

Article

# Supporting information - Polarizability-dependent sorting of microparticles using continuous-flow dielectrophoretic chromatography with a frequency modulation method

Jasper Giesler<sup>1</sup>, Georg R. Pesch<sup>1,\*</sup>, Laura Weirauch<sup>1</sup>, Marc-Peter Schmidt<sup>2</sup>, Jorg Thöming<sup>1</sup> and Michael Baune<sup>1</sup>

<sup>1</sup> University of Bremen, Chemical Process Engineering, Leobener Straße 6, 28359 Bremen, Germany

<sup>2</sup> Brandenburg University of Applied Sciences, Department of Engineering, Magdeburger Straße 50, 14770 Brandenburg an der Havel, Germany

\* Correspondence: gpesch@uni-bremen.de

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## 1. Method

Figure 1 shows the  $\text{Re}(CM)$  and mobility with respect to the frequency. Since the surface conductance (here:  $K_s = 1 \text{ nS}$ ) is not easily measurable, these curves are assumptions and are used as starting point for process parameters. For constant surface conductance and medium conductivity the  $\text{Re}(CM)$  only depends on particle size. The chosen frequency band should include the cross-over frequency of all particles to generate nDEP and pDEP.

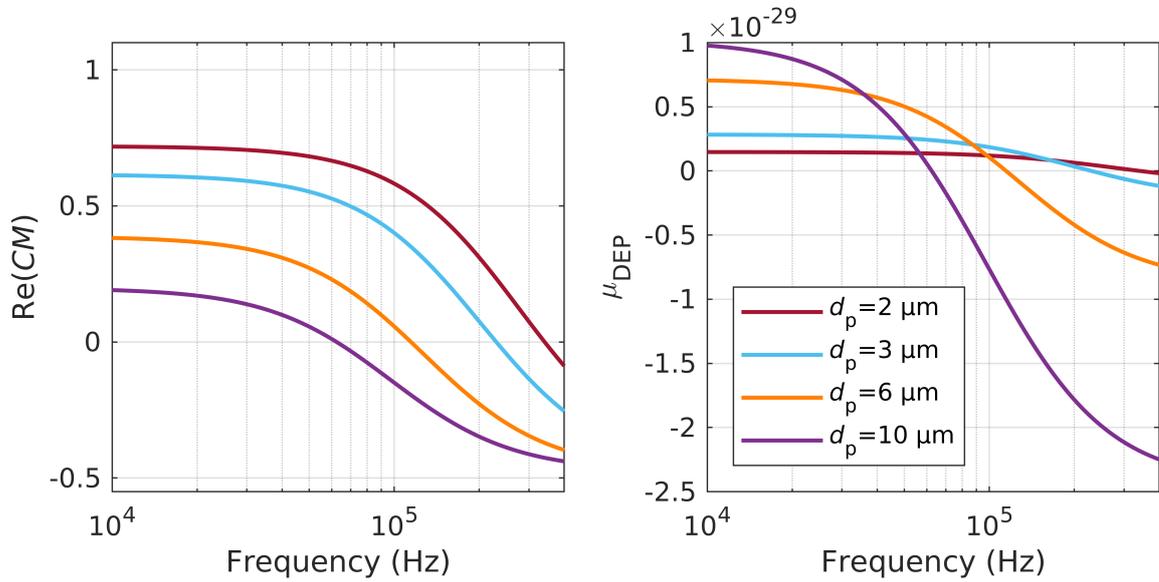
## 2. Device Fabrication

### 2.1. PDMS device

The fabrication of PDMS microfluidic devices (soft lithography) is a well know process, which was described in literature before [1,2]. A SU-8 master mold was produced using a laser printed mask (25.000 dpi KOENEN GmbH, Germany, Figure 2 b) and standard clean room techniques. Afterwards, the PDMS channel can be made by pouring well mixed and degassed PDMS (10:1, base:curing agent, Sylgard 184 Dow corning) onto the master mold. After curing the PDMS at 80 °C for 1 h in a convection oven, the polymer can be cut into pieces as desired using a scalpel. Inlet and outlet, both having an inner diameter of 1.5 mm, were punched using biopsy punches (Rapid-Core, World Precision Instruments, Inc.).

### 2.2. Electrodes

The electrodes were fabricated using physical vapor deposition of first chrome as adhesion layer and second gold as final layer. As substrate 100 mm borosilicate glass wafer were used. Afterwards, using a chrome mask (100.000 dpi bvm.maskshop, Germany), positive photoresist and followed by a wet etching process, the final electrodes were obtained. These electrodes had a width of 100  $\mu\text{m}$  and an identical gap width. To connect the amplifier to the flow cell contact pins were soldered onto the electrode. The full electrode chip has length of 58 mm and a width of 26 mm (Figure 2 a).



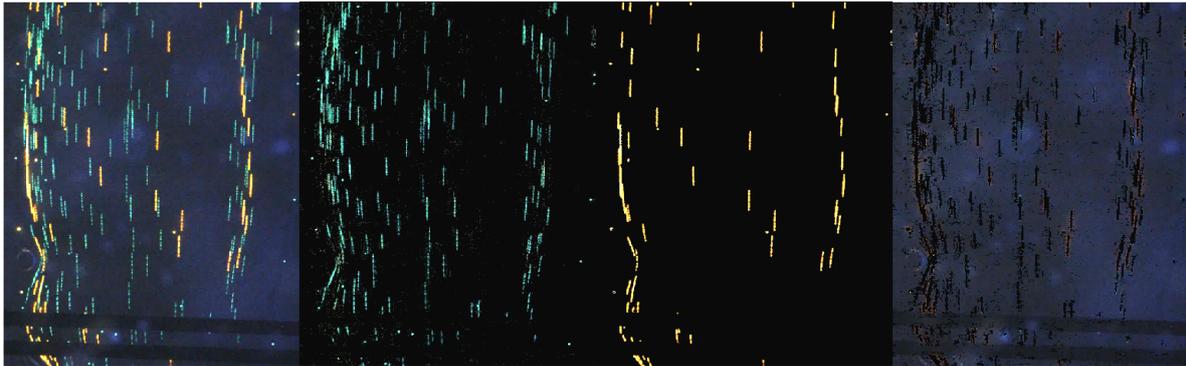
**Figure 1.** Real part of Clausius-Mossotti factor and dielectrophoretic mobility over frequency of the electric field of polystyrene particles ( $d_p = 2 \mu\text{m}$ ,  $3 \mu\text{m}$  and  $6 \mu\text{m}$ ). Particles suspended in DI water as surrounding medium, with  $\sigma_m = 1.2 \mu\text{S cm}^{-1}$  and  $\epsilon_m = 78.5$ ).



**Figure 2.** a) Photography of the used gold electrodes with  $100 \mu\text{m}$  electrode width & gap width b) Photomask for producing the SU8 master mold.

### 26 3. Post-processing

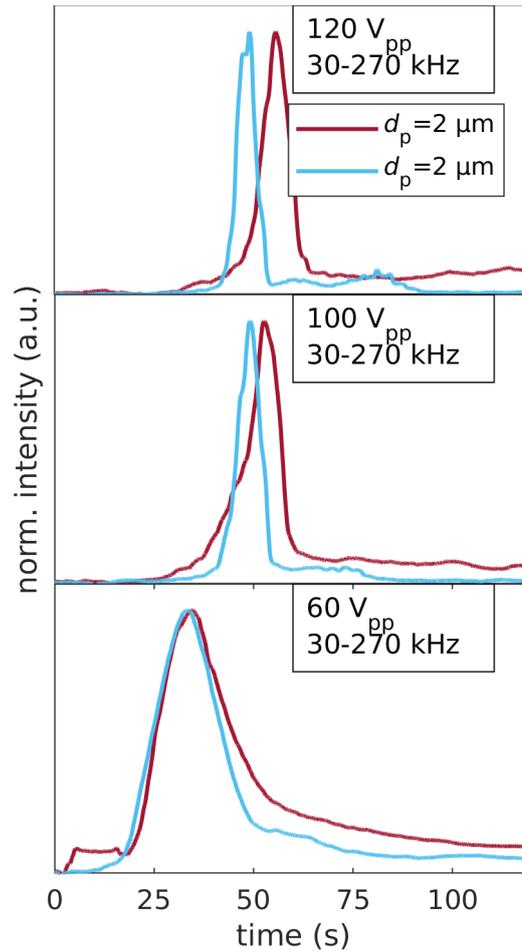
27 A segmentation algorithm was used to extract the fluorescence intensity of each colour of  
28 fluorescence into different images[3]. Afterwards the intensity of each image was calculated and  
29 a moving average was applied to reduce the level of noise of the measured intensities. Finally, the  
30 background signal was subtracted for each colour to eliminate scattered light and permanently adhered  
31 particles from the data. Since all measurements produce slightly different maximum values and each  
32 particle class has a different fluorescence intensity per particle, the Data was normalized by the  
33 maximum intensity value of each experiment.



**Figure 3.** Result of the segmentation process. Original Image, particle typ 1, particle type 2 and background (f.l.t.r)

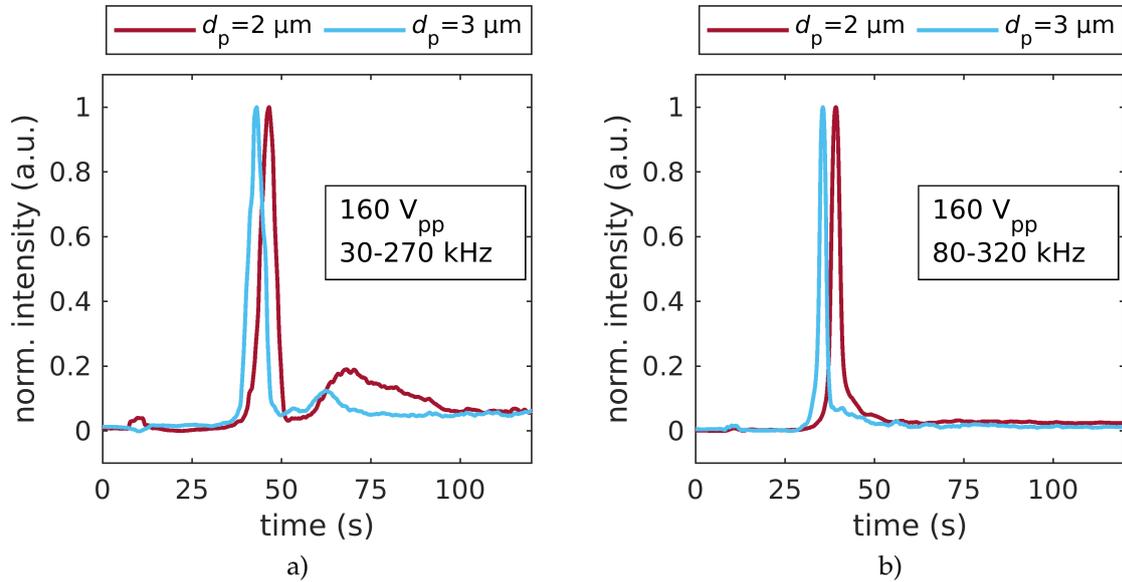
### 34 4. Experimental results

35 The increase in resolution while increasing the applied voltage of the separation of  $2\ \mu\text{m}$  and  $3\ \mu\text{m}$   
36 fluorescent polystyrene particles is illustrated in [Figure 4](#). With increasing voltage, the width decreases  
37 and the peak maxima diverge progressively.

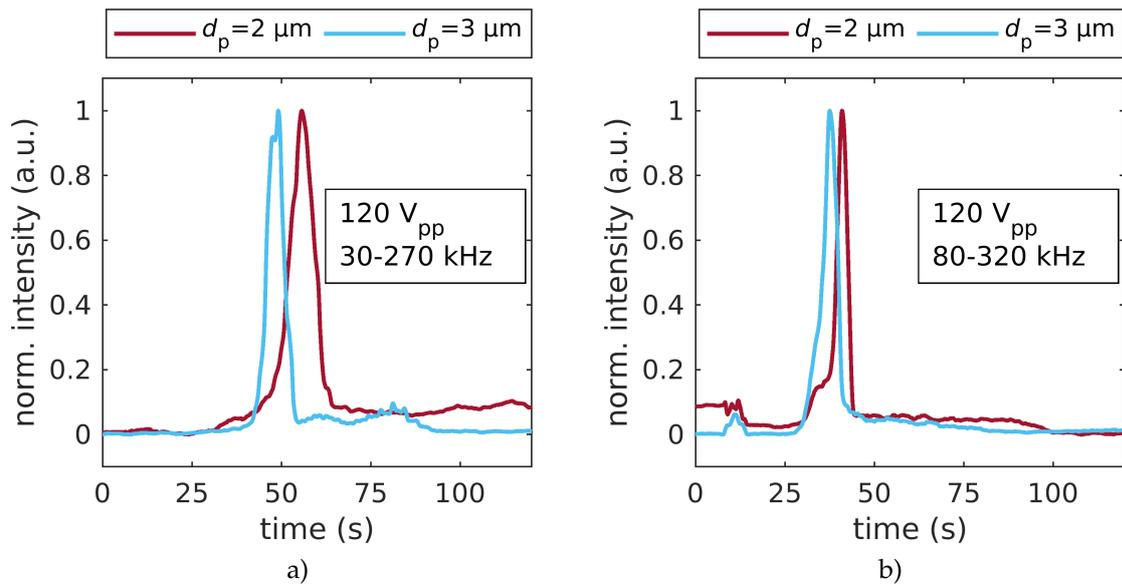


**Figure 4.** Fluorescence intensity over time of 2  $\mu\text{m}$  and 3  $\mu\text{m}$  fluorescent polystyrene particles with using different voltages. With increasing voltage the separation resolution  $R_s$  increases from  $R_s(60 V_{pp}) = 0.051 \pm 0.14$  to  $R_s(100 V_{pp}) = 0.24 \pm 0.07$  and finally to  $R_s(120 V_{pp}) = 0.54 \pm 0.1$  ( $N = 4$  experiments)

38 The variation of the set of frequencies (30 kHz - 270 kHz vs. 80 kHz - 320 kHz) is illustrated in  
 39 [Figure 5](#) for 160  $V_{pp}$ . The decrease in smaller retention time using 80 kHz - 320 kHz is shown in [Figure 6](#).  
 40 The particles elute significantly later, when 30 kHz - 270 kHz are applied. The higher reetention time,  
 41 when using 30 kHz to 270 kHz, is due to less negative dielectrophoretic movement. Therefore, the  
 42 particles are more likley to stay in areas near to the electrode in which only low fluid velocity is present.



**Figure 5.** Fluorescence intensity over time of 2  $\mu\text{m}$  and 3  $\mu\text{m}$  fluorescent polystyrene particles. a) 160 V<sub>pp</sub> at 30 kHz - 270 kHz b) 160 V<sub>pp</sub> at 80 kHz - 320 kHz with a modulation frequency of 300 mHz



**Figure 6.** Fluorescence intensity over time of 2  $\mu\text{m}$  and 3  $\mu\text{m}$  fluorescent polystyrene particles. a) 120 V<sub>pp</sub> at 30 kHz - 270 kHz b) 120 V<sub>pp</sub> at 80 kHz - 320 kHz with a modulation frequency of 300 mHz

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