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

Master Thesis Presentation

Johann A. Brenner

Munich, 31.03.2021

Outline

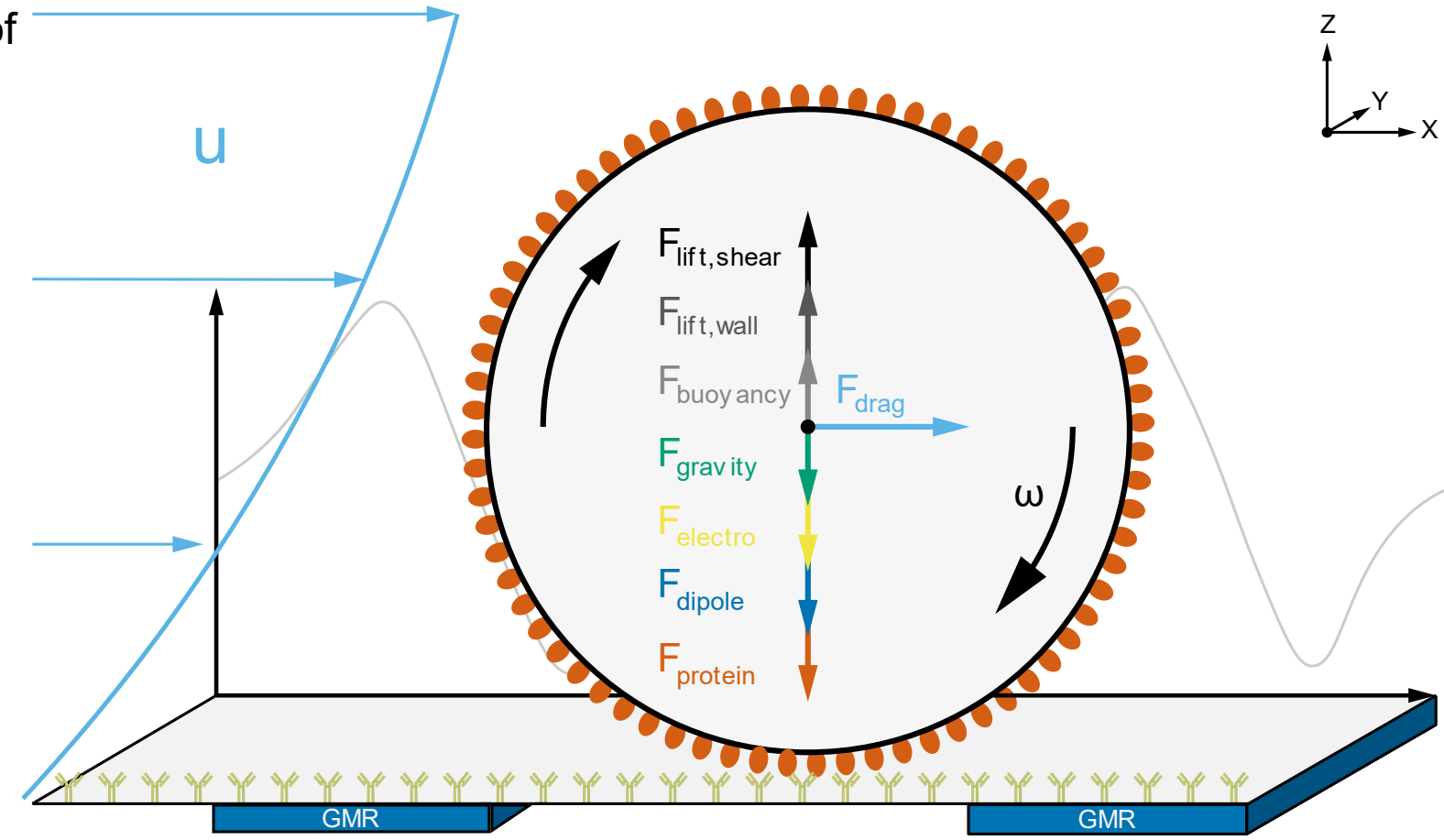
- Theory
  - Immuno-Affinity Measurements in the Magnetic Flow Cytometer
  - Surface Chemistry Methods
- Results
    - Simulation
      - Virtual Prototyping of Cell Signals
      - Calculus Estimation of the Force-Equilibrium in a Microchannel
    - Experimental
      - Magnetic Concentration Measurements
      - Development of a Differential Counting Setup
      - Established Reference Model for Surface Marker Expression Densities
      - Affinity-based Concentration Measurements in the Magnetic Flow Cytometer
- Conclusion

Theory

Immuno-Affinity Measurements in the Magnetic Flow Cytometer

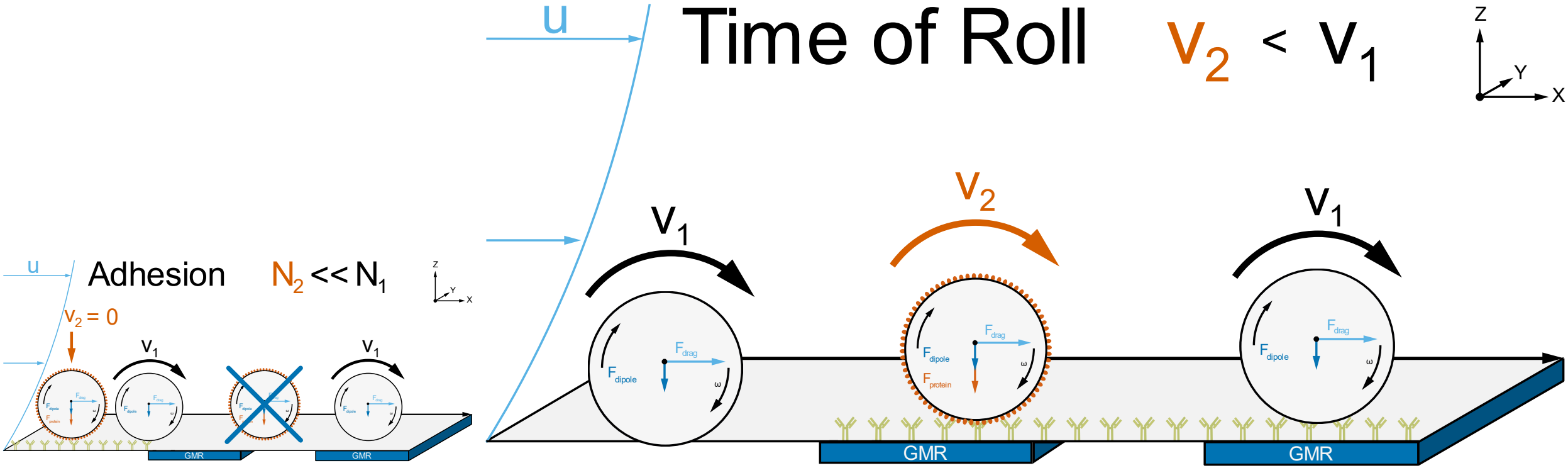
- Magnetic bead rolling on the floor of a microchannel
- Biofunctionalized Channel
- Force-Equilibrium:
  - Hydrodynamics
  - Volume Forces
  - Electro-Magnetism

➔ Change in Velocity or Adhesion



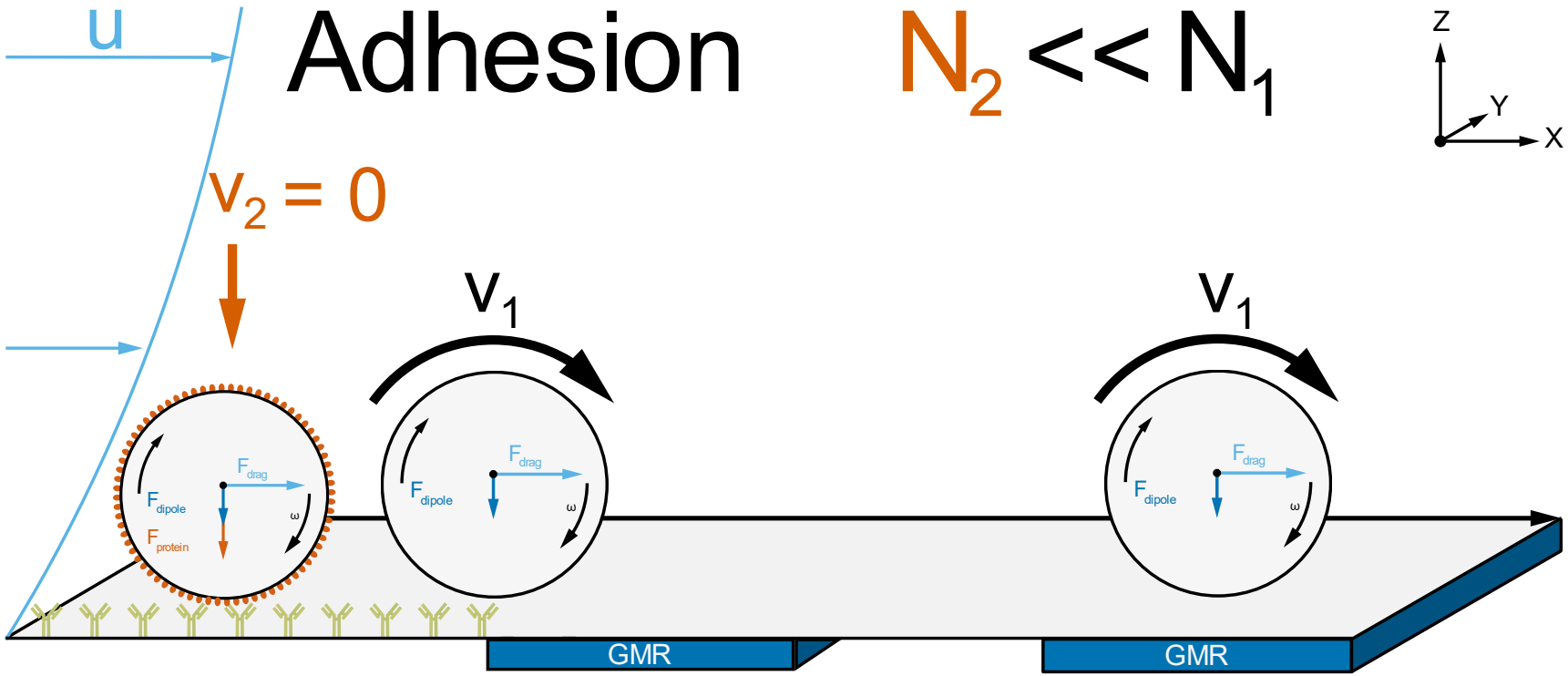
Theory

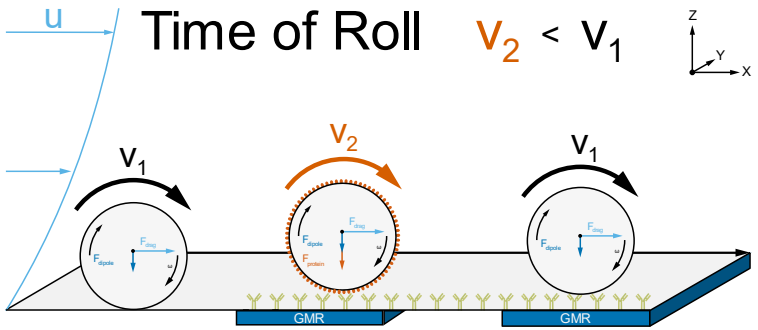
Immuno-Affinity Measurements in the Magnetic Flow Cytometer



Theory

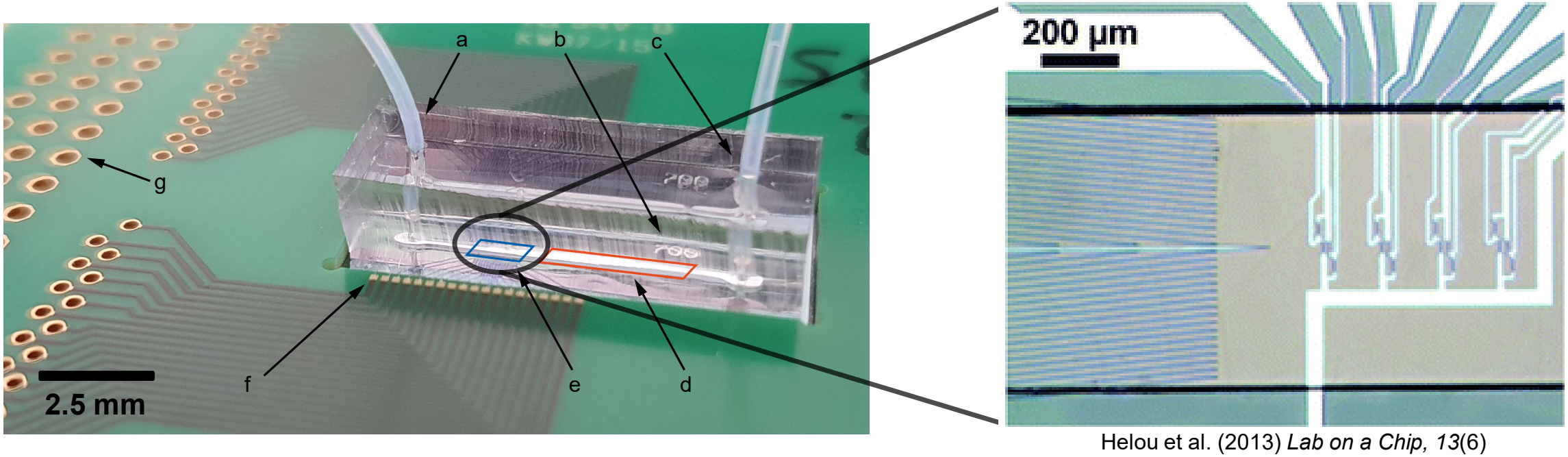
Immuno-Affinity Measurements in the Magnetic Flow Cytometer





Theory

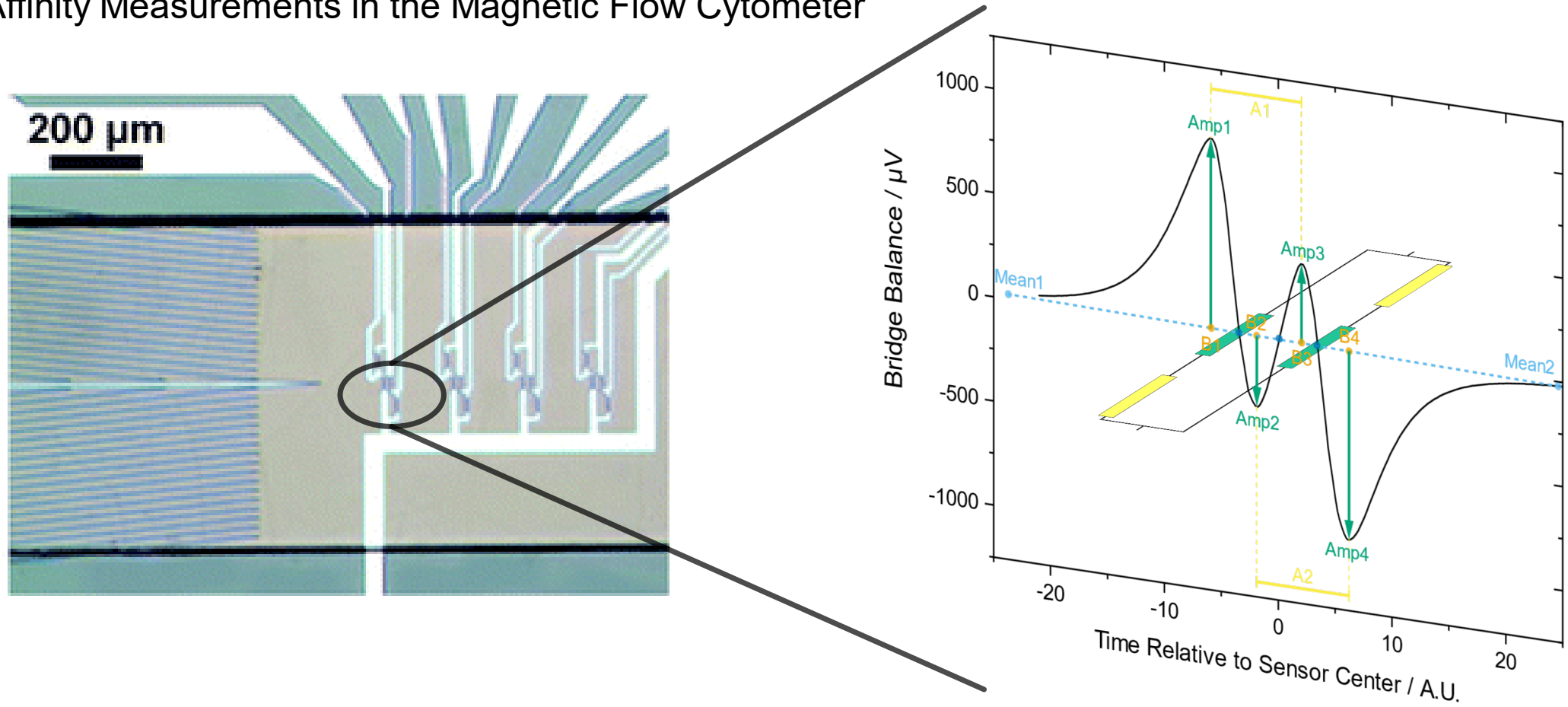
Immuno-Affinity Measurements in the Magnetic Flow Cytometer



Helou et al. (2013) *Lab on a Chip, 13*(6)

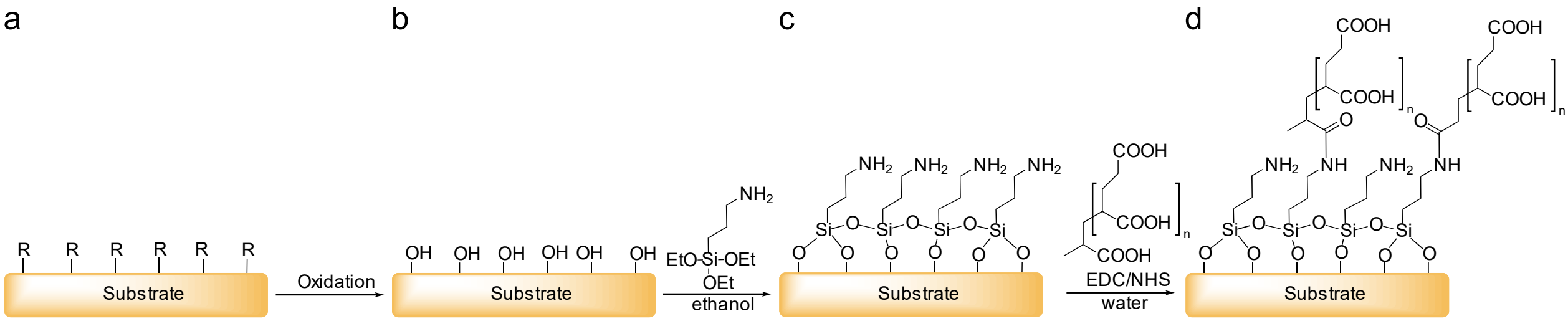
Theory

Immuno-Affinity Measurements in the Magnetic Flow Cytometer



Theory

Surface Chemistry Methods – Covalent Modification

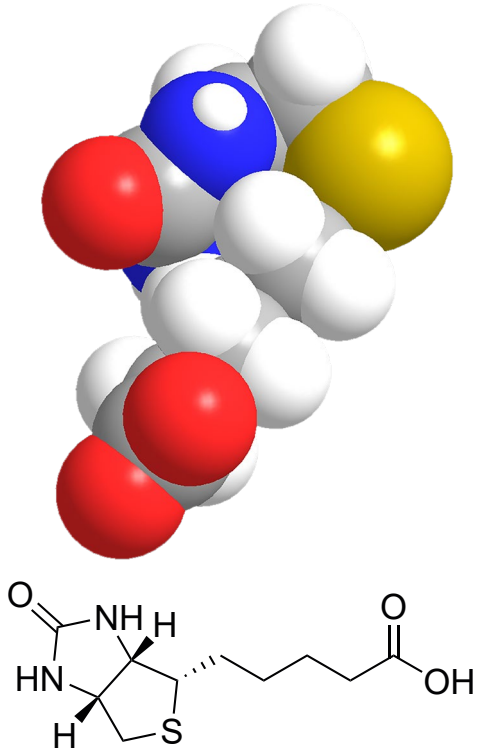


Theory

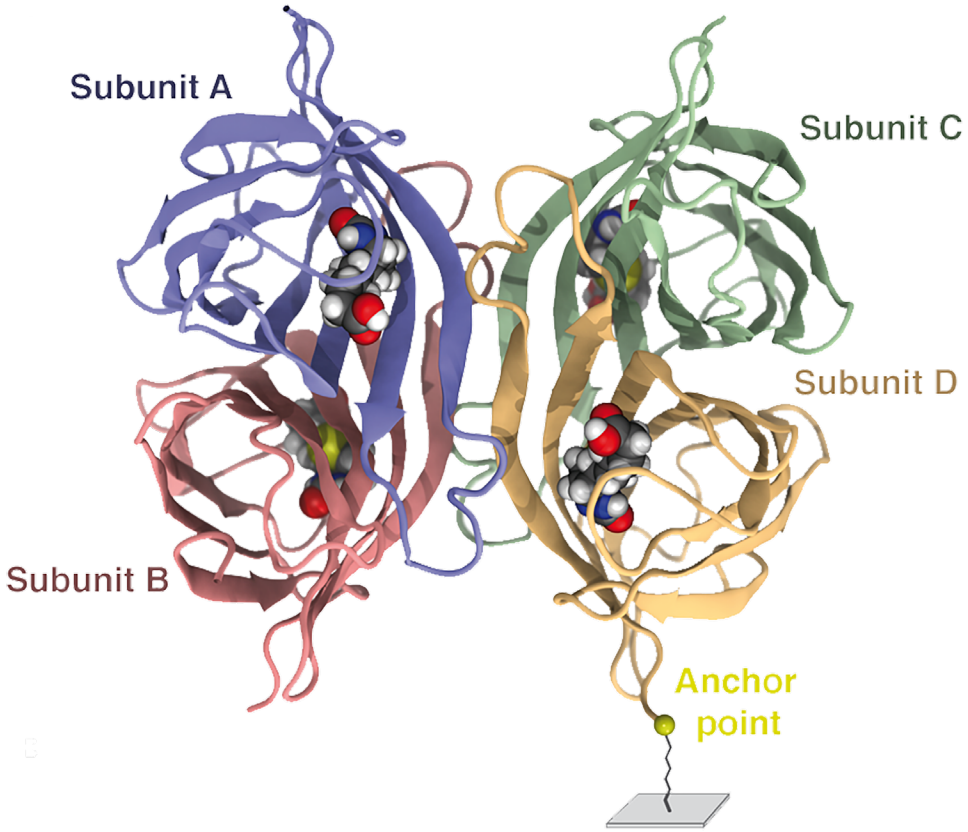
Surface Chemistry Methods – Biotin-Streptavidin

- Biotin-Streptavidin Interaction
- One of the strongest known non-covalent bonds
- Affinity: $K_d = 10^{-13} - 10^{-15}$
- Release force: 100 – 400 pN

➔ Adhesion



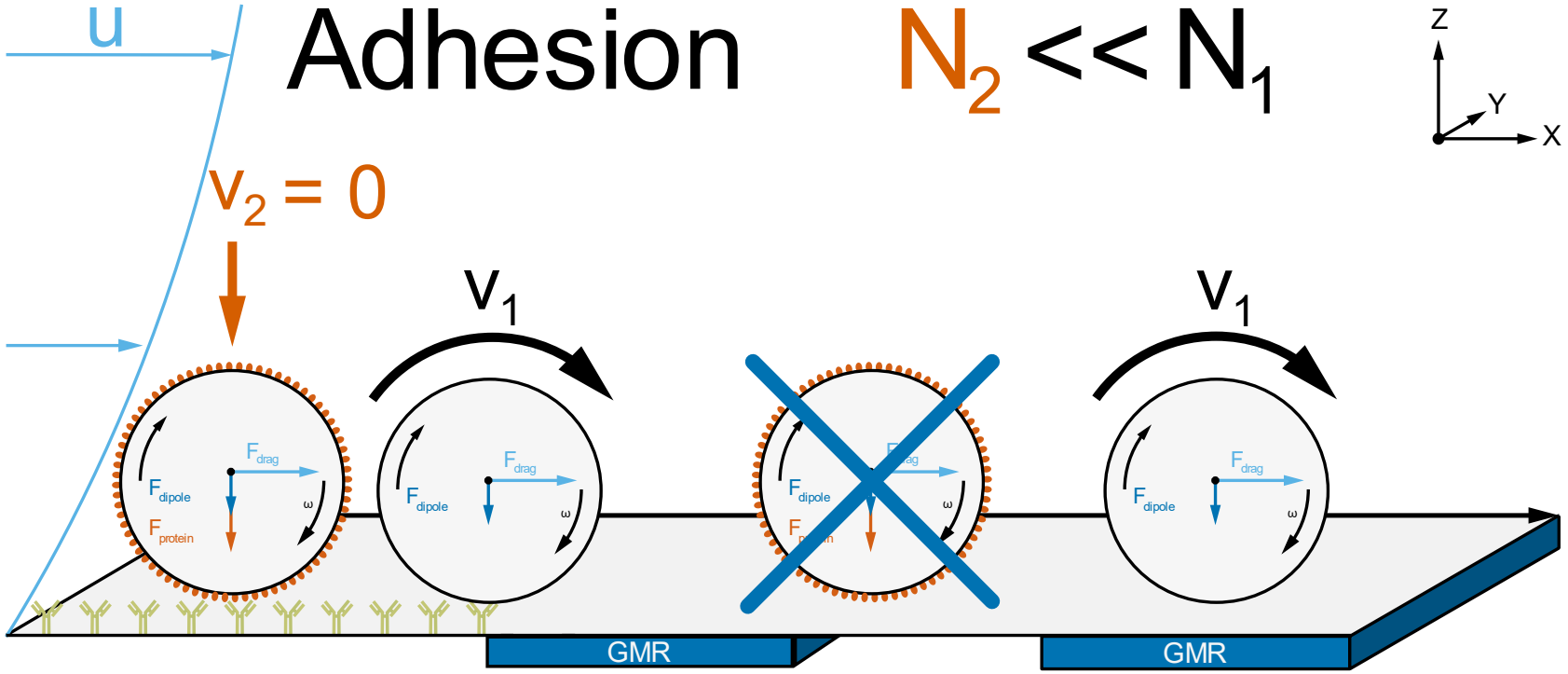
Biotin

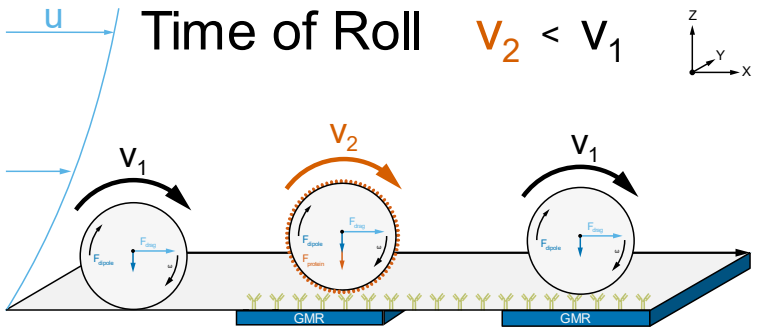


Sedlak et al. (2020) *Sci Adv, 6*(13)

Theory

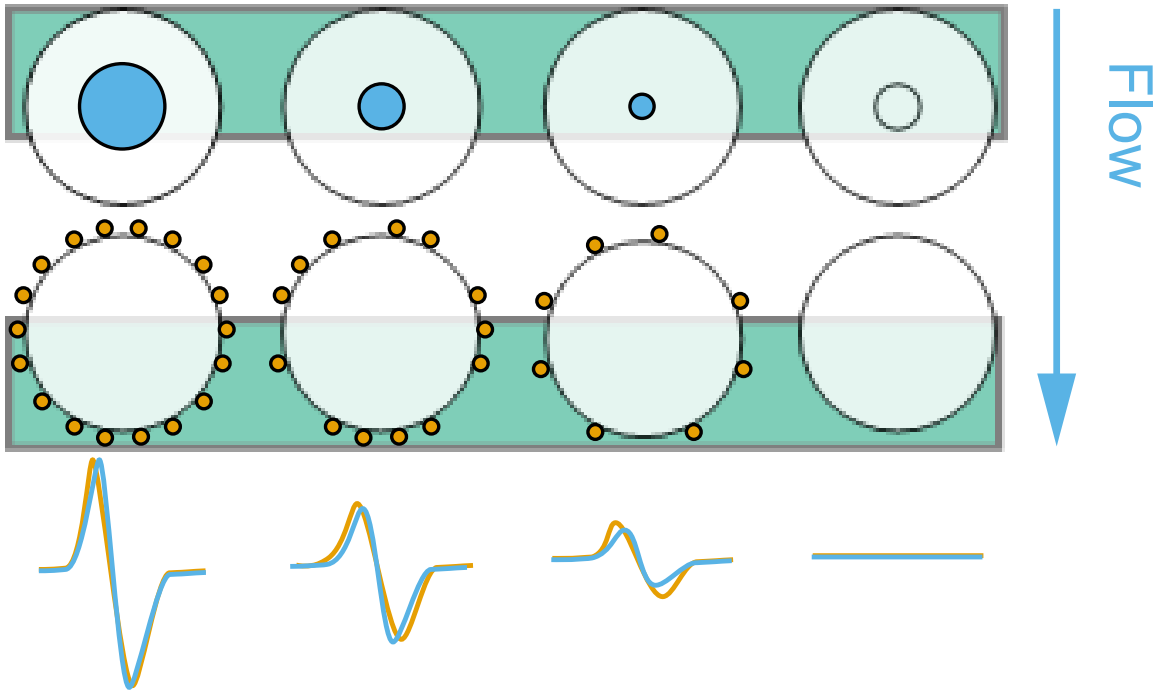
Surface Chemistry Methods – Biotin-Streptavidin



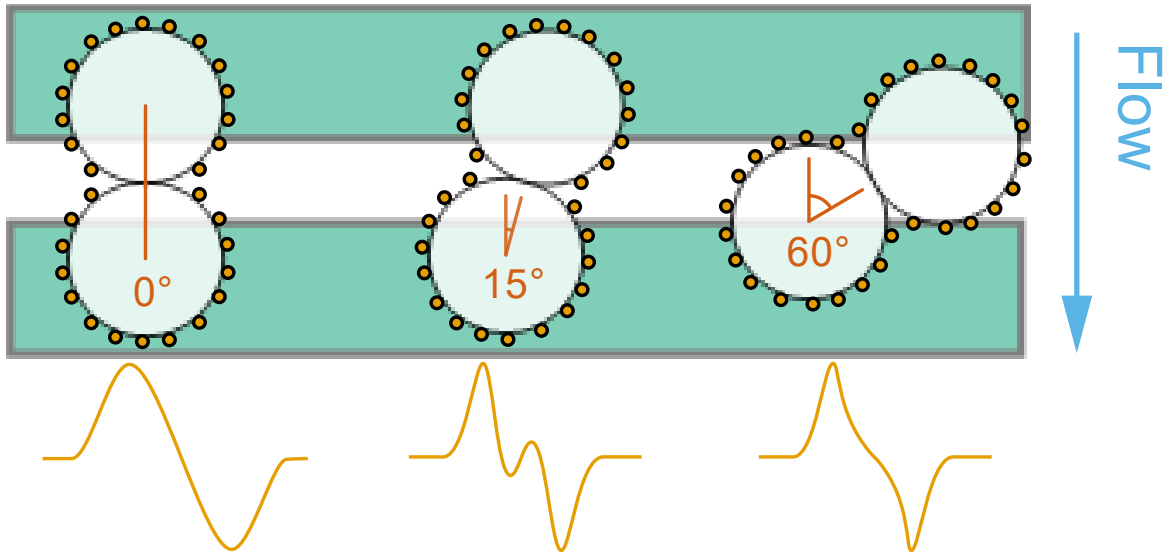


Results

Simulation – Virtual Prototyping of Cell Signals



MNP-Coverage Evaluation

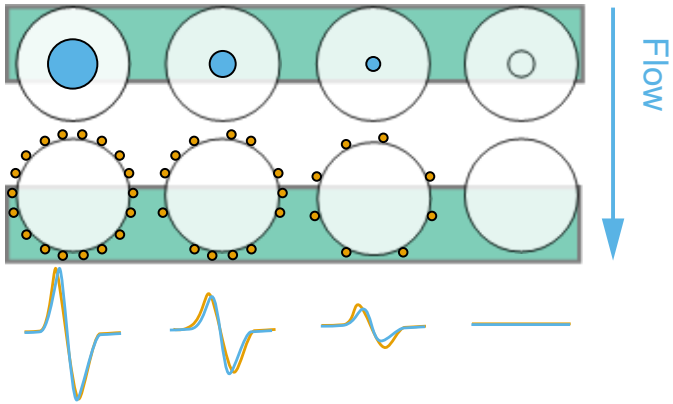


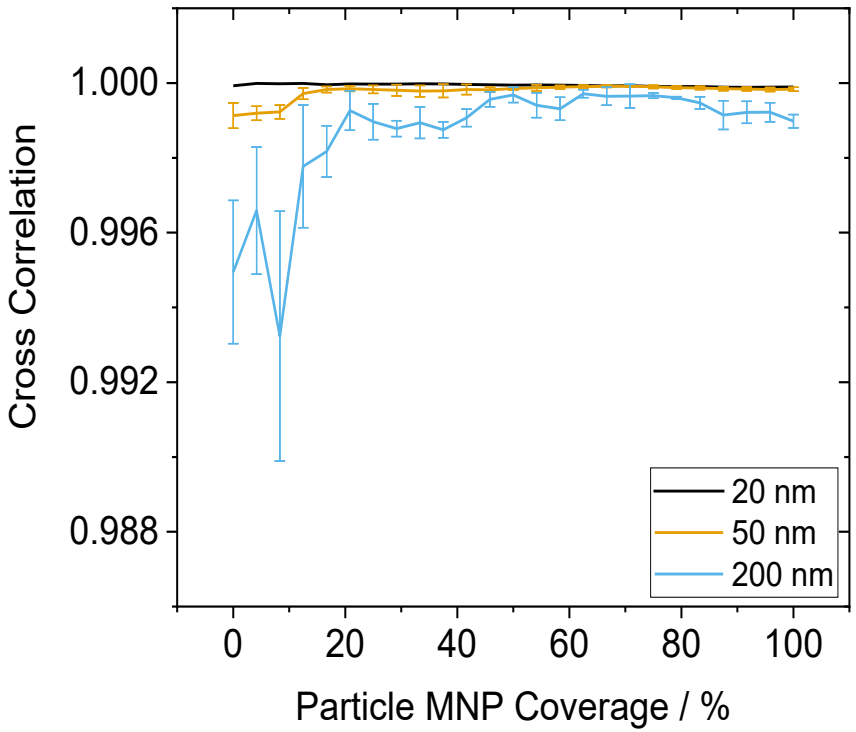
Aggregate Angle Evaluation

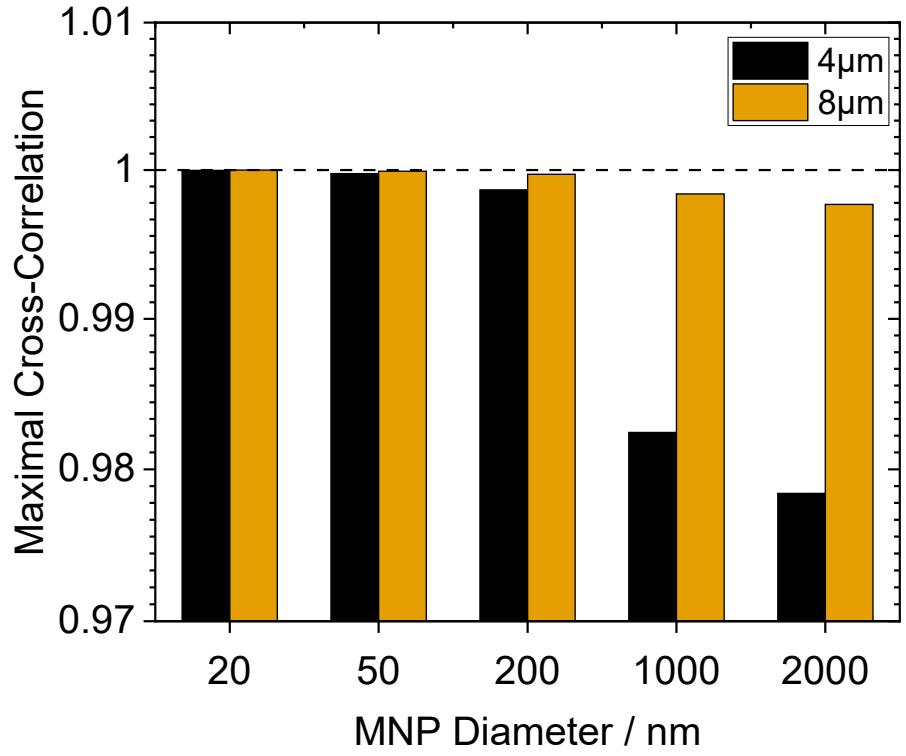
Results

Simulation – Virtual Prototyping of Cell Signals

- Low Coverage:
  - Single Magnetic Momenta cause field inhomogenity
- Signal deterioration for larger MNP diameters



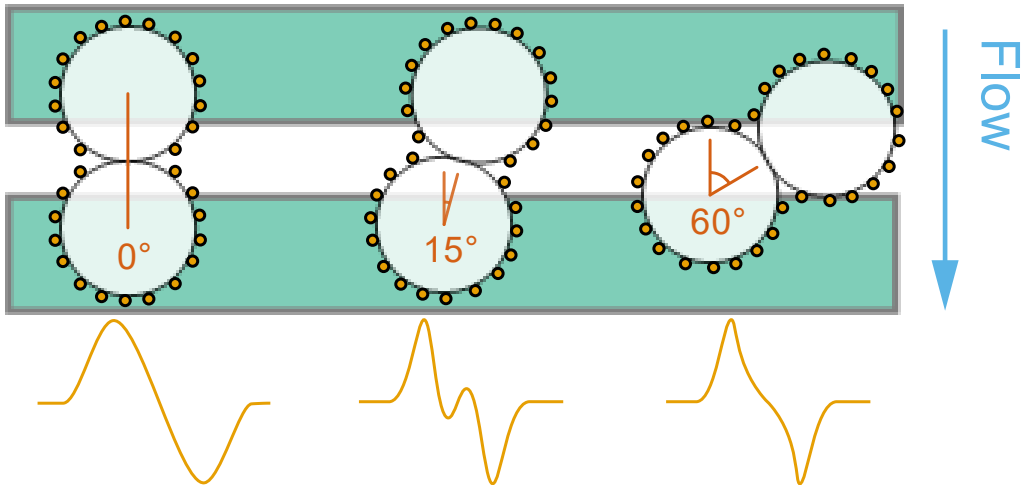


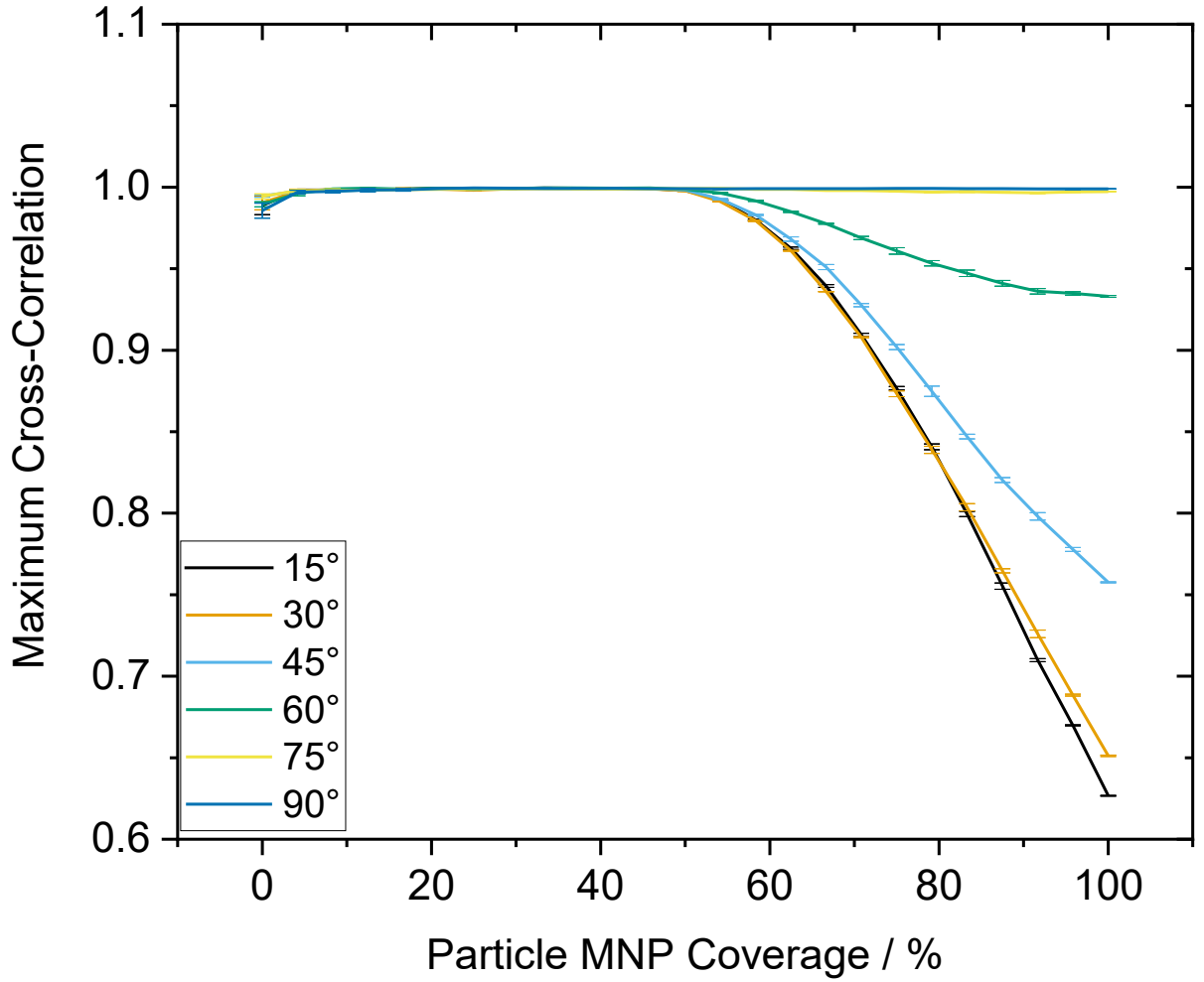


Results

Simulation – Virtual Prototyping of Cell Signals

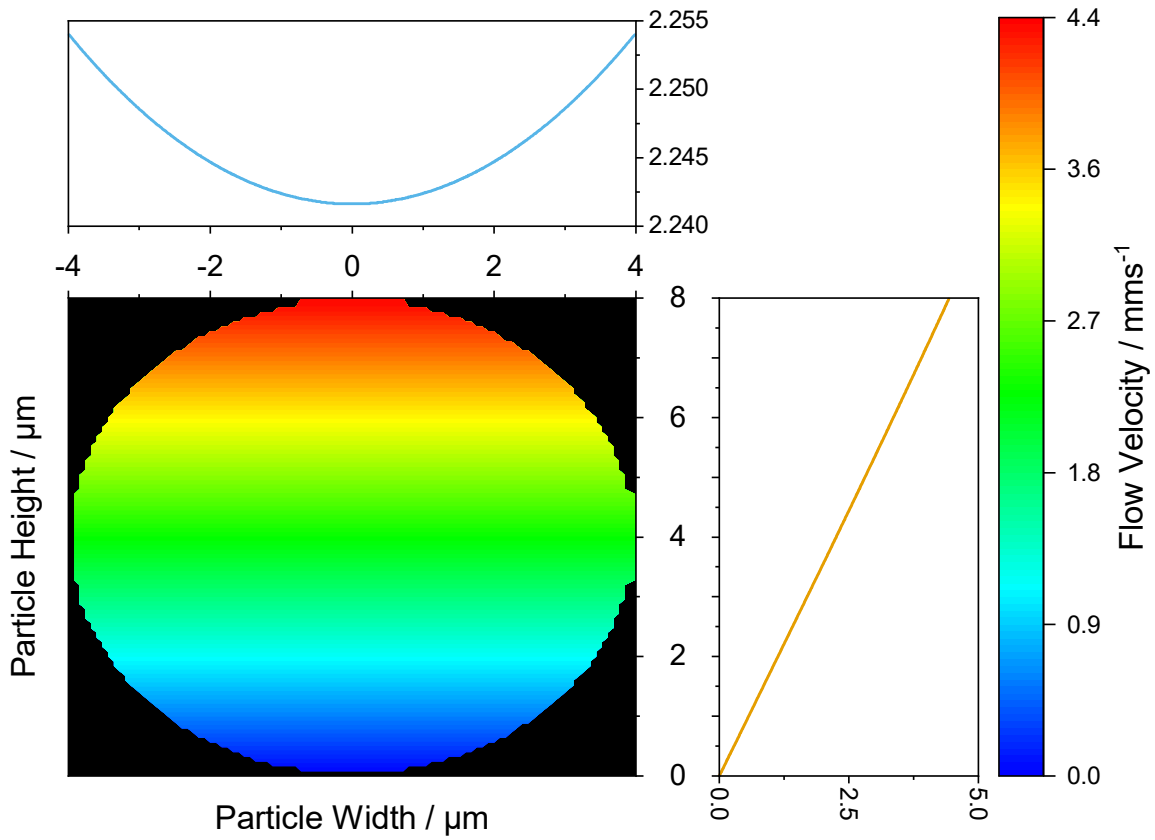
- High correlation in the medium coverage regime due to inhomgeneous field
- Low yaw and high coverage → Two dipoles in superposition
- High yaw and coverage → Aggregate cannot be discriminated by the sensor



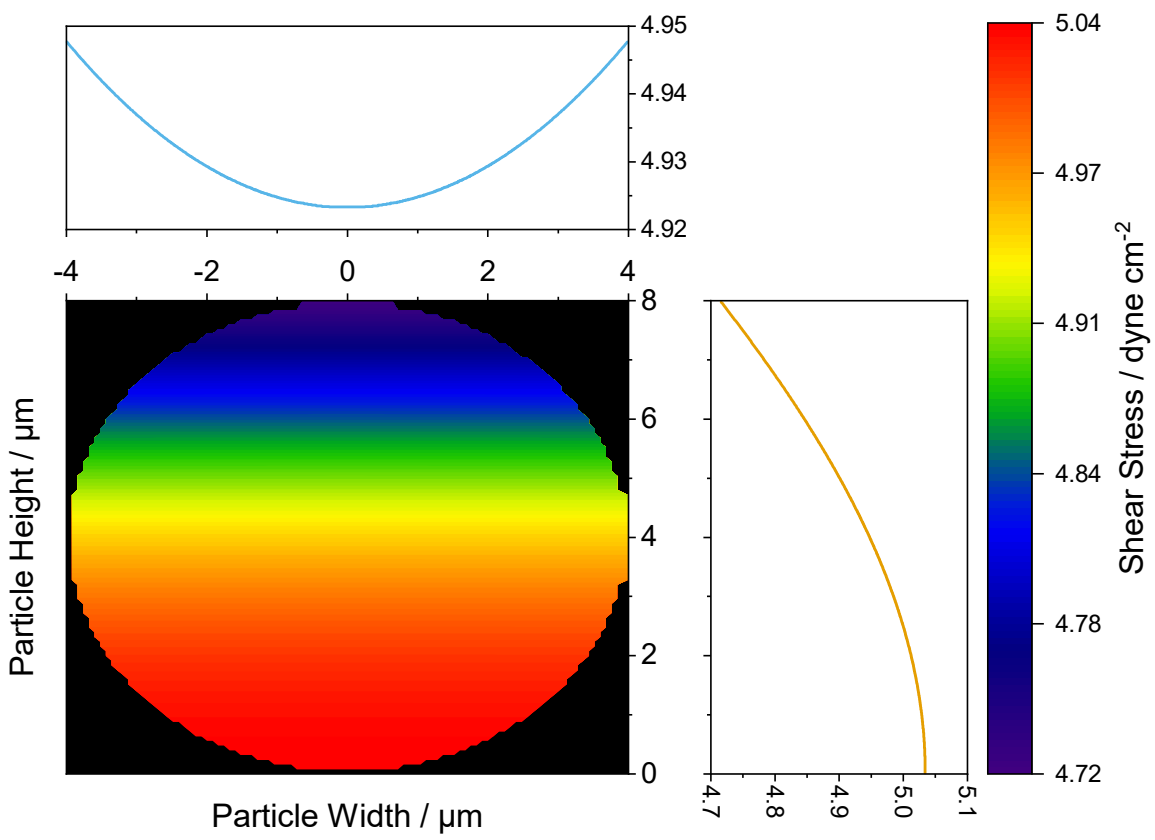


Results

Simulation – Calculus Estimation of the Force-Equilibrium in a Microchannel



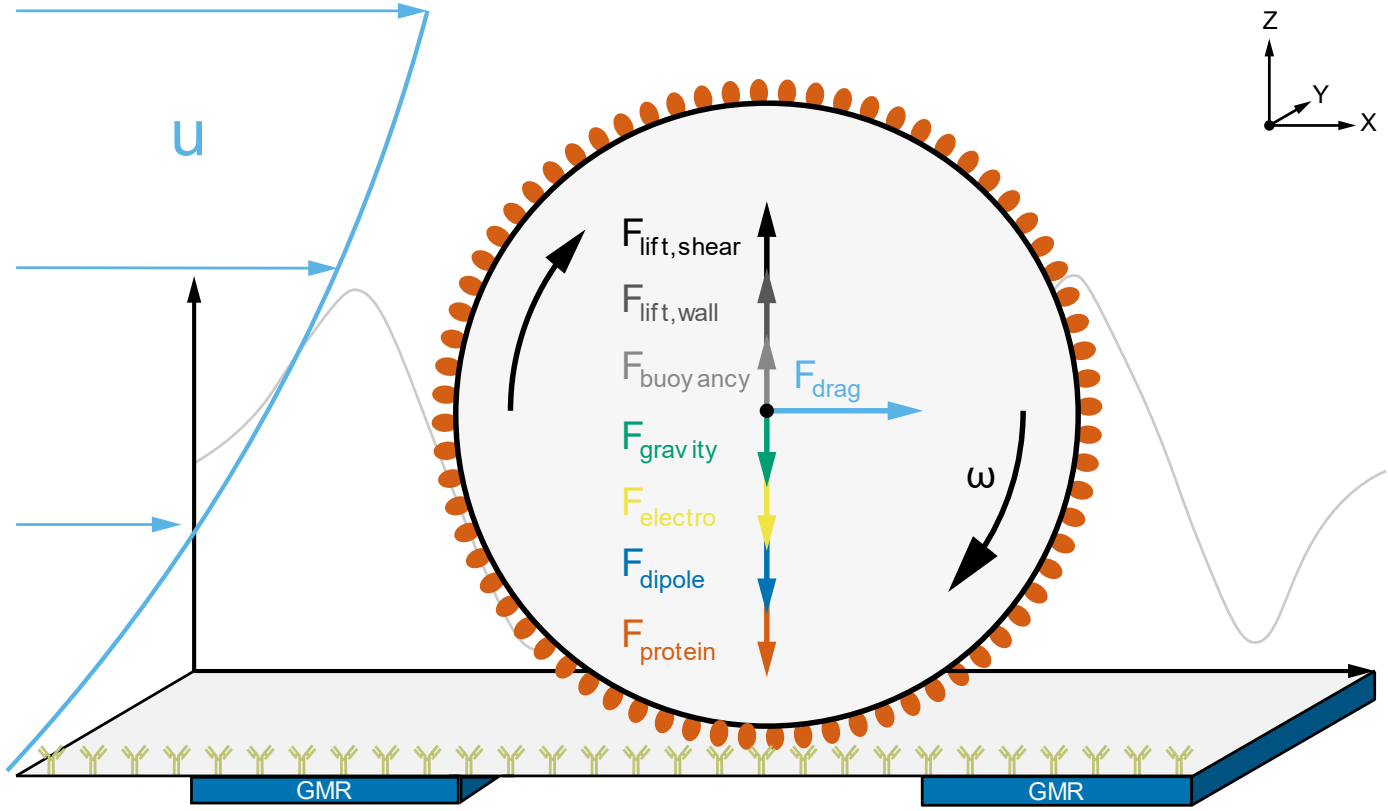
Flow Field in a Particle

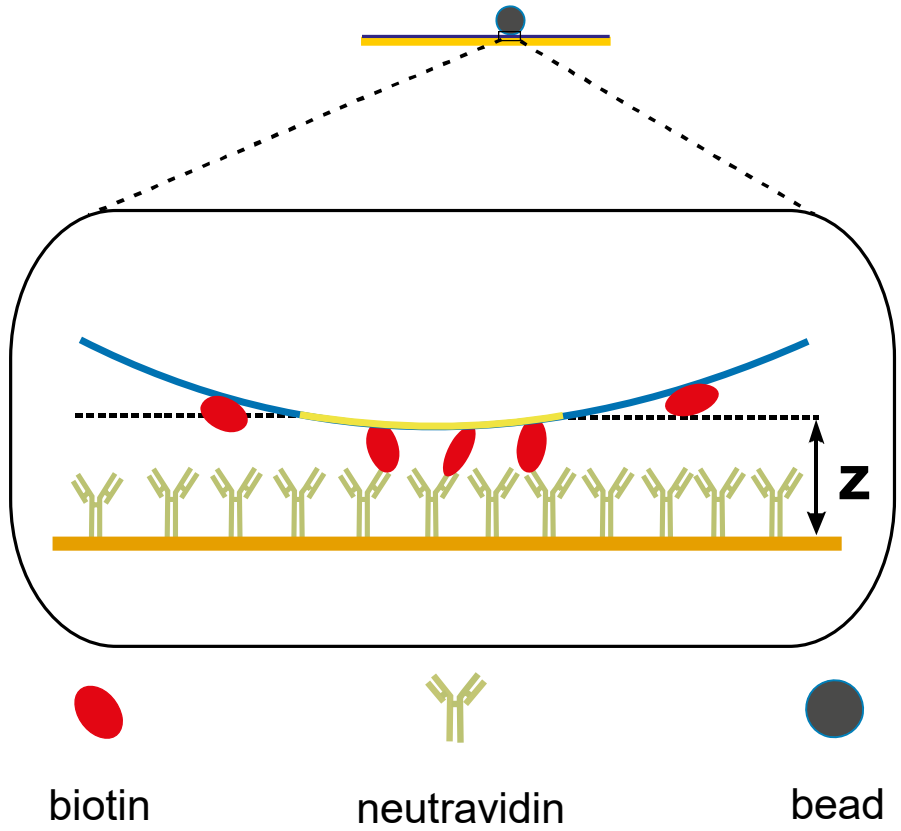


Shear Field in a Particle

Results

Simulation – Calculus Estimation of the Force-Equilibrium in a Microchannel

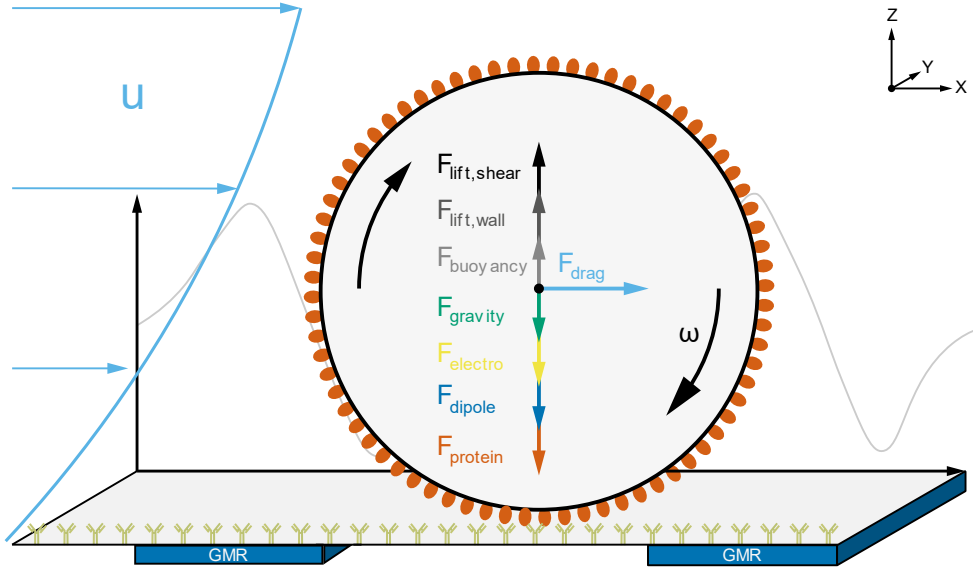




Adopted from Wu and Voldman (2020) *Biosensors and Bioelectronics*, *154*, 112070.

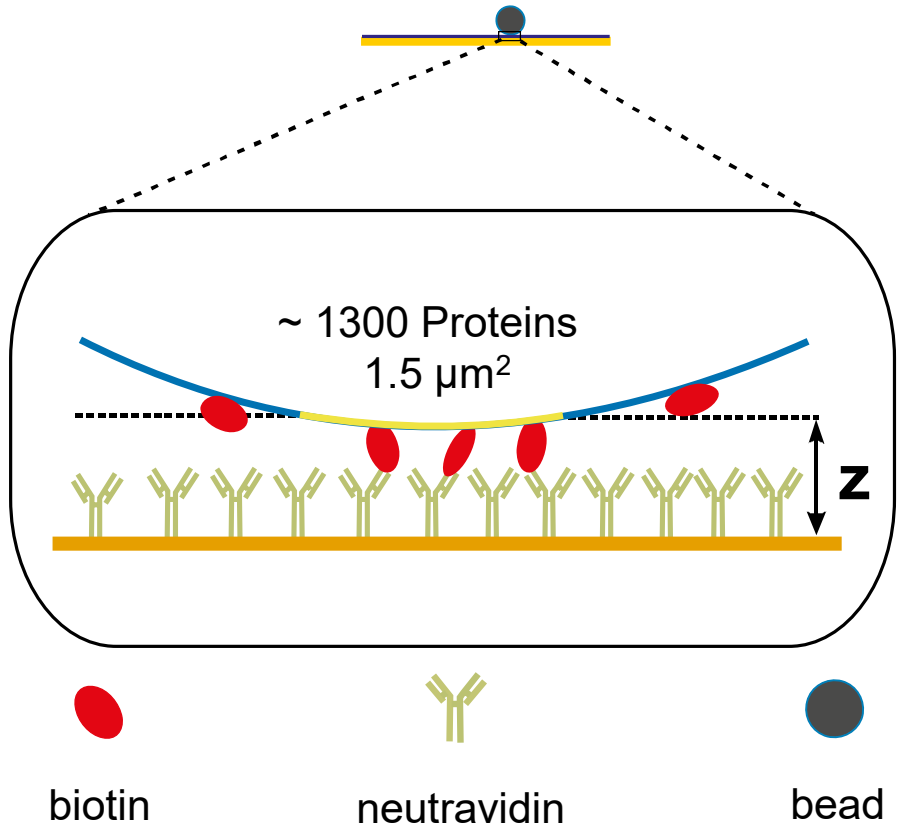
Results

Simulation – Calculus Estimation of the Force-Equilibrium in a Microchannel



$$\mathbf{F}_{\parallel} = \mathbf{F}_{\text{drag}} - C_{\text{rr}} \cdot (\mathbf{F}_{\text{mag}} + \mathbf{F}_{\text{protein}} + \mathbf{F}_{\text{grav}} - \mathbf{F}_{\text{shear}})$$

$$C_{\text{rr}} = \sqrt{\frac{z}{d}} = \sqrt{\frac{30\,\text{nm}}{8\,\mu\text{m}}} = 0.0612$$



Adopted from Wu and Voldman (2020) *Biosensors and Bioelectronics*, *154*, 112070.

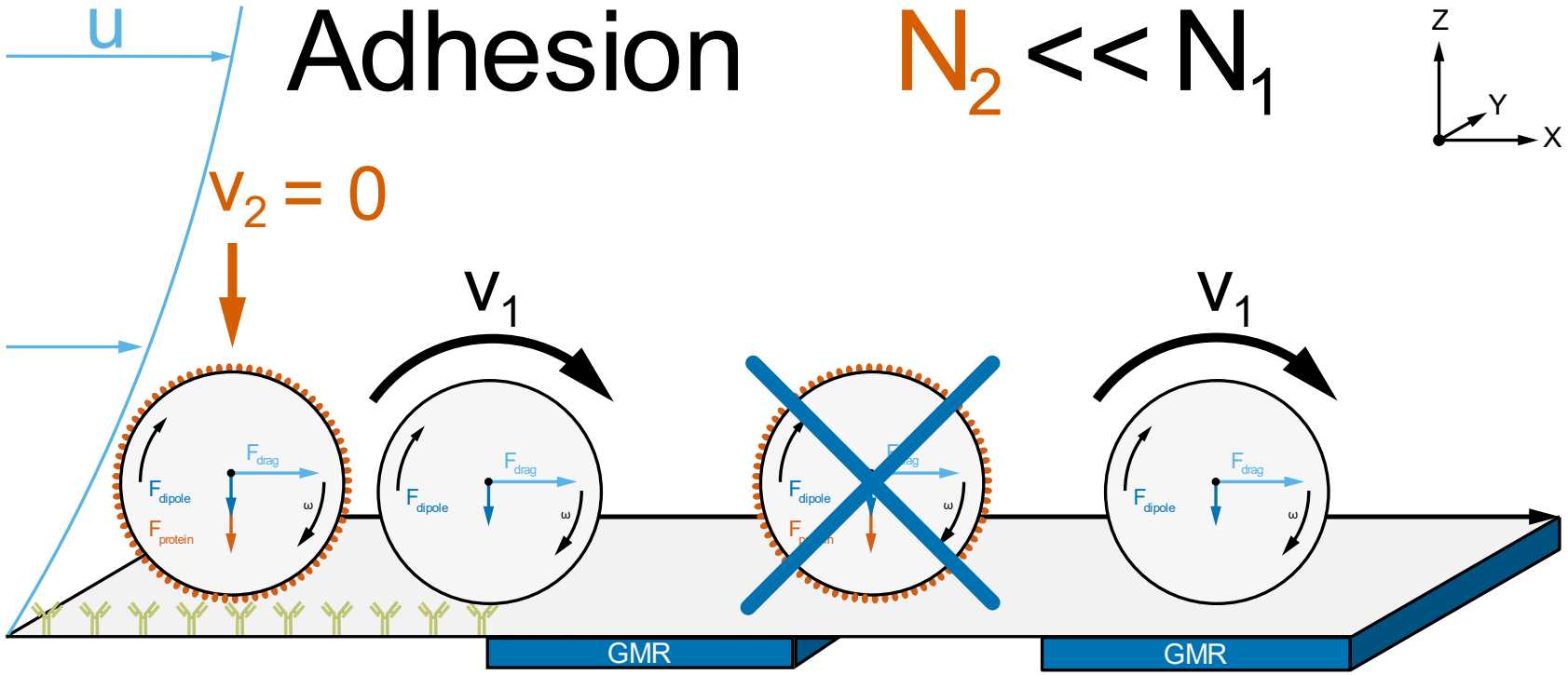
Theory

Surface Chemistry Methods – Biotin-Streptavidin

Predominant Forces
- Stoke‘s Drag: 460 pN
- Dipolar Force: 5 nN
- Protein Force: 5-200 nN

Unfunctionalized Bead
- 150 pN Drag

Bio-Functionalized Bead
- Adhesion caused by 16 - 500 molecules of neutravidin



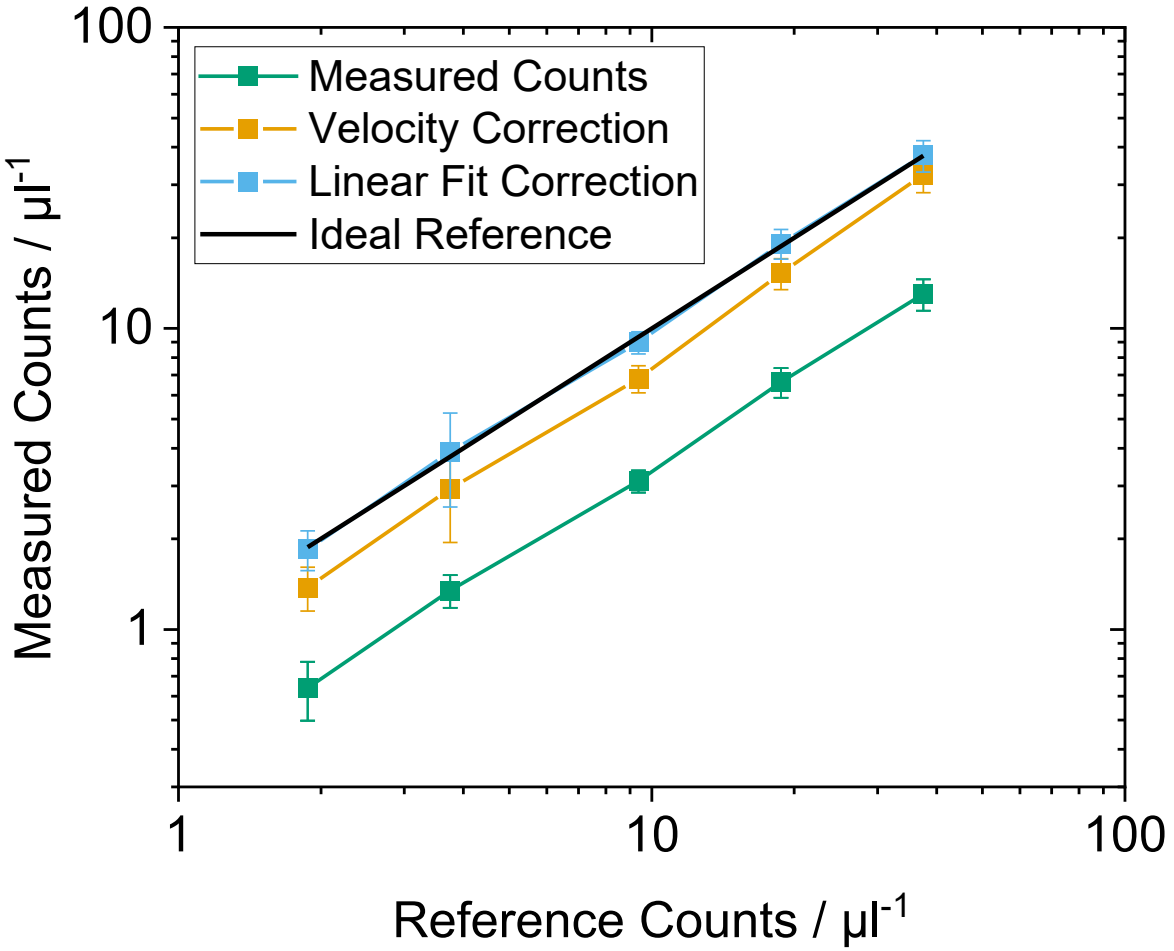
Results

Experimental – Magnetic Concentration Measurements

- Bead stocks characterized in the flow cytometer precisely
- Empirical correction factors to account for bead losses in interconnections and flow profile:

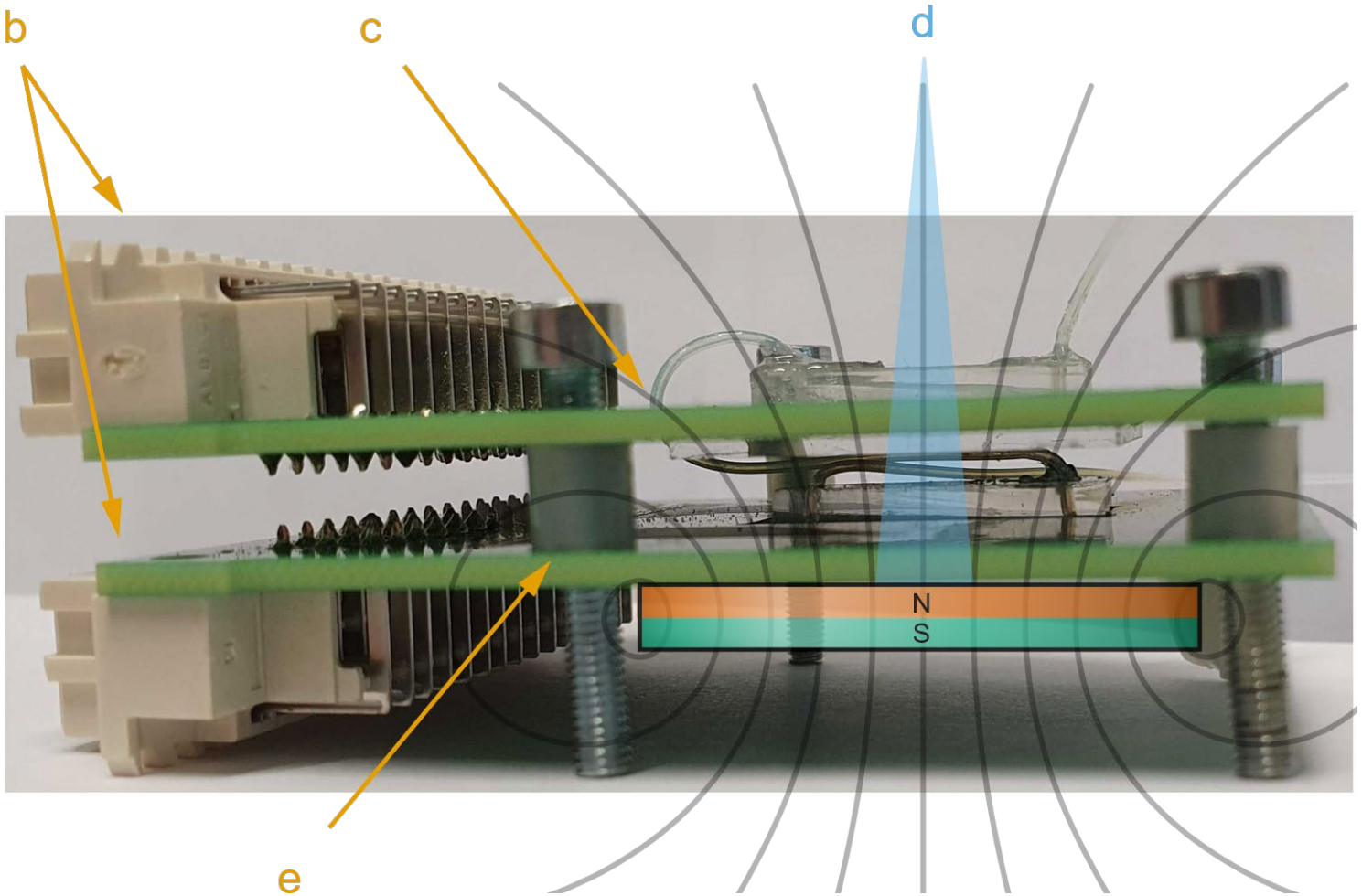
$$C_{\text{const}} = \frac{c_{\text{beads, standard procedure}}}{c_{\text{beads, MRCyte}}}$$

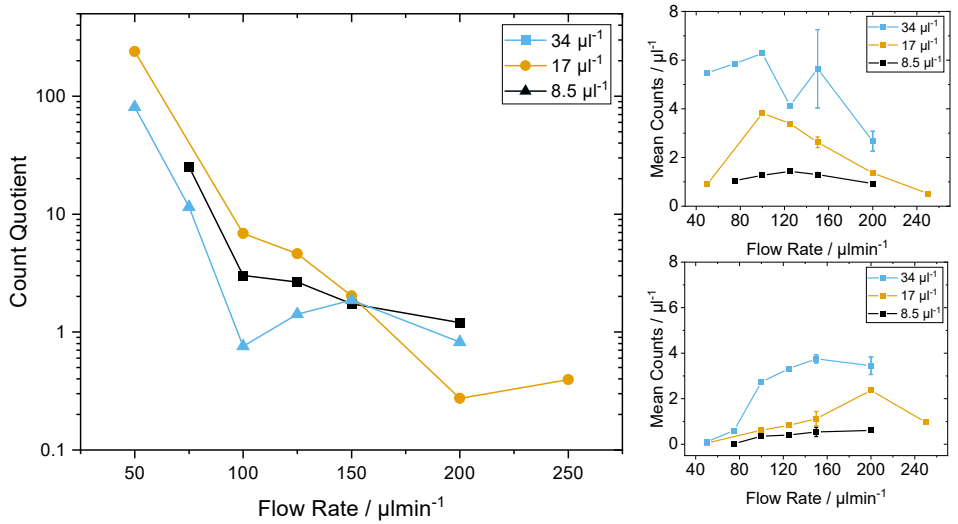
$$C_{\text{velocity}} = \frac{\overline{\mathbf{u}}}{v_c} = \frac{Q}{A \cdot v_c}$$



Results

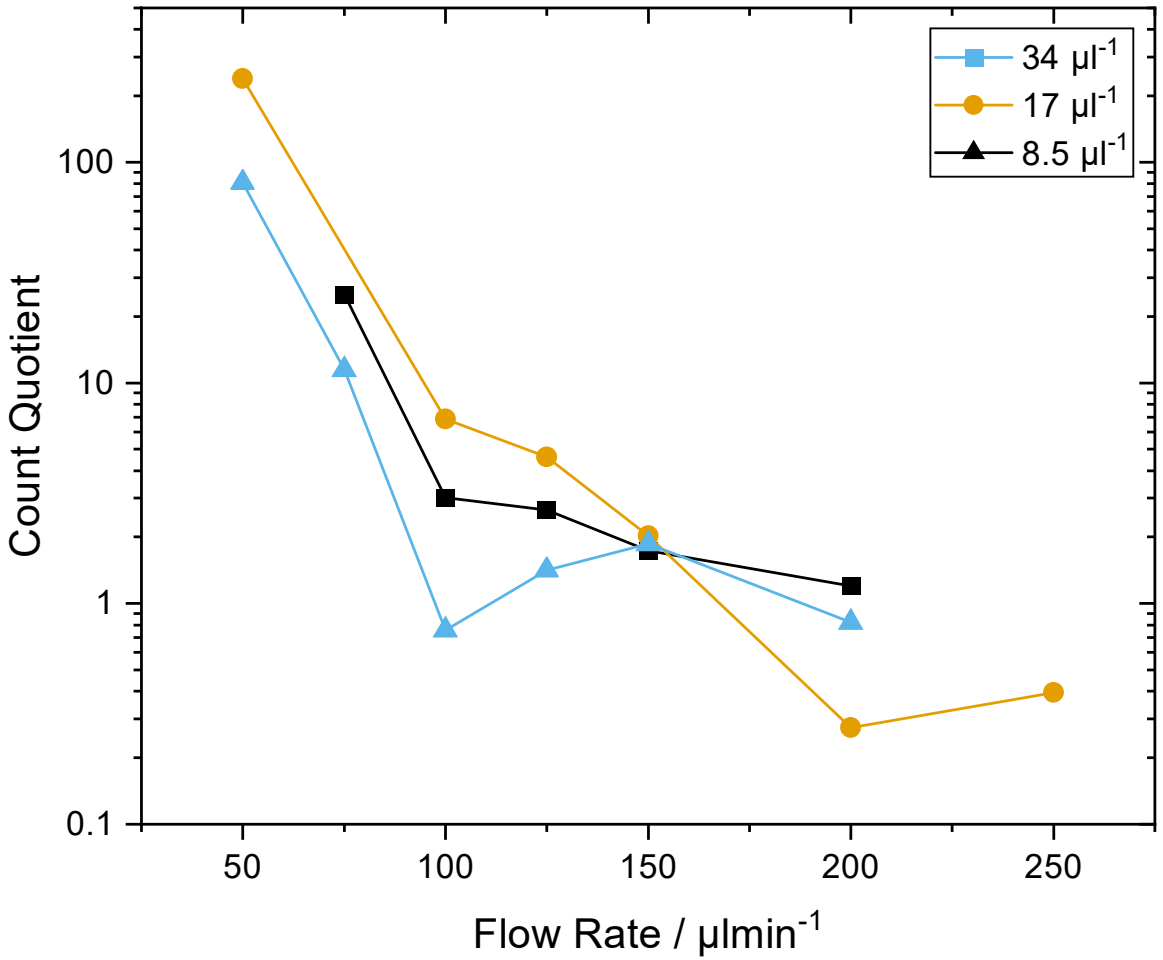
Experimental – Development of a Differential Counting Setup

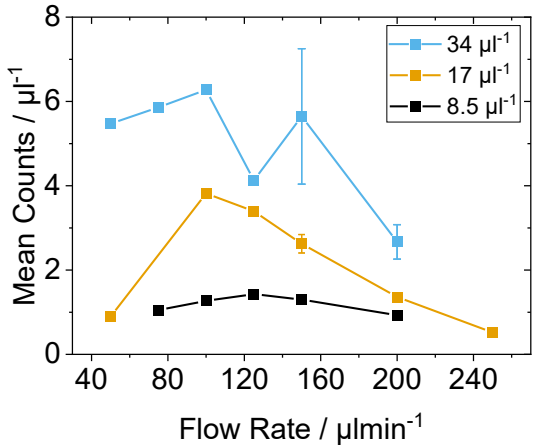


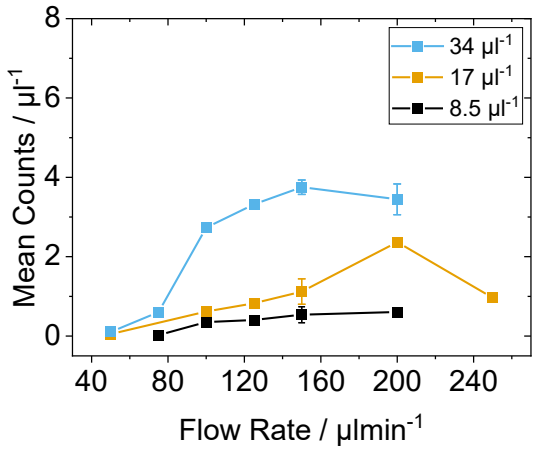


Results

Experimental – Development of a Differential Counting Setup







Results

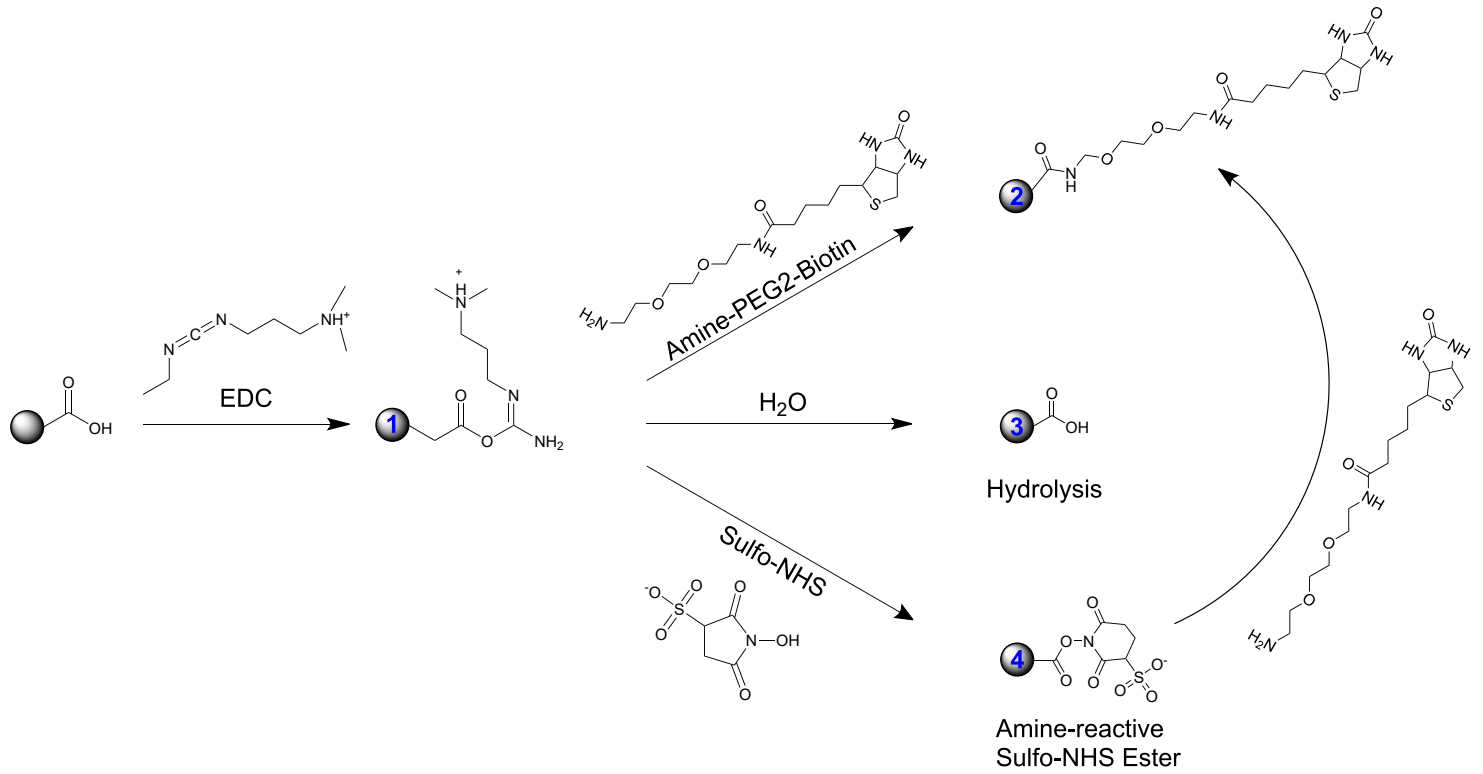
Experimental – Established Reference Model for Surface Marker Expression Densities

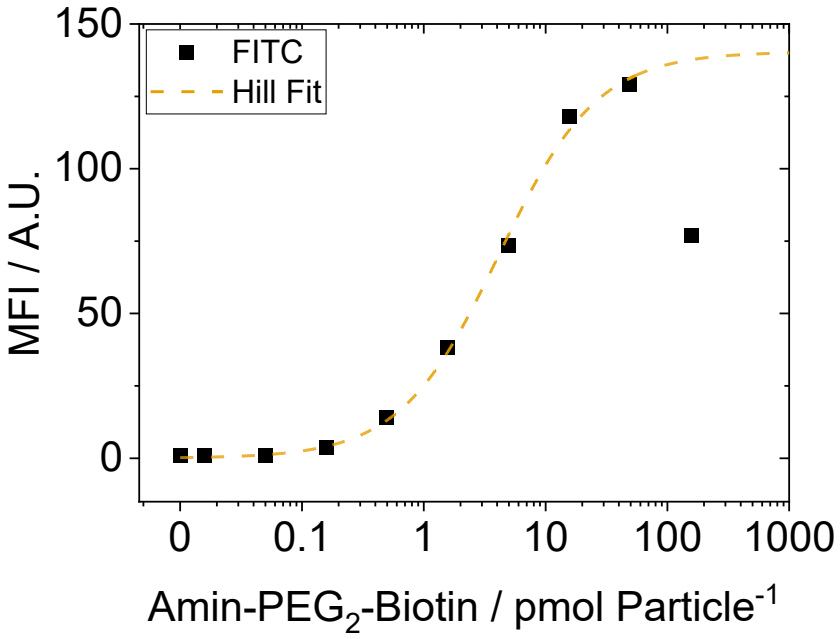
- Proof of concept for the simulations in the magnetic flow cytometer

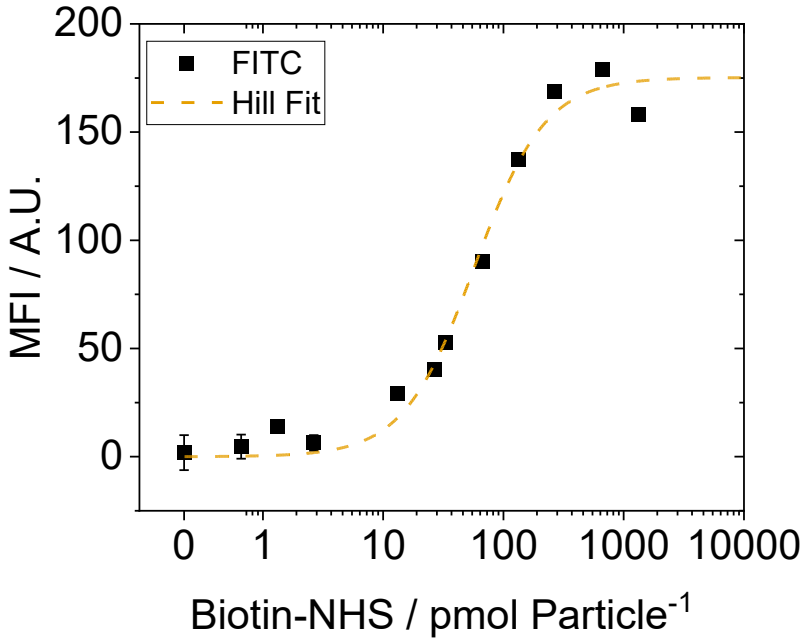
→ **Epitope Density on a cell has to be modulated**

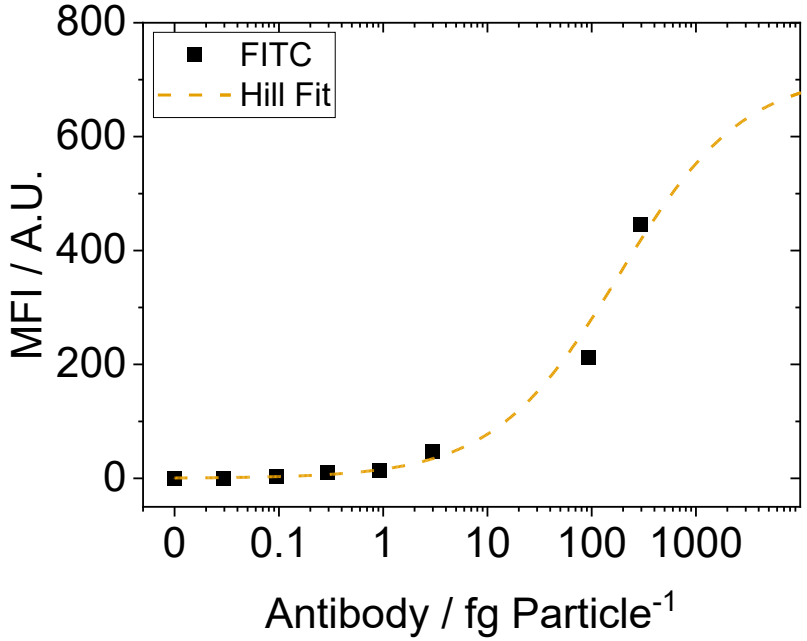
- Development of a methodology to coat beads with highly defined surface markers
- Universally applicable to proteins

→ **Carbodiimide Chemistry**




FITC
Hill Fit
MFI / A.U.
150
100
50
0
0
0.1
1
10
100
1000
Amin-PEG2-Biotin / pmol Particle-1


FITC
Hill Fit
MFI / A.U.
200
150
100
50
0
0
1
10
100
1000
10000
Biotin-NHS / pmol Particle-1


FITC
Hill Fit
MFI / A.U.
800
600
400
200
0
0
0.1
1
10
100
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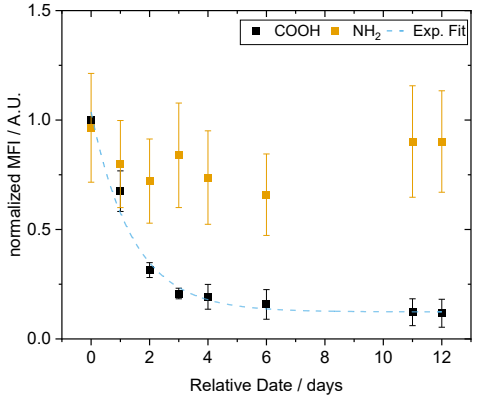
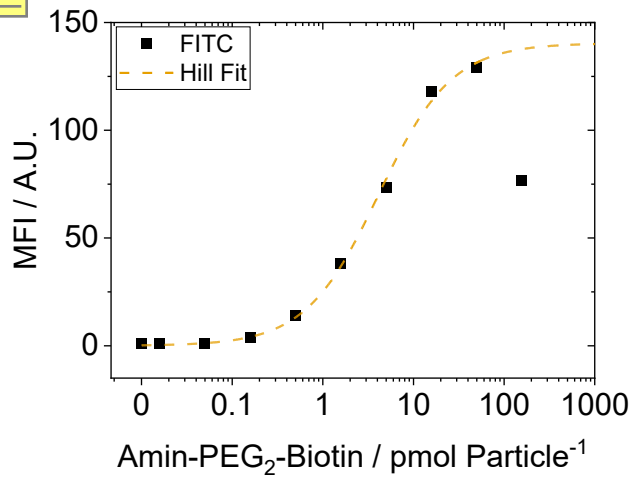
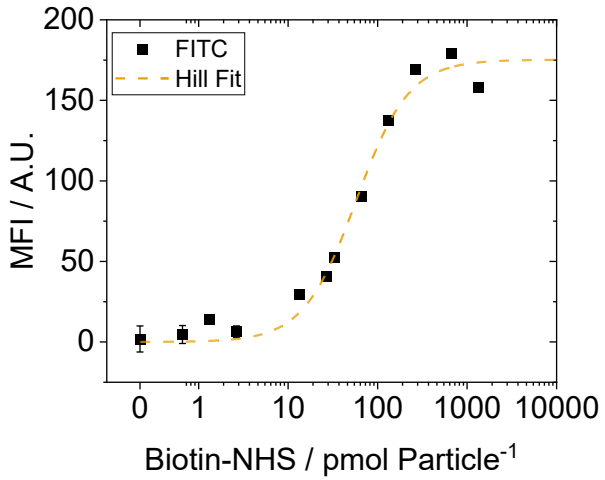
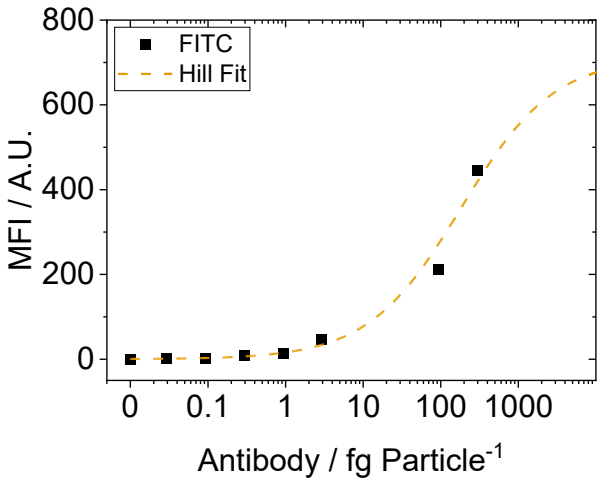
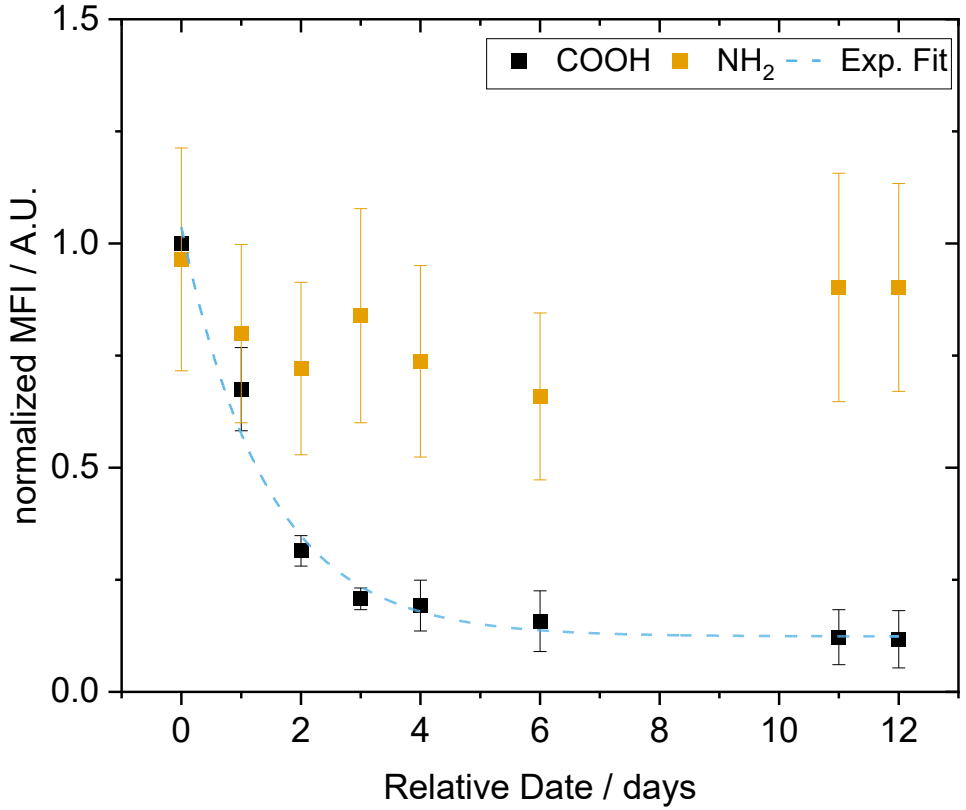
Antibody / fg Particle-1


COOH
NH2
Exp. Fit
normalized MFI / A.U.
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
Relative Date / days


FITC
Hill Fit
MFI / A.U.
Amin-PEG2-Biotin / pmol Particle-1

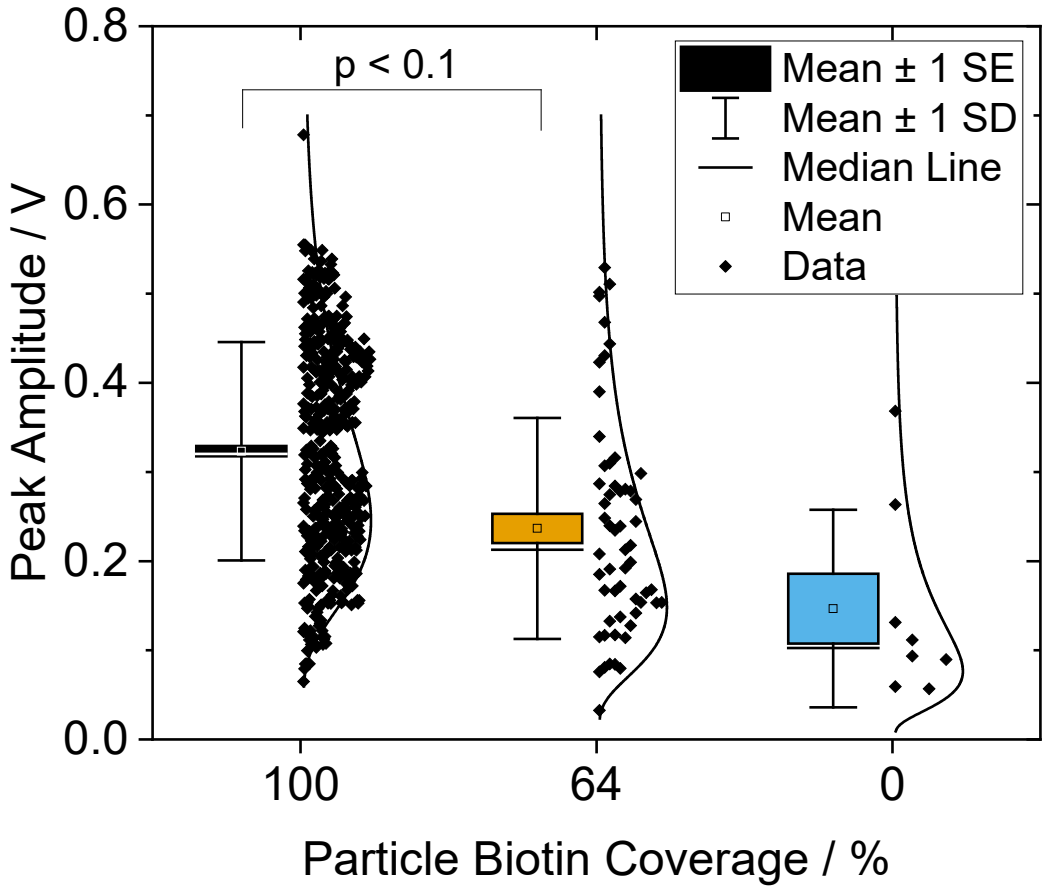

FITC
Hill Fit
MFI / A.U.
Biotin-NHS / pmol Particle-1

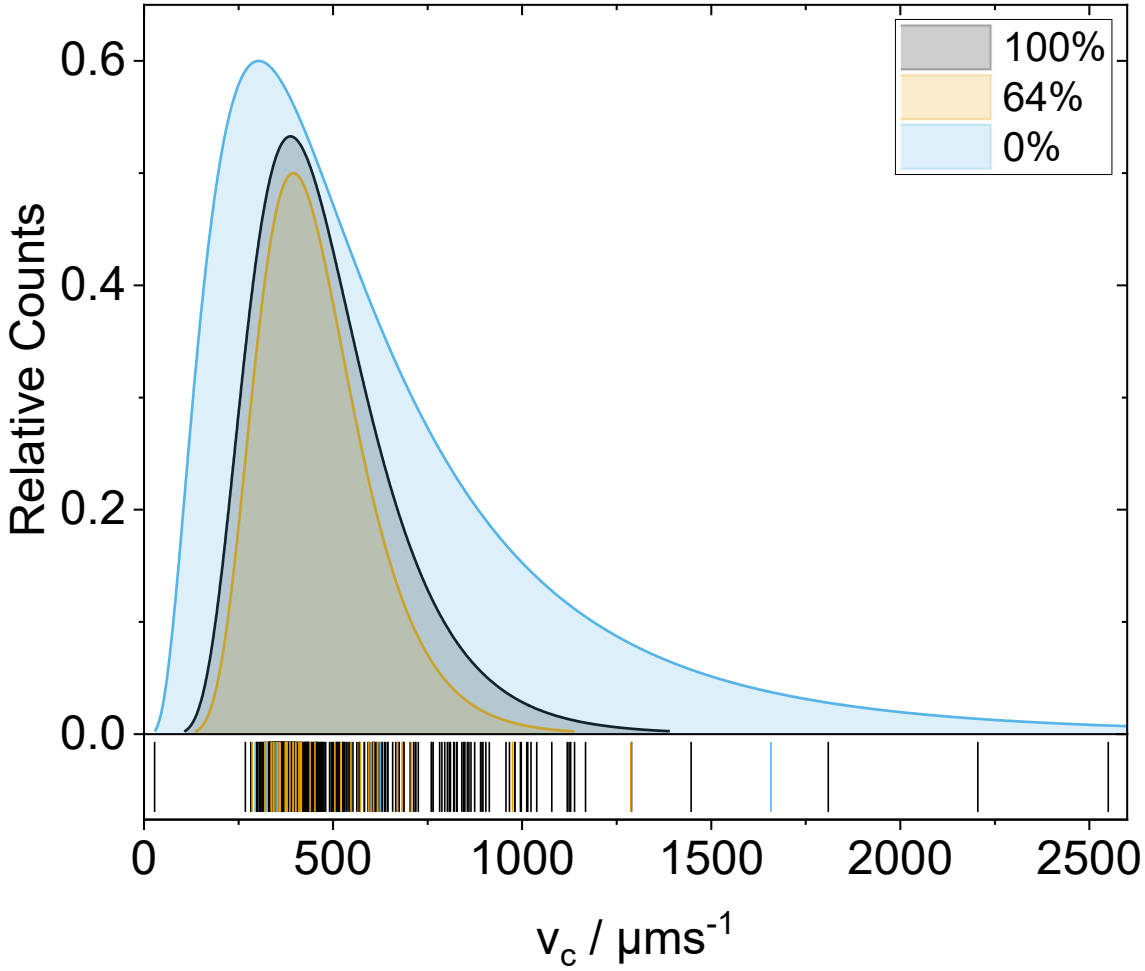

FITC
Hill Fit
MFI / A.U.
Antibody / fg Particle-1


COOH
NH2
Exp. Fit
normalized MFI / A.U.
Relative Date / days

Results

Experimental – Established Reference Model for Surface Marker Expression Densities

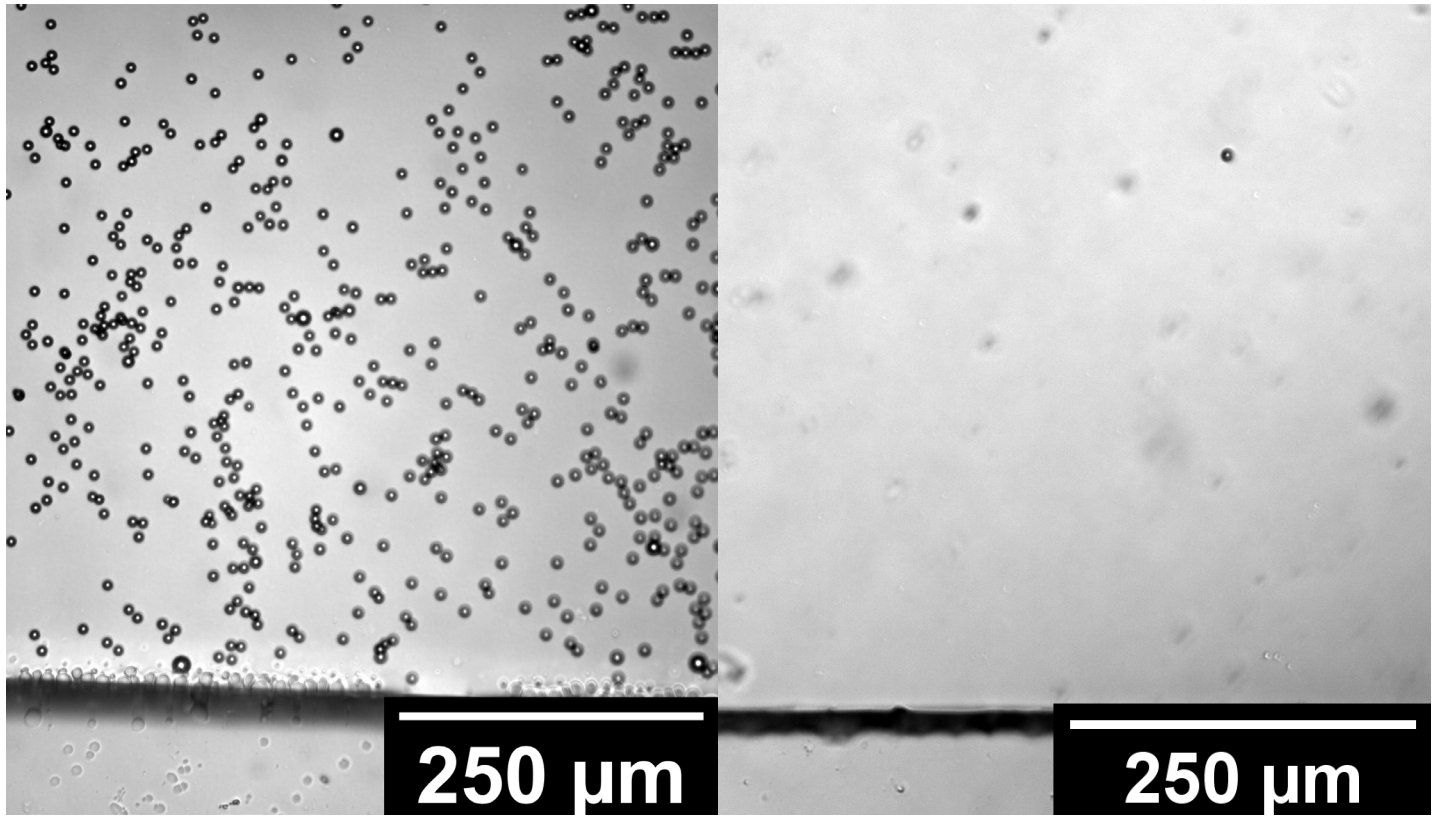




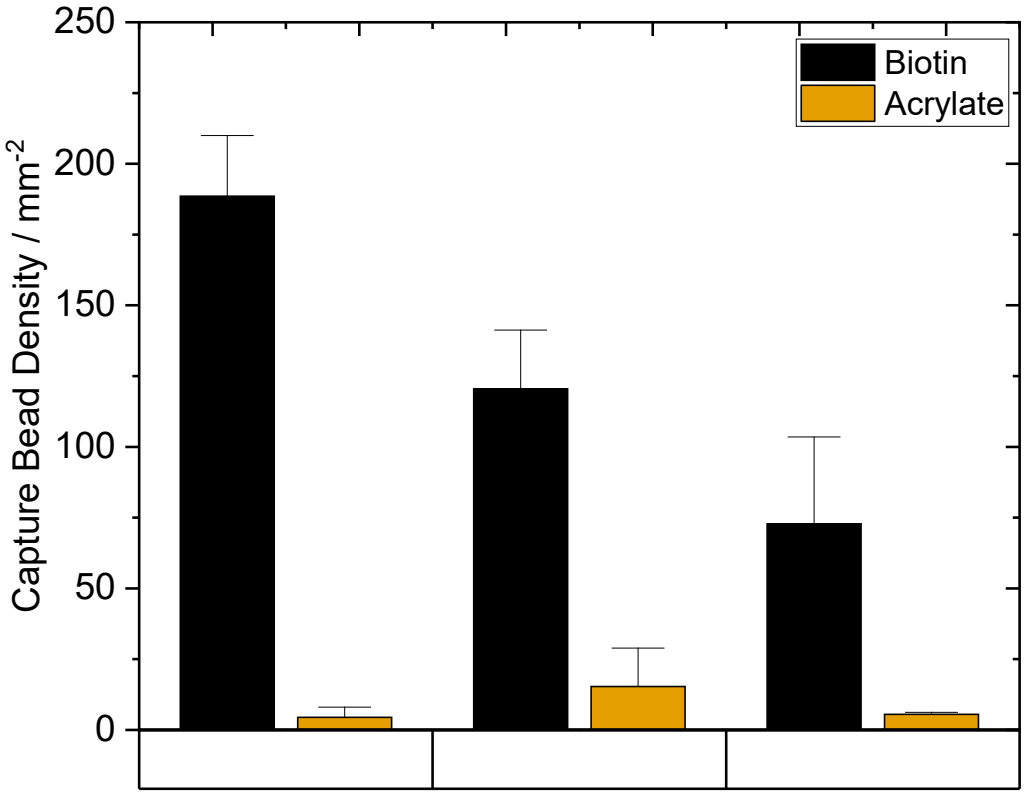
Concenctration measurement of magnetized polystyrene beads

Results

Experimental – Affinity-based Concentration Measurements in the Magnetic Flow Cytometer

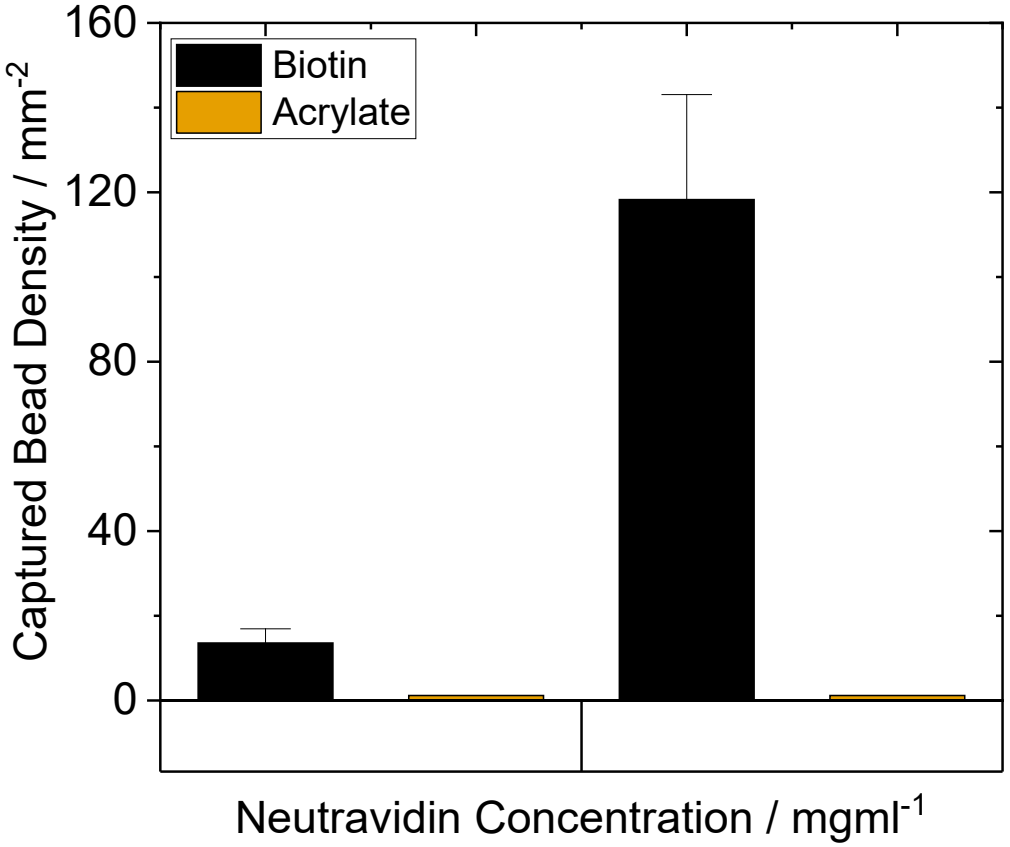


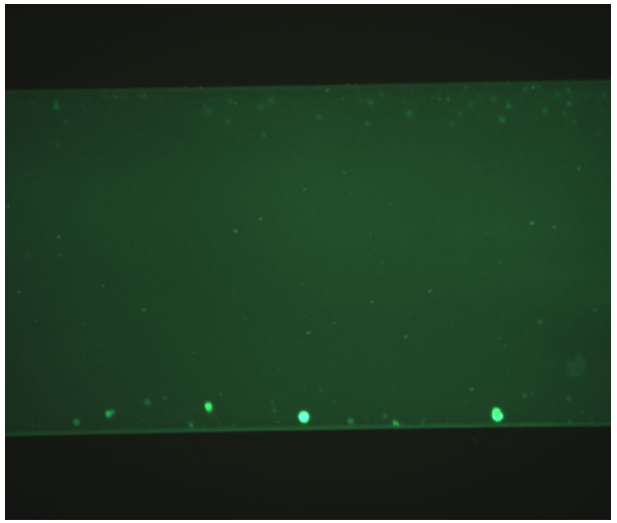
Biotinylated Beads **Plain Beads**

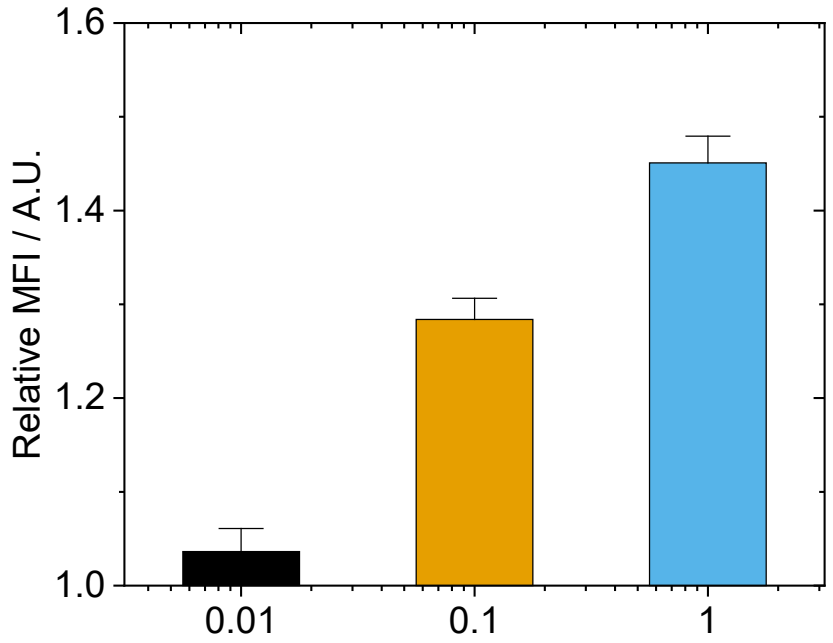


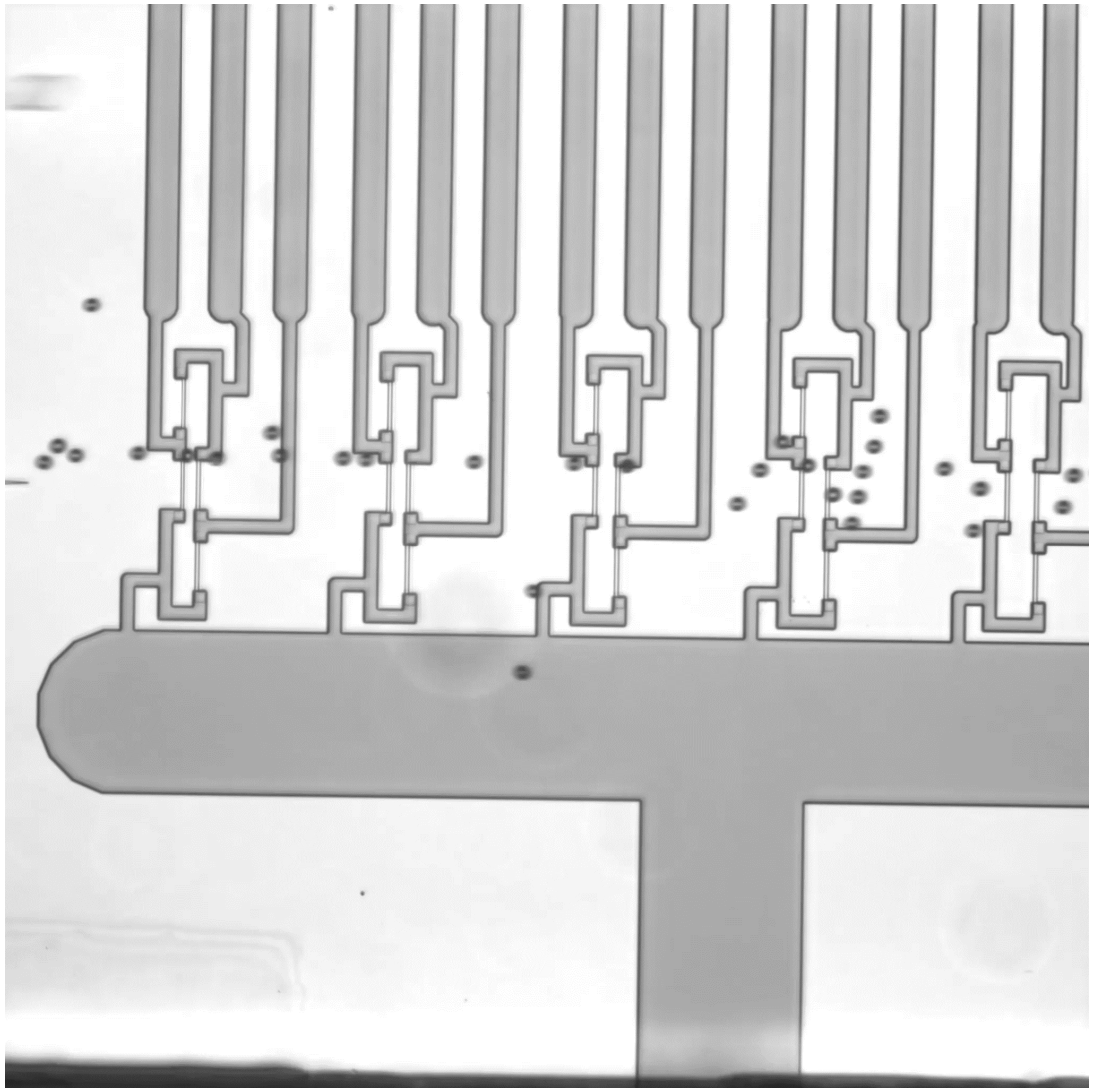
Results

Experimental – Affinity-based Concentration Measurements in the Magnetic Flow Cytometer

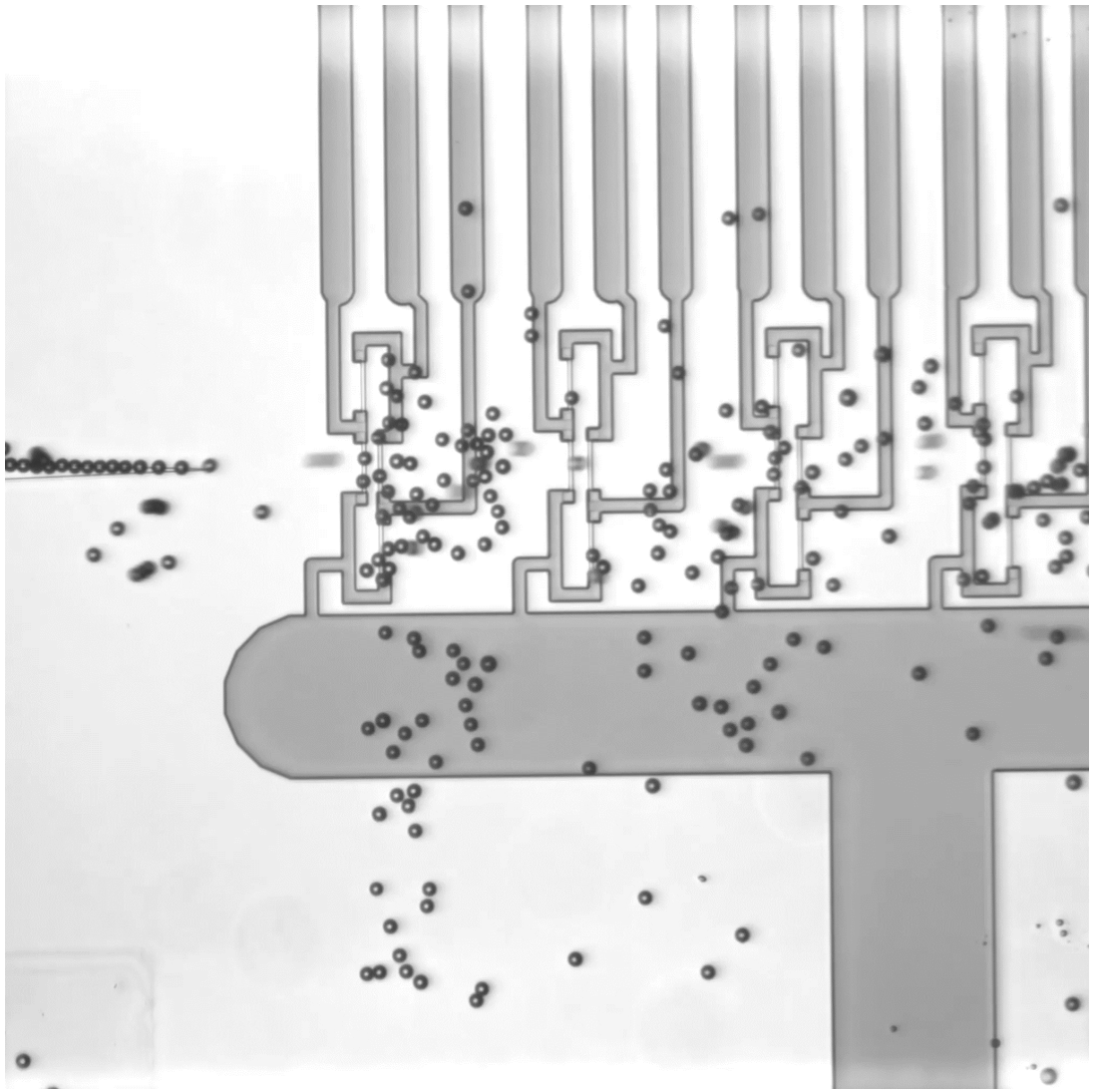








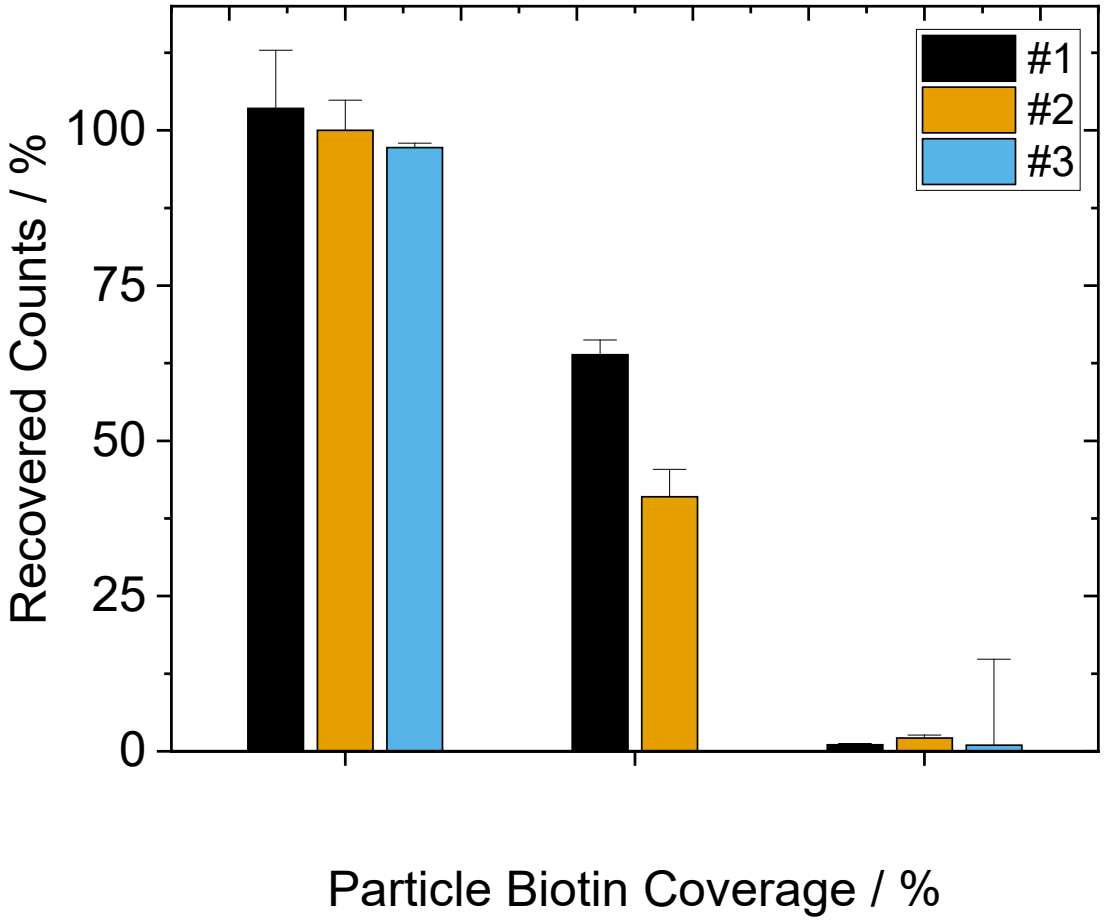
Plain Particles

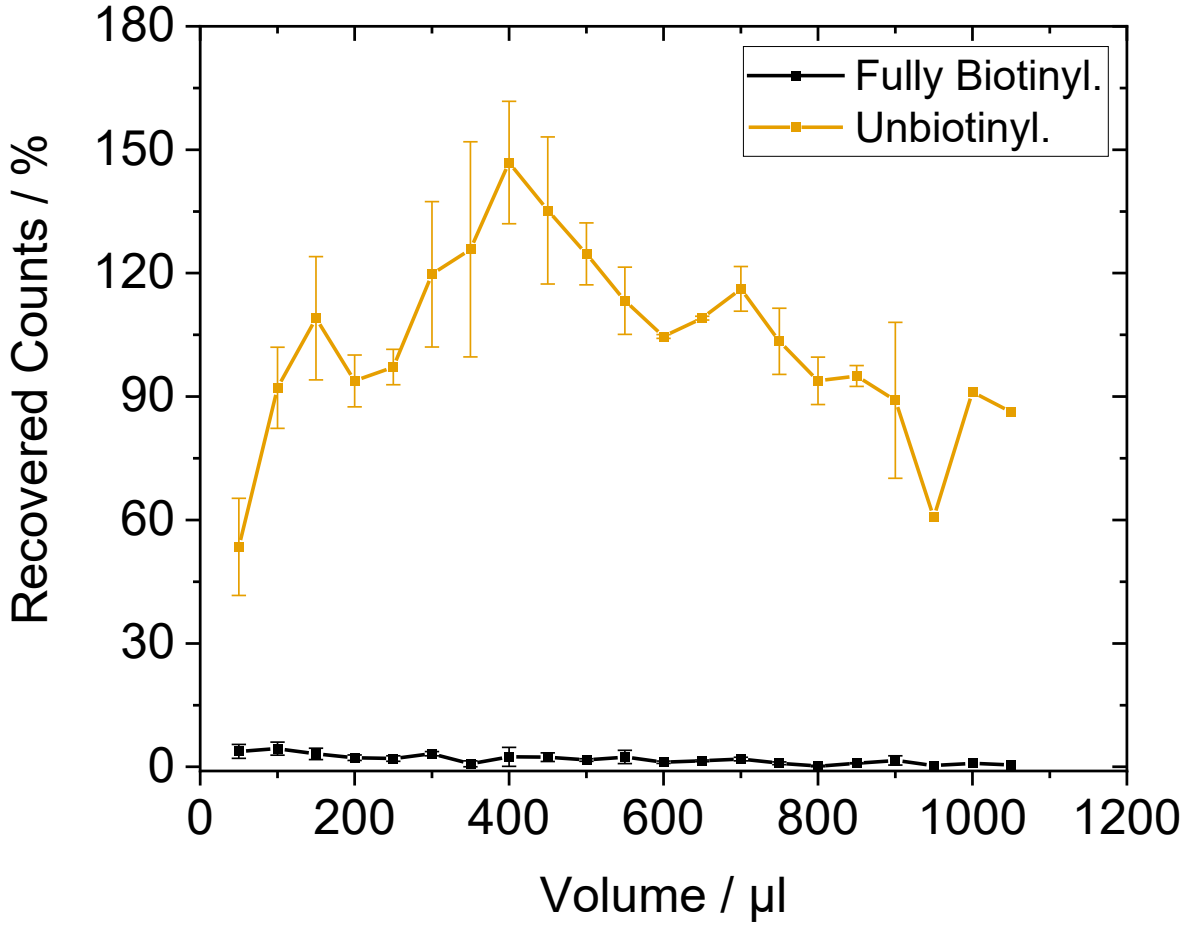


Biotinylated Particles

Results

Experimental – Affinity-based Concentration Measurements in the Magnetic Flow Cytometer





Conclusion

Outcomes of this thesis

1. Established **hydrodynamic** and **magnetic simulations** for physical phenomena of rolling cells
2. Benchmarked **absolute** and **differential concentration measurements** for the magnetic flow cytometer
3. Developed a **reference system** for the variation of the surface receptor density
4. Demonstrated **an affinity-dependent assay** in the magnetic flow cytometer

Thank you, LBE!

Magnetic Field Computations

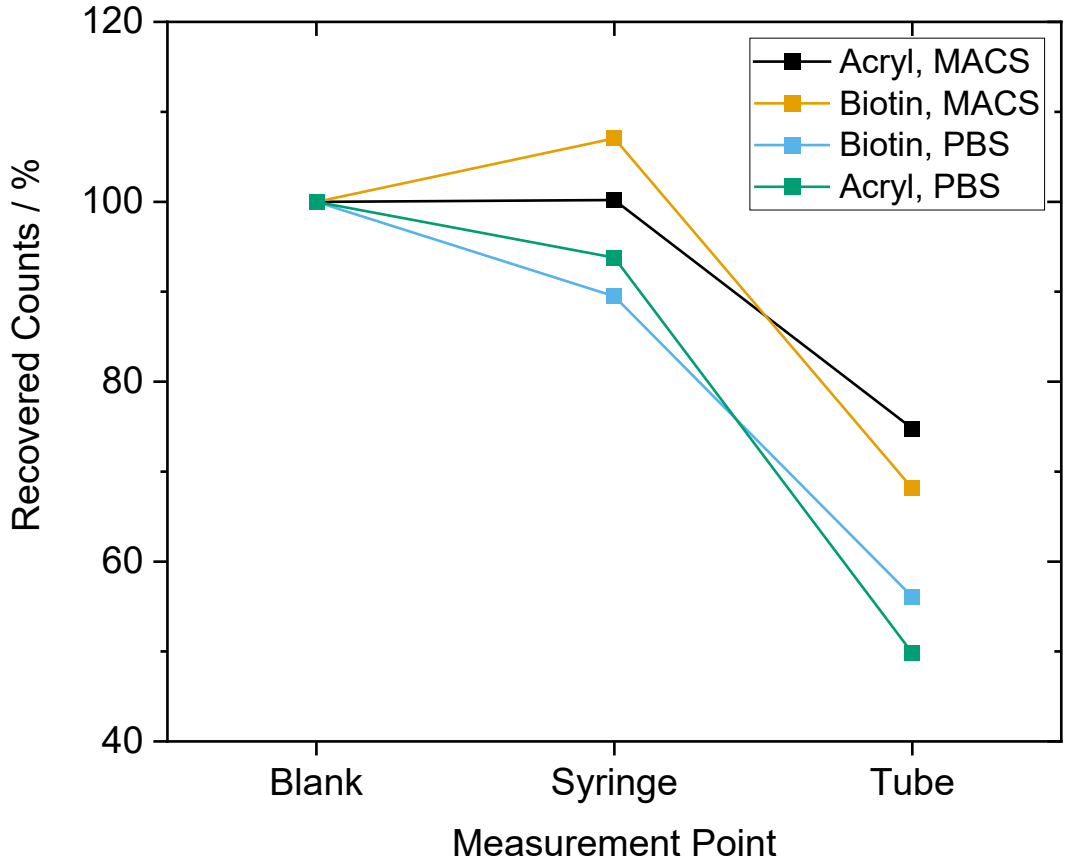
$$\mathbf{B}(t) = \sum_{i=1}^{N} \frac{1}{A_{\text{Sensor}}} \int_{-\frac{l}{2}}^{\frac{l}{2}} \int_{-\frac{w}{2}}^{\frac{w}{2}} \frac{\mu_o}{4\pi} \left(\frac{3\mathbf{r}_i(t)\,(\mathbf{r}_i(t) \cdot \mathbf{m}_i)}{|\mathbf{r}_i(t)|^5} - \frac{\mathbf{m}_i}{|\mathbf{r}_i(t)|^3} \right) \text{dx dy} \qquad 1.1$$

$$\mathbf{R}_{\text{sig}}(t) = -\mathbf{B}(t) \times \frac{S}{100} \times R + R \qquad 1.2$$

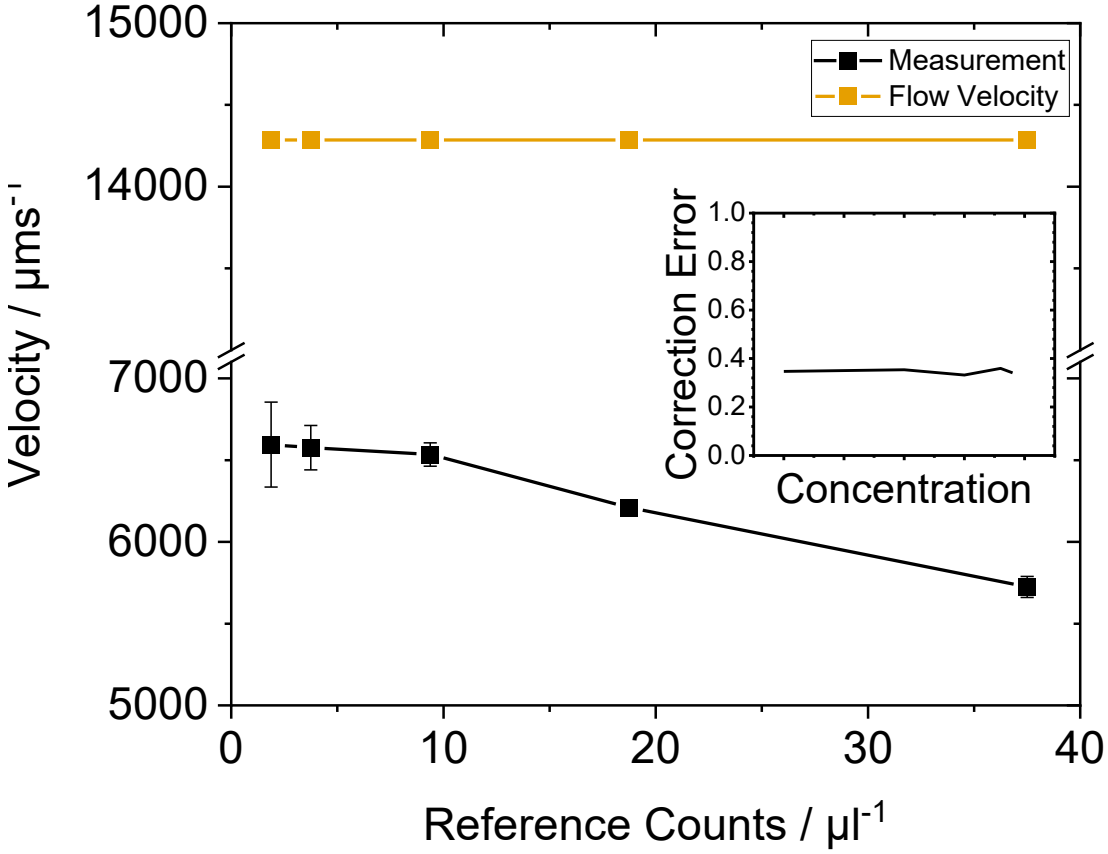
$$\mathbf{V}_{\text{sig}}(t) = \frac{\mathbf{R}_{\text{sig}}(t)}{R + \mathbf{R}_{\text{sig}}(t)} \times V_{\text{p}} - \frac{V_{\text{p}}}{2} \qquad 1.3$$

$$\max\{R_{\text{x y}}(\tau)\} = \max\left\{ \int \mathbf{V}^*_{\text{ref}}(t)\mathbf{V}_{\text{sig}}(t+\tau)\text{dt} \right\} \qquad 1.4$$

Error Sources in the Magnetic Flow Cytometer



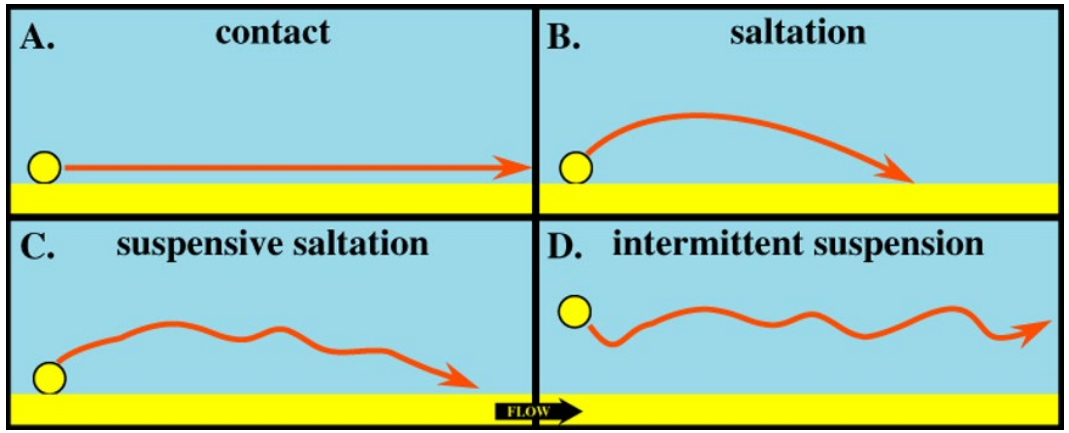
Losses in Interconnections

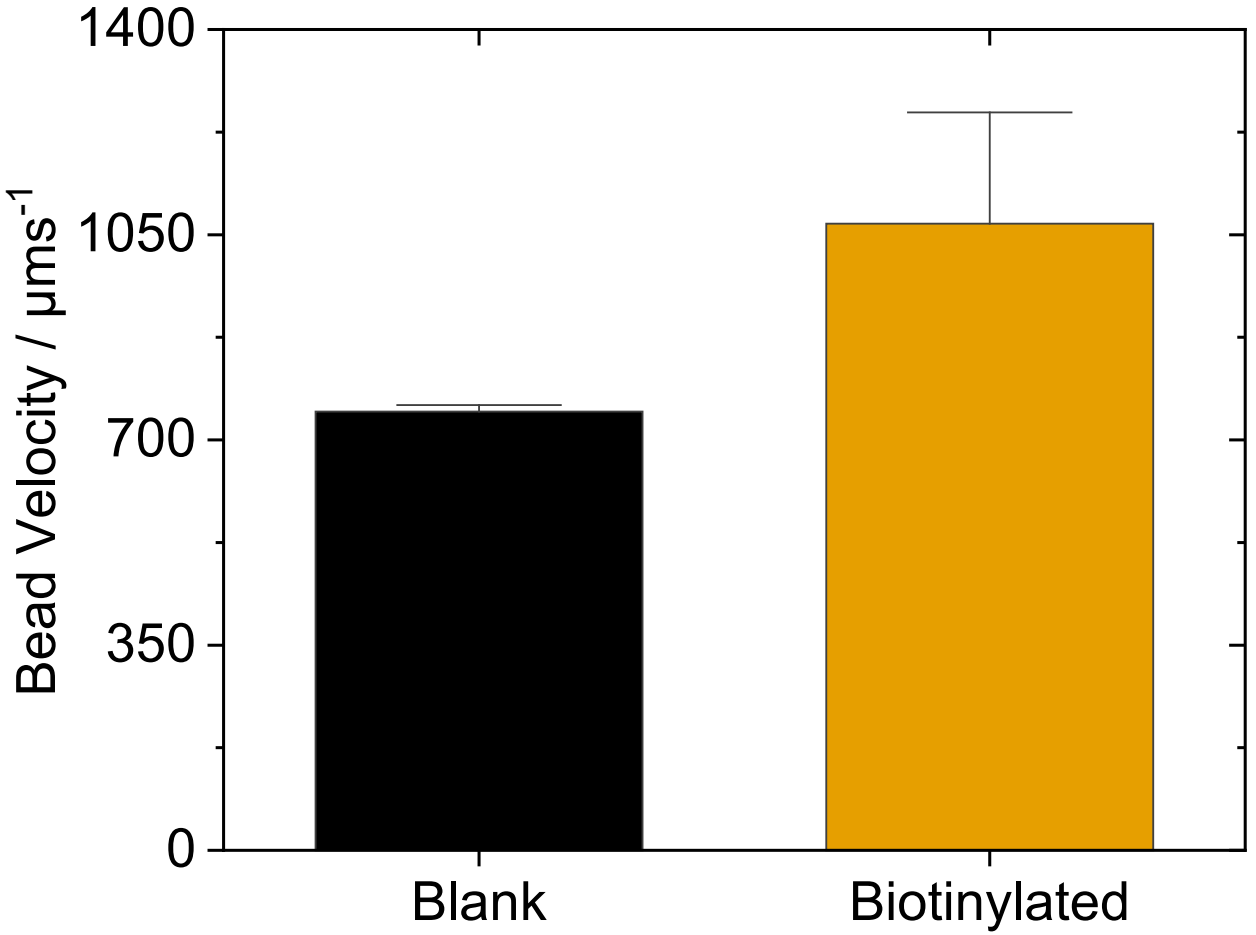


Systematic Error in Velocity Measurements at High Concentrations

Affinity-dependent Bead Velocity

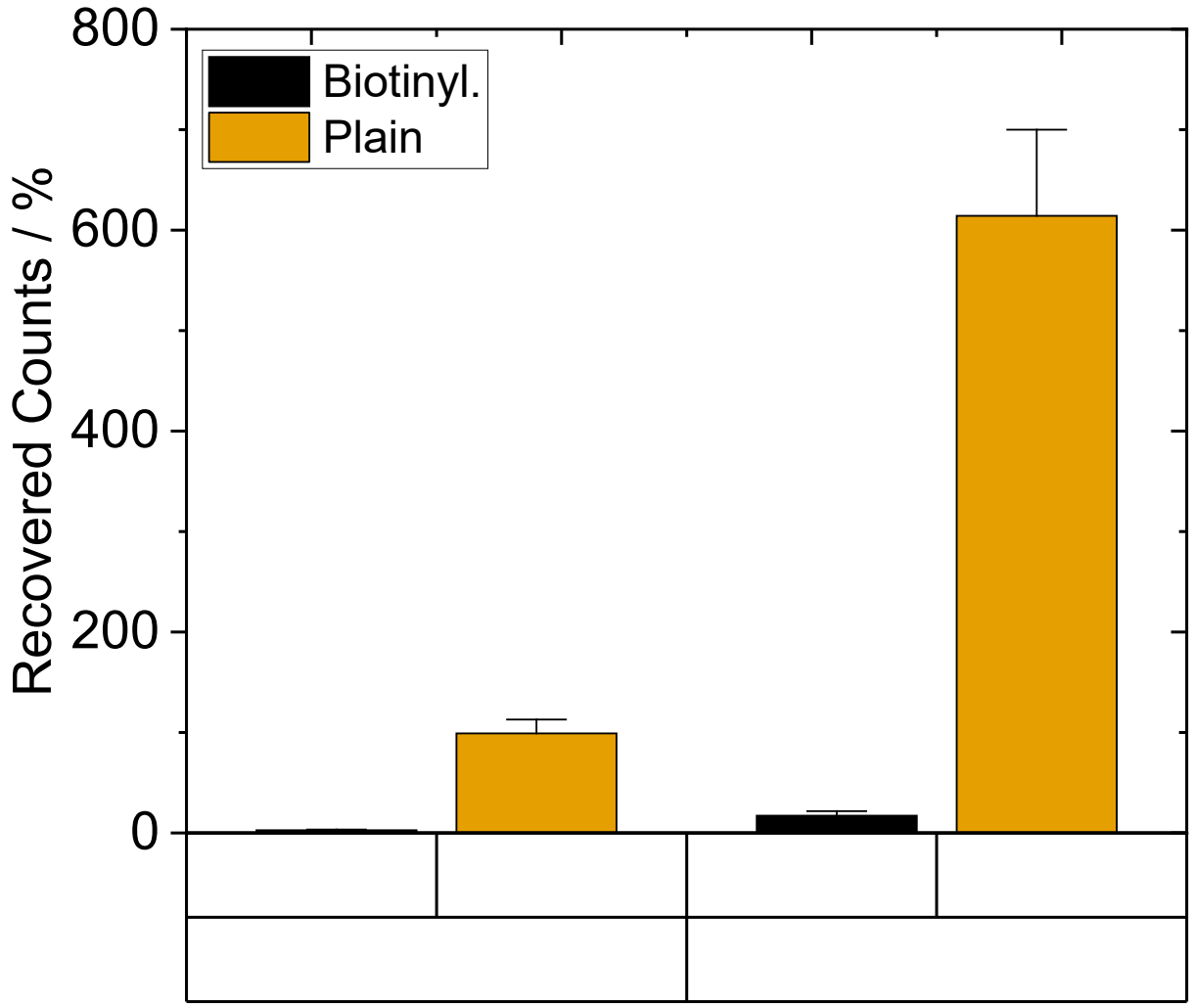
- Magnetically measured bead velocity of biotinlyated bead is statistically higher
- Probably attributed to a saltatory movement after adhesion to the channel (**B,C**)
- After adhesion, a wake forms behind the bead thus causes increasing pressure on the back





Comparison of Empirically Determined Correction Factors

Errors in Affinity-Based Measurements



Error Sources Affinity-Dependent Concentration Measurement

- Inital 100 µl:
  - magnetophoretic structures are filled

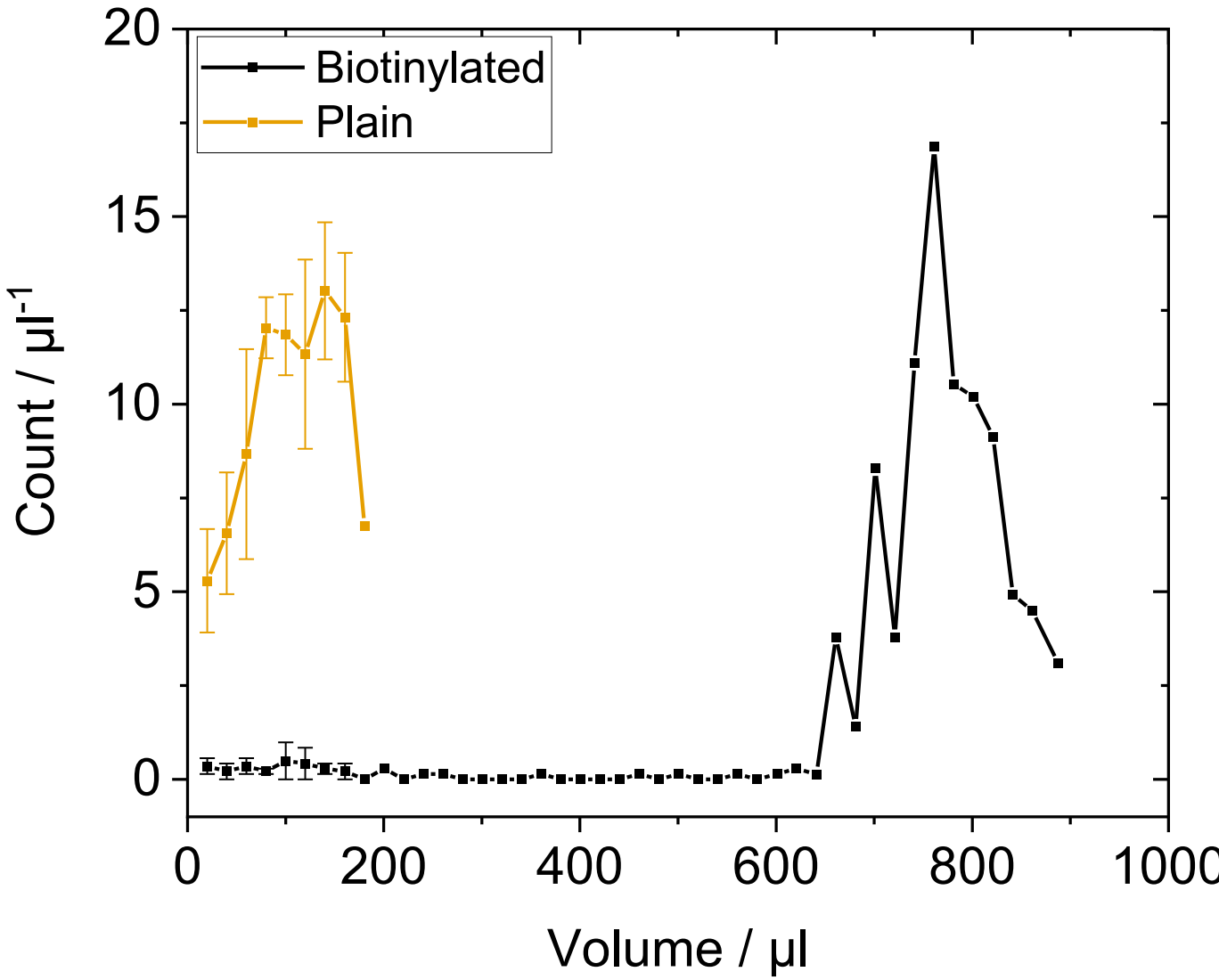
  ➔ Steep rise

- After 700µl:
  - Surface is saturated with biotinylated beads

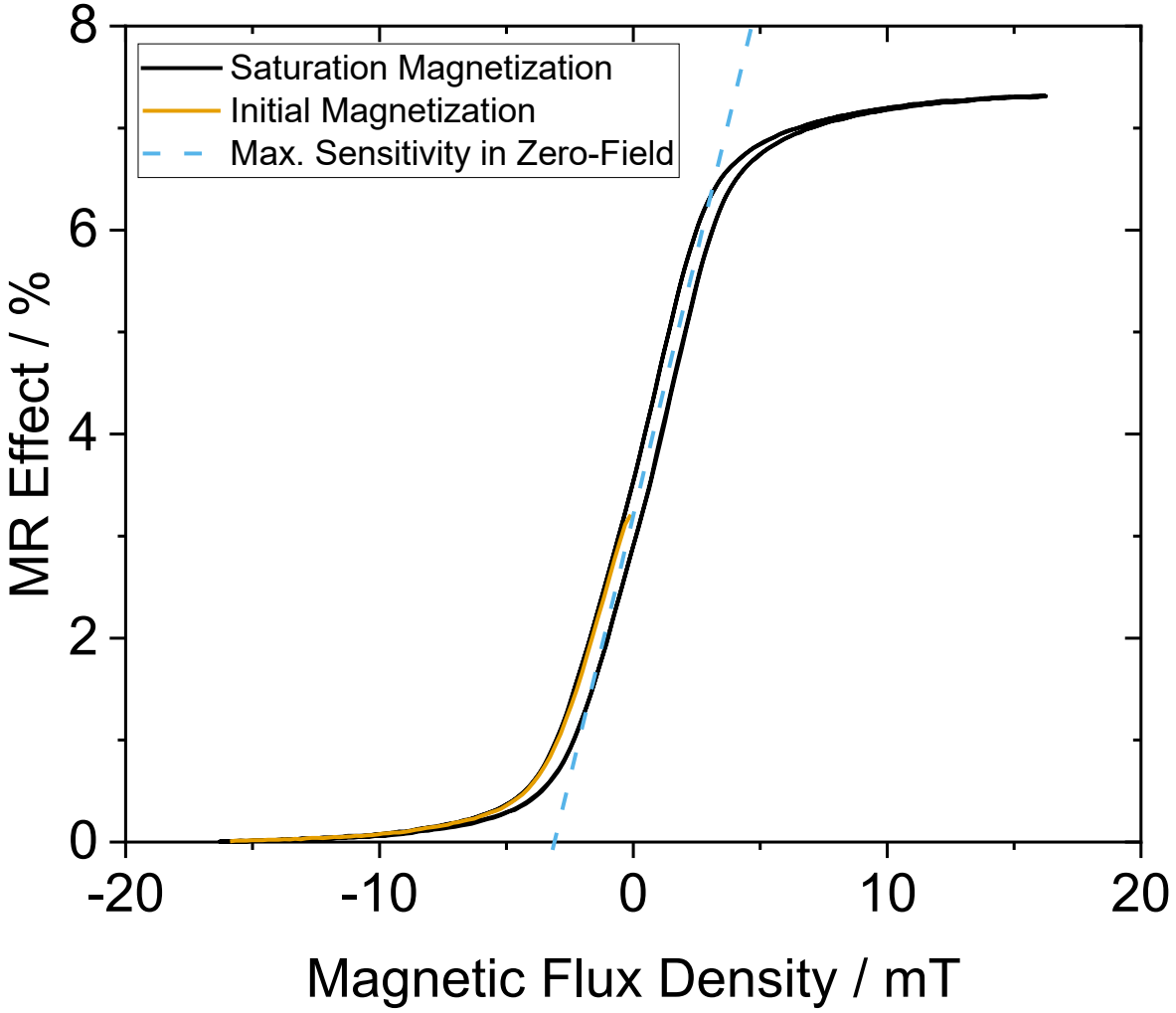
  ➔ Burst-Like Measurement of beads

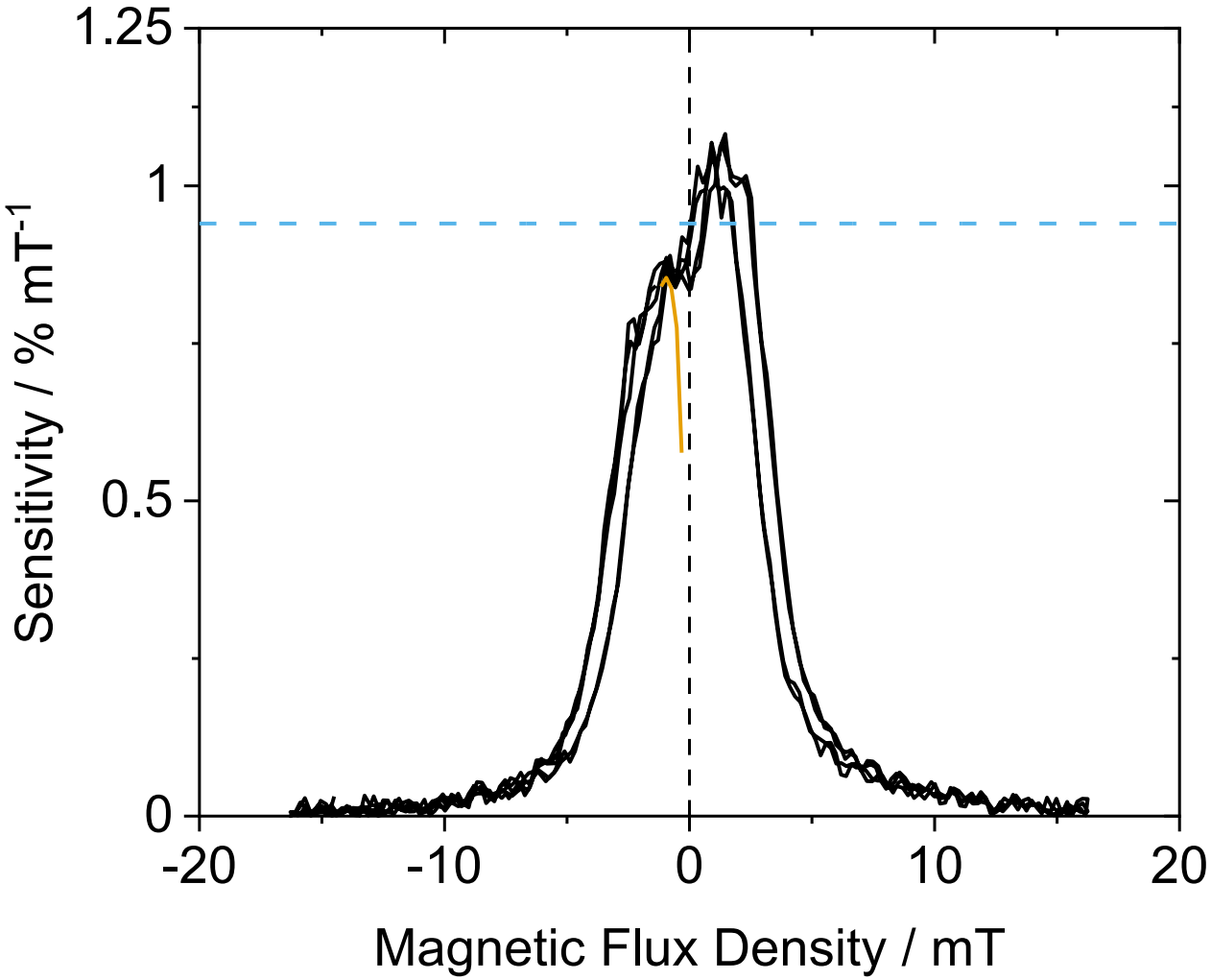
- After 800µl:
  - Empty Syringe and Sedimentation Effects play a role

  ➔ Less beads than expected are measured

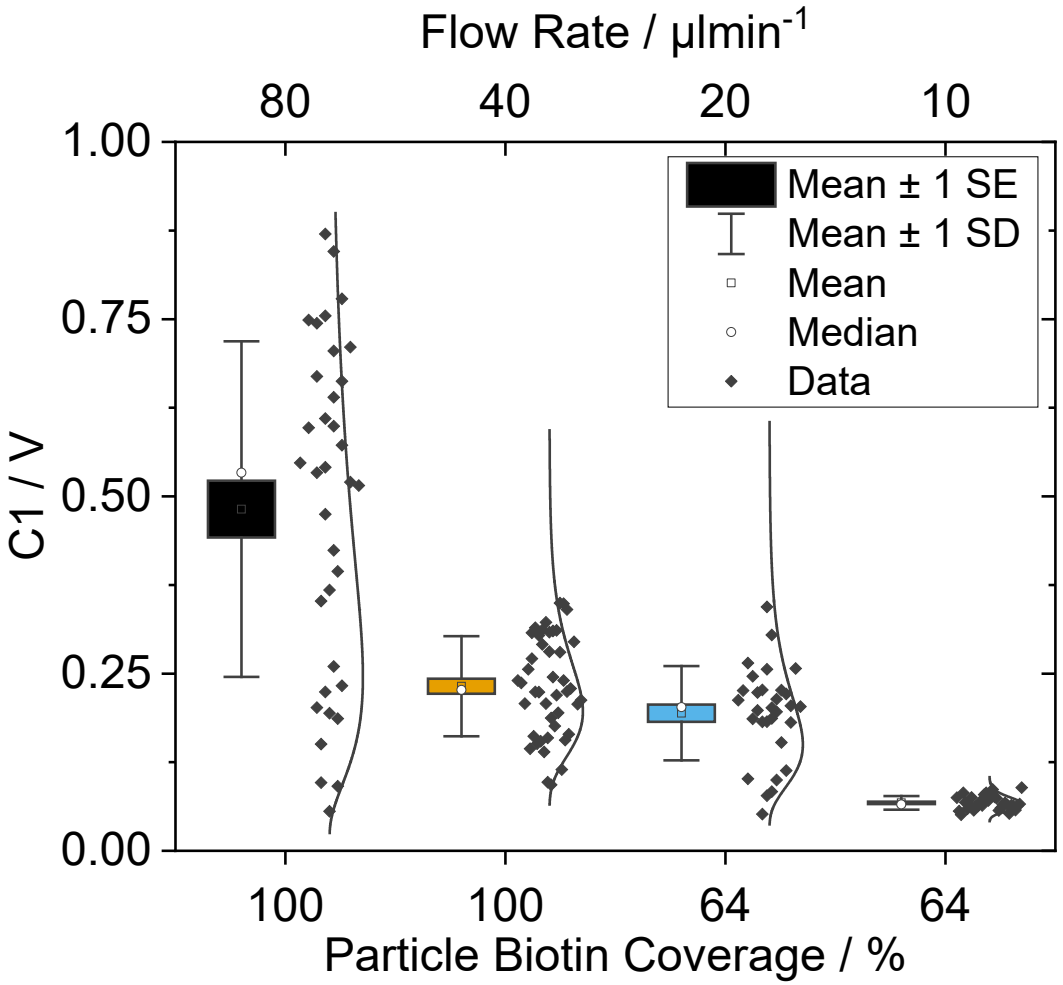


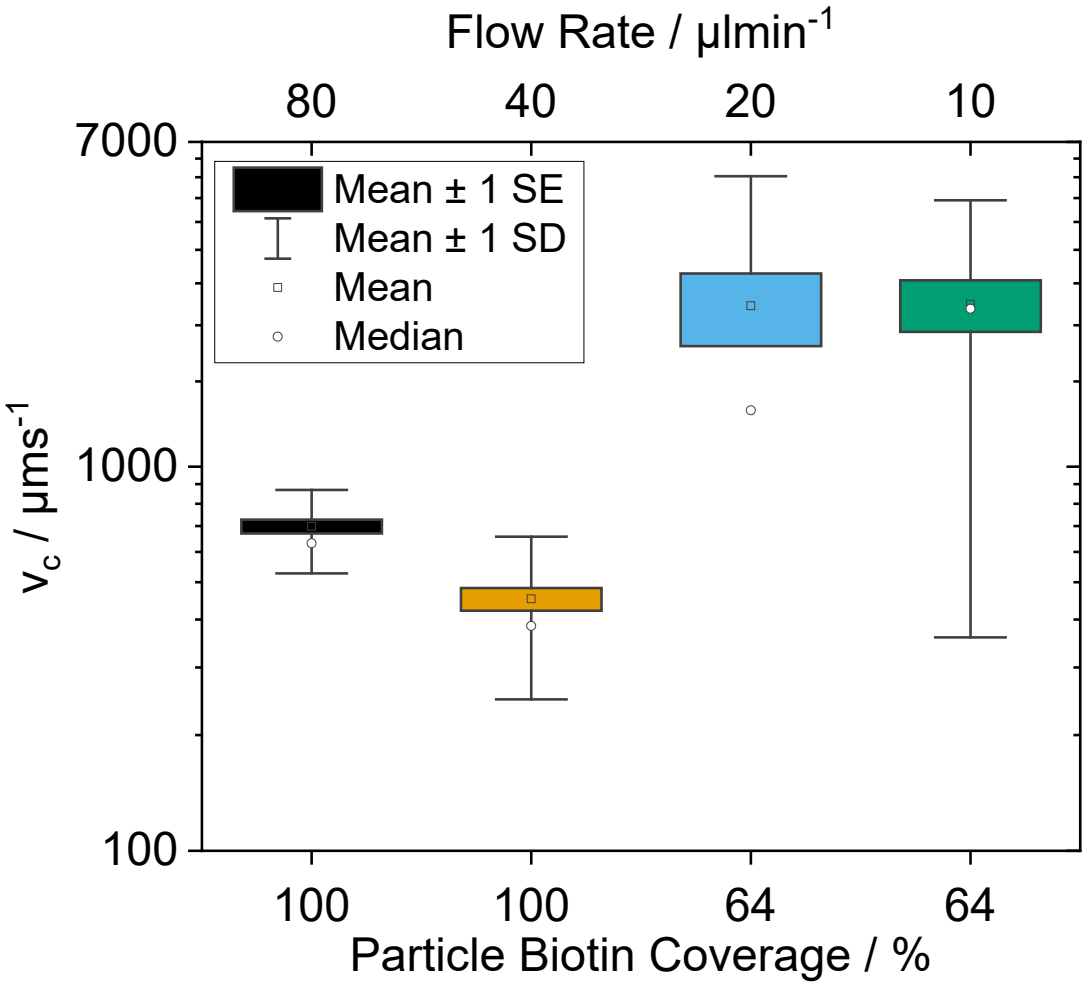
Hysteresis Calibration of the Sensor





Micromod BNF-Dextran

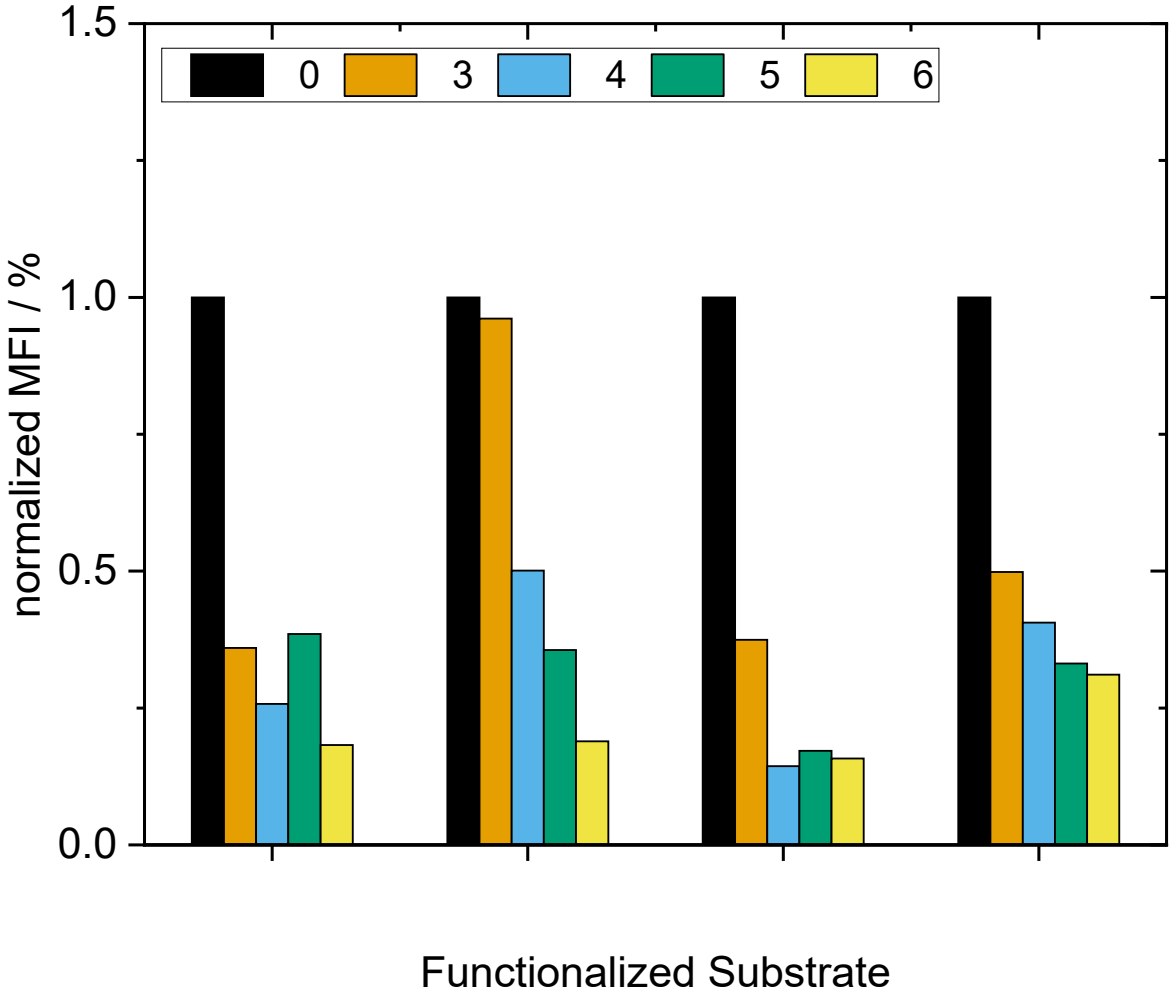




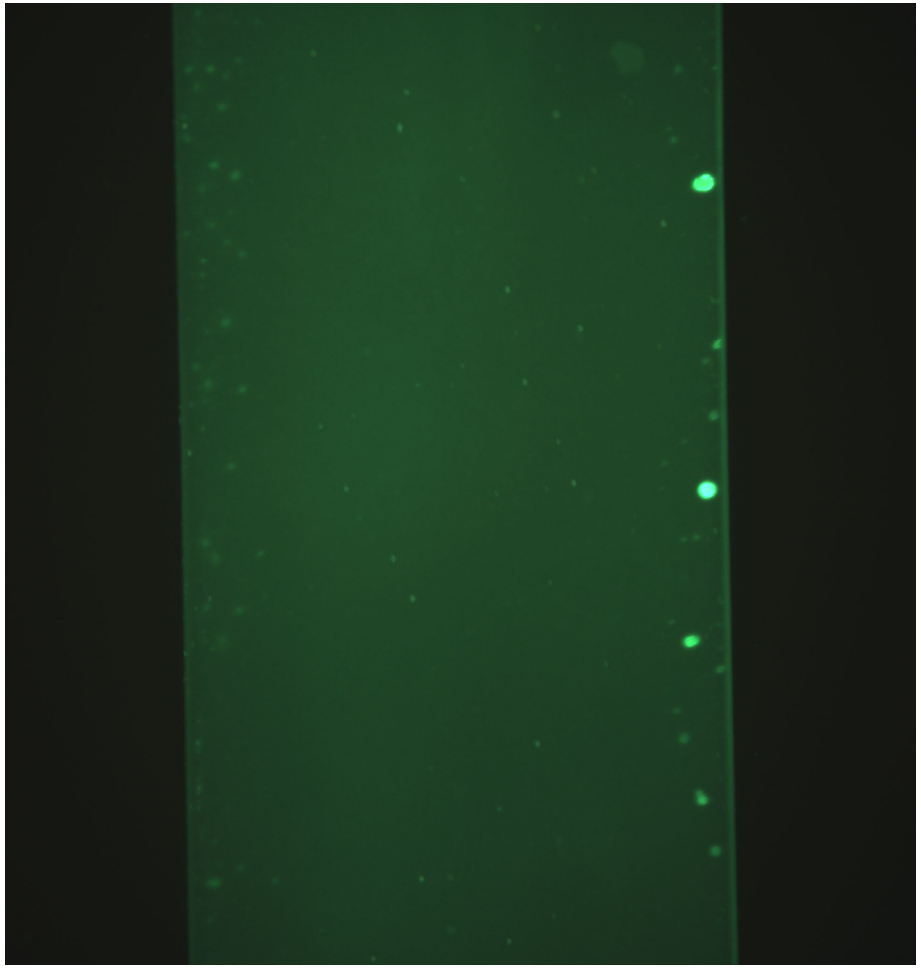
Assessment of Physisorbed Biofunctionalization

- Platereader Experiment with AlOx and SiN surfaces
- Coverage with fluorescent streptavidin
- Washing by 200 µl of buffer with a micropipette

➔ No stability over multiple washing steps
➔ SiN superior to AlOx



Fluorescent Evaluation of the Biofunctionalization



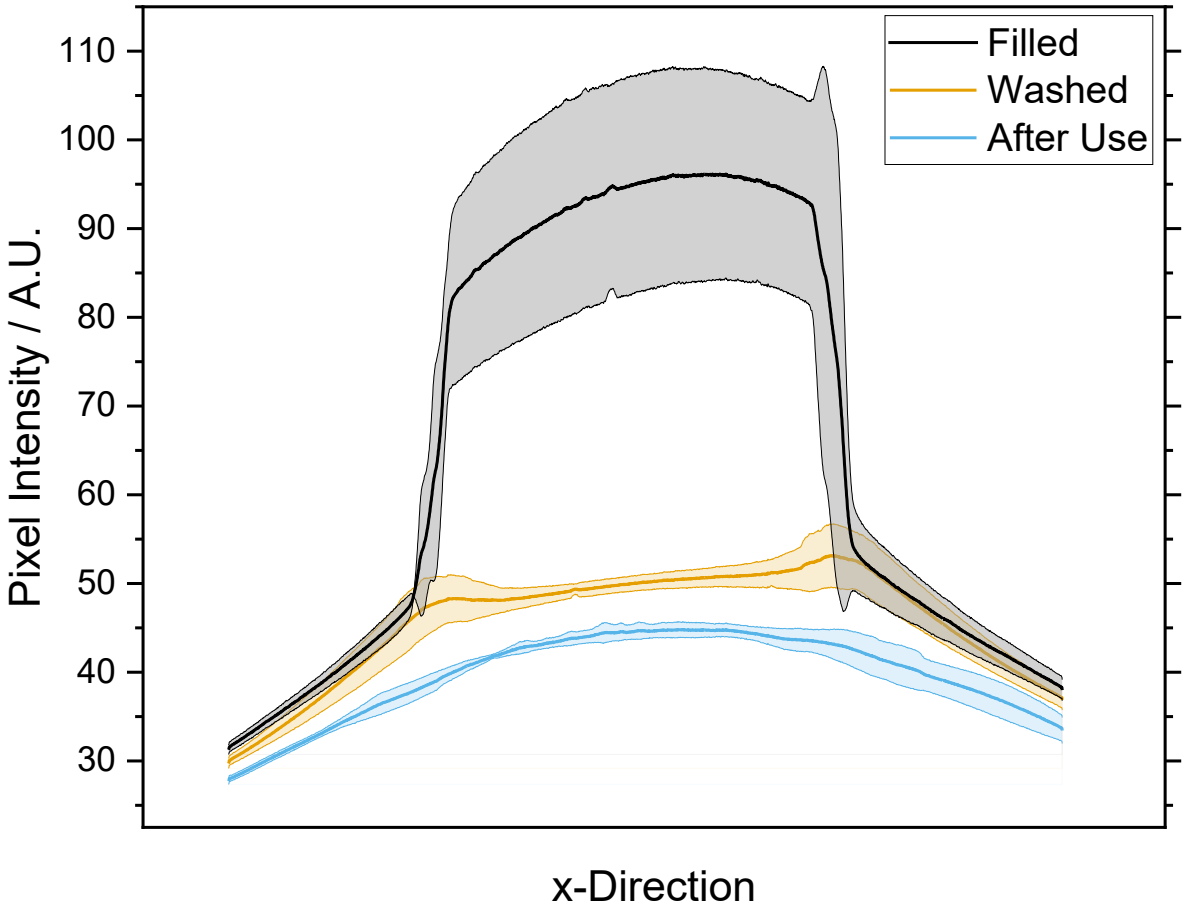
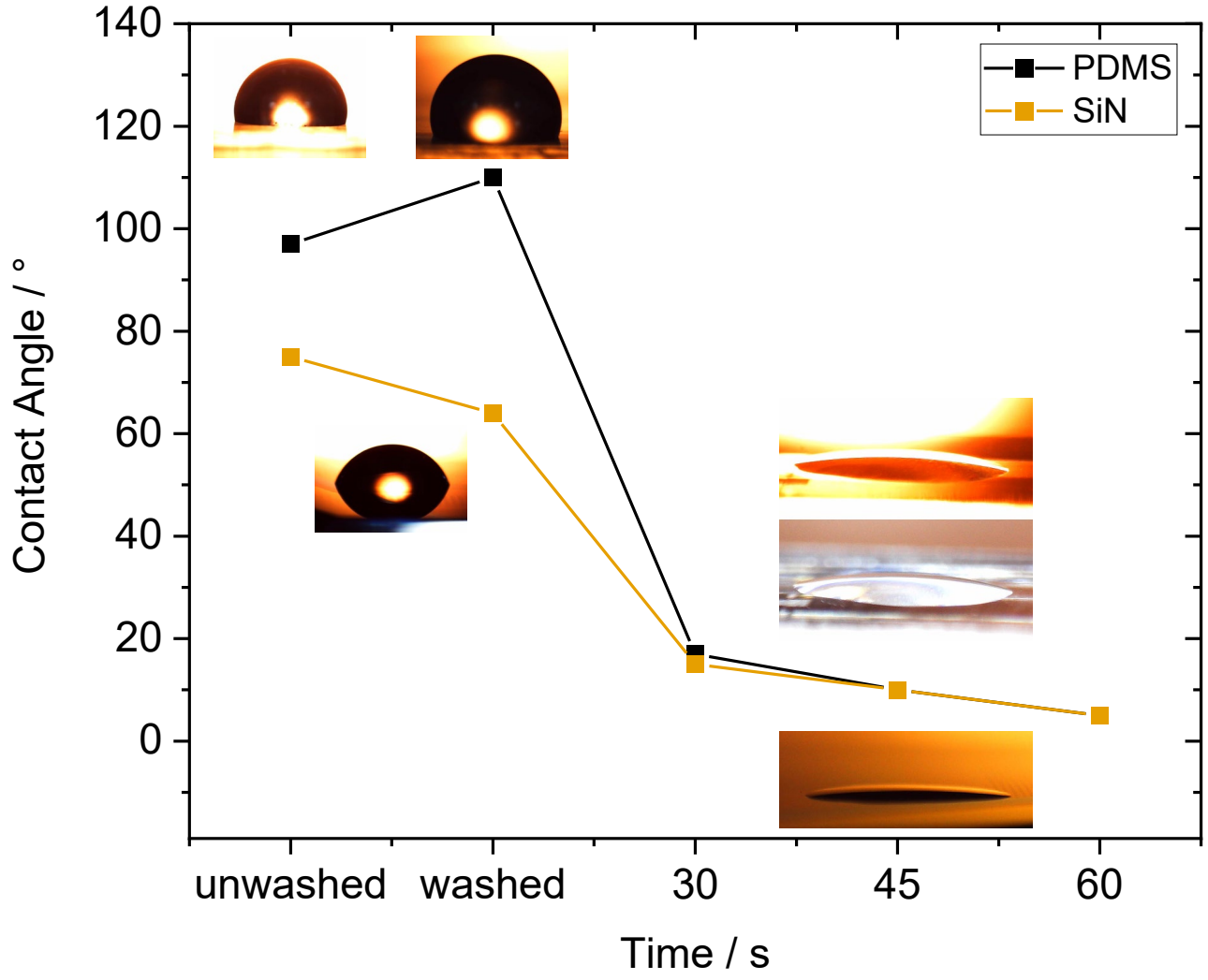


Image Analysis: Integration over vertical Axis
Tool: ImageJ

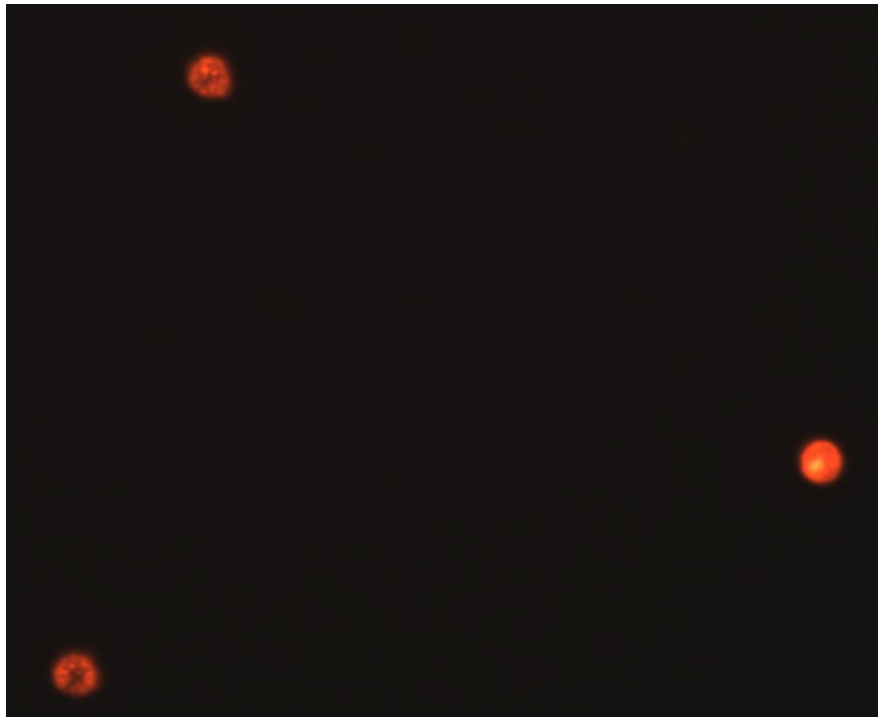
Surface Activation with Plasma

Difference to Piranha not measrurable in contact angle, but in functionalization operation

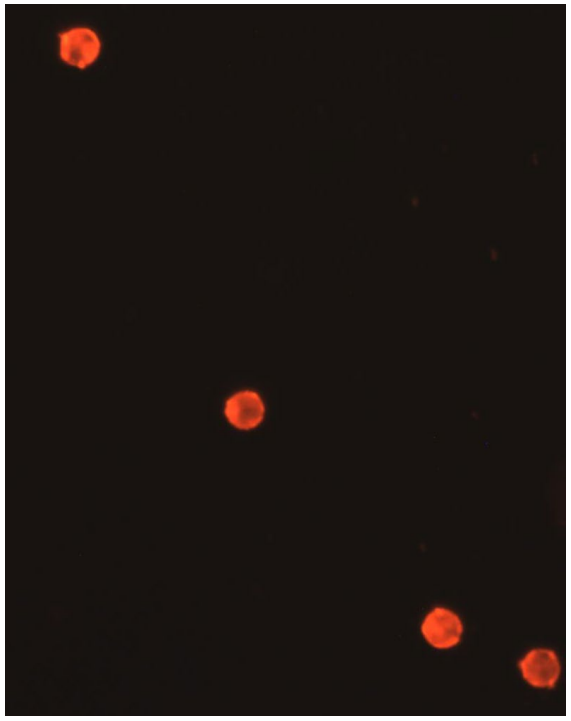


8µm Beads coated with streptavidin-redF-Dextran

Maximum Biotin Coverage



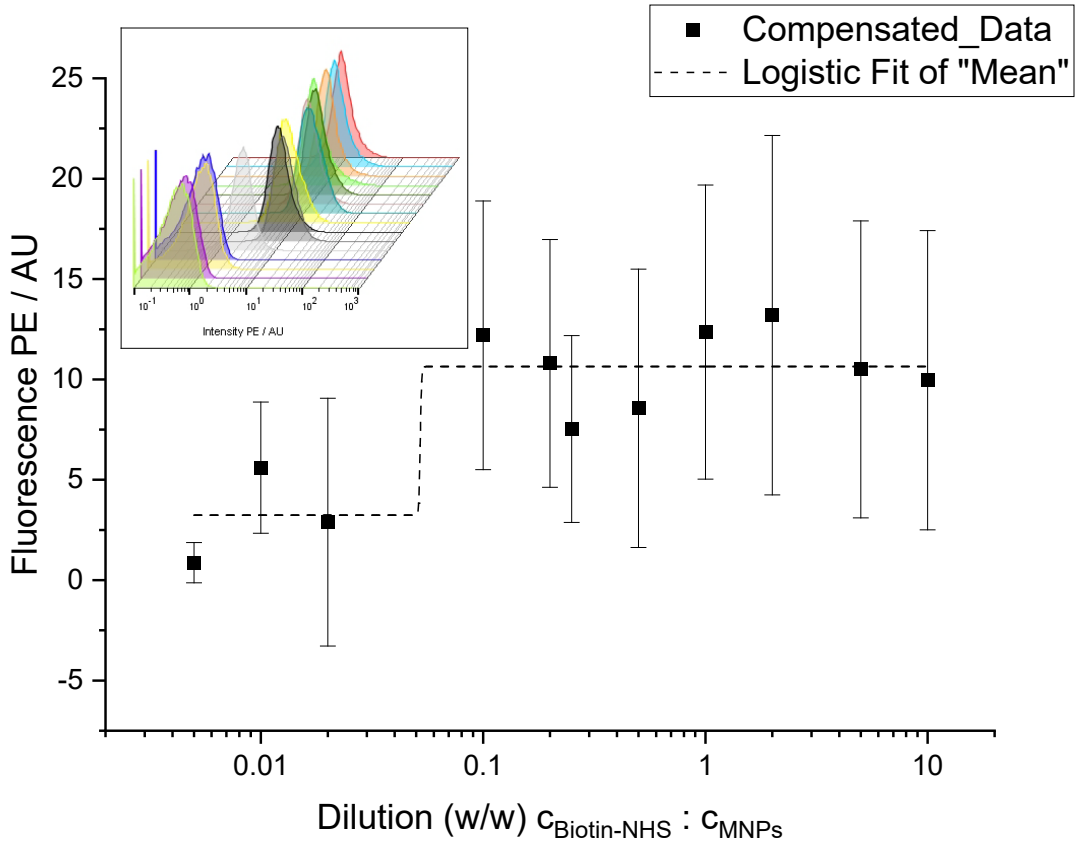
Approx 16% Coverage

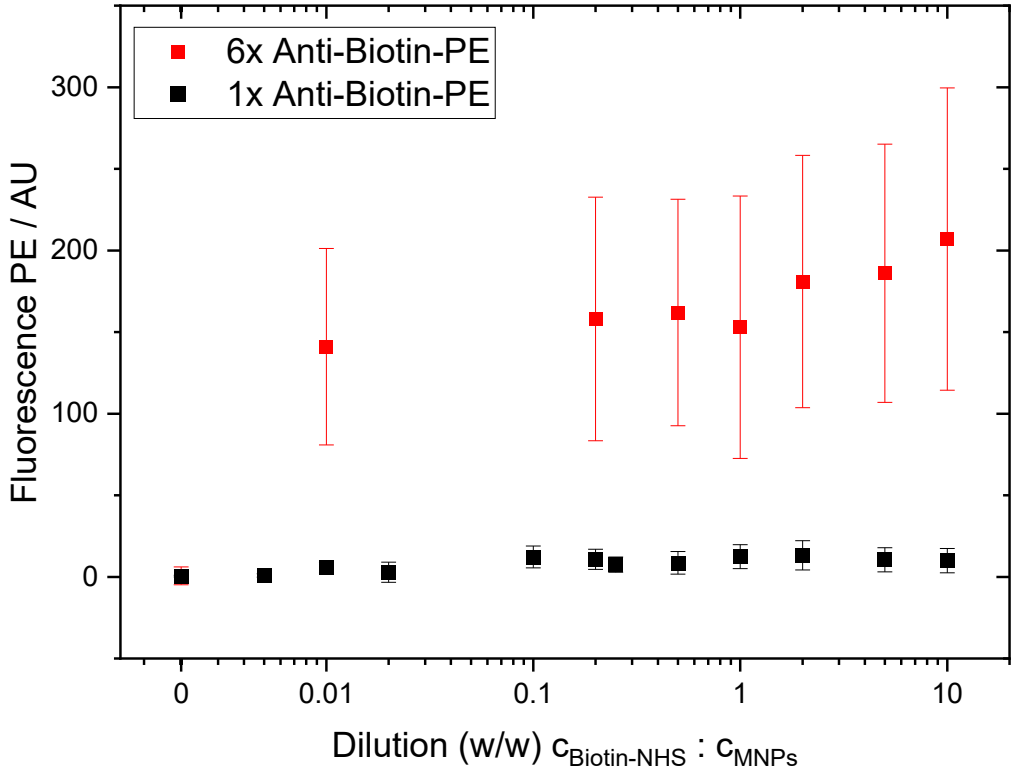


Blank

Unspecific Binding

Anti-Biotin-PE Assessment Not Working

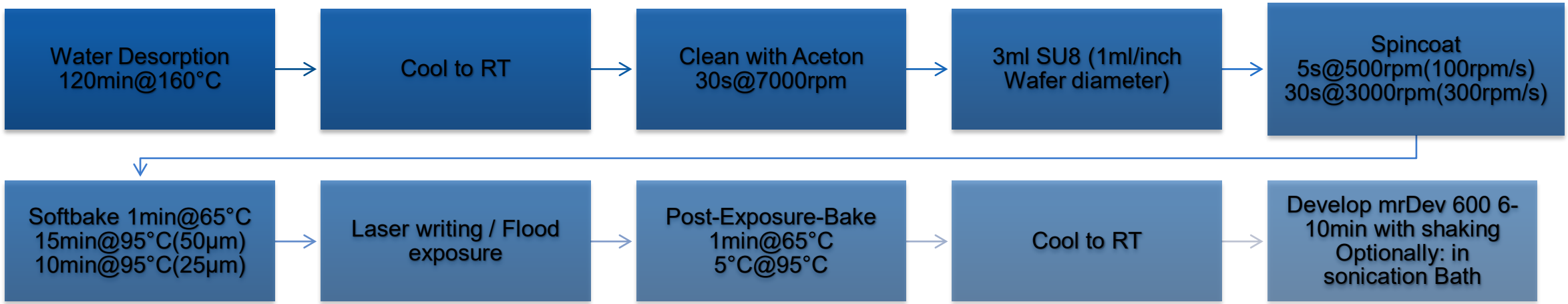




Wafer - Spincoating

- 17.07 3“ SU8-3050 @ 3000 rpm written same day ➔ ~ 98 µm
- 23.07 3“ SU8-3050 @ 3000 rpm written after one week ➔ ~ 94 µm
- 27.07 3“ SU8-3050 @ 3000 rpm written same day ➔ ~ 53 µm
- 29.07 3“ SU8-3025 @ 3000 rpm written two days after ➔ ~ 44 µm

- 4“ desired 150µm Layer ➔ ~ 115µm



Wafer – Laserwriting

Parameter	Value
Spot size	0.01 mm
Overlap	80%
Modulation	100%
Z-Position	-6.782
velocity	40 mm/s (omitted lines in dialog)