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Cornell High Energy Synchrotron Source

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Design, Fabrication and Testing of Dual Input, Mixture Inducing BioSAXS Flow Cell

Abstract

Advancements over the past several decades in microfluidic devices and associated fabrication technologies have drawn considerable interest from the structural biology community. Current boundaries of these technologies are being pressed to the nanoscale level with complex designs that allow for rapid mixing and manipulation of fluids. Common applications exist in fluid dynamics in both laminar and turbulent flow regimes. Another practical application is to combine streams of proteins and ligands, and directly afterwards (within milliseconds) apply an imaging modality to the mixed stream. There has been so much focus on expanding our capabilities that a simplistic and middle-of-the-road approach has been over looked.

BioSAXS is emerging as a popular complimentary technique to crystallography and is well-suited to study protein dynamics. This project aims to develop the necessary technology to study protein dynamics at small time scales (e.g. <10ms after a mixing) at bioSAXS beamlines using proven, cheap and readily accessible microfluidic technology.

At MacCHESS beamline G1, custom bioSAXS compatible flow cells (e.g. single input/output) are made in-house with channel dimensions 1 × 2 mm and are routinely used at flow rates < 10 μL/sec. Based on this successful design, two new designs are presented which feature dual inputs and thus allow for mixing of two input streams within tens of milliseconds of imaging. Design performance was assessed using input streams of NaOH and phenolphthalein in ethanol, as to produce visible color change upon mixing, captured using a high speed camera, and showed the designs to be effective mixers in under 300ms.

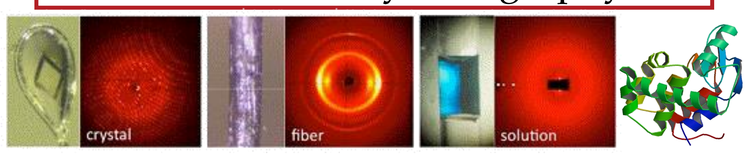
Motivation

Using time controlled mixing and imaging of solutions could give a real time account of the conformational changes that occur during biological reactions and has the potential to take studies of molecular biology to the next level.

Although a device and protocol has been established to achieve this and is capable of imaging on the milli and microsecond timescale, there is a large gap in ability to inducing mixing on larger time scales. The simple features of the flow cell mixers shown in this poster were designed on the micron scale with the intent of providing a feature rich design capable of inducing turbulence. Further, the design can be scaled to smaller dimensions to further reduce sample consumption and shift imaging access closer to the mixing event.

The designs presented here create sufficient obstruction to fluid flow to avoid laminar behavior by increasing the surface area between the two liquids, thus granting more versatility in which larger time scale biological systems can utilize this device. This device could give structural biologists the critical tool required to investigate the dynamics of macromolecules, for example when two distinct proteins or a protein and ligand are brought together, shortly after the mixing event.

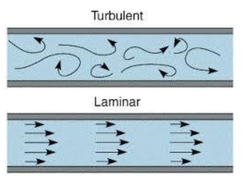
BioSAXS vs Crystallography



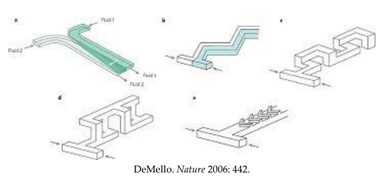
Microcrystallography	SAXS/WAXS
• Very reliable and detailed data allowed by being able to rotate and image 360° of crystal lattice	• Less resolution at atomic scale
• Static representation	• Broader view
• Some biological samples are difficult to crystallize	• Possible to image more realistic natural states and interactions between proteins
	• Simpler sample preparation, and ability to change conditions

Microfluidics of Flow Cells

- Laminar flow, which occurs at low Reynolds's numbers (<1000), is when separate inputs become parallel currents that have only a small amount of mixing along the interface between them. This is what the mixer design attempts to avoid by increasing the Re number above 1000 due to the features of the cells.



- There are several ways to increase the Re number so that turbulent flow is accomplished. Such as geometric features within flow channels designed to obstruct the flow of liquid, outside forces acting upon the liquid within the channel and increasing the flow rate.



DeMello. *Nature* 2006: 442.

- For this project we have chosen to incorporate a simplistic design for ease in fabricating and using as a disposable flow cell to be utilized by MacCHESS users.

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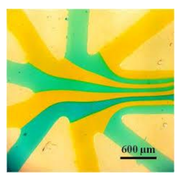
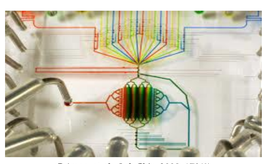
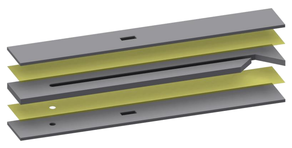


AIChemE, laminar flow

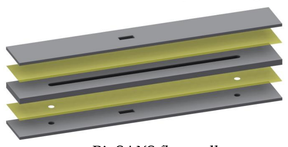


Grimes, et al., *Lab Chip*, 2008: 170(**8**)

Current BioSAXS Flow Cells

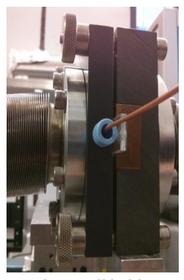


pipette tip compatible
for manual/robotic sample loading

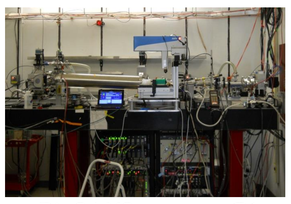


BioSAXS flow cell
for inline sample loading

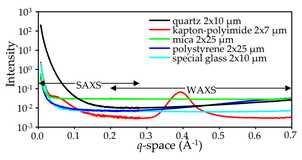
✓ Identical outer dimensions.
✓ Switching flow cells is easy.



inlet to cell holder



BioSAXS setup at G1



Acerbo, et al., *Submitted*, 2014

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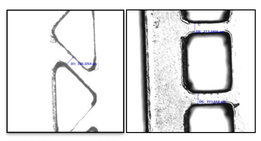
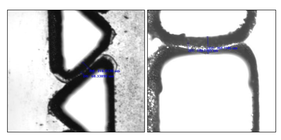


10% power, 800ppi, .5mm z-axis, 2x



30% power, 800ppi, .5mm z-axis, 1x

Optimizing the settings for the Versalaser was a very important aspect of successful fabrication. As shown in the images above, factors such as power level, pulses per inch, and the number of passes with the laser have a great effect on the precision and detail of the features. It was a balancing act between having enough power to fully cut through the layer in one pass, and a low enough power that the material was not melted and details were kept as close to their designed measurements as possible. Although the most well defined details were achieved by 10% power twice over, this was not feasible on large scale printing of the design because the passes would be misaligned. The summary of ideal settings for each PMMA thickness is as follows:

• 2.0 mm	45% power	1000 ppi	1.0 mm z-axis
• 1.5 mm	38.5% power	800 ppi	0.8 mm z-axis
• 1.0 mm	18% power	800 ppi	5.0 mm z-axis
• 0.5 mm	8% power	1000 ppi	3.0 mm z-axis

Ladder Design

In order to determine whether flow in the current design falls in the laminar of turbulent flow regime, Reynolds' equation was used. For each design, an average of 6-7 separate measurements of the mixing features, spaces between them and the width of the channel were taken. The height and length of the channels were assumed to be consistent. Reynolds' equation is as follows:

$$\mathrm{Re} = \frac{\rho \mathbf{v} D_H}{\mu} = \frac{\mathbf{v} D_H}{\nu} = \frac{\mathbf{Q} D_H}{\nu A}$$

❶ portion of channel that does not contain mixing features
❷ portion of channel that contains mixing features

$$❶ = \frac{54.8\mathrm{mm}^3 s^{-1} \times .5136\mathrm{mm}}{1.28\mathrm{mm}^2 s^{-1} \times 1.894\mathrm{mm}^2} = 11.63$$

$$❷ = \frac{28.4\mathrm{mm}^3 s^{-1} \times .3901\mathrm{mm}}{1.28\mathrm{mm}^2 s^{-1} \times .947\mathrm{mm}^2} = 17.64$$

υ is the *kinematic viscosity* (m^2/s): ❶ & ❷ 50% ethanol ≈ $1.280 mm^2/s$

$\mathbf{Q}$ is the *volumetric flow rate* (m^3/s):

❶ (30mm/s)(2mm)(.947mm) = $54.8 mm^3/s$
❷ (30mm/s)(1mm)(.947mm) = $28.4 mm^3/s$

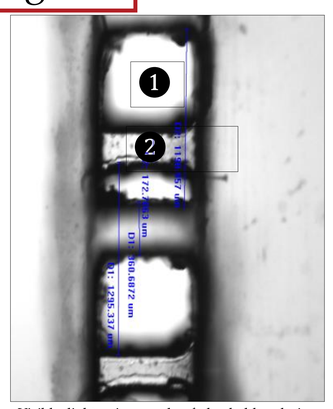
$\boldsymbol{D_H}$ is the *hydraulic diameter* of the pipe(m); For shapes such as squares, rectangular or annular ducts where the height and width are comparable, the characteristic dimension for internal flow situations is taken to be the *hydraulic diameter* and is defined as: $\boldsymbol{D_H = \frac{4A}{P}}$

❶ $= \frac{4(1.894\mathrm{mm}^2)}{1.894\mathrm{mm}} = .5136\mathrm{mm}$ ❷ $= \frac{4(.947\mathrm{mm}^2)}{9.7088\mathrm{mm}} = .3901\mathrm{mm}$

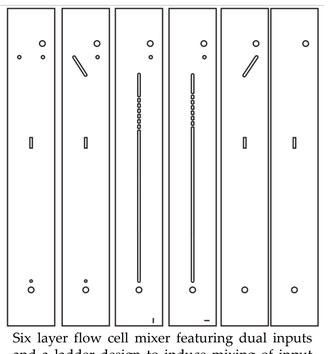
$\boldsymbol{P}$ is the *wetted perimeter*, the inner dimensions of the channel that interacts directly with liquid:
❶ 4(.947mm)+4(2mm)+8(.3705mm)= 14.752mm
❷ 4(.947mm)+4(1mm)+8(.2401mm)=9.7088mm

$\boldsymbol{A}$ is the pipe *cross-sectional area* (m^2):
❶ (.947mm)(2mm)=$1.894 mm^2$ ❷ (.947mm)(1mm)=$.947 mm^2$

Not used specifically during calculations:
$\mathbf{v}$ is the mean velocity of the fluid (SI units: m/s); $\boldsymbol{\rho}$ is the density of the fluid (kg/m^3); $\boldsymbol{\mu}$ is the dynamic viscosity of the fluid (Pa·s = N·s/m^2 = kg/(m·s)).



Visible light micrograph of the ladder design showing bridging features perpendicular to flow direction.

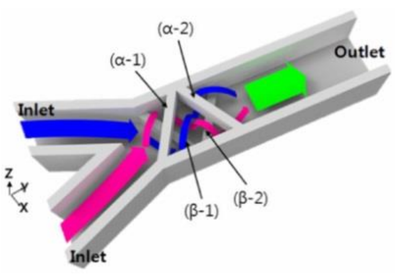


Six layer flow cell mixer featuring dual inputs and a ladder design to induce mixing of input streams.

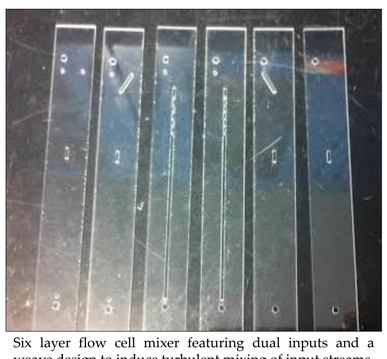
Weave Design

A second mixer design is based on a weave pattern to enhance laminar mixing (shown right). In this design, the bridges in the weave pattern fold the liquid streams and increase their effective boundary surface, and thereby cross-stream diffusion potential.

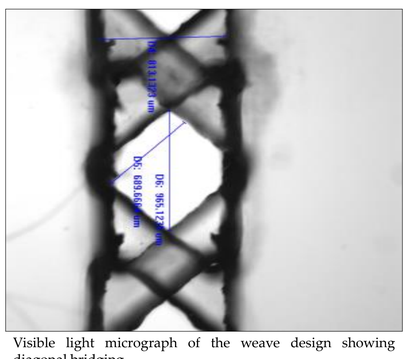
Incorporating such a weave pattern in the bioSAXS flow cell mixer required careful design and fabrication of a flow cell with six layers. Aside from the weave pattern, this design also features inputs that feed into the main channel from the top and bottom of the channel.



Yoo, et al., J Micromech Microeng. 2012: 035007(**22**)



Six layer flow cell mixer featuring dual inputs and a weave design to induce turbulent mixing of input streams.



Visible light micrograph of the weave design showing diagonal bridging.

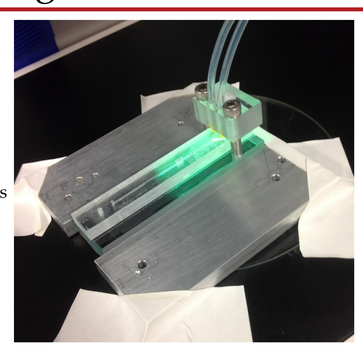
Flow Cell Assembly Protocol

Once the layers have been cut out with the Versa laser, they are brought back to the G1 chemistry lab to be assembled into flow cells with the following protocol:

1. Layers are cleaned with isopropyl alcohol.
2. The first layers to be bonded are 2 and 5, this is because the polystyrene (X-ray transparent window) is to be placed onto the sides that enclose the flow chamber (layers 3 and 4) and need several minutes to bond securely. These layers are 1.5 mm thick and require 9 minutes of chloroform vapor exposure to bond well with the polystyrene.
3. When removing layers 2 and 5, layers 3 and 4 are place into the vapor container, and are 1 mm thick, exposed to vapor for 9 minutes as well, then layers 2-5 are secured and compressed in a vice to ensure tight bonding.
4. Outer layers 1 and 6 must be exposed to vapors for 10 minutes before being added onto the others layers and secured into place using the vice.
5. Ideally compressed gas should be allowed to flow through the cells while they are bonding to avoid residual vapors fouling the X-ray windows, but this was not a priority during assembly because these cells were fabricated primarily for testing of fluid flow and mixing potential. Future sets will be fabricated with sufficient air flow.

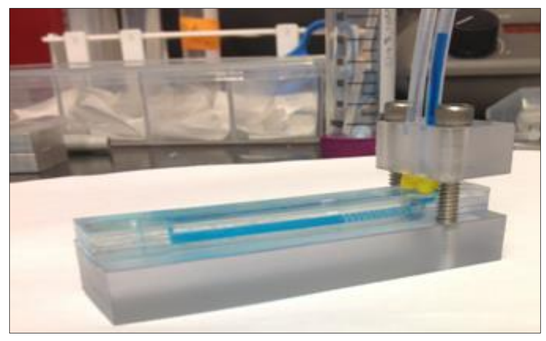
Testing Protocol

Top view image of the testing apparatus secured in place upon the Zeiss Microscope that was used for data collection.

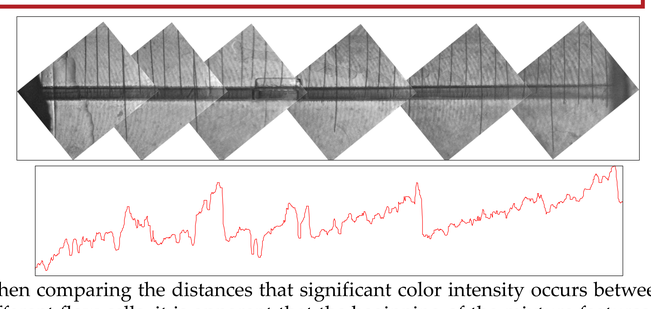


- 1× magnification.
- A PointGrey Flea3 camera was used with image acquisition software by Peter Revesz (CHESS).
- Frame capture speed was effective 17.8 msec between frames, shutter speed was 3 msec, image binning 2×2 and 1000 frames were compiled collected each flow cell test.
- Matlab software to process data was written by Alvin Acerbo.
- Due to the camera only allowing for black and white images, a green (FGV9, Thorlabs) light filter was used to accent the magenta color change of the chemical indicator used.
- Equal parts NaOH (pH 11) and 0.01 M Phenolphthalein in 100% ethanol were used as chemical indicators of molecular diffusion.
- With a dual input Ismatec peristaltic pump, equal amounts of the separated chemical indicators were flowed through the test flow cells at equal rates of approximately 3 cm/sec.
- The apparatus used to secure the input tubes with the test cells, designed and machined by Richard Gillilan, is shown below containing the food dye that was used to estimate the flow rate.

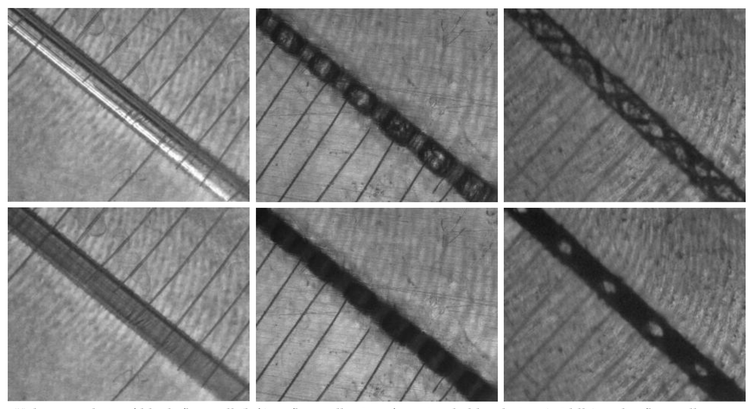
Side view of assembled flow cell with blue dye on one input and water on the other input. The mixing portion of the channel is clearly visible by the blue striation.



Results



- When comparing the distances that significant color intensity occurs between the different flow cells, it is apparent that the beginning of the mixture features has a great influence on the rate of color change, approaching our goal of <100ms.
- It can also be seen that the color change plateaus in the mixture cells over the duration of the run. This could be due to the features mixing to their full ability at certain points along the flow channel.
- Although the Re numbers are relatively low, far beneath the turbulent flow regime, there was still significant mixing of the indicator fluids much earlier in the channel with mixture features than in the featureless channel.



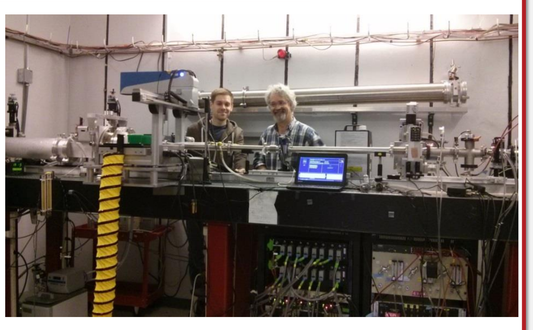
Video snapshots of blank flow cell (left), a flow cell mixer featuring ladder design (middle) and a flow cell mixer featuring a weave design (right). Top images show the channel before liquid is introduced into the system, whereas the bottom images show the flow of liquid through the channel.

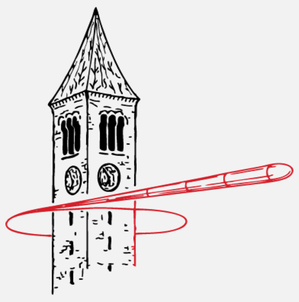
Conclusion

Building off of previous designs that are currently in use at G1 beamline, MacCHESS, the goal of this project was to incorporate mixture inducing features within the flow channel to be able to cause two separate fluids to mix within milliseconds of imaging. We have made progress with this and have further improvements to pursue. The designs presented in this poster show that the combination of horizontal laminar flow, increasing the interface between liquids to span the width of the channel instead of the height, features that obstruct the normal flow and further increase the interface between fluids, and an increased flow rate are effective in causing mixing faster than in the channel with only horizontal laminar flow and no mixture features. In order to address the issues of high sample consumption and shortening the time between initial mixing and imaging, these original designs will be changed and tested thoroughly before being available for use by molecular biologists utilizing the facility here at MacCHESS.

Acknowledgements

I would like to thank Alvin Acerbo for his consistent help and guidance during this project, without which it would not have been possible, and Richard Gillilan for his willingness to take the time to teach me at every opportunity and share some of the valuble wisdom he has accumulated throughout his career.
Special thanks to Beth Rhodes at CNF for training and assistance in the use of the Versalaser.





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