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Magnetically Enhanced Microflow Cytometer for Bead-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.

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List of Abbreviations

A

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1.1. Virtual Prototyping of Cell Signals

During the course

1.1.1. Numerical investigation of immunomagnetic label density and size on quantitative magnetoresistive sensing of single cells and cell aggregates

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

simulation of cell rolling velocity and forces

1.1.2. Single Cell Signal

1.1.3. Cell Aggregates

1.2. Reference Bead Surface Functionalization

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

1.3. Concentration Measurements in MRCyte

Explain v-c

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1.3.2. Count Stability

Measurement over 1h

Concentration Measurement in Diluted Whole Blood

1.3.3. Differential Counting Setup

Sensitivity Calibration

Concentration Measurement in Buffer Solution

1.3.4. Surface Magnetization of Biofunctionalized Beads

Somehow BNF-Dextran showed unspeficity initally, but not anymore later on

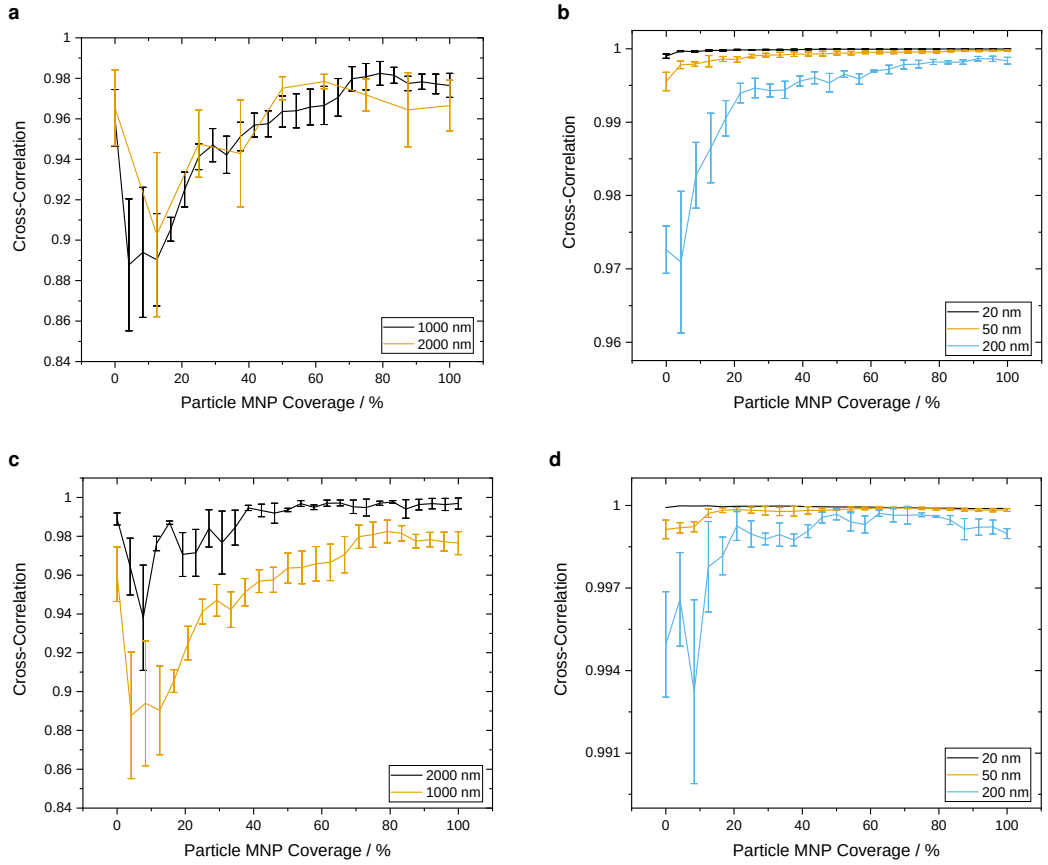

Figure 1: Coverage Dependent Signal Correlation
Mean from 3 differently distributed particles, SEM (**a**) d = 4 µm (**b**) d = 4 µm (**c**) d = 8 µm (**d**) d = 8 µm

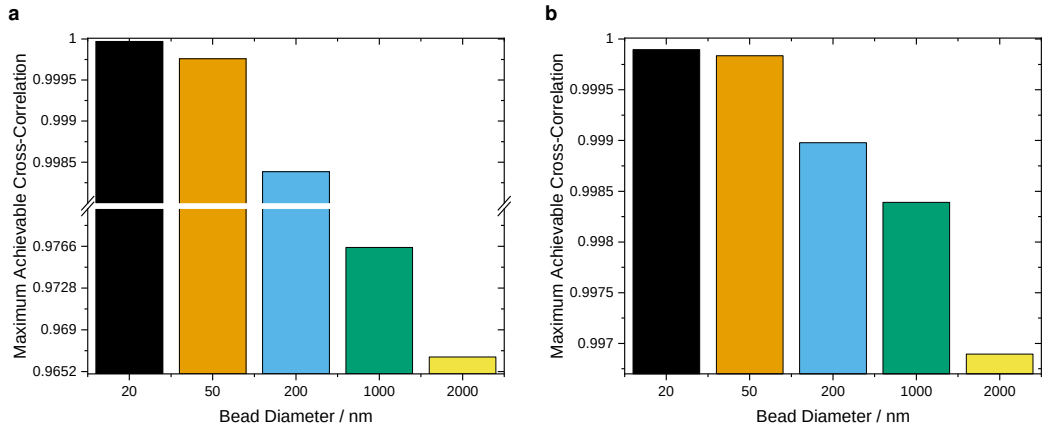

Figure 2: Difference of Cross-Correlation at Maximum Coverage
Mean from 3 different particle distributions at maximum coverage(**a**) d = 4 µm (**b**) d = 8 µm

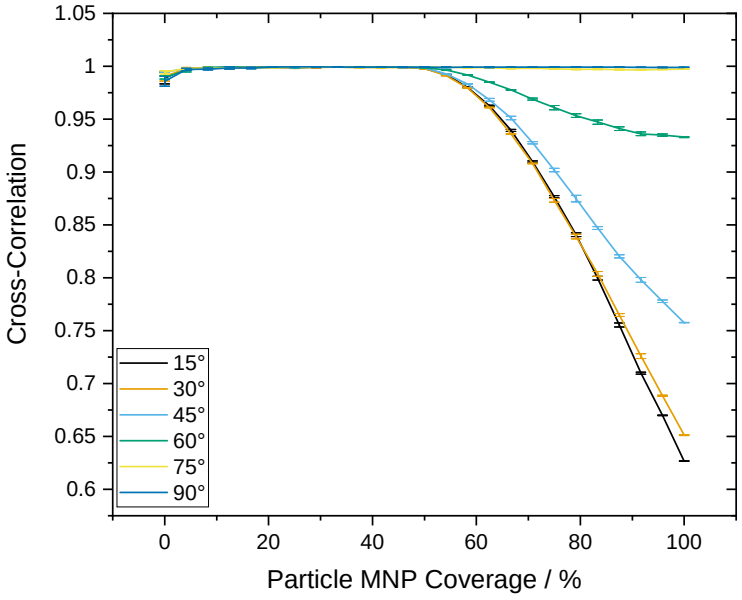

Figure 3: Sensor Signals Correlation between Two Cell Aggregates At Shifting Angles with a Reference Dipole
Mean from 3 differently distributed particles, SEM

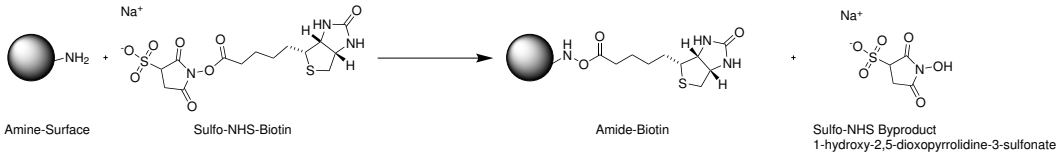

Figure 4: Amine Bead Modification with Sulfo-NHS-Biotin
An amine terminated bead is incubated with sulfo-NHS-Biotin to cover its surface by amide-Biotin. As byproduct the sulfo-NHS-ester 1-hydroxy-2,5-dioxopyrrolidine-3-sulfonate splits off.

1.4. Surface Modification and Biofunctionalization of the Sensor Chip Substrate

1.4.1. Physisorption

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

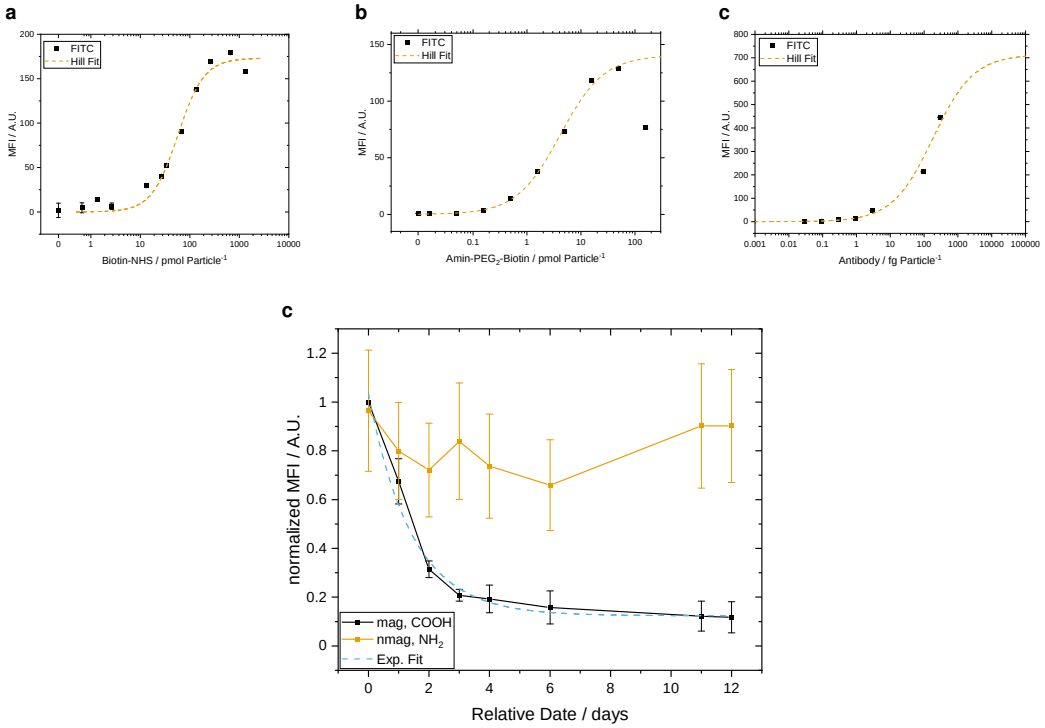

Figure 5: Titration of Biofunctional Molecules on 8 µm Particles
(**a**) NHS-Biotin, MFI, CV, reduced chi square = 275.19597, Hill Fit $y = Vmax * x^n/(k^n + x^n)$, Vmax = 173.077, k = 0.0572831, n = 1.63554 (**b**) Amin-PEG_2-Biotin MFI, CV, outlier neglected Gleichung: $y = Vmax * x^n/(k^n + x^n)$ Vmax 171,02602, k 0,04201, n 0,91338,Chi-Quadr Reduziert 4,07387 (**c**) MFI, CV, reduced chi square = 0.91011, Hill Fit $y = Vmax * x^n/(k^n + x^n)$, Vmax = 713.83643, k = 182.83011 , n = 0.72458 (**d**) MFI, SEM, τ_{decay} = 1.425 57 ± 0.161 88 Equation $y = A \exp^{\frac{-x}{\tau_{decay}}} + y_0$ y0 0.12369 ± 0.01576 A1 0.91263 ± 0.06964 t1 1.42557 ± 0.16188 Reduced Chi-Sqr 0.00542

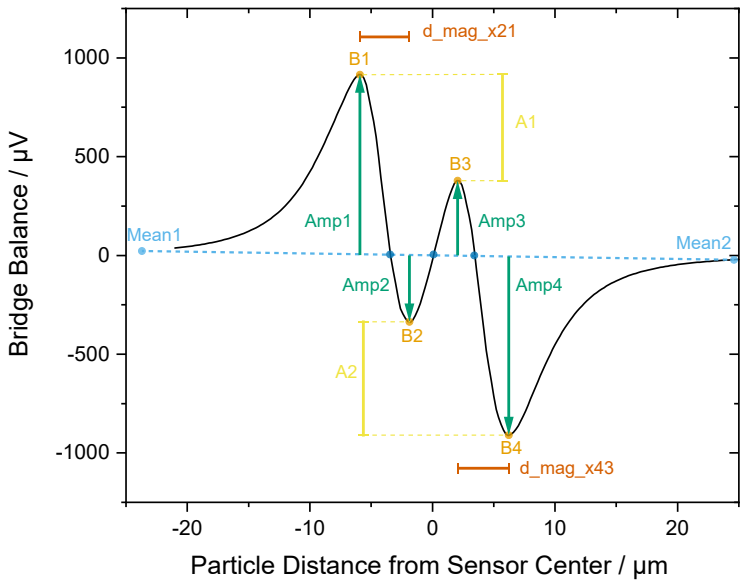

Figure 6: Example Signal of Magnetic Measurement
explain all

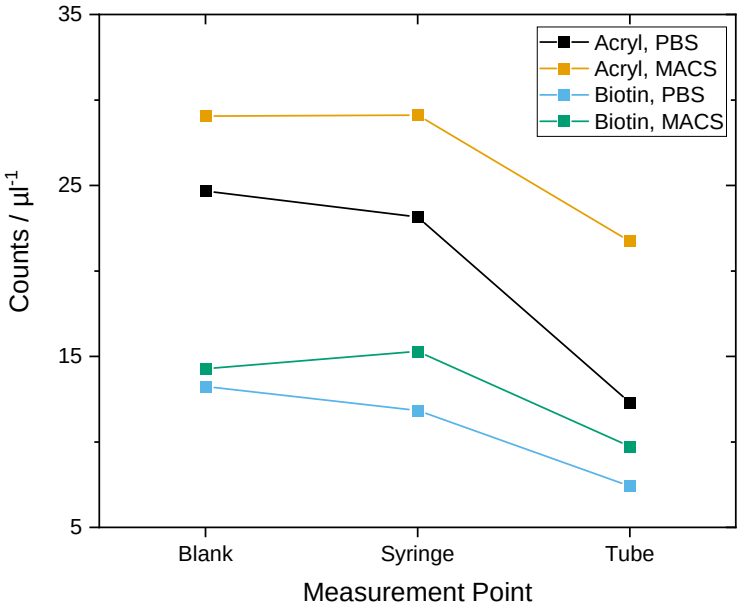

Figure 7: Bead Loss Evaluation in Connectors
Losses in different buffers and bead surfaces.

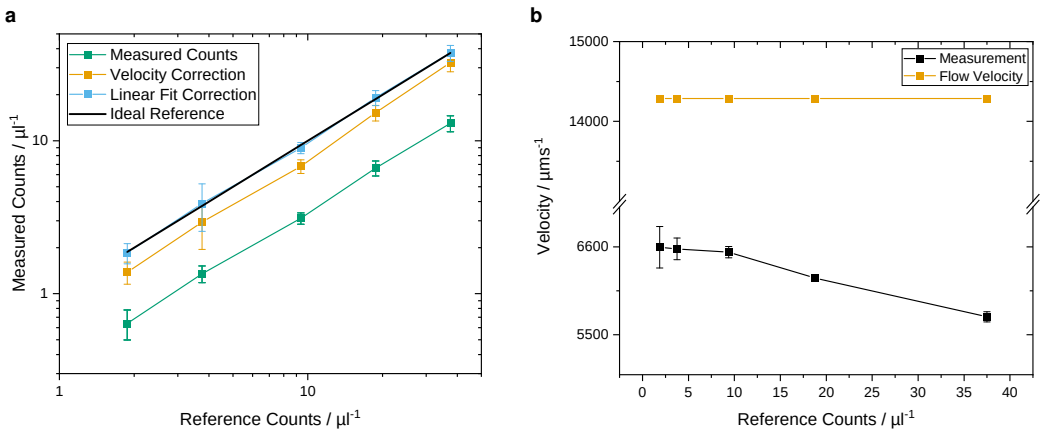

Figure 8: Absolute Concentration Measurements
Mean from 3 independent measurements(**a**) mean, sd (**b**) mean, SEM, Reference Count based error: Liner fit steepness 0.346 22 $\pm$ 0.009 68 –> Correction Factor (inverse) 2.888 33 $\pm$ 0.080 75, Velocity Based Correction: Q/A Dims: 700 µmx50 µm Q = 30 µL min^{-1} –> 2.261 09

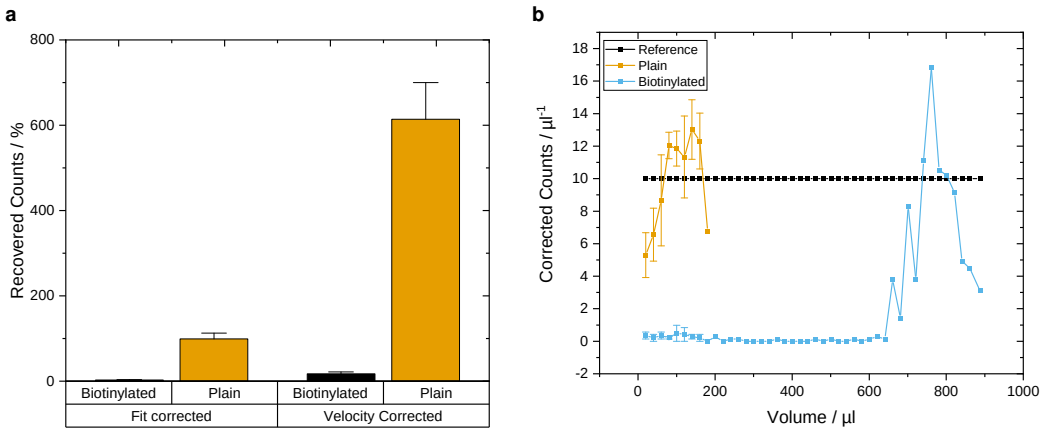

Figure 9: Error Sources in Concentration Measurements
(**a**) mean, SEM Fit factor comparison with protein coated surfaces (**b**) mean, SEM

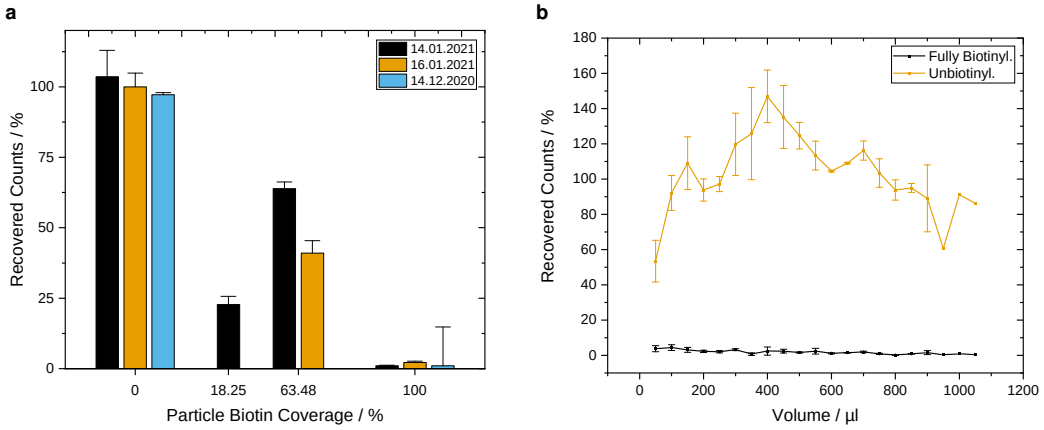

Figure 10: Reproducibility of Concentration Measurements with Saturated Neutravidin Surface
(**a**) 80 µL min^{-1} mean, SEM (**b**) All,mean, SEM,

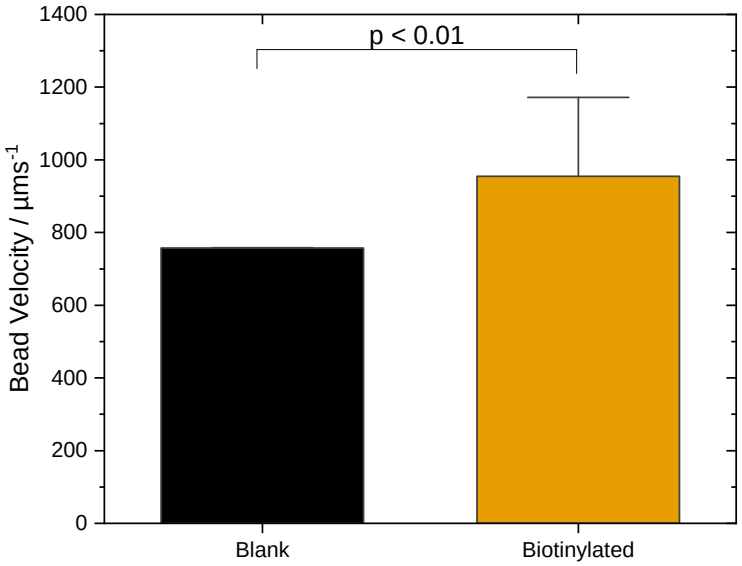

Figure 11: Measured Bead Velocity
p < 0.01

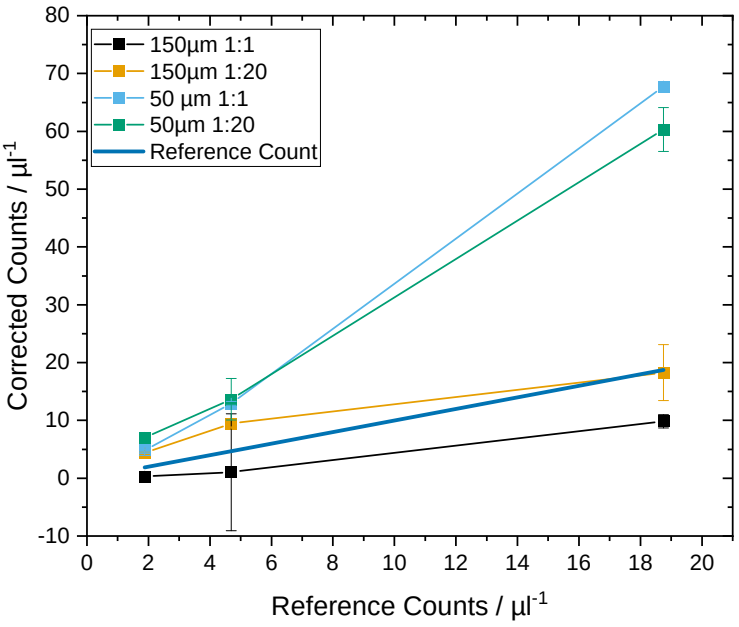

Figure 12: Absolute Concentration Measurement in Blood Samples Under Varying Channel Height
Velocity Correction does not work for high concentrations in 50 µm

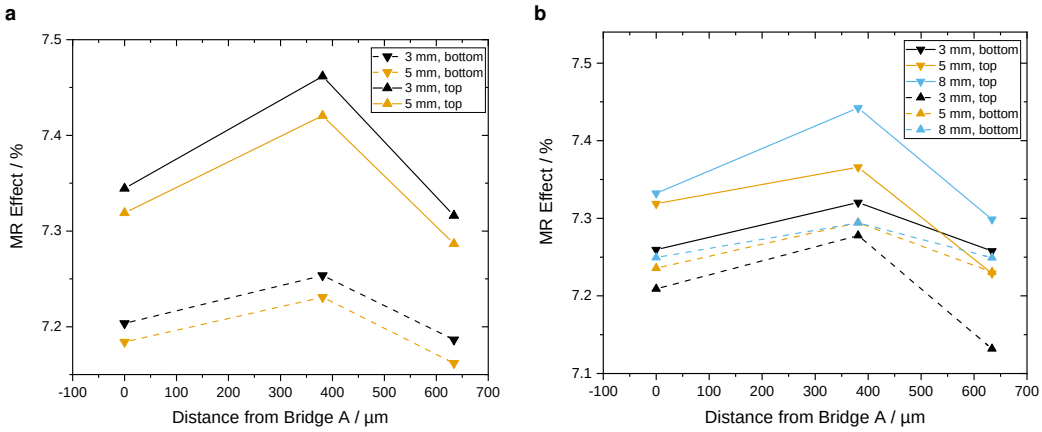

Figure 13: Hysteresis Calibration for Stacked Printed circuit board (PCB)
(**a**) Optimized for top sensor (**b**) Optimized for bottom sensor

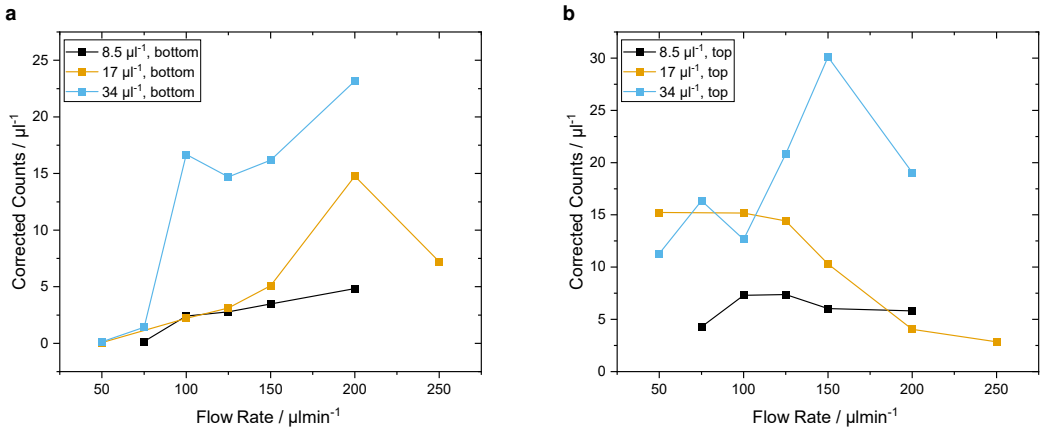

Figure 14: Flow Rate Dependency of Counting Setup
(**a**) Optimized for top sensor (**b**) Optimized for bottom sensor

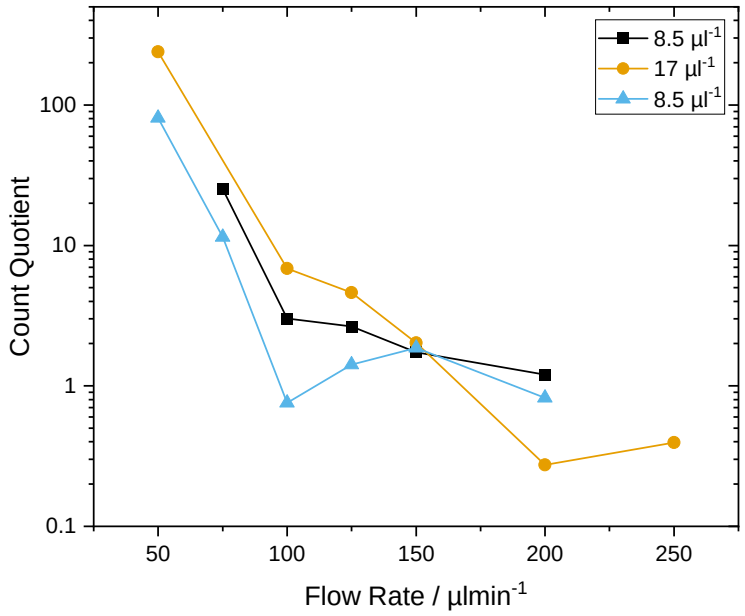

Figure 15: Optimal Differential Counting Flow Rate
Losses in different buffers and bead surfaces.

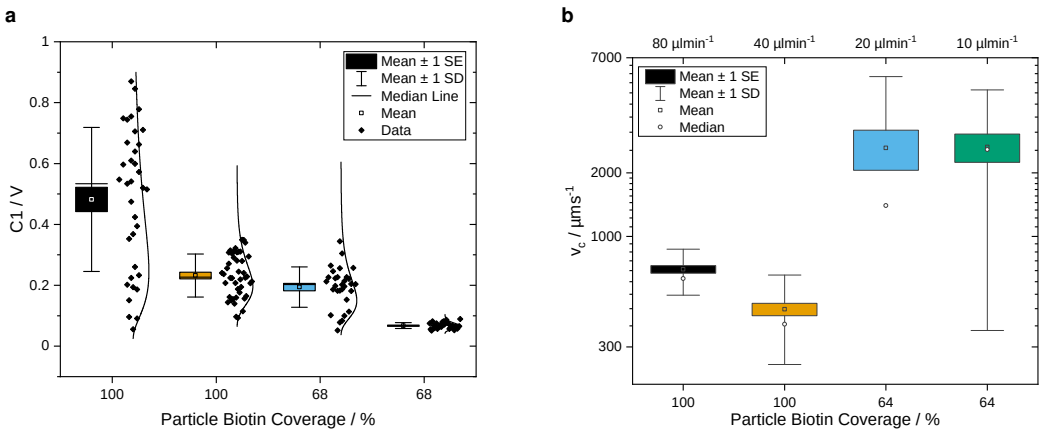

Figure 16: Bead Coverage Assay with BNF-Dextran-redF-100 nm
(**a**) 1. 80 µL min^{-1} 2. 40 µL min^{-1} 3. 20 µL min^{-1} 4. 10 µL min^{-1} (**b**) d = 8 µm

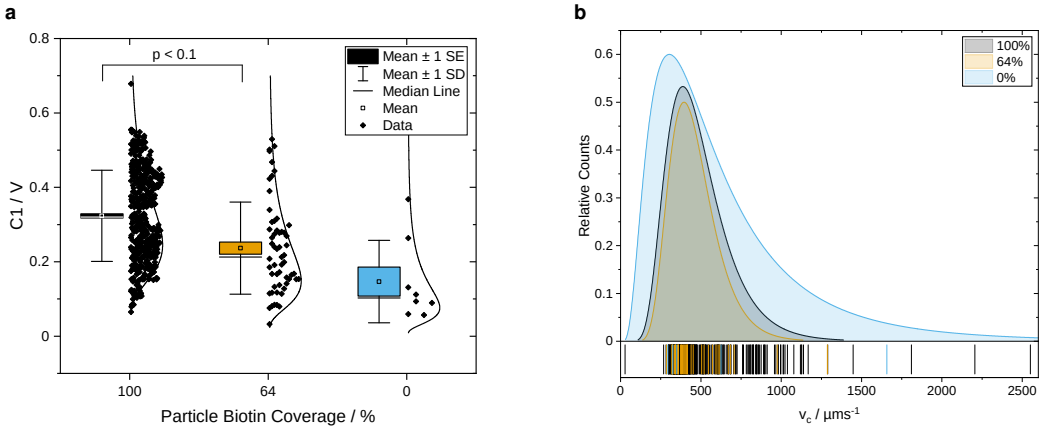

Figure 17: Bead Coverage Assay with OceanNanotec 50 nm
Mean from 3 different particle distributions at maximum coverage, SEM(**a**) d = 4 µm (**b**) d = 8 µm

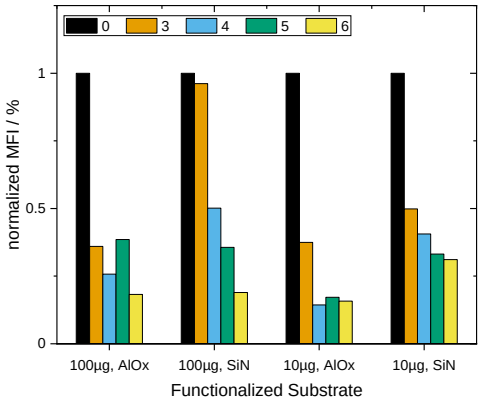

Figure 18: Surface Adsorption Stability of Neutravidin on Silicon nitride (Si_3N_4) and Aluminium oxide (Al_2Ox_3)
Blank with PBS and Blank substrate, corrected, then normalized, absolute protein per ~25 mm

1.4.2. Covalent Attachment

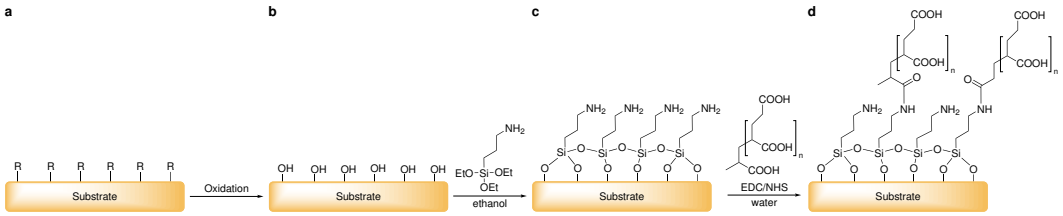

Figure 19: General process chain of chemical surface modification
Any substrate with various surface groups R (**a**) is oxidized to exhibit −OH (hydroxyl) groups.(**b**). Then a silane self-assembled monolayer (SAM) is attached (**c**) and subsequently modified by carbodiimide chemistry with Poly(acrylic) Acid (PAA). (**d**)

Plasma-Based Approach

Water-Based Approach

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

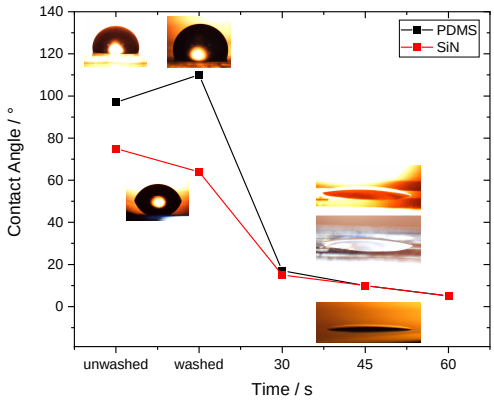

Figure 20: Hydrophbicity Analysis of poly(dimethyl siloxane) (PDMS) under Plasma Exposure
test123

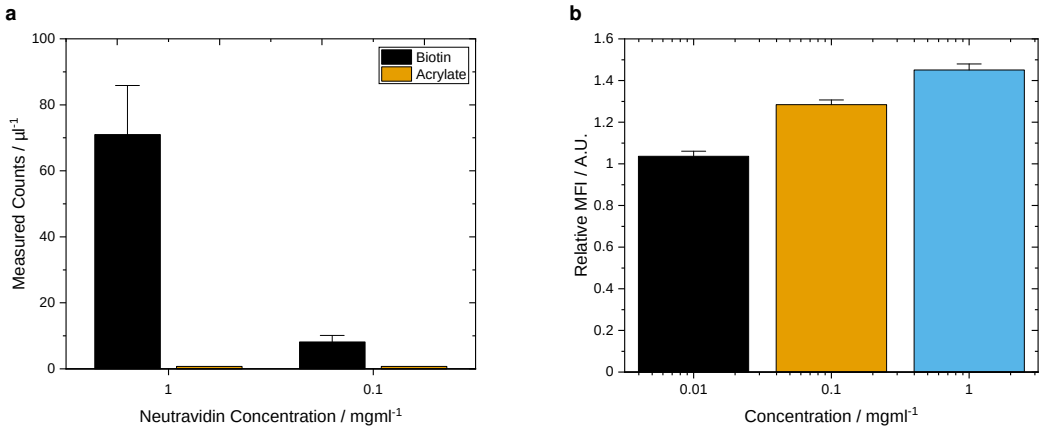

Figure 21: Neutravidin Titration Fluorescence and Bead Capture Assay
Mean from 3 different particle distributions at maximum coverage, SEM(**a**) d = 4 µm (**b**) d = 8 µm

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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