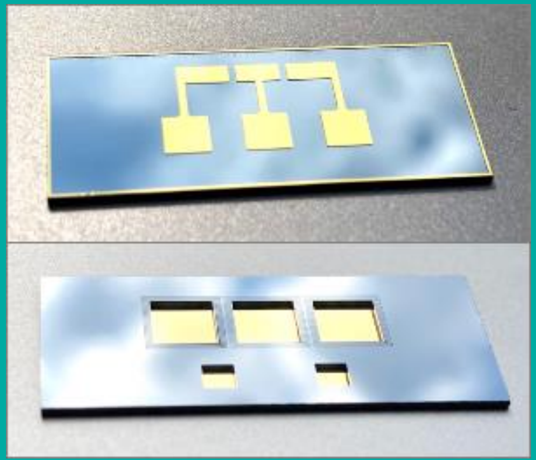
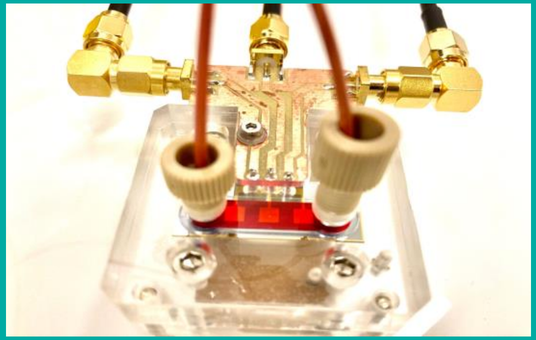
LAB ON CHIP DEVELOPMENTS FOR THE ANALYSIS OF A MECHANISM IN A COMPLEX FLUID





Thérèse LEBLOIS
Université de Franche-Comté
FEMTO-ST Institute
BioInterface and Devices team
Besançon
FRANCE

OUTLINE

- Lab-On-Chip
- Biosensors based on acoustic waves
- Objective: a better understanding of the primary hemostasis
  - ✓ QCM
  - ✓ Quartz on silicon microdevice
- Results
- On-going activities and future plan

WHAT IS OUR MOTIVATION FOR DESIGNING A LAB-ON-CHIP?

→ **Need in the fields of chemistry, agri-food, health and environment**

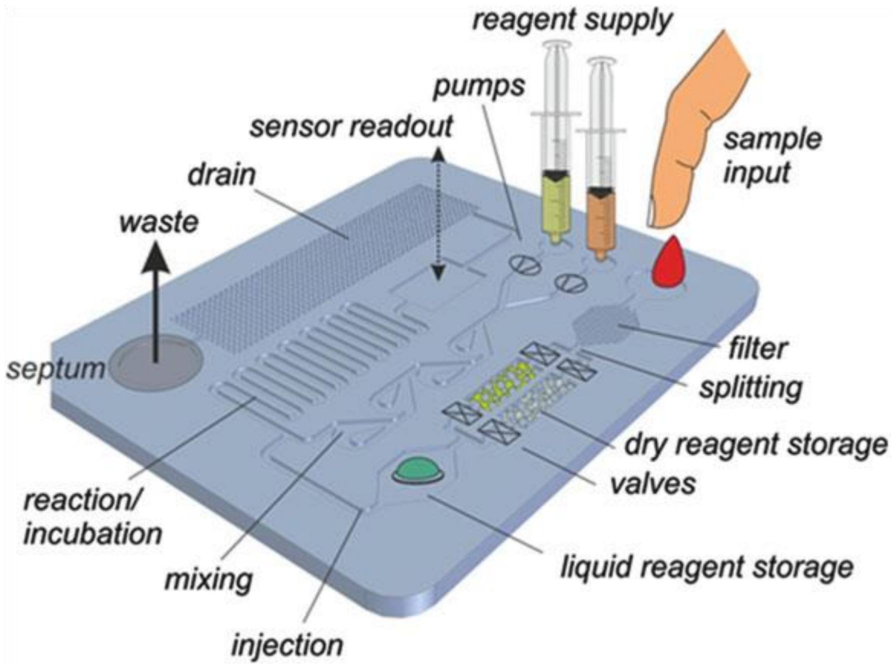
- Faster drug testing with an *in vitro* model before moving to clinical trials
- Better understanding of a physiological, biochemical or chemical mechanisms
- Ability to perform fast heating and cooling at the microscale
- Food quality and production improvement
- Single cell analysis
- Isolation, filtration, sorting of rare elements in complex biofluids
- Detection of biomarkers, contaminants, …with high sensitivity, short diagnostic time; high parallelization of analyses
- Small amounts of expensive reagents and samples, small volume of biofluids

WHAT ARE THE COMPONENTS OF THE LAB-ON-CHIP?

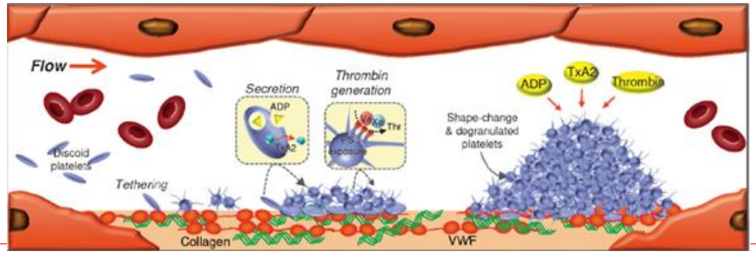
→ **Microfluidics channels and reactors**

→ **Actuators for mixing and sorting**

→ **Sensing (physical /biosensors)**



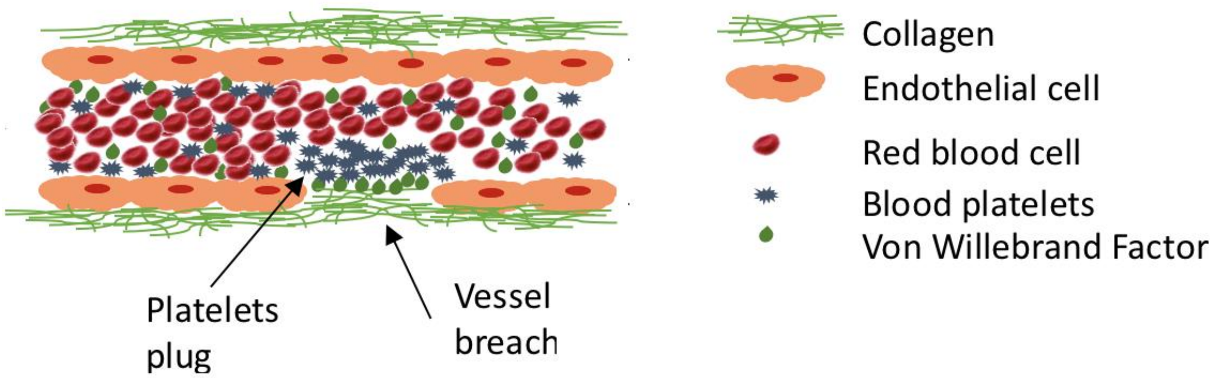
Application: a better understanding of the phenomenon of primary hemostasis - Study is performed in whole blood, therefore in a complex fluid.

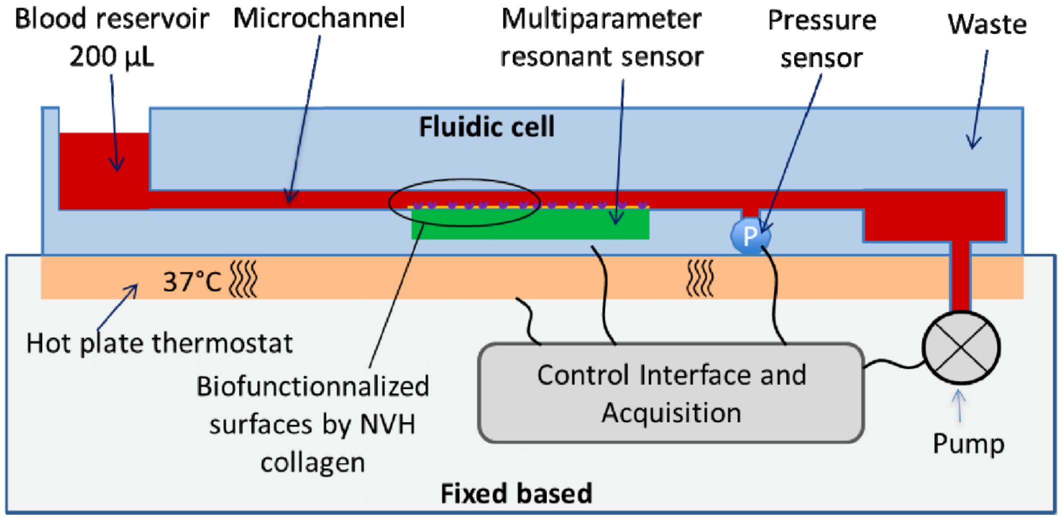


HEALTH: PRIMARY HEMOSTASIS MECHANISM

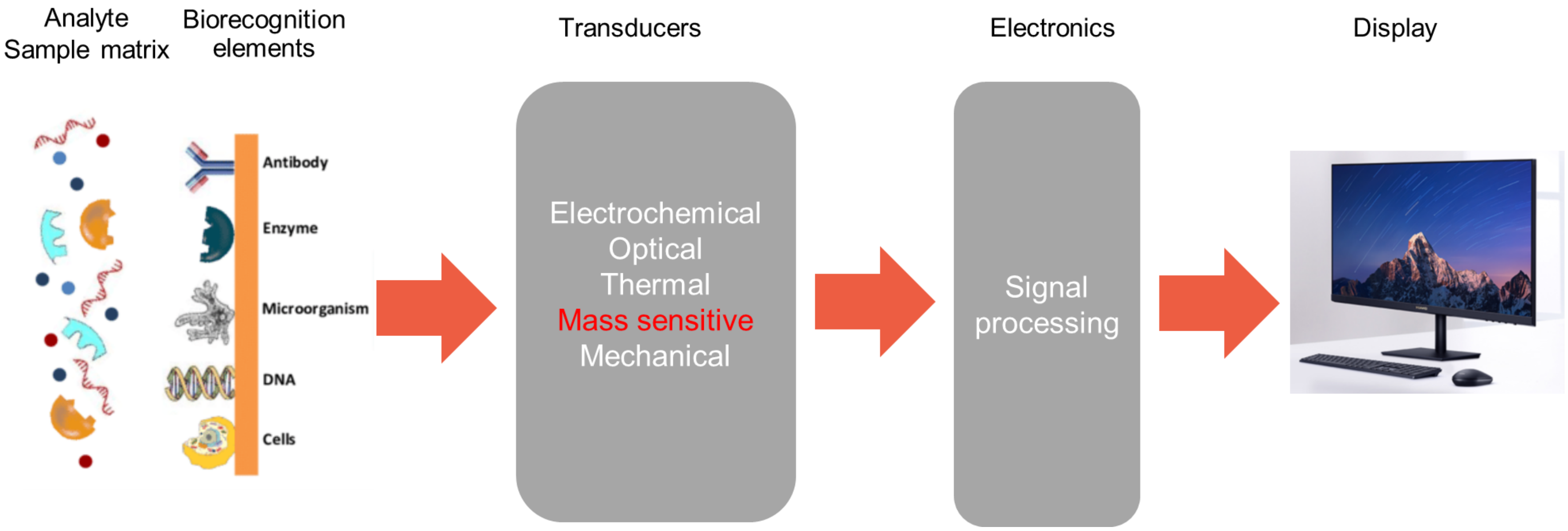
- Hemostasis = a dynamic process, under flow conditions
- Mimic the flow conditions *in vivo*
- Real time measurement
- Low volume of blood required

- Replicate in the microfluidic platform rheological conditions of blood in vessels *in-vivo*
- Functionnalize the surface with collagen to mimic a vessel breach, allow the binds of vWF and to initiate platelets attachment
- Integrate a microbiosensor to detect and follow the interactions at the biointerface





BIOSENSORS



- **Highly specific**
- **Highly sensitive**
- Able to reach **a low LOD**
- Independent of physical parameters (e.g., pH, temperature, etc.)
- Reliable
- **Reusable**
- Low cost

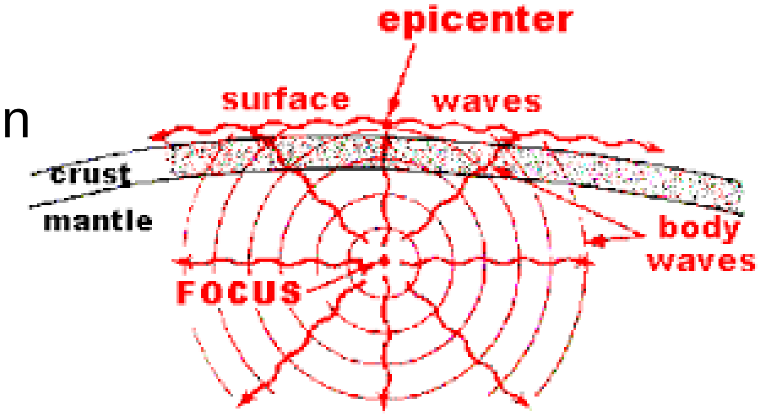
Analytical device that is able to **convert a biological response into an electrical signal.**

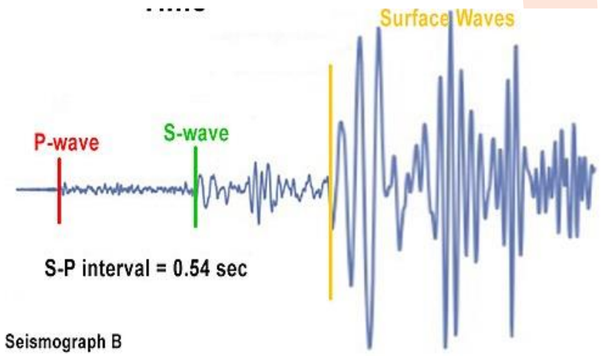
Principle: mass sensing
→ Acoustic transducer

ACOUSTIC WAVE IN SOLID MEDIA : SEISMIC WAVES

Acoustic Waves = Sound waves = **mechanical vibrations** that propagate in a media = fluid (gas or liquid) and solid.

Example of acoustic waves in solid media : Earthquake

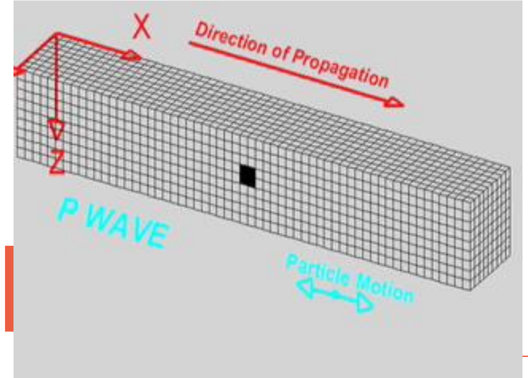


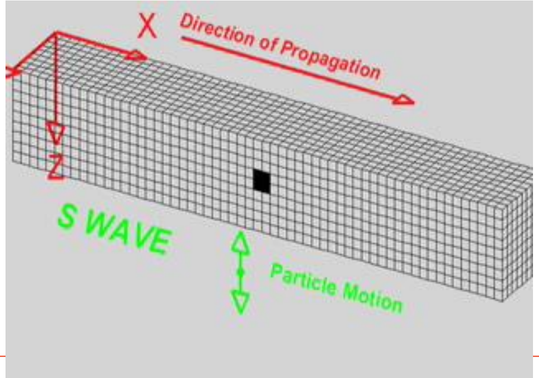


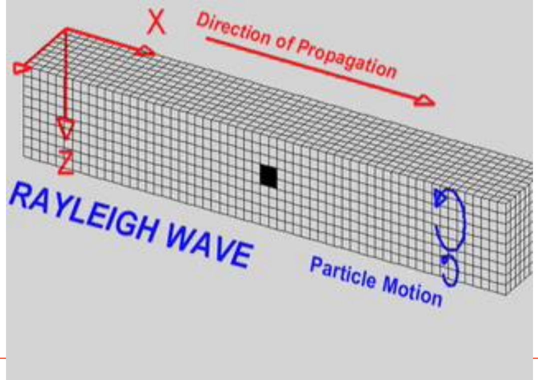
Theory:

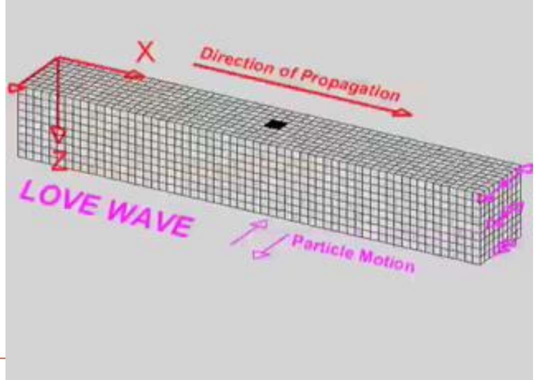
Analytical solution of mechanical vibrations (unguided waves)

+ Boundary condition (guided waves)





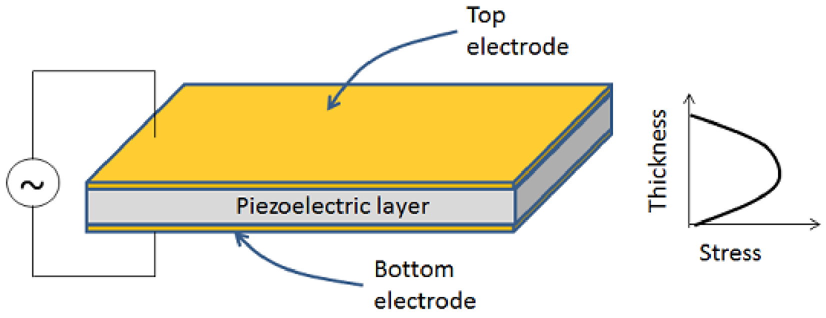




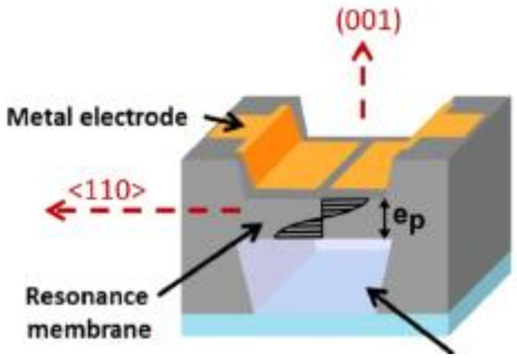
→ **Shear waves in liquid**

TRANSDUCER FOR ACOUSTIC WAVE : BULK ACOUSTIC WAVES

BAW = Parallel Transducer
Two designs



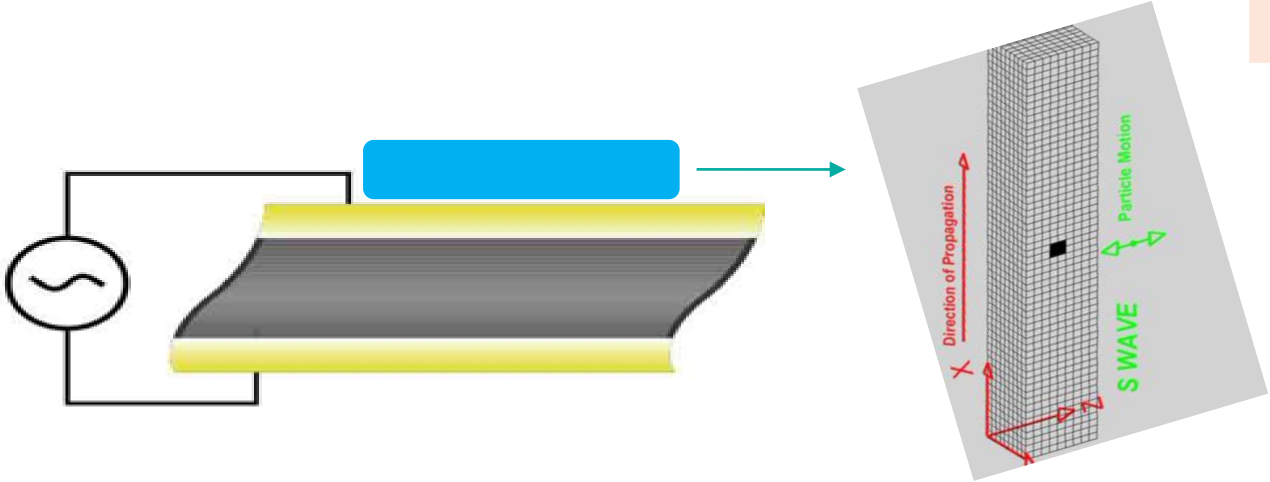
BAW with thickness field Excitation



BAW with lateral field Excitation

3 types of waves: longitudinal / shear waves

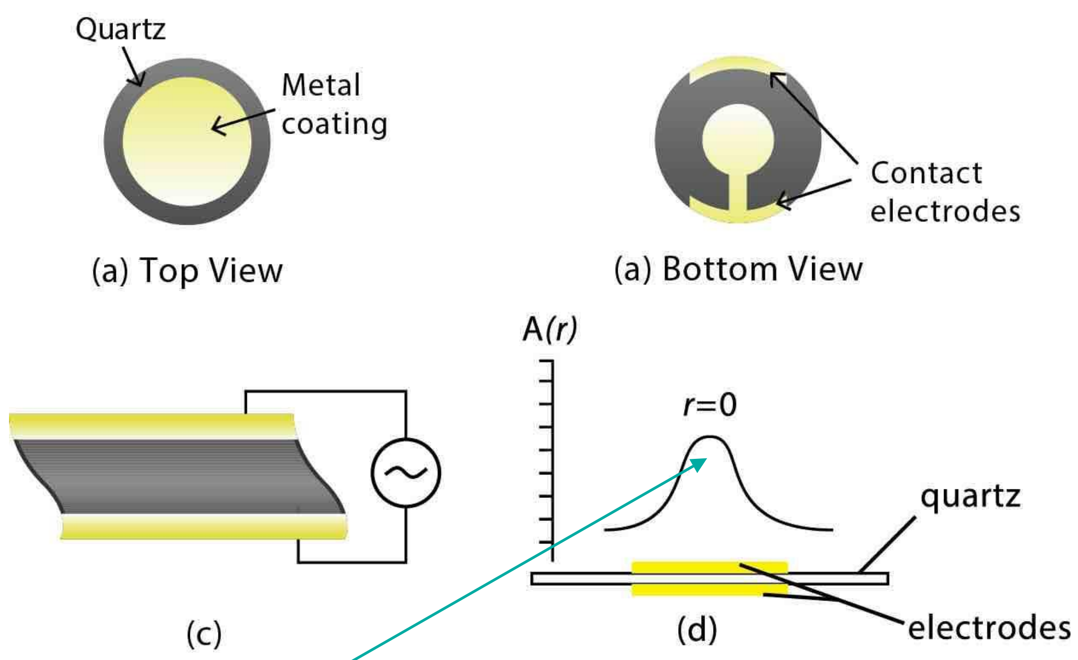
- QCM → **shear waves (in plane displacement)**

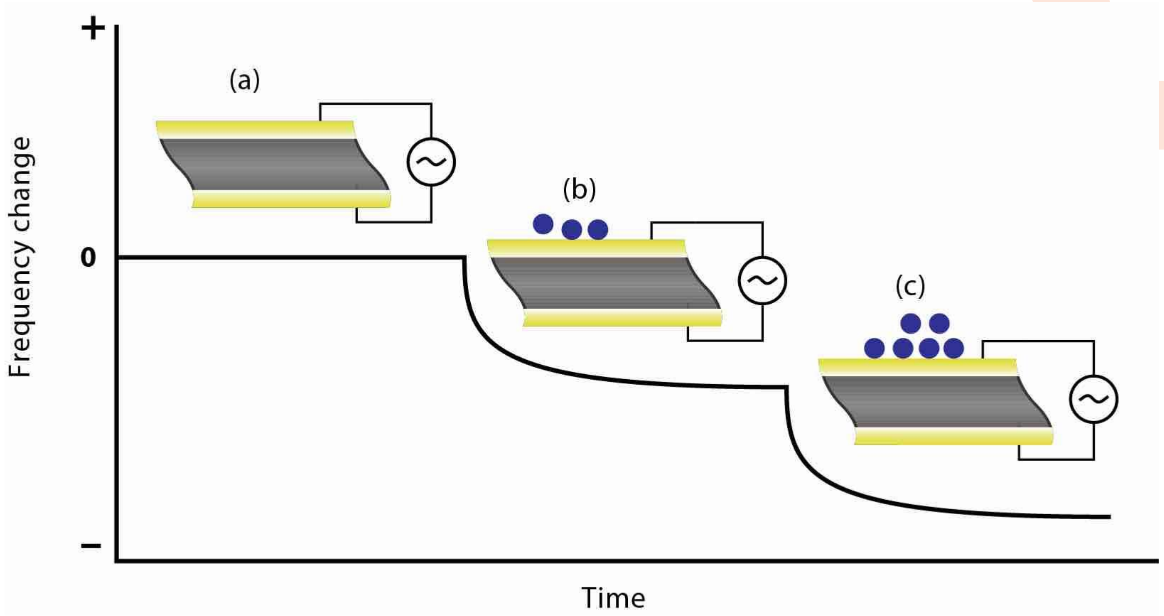


Contact with fluid:
→ **Acoustic energy must not be converted into pressure waves in liquid**
→ **Confine the energy within the plate**
→ **Minimum signal loss in liquid environment**

<u>Frequency</u> = Velocity (Vph) / Wavelength (λ)

COMMERCIAL QCM SENSOR





Peak amplitude (y-axis) at resonance frequency (x-axis)
Q factor: key factor for the performance

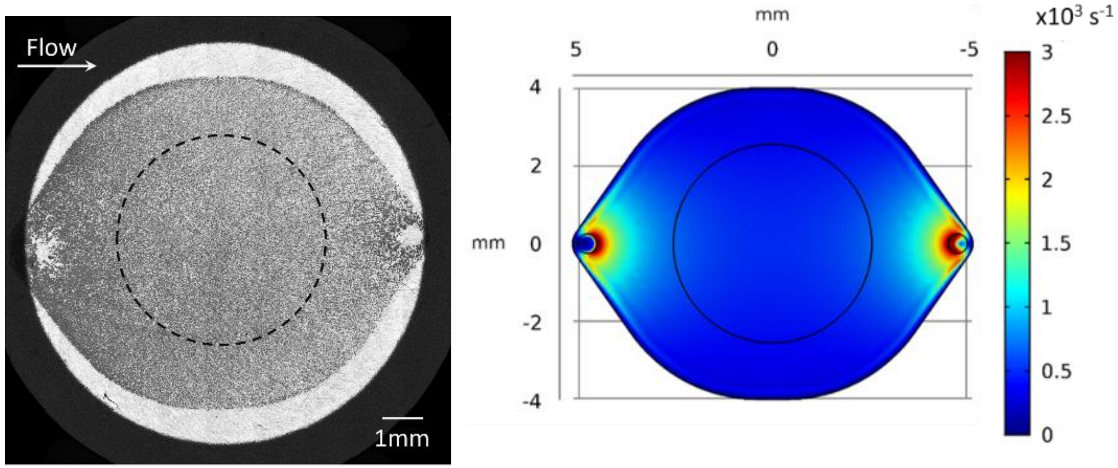
Mass loading → shift in resonance frequency

Viscoelastic material (biomolecules) → shift in frequency and decrease in signal amplitude

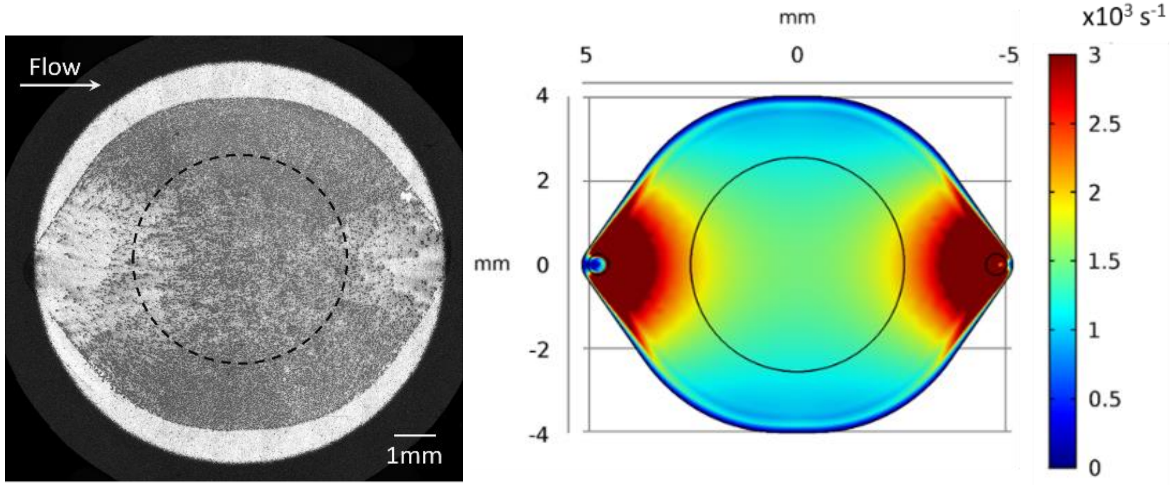
Current commercial QCM sensor has maximum frequency of 10 MHz, with maximum theoretical sensitivity of **4 ng/(cm²·Hz)**

QCM BASED SENSOR FOR PRIMARY HEMOSTASIS STUDY

- Blood perfusion tests results



Shear rate 500 s^{-1}



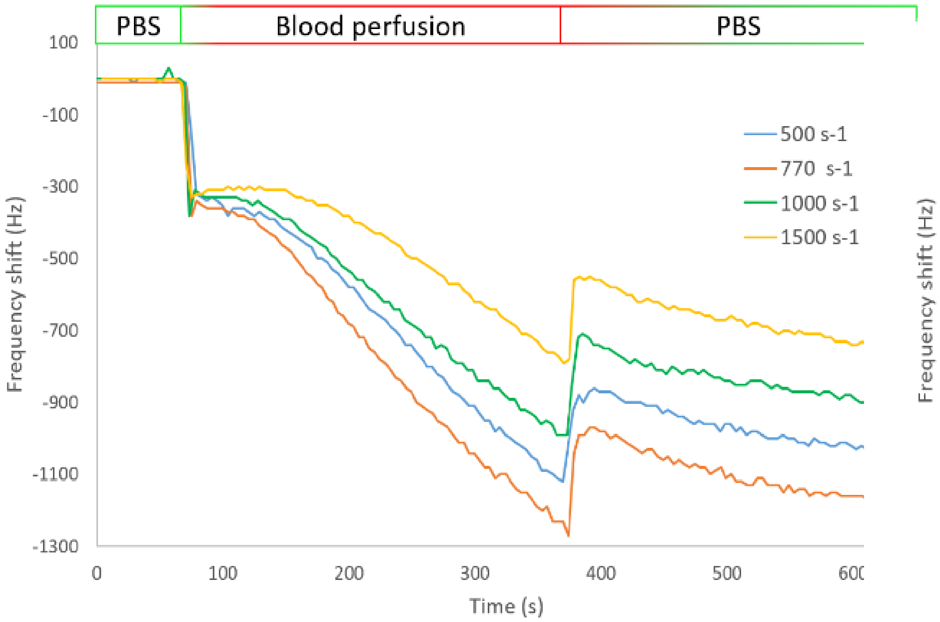
Shear rate 1500 s^{-1}

Shear rate: a key parameter to mimic the physiological behaviour of the device.

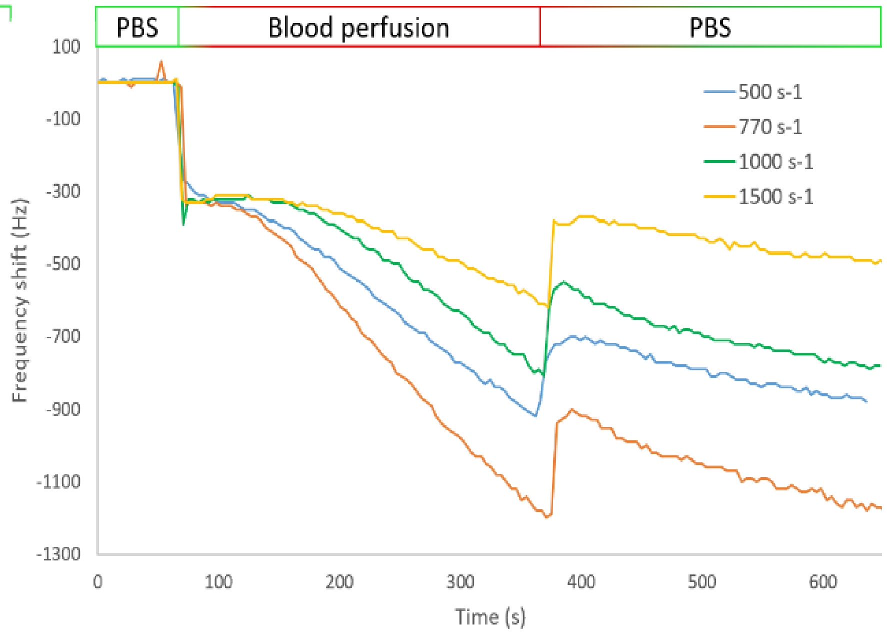
- Blood tests results for two donors

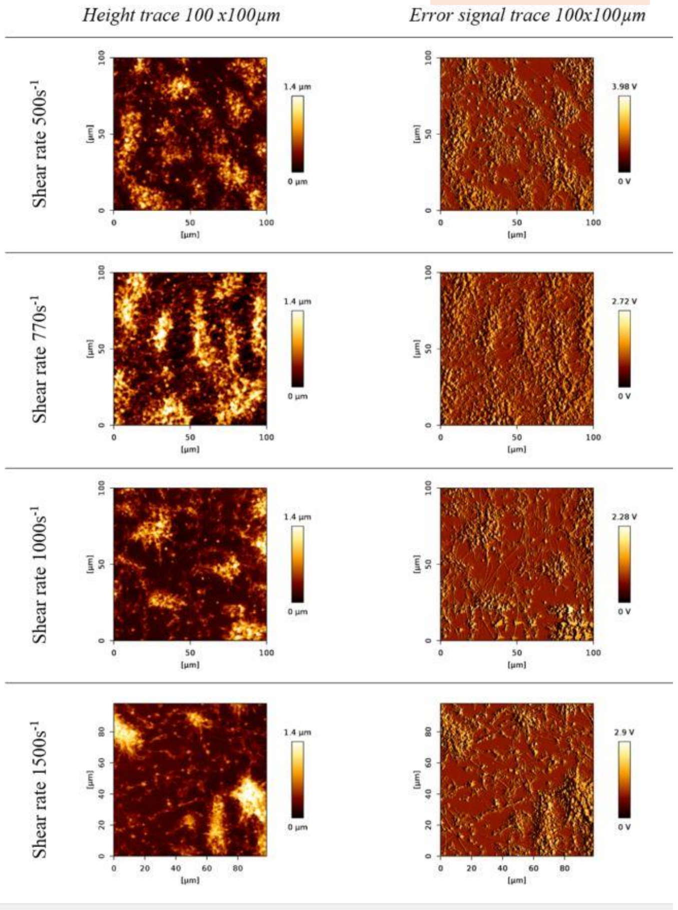
AFM images

Donor 1



Donor 2

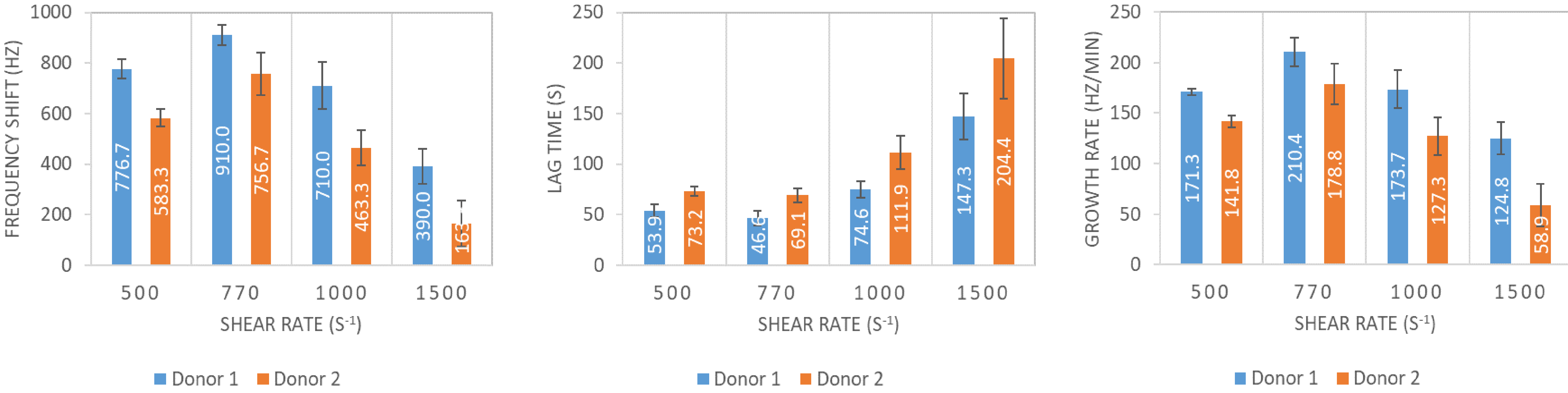




- Criteria
  - frequency shift : After five minutes
  - lag time : corresponds to a frequency shift of 50Hz .

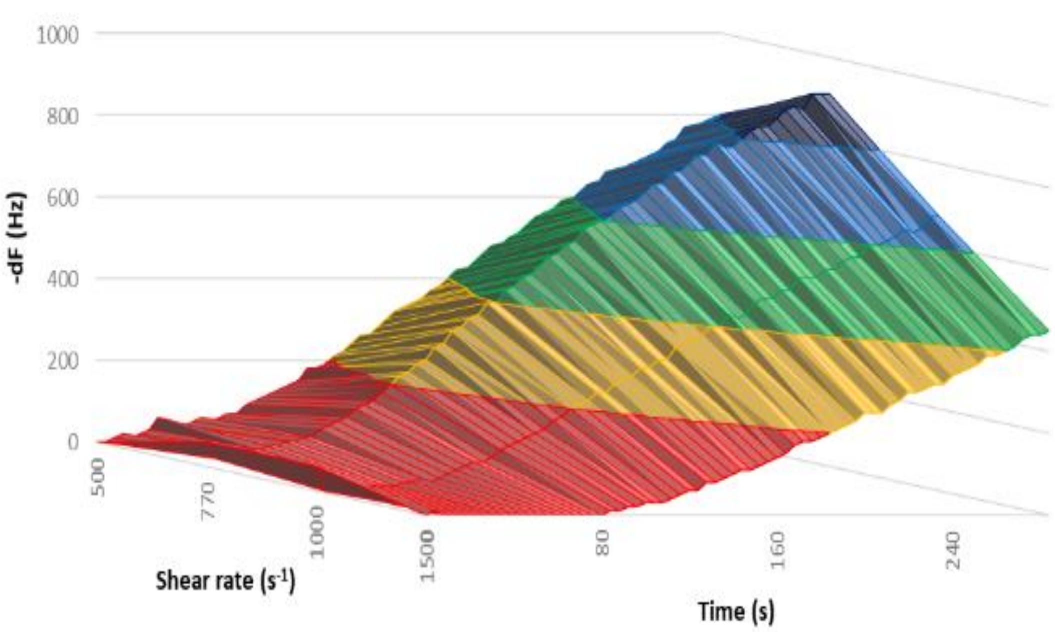
QCM BASED SENSOR: RESULTS

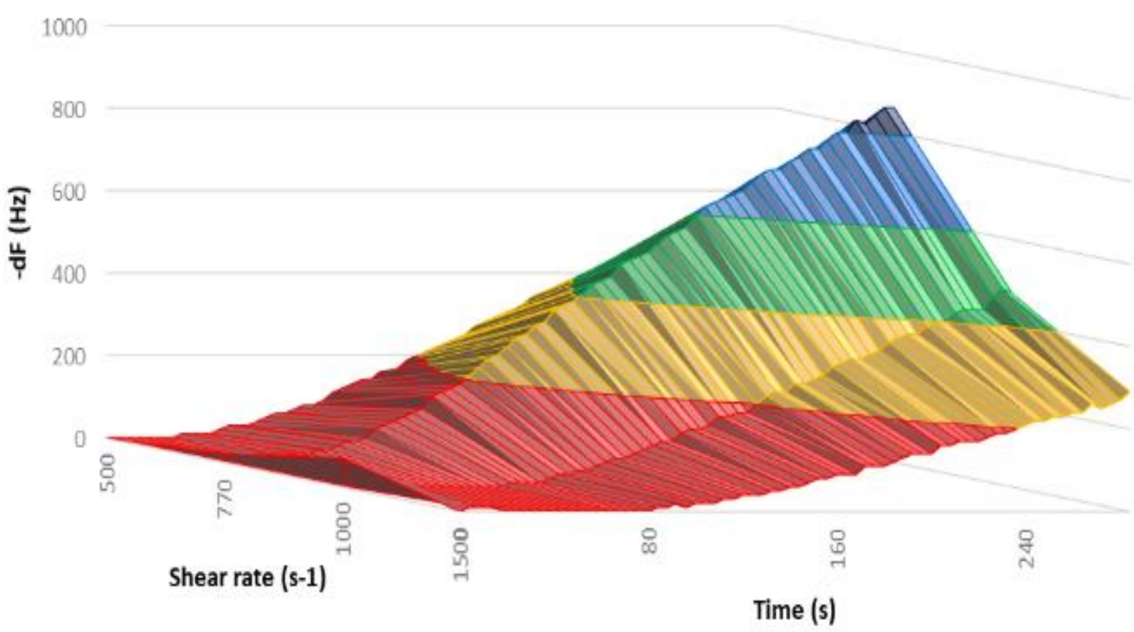
- Quantitative assessment of experimental data



QCM BASED SENSOR: RESULTS

- Blood tests results for two donors





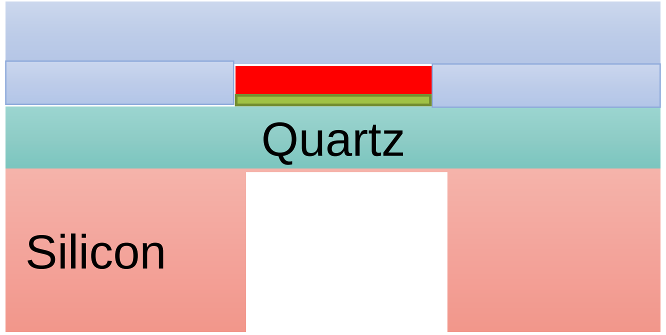
→ We can discriminate the two donors

[1] Oseev, Aleksandr, et al. "Assessment of shear-dependent kinetics of primary haemostasis with a microfluidic acoustic biosensor." IEEE Transactions on Biomedical Engineering (2020).

LIMIATIONS: NEW SENSOR DESIGN

Requirements:

- Ability to evaluate platelets deposits up to 1µm height. (average height values obtained with QCM sensor are in a range 200-400nm after 5 minutes of perfusion)
- Microfluidics integration ability
- Ability to be scaled and multiplied on a single chip
- easier fabrication
- Robust device



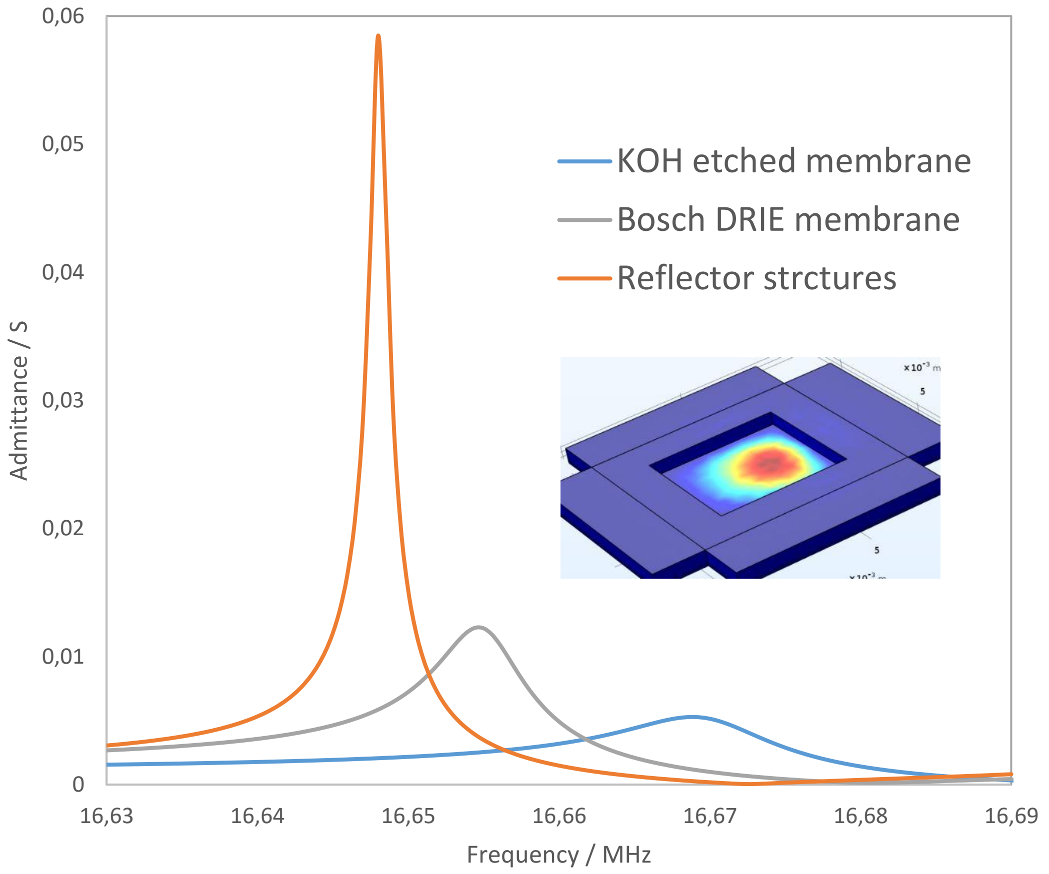
→ **New design of cells Quartz on silicon device**

RESULTS OF THEORETICAL STUDY

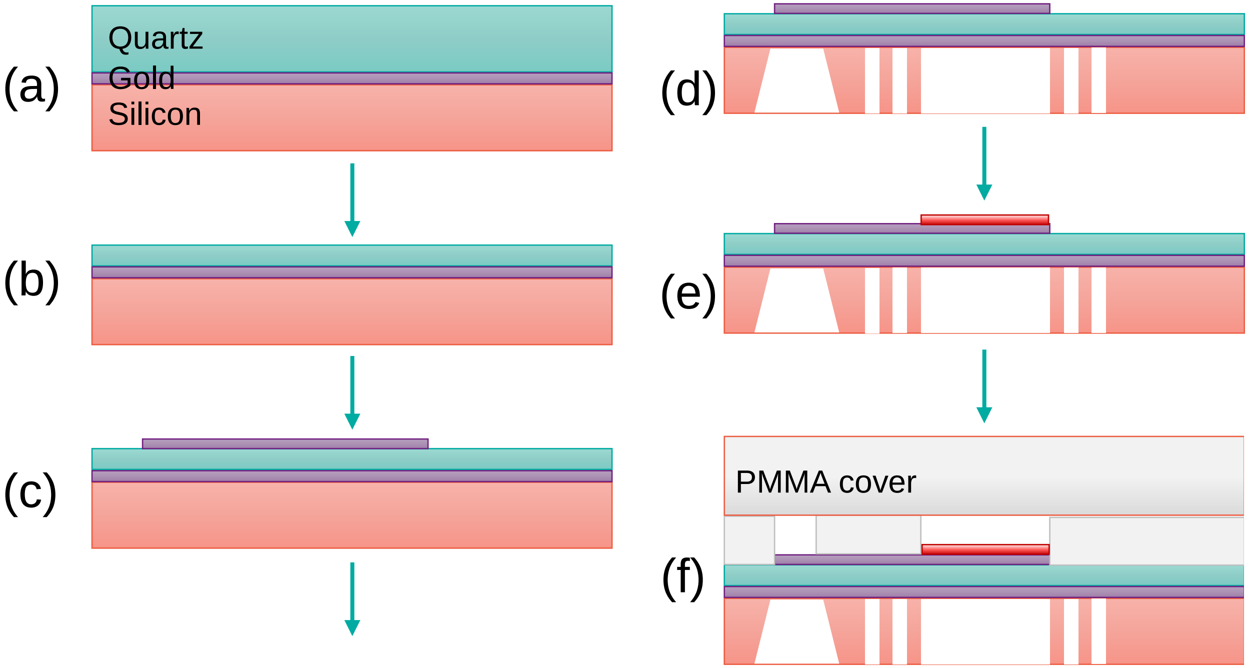
Challenge – dissipation of acoustic energy into a holding silicon wafer leading to significant losses

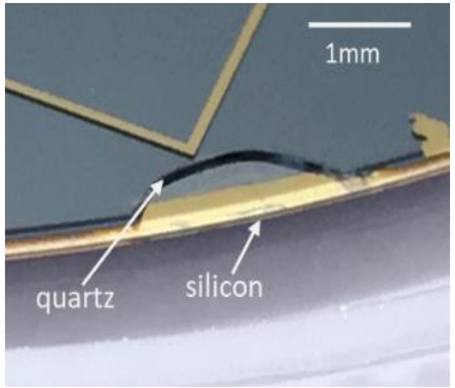
→ Results:

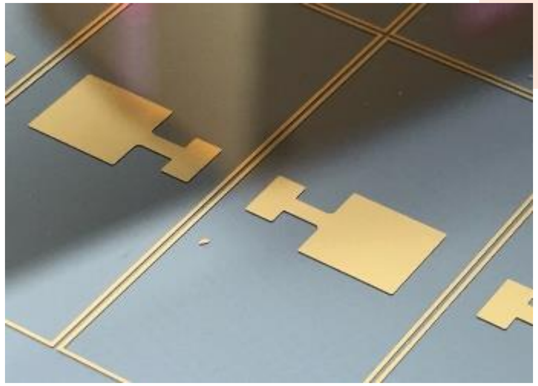
- Determination optimal dimensions
- Evaluation impact of technology
- Design reflector structures that improve Q-factor x10 (comparing to KOH etched membrane)

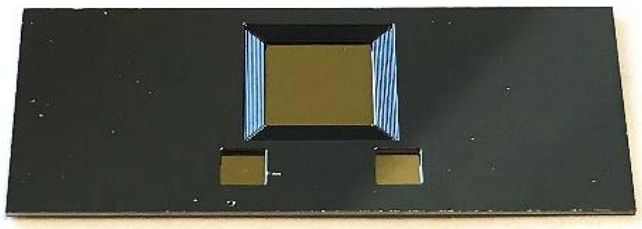


SENSOR PROTOTYPE MICROFABRICATION

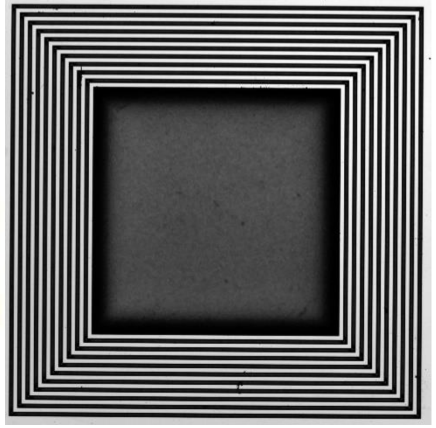




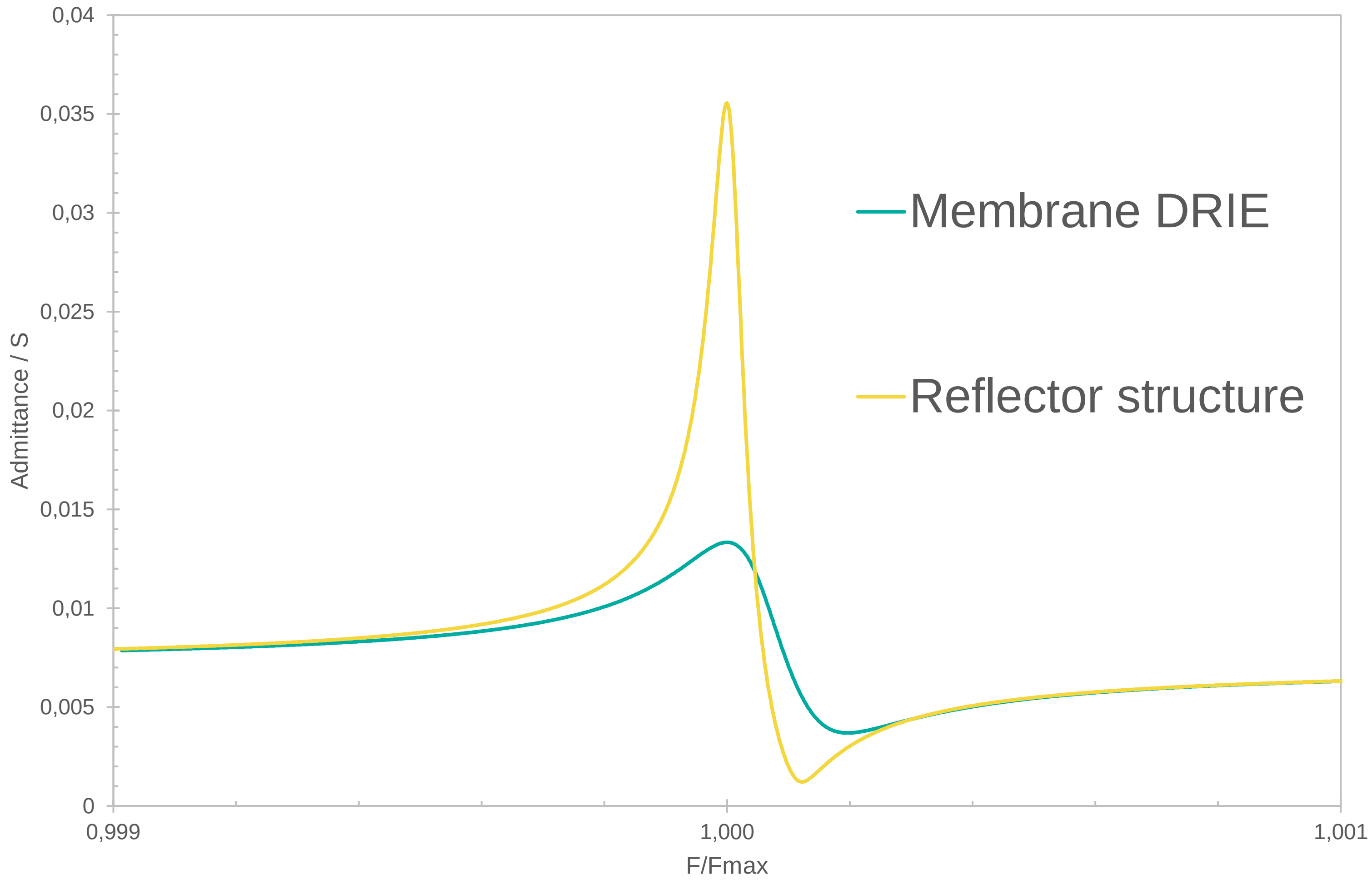




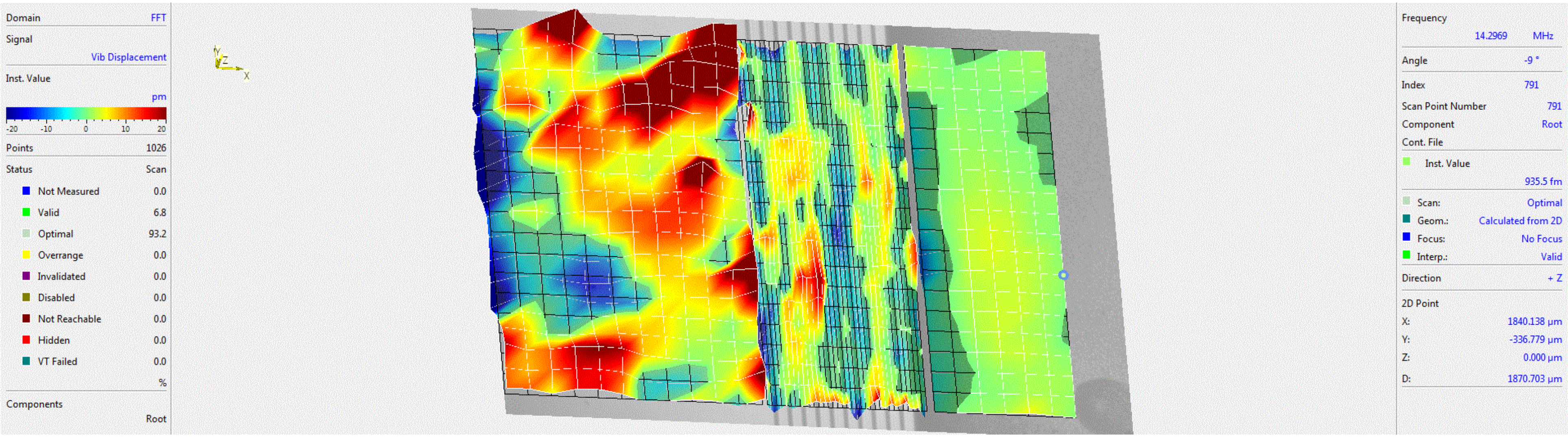
Bottom surface

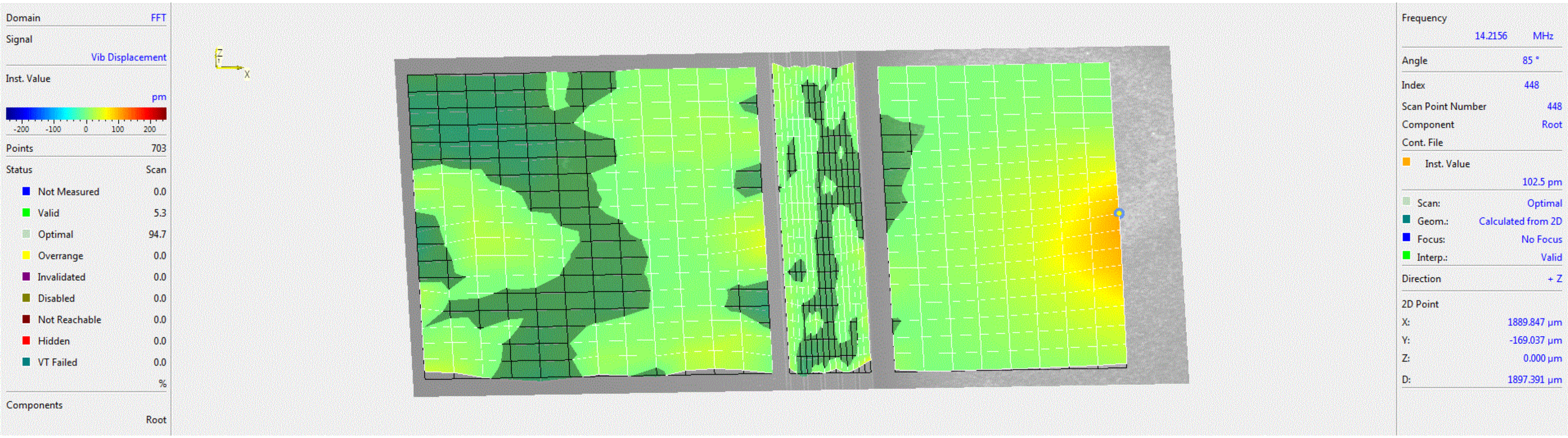


SENSOR CHARACTERIZATION – ADMITTANCE RESPONSE

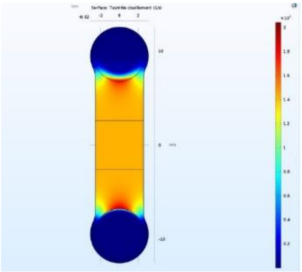


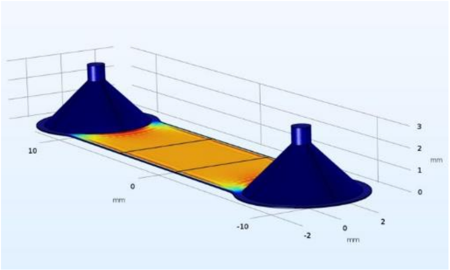
SENSOR CHARACTERIZATION - MEMS VIBROMETER



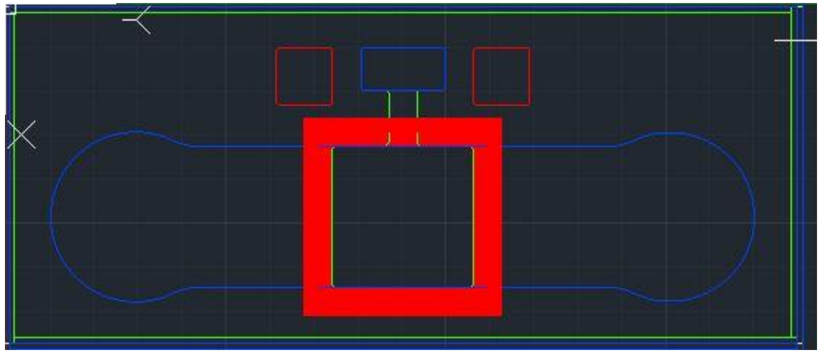


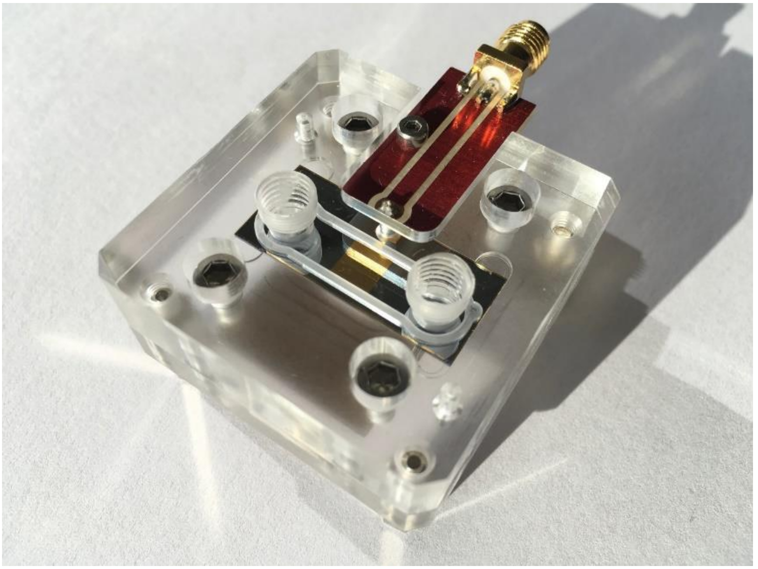
FLUIDIC CELL DESIGN AND REALIZATION



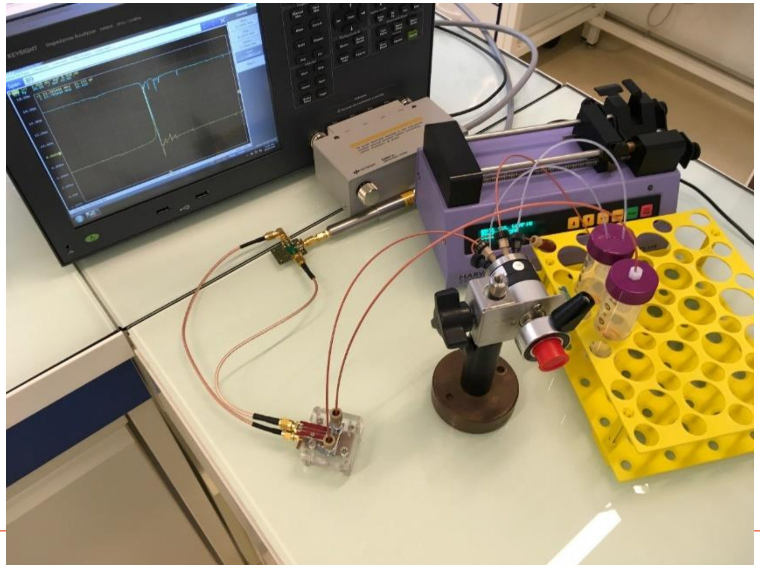


Microfluidic cell with sensor

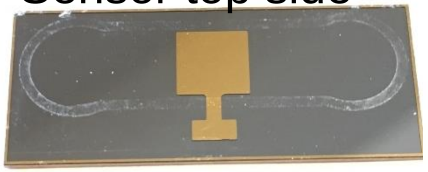




Experimental setup



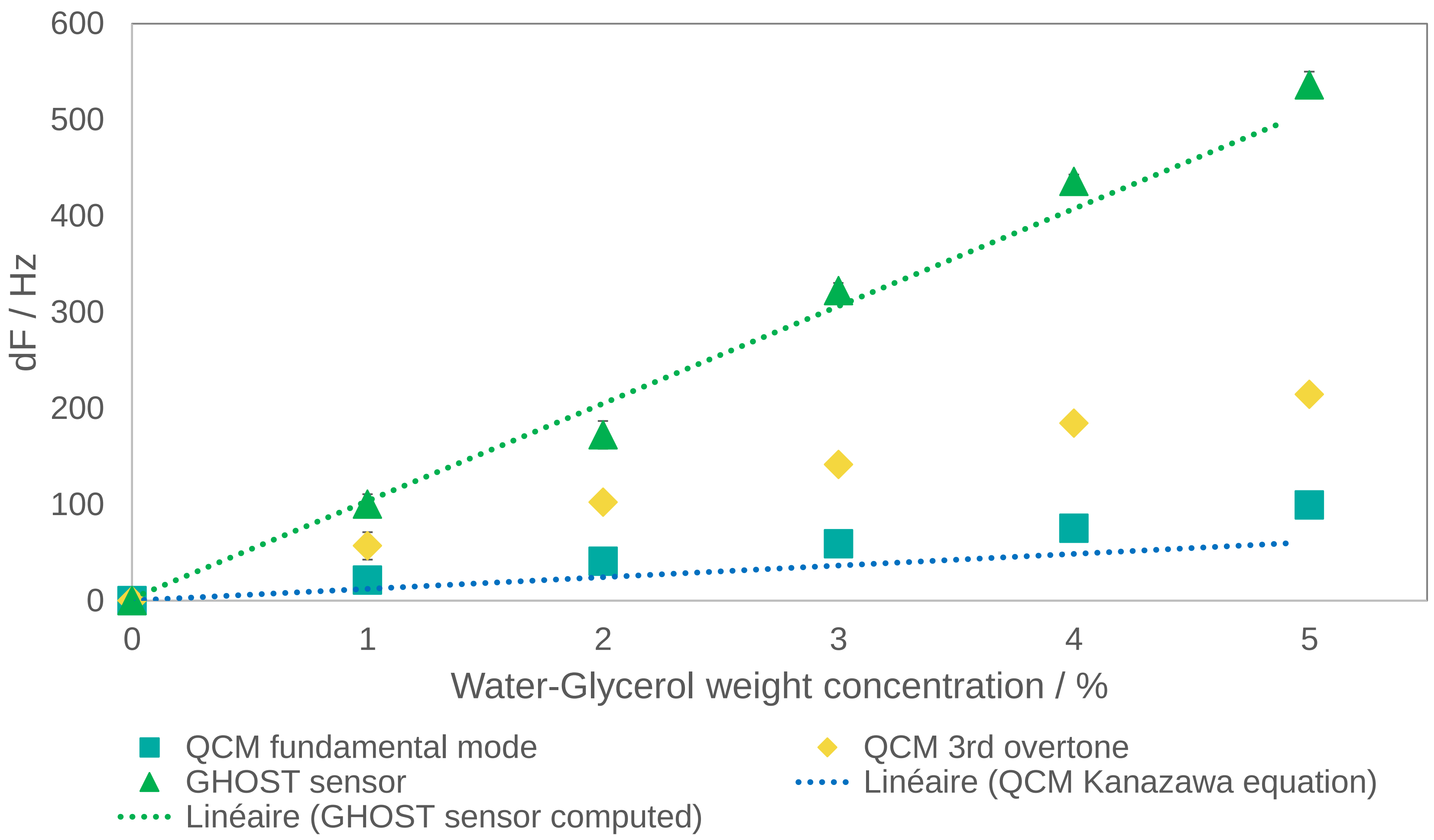
Sensor top side



Sensor bottom side

GHOST SENSOR VS QCM SENSOR RESPONSE

Water/Glycerol Weight %	Density [kg/m3]	Viscosity [Ns/m2]
0	997.38	0.00093505
1	1000.2	0.00096257
2	1002	0.00098074
3	1004.3	0.0010049
4	1006.6	0.00103
5	1009	0.001056

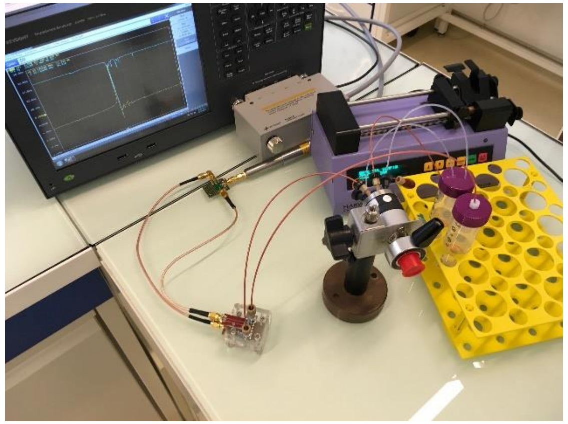


Good sensitivity for the new cell → validation

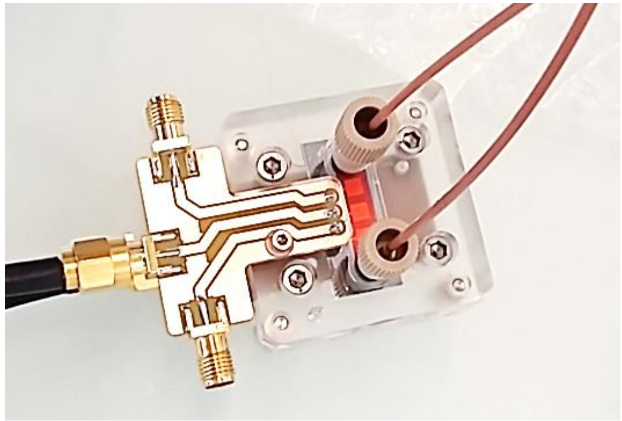
Multiplexed sensor X3

GHOST TESTS– GHOST X3 CHIP – COLLAGEN HORM

- **Experimental set up**

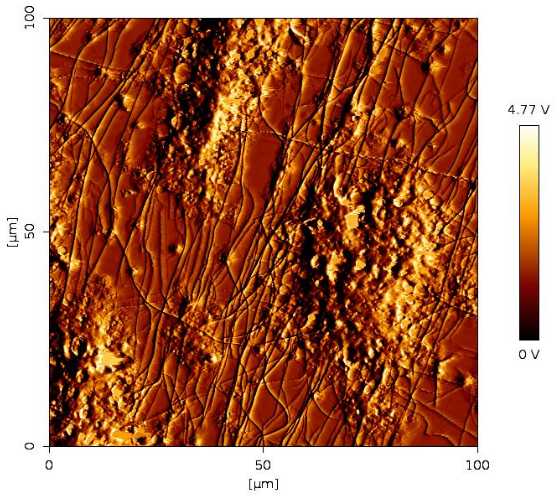


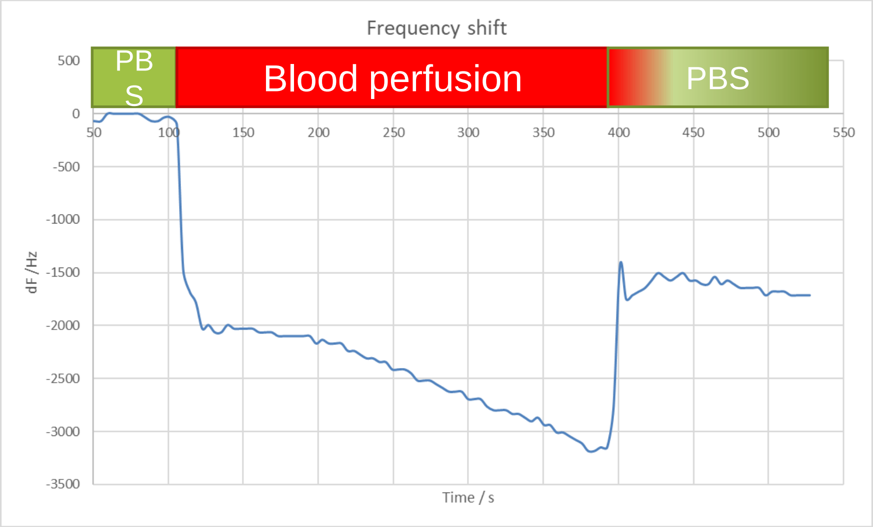
- **Biosensor + microfluidics**

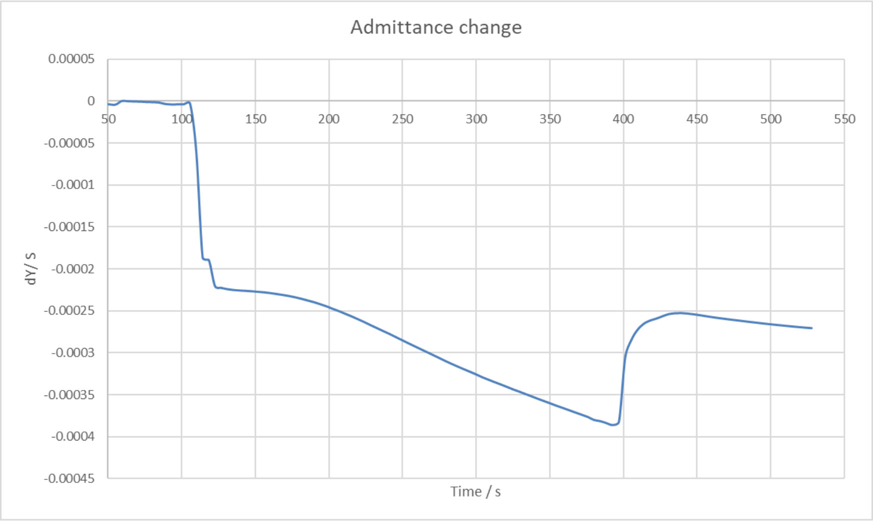


Biointerface :
Gold surface> C11C16 -> SE ->
Collagen type 1: 50µg/mL
-> BSA 0.1% -> Ethanolamine

Tests:
Whole blood
Multiplex X3
Similar to *in vivo* conditions
Time of perfusion: 5min
Temperature: 23°C ± 1°C

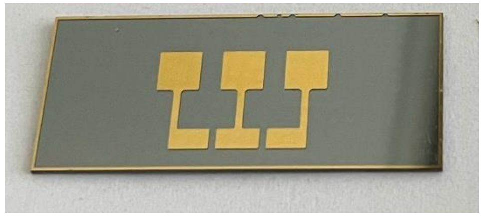


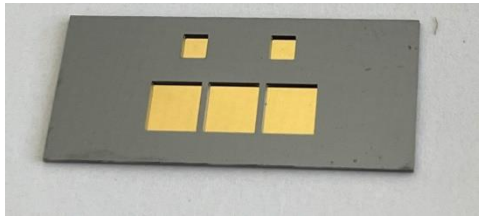




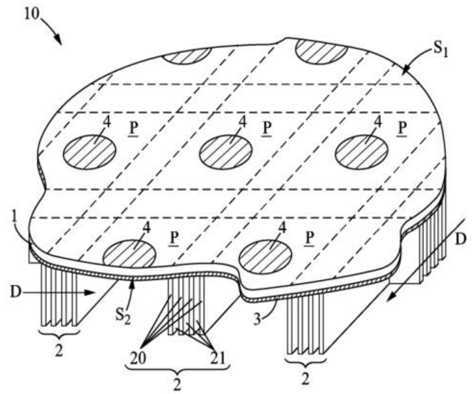
GHOST TESTS– GHOST X3 CHIP

Patent: WO2022128208A1 Acoustic biosensor assay assembly





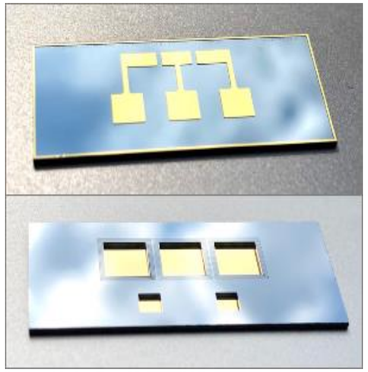
28 mm x 12 mm

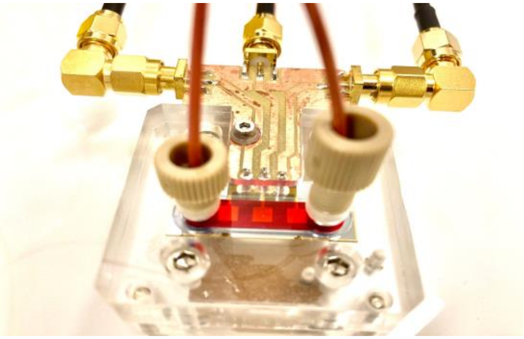


<u>Main Advantages :</u>

- Higher frequency → **<u>higher sensitivity and LOD</u>**
- Multiple sensors **<u>(array/multiplex)</u>** in a single biosensor chip
- **<u>No cross-talked</u>** between sensor for multiple biomarker detection

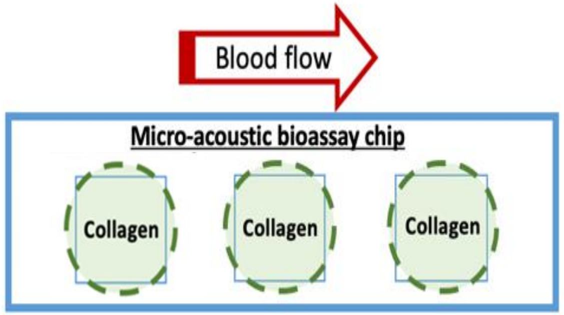
GHOST TESTS– GHOST X3 CHIP : RESULTS





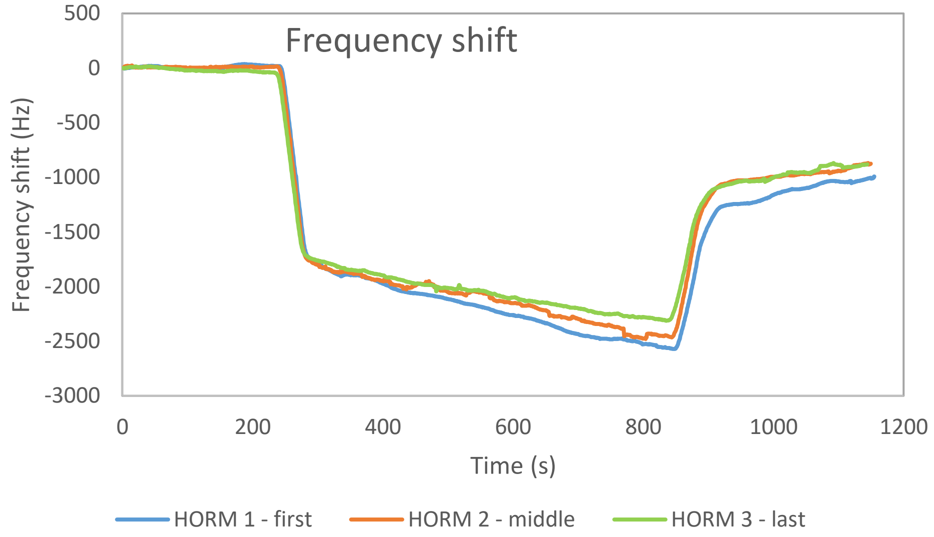
Chip, microfluidics system and electronics

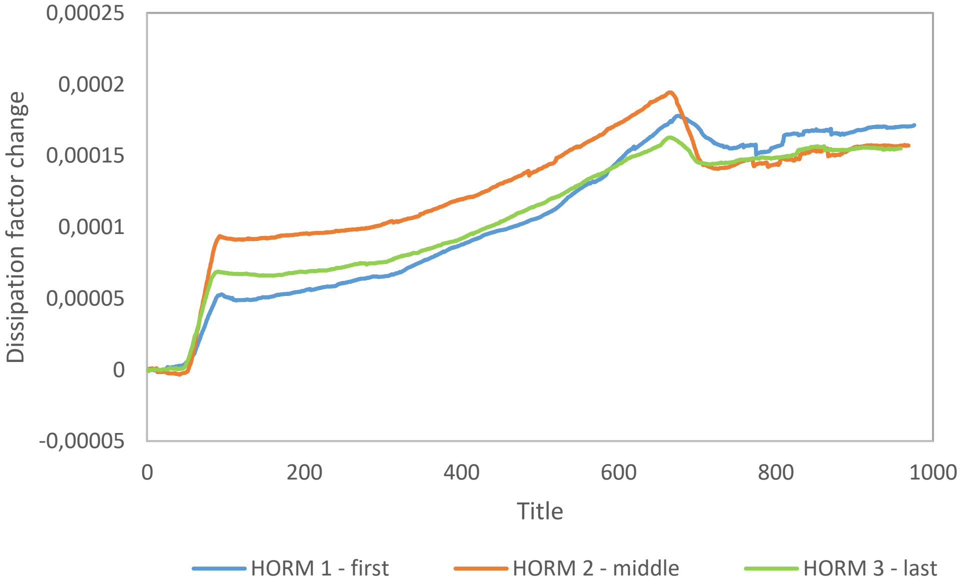
Tests of reproductibility:



Frequency shift and dissipation when whole blood passes over collagen

→ Same response for the 3 cells





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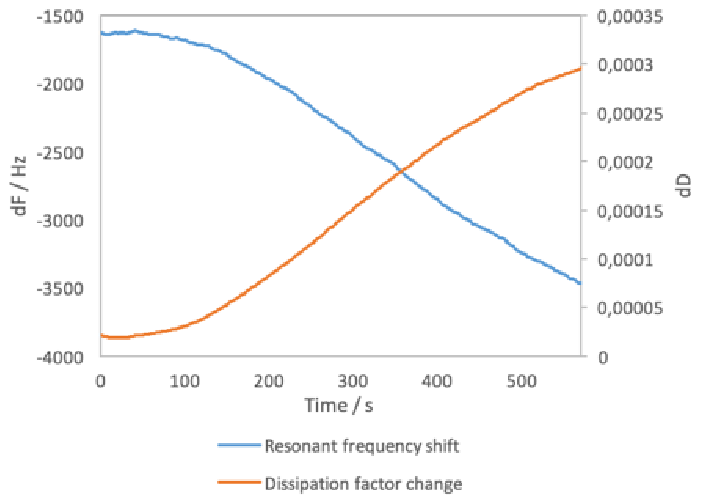
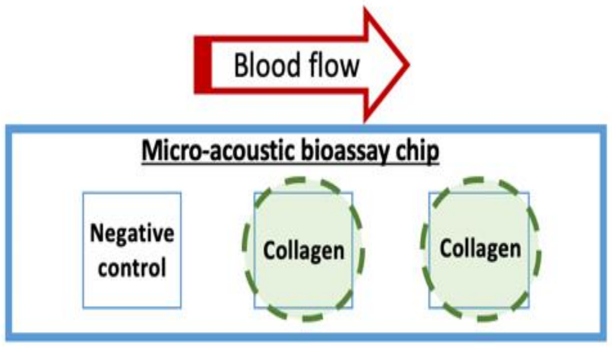
GHOST TESTS– GHOST X3 CHIP : RESULTS

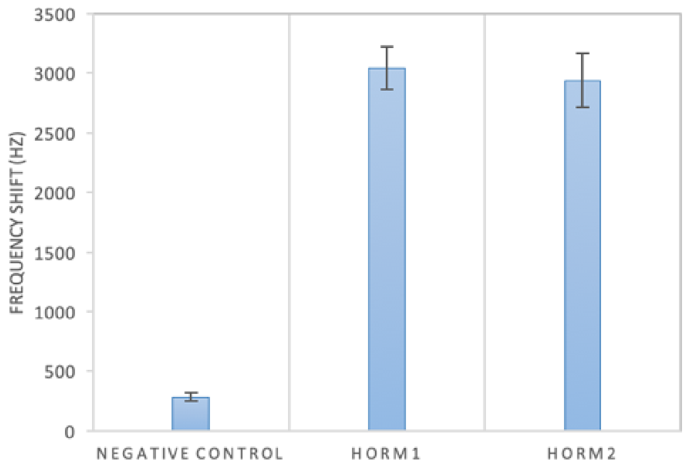
Capture of platelets on collagen type 1 in whole blood

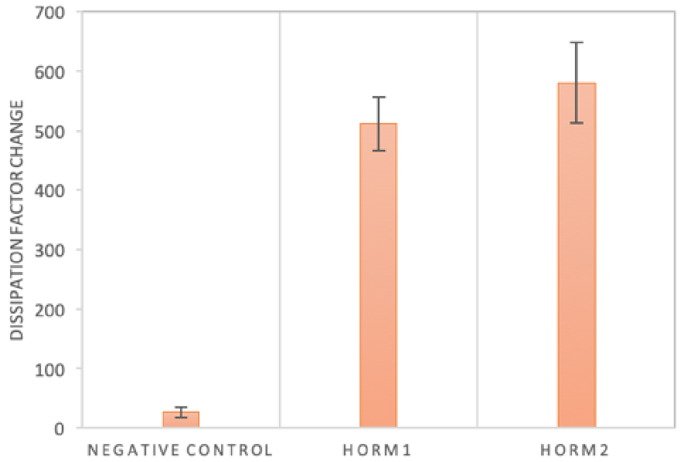


Frequency (blue) and dissipation (orange) responses as a function of time



Platelet capture on collagen type 1. One negative control and two identical collagen surfaces.





→ **Proof of concept and repeatability**
→ **No interference between cells**

ON-GOING ACTIVITIES AND FUTURE PLAN

- Proof of concept for a multiplex biosensor for monitoring a mechanism or detecting a biological element in a complex fluid

- Thinner quartz plate → higher frequency (up to 120MHz) → higher sensitivity

- Higher number of cells → Multiplexing up to 9 sensors/device

- Surface functionalization → biomarkers with higher LOD requirement

- Measurement System : improvement of the electronics to reduce the signal to noise ratio

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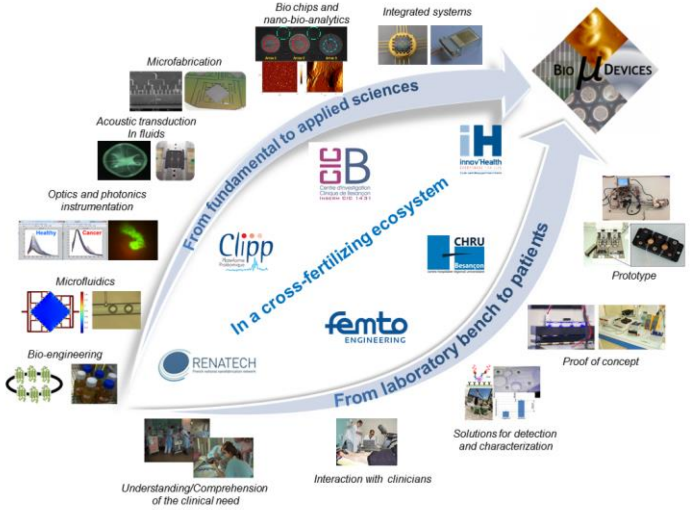

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CHU Centre Hospitalier Universitaire Dijon

Thank you for your attention!

Contact: therese.leblois@femto-st.fr