

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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Contents

1	Introduction and Motivation	4
2	Theoretical Prerequisites	5
2.1	Microfluidics	5
2.1.1	Flow Field inside Microchannels	5
2.1.2	Particles in Microfluidics	5
2.2	Surface Chemistry	5
2.2.1	Silane Chemistry	5
2.2.2	Carbodiimide Crosslinker Chemistry	5
2.2.3	Microscopic Particle Surface Physics	5
2.2.4	The Biotin-Avidin-System	5
2.3	MRCyte	5
2.3.1	Focusing Structures	6
2.3.2	GMR	6
2.3.3	Electrical Circuit	6
2.3.4	Electronic Readout	6
3	Materials and Methods	7
3.1	Microfluidic Fabrication	7
3.1.1	Development of Layout	7
3.1.2	Patterning of Photoresist	7
3.1.3	Soft Lithography	7
3.1.4	Bonding of Microfluidic	7
3.2	Surface Bio-Functionalization	8
3.2.1	Surface Activation	8
3.2.2	Chemical Surface Functionalization	8
3.2.3	Surface Bioconjugation	8
3.3	Magnetic Beadometry	8
3.3.1	Standard Parameters	8
3.3.2	Concentration Measurement	8
3.3.3	Whole Blood Bead Spiking	8
3.3.4	Bead Capture Assay	8
3.3.5	Optical Particle Tracking	8
3.4	Tensiometry	8

4 Results	9
4.1 Signal Similarity For Cells With Varying Bead Coverages	9
4.1.1 Single Cell Signal	9
4.1.2 Cell Aggregates	9
4.2 Reference Bead Surface Functionalization	9
4.2.1 Amine-Surface Biotinylation	9
4.2.2 Carboxy-Surface Biotinylation	9
4.3 Concentration Measurements in MRCyte	9
4.3.1 Count Stability	9
4.3.2 Velocity Measurement	9
4.3.3 2-Chip-Setup for Macro Measurements	9
4.4 Protein Immobilization On The Microfluidic Channel Bottom	9
4.4.1 Physisorption	9
4.4.2 Covalent Attachment	10
5 Discussion	11
6 Outlook	12
List of Abbreviations	14
List of Figures	16
List of Tables	17
Bibliography	19
Statement	19

1. Introduction and Motivation

2. Theoretical Prerequisites

2.1. Microfluidics

conservation of mass, momentum reynolds number

2.1.1. Flow Field inside Microchannels

Navier-Stokes-Approximation for Hagen-Poiseuille

2.1.2. Particles in Microfluidics

Stokes Drag Force Gravity Electro-static interaction Magnetic Force Friction Interface-Forces

2.1.3.

2.2. Surface Chemistry

2.2.1. Silane Chemistry

2.2.2. Carbodiimide Crosslinker Chemistry

EDC-NHS-Activation sulfo-NHS vs. NHS

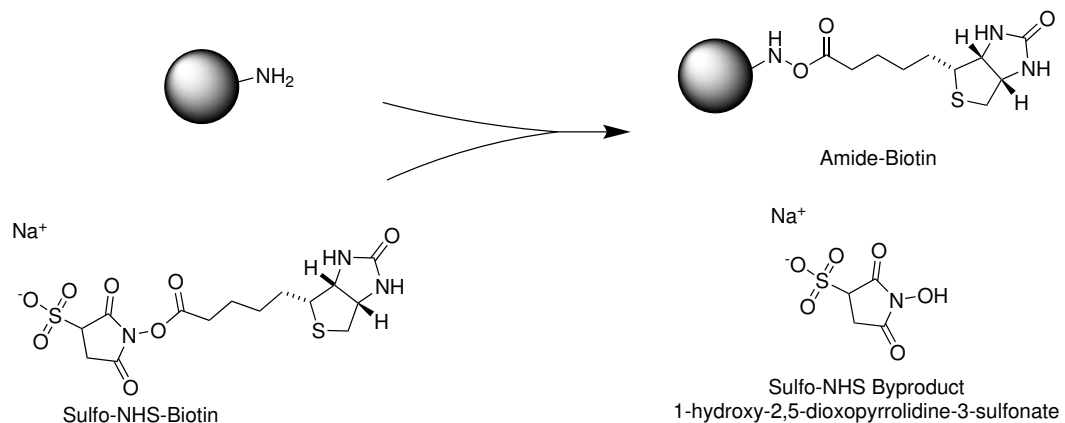


Figure 1 TestSvg

2.2.3. Microscopic Particle Surface Physics

2.2.4. The Biotin-Avidin-System

2.3. MRCyte

Short intro over MRCyte Foto of setup with arrows to necessary parts Microscope Stages PEEK holder Helmholtz coils Kepco MFLI DAQ

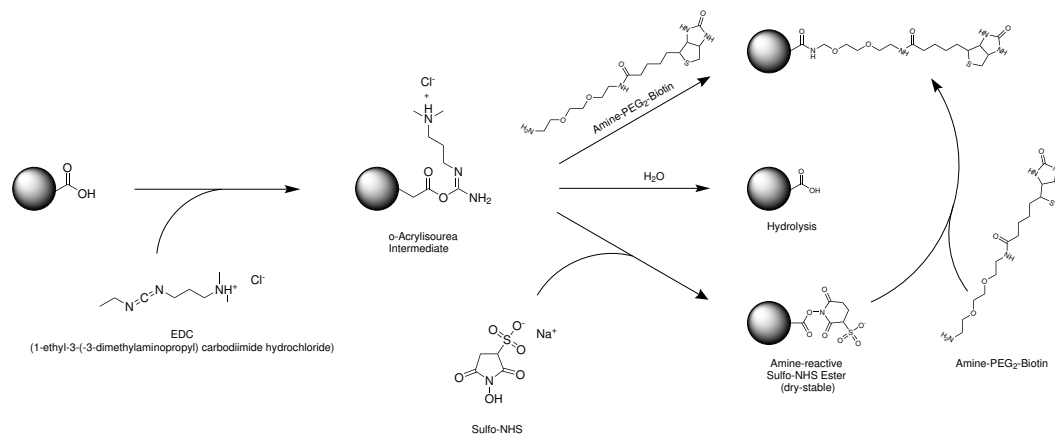


Figure 2 TestSvg

2.3.1. Focusing Structures

test,test Loss because of reduced velocity and magnetic drag

2.3.2. GMR

Different produced GMR stacks Wheatstone Bridge setup Magnet alignment

2.3.3. Electrical Circuit

Ground PCB Stacked PCBs with spacer

2.3.4. Electronic Readout

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Hysteresis Alignment

test,test

Single GMR

test,test

Dual GMR

one MFLI supplies both at same frequency. 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 μ F (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 μ Fs were sonicated in acetone and diH₂O (deionized Water) for 5 min and dried with filtered N₂ (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 μ F 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 O₂ (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.

$$\text{Here goes the mass flow equation} \quad (3.1)$$

Reversible Bonding

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

3.2. Surface Bio-Functionalization

3.2.1. Surface 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

test,test

4.1. Signal Similarity For Cells With Varying Bead Coverages

Cross-Correlation between single dipole with sum magnetic 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

4.2.2. Carboxy-Surface Biotinylation

4.3. Concentration Measurements in MRCyte

4.3.1. Count Stability

Measurement over 1h Measurement of Syringe Tubing Losses

4.3.2. Velocity Measurement

4.3.3. 2-Chip-Setup for Macro Measurements

Sensitivity Calibration

Concentration Measurements

4.4. Protein Immobilization On The Microfluidic Channel

Bottom

4.4.1. Physisorption

Quantification in Plate Reader Trial with Neutravidin + Sensor (Esthis Versuch)

4.4.2. Covalent Attachment

Plasma-Based Approach

Water-Based Approach

Sonicate in Acetone and Water 5' 1:1 HCl (Hydro-Chloric Acid):Methanol H₂SO₄ (Sulfuric Acid) Treat for 30 min in light boiling water

5. Discussion

test,test

6. Outlook

List of Abbreviations

default

μ F - Microfluidic..... 1, 7, 8, 12

A

AAF - Artificial Anti-Ferromagnet..... 1, 12

AFM - Anti-Ferromagnetism..... 1, 12

D

diH₂O - deionized Water..... 1, 7, 12

F

FM - Ferrimagnetism..... 1, 12

FWHM - Full Width at Half Maximum..... 1, 12

G

GMR - Giant Magneto Resistance..... 1, 12

GUI - Graphical User Interface..... 1, 12

H

H₂SO₄ - Sulfuric Acid..... 1, 10, 12

HCl - Hydro-Chloric Acid..... 1, 10, 12

I

IPA - Isopropanol..... 1, 12

M

MACS - MACS running buffer..... 1, 12

MNP - Magnetic Nanoparticle..... 1, 12

N

N₂ - Nitrogen Gas..... 1, 7, 12

NFM - non-ferro-magnetic..... 1, 12

O

O₂ - Oxygen Gas..... 1, 8, 12

P

PBS - Phosphate Buffered Saline	1, 12
PCB - Printed Circuit Board	1, 12
PDMS - Poly(dimethyl siloxane)	1, 7, 12
PM - Paramagnetism.....	1, 12

S

SiN - Silicon Nitride.....	1, 12
SMA - Styrene Maleic Anhydride	1, 12
SPM - Superparamagnetism.....	1, 12

List of Figures

1	TestSvg	5
2	TestSvg	6

List of Tables

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