enLIGHTened biophotonics:
photonic biosensors

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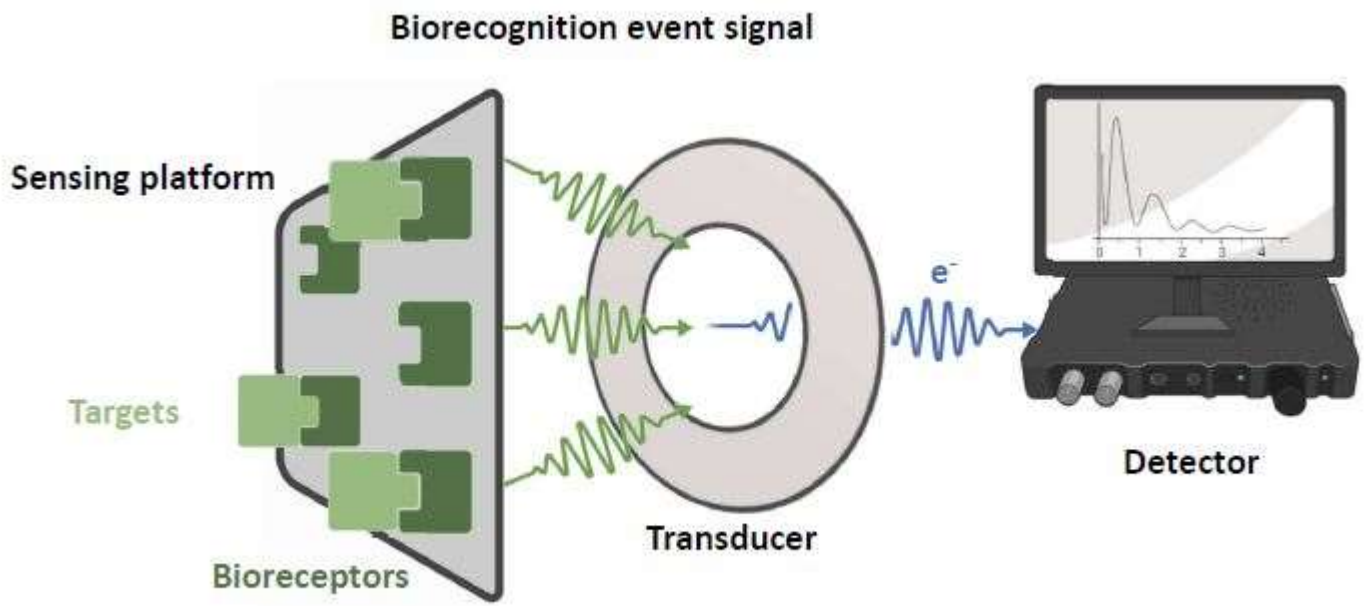
GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA E INNOVACIÓN · AGENCIA ESTATAL DE INVESTIGACIÓN · Unión Europea · FONDO EUROPEO DE DESARROLLO REGIONAL *Una manera de hacer Europa* · GENERALITAT VALENCIANA Conselleria d'Innovació, Universitats, Ciència i Societat Digital · IPN-Bio

This talk may not be the typical one from a physicist…

A **biosensor** is a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transduction element



Well… actually, this is the part for the physicists.

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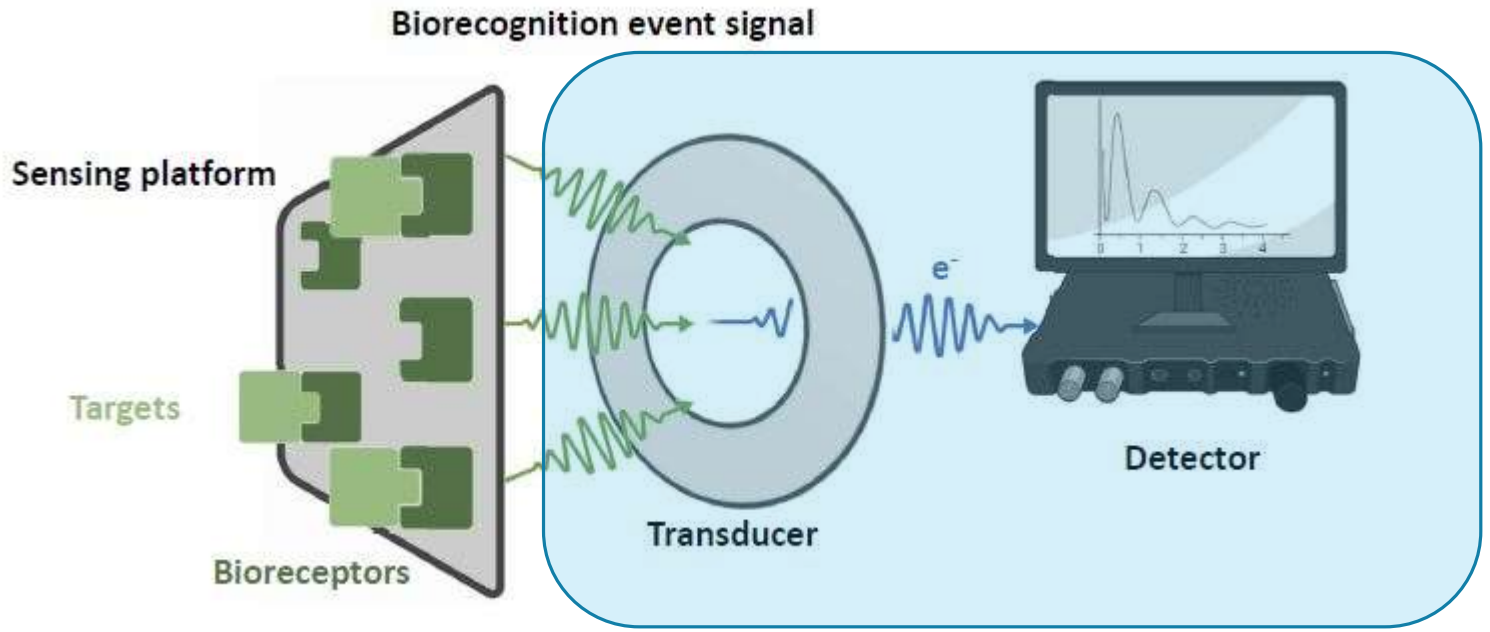


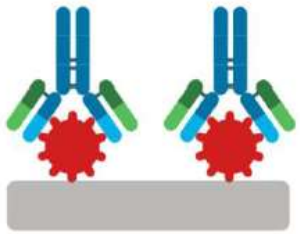
Types of biosensors

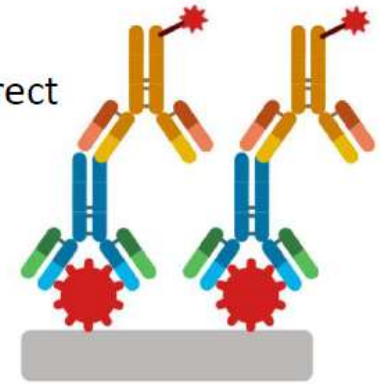
1) Interaction mechanism

- Catalytic
- Affinity

2) Detection

- Direct
- Indirect

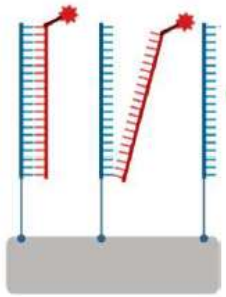




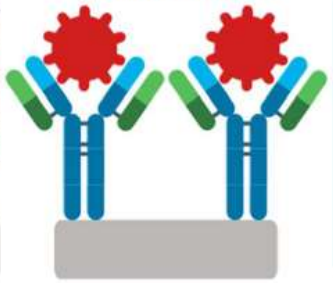
3) Bioreceptor

- Enzymes

- Nucleic acids



- Antibodies



4) Transduction mechanism

- Electrochemical

- Optical

- Magnetic

- Mass-resonators

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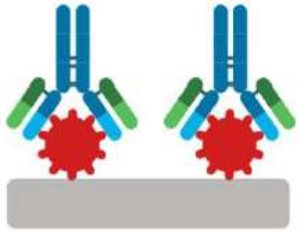

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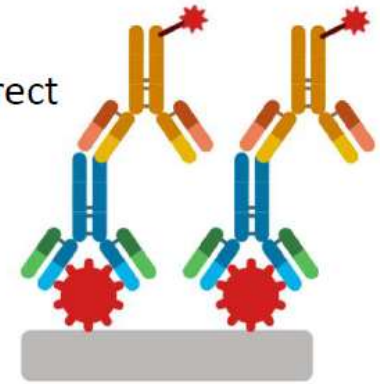

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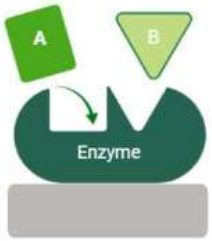

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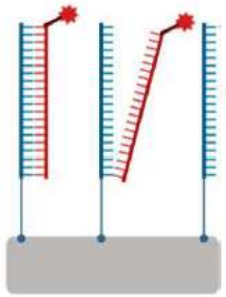

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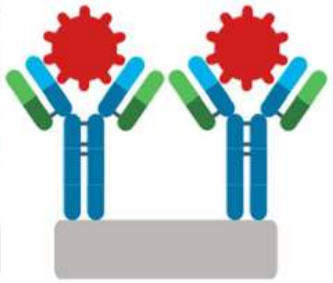

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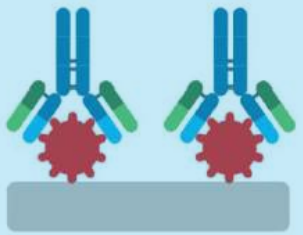
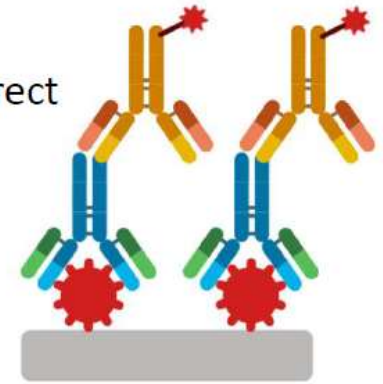


- Indirect



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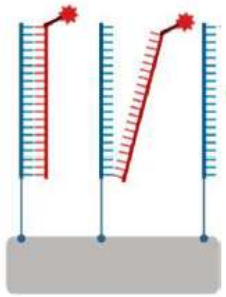

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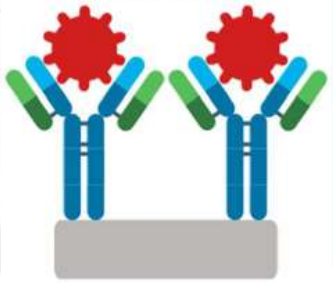

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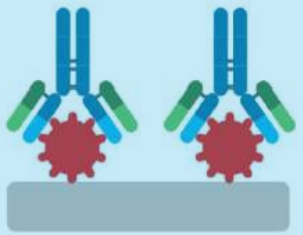

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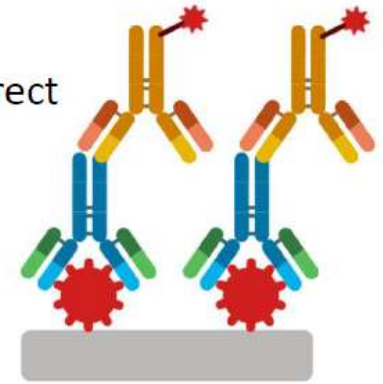

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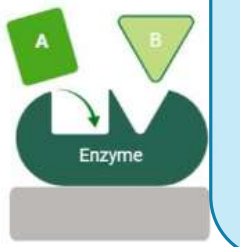

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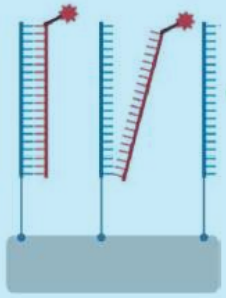

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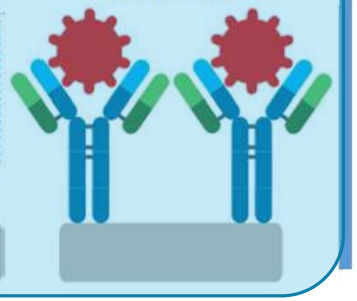

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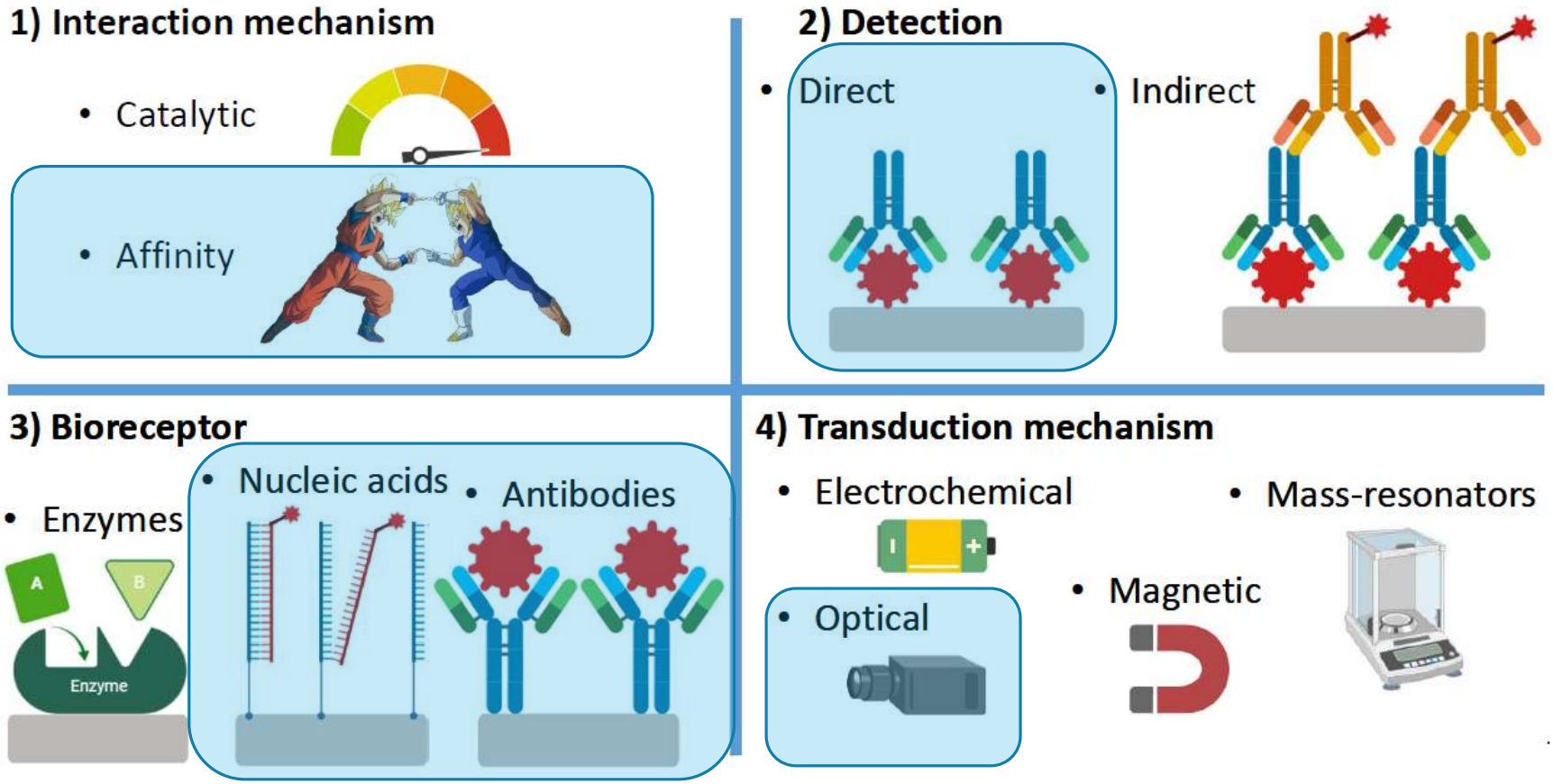
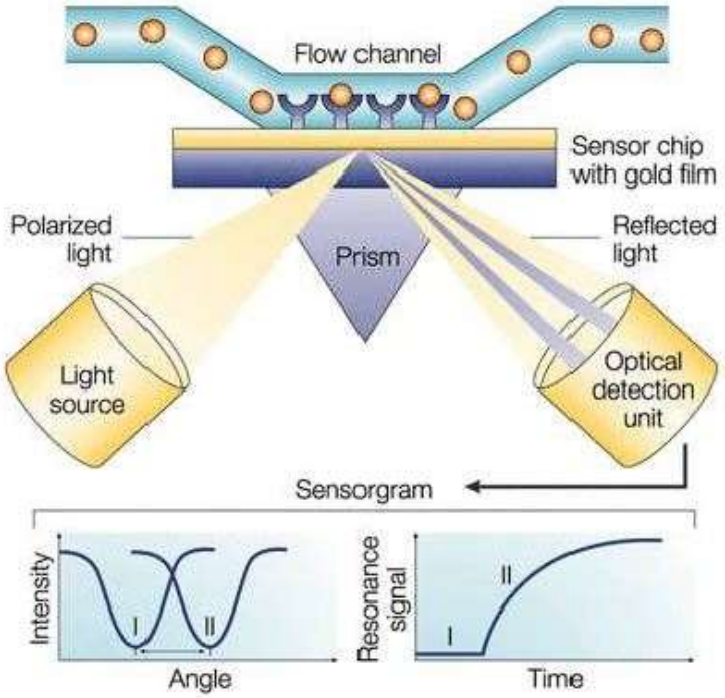


Types of Biosensors: Photonic Biosensors



Surface plasmon resonators

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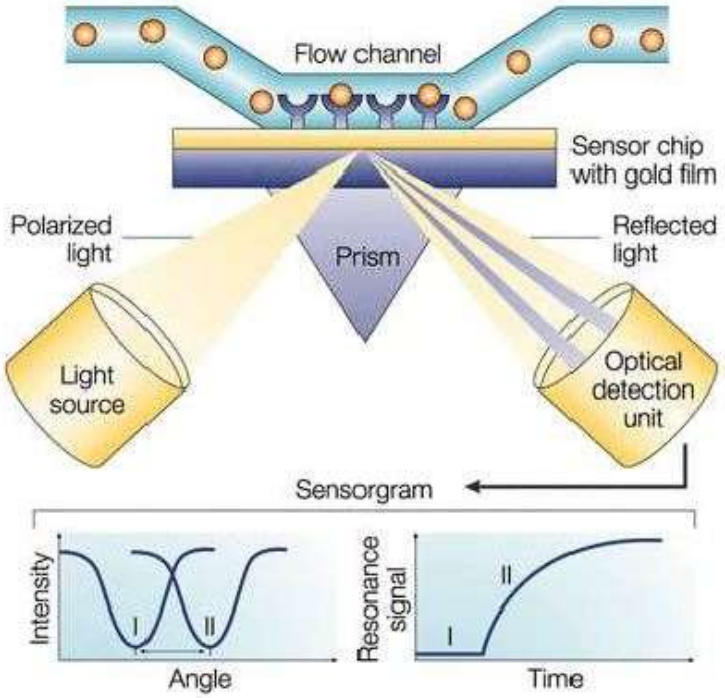
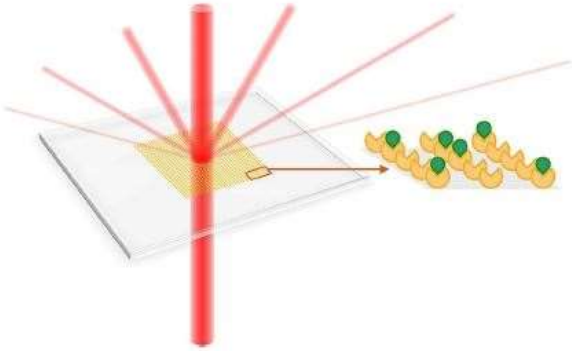


Surface plasmon resonators

Bio-diffractive structures



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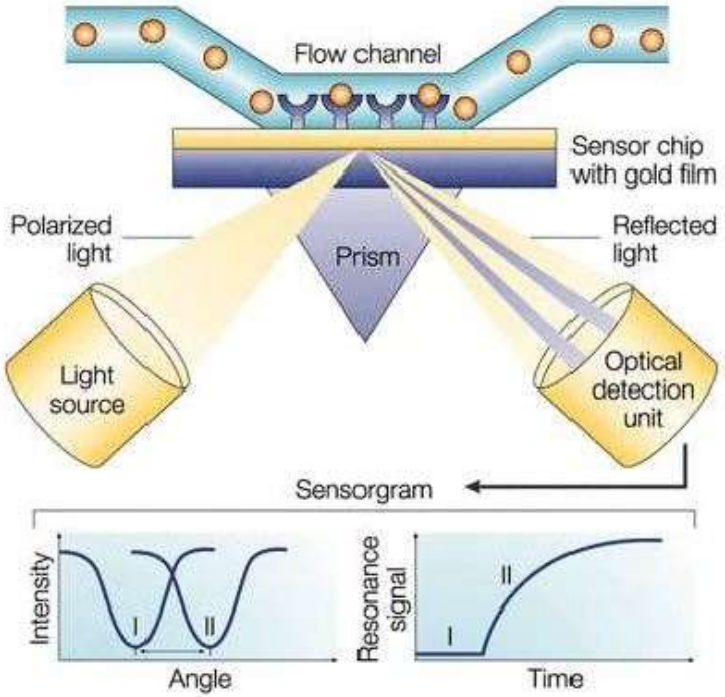

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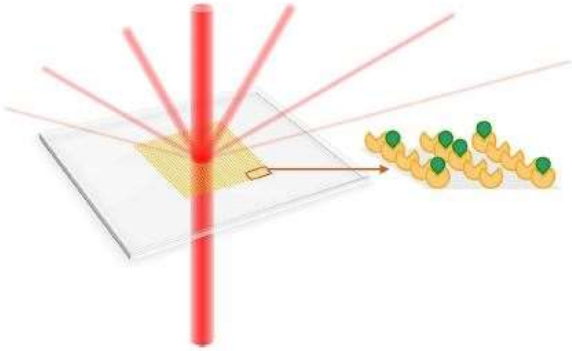
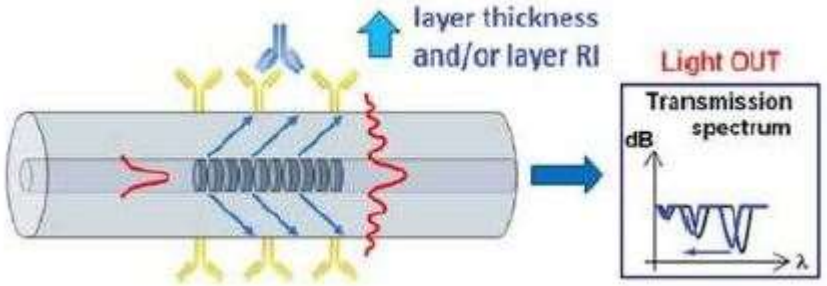
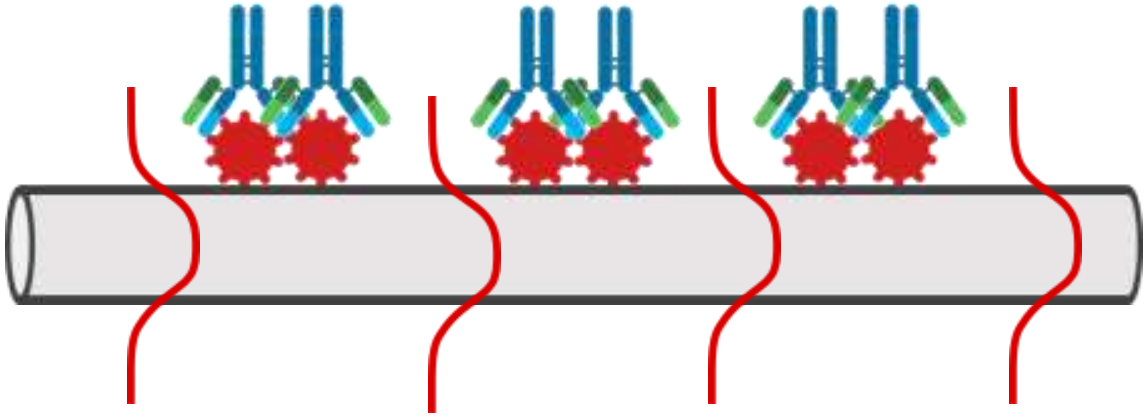
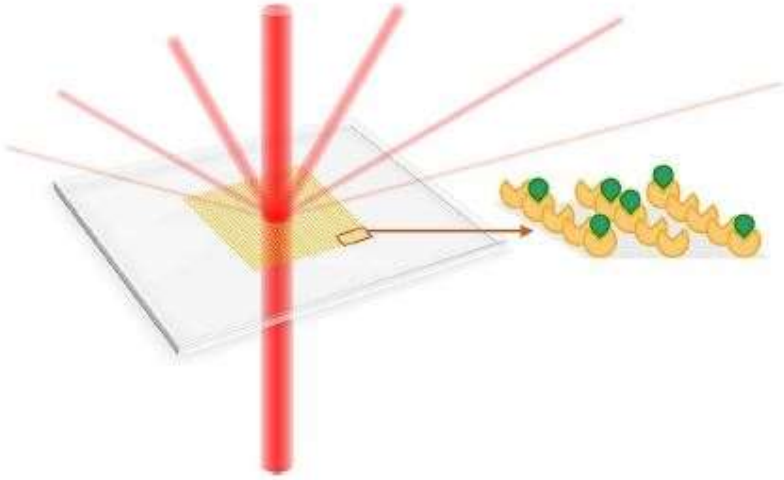


Fiber based biosensors



Diffractive biosensors





How do we functionalize the surface?

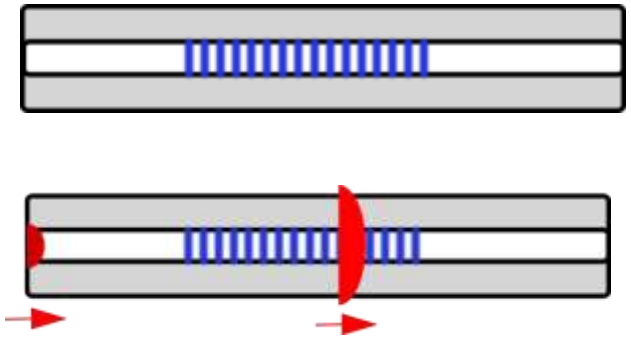
How do we optimize the photonic transducer?

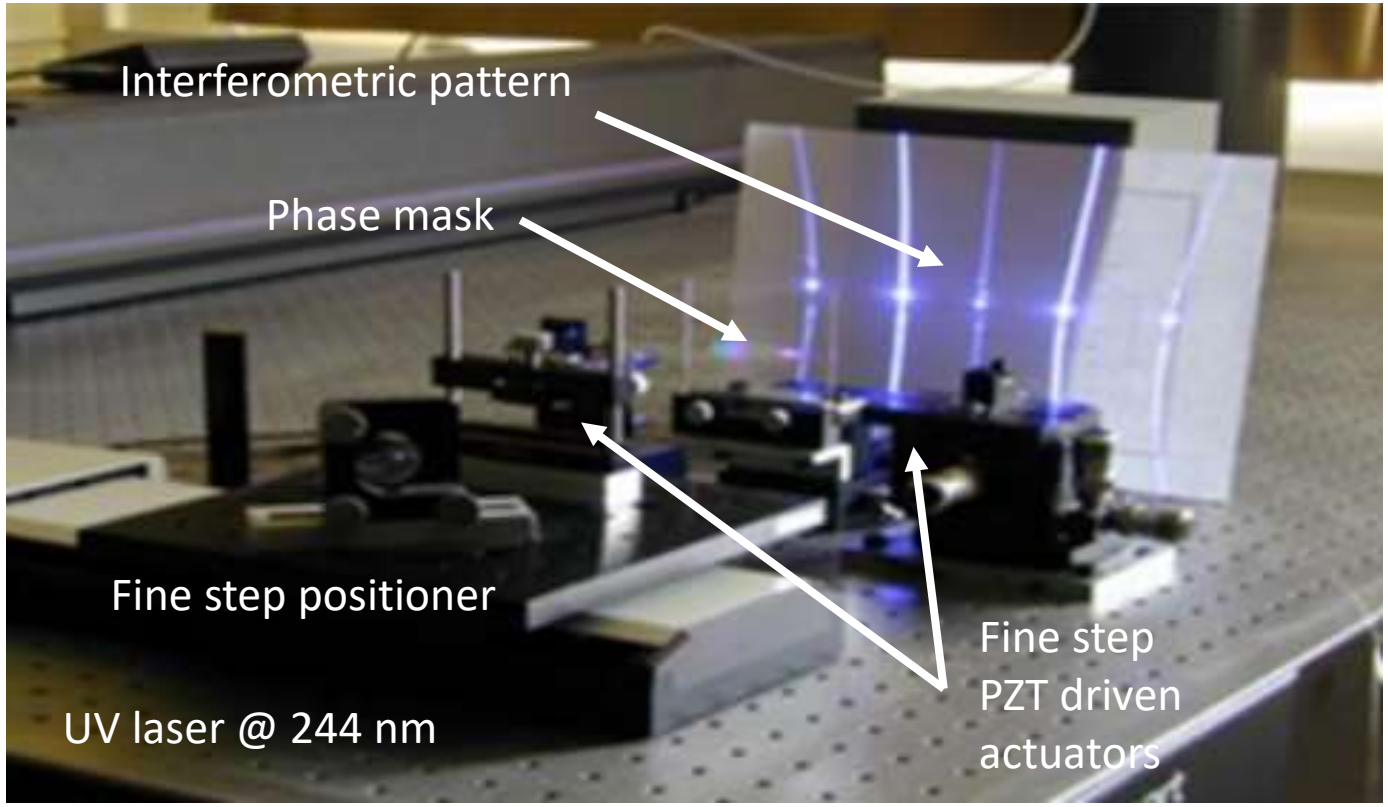
How do we read out and quantify the variations?

1. Overview
2. Narrowband in fiber Long Period Grating
3. Bio-Bragg gratings in tapered fibers
4. UV deactivated, planar molecular gratings
5. Conclusions

Objective: sensing the hybridization of an oligonucleotide (DNA)

Biosensor based on a narrowband LPG (evanescent wave sensor)





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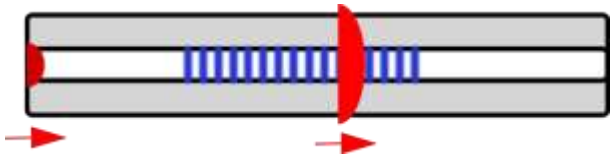
Periodic perturbation in the core index



Coupling to cladding modes: resonances

$$\lambda_R = (n_{co}-n_{cl})\cdot\Lambda$$

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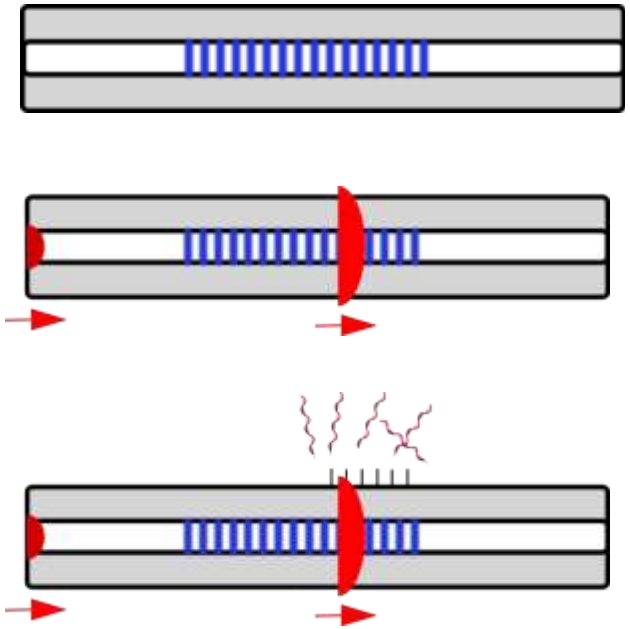

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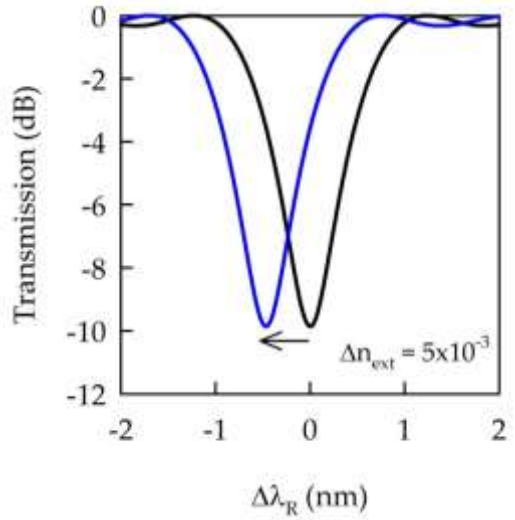

The evanescent tail senses the presence of the analyte: change in the resonance condition

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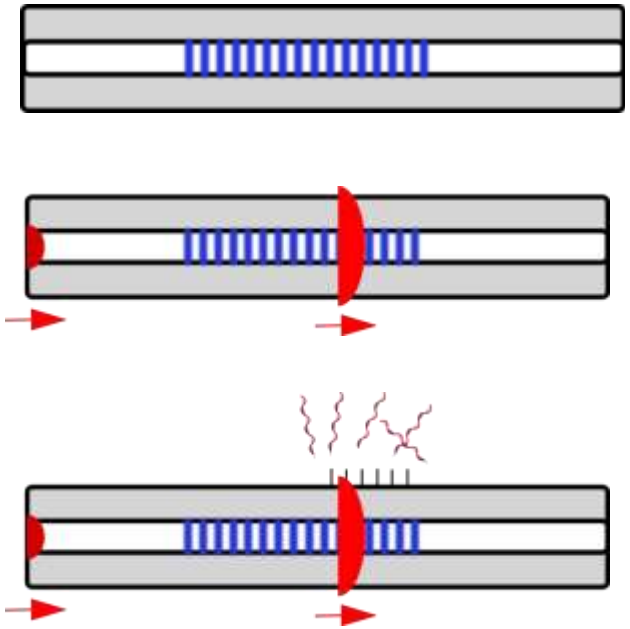

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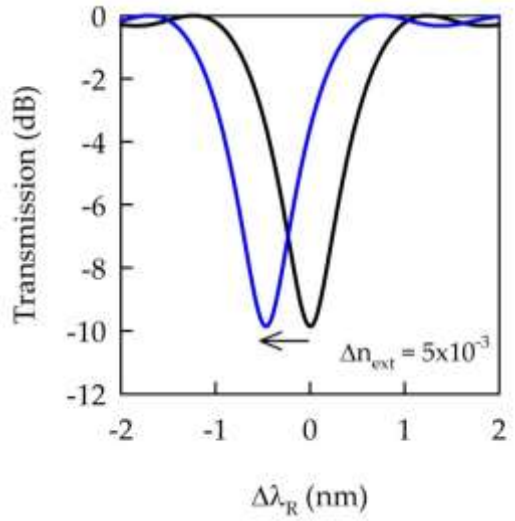
Key point of our proposal:
Narrow-bandwidth LPGs



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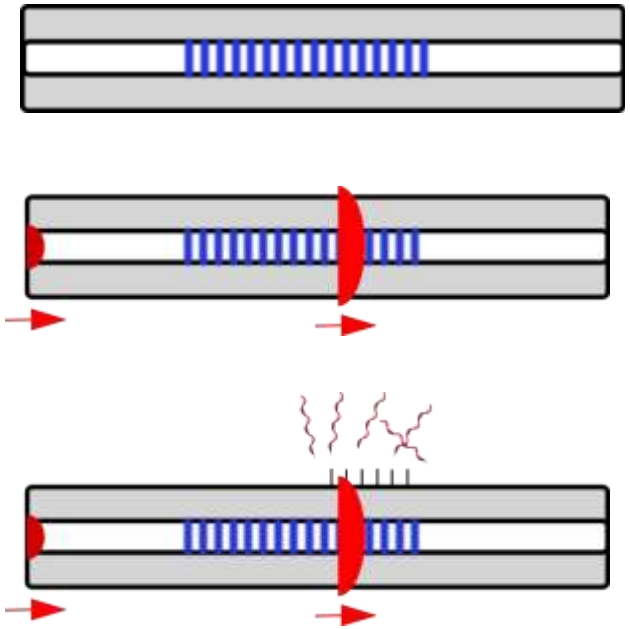

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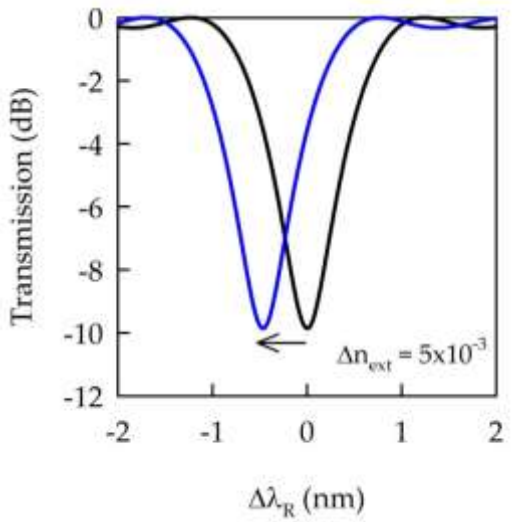


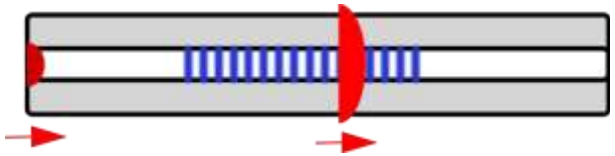
1. Sensitivity

2. Resolution

3. $DL = \frac{R}{S}$

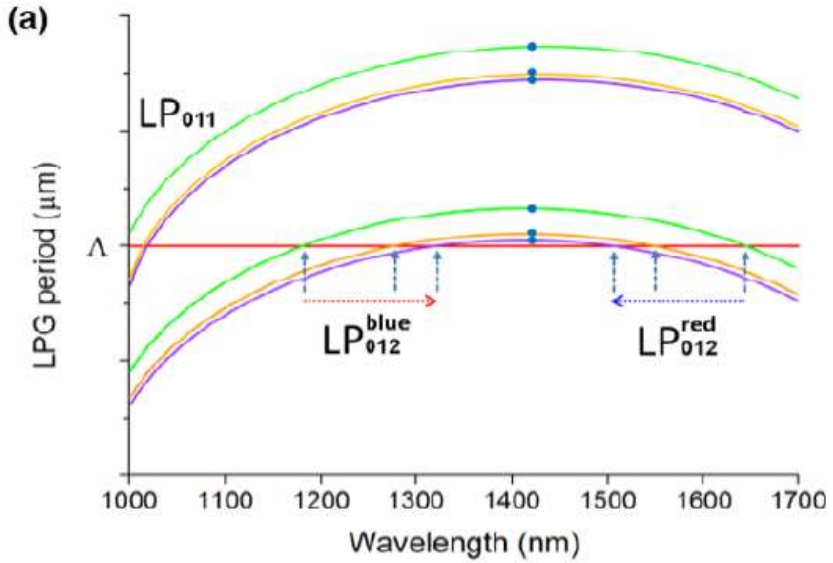
Compromise between both parameters

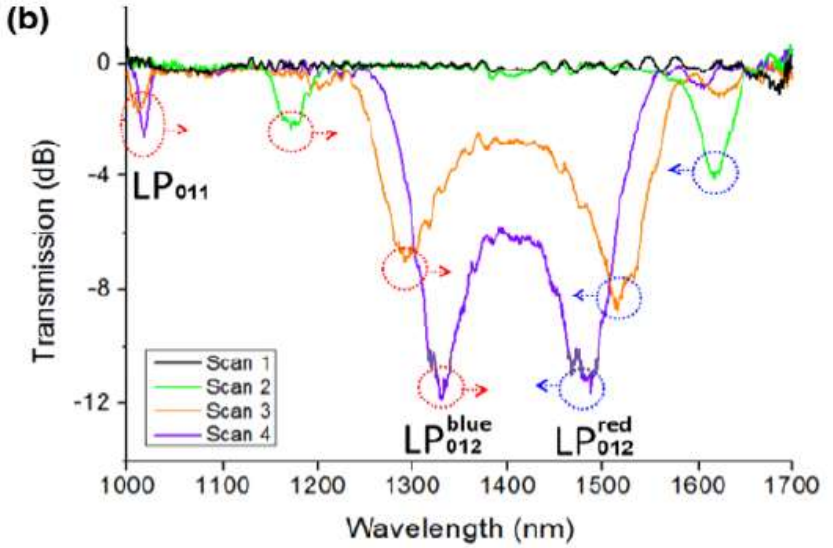
Design of the narrowband LPG



$$\lambda_R = \Lambda \cdot (n_{co} - n_{cl})$$

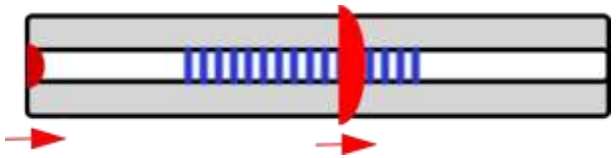
Usual approach: working at the “turning point”





Chen et al., *J Biosens Bioelectron* 2015, 6:2

Fabrication of the narrowband LPG



Narrow bandwidth LPGs

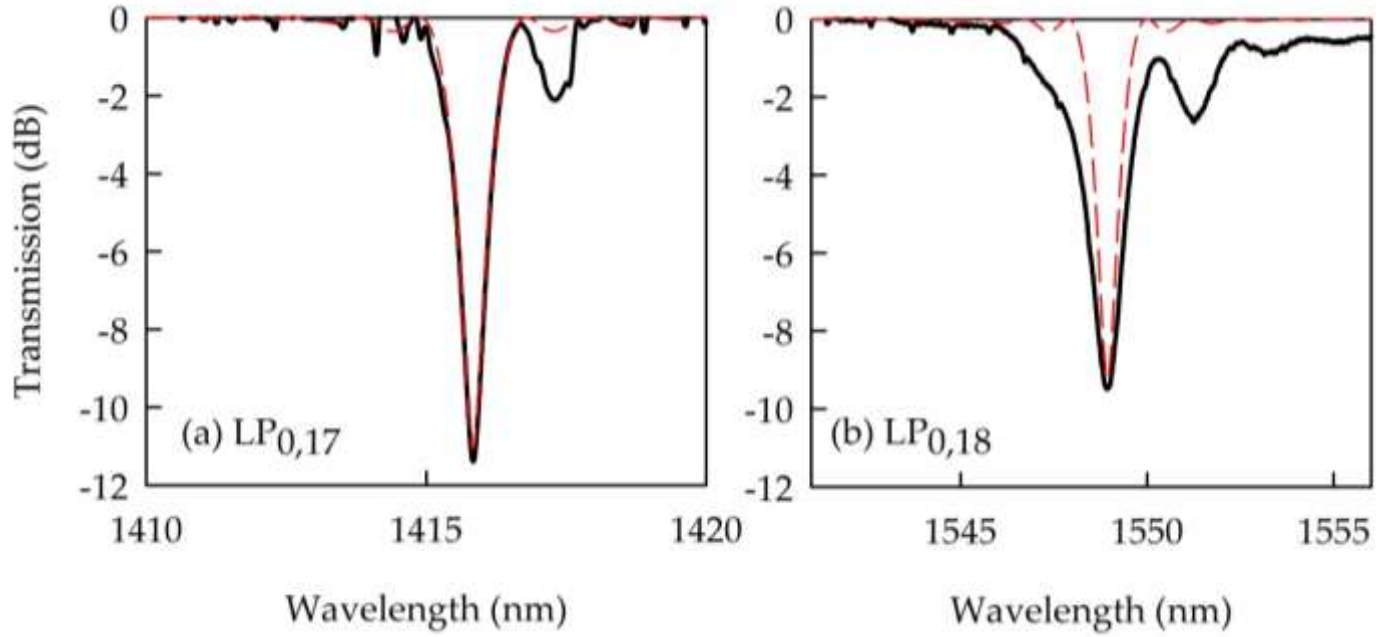
- Long lengths (up to 20 cm)
- High NA (SM1500 Fibercore)
- Low Δn
- Modes $LP_{0,17}$ – $LP_{0,18}$
- Point to point technique

Length: 10 cm

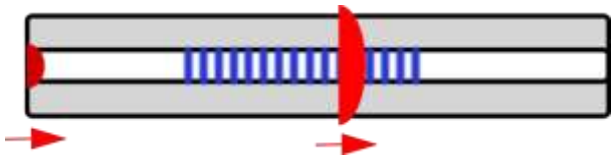
Λ = 52 μm

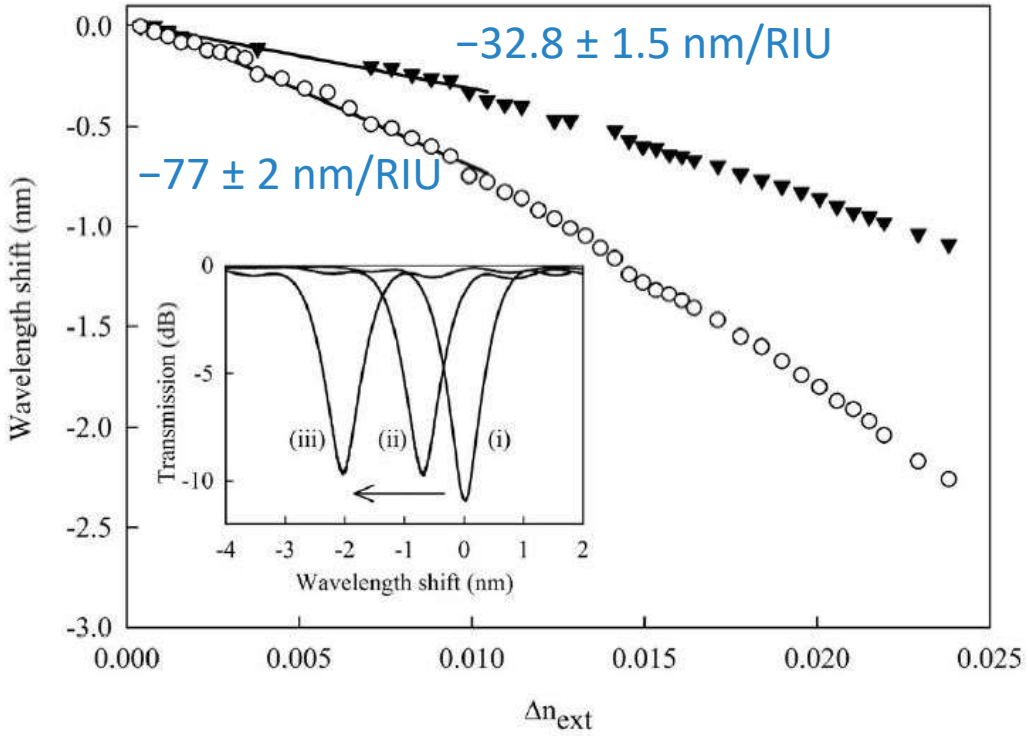
$LP_{0,17}$ – @ 1416 nm; $\Delta\lambda_{3dB}$ = 0.82 nm

$LP_{0,18}$ - @ 1550 nm; $\Delta\lambda_{3dB}$ = 1.52 nm



Fabrication of the narrowband LPG





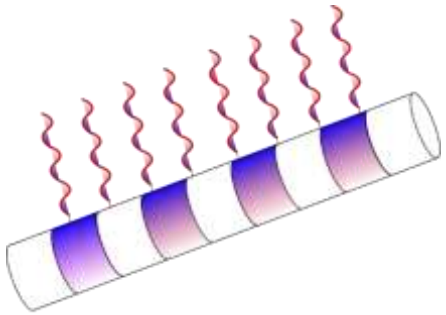
Sensitivity

	S (nm/RIU)	λ_R (nm)
$LP_{0,17}$	-44	1416
$LP_{0,18}$	-94	1550
Chen et al.	-564/+794	

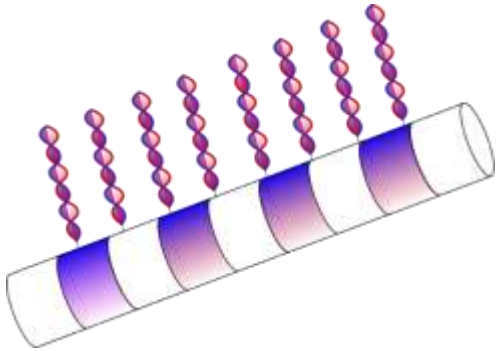
(Calculated)

Triangles @ 1550 nm ($LP_{0,18}$)
Circles @1400 nm ($LP_{0,17}$)

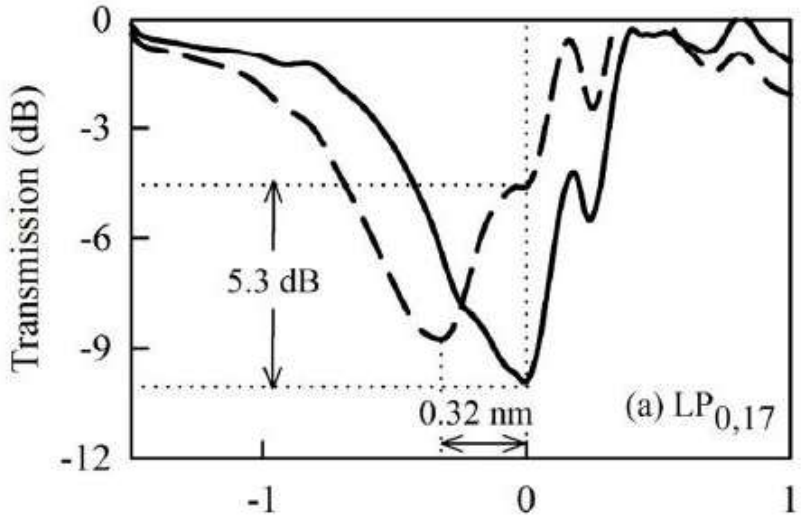
Hybridization of the DNA



Probe: 5'-phosphate-GCA CAG TCA GTC GCC-3'



2 μM complementary: 5'-GGC GAC TGA CTG TGC-3'



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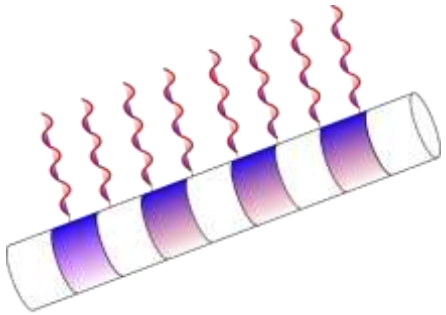

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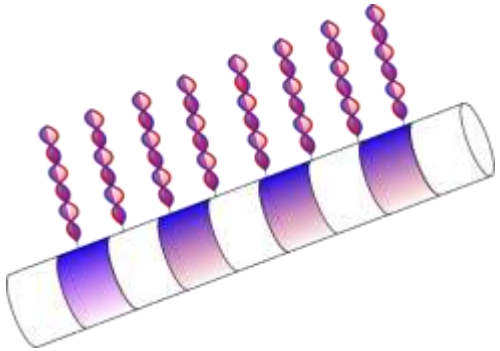
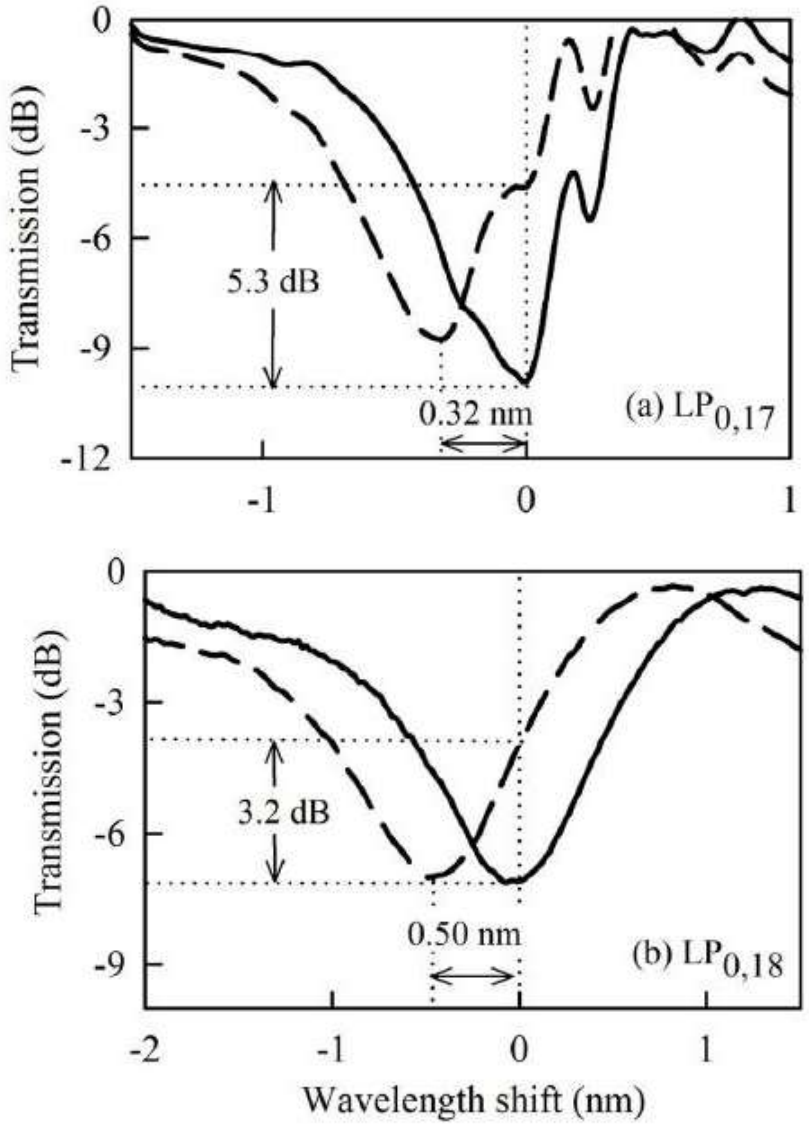
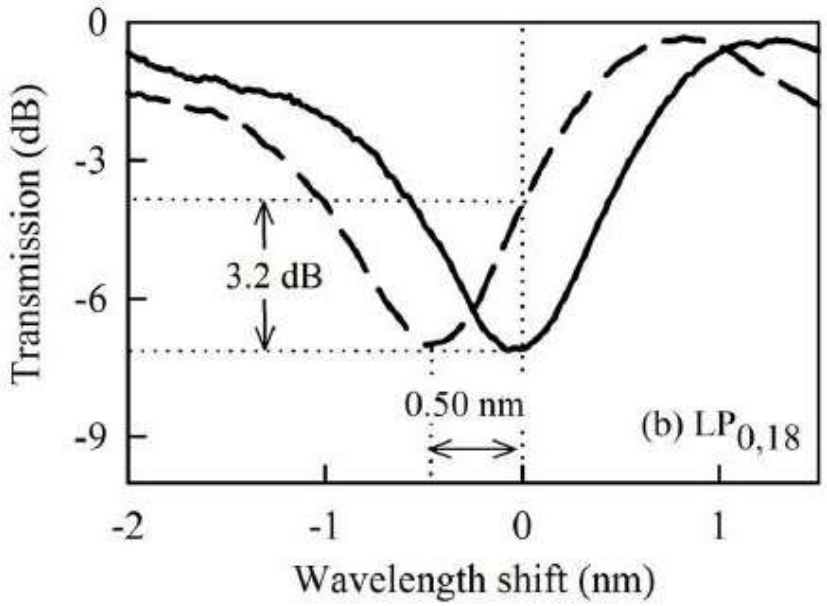


2 μM complementary: 5'-GGC GAC TGA CTG TGC-3'



Hybridization of the DNA



		Sensitivity	Detection Limit
$LP_{0,17}$	Wavelength	160 pm/μM	0.27 μM
	Power	12%/μM	10 nM
$LP_{0,18}$	Wavelength	250 pm/μM	0.24 μM
	Power	11%/μM	10 nM
Chen et al.	Wavelength	522 pm/μM	3.4 μM *(4 nM)*
	Power	2%/μM	50 nM

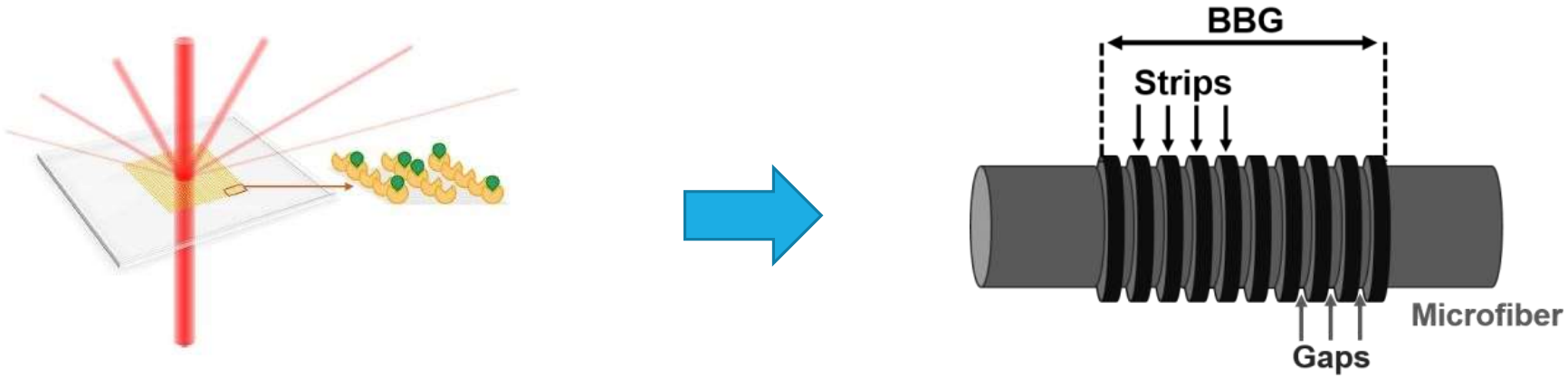
M. Delgado-Pinar et al., *IEEE Sensors Journal*, Vol. 17, pp. 5503-5509, 2017

1. Overview
2. Narrowband in fiber Long Period Grating
3. Bio-Bragg gratings in tapered fibers
4. UV deactivated, planar molecular gratings
5. Conclusions

Bio-Bragg Gratings

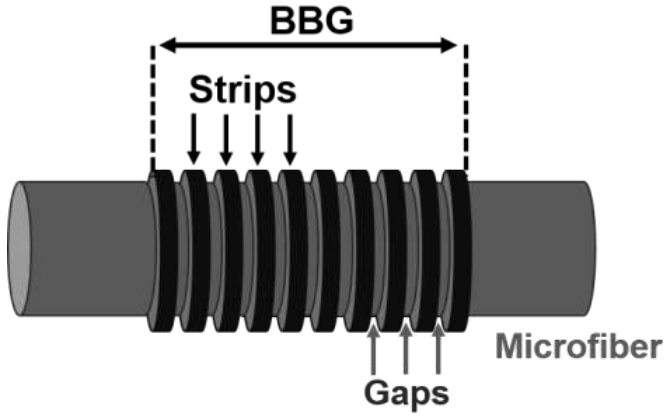
Development of a novel photonic biosensor combining

- Micro-printing of biomolecules on a glass surface [1]
- Optical fiber technology: tapered fibers with large evanescent fields



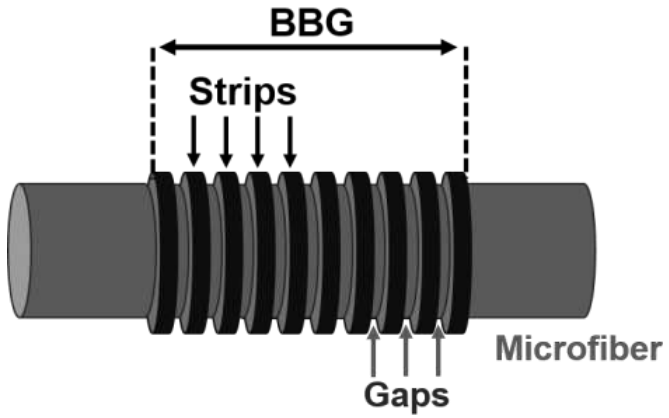
[1] M. Avella-Oliver, M. *et al.*, Anal. Chem. 89, 9002–9008 (2017) https://doi.org/10.1021/acs.analchem.7b01649

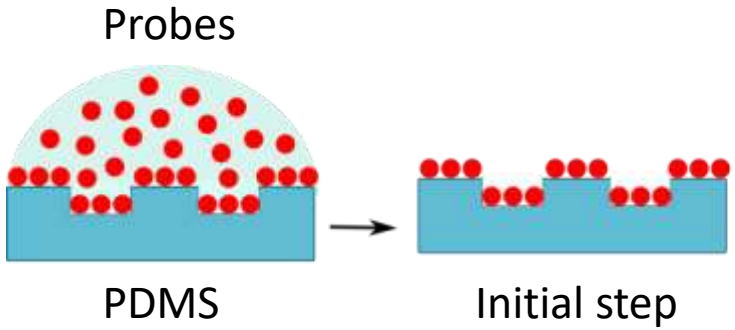
Bio-Bragg Gratings: microstamping

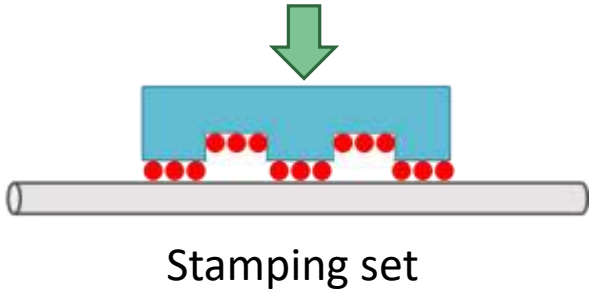


Microfiber: tapered fiber from SMF28
Diameter: 2-5 μm

Bio-Bragg Gratings: microstamping

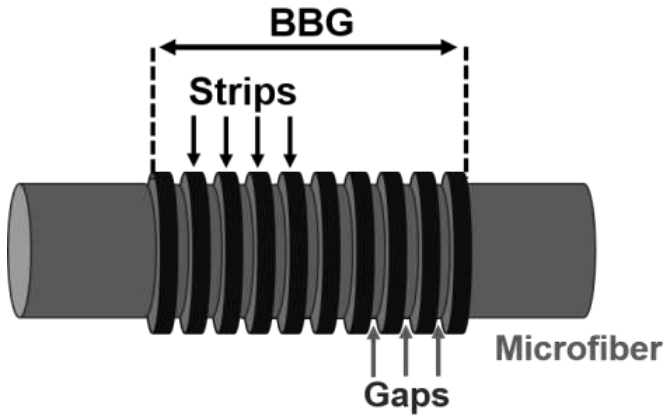


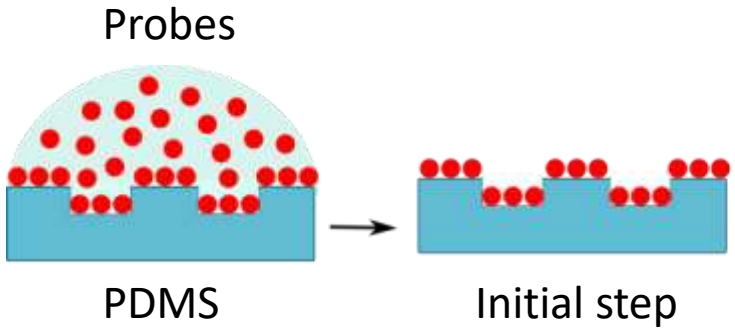


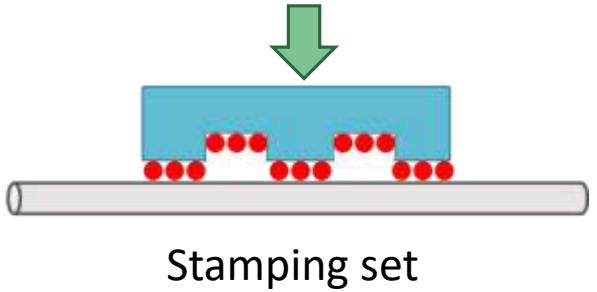


Microfiber: tapered fiber from SMF28
Diameter: 2-5 μm
Biomolecules: BSA/aBSA
PDMS stamp fabricated from a silicon grooved nanostructure, Λ = 555 nm

Bio-Bragg Gratings: microstamping





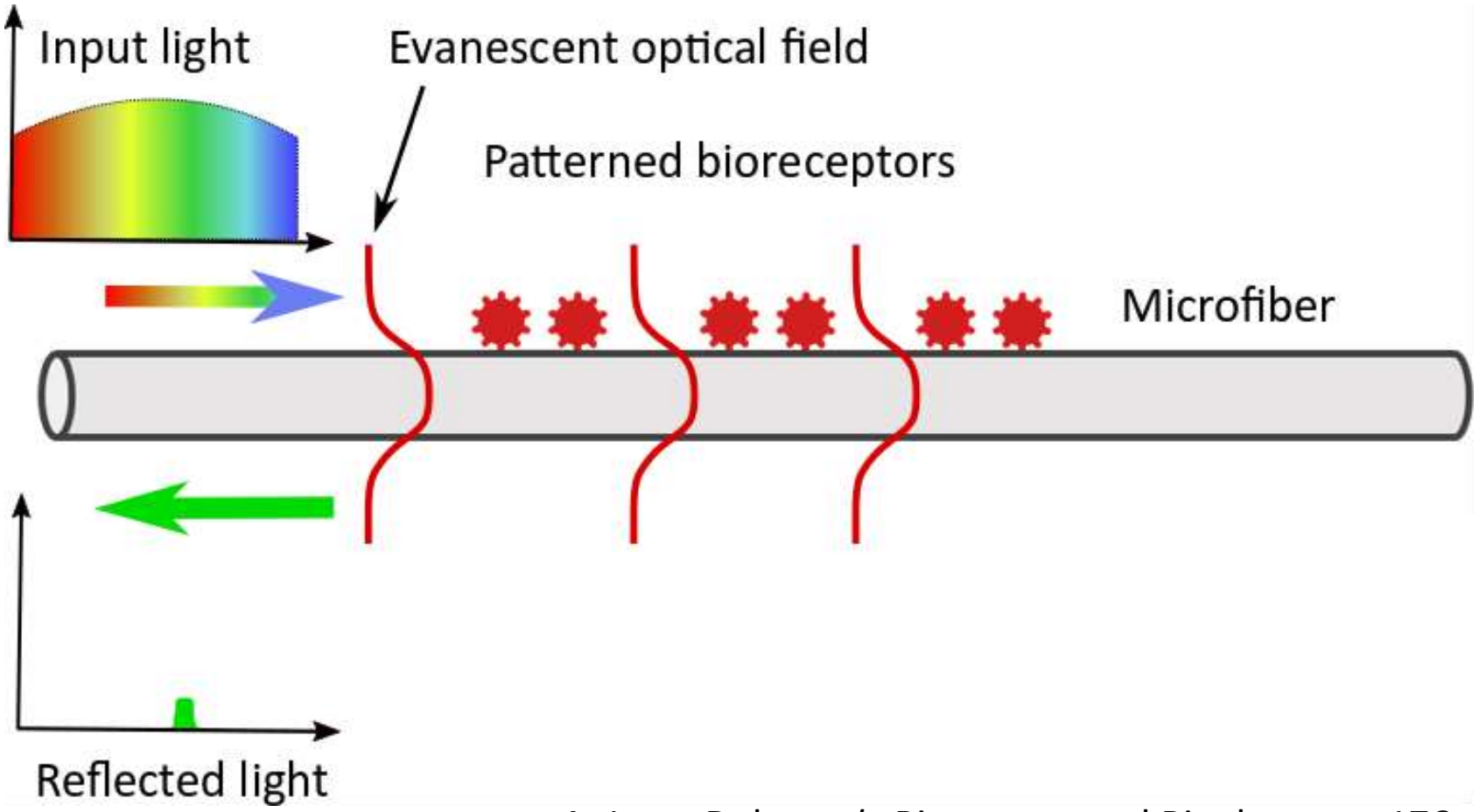


Structured pattern of BSA molecules along the tapered fiber

Microfiber: tapered fiber from SMF28
Diameter: 2-5 μm
Biomolecules: BSA/aBSA
PDMS stamp fabricated from a silicon grooved nanostructure, Λ = 555 nm

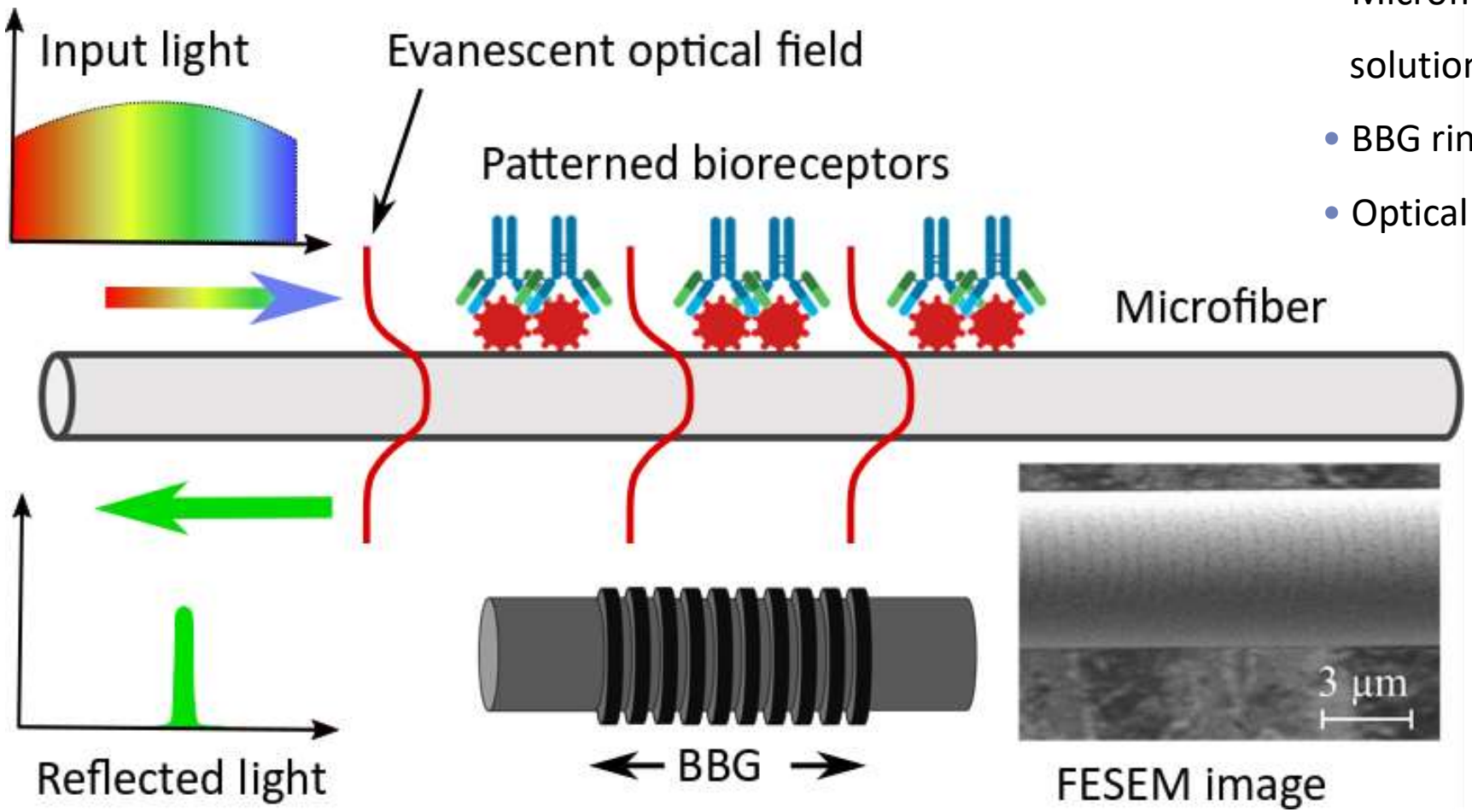
Bio-Bragg grating

Bio-Bragg Gratings: operation principle



A. Juste-Dolz *et al.,* Biosensors and Bioelectron., 176, 112916 (2021)

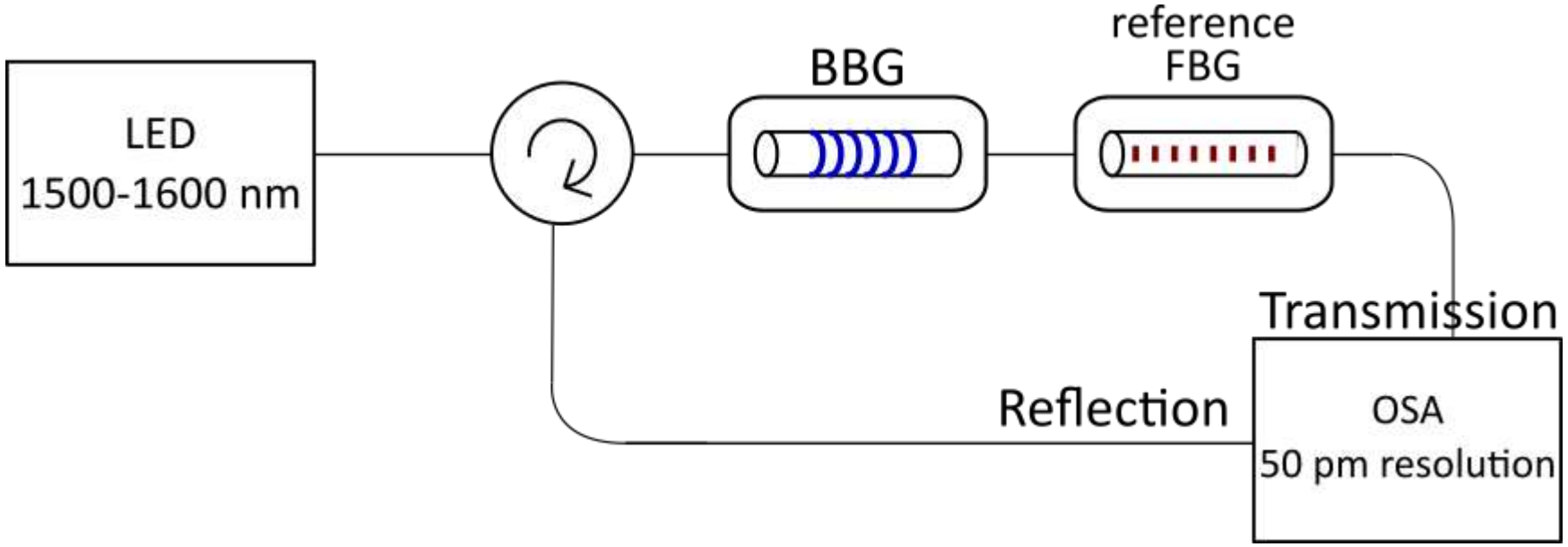
Bio-Bragg Gratings: operation principle



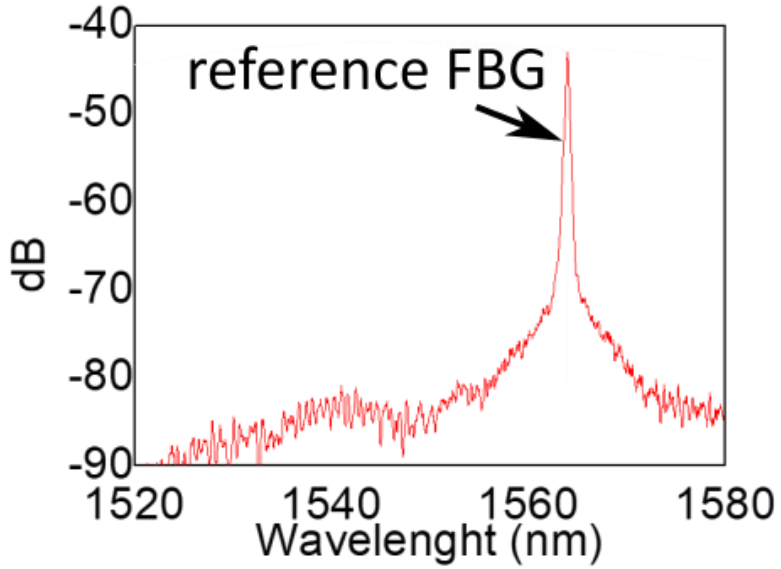
Immunoassay

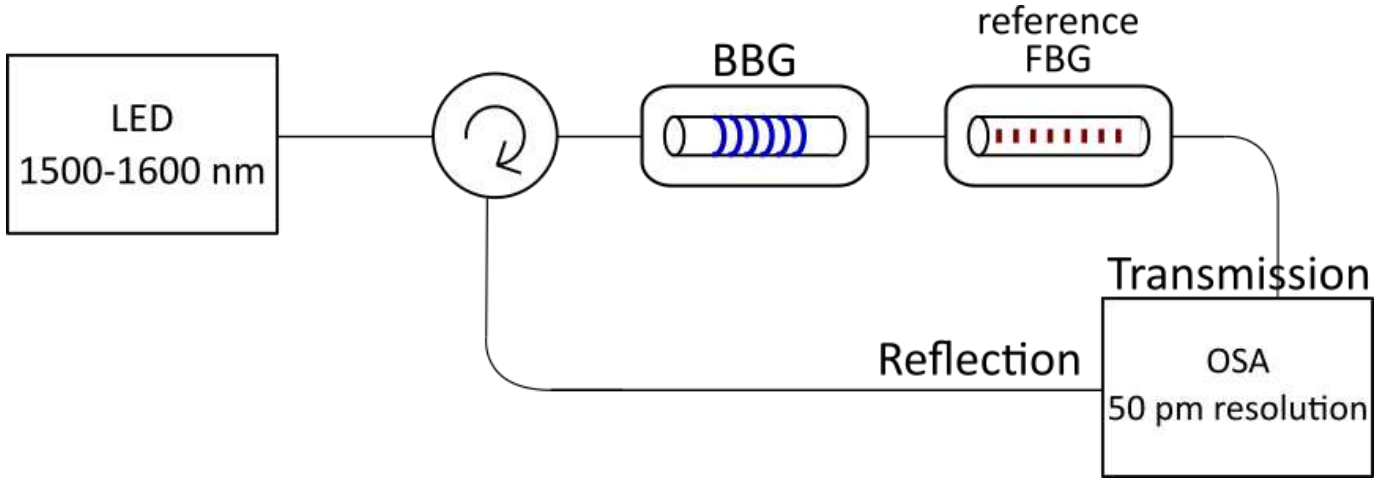
- Microfiber immersed in PBS-T solutions of aBSA for 30’
- BBG rinsed with PBS-T and air dried
- Optical response measured in air

Bio-Bragg Gratings: characterization



Bio-Bragg Gratings: characterization





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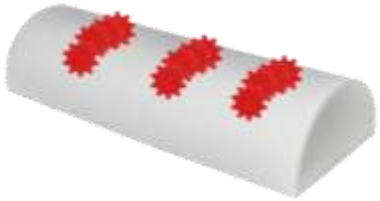
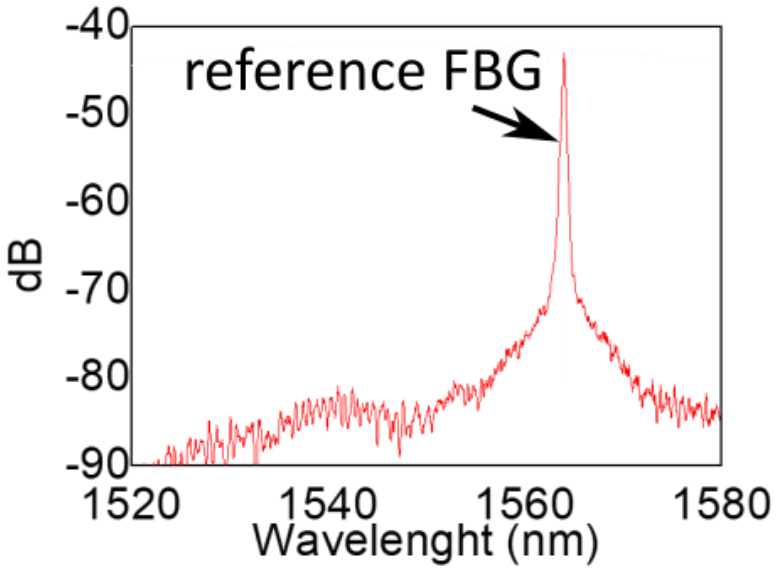
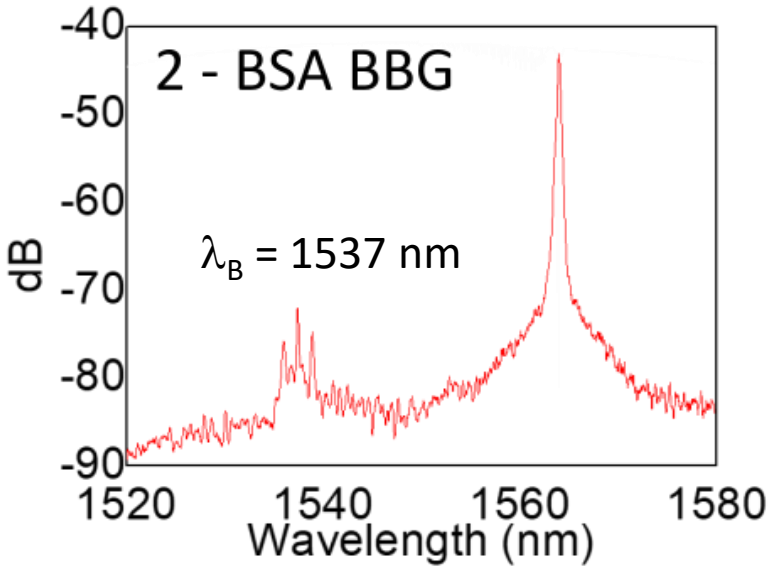
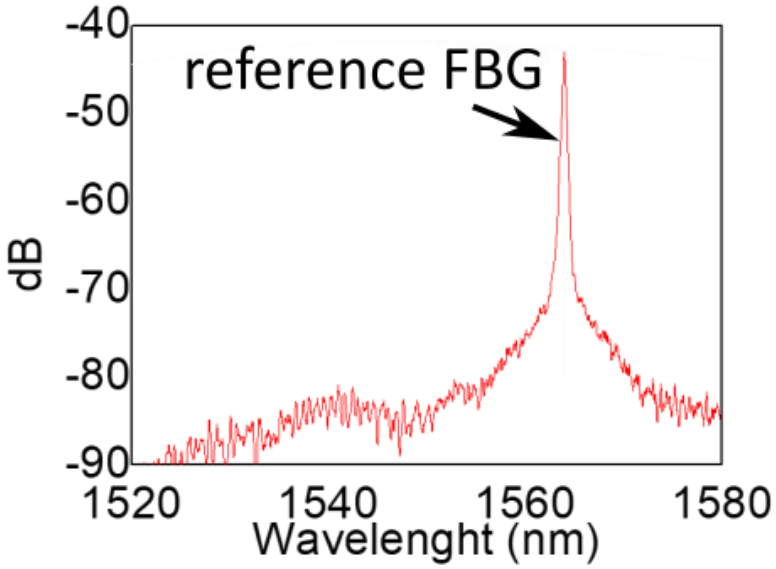
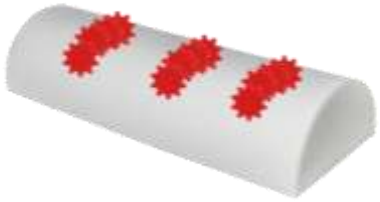


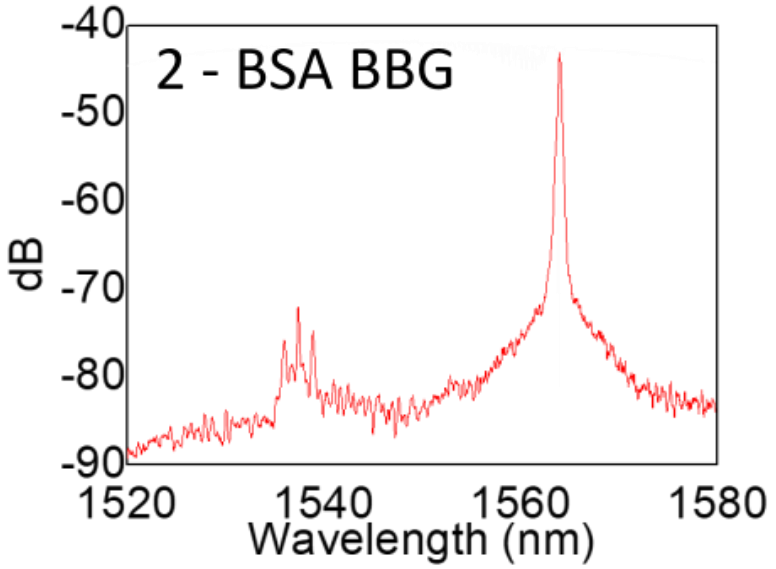


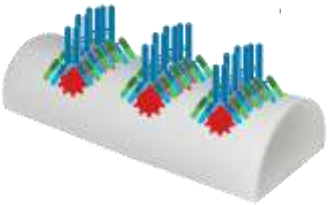


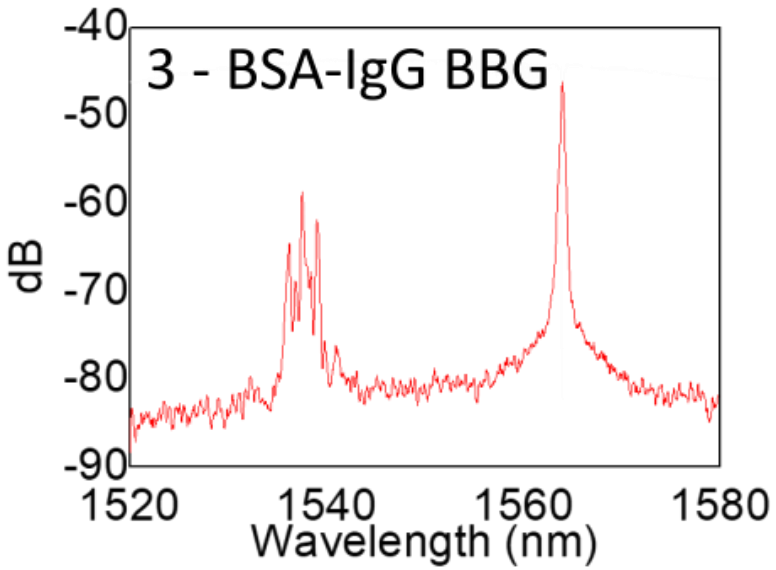
Bio-Bragg Gratings: characterization











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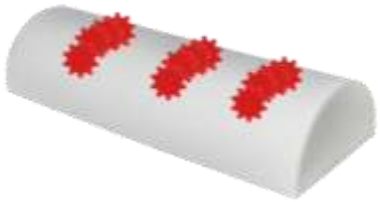
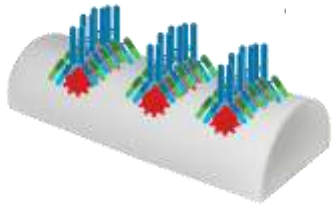
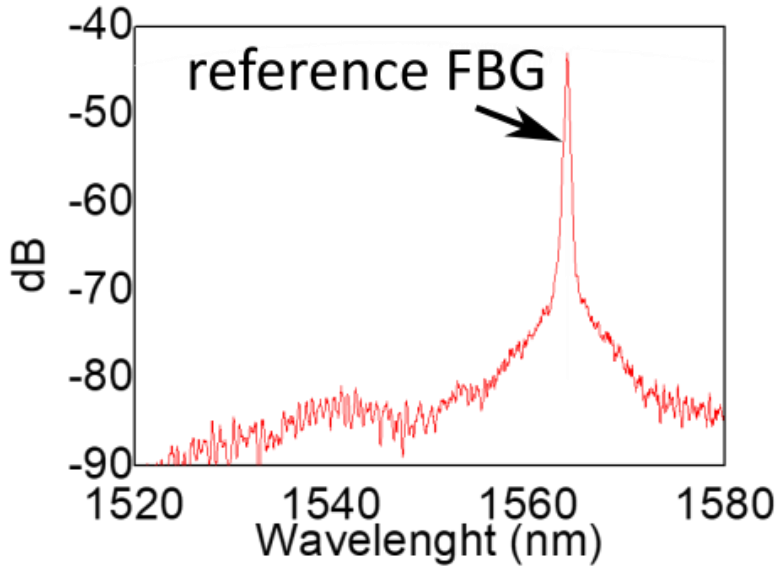
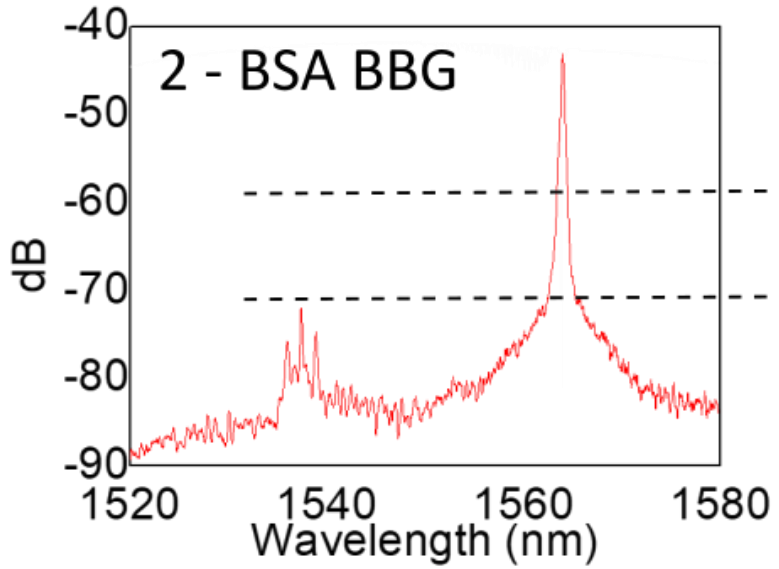
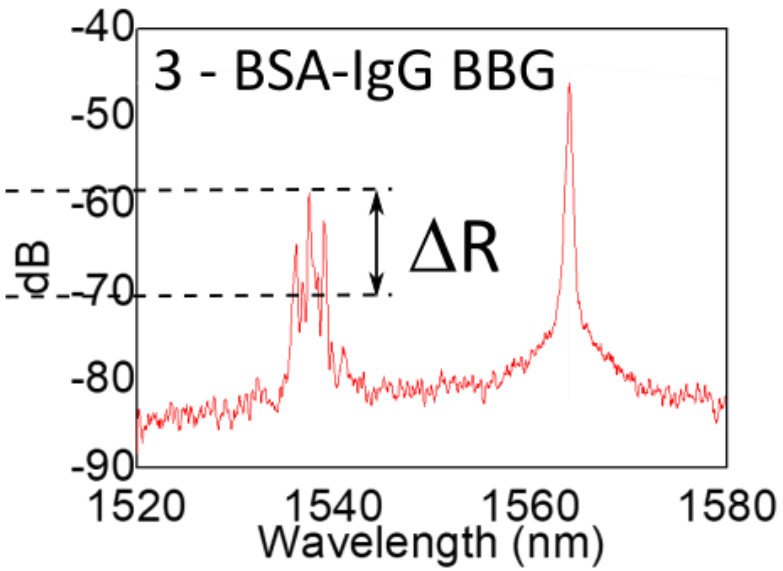


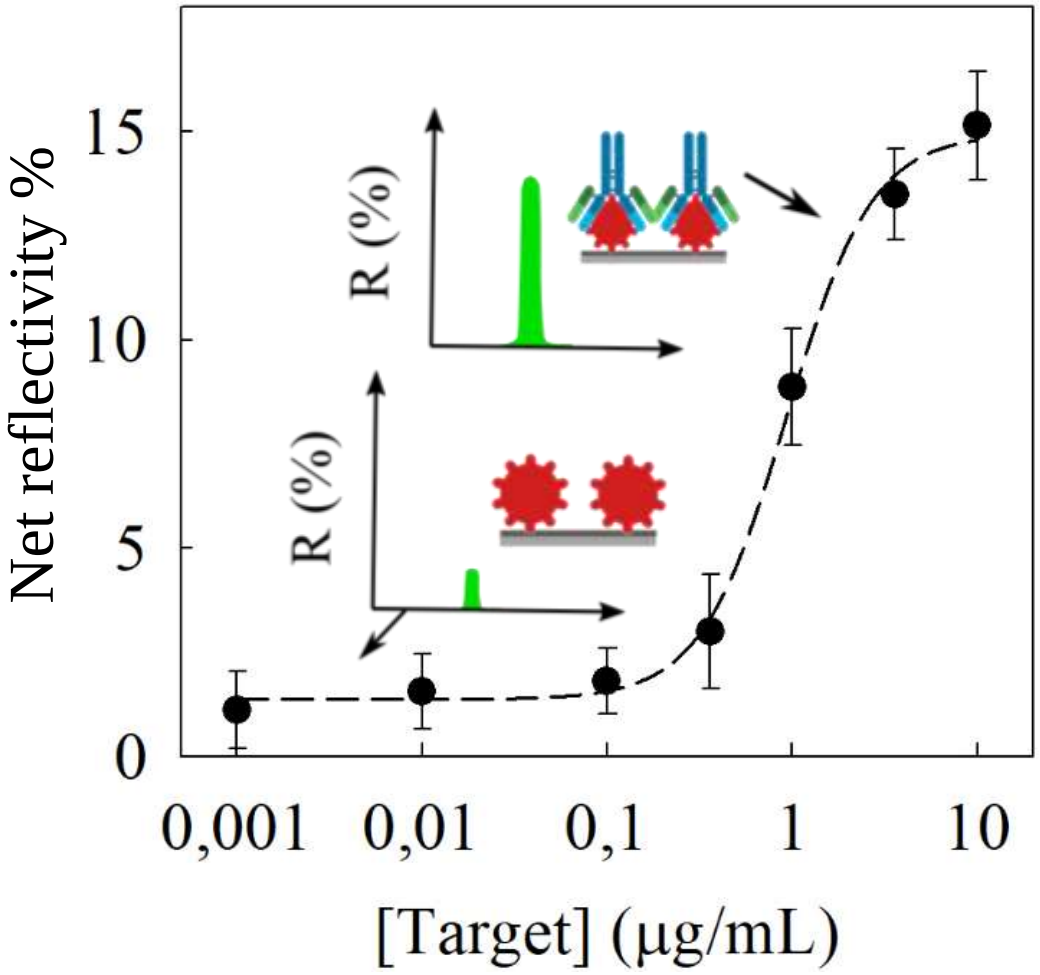








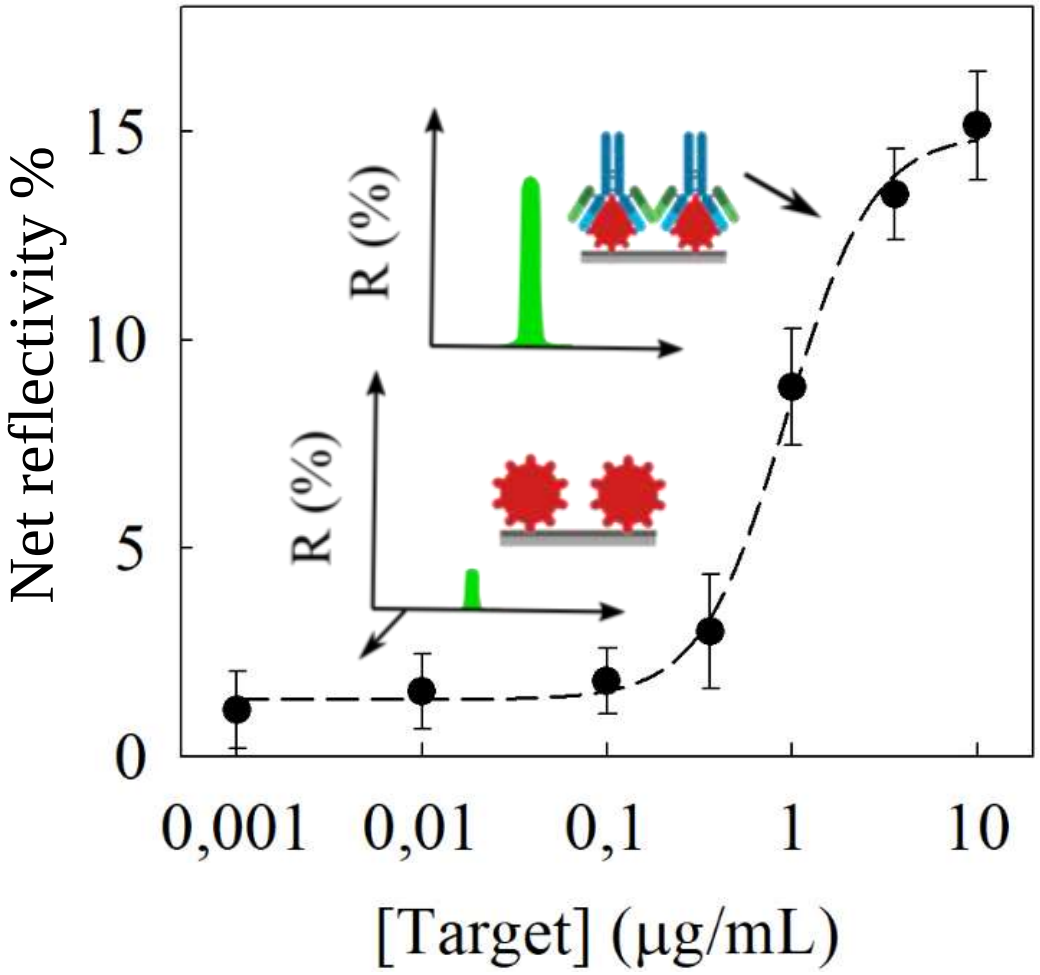
Bio-Bragg Gratings: immunoassay



A set of 3 µm microfibers were individually fabricated and tested (3 devices per concentration) to perform a BSA-IgG immunoassay in label-free conditions, PBS-T buffer.

Experimental data are fitted to a sigmoidal (logistic four-parameters) regression, $R^2 = 0.997$.

Bio-Bragg Gratings: immunoassay



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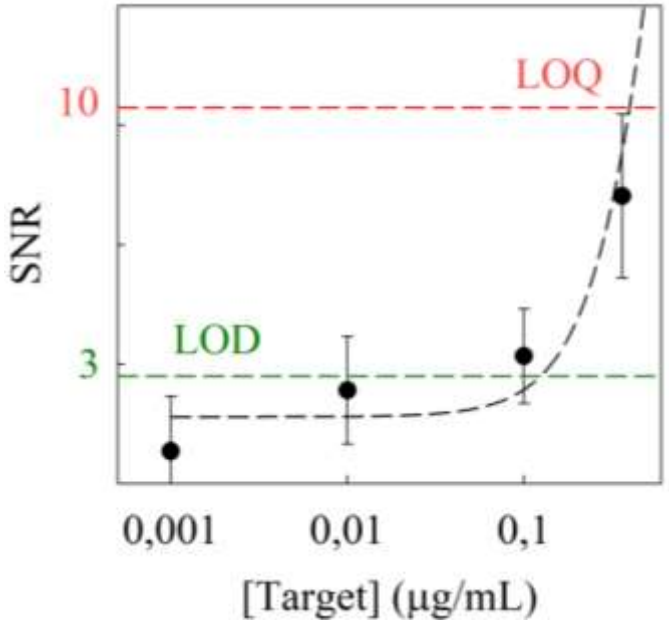


LOD 0.1 µg/mL
LOQ 0.4 µg/mL

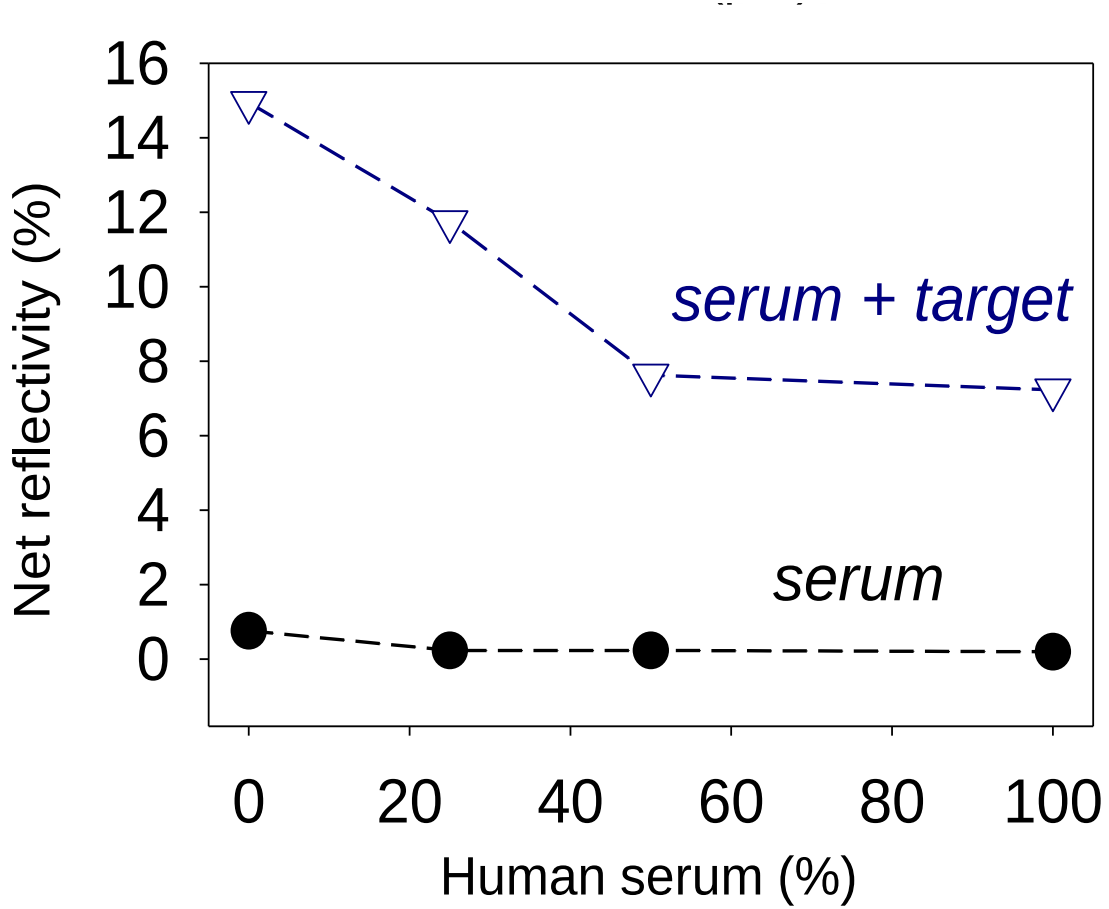
Bio-Bragg Gratings: immunoassay

Non specific bindings

Comparison between solutions of BSA (10 μg/mL) in PBS-T, and dilutions of human serum in PBS-T (7% of non-specific proteins, potentially interfering lipids, etc.)

Net reflectivity drops by half when pure human serum was used.

Serum dilutions without targets do not increase the reflectivity achieved by the BSA-BBG.



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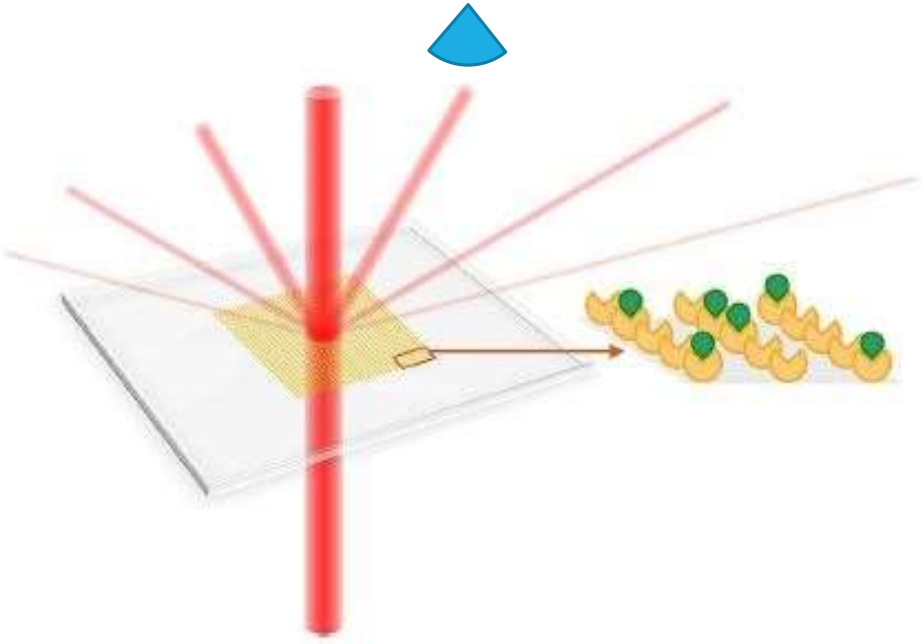
Planar molecular gratings

chip compatible: integrated photonic circuits

Intensity ⟶ Antibody concentration



Planar molecular gratings

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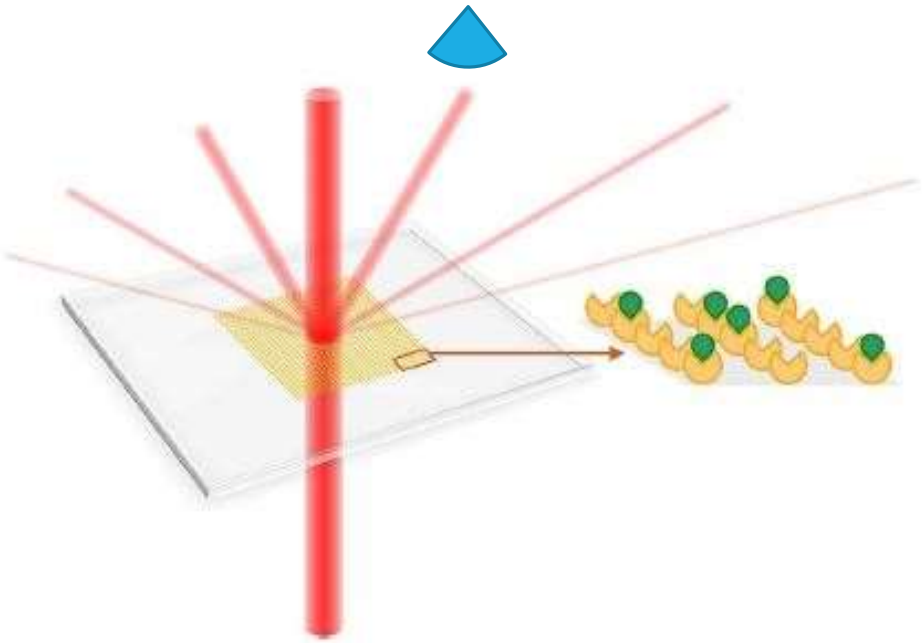
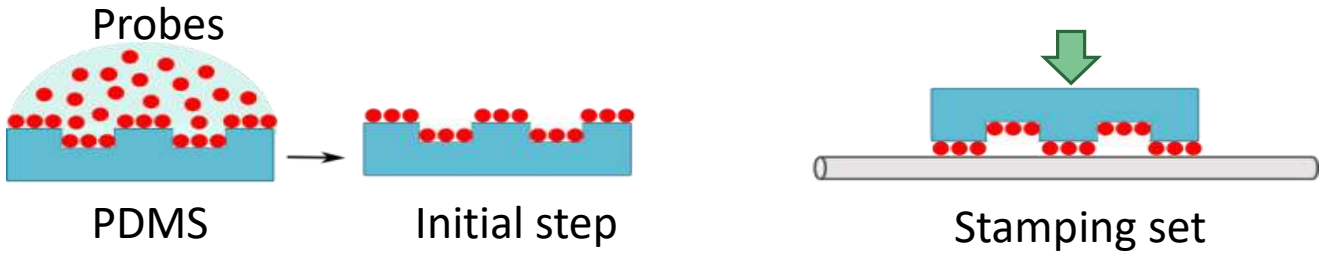


Microstamping /microcontact printing



M. Avella-Oliver, M. *et al*., Anal. Chem. 89, 9002–9008 (2017)

Planar molecular gratings

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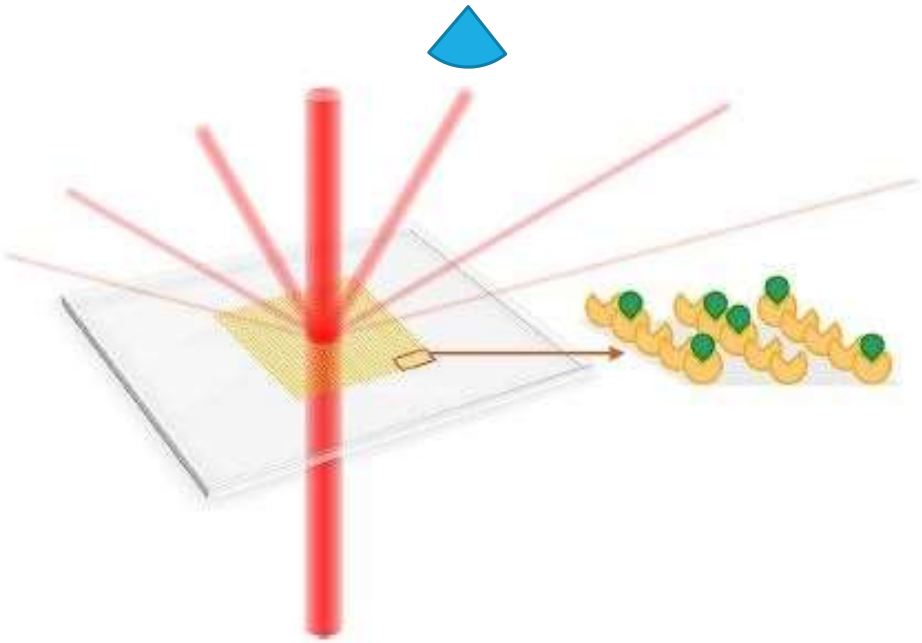

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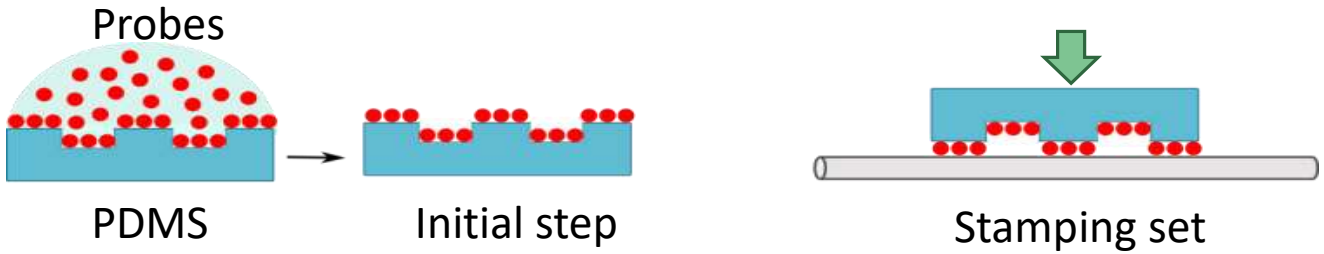
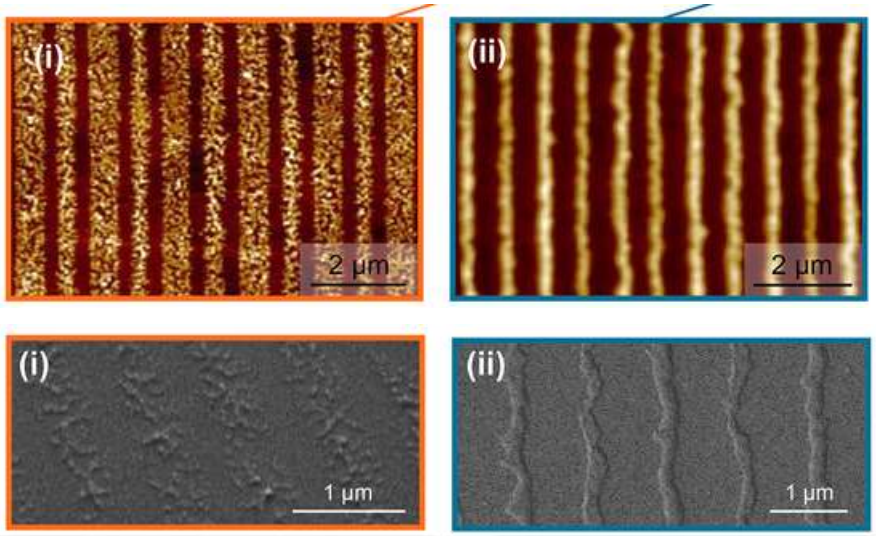


AFM images

FESEM images



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Planar molecular gratings

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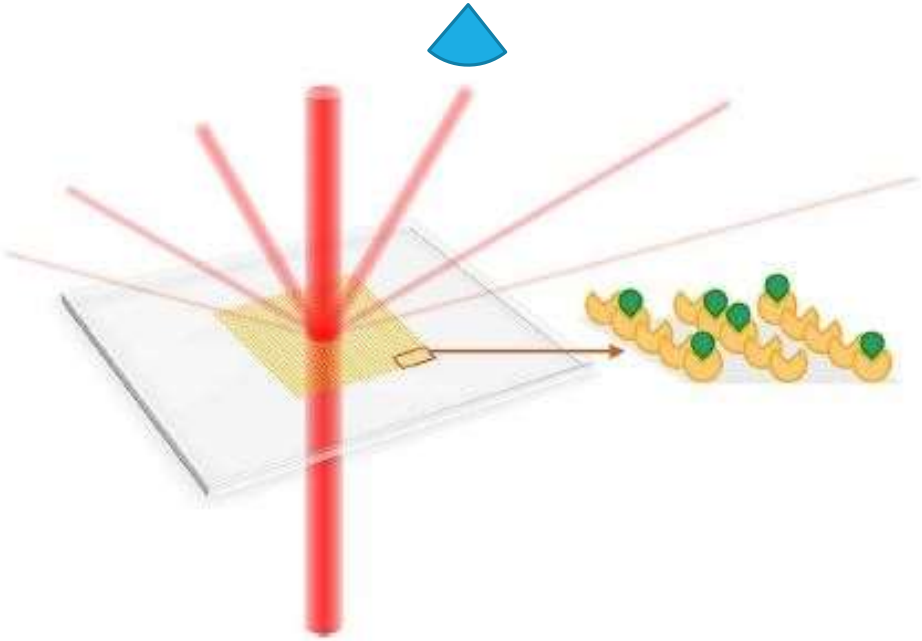
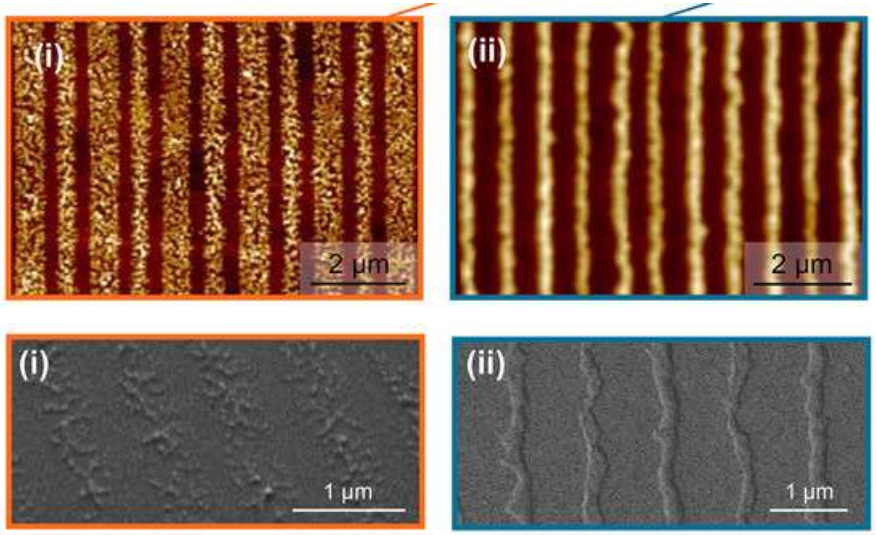


Poor quality of the diffracted beam (chirp in the fiber-BBG)

We can improve this!

AFM images

FESEM images



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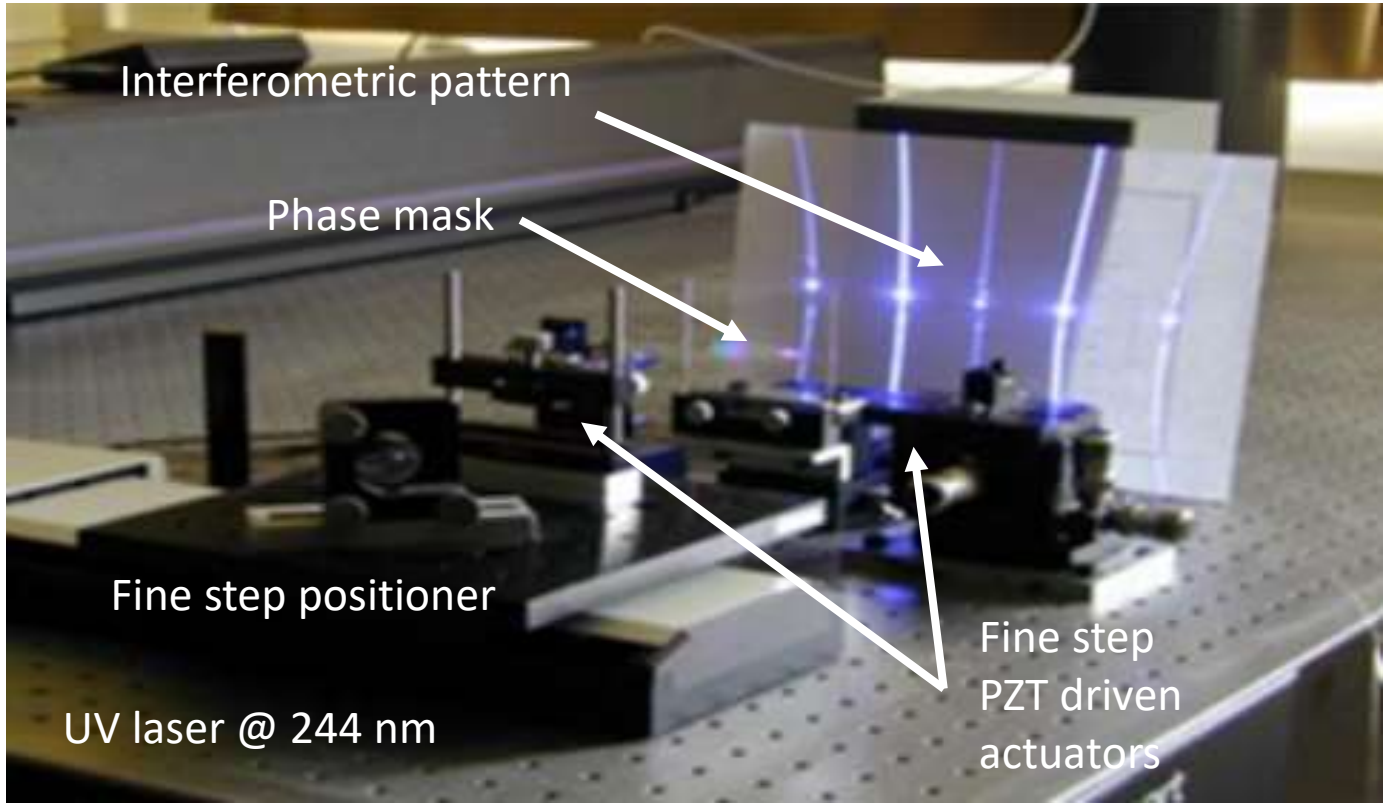
UV radiation system to write in-fiber FBGs

A. Juste-Dolz *et al.*, *ACS Appl. Mater. Interfaces* 2022, 14, 36, 41640–41648

Phase mask period: 710 nm

Controllable fluence of UV by means of the emitted power and the sweeping speed of the UV beam along the phase mask.

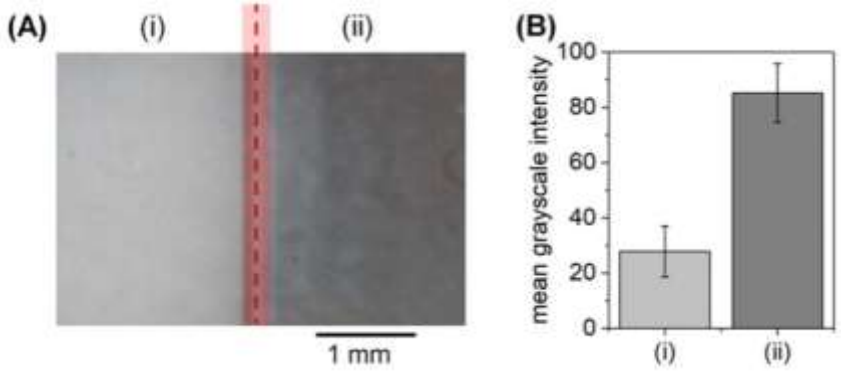


UV radiation system to write in-fiber FBGs

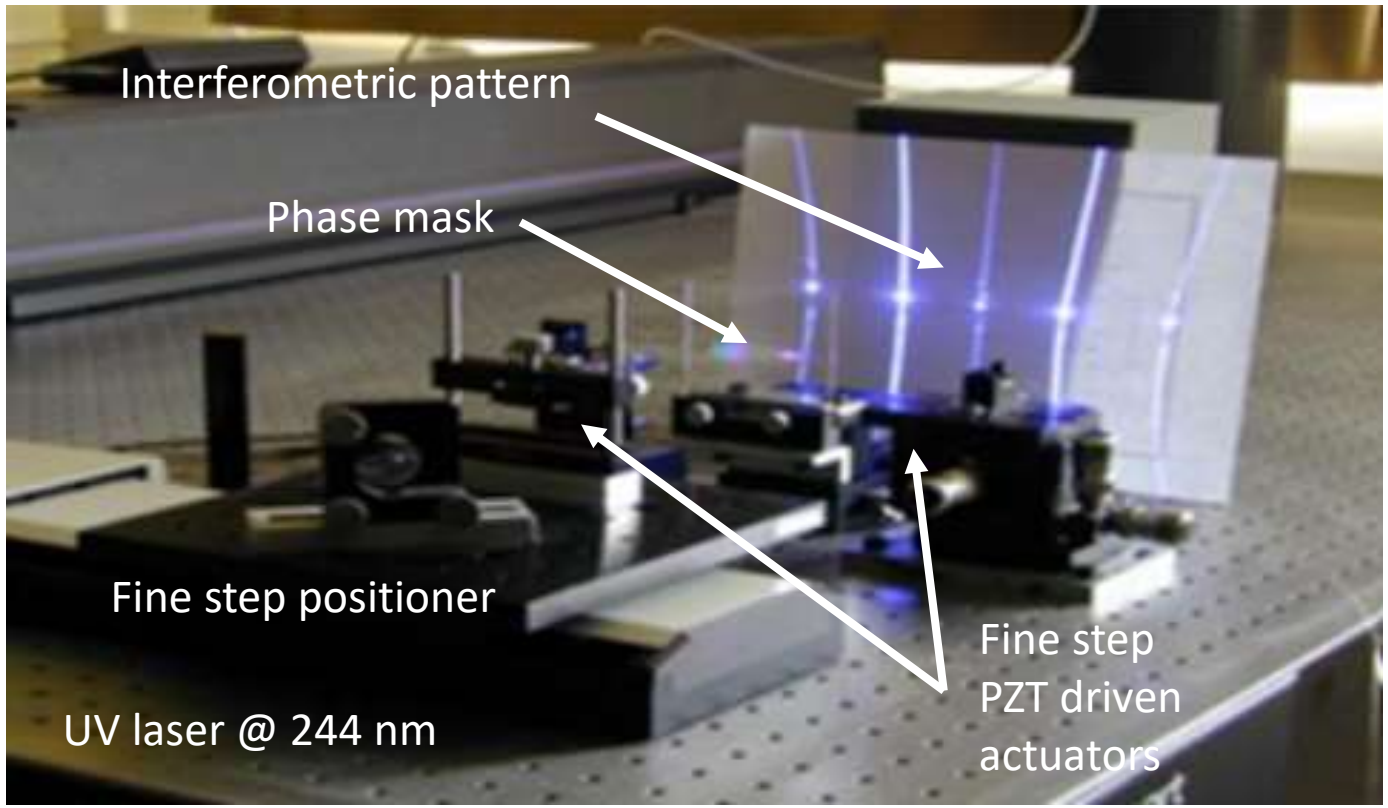
Phase mask period: 710 nm

Controllable fluence of UV by means of the emitted power and the sweeping speed of the UV beam along the phase mask.

Biological molecules can be deactivated by means of UV radiation



A. Juste-Dolz *et al.*, *ACS Appl. Mater. Interfaces* 2022, 14, 36, 41640–41648



Gold labelled BSA/aBSA immunoassay; (i) fluence 66 J/cm^2 – (ii) non irradiated

UV radiation system to write in-fiber FBGs

A. Juste-Dolz *et al*., *ACS Appl. Mater. Interfaces* 2022, 14, 36, 41640–41648

Expectatives:

better uniformity of the strips than when using microcontact printing

better beam quality

UV radiation system to write in-fiber FBGs

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improved performance?

UV radiation system to write in-fiber FBGs

Expectatives:

better uniformity of the strips than wen using microcontact printing
better beam quality

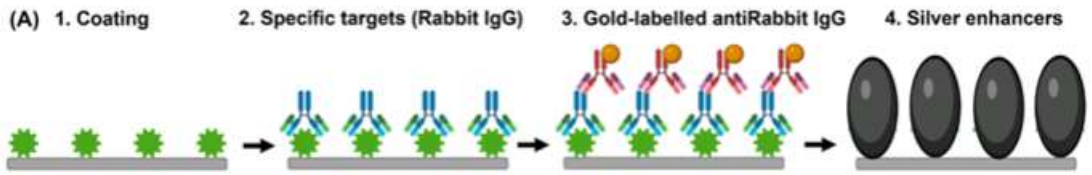
¿improved performance?

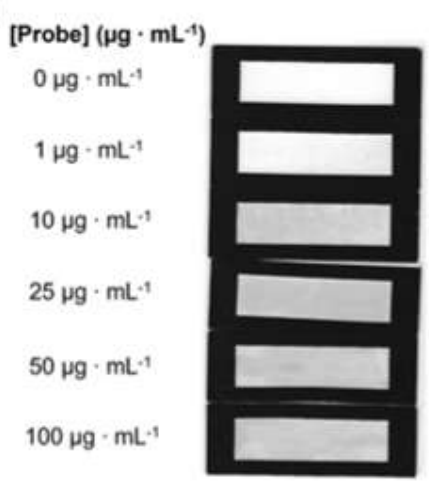
Work to do:

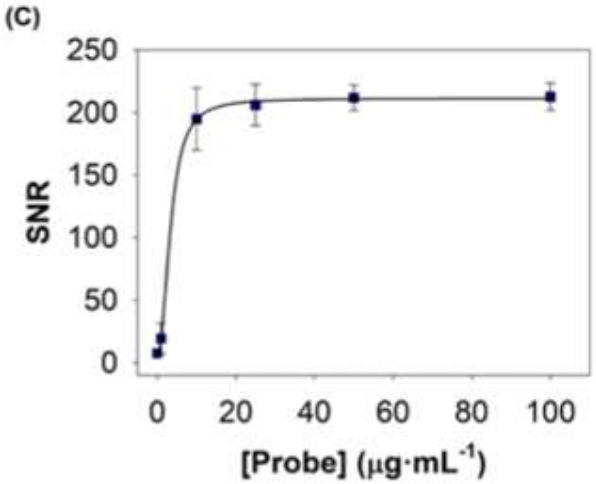
optimization of the fabrication parameters
immunoassays for demonstrating the performance of the biosensor

Fabrication: optimization of parameters

BSA coating: optimization of the concentration covering the silica substrate (biolayer)





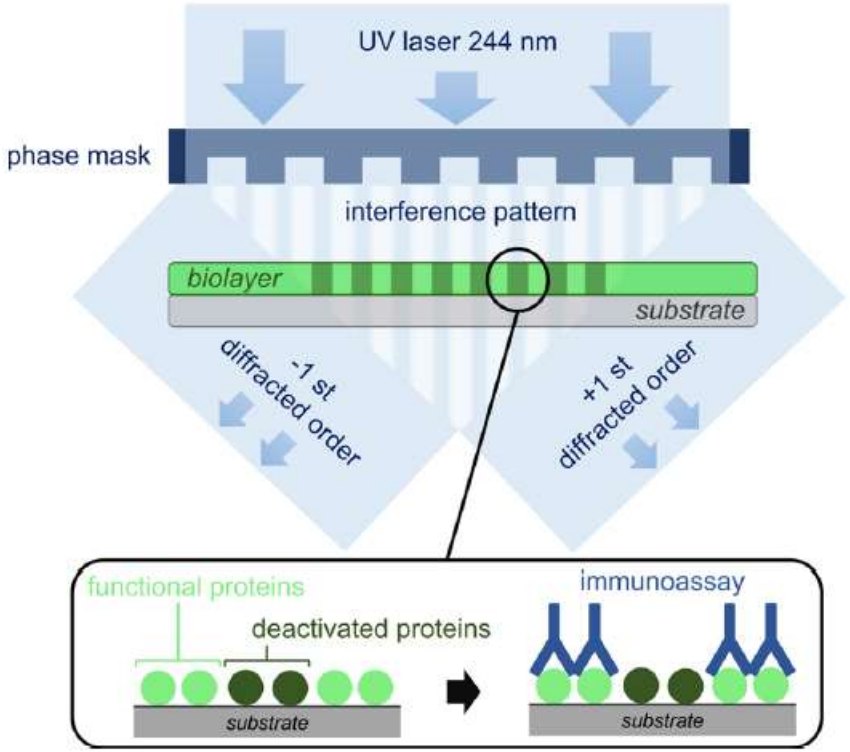


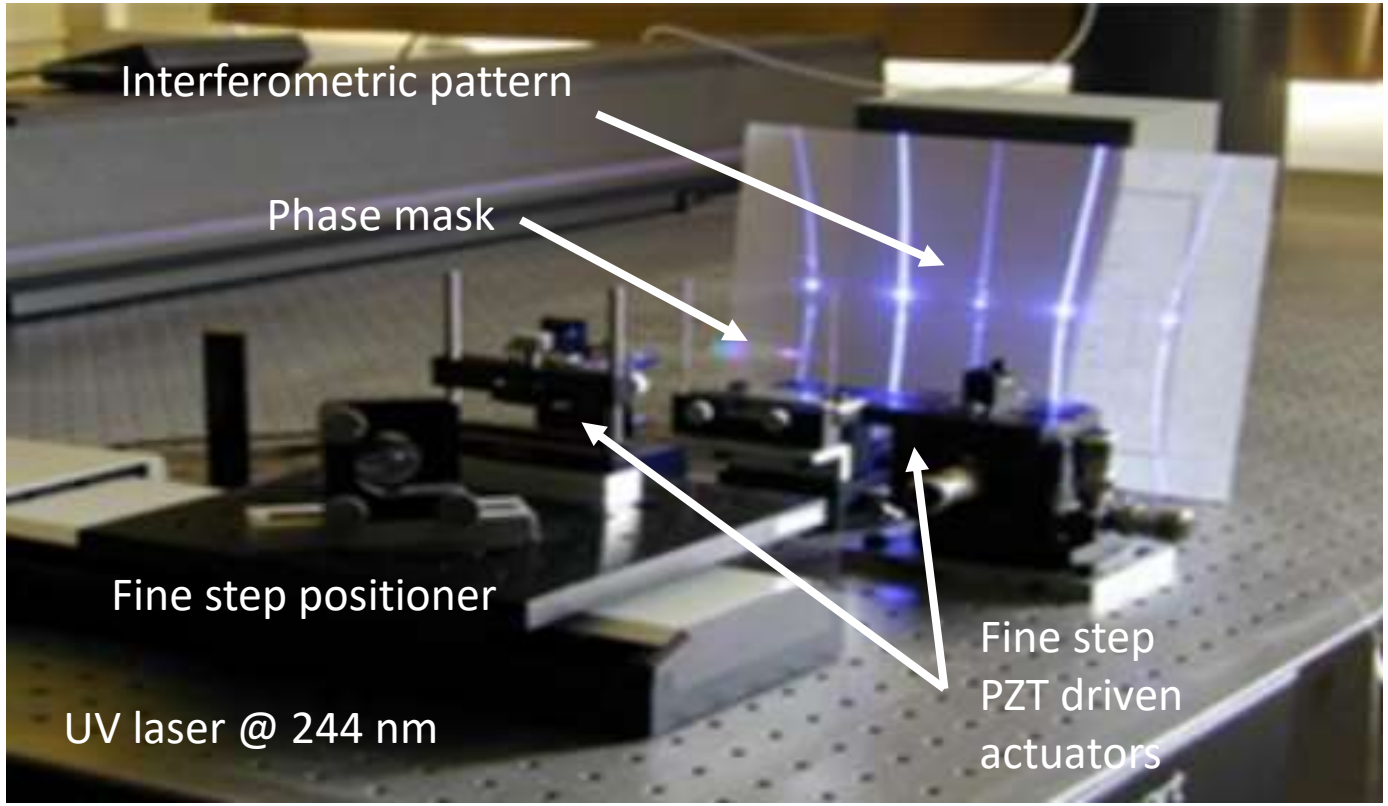
Different BSA concentrations: 0-100 µg/mL

Specific rabbit, gold labelled IgGs: 10 µg/mL

Not irradiated

UV radiation of uniform biolayers

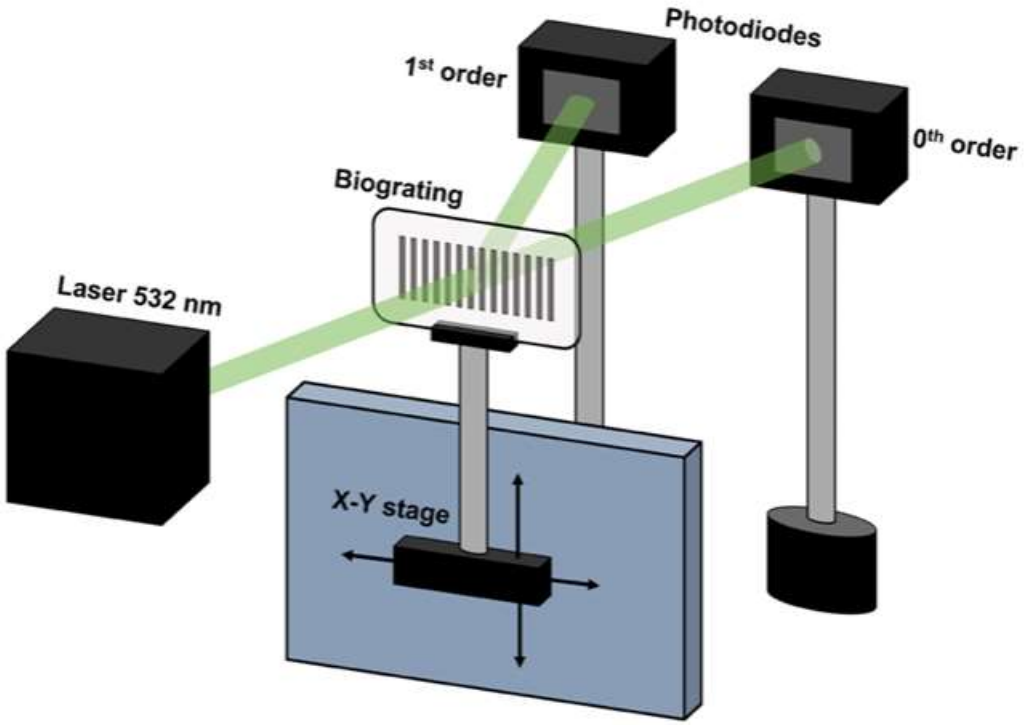




Control of the laser power and the sweeping speed for getting different UV fluences

Fabrication: optimization of parameters

Interrogation setup of the molecular gratings

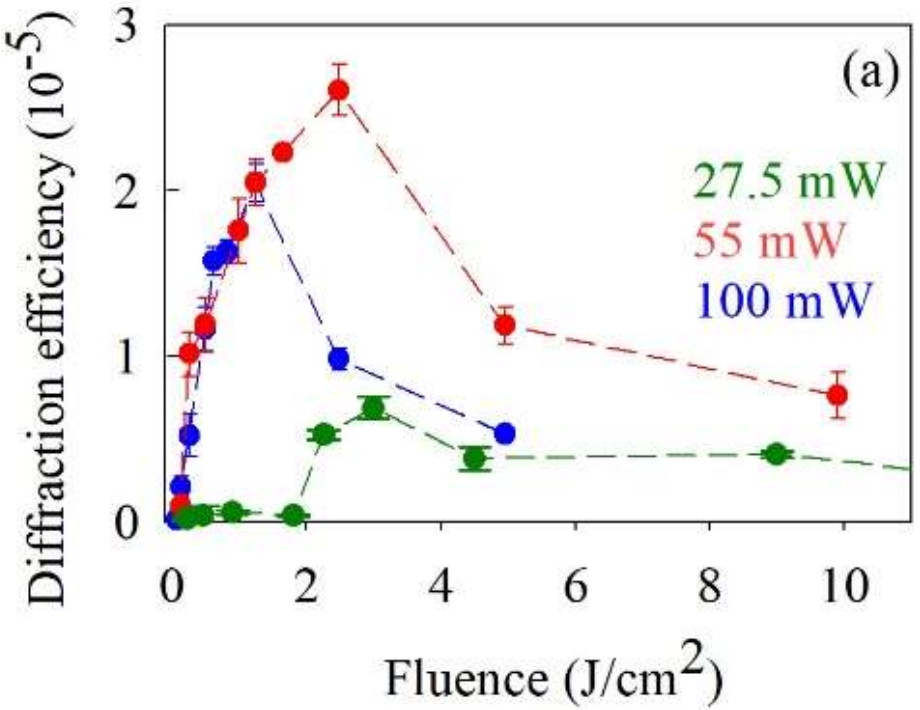


Unlabelled IgGs of different concentrations

Measurement the diffraction efficiency of the first order

Fabrication: optimization of parameters

Deactivation of BSA molecules: optimization of the UV fluence



Diffraction efficiency after incubating IgGs 10 μg/mL

Different powers, different speeds.

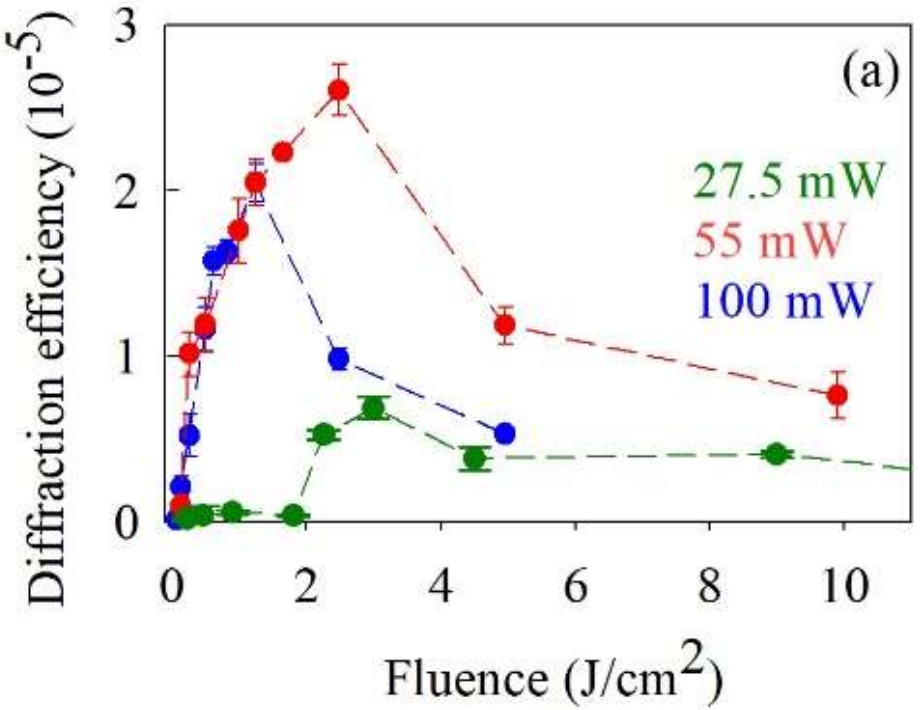
Low power regime: molecules were not deactivated even at low speeds.

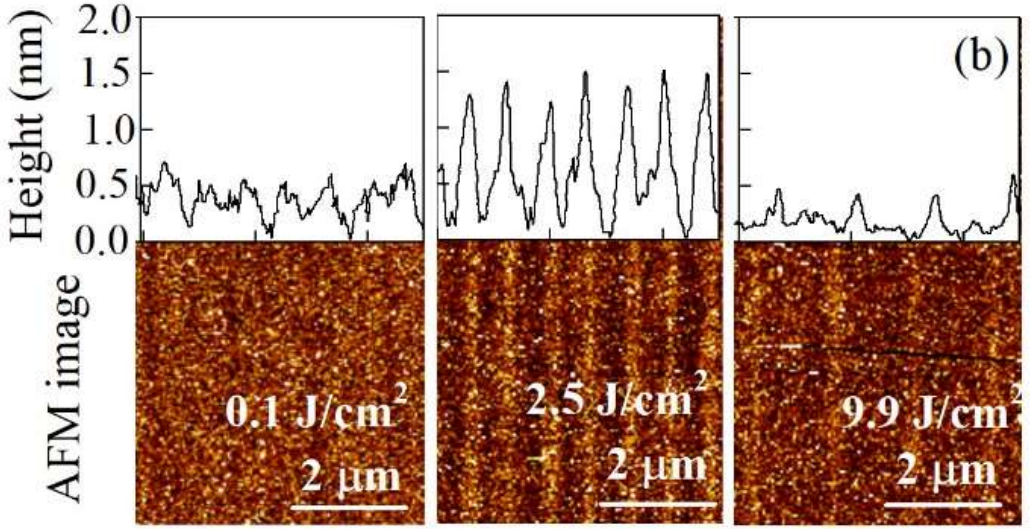
High power regime: molecules were over-deactivated, even though no ablation was detected.

Medium power regime: best diffraction results.

Fabrication: optimization of parameters

Deactivation of BSA molecules: optimization of the UV fluence



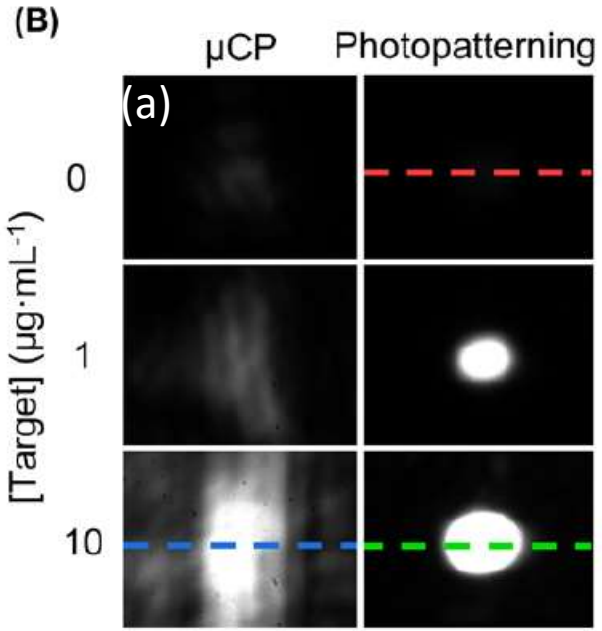


Period: 710 nm /1420 nm

Topological characterization: AFM images (55 mW)

Fabrication: optimization of parameters

Characterization of the molecular grating performance



Left: microcontact printing
Right: UV deactivation

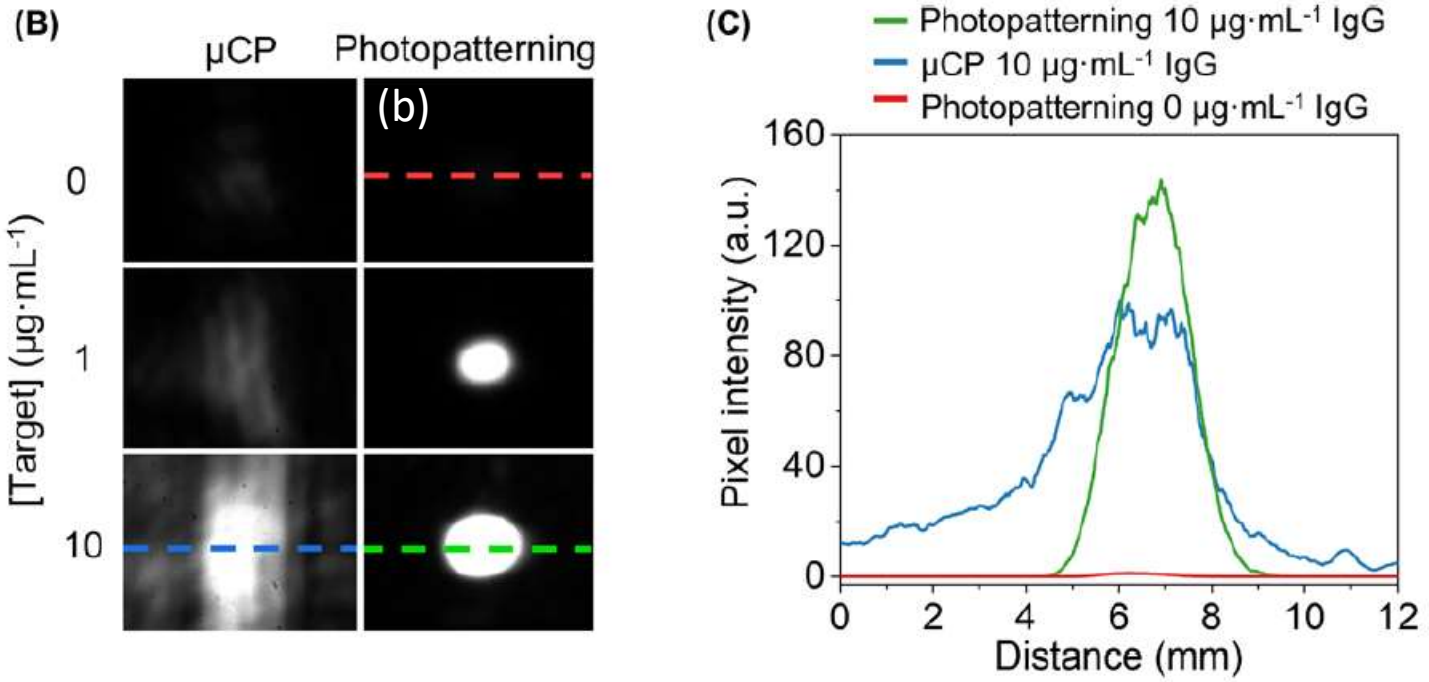
(a) BSA pattern: there exists a “zero response” for μcontact printing

The first-order beam is more defined when UV-deactivation is employed

(some images are saturated to fix the interrogation parameters)

Fabrication: optimization of parameters

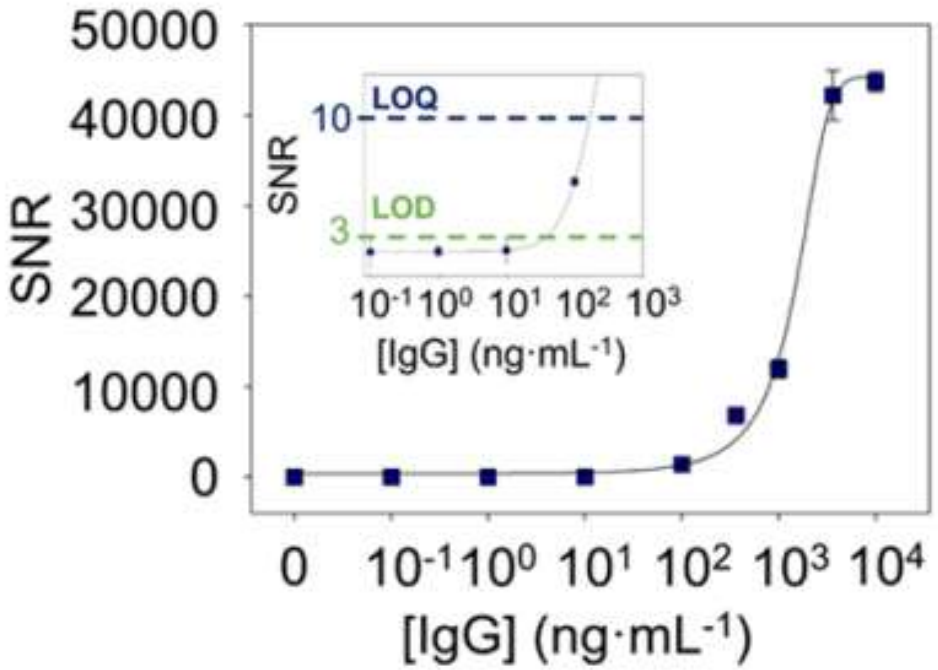
Characterization of the molecular grating performance



(b) uniform BSA biolayer: there is not a topological change: this will result in an improved detection limit.

Fabrication: optimization of parameters

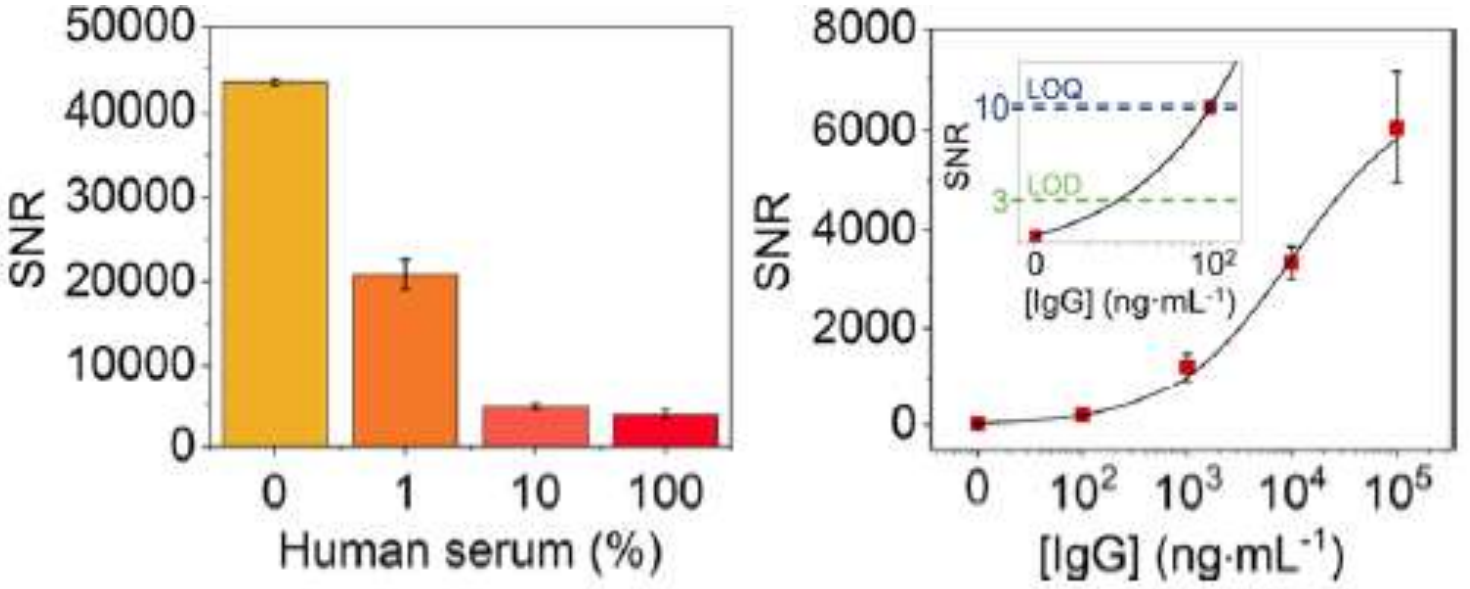
Immunoassay: LOD and LOQ



LOD: 53 ng/mL (0.4 nM)
LOQ: 164 ng/mL (1.1 nM)
anti-BSA IgG in PBS-T

Fabrication: optimization of parameters

Immunoassay: human serum



LOD: 36 ng/mL (0.3 nM)
LOQ: 100 ng/mL
anti-BSA IgG in human serum

Lower than in buffer!

LOD: 53 ng/mL (0.4 nM)
LOQ: 164 ng/mL (1.1 nM)
anti-BSA IgG in PBS-T

1. Overview
2. Narrowband in fiber Long Period Grating
3. Bio-Bragg gratings in tapered fibers
4. UV deactivated, planar molecular gratings
5. Conclusions

1. Design of diffractive biosensors: three proposals

Narrow LPG

Bio-Bragg grating

UV molecular grating

2. Use of resonant phenomena/diffractive elements: avoid the ambiguity inherent to interferometric designs

3. Label-free performance

State of the art

Technique	Target	LOD	Matrix	Reference
SPR	ssDNA	0.1 nM	Buffer	An, N. et al., *Talanta* 2021, 231, 122361.
SPR	HSA	100 ng/mL	Buffer	Makhneva, E. et al., *Anal. Bioanal. Chem.* 2019, 411 (29), 7689–7697.
Focal molography	IgG	1.3 nM	Human Plasma	Gatterdam, V. et al., *Nat. Nanotechnol.* 2017, 12 (11), 1089–1095.
Turning point LPG	DNA	4 nM	Buffer	Chen et al., *J Biosens Bioelectron* 2015, 6:2
Narrow LPG	**DNA**	**10 nM**	**Buffer**	**M. Delgado-Pinar et al., *IEEE Sensors Journal*, Vol. 17, pp. 5503-5509, 2017**
Bio-Bragg grating	**IgG**	**100 ng/mL**	**Buffer**	**A. Juste-Dolz et al., *Biosensors and Bioelectron.*, 176, 112916 (2021)**
UV molecular grating	**IgG**	**53 ng/mL (0.4 nM)**	**Buffer**	**A. Juste-Dolz et al., *ACS Appl. Mater. Interfaces* 2022, 14, 36, 41640–41648**
UV molecular grating	**IgG**	**36 ng/mL (0.3 nM)**	**Human Serum**	**A. Juste-Dolz et al., *ACS Appl. Mater. Interfaces* 2022, 14, 36, 41640–41648**

Many thanks!

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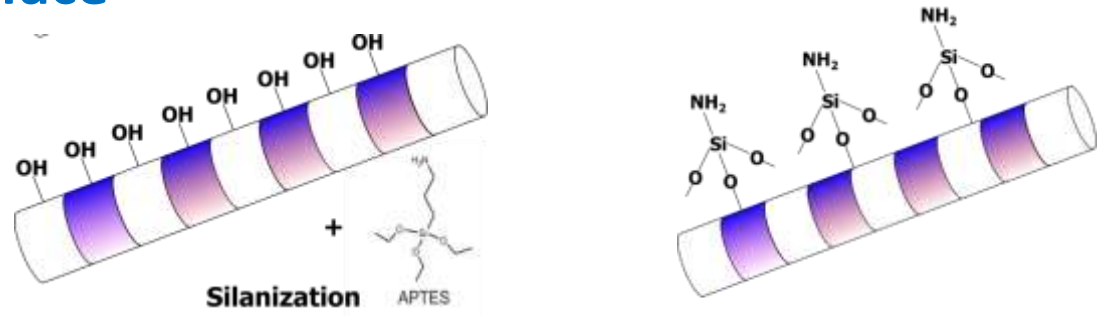
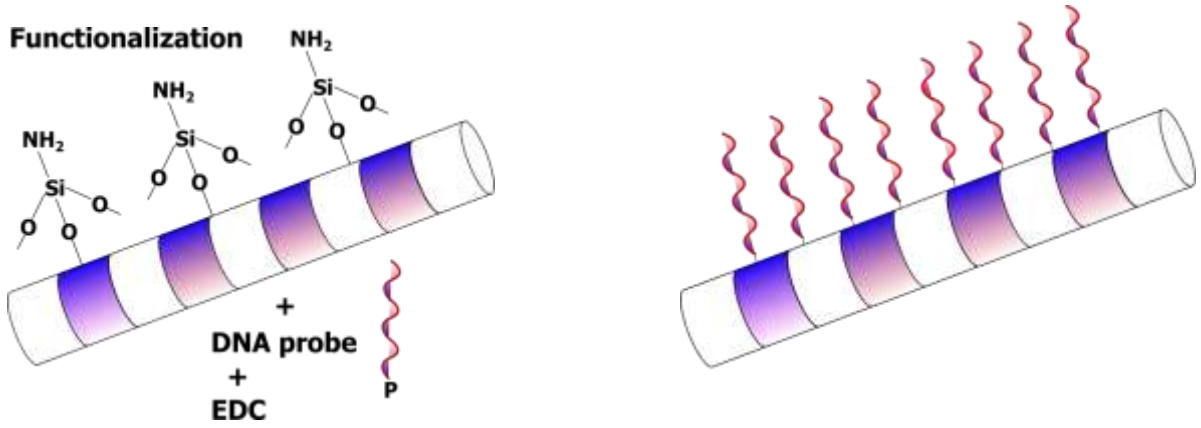
Functionalization of the surface

silanization



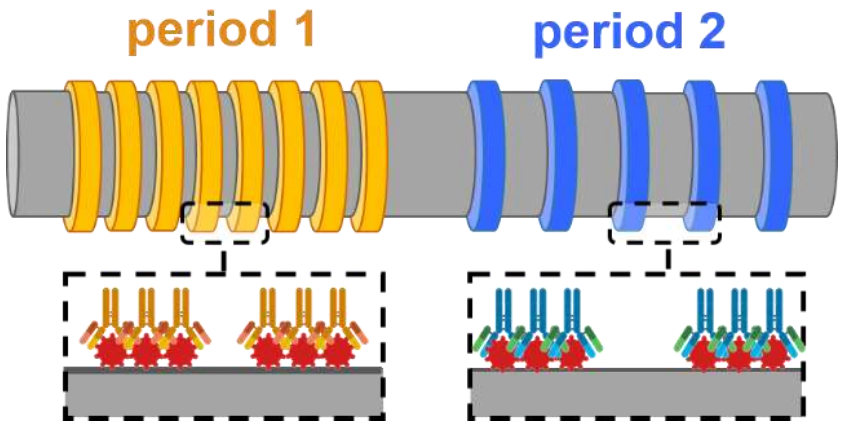
functionalization

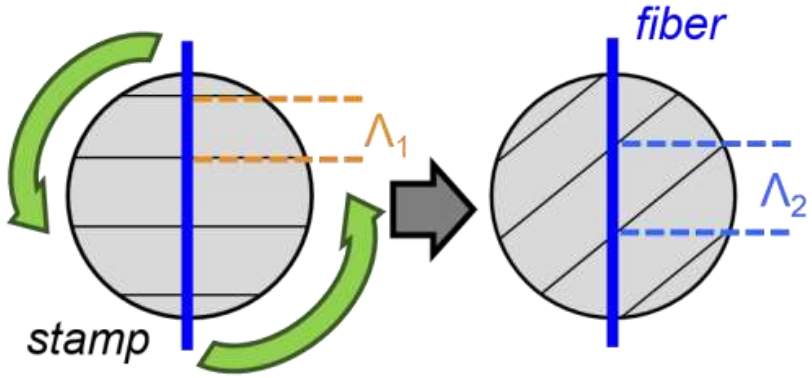


Process described in Chen et al.

Probe: 5'-phosphate-GCA CAG TCA GTC GCC-3'
Complementary: 5'-GGC GAC TGA CTG TGC-3'

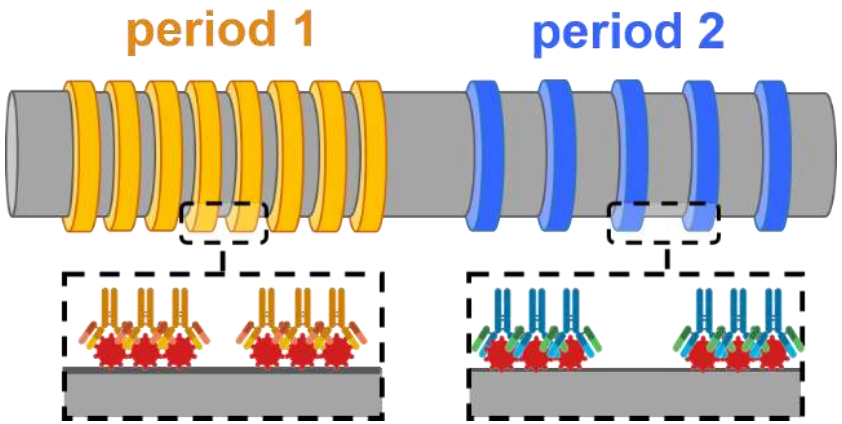
Bio-Bragg Gratings: multiplexation

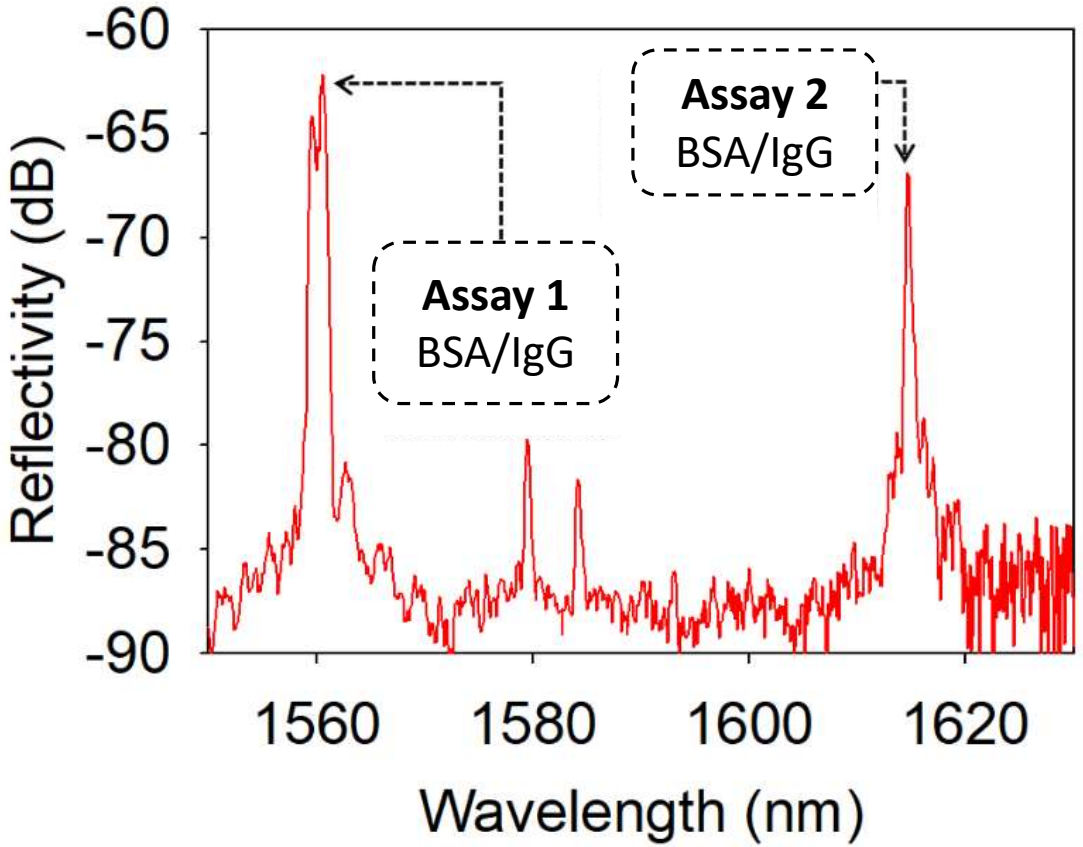




Bio-Bragg Gratings: multiplexation

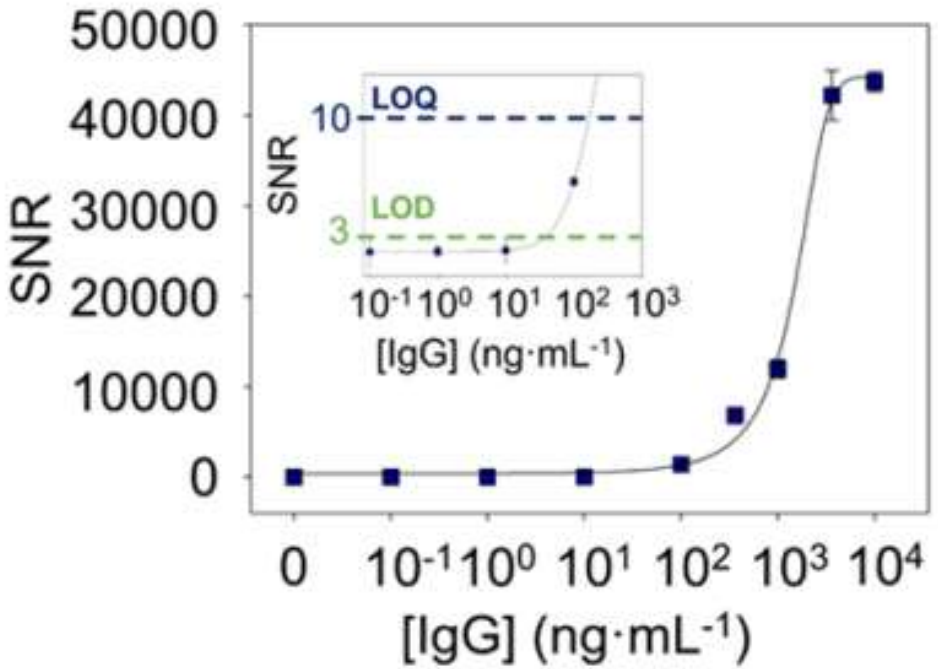
[IgG]= 10 μg/mL; cascaded BBGs of 5 mm length each

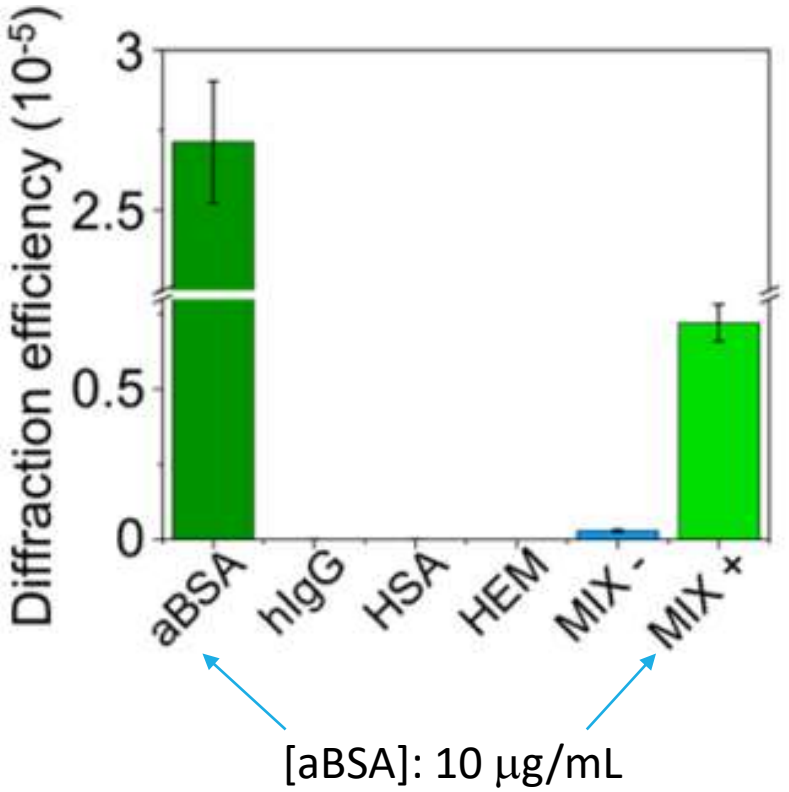




Fabrication: optimization of parameters

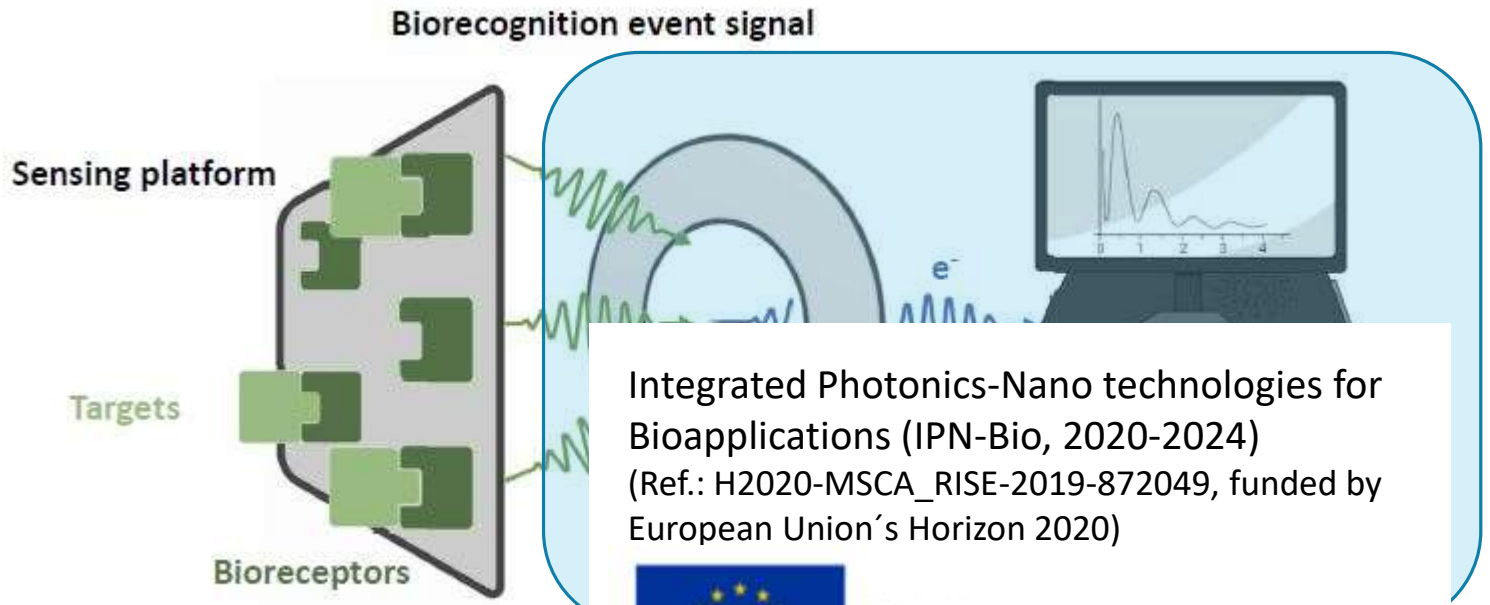
Immunoassay: specificity





Well… actually, this is the part for the physicists.

A **biosensor** is a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transduction element



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