=161$ nm
L	(a-Si ₃ N ₄)	$n_L=1.757$	$t_L=310$ nm
P	(PPAA polymer)	$n_P=1.514$	$t_P=20$ nm

The PPAA polymer is needed to functionalize the sensor surface with the proper markers receptors

PPAA is deposited by plasma polymerisation



Side product of the research – Thermal stability of BSW

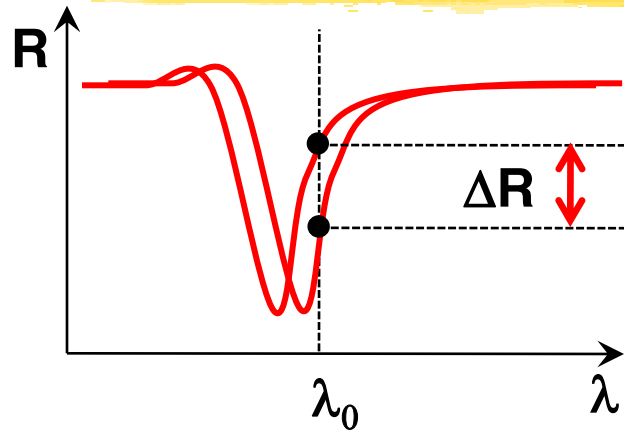
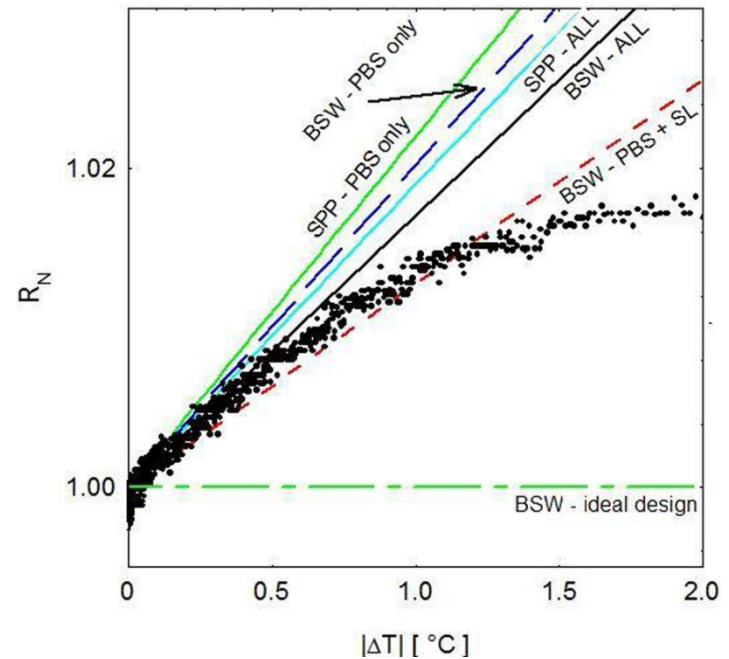
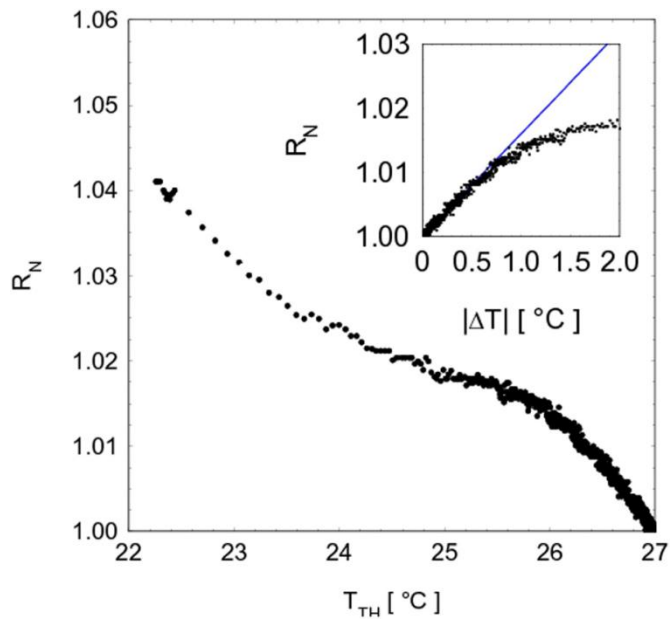


TABLE I. Complex thermo-optic coefficients used in the numerical simulations.

Material	$d\tilde{n}/dT$ ($10^{-4} \text{ } ^\circ\text{C}^{-1}$)	λ (nm)	Ref.
PBS	-0.76	1475	12
a-Si:H	+2.3	1550	13
a-Si ₃ N ₄ :H	+0.2	1550	14
PAA	-2.0	1550	15
Au	6.2+j(-1.8)	810	16



Bio Sensing - Demonstration of selectivity – Antibody pairing



Application to cancer biomarkers detection



BSW application to the detection of cancer biomarkers

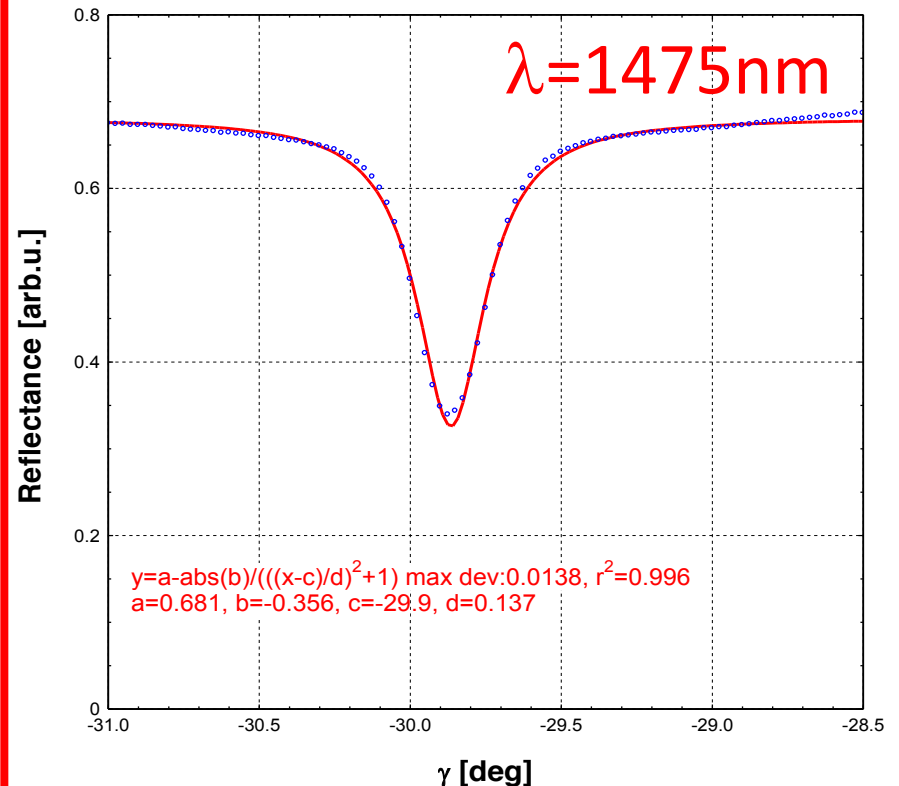
1DPC

Glass / (HL)⁸ / P / PtA(AF546) / air

H	(a-Si)	$n_H=3.486$	$t_H=168$ nm
L	(a-Si ₃ N ₄)	$n_L=1.757$	$t_L=323$ nm
P	(PPAA polymer)	$n_P=1.514$	$t_P=20$ nm

The PPAA polymer is needed to functionalize the sensor surface with the proper markers receptors

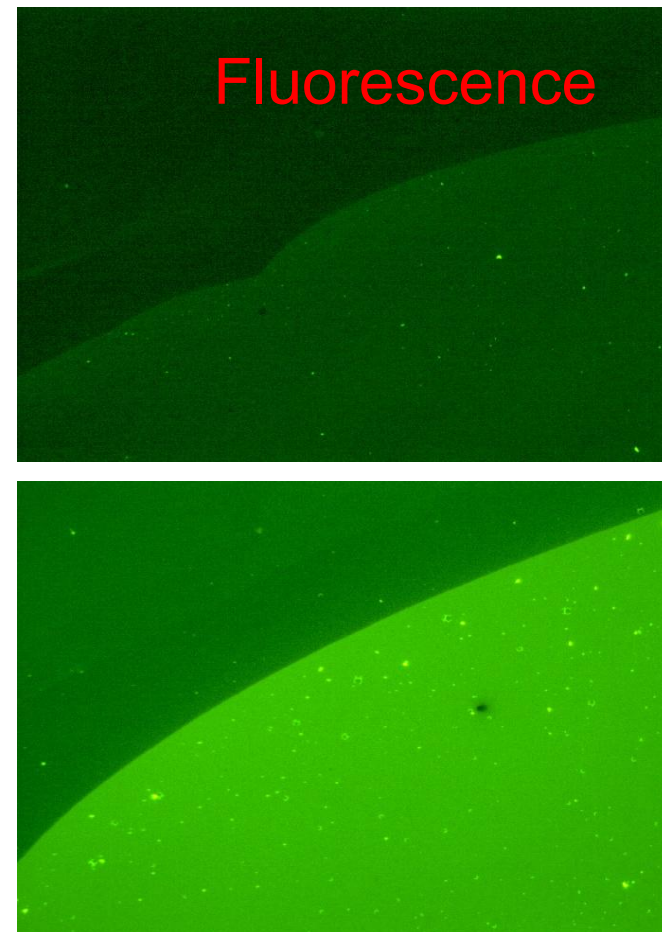
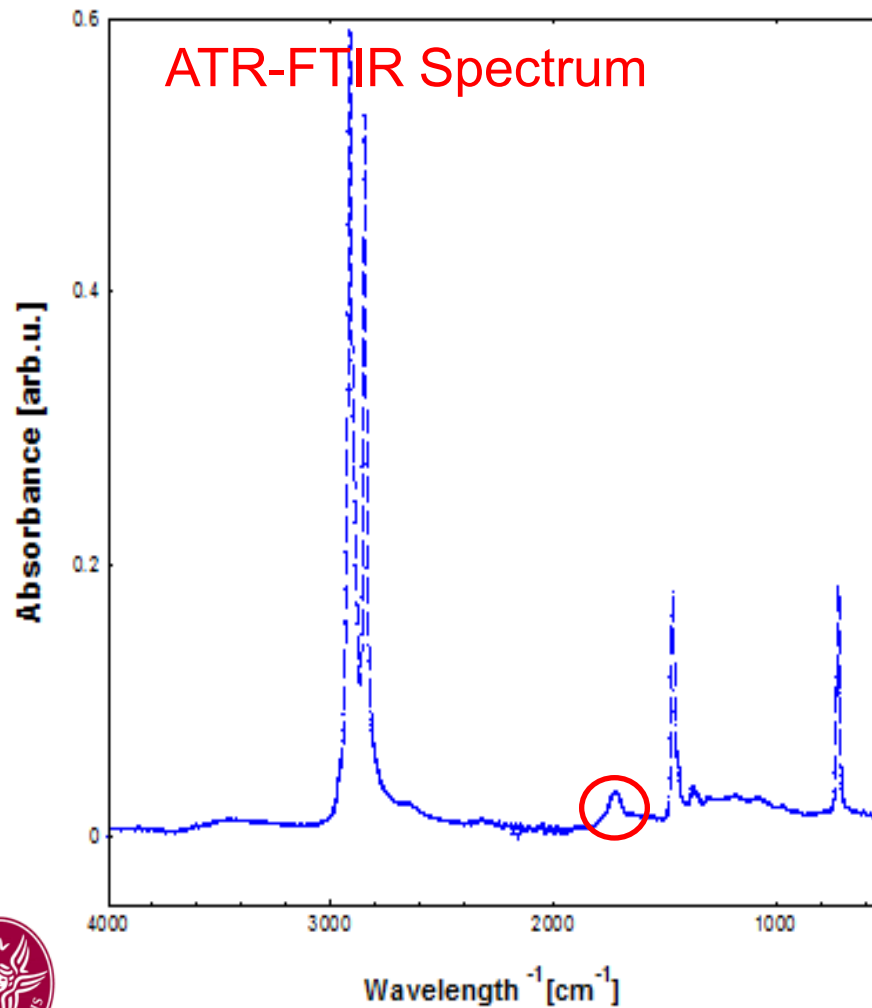
PPAA is deposited by plasma polymerisation



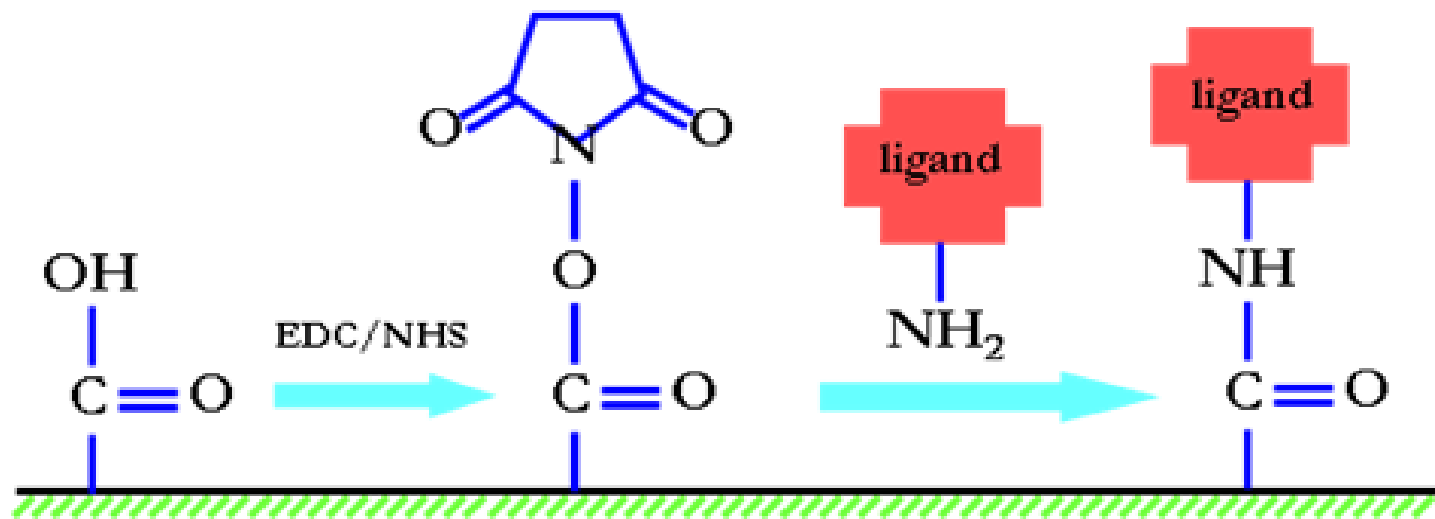
PBS - pH 8



BSW application to the detection of cancer biomarkers



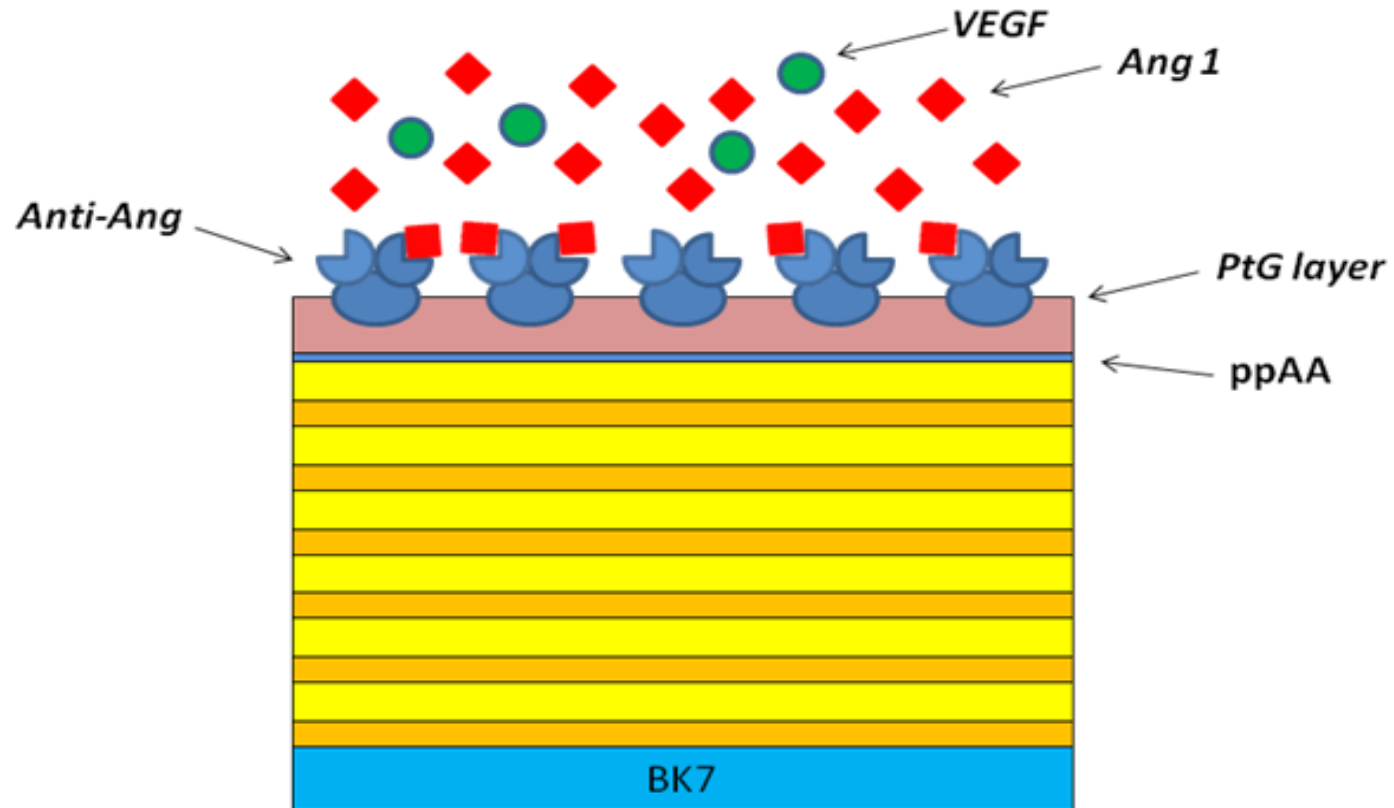
BSW application to the detection of cancer biomarkers



The carboxylic COOH groups of PPAA must be activated with EDC/NHS

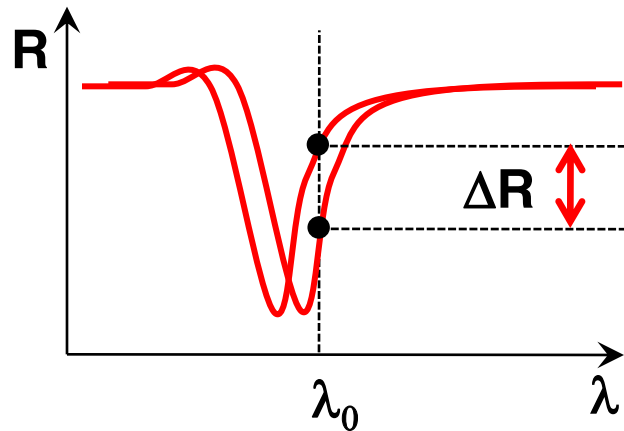


BSW application to the detection of cancer biomarkers

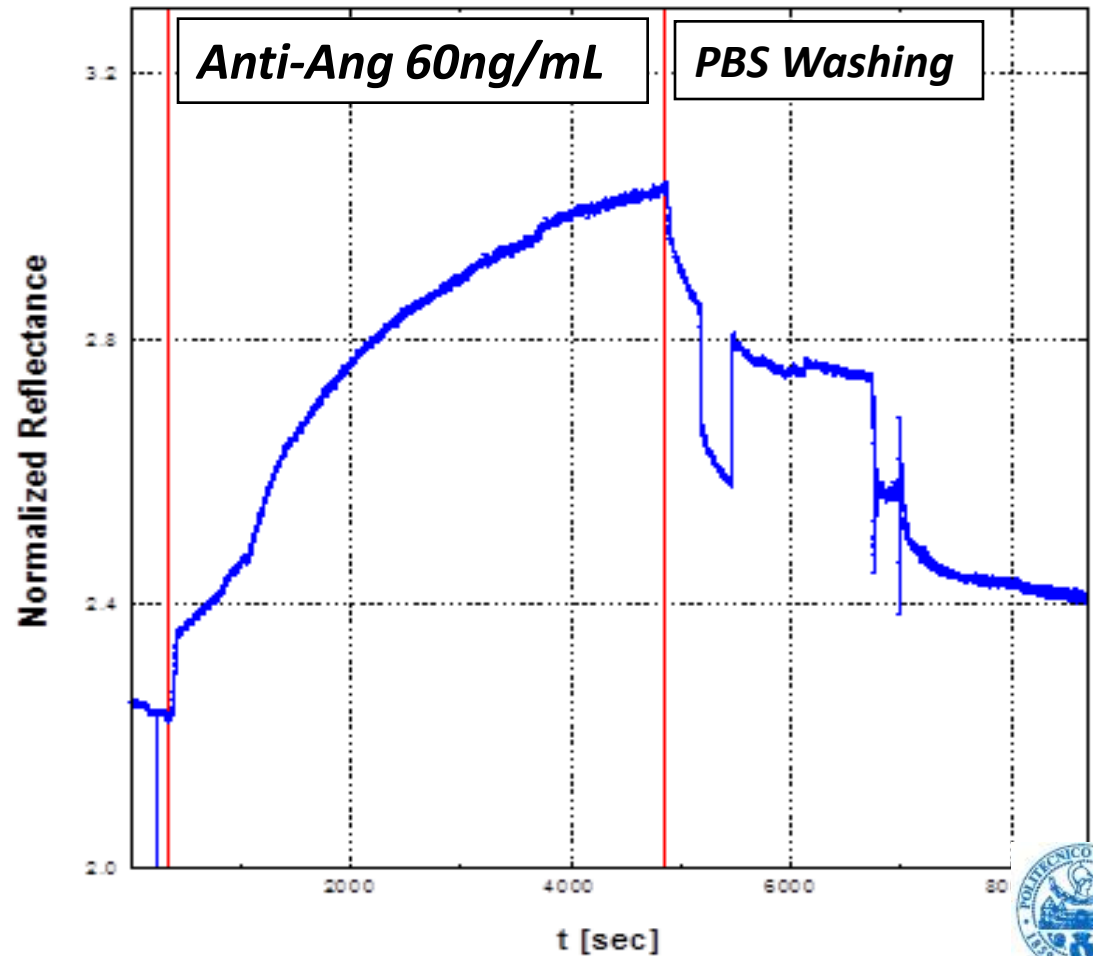


Recognition protocol

BSW application to the detection of cancer biomarkers



The surface was incubated before with Protein G with concentration $100 \mu\text{g/mL}$



BSW application to the detection of cancer biomarkers

