Material Characterization with PZE actuated Suspended Microchannel Resonators

Semester Project @ Advanced NEMS Lab

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ABSTRACT

SMRs have been used in numerous applications for life science where most samples are contained in liquid. In this work we investigate how a biological medium, such as phosphate buffer solution (PBS), affects the Signal to Noise ratio (SNR) of PZE actuated SMRs. We report that SNR is directly linked to the conductivity of the solution, that is inevitable for biological applications. Furthermore, we look into a novel application for these devices in the food industry, namely the characterization of olive oil. We prove that it is possible to distinguish two different olive oils by analysing the quality factor and resonance frequency, that are linked to the viscosity and density of the fluid.

Acknowledgments

I would like to thank Guillermo Villanueva for giving me the opportunity to work on this project and for his trust and supervision. A special thanks goes to Damien Maillard, for his guidance, patience and for being always available to help me and providing invaluable knowledge on the device. I would also like to thank Jonathan Cottet for providing us with the protocol for making the PBS compensated with Glucose solution. Finally, I would like to thank Brahim Ben Hamouda, Guillermo Villanueva, Joaquim Cruz and Michele Martemucci that gifted me with samples of olive oil from their home towns.

Contributions

Wire bonding of the SMRs was performed by Damien Maillard. The devices used in the experiments were fabricated by Damien Maillard and Annalisa De Pastina. Matlab scripts for Lorentzian fitting were taken from the lab computer. I contributed to the project by performing the experiments.

Contents

1 Introduction

The concept of suspended microchannel cantilever (SMR) was first introduced in 2003 by *Burg and Manalis*, which wrote about a resonant mass sensor for label free biomolecular detection in subnanoliter fluids [1]. SMRs are resonating cantilevers with a microfluidic channel embedded to remove the viscous damping of the fluid and allow for operation in vacuum. The main advantage, is that these devices exhibit high Quality Factor (15000 was achieved in vacuum) and can resolve very small mass changes with a precision of 300 attograms [2].

Said devices have numerous applications in life science where most samples need to be contained in liquid. They have been used for quantifying particle coating [3], monitor cell growth [4][5], count bacteria [2], characterize the deformability and surface friction of cancer cells [6], measure single cell density [7], and many other applications.

However, these devices can also be used for characterizing liquids for food industry applications. In this project, we explore a novel application in this field, by using SMRs for characterizing olive oil. We chose olive oil because its production and marketing has been involved in scandals by the *agro-mafia*. An article by Forbes states that olive oils that are sold as italian are probably mixed with other olive oils of dubious provenance, unless if they are bought directly from a producer or a certified distributor. So where does olive oil marked "Italian extra virgin" come from? An undercover operation by the italian police - dubbed operation "Mamma mia" - showed that thousands of tons of low quality oils from Spain and Greece were passed off as Italian extra virgin olive oil [8]. Since SMRs can detect very small differences in density and viscosity of fluids, here we investigate if it is possible to distinguish olive oils based on geographical provenance.

Furthermore, different actuation and transduction principles for these devices have been implemented in literature. The novelty of our project, is in the use of piezoelectric (PZE) electrodes for this purpose. However, it was noticed in previous experiments conducted in Advanced NEMS laboratory that when using the SMRs for detecting bacteria using PZE detection, the signal to noise ratio (SNR) at the output decreases compared to optical detection. Here, we also aim to investigate how mediums in which biological matter is suspended, affect the output SNR.

In summary, with this project we aim to look into two main applications for the SMRs: biology and food industry. The aims of this project are:

- investigate how biological mediums affect the SNR in PZE actuated SMRs
- look into a novel application for these devices, namely characterize olive oils from different geographical regions

2 Background

In this section we discuss the advantages of PZE detection and actuation compared to optical and electrostatics, that constitutes the main novelty for the devices that we are using. We then discuss how the Quality Factor is affected by fluid viscosity since we use this parameter to characterize olive oils. Finally, we talk about the temperature responsivity of the device, as it is a key parameter for changing the density and viscosity of fluids in our experiments.

Actuation and Detection Principle for SMRs

Different methods can be used to actuate and transduce the motion of the resonator. The most common method for transduction is optical detection while for actuation it is electrostatic or piezoelectric.

In optical detection, a laser is shined on the tip of the cantilever and reflected onto a position sensitive photodetector. Another commonly used optical method is Laser Doppler Vibrometry (LDV) implemented with an interferometer that measures minute frequency shifts in the backscattered light from the SMR. The cantilever is actuated by electrostatic forces or by a piezo-shaker integrated underneath the chip. In the case of electrostatic actuation two metal electrodes are deposited in proximity and under the resonator which moves due to Coulombic attraction between oppositely charged plates (Fig 1a) [9][2][10].

De Pastina et al developed a novel fabrication method for integrating Aluminium Nitride PZE electrodes on top of the cantilever. AlN 300nm covers 25% of the cantilever length to allow visual access for optical inspection. Each resonator has two independent electrodes: one for actuation and the other for detection (Fig 1b). However, depositing AlN on top of the cantilever causes an added mass, leading to a trade off between the added mass and PZE efficiency [11]. For our experiments we use these SMRs.

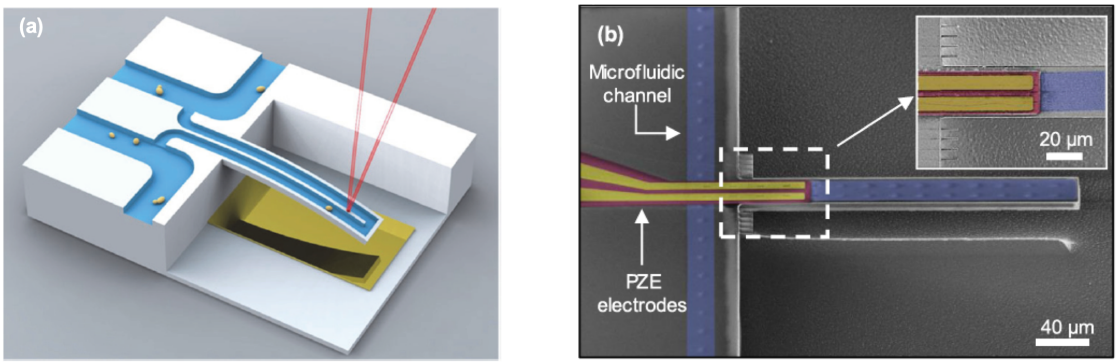

Figure 1: **(a)** Cantilever actuated by electrostatic forces and deflection obtained by optical means [10]. **(b)** Scanning electron microscopy image of SMR with integrated piezoelectric electrodes for actuation and detection [11].

The advantage of having a piezoelectric readout, is that no optical alignment is required and the electrodes are integrated directly on the chip, eliminating the need of an external optical detection setup. When using optical readout, the resonance frequency amplitude is dependent on the position of the laser beam, and the optical alignment can be very sensitive to external vibrations. Moreover, the laser beam will heat the tip of the cantilever causing a shift in the resonance frequency, as is further discussed in the temperature responsivity section.

Quality Factor to measure Fluid Viscosity

As discussed in the introduction, the advantage of having a microfluidic channel embedded into the cantilever is to remove viscous damping and enhance quality factor. For solid cantilevers, the quality factor rarely exceeds a few hundreds when operated in air and even drops to 10 in water. When in gas, reducing the dimension of the cantilever comparable to the mean free path of the gas molecules can significantly improve the quality factor since we enter the molecular regime and have a Knudsen number greater than 1 [12]. However in fluids, the viscous damping is unavoidable and the quality factor decreases monotonically as viscosity of the fluid increases.

Burg et al in 2007, show that using a hollow cantilever they could achieve high quality factors of 15000. Furthermore, they obtain the same quality factor when the channel is filled with air or with water, with different resonance frequencies (Fig 2 *left*) [2]. However, in 2009 the same authors discuss that damping in liquid filled cantilevers can increase or decrease in a non-monotonic fashion as viscosity is increased. For 8 μm cantilevers surrounded by vacuum, they obtained Q = 9172±15 when the channel was filled with nitrogen gas, Q= 5653±11 when filled with pure water, and unexpectedly the quality factor increased to Q=7371±12 when the channel was filled with 72% glycerol in an aqueous solution, showing a reduction in damping despite a 30 fold increase in viscosity. Fig 2 *right* shows a plot of quality factor as a function of fluid viscosity, with alternating regimes of increasing and decreasing dissipation. A theoretical explanation for this phenomenon is proposed in the paper [13].

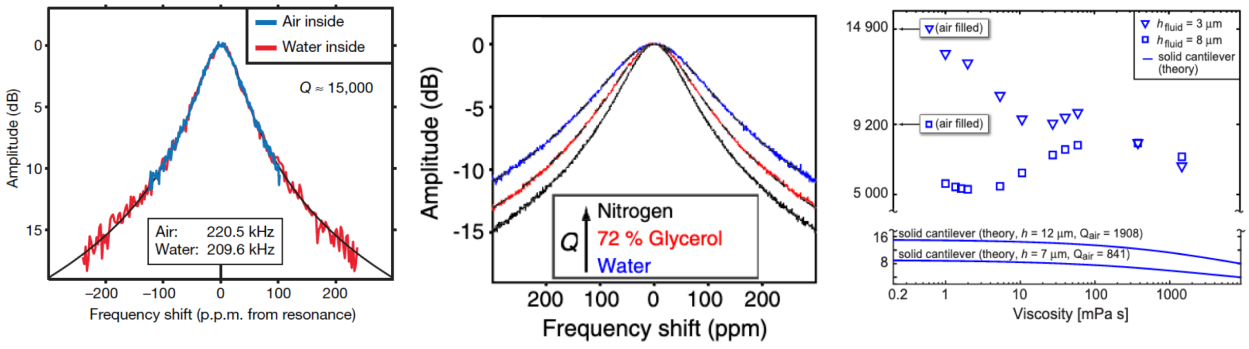

Figure 2: *Left*: Quality Factor measurements with air and water inside the channel [2]. *Center*: Quality factor change with viscosity of the fluid. *Right*: Non-monotonic behaviour of quality factor with fluid viscosity [13].

Temperature Responsivity

SMRs can be used also for temperature related applications, since the resonance frequency shifts due to temperature. Possible applications are the monitoring of a chemical reaction (exothermic or endothermic) in nanoliter volumes.

Shifts in resonance are caused by two main thermal effects:

1. In solids, changes in temperature cause variations in the Young's Modulus of the Silicon Nitride.

2. In fluids, changes in temperature cause changes in density.

Temperature can also induce stress however this phenomenon is neglected in singly clamped beams. The temperature responsivity of the resonance frequency for the SMRs in vacuum is given by:

$$\frac{1}{f}\frac{\delta f(T)}{\delta T} = \frac{1}{2}\beta_{SiNx} + \frac{1}{2}\alpha_{SiNx} - \frac{1}{1+\gamma\frac{\rho_s}{\rho_f}}\left(\frac{3}{2}\alpha_{SiNx} + \frac{1}{2}\frac{1}{\rho_f}\frac{\delta\rho_f(T)}{\delta T}\right) \tag{1}$$

where $\beta_{SiNx} = \frac{1}{E}\frac{\delta E(T)}{\delta T}$ is the Young's modulus variation with themperature and α_{SiNx} is the thermal expansion coefficient of Silicon Nitride, ρ_f and ρ_s are the density of the solid and the fluid and $\gamma = \frac{A_s}{A_f}$ is the ratio of the cross sectional area of the solid and fluid [14] [9].

Fig 3 shows that when the devices are empty, the resonance frequency decreases due to the softening of the SiN. The β_{SiNx} term dominates the temperature responsivity. When the device is filled with water, the frequency increases with temperature. This is due to the change in density of the fluid and $\frac{1}{\rho_f}\frac{\delta\rho_f(T)}{\delta T}$ becomes the dominant term [14].

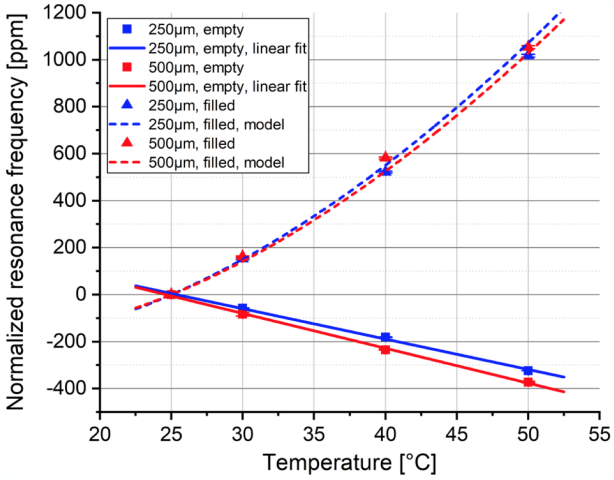

Figure 3: Frequency behaviour with respect to temperature for 250 μm and 500 μm cantilevers when empty and filled with water [14].

3 Methods

Experimental Set up

The experimental set up is shown in Fig 4, and can be subdivided in 4 main blocks: (1) the electrical interface with the chip, (2) the fluidic connector, (3) the temperature control, (4) the external control of fluids through a syringe pump.

Electrical Interface: The SMR chip is wire bonded to a PCB with 8 pads connected to SMA connectors. Only 4 of these pads are wire bonded: two are connected to the PZE electrodes of the 500 μm cantilever, and the other two to the 250 μm cantilever. The PCB is screwed to a 3D printed holder. The chip is on a copper base and is kept in place with double side scotch tape. The SMAs are connected to the Lock in amplifier (*UHFLI 600 MHz Lock-in Amplifier, Zurich Instruments*) via coaxial cables. The lock in amplifier, enables the actuation of the PZE electrodes and the detection of the output signal resonance frequency, phase, quality factor etc. We use two of the cantilevers for actuation: the 500 μm is used for measurement while the 250 μm is actuated with a 180^o phase shift for compensating the parasitic capacitance and obtain a balanced signal.

Fluidic Connector: The chip is connected to the fluidics via a connector shown in Fig 4a. Since exchanging fluids in the SMR can be a lengthy process due to the high fluidic resistance of the microchannel, the inlet is connected in parallel to two channels. One of these channels is the *bypass channel* that allows the quick exchange of solutions through the microfluidic tubes and connector. When the bypass channel is then closed, the fluid will flow in the high fluidic resistance microchannel and the solution will be exchanged. Furthermore, a PTFE tube is used to link the connector to a vacuum pump to achieve vacuum in the cavity where the SMR is present.

Temperature Control: The set up is able to control temperatures between 25^oC and 50^oC using a Peltier module (*RC3-4, Marlow Industries Inc.*), a thermistor (*PR103J2, U.S. Sensor Corp*), and a Laser Diode Temperature Controller (*LDT-5910C, Newport Corporation*). The thermistor is inside a cavity present in the copper plate on which the SMR is placed, therefore in these experiments we assume that the temperature of the SMR is the same as the copper plate. The peltier module is used to heat and cool down the chip and is placed beneath the Cu plate in a 3D printed holder.

External control of fluid parameters: Liquids are filled in a syringe and pushed through a pressure sensor, into the inlet of the connector by using a syringe pump (*NEMESYS, Cetoni*). Flow rates are controlled by *Qmix Elements* software using the feedback from the pressure sensor. Flow rates are set to keep the pressure constant below 1 bar, depending on the fluid.

The experimental setup used in this project was developed in Damien's master thesis and more information can be found in the paper [14].

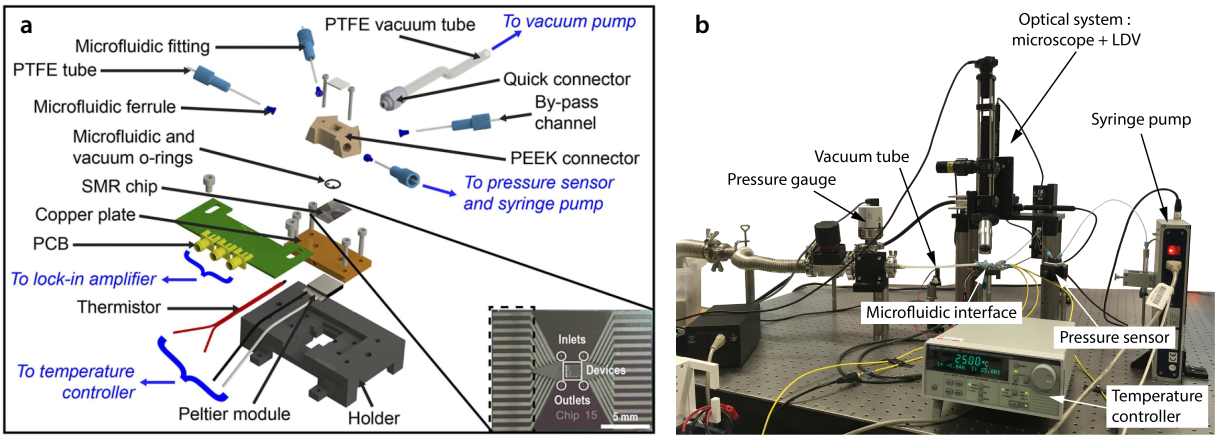

Figure 4: Experimental Set up: Overview of the experimental platform used to interface the SMR chip to the electronics, vacuum, fluidics and temperature control [14].

Measured Fluids

We performed two main types of experiments: the first aimed to study how PZE affects the SNR for biological applications and the second aimed to characterize olive oil.

(1) SMRs for biosensing

We study how the readout from the PZE electrodes is affected by the conductivity of the solution. Conductive solutions are unavoidable in physiological monitoring since bacteria, cells and other bio-particles are suspended in salty solutions such as PBS or DMEM (medium for cells).

Solutions of 1x, 2x and 10x PBS were prepared using *phosphate buffer saline tablets by Sigma Aldrich*. A stock solution of 10x PBS was prepared (1 tablet in 20 ml of DiH20) and 1x and 2x concentrations were obtained by diluting the stock solution with DiH_2O. The conductivity was measured for the different PBS concentrations (*Mettler Toledo SevenEasy Conductivity Meter*). The buffer helps to maintain a constant pH (at 7.4) and osmolarity (at 300 mOsm/kg) which is crucial in life science applications.

To decrease the conductivity of the 1xPBS solution, but maintain the same pH and osmolarity another solution consisting of PBS compensated with Dextrose was made. The solution was prepared by adding 2.44g of dextrose in 50 ml falcon tube with 5ml of 1x PBS and 45 ml of DiH_2O. The new solution has a conductivity of 0.1696 S/m - 10 times lower than 1x PBS that has a conductivity of 1.46 S/m - and a measured osmolarity of 303 mOsm/kg.

The solutions were then introduced in the syringe pump and allowed to flow through the 500 μm SMR. The resonance frequency, signal standard deviation, signal average and resonance frequency stability over 20 seconds were measured for different actuation voltages (20mV, 40mV, 60mV, 80mV

Table 1: Actuation and balancing Voltages for the first configuration (C1) of SMR with the fluidic access from the top and second configuration (C2) with fluidic access from the bottom. C1 was used for conductivity experiments while C2 for glucose and olive oil experiments.

V actuation	20 mV	40 mV	60 mV	80 mV	100 mV
V balancing (C1)	18.23 mV	36.46 mV	54.69 mV	72.92 mV	91.15 mV
V balancing (C2)	37 mV	74 mV	111 mV	148 mV	-

and 100mV). For these experiments fluids were introduced at a flow rate of 5 μl/min and pressures were kept constant at 0.3 bars. Fluids were fully exchanged in less than 120 minutes. Balancing was performed by actuating the 250 μm SMR with a 180^o phase shift. The corresponding actuation and balancing voltages are shown in Table 1.

Moreover, microfluidic tubes, pressure sensor and syringe were thoroughly washed with DiH_2O when changing the solution to avoid the contamination between fluids.

(2) SMRs for Olive oil characterization

Olive oils from Tuscany, Puglia, different regions of Spain, Tunisia and commercial olive oils with unknown provenance were used for these experiments. However, only the olive oil from Spain and from MBudget succesfully entered in the SMR, since a shift in frequency was visible.

Olive oils were introduced in the SMR using the same technique discussed above. Flow rates were set at 5 μl/min and a pressure was kept constant a 1 bar. The SMRs were actuated at 50mV and the temperatures were swept from 25 ^{o}C to 50 ^{o}C in steps of 5 ^{o}C by using the temperature controller. Resonance frequency was recorded and a matlab script was used to fit the lorentzian and extract the quality factor. Exchanging between water and olive oil took approximately 2-3 hours however, due to the high viscosity, it took more than 12 hours to exchange between different types of olive oils. Furthermore, olive oils from Tuscany, Tunisia, Puglia and Spain didn't succesfully enter the SMR although they were left to flow overnight.

Due to the apolarity of olive oil, it wasn't possible to fully wash the setup with water as in the previous experiments. For this reason we washed the fluidic fittings, pressure sensor and syringe with acetone and then cleaned them with dry paper multiple times. Nonetheless, we cleaned the micro-channel of the SMR by flowing water and waiting to reach the baseline in frequency to confirm that the olive oil was fully removed from the channel.

SMR Configurations

We used two different inlet configurations of SMRs for our experiments. The first configuration (C1) has fluidic access opened from the top of the wafer and was used for the conductivity measurements. The second configuration (C2), has fluidic access from the backside of the wafer and was used for the glucose compensated solution and olive oil experiments.

4 Results and Discussion

SMRs for biosensing results

As already discussed, conductive solutions are unavoidable in physiological monitoring. We measured the conductivity of 1x, 2x, and 10x concentration of PBS and the results are shown in Table 2. We notice in Fig 5a, that conductivity increases linearly ($r^2 = 0.9968$) with concentration of PBS.

We examine the output noise response dependance to conductivity for the first configuration SMR (C1). Fig 5(b,c) shows the SNR behaviour with different conductivities for different actuation voltages. We can notice that as the actuation voltage increases, the amplitude also increases, improving the SNR. Furthermore, we see that an increase in conductivity leads to a reduction in the SNR. For example, the conductivity increases 10000 times from DiH_2O to 1x PBS and the SNR reduces 5.4 times for 100 mV actuation voltage. These results show a clear dependence of the noise to the conductivity of the solution. Moreover, it can be noticed that we are missing a data point for 10x PBS actuated at 20 mV in Fig 5(b,c). This is because the signal was entirely screened by the noise and we couldn't record a value.

To further study the behavior of the device with conductivity, we used a solution that is 10 times less conductive than 1x PBS. The solution was made by diluting PBS in DiH_2O (10% PBS and 90% DiH_2O). The buffer keeps the pH constant at 7.4, and the osmolarity was compensated by adding dextrose. Since the solution has the same osmolarity and pH of 1x PBS, it can be used as biological medium for bacteria measurements. A cantilever with a backside configuration was used for these experiments so results are not directly compared to the ones obtained in Fig 5(b-c).

As shown in Fig 5(d), we see that for the glucose compensated solution, the SNR doesn't change with actuation voltage (SNR = 15.29 - 15.82) as in the previous experiments. However, when flowing 1x PBS solution in the same cantilever, the SNR increases with actuation voltage. Furthermore, we notice that 1x PBS for the backside channel configuration, exhibits an SNR 8.4 times higher than the one obtained for the front side cantilever, and even better than distilled water. Further experiments should be conducted to determine the validity of these results since they are in contradiction. The use of a different cantilever configuration might have affected the consistency of the results.

Table 2: Conductivity measured for distilled water, 1x, 2x, 10x PBS and isopropanol (IPA).

	Di Water	1x PBS	2x PBS	10x PBS	IPA
Conductivity (S/m)	$1.94{\cdot}10^{-4}$	1.518	2.66	10.54	$7{\cdot}10^{-6}$

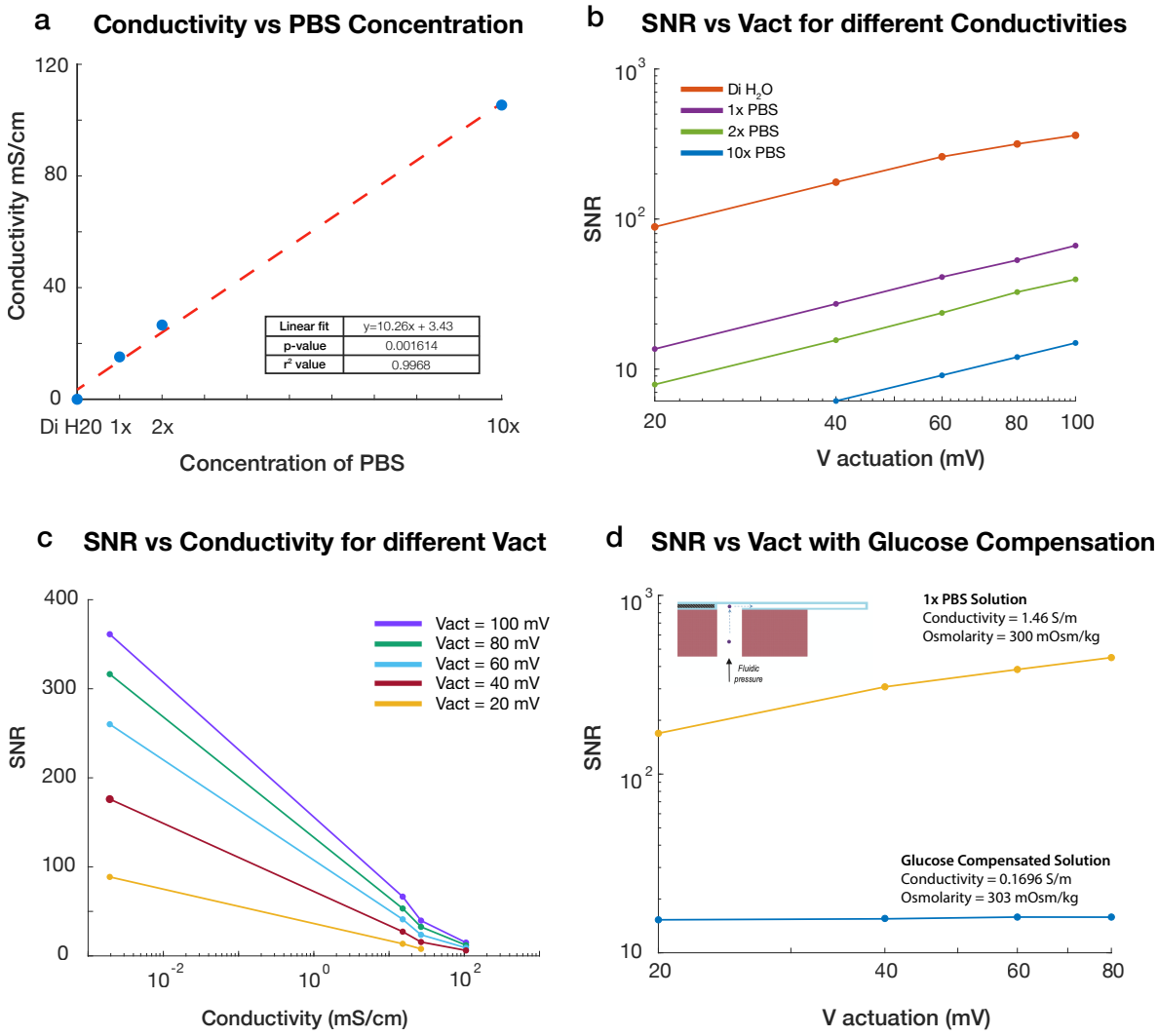

Figure 5: (a) Conductivity of PBS vs concentration, (b) SNR vs actuation voltage for Di water, 1x PBS, 2x PBS and 10x PBS, (c) SNR vs conductivity of the solution for different actuation voltages, (d) SNR vs actuation voltage in for glucose compensated solution and 1x PBS in the backside channel configuration.

SMRs for Olive oil characterization

We characterized olive oils from MBudget and Spain with the SMRs by considering how viscosity and density changes with temperature. In our results, we record the quality factor to measure qualitatively oil viscosity and resonance frequency to find quantitatively the oil density. We sweep the temperature from 25 ^{o}C to 50 ^{o}C to change these two parameters.

Results are shown in Fig 6. We can notice by looking at the normalized resonance frequency, that olive oil from MBudget and Spain both increase linearly with Temperature with a 74.55 ppm/C slope. We can see that the oil behaves differently from water, that has a quadratic increase in frequency with Temperature.

The density of olive oil was computed using:

$$f_r(T) = f_{empty}(T) \cdot \sqrt{\frac{1}{1 + m_f m^{-1}}} \tag{2}$$

which is rearranged to find the density of the fluid:

$$\rho_f = \left[\left(\frac{f_E(T)}{f_R(T)} \right)^2 - 1 \right] \cdot \rho_s \cdot \gamma \tag{3}$$

where $f_E(T)$ and $f_R(T)$ are the resonance frequency at temperature T for empty and filled cantilevers, $\gamma = \frac{A_s}{A_f} = 0.83$, ρ_f and ρ_s are the density of the fluid and the solid.

Results of the computed density are shown in Fig 6c, where we notice a 0.16% difference in density between Spanish and MBudget olive oil. Furthermore, in both cases there is a -0.6 kg/m^3C density change with temperature in the range between 25 ^{o}C and 50 ^{o}C.

Fig 6b shows the change in quality factor with temperature. As discussed in the background section, the quality factor is related to fluid viscosity. We computed the quality factor using a matlab script that fits a lorentzian to the resonance peak. We notice that both olive oils have similar quality factor at room temperature (4787 for MBudget and 4788 for Spanish olive oil) but the quality factor for spanish olive oil changes between 4788 and 5167 while for MBudget it changes between 4787 and 4952 in the range 25-50 ^{o}C.

In conclusion, we can distinguish 2 different olive oils by considering viscosity and density. However, olive oil characterization might not be a main application for SMRs due to the long times for the high viscosity fluid to enter the microchannel.

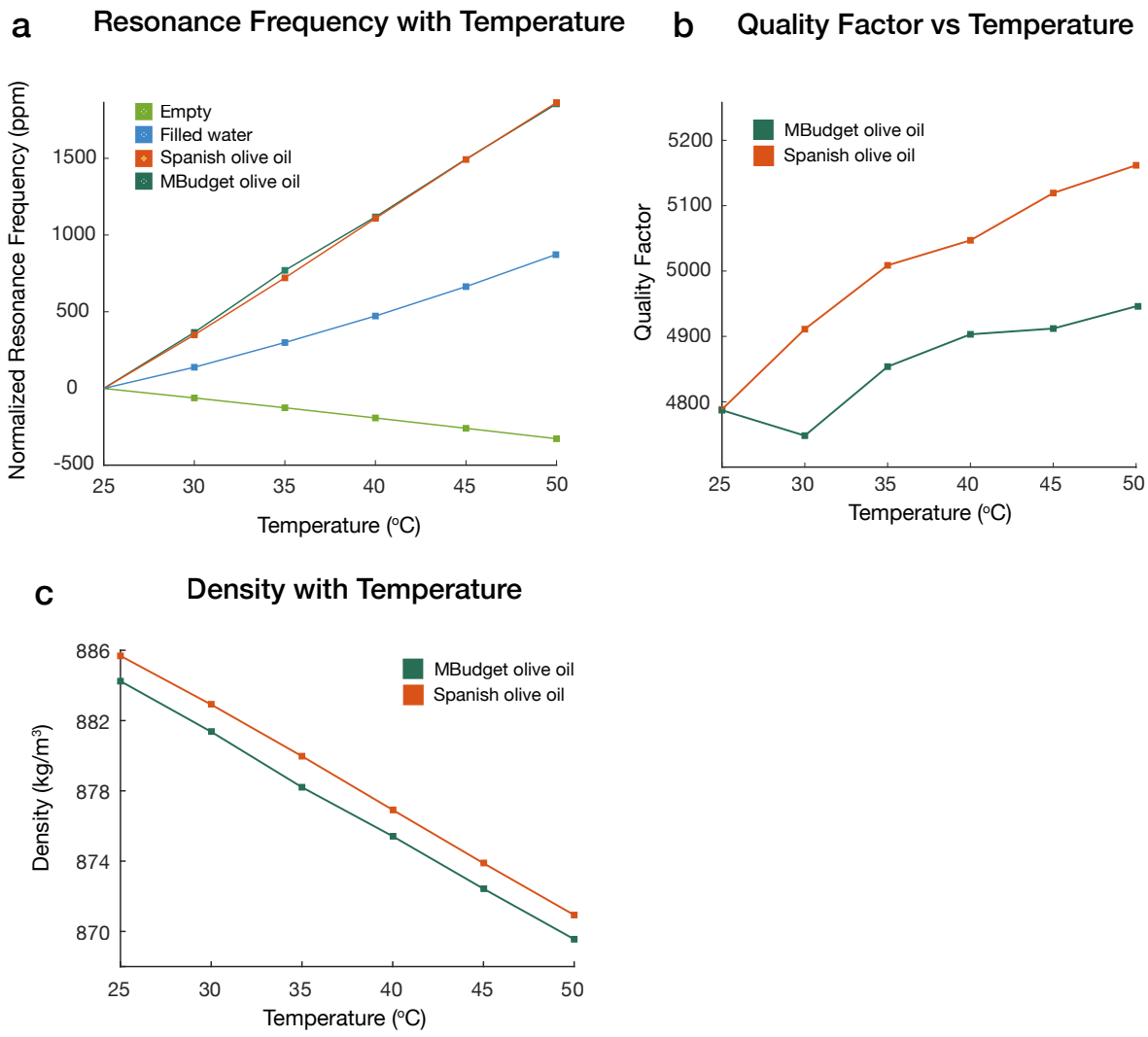

Figure 6: (a) Normalized resonance frequency with temperature for empty cantilever, filled with water and olive oil from Spain and MBudget, (b) Quality factor behaviour with temperature for different olive oils, (c) Extracted density plot with temperature.

5 Conclusion

We have proven that PZE actuated SMRs have a SNR dependent on the conductivty of the solution. We have additionally proven that SMRs can be used to characterize and distinguish two different types of olive oils.

However, the origin of the noise is not yet understood and further experiments should be conducted to assess the source. Furthermore different families of olive oils should be tested to fully see if we can distinguish olive oils based on geographical provenance.

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