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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
Master of Science (M.Sc.)
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Contents

1. Theoretical Prequisites

The main measurement principle by a GMR (Giant Magneto Resistance)-Sensor has been already described and characterized exhaustively by Helou [1], Reisbeck [2] and Brenner [3]. Therefore, this theoretical part will focus on (bio-)physical aspects of a cell rolling motion inside a microfluidic channel and surface modification chemistry.

1.1. Microfluidics

The main experiments of this work were carried out in microfluidic environments, which exhibit favorable properties compared to common turbulent systems. From a fluid-mechanical standpoint, shrinking the scales makes interfacial as well as electrokinetic phenomena much more significant, and reduces the importance of pressure and gravity.[4] However, electodynamics, chemistry and fluid dynamics are incetricably intertwined, so that fluid flow can create electric fields (and vice versa), with a degree of coupling driven by the surface chemistry. Many of the resulting phenomena arise or can explained by Cauchy-Momentum equation (eq. 1.3) and the resulting Navier-Stokes equation for incompressible fluids (eq. 1.4).

$$\frac{\partial}{\partial t}\iiint \rho \mathrm{dV} = -\iint \rho \mathbf{u} \cdot \vec{\mathbf{n}} \mathrm{dA} \tag{1.1}$$

$$\nabla \cdot \mathbf{u} = 0 \tag{1.2}$$

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = \nabla \cdot \boldsymbol{\tau} + \sum_i \mathbf{f}_i \tag{1.3}$$

$$\underbrace{\rho \frac{\partial \mathbf{u}}{\partial t}}_{\text{Transient}} + \underbrace{\rho \mathbf{u} \cdot \nabla \mathbf{u}}_{\text{Convection}} = \underbrace{-\nabla p}_{\text{Pressure}} + \underbrace{\eta \nabla^2 \mathbf{u}}_{\text{Viscous}} + \underbrace{\sum_i \mathbf{f}_i}_{\text{Body Forces}} \tag{1.4}$$

conservation of mass, momentum reynolds number

1.1.1. Flow Field inside Microchannels

The foremost characteristic of a microchannel is the laminar flow behavior, which causes deterministic pathlines. Mathematically this is described by the reynolds number, which compares the intertia to shear forces. If it results below a certain threshold of 2000, laminar flow can be assumed. This holds true for the utilized microfluidic with the dimensions 12 000 µm x 700 µm x 150 µm (l x w x h) and aequous buffer solutions, where the channel width was used as characteristic length l. Hence, the Navier-Stokes equation can be applied to our system.

$$Re \;=\; \frac{2\rho|\overline{u}|l}{\eta} \tag{1.5}$$

The step from the Cauchy momentum equation to the Navier-Stokes equation is complex and harbors several sources of error. First, an incompressible newtonian fluid as well as channel is assumed. The used water suspensions can be approximated with negligible compressibility, which is not true for the real case. Also, for blood or other shear-thinning fluids some deviations are prone for high errors. This happens due to the fact that the $\boldsymbol{\tau}$ (surface stress tensor) is decomposed into pressure and viscous contributions as shown in the equations 1.6. Then, the divergence relation of the respective viscous stress (eq. 1.7) does not hold for non-uniform viscosity η.

$$\boldsymbol{\tau} = \boldsymbol{\tau}_{viscous} + \boldsymbol{\tau}_{pressure} = 2\eta\epsilon - p\mathbf{I}_{3\times3} \tag{1.6}$$

$$\nabla \cdot \boldsymbol{\tau}_{viscous} = \nabla \cdot 2\eta\epsilon = \nabla \cdot \eta\nabla\mathbf{u} \overset{only\ if\ \eta}{\underset{uniform}{=}} \eta\nabla^2\mathbf{u} \tag{1.7}$$

Second, the channel height varies in reality as a result of fabrication inaccuracies. In the model case of a flow through a rectangular channel, no analytical solution of the Navier-Stokes equation exists, but a Fourier Series expansion if channel width is larger than channel height. [5] The equation 1.8 shows that height deviations can have prominent influence on a channel velocity simulation as it is proportional to h^2. Further, the flow rate (which is the velocity integral over the channel cross section) depends even on h^3.

$$u_x(y,z) = \frac{4h^2\Delta p}{\pi^3\eta l}\sum_{n,odd}^{\infty}\frac{1}{n^3}\left(1 - \frac{\cosh(n\pi\frac{y}{h})}{\cosh(n\pi\frac{w}{2h})}\right)\sin(n\pi\frac{z}{h}) \tag{1.8}$$

Third, the transient term (eq. 1.4) was neglected in all simulations, but a connected syringe pump possesses a slow rise time (Fig. 1a) and a remaining “pulsation error” in steady state (Fig. 1b). In effect, another error adds to the simulation, which is only valid after several ten seconds of the last flow rate change.

For later studies in a matlab model, the flow velocity and shear stress computations

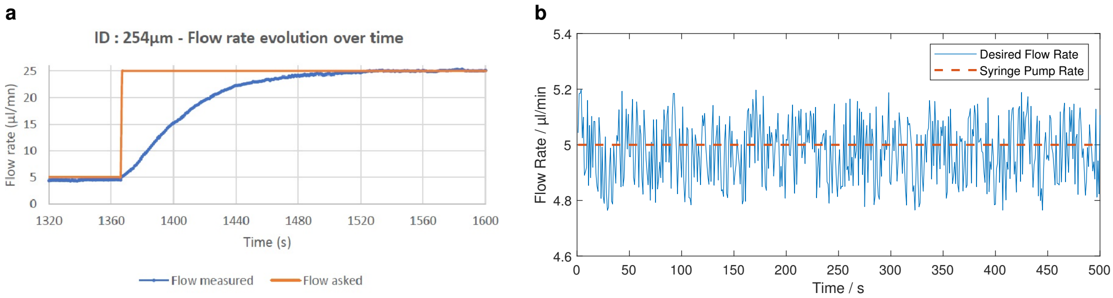

Figure 1: Syringe Pump error sources
Set flow rate: ▬▬ , Real Flow Rate: ▬▬ **a,** Transient step answer of a syringe pump through a microtube with 254 µm inner diameter. **b,** Steady state flow rate error around the desired 5 µL min^{-1} dispensing rate. A sinusoidal behaviour caused by the microstepping can be observed. [6]

were carried out with the error sources considered.

1.1.2. Particles in Microfluidics

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

1.1.3.

1.2. Surface Chemistry

1.2.1. Silane Chemistry

1.2.2. Carbodiimide Crosslinker Chemistry

EDC-NHS-Activation sulfo-NHS vs. NHS

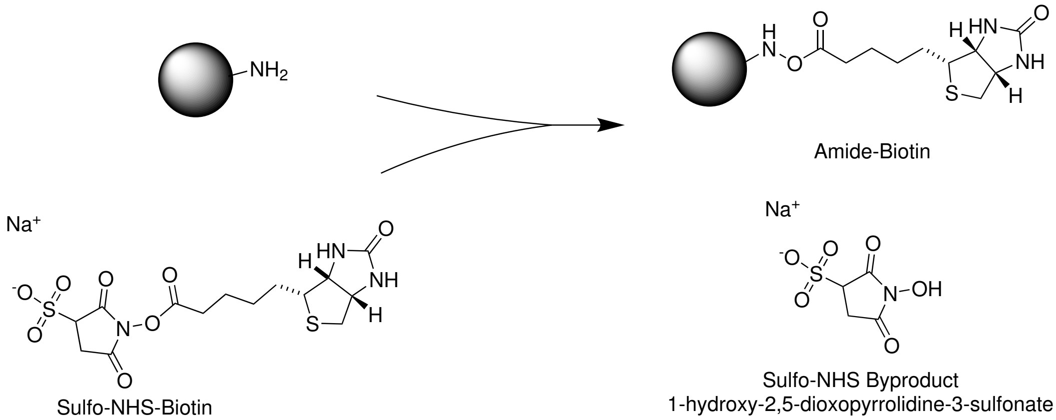

Figure 2: TestSvg

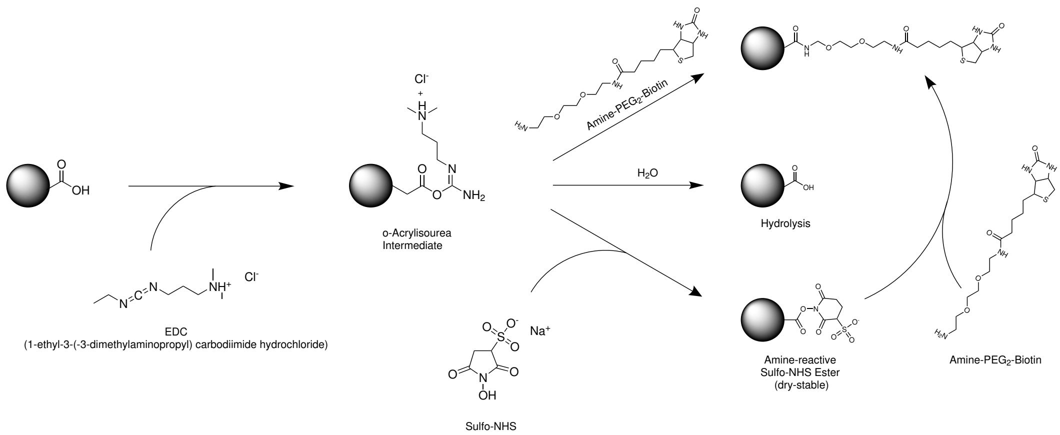

Figure 3: TestSvg

1.2.3. Microscopic Particle Surface Physics

1.2.4. The Biotin-Avidin-System

1.3. MRCyte

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

1.3.1. Focusing Structures

test,test Loss because of reduced velocity and magnetic drag

1.3.2. GMR

Different produced GMR stacks Wheatstone Bridge setup Magnet alignment

1.3.3. Electrical Circuit

Ground PCB Stacked PCBs with spacer

1.3.4. Electronic Readout

test,test

Hysteresis Alignment

test,test

Single GMR

test,test

Dual GMR

one MFLI supplies both at same freuqency. Aux Trigger tested, but no advantage.

List of Abbreviations

Symbols

τ - surface stress tensor

η - dynamic viscosity

μF - Microfluidic

ρ - density

$\sum_i \mathbf{f}_i$ - body forces

A

AAF - Artificial Anti-Ferromagnet

AcOH - Acetic Acid

AFM - Anti-Ferromagnetism

APTES - (3-aminopropyl)triethoxysilane

D

diH_2O - deionized water

E

EDC - 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EtOH - Ethanol

F

FM - Ferrimagnetism

FWHM - Full Width at Half Maximum

G

GMR - Giant Magneto Resistance

GUI - Graphical User Interface

H

H_2O_2 - Hydrogen Peroxide

H_2SO_4 - Sulfuric Acid

HCl - Hydrochloric Acid

HF - Hydrofluoric Acid

I

IPA - Isopropanol

M

MACS - MACS running buffer

MeOH - Methanol

MES - 2-(N-morpholino)ethanesulfonic Acid

MNP - Magnetic Nanoparticle

N

N_2 - Nitrogen Gas

NFM - non-ferro-magnetic

NHS - N-hydroxysuccinimide

O

O_2 - Oxygen Gas

P

PAA - Poly(acrylic) Acid

PBS - Phosphate Buffered Saline

PCB - Printed Circuit Board

PDMS - Poly(dimethyl siloxane)

Piranha - H_2O_2:H_2SO_4

PM - Paramagnetism

S

SiN - Silicon Nitride

SMA - Styrene Maleic Anhydride

SPM - Superparamagnetism

U

u - flow field

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Statement

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Munich, December 4th, 2020, Signature