2019 UST Global Research Internship

Study and optimization of a microfluidic device for cell separation

KIST School (Bio-Medical)

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Contents

Abstract

Diabetes Mellitus I is one of the deadliest autoimmune diseases. The transplantation of islet cells shows great results in combating this disease. They can be obtained by separation from pancreatic acinar tissue with microfluidic devices. In contrast to their great recovery results, they lack in throughput and atomization. In-depth study of their mechanism and the related literature will allow the designing of a new and optimized device of great potential and applicability.

Sorting of pancreatic islets from acinar tissue

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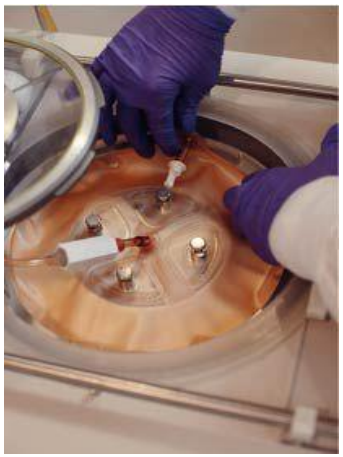
Weekly report No. 2

Density gradient centrifugation

- Conventional method
- Based on the marginal density difference between islets and acinar tissue
- Pancreas from donor is treated with enzymes (collagenase, protease)
- Incubation in University of Wisconsin (UW) solution after enzymatic digestion
- Top-loading in centrifugal bag (COBE 2991 cell processor)
- Gradient maker: 5 mL 25% Ficoll 400 solution (polysaccharide)
- Centrifugation of the bag using the COBE device
- Cells are allowed to float to positions in the gradient where the density of the media is identical to that of the cell

Density gradient centrifugation Results

- Acinar tissue sinks to the bottom (heavy)
- Islets float on top (light)
- Recovery: Islet pellet is suspended in solution and incubated until medical assessment
- Alternative: Hand-picking, can cause physical problems to the operator



Density gradient centrifugation

Pancreas source	Density of Islet cell (g/cm^3)	Density of Acinar tissue (g/cm^3)
Human	1.0370	1.0960
Mouse	1.0700	1.1000
Porcine	1.0645	1.0850

Advantages

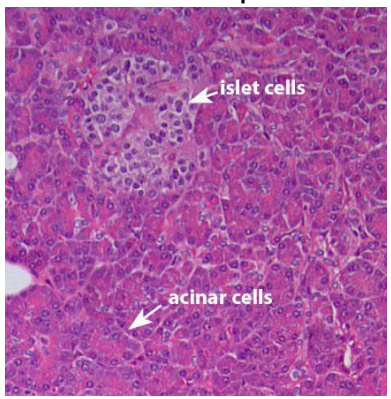
- Time efficient
- Easily automated
- Purity: 87.8%
- Recovery: 85.1%

Disadvantages

- High shear force on islets (damage)
- Miniscule density difference between islets and acinar tissue
- Islets can get trapped in acinar layer
- Product loss
- High-volume transplant plugs (autoimmune response)

Microfluidic chips

- Hydrodynamic resistance and size and deformability-based methods
- Array of channels on a fabricated chip
- Threshold bypass pressure measurement: allows only acinar tissue to pass through a constriction → geometry of the device
- Acinar tissue: small and deformable
- Islets: large and rigid
- Comparison with conventional methods:



	Density Gradient Centrifugation	Microfluidic filtration
Purity	87.8%	99.0%
Viability	96.5%	95.0%
Yield	85.1%	98.0%

Two-cycle operation device for the separation of islets from acinar tissue

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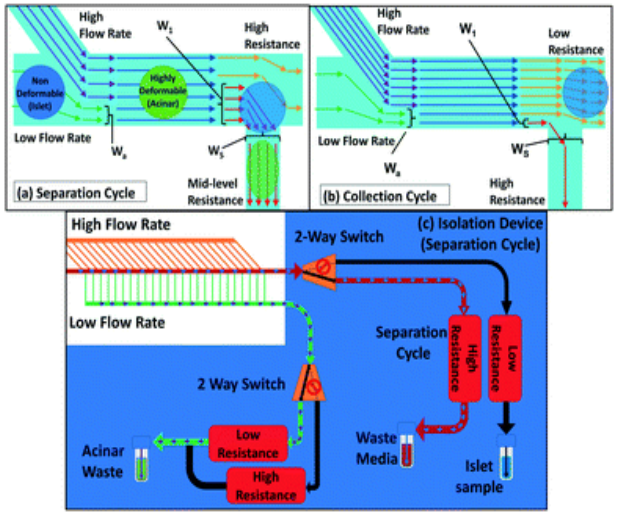
Week No.3

Two-cycle operation
Separation:

- Acinar cells get deformed and pass through constrictions
- Islets get trapped at the junctions (occlusion)
- Main channel: High resistance
- Branching channels: Medium resistance
- At a critical occlusion level the resistance profile is switched
- Variation of flowrate changes the resistance accordingly



Switching between modes
First suggestion: pressure control

- Increasing the outlet pressure in main channel:
  - Flow rate in branching channels increases relative to that in the main channel
  - Lower induced hydrodynamic resistance in the branching channels
- Lowering the outlet pressure in the main channel:
  - Flow rate in branching channels decreases relative to that in the main channel
  - Higher induced hydrodynamic resistance in the branching channels

Switching between modes
Second suggestion: use of valves

- Use of manual or electromagnetic device
- Electrical switches: Generated magnetic field controls the opening of the valve and the fluid flow appropriately
- Rapid switching ability
- Electrical signal controls the valve
- Resistance changes with the opening of the valve

Resistance control using valves

- Fully open: least possible resistance
- Increasing resistance with increasing closing percentage of the valve
- At a certain point, carrier fluid allowed to pass through but islets remain trapped at the junctions
- Fully closed: Maximum resistance, flow is shut off, procedure stops
- Opening is controlled by electric switches

microVISC by Rheosense

Global Research Internship

Week No. 4

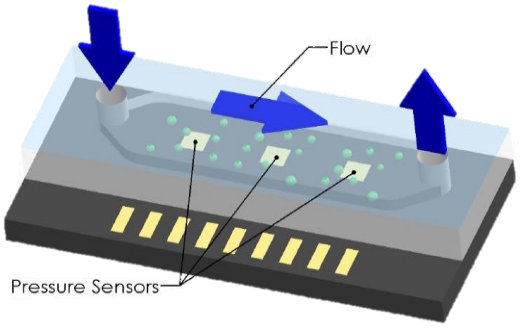
microVISC: Basic info

- Accurate and repeatable measurement of viscosity
- Newtonian and non-Newtonian
- Wide temperature range
- Microfluidic glass channel with rectangular slit
- Channel width >> channel depth
- Si pressure sensor array: measures pressure drop

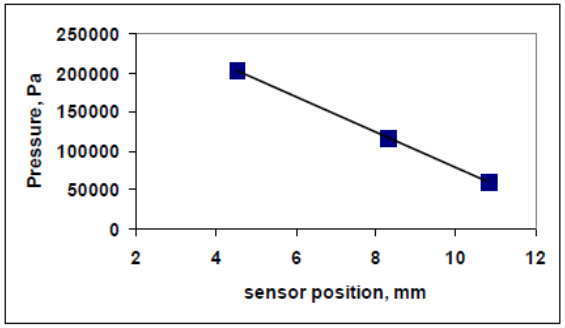
Technical specifications

Property	Value
Device dimensions	14.5 x 21.6 x 7.1 cm^3
Channel depth	50 or 100 µm
Accuracy	± 2%
Repeatability	± 1%
Flow rate range	0.5 – 450 µL/min
Temperature range	18 – 40 °C
Humidity range	0 – 90 %
Battery life	100 measurements per charge
Pipette volume	400 µL
Weight	0.8 kg
Waste volume	40 mL
Shear rate range	6.5 – 5,850 or 1,7 – 1,453 s^{-1}
Viscosity range	0.2 – 100, 60-5,000, 500 – 20,000 mPa·s

Theory of operation



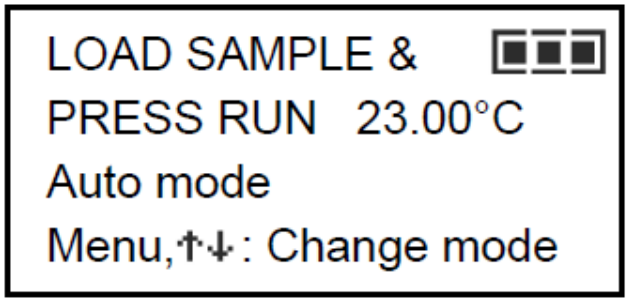
- Sensor measure pressure drop
- Three equations:
  - $\dot{\gamma}_{app} = \frac{6Q}{wh^2}$ apparent shear rate
  - $\tau = -slope\,\frac{wh}{(2w+2h)}$ wall shear stress
  - $\eta = \frac{\tau}{\dot{\gamma}_{app}}$ viscosity



- Above approach is good enough for Newtonian fluids
- Non-Newtonian fluids: viscosity varies with applied shear rate
- Weissenberg-Rabinowitsch correction:
  - $\dot{\gamma} = \frac{\dot{\gamma}_{app}}{3}\left(2 + \frac{d \ln \dot{\gamma}_{app}}{d \ln \tau}\right)$

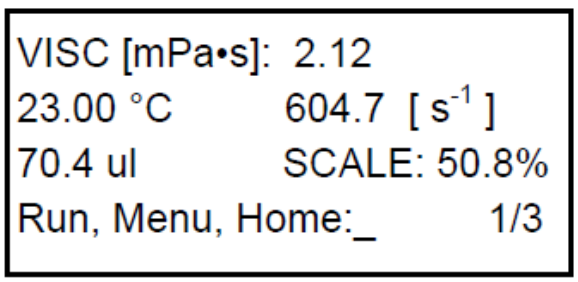
Modes of operation: automated

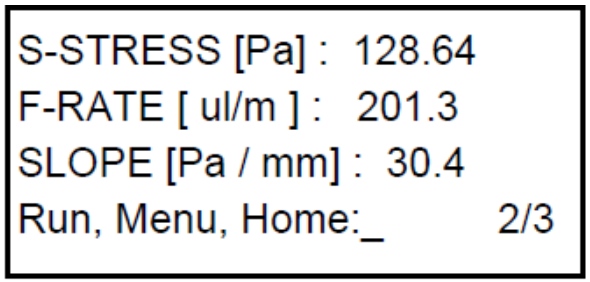
- Sample is loaded into the zero dead volume pipette
- Bubbles lead to errors
- Pipette is mounted in the unit with the tip into the inlet port and the flange firmly in place
- Pusher moves to the plunger
- Priming: channel is filled with the fluid before an accurate baseline can be measured

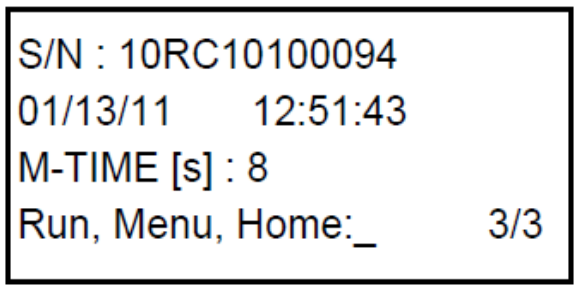


Modes of operation: automated

- Once viscosity value is stabilised, the measurement comes to an end
- Results are showcased in three pages:







- Advanced mode: the user may set parameter values
- Cleaning mode: uses solvents to clean the cartridge and remove sample residues

Contribution to an experiment

- Surface photografting of acrylic acid on PDMS with absorbed benzophenone
- Different UV light intensities: 12% and 30%
- Preparation of pH 10 NaOH solution by diluting already made pH 14.0 solution using a pH-meter and arbitrary amount of deionised water
- Rinsing: 100% ethanol for 30 minutes and continuous shaking
- After 15 minutes each fragment was flipped using lab forceps
- Fragments were treated with pH 10 NaOH solution for 20 minutes and were flipped similarly after 10 minutes
- After the dyeing process, they will be examined under SEM

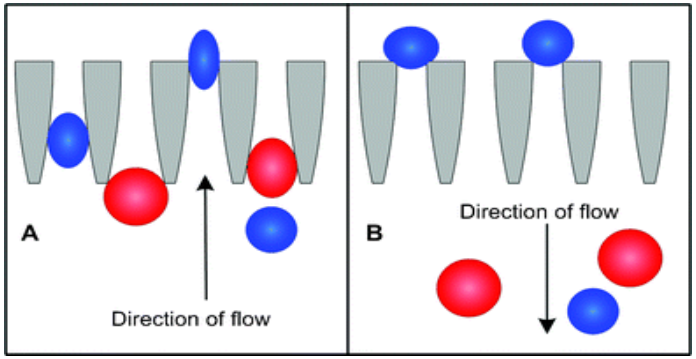
In-depth study of the sorting mechanism of islet cells

Global Research Internship

Week No.5

Explaining the estimation of correlation factors

- Separation of mouse lymphoma cells (MLCs) from human peripheral blood mononuclear cells (PBMCs) using funnel ratchets
- Size-based separation
- Distribution of MLCs compared with similarly sized polystyrene microparticles under identical conditions
- Microparticle sizes strongly correlated to the trapping funnel size
- Pearson correlation coefficients:
  - Microparticles: 0.93
  - MLCs: 0.65

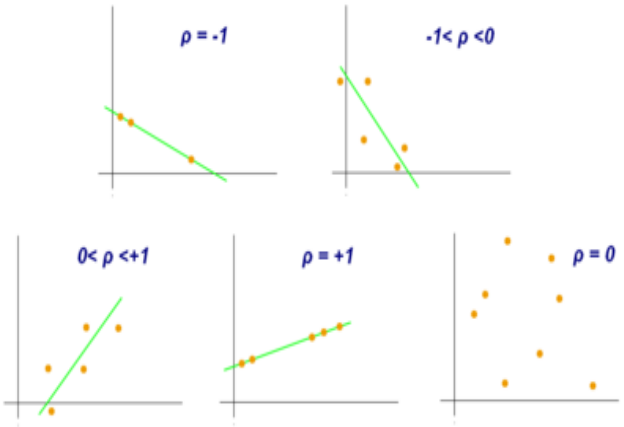


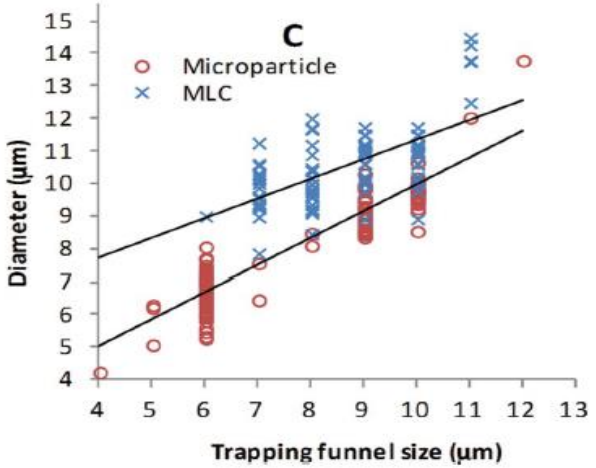
Explaining the estimation of correlation factors

- Population formula: $\rho_{x,y} = \frac{cov(x,y)}{\sigma_x \sigma_y}$
- Cov(x,y): covariance of x and y and σ_x, σ_y standard deviation of x and y respectively
- Sample formula: $r_{x,y} = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2}\sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}$
- X,y the set of variables and $\bar{x}, \bar{y}$ the mean of x and y respectively
- -1: total negative correlation
- +1: total positive correlation

Explaining the estimation of correlation factors

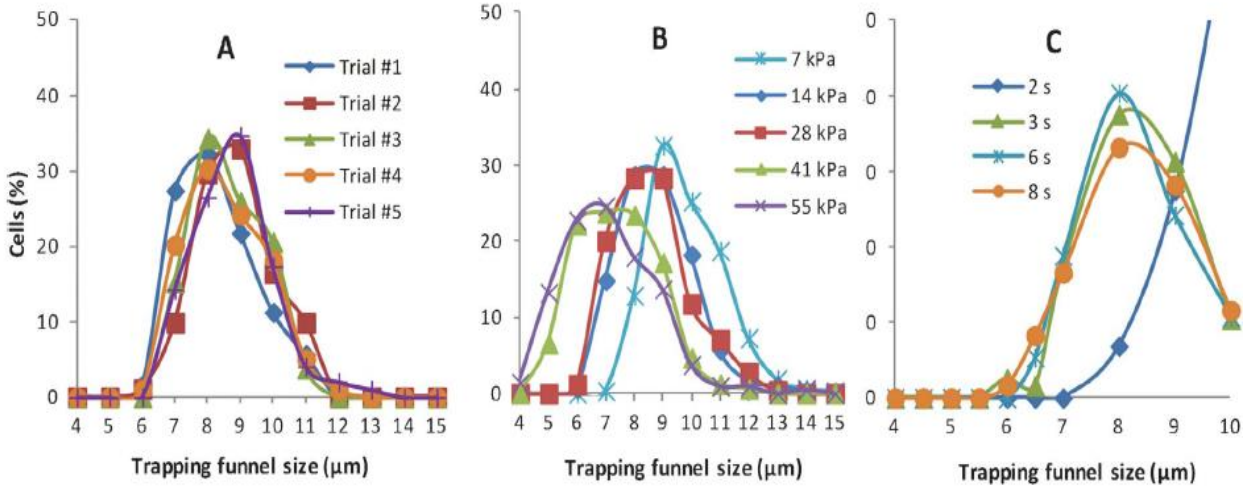
- 0: no correlation at all
- In this case, both showed positive correlation
- MLCs: reduced correlation → variability in the deformability





What is the y-axis on figure 7c?

- Part of a group of three graphs investigating the device's operating parameters
- In particular: effect of oscillatory pressure timing (intervals of forward pressure)

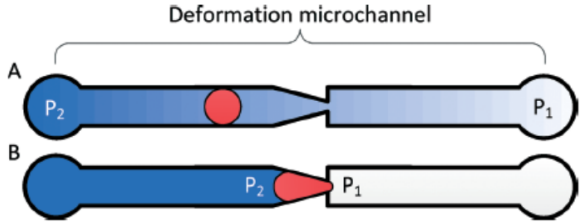


What is the y-axis on figure 7c?

- Y-axis: % of the cell distribution after the sorting process
- Figure C: values have been (accidentally) distorted as if they were 0
- Minimum 3 s time interval for a characteristic distribution
- Further increase in interval of forward pressure leads to steady-state distribution

Multiplexed fluidic plunger mechanism for the measurement of red blood cell deformability

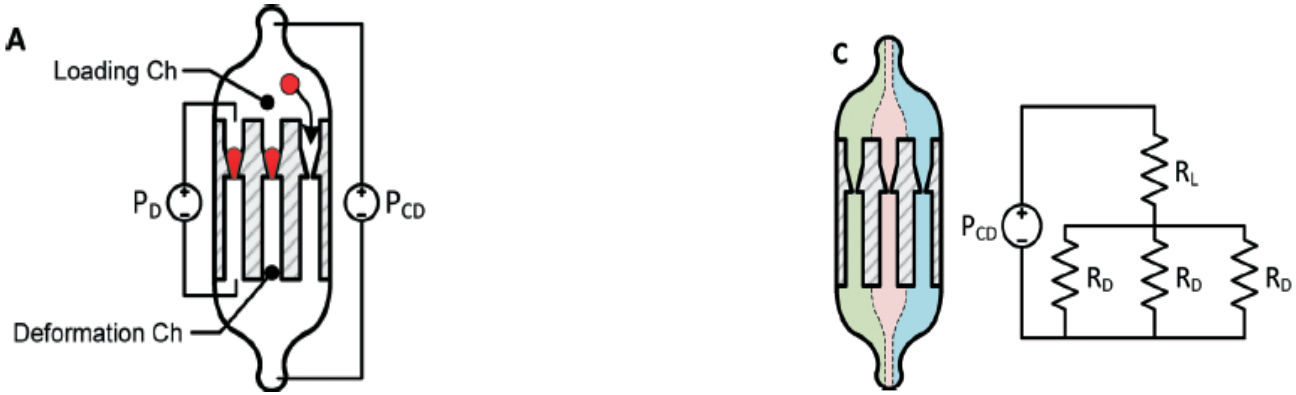
- Pressure for one channel occupied
- Multiplexed fluidic plunger (MFP) measures the deformability of red blood cells (RBCs).
- Constriction of cells through a channel → deformability pressure gets calculated



- Cell <u>not</u> trapped in a constriction: applied pressure P_{CD} distributed across the microchannel, $P_D = P_{CD}$
- Cell trapped: applied pressure is focused across the cell

Pressure for one channel occupied

- Electrical circuit analogy and Kirchhoff's first law (current divider)



- If the constriction channel is unoccupied, only the resistance of the loading channel is considered
- Trapped cell: deformation resistance R_D
- In case of one channel out of N in total occupied:

$$R = R_D + (N-1)R_L$$

Pressure for one channel occupied

- Factor (N-1) decreases as more channels get occupied (N-2 for 2 channels, N-3 for 3… and so forth)
- Assuming consistent flow rate and using Poiseuille's law of fluid flow:

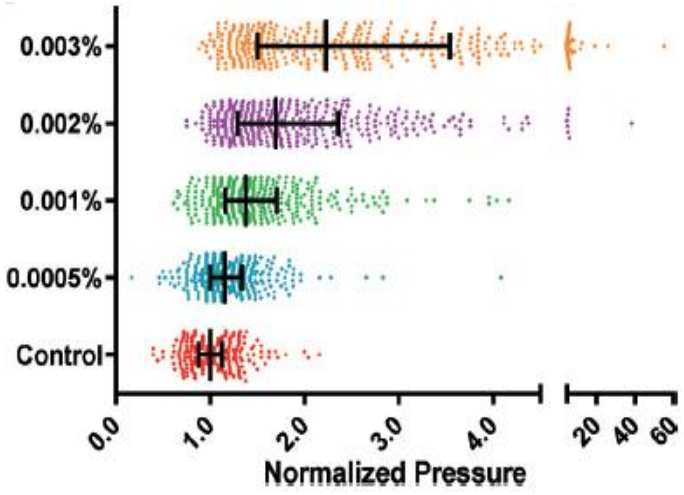
$$F_D = F_{CD} \Leftrightarrow \frac{P_{D,1}}{R_{D,1}} = \frac{P_{CD}}{R_{CD}} \Leftrightarrow \frac{P_{D,1}}{R_D} = \frac{P_{CD}}{R_D + (N-1)R_L}$$

$$\Rightarrow \boxed{P_{D,1} = P_{CD}\frac{R_D}{R_D + (N-1)R_L}}$$

- When all the channels get occupied (N – N = 0), the equation becomes: $P_{D,N} = P_{CD}$

What does the differently coloured bar mean?

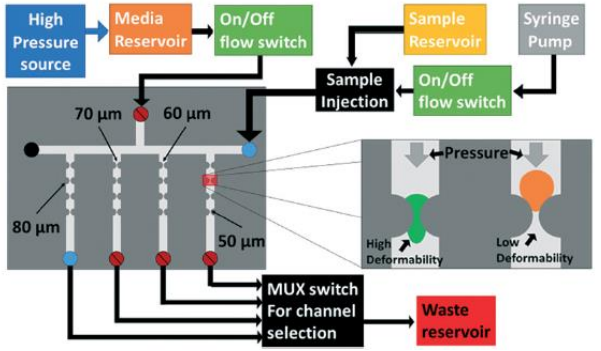
Evaluation of the sensitivity of the device
Deformability profiles



- Treatment with glutaraldehyde (GTA)
- Fixative agent, induces cross-linking → reduction in deformability depending on the concentration
- Higher concentration: less deformable particles and wider pressure range (with statistical significance, $p<0.005$)
- Different colours suggest different deformability

Deformation measurement of 4 different aggregates at the same time

- Cell aggregates are individually translated in a batch flow microfluidic device towards the constriction
- Preferred to the continuous one, limiting the applied pressure to the minimum level



- <u>With 4 at a time:</u>
  - Pressure measurement for a wide range of aggregates
  - Reducing measurement time and increasing throughput
  - Boosting cost-effectiveness

Side task: Review of a proposal

- Review of a scientific proposal titled: “Affinity capillary electrokinetic methods for selective analysis of biopolymers and metabolites and for study of their interactions” submitted by Misksik I. and Kasicka V.
- Very good proposal
- Minor language errors
- Comments: Addition of some sections containing cost analysis (materials, equipment) expected results and problems and some comments about the theoretical approach

Numbering-up strategy for HDF cell sorting

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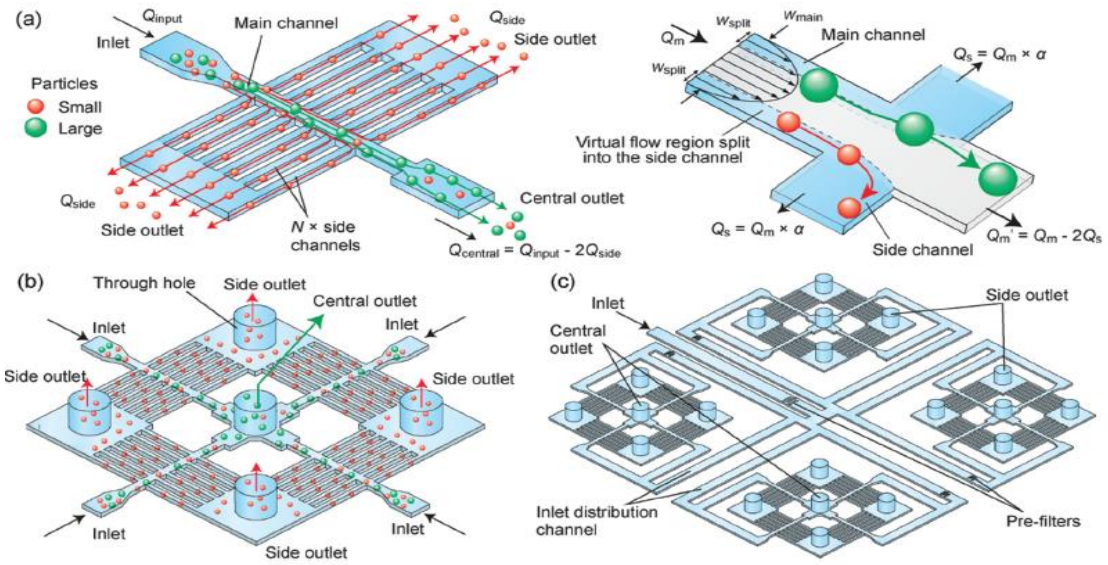
Week No. 6

Device design
Latest experimental results

- 4 separation units arranged in a square
- 4 squares in parallel with one inlet port only
- Inlet port connected to the units by a branched distribution network
- Unit throughput → low (tens of μL min^{-1})



Device design

- One central outlet C1 and two side outlets S1 and S2
- Large particles: main channel, exit through C1
- Small particles: side channels, exit through S1 and S2
- Particles with radius > cut-off width (w_{split}) do not enter the side channels → separation size
- Cut-off width: determined by the width of main channel and ratio a of flow rate split into the side channels → tuning of separation size
- Side channels: Narrow and broad segments → constant fluid flow rate

Design parameters
New points in the design

Microdevice	Separation units	w_{main} [µm]	w_{split} [µm]	α [%]	N	d_{sep} [µm]	d_{coll} [µm]	Q_c/Q_{in} [%]	Q_s/Q_{in} [%]	Concentration factor
I	64	25	2.5	2.8	37	20	100-110	11.9	44.1	8.4 – fold
II	128	25	3.5	5.3	15	20	100-110	18.4	40.8	5.4 – fold

- No sheath flow (focusing flow) required
- Only a syringe pump is needed for operation
- Separation is independent of fluid flow rate
- Square microposts (20x20 µm) as pre-filters at the inlet to prevent clogging

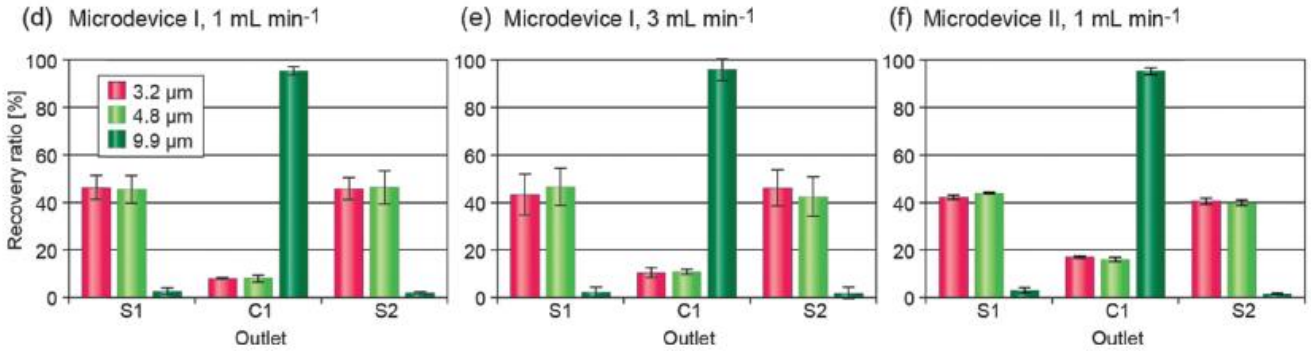
Recovery and purity

- Functionality: 88% of inlet flow is distributed into the side channels
- 1-2% discrepancy with theoretical data
- Diameter of sorted particles: 3.2, 4.8 and 9.9 μm
- Small particles (3.2 and 4.8) → flow through the side channels and collected from S1 and S2
- Large particles (9.9) → flow through main channel and collected from C1
- Some of the smaller particles were collected from C1 ➔ no perfect separation of smaller particles in one round

Recovery and purity
Fluorescent microparticles

	Recovery	Purity [Concentration factor]
Large particles (device I)	95% (C1)	8.8-fold
Small particles (device I)	90% (S1,S2) or 10% (C1)	8.8-fold
Large particles (device II)	95% (C1)	5.6-fold
Small particles (device II)	82% (S1,S2) or 18% (C1)	5.6-fold

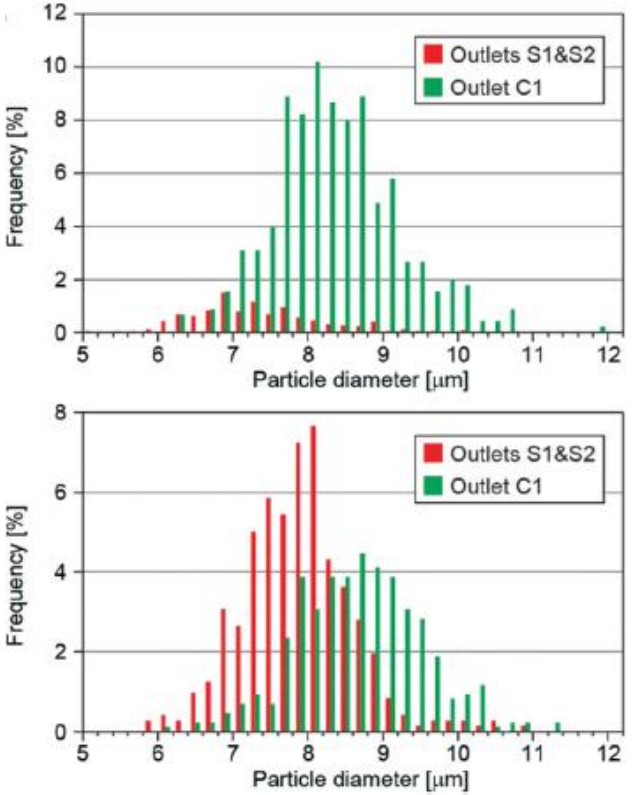
Table with sorting results



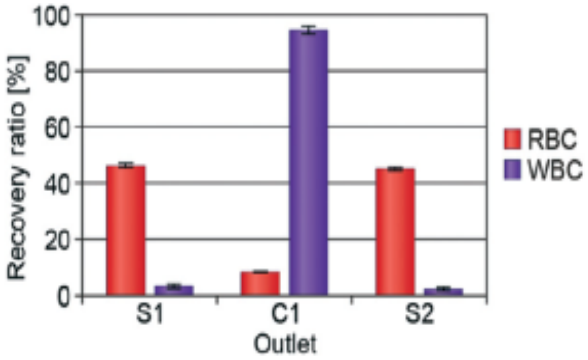
Graphs of sorting results

Recovery and purity
Polydispersed particles

- Average diameter: 8.5 μm
- Device I: 95% recovered from outlet C1
- Device II: Recovery in fractions
  - Outlet S1, S2: Particles with diameter 8.1 μm
  - Outlet C1: Particles with diameter 8.7 μm
- Same purity values (concentration factor)



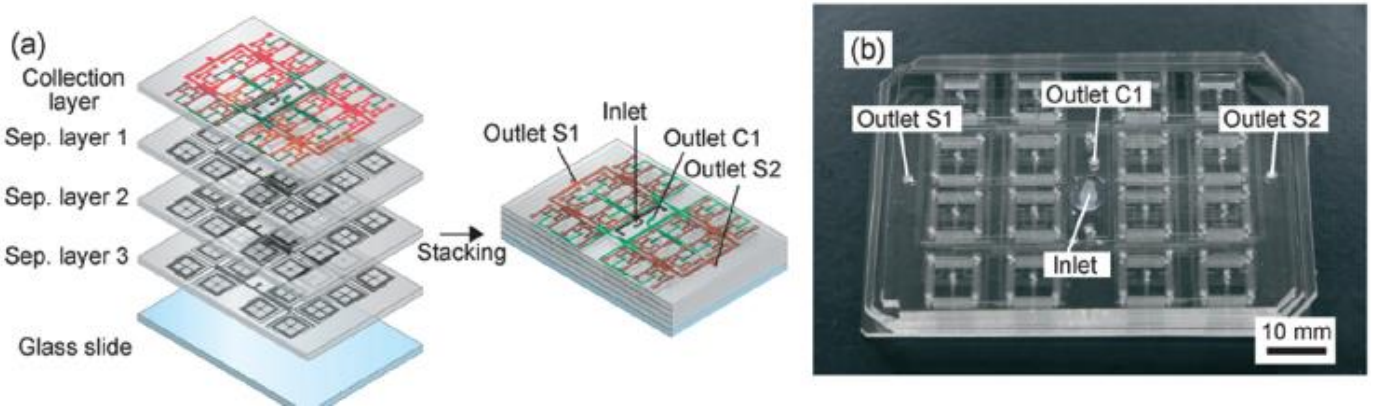
Recovery and purity
White blood cells sorting



- RBCs: behaviour as spherical particles with 3 μm diameter
- WBCs → flow through main channel, collection from outlet C1
- RBCs → flow through side channels, collection from outlets S1, S2
- Recovery values:
  - WBCs: 94% from outlet C1
  - RBCs: 91% from outlets S1, S2 (or 9% form C1)
- 4 consecutive rounds of sorting yield 75% WBCs population
- 4,000 – fold increase in purity after 4 consecutive rounds

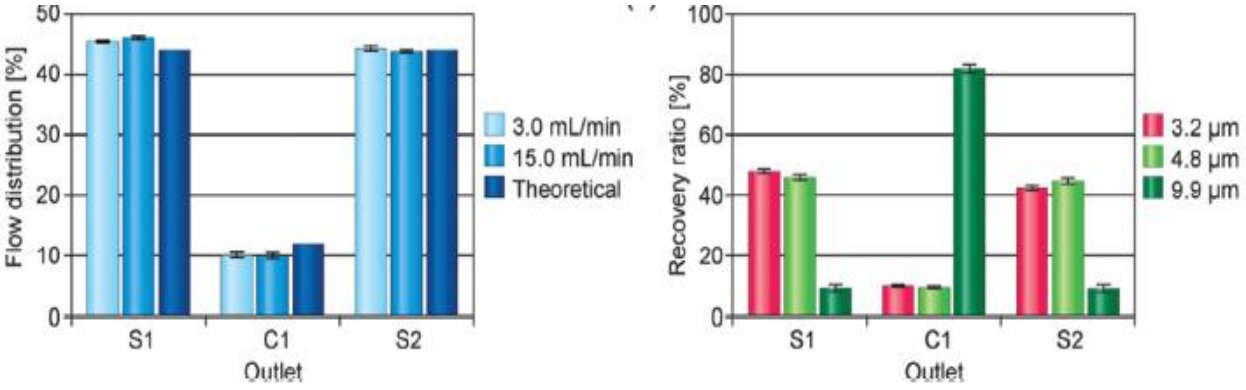
Advancement in throughput
Multi-laminated device

- Parallelised squares → up to 3 mL min^{-1}
- Vertical connection of layers through holes, increasing the number of units without degrading separation
- 3 separation layers and one collection layer
- Maximum throughput of 15 mL min^{-1}



Advancement in throughput
Multi-laminated device

- Flow distribution was not affected by the increased inlet flow rate
- Slight increase in fraction of large particles collected from side outlets S1 and S2
- More than 80% of particles recovered at outlet C1



Conclusions

- Great potential of biological applications
- Maximum throughput of 15 mL min^{-1}
- Adjusting the geometry: tuning of the separation size
- Multiple rounds lead to very pure results
- Results competitive to those of conventional methods in labs
- Easy to operate
- Experimental application of the presented suggestions

Plan after the internship

- Return back and continue my undergraduate studies
- Utilising the knowledge acquired at Dr. Chun's Lab as much as I can
- Focusing on biological applications on a post-studies level
- Considering the UST PhD programmes for future enrollment

Thank you!