Development of a specific microfluidic biosensor for the detection of pathogenic bacteria

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Introduction

The detection of pathogenic bacteria has become a major issue in the food and healthcare industries. To prevent those contamination, it is necessary to identify them as soon as possible. The actual detection time is around 48 to 72 hours. Therefore, new biosensors have been developed to improve specific detection [1] and to reduce the detection time. Among these, piezoelectric offer a promising solution due to their high sensitivity and reliability.

Biosensor

We are aiming at developping an acoustic biosensor using the Bulk Acoustic Waves (BAW) in Lithium Niobate (LN), with two sides covered with electrodes. In addition, one electrode will be functionnalized with antibodies against the targeted bacteria. Due to the pyroelectric property of LN, a room temperature protocol needs to be developed.

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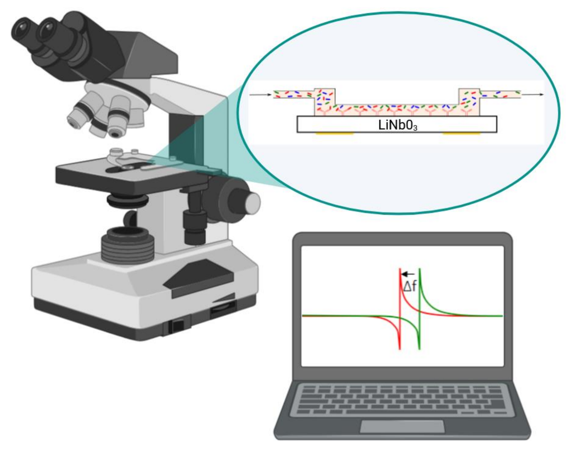
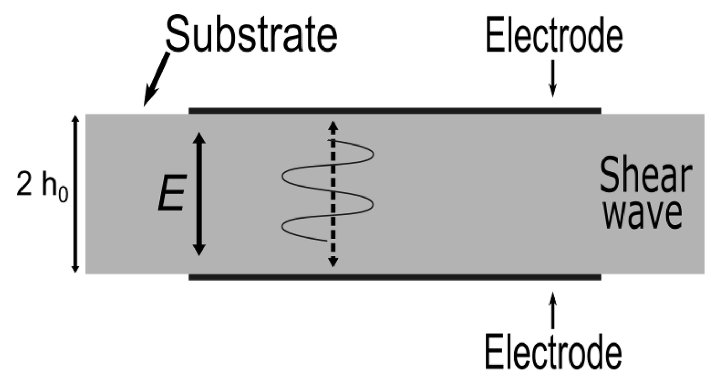
Moreover, a fluidic chamber will be designed to perform in-flow detection.



Transduction

The Quartz Crystal Microbalance (QCM) is widely used to measure very low mass variation (at 5 MHz, 17.7 ng/(cm².Hz) and at 10 MHz, 4.4 ng/(cm².Hz)). Similar acoustic biosensors have been developed with AsGa and ZnO to lower LOD. In this study, we focused on LN shear waves. Indeed, piezoelectric constants of LN are high and shear waves are less attenuated than longitudinal ones by the fluidic medium on the surface. The excitation of these waves are performed using Thickness Field Excitation (TFE).

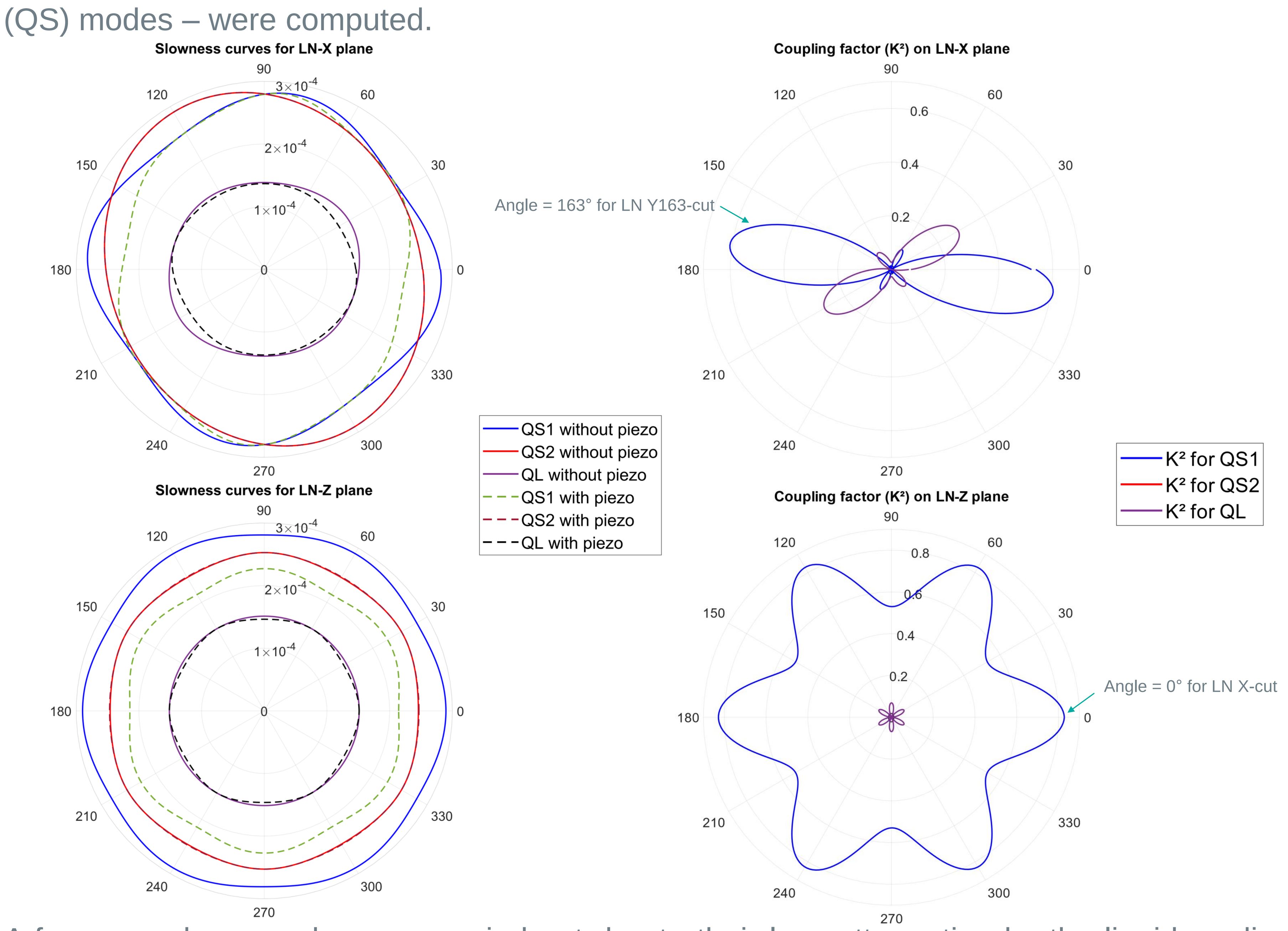
Piezoelectric constants (C/m²)	Crystalline class	e_{11}	e_{14}	e_{15}	e_{22}	e_{31}	e_{33}
Quartz (SiO_2)	32	0,171	-0,0406	0	0	0	0
AsGa	-43m	/	-0,16	/	/	/	/
ZnO	6mm	/	/	-0,59	/	-0,61	1,14
$LiNbO_3$	3m	0	0	3,7	2,5	0,2	1,3



Thickness Field Excitation Mode

Determination of LN cuts in TFE configuration

To identify the most efficient LN cuts for the transducer, the electromechanical coupling factors K² were determined. To obtain those results, the propagation slowness curves with and without piezoelectricity for 3 modes – quasi-longitudinal (QL), low and fast quasi-shear (QS) modes – were computed.



A focus on shear modes was carried out due to their less attenuation by the liquid medium on the surface.

=> The maxima of K² for shear modes without longitudinal wave excitation are obtained in the following directions:

θ = 163° ± 180° on X plane and **θ = 0° ± 60°** on Z plane

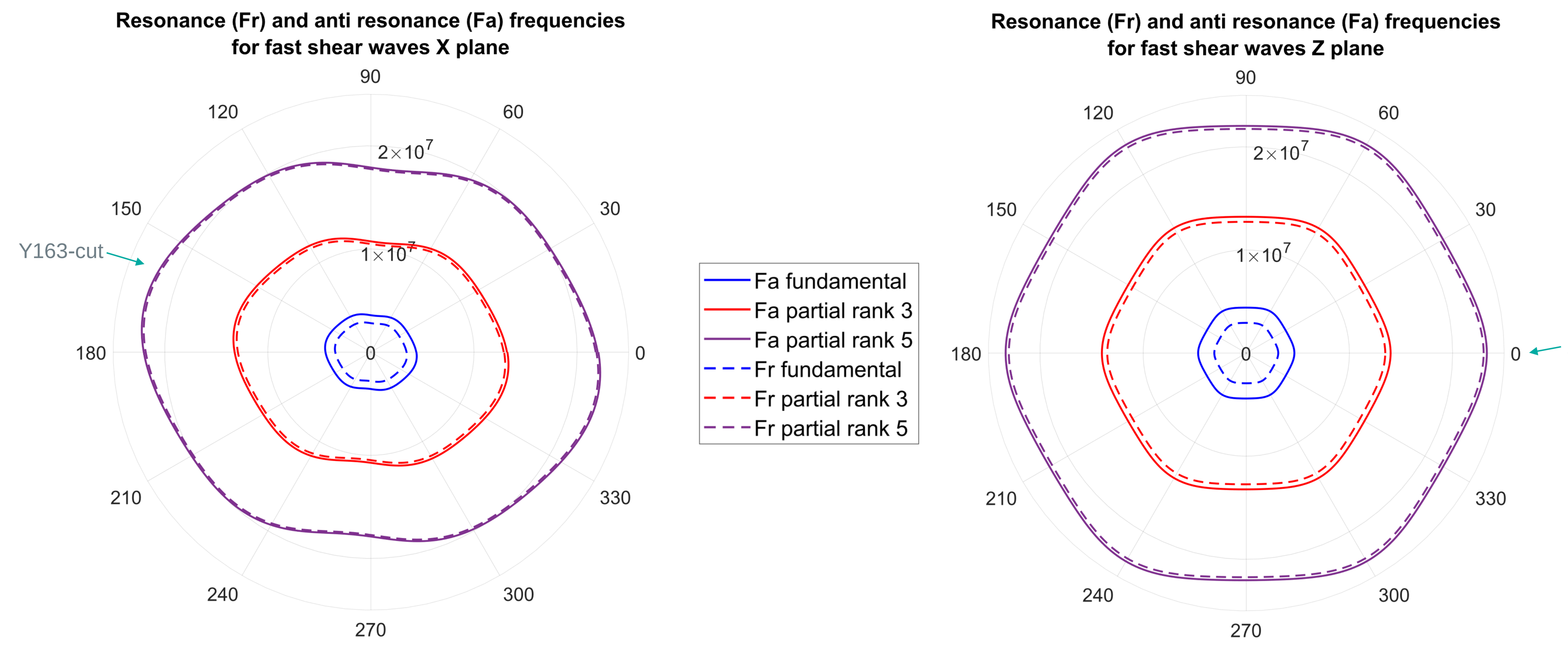
=> For TFE configuration, the associated cuts are **Y163-cut** and **X-cut** respectively

Frequencies

According to the size of the piezoelectric device, the theoretical anti-resonance Fa and resonance frequencies Fr were determined.

$$Fa = (2p+1)\frac{1}{4}\frac{Vpiezo}{h_0}$$

$$Fr = Fa\left(1 - \left(\frac{4K^2}{(2p+1)^2\pi^2}\right)\right)$$



For Y163-cut, the frequencies are:

Y163-cut	Fr (in MHz)	Fa (in MHz)
Fundamental	3.4985	4.4569
Partial 3	13.051	13.371
Partial 5	22.093	22.284

For X-cut, the frequencies are:

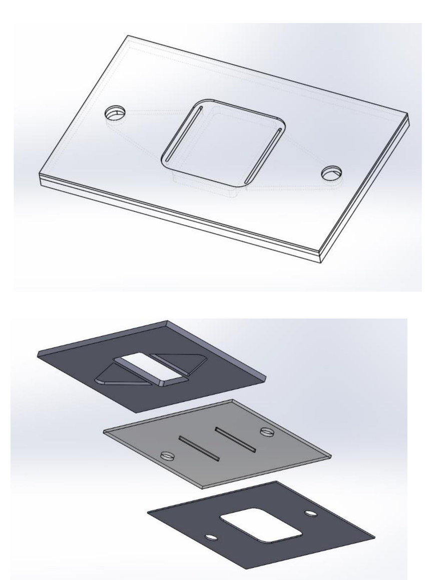
X-cut	Fr (in MHz)	Fa (in MHz)
Fundamental	3.1002	4.6649
Partial 3	13.473	13.995
Partial 5	23.012	23.325

The 3 and 5 partial ranks will be used for detection according to their high quality factor.

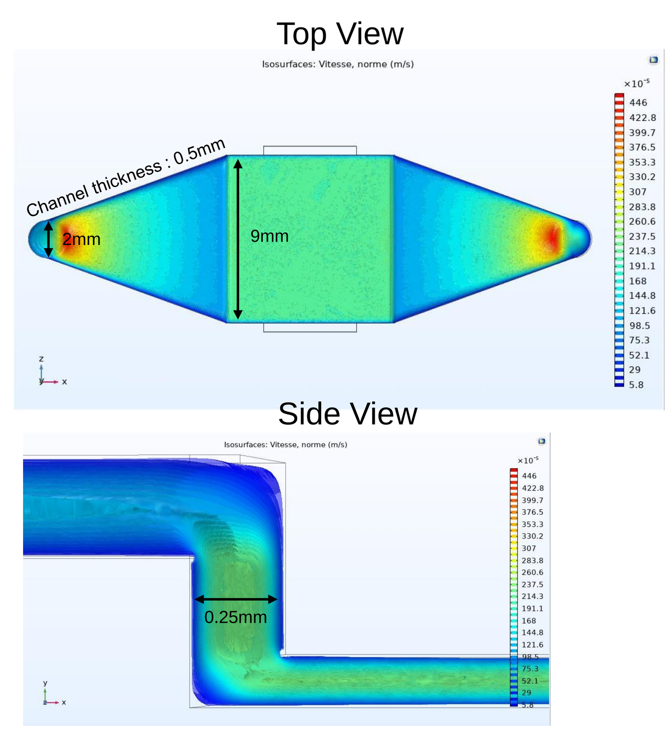
Fluidic chamber

A fluidic chamber was designed and fabricated in order to carry out in-flow detection. This chamber has to allow an optical access for monitoring the biological interactions as well as ensure laminar flow below the optical window. Different designs were simulated by finite element method (COMSOL Multiphysics®) to minimize turbulences.

Design

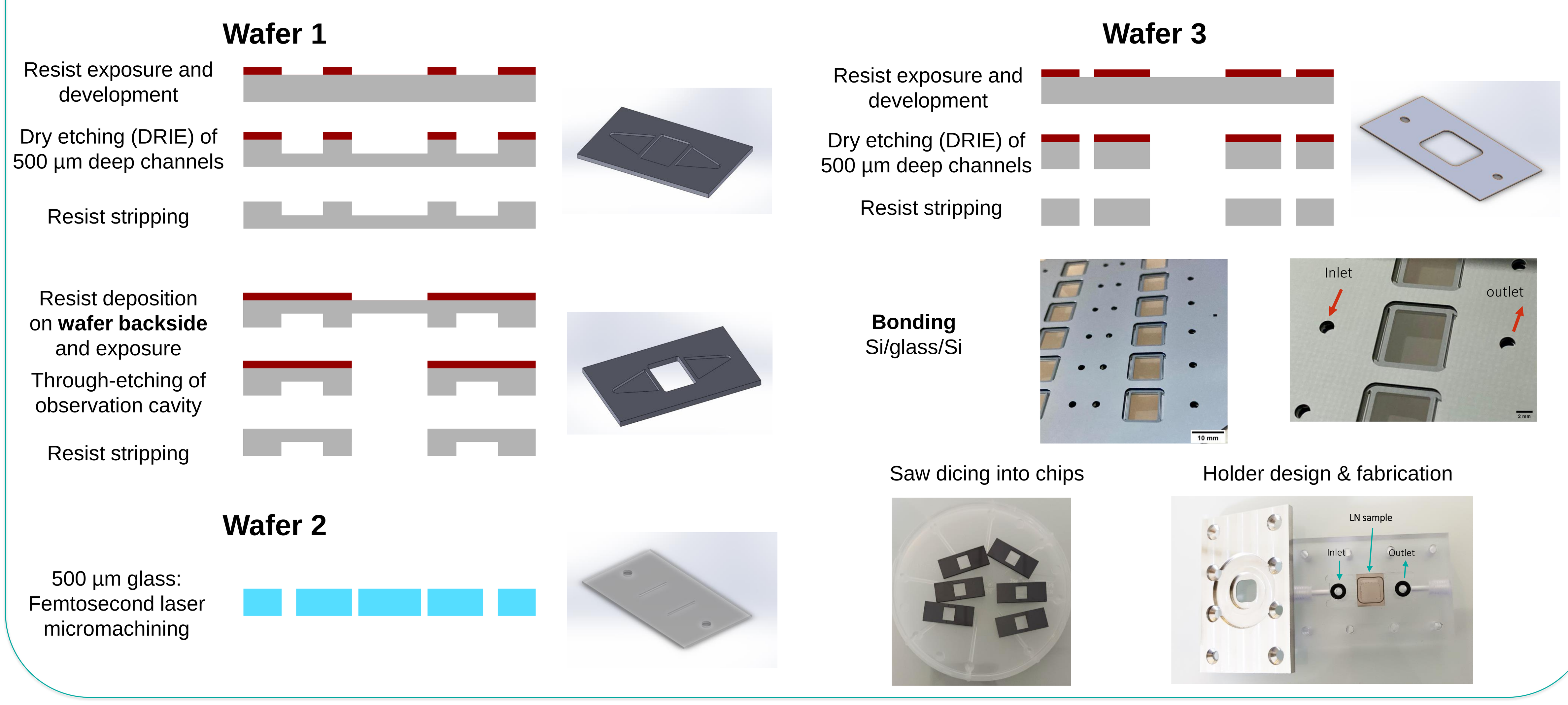


FEM Simultations



Flow chart

The wafer stack is realized by multiwafer bonding of silicon and glass, structured by double-sided plasma etching and laser ablation, respectively. The 1750 µm thick chips, separated using saw dicing, provide a 9 mm x 9 mm observation chamber.



Conclusion

Validation of 2 cuts in TFE configuration for pathogenic bacteria detection.
Identification of the resonance frequencies for the selected waves.
Fabrication of a fluidic chamber using Si double-side dry-etching, glass femtosecond laser micromachining and multi-wafer bonding.

Perspectives

Fabrication and characterization of the X-cut and Y163-cut transducers.
Design of Lateral Field Excitation resonators.
Tests of biosensors in biologic fluids using the fluidic chamber.

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[1] Ali, A. A., Altemimi, A. B., Alhelfi, N. & Ibrahim, S. A. Application of Biosensors for Detection of Pathogenic Food Bacteria: A Review. *Biosensors* **10**, 58 (2020).