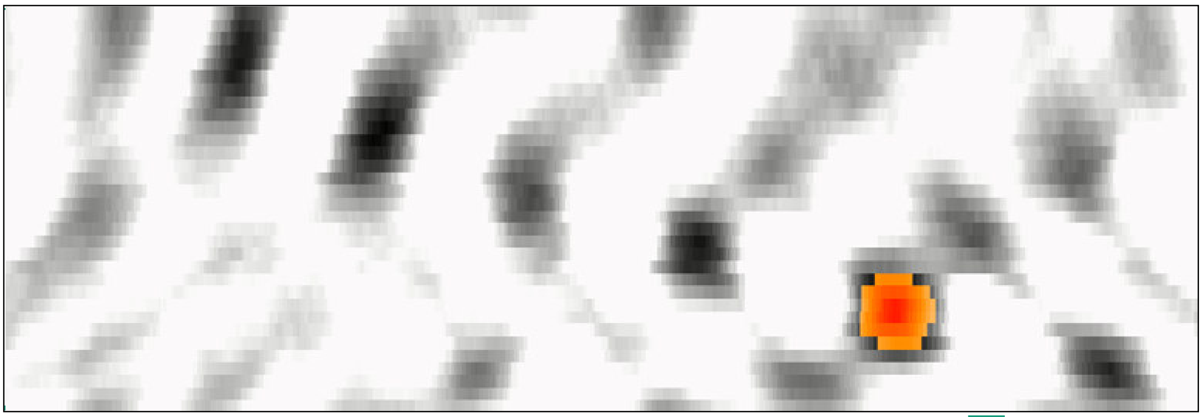
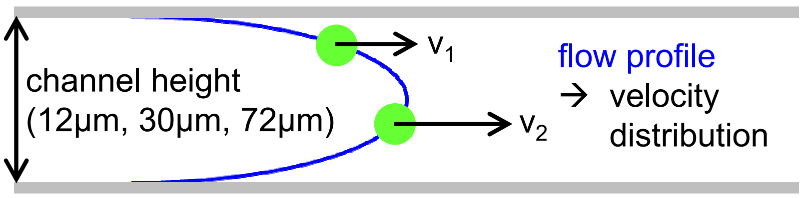
TRANSPORTPHÄNOMENE FÜR PARTIKEL UND ZELLEN IN MIKROFLUIDISCHEN STRÖMUNGEN

Michael Baßler



Fraunhofer IMM

Transport in microchannels



particle velocity depends on

- flow rate and channel dimensions
- flow regime (laminar), viscosity (Newtonian / non-Newtonian)
- position in flow profile (distance to wall)
- particle size, shape (spheres) and deformability (rigid, soft)

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Tubular pinch effect – equilibrium position

Segré-Silberberg effect (nature, 1961)

- spheres: 0.8 mm, 1.6 mm
- tube: 11.6 mm
- d/R = 0.07, 0.14

→ equilibrium position: ~0,6*R (independent of experimental parameters)

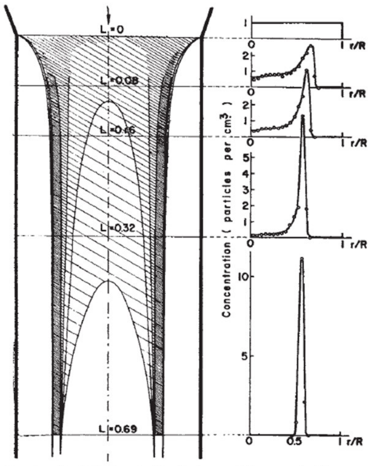

Fig. 2. The 'tubular pinch' effect: particle concentration as a function of radial and longitudinal position in tube. Initial concentration 1 part./cm.³. Concentrations are indicated by shading; closest shading (more than 2 part./cm.³); next (1–2 part./cm.³); next (0·5–1 part./cm.³); lightest shading (0–0·5 part./cm.³)

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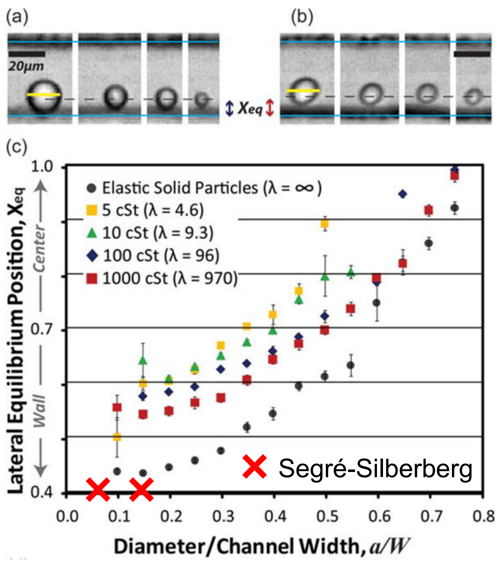
Size dependence of equilibrium position

Hur et al. (LabChip 2011)

- rigid particles vs. droplets
- larger object closer to center
- droplets slightly closer to center



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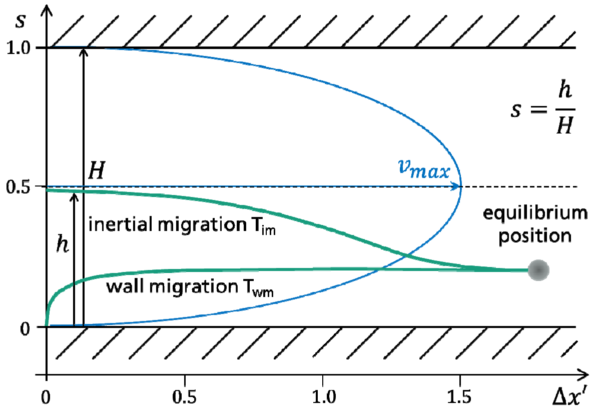
Travel length to equilibrium position

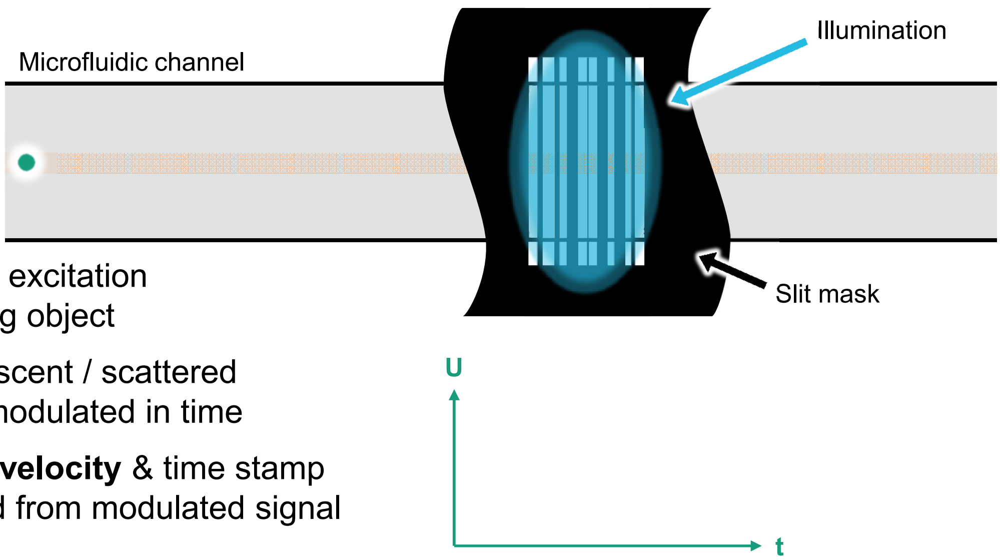
- Ho & Leal
  (J. Fluid Mech. 1974):

$$\Delta x = \frac{\Delta x' H^4}{Re(R_P)^3} = 36\pi \int_{s_0}^{s} \frac{s'(1-s')}{G(s')} ds'$$

- Some particles may ride in the center
- wall migration more efficient



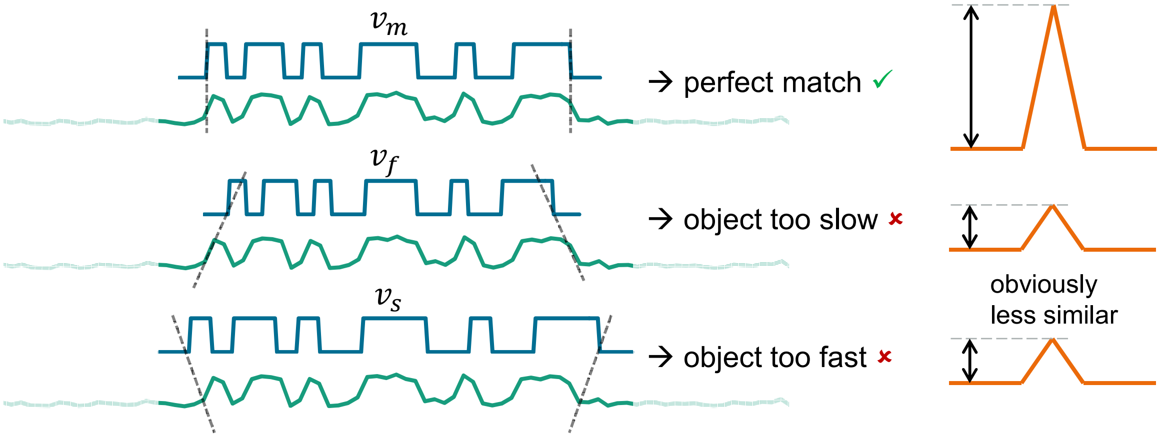
Flow cytometry: spatially modulated excitation



- patterned excitation vs. moving object
  - → fluorescent / scattered light modulated in time
- intensity, **velocity** & time stamp recovered from modulated signal

Data processing

- object velocity recovered by correlating the signal with a set of stretched variations of the known sequence



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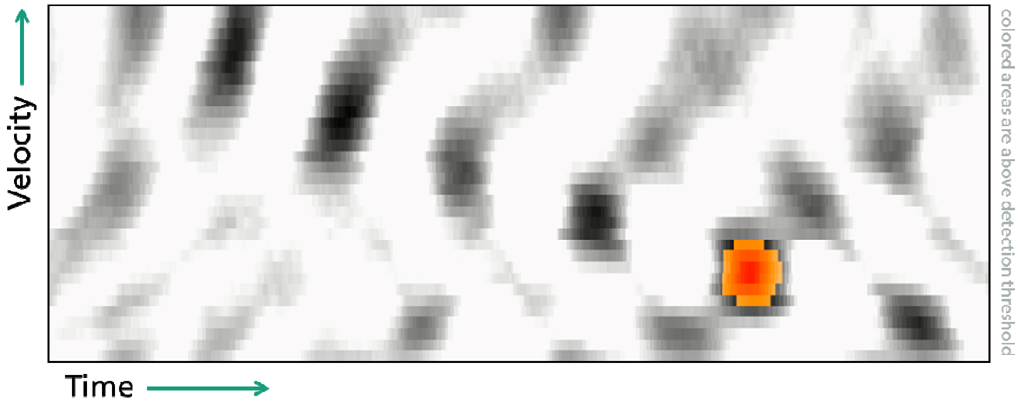
Data processing



- individual objects are recorded as (intensity, velocity, time)-tuples
- further analysis operates on these tuples

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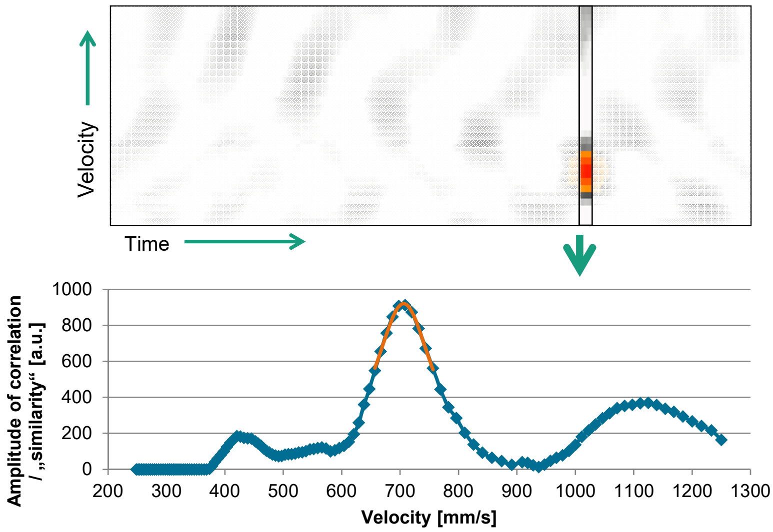
Recovery of velocity and intensity

- extract „time-slice“ containing event
- fit Lorentz distribution

$$\frac{1}{\pi} \cdot \frac{w}{w^2 + (x - c)^2}$$

- recover velocity and intensity from fit



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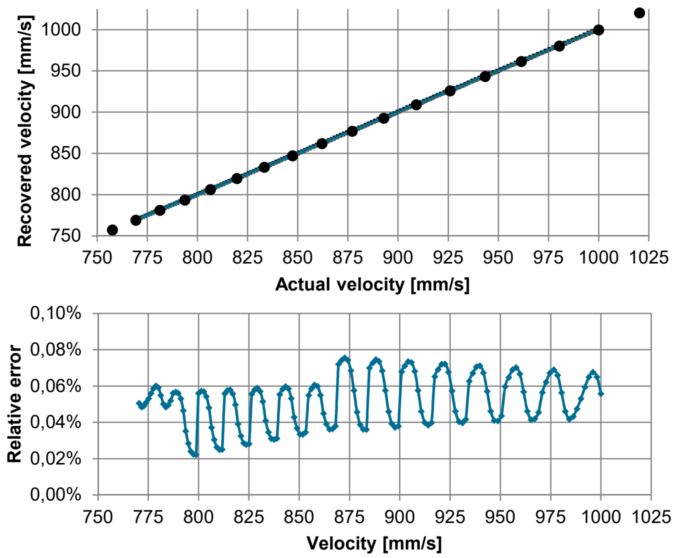
Precision

- velocity recoverable with high precision
- error below 0.1% (using the Lorentz fit) !
- higher density of velocity channels → enhanced precision
- amplitude carries error of ~2%



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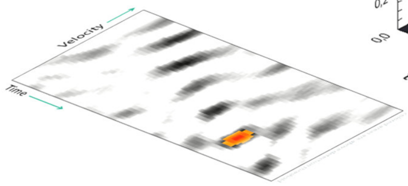
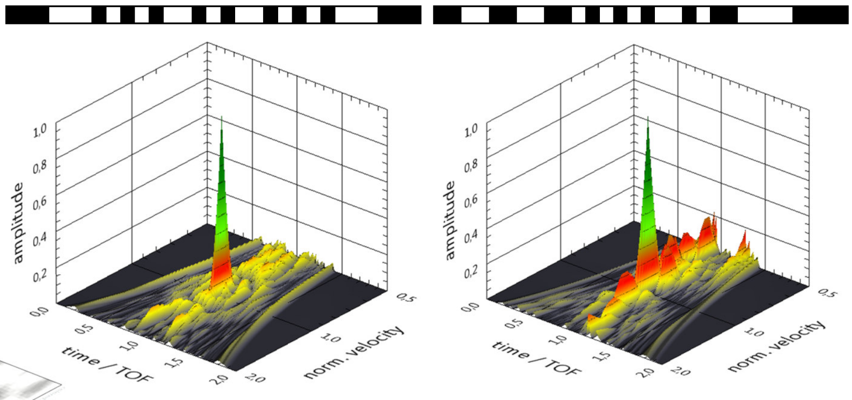
Side lobes and optimized codes

- 2D-Q-factor

$$Q_{2D} = \frac{Gain_{SNR}}{\max(SL)}$$



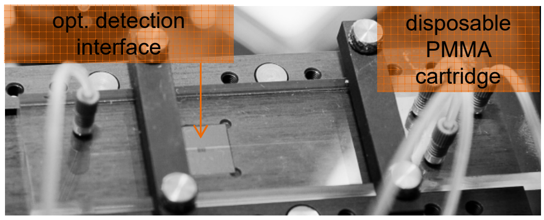


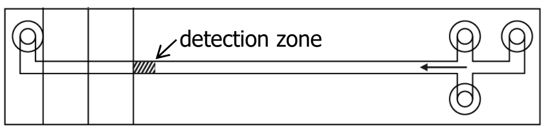
$Q_{2D}(QP29) = 27$ $Q_{2D}(MF30) = 11$

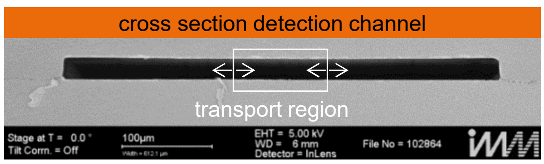
Microfluidic channel

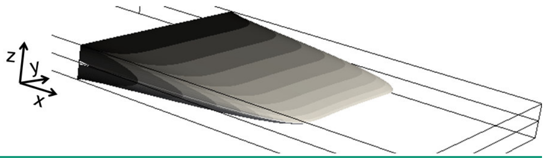
- material: PMMA
- one sample port and two lateral sheath ports
- channel dimensions:
  width 500 µm
  depth 12µm, 30 µm, 72 µm
  length 50 mm

→ partices exposed to 1-dimensional flow profile in transport region !





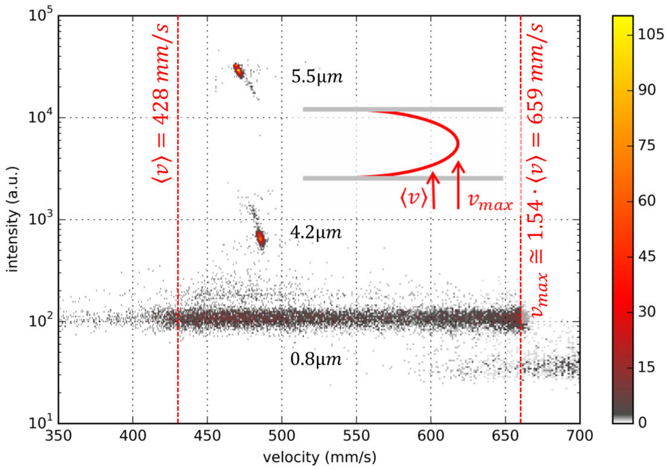




Particle scatter plot

- channel: 12µm (x 500µm)
- flow rate: 200µl/min
- flourescent particles: excitation 488nm
  emission 554nm

- narrow distribution for 5.5µm and 4.2µm
- wide distribution for 0.8µm



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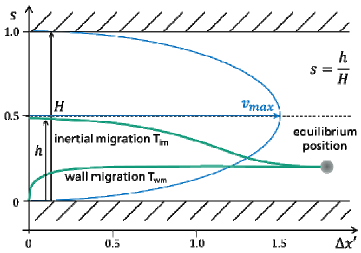
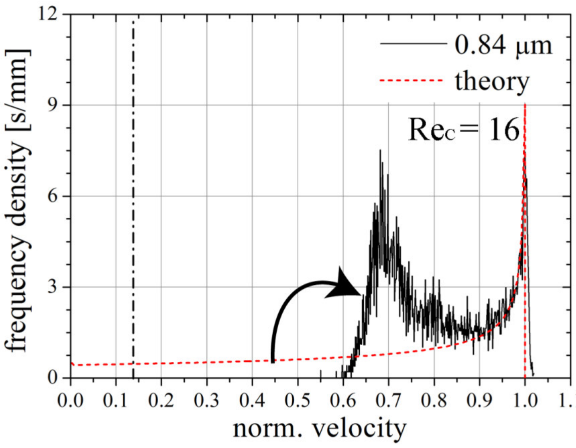
Velocity distribution for 0.8µm particles



- Ho & Leal: $(Re = 16)$
  $\Delta x = 1.69\ mm$
- our result at $\Delta x = 47.5\ mm$!



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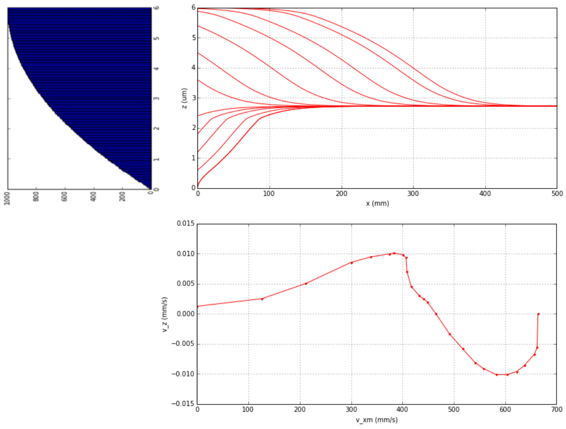
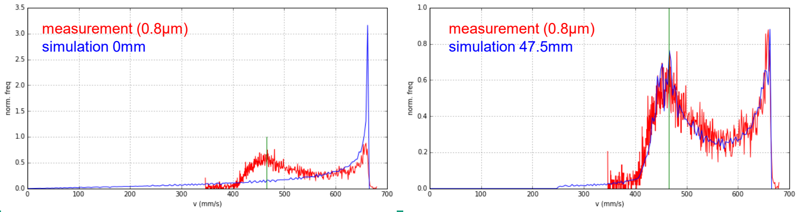
Particle migration (Monte Carlo simulation)

- measurement: 11,163 particles
- simulation: ~130,000 particles

- equilibrium position to be expected at x>350mm (99% of particles in main peak)





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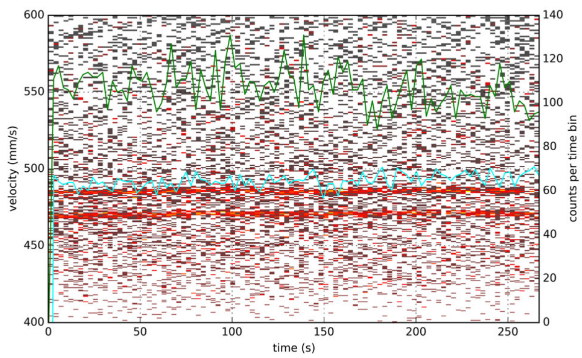
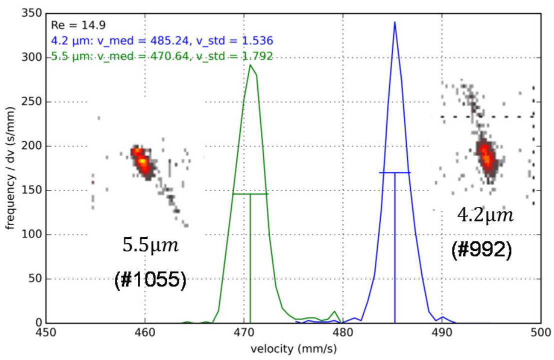
Velocity distribution for 5.5µm and 4.2µm particles

- perfectly constant flow rate
- size distribution within each population





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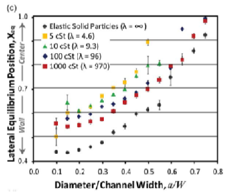
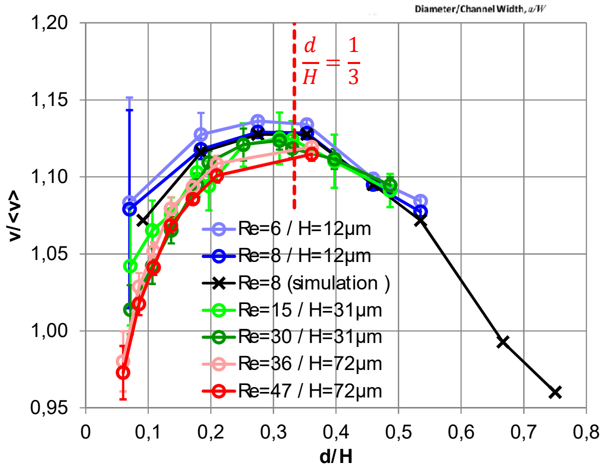
Size dependence of particle velocity



- channel height (µm):
  12, 30, 72
- particle diameter (µm):
  0.8 – 6.4, 2.1 – 15.1, 4.3 – 26.0
- maximum particle velocity at

$$\frac{d}{H} \approx \frac{1}{3}$$

- estimated resolution beyond 4.2µm: 50nm



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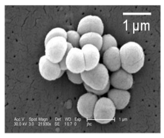
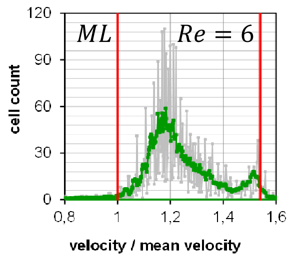
Micrococcus Luteus (bacterium)

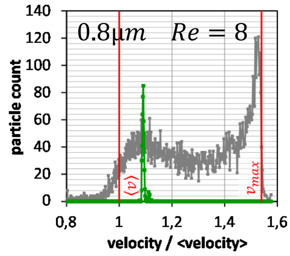




- culture, small (<1µm), sperical
- „rapid“ alignment
- clearly distinct equilibrium velocity:

$$\frac{v_{ML}}{\langle v \rangle} \approx 1.17 \qquad \frac{v_{0.8\mu m}}{\langle v \rangle} \approx 1.07$$

- difference: deformability!
- opportunity: measure size <u>and</u> velocity → asses deformability !



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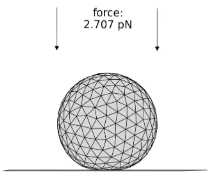
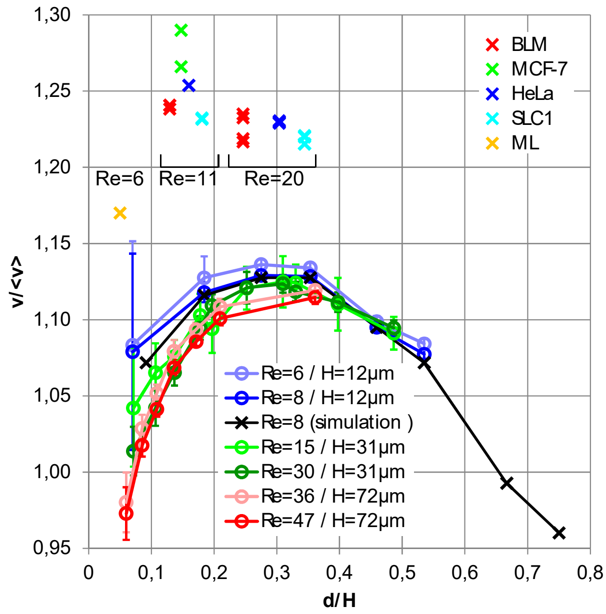
Velocity for deformabable objects (cells)



- bacterium:
  Micrococcus Luteus <1µm
- tumor cell line:
  BLM 12.8±0.5µm
  MCF-7 14.6±0.5µm
  HeLa 15.8±1µm
  SCL1 17,9±0.5µm



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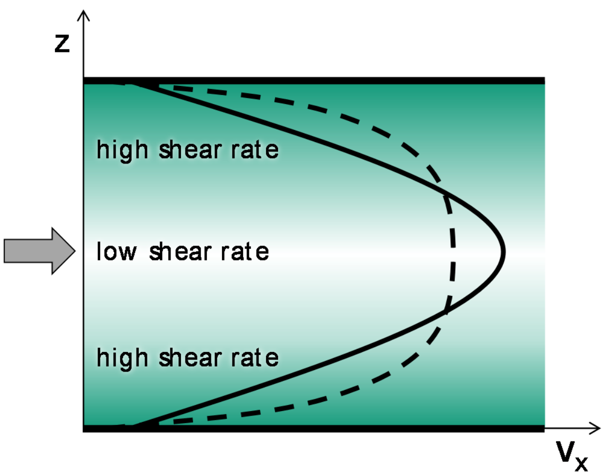
Newtonian and non-Newtonian fluids



- Newtonian fluids
  - parabolic flow profile
  - constant viscosity over channel height z
- shear-thinning fluids
  - lower viscosity at the wall
  - higher viscosity in the middle
  - flattened flow profile
  - lower maximum velocity

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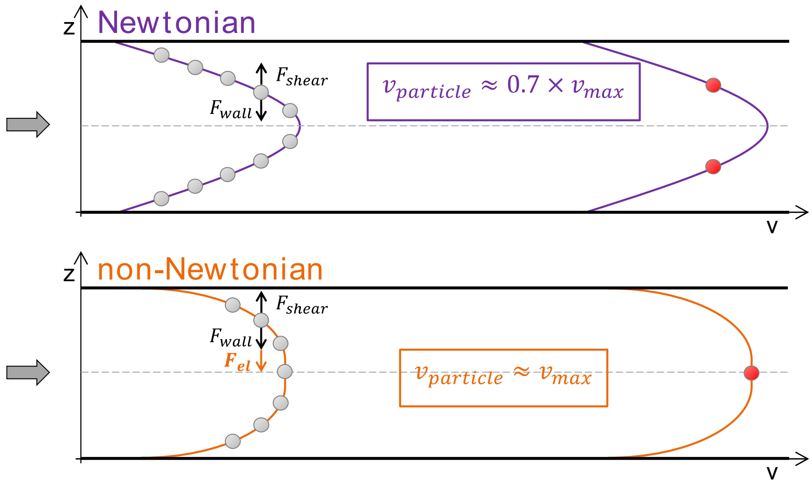
Objects in Newtonian and non-Newtonian fluids



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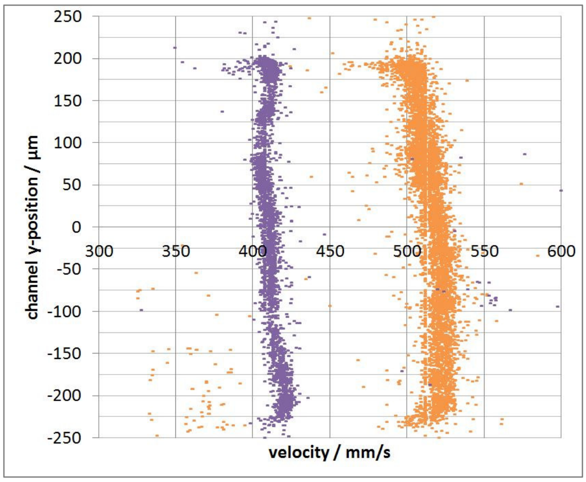
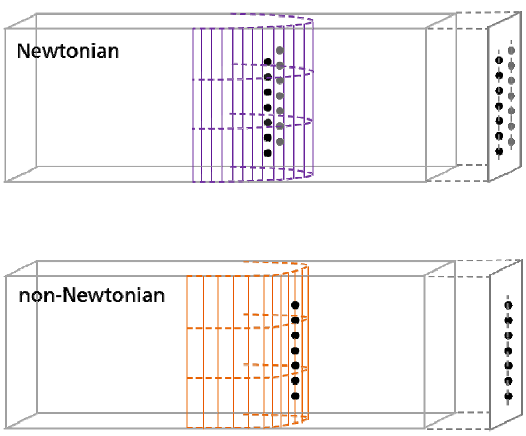
Alignment of microparticles in single-component microflows





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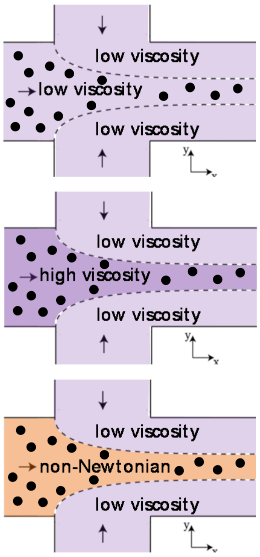
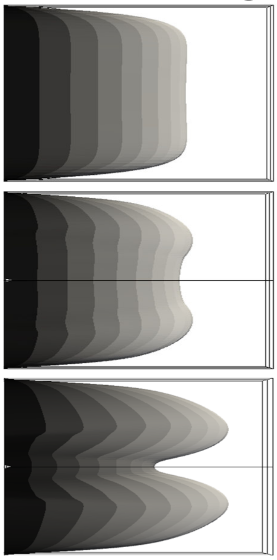
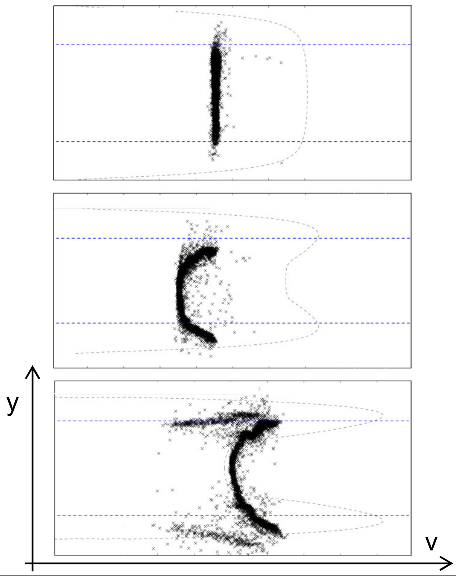
Alignment in various flow regimes







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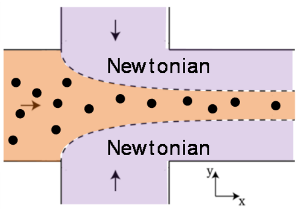
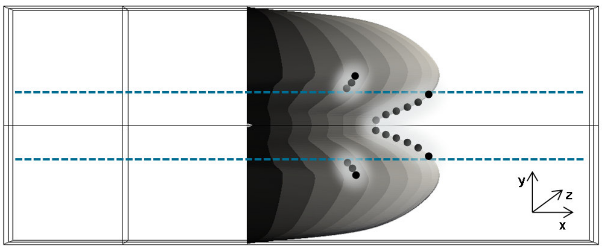
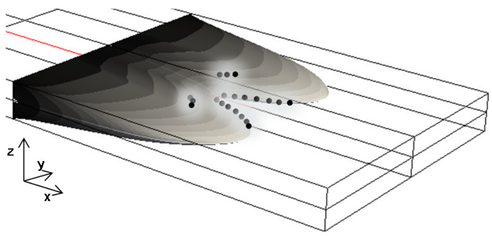
Alignment of microparticles in *Newtonian* and *non-Newtonian* multi-component microflows



- 2D v-shaped flow profile





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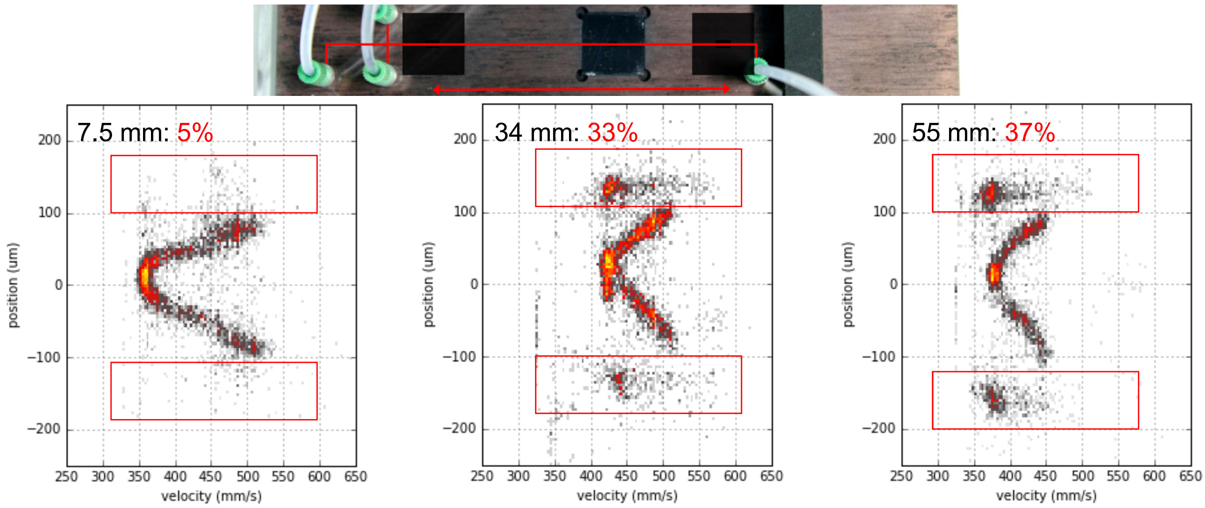
Particle transfer along channel length



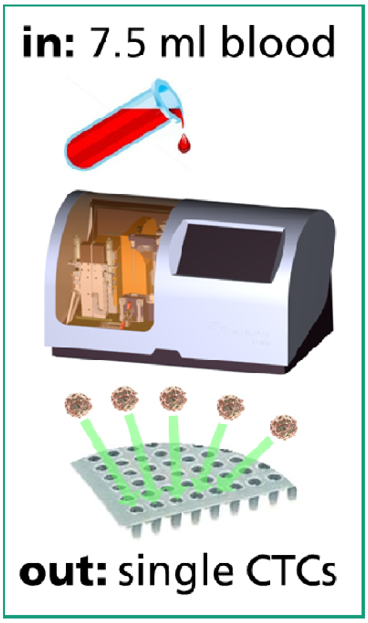
Summary: particles in microflows

- equilibrium velocity depends on
  - size
  - deformability
  - rheology
- equilibrium velocity „easy“ to measure
  → access to physical particle/cell properties
- Particle migrate in complex flow profiles
  → high potential for sorting and/or enrichment

CTCelect – fully automated microfluidic system for isolation of circulating tumor cells

Get access to single CTCs

- Liquid Biopsy – Circulating Tumor Cells (CTCs)
- fully automated device for isolating
  single CTCs from blood primary tubes
  - no manual sample preparation
  - high reproducibility
- provide viable CTCs ready-to-use for single cell analysis: NGS, RT qPCR, …



Technical challenge: number of CTCs

- CTCs in blood: 1-10 CTCs / mL blood
- sample volume: 7.5 ml EDTA blood
  - ➔ 7-75 CTC expected

- 7.5 ml blood are containing
  - ~ 3.5 x 10^{10} erythrocytes
  - 2.8-4.9 x 10^{7} leukocytes
  - 2.1-2.8 x 10^{9} thrombocytes
  - **In sum: ~3.7 x 10^{10} "cells"**

Finding the needle
in the hay stack!

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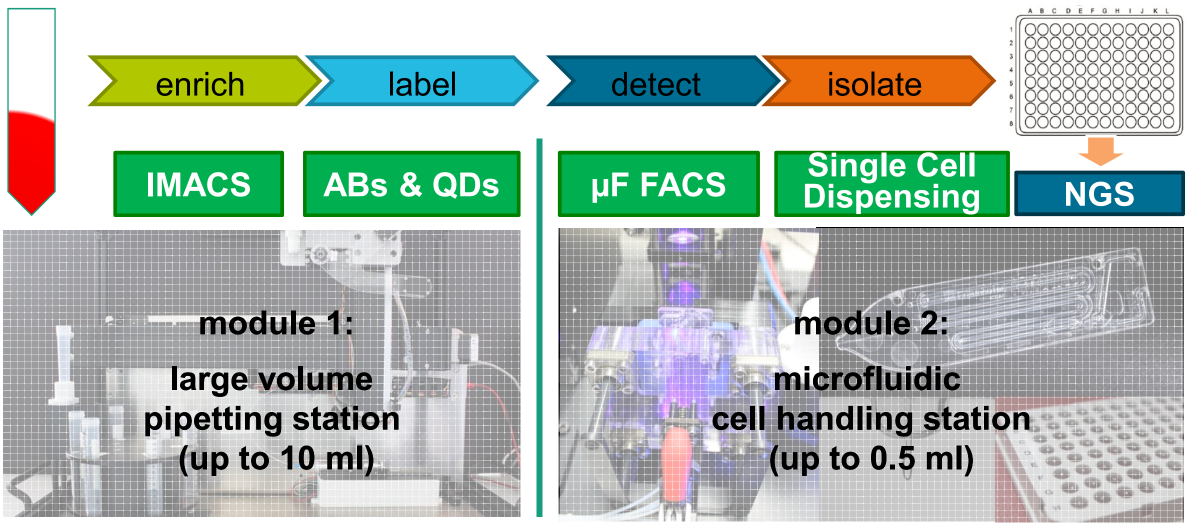
The CTCelect strategy



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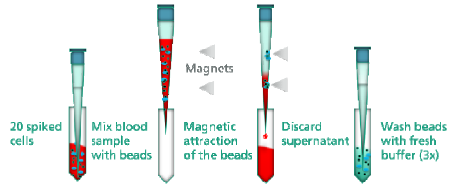
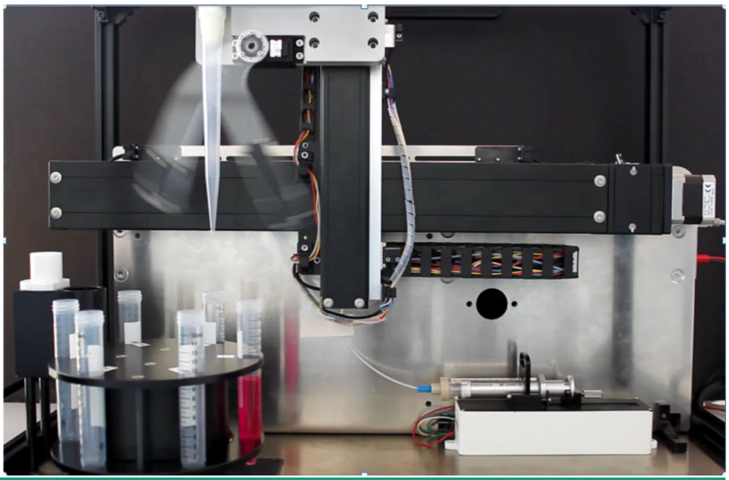
Automated liquid handling and immunomagnetic separation



- fully automated
- max. 7.5 mL sample
- optimized reagent kit

- performance:
  20 MCF7 cells in
  7.5 mL whole blood

→ cell recovery 93%



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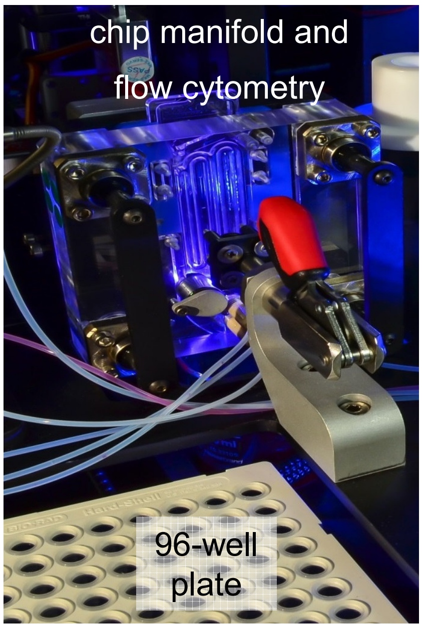
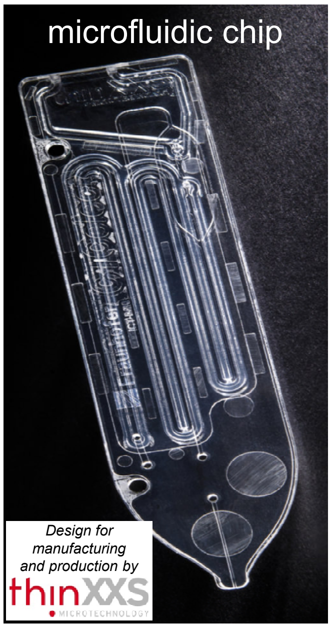
Microfluidic cell handling

- flow cytometry
- single cell dispensing





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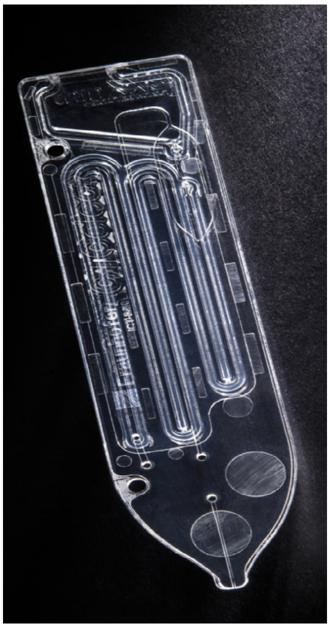
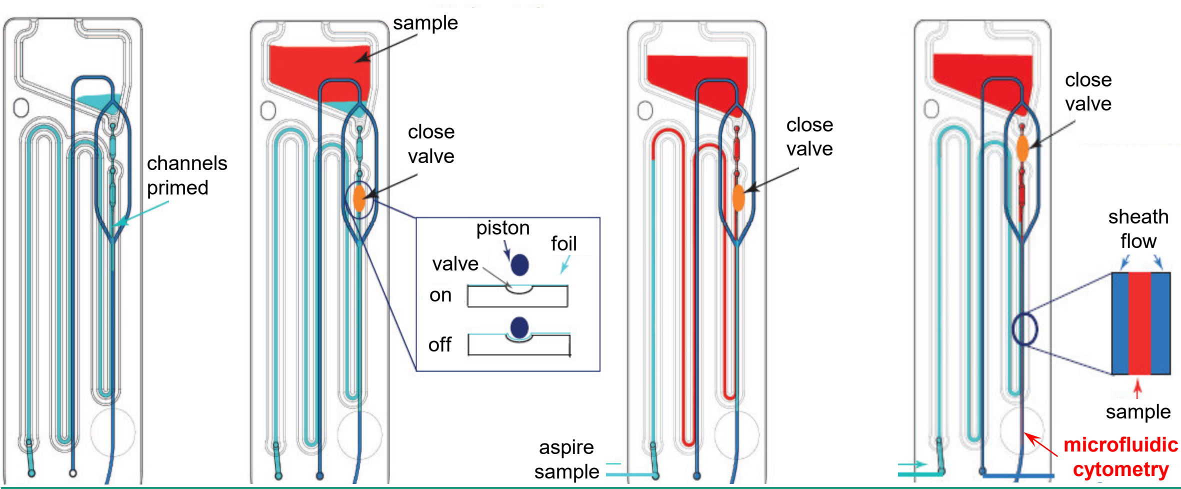
Main features of microfluidic chip

- sample reservoir
- storage meander
- two membrane valves
- flow cytometry channel
- sheath flow
- dispensing nozzle



Microfluidic protocol

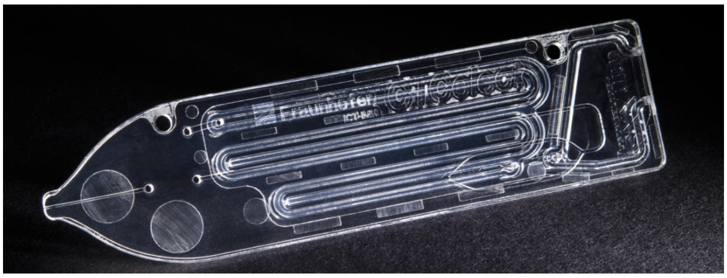
- **cell recovery 91%**

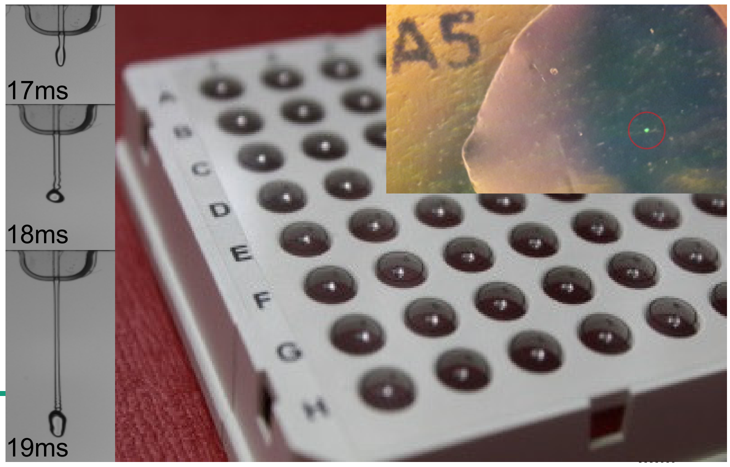


Microfluidic single cell dispensing

- real time data processing by FPGA
- in case of CTC: FPGA triggers dispenser (delay depends on velocity)
- feasable droplet size 0.3 µl – 3 µl
- droplets always aligned to cavities

→ **cell recovery 89%**





Summary – system performance

immunomagnetic enrichment:	93%
microfluidic cytometry:	91%
after single cell dispensing:	89%
total cell recovery:	**75%**
purity (probability for background of one, two or three lymphocytes)	**10%, 1%, 0.1%**

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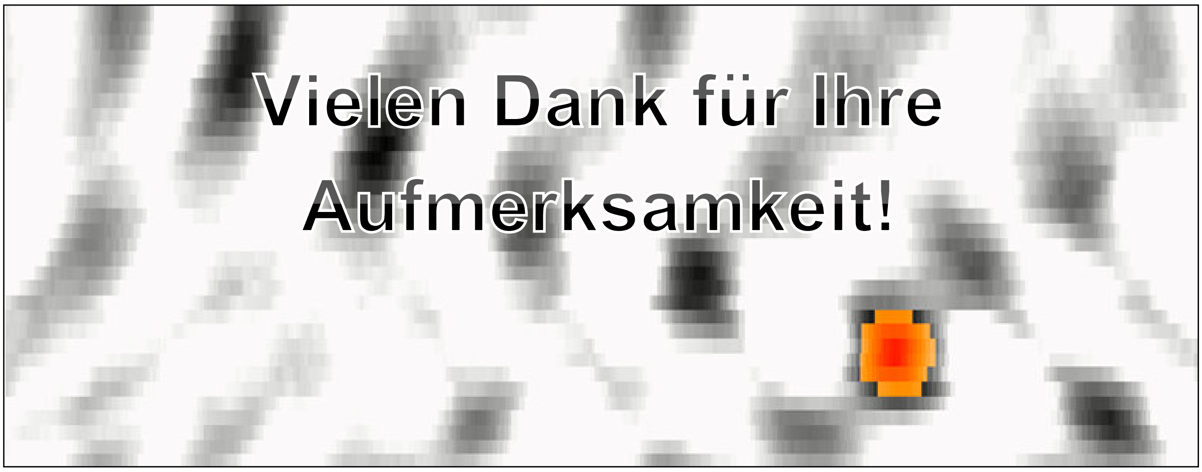
Vielen Dank für Ihre
Aufmerksamkeit!