DEPARTMENT OF Biomedical Engineering
UNIVERSITY OF WISCONSIN–MADISON

Microfluidic Cell Sorter

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Client: Skala Lab - Melissa Skala, Emmanuel Contreras Guzman

Team: Josh Zembles, Sara Wagers, Caleb Heerts, Hunter Hefti

Abstract

- Current microfluidic chips move cells too fast past the detector
    - Goal is to design a microfluidic plate that will sufficiently slow cells down
    - Need to consistently hold an x, y, z location for cells
- Two designs selected for additional testing
    - Funnel Design
    - Inertial Ordering (AKA Snake Design)
- Flow simulations utilizing SolidWorks
- Results:
    - Funnel shows promise, further experimentation needed
    - Snake design hiccups, little control over centering cells
- Future:
    - Alterations to designs
    - Prototyping

Client Background

- Dr. Melissa Skala
  - Department of Biomedical Engineering
  - Morgridge Institute for Research
- Emmanuel Contreras
  - Morgridge Institute for Research

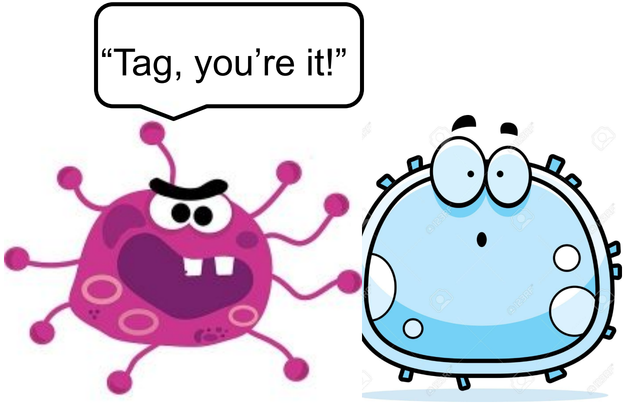
College of Engineering
UNIVERSITY OF WISCONSIN–MADISON

MORGRIDGE
INSTITUTE FOR RESEARCH

Current devices move cells too quickly to be analyzed

Background - Cell Sorting

- Process of separating cells by size or type for further analysis
- Usually accomplished via an innate system of size identification or via labelling/tagging
- Often important as a source of cell identification and for stem cell research



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Design Criteria

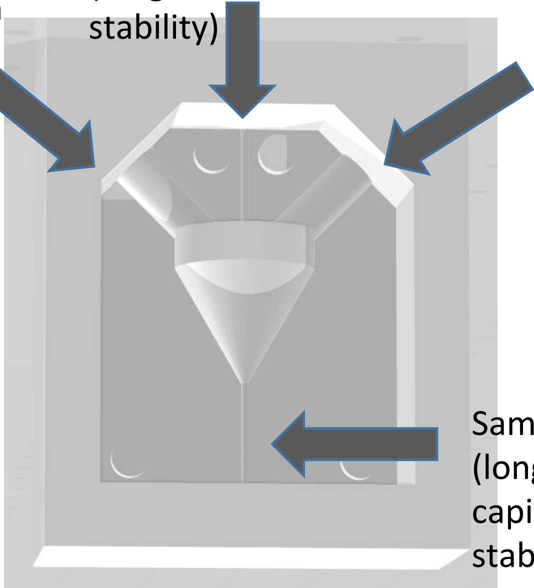
- Sufficiently slow cells down (Flow speed of ~1 mm/s)
    - 100's of ms over detector
- Single-file cell flow through interrogation window
- Cells held in a fixed x, y, z location
- Flow in PBS (Phosphate-Buffered Saline)
- Flow cell has to fit the microscope stage insert
- Bottom side of the flow cell would need to have ~150 micron glass thickness and accommodate the ~1 inch wide objective lens with a working distance of 0.2 mm.

Background - Previous Designs

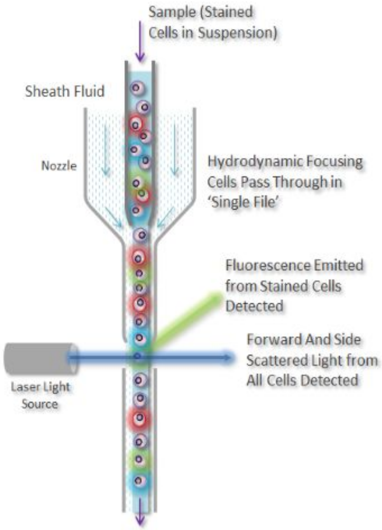
Sheath Fluid

Sample Inlet (longer for needle stability)

Burp Line



Sample Inlet (longer for capillary tube stability)



Flow Cytometry fluidics[5]

Clients initial design

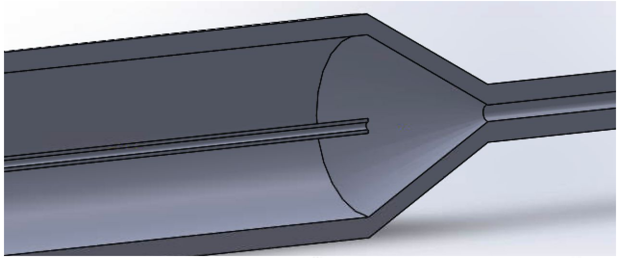
- Uses sheath flow to center cells
- Same methods used in Flow Cytometry

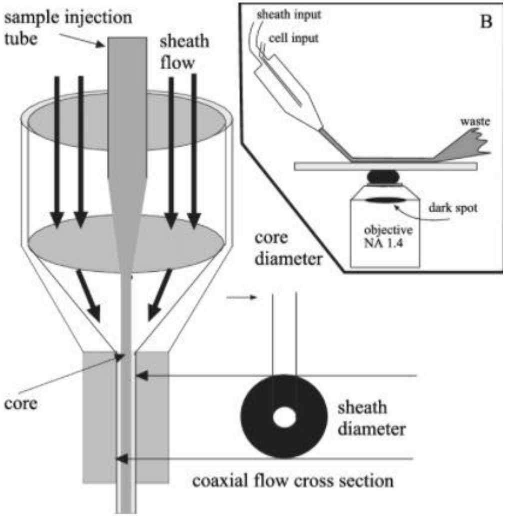
Cons

- Currently too fast
- Too crowded to read each cell individually
- Created bubbles in the line

Funnel Concept and Design

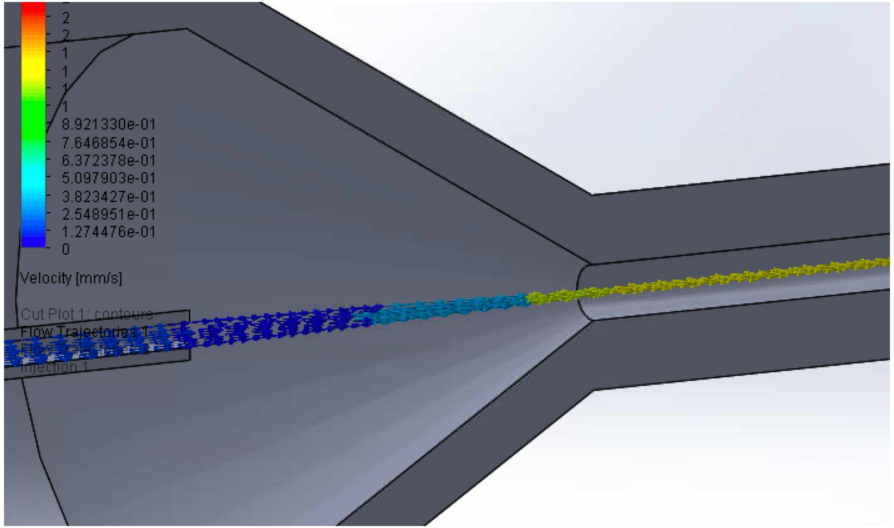
- Based on previous in-lab designs
- 3D cone-shaped cellular inlet
- Allows sheath flow to surround cell injection site
- Cell centering is more consistent



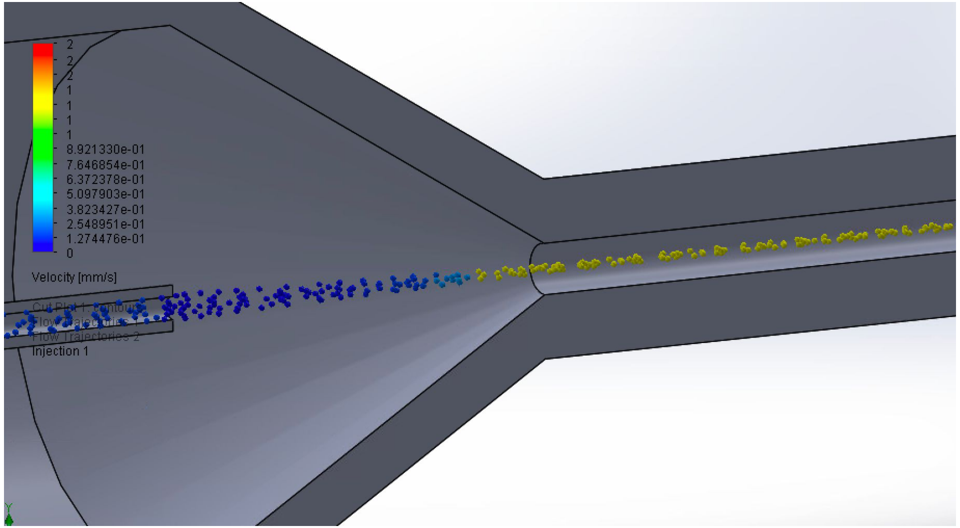


Funnel Results

Flow Trajectory



Particle Tracker



Funnel Testing and Results

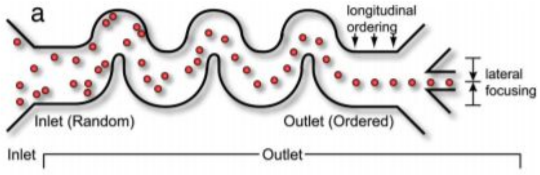
- Velocity of the fluid in the channel at different Sheath Flow speeds
- During testing it was found that the inlet velocity of the cells had no significant effect on the velocity of fluid in channel

Velocity (Cells) [mm/s]	0.1	0.55	1	0.1	0.55	1	0.1	0.55	1
Velocity (Sheath Flow) [mm/s]	0.01	0.01	0.01	0.055	0.055	0.055	0.1	0.1	0.1
Velocity in the channel [mm/s]	1	1	1	7	7	7	13	13	13

Velocity (Cells) [mm/s]	0.1	0.1	0.1	0.1	0.1
Velocity (Sheath Flow) [mm/s]	0.001	0.005	0.01	0.015	0.02
Velocity in the channel [mm/s]	0.148	0.656	1	2	3

Snake Concept and Design

- Initial discovery
- Inertial Ordering
  - Centering cells using properties of fluid motion
  - Focuses laterally
- Variability
  - Different papers, different designs
- Symmetry
  - Symmetrical curvature vs asymmetrical



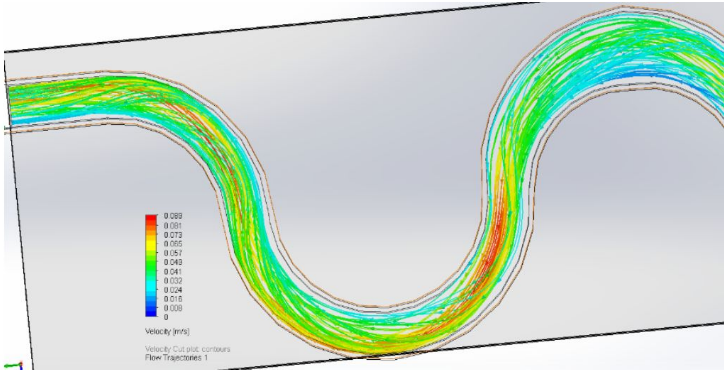
D. D. Carlo, D. Irimia, R. G. Tompkins, and M. Toner, "Continuous inertial focusing, ordering, and separation of particles in microchannels," *Proceedings of the National Academy of Sciences*, vol. 104, no. 48, pp. 18892–18897, 2007.

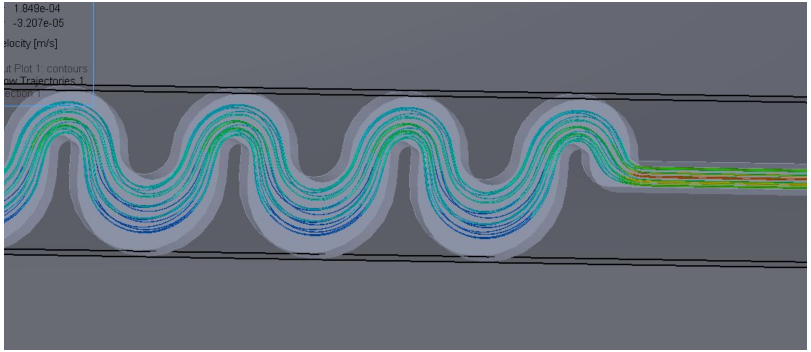
Snake Testing and Results

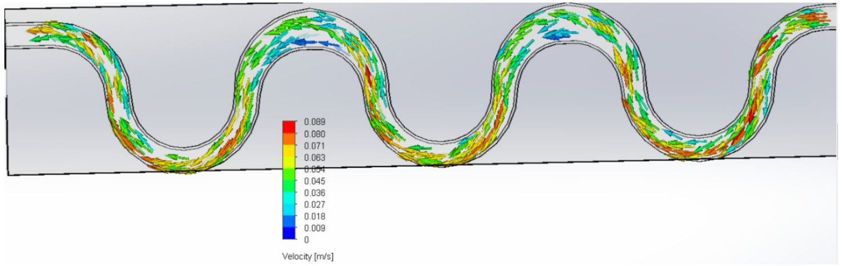
- Sheath fluid?
- Technical issues
- Multiple iterations
- Fluid simulation testing
  - Comsol vs SolidWorks
    - Technical issues
- Particle simulations

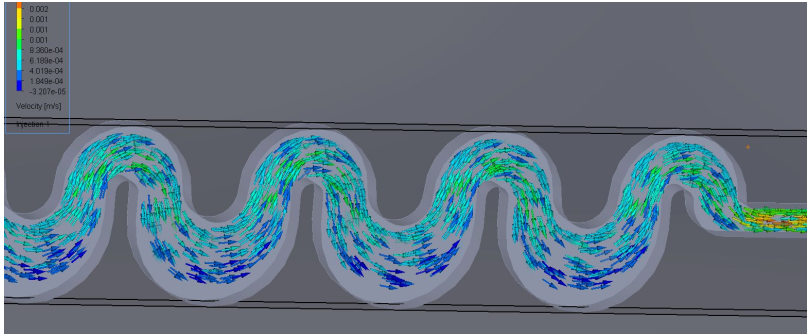
- Flow simulation reveals faster flow at center
- Particle simulation gives mixed results
  - Mostly appears to be little or no centering

Snake Results









Mathematical Considerations

- Reynolds Number
  - Ratio of inertial to viscous forces
  - Channel Reynolds Number (R_c)

$$R_c = \frac{U_m D_h}{\nu}$$

  - Particle Reynolds Number (R_p)

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

- Dean Number
  - $De = Re(D_h/2r)^{1/2}$
  - Describes the relationship between viscous and centrifugal forces in a curved channel

Future Work

- Optimize dimensions
- Create turbulence-free connection
- Fabricate prototypes in the Morgridge Center Fab Lab
- Test the designs with polystyrene beads
- Decide on one final design
- Conduct thorough efficacy testing with cells

Acknowledgements

- Professor Justin Williams
- Skala Lab members: Melissa Skala, Emmanuel Contreras, Kayvan Samimi, and Andrea Schiefelbein

References

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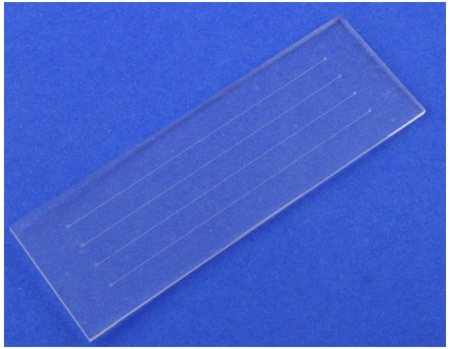
8. "Continuous inertial focusing, ordering, and separation of particles in microchannels" https://www.pnas.org/content/pnas/104/48/18892.full.pdf

Questions / Comments?

Background - Competing Designs

Microfluidic Cell sorter: On-chip Sort[3]



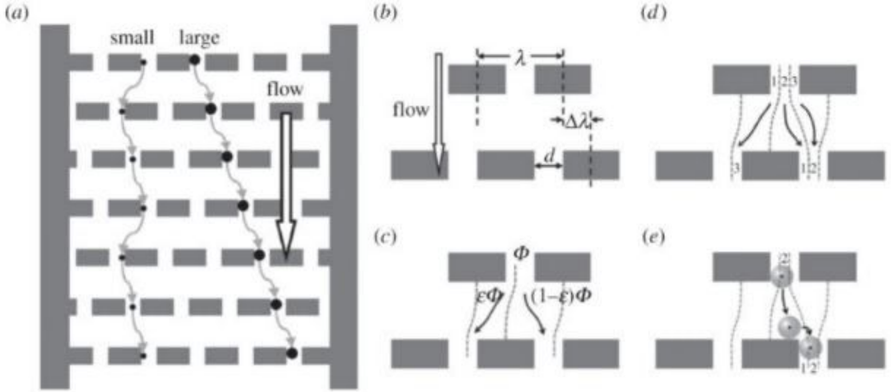
Microfluidic ChipShop[4]

- Cell sorting and Flow Cytometers
- Expensive
- All-in-one device
- Unable to detect decay time which is not standard

- A straight channel chip from Microfluidic ChipShop
- Cells are not focused

Design 1: Plinko

- Cell centering through seemingly random motion
- Allows for relatively precise cell centering
- Potential to slow flow down by widening channel



Sturm *et al.*, *Interface Focus*, **2014**;4(6):20140054. doi:10.1098/rsfs.2014.0054.

Design 2 - Funnel

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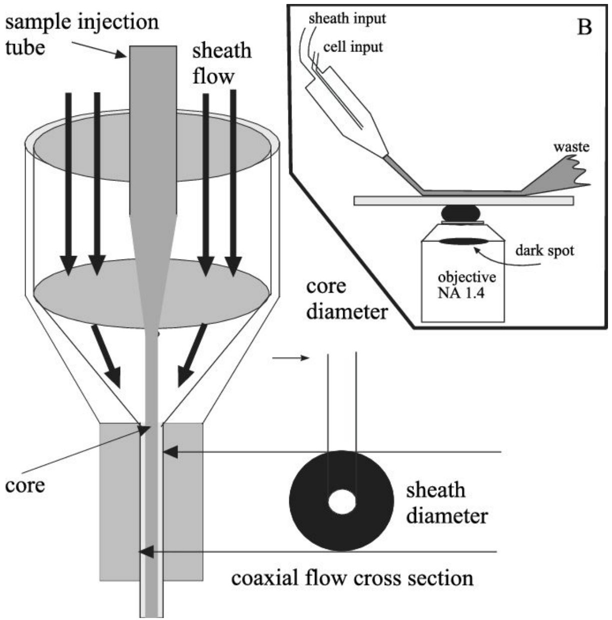
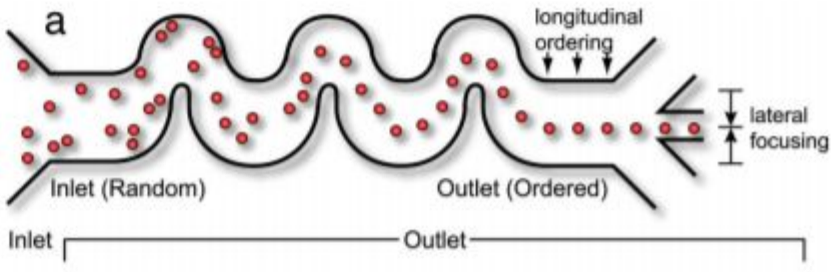
- Allows sheath flow to surround cell injection site
- Cell centering is more consistent



https://www.researchgate.net/figure/Shown-here-is-the-basic-structure-of-a-typical-flow-cell-Sheath-fluid-flows-through-a_fig1_230745384

Design 3 - Snake

- Relies on properties of entry and diffusion
- Cells laterally focus themselves
- Potential to reduce flow via outlets



https://www.pnas.org/content/pnas/104/48/18892.full.pdf

Design Matrix

Design Criteria	Plinko		Funnel		Snake	
Speed Reduction (25)	5/5	25	3/5	15	4/5	20
Positioning (25)	3/5	15	3/5	15	4/5	20
Ease of Fabrication (20)	3/5	12	5/5	20	4/5	16
Reusability/Sterility (15)	4/5	12	5/5	15	5/5	15
Manufacturing Cost (10)	5/5	10	5/5	10	5/5	10
Safety (5)	5/5	5	5/5	5	5/5	5
Total (100)	79		80		**86**	