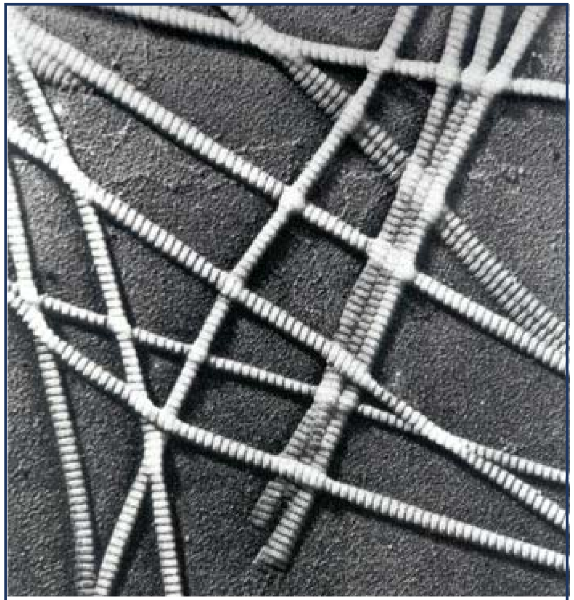
Biosensors,
biocompatibilization of materials,
and **surface science**

require the investigation of

the **sorption processes** and
the **3-D organization**
of proteins at solid/liquid interface.

Many **qualitative** methods
but
few **quantitative** methods
and
even fewer **real-time** measurements !



http://www.aad.org/education/CollagenFigure1.htm

Collagen

Fibrillar protein of the extracellular matrix

Triple helix 300 nm long
1.5 nm diameter
Weight ~ 300 kDa

Forms elastic fibers

Fibrinogen

Blood protein

Three globular domains linked by fibrillar segments
Weight ~ 340 kDa

Causes blood clotting

How to investigate in liquids the behavior of adsorbed proteins ?

A novel biosensor platform integrating **Love mode acoustic wave** and **surface plasmon resonance** for the investigation of protein layers

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OUTLINE

- Introduction
- Love mode surface acoustic wave (SAW)
  - Structure and characteristics
  - Sensitivity enhancement
- Surface plasmon resonance (SPR)
- Combined SAW/SPR
  - Technique
  - Method
  - Application to collagen and fibrinogen
- Conclusions

SURFACE ACOUSTIC WAVE SAW
SURFACE PLASMON RESONANCE SPR

Label-free sensing on a common surface

- increased system integration
- fast analysis
- low analyte volume
- identical event
- simplicity of sample preparation

Real time measurement

- adsorption kinetic
- dynamic evolution of the layer

LOVE MODE SAW SENSOR: STRUCTURE

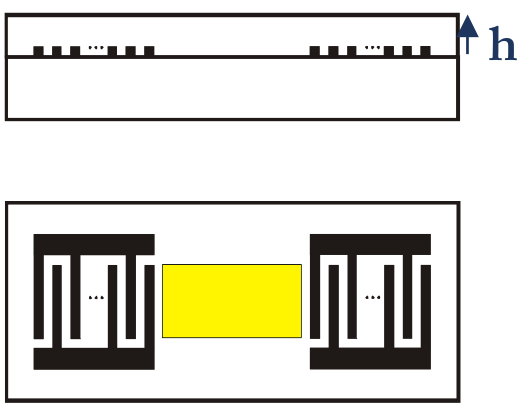
Overcoated SAW delay line is a mass sensitive device.

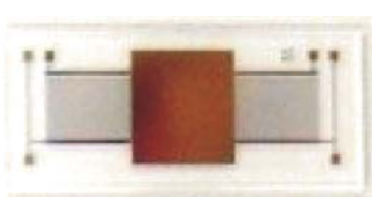
Shear horizontal polarized acoustic wave that results in low loss with liquid loading.

Substrates: **α-SiO$_2$**, $LiNbO_3$, $LiTaO_3$

Guiding layers: **SiO$_2$**, polymers/epoxies, ZnO

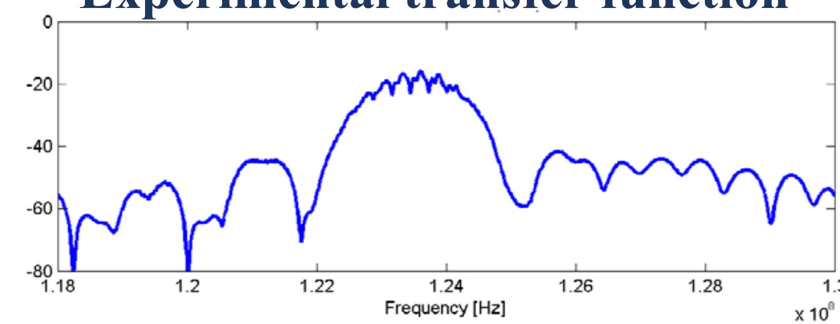
Delay line structure



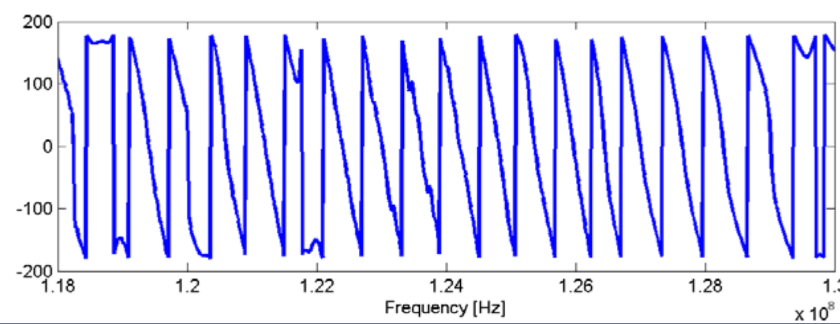


Experimental transfer function

I.L.



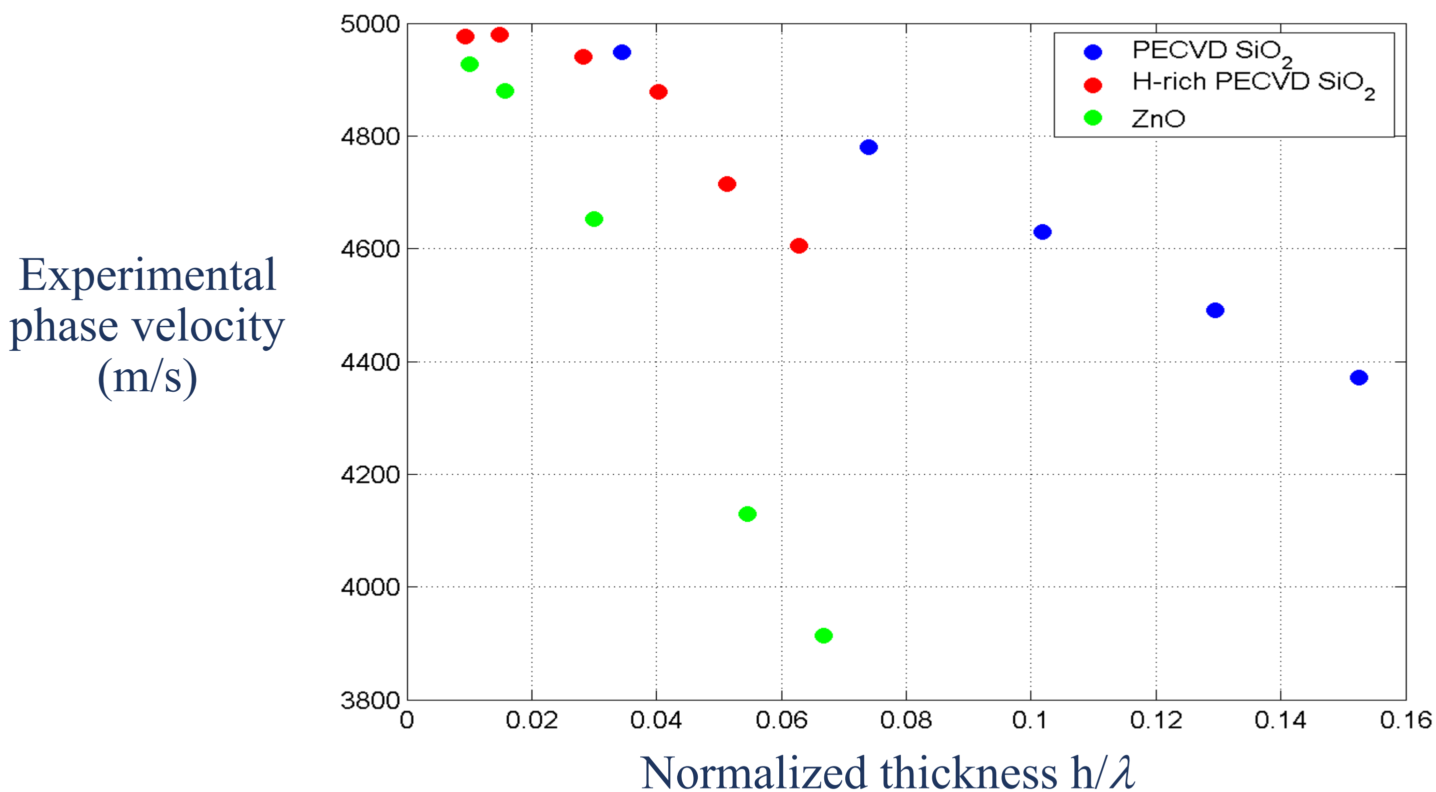
Phase ϕ



LOVE MODE SAW SENSOR: CHARACTERISTICS

The dispersion of Love mode is ruled by
- the acoustic velocity $V^2 = \mu / \rho$
- the acoustic impedance $Z^2 = \rho * \mu$

Mechanical properties are function of
- the selected guiding materials;
- the structure (processing parameters, *i.e.* PECVD).



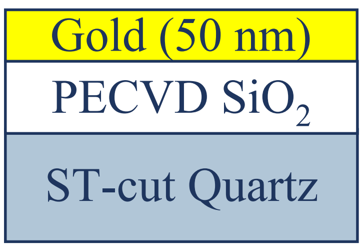
LOVE MODE SAW SENSOR: MASS SENSITIVITY

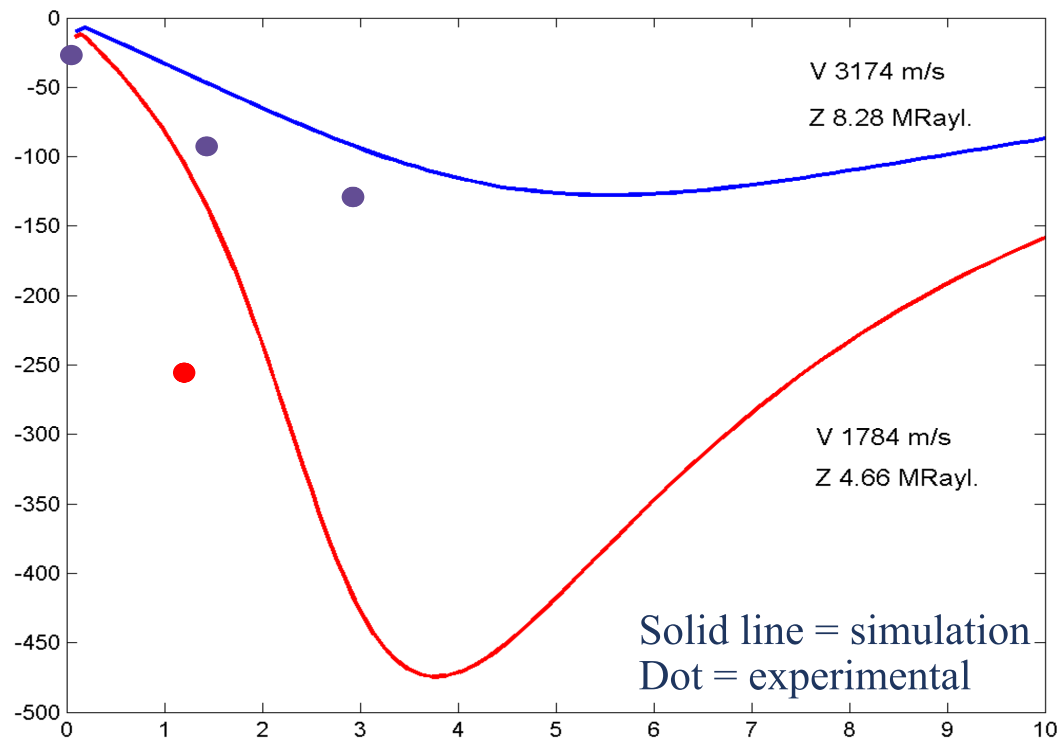
Mass sensitivity

$$S = \frac{\lambda}{360^\circ D} \cdot \frac{\Delta\phi}{m}$$

λ acoustic wavelength
D sensing length
m surface density

Strong dispersion = high sensitivity:
- H-rich PECVD SiO_2 (reduced stiffness);
- Gold (low velocity).

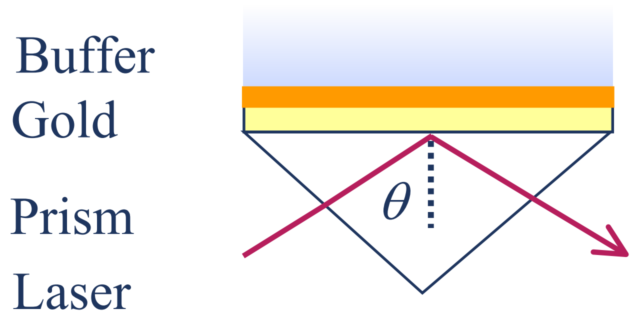


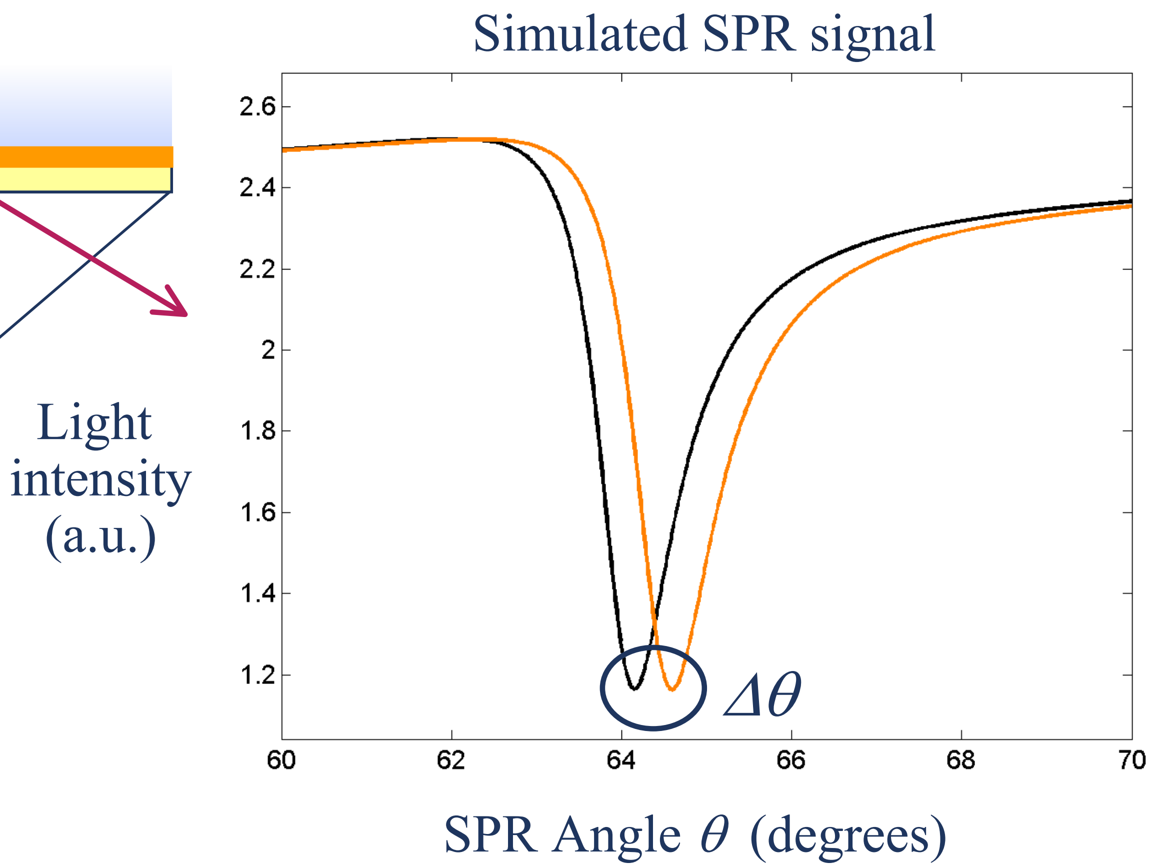


SURFACE PLASMON RESONANCE SENSOR

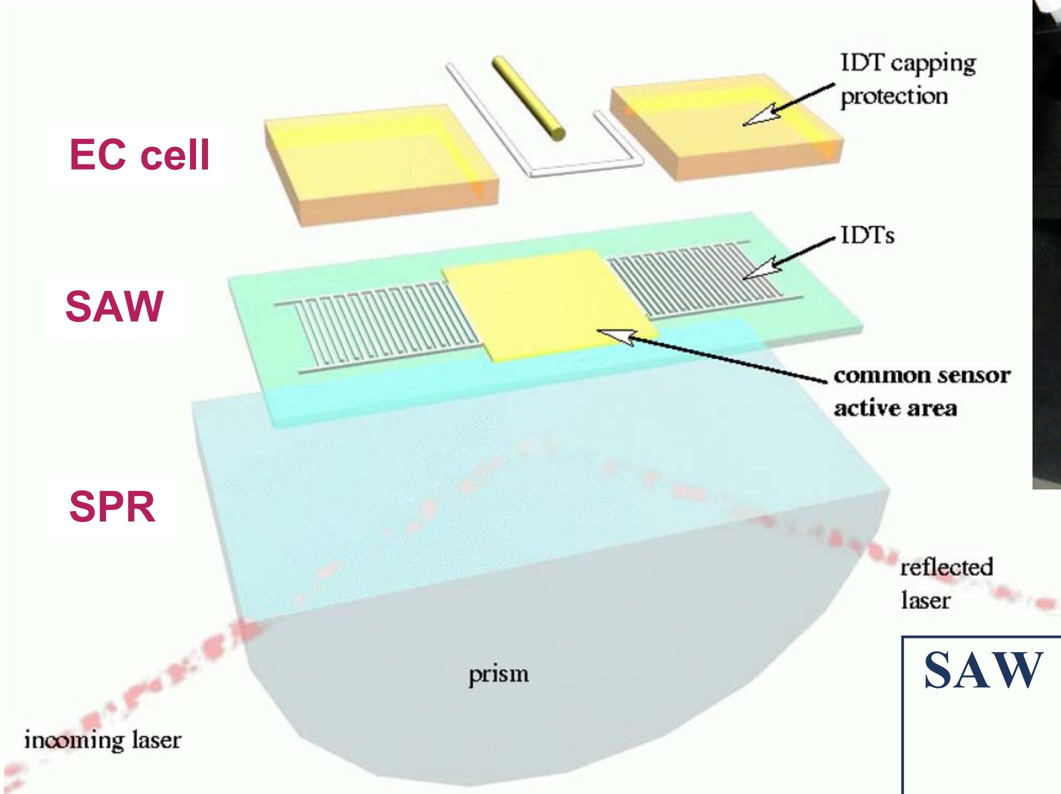
Surface Plasmon Resonance (SPR) is an **optical method** where collective electromagnetic modes are excited by a light source at metal-dielectric interface.

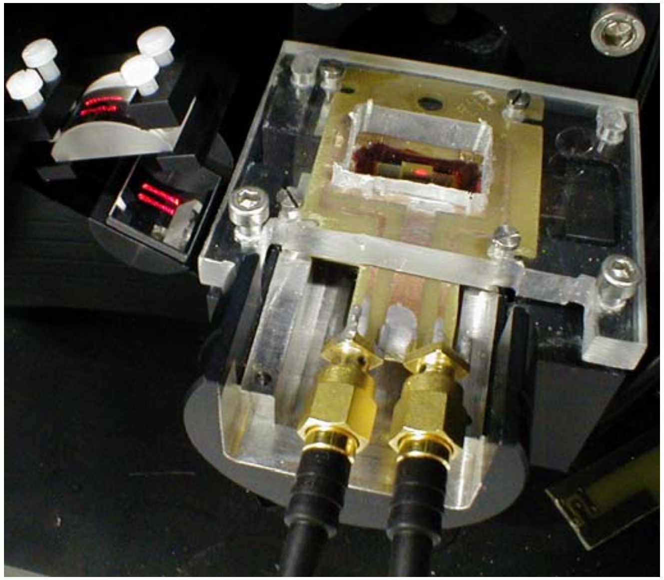
SPR is sensitive to the **thickness *d*** and the **refractive index *n*** of dielectric added layers above specific metals (gold, silver, …).





COMBINED SAW/SPR: SET-UP



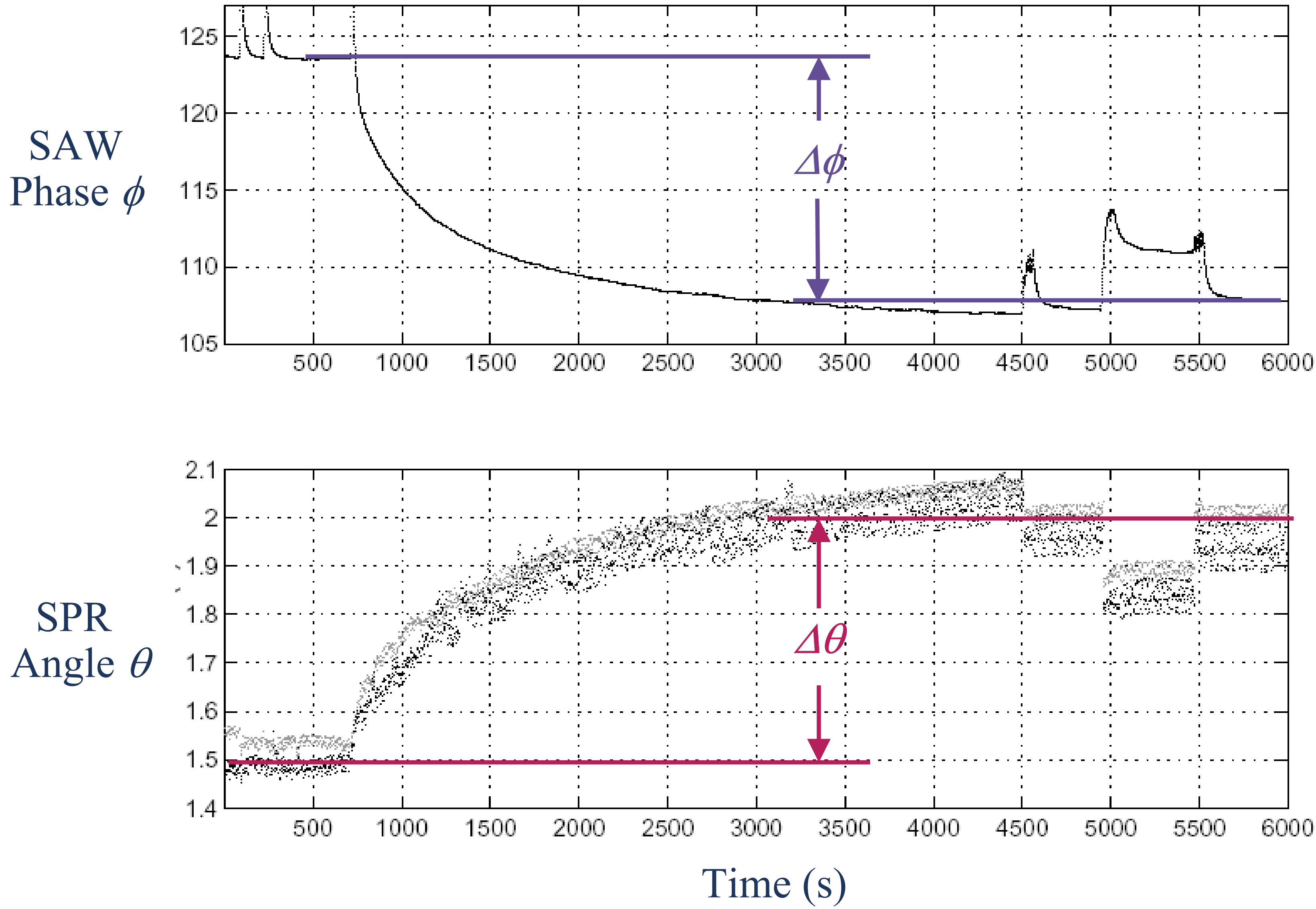


SAW	ST-cut quartz substrate 1.2 µm H-rich SiO_2 10 nm Ti/ 50 nm Au wavelength 40 µm SU8/glass IDT capping Cu-calibrated mass sensitivity of –260 cm^2/g

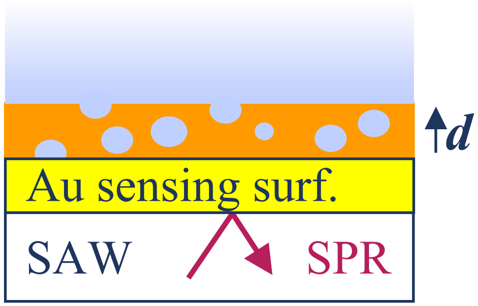
SPR	modified IBIS II SPR (IBIS Technologies BV) 670 nm laser light Kretschmann configuration

COMBINED SAW/SPR: EXPERIMENTAL EXAMPLE

Collagen 30 μg/ml adsorption on hydrophobic surface



SAW/SPR MODELING



d = thickness of the layer (protein + water)
x = proportion of proteins in the layer
ρ = density
n = refractive index

Linear combination of the protein and the water:

density $$\rho(x) = x\,\rho_{PROTEIN} + (1-x)\,\rho_{WATER}$$

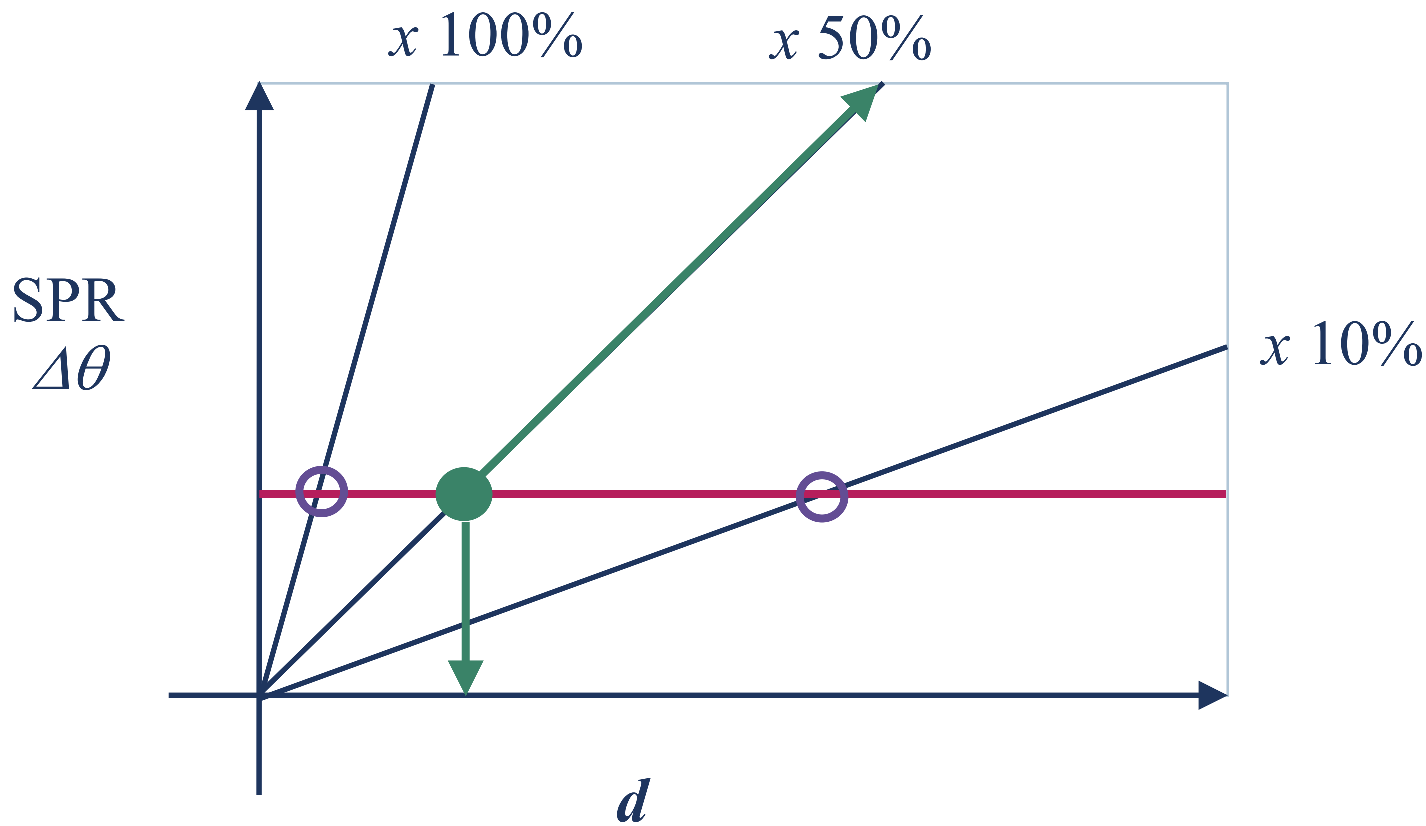
refractive index $$n(x) = x\,n_{PROTEIN} + (1-x)\,n_{WATER}$$

The **SAW shift** $\Delta\phi$ gives the surface density ***m*** of the layer:

$$m = \left(\frac{\lambda}{360^\circ\ DS}\right)\Delta\phi = \rho(x)\,d$$

The **SPR shift** $\Delta\theta$ is simulated from ***d*** and ***n(x)***.

SAW/SPR MODELING



1) Simulated SPR shifts $\Delta\theta\,(d,x)$

2) Experimental SPR shift

3) Allowed values for SAW shifts $\Delta\phi$ (d,x)

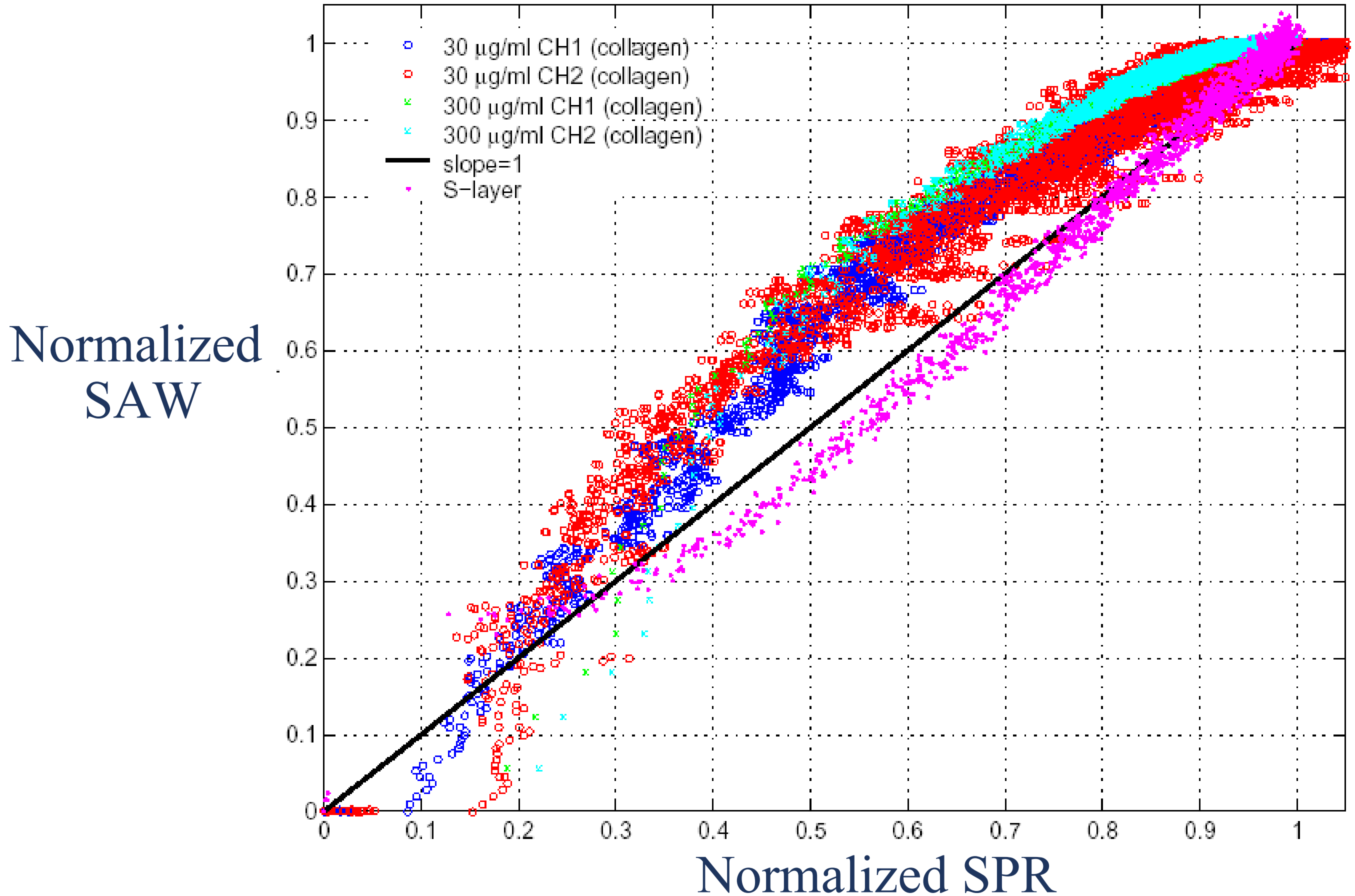
4) Extraction of thickness ***d*** and protein content %

EXPERIMENTAL RESULTS

In-situ measurements of collagen and fibrinogen adsorbed on hydrophobic surfaces:

	Analyte (µg/ml)	Surface density m (ng/cm^2)	Thickness d (nm)	Protein content x (%)
STRONG HYDRAT.	Collagen 30 µg/ml	1750 ± 150	16 ± 3	25 ± 15
	Collagen 300 µg/ml	2100 ± 200	19 ± 3	35 ± 10
STACKED LAYERS	Fibrinogen 46 µg/ml	750 ± 100	6 ± 1.5	50 ± 10
	Fibrinogen 460 µg/ml	1500 ± 500	13 ± 2	50 ± 10
"DRY" LAYERS FOR SAW CALIB.	S-layer	560 ± 20	4.7 ± 0.7	75 ± 15
	CTAB	135 ± 15	1 ± 0.1	100

EXPERIMENTAL RESULTS: ADSORPTION KINETICS



The kinetic of the adsorption monitored by the two techniques is different:

- wrong model ? **Viscous effects** neglected in the SAW !
- mass over/underestimation.

DISCUSSION: DRAWBACKS OF THE TECHNIQUES

SAW drawbacks

- high temperature sensitivity
- rigid mass and viscous effects combined
- signal distortion due to interferences
- different phase and group velocities

SPR drawbacks

- temperature dependence
- signal distortion due to birefringence
- optical interferences for multiple layers
- data extraction through modeling
- limited to metal surfaces (gold, silver)

Combined technique

- no interferences between SAW and SPR
- complementary information allowing extraction of coupled parameters (ρ, $n \rightarrow x$, d) on a same layer

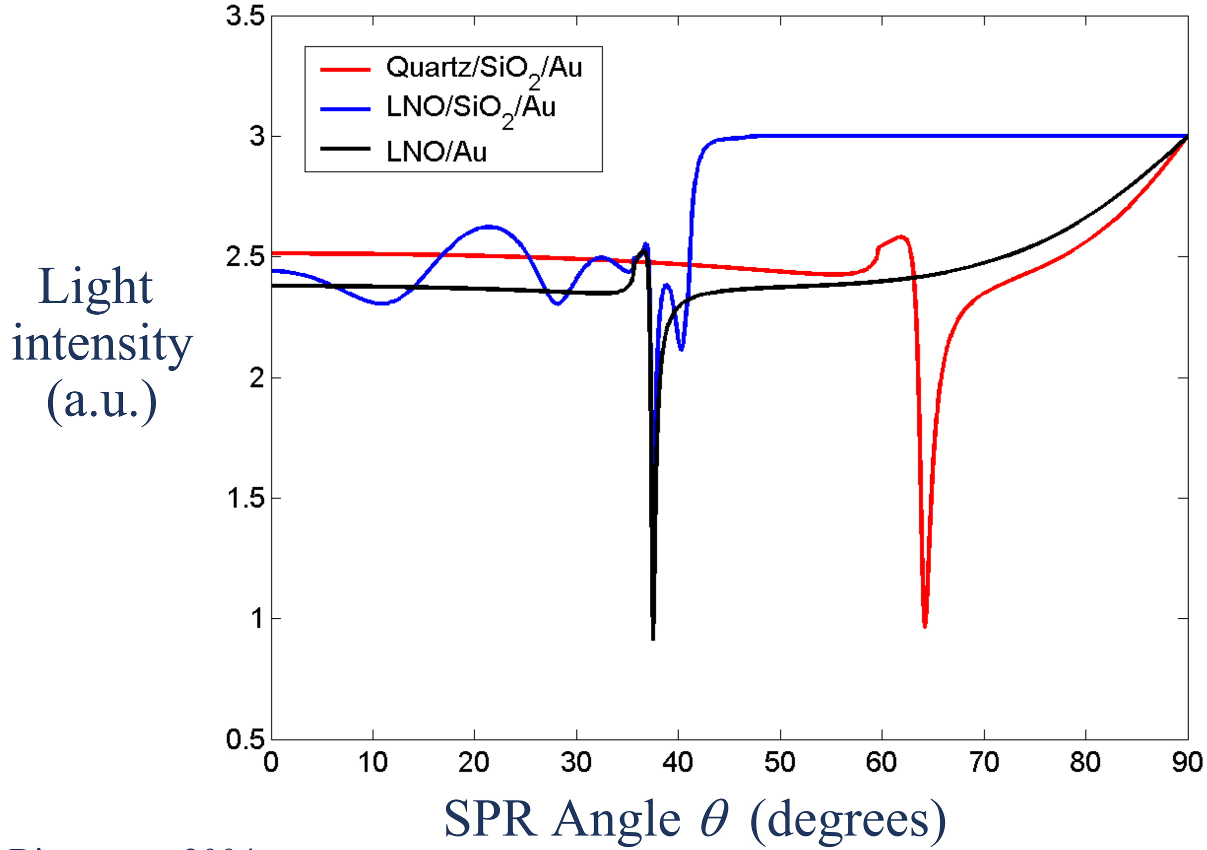
DISCUSSION: LOVE MODE SAW MATERIAL COMBINATIONS

SPR angle is function of ***n*** and of the light wavelength.

Interferences and total internal reflection effects must be considered for a stack of layers.

SPR sensitivity higher for θ closer to 90°.

Conductive layers influence the SAW transfer function by shortcutting the transducers.



CONCLUSIONS

- Combined SAW/SPR technique:
  - provides information about the **thickness** and **water content** of protein layers;
  - **real time measurements**, with some insights into the adsorption kinetics;
  - **label-free** acoustic and optical method.

- Ways to increase the Love mode SAW mass sensitivity:
  - H-rich PECVD SiO2
  - Gold

- Experimental results on collagen and fibrinogen adsorption demonstrate the potential and the limits of the combined technique.

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