BME Design-Fall 2020 - Josh Zembles
Complete Notebook

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Team contact Information

Sara Wagers - Dec 08, 2020, 11:04 PM CST

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Project description

Sara Wagers - Oct 07, 2020, 1:06 PM CDT

Course Number:

400

Project Name:

Microfluidic Cell Sorter

Short Name:

Cell Sorter

Project description/problem statement:

The Skala lab has developed label-free optical signals to sort T-cells by activation state. The next step in their research requires a microfluidic chip to flow the cells at speeds that allow 100's of ms integration time on the detector. The device can be commercial or newly designed, and requires a number of specifications in order to integrate with their system. The function of the device should create single-file cell flow through the interrogation window with a stable core diameter of 20 um to 50 um while ensuring that stability is first maintained in the z direction. Cells should flow through the microfluidic device along with a PBS sheath fluid at a flow speed of 1 mm/s and up to 10x faster.

About the client:

Dr. Melissa Skala works out of the Skala Lab which is associated with the Morgridge Institute for Research. The lab is known for photonics based technologies which they use to discover cancer solutions. Quality control of T-cell and stem cell therapies plays a large roll in this research. Emmanuel Contreras Guzman and Kayvan Simimi were designated from the lab to aid in the improvement of the T-cell sorting mechanism and are the main side contacts of the project.

2020/09/11- Advisor Meeting 1

Hunter Hefti - Oct 05, 2020, 3:48 PM CDT

Title: Advisor Meeting

Date: 09/11/2020

Content by: Sara Wagers

Present: Justin Williams, Sara, Josh, Hunter, Caleb

Goals: Record meeting notes for Advisor meeting

Content:

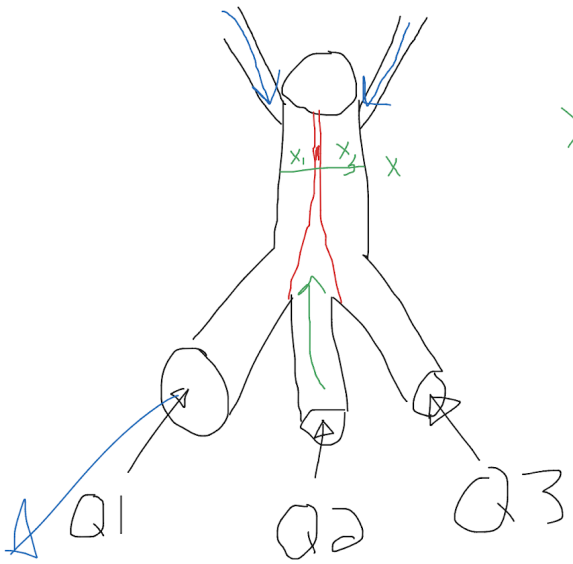
- Actual project seems different than the prompt
    - Focus on the flow mechanism to get the cells in front of the laser
- Challenge with this project is that most devices are continuous flow
    - Makes it difficult to keep the cells there for a long enough time
    - Williams has a paper he can send us
- Another challenge is getting the cells in the center of the channel
    - Cells tend to go to the sides where velocity of flow is the lowest
- The other would be stop-flow microfluidics
    - Can stop the flow momentarily
    - Might not be any commercial devices out there

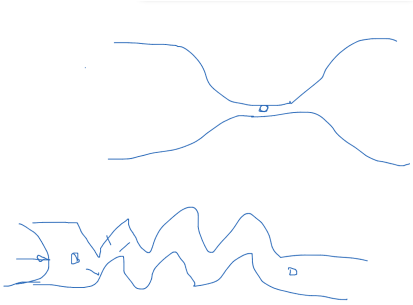
2 things for us to work on

- Getting the cells centered
    - Design the geometry of the device
- Pumping mechanism
    - Need to be able to stop and start it at an appropriate time
    - Slowing it down would be easier probably

Hydrodynamic focusing

- Sheath flow?
- Use fluid to push other fluids around





Sheath flow is somewhere that we can start

Can we use sheath flow but have 2 more inlets that oppose the flow to slow it down?

- Would make it very unstable and hard to keep laminar flow
    - Maybe ok if really slow? But then you can't do a lot to the flow

We should look into the non-sheath methods as well

Where can we fabricate

- Makerspace has some microfluidic printing capabilities
    - Online ordering system
- Morgride also has some?
    - Investigate, might still be running?
    - Dr. WIlliams can put us in contact with the guy

Can be prototyped larger, everything with laminar flow can be scaled very easily

Meeting sunday at 11 to talk about PDS

Conclusions/action items:

2020/09/18 - Advisor Meeting 2

Hunter Hefti - Oct 05, 2020, 3:50 PM CDT

Title: Advisor Meeting 2

Date: 09/18/2020

Content by: Sara Wagers

Present: Justin Williams, Josh, Sara, Caleb, Hunter

Goals: Meeting notes

Content:

- PDS is going to evolve, but looks fine so far
- Production characteristics
    - Manufacturability of these things might be a challenge
        - Depending on how reusable and how many they want
- How can we make these?
    - Commercial vendors, upload your design and they make it for you
        - Look at the specifications of what they can make, how small can they make details
        - Materials
            - Glass, pdms
        - There might be microfluidic printers that can make these
            - The makerspace has some
                - The resin printers are the most common way to make microfluidic devices
                    - Accuracy wasn't great for us in the past
                        - We might have to do prototyping in a larger scale
            - The WID has one too
        - Glass microfabrication techniques
            - Special glass that turns to liquid in the presence of UV light
        - Only need glass on the bottom
            - Norlan optical material glue???
                - Has the same index of refraction of glass
            - If using glass and pdms, you can bond them together with oxygenated plasma(?)
                - We have a plasma chamber in the teaching lab or go to farm and fleet to buy a cattle prod and make your

own

- Caleb's design idea
    - Will it hurt laminar flow?
        - Slowing it down will help, but widening it may hurt the laminar flow
    - Potentially viable!
        - Depending on how small the restrictions are, it will increase the fluidic resistance
        - If the holes are too small, the fluid in the inner part will not reduce its velocity
    - Might be hard to fabricate
    - There are some ideas like that
        - The put posts on the side instead of putting slots
        - Make a wall of posts instead of a wall with slots in it
            - Easier to fabricate and more structurally stable
        - Microfluidic plinko game
            - Use the concept of plinko to focus the cells
                - Oblong and angled pegs that can help to push the cells in the direction they want them to go
            - If we designed something where the cells bounce around in a deterministic way, they will be slowed down by the end and all end up in the same spot
                - Fluid will go through without disruption in their laminar flow streams but the cells are big so they bounce around and get slowed down
- Tesla mixer
    - Opposing flows
    - You can get some very chaotic flows in a microfluidic device just by running two flows against each other

- For prototyping
    - Does solidworks work well?
        - Designing in solidworks works well
        - It has some fluid modeling capabilities that should work for some simple microfluidics
            - Tutorials online should help
    - Can upload the solidworks into a different fluid modeling software if needed

How are you going to connect the pump to the device in a way that is easy, leakproof, etc???

- People often think about this too late
- If 3D printing people usually just print a luer lock onto the piece
    - There are files online for these

- Touch base on wednesday about whether or not friday meeting>
    - He should have a lot of time on thursday

Conclusions/action items:

2020/10/09 - Advisor Meeting 3

Sara Wagers - Dec 06, 2020, 12:34 PM CST

Title: Advisor Meeting 2

Date: 10/09/2020

Content by: Sara Wagers

Present: Justin Williams, Josh, Sara, Caleb, Hunter

Goals: Meeting notes

Content:

- Presentation feedback
    - Good presentation
    - Going forward -- Testing
        - What materials do we need and stuff?
        - Easy way to do it without even using a pump - gravity
- Fabricating
    - Makerspace
        - Dolomite
    - Beebe lab does it
    - Outsourcing to a company, plenty that make custom microfluidics
        - Look around and see where we can send the solidworks design
- Could start something based on the design in the paper
    - If we can replicate what they do then were doing something wrong with the pump or the connection
    - Good to just get experience with getting fluid through it

- PMMA for microfluidics?
    - Someone told Caleb that it fits within the wavelength needs
    - Beebe lab has the capability to do it
        - A mold casting technique
    - There is a lot of interest in using PMMA for microfluidics
        - Clear, inert, easy to work with
        - Might just advertise it as medical grade acrylic

- Next week meeting
    - Verify when sending the progress report if meeting is needed

- Plan going forward
    - Create solidworks things
        - Different designs based on the snake?
            - Spiral?
    - Research more literature on inertia stuff
        - calculations/math considerations
    - Research the connections/fittings

Conclusions/action items:

2020/10/30 - Advisor Meeting 4

Sara Wagers - Dec 06, 2020, 12:35 PM CST

Title: Advisor Meeting 2

Date: 10/30/2020

Content by: Sara Wagers

Present: Justin Williams, Josh, Sara, Caleb, Hunter

Goals: Meeting notes

Content:

- Show and tell looks good
- Prelim report
    - Looks great overall
    - Testing needs to be expanded for the final report
        - And plan for data analysis
- Where are we?
    - Solidworks roadblocks
    - Another group is using comsol
        - Maybe look into it?
- What about making devices?
    - 3d printer in the morgridge
        - Higher resolution - Viper
        - Really slick and nice
        - Directly print the luer lock connectors too
            - Pretty useful
            - Maybe even printed it directly onto a glass slide to avoid needing to bond
            - Williams will send us dissertation from the student who did it
                - Pedro resto
- What can the simulation help us with?
    - Iterate things like the flow speeds
    - Powerful tool to run through various design iterations
        - Test different widths and lengths and such
            - Then we can start plotting things like velocity vs height

Conclusions/action items:

2020/11/06 - Advisor Meeting 5

Sara Wagers - Dec 06, 2020, 12:36 PM CST

Title: Advisor Meeting 2

Date: 11/6/2020

Content by: Sara Wagers

Present: Justin Williams, Josh, Sara, Caleb, Hunter

Goals: Meeting notes

Content:

- Solidworks issues
- Thesis from grad student
    - Williams only has a hard copy so he can't send that to us
    - Will ask him if he can send something
- 3D printer material toxic to cells
    - Maybe just use it for prototyping
        - Can test the fluid properties
        - Use beads to simulate the cells
    - They want to reuse the cell populations after sorting

- Tong lecture next week??
    - Touch base on thursday about meeting
- Outreach
    - We still have to submit a plan
    - Talk to Tracy Pucinelli about it
        - See her email
    - Try to figure something out earlier
    - Microfluidics lends itself well to outreach
        - Principles are pretty easy to demonstrate with larger devices
            - More viscous fluid
            - Maple syrup in a large device can retain laminar flow
            - Microfluidics in nature!
                - How organisms use laminar flow to survive
                    - Bacteria and insects?
                    - Paper by purcell -- life at low reynolds number
- All campus resources closed to undergrads after thanksgiving

Conclusions/action items:

2020/11/20 - Advisor Meeting 6

Sara Wagers - Dec 06, 2020, 12:38 PM CST

Title: Advisor Meeting 2

Date: 11/20/2020

Content by: Sara Wagers

Present: Justin Williams, Josh, Sara, Caleb, Hunter

Goals: Meeting notes

Content:

- Poster session in 2 weeks!!!!!
    - Put together a poster like normal
    - Video presentation -- powerpoint but walking through the poster

- Tracy Puccinelli to send out stuff about the outreach again
    - Look at the one she sent in october
    - Direct questions about it to her

- Problems with fluid modeling
    - Modeling not turning out exactly like we want it to
        - Models are done, but cells aren't aligning exactly how we want it to
            - Put cells in --- non-newtonian behavior
    - Assume that the particles are going to be centered
        - Useful things to measure are flow rates and velocities in the zone, etc
        - May take more help and tweaking to get it to work
            - Read Dino Di Carlo paper https://www-pnas-org.ezproxy.library.wisc.edu/content/104/48/18892
                - Might be some hints and info in here about how they modeled it
    - Good that the model is working and we have some results
        - Would be better if they were what we wanted
        - The goal this semester is to make sure that we have enough info and results to be able to hit the ground running and know our direction to go in next semester
- Josh Parametric Study
    - Problem with knowing the centering of the cells

        - The client wants to know how centered the cells are/ where the cells are within the channel
        - Want to be able to get a graph showing locations of the cells
    - Useful to be able to compare the designs
        - Can show the client what the trade-offs are between the different designs

- Testing
    - Might not be able to get something to the client to be made in time for the semester
    - Its alright --- just make sure that we have the fluid simulations down
    - Campus to close next week - gotta get our stuff done
        - Morgridge is a different entity so it might still be functioning
    - Get a deep analysis/protocol
        - Show that the design is printable and will work
            - It is in a reasonable place to be able to send off to the client or a company to make it for us -- be ready for next semester (or even over break)
        - Give specs of the printer
        - Or provide other commercial entities

No meeting next friday -- if we want feedback prior to poster session

He has cleared schedule on the next monday (not 10 am)

Let him know when sending progress report!

Conclusions/action items:

2020/09/10 - Client Meeting

Hunter Hefti - Sep 10, 2020, 12:49 PM CDT

Title: Client Meeting 1

Date: 09/10/2020

Content by: Entire Team

Present: Entire Team

Goals: Get questions answered and develop an initial idea of the needs of the project

Content:

OVERVIEW

Fluorescent lifetime imaging

- Non-invasive and real time to look at cells
- NADH and FAD

Flow cytometry

First prototype

Pdms

Funnel too big - couldn't focus, bubbles

Second

Smaller funnel and burp line

Firefly sci

Cells are 10 micron diameter

Difference between the design and normal flow cyto

- Weaker signals
- Trying to detect decay time which is not standard
- Aiming to get 10 milliseconds for the time the cell is in front of detector
    - Interrogation path
-

Distance from the objective is important

= confine the cells in x, y, and z

Speed range = 1mm/s

Build from scratch, find a commercial solution or modify one

Fluid dynamics computations would be great! (solidworks, auto desk)

Flow in PBS probably

- What's the budget for the project? (Is this lenient if necessary)
    - $2000?

    - Email them when we want to buy stuff
        - Andrea, cc melissa
- What exactly are we designing/ how is it playing into the sorter that you've already created?
    - Seems this has been answered already?
- Is there any documentation you'd like us to read of what has been done already?
    - Do you know of things that don't/won't work?
        - Coverglass, quartz are good materials. PDMS probably doesn't work. Must cooperate with UV light penetration
        - Possibly could be a square channel versus a circle channel. Don't have to worry about aberration?
        - Mating of pdms and glass capillary was a source of turbulence. Flow was messing up and the fluid was mixing at a strange place. Cells were moving around
        - Heating and pulling a larger capillary into a smaller diameter. There's a chemistry lab that will do glass work available
-
- Do you have any samples you can provide/ show?
-
- Materials for us to test with? Do we need to test with actual cells?
    - They have beads
-
- What are you ideally looking to be accomplished this semester?
-

  Some cells were moving faster than others?

Conclusions/action items:

2020/10/02 - Client Meeting 2

Hunter Hefti - Oct 05, 2020, 3:44 PM CDT

Title: Client Meeting #2

Date: 10/02/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Emmanuel, Kavyan, and Melissa

Goals: Discuss design ideas and where to go moving forward

Content:

Do you use a specific concentration? Can we mess with the dilution?

The funnel design is pretty standard for centering cells

- Simulation to characterize relationship between centering and speed of flow
- Get an idea of what pressures we would need

- Good idea to make a spreadsheet with different papers used for design ideas
- Also keep a spreadsheet/list of the companies that could potentially make this for them

Snake idea

- Simulate some stuff with number of turns or angles
- Would be great to play around with it
- Could make a couple of different prototypes
    - Testing is fun and can be easy

Autodesk

- They have access in their lab, good for modeling

Pdms issues

- Long term, sterility is an issue
- Optically, as long as the bottom is glass, its fine
- Mating of pdms and quartz capillary introduced turbulence

What part of the bottom needs to be glass?

- Just the bottom side of the square channel
    - For epi-flourescence microscopy it just bounces up and back down

New material?

- Just needs to not absorb uv light
- Shouldn’t be too scattering and needs to let the 450 nm light through, a weaker light
- Is it possible to image through pdms?
    - Not sure, haven’t tried yet
    - Maybe if its thin enough

Conclusions/action items:

2020/10/16 - Client Meeting 3

Sara Wagers - Dec 08, 2020, 10:49 PM CST

Title: Client Meeting #3

Date: 10/16/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Skala Lab Team

Goals: Discussion and status update

Content:

- What we've been up to
    - Making the designs in onshape
    - Downloading into solidworks for simulations
        - Do some research to make sure that we can do the modeling from the onshape
        - Emmanuel is suggesting to use solidworks
            - Test out onshape before investing too much time into it
- Snake design
    - Not a lot of information in the literature about the dimensions of the design
        - Shot in the dark?
            - Check out the paper that emmanuel sent in slack
                - Might have the turn radius?
                - At least would be able to see the number of turns
        - Look into the supplemental information on that paper
            - Get the aspect ratio even if not the exact dimensions
    - Square vs round channel
        - Square channel is ideal for the imaging
            - Round scatters light more
- Connectors
    - What did they use?
        - Sheath doesn't matter, low resistance
            - Whatever Luer connector
        - For the cells
            - ½ inch needle (.09 mm inner diameter)
            - Cylindrical, round end -- not sharp

        - Check out the paper on slack for some dimensions
            - Chip fabrication and assembly
            - https://www.nature.com/articles/s41592-020-0831-y#Sec21
- Makerspace 3d printer
    - Dolomite printer

- They still have the initial device
    - We could use it to test or even to just integrate the snake part afterwards
        - Add the snake before or after the sheath fluid
            - Goal is better confinement of the cells laterally and vertically

- Reached out to groups about pricing
    - A lot of them weren't actually custom
    - Some were like $5-20000, not great
    - Are there manufacturers of the mold and not the chip????
        - Could be a cheaper route
        - They could get us some higher resolution
            - The one kayvan used is the highest resolution 3D resin printer on campus, so won't get any better unless we outsource

- Funding for the makerspace stuff
    - The lab has a funding string we can use
- Try out the simulations before we get stuff printed

- Morgridge printing
    - Just give them the sdl file and they print it
    - Viper something (fablabs)
    - Resolution is something under 25 micron

Conclusions/action items:

Next Steps:

- Get some specifics on the models
    - Pick some dimensions and get some simulations going
- Research the dolomite printer
    - What is the resolution that it can do
    - Contact the makerspace and ask them more about it

2020/10/23 - Client Meeting 4

Sara Wagers - Dec 08, 2020, 10:50 PM CST

Title: Client Meeting #4

Date: 10/23/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Skala Lab

Goals: Status update and discussion

Content:

- Sharing progress update
    - Contacting companies about molds
        - Look at resolution and materials
        - Non-stick coatings for pdms
    - Dolomite
        - Waiting to hear back from them
            - Can we go in person to ask them about it?
        - Don’t think that the resolution will be high enough
            - What is the vertical resolution?
                - Not seeing it right away, we can find more info about it later
    - How are the designs and simulations coming?
        - For some of these we can do a 2D projection and then just pull it out to make the design simpler
        - Josh design in Solidworks
            - Figure out what is making the weird flow thing
            - Modify to get a square channel to be used for imaging
                - Should be a pretty easy switch
                - Sample should be confined to 50 micron
                    - Whole capillary could be wider (like 100 micron or something) with the sheath fluid
            - Need to bring some pressures down -- way too fast
                - Start with the same pressure for needle and the sheath flow
        - Snake designs
            - Still need some work to get the simulations going
            - They want some pictures of the designs in the slack channel

- Maybe also post some of the dimensions
    - Width, height, turning radius
    - schematics

Conclusions/action items:

Add pictures and dimensions of the designs to the slack channel to get help from Kayvan and Emmanuel.

2020/10/30 - Client Meeting 5

Sara Wagers - Dec 08, 2020, 10:52 PM CST

Title: Client Meeting #5

Date: 10/30/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Skala Lab

Goals: Status update and discussion

Content:

- Hows it going?
    - Technical issues
        - Josh tried changing geometry but now it doesn't work
            - Is this because of the citrix thing????
        - Caleb downloading issues
            - Installing solidworks issues
                - Maybe try using citrix?
                - Kayvan can try setting up remote connection to one of their lab computers
                    - Potential option
        - Hunter's solidworks problems
            - 3 or 4 iterations of the model because the simulation keeps messing up all of the geometries
        - If we can upload them to the google drive Emmanuel can try to load them on their end and see if there is an issue with the design itself
            - Make a designs folder
    - Comsol???
        - Advisor suggested it
            - Haven't used much before
            - But can download software directly on computer
        - Import solidworks into comsol
    - Solidworks
        - Rob in the fablab knows a lot about solidworks and microfluidic design/fabrication
- What is the roadmap like?
    - Current plan is to do the modeling and simulations
        - If we want to troubleshoot they can set us up with the right people
    - Possibly print a mold in the Viper - are they able to test for us

- Viper printing
    - The worst resolution is the height?
    - Things can collapse if not properly supported
        - Same design can turn out differently based on how it is rotated
    - Process
        - Just give them the stl file and dimensions
        - Usually gets done in about a week?
    - Design needs to be a negative to form the mold
        - Material is maybe toxic to cells so we need to make the mold for pdms
- If we need anything from them just let them know
    - Don’t waste hours trying if we can ask them for help/troubleshooting

Conclusions/action items:

First order of business is sharing with them to see what the problem is. Continue work on simulations.

2020/11/09 - Client Meeting 6

Sara Wagers - Dec 08, 2020, 10:53 PM CST

Title: Client Meeting #6

Date: 11/9/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Skala Lab

Goals: Status update and discussion

Content:

- Caleb's model -- Snake
    - Got it running well
        - Cells are focused up near the top wall of the curve
        - Still doing some degree of focusing
    - Will try changing the radius and rebuilding to see if it helps
        - New one will be at the correct size and scaling in case that is affecting it
    - Look at the inlet velocity
        - Will changing that change the focusing at all
    - Is there a sheath fluid too or just the cells?
        - Will it give us the right confinement of the cells without a sheath fluid?
            - If we need to add sheath, do we add before or after the curves
            - Sort of combining the two designs together????

- What is the speed that the pump can do?
    - 0 to 1 bar of pressure (1000th of a bar steps)
    - Tubing - 1 mm diameter?
    - Velocity anywhere from 0 to fairly fast, but looking at the slower end

- Joshs 3d funnel
    - Working better now
        - 32 gauge needle (.09 mm inner diameter)
    - Sheath flow
        - Right now a 1 to 10 ratio
        - If we decrease the width of the funnel and decrease the amount of sheath fluid, can impact the speeds and such

        - Interested to know what pressure needs to be applied to push the fluid
            - If sheath pressure is too high and cells too low there can be back flow
            - If raise one need to raise the other
- Create a graph with some of these measurements
    - 3D plot or something
        - X and y like pressure at each inlet, z would be diameter of the core

- Try a 2D version of the funnel model
    - 50 micron height
    - Needs to be confined to a 10 micron height
        - So if it can be in the center, then its fine, but

- Can the simulation give us an outlet density?

Conclusions/action items:

Next steps are to share the designs with Emmanuel so he can give more specific feedback.

2020/11/16 - Client Meeting 7

Sara Wagers - Dec 08, 2020, 10:54 PM CST

Title: Client Meeting #7

Date: 11/16/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Skala Lab

Goals: Status update and discussion

Content:

- Josh trying to do the parameter study
    - Making progress but haven't been totally successful yet
    - See if there is a way to run the simulation with multiple parameters
- Caleb and Hunter model
    - How is the height focusing?
        - Not great vertical focusing

For next time:

Redesign without the scaling

- Try making the necks narrower on the turns?
- Are the turns focusing or is it just the sheath

Try the snake without the sheath fluid

- The idea is to try to get confinement but at a lower speed

Conclusions/action items:

Redesign without the scaling

- Try making the necks narrower on the turns?
- Are the turns focusing or is it just the sheath

Try the snake without the sheath fluid

- The idea is to try to get confinement but at a lower speed

2020/11/23 - Client Meeting 8

Sara Wagers - Dec 08, 2020, 10:55 PM CST

Title: Client Meeting #8

Date: 11/23/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Skala Lab

Goals: Status update and discussion

Content:

- Update on the simulation
    - Josh trying to find a way to show where the particles are ending up to see how confined it is
    - Trying at different speeds to see if he can put it together
    - How to measure speeds in the middle of the channel?
- Caleb's
    - Kayvan read through the paper and might be able to help
        - Compared three designs
        - Something about reynolds number
            - Faster speed and bigger particles get better confinement to a point
    - Want to add a bunch more turns
    - Radius of curvature -- this is the point of the last figure in the paper
        - Interested in the circle outcome (in the figure)
        - Y axis is the ratio or the radius of the particle and radius of the channel?
        - X axis is the Dean number
            - Ratio of channel diameter to radius of curvature of the turns
            - Want to be between 1 and 10 or 20
                - 1 is a tighter turn
                - Bottom turn would have more like 10
        - Don't have to hit an exact number --- it is a rough range to aim for
            - Probably why they don't give the exact numbers
    - Let's try to get the tighter turn closer to a 1:1 ratio of channel diam to radius of curvature
    - Channel size
        - Particle can be up to 50% of the channel
        - No larger than 100 micron

Conclusions/action items:

Deliverables for next time

- Josh:
    - 3d plot of velocity to velocity of particles
    - Maximize confinement
- Caleb
    - At least reproduce what this paper is doing
        - Try to get the right particle sizes and turn sizes

2020/12/07 - Client Meeting 9

Sara Wagers - Dec 08, 2020, 10:56 PM CST

Title: Client Meeting #9

Date: 12/7/2020

Content by: Sara Wagers

Present: Caleb, Josh, Hunter, Sara, Skala Lab

Goals: Status update and discussion

Content:

- Fix the turn radius/channel width ratio in hunter's snake
- Put all of these designs in the shared drive
    - Emmanuel can try to work on them over the break
- Josh's funnel
    - Changing the cell velocity doesn't really affect the speed of them in the channel
        - The sheath flow is more responsible for the speed in the channel
        - How does this translate to pressure?
            - Can take a look at that
            - If we have a big pressure difference we could have backflow
            - Having things in terms of pressure sort of normalizes it because velocity without knowing area isn't super meaningful
- Looking at the results of the simulation isn't a very vigorous way to look at the confinement of the cells
    - Need to look at the velocity across the channel
    - Email Rob for help with this
        - Attach the simulation file and everything in one place
        - Kayvan will send an email to introduce

- Tasks for next semester
    - Particle velocity profile
        - See if we can export the distribution of particles in cross section
            - Will tell us about the focusing
    - Inertial project
        - Figure out how to match what they're doing in the paper
        - Get a simulation that reproduces it -- prove that it works

        - Get in touch with Rob
            - Try to get that stuff sorted out before the start of the next semester
        - Get a design ready for fabrication for the next semester
        - Rename files in the google drive with the date

Conclusions/action items:

- Get in touch with Rob
    - Try to get that stuff sorted out before the start of the next semester
- Get a design ready for fabrication for the next semester
- Rename files in the google drive with the date

2020/10/7 Design Matrix

Sara Wagers - Oct 07, 2020, 11:06 AM CDT

Title: Design Matrix

Date: 10/7/2020

Content by: Team

Goals: To weigh the criteria of the different designs to decide which to pursue further.

Content:

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**	

Conclusions/action items:

This table is not the only deciding factor in moving forward. After talking to the client, they like the snake idea but were very open to further exploring multiple options.

2020/09/18 Product Design Specifications

Sara Wagers - Oct 07, 2020, 11:04 AM CDT

Title: PDS

Date: 9/18/2020

Content by: Team

Goals: To outline the specifications for the design.

Content:

Microfluidic Cell Sorter

Project Design Specifications

Team:

Josh Zembles

Sara Wagers

Caleb Heerts

Hunter Hefti

Date:

September 18th, 2020

Function: The Skala lab has developed label-free optical signals to sort T-cells by activation state. The next step in their research requires a microfluidic chip to flow the cells at speeds that allow 100's of ms integration time on the detector. The device can be commercial or newly designed, and requires a number of specifications in order to integrate with their system. The function of the device should create single-file cell flow through the interrogation window with a stable core diameter of 20 um to 50 um while ensuring that stability is first maintained in the z direction. Cells should flow through the microfluidic device along with a PBS sheath fluid at a flow speed of 1 mm/s and up to 10x faster.

Client Requirements: There are a number of specifications that need to be considered in order to ensure that our design is fully compatible with the equipment used by the Skala Lab:

- The device should be able to fit within their microscope’s stage insert
- The bottom of the flow cell must have 150 micron glass thickness while accommodating the 1 inch wide objective lens at a working distance of 0.2mm.
- This device should be created with a budget of $2000 in mind, aiming to save money as compared to custom microfluidics and the cost of flow cytometers.

Design Requirements:

1. Performance Requirements: The device must be able to maintain sufficient pressure to flow the cells and media through the channel at a consistently low flow rate. Ideally, the device will be effectively integrated with the pump system that the Skala Lab has already set up. The microfluidic chip should maintain consistent performance over time as it is intended to be a reusable device.

2. Safety: There are limited safety concerns regarding the development of this device. The device should pose no threat to the user if used correctly as all cells and fluids should be contained within the channel. When operating the device or handling any associated cell cultures, typical safety protocols should be adhered to.

3. Accuracy and Reliability: This device must operate accurately to ensure that cells are within the interrogation window for a suitable amount of time. The channel must reliably create a single-cell flow of 1 mm/s and must also limit the variance in z-direction of the cells as they flow through. An accurate device will ensure that experimental data is useful within and between experiments.

4. Life in Service: The life of a flow cell is vague as the potential for reuse is essential to its design. Laboratory glassware can be used indefinitely as long as proper maintenance is applied to keep the material clean. The design will likely be made from glass or quartz as listed below. These items are not particularly prone to a quick expiration. Prototype designs should have a lifespan of at least a few weeks in order for testing to be completed while the final design should have a lifespan that exceeds 10 years if necessary and if proper maintenance is applied.

5. Shelf-Life: In conjunction with the life in service, the flow cytometer cell should be designed in such a way that parts do not degrade while in use. As such, while not in use, the cell should be able to withstand an extended period of resignation in storage that surpasses the lifespan of a cell that is in continuous circulation. This assumes that, prior to storage, proper sterilization techniques using ethanol are employed to prevent mineral build-ups or the proliferation of any residual cells.

6. Operating Environment: Elements of the cell will be exposed to a pulsed laser and should be able to withstand such exposures. Placement under a microscope or under other varieties of imaging equipment may also be possibilities. Pumps are used to produce the pressure that powers the transport mechanisms responsible for pushing fluid and cells through the cell which should also be accounted for. General lab temperatures and light exposures should also be accounted for if necessary.

7. Ergonomics: The microfluidic cell functions similar to a glass slide used for microscope viewing and can be placed over the laser in a manner that is similar. The human hand is capable of picking up objects that are 1 mm thick with relative ease and only two fingers will be required to pinch together enough strength to pick up and hold the cell. Other elements such as the pump have already been designed ergonomically in a fashion that allows for the control of pressure and flow to remain in the hands of the user.

8. Size: The objective access window that is meant to carry the Quartz/Glass capillary is roughly 3.5 cm long while the PDMS that currently acts as the inlet and outlet are nestled at either end of the tube. The size of the current cell is about as thick as a 1mm glass slide but can likely be thicker up to ~ 2.5 mm while the whole of the device is 9.6-9.75 x 2 cm in overall size. The current laser is set up to accommodate objects roughly this size so the length of the overall cell should not exceed 10 cm in length and not much more than 2 cm in width.

9. Weight: A reasonable weight to set the design of the cell can be estimated as less than 15 grams. Glass can be reasonably approximated as having a density of 2.5g/cm^3 while quartz has a density of 2.43g/cm^3 and PDMS has a density of 0.965g/cm^3. Using all of these measurements in various combinations using the estimated maximal size of the object above, all calculations yield potential weights that are near or smaller than 15 grams. A device made entirely of PDMS would weigh approximately 5 grams. As such, the weight of the cell is expected to fall near one of these measurements.

10. Materials: The materials used for the design should be biocompatible or bioinert. They should not interact with the cells, cell media, or other solutions such as PBS, DI water, or clean water in order to stop any contamination from occurring. Additionally, the materials used should allow light to pass through uninterrupted for measurements being taken. Materials suggested by the client include either quartz or glass, however for prototypes, PDMS may be used due to its ease of fabrication. The material should be able to be reused and cleaned either with ethanol or an autoclave.

11. Aesthetics: The focus of this design is more on functionality. Being able to align the cells with a certain speed is the main importance meaning aesthetics aren’t a major concern. The materials shouldn’t be sharp when touched and the design as a whole should be relatively small to fit on the stage of the lab’s microscope. Additionally, the material chosen must be transparent to allow light to pass through.

Production Characteristics:

1. Quantity: For the semester, only one product is needed, but if a successful design is found, then more could be produced for analyzing multiple groups of cells at once.

2. Target Product Cost: The client has set a budget of $2000 for the prototype. They are hoping to create a device more cost effective than a custom flow cytometer that can be produced with prices ranging upwards of $4000 [1].

Standards and Consumer Characteristics:

1. Standards and Specifications: There are no federal regulations concerning this device since it is being specifically designed for the clients use. However, the device needs to be sterilized to ensure no contamination.

2. Patient or User-related Concerns: It is incredibly important that this device will maintain sterility and work accurately as it will be used for research experiments. Care should be taken to ensure that cells from different batches are separated and treated as such.

3. Competition: Currently most cell sorting microchips [2] use weight or size as the factor to separate different cells. These kinds of chips will not work since they depend on multiple types of cells while the clients have one type and are either fluorescent or not. The cell sorting techniques that are based on fluorescence are an all-in-one machine. The client only wants the microchip which allows cells to be centered in a stream so their custom laser can be used to identify each cell. Microchips that consist of small channels are available on the market that allow for a stream of cells to flow through a narrow channel under a microscope [3]. However, these cells are not centered within the channel for the laser.

References:

[1] “Custom Quartz Flow Cell Manufacturing,” *FireflySci Cuvette Shop*. [Online]. Available: https://www.fireflysci.com/custom-quartz-flow-cell-manufacturing. [Accessed: 17-Sep-2020].

[2] Microfluidic Chip-Based Gentle Cell Sorter, Single Cell & Cluster Dispenser | On-chip Bio. 2020. *On-Chip Sort CONSUMABLES : MICROFLUIDIC CHIP | Microfluidic Chip-Based Gentle Cell Sorter, Single Cell & Cluster Dispenser | On-Chip Bio*. [online] Available at: <https://on-chipbio.com/product-onchip_sort/microfluidic-chip/> [Accessed 17 September 2020].

[3] “Straight Channel Chips - Glass,” *microfluidic ChipShop*. [Online]. Available: https://www.microfluidic-chipshop.com/catalogue/microfluidic-chips/glass-chips/straight-channel-chips-glass/. [Accessed: 17-Sep-2020].

Conclusions/action items:

These specifications are temporary and will be adjusted as we progress through the project to better suit the needs of the client and realistic goals for the semester.

2020/10/29- Show and Tell Pitch

Sara Wagers - Dec 08, 2020, 11:03 PM CST

Title: Show and Tell Pitch

Date: 10/29/2020

Content by: Team

Goals: Our pitch for the show and tell in order to receive feedback and guidance from other teams.

Content:

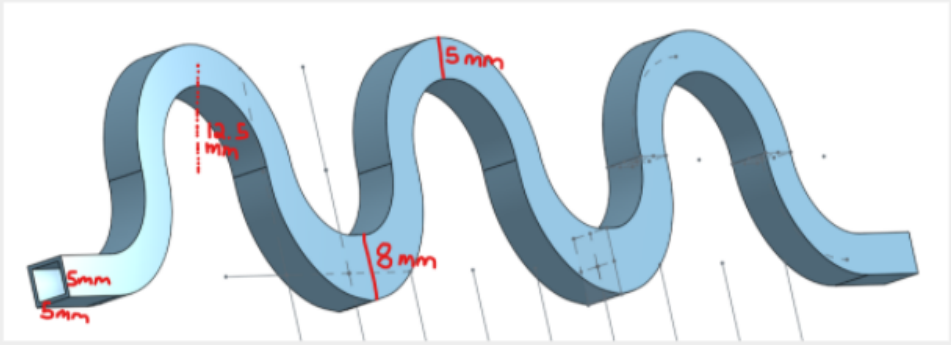
Our client, the Skala lab, is asking us to design a microfluidic chip to aid in their current method of cell sorting. Their current system utilizes label-free optical signals which sort T cells by activation state. This method would improve monitoring and quality control of T cell manufacturing. For this system to work, cells need to flow through the microfluidic chip at the source of the optical signals at a controlled rate and in a single file line in order to maximize the effectiveness of the sorting system. Redesigning the microfluidic chip would serve the purpose of aiding in focusing the flow of the cells at the center of the channel at a rate that allows for the 100's of ms integration time that the detector requires to function correctly.

The current solution to this problem is to design a typical flow cell that includes one or more features that would physically limit the cells motion in either axis and require them to fix into a central orientation. Two features which we are heavily focusing on include a modification of the sheath fluid inlet into a cone shape and an insert of a serpentine channel which would generate inertial lift forces on the fluid that would force the cells into the center. While the cone is a rather common modification, variations of the snake-like channel design are less so, and analysis of the various dimensions involved will require multiple simulations prior to fabrication.

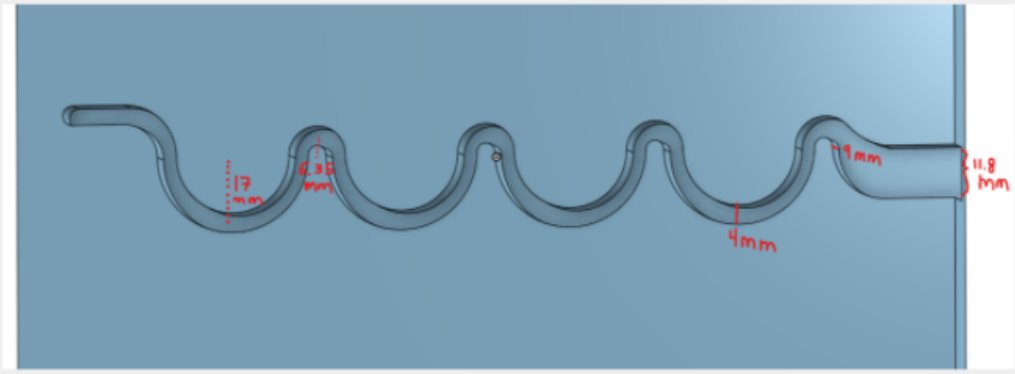
So far we have been working on developing CAD models of potential designs. We are hoping to develop fluid flow simulations using the CAD models in Solidworks. We are currently working through some of the issues with the models to be able to properly run the simulations. We have also been investigating different manufacturers and materials to be used when fabricating a prototype.

We would like help with ideas about fabrication methods and possible materials to consider for the device. Current options that are being considered are mostly commercial while the use of 3D printers is being offered by the MakerSpace and alternative printers are available from Morridge. The bulk of the device will likely be made with PDMS. Further input on fluid simulations from anyone with SolidWorks experience on the subject would also be appreciated with emphasis on generating fluid simulations involving a particle stream.

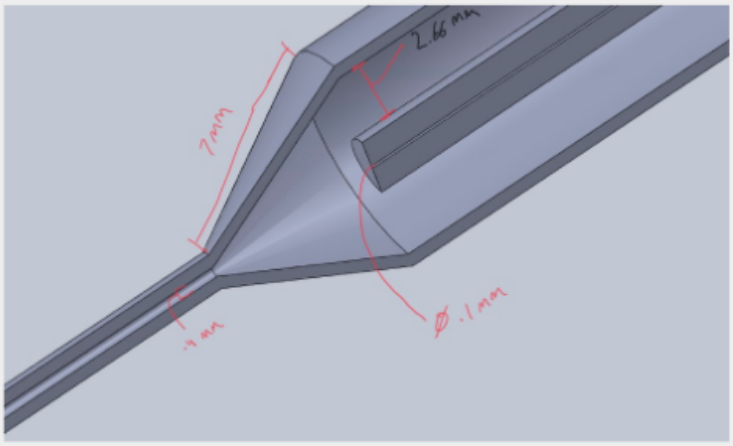
Snake Design (Inertial Lift)



Dimensions scaled up for initial testing and prototyping



Funnel/Cone Design



Conclusions/action items:

This will be posted on Piazza for feedback from other teams.

2020/10/05 Expense Spreadsheet

Caleb Heerts - Oct 05, 2020, 3:48 PM CDT

Title: Cost Spreadsheet

Date: 10/5/2020

Content by: Caleb

Present:

Goals:

Content:

Expenses

Item	Description	Manufacturer	Part Number	Date	QTY	Cost Each	Total	Link
Component 1								
Component 2								
Component 3								
TOTAL:								

Conclusions/action items:

2020/10/7 Material Considerations

Sara Wagers - Oct 07, 2020, 12:43 PM CDT

Title: Material Considerations

Date: 10/7/2020

Content by: Sara

Goals: initial thoughts about materials to use in the device

Content:

In creating a microfluidic device, the optical properties of the device need to be considered. Due to the experimental set-up, the materials selected need to allow for a laser of up to 450 nanometer light to shine through without causing too much light scattering for the epi-fluorescence microscope. Materials suitable to provide the desired optical properties include glass and quartz, so the bottom face of the device should be made from one of these materials or one with similar properties.

While it is crucial that the laser can shine through the bottom surface of the device, the other surfaces can be made of other materials. A popular material for microfluidic applications is PDMS due to its biocompatibility and ease of fabrication. PDMS can be mated to a glass surface through plasma bonding, a technique that the team has the capability to perform in the ECB tissue lab.

It is important to also note that there are limitations associated with PDMS. In the long term, sterility and longevity of the material can be an issue. This may not be a large concern as the clients would be able to fabricate a number of these devices for use in the lab. Additionally, the clients have highlighted that an issue in past designs was turbulence created at the mating site of PDMS and the quartz capillary. Due to these issues with PDMS, the team plans to conduct further research into alternative material choices.

info about PDMS:
https://www.elveflow.com/microfluidic-reviews/general-microfluidics/the-polydimethylsiloxane-pdms-and-microfluidics/

Conclusions/action items:

We need to do some more research before deciding if the device should be made from PDMS/Glass or another material

2020/12/08 - Final Poster

Sara Wagers - Dec 08, 2020, 10:59 PM CST

Title: Final Poster

Date: 12/8/2020

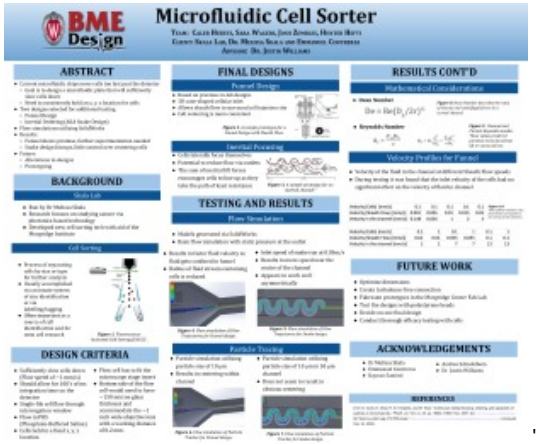
Content by: Team

Goals: To present our work for the semester in a detailed poster.

Content:

See attachment for poster

Sara Wagers - Dec 08, 2020, 10:58 PM CST



Final_Poster.pdf(834.9 KB) - download

2020/09/17 Background Microfluidic Cell Sorting

Josh Zembles - Sep 17, 2020, 8:38 AM CDT

Title: Background Microfluidic Cell Sorting

Date: 2020/09/17

Content by: Josh Zembles

Present: Josh

Goals: To gain basic background knowledge of current cell sorting techniques.

Content:

12 different types of cell sorting mechanics that could be useful to keep in mind for this project.

https://www.elveflow.com/microfluidic-reviews/microfluidics-for-cell-biology/label-free-microfluidic-cell-separation-and-sorting-techniques-a-review/

Most of these designs deal with actively separating the cells, however we only want to center the cells in the middle.

Number 11 shows an acoustophoresis design that centers the cells within the channel using ultrasonic waves. We could potentially modify this design to incorporate the sheath design that mostly centers the cells already, then have the flow go through the ultrasonic waves right before the imaging laser to get them centered.

Conclusions/action items:

2020/10/1 Plinko design concept

Josh Zembles - Oct 07, 2020, 12:17 PM CDT

Title: Plinko design concept

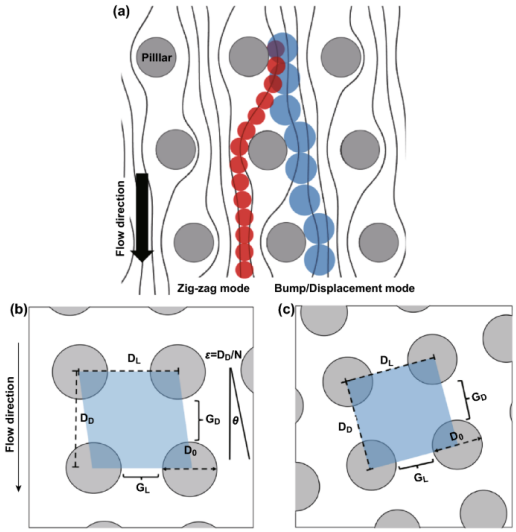
Date: 10/1/2020

Content by: Josh

Goals: To understand the mechanism of the plinko design

Content:

The plinko design is based on the Deterministic Lateral Displacement concept. It uses the physical size of cells to sort a population of different sized cells. The larger cells would move towards the side while the smaller cells are able to move with more agility to move through the channel without moving to the side. We would take the aspect of larger cells drifting towards one side and implement it into a design. The size of the cell that moves sideways in the diagram can be changed based on the configuration and size of the pillars. Configuring the "larger cell" to be the size of the T-cells being used would allow them to move sideways when flowing through. The idea would be to move the cells inward toward the center of the channel by having the left half of the pillars moving the cells to the right and the right side moving them to the left.



Conclusions/action items:

https://link.springer.com/article/10.1007/s40820-019-0308-7

2020/10/6 Flow Cytometry Background

Josh Zembles - Oct 06, 2020, 8:53 PM CDT

Title: Flow Cytometer Background

Date: 10/6/2020

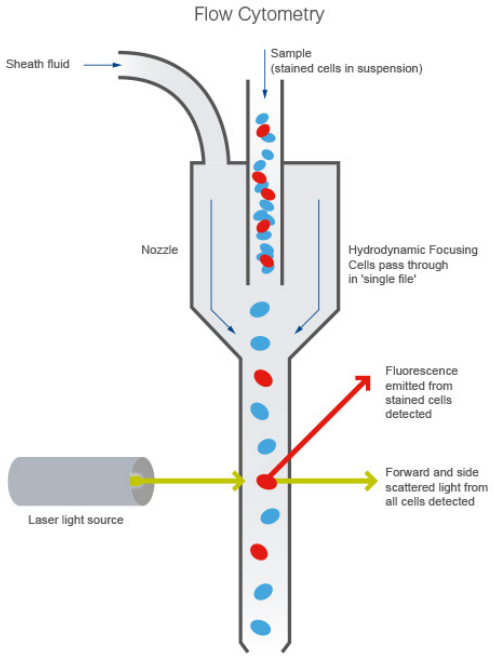
Content by: Josh

Present: Josh

Goals: To understand how flow cytometry works.

Content:

One design uses the same mechanism as flow cytometers use to focus cells within streams.



The design uses a sheath flow around the stream containing cells to keep the cells centered within the channel. This happens because the no-slip boundary condition along the channel says that flow close to the edges are slower and increases as it goes to the center.

Conclusions/action items:

Understanding the mechanism can help guid our design. More research into the flow dynamics and factors that affect the system will provide a better design for the client.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3251643/

2020/09/17 Microfluidic Chip Cell Sorter

Josh Zembles - Oct 06, 2020, 7:22 PM CDT

Title: Microfluidic Chip Cell Sorter

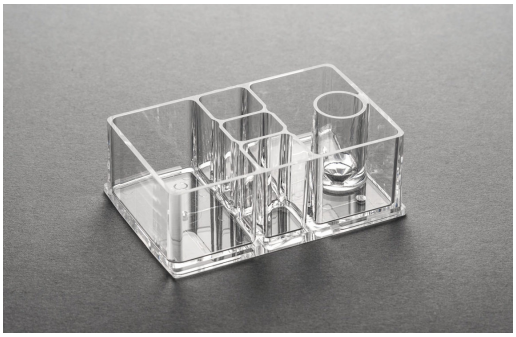
Date: 09/17/2020

Content by: Josh

Present: Josh

Goals: Research on competing design

Content:



Microfluidic Chip Cell Sorter by On-Chip

This is an all-in-one machine that separates cells based on their fluorescence. These kinds of machines, however, are expensive and do not meet the specificity required by the client. The client uses their own set-up with a specialized laser configuration and has asked us to develop a device that allows them to use their laser.

Conclusions/action items:

A similar concept of the Microfluidics chip could be used to influence our device.

https://www.microfluidic-chipshop.com/catalogue/microfluidic-chips/glass-chips/straight-channel-chips-glass/

2020/09/17 Microfluidic Channel

Josh Zembles - Oct 06, 2020, 7:41 PM CDT

Title: Microfluidic Channel

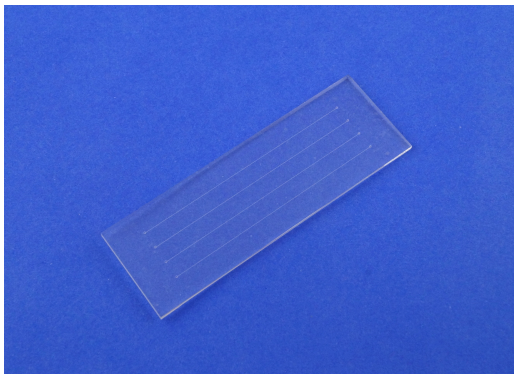
Date: 09/17/2020

Content by: Josh

Present: Josh

Goals: Research on competing designs

Content:



Microfluidic channel by Microfluidic ChipShop

This product shows options that allow cells to travel down a narrow channel. However, cells that go through the channel are not centered within the channel. The client requires cells to be sent through the channel one-by-one in a single file in the center so that the laser can detect each cell.

There are some other options from the website that the client has tried that allow cells to be centered using sheath flow around the stream of cells.

Conclusions/action items:

A similar concept of the Microfluidics channel could be used to move cells through other parts of the device that does not need to be centered.

https://www.microfluidic-chipshop.com/catalogue/microfluidic-chips/glass-chips/straight-channel-chips-glass/

2020/12/1 Funnel Design

Josh Zembles - Dec 08, 2020, 6:51 PM CST

Title: Funnel Design

Date: 12/1

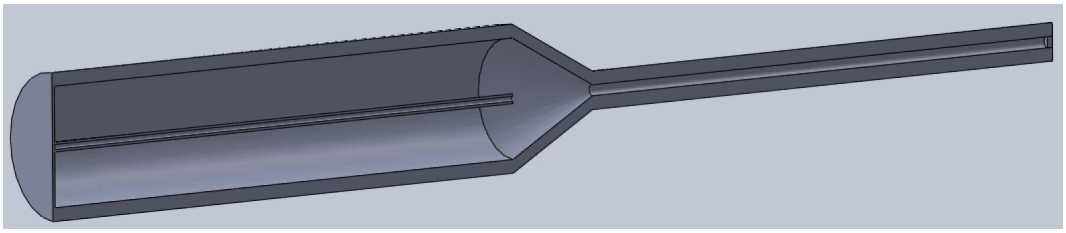
Content by: Josh

Goals: To design and draw the 3D Funnel Design in SolidWorks

Content:

Attached is the solid works designs of the 3D Funnel that is used in the simulation testings for the final report.

Cross section view of the solidworks model



Conclusions/action items:

Josh Zembles - Dec 08, 2020, 6:51 PM CST

Funnel_Design2_12.8.20.SLDPRT(219.5 KB) - download

2020/12/8 Funnel Design simulations

Josh Zembles - Dec 08, 2020, 6:59 PM CST

Title: Funnel Design simulations

Date: 12/8

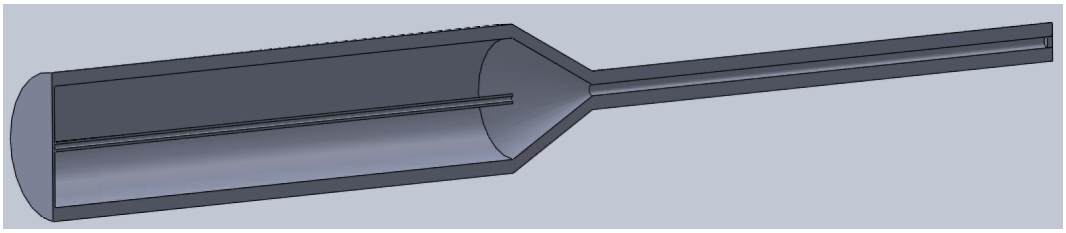
Content by: Josh

Goals: To simulate the 3D Funnel Design in SolidWorks

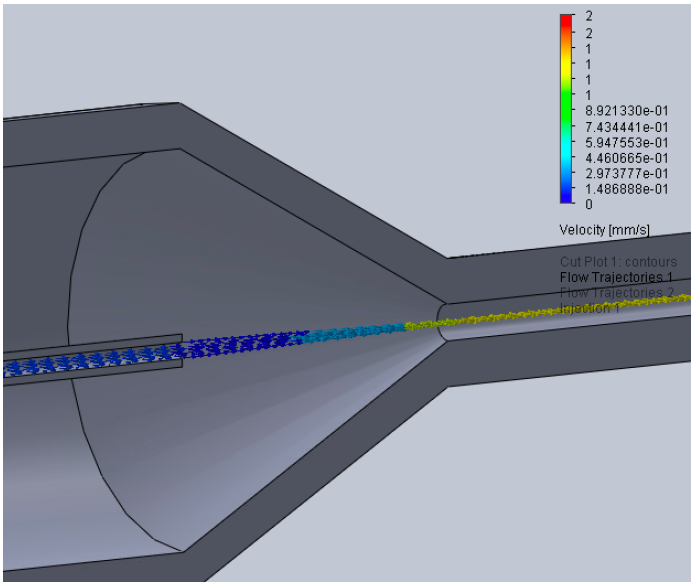
Content:

Attached is the solid works designs of the 3D Funnel that is used in the simulation testings for the final report.

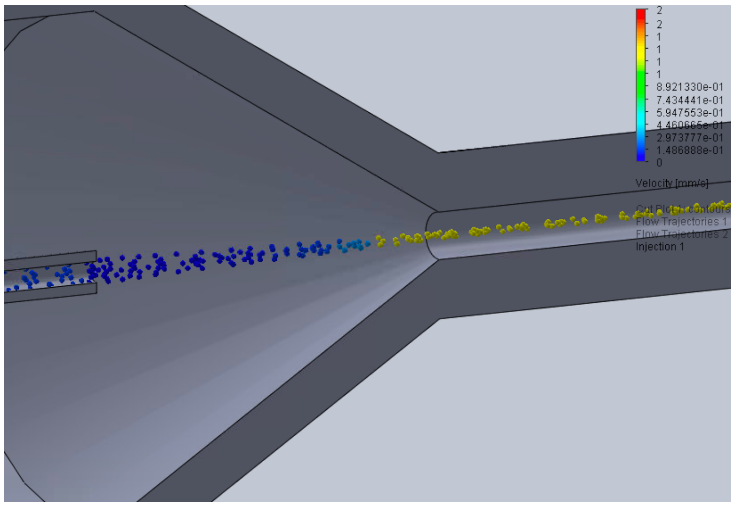
Cross section view of the solidworks model



The simulation of the Funnel design, as seen in figure below, shows that it was able to reduce the diameter of the core flow as it enters the channel. It was also shown that as the core fluid travels into the channel from the needle, it increases its velocity. As of now, the team was not able to quantify the reduction in the core diameter other than by looking at the streamlines of the flow. Having a better understanding of the reduction of core diameter would give better insight on where the cells would be traveling down the channel. The cells would most likely stay within the flow of the core fluid, however to provide more evidence of this, particle tracker simulations were used.



The particle study shows what cells would behave like as they traveled through the device and it shows the cells being confined along with the core flow. Another issue the team had was quantifying where the cells were actually located within the channel. We could not find a way to look at where the cells were passing through the cross section of the channel other than looking at the results of the particles traveling down the device. We are hoping to be able to find a way to look at how close to the center of the circular channel the cells would be located.



We first varied the velocity of both inlets to gain an idea of the effects that it would have, shown in figure **J4**. The results revealed that the velocity of the sheath flow had a much greater impact on the flow in the channel while changing the inlet velocity of the cells had little to no impact. Following these results, we fixed the velocity for the cells and varied the sheath flow velocity to understand how it would need to be adjusted to get a final channel velocity of around 1-2 mm/s. The results conclude that the velocity of the fluid in the channel was dependent on the sheath flow velocity. Another factor that would affect the velocity is the volume of fluid in the sheath flow. This could be varied if more control was needed.

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

Josh Zembles - Dec 08, 2020, 6:53 PM CST

DATA

Funnel_Design2_12.8.20.SLDPRT(219.5 KB) - download

2020/9/9 Green Pass Permit

Josh Zembles - Oct 06, 2020, 7:59 PM CDT

Title: Green Pass Documentation

Date: 9/9/2020

Content by: Josh

Goals: To document the completion of the Green Permit

Content:

Conclusions/action items: I have completed the Green Pass to be able to work in the lab for testing and development of our prototype.

2020/9/9 Biosafety Training

Josh Zembles - Oct 06, 2020, 7:59 P

Title: Biosafety Training Documentation

Date: 9/9/2020

Content by: Josh

Goals: To document the completion of the biosafety training course

Content:

University of Wisconsin-Madison

This certifies that JOSH ZEMBLES has completed training for the following course(s):

Course Name	Curriculum or Quiz Name	Completion Date	Expiration Date
Biosafety Required Training	Biosafety Required Training Quiz	10/22/2018	

Data Effective: Mon Oct 22 15:43:56 2018
Report Generated: Wed Dec 12 01:37:08 2018

Conclusions/action items: I have completed biosafety training to be able to work in the lab for testing and development of our prototype.

2020/10/4 Flow Controls

Sara Wagers - Oct 07, 2020, 11:40 AM CDT

Title: Flow Controls

Date: 10/4/2020

Content by: Sara

Goals: To investigate parameters of our device that can be controlled in order to reduce the flow through the device.

Content:

https://www.elveflow.com/microfluidic-reviews/microfluidic-flow-control/flow-control-in-microfluidics-device/

- This is a useful source that breaks down some of the mathematics and calculations involved in microfluidics
    - navier stokes - we learned this in transport
    - pressure equations
- it also has information about different types of pumps and other variables that can influence the flow through a system
- large list of references that can lead us to more specific information

https://store.micronit.com/flow-splitter

- This device can split the tubing from the microfluidic pump into two
    - This could potentially help to put less fluid into the device while allowing the pump to work at the necessary speed.

Conclusions/action items:

It is important to consider that there are a number of variables affecting our flow. We are able to play around with a few different things to decide how to get the flow down to the rate that we want it to be at. These are helpful resources to look at for that.

2020/10/5 Microfluidic Cross-Filtration

Sara Wagers - Oct 07, 2020, 11:36 AM CDT

Title: Self-Focusing Microfluidic - Cross Filtration

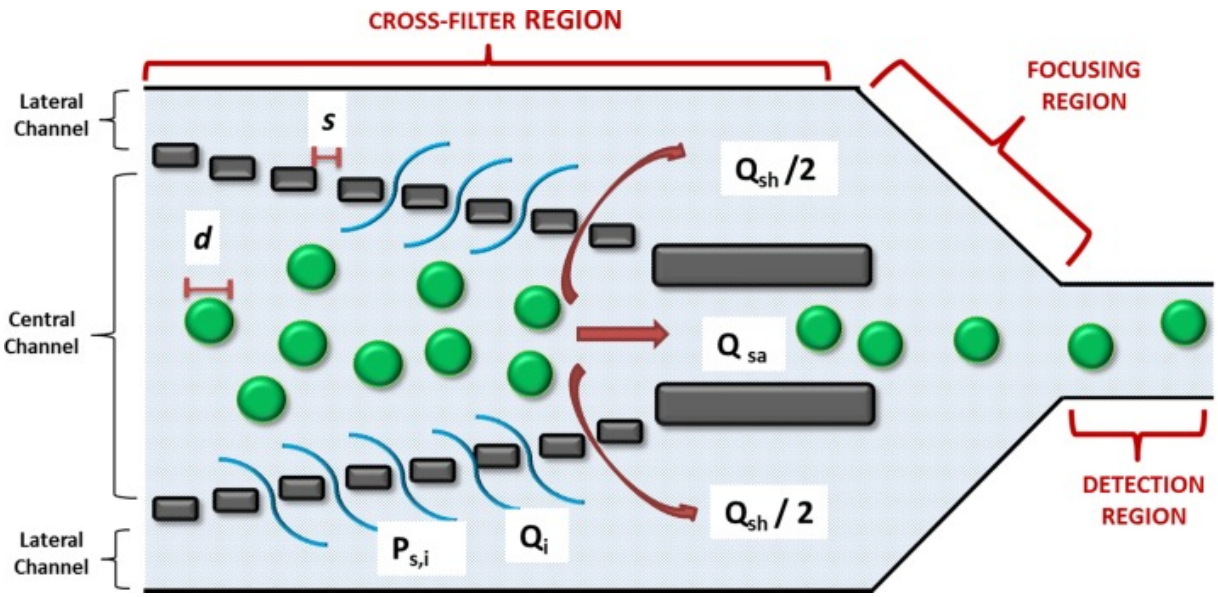
Date: 10/5/2020

Content by: Sara

Goals: To look into a possible technique for centering the cells.

Content:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4654736/



- This design is again similar to Caleb's idea of letting some of the flow out
    - by making the posts in the formation above, the cells will become more focused in the center
        - it still looks like they might not be perfectly centered, but might be good enough

- How do we get them to be centered in the z-axis?

- The paper includes lots of calculations and images that will be helpful in analyzing whether it is a suitable design for our purposes.

Conclusions/action items:

This could be a potential design idea or moficiation of one of the other designs to help with the focusing of the cells.

2020/10/07 Fluid Separating Analysis

Sara Wagers - Oct 07, 2020, 11:47 AM CDT

Title: Analysis of Fluid Separating Microfluidic

Date: 10/7/2020

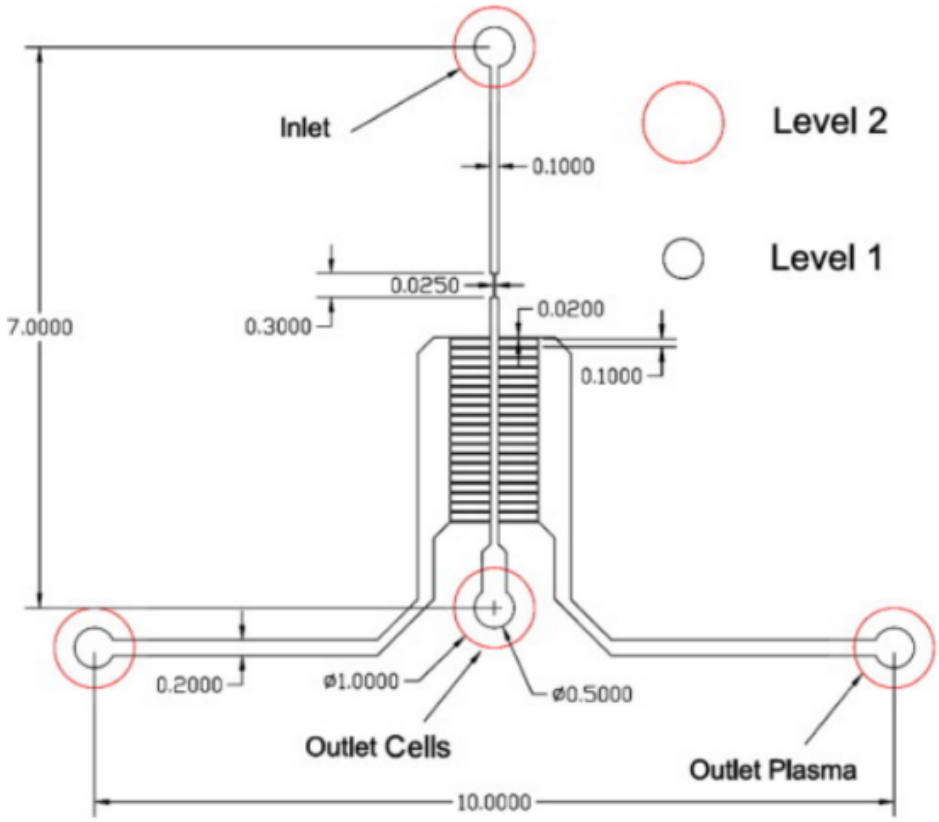
Content by: Sara

Goals: To look at this paper as a potential design consideration and idea

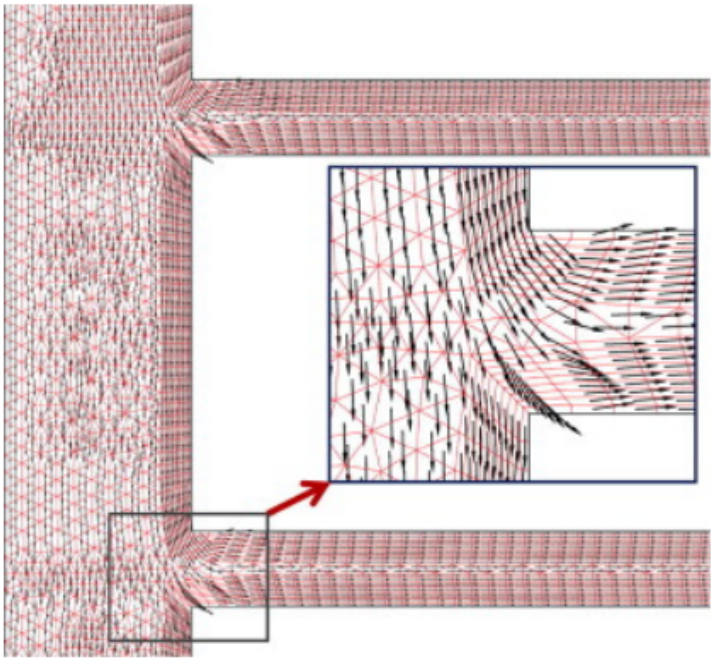
Content:

https://www-sciencedirect-com.ezproxy.library.wisc.edu/science/article/pii/S0307904X11003891

- separating blood with a microfluidic device
    - the channel has a number of bifurcations off of the main stream
        - could reduce the volume of fluid through the main stream, lowering the velocity
    - idea of the device is to get a higher concentration of particles going through the main stream.



- They also did calculations and CFD on the system



- The bifurcations could create an opportunity for more turbulent flow to arise at the corners
    - modification and simulations should account for this possible issue
    - may not affect the overall fluid dynamics through the main channel?
        - does it matter if the cells come out of it in laminar flow?
- The researchers also looked into the pressure profiles throughout the system
    - this is an important consideration

Conclusions/action items:

This design is another possible idea to consider from separating microfluidics. While we will not be trying to separate something, it could help us achieve the flows that we want.

2020/9/30 Microfluidic Separating - Plinko

Sara Wagers - Oct 07, 2020, 11:19 AM CDT

Title: Microfluidic Plinko and Separators

Date: 9/30/2020

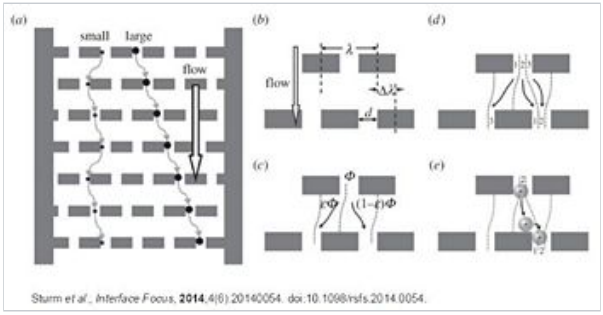
Content by: Sara

Goals: To look into the possibility of using the microfluidic plinko idea

Content:

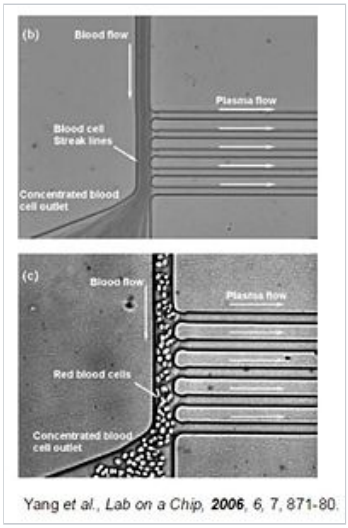
https://openwetware.org/wiki/Microfluidic_Red_Blood_Cell_Separation_for_Rapid_Blood_Testing_-_Rune_Percy,_Alex_Smith

- Desribes the "Plinko" type device that was discussed in an advisor meeting
    - DLD = Deterministic Lateral Displacement
        - the particles will be sorted based on their size as they bounce through the plinko game
- Something to note is that they recommend a higher flow velocity in order to reduce diffusion effects for a successful separation of the particles
    - Because we aren't using this for separating, the effects of diffusion should not matter as much so we should be able to keep the velocity low
        - as long as we can get all of the particles to end up where they need to be eventually



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

- This article also describes a different technique for separation that uses a cross flow design
    - the example is separating blood cells out of plasma
        - relates to Caleb's idea of letting out some of the fluid to reduce the velocity of the flow in the wider channel



Yang et al., Lab on a Chip, **2006**, 6, 7, 871-80.

Conclusions/action items:

These designs were originally created to be separators for different sizes of cells, but we could potentially use them as a method to slow down the flow of the cells. We will need to conduct simulations in order to determine if they have the intended effect.

2020/10/16 Microfluidic Bioparticle Separator

Sara Wagers - Oct 16, 2020, 12:23 PM CDT

Title: Continuous Flow Microfluidic Bioparticle Concentrator

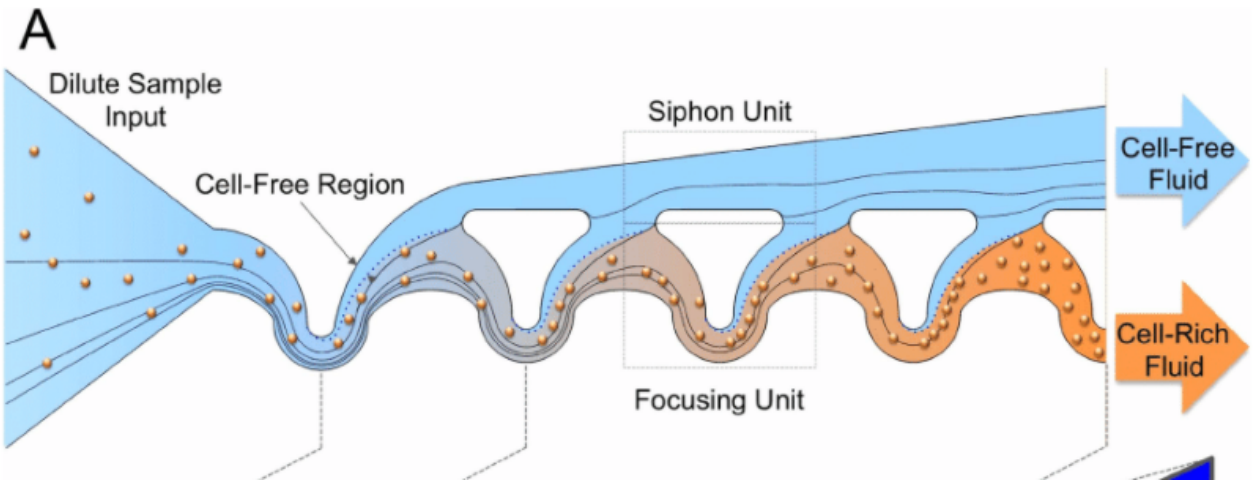
Date: 10/16/2020

Content by: Sara

Goals: A potential derivative of the snake idea to investigate further

Content:

https://www.researchgate.net/publication/278044081_Continuous_Flow_Microfluidic_Bioparticle_Concentrator



Conclusions/action items:

These designs were originally created to be separators for different sizes of cells, but we could potentially use them as a method to slow down the flow of the cells. We will need to conduct simulations in order to determine if they have the intended effect.

2020/10/5 Software for simulations

Sara Wagers - Oct 07, 2020, 10:06 AM CDT

Title: Simulation Software Ideas

Date: 10/5/2020

Content by: Sara

Goals: To look into and compare some potential software platforms to aid in modeling the microfluidics.

Content:

Solidworks

- familiar to us
    - we have modeled turbulent flow at a larger scale, and the microfluidic laminar flow should be much easier
- could also use OnShape (an online version of Solidworks with better sharing capabilities)

COMSOL

- Also somewhat familiar, but a bit more challenging to get down
- used more frequently for modeling laminar flow
    - there are many tutorials online to help
- https://www.comsol.com/models/microfluidics-module
    - they have a gallery with different microfluidics applications that the software is capable of producing

Autodesk

- suggested by Emmanuel as something they have used in the past for simulations
    - they would be able to help us more if they are familiar
- https://www.autodesk.com/products/cfd/overview
- will probably work very similarly to the other softwares

Flow3D

- https://www.flow3d.com/industries/micro-bio-nano-fluidics/
- a different software that we have not considered before
- we have access to it through the CAE software library

Conclusions/action items:

These should all be very viable possibilities for software to use in modeling our system. It may be beneficial to try out the different types to decide what we like or to compare results from using different simulations.

2020/09/23 Training Documentation

Sara Wagers - Sep 23, 2020, 9:49 AM CDT

Title: Training Documentation

Date: 9/23/20

Content by: Sara

Goals: to document the completion of trainings that are required to use the tissue engineering laboratory and lab spaces that may be needed for testing of our prototypes.

Content:

University of Wisconsin-Madison

This certifies that SARA WAGERS has completed training for the following course(s):

Course Name	Curriculum or Quiz Name	Completion Date	Expiration Date
ANIMAL CONTACT RISK QUESTIONNAIRE	ENVIRONMENTAL & OCCUPATIONAL HEALTH	4/17/2020	4/16/2021
ANNUAL HERPES B SAFETY TRAINING	ANNUAL HERPES B SAFETY TRAINING QUIZ	4/19/2019	
ANNUAL HERPES B SAFETY TRAINING	ANNUAL HERPES B SAFETY TRAINING QUIZ 2020	3/30/2020	
BIOSAFETY 102: BLOODBORNE PATHOGENS FOR LABORATORY AND RESEARCH	BLOODBORNE PATHOGENS SAFETY IN RESEARCH QUIZ	4/29/2019	
BIOSAFETY 102: BLOODBORNE PATHOGENS FOR LABORATORY AND RESEARCH	BLOODBORNE PATHOGENS SAFETY IN RESEARCH QUIZ 2020	3/30/2020	
BIOSAFETY 105: BIOSAFETY CABINET USE	BIOSAFETY 105: BIOSAFETY CABINET USE QUIZ	5/20/2019	
BIOSAFETY 106: AUTOCLAVE USE	BIOSAFETY 106: AUTOCLAVE USE: SAFETY AND EFFICACY - VERIFICATION QUIZ	5/20/2019	
BIOSAFETY 107: CENTRIFUGE SAFETY	BIOSAFETY 107: CENTRIFUGE SAFETY VERIFICATION QUIZ	5/21/2019	
BIOSAFETY REQUIRED TRAINING	BIOSAFETY REQUIRED TRAINING QUIZ	10/21/2018	
CHEMICAL SAFETY: FUME HOOD SAFETY TRAINING	FUME HOOD FINAL QUIZ	5/21/2019	
DISPOSING OF HAZARDOUS CHEMICALS	FINAL QUIZ	12/11/2019	
RISK COMMUNICATION IN ANIMAL FACILTIES	RISK COMMUNICATION IN ANIMAL FACILITIES QUIZ	4/16/2019	
SAFETY FOR PERSONNEL WITH ANIMAL CONTACT	ANIMAL CONTACT PERSONNEL QUIZ	4/25/2019	
STEM CELL ETHICS AND POLICY TRAINING	ASSURANCE	5/20/2019	

Data Effective: Wed Jun 17 9:39:03 2020
Report Generated: Wed Sep 23 09:47:47 2020

Conclusions/action items:

I have completed general biosafety training and training necessary to work with cells.

2020/09/23 Green Permit

Sara Wagers - Sep 23, 2020, 9:46 AM CDT

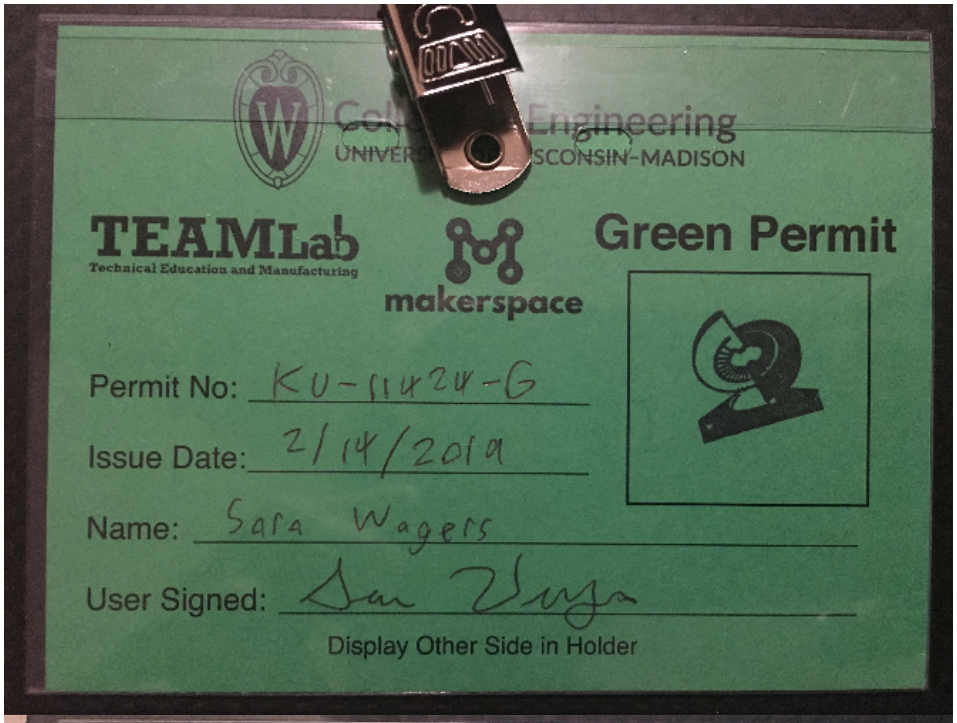
Title: Green Permit Documentation

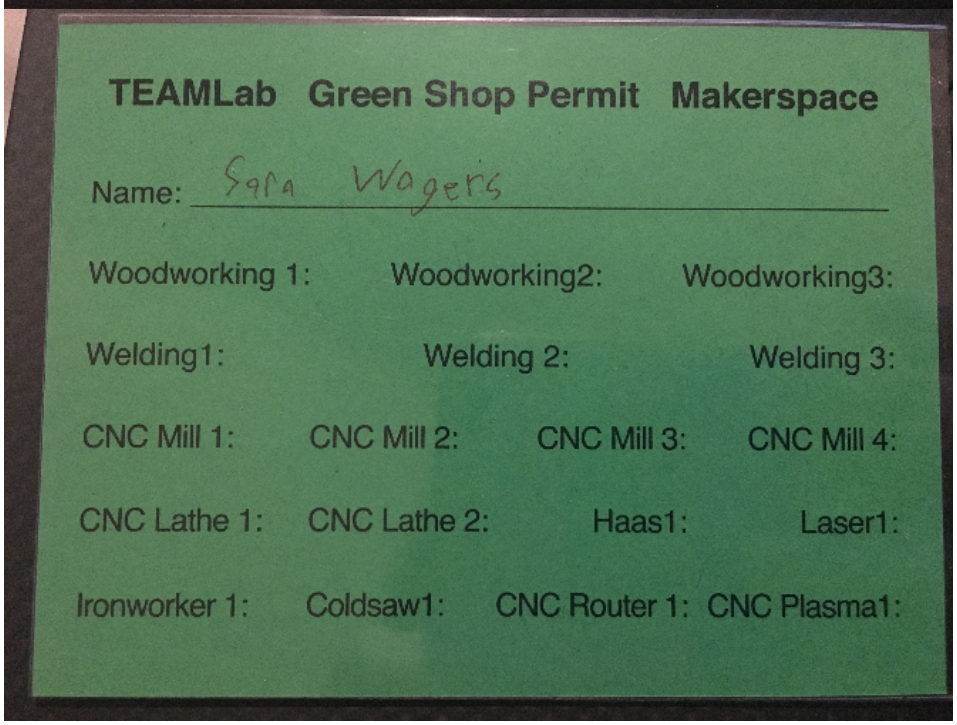
Date: 9/23/20

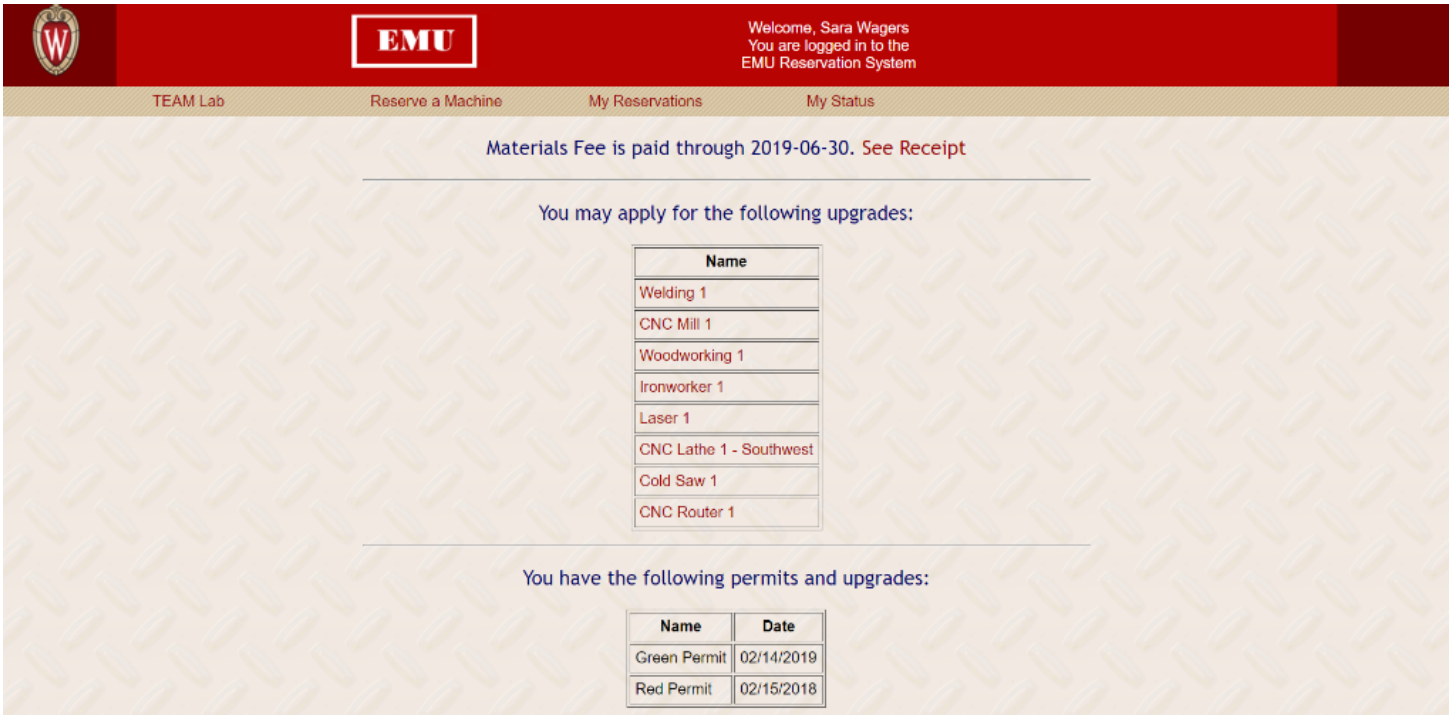
Content by: Sara Wagers

Goals: To document the completion of the green permit

Content:







Conclusions/action items: I have completed the green permit training and will be able to use the tools in the shop to fabricate our device. .

2020/9/12 - Initial presentation

Caleb Heerts - Sep 12, 2020, 12:40 PM CDT

Title: Initial presentation

Date: 9/12/20

Content by: Caleb

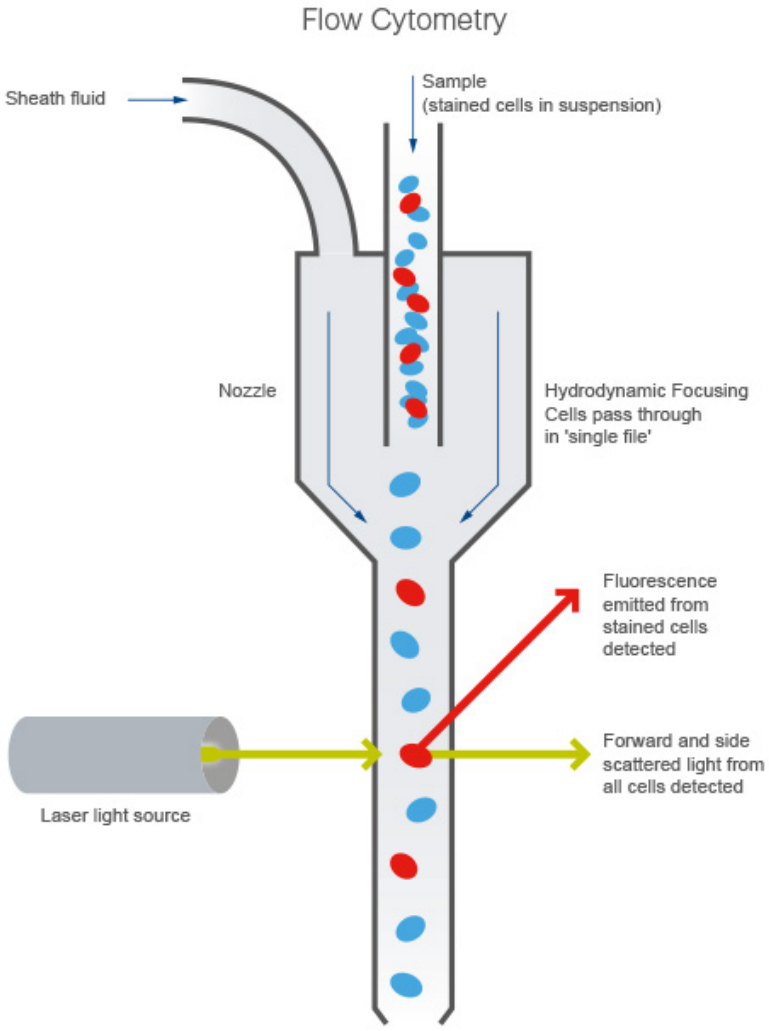
Present: Caleb

Goals: Go over initial presentation info

Content:

https://docs.google.com/presentation/d/18dRaiYMPJV8gdwqXjbExOyJwMrP0juIu/edit#slide=id.p2

- Skala Lab is using Fluorescent Lifetime Imaging (FLIM) to image cellular metabolism on a cell by cell basis and in real time
    - Using light to hit cells and identify the light that passes through/ bounce back
    - Won't need to design this part for the project
- Currently using sheath flow methods for analyzing cells
    - too fast, need the cells to slow down to be analyzed
    - Can't turn down pump as cell flow will stop or become less centralized



    - They've tried a few designs with sheath flow
        - first design had bubbles messing up the flow and it was hard to keep the needle with cells stable
        - Haven't tried the second design
        - need the sheath to be made of either quartz or class so they can image through it
            - not opposed to square or circle sheaths, but need cells to be held fairly in place so they can measure them

- Custom designs from external companies are very expensive -> 4000$ per piece
-

Design Criteria:

Design/Source a flow cell for a flow cytometer given the following constraints:

- Single-file cell flow through interrogation window
- Stable core diameter (20 um to 50 um) (stability in Z more important than X and Y)
- Flow speed of ~1 mm/s, up to 10X faster (at best)
- Flow in PBS (Phosphate-Buffered Saline)
- 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.
- Entire flow cell would have to fit the microscope stage insert.

CFD simulations for various designs
Explore options for sorting cells at the outlet of the flow cell

"We want single-file cells and stable flow through the center of the interrogation region. Z direction confinement (20 um) and stability is more important than XY plane confinement. That is because the pinhole rejects light from outside of that depth window. We can adjust excitation spot to be oblong in XY but we can't do much about Z"

Here's a website they got a quote about a customer flow cytometer from: https://www.fireflysci.com/custom-quartz-flow-cell-manufacturing

Conclusions/action items:

Look up microfluidic literature and see what kind of work/ designs are out there. Get more familiar with the devices

2020/09/12 - Other designs (from Williams)

Caleb Heerts - Sep 12, 2020, 5:11 PM CDT

Title: Other methods besides sheath flow for the ordering of cells

Date: 9/12/20

Content by: Caleb

Goals: Learn about methods to order cells in microfluidic devices

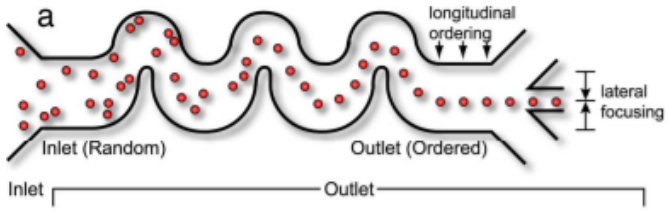
Content:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5505260/

- Flow cytometers are well-established and researched for collecting data and sorting cells
    - good for analyzing intact cells (versus breaking them apart for analysis)
    - Can usually only analyze cells up to 20 microns in diameter
- Measuring NADH (plays a role as an electron carrier and is involved in metabolic pathways such as glycolysis) is used to study cell metabolism
    - Found bound in mitochondria or free in cytoplasm/ mitochondria
- Flow cytometer testing done with "Hydrodynamic focusing is the process by which two fluids under laminar flow and in common containment remain as separate streams based on differences in density, viscosity, the dimensions of the containment vessel, and/or velocity. This process is commonly used in flow cytometry to alter sample stream dimensions within a flow cell without constructing multiple flow cells of different dimensions."
- Need to watch out for shear forces as a result of flow in microfluidics -> could tear the cells apart

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

- Particles under laminar flow follow fluid streamline
- Particle inertia can be used to align the cells through a differently shapped channel

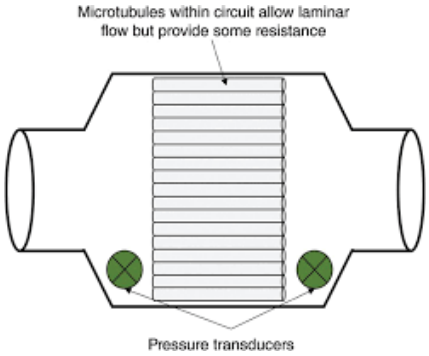


- Faster velocities will create a tighter ordering of the cells (although they will be moving faster)
    - need to watch out for shear forces as the cells speed up as well

Conclusions/action items:

Could we perhaps widen the middle section where the cells are analyzed by splitting it into multiple small compartments (but flow will be reduced overall? Widen the overall area that water is going through, but keep the channel size the same (just allow for multiple channels)

Ex (kind of)


Microtubules within circuit allow laminar flow but provide some resistance
Pressure transducers

2020/09/23 - My design ideas

Caleb Heerts - Se

Title: Other methods besides sheath flow for the ordering of cells

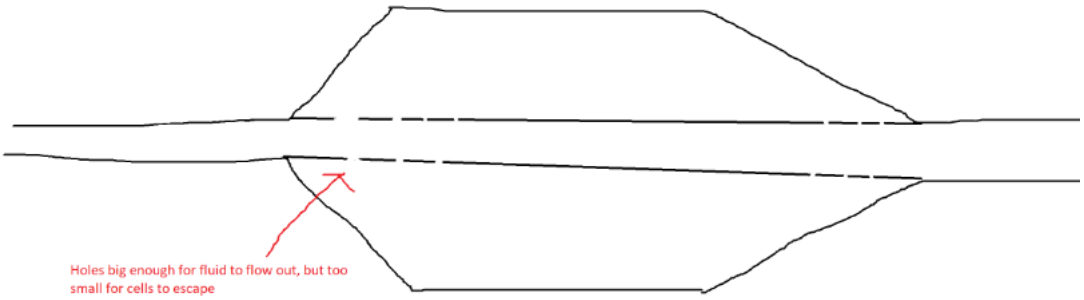
Date: 9/23/20

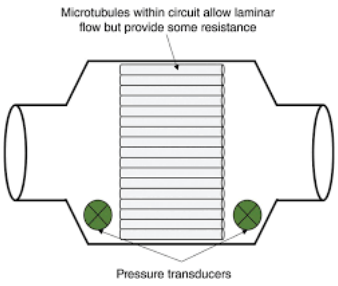
Content by: Caleb

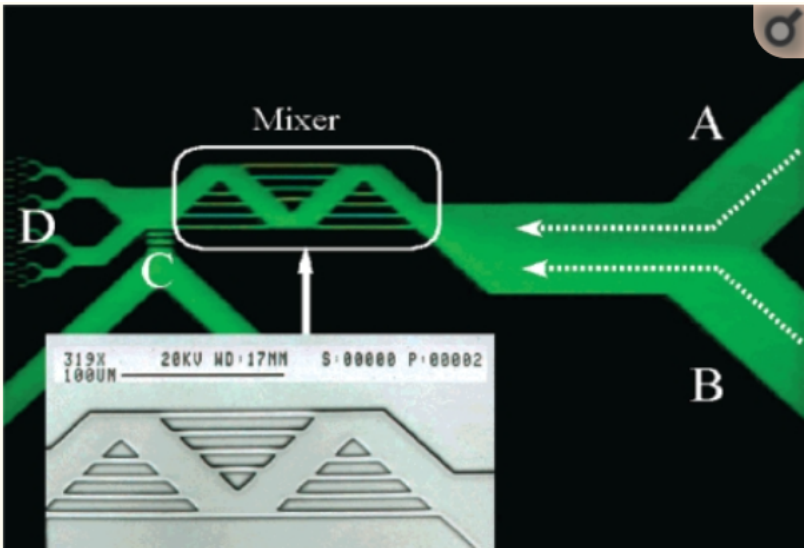
Goals: Brainstorm some ideas for the microfluidic device and constraints

Content:

Design 1:

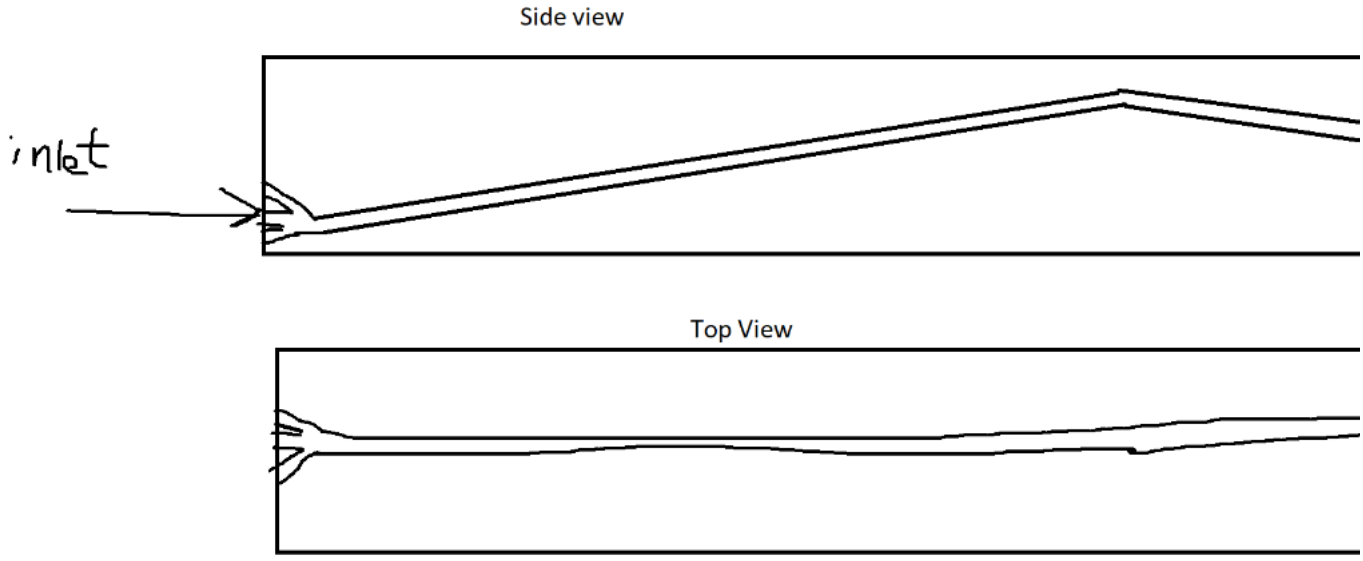


- 

- Similar to this:
- Goal: spread out flow coming in to a greater volume to slow it down, but keep the cells in the same place
- Holes allow for liquid to flow out of main center channel, but cells are stuck in the main center tube for analysis
    - Inner tube could have a fence/ grate like architecture to keep fabrication easier
- See here something similar: 

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3116190/ (specifically cite 62)

Design 2



-
- Use of gravitational forces to slow down fluid as it climbs uphill
  - worry about slowing down too much (lose laminar flow/ potential of backflow?)
- Have to remove some of the excess material off the bottom to give a consistent Geometry for the laser to pass through
  - can we have an angle?
  - Do we have to have the main catheter line exposed for the laser?

Conclusions/action items:

2020/10/04 Microfluidic companies

Caleb Heerts - Oct 16, 2020, 1:26 PM CDT

Title: Microfluidic companies

Date: 10/4/2020

Content by: Caleb

Content:

Elveflow Microfluidics	Our core expertise is premium flow control equipment, we have a broad range of pumps, valves, sensors used for many various applications.	referred us to Darwin Microfluidics
Darwin Microfluidics	offer a wide range of chip solutions in various materials (polycarbonate, COC, COP, etc.).	PMMA may work as a material
Dolomite Microfluidics	we fabricate glass or quartz chip using an isotropic wet etching process. We do not work with PDMS. Custom glass devices are made in wafers of glass that are 100mm x 100mm square in size. These are then processed and fused together to create a complete wafer, but then cut into pieces to make your chips. The cost of the first prototype wafer can vary between $5,000 to $20,000 depending on the design and the processes needed. These costs include the costs of tooling that needs to be made only one time. The cost of making more of exactly the same wafers after this then falls very much. The only materials that we fabricate chips from are glass and quartz.	if you are looking at only one chip, the cost would be quite high. If you need several chips, then it becomes more cost efficient. Note also that we cannot do rectangular or square channels as we use wet-etching with HF technique to create channels in the glass.

Conclusions/action items:

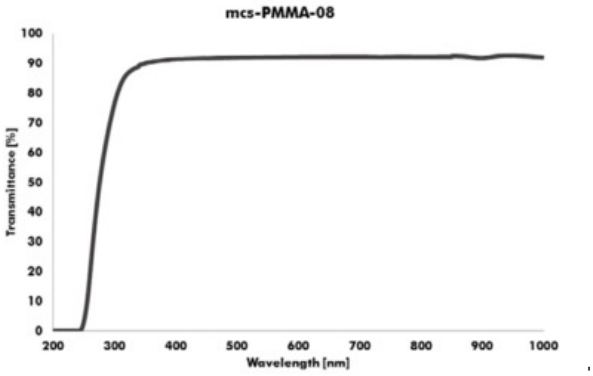
Caleb Heerts - Oct 05, 2020, 3:52 PM CDT

INFORMATION SHEET

Title	Glass chip design guide

Dolomite_Microfluidics_Manufacturing.pdf(913.7 KB) - download

Caleb Heerts - Oct 07, 2020, 12:11 PM CDT



transmittance_of_pmma.png(77 KB) - download

2020/10/5 Microfluidic sorting (Inertial Sorting)

Caleb Heerts - Oct 12, 2020, 4:52 PM CDT

Title: Microfluidic Sorting techniques

Date: see above

Content by: Caleb H

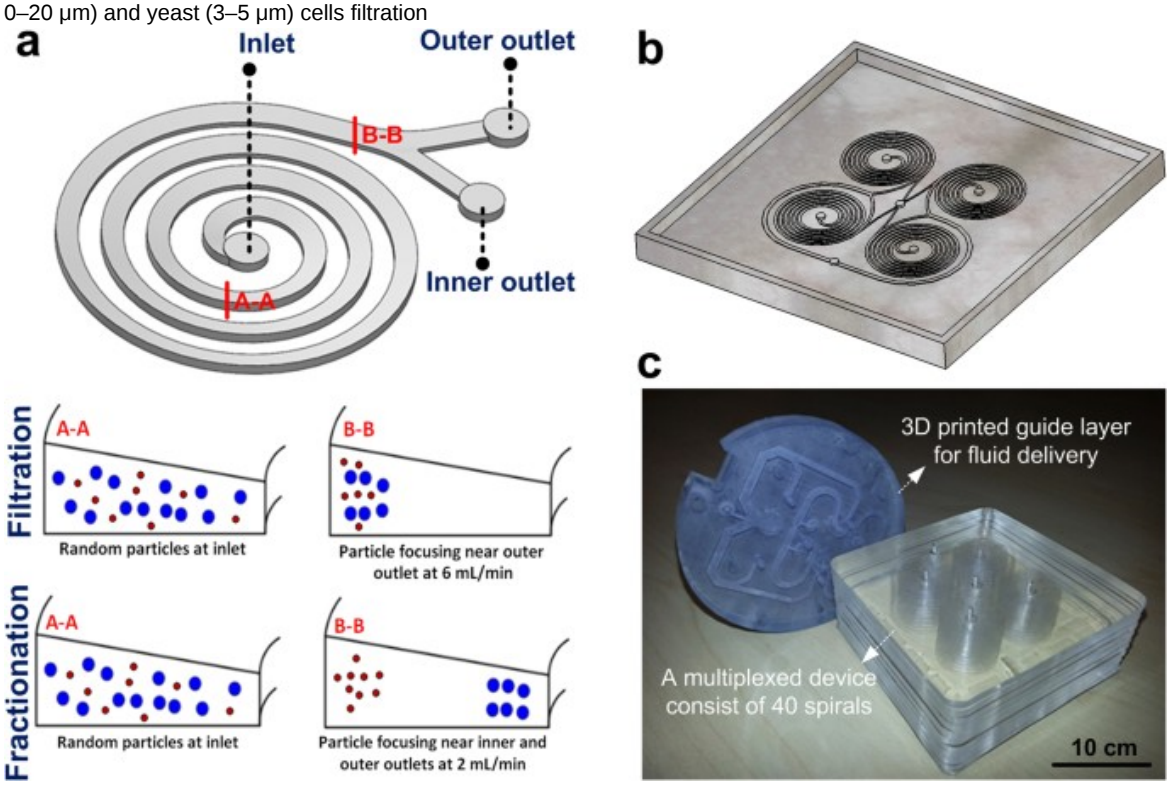
Goals:

Content:

https://www.elveflow.com/microfluidic-reviews/microfluidics-for-cell-biology/label-free-microfluidic-cell-separation-and-sorting-techniques-a-review/ (many options here)

https://www.nature.com/articles/srep11018 (inertial size sorting)

- 500mL/min for the stacked channels
- 0–20 μm) and yeast (3–5 μm) cells filtration



- Spiral filtration method
- Used PDMS as a material
- The spiral device used in this study for mammalian cell retention and fractionation was an 8-loop spiral microchannel with one inlet and two outlets with radius increasing from 8 mm to 24 mm for efficient cell migration and focusing

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

- Square channels used
- 9-micro m-diameter particles in a square channel (50 micro m)
- . The equilibrium position for particles is ~9 micron from the channel edge

https://www.biorxiv.org/content/10.1101/862227v2.full

- Used soft lithography with PDMS and a mylar mask
-

Conclusions/action items:

11/5/20 - Snake Design - SolidWorks

Title: Snake Design Solidworks and Analysis pictures

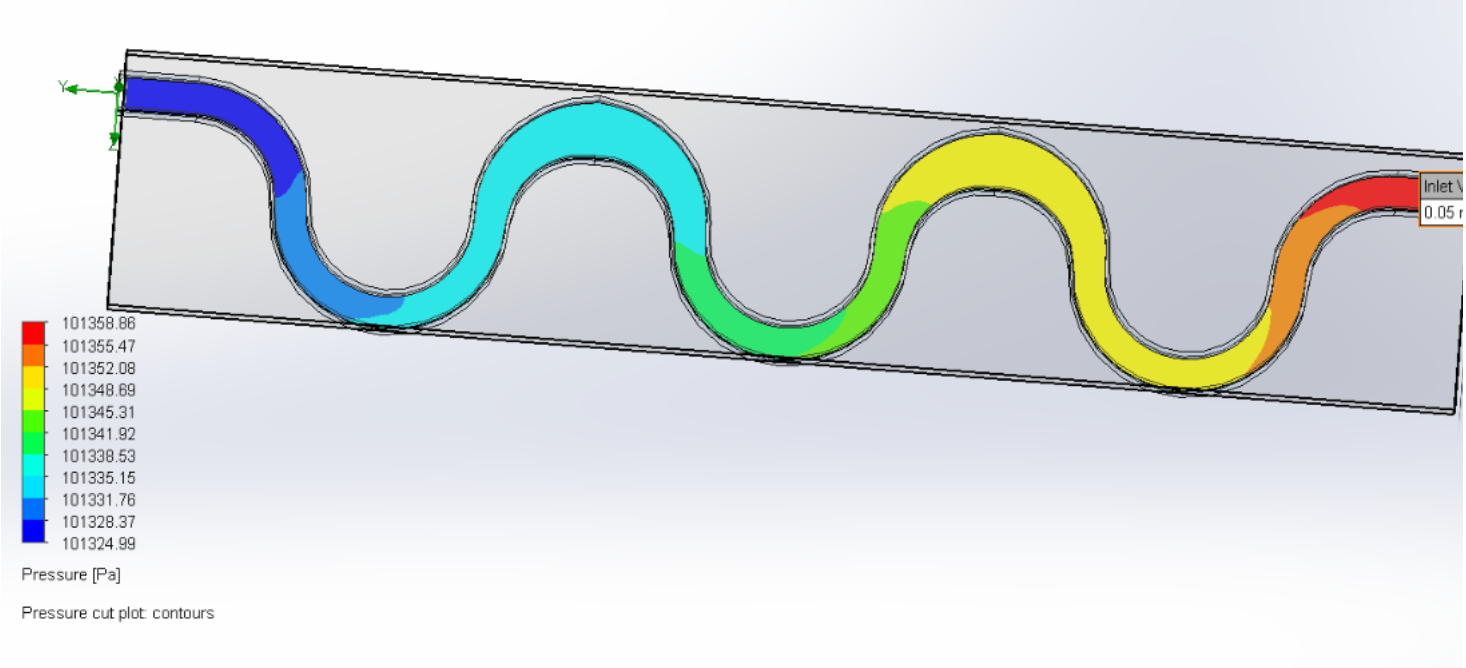
Date: 11/5

Content by: Caleb

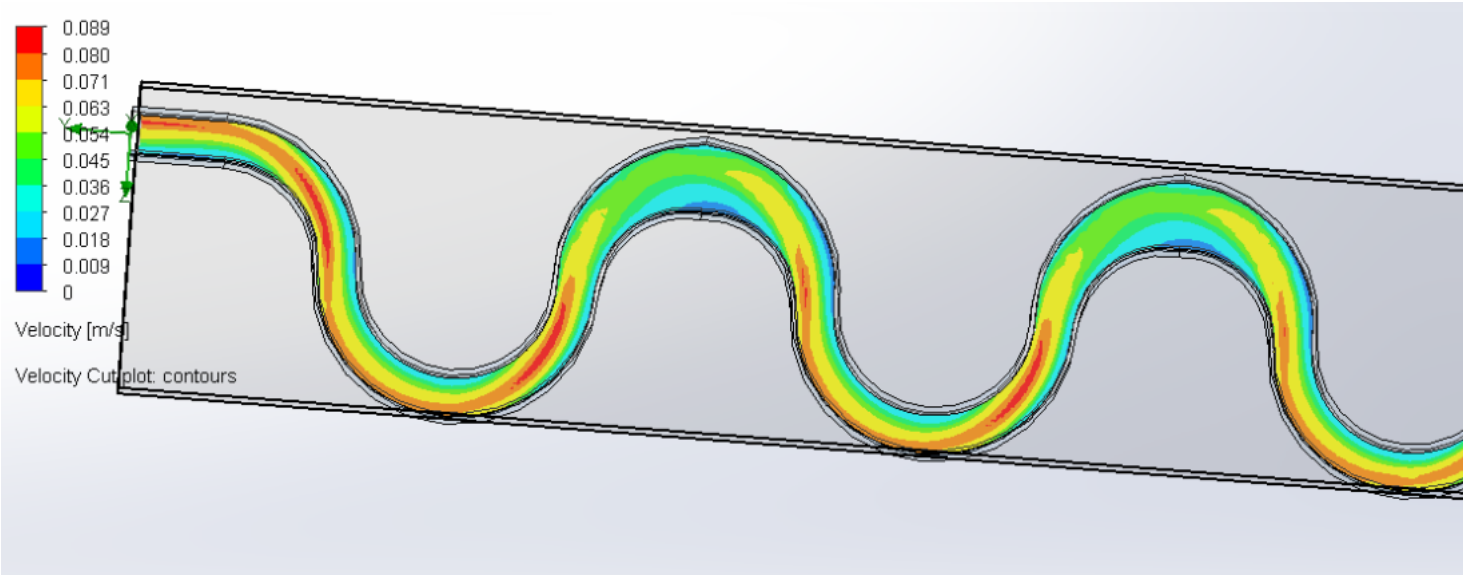
Goals: Look at the snake design and analyze the fluid flow

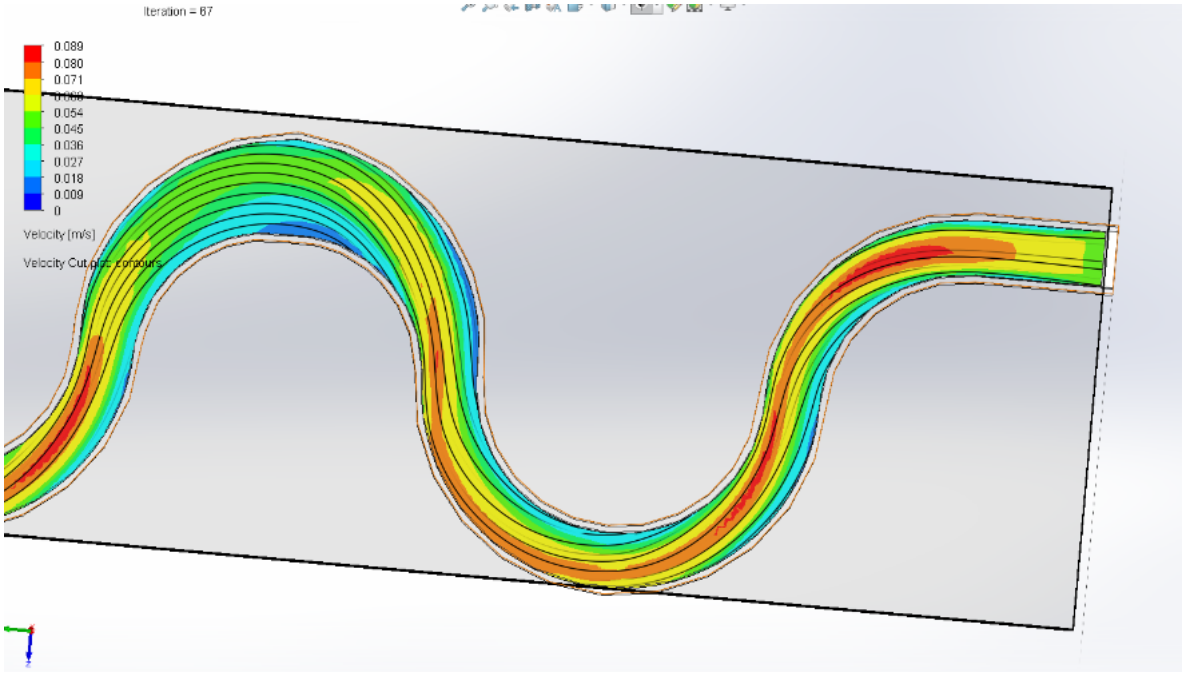
Content:

Pressure Cut Plot:



Velocity Cut Plot:

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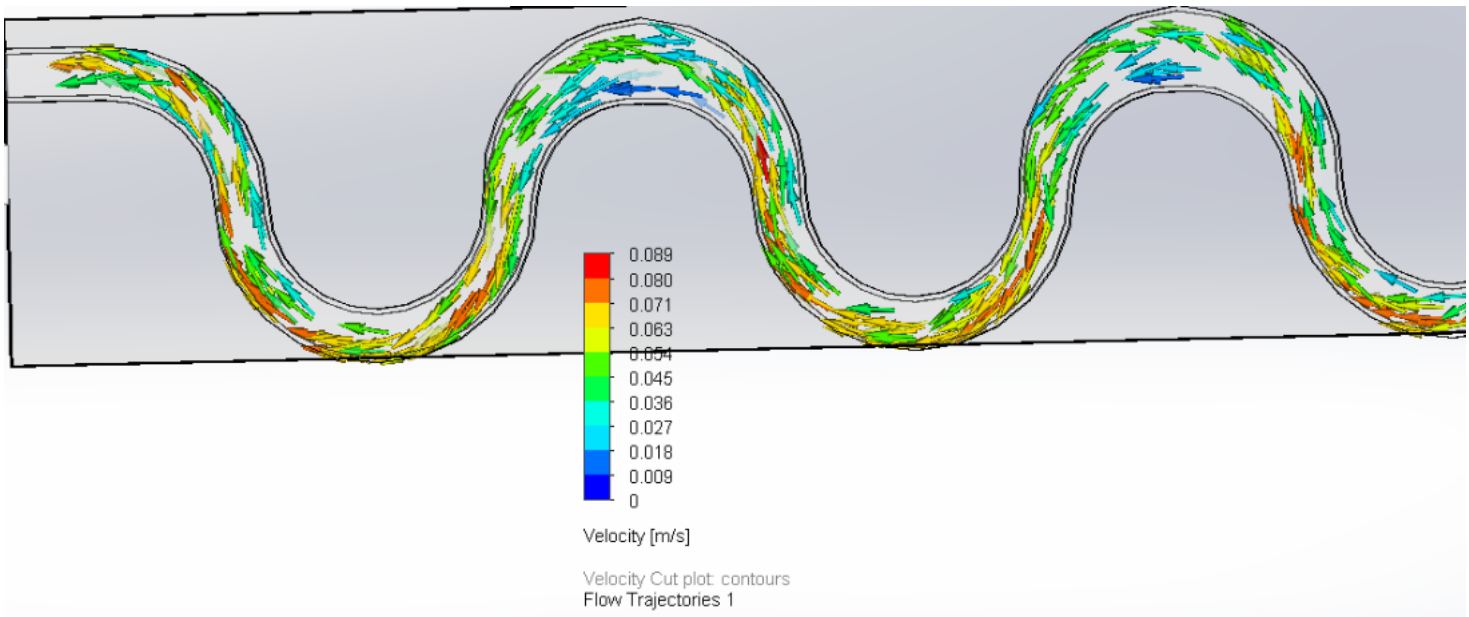
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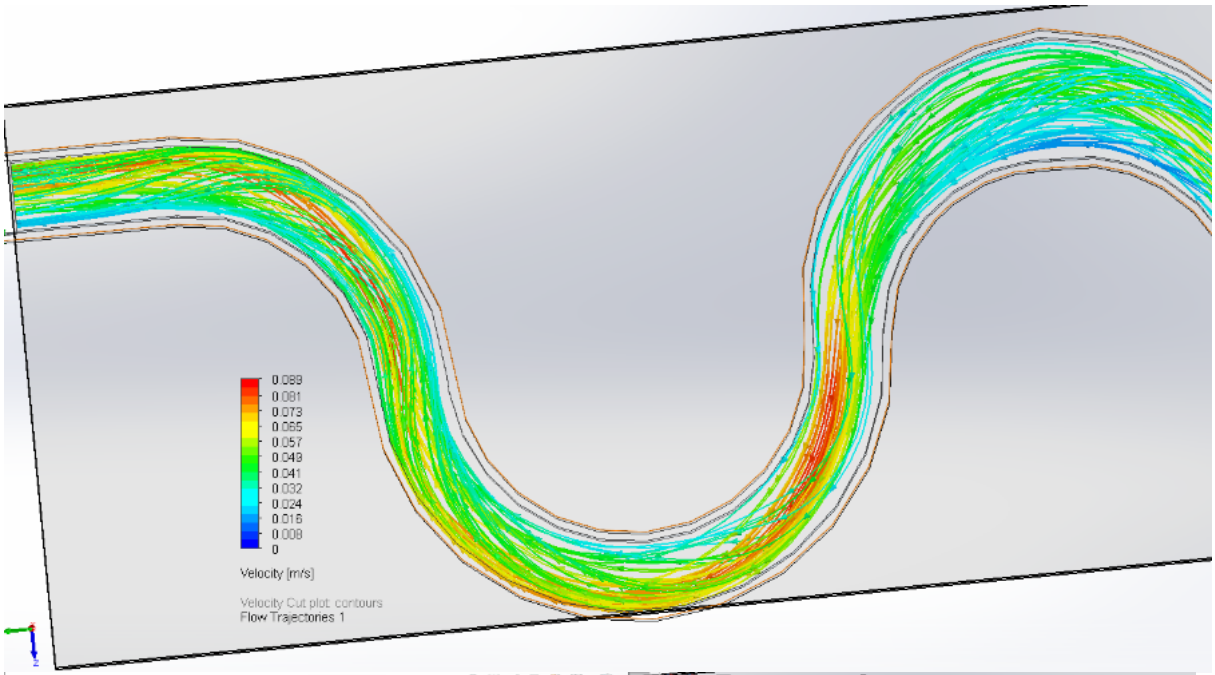
Velocity Cut plot: contours
Flow Trajectories 1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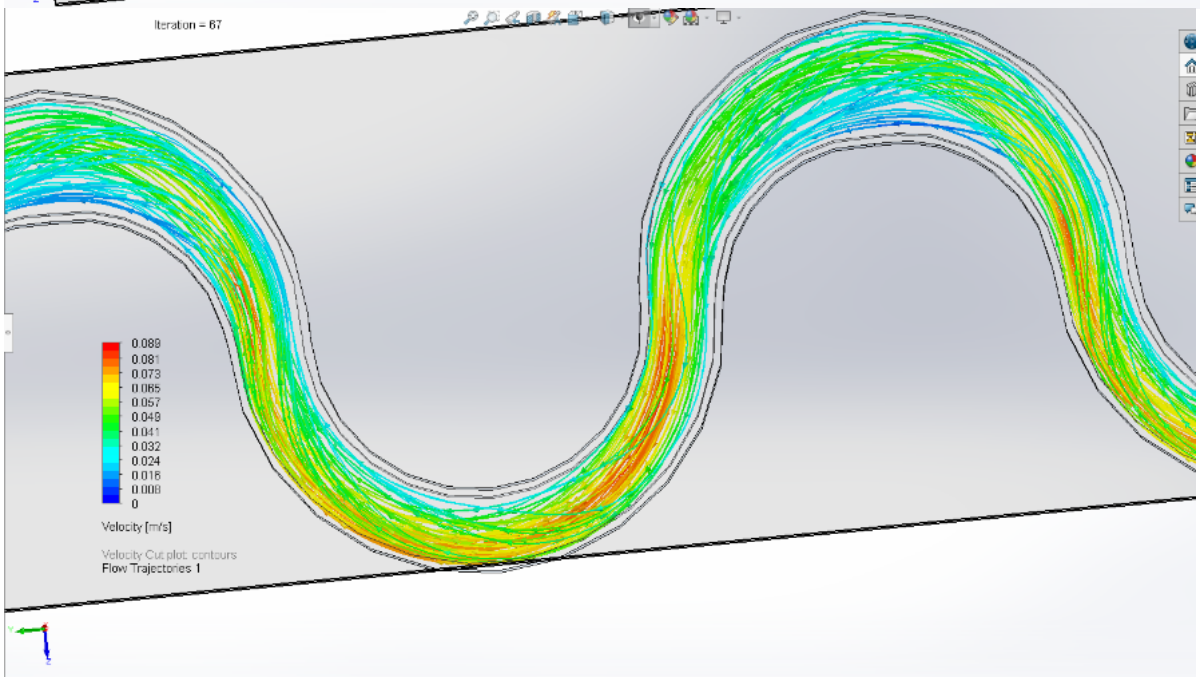
Flow Trajectories 1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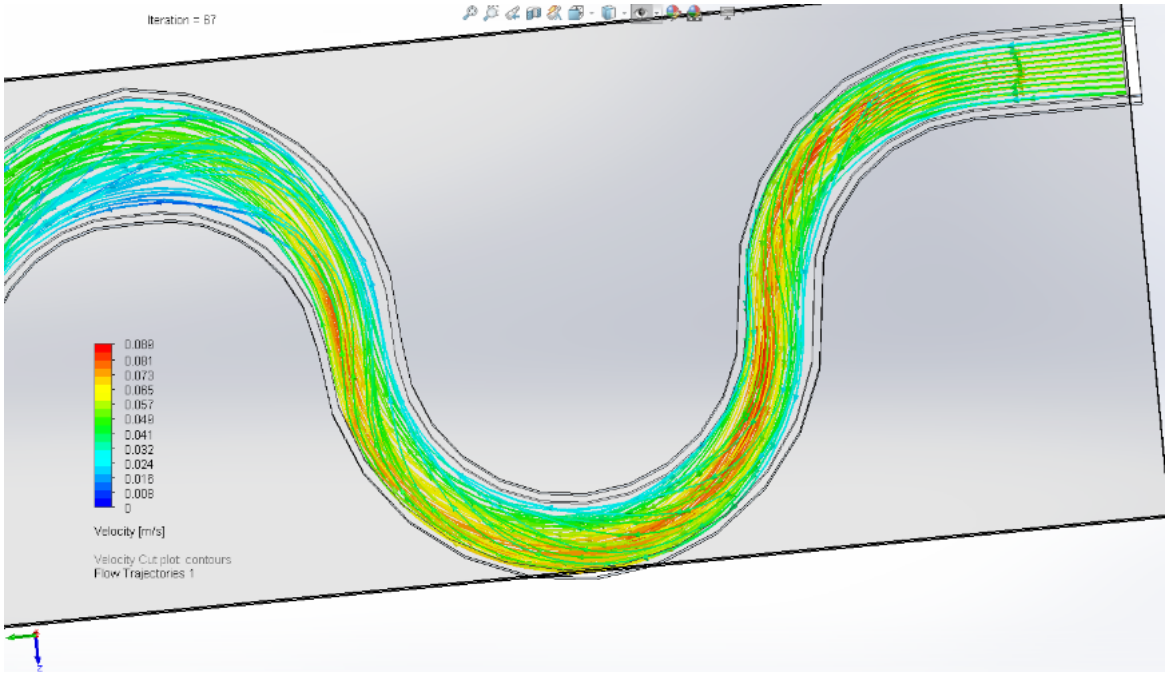
Velocity [m/s]
Velocity Cut plot: contours
Flow Trajectories 1



Conclusions/action items:

Caleb Heerts - Nov 05, 2020, 8:46 PM CST

Snake.sldprt(198.3 KB) - download

Caleb Heerts - Nov 05, 2020, 8:46 PM CST

Snake_project_folders.html(543 Bytes) - download

12/5/20 - Snake Designs - SolidWorks

Caleb Heerts - Dec 09, 2020, 12:40 PM CST

Title: Snake Design Solidworks and Analysis pictures

Date: 11/5

Content by: Caleb

Goals: included are the other snake designs

Content:

Conclusions/action items:

Caleb Heerts - Dec 09, 2020, 12:40 PM CST

Snake_2.SLDPRT(96.5 KB) - download

Caleb Heerts - Dec 09, 2020, 12:40 PM CST

Snake_4.SLDPRT(168.6 KB) - download

Caleb Heerts - Dec 09, 2020, 12:40 PM CST

Snake.sldprt(167.4 KB) - download

Caleb Heerts - Dec 09, 2020, 12:40 PM CST

Snake_project_folders.html(543 Bytes) - download

Caleb Heerts - Dec 09, 2020, 12:40 PM CST

Snake3.SLDPRT(260.6 KB) - download

Caleb Heerts - Dec 09, 2020, 12:40 PM CST

Snake3_project_folders.html(538 Bytes) - download

2020/10/04 Biosafety Training

Caleb H

Title: Biosafety Training Documentation

Date: 10/4/2020

Content by: Caleb H

Goals: To verify the completion of the biosafety training course

Content:

University of Wisconsin-Madison

This certifies that CALEB HEERTS has completed training for the following course(s):

Course Name	Curriculum or Quiz Name	Completion Date	Expir
Biosafety Required Training	Biosafety Required Training Quiz	3/16/2019	
Chemical Safety : Hazard Communication with Globally Harmonized System (GHS)	Hazard Communication with GHS Update Quiz	9/10/2017	

Data Effective: Sat Mar 16 15:58:39 2019
Report Generated: Wed Oct 9 01:39:35 2019

Conclusions/action items: I have completed biosafety training to be able to work in the lab for testing and development of our prototype.

2020/10/04 Green Pass Permit

Caleb Heerts - Oct 04, 2020, 9:23 PM CDT

Title: Green Pass Documentation

Date: 10/4/2020

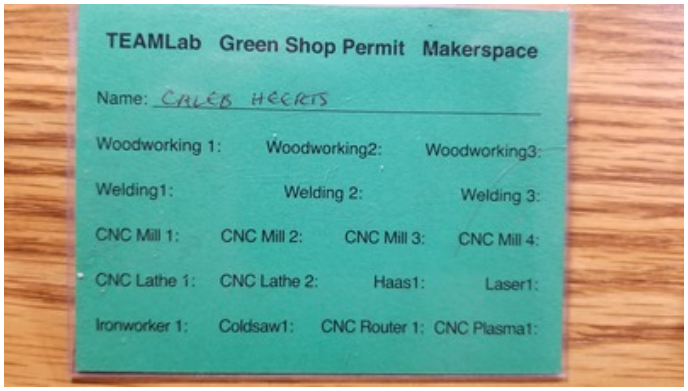
Content by: Caleb H

Goals: To document the completion of the Green Permit

Content:

Conclusions/action items: I have completed the Green Pass to be able to work in the lab for testing and development of our prototype.

Caleb Heerts - Oct 09, 2019, 1:49 AM CDT



greenpass.png(14.9 MB) - download

Caleb Heerts - Oct 09, 2019, 1:50 AM CDT

greenpass2.png(18.8 MB) - download

10/05/2020 - Multiphoton Flow Cytometry to Assess Intrinsic and Extrinsic Fluorescence in Cellular Aggregates: Applications to Stem Cells

Hunter Hefti - Oct 06, 2020, 10:06 AM CDT

Title: Multiphoton Flow Cytometry to Assess Intrinsic and Extrinsic Fluorescence in Cellular Aggregates: Applications to Stem Cells

Date: 10/05/2020

Content by: Hunter Hefti

Present: N/A

Goals: Summarize findings regarding flow cytometry

Content:

The article describes a process of flow cytometry that is quite similar to the process described by the Skala lab. NADH and FAD are both used for detection by a laser. In all likelyhood, this similiarity is due to the heavy involvement of UW's department of Biomedical Engineering as both our advisor and a few notable professors in the program were involved in the creation of this paper. Details of a "novel" (2010) system of multiphotonic detection of fluorescent characterization with a cell sample are described alongside methods for the program. The main focus is not on the design of the flow cell but rather the process of cytometry whereby a laser is shown through cells and a program utilizes the resulting optical feedback to make characterizations of these cells.

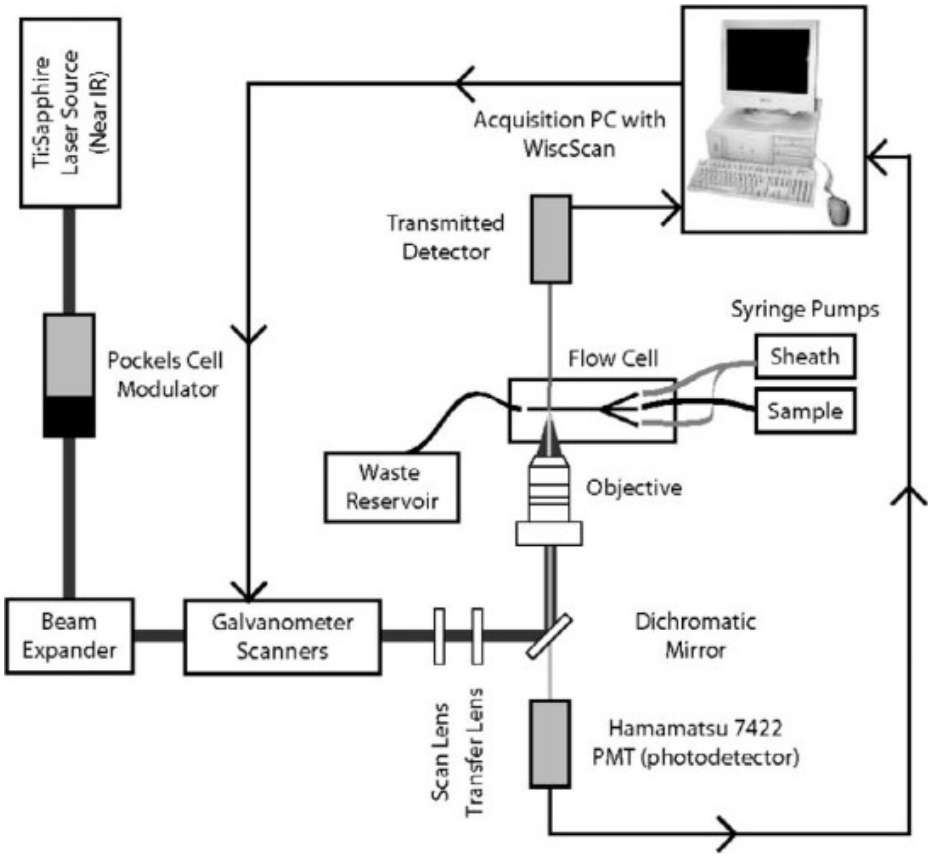


Figure 1. A diagram displaying the fuction of a typical flow cytometry system

Conclusions/action items:

This type of program is likely what eventually derived the one that Skala lab uses for their cell sorting program. Coming back to this article will be useful to make determinatios about flow cells and flow cytometry.

D. G. Buschke, J. M. Squirrell, H. Ansari, M. A. Smith, C. T. Rueden, J. C. Williams, G. E. Lyons, T. J. Kamp, K. W. Eliceiri, and B. M. Ogle, “Multiphoton Flow Cytometry to Assess Intrinsic and Extrinsic Fluorescence in Cellular Aggregates: Applications to Stem Cells,” *Microscopy and Microanalysis*, vol. 17, no. 4, pp. 540–554, 2010.

10/06/2020 - Guidelines for the use of flow cytometry and cell sorting in immunological studies

Hunter Hefti - Oct 06, 2020, 12:38 PM CDT

Title: Guidelines for the use of flow cytometry and cell sorting in immunological studies

Date: 10/06/2020

Content by: Hunter Hefti

Present: N/A

Goals: Summarize findings that regard the use of flow cytometry and cell sorting

Content:

This article is an extremely long description of the guidelines that should be utilized when making a flow cytometer and when carrying out experiments regarding the marriage between flow cytometry and cell sorting. The article goes into a lot of depth into the materials that should be used for manufacturing these objects, the types of experiments that can be carried out, a few diagrams that depict the work flow of the cytometer. A few pictures are included that demonstrate the amount of work that went into making this comprehensive review of the system.

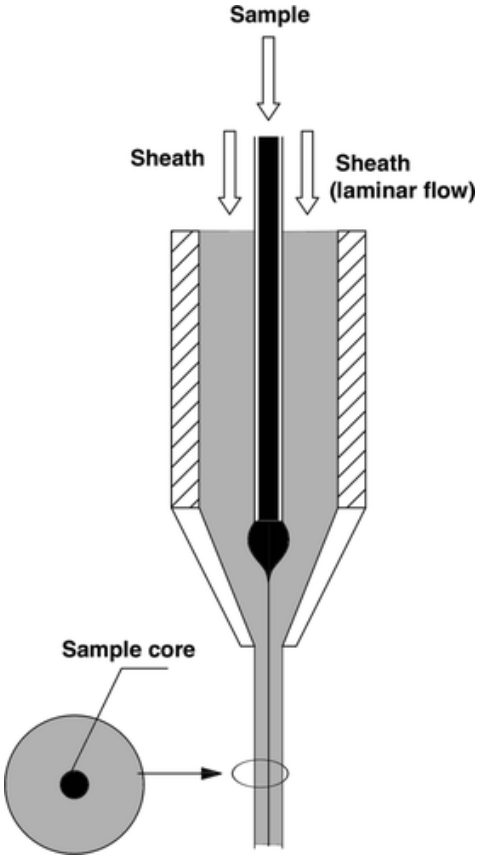


Figure 1. A simple rendering of a type of cell inlet utilizing sheath flow

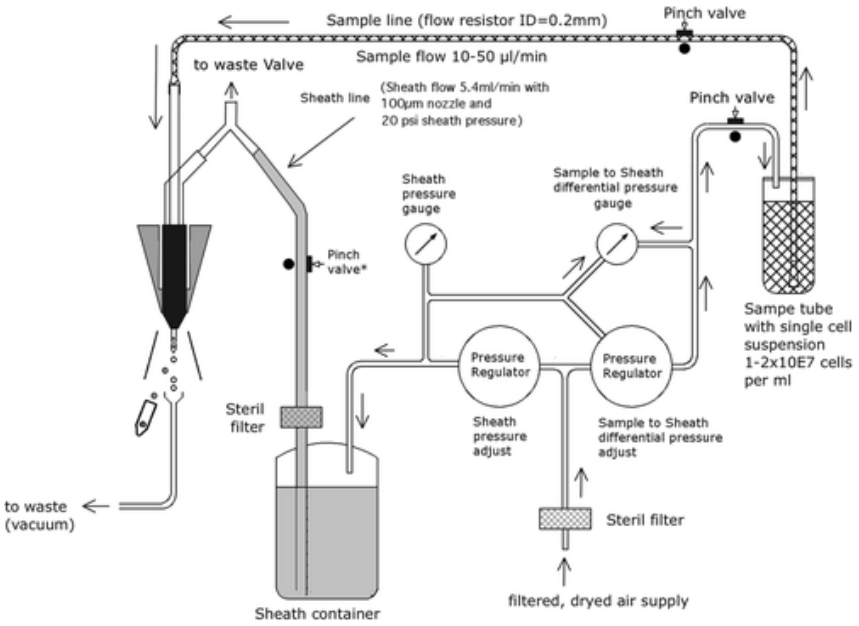


Figure 2. A schematic of a set up of valves and pumps that would be used to regulate the flow within the cell sorter

Other suggestions laid down by the article include considerations of buffer solutions, the types of media that various cells can interact with, and solution instructions for sheath fluids and other various types of fluid mechanic concepts that would be useful to research further.

Conclusions/action items:

Not every detail from the article could possibly have been summarized so this resource should be reflected on frequently when during the project when deciding if certain standards are being met.

https://onlinelibrary.wiley.com/doi/full/10.1002/eji.201646632

10/06/2020 - Fluidics

Hunter Hefti - Oct 06, 2020, 12:46 PM CDT

Title: Fluidics

Date: 10/06/2020

Content by: Hunter Hefti

Present: N/A

Goals: Describe basics of fluidics using paper

Content:

Fluidics is a paper that seeks to summarize various methods for utilizing flow cytometry and cell sorting to develop a method for label free cell differentiation. This paper is extremely comprehensive and, similar to the last paper, will not be summarized in full. The most interesting and relevant topic is the mention of the inertial ordering method which was developed and described in another entry of this notebook. Various other papers are listed which attempt to describe the same system and an explanation for the physics and the "path of least resistance" concept is also provided in more detail than is described by most other papers. Overall, this should be used as a reference resource when coming up with designs or alterations to the design as much of what has been already tested in this field is described or linked by this article.

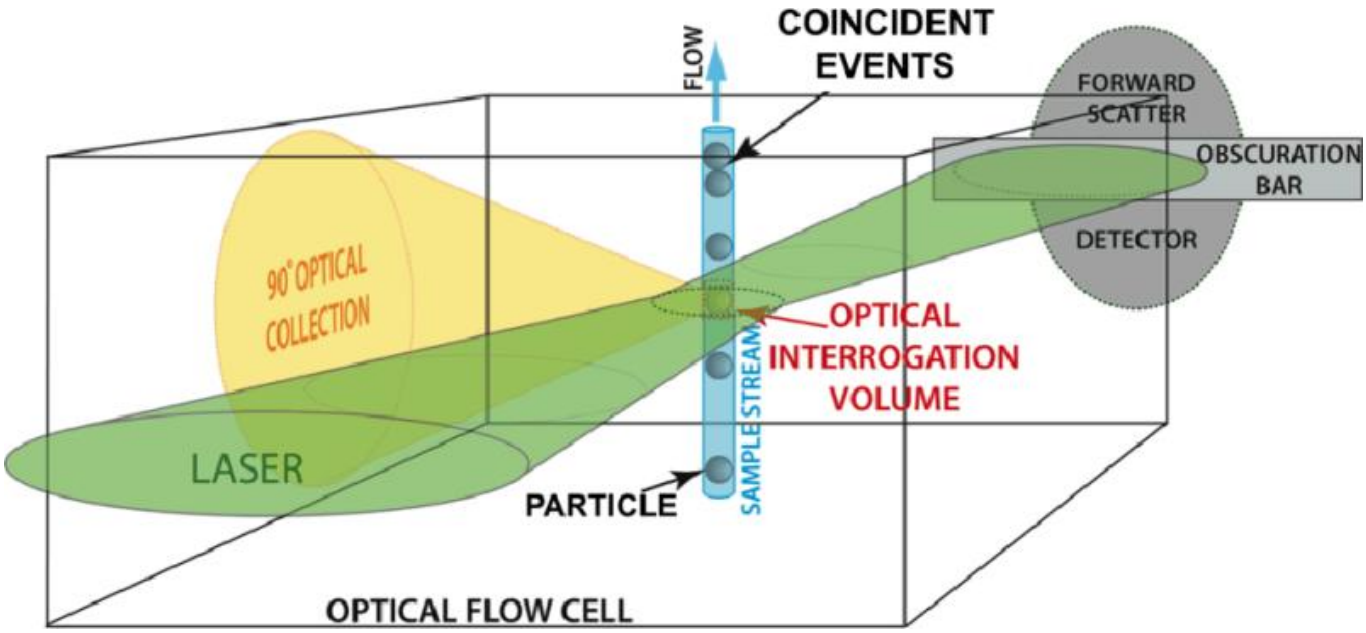


Figure 1. A type of laser optical flow cytometry in which forward and side scatter are analyzed to determine the type of cell and the contents of the cell for future sorting

Conclusions/action items:

This is another useful paper that includes articles that would be worth researching well into the future. Fluidics describes some basic mechanics of the fluid flow system that is central to cytometry and cell sorting and should be considered when reviewing the success of our eventual design. An article by Hurl should be further researched and includes further information on intertial cell ordering.

P. P. A. Suthanthiraraj and S. W. Graves, “Fluidics,” *Current Protocols in Cytometry*, vol. 65, no. 1, 2013.

12/06/2020 - Reynolds Number

Hunter Hefti - Dec 06, 2020, 10:01 PM CST

Title: Reynolds Number and it's use in the Snake Design

Date: 12/06/2020

Content by: Hunter Hefti

Present: N/A

Goals: Explain the use of Reynolds number

Content:

$$R_c = \frac{U_m D_h}{\nu} \qquad [1]$$

and

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

Equation 1 details the Reynold's number associated with the channel while Equation 2 details the particle Reynold's number. The latter of these is the most integral to the design of the snake. The kinematic viscosity of water is $1.787*10^{-6}$ m^2 s^{-1} while the velocity of the fluid was 0.05m^2/s. Ultimately, the best particle Reynold's number turned out to be 0-0.2 according to one of our main sources. With this in mind, our client drew up a reasonable particle diameter of 10um and a channel diameter of 50um. Ideally, the result should be more centered.

Conclusions/action items:

The continued use of these calculations will be critical.

10/04/2020 - Continuous inertial focusing, ordering, and separation of particles in microchannels

Hunter Hefti - Oct 04, 2020, 4:52 PM CDT

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

Date: 10/04/2020

Content by: Hunter Hefti

Present: N/A

Goals: Summarize parts of the paper that provide inspiration for the snake design

Content:

The main portion of the article focuses on the testing of an inertial ordering system in which flow orients itself central to the channel across the channel and lengthwise. A principle of centripetal forces with respect to curvature in a pipe can be described by the deans number. When a certain amount of symettrical curves are introduced, turbulent flow allows for the centering of stream lines to two separate flows with focusing becomin more complex as Deans number is increased. The asymetric flow system focuses the channel better twoards a central streamline. Expermentation with particules of 10um was induced to observe this effect and is shown in the figure below.

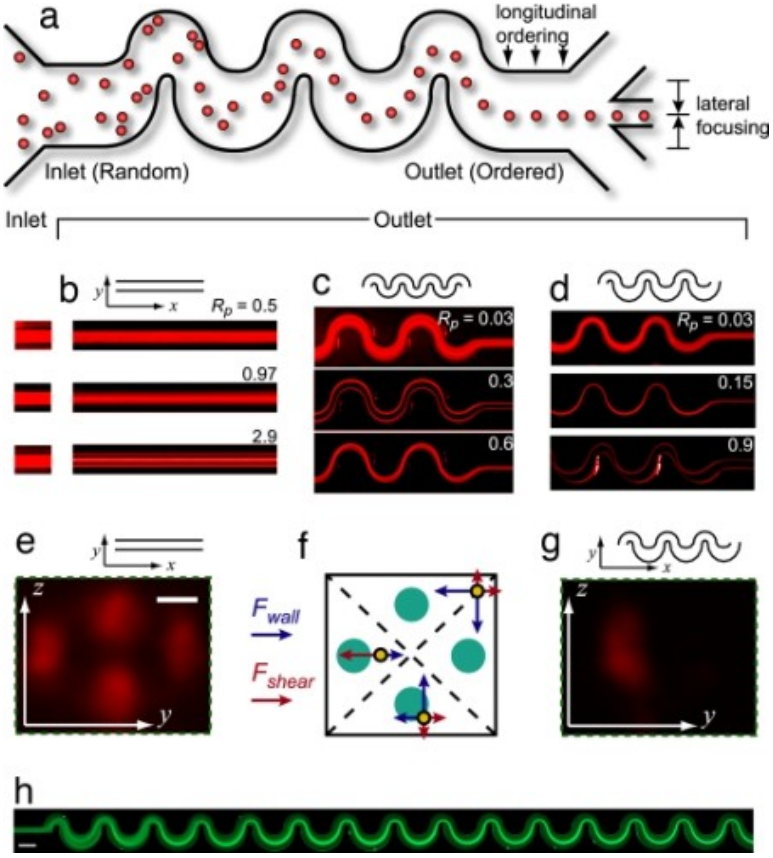


Figure 1. A compartive image of a rectangular channel, a symmetric curve design, and an asymmetric curve design as they focus cells down a channel.

The second figure included in this entry reflects the results of the experimentation with cell centering and compares the asymmetric design with a typcial flow through a rectangular channel. Results are observably different and reflect a positive aspect of hte inertial lift principle that is involved in straightening out the cells.

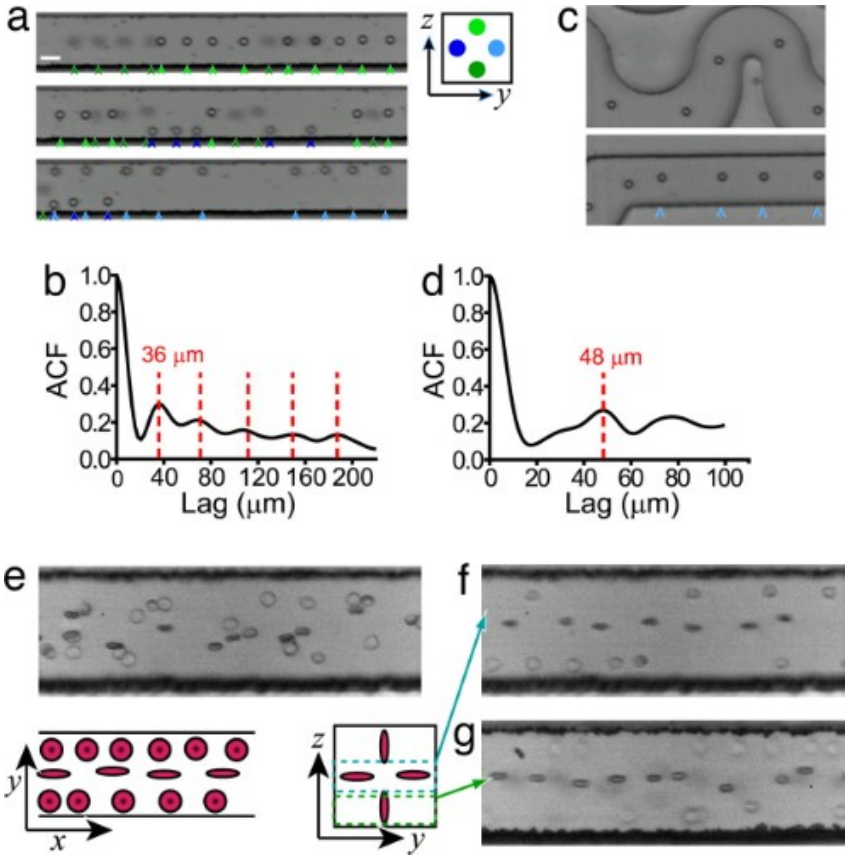


Figure 2. A comparision between the left's rectangular channel and the right's asymmetrical curve channel. Bottom images demonstrate the 10um cells as they pass through the outlet channel.

SU-8 master molds were generated using soft-lithography techniques followed by a PDMS pour which formed the inlet and outlet portions as well as the surrounding channel. The resulting device was plasma bonded to a glass slide for viewing under a microscope.

Conclusions/action items:

This experimentation is heavily noteworthy for functiionally centering the cells one at a time. Flow speed is unaccounted for, but the ability for Skala lab to vary flow velocities using the pump might imply an advantage towards this sort of design to aid in the focusing of cells down the channel.

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.

10/04/2020 - Intelligent image-based deformation-assisted cell sorting with molecular specificity

Hunter Hefti - Oct 06, 2020, 12:49 PM CDT

Title: Intelligent image-based deformation-assisted cell sorting with molecular specificity

Date: 10/04/2020

Content by: Hunter Hefti

Present: N/A

Goals: Review paper posted on slack channel regarding design with curving channels

Content:

The paper aims to find a method of label-free cell sorting utilizing real-time fluorescence and deformable cytometry with sorting based on standing surface acoustic waves and transfer molecular specificity to image-based sorting using an efficient deep neural network. This method resulted in the creation of the soRT-FDC chip as pictured below. Sheath fluid in this model did not enter to transport the sample blood cells down the view channel until after the sample had passed through a serpintining channel of curves that are intended to focus the cells longitudinally. Once the sheath fluid contacted the focused cells, the flow runs down a 20-µm- or 30-µm-wide and 880-µm-long channel where the observations are taken. The soRT-FDC chip consists of a polydimethylsiloxane (PDMS) replica bonded to a lithium niobate substrate with chromium-gold IDTs deposited on its surface. PDMS replicas were fabricated according to well-established soft-lithography processes.

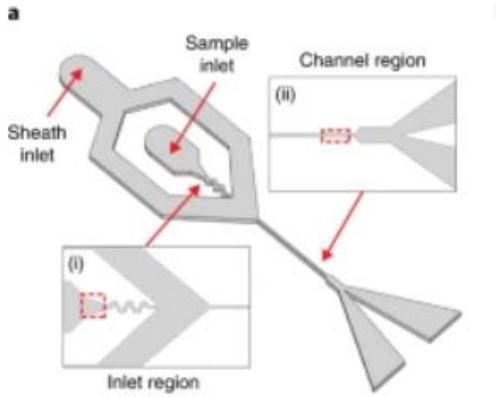


Figure 1. The soRT-FDC flow cytometer chip with the inlet and channel regions highlighted

Conclusions/action items:

The inclusion of the curved cell inlet portion which is designed to focus cells longitudinally was mentioned frequently by our advisor, Justin Williams, who suggested that randomized properties of particle movement would restrict cells to the path of least resistance. Adding sheath fluid prior to the serpentine may focus the cells in a way that the Skala lab would desire and would be useful to model.

A. A. Nawaz *et al.*, "Intelligent image-based deformation-assisted cell sorting with molecular specificity," *Nature Methods,* vol. 17, no. 6, pp. 595-+, Jun 2020, doi: 10.1038/s41592-020-0831-y.

10/05/2020 - Continuous Label-Free Electronic Discrimination of T Cells by Activation State

Hunter Hefti - Oct 06, 2020, 12:05 PM CDT

Title: Continuous Label-Free Electronic Discrimination of T Cells by Activation State

Date: 10/05/2020

Content by: Hunter Hefti

Present: N/A

Goals: Inquire into a recent paper that appears to accomplish something similar to our project.

Content:

This paper describes the function of a device that utilizes dielectrophoresis to separate particles by size and label. A method is employed which marks cells based on the type and size of T-cell that the researchers wish to sort by. Cell separation is achieved by the ability for the DEP method to seaparate from activated and naive T-cell types in which a channel allows passage of cells through the DEP objective window and separated cells are ushered through a coulter counter to determine the number of separated cells that have been differentiated from the back.

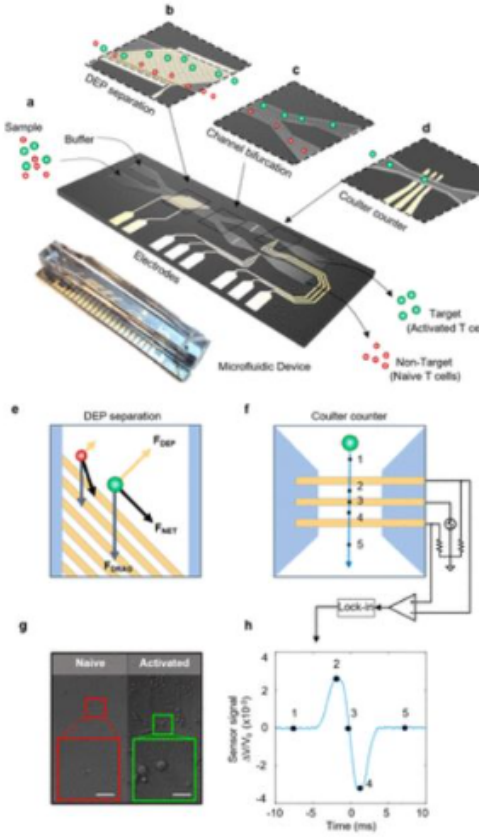


Figure 1. Display of the set up of the device that was created for the purposes of label-free cell sorting

It was maufactured using SU-8 photoresist and silicon wafers to procure a master mold. Following this, the basic structure was cured utilizing PDMS which was thinly spread across the wafer and allowed to sit overnight.The PDMS was then plasma bonded to a silicon plate which connected to the DEP device that allowed for cell separation.

Conclusions/action items:

While an interesting approach, the Skala lab is attempting to utilize a specific system of cell sorting that involves software usage rather than exploiting properties of cells under DEP. This device does not allow for cellular centering in the channel nor is a viewing objective even necessary. So while extremely interesting and a possible alternative approach to the entire sorting process, our project requires improvements to a system that this does not seek to mimic.

P. Han, S. Yosinski, Z. A. Kobos, R. Chaudhury, J. S. Lee, T. M. Fahmy, and M. A. Reed, “Continuous Label-Free Electronic Discrimination of T Cells by Activation State,” *ACS Nano*, vol. 14, no. 7, pp. 8646–8657, 2020.

10/06/2020 - On chip

Hunter Hefti - Oct 06, 2020, 10:17 AM CDT

Title: On-chip Sort

Date: 10/06/2020

Content by: Hunter Hefti

Present: N/A

Goals: Describe a competing design

Content:

Company that makes this chip advertises it as "damage free" cell sorting which operates a minimal volume of 20ul using a "flow shift" method for control. They report a high sterility and advertise easy storage and clean up with no contact with other devices being a main assumption. Unlike many other typical devices, this one is entirely made up of plastic but can be modified to fit the type of set up that the lab has, with accomadations being made for types of lasers and laser placement as well as guidelines for utilizing different colored lasers to observe different effects on different types of cells.

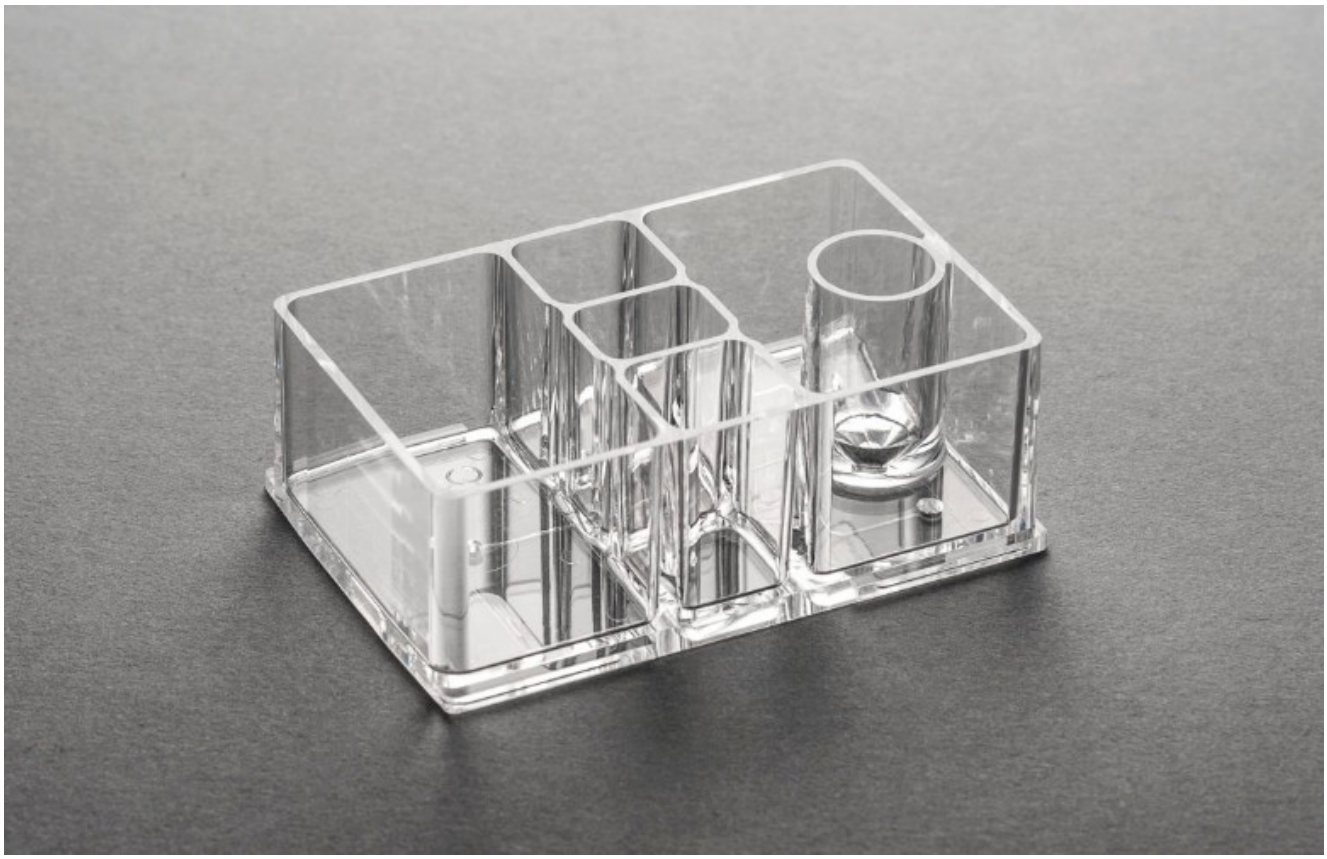


Figure 1. A photo of the on-chip sort design

Conclusions/action items:

This does not show immediate promise as the design does not seem to fully accomodate for everything that the Skala lab currently has set up.

https://on-chipbio.com/product-onchip_sort/

10/06/2020 - Straight Channel Glass Chip

Hunter Hefti - Oct 06, 2020, 10:21 AM CDT

Title: Microfluidic Chip Shop

Date: 10/06/2020

Content by: Hunter Hefti

Present: N/A

Goals: Describe another company that makes microfluidic chips.

Content:

The Microfluidic ChipShop is an online company that specializes in the creation of flow chips that are made to the specifications of the customer. One of their more popular products, the Straight Channel Glass Chip does not directly include a sheath fluid insert or a cellular inlet space that is separate from the rest of the chip, but the company does ensure size quality. In other words, the objective space in which the laser would pass through the chip would render quality optical feedback that would ensure a proper analysis of the cells once they have made it inside of the channel.

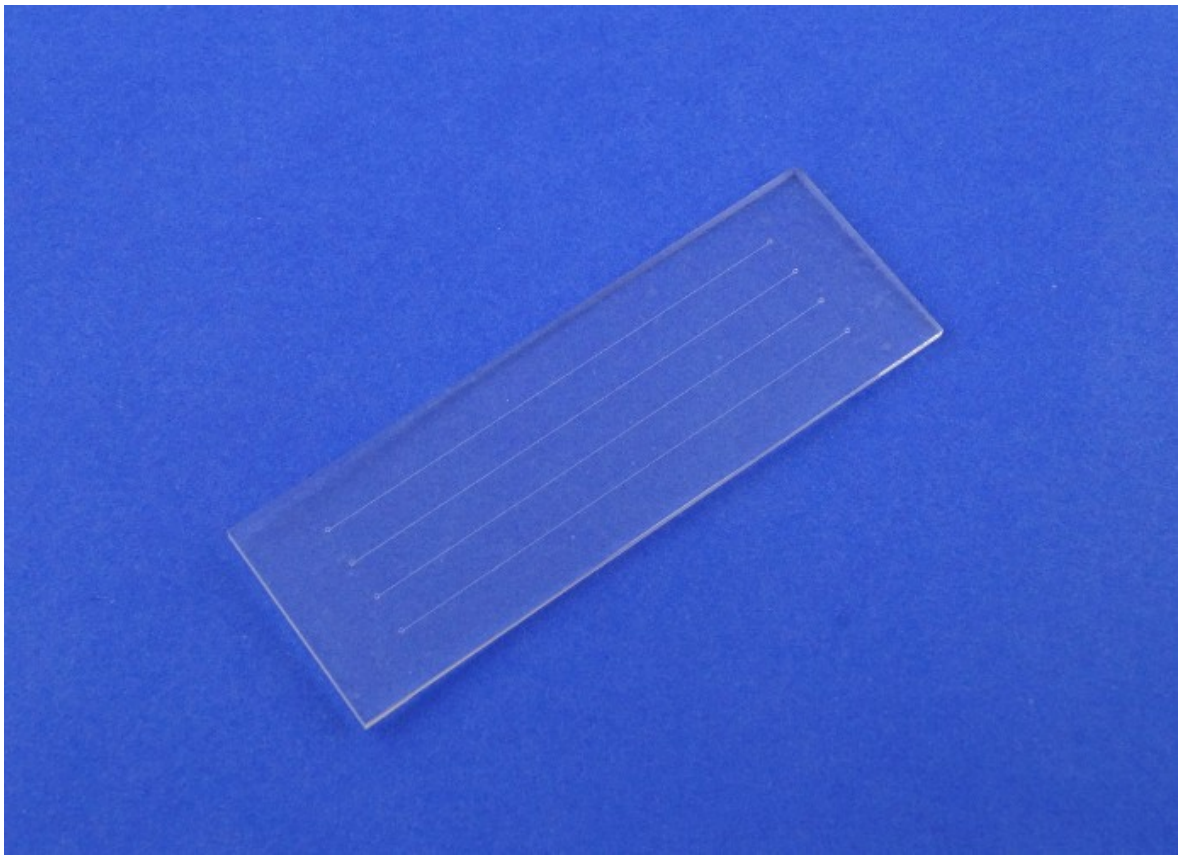


Figure 1. An example of the stright channel chip in which cells enter microtubules and are propelled across by microfluidic properties.

Conclusions/action items:

This design also does not properly take advantage of the Skala lab set up and marks a relatively easy to manufacture and procure design that is not the main focus of this design project.

https://www.microfluidic-chipshop.com/catalogue/microfluidic-chips/glass-chips/straight-channel-chips-glass/

10/04/2020 - Plinko Design

Hunter Hefti - Oct 05, 2020, 3:25 PM CDT

Title: The Plinko Design

Date: 10/05/2020

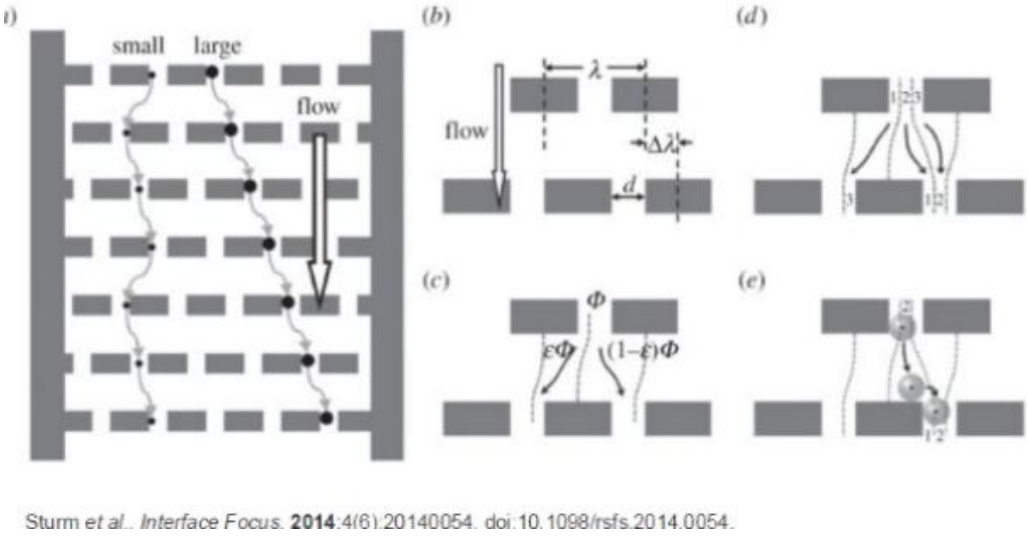
Content by: Hunter Hefti

Present: N/A

Goals: Establish a description of the plinko design with some basic explanation of its theory

Content:

The plinko design was based off of an idea that was conjured up and suggested by our advisor, Professor Justin Williams. Named after the Price is Right game of the same name, the Plinko design would consist of a channel containing multiple rodlike inserts organized in a seemginly random but precisely calculated fashion. Mathematically, Plinko is not as random as it initially appears. Balls or pucks or chips are seen to enter from the top of the plinko board and are observed to fall from peg to peg until bouncing into a particular slot at the base of the board. This pattern of bouncing and dropping is actually subject to the size and weight of the object and has been carefully mathematically analyzed to determine the predictability of the objects final destination. As such, it was hypotesized that the use of such a plinko concept would allow for precise placement of rods so as to decrease the velocity of flow by widening the channel while ensuring that cells were properly relocated towards the center of the device.



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

Figure 1. A type of plinko design used for sorting cells of various sizes. The paper was accompanied with precise calculations for determining where the cells would end up once they entered.

This type of design as been used before for cell sorting applications. Primarily, the plinko design is good at determining the location of objects when the shape and size are varied as variations in these parameters allow for easy differentiation in the location that the board motivates these objects to move. The type of cell sorting that is usually accomplished with the plinko design is almost strictly in line with sorting cells by size rather than by type or by internal mechanism. In other words, cytometry wouldn't have a use in this method as the type of cell sorting that can be accomplished using cytometry would not be reflected in this design.

Conclusions/action items:

Kayvan shot this idea down pretty quickly. Given that the main function of the typical plinko design was to sort based on observable physical characteristics, the idea of centering cells using the plinko design while adjusting flow speed using the outlet flow from the sides of the channel seemed inconsequential and redundant. Flow speed will be controllable via the pumps, so building a reliable design which can center a single file line of cells will be more critical. This is not the obvious choice for that function.

10/04/2020 - Funnel Design

Hunter Hefti - Oct 06, 2020, 8:53 AM CDT

Title: The Funnel Design

Date: 10/05/2020

Content by: Hunter Hefti

Present: N/A

Goals: Describe function of the funnel design in the context of its attempts to meet the goals.

Content:

Most flow cytometer chips will employ the use of some form of cone shaped or triangular design in order to bolster the ability for the sheath fluid to improve the flow of the cells down the channel. As such, the Skala lab built many of their first prototypes based around a cone shaped design. In a typical flow chip, the sheath fluid must converge upon the incoming cell sample and push it downstream in a controlled manner via laminar flow. Many designs utilize a two dimensional approach where flow converges from two separate directions. The funnel is intended to eliminated the sheath flow convergence point by allowing for a continuous stream of PBS down all sides of the channel. This approach will allow for the cell sample to immediately be focused as sheath fluid surrounds the cell flow on all sides. Such a design would greatly benefit from the elimination of any possible turbulent flow that might result if a two dimensional design were to include more than one sheath fluid entry point.

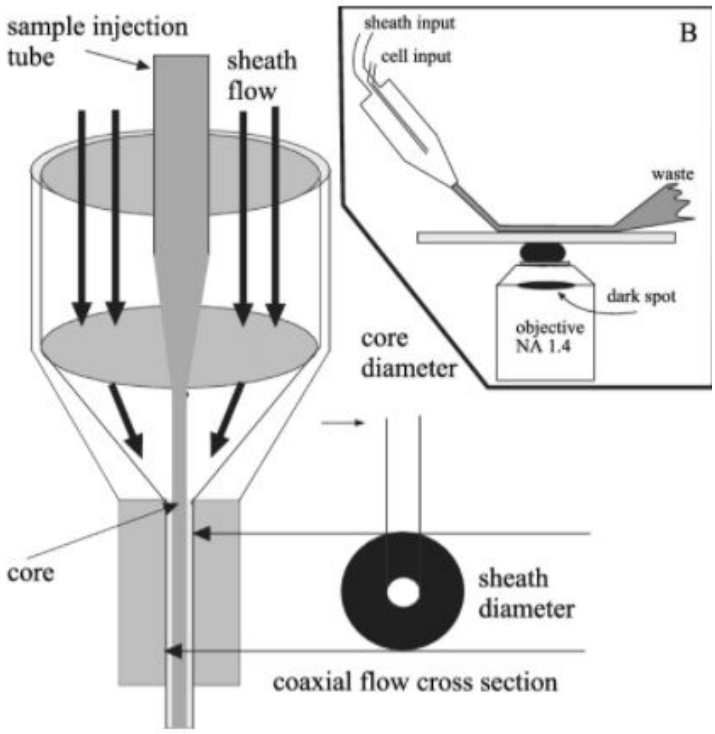


Figure 1. An image involving a funnel type design in which a core diameter is displayed as a result of the introduction of three dimensional sheath fluid focusing.

The above image depicts a typical design for a funnel inlet. Sheath fluid is permitted to flow around all sides of the cell input and is able to fill the inlet prior to reaching the cell inputs termination point. In this example, the inlet is set at an angle so there is potential for some turbulence at the kink in the channel. Such changes in flow would not be beneficial so the design would have to incorporate some two dimensional plane or else decrease the turbulence that would occur. During flow simulations, these parameters would be adjusted to maximize the ability for the funnel to correctly do its job.

Conclusions/action items:

The funnel design was recognized to be the most common type of cellular inlet design by the Skala lab team. They, themselves, have adapted the design to fit many of their prototypes. They are interested to know how simulations go for testing out different variations on this concept and, if possible, would like to see work on such a design carried out alongside the production of the final design.

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

10/04/2020 - Snake Design

Hunter Hefti - Oct 06, 2020, 9:47 AM CDT

Title: The Snake

Date: 10/06/2020

Content by: Hunter Hefti

Present: N/A

Goals: Describe the benefits of the snake design and how it wouldl seek to accomplish its goals

Content:

Our advisor was very keen to aid us in brainstorming ideas. He suggested early on in the process that we could utilize inertial lift forces to require cells to choose a path of least resistance that would center them in the channel. Following some research, it was discovered that a few types of cell sorting mechanisms utilized an initial cell focusing techinque which utilized a serpintining set of asymmetric curves that forced the cells into a linear formation prior to sheath fluid contact. One paper even described experimental results of symmetric vs asymmetric curvature and its effects on the streamlines that effected particle output. The snake design is modelled after these assumptions with the caveat that sheath fluid is added prior to the curved channel design. This should allow all flow to have focused streamlines that should aid in the centering of the cells throughout the length of the objective channel. An image taken from the aforementioned paper is included and the paper is cited earlier in this notebook.

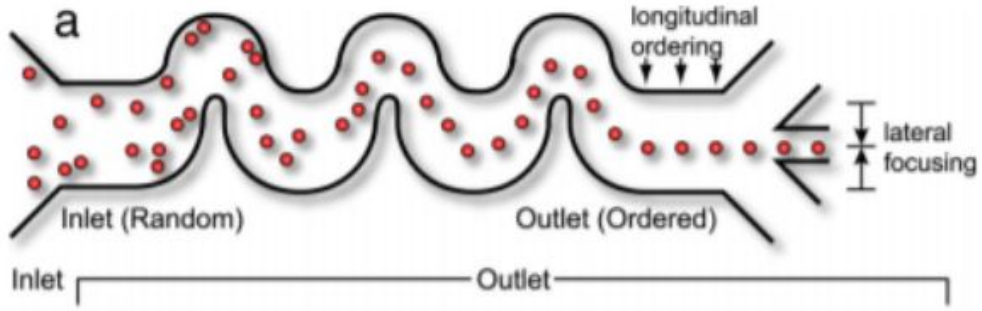


Figure 1. A design that utilizes the asymmetric curved cellular inlet. This design also features outlets for fluid which can possibly enable the design to reduce flow speed and slow the cell flow down.

Intertial lift forces are the result of the flows alterations in direction and velocity that occur at the points where the curvature requires it. In order for the cells to properly move through the system and reach the final glass objective channel, the streamlines will have to align at least parallel so as to take a central path that avoids contact with the walls of the channel. Asymmetric curvature designs allow the focusig of these streamlines to be exactly central under varying conditions. So the design of the snake will require much simulation in order to find the precise shape and number of curves to optimally focus the cells.

Conclusions/action items:

The Skala lab members found this design to hold the most promise as far as simulation options go. The snake was observed in papers that had been reviewed by members of the lab so this will be the main focus of most of our production, experimentation, simulation, and research prospects.

10/23/2020 - OnShape Design

Title: The Original OnShape Design

Date: 10/23/2020

Content by: Hunter Hefti

Present: N/A

Goals: Describe initial design and the reason for leaving OnShape behind

Content:

The intiial test of the feasability of the design was attempted using OnShape. OnShape is very similar to SolidWorks but it is completely online which allows members to share and collaborate drawback to OnShape is that it lacks a built in method for flow simulations, so the usefulness of the program was debatable. Several snake models were made using OnShape so that the tean would look like. But considering the use of OnShape was very limited, the Snake had to be completely redone in SolidWorks anyway.

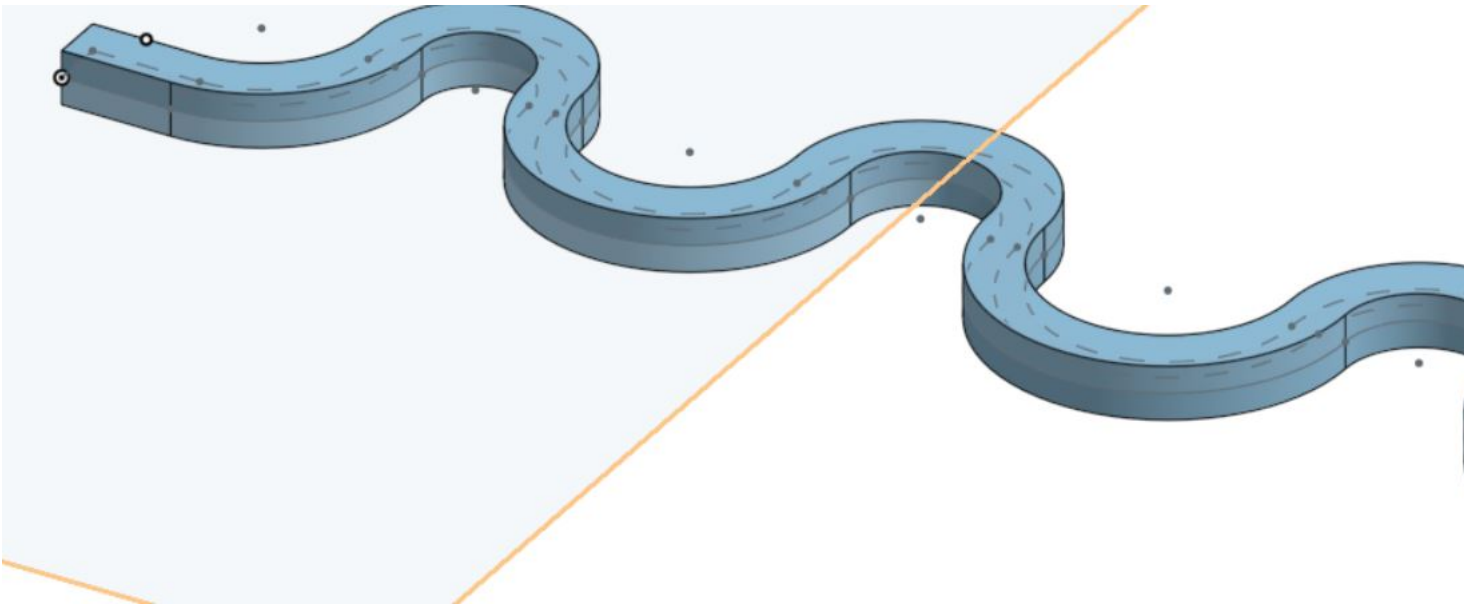


Figure 1 - The initial OnShape design

Upon consultation with the client regarding these initial designs, the one featured above was sruitinized for having circular inlets and outlets. The clients determined that a square channel woul forward, designing square channels would be the pressing design choice that linked the rest.

Conclusions/action items:

While OnShape was useful for gaining insight into the clients wishes, it required excess simulation software to work and so was scrapped.

10/30/2020 - Snake1

Title: Snake1

Date: 10/30/2020

Content by: Hunter Hefti

Present: N/A

Goals: To present the first SolidWorks model that attempted to mimic OnShape's design

Content:

The Snake1 design was closely modeled after the initial OnShape design. It was created to observe the effect of adding particles into a semetrically curved aparatus. The initial set up of the flu however. Solidworks was not working well with the laptop and many updates and downloads were necessary to discover a solultion. After this, a few mistakes in the initial design prevented ac resolved by the design of Snake3.

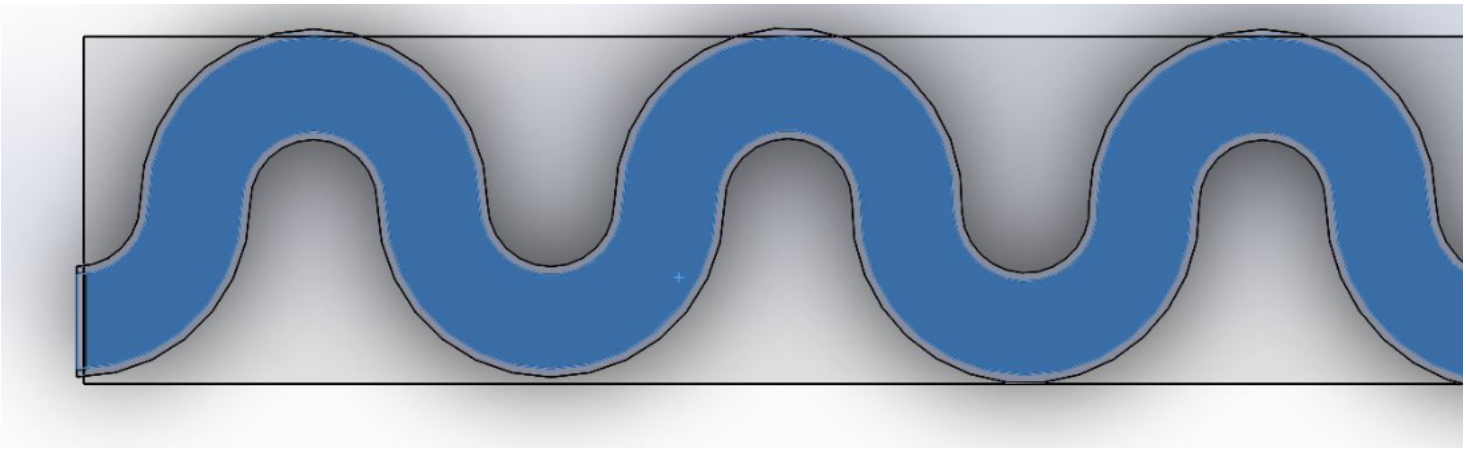


Figure 1 - Snake1 design

The eventual result was a complete lack of focusing. this may have been due to the symmetry or it could have been another error with the initial values set for the speed.

Conclusions/action items:

Snake1 was intended to demonstrate the lack of focus. It did. But it is unclear if the lack of focus was due to design error or due to legitimate physical properties of the curvature shape.

11/05/2020 - Snake2

Hunter Hefti - Dec 07, 2020, 1:36 PM CST

Title: Snake2

Date: 11/05/2020

Content by: Hunter Hefti

Present: N/A

Goals: Describe the evolution of the snake design that incorporated sheath fluid

Content:

The second design was conceived at the same time as the Snake1 design. Snake2 was intended to show the viability of adding sheath fluid immediately following the snake channel. The idea was that one inlet would allow for the introduction of particles that would pass through the inertial focusing channel before contacting the sheath fluid at the outlet and speeding out towards the observation chamber. The beginngings of the new method of design (extruded cuts into a sheet of material rather than generating a stand-alone model) are also displayed. Unfortunately, the fluid simulation was still not properly in place and the design was generated with awkward proportions.

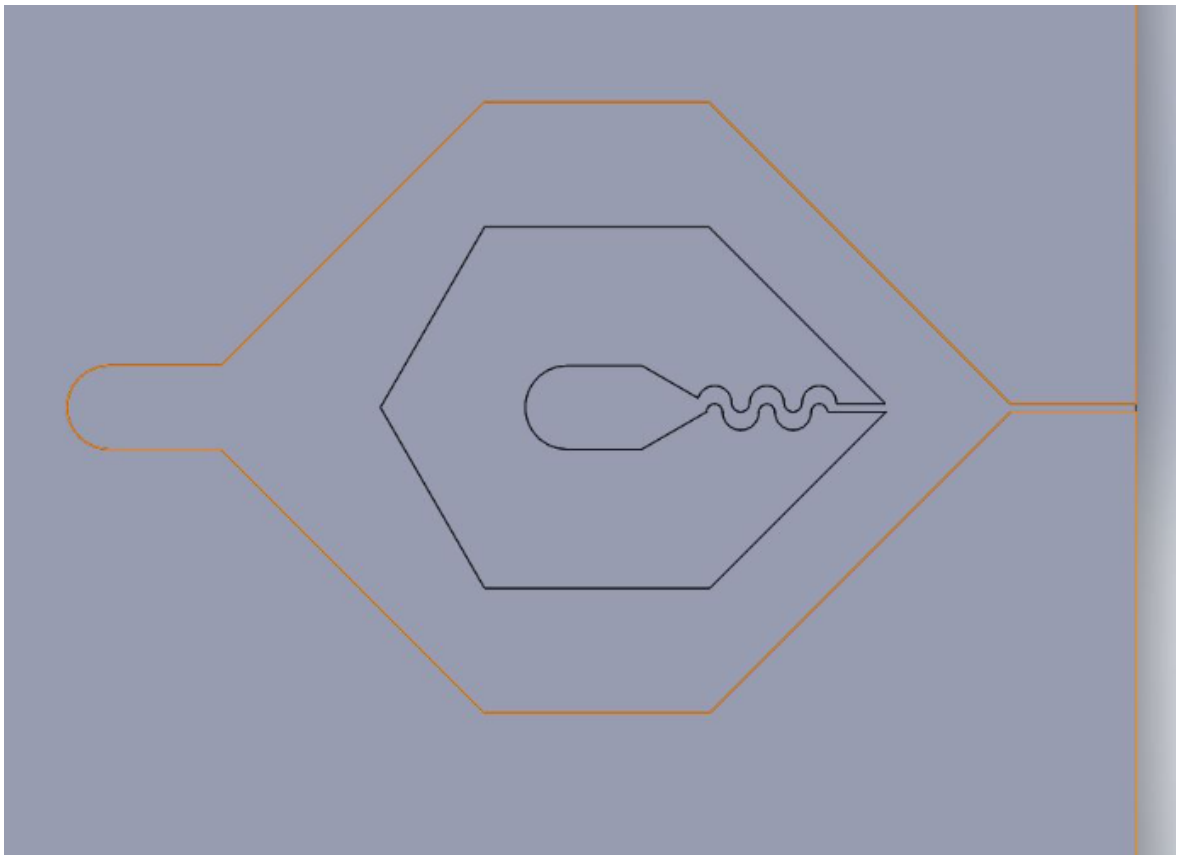


Figure 1 - Snake2 design adds extra room for sheath fluid to enter from the back and navigate to the inertial flow exit

Conclusions/action items:

Snake2 allowed for a template to build Snake3 and make alterations to observe Snake4 and Snake5, the last of which would be the working model that had been the objective of designing Snake2.

11/16/2020 - Snake3-5

Title: Snake3-Snake5

Date: 11/05/2020 - 11/18/2020

Content by: Hunter Hefti

Present: N/A

Goals: To describe the outcome of Snake3 and its subsequent improvements in Snake4 and Snake5

Content:

Snake 3-5 refers to a set of three attempts to perfect the design of Snake2 with proper simulation boundaries in place. The channel, in all cases, was generated without properly scaling the de the purpose of this design was merely to see whether sheath fluid would be an important edition or not. Snake3 fell into trouble with improper mating while Snake4 was redesigned when the sl Pictures below are from Snake5 and illustrate the qualitiative results of our simulation testing on this design. When presented in this form to the client, it was remarked that the centering all see fluid. Thus, Snake6 needed to be generated to see if the snake would actually improve centering all by itself or if it was all a matter of the sheath fluids assistance.

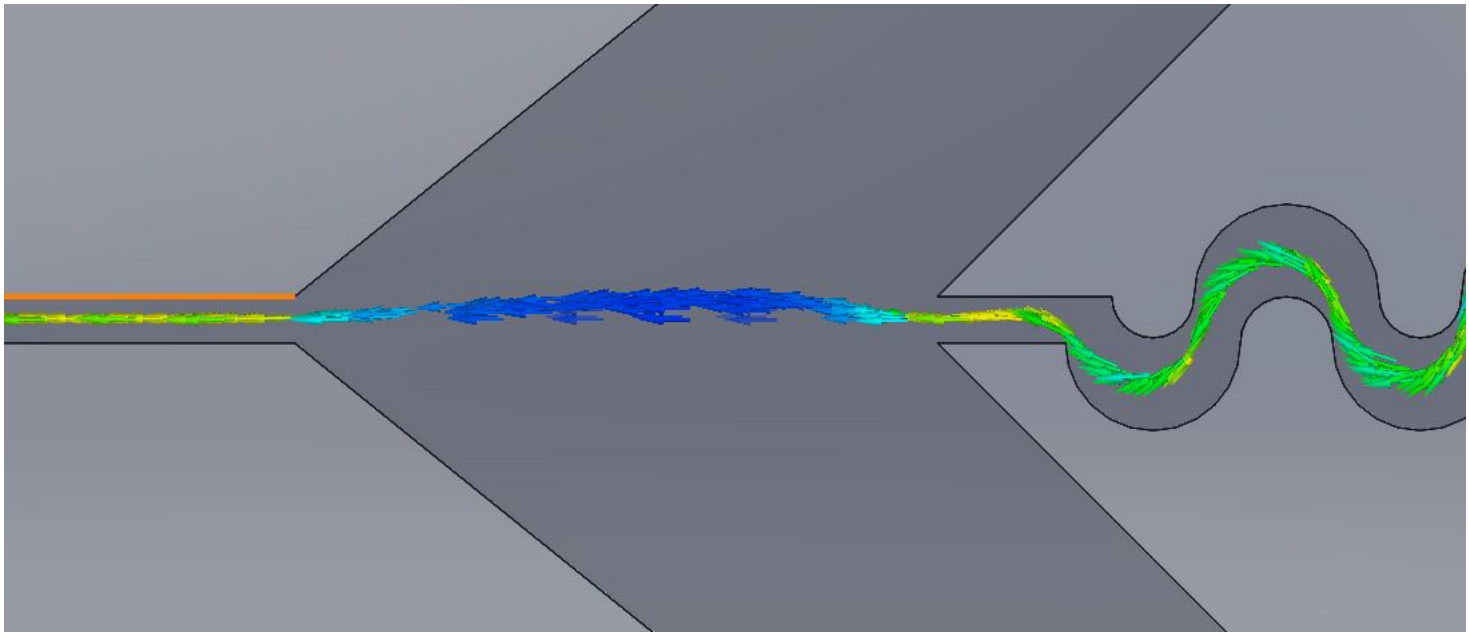


Figure 1 - Snake5 when sheath fluid is entirely removed. This is the particle simulation results and demonstrates a divergences at the exit of the snake.

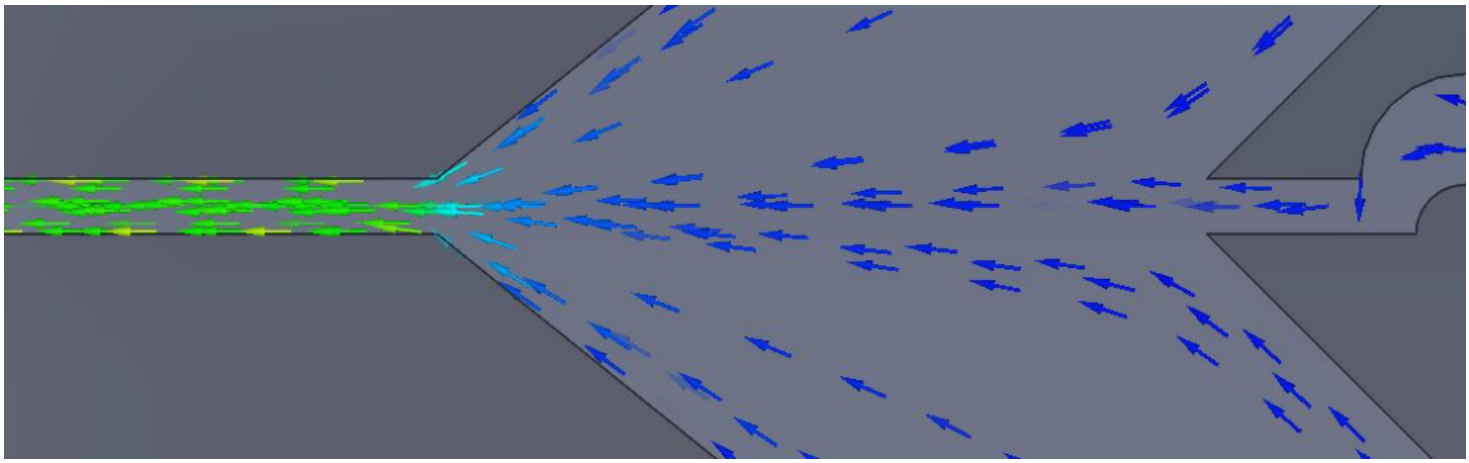


Figure 2 - Snake5 when sheath fluid is implemented. These are the flow trajectories which show sheath fluid running into the side of the channel and converging at the exit.

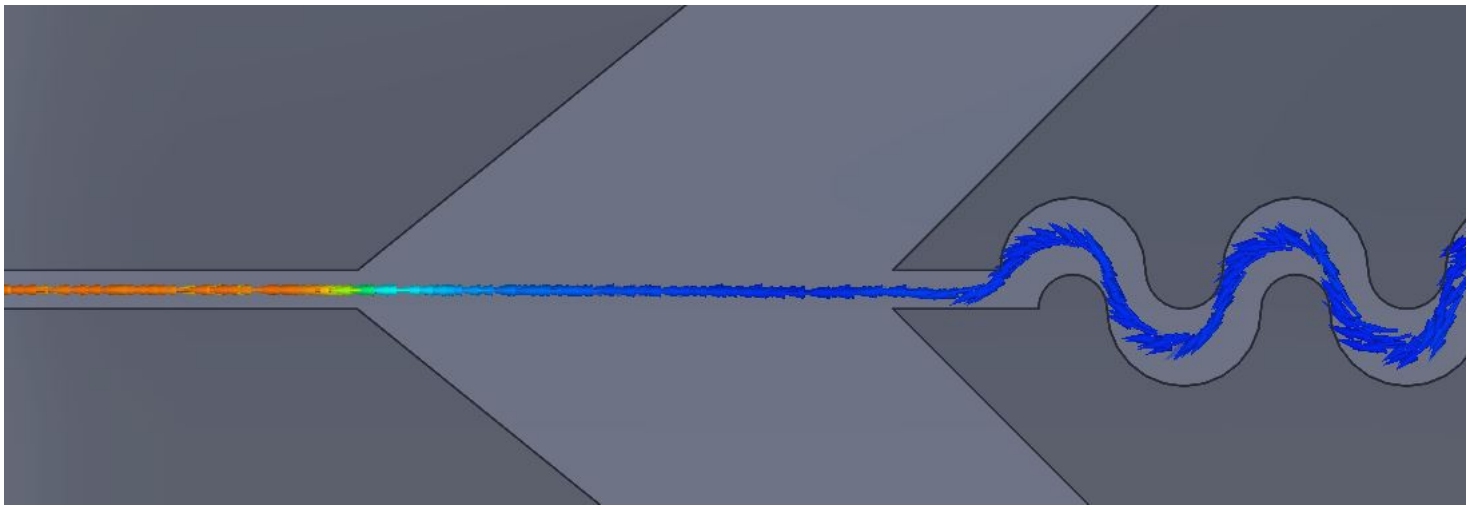


Figure 3 - Snake5 with sheath fluid, observing the particle simulation. Centering is observed but this is likely only due to the presence of the sheath fluid.

Conclusions/action items:

Snake5 proved to be valuable in helping identifiy errors in the design and knowing that the goal should be to elongate the design to allow for more inertial focusing.

12/02/2020 - Snake6

Title: Snake6

Date: 12/02/2020

Content by: Hunter Hefti

Present: N/A

Goals: Discuss the results of the 6th iteration of the snake design

Content:

The Snake6 design was an attempt to generate an exact mimic of the design that was generated by the Di Carlo group in 2007. This was done using the built in scale bar seen in figure 1 belo approximately 100 microns in length. However, it nearly matches the top radius of curavture which is theoretically supposed to be 50 micron. The former measurement was used for analyis wh width of the radius of the top channel. Aside from this, the results were not overly promising. In both the flow trajectory and particle simulations, speed seemed to increase at the center of the c that actually occured with the particles. With only qualitative analysis to go off of, this does not imply a complete lack of focus. However, the inability to see rapid change in focus certainly does will take place in the future.

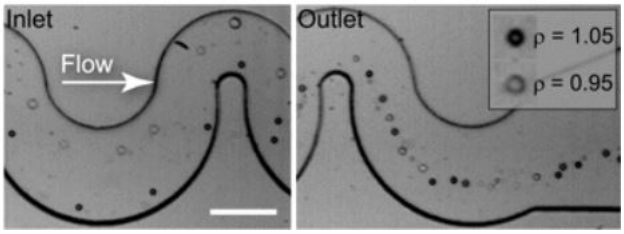


Figure 1 - Figure depicted in the Di Carlo paper which revolutionized this idea. Scale bar is listed to be 100 micron.

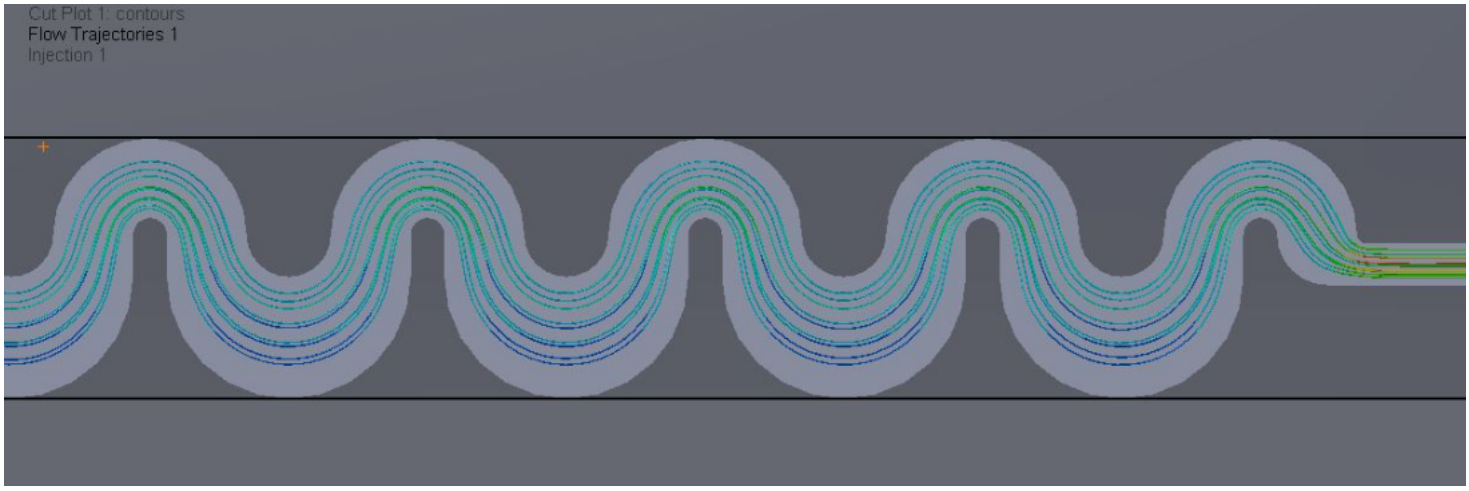


Figure 2 - The flow trajectory for the model. Increase in red coloration indicates faster velocity.

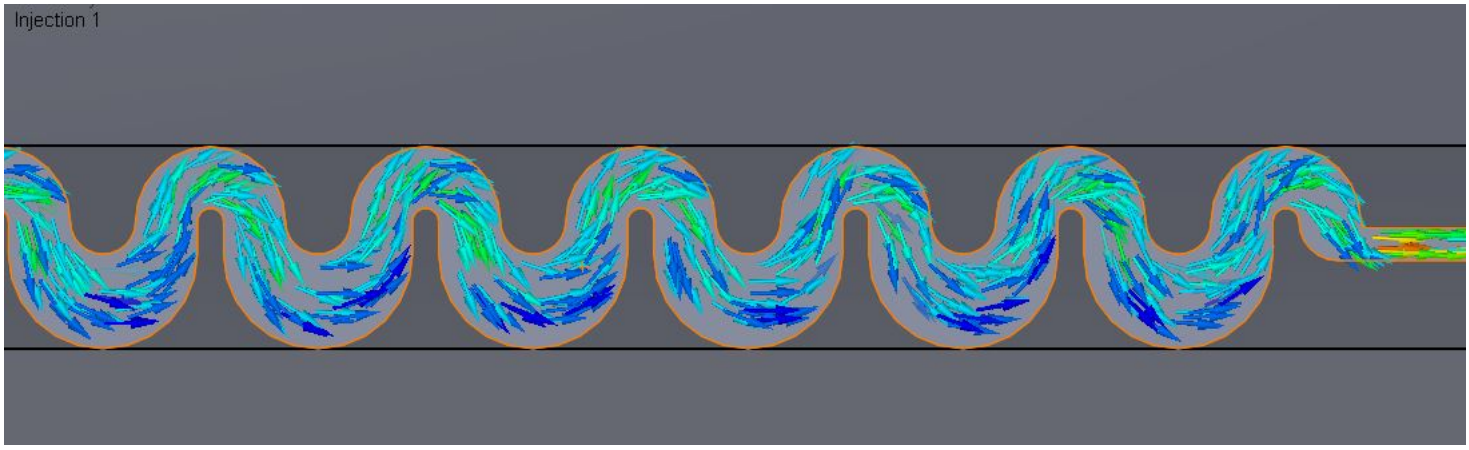


Figure 3 - Particle trajectories for the Snake6 simulation using 10 micron diameter particles. There does not appear to be any focusing

Conclusions/action items:

The clients noted that one of the design specifications, namely, the 1:1 curvature radius was not met. This should be improved in a further design. Alongside this, generating a different design center of the channel would be a nice addition. Essentially, just further testing is needed.

10/04/2020 - Biosafety Training

Hunter Hefti - Oct 04, 2020, 12:59 PM CDT

Title: Biosafety Training Documentation

Date: 10/04/2020

Content by: Hunter Hefti

Present: N/A

Goals: Establish existing biosafety training documentation that will allow me to work in general bio labs

Content:

Biosafety Required Training	Biosafety Required Training Quiz	2/25/2019	

Data Effective: Mon Feb 25 16:04:25 2019
Report Generated: Mon Feb 25 20:52:31 2019

Conclusions/action items:

Expires after five years or upon Wiscard Expiration, neither of which take place during the current semester.

10/04/2020 - Bloodbourne Pathogen

Hunter Hefti - Oct 04, 2020, 1:01 PM CD

Title: Bloodborne Pathogen Training

Date: 10/04/2020

Content by: Hunter Hefti

Present: N/A

Goals: Establish current training for bloodborne pathogens, granting permission to work with blood in designated lab spaces

Content:

BIOSAFETY 102: BLOODBORNE PATHOGENS FOR LABORATORY AND RESEARCH	BLOODBORNE PATHOGENS SAFETY IN RESEARCH QUIZ 2019	11/21/201

Conclusions/action items:

The training actually does expire one year from its completion date. It will be renewed at some point prior to expiration, however.

10/04/2020 - Green Pass

Hunter Hefti - Oct 04, 2020, 1:02 PM CDT

Title: Green Pass - Team Lab Certification

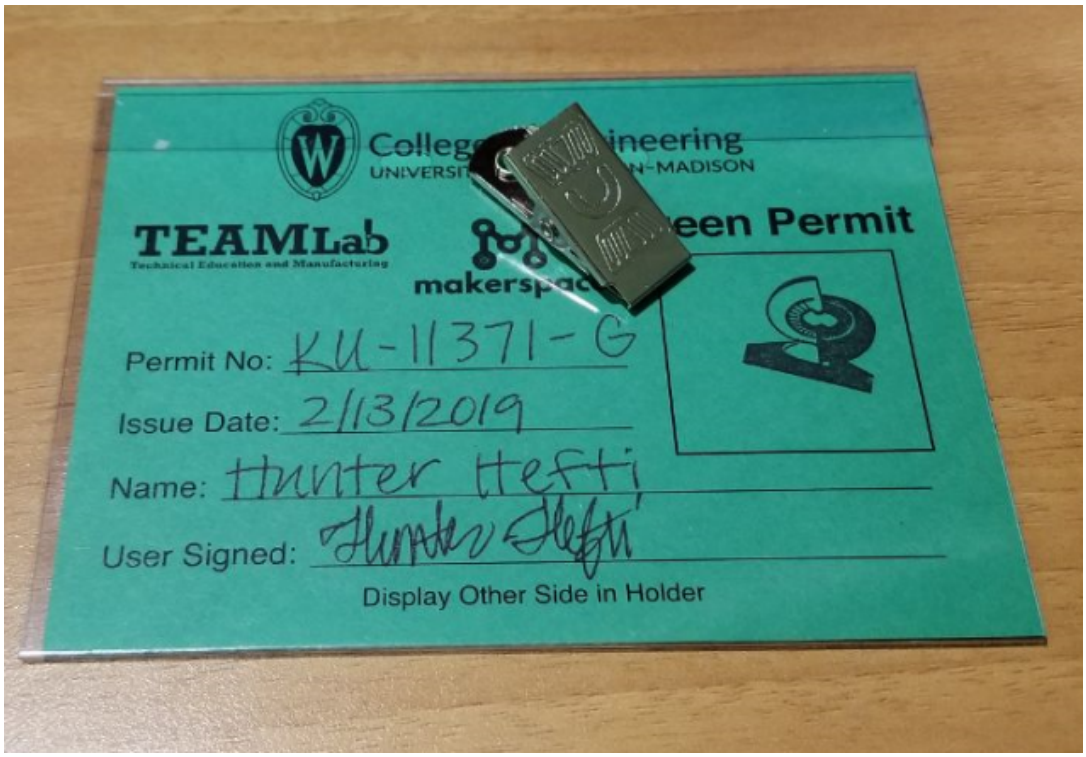
Date: 10/04/2020

Content by: Hunter Hefti

Present: N/A

Goals: Establish current training allotments with the Team Lab in the basement of ECB (which is no longer in existance)

Content:



Conclusions/action items:

This also has no set expiration date, but I am limited to the use of only a few machines in the team lab. If welding or some advanced form of machine is needed, more training must be acquired.

2014/11/03-Entry guidelines

John Puccinelli - Sep 05, 2016, 1:18 PM CDT

Use this as a guide for every entry

- Every text entry of your notebook should have the **bold titles** below.
- Every page/entry should be **named starting with the date** of the entry's first creation/activity, subsequent material from future dates can be added later.

You can create a copy of the blank template by first opening the desired folder, clicking on "New", selecting "Copy Existing Page...", and then select "2014/11/03-Template")

Title: Descriptive title (i.e. Client Meeting)

Date: 9/5/2016

Content by: The one person who wrote the content

Present: Names of those present if more than just you (not necessary for individual work)

Goals: Establish clear goals for all text entries (meetings, individual work, etc.).

Content:

Contains clear and organized notes (also includes any references used)

Conclusions/action items:

Recap only the most significant findings and/or action items resulting from the entry.

2014/11/03-Template

John Puccinelli - Nov 03, 2014, 3:20 PM CST

Title:

Date:

Content by:

Present:

Goals:

Content:

Conclusions/action items: