Electronic Supplementary Information

Integration of digital microfluidics with whispering-gallery mode sensors for label-free detection of biomolecules

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Spin coating parameters for cyanoethyl pullulan (CEP)

Cyanoethyl pullulan (CEP or Cyanoresin-S) dissolved in propylene carbonate was coated as a dielectric layer on the bottom substrate. Fig. S1 shows the spin curves for three concentration of CEP in propylene carbonate. Depending on the desired layer thickness, a suitable concentration can be chosen. We used the following program to apply a 7 µm CEP layer from 30 wt% CEP in propylene carbonate:

1) Pour a portion to cover ¼ of the wafer or chip
2) Spin at 500 rpm with ramp 100 rpm/s for 30 seconds to dispense over the whole chip
3) Accelerate to 3100 rpm with ramp 1000 rpm/s for 60 seconds
4) Stop and let the chip rest for 30 seconds
5) Baking at 100 °C for several hours. It depends on layer thickness, (vacuum) oven or hotplate

More details about the handling of CEP can be found in an article by Chen et. al.[1]

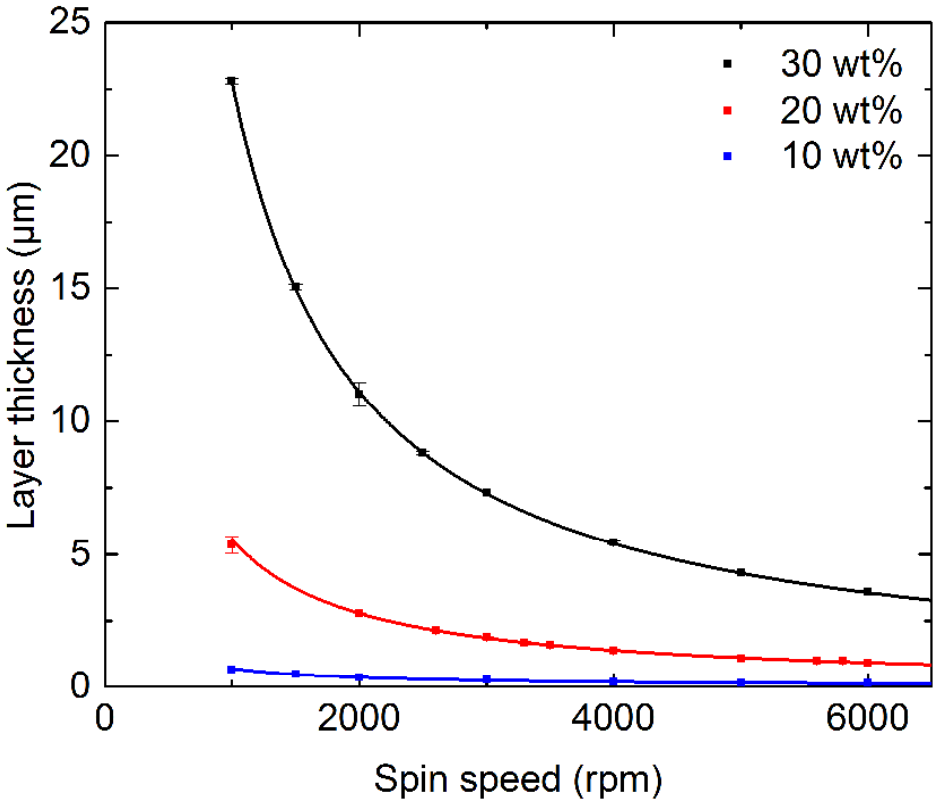

Fig. S1 Spin curves of cyanoethyl pullulan (CEP) in propylene carbonate. The highly concentrated solution with 30 wt% concentration was spun at 3100 rpm to achieve the target thickness of 7 µm.

Operation of DMF pixels with microwells

In the main article, three levels of actuation voltage are discussed depending on the kind of droplet movement. Movement of droplets on unstructured surface was possible with 70 V, while moving droplets over microwell boundaries required higher activation voltages. Moving droplets into the microwells required as high as 90 V to overcome the boundaries, while moving them out required an even higher voltage of 120 V. We attribute the differences in the driving voltages to the absolute interface energy differences of droplets, which is proportional to their total surface area. Moving a droplet into or out of a microwell demands squeezing through the microwell boundary, effectively increasing its surface area. This requires an additional energy proportional to the increase in surface area, deformation energy. Therefore, higher actuation voltages are needed in comparison to the standard voltages used to move droplets over the unstructured area of the integrated DMF system. Fig. S2A – S2C shows the three states of a droplet: outside a microwell, transiting over a boundary, and inside a microwell. Moving the droplet into the microwell (1 →2) causes a smaller deformation than moving a droplet out (3 → 1). Therefore, 90 V is sufficient to move a droplet into the microwell, while 120 V is needed to move it out.

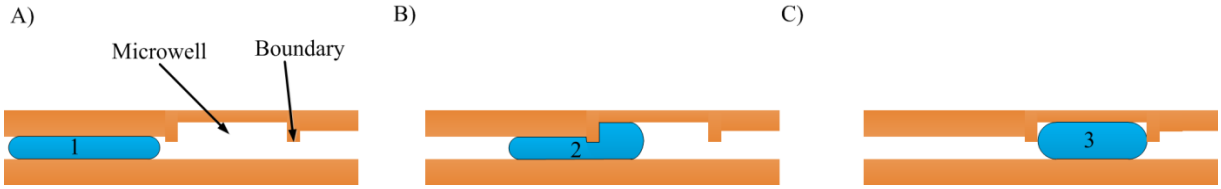

Fig. S2 Microwell operation. Droplets are deformed while moving over microwell boundaries. The additional energy needed depends on the extent of deformation, which is smaller for moving a droplet into a microwell than the opposite direction. Accordingly, the smaller actuation voltage (90 V) is sufficient to move a droplet into a microwell, than out (120 V).

Chip-characterization, droplet mobility and biofouling

For chip-characterization and mobility improvement, different fluids and additives (e.g. surfactants) were compared on the same device with pure distilled water (see Fig. S3) under identical operating conditions: driving voltage, driving frequency and droplet volume. The top-substrate is a normal ITO-glass coated with Teflon.

The droplet mobility can be characterized by its maximum speed or the time the droplet needs to arrive at the final position. Speed is the derivation of the capacitance curve.[2] The final position is reached at the capacitance curve's plateau. The plateau is very stable even though setting a specific time for reaching the plateau is ambiguous. Fig. S3B and Fig. S3C show the mobility and the stability of the mobility (biofouling) of a pure PBS droplet (see Fig. S3C) and a PBS droplet with Streptavidin and 0.2 vol% Pluronic L64).

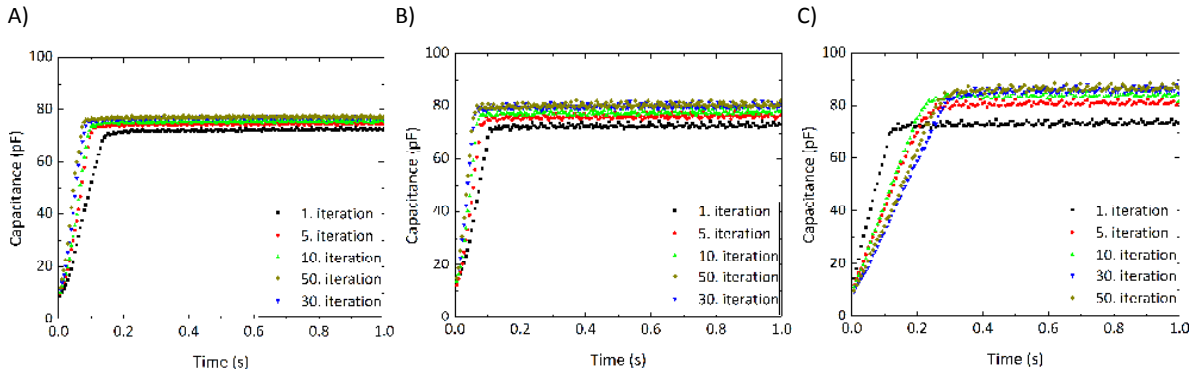

Fig. S3 Mobility measurements. A) Distilled water (reference.) B) PBS. C) PBS with streptavidin and 0,2v% Pluronic L64. All measurements were conducted at uniform settings: Actuation at 65 V, frequency of 1 kHz, using 1.5 µL droplet volumes, chips separated apart by 190 µm and with 7 µm CEP dielectric layer at the bottom substrate.

Contact angle measurements

Contact angle measurements were performed to investigate the influence of Pluronic L64 in combination with different solutions on surface biofouling. This information is important as it affects droplet mobility similar to the case of streptavidin mentioned in the main article. Fig. S4 shows the measurement results. A distilled water droplet (2µl) on a plane, unmodified Teflon AF surface (spots 1-3, red bars) has a contact angle of about 120°. On the functionalized surfaces (spots 4-6, red bars), the contact angle is about 105°. The spots were treated in three combinations of surface treatment and the contact angle of distilled water was measured again (green bars). The

surface treatments include deposition of solutions containing streptavidin with or without Pluronic L64 for different time durations. The treatments performed on the spots before contact angle measurements are as listed below:

Spots 1 and 4: 20 min treatment with a PBS-streptavidin solution with 0.1% Pluronic L64

Spots 2 and 5: 1 min treatment with PBS-streptavidin solution without Pluronic L64.

Spots 3 and 6: 20 min treatment with PBS-streptavidin solution without Pluronic L64.

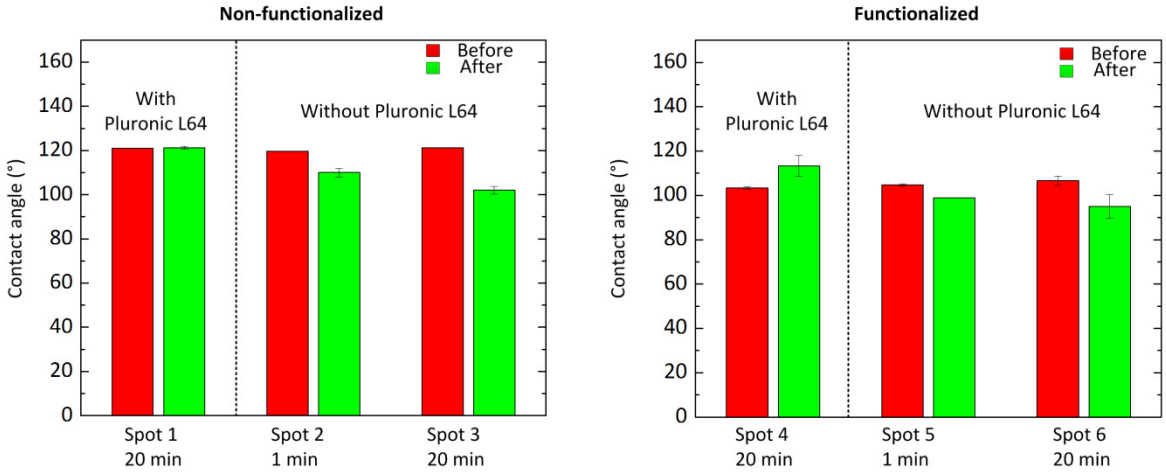

Fig. S4 Contact angle of 2 µl DI-water on non-functionalized and functionalized spots of the top-substrate measured before (in red) and after (in green) treatment with streptavidin containing buffer solutions. Reduced contact angles were measured for spots treated in the absence of Pluronic L64, which is an indication of biofouling. This effect was absent in the prescence of Pluronic L64.

The results on spot 1 show that Pluronic L64 suppresses unspecific binding of the Streptavidin to the Teflon: The contact angle is not affected by exposure to Pluronic L64-containing PBS-streptavidin solution for 20 minutes, i.e., biofouling does not occur. Spots 2 and 3 were taken for streptavidin solution without Pluronic L64 and show an increasing biofouling, i.e., a decreasing contact angle with longer incubation time. Moving of droplets over these spots was impossible. Before surface treatment, the functionalized spots 4-6 exhibit generally a lower contact of approximately 105 ° due slightly increased hydrophlilicity upon biotinylation. After surface treatment with pure PBS-streptavidin solution, i.e., without Pluronic L64, spots 5 and 6, the contact angles reduce even further, indicating strong biofouling. This is a similar observation as for the case of the non-functionalized surface tests. In contrast to that, the treatment on spot 4 with Pluronic L64 in the streptavidin-containing solution increased the contact angle to about 115°. The contact angle increase is attributed to a non-specific adsorption of Pluronic L64. Further experiments are needed to fully understand the exact mechanisms of biofouling and the dependence on an applied electric field.

Surface functionalization

The surface was incubated for 2 hours with 1.4 mM biotinylated BSA and blocked with 1% BSA (bovine serum albumin) in PBS (phosphate-buffered saline) for 20 minutes to avoid non-specific absorption. To verify the surface functionality Cy3-labelled streptavidin was made to react with the functionalized surface for 30 minutes. As a control, another substrate was coated with Teflon and the same procedure was repeated on it except the biotinylation. Fig. S5 shows the fluorescence images with the red surface showing the area that is covered with streptavidin, verifying the functionality of the surface functionalization protocol.

Table S1: Description of samples used for verification of the functionalization protocol.

Sample label	**Functional layer**	**Fluorescent marker**
Functionalized	BSA-biotin	Cy3_streptavidin
Control 1	none	Cy3_streptavidin
Control 2	none	none

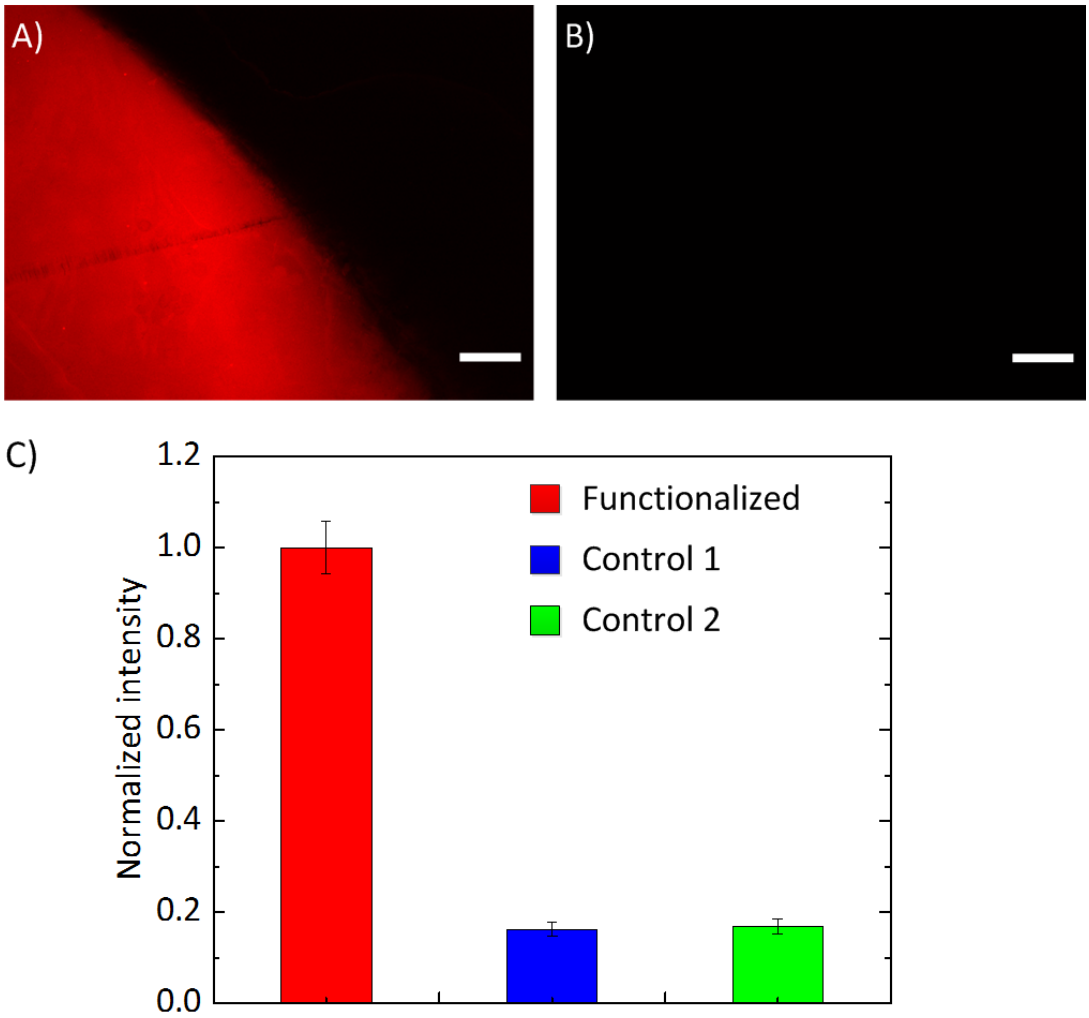

Fig. S5 Surface functionalization of Teflon coated PMMA surface. A) The red spot shows fluorescence from the area functionalized with BSA biotin and stained with Cy3-streptavidin. B) The control sample stained with Cy3-streptavidin without being functionalized, shows low fluorescence. C) Graph depicting the normalized, integrated fluorescence of tested substrates.

PBS 1× mixed with surfactant (0.1 vol% Pluronic L64) was used as a buffer solution and for preparation of all analytes. Addition of the surfactant facilitates the mobility of droplets on the microfluidic chip.

References

1. J. Chen, Y. Yu, K. Zhang, C. Wu, A. Q. Liu and J. Zhou, *Sens. Actuators, B*, 2014, **199**, 183-189.
2. R. Fobel, C. Fobel and A. R. Wheeler, *Appl. Phys. Lett.*, 2013, **102**, 193513.