MULTIPLEXED BIOSENSOR USING QUARTZ-ON-SILICON MICROACOUSTIC (QSIM) TECHNOLOGY FOR IN-VITRO LABEL-FREE INVESTIGATION OF HEMOSTASIS

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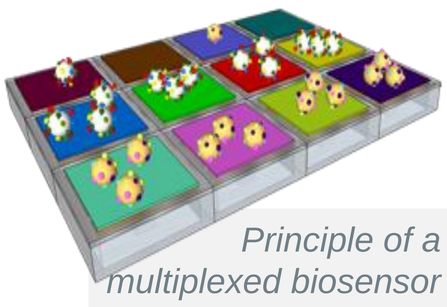


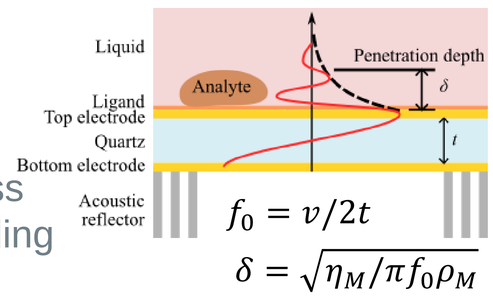
Principle of a multiplexed biosensor

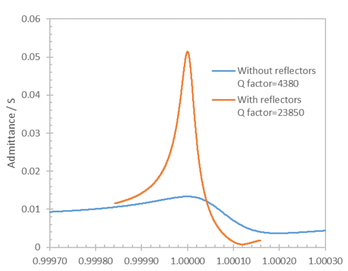
Introduction

We report on the development of a new type of **micro-acoustic biosensor** for medical diagnosis that combines the advantages of microsystems (compactness, batch fabrication) and the performance of monocrystalline quartz biosensors. The technology allows building **multiplexed sensor** for robust assessment of **non-purified biological sample** with redundancy and negative control. In this **proof-of-concept**, the biosensor is used for the **label-free assessment of primary hemostasis**, performing **real-time** monitoring of platelet plug formation **using whole blood**.

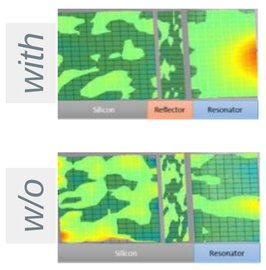
1. Sensing unit design

- Miniaturization of **quartz crystal microbalance (QCM)** biosensor
- Resonance peak sensitive to mass and viscoelasticity of surface loading
- Integration of acoustic reflectors:
  - ► Reduces anchor dissipation losses for high Q-factor (**Q>1000 in water** and >20000 in air)
  - ► **Localize the acoustic energy in the resonator** for dense integration of sensing units without cross-talk

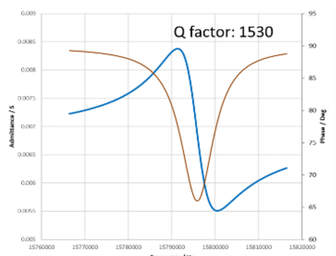




Admittance in air with or w/o reflector

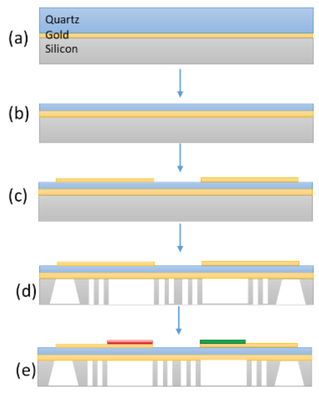


Wave amplitude with or w/o reflector

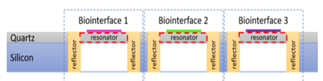


Complex admittance in water with reflector

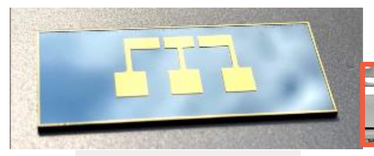
2. Fabrication



- Quartz-on-silicon wafer fabrication with gold **thermocompression bonding**
- Control of quartz thickness *t* by **polishing** for choosing f_0
- Patterning of gold top electrodes
- **Etching** of resonator membrane and reflectors in silicon layer with DRIE
- **Grafting** of top gold surface with ligand



QSiM sensor chip integrating 3 sensing units

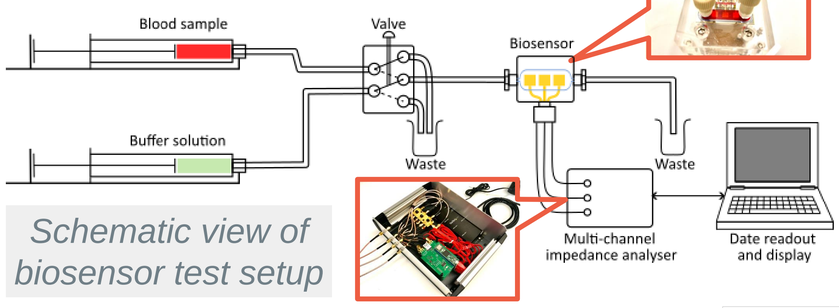


Chip top view

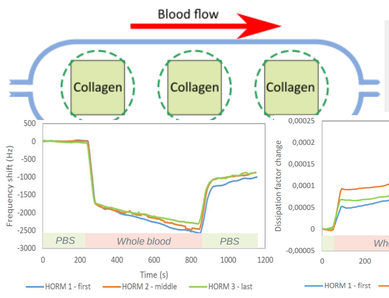
Chip bottom view (zoom on Si reflector)

3. Sensor characterization

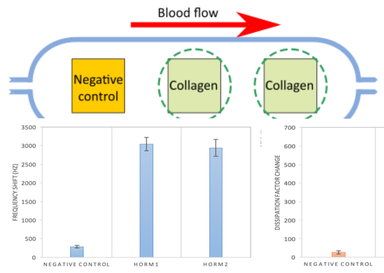
- Sensor chip combines **3 sensing units** in line with a **microfluidic circuit** machined in **PMMA**
- Each sensing unit is **independently grafted** with a specific ligand
- Real-time **measurement** of sensing unit **resonating frequency** (f_0) and **dissipation factor** (D) using OpenQCM electronics
- **Excellent repeatability** between the 3 sensing units for platelet capture with the same collagen with small effect of in line arrangement of units
- **Low crosstalk** as shown by comparing specific and non-specific binding platelet aggregation using collagen and BSA (negative control)



Schematic view of biosensor test setup



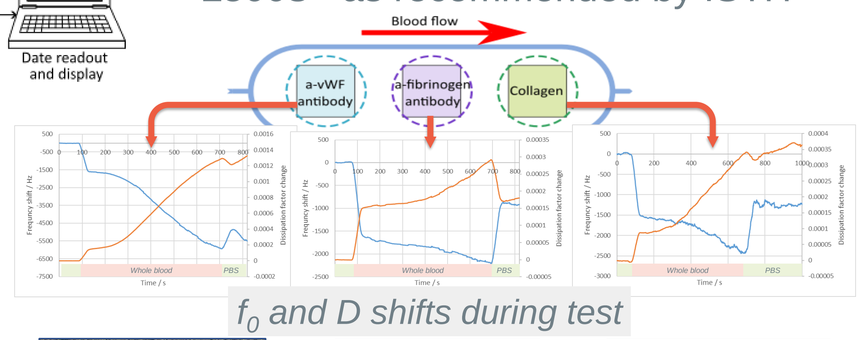
f_0 and D shifts during test



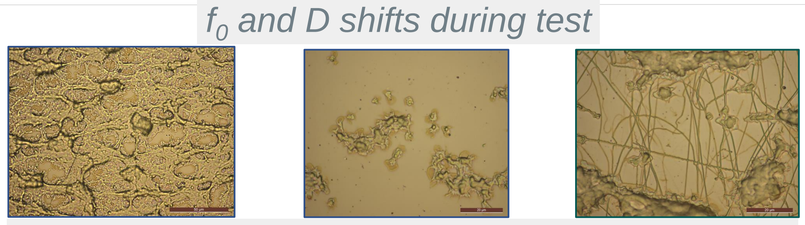
f_0 and D shifts at the end of test

4. Multiplexed biosensor test

- Tests performed with PBS (for surface flush) and anticoagulated **whole blood** from healthy donors
- During test, blood flows on the chip surface for **10 min** at a **shear rate** of **1500s^{-1}** as recommended by ISTH



f_0 and D shifts during test



Optical image of sensor surface at the end of test

- Multiplexed sensor responses (which are confirmed by optical image) show that the vWF and the collagen are able to hold platelet, but fibrinogen can't, probably because of the high shear rate (1500s^{-1}) used in the test

Conclusion

- The multiplexed QSiM sensor with 3 sensing units showed good repeatability and low crosstalk
- Some hemostasis deficiencies corresponding to specific biological mechanism may already be identified with the sensor
- Multiplexed sensing units with different shear rates would allow investigating simultaneously more mechanisms of hemostasis and could help provide a better diagnosis of hemostasis deficiencies
- The QSiM biosensor technology will open new opportunities for multiplexed label-free biosensor in medical diagnosis

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Acknowledgment: This research was funded by ANR GHOST no. ANR-17-CE19-0026-01. The work was partly supported by the French Renatech network and its FEMTO-ST technological facility