# Ecole Thématique Plasmonique Moléculaire et Spectroscopies Exaltées 2016

## Plasmonic biosensing

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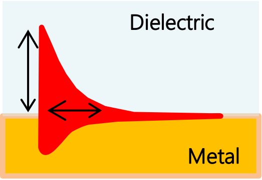
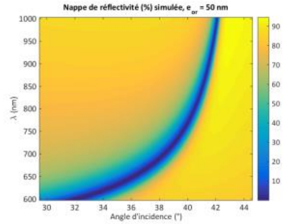
20-24 juin 2016 Toulouse (France)

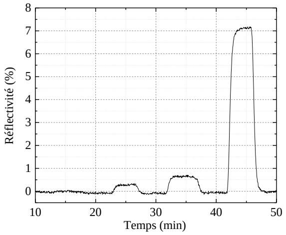
- Principle of an SPR biosensor.



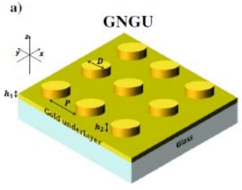

- Instrumental design– state of the art.



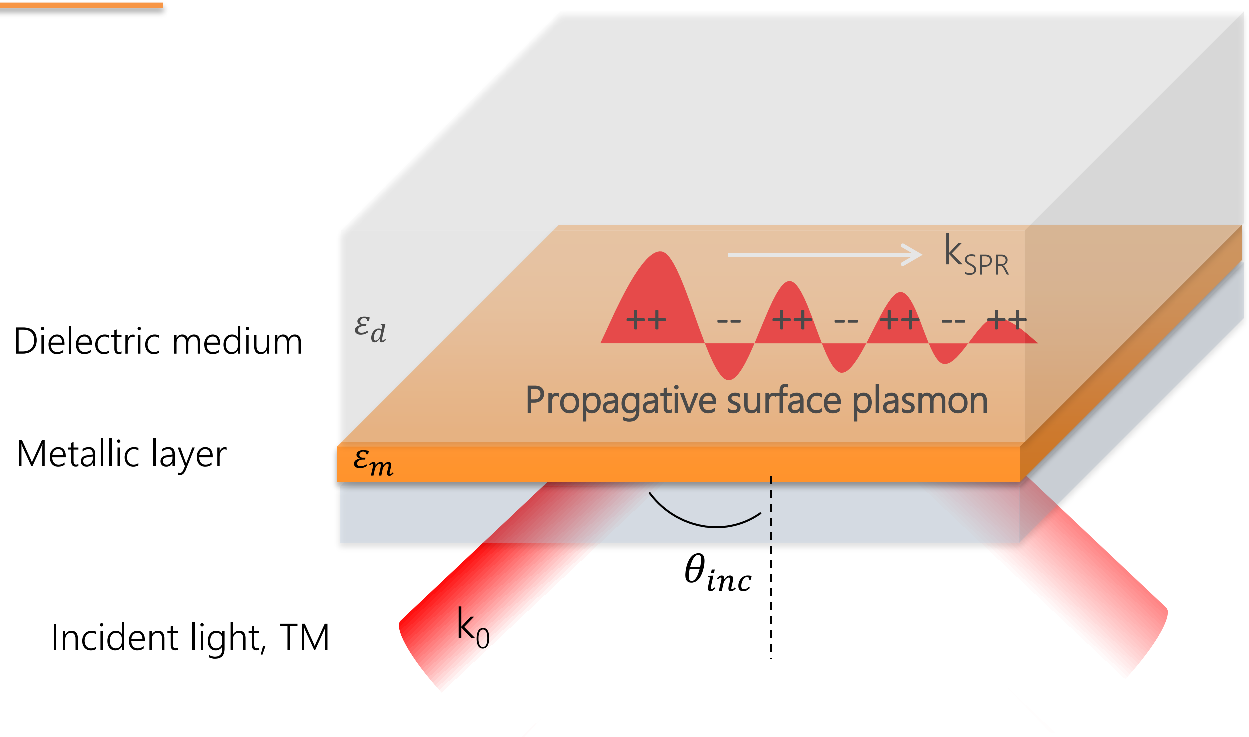

- Shot noise and limit in resolution.




- Perspectives of SPR biosensing.



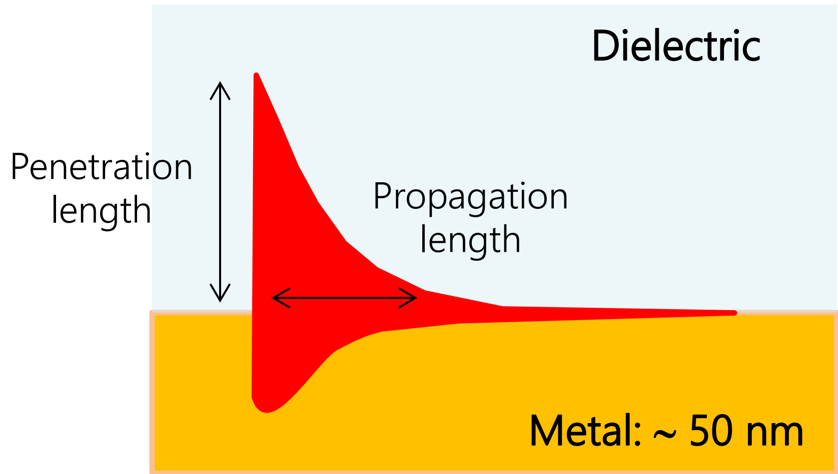
# Surface plasmon resonance (SPR)

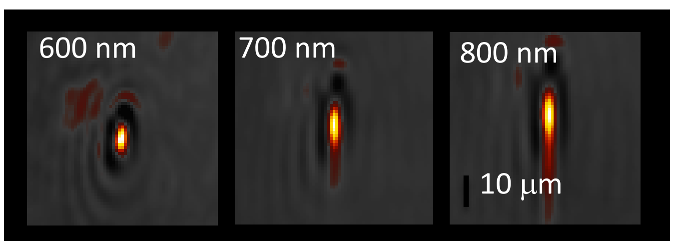


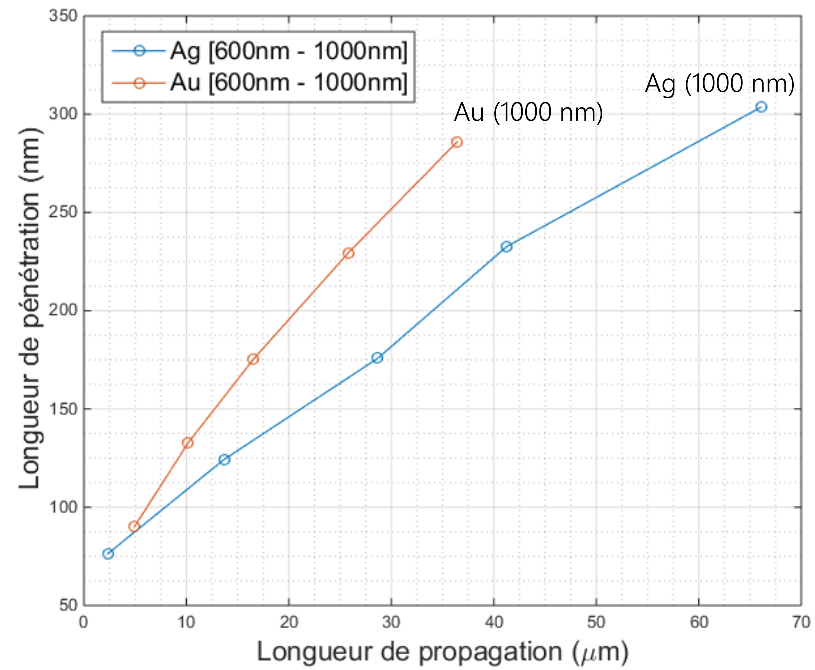

**Surface plasmon:** guided mode in the metal layer. The coupling between the incident TM light and the plasmon is maximum when:

$$k_{0,x} = \frac{2\pi}{\lambda_{inc}} n.\sin(\theta_{inc}) = k_{SPR} = \frac{2\pi}{\lambda_{inc}} Re\left\{\sqrt{\frac{\varepsilon_m \cdot \varepsilon_d}{\varepsilon_m + \varepsilon_d}}\right\}$$

# Properties of surface plasmon

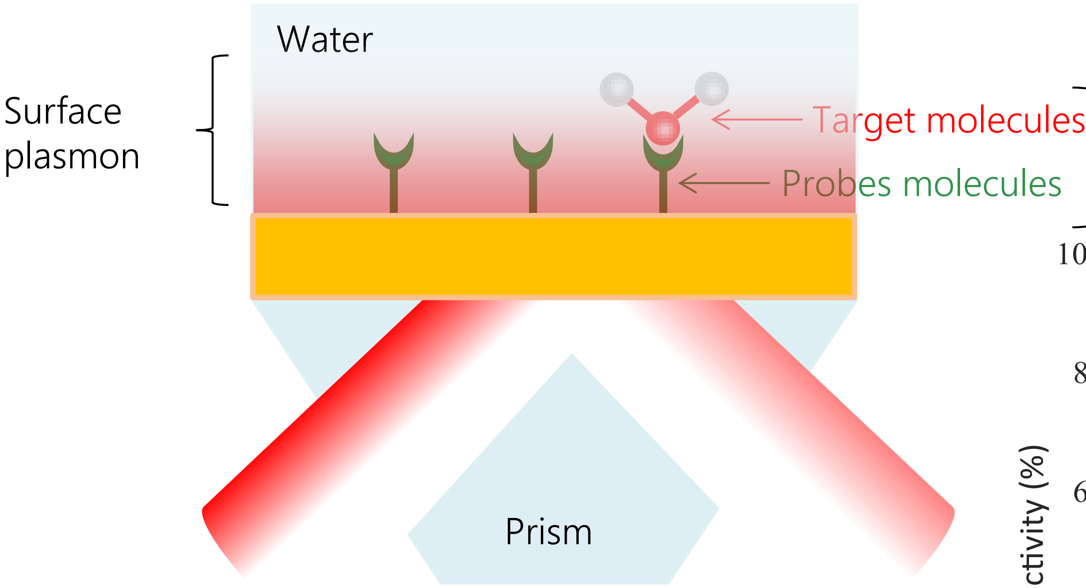


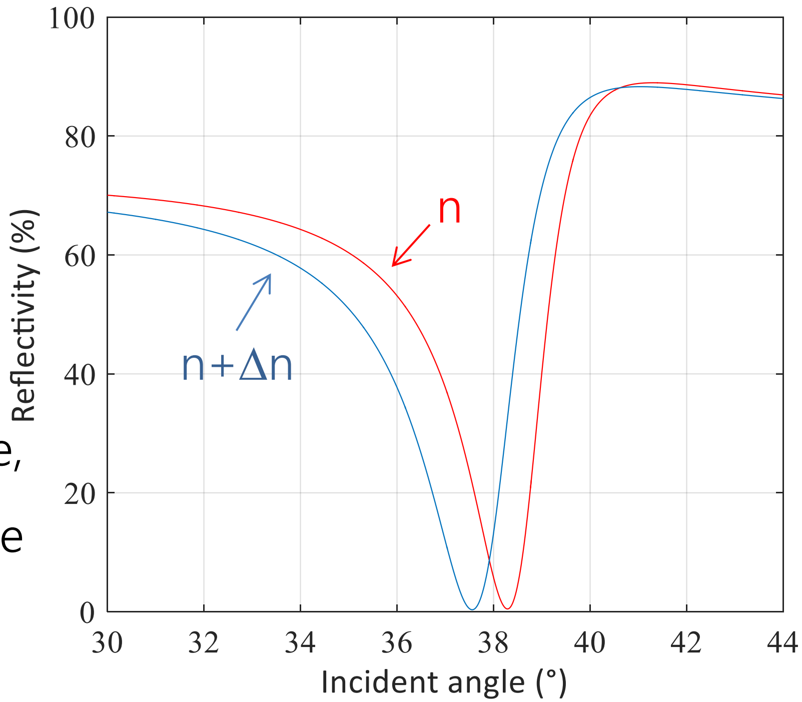




The plasmon are evanescent waves with typical size of a few 100s nm in the dielectric and a few 10s μm on the metallic surface.

# Principle of an SPR biosensor



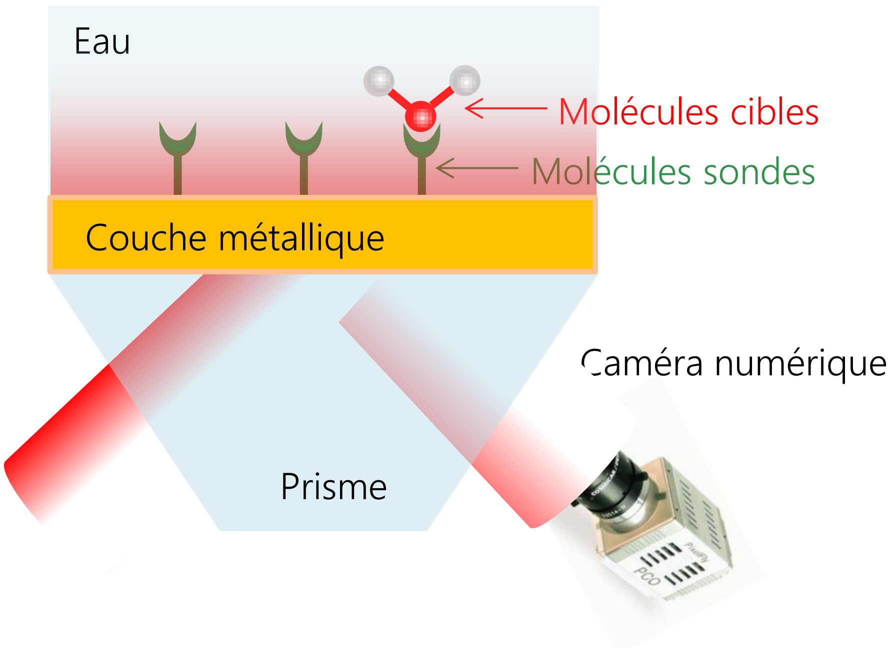

Refractive index change Δn (RIU)

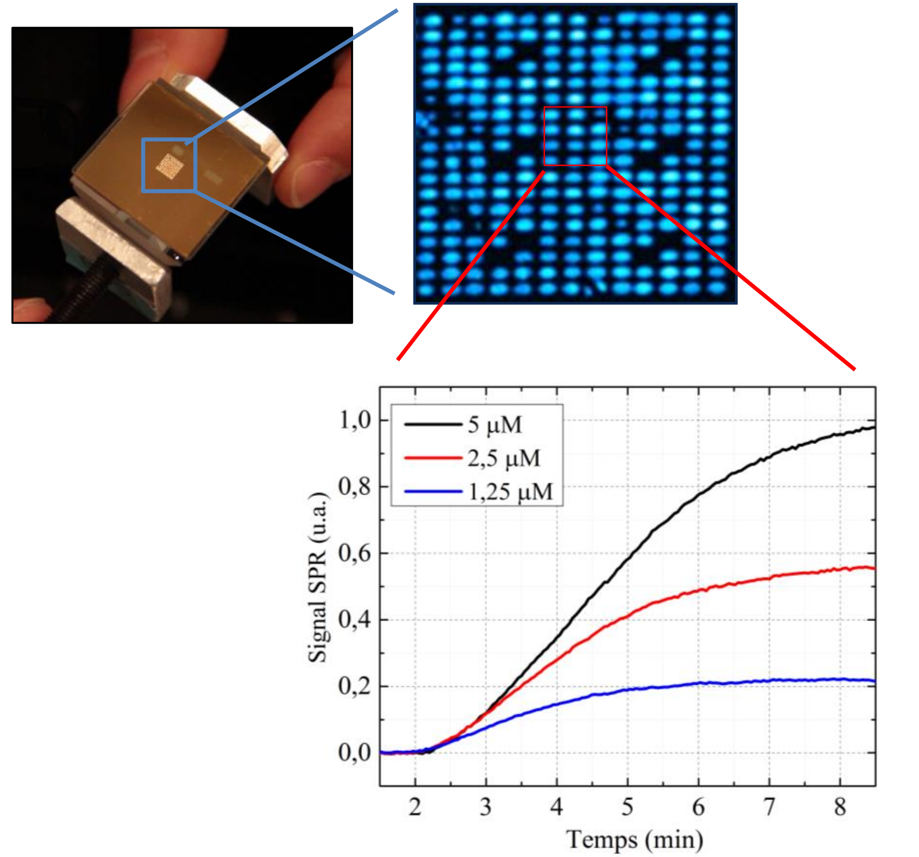



Binding of a molecular layer at the metal surface, (inside the plasmon evanescent wave) change the SPR coupling condition.

→ SPR biosensors are based on the detection of this shift of the SPR resonance.

# Biochips and SPR imagery




Using a digital camera, an image of the whole surface of the biochip can be acquired. Kinetics of the interaction between the target molecule and the different probes can be followed in real time.

# Performances of SPR biosensors

| Advantages | | |
|---|---|---|
| √ **Label free**<br>⇒ Easy to implement<br>⇒ Rapid | √ **Real-time Imaging**<br>⇒ Parallel detection of multiple interactions<br>⇒ Determination of affinity constants | √ **Quantitative**<br>⇒ Proportionality of the SPR signal with the amount of captured molecules. |

| Disadvantages | |
|---|---|
| x **Specificity**<br>⇒ Specificity relies entirely on the probe and the surface chemistry. | x **Mass detector**<br>⇒ Very difficult to detect small molecules (< kDa) |

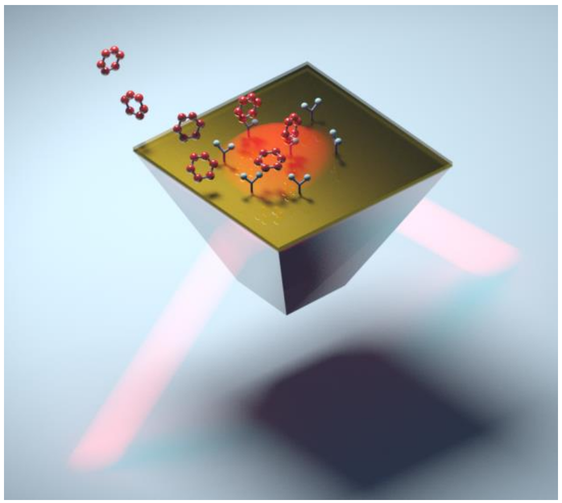
Sensitivity of SPR biosensors is given in term of change of refractive index (unit: RIU). The minimum change that can be detected is around **$10^{-6}$ to $10^{-7}$ RIU**

Limit of detection:

~ **nM** for large molecules > 10 kDa

~ **µM** for small molecules of a few 100s Da

# Possible interrogation modes



Change of the dielectric index

Adsorption of a biomolecular layer

Change of resonance conditions

Reflectivity

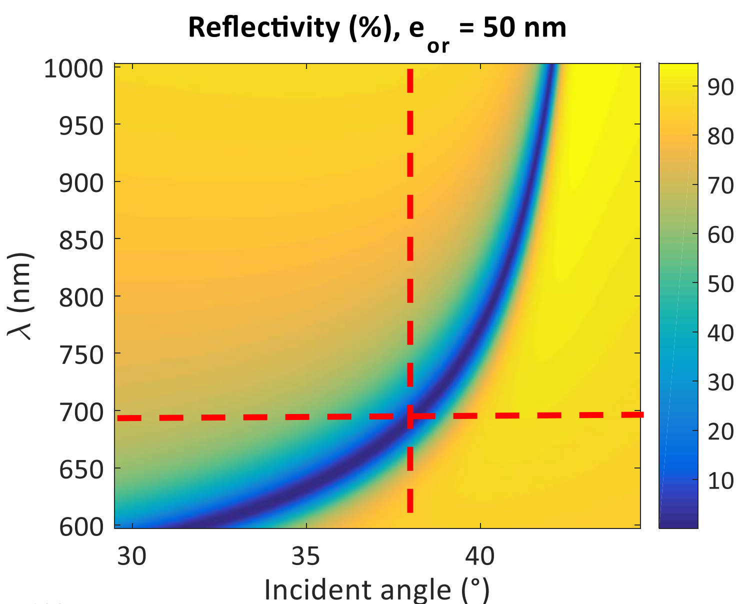
Resonance wavelength

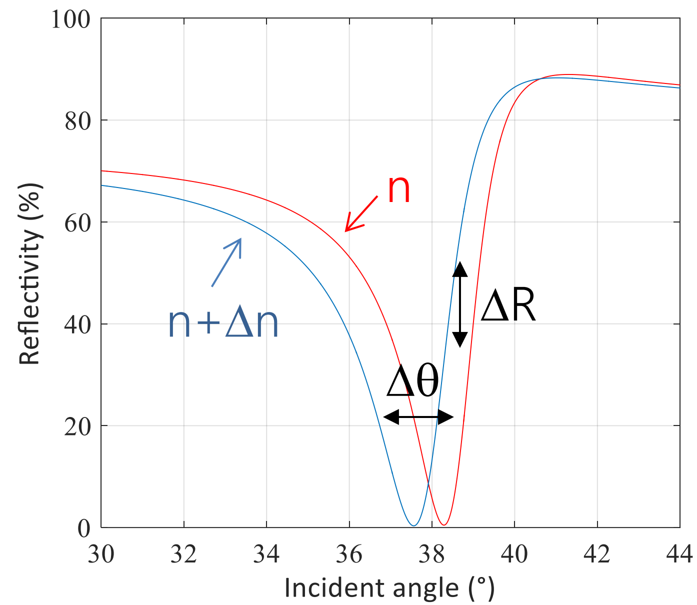
Resonance angle

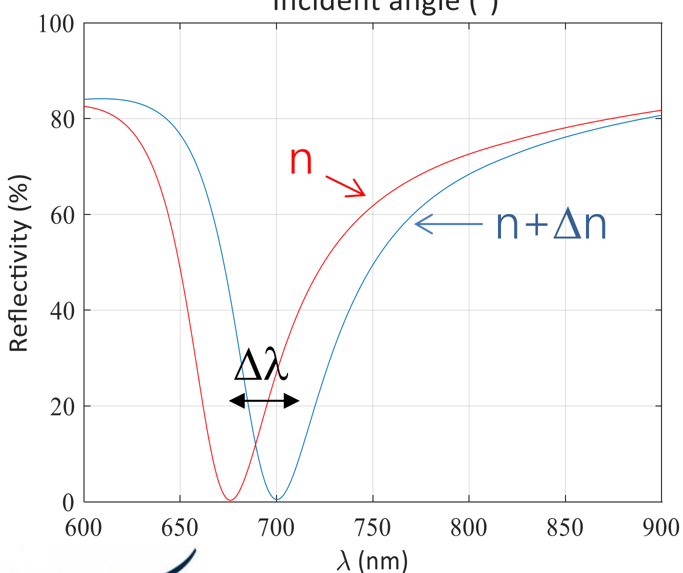
Phase

$$k_{0,x} = \frac{2\pi}{\lambda_{inc}} n.\sin(\theta_{inc}) = k_{SPR} = \frac{2\pi}{\lambda_{inc}} Re\left\{\sqrt{\frac{\varepsilon_m \cdot \varepsilon_d}{\varepsilon_m + \varepsilon_d}}\right\}$$

# Possible interrogation modes



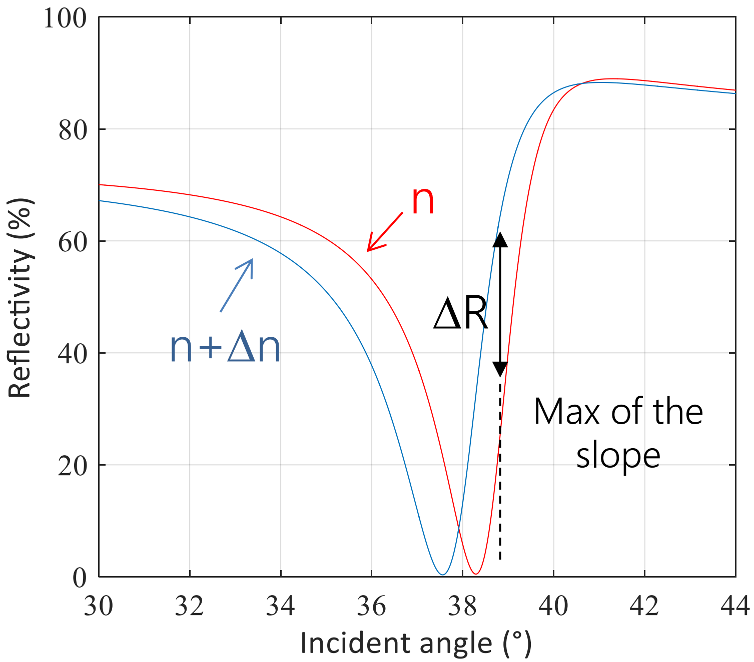



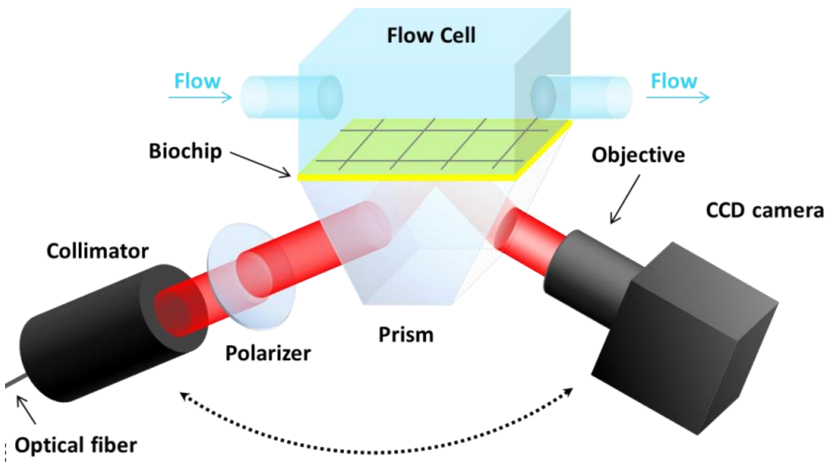




The shift in the SPR resonance, after a biomolecular interaction at the metallic surface, can be detected as:

- An angular shift $\Delta\theta$, at a given $\lambda_{inc}$
- A spectral shift $\Delta\lambda$, at a given $\theta_{inc}$
- A change in reflectivity $\Delta R$, at a given $\theta_{inc}$ and $\lambda_{inc}$

# Reflectivity interrogation mode



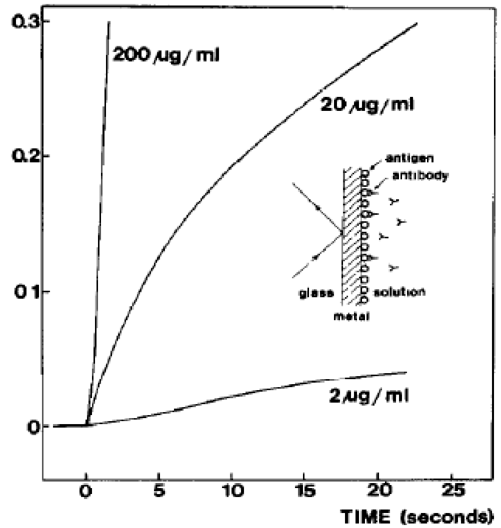




- Angle of incidence is chosen in order to maximize the change in reflectivity ΔR.
- Direct, real time measurement over all biochip.

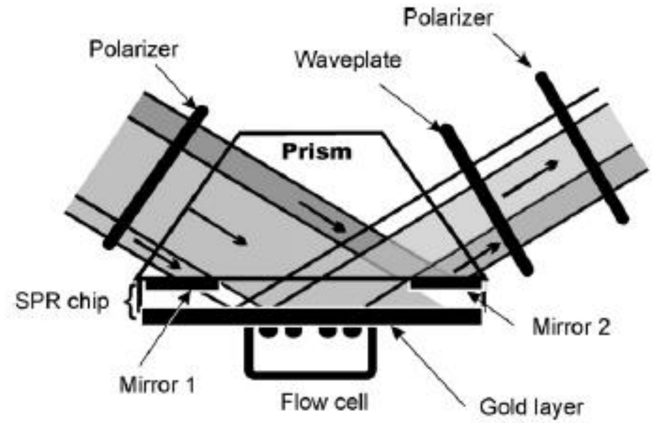
✓ Fast imagery, no mechanical displacement, simple instrumental setup.

✗ Limited dynamic.

# Reflectivity interrogation mode

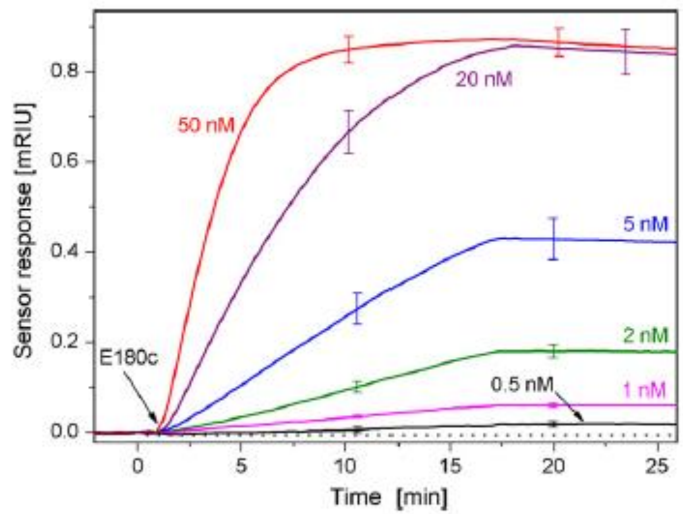



B. Liedberg and al Sensors and Actuators(1983)



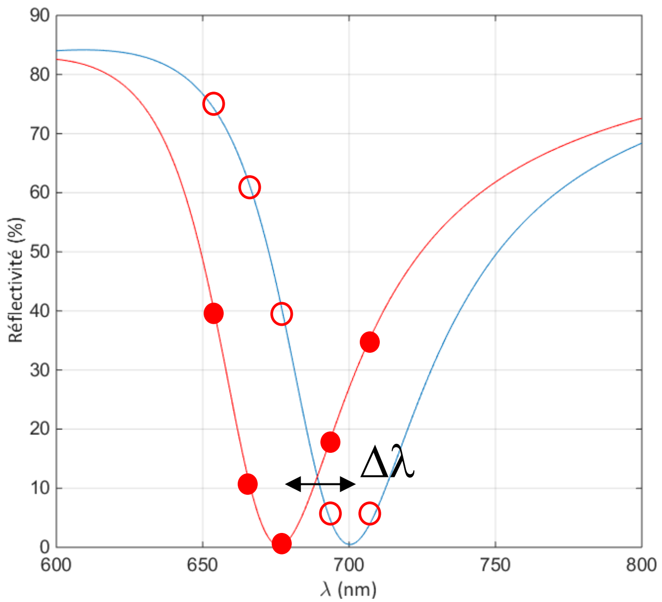


OpenPlex @Horiba Scientific




M. Piliarik and al. Biosensors and Bioelectronics (2009)

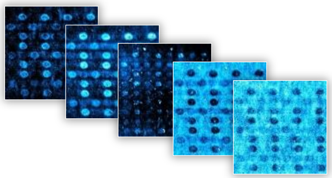
# Spectral interrogation mode




Acquisition of the complete spectral resonance using a white light and a spectrometer.

OR

N (~ 5 à 10) discrete measurements at N incident wavelengths.

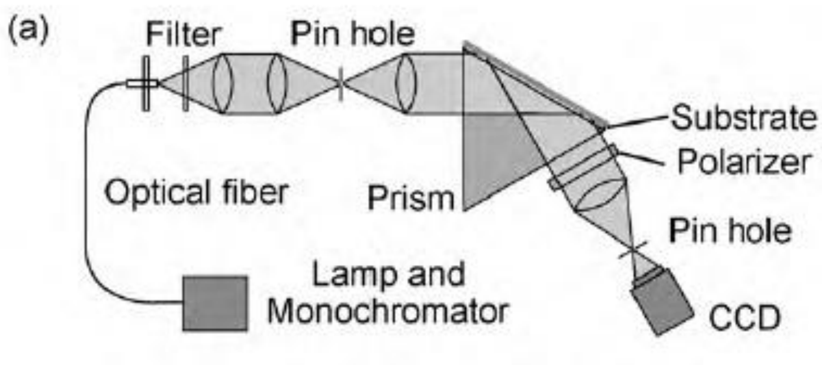


→ The spectral SPR resonance can be reconstructed by fitting the acquired data points (polynomial, pseudo-lorentzien). Following the position of the minimum allows to obtain the spectral shift Δλ due to the biomolecular interaction.

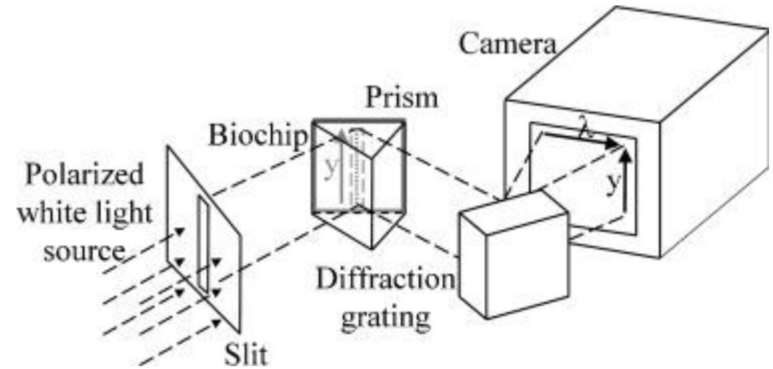
✓ Linear signal, good dynamic

✗ Slow acquisition time or loss of imaging capability (if spectroscopy).

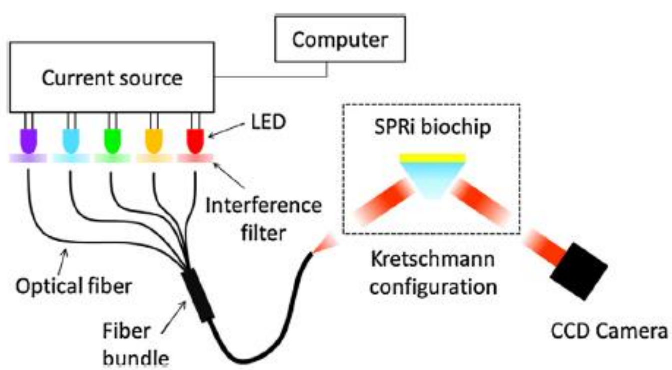
# Spectral interrogation mode



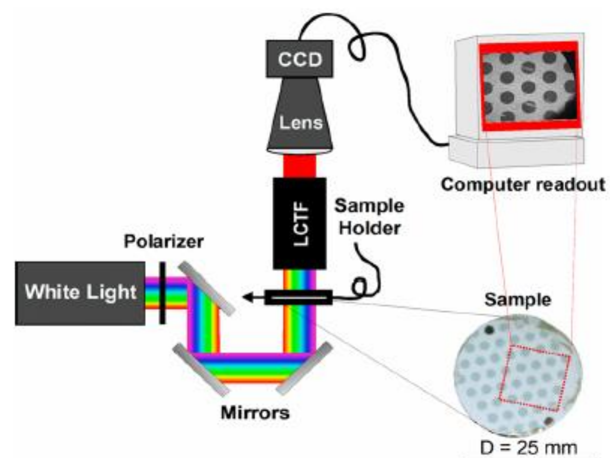

S. Otsuki and al. Biosensors and Bioelectronics (2010)




F. Bardin and al. Biosensors and Bioelectronics (2009)

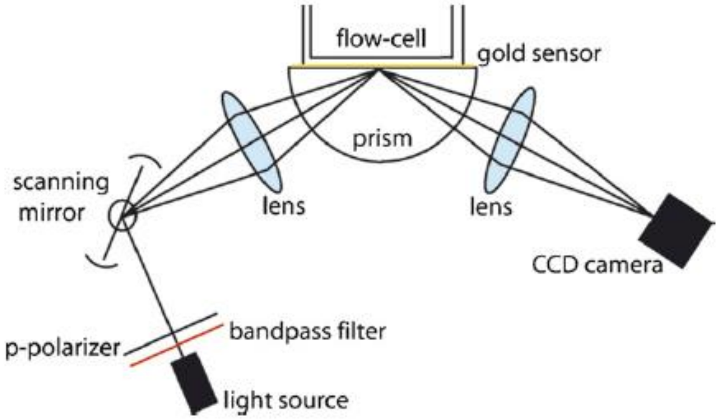



A. Sereda and al. Sensors and Actuators B (2015)

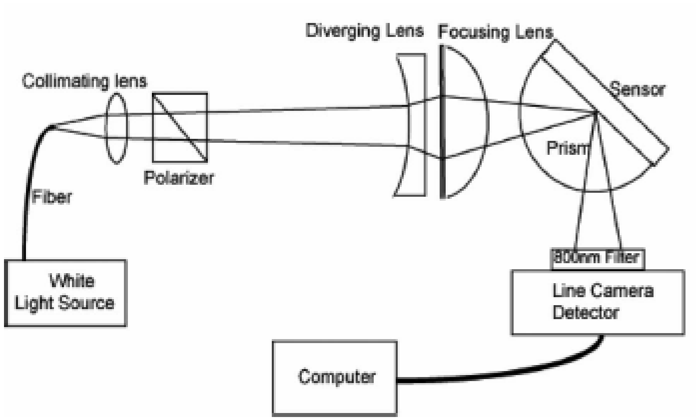



J.A. Ruemmele and al. Analytical Chemistry (2013)

# Angular interrogation mode



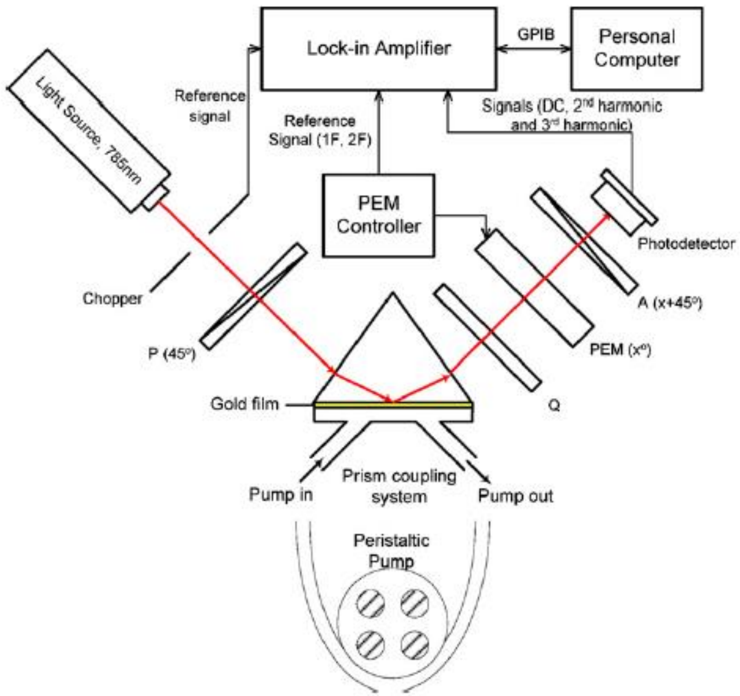

J.B. Beusink and al. Biosensors and Bioelectronics (2008)

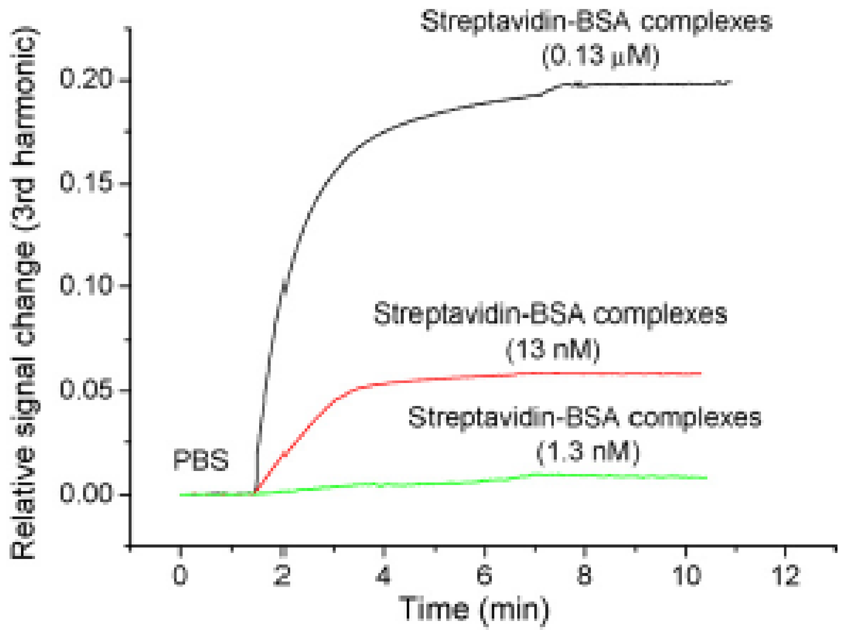



J. Guo and al. Optics letter (2008)

Biacore S200 @ GE Healthcare

# Phase interrogation mode



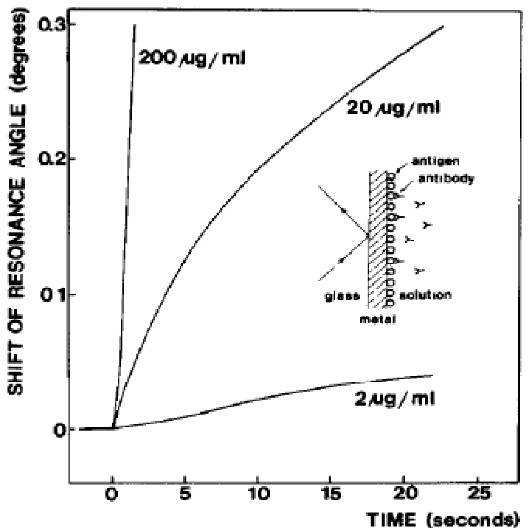




W.C. Law and al. Biosensors and Bioelectronics (2007)

# Comparison of the different instrumental modes

| Configuration | Resolution | Dynamic | Imaging capability ? | Speed | Mechanical movement ? |
| --- | --- | --- | --- | --- | --- |
| Reflectivity interrogation | $10^{-6}$ RIU | $10^{-3}$ RIU | Yes | + | No |
| Angular interrogation | $10^{-7}$ RIU | $10^{-2}$ RIU | Yes | - | Yes |
| Spectral interrogation (spectral sweep) | $10^{-7}$ RIU | $10^{-2}$ RIU | Yes | - | Yes |
| Spectral interrogation (spectroscopy) | $10^{-7}$ RIU | $10^{-2}$ RIU | No | + | No |
| Phase interrogation | $10^{-7}$ RIU | < $10^{-3}$ RIU | Yes | + | No |

# Comparison of the different instrumental modes

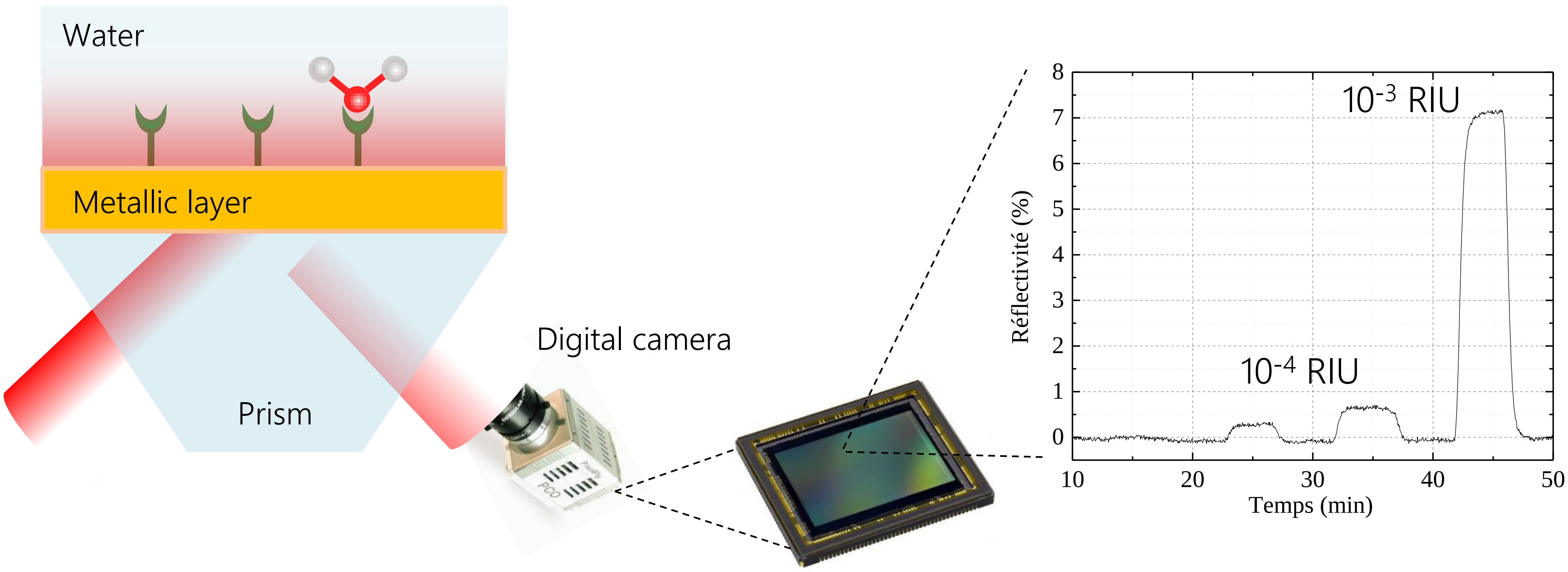



B. Liedberg and al Sensors and Actuators(1983)

on this surface By choosing an angle of incidence half way down the reflectance minimum and measuring the intensity of the reflected light at that constant angle, changes in the refractive index of about $10^{-5}$ are easily detected This is due to the sharpness of the resonance minimum The sensitivity is hence very high, even compared with ellipsometry

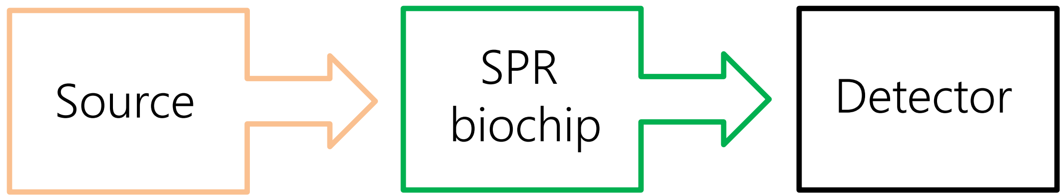
# Fundamental limit of SPR systems

For all optical configurations of SPR biosensors, the SPR signal always relies on one or many flux measurement on a mono-detector (pixel).



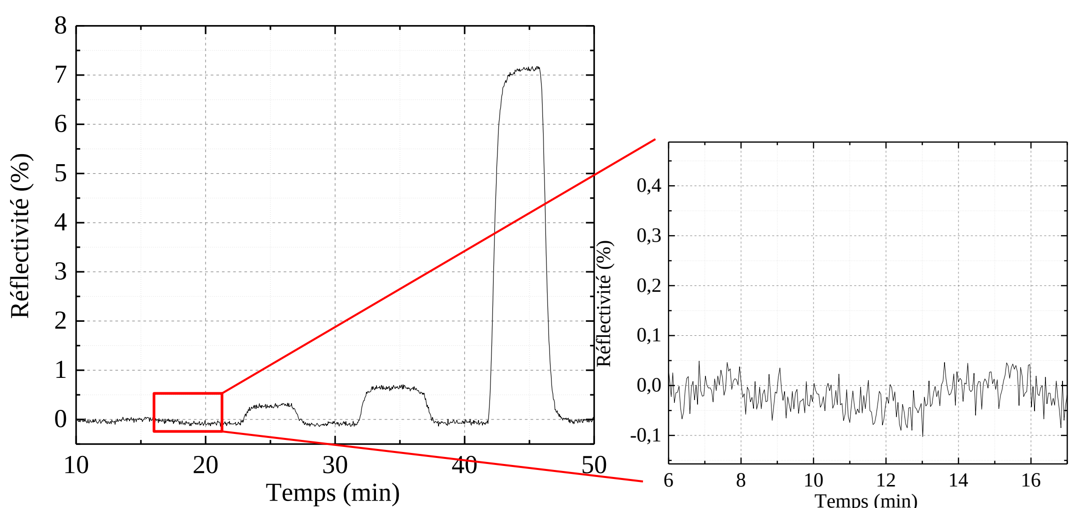

The resolution, i.e. the smallest variation Δn that can be measured, depends on the accuracy on which the flux is measured on the detector.

# Fundamental limit of SPR systems - noise




$F_0(\lambda_{inc}, \theta_{inc})$ R(n) $F_{det} = R.F_0$

For all optical detector, close to saturation: CCD, CMOS, photodiode, the dominant noise is shot noise.




$\sigma_{shot} = \sqrt{N_{e^-}} \propto \sqrt{R.F_0}$

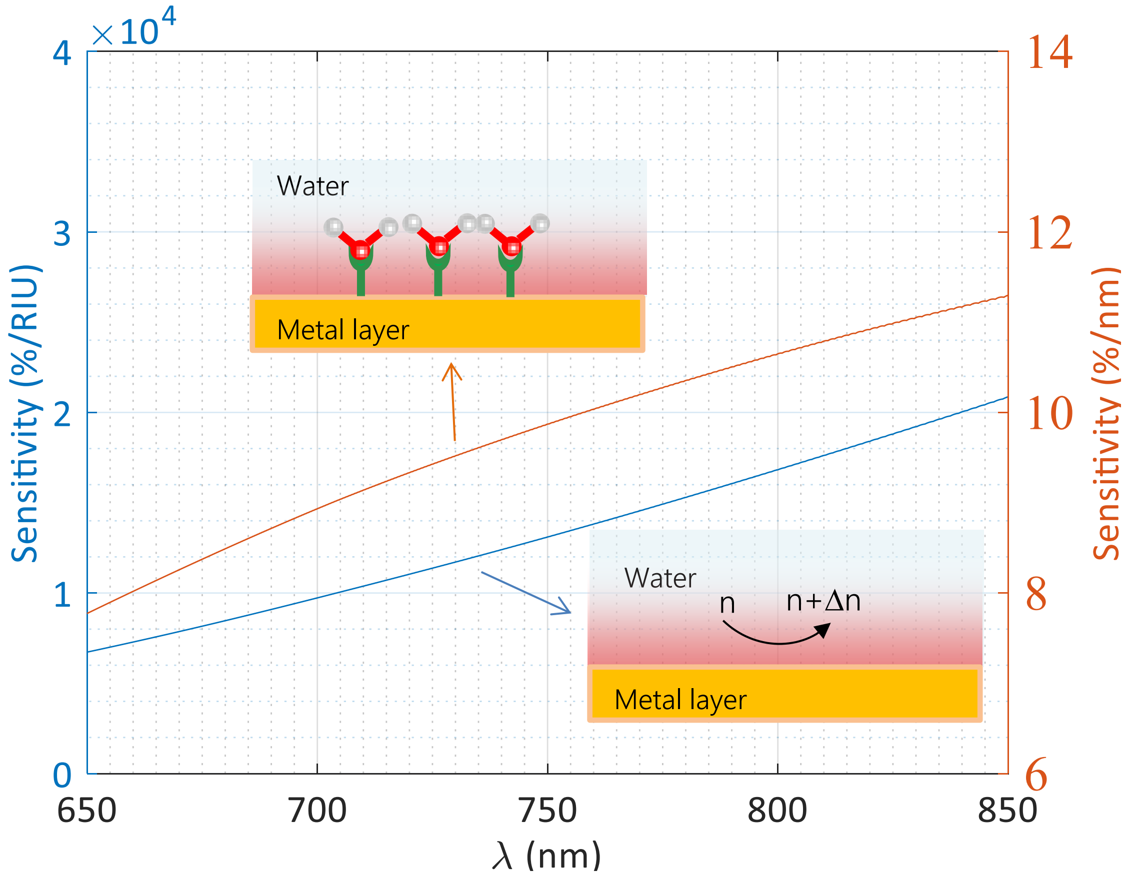
For 1 pixel and 1 measurement :

$\sigma_{shot} \sim 0{,}5\ \%$ à 1%

(true for all λ)

# Fundamental limit of SPR systems - sensitivity

The shift of the SPR resonance for a step of index or the binding of a biomolecular layer depends on the illumination wavelength.



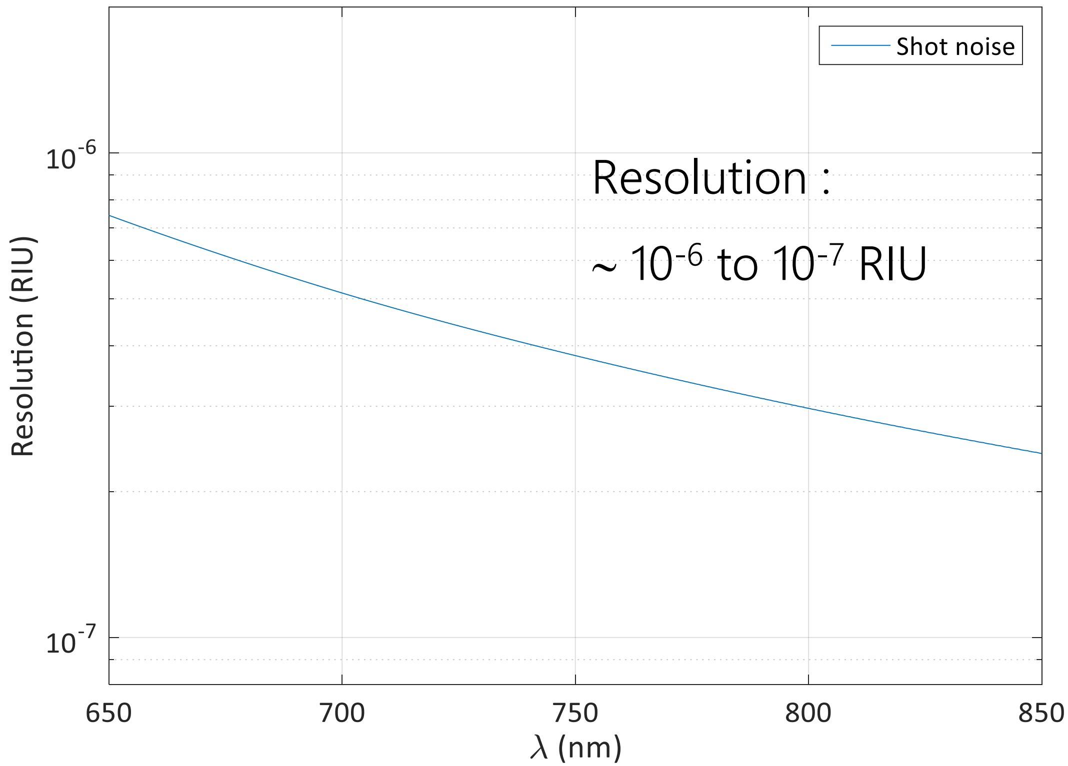

Case of a 50 nm of gold on 3 nm of Titane

# Fundamental limit of SPR systems - resolution

In fact, during an SPR measurements, a number of pixels and images are averaged :

$$\sigma_{shot}^{moyenne} = \frac{\sigma_{shot}}{\sqrt{N_{pixel} . N_{image}}}$$

Typically, in most SPR systems : $N_{pixel}.N_{image} \sim 10^3$ à $10^4$

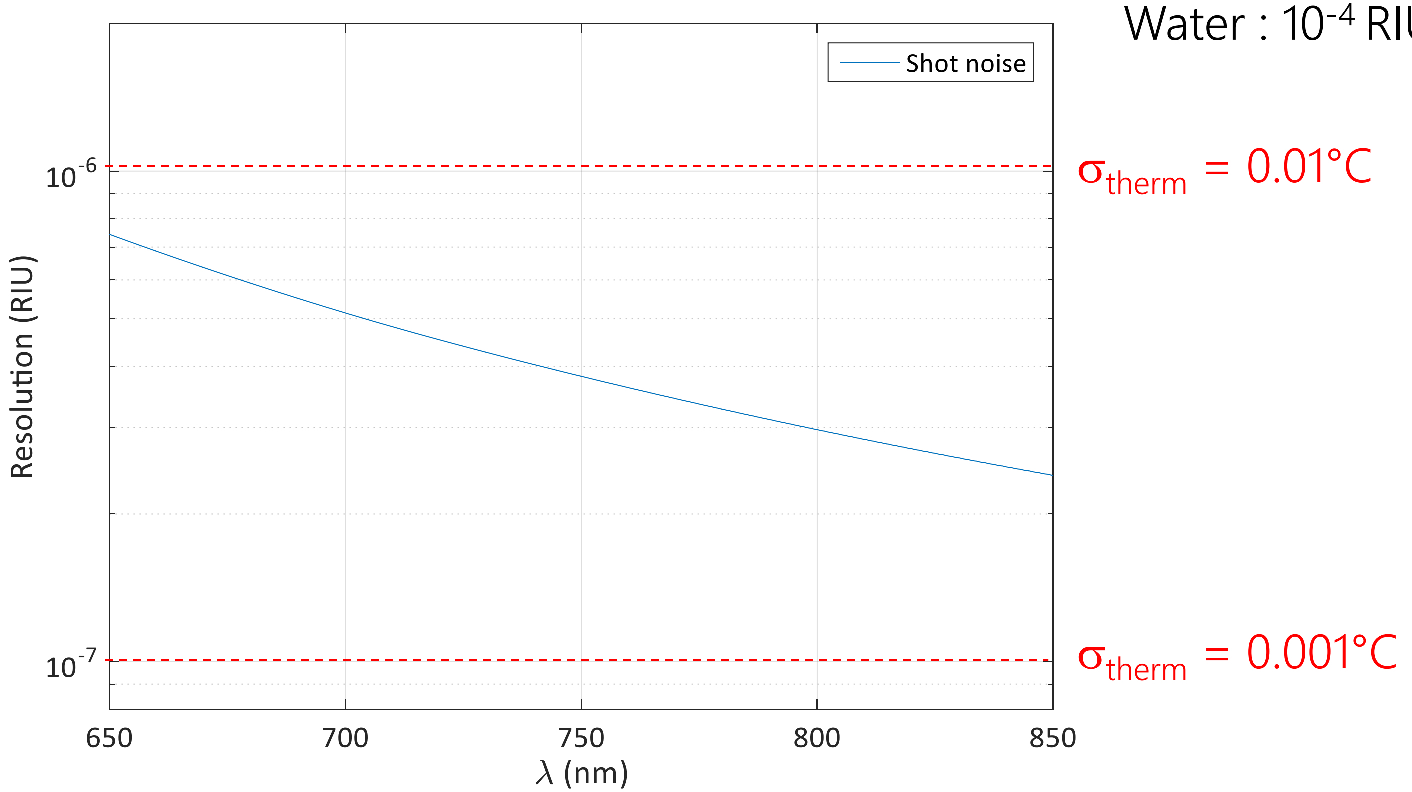


# Fundamental limit of SPR systems - resolution

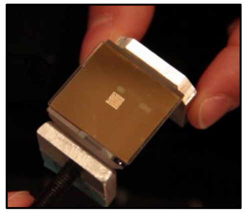
Refractive index of most materials depends on temperature. In particular, fluctuation of the temperature of the water medium above the biochip induce thermal noise

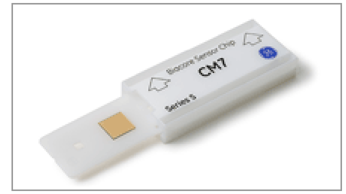
→ SPR setup must be thermally stabilized( better than 0.01°C)

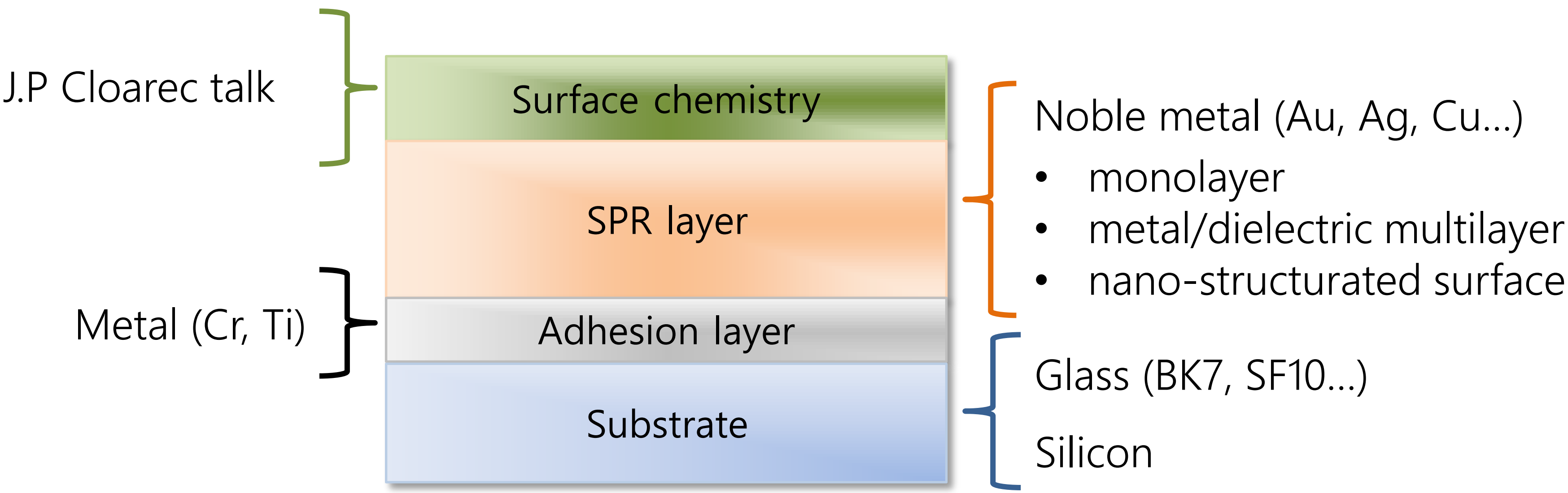
Water : $10^{-4}$ RIU/°C



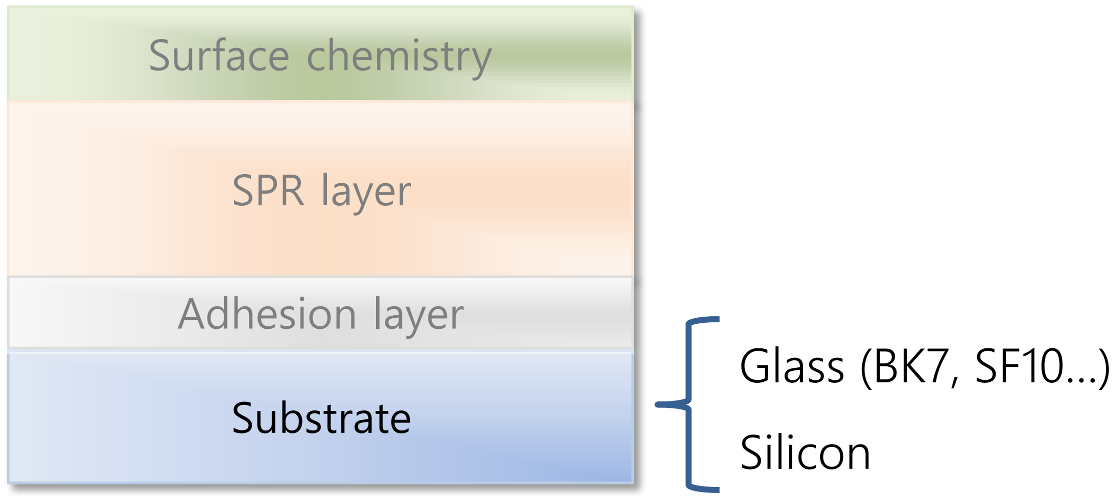
# SPR biochip – possible improvements ?







# SPR biochip – substrate

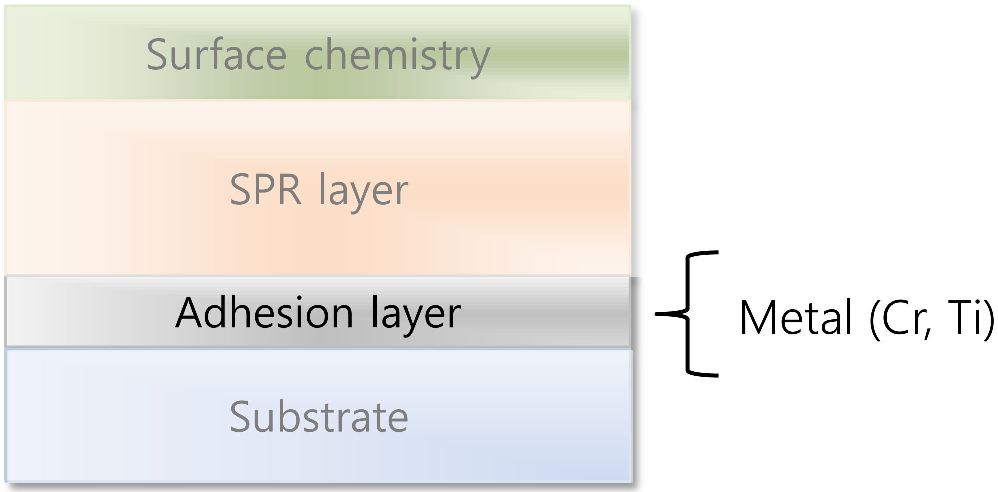



Choice of the substrate material is governed by the light – plasmon coupling condition :

$$\frac{2\pi}{\lambda} n_{substrate} sin\theta = \frac{2\pi}{\lambda} Re\left\{\sqrt{\frac{\varepsilon_m \cdot \varepsilon_d}{\varepsilon_m + \varepsilon_d}}\right\}$$

Geometry of the substrate is usually optimized for imaging quality.

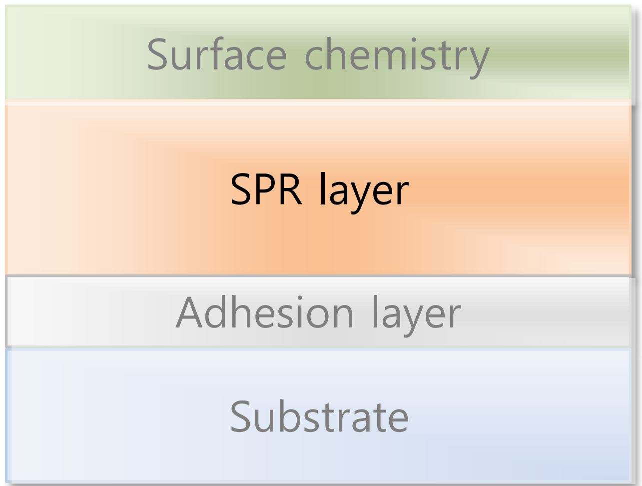
# SPR biochip – adhesion layer




< 5 nm of Chromium or Titanium : allows good adhesion of the metallic layer on glass (mechanical, chemical and thermal resistance).

Highly absorbing layer→ drop in sensitivity of the SPR setup.

# SPR biochip – metallic monolayer




Noble metal (Au, Ag, Cu…)

- **monolayer**
- metal/dielectric multilayer
- nanostructured surface

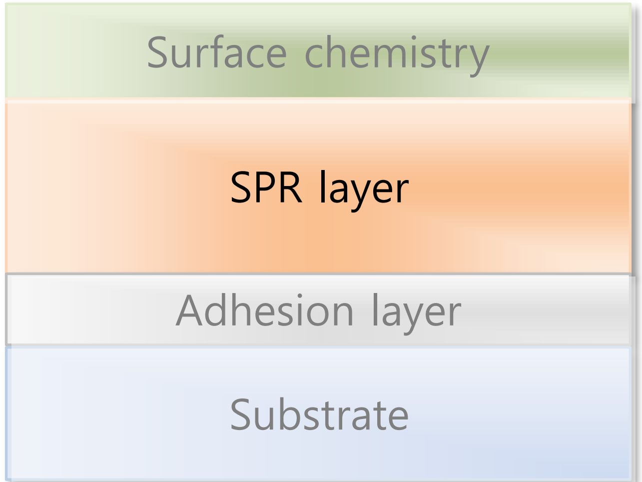
Simple and economical to produce (~ 10 €/sample), good reproducibility.

Good homogeneity over large surface ($cm^2$)

Silver biochips still non-standard due to passivation problem.

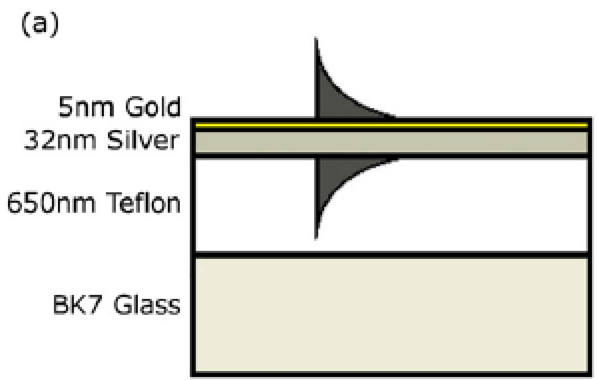
Resolution ~$10^{-7}$ RIU seems difficult to improve significantly

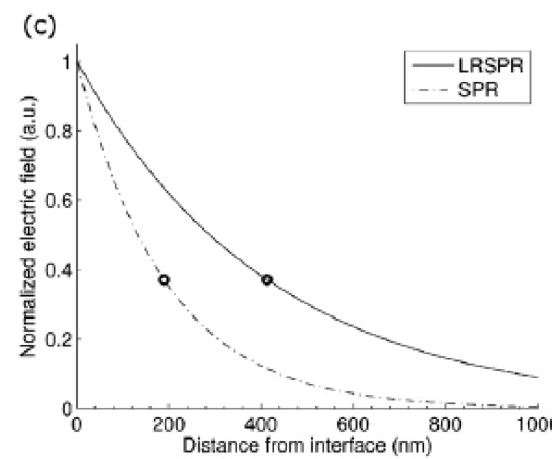
# SPR biochip – metal/dielectric multilayer




Noble metal (Au, Ag, Cu…)

- monolayer
- **metal/dielectric multilayer**
- nano-structurated surface



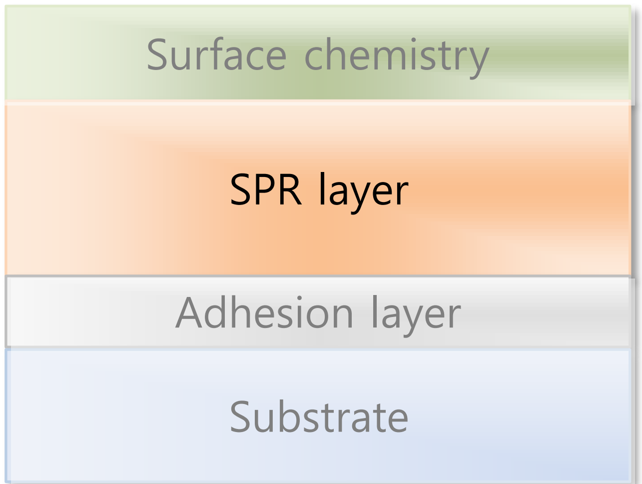




V. Chabot and al. Sensors and Actuators B (2012)

✔ Allows tuning of the penetration depth (cellular imaging)

✘ No significant gain in resolution

# SPR biochip – nano-structured metallic layer

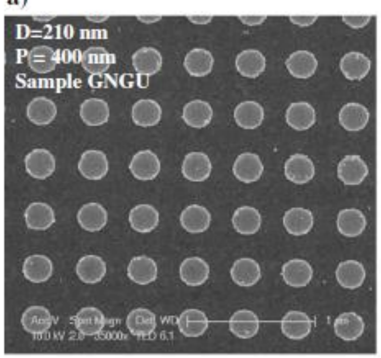


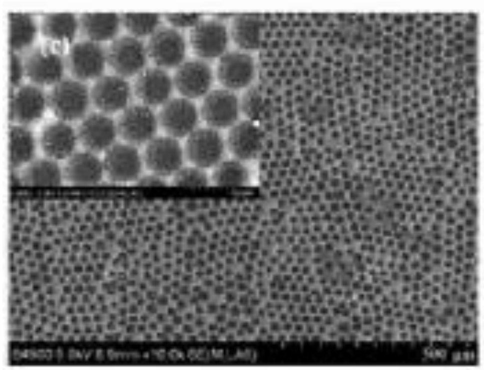

Noble metal (Au, Ag, Cu…)

- monolayer
- metal/dielectric multilayer
- **nano-structurated surface**

How can structuration of the metallic surface improve SPR biosensing ?

a)

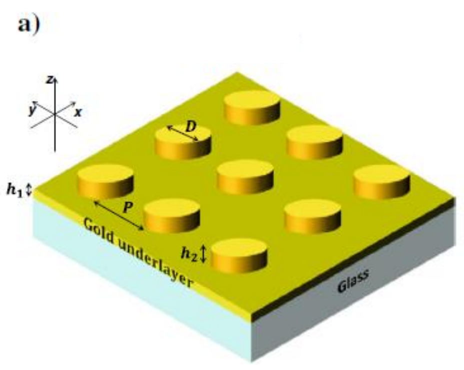


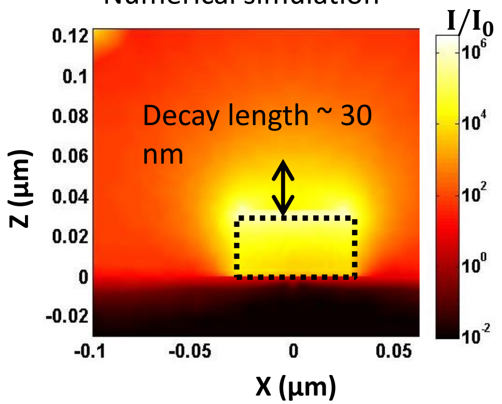



Bringing together the high sensitivity of PSP and the EM localization of LSP.

# SPR biochip – nano-structured metallic layer

- Confinement of the plasmonic evanescent wave ~10s nm




M. Sarkar and al. Plasmonics (2015)

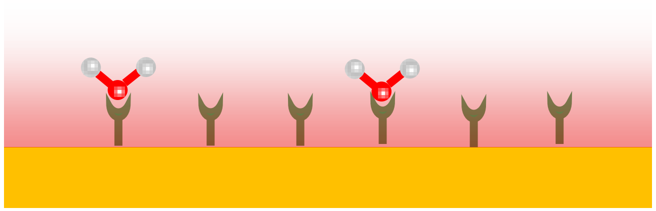
$$Signal \propto \int_0^{e_{bio}} \Delta n_{bio} . |E(z)|^2 dz \qquad Noise \propto \int_{e_{bio}}^{\infty} \Delta n_{noise} . |E(z)|^2 dz$$

→ higher sensitivity to the binding of nanometer thick biolayer.

# SPR biochip – nano-structured metallic layer

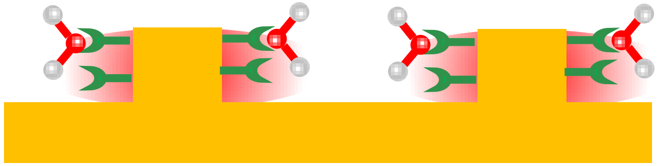
Spatially localized electromagnetic field along the nanostructure

Flat gold



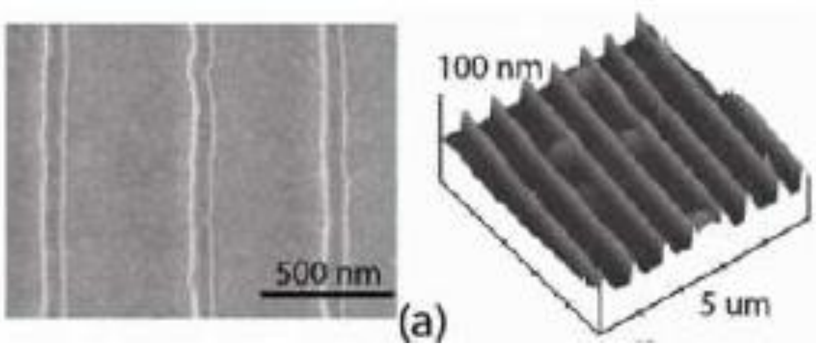
$$Signal \propto \frac{A_{with\ target}}{A_{total}} \ll 1$$ at low concentration of target molecules

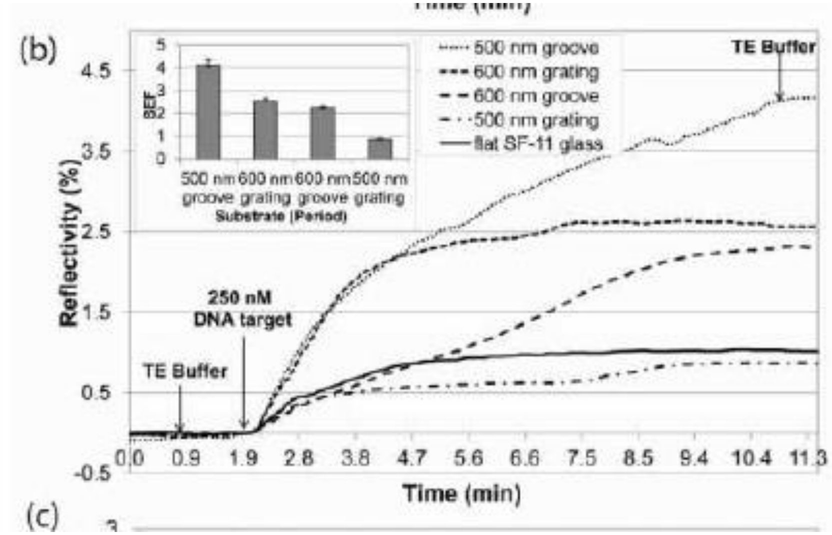
Nanostructurated surface



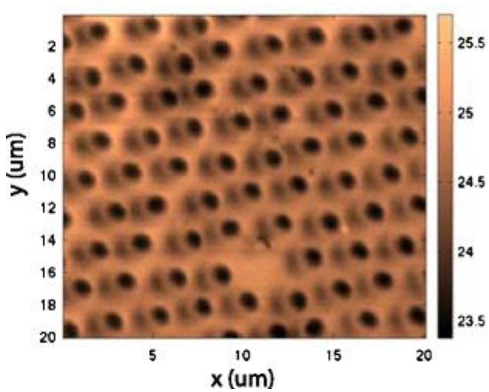
$$Signal \propto \frac{A_{with\ target}}{A_{LSP}} \sim 1$$ → lower limit of detection

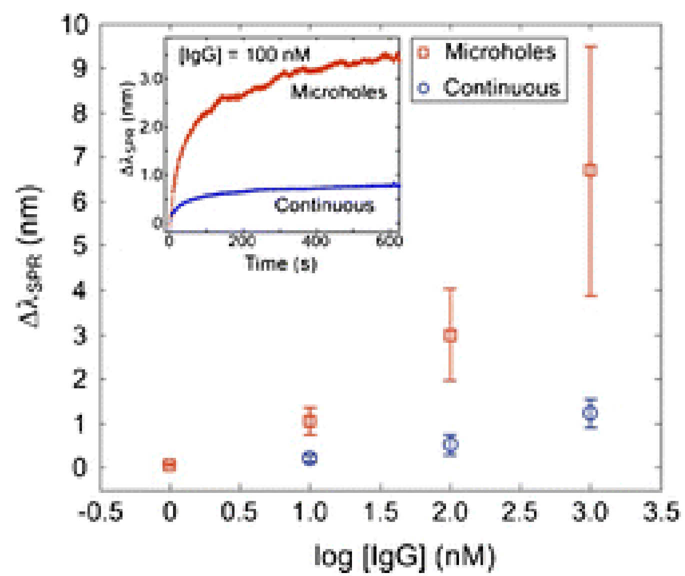
# SPR biochip – nano-structured metallic layer



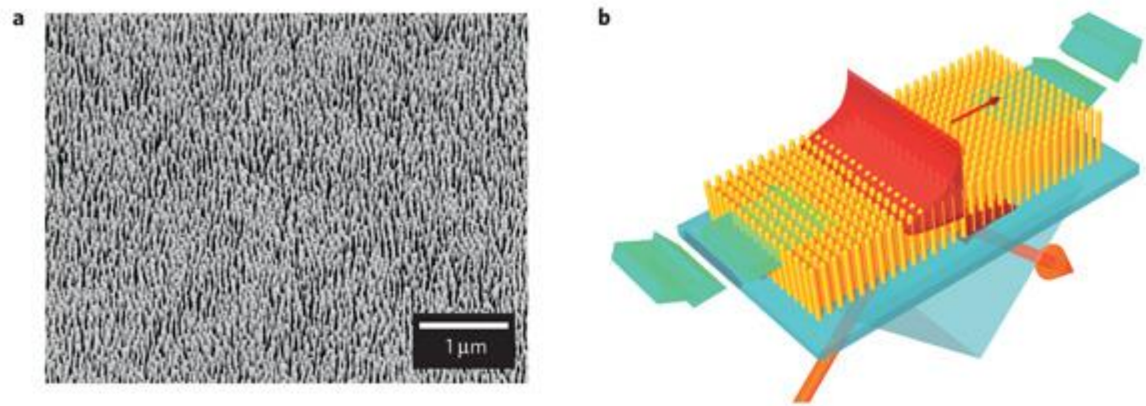

*L. Malic and al. Optics Express 2009*

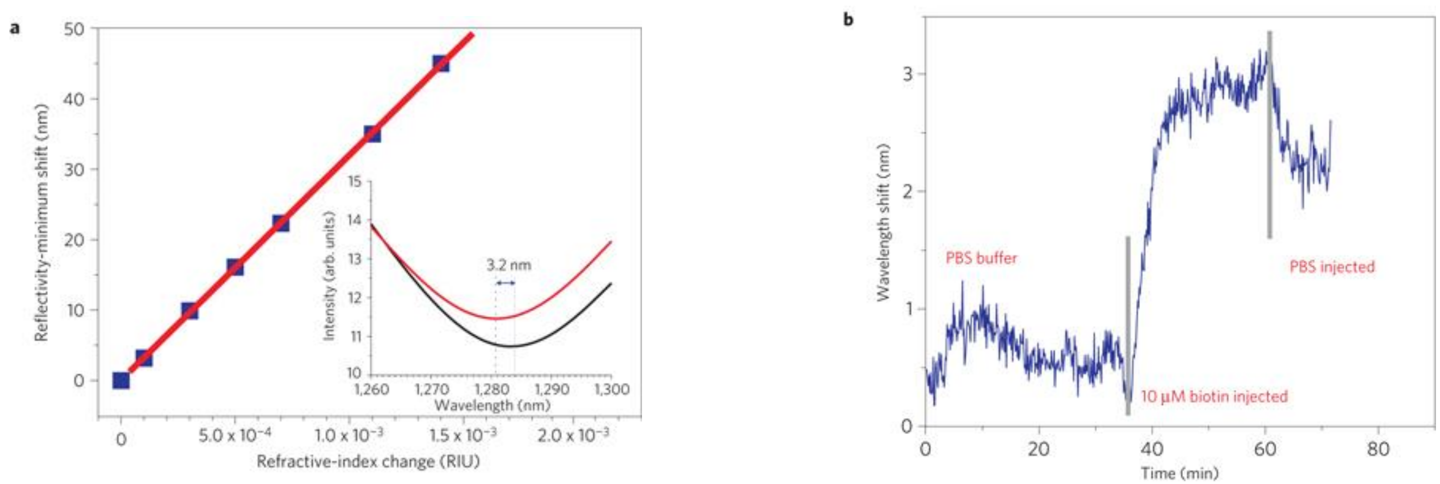



*L. Live and al. Bioanalytic. Chem 2012*

# SPR biochip – nano-structured metallic layer




*P. Pastkovsky and al. Nature Materials 2009*

# SPR biochip – Christmas wish list

What would be an ideal nano-structurated biochips for SPR instruments ?

- Directly compatible with SPR instruments already in used.
- Large nano-structurated area (> $mm^2$) or a significant number of nanostructured areas (>100 x 100 $\mu m^2$) on a single biochip.
- Homogenous plasmonic response across the biochip.
- Must be available in (very) large quantity for 'real' biological studies
- Not cost-prohibitive.

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# Thank you for your attention