Med Uni Graz

Pioneering Minds

COMBINING NANOTECHNOLOGY AND MILLIFLUIDICS: SORTING OF YEAST CELLS ACCORDING TO THEIR AGE

BioNanoNet Annual Forum &

BNN Networking Session

13.09.2023 Wien

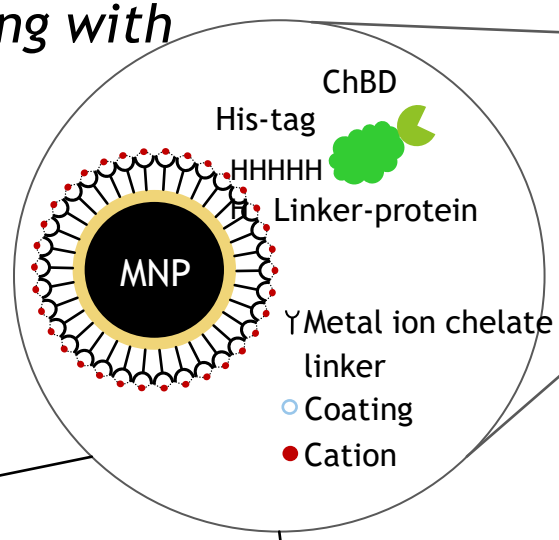
Sebastian Schwaminger

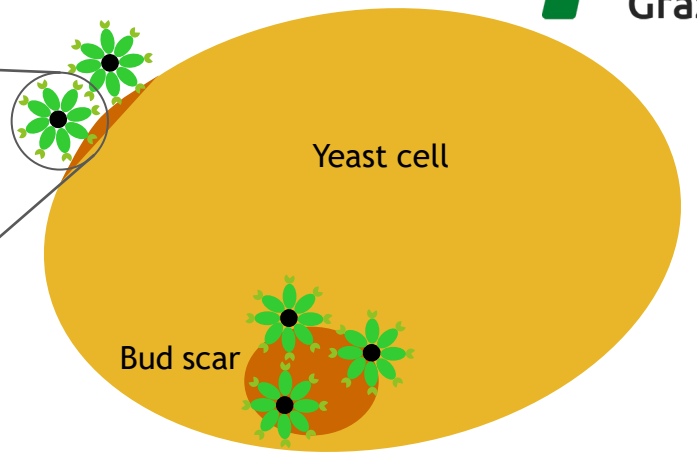
Nano Medicine AUSTRIA

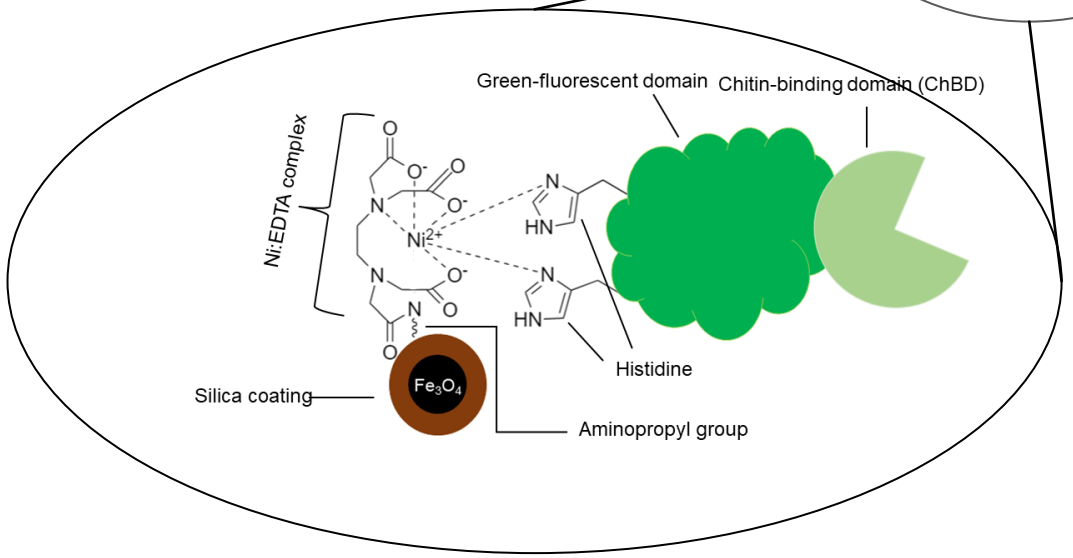
Project idea

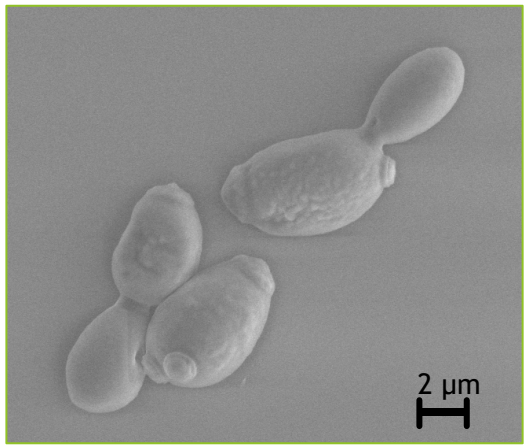
Age dependent magnetic labeling with coated magnetic nanoparticles

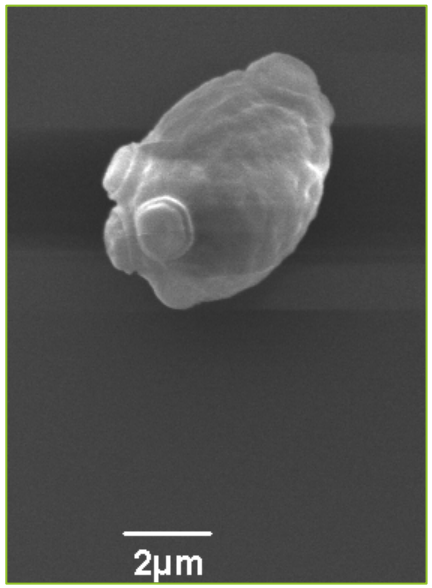
- → Adsorption
- → Agglomeration
- → Specificity











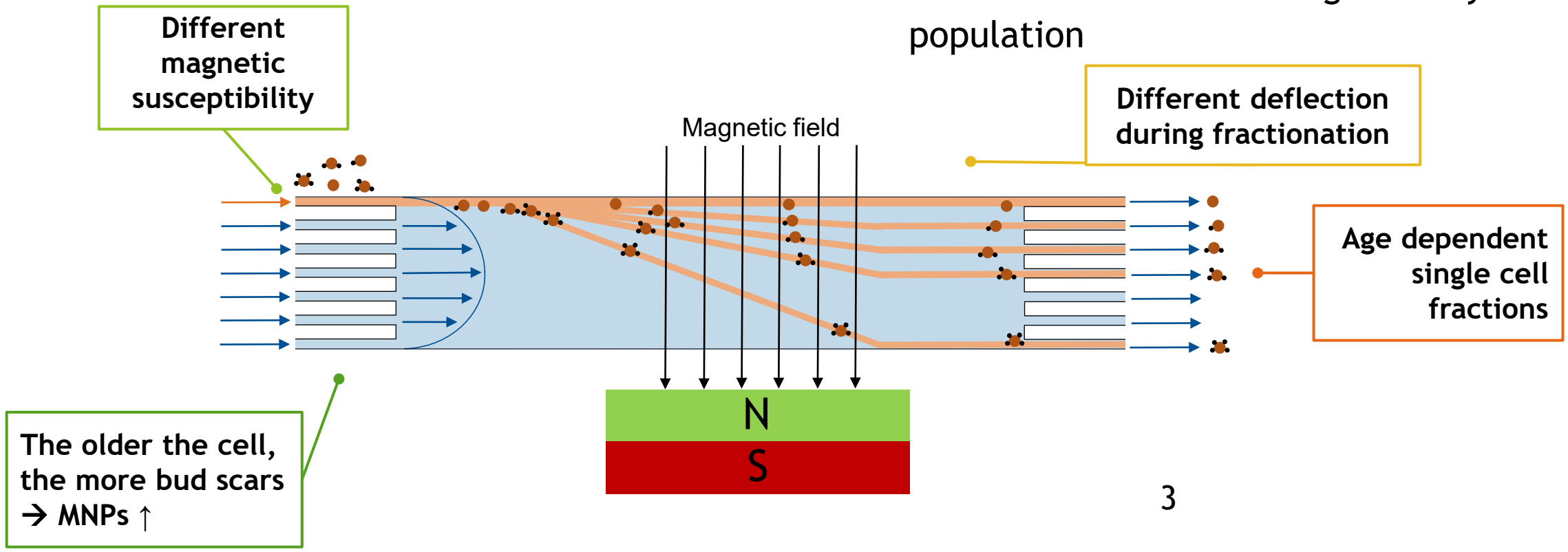
Project idea

Magnetically based cell deflection

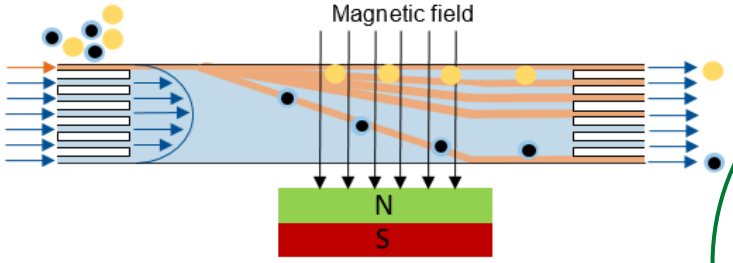
→ Micro- (milli)fluidic chip
→ Construction
→ Design

Advantages:

- Small sample volume
- Less reagent consumption
- Non-invasive
- Fractionation of a heterogeneous yeast population



Approach

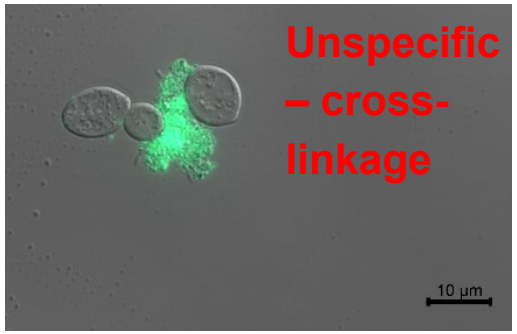


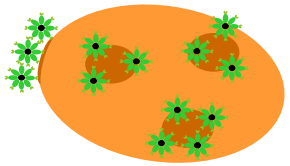
Fractionation

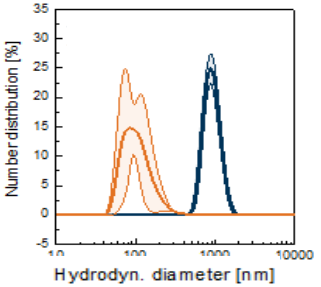
Agglomeration

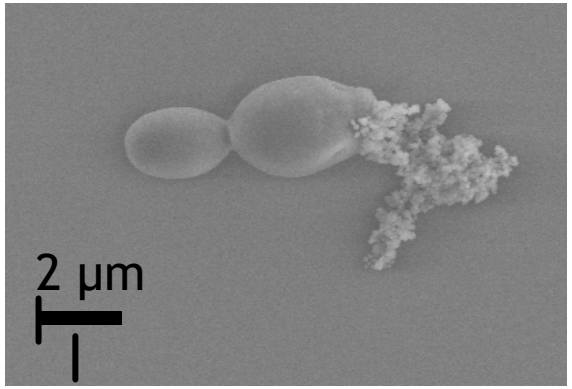
Synthesis & Adsorption

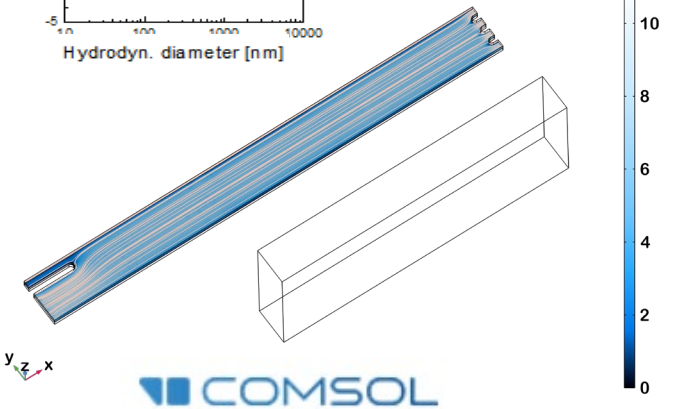
Simulation





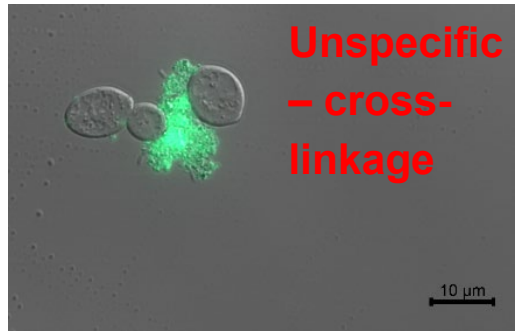




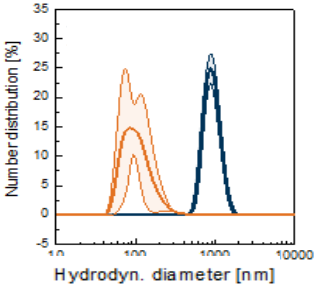


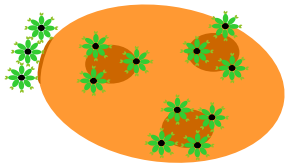
Materials

Med Uni Graz

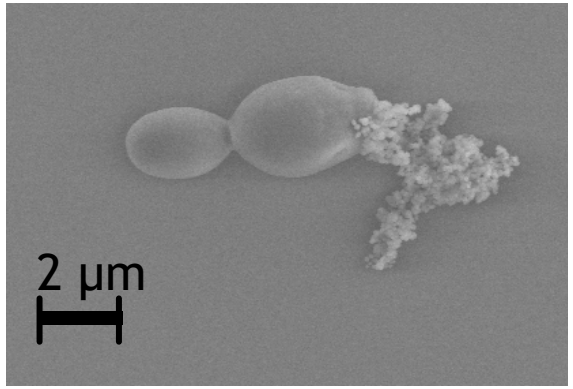


Agglomeration



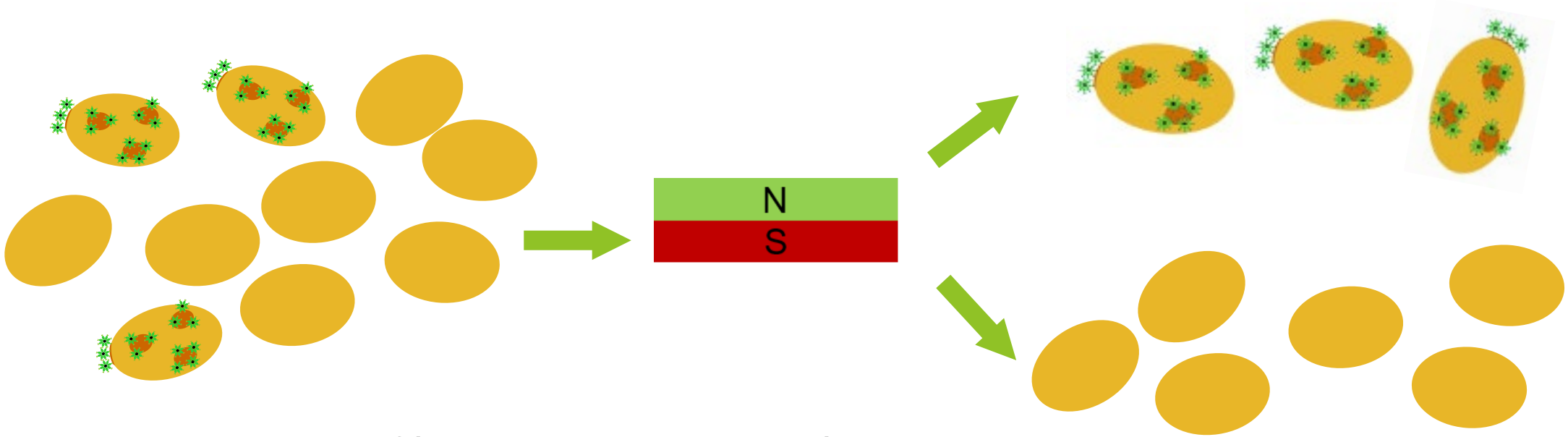


Synthesis & Adsorption



COMSOL

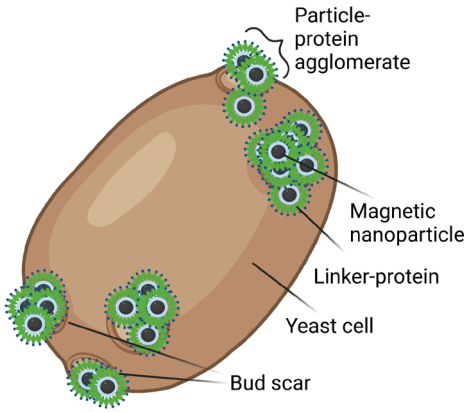
Magnetic separation

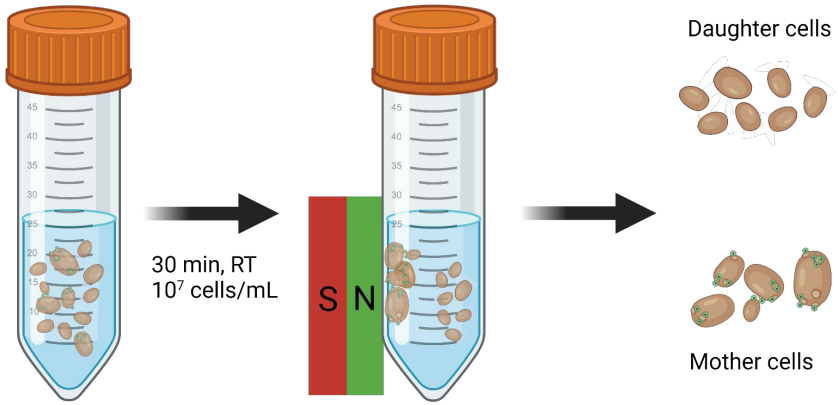


Magnetic separation of heterogeneous yeast culture

- → **Complex system**
- → **Modell system for set-up evaluation and first optimization**

Magnetic labeling of *S. pastorianus*

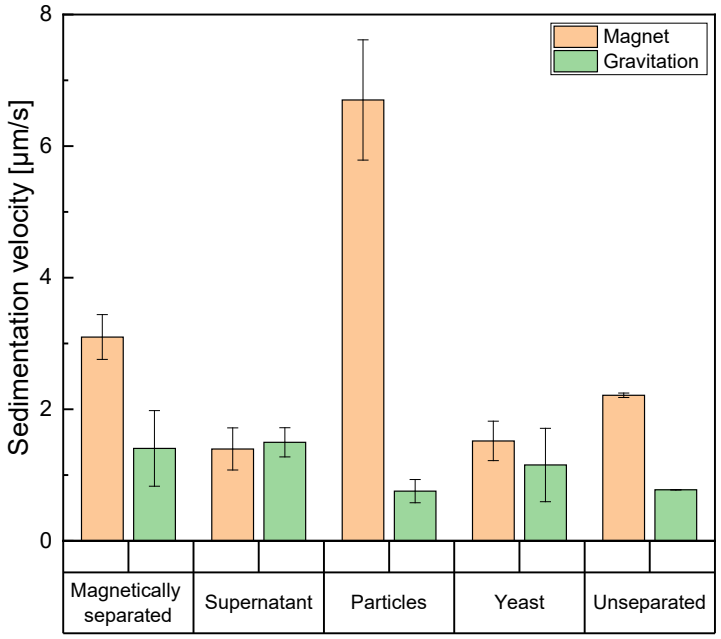




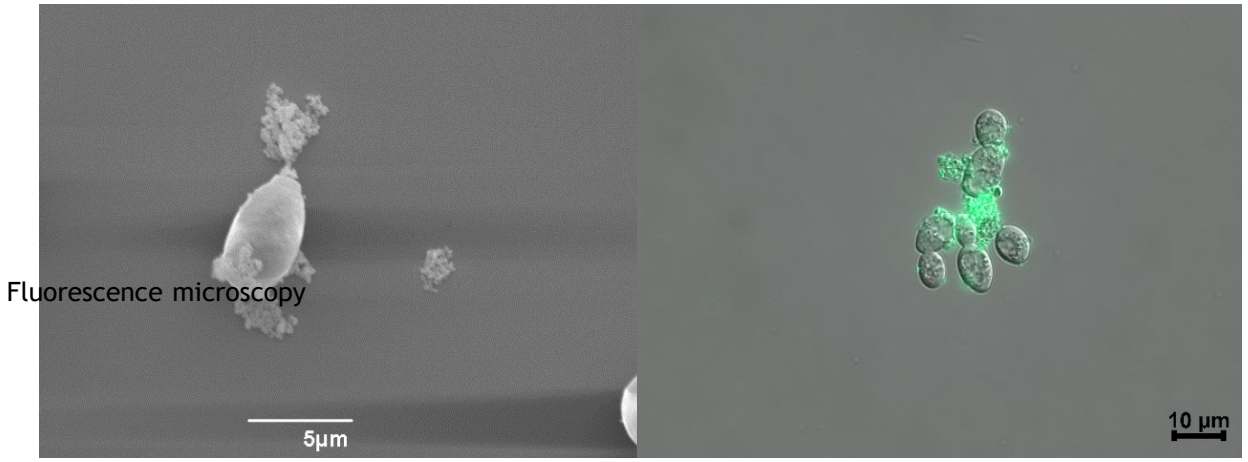
- Incubation of yeast cells with nanoparticles
- Magnetic separation

- Effective method for yeast cell rejuvenation

Magnetic separation verification



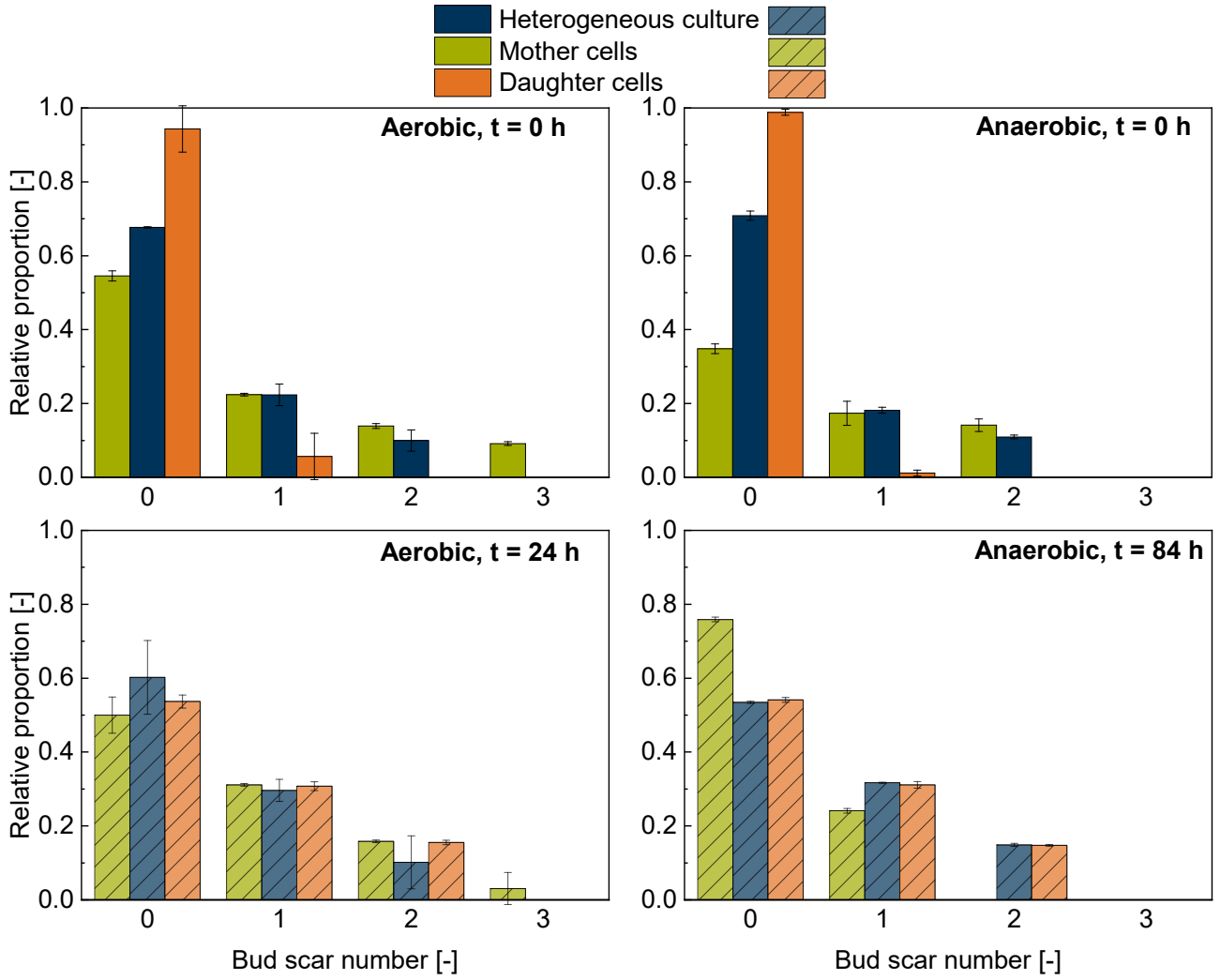
Magnetic sedimentation experiments (LUMiReader, 50 mT field), OD 2, 0.08 g/L MNP



Scanning Electron Microscopy

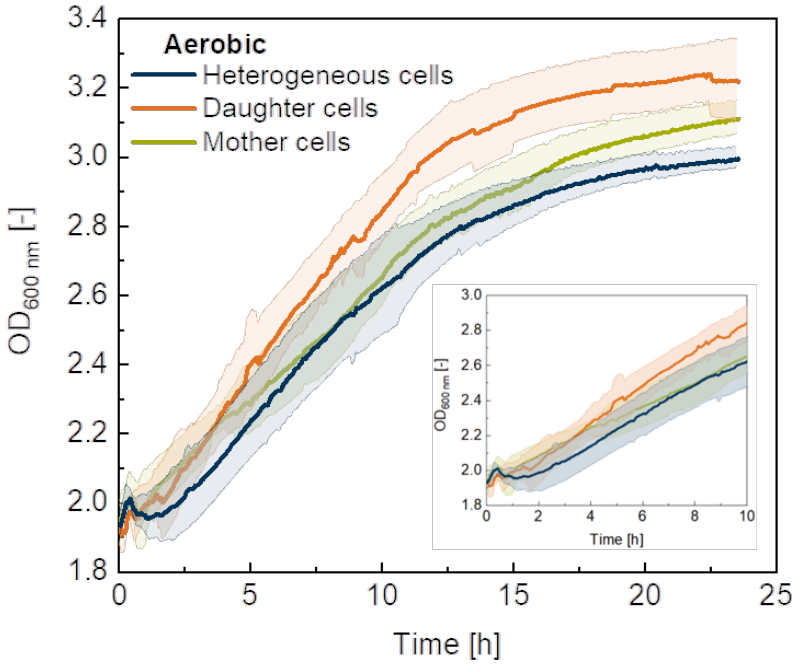
Characterization of yeast cells (Cytometry)

- Magnetic separation for both cultivations (aerob, anaerob) successfull
- Enrichment of daughter cell fraction to almost 100%
- After cultivation end (aerob t = 24 h, anaerob t = 84 h) alignment of age distribution for aerobic conditions, for anaerobic conditions increased daughter cell content for mother cell cultivation
- Not completely in stationary phase



Growth rates

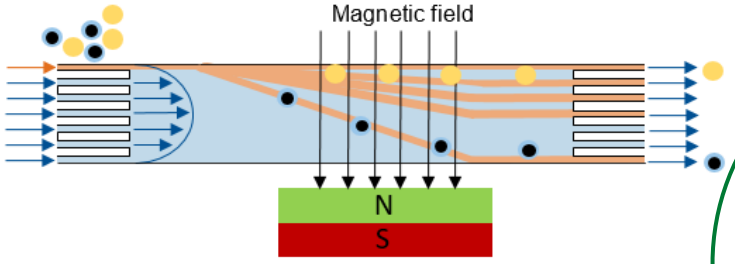
- Fluctuation in lag phase
- Daughter cells grow to higher OD compared to mother cells ▫ More efficient metabolism?
- High growth rate differences
- Aerob: 22% lower growth rate for mother cells compared to mother cells
- Magnetic separation does not influence viability of yeast cells
- High viabilities for daughter cells



Growth rate	Heterogeneous [1/h]	Daughter cells [1/h]	Mother cells [1/h]
Aerobic	0.031 ± 0.00	00.37 ± 0.00	0.029 ± 0.00

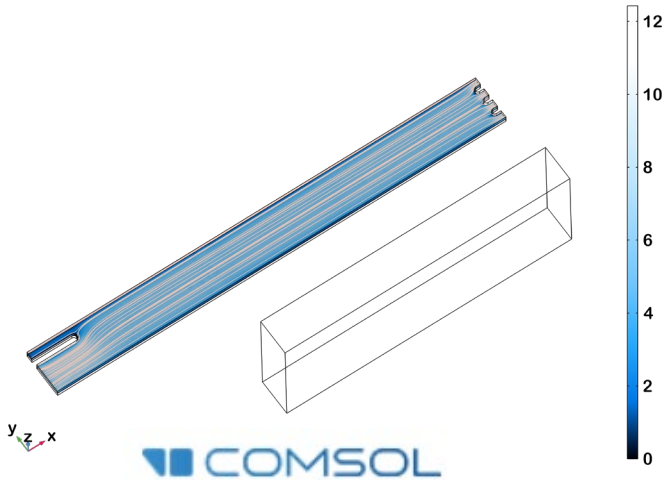
Aerobic		
	Viability t_0 [-]	Viability t_{24} [-]
Heterogeneous culture	0.98 ± 0.00	0.99 ± 0.00
Mother cells	0.93 ± 0.00	0.99 ± 0.00
Daughter cells	0.98 ± 0.00	0.99 ± 0.00

Model system



Fractionation

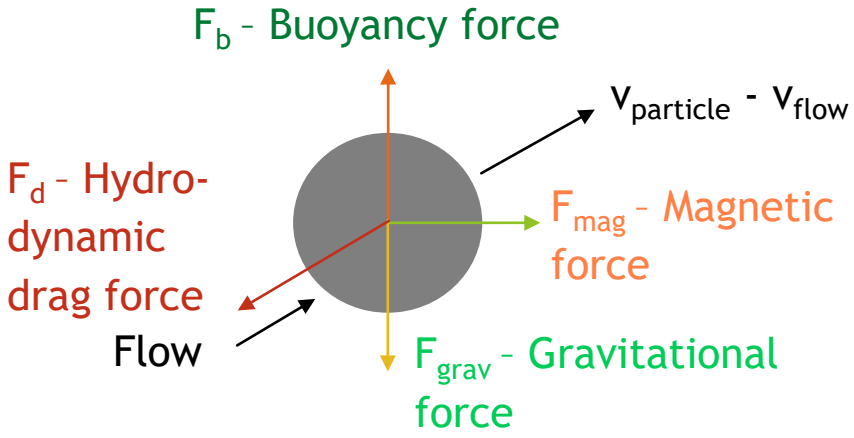
Simulation



Magnetophoresis in microfluidics

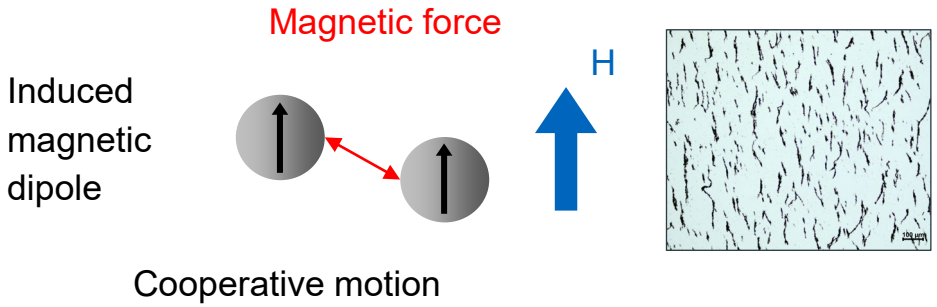
Parameters

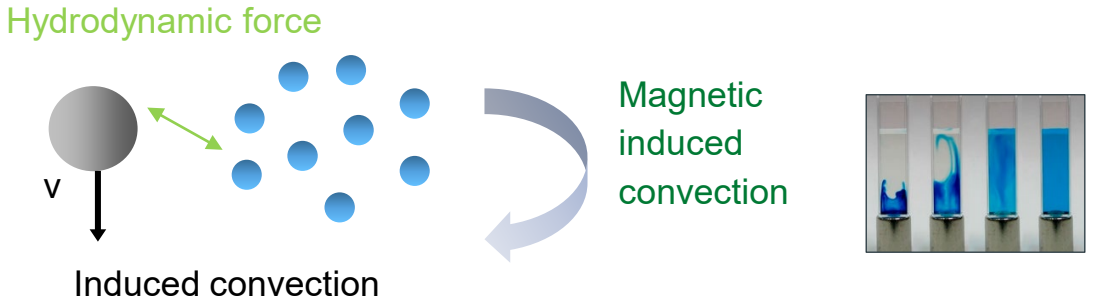
- Magnet distance
- Concentration
- Channel width
- Viscosity
- Channel height



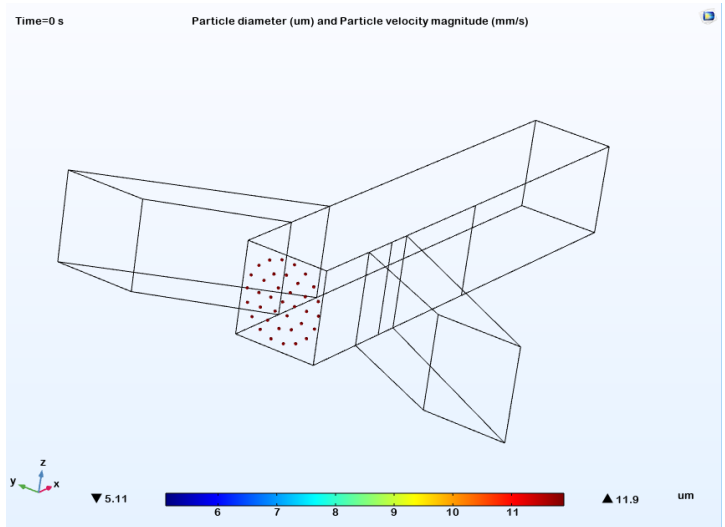
$$Gr_m = \frac{\rho * \nabla B * \left(\frac{\partial M}{\partial c}\right) * (c_s - c_\infty) * L_c^3}{\eta^2}$$

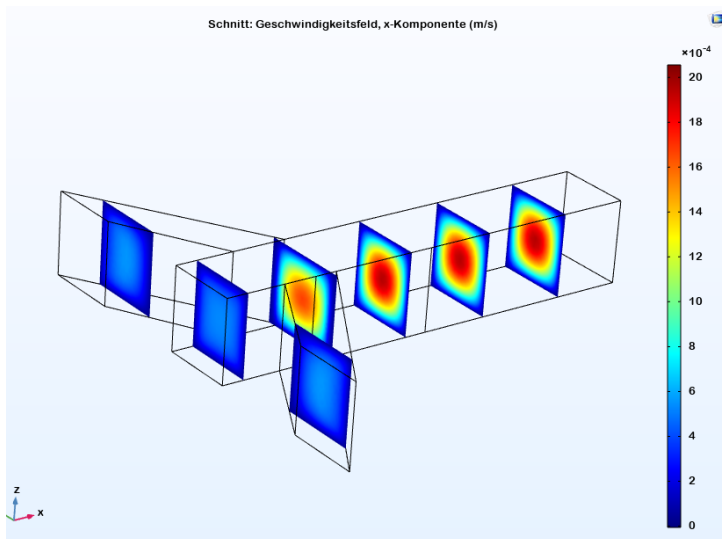
Cooperative Magnetophoresis

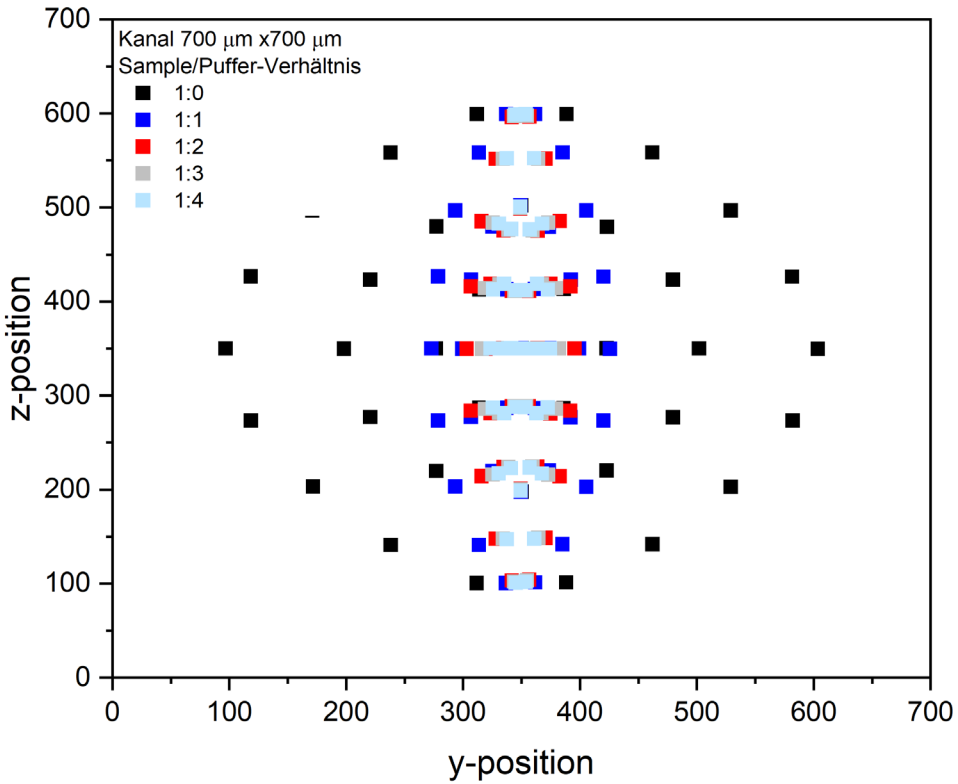




Hydrodynamic focusing



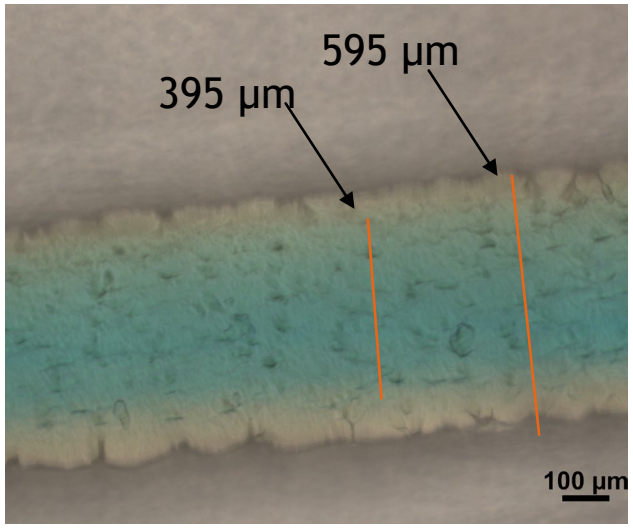


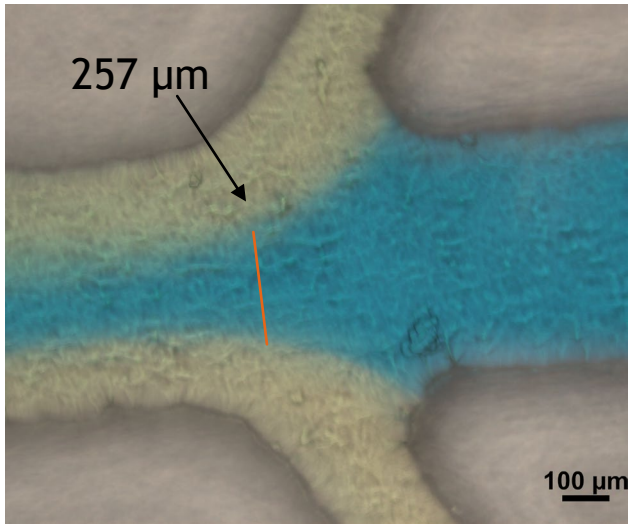


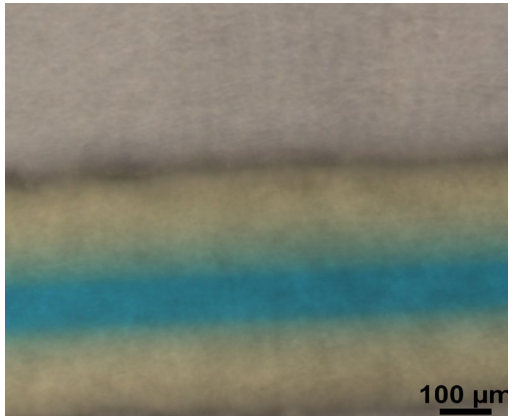


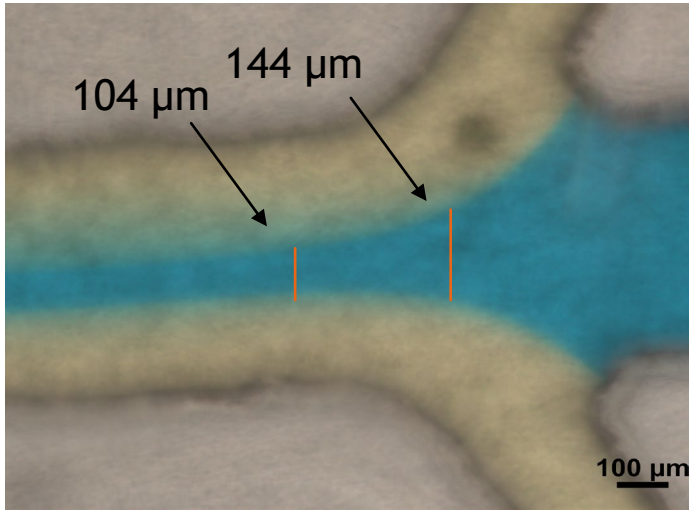
Hydrodynamic focusing

- 1:1 sample to buffer ratio
- Successful focusing
- Partically diffusion after 2 cm
- 1:2 sample to buffer ratio
- Successful focusing over complete channel





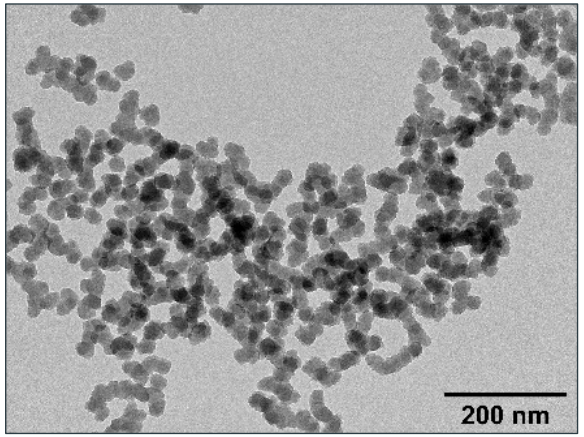


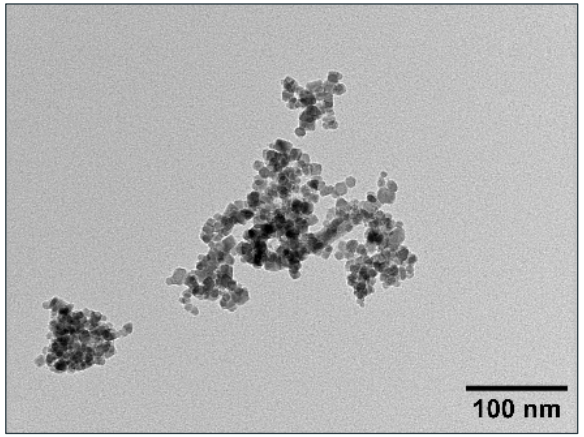


Model system development

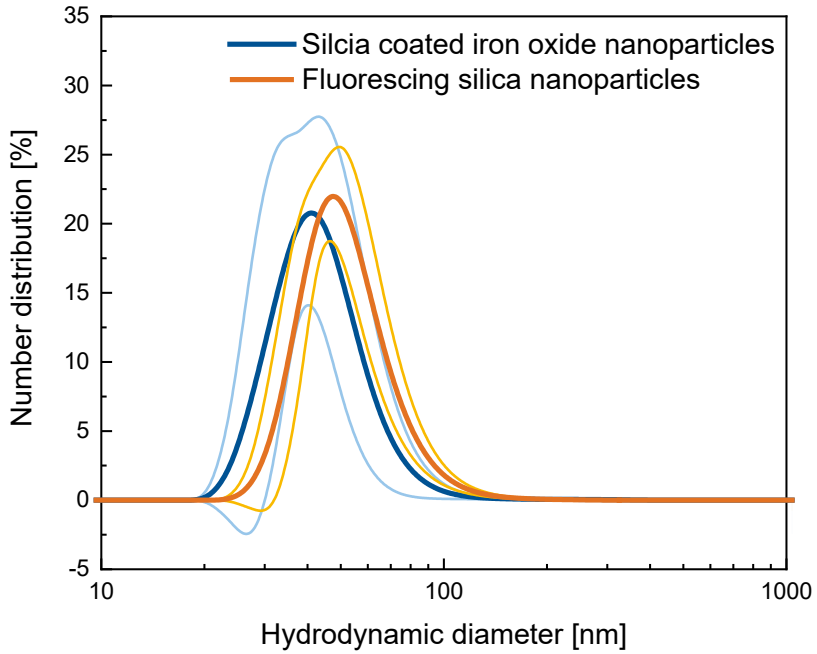
Fluorescent silica nanoparticles
→ Non-magnetic
→ Fluorescence detection

Silica coated iron oxide nanoparticles
→ Superparamagnetic
→ Detection via UV-Vis



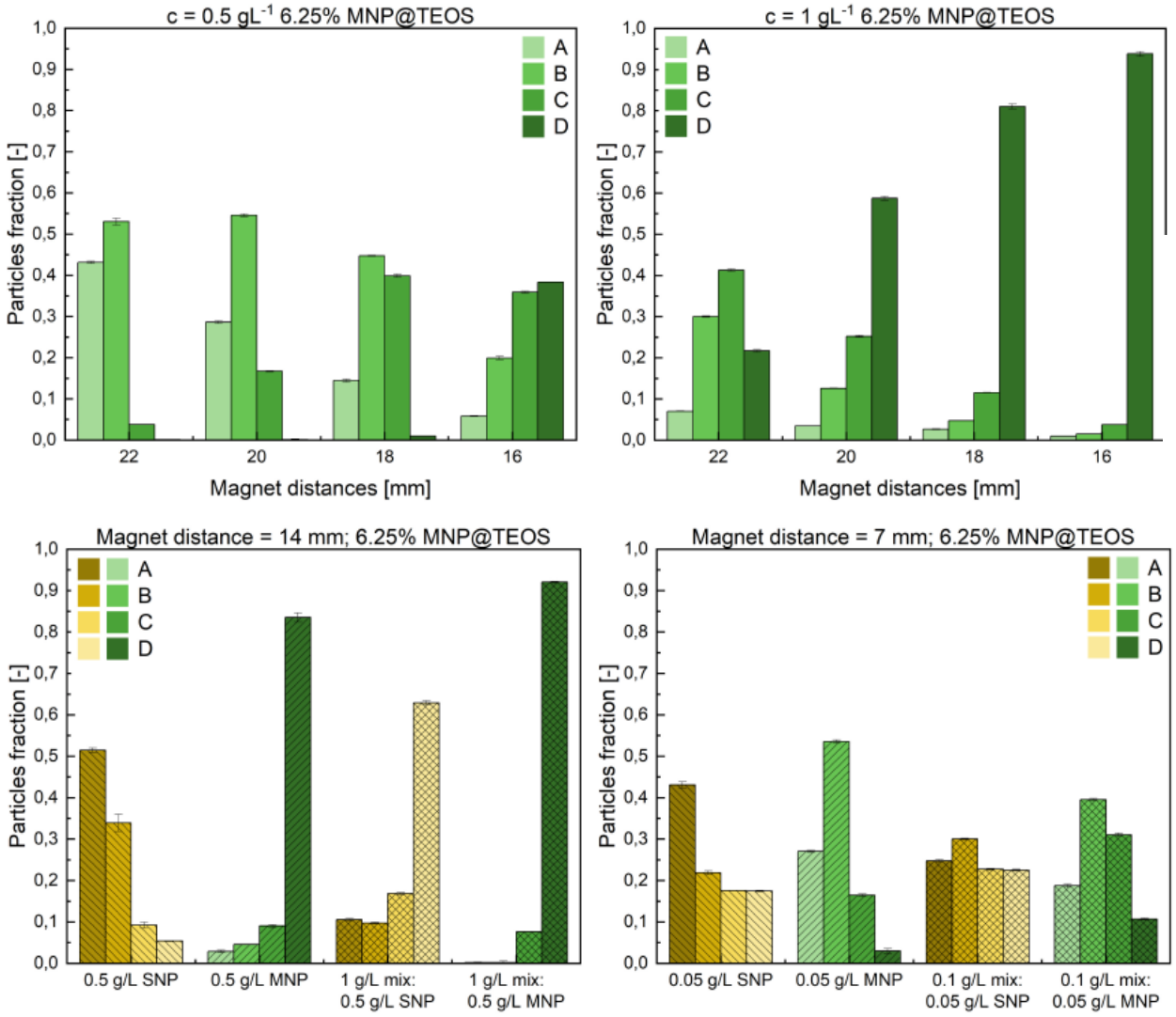


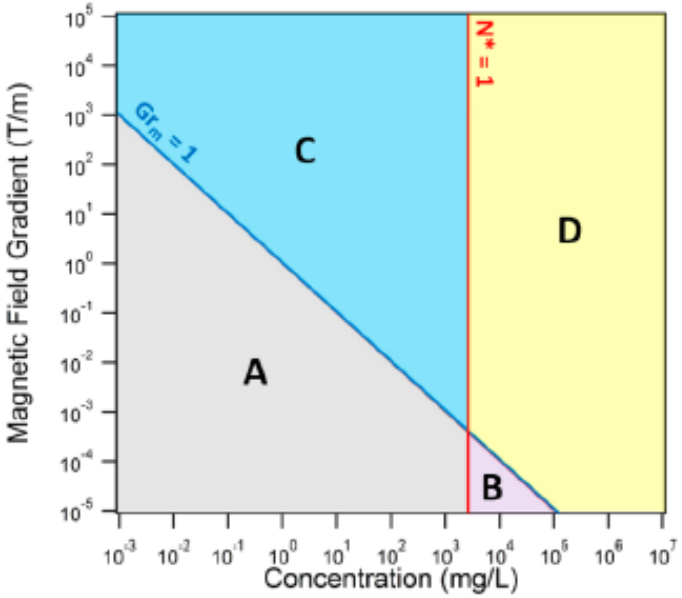
	Zeta potential [mV]	Diameter [nm] TEM
Silica coated iron oxide nanoparticles	-24.26 ± 0.61	11.68 ± 3.57
Fluorescing silica nanoparticles	-43.68 ± 3.12	26.58 ± 5.01



Nanoparticle model system
→ Easy to synthesize
→ Comparable particle species
→ Fast analysis
→ Colloidal stable
→ Easier modeling

Challenges of fractionation





A Classical (non-interacting)

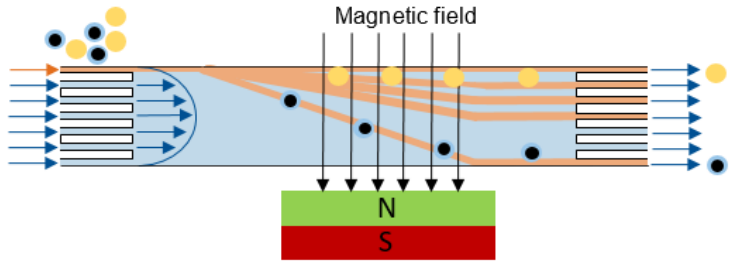
B Cooperative magnetophoresis

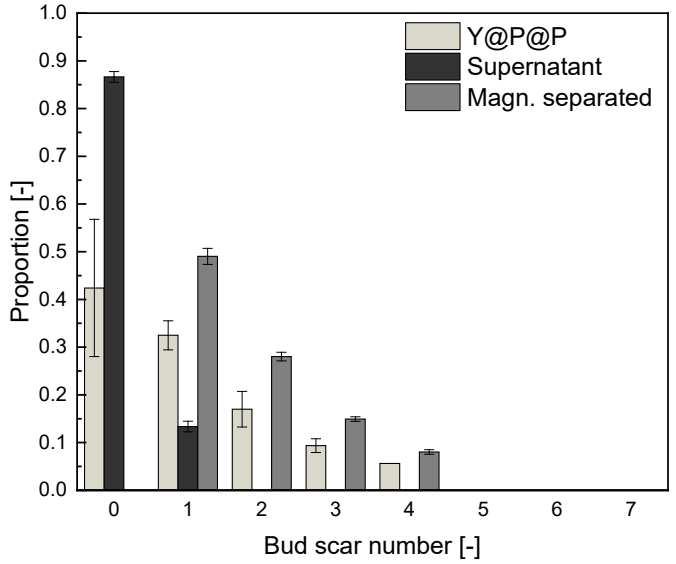
C Induced convection

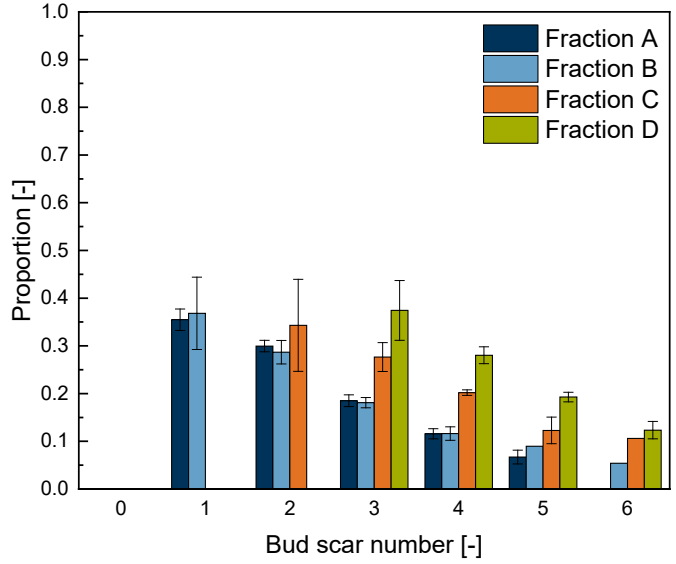
D Induced convection and cooperative magnetophoresis

Fractionation process

- Magnetic pre-separation to separate mother from daughter cells
- Complete removal of daughter cells
- No complete fractionation but significant changes in the heterogeneiety of the cell culture
- Older cells (> 2 budding scars) are mostly influenced by magnetic field



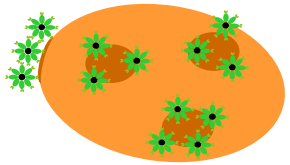


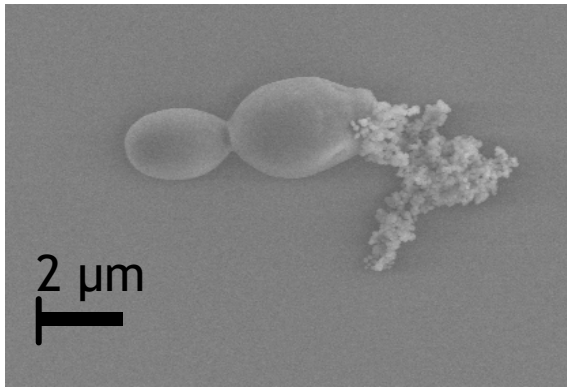


Results and outlook

Results

- Magnetic Separation process for daughter and other cells works
- Enrichment of older cells is possible with a millifluidic flow cell
- Differences between cellular ages can be shown by growth rates
- Millifluidic challenges due to magnetic convection





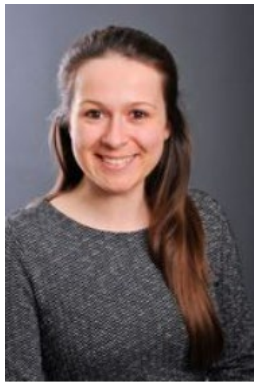
Outlook

- Optimization of magnet distance and flow rate to improve efficiency/time, sedimentation and classification and identification of metabolic and genomic differences of yeast cells of different ages

Where to use this technology?

- We are looking for applications in pharmaceutical technology (especially for Pichia pastoris yeasts)
- CORNET calls (e.g. March 2024) we need an Austrian industrial partner
- IHI calls for sampling and diagnostics (IHI call 5)
- Horizon Europe calls

Acknowledgements



Leonie Wittmann



Marco Eigenfeld



Thomas Becker

Team Nanos



Marco Reindl



Verena Zach

Med Uni Graz

Med Uni Graz

brau & getränke technologie

Nano Medicine AUSTRIA

DFG Deutsche Forschungsgemeinschaft

Med Uni Graz Otto-Loewi Research Center