

# Time-resolved measurements of DNA interactions in an electrowetting-on-dielectric system using confocal microscopy

**Lorenz T. Sparrenberg, Benjamin Greiner, Christian Mueller and Harald P. Mathis**

Fraunhofer Institut for Applied Information Technology FIT; BioMOS; Schloss Birlinghoven, D-53754 Sankt Augustin, Germany

E-mail: [lorenz.sparrenberg@fit.fraunhofer.de](mailto:lorenz.sparrenberg@fit.fraunhofer.de)

**Abstract.** To identify new drug candidates a deep and profound knowledge of molecule interactions is needed. In the current work, a combination of an electrowetting-on-dielectric (EWOD) system with a confocal microscopy is presented for the first time. The aim of this research is to attain time-resolved information about nucleic acid interactions at a single molecule level. Confocal microscopy is a promising technique for reaction analysis on a molecular scale. But the liquid handling of the needed tiny volumes of highly diluted solutions is very challenging. An EWOD based system for droplet handling can address this demand. In this paper the development of an EWOD system for droplet handling in nanoliter scale is discussed and the combination of the EWOD system with a confocal microscope, to investigate nucleic acid interactions, is evaluated.

## 1. Introduction

Time-resolved information about molecular interactions are very important in biochemistry and pharmacology. To identify new drug candidates a deep and profound knowledge of drug-target interactions is needed. Methods based on fluorescence-fluctuation-spectroscopy (FFS) has become a standard in the analysis of molecular processes [1]. FFS is based on fluorescence fluctuations of molecules in tiny detection volumes [2]. A combination of confocal microscopy and improved photo detectors allows the detection of photon events originating from single molecules in highly diluted solutions [3]. To investigate time-resolved interactions in highly diluted nucleic acid solutions the liquid handling becomes a severe challenge. Tiny amounts of reagent solution have to be placed in the focus of a microscope at a defined starting point. By means of manual pipetting it is almost impossible to execute this task. Therefore a precise and reproducible handling technique for small liquid amounts in nanoliter scale is needed. An electrowetting-on-dielectric (EWOD) system for liquid-handling can address these demands. Electrowetting means to exert forces on phase interfaces such as liquid-gas or liquid-liquid interfaces by electric fields and was first described by Lippmann in 1875 [4]. This effect can be used to transport small droplets over an electrode array [5]. In recent years EWOD systems have drawn a lot of attention because they enable the manipulation of droplets in nanoliter scale [6]. Fundamental microfluidic operations with droplets have been demonstrated with EWOD systems (i.e. droplet transport, droplet splitting, create droplet from reservoir) [7]. It has been shown that EWOD

systems can transport not only pure water but also protein solutions [8], nucleic acid solutions [9] and even entire mammalian cells [10]. In the current work an EWOD system for droplet manipulation is developed and its potential to investigate time-resolved molecule interactions is evaluated in combination with a confocal microscope.

## 2. Experimental details

### 2.1. EWOD device fabrication

For the production of the electrowetting-on-dielectric (EWOD) device standard microfabrication techniques are used. Both quartz and silicon wafers are used as substrates. Silicon wafers have excellent properties for photolithographic processes but because of their strong reflective behaviour fluorescence measurements with high signal to noise ratio are difficult. Hence, quartz wafers are employed for the combination of EWOD and confocal microscopy. Electrodes are formed by means of photolithography and consist of 20 nm chromium and 70 nm platinum. In figure 1, a photo emulsion mask with the electrode structures is displayed. Reservoirs are

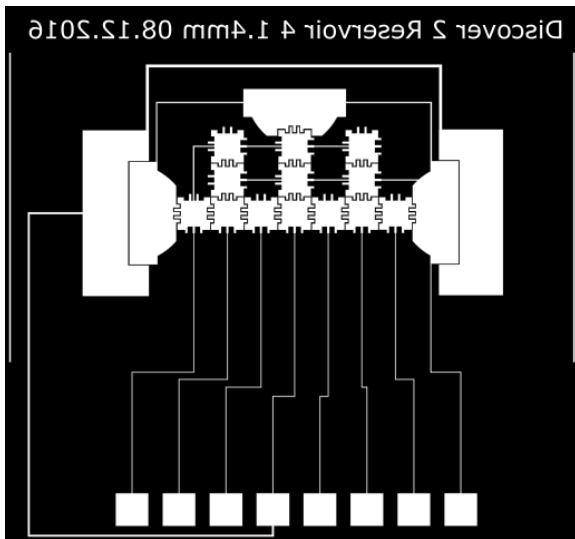


Figure 1: Photo emulsion mask of the EWOD structure with 10 nm resolution. Each electrode of the array has a width of 1.4 mm and interdigital structures to ensure droplet contact to adjacent electrodes. The bottom line of electrodes are the contact electrodes where the voltage is connected.

situated on top of the large electrodes on the left and the right side. From there small droplets can be taken. The electrode array at the bottom of the mask is used to contact the electrodes to the voltage supply. To reduce the number of contact electrodes some electrodes are linked together. As dielectric layer aluminum oxide is deposited by a sol-gel technique [11]. After a final tempering of the microfluidic structure at 450 °C a homogenous crack-free aluminum oxide layer of 400 nm thickness is obtained. The deposited aluminum oxide layer is smooth with no continuous pores. For guaranteeing a defined distance between electrodes and cover lid a frame of SU-8 with a thickness of 100  $\mu\text{m}$  is deposited. Small holes to fill the reservoirs with liquid are bored by an ultrasonic drill. As cover lid and simultaneously grounding electrode a glass plate with an indium tin oxide (ITO) layer is used. Finally, an approximately 50 nm thick hydrophobic layer of teflon is spin coated on both the device and the cover lid. To improve quality assurance interferometry measurements are taken. For a better handling a holder for the components of the EWOD device is printed by a 3D-printer. The needed voltage square wave is generated by a function generator and subsequently amplified to the required voltage level. An Arduino controlled in-house build circuit switches the voltage signal between adjacent electrodes. Non activated electrodes are connected to ground. The circuit is designed to cover a voltage range up to  $\pm 175$  V. EWOD experiments were performed with a voltage of 80  $V_{pp}$  with a frequency of 250 Hz (square wave). The droplets consist of DI-Water or 10 ng/ $\mu\text{l}$  solution with 0.01 wt%

TWEEN<sup>®</sup> 20 to reduce unspecific biomolecule adsorption to the teflon surface [9]. N-dodecane is used as ambient fluid to reduce friction forces and to stabilize the cassie-baxter state of the droplet on the surface and thus reducing contact angle hysteresis [12, 13].

## 2.2. Combination of EWOD device and microscope

The evaluation of the assembled EWOD device and microscopy system is conducted in two steps. A first simplified experiment is performed as a proof of concept. In this first experiment the number of involved components is reduced to a necessary minimum. Two droplets are merged by means of EWOD. One droplet consists of DI-water and the other one contains the fluorescence dye Alexa488 ( $1 \times 10^{-9}$  M). Additionally, no ambient fluid but air is used in the EWOD system to avoid liquid-liquid interfaces that might influence the measurement. The water droplet is firstly moved to the detection spot. After this the droplet with Alexa488 dye is merged with the previous droplet. For fluorescence measurement a 63x oil emulsion objective (Zeiss) is used. Fluorescence excitation is done at 488 nm wave length.

In the next evaluation step a small droplet of DNA solution (10 ng/ $\mu$ l DNA with 0.01 wt% TWEEN<sup>®</sup> 20, 1:1000 Sybr Green I) is moved in and out of the detection spot of the microscope. The purpose of this evaluation is to point out the reproducible movement of nucleic acid solutions by means of electrowetting while measuring the fluorescence signal.

## 3. Results and discussion

### 3.1. Evaluation of EWOD device for liquid handling

To monitor the quality of the EWOD device interferometry measurements of the electrode structures are taken. The interferometry measurements ensure the integrity of the structures and the overlaying dielectric layer, an exemplary measurement is displayed in figure 2.

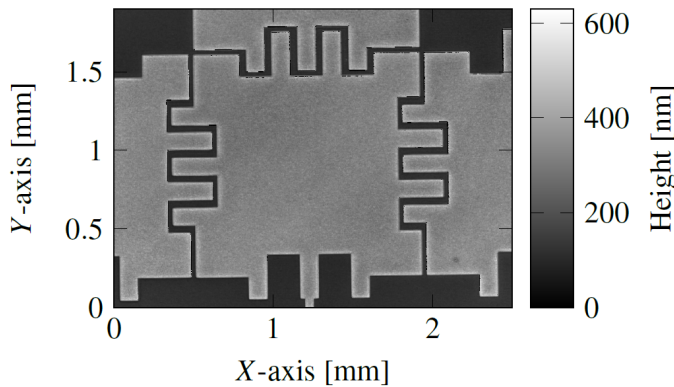


Figure 2: Interferometry measurement of an EWOD electrode ( $1.4 \text{ mm} \times 1.4 \text{ mm}$ ). 20 nm chromium and 35 nm platinum are sputtered onto a quartz substrate. The surface is covered by an aluminum oxide layer of 600 nm thickness.

As it can be seen there are no cracks or obvious pores in the dielectric layer. The height difference between electrode and substrate originates from the different reflective properties of platinum and quartz that influence the measurement of the transparent  $\text{Al}_2\text{O}_3$  layer. The functionality of the EWOD device is tested by a series of droplet manipulation experiments. A sequence of droplet displacements can be seen in figure 3. A solution of 10 ng/ $\mu$ l with 0.01 wt% TWEEN<sup>®</sup> 20 is used. The switching time of the voltage is 500 ms. **A)** A 300 nl droplet is taken from a reservoir at the bottom of the image. **B)** The droplet is moved to a central electrode and **C)** is divided into two even droplets of 150 nl. **D–E)** The small droplets are transported to electrodes at the right side of the structure, respectively. **F–H)** The droplets are merged and moved back to the reservoir. The same operation can be shown with DI-water. In this case the spherical droplet shape is less deformed while the droplet displacement from electrode to electrode is more precise. This is due to a higher surface tension of DI-water compared to DNA

solutions with the addition of the detergent TWEEN<sup>®</sup> 20 which significantly lowers the surface tension.

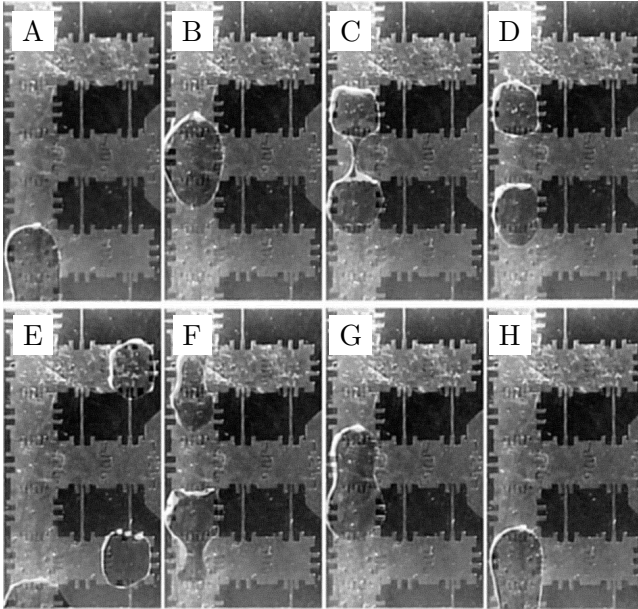


Figure 3: Transport of DNA solution using EWOD. The created droplet has a volume of approximately 300 nl and after the splitting a volume of ca. 150 nl. The droplet volume consists of 10 ng/ $\mu$ l DNA solution in water with 0.01 wt% TWEEN<sup>®</sup> 20 added. Electrical parameters:  $U = 80 V_{pp}$ ,  $f = 250$  Hz square wave, switching time  $t = 500$  ms.

It should be noted that DI-water droplets can be switched more rapidly (250 ms) without any errors. Moreover, an electrowetting system driven with pure water is less prone to dielectric breakdown because of the lower conductivity of DI-water compared to electrolyte solutions. The EWOD system can be driven for several hours at voltages from 80  $V_{pp}$  to 100  $V_{pp}$ . At higher voltages defects at the electrode edges occur, which finally lead to a dielectric breakdown because of a higher density of the electric field lines there. Good repeatability of the droplet movement indicates that the addition of 0.01 wt% TWEEN<sup>®</sup> 20 to the solution minimizes the adsorption of nucleic acids onto the teflon layer.

### 3.2. Combination of EWOD device and microscopy

In a first experimental setup of the assembled liquid handling system and the confocal microscope two droplets (DI-water and Alexa488 solution) have been merged in the focus of the confocal microscope using EWOD technique. The measured fluorescence intensity over time is shown in figure 4. After a short lag time of about 3 min, a sigmoidal increase of fluorescence intensity until minute 12 can be seen (data points), after which a linear signal increase can be observed. The results indicate the diffusion of Alexa488 molecules from the highly concentrated droplet volume to the low concentrated droplet volume until an equilibrium is reached. The linear increase of the intensity is due to evaporation. Thus leading to an observable increase of concentration over time. The dotted line displays the corrected intensity and shows a plausible curvature for free molecule diffusion. For the modeling of the evaporation a cylindrical geometry of the merged droplet is assumed.

At first glance the large time scale of the measurement surprises. But it has to be considered that after merging almost exclusively diffusion is responsible for molecule transport within the droplet volume over a distance of about 0.5 mm. For comparison simulation data for a solely diffusion driven mixing of droplet volumes is added to the graph. The diffusion is modeled as two volumina with infinite extension. At the very beginning the molecule distribution at the transition line from one volume to the other is like a step function. From now on the mixing of

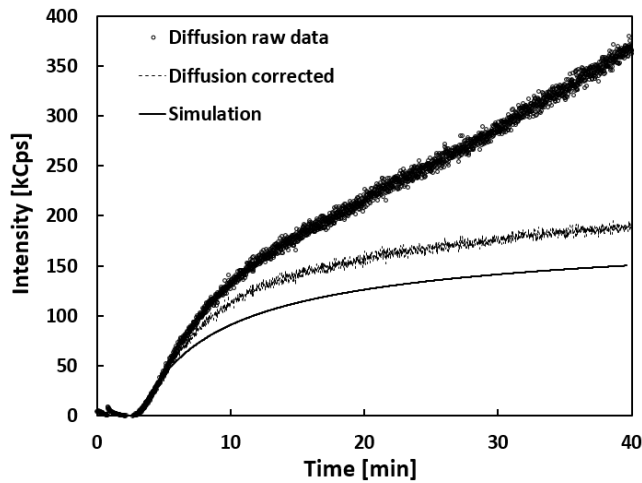


Figure 4: Diffusion of Alexa 488 after fusion of two droplets. Fluorescence measurement with confocal microscope using 63x oil emulsion objective.

the two volumes is only driven by diffusion. As it can be seen the time scales match the previous data curves. The discrepancy between measurement and simulation can be explained by a small convective mixing term in the droplet experiment which was not considered in the model of the molecule diffusion. Another uncertainty is related to the evaporation effect and its modelling. The evaporation can be suppressed by using silicone oil or dodecane as ambient fluid. The intensity measurements during several movement sequences of DNA solution in and out of the detection volume of the confocal microscopy can be seen in figure 5. The intensity

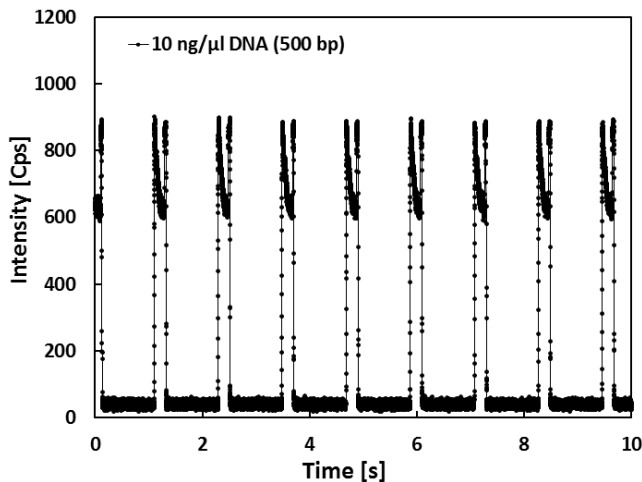


Figure 5: Movement of DNA solution in and out of the detection volume of the microscope. 10 ng/ $\mu$ l DNA solution with 0.01 wt% TWEEN<sup>®</sup> 20 added, 10x objective

signal sharply increases from 50 Cps to 900 Cps each time the droplet is transported into the detection volume. Another peak is found whenever the droplet is moved out of the detection spot. Between these two sharp peaks an intensity decrease from 900 Cps to 600 Cps is observable. The signal intensity of 50 Cps corresponds to the background noise of the measurement in the EWOD device. The intensity drop from 900 Cps to 600 Cps between the droplet movements is presumably due to bleaching of the fluorescence dye. Because of the highly reproducible intensity curves for each droplet movement a good mixing of the droplet volume can be assumed. The excellent reproducibility of the peaks allows the derivation of underlying models to describe the bleaching of the fluorescence molecules. It can be concluded that the experiments prove the feasibility of time resolved measurements in droplets after transporting via electrowetting.

#### 4. Conclusion

In this work an EWOD based liquid handling technique has been developed and combined with a confocal microscope. The developed electrowetting system for liquid handling can manipulate DI-water droplets and DNA solutions of 10 ng/ $\mu$ l concentration repeatably and reliable. Additionally, standard operating modes like droplet transport, droplet merging, droplet splitting and taking droplets from a reservoir has been shown. Thus, a powerful liquid handling system has been established. The proof-of-concept i.e. the combination of an EWOD device with a confocal microscope was displayed by the merging of two droplets and the measuring of fluorescence increase due to molecule diffusion from one droplet to another. In addition, a small droplet of DNA solution was moved in and out of the detection spot of the microscope. The measured intensity signals are reproducible without any signal loss from peak to peak. This indicates the good suitability of the combined system for further molecule interaction measurements.

In this work the potential of EWOD based liquid handling techniques combined with high resolution confocal microscopy has been shown. Further experiments are planned to investigate the interaction of nucleic acids on a single molecule level. This knowledge may help to profoundly increase the understanding in biological mechanisms and interactions. The identification of new drug candidates and the development of new therapeutics will benefit from these insights.

#### 5. References

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