Integrated microfluidics / lab-on-chip systems for point-of-care medical diagnostic applications

EK MFA

ÓBUDAI EGYETEM
ÓBUDA UNIVERSITY

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www.biomems.hu, www.mems.hu

The aim of my research

- design and development of integrated microfluidic systems for sample transporters using fast and reliable diagnostic tool
- screen for urine bacterium on a targeted microfluidic platform
- study and determine the geometry and material structure of the microfluidic system; promoting compatibility with industrial technologies

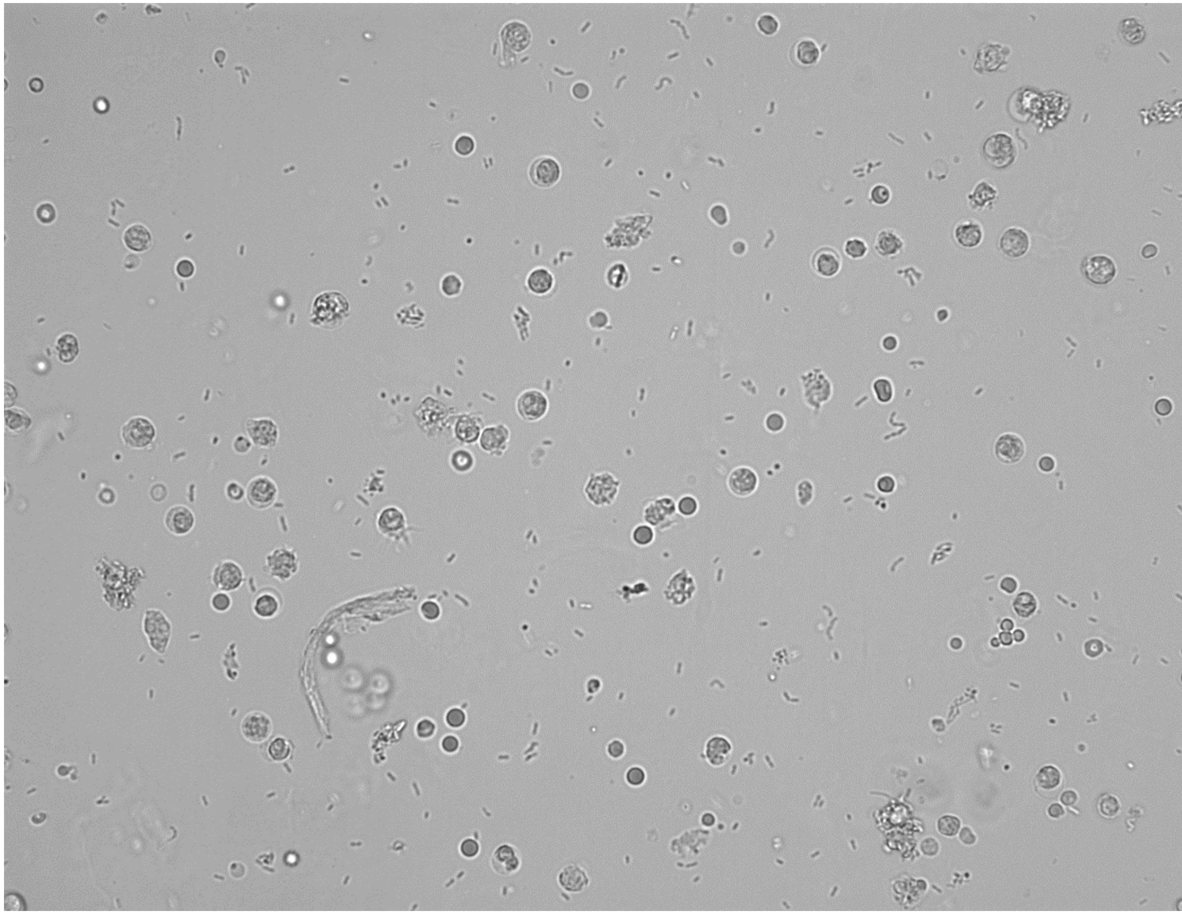
Main tasks of this semester

Investigation and application of hydrodynamic size and morphology based separation possibilities, such as:

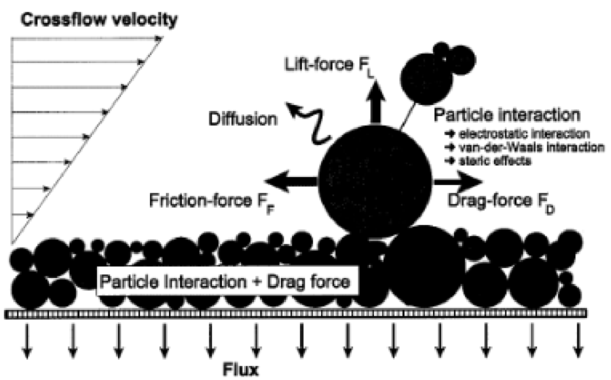
- **Crossflow filtration**
- **Lateral focusing** – Lateral concentration of the sample

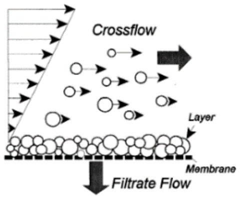
Cell separation

- RBC (7-10µm)
- Leukocyte (8-15µm)
- Epithelial (~40-60µm)
- Small round epithelial (~10-40µm)
- Hyalin cylinder (~10-40-60µm)
- Phatological cylinder (~10-40-60µm)
- Bacterium
  - Stick (BACr) (~2-4µm)
  - Coccus (BACc) (~1-2µm)
- Crystals (~5-20-40µm)
  - CaOxd
  - CaOxm
  - Triple phosphate
  - Uric acid crystal
- Fungi (~7-50µm)
- Neck
- Sperm (~20-30µm)

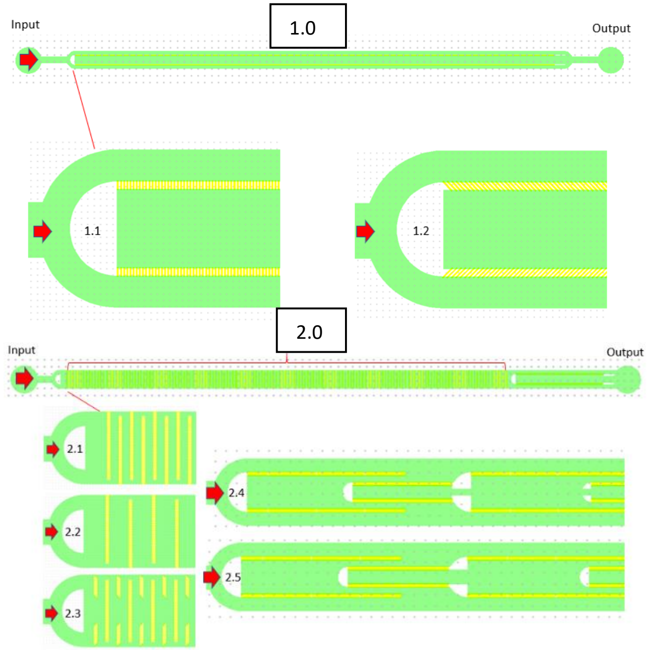


I. Cellseparation (theory)





[S. Ripperger et. al]



Experiment (previous semester)

Measurement procedure

Bead mixture (~2 ml; 0,01% Tween 20):

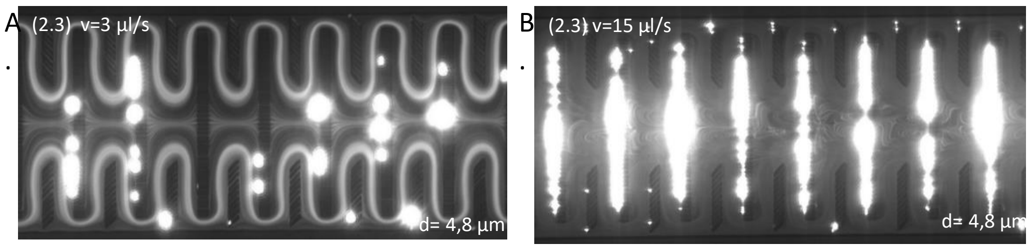
15 ul (d=4,8 um) Beads – Green [FITC]

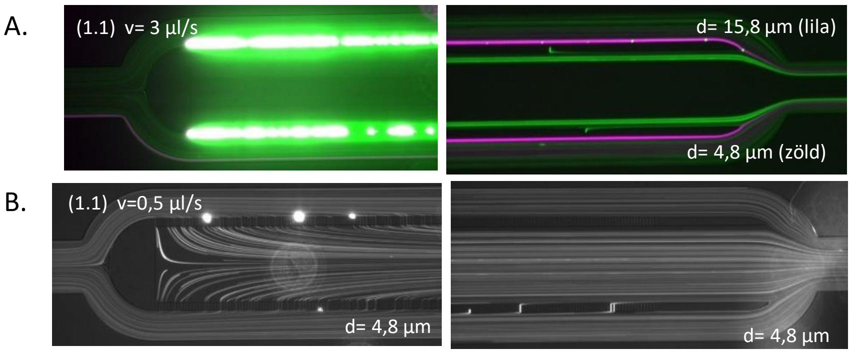
35 ul (d=15,8 um) Beads – UV [DAPI]

1950 ul dH2O

0,2 ul Tween 20

- Crossflow - chip treatment with Pluronic ~ 1,5 h
- Crossflowban flow rate: 0,5-1ul/s, 3ul/s
- Lateral focuing, flow rate: 0.5-1-3-6-9-15 ul/s





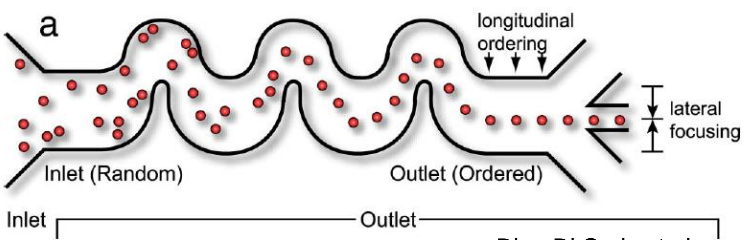
Type 1.1 chip testing (A.) 3 µl/s; és (B.) 0,5 µl/s - flow rate, at different wavelength FITC -4.8 um bead (green); DAPI – 15.8 um (purple)

II. Lateral focusing (theory)

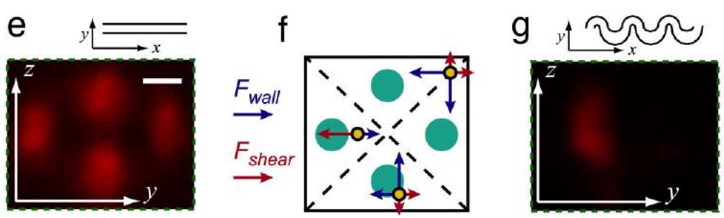
Continuous inertial focusing, ordering, and separation of particles in microchannels

Dino Di Carlo, Daniel Irimia, Ronald G. Tompkins, and Mehmet Toner*

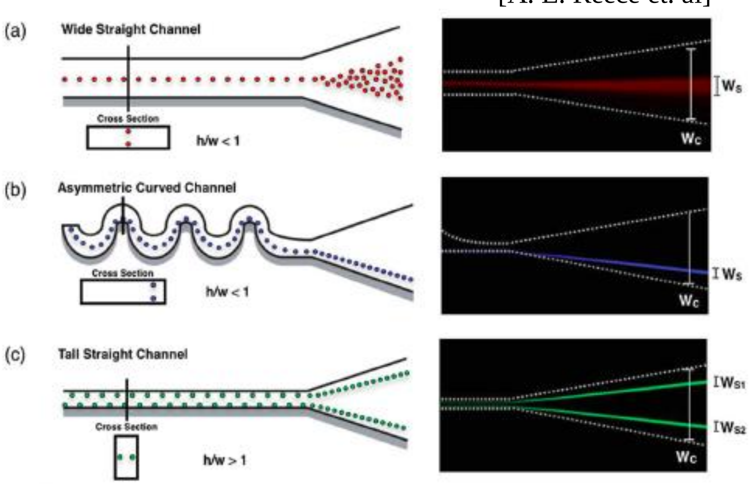
$$R_p = R_c \frac{a^2}{D_h^2} = \frac{U_m a^2}{\nu D_h}.$$



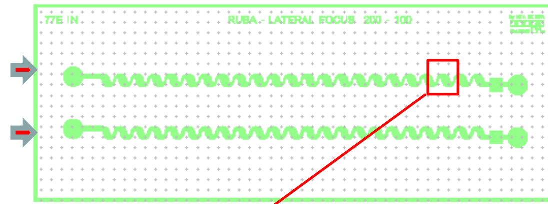
Dino Di Carlo et al.



[A. E. Reece et. al]

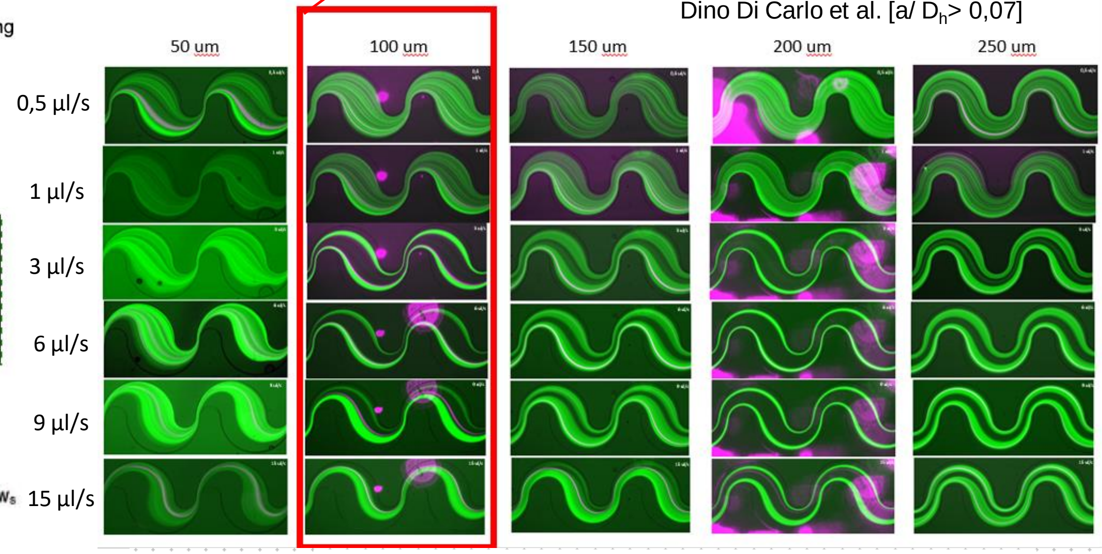


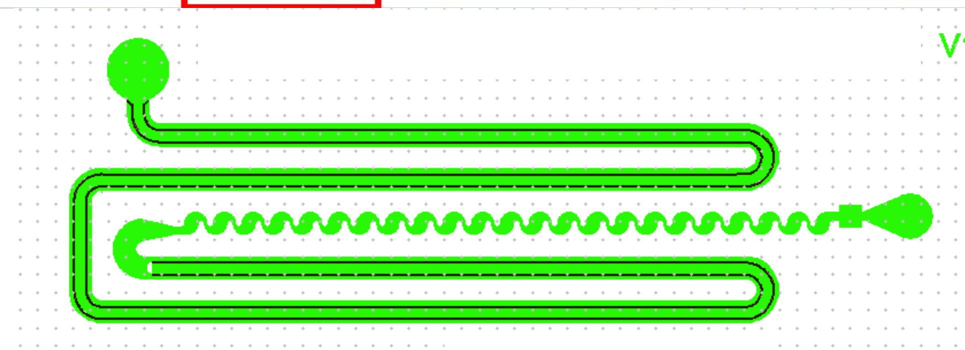
Experiment (previous semester)



w (µm)	4,8 µm/Dh	15,8 µm/Dh
50	0,096	0,316
100	0,072	0,237
150	0,064	0,211
200	0,06	0,1975
250	0,058	0,1896

Dino Di Carlo et al. [a/ D_h> 0,07]

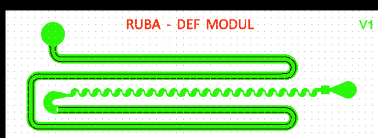


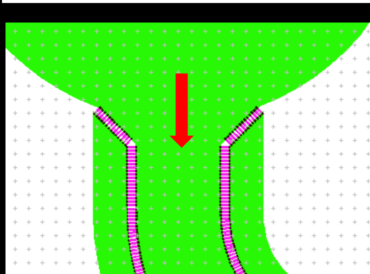


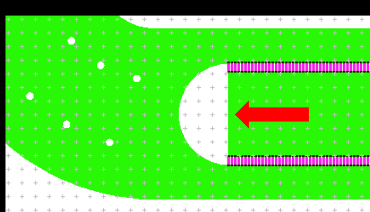
Experiment with Escherichia coli

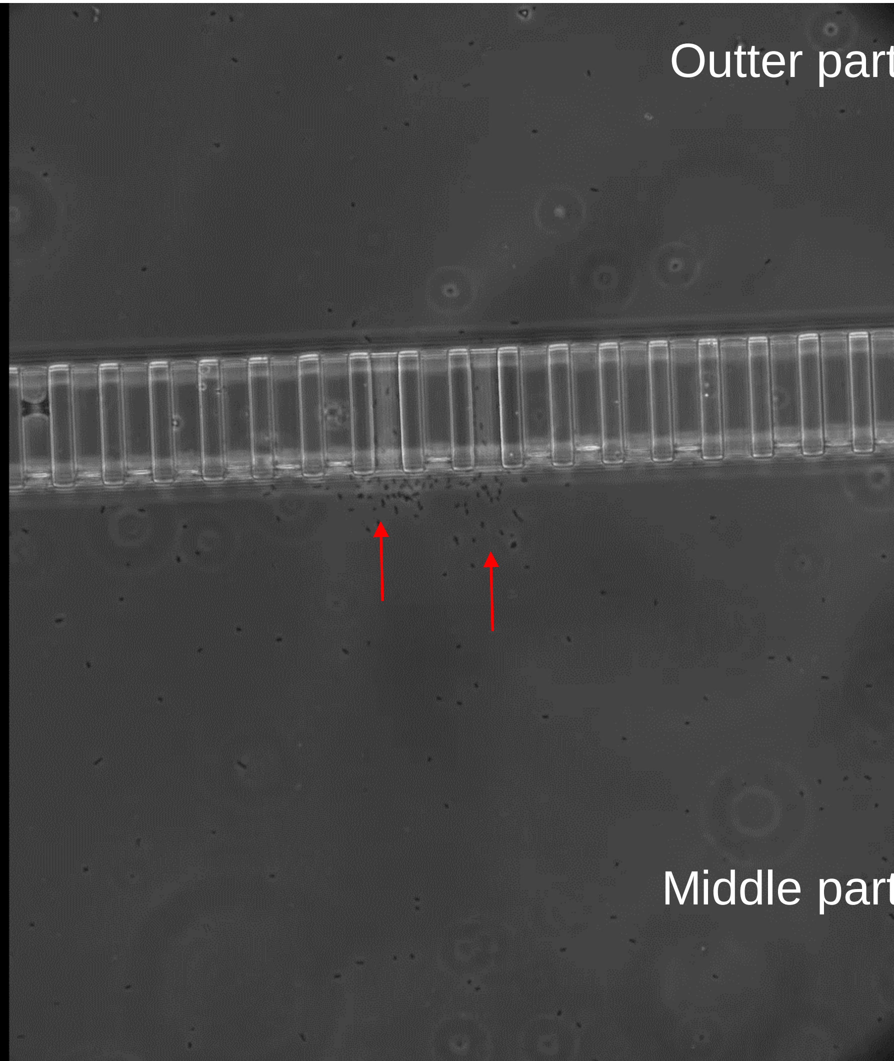
Height: 50 - 10 µm

- Surface treatment ~ 1h 4% Pluronic L35; then rised with dH2O
- 4 MCF E.coli (kb. 10^7 – 10^9 baci)-> 10x dilution in PBS



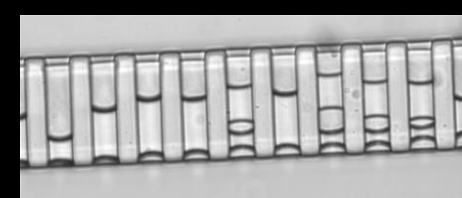


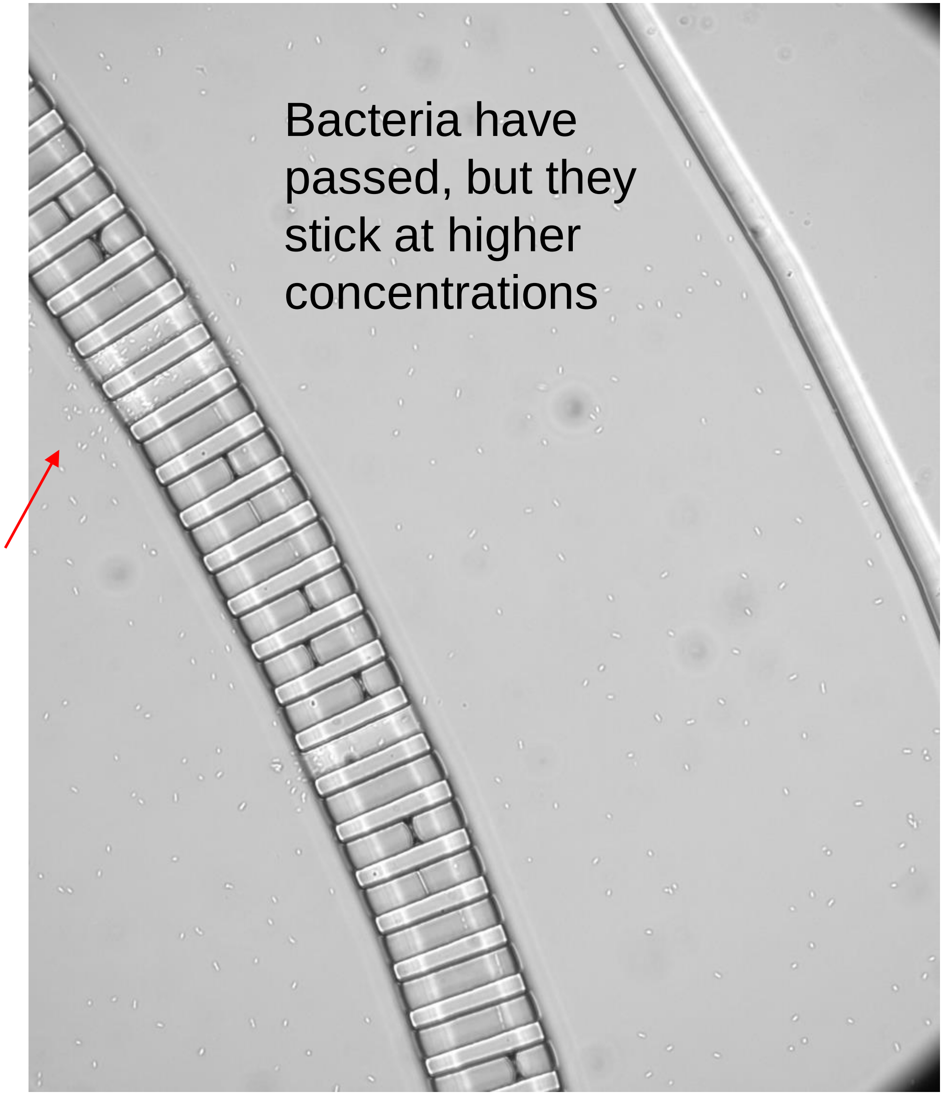


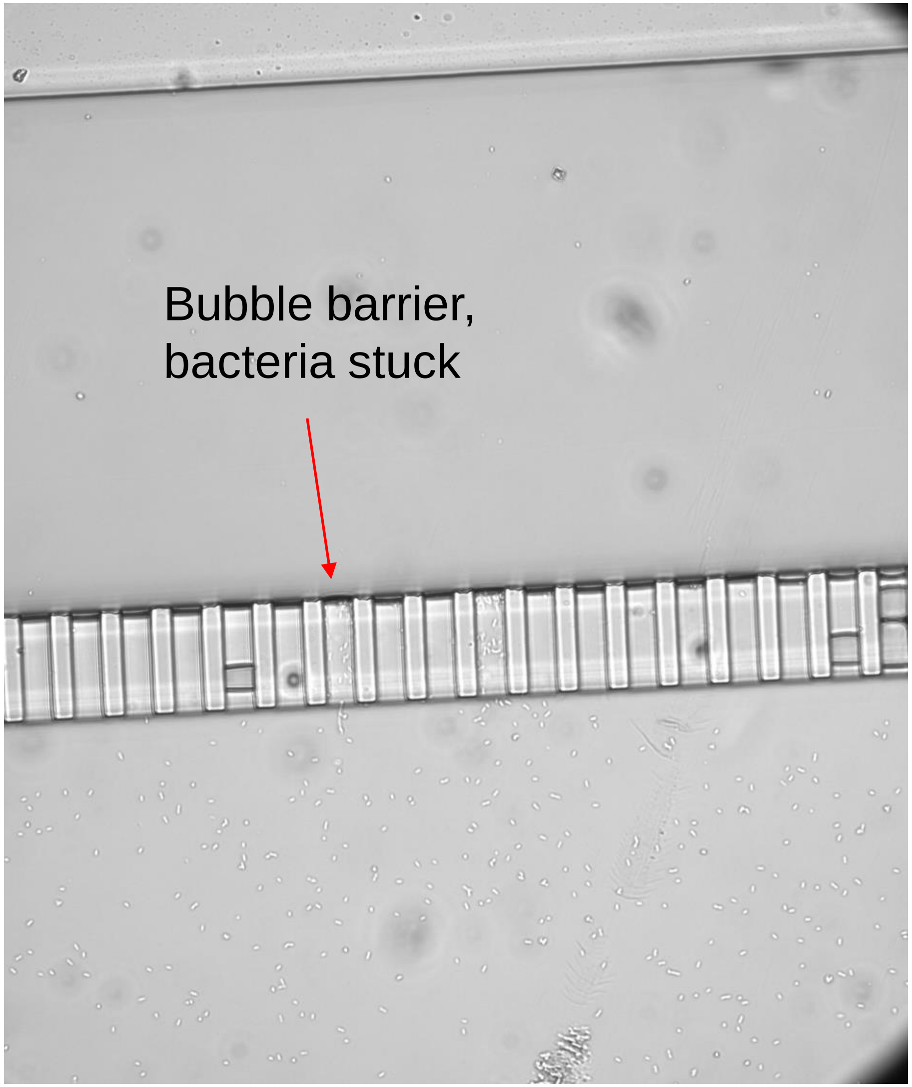


- Flow: from the right to the left
- Lot of bacteria sticks to the surface instead of passing through

4% Pluronic L35 - PDMS




Bacteria have passed, but they stick at higher concentrations


Bubble barrier, bacteria stuck

Further surface treatment needed

- Dissolve 8.7g PVA (powder) in 100 ml dH2O (stirring, boiling…. 25°C -> 100°C->65°C)
- Flushing PDMS with IPA
- Blow off with N2 and place on a hot (110 °C for 15 min)
- O2 plazma treatment of PDMS (100 W, 1 min)
- Soak for 10 min in 87% PVA
- Blow off with N2 and place on a hot plate at 110 °C for 10 min

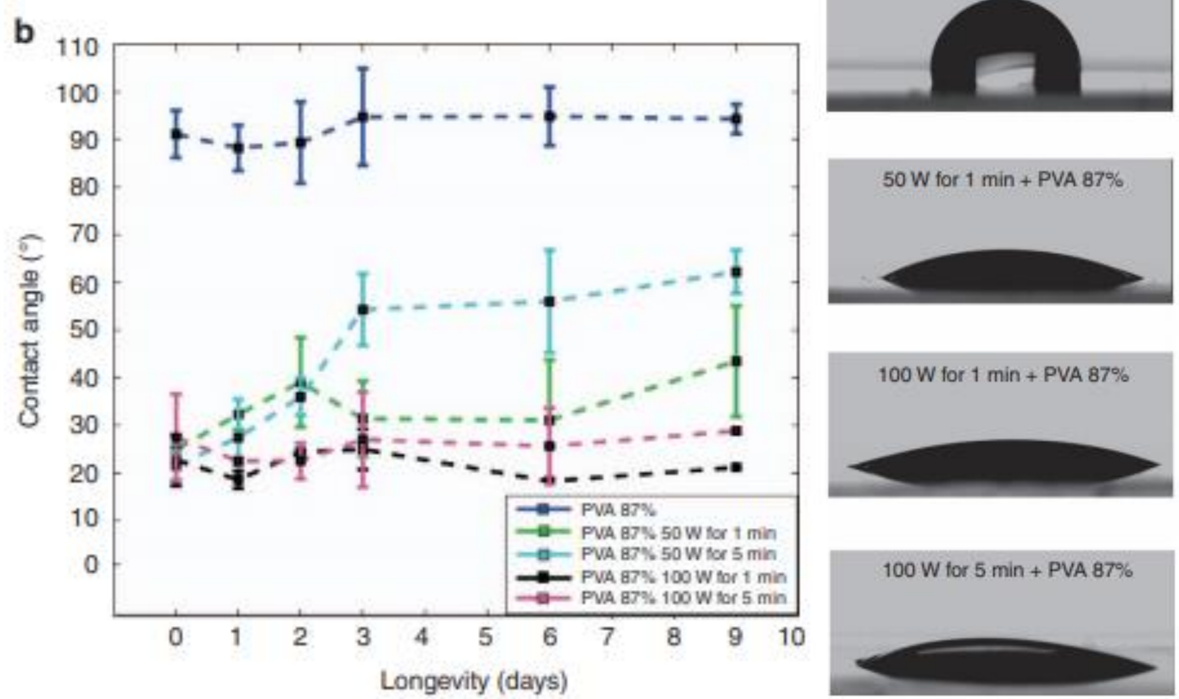
ARTICLE

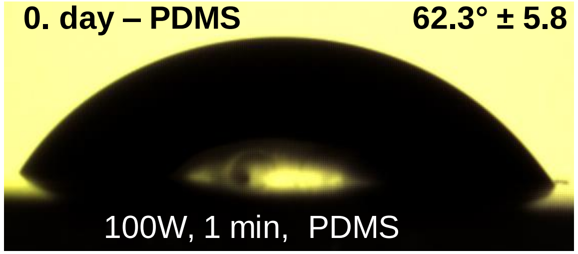
Hydrophilic surface modification of PDMS for droplet microfluidics using a simple, quick, and robust method via PVA deposition

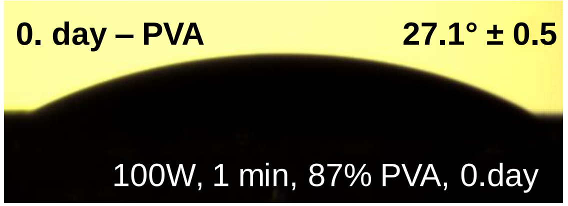
Tatiana Trantidou[1], Yuval Elani[1,2], Edward Parsons[3] and Oscar Ces[1,2]

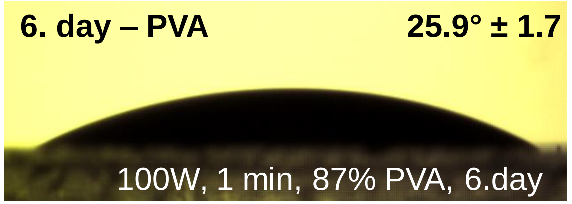
Microsystems & Nanoengineering (2017) 3, 16091; doi:10.1038/micronano.2016.91

www.nature.com/micronano

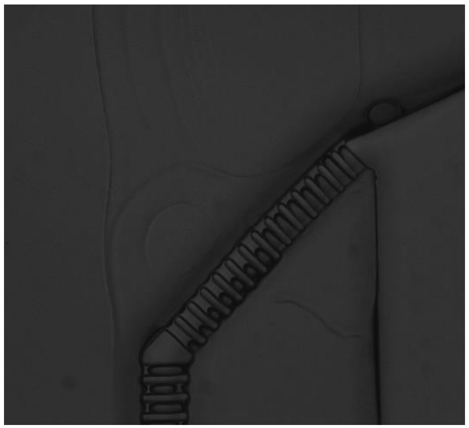


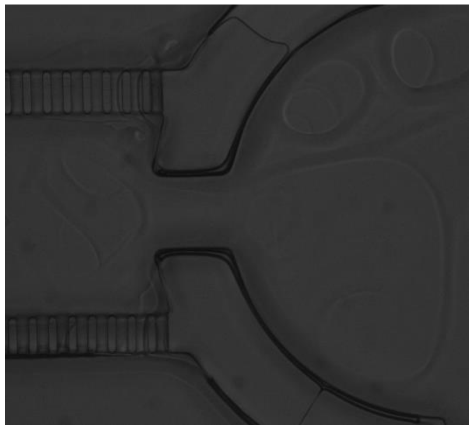


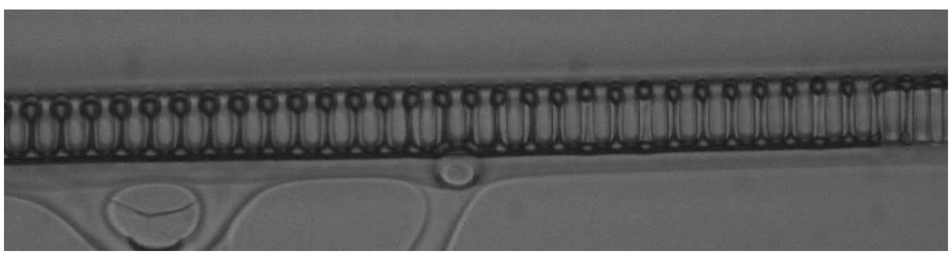


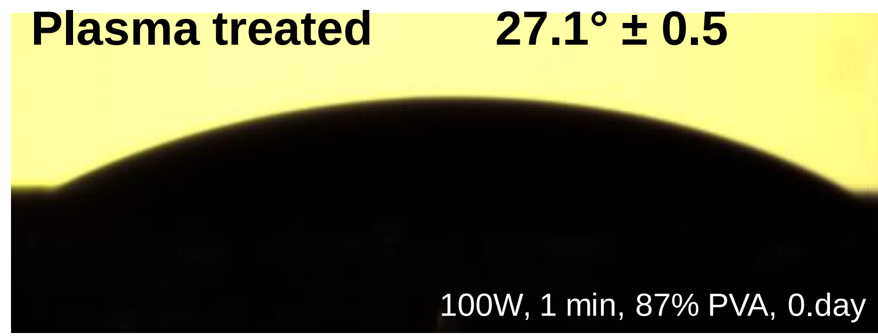


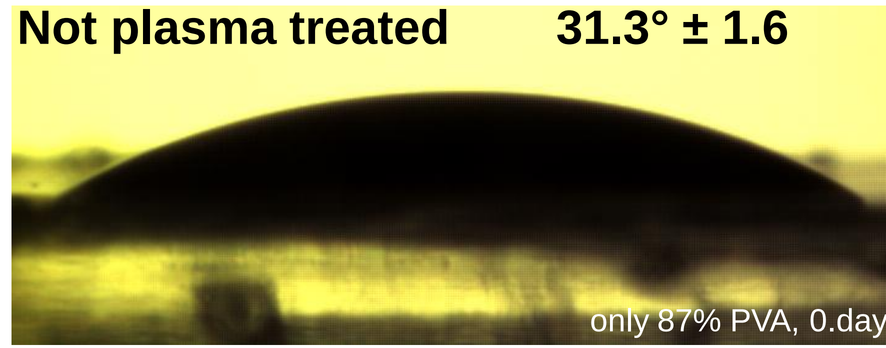
100-10 um-V2, O2 plazma (100W, 1min, ~25 min PVA treatment)











	Contact angle	Dispersion
21% PVA	25,4	4,7
43,7% PVA	20,8	1,6
87% PVA	26,2	0,6

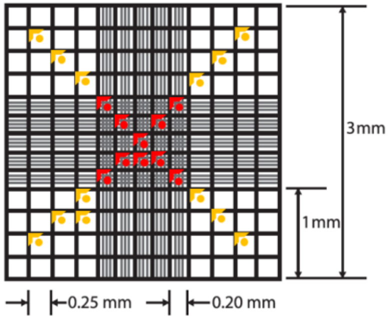
80 C°, 5 min soaking, venting; 10 min 80C°

No further changes brought better results; crossflow measurements were made right after plasma treatment, in water filled channels.

Mix 5 ml	(+ **0.5 ul Tween 20)**
4837,5 ul	dH2O
37,5 ul	15,8 um – UV ; DAPI
37,5 ul	6 um – Pink; Cy3
37,5 ul	1,97 um – L. Yellow; DAPI

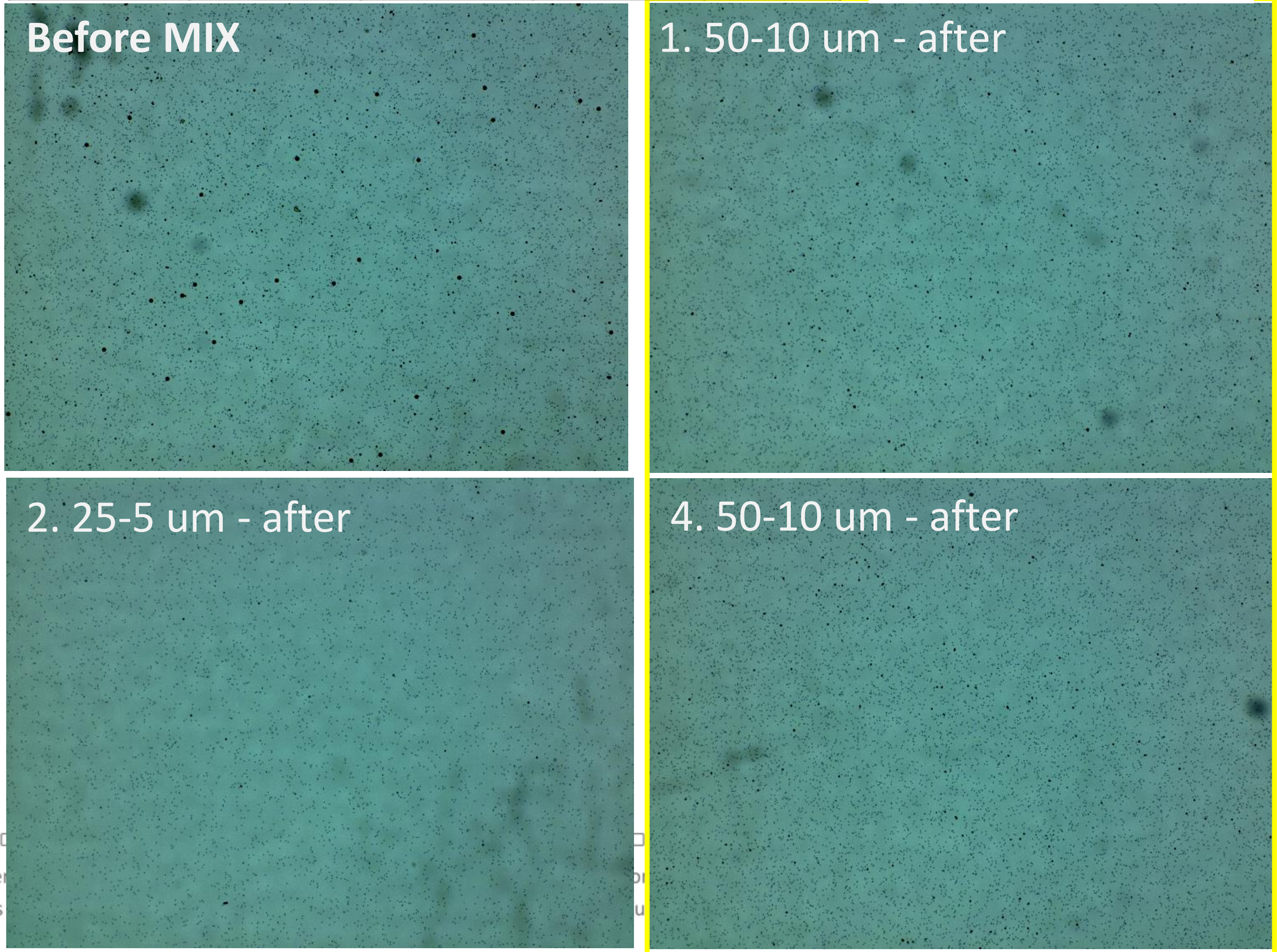
C-Chip (DHC-N01)

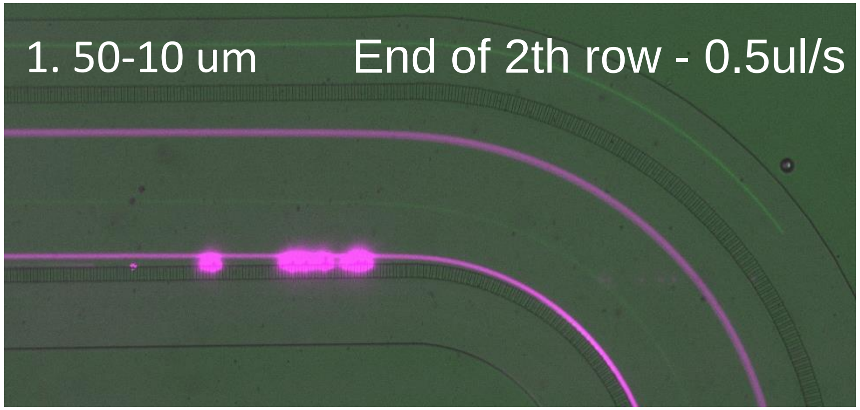
Crossflow parameters (V1 - height)	15,8 um (Σ pieces in 10 squares)	6 um (Σ pieces in 10 squares)	1,97 um (Σ pieces in 10 squares)	Filtered sample volume approx.
Before MIX	3	17	921	
1. 50-10 um	0	14	779	0.7 ml
2. 25-5 um	0	3	360	0.65 ml
4. 50-10 um	0	15	636	0.6 ml

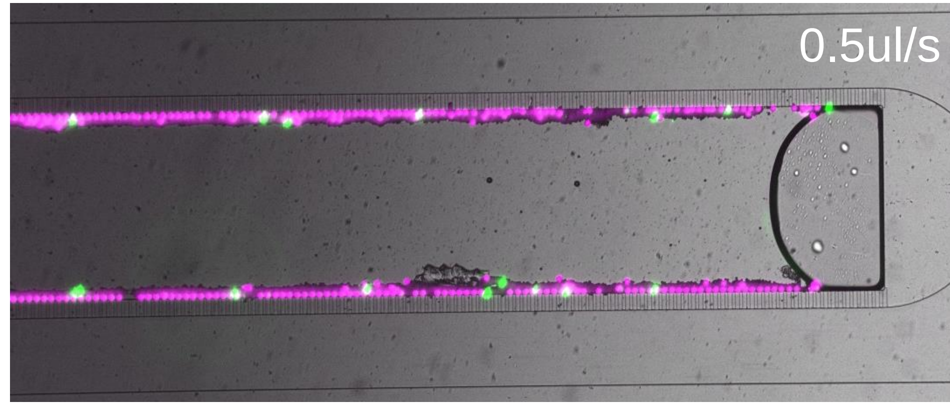


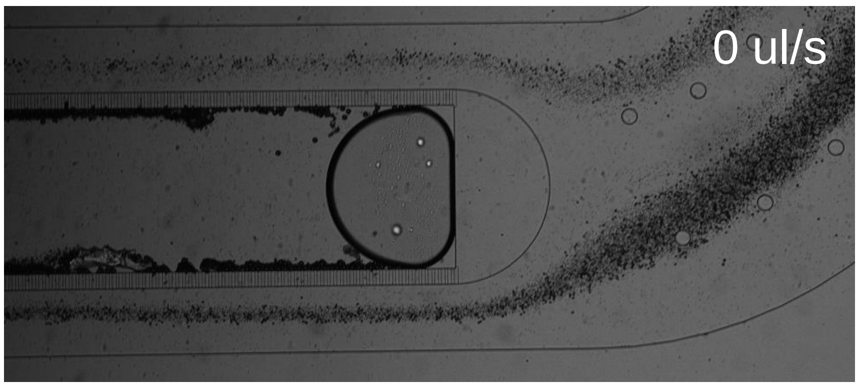
LUNA (min. detect. 3 um)

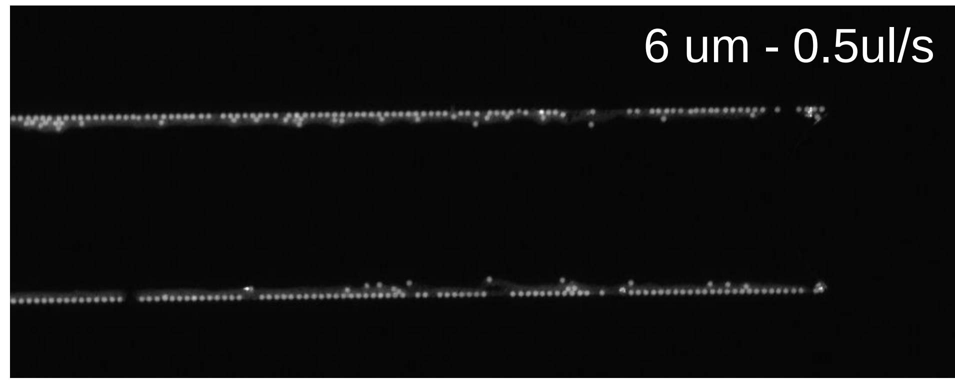
Crossflow type (V1 - height)	Average (3) (cells/ml; average cell size; cell number)
Before MIX	4,33 x 10^5; 5,7667 um 186 cells
1. 50-10 um	2,14 x 10^5; 5,1333 um 120 cells
2. 25-5 um	2,79667 x 10^4; 4,5667 um 12 cells
4. 50-10 um	2,3 x 10^5; 5,0333 um 98,667 cells

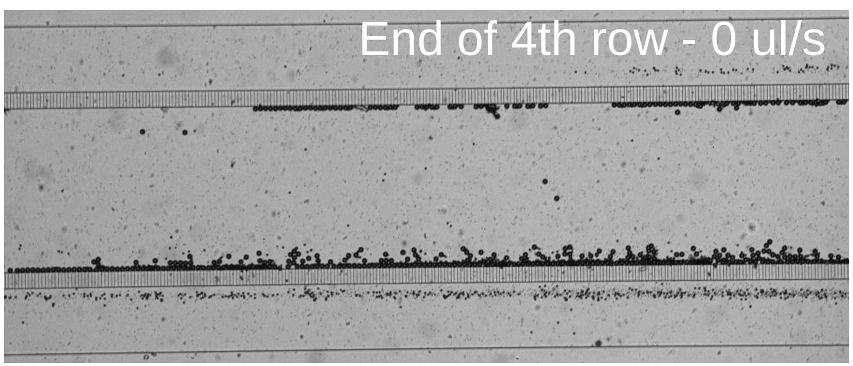


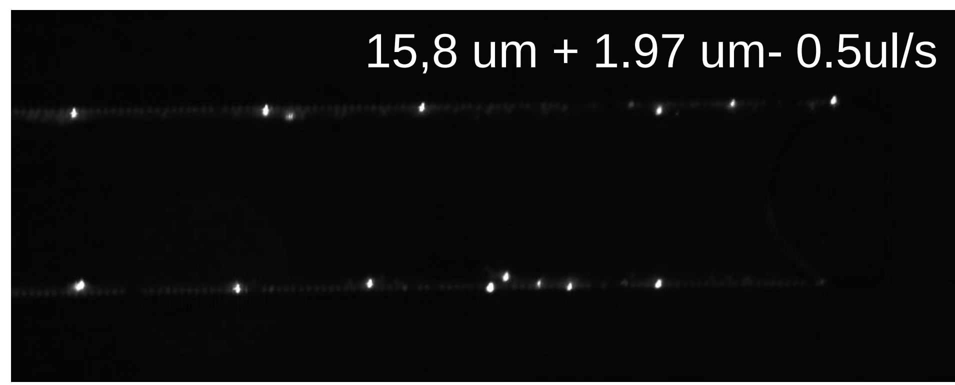

1. 50-10 um
End of 2th row - 0.5ul/s


0.5ul/s

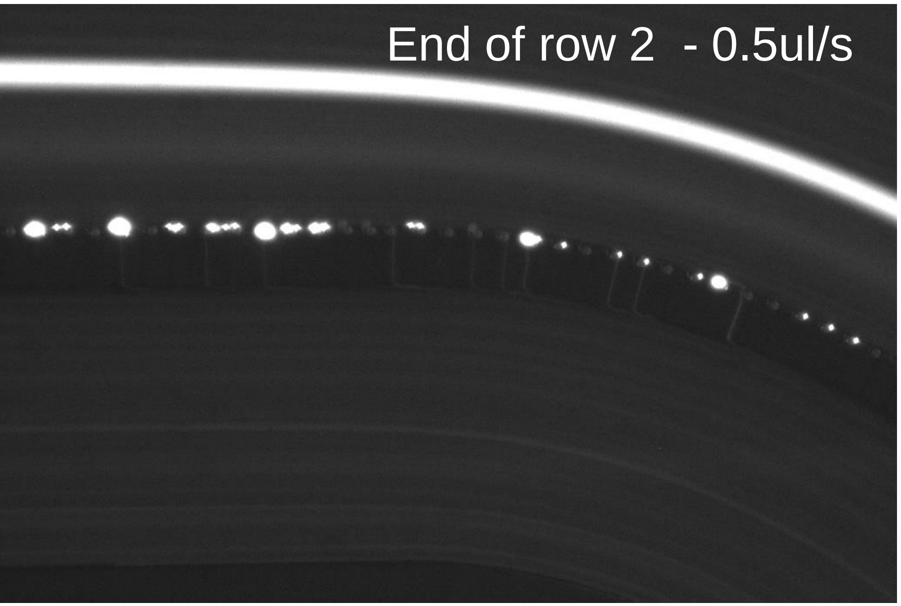

0 ul/s

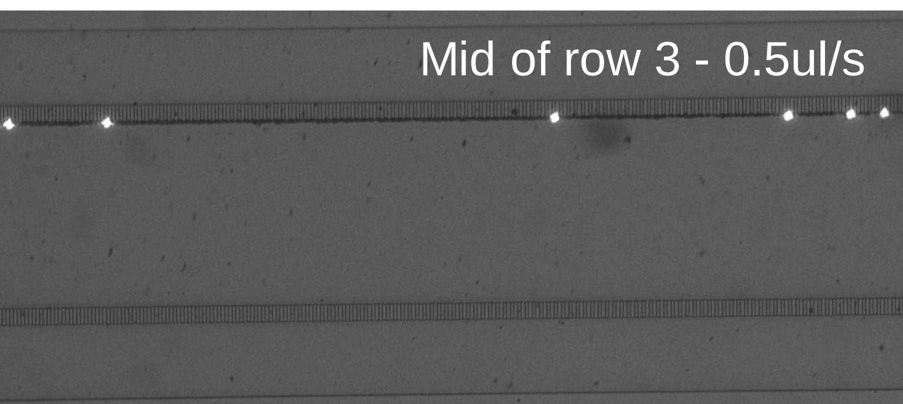

6 um - 0.5ul/s

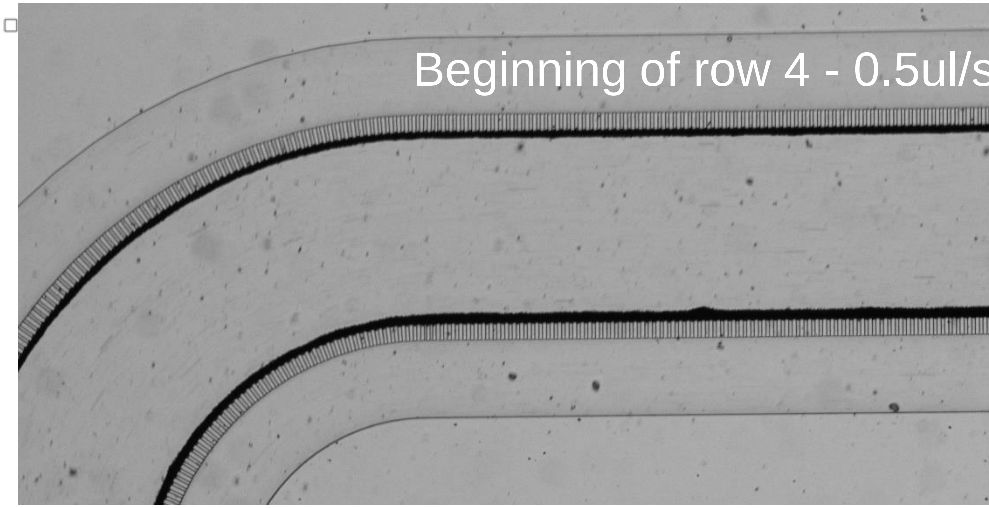

End of 4th row - 0 ul/s

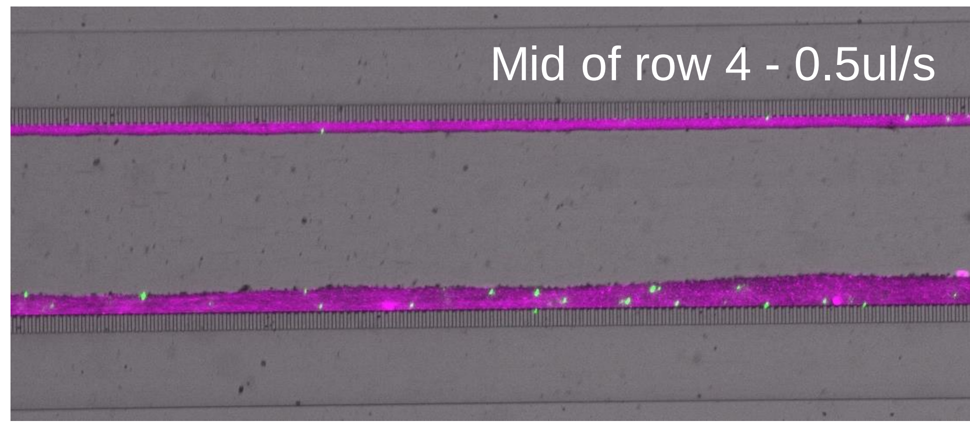

15,8 um + 1.97 um- 0.5ul/s

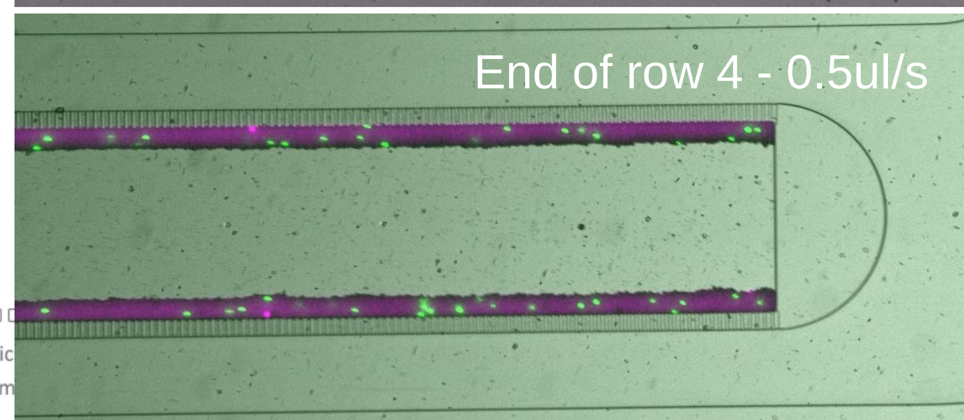

2. 25-5 um
Beginning of row 2 - 0.5ul/s


End of row 2 - 0.5ul/s


Mid of row 3 - 0.5ul/s


Beginning of row 4 - 0.5ul/s


Mid of row 4 - 0.5ul/s

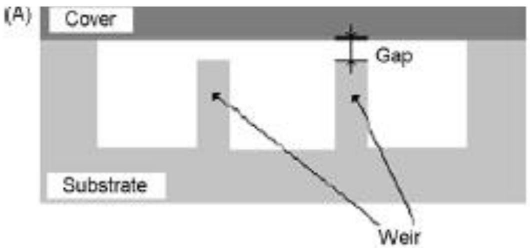

End of row 4 - 0.5ul/s

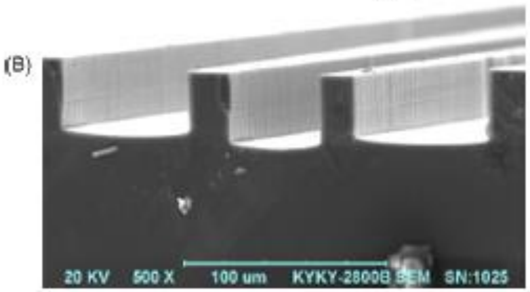
Microfluidic chip for blood cell separation and collection based on crossflow filtration

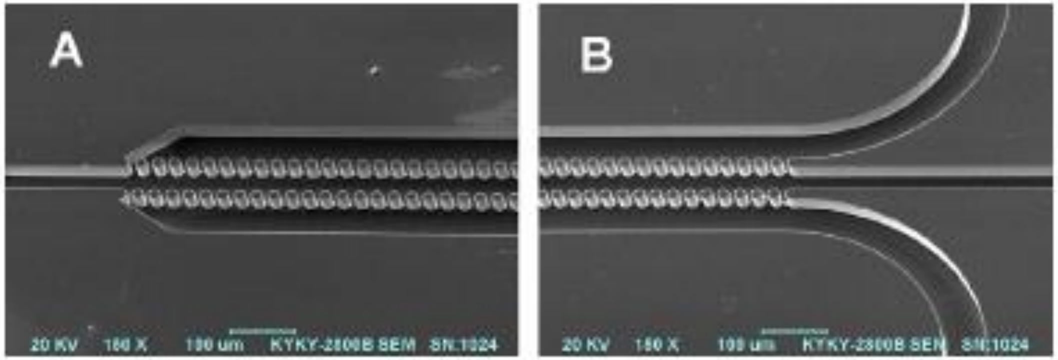
Xing Chen *, Da Fu Cui **, Chang Chun Liu, Hui Li

The State Key Laboratories of Transducer Technology, The Institute of Electronics, Chinese Academy of Sciences, 100080 Beijing, China

Available online 6 August 2007







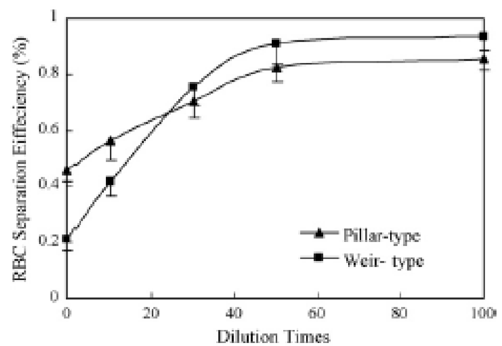


Fig. 4. Effect of variation of cell concentration on the removing efficiency of RBC.

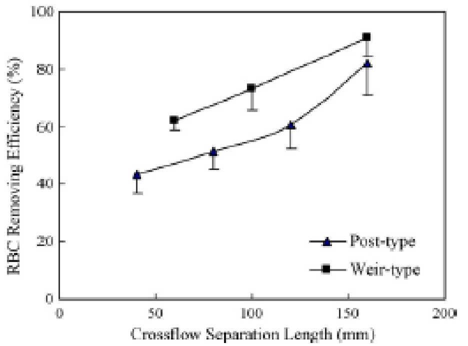
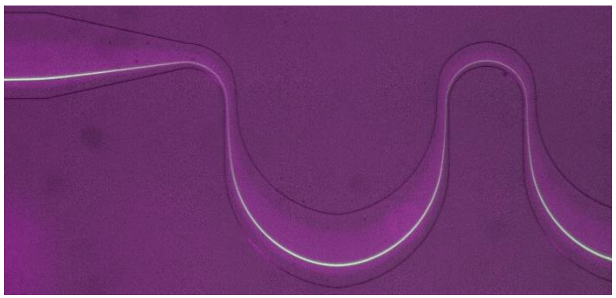


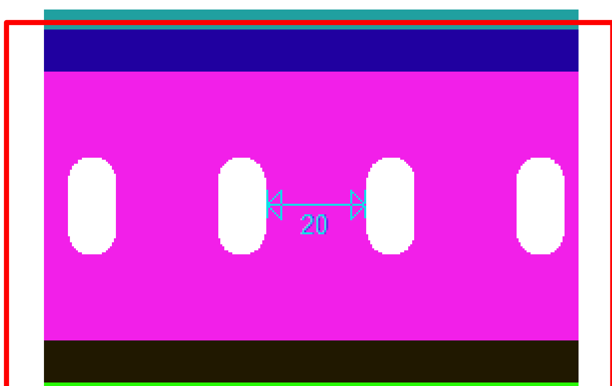
Fig. 5. Effect of variation of separation length on the removing efficiency of RBC.

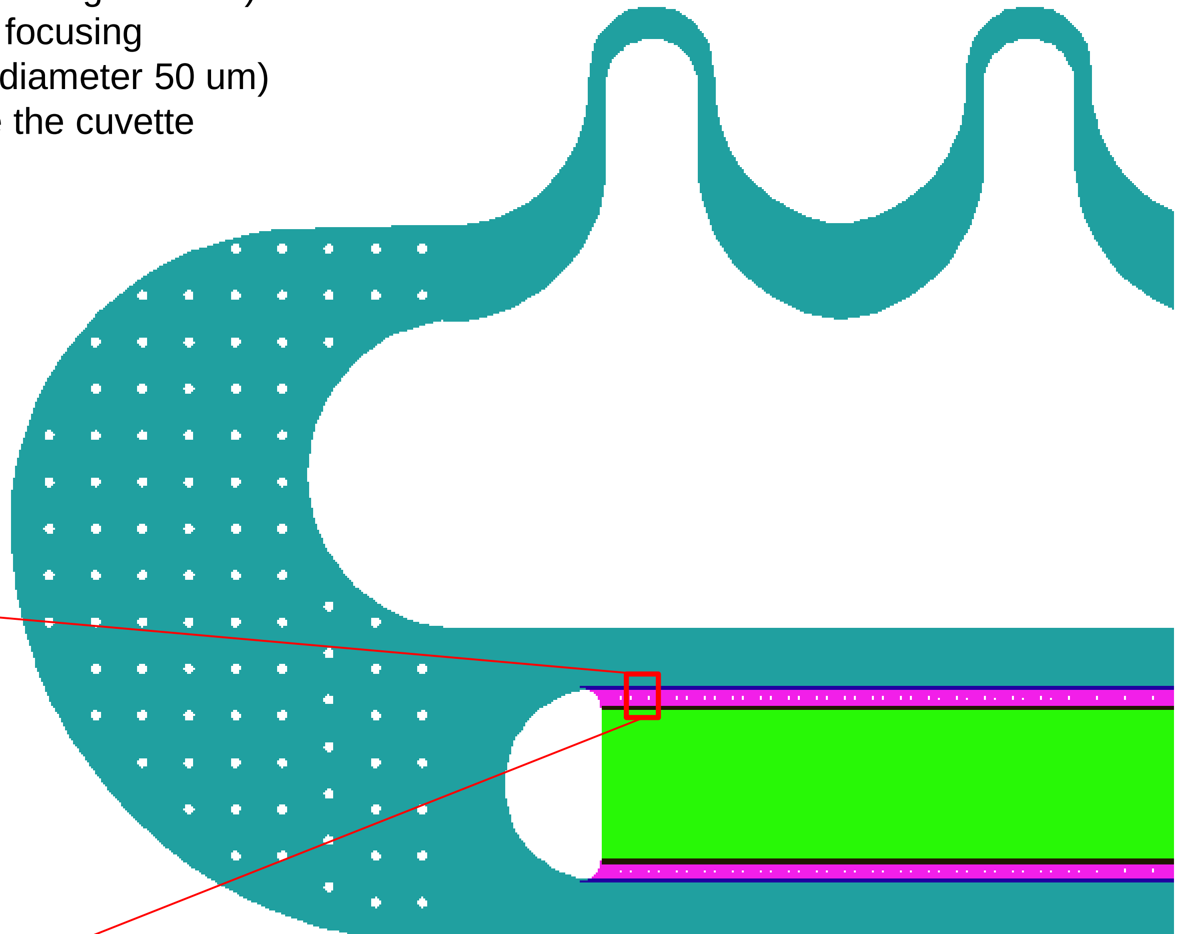
- <u>**Weir type**: h. 26,6 um; w: 20um -> gap 3.5 um</u>
  <u>**Circle type:** d= 20 um; gap: 6.5 um</u>
- <u>**Parameters:**</u> H:30um x W: 60um x L:160 mm; **Branch** H:30um x W: 60umx L:160 mm; **Flow rate:** 4,6 ul/min; 3 ul/min
  - 100x dilution RBC separation efficiency -> circle: 85.3%;
  weir: 93.3% -> after two round of filtration RBC's are removed more than 95%

Weir-type crossflow

- Reduce filter height (to ~4-5 um height)
- Reduce column spacing (only supporting function)
- Installation of a new type of lateral focusing
  (M25-K50 : height 25 um – critical diameter 50 um)
- A waste channel is required before the cuvette







Continuous inertial focusing, ordering, and separation of particles in microchannels

Dino Di Carlo, Daniel Irimia, Ronald G. Tompkins, and Mehmet Toner*

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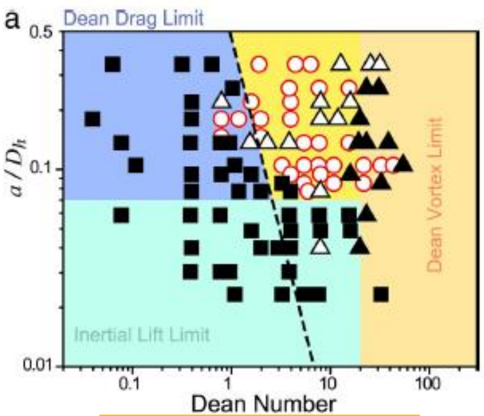
Edited by Jerry P. Gollub, Haverford College, Haverford, PA, and approved October 8, 2007 (received for review May 25, 2007)



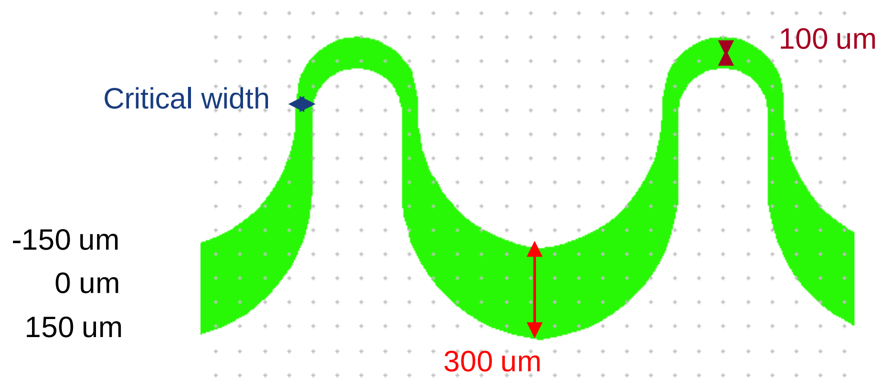
a/Dh > 0.07

$$\frac{F_z}{F_D} \sim \frac{1}{\delta}\left(\frac{a}{D_h}\right)^3 R_c^n, \quad (n < 0).$$

Fd Fz	Dean drag force Inertia lift force
if Fd>> Fz if Fd<< Fz	Dean flow is dominant - mixing Focusing is done by inertia lift forces alone, this requires an appropriate channel length

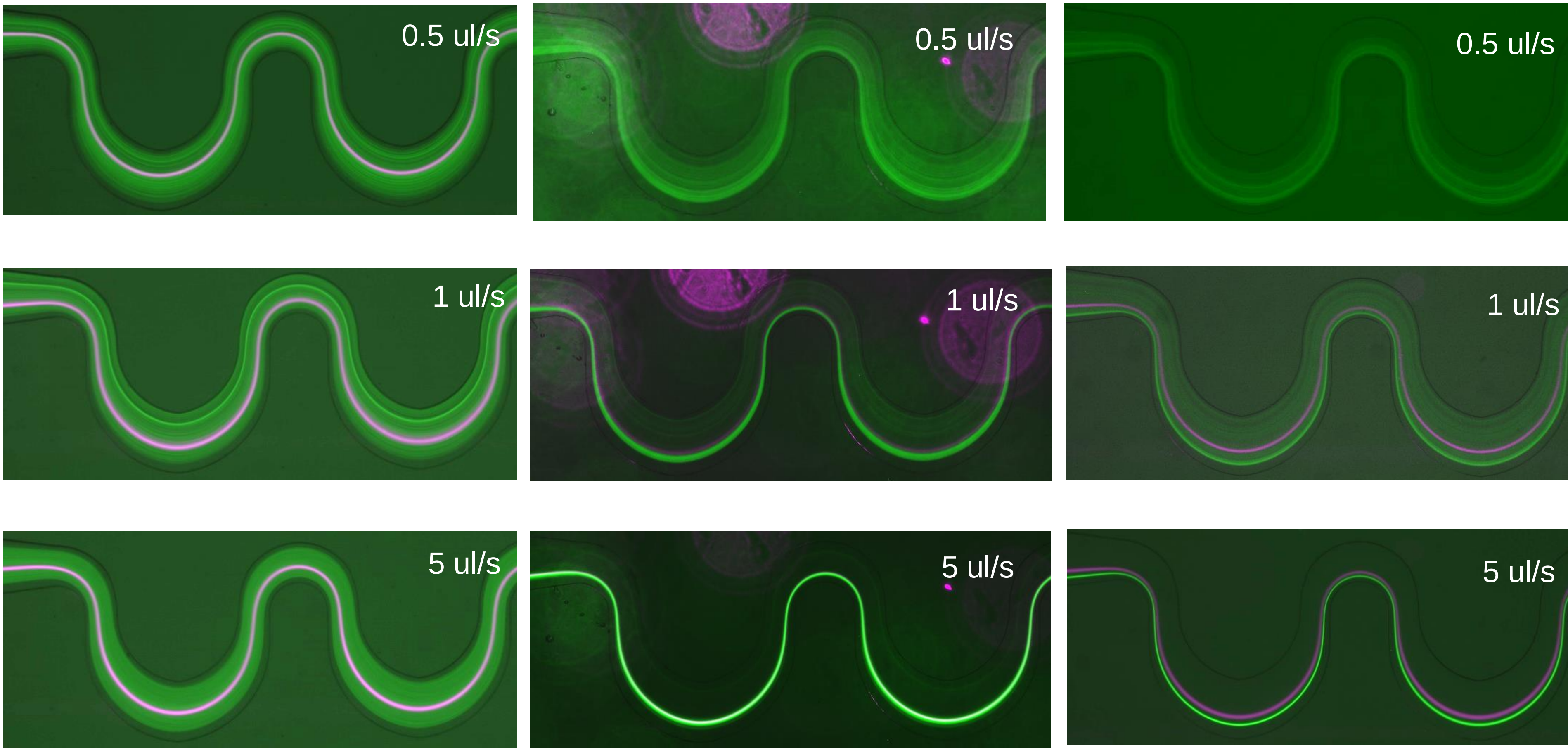
Lateral focusing

a: 15.8 um; 4.8 um; 1.98 um; 1 um

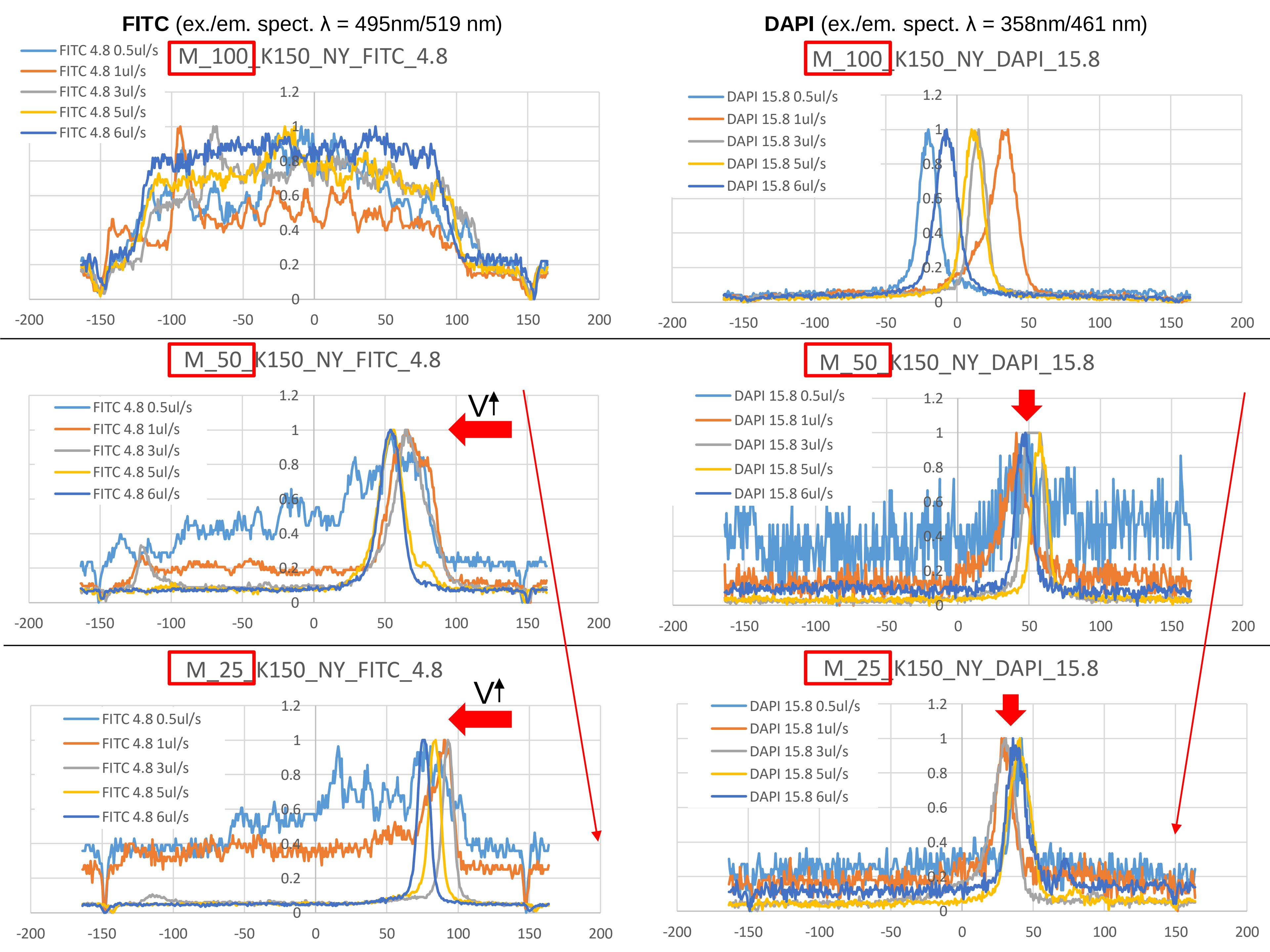


Pressed system			Theory		By experiment		Theory	
Height (um)	Critical width (um)	Dh	15.8/Dh	4.8/Dh	15.8/Dh	4.8/Dh	1.98/Dh	1/Dh
100	50	66,7	0,237	0,072			0,030	
100	100	100,0	0,158	0,048	2 ul/s		0,020	
100	150	120,0	0,132	0,04	0.5 ul/s		0,017	
50	50	50,0	0,316	0,096			0,040	
50	100	66,7	0,237	0,072	1 ul/s	3 ul/s	0,030	
50	150	75,0	0,211	0,064	1 ul/s	6ul/s	0,026	
25	50	33,3	0,474	0,144	0.5 ul/s	0.5 ul/s	0,059	
25	100	40,0	0,395	0,12	0.5 ul/s	2 ul/s	0,050	
25	150	42,9	0,369	0,112	0.5 ul/s	5 ul/s	0,046	
20	50	28,6	0,553	0,168			0,069	0,035
15	50	23,1	0,685	0,208			0,086	0,043
10	50	16,7	0,948	0,288			0,119	0,060
5	50	9,1	1,738	0,528			0,218	0,110

V1_M100_K150_NY | V1_M50_K150_NY | V1_M25_K150_NY



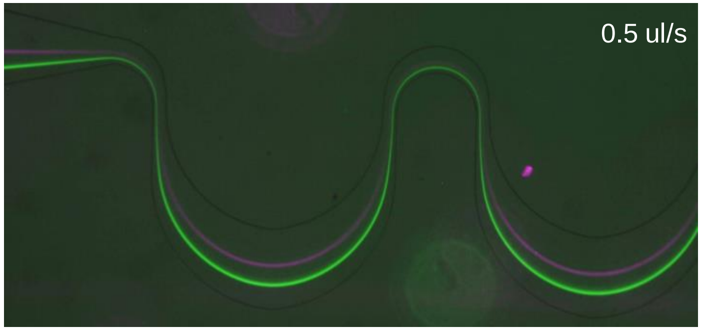
d= 4.8 um (green);
d= 15.8 um (purple)

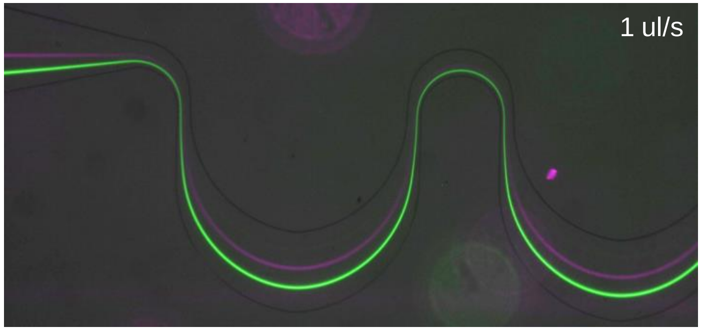


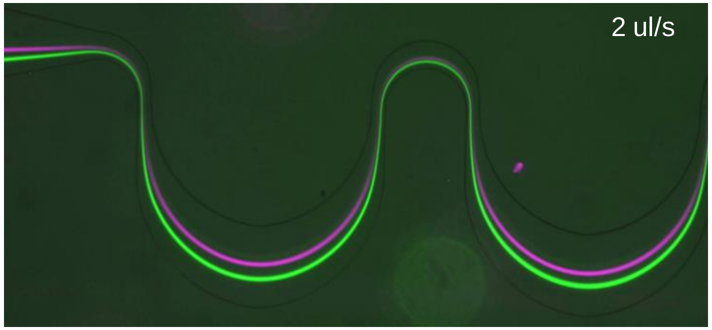
FITC (ex./em. spect. λ = 495nm/519 nm)
DAPI (ex./em. spect. λ = 358nm/461 nm)
M_100_K150_NY_FITC_4.8
FITC 4.8 0.5ul/s
FITC 4.8 1ul/s
FITC 4.8 3ul/s
FITC 4.8 5ul/s
FITC 4.8 6ul/s
M_100_K150_NY_DAPI_15.8
DAPI 15.8 0.5ul/s
DAPI 15.8 1ul/s
DAPI 15.8 3ul/s
DAPI 15.8 5ul/s
DAPI 15.8 6ul/s
M_50_K150_NY_FITC_4.8
V↑
M_50_K150_NY_DAPI_15.8
M_25_K150_NY_FITC_4.8
V↑
M_25_K150_NY_DAPI_15.8

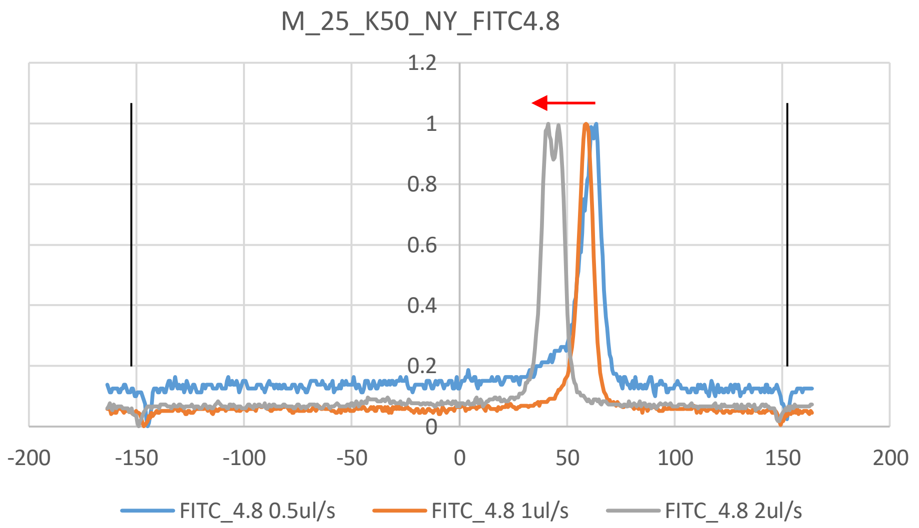
V1_M25_K50_NY

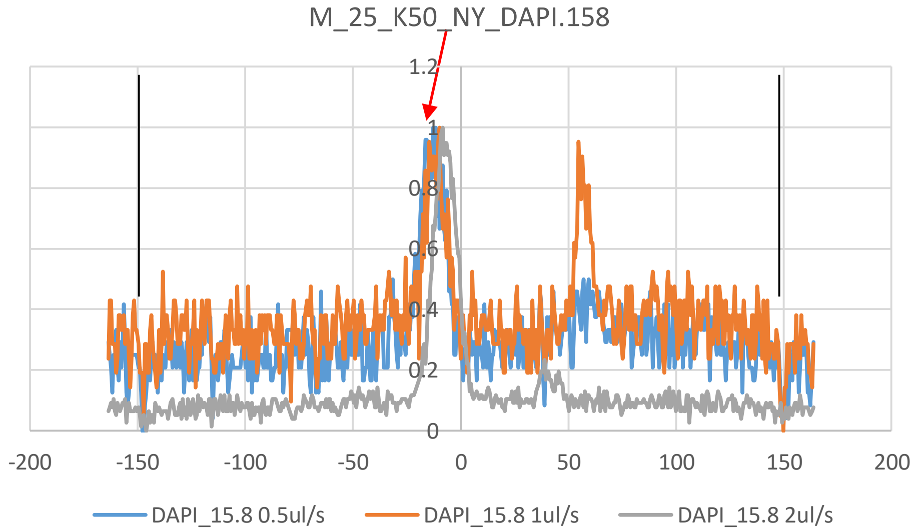
d= 4.8 um (green)
d= 15.8 um (purple)



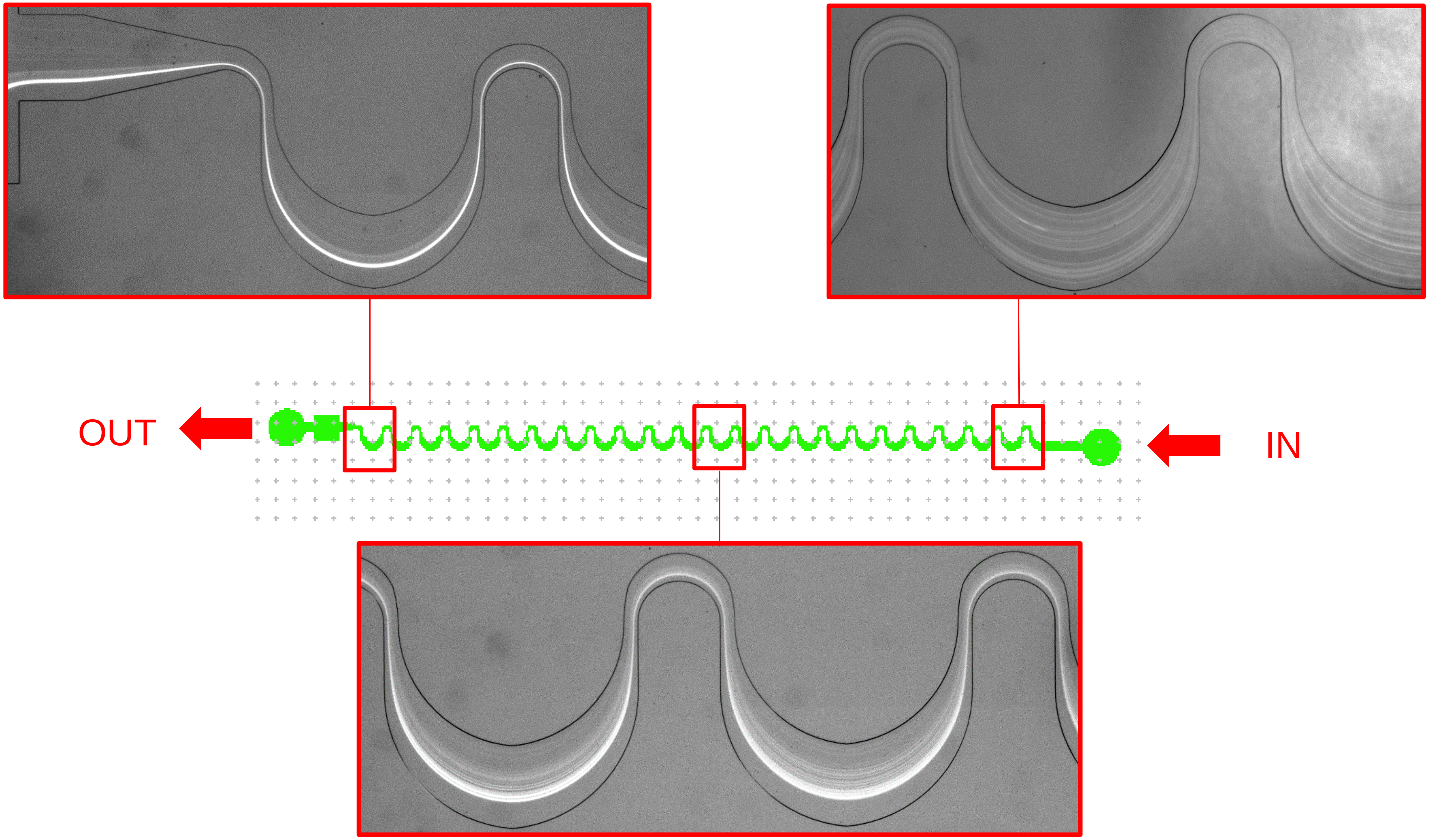








V1_M25_K50_NY **FITC_4.8 µm_0.5 µl/s**



a/Dh > 0.07

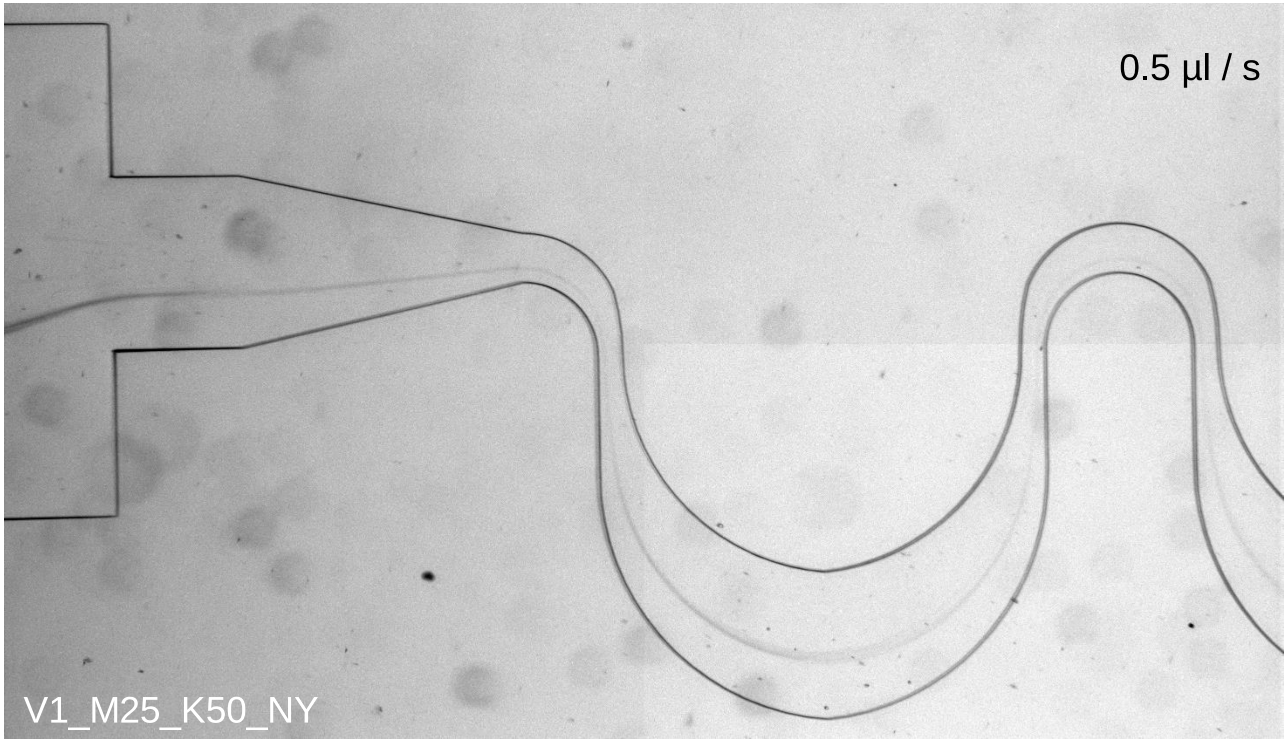
Pressed system

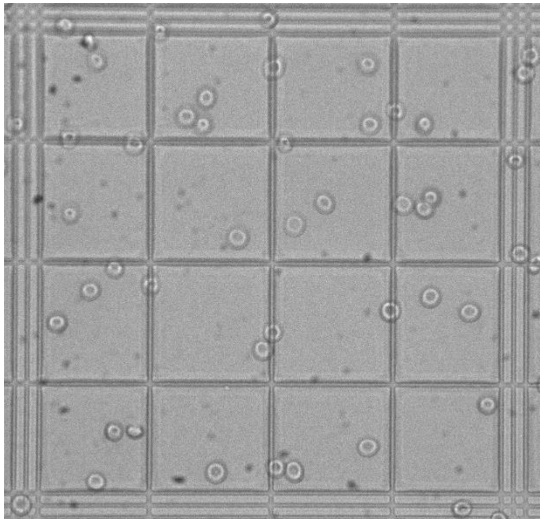
h	Critical w	Dh	Theory 7,2/Dh	Theory 2,2/Dh
25	50	33,3	0,216	0,066

RBC – Diameter: ~7,2 µm, thickness: 2,2 µm

Blood – 100x dilution

- 1 ml PBS
- 10 µl – blood





	RBC/10 square
RBCs	43,9

Summary

- Beads with 4.8 um diameter and RBS's were focused in a single poin in lateral focusing channel (height 25 um – width 50um; flow rate: 0.5 ul/s). Strickter crossection are considered.
- Effective testing of crossflow filter was made by checking filtrate. Target get lost by drastic height reduction. 50 µm high fluid is required, but reduction of the filter height should be reconsidered.
- Weir type filter design is in progress..
- Biochamber has arrived, installation is in progress

Plan

- Further experiments with Crossflow
- Optimization of lateral focus for 1.97 um beads
- Make new mask design
- COC-PDMS hibrid bonding tests (GPTMS + APTMS, TMSPMA)
- Start of biological measurement