An Electrowetting on Dielectrics Based Lab-on-a-Chip Utilizing an Integrated High Fundamental Frequency Quartz Crystal Resonator as a Biosensor

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Abstract

We demonstrate the operation of an Electro Wetting on Dielectrics (EWOD) hybrid lab-on-a-chip system by utilizing a High Fundamental Frequency (HFF; 50MHz) Quartz Crystal Microbalance (QCM) resonator as mass-sensitive sensor. In a first experiment we have tested the reversible formation of a phospholipid monolayer out of an aqueous buffer suspension of phospholipid vesicles onto the integrated sensor, coated by a Self-Assembled Monolayer (SAM) of 1-octadecanethiol on a gold-covered surface. The altered mass load resulted in a shift of the resonance frequencies. In the second experiment, the formation of a protein multilayer composed of streptavidin and biotinylated immunoglobulin G was monitored. The original resonance frequency was recovered by the complete lipid removal by the detergent octyl-D-glucopyranoside. Using these sample applications, we were able to demonstrate and verify the feasibility of our prototype combining a mass-sensitive QCM sensor with digital microfluidics based on EWOD.

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Keywords: Introduction

1. Introduction

The realization of a lab-on-a-chip platform, which is designed to perform and detect (bio-)chemical reactions by directing microliter volumes of different reagents and reaction products, is one of the major goals within...
microfluidics. The fluids can either be contained in fluid channels and driven as a continuous flow or separated into discrete droplets which are individually addressed and actuated. The latter holds the advantage of more degrees of freedom and a higher flexibility to react on real-time measurement results. Furthermore, measurements can be performed on precisely controlled sample droplets out of a microfluidic stream saving the rest of the sample fluid for further operations. Essential for this so called digital microfluidics is the realization of the single droplet actuation. It affects all design requirements for the integration of further microfluidic components and sensor elements.

The intention of our contribution is to show the feasibility of integrating a Quartz Crystal Microbalance (QCM) into a digital microfluidics platform utilizing Electro Wetting on Dielectrics (EWOD) as actuation mechanism. The QCM is a mass-sensitive sensor which is (in a functionalized condition) able to indicate the binding of biomolecules by a shift of the resonance frequency [1].

1.1. Actuation

The chosen mechanism for the droplet actuation is EWOD, which can be used for manipulations (creation, transport, mixing and divisions) and accurate handling of multiple small volume droplets [2, 3, 4] on a common electrode array. Different (bio)-chemical reactions can be performed on the same chip by simply changing the sequence of droplet manipulations.

A droplet confined between two plates adopts a shape with minimal total surface energy. In other words, the shape and the contact angle $\theta$ of a droplet ($L$) in contact with a hydrophobic surfaces ($S$), surrounded by an ambient fluid (liquid or gas, $G$) is predetermined through the balance of surface tensions $\gamma$, given by the Young’s equation

$$\gamma_{LS}(\cos \theta) = \gamma_{SG} - \gamma_{SL}$$

(1)

Different contributions to the total energy, e.g., electric potential differences, surfactants as well as gravitation change the actual droplet shape [5]. For small droplets with a high surface to volume ratio, gravitation can be neglected. Thus, the shape can be approximated by a sphere which is cut off by the underlying surface. Applying a voltage $V$ between two electrodes, which are electrically isolated from the droplet by a dielectric layer, changes the charge distribution at the droplet surface, and lowers the effective surface energy of the solid/liquid interface proportional to the specific capacitance $c$ of the isolating layer, described by the Lippmann-Young equation.

$$\gamma_{SL}(V) = \gamma_{SL} - \frac{cV^2}{2}$$

(2)

As a result a pulling force towards the energized electrode and a consecutive transportation, see Fig. 1, is achieved.

Fig. 1: Automated droplet actuation across several electrode lines of the EWOD base plate. The saw tooth design ensures the overlap of the droplet with at least two neighbouring electrodes.
1.2. Sensor principle

As a sensor a QCM resonator with a thickness shear mode resonance frequency of $f_0 \approx 50\text{MHz}$ was utilized. A change in mass load per unit area $\Delta m$ is the main detectable effect of biochemical binding interactions under study. According to the Sauerbrey relation [6], a change in surface mass deposition, originating, e.g., from biochemical binding to an interface film on the sensor surface, results in a corresponding shift of the fundamental resonance frequency $\Delta f$. The Sauerbrey relation is given below in (3), where $\rho$ denotes the mass density (2.648 g/cm$^3$) and $\mu$ the shear modulus (29.47 GPa) of the QCM. A crucial factor for the performance of QCM systems is the fundamental resonance frequency, $f_0$, of the piezoelectric sensor due to the quadratic dependency of frequency shift:

$$\Delta f = -2f_0^2 \Delta m / \sqrt{\rho \mu}$$

(3)

Following the resonance condition (4), the fabrication of TSM resonators with higher values of $f_0$ is basically achieved by reducing the plate thickness $h$ of the sensor.

$$f_0 = \sqrt{\mu / \rho} / 2h$$

(4)

For practical purposes, the minimum thickness is constrained by mechanical stability issues. Most applications utilize either 5 MHz or 10 MHz resonators, with a corresponding plate thickness of 0.33 mm or 0.17 mm which provides a sufficient mechanical stability. Occasionally, sensors with resonance frequencies between 20 MHz and 30 MHz are used (e.g. [7] and [8]). These sensors are smaller in diameter (typically about 8 mm) and already rather thin and fragile.

An alternative approach to realize higher fundamental frequencies is to utilize quartz resonators with an “inverted mesa” structure. These quartz disks with a diameter and thickness of about 5 mm and 0.1 mm, respectively, have a membrane of reduced thickness only in their small central circular area of about 5 mm$^2$. The surrounding thicker material provides a better mechanical stability and the small membrane can become as thin as 8.3 μm, resulting in a high fundamental frequency (HFF) up to $f_0 = 200\text{MHz}$.

2. Realization

2.1. Functionalized QCM

The integration of a QCM sensor into an EWOD platform requires adjustments to the electrode design and the applied coating layers. As described in section 1.1, for a reliable EWOD actuation the coating of all surfaces contacting the droplet has to be hydrophobic with a low hysteresis angle. Furthermore, the droplet has to be in contact with an external electric field applied via two electrodes isolated from the droplet by a dielectric layer. The first step of integrating a QCM sensor is to bring a hydrophobic layer to its surface which on the one hand, allows for actuation and enables the binding of the bio-molecules of interest on the other hand. This layer was found in form of a self assembled monolayer (SAM) of 1-octadecanethiol, obtained from Sigma Aldrich, bound on a gold layer (3 nm Cr as an intermediate adhesion layer and 50 nm Au) deposited by e-beam PVD onto a glass substrate into which the QCM is integrated, see Fig.2. The functionalized surface shows hydrophobic properties with a contact angle of $101\pm1^\circ$ and a low hysteresis angle $a$ (a represents the “pinning” of the contact line up to a certain unbalance of the surface tensions). Furthermore, it allows for the formation of a phosphor-lipid monolayer out of an aqueous buffer suspension, which serves as a sample reaction for bio-chemical bindings in general. A solution of phosphor-lipids which carried biotin residues on 20% of the phospholipid head groups in Phosphate-buffered saline (PBS) were prepared as described in [1].

As a second step the functionalized QCM has to be bonded as planar as possible into the EWOD platform, in our case into the base plate. First tests confirmed the feasibility of the surface tension driven transportation.
2.2. Electrowetting platform

The EWOD platform consists of two electrode arrays photo-lithographically structured onto glass substrates. Each of them allows for EWOD actuation in along one axis perpendicular to each other. The HFF-QCM sensor is integrated into the base plate and is covered by a 3 nm chromium adhesion layer plus a conductive 50 nm gold layer. The latter is suitable for the formation of the hydrophobic SAM, compare with 2.1. The top plate consists of an electrode array structured co-planar on the substrate with a saw-tooth like separation line, which ensures the overlap of the droplet with at least two electrodes at an arbitrary position. The electrode array is electrically isolated by a 150 nm thick Al₂O₃ layer combined with a hydrophobic coating of 1.6 μm Polytetrafluorethylene (Teflon AF, obtained from DuPont, PTFE). The breakdown voltage of the realized isolation layer exceeds 100 V, resulting in a reduction of the surface energy of 0.11 Jm⁻² which provides forces exceeding the minimal actuation forces of PBS on PTFE.

The droplet is squeezed in between base and cover plate positioned in a 2mm distance, as illustrated in Fig.3 and is transported by means of EWOD actuation to and off the sensor field.

Fig. 2: a) hydrophobic top electrode of the EWOD platform with integrated QCM sensor coated by a 1-octadecanethiol SAM, b) PBS droplet containing phosphor lipids resting on the QCM for biochemical bindings with the SAM

Fig. 3: Illustration of a sample droplet in-between base and cover plate. The base plate consist of a) 50 nm Au coated by a 1-octadecanethiol SAM, b) glass substrate and e) the HFF-QCM sensor. The cover plate enabling EWOD actuation is a structured 50 nm thick Au-electrode array, coated by c) an Al₂O₃ layer and d) a hydrophobic 1.6 μm PTFE layer
3. Measurements

The consecutive and reversible binding of the sequence Phospholipids on a SAM of 1-octadecanethiol and Streptavidin on biotin carrying phospholipids was shown in [1]. In that contribution, the reagents were provided in a continuous flow whereas in our contribution the sample liquid is provided to the QCM sensor element in terms of single droplets, which allows for an individual control and manipulation prior and posterior to the measurement. A series of phosphate buffered saline (PBS) droplets (P1-P3 in Fig. 4) of 10 μl volume were transported to and away from the QCM sensor and initial reverence spectra were taken at ambient temperature of 24.1°C to determine the fundamental frequency and corresponding quality factor $Q = f_0 / \Delta f_{\text{FWHM}}$ (50,777 MHz and 394, respectively) without an additional mass load.

Subsequently a series of droplets containing biotin carrying phospholipids (L1-L9) was directed over the QCM sensor. In agreement with theory, a shift to lower resonance frequencies of the QCM was determined via impedance measurements and a stable level was reached after 8 minutes indicating the formation of a phospholipid monolayer, which carries biotin residues on 20% of the phospholipid head groups [9]. Measurements on PBS (P4-P6) confirmed the stability of the formed monolayer.

A multilayer formation was achieved by directing a series of streptavidin enriched droplets (S1-S4), which allows for bio-specific binding to the Biotin-presenting phospholipid coating of the QCM [10]. An additional decrease of ~20 kHz in the resonance frequency of the QCM was determined; see Fig 4. After the first three measurements in a time interval of 3 minutes the signal did not decrease further. Terminatory, two PBS droplets (P7, P8) were placed on top of the sensor to confirm the multilayer formation as the origin of the frequency shift and to rule out shifts due to a different viscosity of the dissolution.

![Shift of resonance peak due to biological binding](image)

Fig. 4: First preliminary results obtained with samples moved to QCM-sensor: A series of phosphate buffered saline (PBS; P1-P3) droplets containing phospholipids (L1-L8), followed by streptavidin (S1-S4) enriched droplets enabling biochemical binding to the lipids were directed onto the QCM sensor. Intermediate measurements on PBS Droplets (P4-P6 and P7 and P8) were performed to ensure shift due to binding.

In a second experiment we brought a droplet detergent of octyl-D-glucopyranoside to the surface coated with phospholipids. The binding tail was removed and the original resonance frequency restored. After the measurements, which indicated the presence of one of both substances, we tried to direct the droplets away from the sensor to use them for further reactions. In the case of a positive detection of bindings the hydrophobic properties of the functionalized QCM get lost. Thus, we were not able to drag the droplet away from the QCM by means of...
electro wetting actuation. A chemical solution of that sticking droplet is currently investigated. The approaches of either washing away the binding molecules or the new formation of a hydrophobic surface are most promising.

4. Conclusion

We presented the integration of a functionalized QCM sensor with high fundamental frequency into a platform designed for EWOD actuations. The frequency shift of the resonance frequency was detected, indicating the binding of bio-molecules out of single droplets to the functionalized surface. Thus, the applicability of functionalized QCM sensors in digital microfluidics was shown. The drawback of this detection method is that after the first binding of lipids the hydrophobic layer gets more and more hydrophilic. Therefore, the QCM sensor principle has to be applied to a different bio-chemical system where the hydrophobic properties are conserved during the detection process. Currently, we are investigating chemical reactions which restore the original or form a new hydrophobic layer.

Acknowledgements

This work was supported by the Austrian Science Fund FWF under contract no. L442-N14.

References