Heinz Nixdorf-Lehrstuhl für Biomedizinische Elektronik
Fakultät für Elektrotechnik und Informationstechnik
Technische Universität München

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.)
of the Department of Electrical and Computer Engineering
at the Technical University of Munich.

Supervised by Dr.-Ing. Mathias Reisbeck
Prof. Dr. rer. nat. Oliver Hayden

Submitted by Johann Alexander Brenner
Weisbergerstraße 5a
85053 Ingolstadt
03662733

Submitted on December 4th, 2020 at Munich

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.

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

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

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

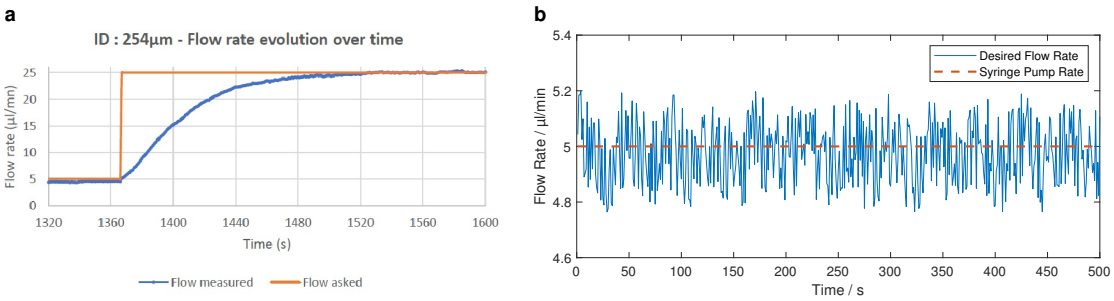


Figure 1: Syringe Pump error sources
Set flow rate: , Real Flow Rate: **a,** Transient step answer of a syringe pump through a microtube with 254 µm inner diameter. **b,** Steady state flow rate error around the desired 5 µL min^{-1} dispensing rate. A sinusoidal behaviour caused by the microstepping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

Molecules can be immobilized through various mechanisms on surfaces to achieve a desired functionality. The most simple is physisorption. Here, a biomolecule is bonded only by weak elektrostatic, van-der-Waals or dipole-dipole interaction with a adsorption enthalpy below 50 kJ mol^{-1}. In contrast, this yields fast reaction rates, because no activation energy has to be overcome. Although a large number of molecules can be captured with this method, several drawbacks have been identified. [7], [8] For example, immobilized receptors can start to desorb or change their position, which in turn reduces sensitivity or causes false-positive results. [9], [10]

Therfore, most functionalization approaches rely on chemisorption where molecules are covalent bound to a surface. Due to the higher activation energy barrier this bonding mechanism works slower in comparison to physisorption though higher temperatures or catalysators can promote an equilibrium. One of the most well-known strategies to achieve reproducible thin films on surfaces is the formation of SAMs (self-assembled monolayers)

[11]

Weiterhin kann es zu Verschiebungen der adsorbierten Rezeptoren kommen, wodurch es zu falsch-positiven Ergebnissen kommen kann.[9] Daher basieren die meisten Funktionalisierungsmethoden nicht auf der Physisorption, sondern auf der Chemisorption. Hierbei werden Moleküle kovalent an die Oberfläche gebunden. Aufgrund der dabei zu überwindenden Aktivierungsbarriere läuft diese Reaktion langsamer im Vergleich zur Physisorption ab. Bei erhöhten Temperaturen kann das Einstellen des Gleichgewichtes jedoch begünstigt werden. Eine der bekanntesten Strategien dünne Filme auf Oberflächen zu erzeugen, ist die Bildung von selbstorganisierenden Monoschichten (SAM),[46-48] wobei eine kovalente, sehr stabile Bindung zwischen den organischen Komponenten und dem Substrat ausgebildet wird.[49] SAM bilden sich spontan beim Eintauchen in oberflächenaktive Lösungen. Hierzu gehören die Bindung von Alkanthiolen auf Goldoberflächen und die Bindung von Silanen auf Siliziumoberflächen (Abbildung 6).

1.2.1. Silane Chemistry

By the use of silane chemistry a surface is rendered organofunctional with alkoxysilane molecules. Since glass, silicon, alumina, titania, and quartz surfaces, as well as other metal oxide interfaces, are rich in hydroxyl groups, silanes are particularly useful for modifying these materials. These groups attack and displace the alkoxy groups on the silane thus forming a covalent $-\mathrm{Si}-\mathrm{O}-\mathrm{Si}-$ bond. [12]

A silane molecule contains a central silicon atom bonded to two types of groups - alkoxy groups and organo-functional groups. These two types exhibit different reactivity allowing sequential reactions and have the ability to form a durable bond between organic and inorganic materials. The final result of reacting an organosilane with a substrate ranges from altering the wetting or adhesion characteristics of the substrate, utilizing the substrate to catalyze chemical transformation at the heterogeneous interface, ordering the interfacial region, and modifying its partition characteristics. Significantly, it includes the ability to effect a covalent bond between organic and inorganic materials. Especially in optical or biological sensors, silane modifications open a broad range of applications. [13]

Die Einführung von neuen Funktionalitäten durch die Silanisierung der Oberfläche hat eine große Bedeutung in der Biosensorik. Die Hydroxylgruppen können hierbei durch

die Reinigung der Oberfläche mit Piranha-Lösung oder UV/Ozon aufgebaut werden kann. Die so erzeugten reaktiven Hydroxylgruppen reagieren anschließend in einer Kondensationsreaktion mit dem Silan (Abbildung 7). Häufig beschrieben wird die Umsetzung von Glasoberflächen oder Siliziumoberflächen mit 3-Aminopropyltrimethoxysilan (APTMS) oder Aminopropyltriethoxysilan (APTES).

Unterschiede bei der Verwendung von APTES und APTMS liegen in der Reaktivität der Verbindungen. Aufgrund der großen Reaktivität von APTMS werden die Reaktionen in reinen organischen Lösungsmitteln durchgeführt. Dadurch lassen sich dünnere und kontrollierte Schichten mit Aminopropylgruppen erzeugen. Bei der Bildung von Monoschichten mit APTES muss die Reaktion mit Wasser katalysiert werden.[51] Weiterhin wird in der Literatur beschrieben, dass es zu Reaktionen zwischen den Aminogruppen des Silans und den Hydroxylgruppen der Oberfläche kommen kann, wobei keine geordnete Schicht erhalten muss die Reaktion mit Wasser katalysiert werden.[51] Weiterhin wird in der Literatur beschrieben, dass es zu Reaktionen zwischen den Aminogruppen des Silans und den Hydroxylgruppen der Oberfläche kommen kann, wobei keine geordnete Schicht erhalten

Oxidation

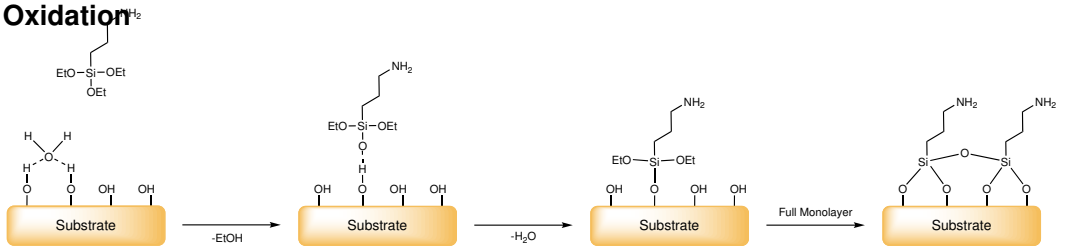


Figure 2: APTES

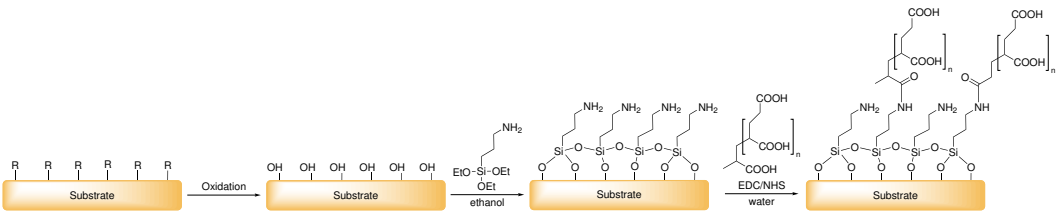
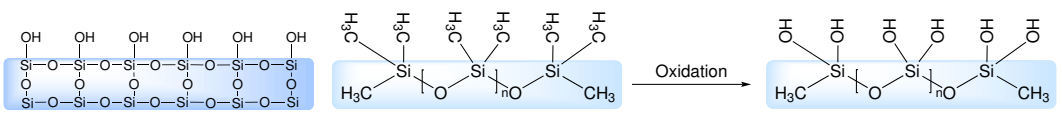


Figure 3:

a **b**



Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

Nam dui ligula, fringilla a, euismod sodales, sollicitudin vel, wisi. Morbi auctor lorem non justo. Nam lacus libero, pretium at, lobortis vitae, ultricies et, tellus. Donec aliquet, tortor sed accumsan bibendum, erat ligula aliquet magna, vitae ornare odio metus a mi. Morbi ac orci et nisl hendrerit mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper vestibulum turpis. Pellentesque cursus luctus mauris.

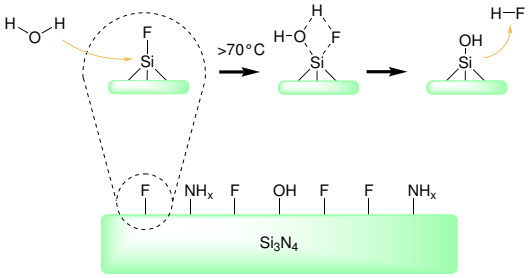


Figure 5: Silicon nitride etching with hydrofluoric acid

1.2.2. Carbodiimide Crosslinker Chemistry

EDC-NHS-Activation sulfo-NHS vs. NHS

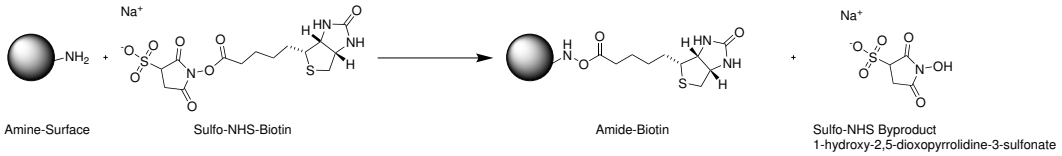


Figure 6: TestSvg

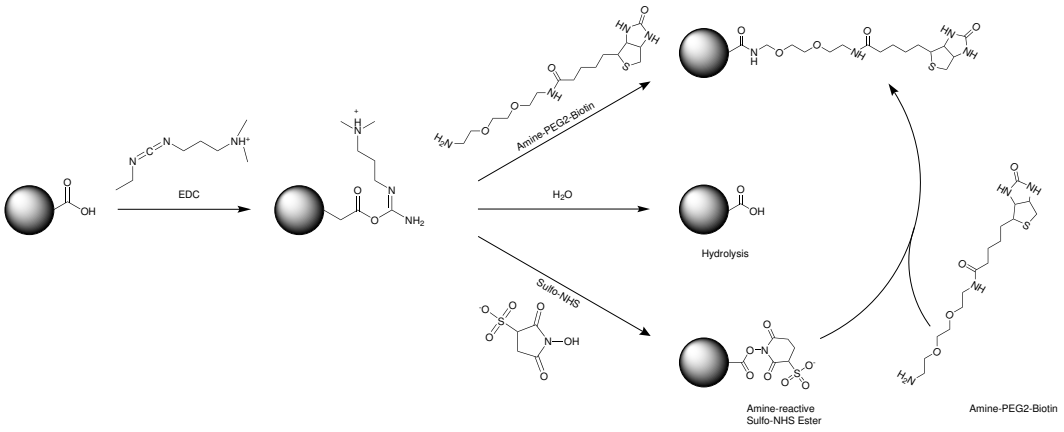


Figure 7: TestSvg

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.

2. Results

test,test

2.1. 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

2.1.1. Single Cell Signal

2.1.2. Cell Aggregates

2.2. Reference Bead Surface Functionalization

2.2.1. Amine-Surface Biotinylation

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

Magnetic Polystyrene Bead
Non-Magnetic Polystyrene Bead

2.2.2. Carboxy-Surface Biotinylation

2.3. Concentration Measurements in MRCyte

2.3.1. Count Stability

Measurement over 1h Measurement of Syringe Tubing Losses

2.3.2. Velocity Measurement

2.3.3. 2-Chip-Setup for Macro Measurements

Sensitivity Calibration
Concentration Measurements

2.4. Protein Immobilization On The Microfluidic Channel Bottom

2.4.1. Physisorption

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

2.4.2. Covalent Attachment

Plasma-Based Approach

Water-Based Approach

Sonicate in Acetone and Water 5’ 1:1 HCl (hydrochloric acid):Methanol H_2SO_4 (sulfuric acid) Treat for 30 min in light boiling water

List of Abbreviations

Symbols

$\boldsymbol{\tau}$ - 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

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

SAM - self-assembled monolayer

Si_3N_4 - silicon nitride

SMA - styrene maleic anhydride

SPM - superparamagnetism

U

u - flow field

List of Figures

List of Tables

Bibliography

[1] M. Helou, “Magnetic flow cytometry,” PhD Thesis, 2014.

[2] M. Reisbeck, “Integration und quantitative analyse in der magnetischen durchflusszytometrie,” Thesis, 2019.

[3] J. Brenner, “Superparamagnetic nanoparticles in picoliter droplets for measurements with spin valves,” Bachelor Thesis, 2018.

[4] B. Kirby, *Micro- and Nanoscale Fluid Mechanics*. 2010, ISBN: 9780511760723. DOI: 10.1017/cbo9780511760723.

[5] H. Bruus, *Theoretical Microfluidics*. Technical University of Denmark: Oxford University Press, 2008, ISBN: 978–0–19–923508–7.

[6] “Syringe pumps.” (2021), [Online]. Available: https://www.fluigent.com/resources/microfluidic-expertise/what-is-microfluidic/system-comparison-for-microfluidic-applications/.

[7] W. Putzbach and N. Ronkainen, “Immobilization techniques in the fabrication of nanomaterial-based electrochemical biosensors: A review,” *Sensors*, vol. 13, no. 4, pp. 4811–4840, 2013, ISSN: 1424-8220. DOI: 10.3390/s130404811.

[8] R. Funari, B. Della Ventura, C. Altucci, A. Offenhäusser, D. Mayer, and R. Velotta, “Single molecule characterization of uv-activated antibodies on gold by atomic force microscopy,” *Langmuir*, vol. 32, no. 32, pp. 8084–8091, 2016, ISSN: 0743-7463. DOI: 10.1021/acs.langmuir.6b02218.

[9] A. Ymeti, J. Kanger, J. Greve, G. Besselink, P. Lambeck, R. Wijn, and R. Heideman, “Integration of microfluidics with a four-channel integrated optical young interferometer immunosensor,” *Biosensors and Bioelectronics*, vol. 20, no. 7, pp. 1417–1421, 2005, ISSN: 0956-5663. DOI: https://doi.org/10.1016/j.bios.2004.04.015.

[10] M.-J. Bañuls, R. Puchades, and Á. Maquieira, “Chemical surface modifications for the development of silicon-based label-free integrated optical (io) biosensors: A review,” *Analytica Chimica Acta*, vol. 777, pp. 1–16, 2013, ISSN: 0003-2670. DOI: 10.1016/j.aca.2013.01.025.

[11] N. Lange, “Selective chemical modification of silicon nitride surfaces for novel biosensor application,” Thesis, 2017. DOI: http://dx.doi.org/10.17169/refubium-11275.

[12] B. Seed, “Silanizing glassware,” *Current Protocols in Cell Biology*, vol. 8, no. 1, A.3E.1–A.3E.2, 2000, ISSN: 1934-2500. DOI: 10.1002/0471143030.cba03es08.

[13] GELEST, *Silane coupling agents*, Catalog, 2014.

[14] A. P. Guimarães, *Principles of Nanomagnetism*. Springer International Publishing, 2017. DOI: 10.1007/978-3-319-59409-5.

[15] micromod Partikeltechnologie GmbH, *Technical data sheet - nanomag®-d-spio 50nm*, 2018.

[16] M. J. Owen and P. J. Smith, “Plasma treatment of polydimethylsiloxane,” *Journal of Adhesion Science and Technology*, vol. 8, no. 10, pp. 1063–1075, 1994. DOI: 10.1163/156856194X00942.

[17] C.-G. Stefanita, *Magnetism*. Springer Berlin Heidelberg, 2012. DOI: 10.1007/978-3-642-22977-0.

[18] K. H. J. Buschow and F. R. de Boer, *Physics of Magnetism and Magnetic Materials*. Springer US, 2003. DOI: 10.1007/b100503.

[19] G. C. Papaefthymiou, “Nanoparticle magnetism,” *Nano Today*, vol. 4, no. 5, pp. 438–447, Oct. 2009. DOI: 10.1016/j.nantod.2009.08.006.

[20] P. Gravesen, J. Branebjerg, and O. S. Jensen, “Microfluidics-a review,” *Journal of Micromechanics and Microengineering*, vol. 3, no. 4, pp. 168–182, Dec. 1993. DOI: 10.1088/0960-1317/3/4/002.

[21] W. J. Dressick, C. S. Dulcey, J. H. Georger, G. S. Calabrese, and J. M. Calvert, “Covalent binding of pd catalysts to ligating self-assembled monolayer films for selective electroless metal deposition,” *Journal of the Electrochemical Society*, vol. 141, no. 1, pp. 210–220, 1994, ISSN: 0013-4651. DOI: 10.1149/1.2054686.

[22] K. C. Andree, A. M. Barradas, A. T. Nguyen, A. Mentink, I. Stojanovic, J. Baggerman, J. van Dalum, C. J. van Rijn, and L. W. Terstappen, “Capture of tumor cells on anti-epcam-functionalized poly(acrylic acid)-coated surfaces,” *ACS Appl Mater Interfaces*, vol. 8, no. 23, pp. 14 349–56, 2016, ISSN: 1944-8252 (Electronic) 1944-8244 (Linking). DOI: 10.1021/acsami.6b01241.

[23] R. Hicsanmaz, “Setup and assessment of laser lithography for the fabrication and integration of biosensor and microfluidic devices,” Technical University Munich, 2020.

[24] K. C. Andree, A. M. Barradas, A. T. Nguyen, A. Mentink, I. Stojanovic, J. Baggerman, J. van Dalum, C. J. van Rijn, and L. W. Terstappen, “Capture of tumor cells on anti-epcam-functionalized poly(acrylic acid)-coated surfaces,” *ACS Appl*

Mater Interfaces, vol. 8, no. 23, pp. 14 349–56, 2016, ISSN: 1944-8252 (Electronic) 1944-8244 (Linking). DOI: 10.1021/acsami.6b01241.

[25] G. Antonacci, J. Goyvaerts, H. Zhao, B. Baumgartner, B. Lendl, and R. Baets, "Ultra-sensitive refractive index gas sensor with functionalized silicon nitride photonic circuits," *APL Photonics*, vol. 5, no. 8, 2020, ISSN: 2378-0967. DOI: 10.1063/5.0013577.

[26] A. Arafat, M. Giesbers, M. Rosso, E. J. R. Sudhölter, K. Schroën, R. G. White, L. Yang, M. R. Linford, and H. Zuilhof, "Covalent biofunctionalization of silicon nitride surfaces," *Langmuir*, vol. 23, no. 11, pp. 6233–6244, 2007, ISSN: 0743-7463 1520-5827. DOI: 10.1021/la7007045.

[27] A. Arafat, K. Schroen, L. C. de Smet, E. J. Sudholter, and H. Zuilhof, "Tailor-made functionalization of silicon nitride surfaces," *J Am Chem Soc*, vol. 126, no. 28, pp. 8600–1, 2004, ISSN: 0002-7863 (Print) 0002-7863 (Linking). DOI: 10.1021/ja0483746.

[28] M. J. Banuls, V. Gonzalez-Pedro, C. A. Barrios, R. Puchades, and A. Maquieira, "Selective chemical modification of silicon nitride/silicon oxide nanostructures to develop label-free biosensors," *Biosens Bioelectron*, vol. 25, no. 6, pp. 1460–6, 2010, ISSN: 1873-4235 (Electronic) 0956-5663 (Linking). DOI: 10.1016/j.bios.2009.10.048.

[29] B. Baur, G. Steinhoff, J. Hernando, O. Purrucker, M. Tanaka, B. Nickel, M. Stutzmann, and M. Eickhoff, "Chemical functionalization of gan and aln surfaces," *Applied Physics Letters*, vol. 87, no. 26, p. 263 901, 2005, ISSN: 0003-6951. DOI: 10.1063/1.2150280.

[30] J. Diao, D. Ren, J. R. Engstrom, and K. H. Lee, "A surface modification strategy on silicon nitride for developing biosensors," *Anal Biochem*, vol. 343, no. 2, pp. 322–8, 2005, ISSN: 0003-2697 (Print) 0003-2697 (Linking). DOI: 10.1016/j.ab.2005.05.010.

[31] T. Ghonge, H. Ceylan Koydemir, E. Valera, J. Berger, C. Garcia, N. Nawar, J. Tiao, G. L. Damhorst, A. Ganguli, U. Hassan, A. Ozcan, and R. Bashir, "Smartphone-imaged microfluidic biochip for measuring cd64 expression from whole blood," *Analyst*, vol. 144, no. 13, pp. 3925–3935, 2019, ISSN: 1364-5528 (Electronic) 0003-2654 (Linking). DOI: 10.1039/c9an00532c.

[32] M. Hofstetter, J. Howgate, M. Schmid, S. Schoell, M. Sachsenhauser, D. Adigüzel, M. Stutzmann, I. D. Sharp, and S. Thalhammer, "In vitro bio-functionality of gal-

lium nitride sensors for radiation biophysics," *Biochemical and Biophysical Research Communications*, vol. 424, no. 2, pp. 348–353, 2012, ISSN: 0006-291X. DOI: 10.1016/j.bbrc.2012.06.142.

[33] D. Kim and A. E. Herr, "Protein immobilization techniques for microfluidic assays," *Biomicrofluidics*, vol. 7, no. 4, p. 41 501, 2013, ISSN: 1932-1058 (Print) 1932-1058 (Linking). DOI: 10.1063/1.4816934.

[34] J. Klug, L. A. Pérez, E. A. Coronado, and G. I. Lacconi, "Chemical and electrochemical oxidation of silicon surfaces functionalized with aptes: The role of surface roughness in the aunps anchoring kinetics," *The Journal of Physical Chemistry C*, vol. 117, no. 21, pp. 11 317–11 327, 2013, ISSN: 1932-7447 1932-7455. DOI: 10.1021/jp212613f.

[35] N. Lange, P. M. Dietrich, A. Lippitz, N. Kulak, and W. E. S. Unger, "New azidation methods for the functionalization of silicon nitride and application in copper-catalyzed azide-alkyne cycloaddition (cuaac)," *Surface and Interface Analysis*, vol. 48, no. 7, pp. 621–625, 2016, ISSN: 01422421. DOI: 10.1002/sia.5950.

[36] A. P. Le Brun, S. A. Holt, D. S. Shah, C. F. Majkrzak, and J. H. Lakey, "The structural orientation of antibody layers bound to engineered biosensor surfaces," *Biomaterials*, vol. 32, no. 12, pp. 3303–11, 2011, ISSN: 1878-5905 (Electronic) 0142-9612 (Linking). DOI: 10.1016/j.biomaterials.2011.01.026.

[37] L. H. Liu, D. J. Michalak, T. P. Chopra, S. P. Pujari, W. Cabrera, D. Dick, J. F. Veyan, R. Hourani, M. D. Halls, H. Zuilhof, and Y. J. Chabal, "Surface etching, chemical modification and characterization of silicon nitride and silicon oxide–selective functionalization of si3n4 and sio2," *J Phys Condens Matter*, vol. 28, no. 9, p. 094 014, 2016, ISSN: 1361-648X (Electronic) 0953-8984 (Linking). DOI: 10.1088/0953-8984/28/9/094014.

[38] M. E. Marques, A. A. P. Mansur, and H. S. Mansur, "Chemical functionalization of surfaces for building three-dimensional engineered biosensors," *Applied Surface Science*, vol. 275, pp. 347–360, 2013, ISSN: 01694332. DOI: 10.1016/j.apsusc.2012.12.099.

[39] A. Psarouli, A. Bourkoula, P. Petrou, K. Misiakos, N. Chaniotakis, and S. Kakabakos, "Covalent binding vs. adsorption of biomolecules on silicon nitride planar waveguides," *Procedia Engineering*, vol. 25, pp. 350–353, 2011, ISSN: 18777058. DOI: 10.1016/j.proeng.2011.12.086.

[40] M. Rosso, *Modification of Silicon Nitride and Silicon Carbide Surfaces for Food and Biosensor Applications*. 2009, ISBN: 978-90-8585-379-4.

[41] P. Saengdee, C. Promptmas, S. Thanapitak, A. Srisuwan, A. Pankiew, N. Thornyanadacha, W. Chaisriratanakul, E. Chaowicharat, and W. Jeamsaksiri, "Optimization of 3-aminopropyltriethoxysilane functionalization on silicon nitride surface for biomolecule immobilization," *Talanta*, vol. 207, p. 120 305, 2020, ISSN: 1873-3573 (Electronic) 0039-9140 (Linking). DOI: 10.1016/j.talanta.2019.120305.

[42] S. Tan, L. Wang, J. Yu, C. Hou, R. Jiang, Y. Li, and Q. Liu, "Dna-functionalized silicon nitride nanopores for sequence-specific recognition of dna biosensor," *Nanoscale Research Letters*, vol. 10, no. 1, 2015, ISSN: 1556-276X. DOI: 10.1186/s11671-015-0909-0.

[43] T. D. To, A. T. Nguyen, K. N. T. Phan, A. T. T. Truong, T. C. D. Doan, and C. M. Dang, "Modification of silicon nitride surfaces with gopes and aptes for antibody immobilization: Computational and experimental studies," *Advances in Natural Sciences: Nanoscience and Nanotechnology*, vol. 6, no. 4, 2015, ISSN: 2043-6262. DOI: 10.1088/2043-6262/6/4/045006.

[44] P. Vermette, T. Gengenbach, U. Divisekera, P. A. Kambouris, H. J. Griesser, and L. Meagher, "Immobilization and surface characterization of neutravidin biotin-binding protein on different hydrogel interlayers," *Journal of Colloid and Interface Science*, vol. 259, no. 1, pp. 13–26, 2003, ISSN: 00219797. DOI: 10.1016/s0021-9797(02)00185-6.

[45] C. R. Vistas, A. C. P. Águas, and G. N. M. Ferreira, "Silanization of glass chips—a factorial approach for optimization," *Applied Surface Science*, vol. 286, pp. 314–318, 2013, ISSN: 01694332. DOI: 10.1016/j.apsusc.2013.09.077.

[46] C. Wang, Q. Yan, H.-B. Liu, X.-H. Zhou, and S.-J. Xiao, "Different edc/nhs activation mechanisms between paa and pmaa brushes and the following amidation reactions," *Langmuir*, vol. 27, no. 19, pp. 12 058–12 068, 2011, ISSN: 0743-7463 1520-5827. DOI: 10.1021/la202267p.

[47] M. Yüce and H. Kurt, "How to make nanobiosensors: Surface modification and characterisation of nanomaterials for biosensing applications," *RSC Adv.*, vol. 7, no. 78, pp. 49 386–49 403, 2017, ISSN: 2046-2069. DOI: 10.1039/c7ra10479k.

[48] K. AbuZineh, L. I. Joudeh, B. Al Alwan, S. M. Hamdan, J. S. Merzaban, and S. Habuchi, "Microfluidics-based super-resolution microscopy enables nanoscopic characterization of blood stem cell rolling," *Sci Adv*, vol. 4, no. 7, eaat5304, 2018,

ISSN: 2375-2548 (Electronic) 2375-2548 (Linking). DOI: 10.1126/sciadv.aat5304.

[49] B. Alberts, A. Johnson, J. Lewis, D. Morgan, M. Raff, K. Roberts, and P. Walter, *Molecular Biology of the Cell*, ISBN: 0815345240.

[50] K.-C. Chang and D. A. Hammer, "The forward rate of binding of surface-tethered reactants: Effect of relative motion between two surfaces," *Biophysical Journal*, vol. 76, no. 3, pp. 1280–1292, 1999, ISSN: 0006-3495. DOI: 10.1016/s0006-3495(99)77291-7.

[51] R. Cheng, T. Zhu, and L. Mao, "Three-dimensional and analytical modeling of microfluidic particle transport in magnetic fluids," *Microfluidics and Nanofluidics*, vol. 16, no. 6, pp. 1143–1154, 2013, ISSN: 1613-4982 1613-4990. DOI: 10.1007/s10404-013-1280-z.

[52] S. Choi, O. Levy, M. B. Coelho, J. M. Cabral, J. M. Karp, and R. Karnik, "A cell rolling cytometer reveals the correlation between mesenchymal stem cell dynamic adhesion and differentiation state," *Lab Chip*, vol. 14, no. 1, pp. 161–6, 2014, ISSN: 1473-0189 (Electronic) 1473-0189 (Linking). DOI: 10.1039/c3lc50923k.

[53] M. Dembo, D. C. Torney, K. Saxman, and D. Hammer, "The reaction-limited kinetics of membrane-to-surface adhesion and detachment," *Proceedings of the Royal Society of London. Series B. Biological Sciences*, vol. 234, no. 1274, pp. 55–83, 1997, ISSN: 0080-4649 2053-9193. DOI: 10.1098/rspb.1988.0038.

[54] S. J. DeNardo, G. L. DeNardo, A. Natarajan, L. A. Miers, A. R. Foreman, C. Gruettner, G. N. Adamson, and R. Ivkov, "Thermal dosimetry predictive of efficacy of 111in-chl6 nanoparticle amf–induced thermoablative therapy for human breast cancer in mice," *J Nucl Med*, vol. 48, no. 3, pp. 437–44, 2007, ISSN: 0161-5505 (Print) 0161-5505 (Linking).

[55] B. Doffek, "Magnetic flow cytometry for thrombocyte analysis," Thesis, 2015.

[56] C. Dong and X. X. Lei, "Biomechanics of cell rolling: Shear flow, cell-surface adhesion, and cell deformability," *Journal of Biomechanics*, vol. 33, no. 1, pp. 35–43, 2000, ISSN: 00219290. DOI: 10.1016/s0021-9290(99)00174-8.

[57] M. Ermis, E. Antmen, and V. Hasirci, "Micro and nanofabrication methods to control cell-substrate interactions and cell behavior: A review from the tissue engineering perspective," *Bioact Mater*, vol. 3, no. 3, pp. 355–369, 2018, ISSN:

2452-199X (Electronic) 2452-199X (Linking). DOI: 10.1016/j.bioactmat.2018.05.005.

[58] M. A. M. Gijs, "Magnetic bead handling on-chip: New opportunities for analytical applications," *Microfluidics and Nanofluidics*, 2004, ISSN: 1613-4982 1613-4990. DOI: 10.1007/s10404-004-0010-y.

[59] C. Grüttner, K. Müller, J. Teller, F. Westphal, A. Foreman, and R. Ivkov, "Synthesis and antibody conjugation of magnetic nanoparticles with improved specific power absorption rates for alternating magnetic field cancer therapy," *Journal of Magnetism and Magnetic Materials*, vol. 311, no. 1, pp. 181–186, 2007, ISSN: 03048853. DOI: 10.1016/j.jmmm.2006.10.1151.

[60] H. Happel John; Brenner, *Low Reynolds number hydrodynamics*, ser. Mechanics of fluids and transport processes. 1981, ISBN: 978-94-009-8352-6. DOI: 10.1007/978-94-009-8352-6.

[61] U. Hassan, T. Ghonge, J. Reddy B., M. Patel, M. Rappleye, I. Taneja, A. Tanna, R. Healey, N. Manusry, Z. Price, T. Jensen, J. Berger, A. Hasnain, E. Flaugher, S. Liu, B. Davis, J. Kumar, K. White, and R. Bashir, "A point-of-care microfluidic biochip for quantification of cd64 expression from whole blood for sepsis stratification," *Nat Commun*, vol. 8, p. 15 949, 2017, ISSN: 2041-1723 (Electronic) 2041-1723 (Linking). DOI: 10.1038/ncomms15949.

[62] M. Hejazian, W. Li, and N. T. Nguyen, "Lab on a chip for continuous-flow magnetic cell separation," *Lab Chip*, vol. 15, no. 4, pp. 959–70, 2015, ISSN: 1473-0189 (Electronic) 1473-0189 (Linking). DOI: 10.1039/c4lc01422g.

[63] M. Helou, M. Reisbeck, S. F. Tedde, L. Richter, L. Bär, J. J. Bosch, R. H. Stauber, E. Quandt, and O. Hayden, "Time-of-flight magnetic flow cytometry in whole blood with integrated sample preparation," *Lab on a Chip*, vol. 13, no. 6, 2013, ISSN: 1473-0197 1473-0189. DOI: 10.1039/c3lc41310a.

[64] Y.-C. Hsiao, R. Khojah, X. Li, A. Kundu, C. Chen, D. B. Gopman, A. C. Chavez, T. Lee, Z. Xiao, A. E. Sepulveda, R. N. Candler, G. P. Carman, D. Di Carlo, and C. S. Lynch, "Capturing magnetic bead-based arrays using perpendicular magnetic anisotropy," *Applied Physics Letters*, vol. 115, no. 8, 2019, ISSN: 0003-6951 1077-3118. DOI: 10.1063/1.5085354.

[65] J. J. S. Jr, "A method of calibrating helmholtz coils for the measurement of permanent magnets,"

[66] G. Kokkinis, S. Cardoso, F. Keplinger, and I. Giouroudi, "Microfluidic platform with integrated gmr sensors for quantification of cancer cells," *Sensors and Actuators B: Chemical*, vol. 241, pp. 438–445, 2017, ISSN: 09254005. DOI: 10.1016/j.snb.2016.09.189.

[67] J. M. Koo and C. Kleinstreuer, "Liquid flow in microchannels: Experimental observations and computational analyses of microfluidics effects," *Journal of Micromechanics and Microengineering*, vol. 13, no. 5, pp. 568–579, 2003, ISSN: 0960-1317. DOI: PiiS0960-1317(03)57671-9Doi10.1088/0960-1317/13/5/307.

[68] H. G. Kye, B. S. Park, J. M. Lee, M. G. Song, H. G. Song, C. D. Ahrberg, and B. G. Chung, "Dual-neodymium magnet-based microfluidic separation device," *Sci Rep*, vol. 9, no. 1, p. 9502, 2019, ISSN: 2045-2322 (Electronic) 2045-2322 (Linking). DOI: 10.1038/s41598-019-45929-y.

[69] T. Li, J. Chen, Y. Han, Z. Ma, and J. Wu, "Study on the characteristic point location of depth average velocity in smooth open channels: Applied to channels with flat or concave boundaries," *Water*, vol. 12, no. 2, 2020, ISSN: 2073-4441. DOI: 10.3390/w12020430.

[70] A. Liakopoulos, F. Sofos, and T. E. Karakasidis, "Friction factor in nanochannel flows," *Microfluidics and Nanofluidics*, vol. 20, no. 1, 2016, ISSN: 1613-4982. DOI: 10.1007/s10404-015-1699-5.

[71] F. Liu, L. Ni, and J. Zhe, "Lab-on-a-chip electrical multiplexing techniques for cellular and molecular biomarker detection," *Biomicrofluidics*, vol. 12, no. 2, p. 021 501, 2018, ISSN: 1932-1058 (Print) 1932-1058 (Linking). DOI: 10.1063/1.5022168.

[72] H. Y. Liu, C. Koch, A. Haller, S. A. Joosse, R. Kumar, M. J. Vellekoop, L. J. Horst, L. Keller, A. Babayan, A. V. Failla, J. Jensen, S. Peine, F. Keplinger, H. Fuchs, K. Pantel, and M. Hirtz, "Evaluation of microfluidic ceiling designs for the capture of circulating tumor cells on a microarray platform," *Adv Biosyst*, vol. 4, no. 2, e1900162, 2020, ISSN: 2366-7478 (Print) 2366-7478 (Linking). DOI: 10.1002/adbi.201900162.

[73] R. Liu, C. H. Chu, N. Wang, T. Ozkaya-Ahmadov, O. Civelekoglu, D. Lee, A. K. M. Arifuzzman, and A. F. Sarioglu, "Combinatorial immunophenotyping of cell populations with an electronic antibody microarray," *Small*, vol. 15, no. 51, e1904732, 2019, ISSN: 1613-6829 (Electronic) 1613-6810 (Linking). DOI: 10.1002/smll.201904732.

[74] J. Loureiro, C. Fermon, M. Pannetier-Lecoeur, G. Arrias, R. Ferreira, S. Cardoso, and P. P. Freitas, "Magnetoresistive detection of magnetic beads flowing at high speed in microfluidic channels," *IEEE Transactions on Magnetics*, vol. 45, no. 10, pp. 4873–4876, 2009, ISSN: 0018-9464. DOI: 10.1109/tmag.2009.2026287.

[75] M. Madadelahi, L. F. Acosta-Soto, S. Hosseini, S. O. Martinez-Chapa, and M. J. Madou, "Mathematical modeling and computational analysis of centrifugal microfluidic platforms: A review," *Lab on a Chip*, vol. 20, no. 8, pp. 1318–1357, 2020, ISSN: 1473-0197. DOI: 10.1039/c9lc00775j.

[76] R. P. McEver and C. Zhu, "Rolling cell adhesion," *Annu Rev Cell Dev Biol*, vol. 26, pp. 363–96, 2010, ISSN: 1530-8995 (Electronic) 1081-0706 (Linking). DOI: 10.1146/annurev.cellbio.042308.113238.

[77] D. P. McIntyre, A. Lashkaripour, and D. Densmore, "Rapid and inexpensive microfluidic electrode integration with conductive ink," *Lab on a Chip*, 2020, ISSN: 1473-0197 1473-0189. DOI: 10.1039/d0lc00763c.

[78] A. Munaz, M. J. A. Shiddiky, and N. T. Nguyen, "Recent advances and current challenges in magnetophoresis based micro magnetofluidics," *Biomicrofluidics*, vol. 12, no. 3, p. 031 501, 2018, ISSN: 1932-1058 (Print) 1932-1058 (Linking). DOI: 10.1063/1.5035388.

[79] N.-T. Nguyen, "Micro-magnetofluidics: Interactions between magnetism and fluid flow on the microscale," *Microfluidics and Nanofluidics*, vol. 12, no. 1-4, pp. 1–16, 2011, ISSN: 1613-4982 1613-4990. DOI: 10.1007/s10404-011-0903-5.

[80] N. Pamme, "Magnetism and microfluidics," *Lab Chip*, vol. 6, no. 1, pp. 24–38, 2006, ISSN: 1473-0197 (Print) 1473-0189 (Linking). DOI: 10.1039/b513005k.

[81] J. W. Perthold and C. Oostenbrink, "Simulation of reversible protein–protein binding and calculation of binding free energies using perturbed distance restraints," *Journal of Chemical Theory and Computation*, vol. 13, no. 11, pp. 5697–5708, 2017, ISSN: 1549-9618 1549-9626. DOI: 10.1021/acs.jctc.7b00706.

[82] A. Pierres, D. Touchard, A.-M. Benoliel, and P. Bongrand, "Dissecting streptavidin-biotin interaction with a laminar flow chamber," *Biophysical Journal*, vol. 82, no. 6, pp. 3214–3223, 2002, ISSN: 0006-3495. DOI: 10.1016/s0006-3495(02)75664-6.

[83] E. Räth, "Affinity-based cell rolling assays in magnetic flow cytometry," Thesis, 2020.

[84] M. Reisbeck, M. J. Helou, L. Richter, B. Kappes, O. Friedrich, and O. Hayden, "Magnetic fingerprints of rolling cells for quantitative flow cytometry in whole blood," *Scientific Reports*, vol. 6, no. 1, 2016, ISSN: 2045-2322. DOI: 10.1038/srep32838.

[85] J. Schütt, R. Illing, O. Volkov, T. Kosub, P. N. Granell, H. Nhalil, J. Fassbender, L. Klein, A. Grosz, and D. Makarov, "Two orders of magnitude boost in the detection limit of droplet-based micro-magnetofluidics with planar hall effect sensors," *ACS Omega*, vol. 5, no. 32, pp. 20 609–20 617, 2020, ISSN: 2470-1343 2470-1343. DOI: 10.1021/acsomega.0c02892.

[86] J. Schütt, D. I. Sandoval Bojorquez, E. Avitabile, E. S. Oliveros Mata, G. Milyukov, J. Colditz, L. G. Delogu, M. Rauner, A. Feldmann, S. Koristka, J. M. Middeke, K. Sockel, J. Fassbender, M. Bachmann, M. Bornhäuser, G. Cuniberti, and L. Baraban, "Nanocytometer for smart analysis of peripheral blood and acute myeloid leukemia: A pilot study," *Nano Letters*, 2020, ISSN: 1530-6984 1530-6992. DOI: 10.1021/acs.nanolett.0c02300.

[87] F. Shamsipour, A. H. Zarnani, R. Ghods, M. Chamankhah, F. Forouzesh, S. Vafaei, A. A. Bayat, M. M. Akhondi, M. Ali Oghabian, and M. Jeddi-Tehrani, "Conjugation of monoclonal antibodies to super paramagnetic iron oxide nanoparticles for detection of her2/neu antigen on breast cancer cell lines," *Avicenna J Med Biotechnol*, vol. 1, no. 1, pp. 27–31, 2009, ISSN: 2008-2835 (Print) 2008-2835 (Linking).

[88] S. S. Shevkoplyas, A. C. Siegel, R. M. Westervelt, M. G. Prentiss, and G. M. Whitesides, "The force acting on a superparamagnetic bead due to an applied magnetic field," *Lab Chip*, vol. 7, no. 10, pp. 1294–302, 2007, ISSN: 1473-0197 (Print) 1473-0189 (Linking). DOI: 10.1039/b705045c.

[89] C. Sommer, "Die größenabhängigkeit der gleichgewichtsgeschwindigkeit von partikeln beim transport in mikrokanälen," Thesis, 2014.

[90] D. Song, R. K. Gupta, and R. P. Chhabra, "Drag on a sphere in poiseuille flow of shear-thinning power-law fluids," *Industrial and Engineering Chemistry Research*, vol. 50, no. 23, pp. 13 105–13 115, 2011, ISSN: 0888-5885 1520-5045. DOI: 10.1021/ie102120p.

[91] H. C. Tekin, M. Cornaglia, and M. A. Gijs, "Attomolar protein detection using a magnetic bead surface coverage assay," *Lab Chip*, vol. 13, no. 6, pp. 1053–

9, 2013, ISSN: 1473-0189 (Electronic) 1473-0189 (Linking). DOI: 10.1039/c3lc41285g.

[92] A. E. Urusov, A. V. Petrakova, M. V. Vozniak, A. V. Zherdev, and B. B. Dzantiev, "Rapid immunoenzyme assay of aflatoxin b1 using magnetic nanoparticles," *Sensors (Basel)*, vol. 14, no. 11, pp. 21 843–57, 2014, ISSN: 1424-8220 (Electronic) 1424-8220 (Linking). DOI: 10.3390/s141121843.

[93] C. Wang, S. Zhao, X. Zhao, L. Chen, Z. Tian, X. Chen, and S. Qin, "A novel wide-range microfluidic dilution device for drug screening," *Biomicrofluidics*, vol. 13, no. 2, p. 024 105, 2019, ISSN: 1932-1058 (Print) 1932-1058 (Linking). DOI: 10.1063/1.5085865.

[94] H. Wang and Y. Wang, "Measurement of water flow rate in microchannels based on the microfluidic particle image velocimetry," *Measurement*, vol. 42, no. 1, pp. 119–126, 2009, ISSN: 02632241. DOI: 10.1016/j.measurement.2008.04.012.

[95] H. Watarai and M. Namba, "Capillary magnetophoresis of human blood cells and their magnetophoretic trapping in a flow system," *Journal of Chromatography A*, vol. 961, no. 1, pp. 3–8, 2002, ISSN: 00219673. DOI: 10.1016/s0021-9673(02)00748-3.

[96] R. Wirix-Speetjens, W. Fyen, X. Kaidong, B. Jo De, and G. Borghs, "A force study of on-chip magnetic particle transport based on tapered conductors," *IEEE Transactions on Magnetics*, vol. 41, no. 10, pp. 4128–4133, 2005, ISSN: 0018-9464. DOI: 10.1109/tmag.2005.855345.

[97] D. Wu and J. Voldman, "An integrated model for bead-based immunoassays," *Biosensors and Bioelectronics*, vol. 154, 2020, ISSN: 09565663. DOI: 10.1016/j.bios.2020.112070.

[98] T. Yago, J. Wu, C. D. Wey, A. G. Klopocki, C. Zhu, and R. P. Mcever, "Catch bonds govern adhesion through l-selectin at threshold shear," *Journal of Cell Biology*, vol. 166, no. 6, pp. 913–923, 2004, ISSN: 1540-8140. DOI: 10.1083/jcb.200403144.

[99] M. Yashima, Y. Ando, and Y. Tabira, "Crystal structure and electron density of alpha-silicon nitride: experimental and theoretical evidence for the covalent bonding and charge transfer," *The Journal of Physical Chemistry B*, vol. 111, no. 14, pp. 3609–3613, 2007, ISSN: 1520-6106. DOI: 10.1021/jp0678507.

[100] R. Yokokawa, Y. Sakai, A. Okonogi, I. Kanno, and H. Kotera, “Force measurement and modeling for motor proteins between microsphere and microfluidic channel surface,” ISBN: 978-0-9798064-3-8.

[101] T. Zhu, D. J. Lichlyter, M. A. Haidekker, and L. Mao, “Analytical model of microfluidic transport of non-magnetic particles in ferrofluids under the influence of a permanent magnet,” *Microfluidics and Nanofluidics*, vol. 10, no. 6, pp. 1233–1245, 2011, ISSN: 1613-4982 1613-4990. DOI: 10.1007/s10404-010-0754-5.

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