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Magnetically Enhanced Microflow Cytometer for Bead- and Cell-based Immunoaffinity Measurements in Whole Blood Samples

Scientific thesis for the attainment of the academic degree
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of the Department of Electrical and Computer Engineering
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conservation of mass, momentum reynolds number

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test,test Loss because of reduced velocity and magnetic drag

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Ground PCB Stacked PCBs with spacer

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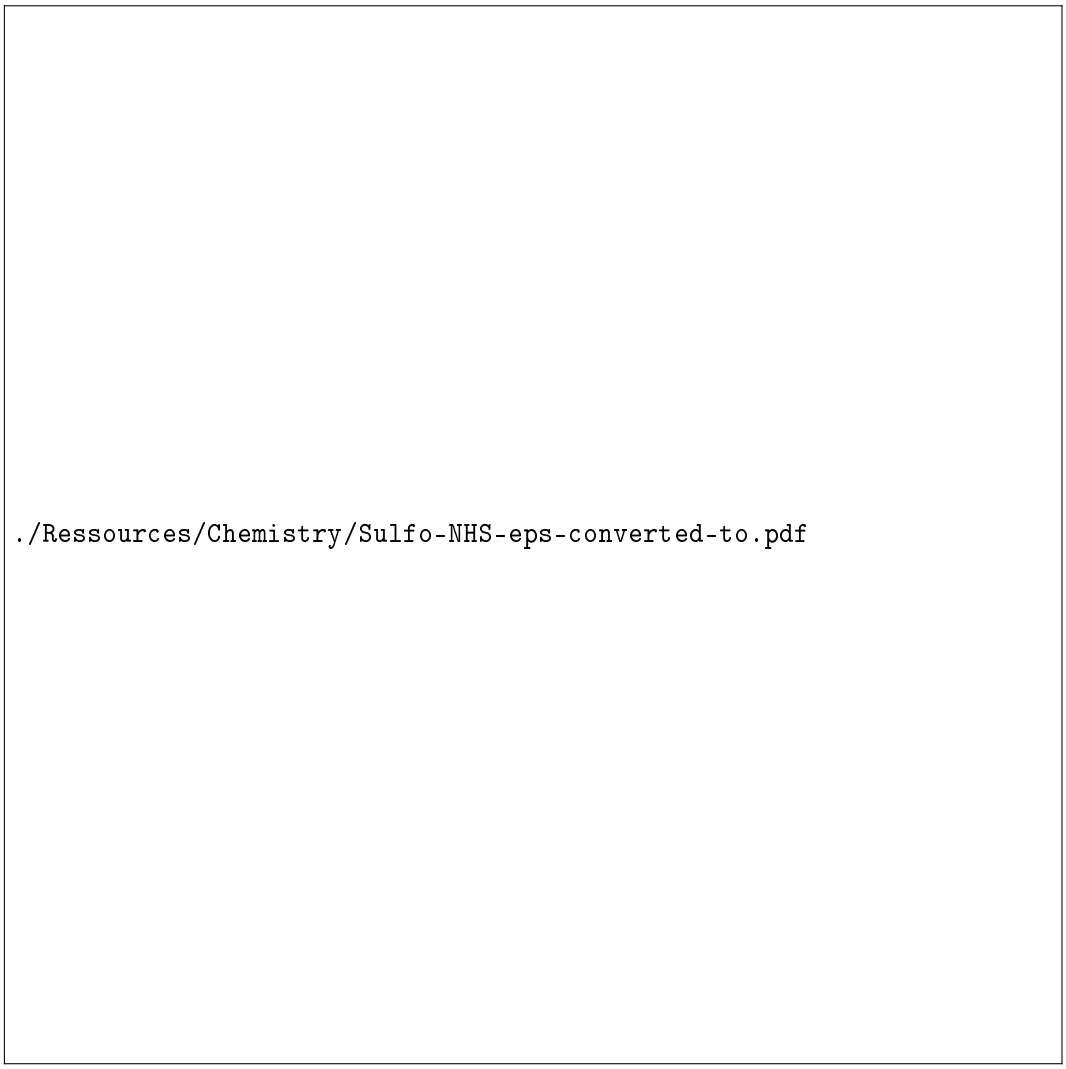

Figure 1 TestSvg

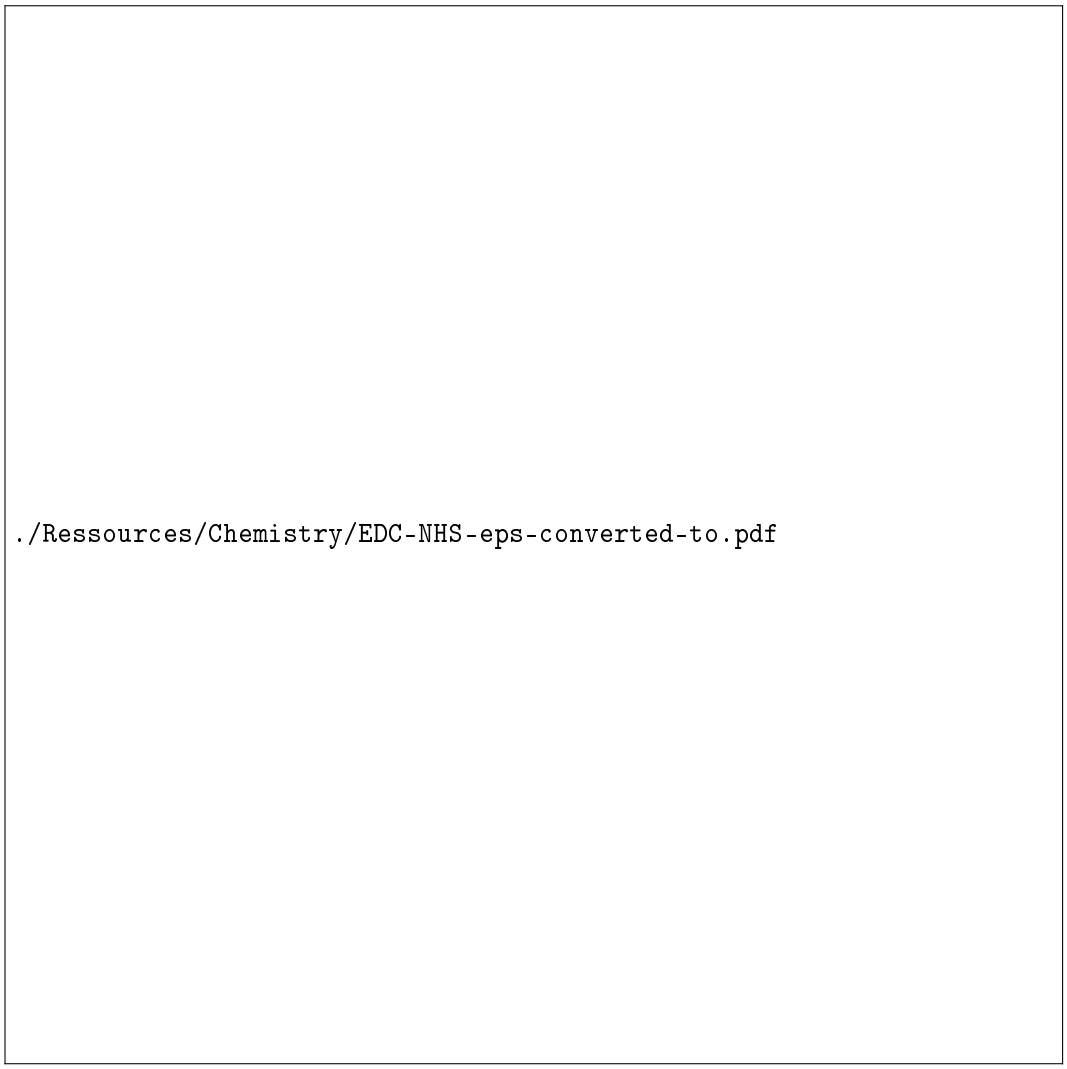

Figure 2 TestSvg

Hysteresis Alignment
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Single GMR
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Dual GMR
one MFLI supplies both at same freuqency. Aux Trigger tested, but no advantage.

3. Materials and Methods

3.1. Microfluidic Fabrication

3.1.1. Development of Layout
3.1.2. Patterning of Photoresist
3.1.3. Soft Lithography

The fabricated wafer was placed the center of a 90 cm petri dish. A PDMS (Poly(dimethyl siloxane)) mold was created by vigorous mixing of the pre-polymer base with its curing agent (Sygard 184, Dowsil) in a ratio of 10:1 (w/w). For 3" wafers, thin channels were casted from 15 g, normal channels from 20 g PDMS in the petri dish. Gas bubbles were removed from the mixture in a desiccator for 20 min at 2 hPa , and the clear PDMS was cured in an oven (Um, Memmert) for 1 h at 60 °C. After curing, the PDMS mold was released from the petri dish carefully, taken off the wafer and stored in a clean petri dish upon further processing.

3.1.4. Bonding of Microfluidic

Under laminar flow, crosslinked molds were cut into pieces with the respecting single µF (Microfluidic) with a razor blade. Holes for in- and outlet were punched through the containing channels with a biopsy puncher (ID 0.5 mm, WellTech). The substrates and µFs were sonicated in acetone and diH_2O (deionized Water) for 5 min and dried with filtered N_2 (Nitrogen Gas) completely. For the bonding of PDMS to various substrates different protocols have been established:

PDMS Glueing

Here, a micron-height layer of uncured PDMS was used as an adhesive layer between µF and substrate. Approx. 3 mL were poured onto a 3" wafer and spun down for 5 min at 6000 $\frac{1}{\text{min}}$. The microchannel was placed on the substrate by visual control of a stereo microscope (SMZ800, Nikon) with 8-fold magnification. Subsequently, the bonding process could be finished by a 1 h bake at 60 °C or over-night at room temperature.

Plasma Bonding

Due to the chemical nature of glass (or more generally oxides) and PDMS, the respective parts can be activated by the exposure to a controlled oxygen plasma which generates additional silanol (Si-OH) groups on their surfaces and removes impurities at the

same time. Bringing the activated surfaces in contact triggers the formation of covalent bonds almost immediately. First, the acetone-wiped substrates and the microchannels were centered inside the plasma cleaner (Zepto, Diener). Second, vacuum was applied to a final pressure <0.2 hPa. Third, the chamber was flushed with pure O_2 (Oxygen Gas) until a chamber pressure from 0.7 hPa to 0.8 hPa had been stabilized. Fourth, the plasma process was executed with 30 W (Power-Poti: 100) for 45 s to 60 s (Time-Poti: 15-20). Upon finish, the chamber was flushed for 5 s and ventilated. Immediately, the corresponding workpieces were brought into contact and pressed together gently. To ensure a durable bond, the assembled workpieces were baked for 1 h at 60 °C.

$$Heregoesthemassflowequation \tag{3.1}$$

Reversible Bonding
To bond the µF to a substrate reversibly and without residues, the channel can be brought into contact with the bottom part without any adhesinon agent. For low-pressure as well as vacuum driven flows, this method is preferrable due to its time and work efficiency.

3.2. Surface Bio-Functionalization

3.2.1. Surface Activation

To functionalize any surface with $-$OH

Hydrochloric-Sulfuric Acid Activation
Piranha Activation
Hydrofluoric Acid Activation

3.2.2. Chemical Surface Functionalization

3.2.3. Surface Bioconjugation

3.3. Magnetic Beadometry

3.3.1. Standard Parameters

3.3.2. Concentration Measurement

3.3.3. Whole Blood Bead Spiking

3.3.4. Bead Capture Assay

3.3.5. Optical Particle Tracking

3.4. Tensiometry

4. Results

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4.1. Signal Similarity For Cells With Varying Bead Coverages

Cross-Correlation between single dipole with sum magentic moment and surface covered with randomly distributed magnetic particles

4.1.1. Single Cell Signal

4.1.2. Cell Aggregates

4.2. Reference Bead Surface Functionalization

4.2.1. Amine-Surface Biotinylation

Streptavidin-Atto488 reference calibration Anti-Biotin-PE working? BNF-Dextran-Streptavidin unspecific binding?

Magnetic Polystyrene Bead
Non-Magnetic Polystyrene Bead

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Sensitivity Calibration
Concentration Measurements

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4.4.2. Covalent Attachment

Plasma-Based Approach

Water-Based Approach

Sonicate in Acetone and Water 5' 1:1 HCl (Hydro-Chloric Acid):Methanol H_2SO_4 (Sulfuric Acid) Treat for 30 min in light boiling water

5. Discussion

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6. Outlook

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Statement

I declare that I have authored this thesis independently, that I have not used other than the declared sources / resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Munich, December 4th, 2020, Signature