Quantifying µ-Scale Mixing Time Using Sodium Hydroxide and Phenolphthalein

Using Dolomite's Micromixer System

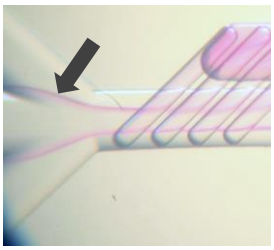
dolomite

Introduction to Micromixing Technology

Microfluidic research has resulted in commercialization of microreactor technologies, with wide adoption across multiple disciplines, and in many labs. Broadly, micromixing addresses (1) chemical applications in chemical synthesis, polymerization, micro process engineering and extraction, (2) bio/pharma applications for DNA analysis, biological screening enzyme assays, protein folding. Pharmaceutical technology relies on accurate blending at various stages, and (3) detection/analysis of chemical or biochemical content combined with NMR, FTIR, or Raman spectroscopies. Achieving good mixing of fluids thus emerges as the most common unit-operation in a chemical process and is thus a key component in any multicomponent fluidic test system, where it is necessary to mix small reagents volumes in a controlled but short period of time.

Bulk mixing – Active mixing strategies are essential to ensure completeness of mixing. Chemical inhomogeneity may still remain in bulk methods.

Miniaturizing the size and volume of the mixer enables more efficient and complete mixing by bringing concentration gradients closer together, as well as presenting an extremely high surface to volume ratio for reactions. The flow is laminar diffusion dominated, and to exploit benefits of miniaturization, strategies in addition to simple scaling down must be considered. The small dimensions mean laminar flow, which means diffusion dominated mixing. In effect, relatively long mixing times in typical channels of ~100um diameter warrant the use of augmented mixing to make the miniaturization attractive.



Micromixing – Reliable and repeatable mixing. Continuous flow ensures all species at the reaction front (black arrow) undergo very similar outcomes.

Further, many practical applications involve the presence of macromolecules whose mixing is particularly inefficient as their large size causes diffusion coefficients one or two orders of magnitude lower than that of most liquids.

Passive mixing requiring no additional energy input involves a split-and-recombine approach, which encourages uniform mixing. The added ability to ramp up flow rates introduces the significant benefits of chaotic advection to augment mixing – chaotic advection refers to swirling, churning and rolling flow patterns. These key features are harnessed in the design of the Dolomite micromixer microfluidic device, enabling fast mixing. A typical micromixer system is composed of the microfluidic device, precision pumping, fluidic elements and relevant software. Automated control of the liquid reagent addition is possible by varying dispense volume or flow rate over a time period. It is also possible to generate advanced flow rate profiles.

Summary

This application note evaluates mixing using a rapid chemical reaction causing a colour change as the indicator of mixing. Observations of colour or intensity variations of a dye or pH indicator on the microscale is quantified as it is mixed with a strong alkali. Reaction times under different conditions are parametrically investigated. For example, with a high flow rate ratio and two fluids with viscosities similar to water, perfect mixing can be achieved in 2.3 milliseconds (12 stages).

The setup section for the micromixer system illustrates the necessary components, including visual diagnostics. The working fluids are sodium hydroxide (0.1M NaOH) (aq) solution and 0.5% w/v phenolphthalein (methanol) solution. When mixed on-chip, pink streaks form at the interface of the two solutions. Gradually, the streaks diffuse into a uniform colour, at which point the solutions are considered mixed. Detailed images and timescale information are used to investigate the actual mixing process with the fluid color change from clear to pink indicating the completeness of mixing.

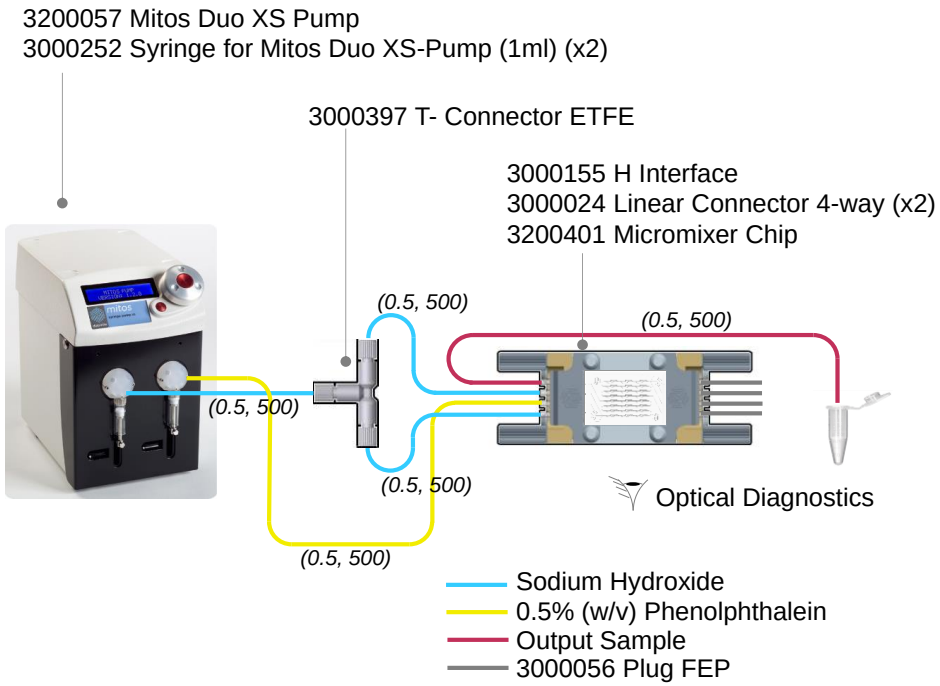
Rapid mixing times were observed with flow rates in the range of a few microliters per minute, up to millilitres per minute. Successful mixing was achieved at all flow rates. The dependence on the flow rate is further investigated to reveal that the path length required for full mixing first increases with increasing flow rate. At a certain point, when the flow rate is sufficienctly high, the flow transitions from laminar to chaotic – mainly due to the swirling flows around corners. At this flow rate, and progressively higher rates, the mixing impoves exponentially with increasing flow rate. The increased mixing results in a shorter path length (and fewer mixing stages) required for the two input fluids to mix completely.

The system highlights that perfect mixing is achieved at both high as well as low flow rate ratios. The microfluidic device, and other wetted parts offer excellent chemical stability, high visibility (excellent access for optics), and good optical transmission. The micromixer system performs exceptionally fast, works in continuous flow mode and achieves total mixing of two or three fluid streams within milliseconds.

The mixing demonstrated with a simple pH test here is shown to be extensible to more complex multi-component fluid chemistry. While the work demonstrated for this note focusses on liquid-liquid mixing, the same approach conceptually extends to gas-liquid mixing and gas-gas mixing.

Setup and System Configuration

The micromixer system is composed of the microfluidic device, precision pumping, fluidic elements and relevant software. For this application note, a syringe pump was used, but other options can be used, including the Mitos P Pump, with the optimum choice depending on a range of factors discussed in Appendix x below. The Micromixer Chip (Part No.3200401) is a lamination-based compact glass microfluidic device that allows rapid mixing of two or three fluid streams in each of the two independent mixing geometries. The Micromixer Chip is used with a Linear Connector 4-way (Part No. 3000024) and a H Interface (Part No. 3000155) to enable fluidic connection between the tubing and chip. A Mitos Duo XS Pump (Part No. 3200057) each fitted with a Syringe for Mitos Duo XS Pump, 1ml (Part No. 3000252) was used to deliver the two fluids at volumetric flowrates to the chip. FEP tubing (1/16" x 0.25mm, 10 metres) from the Syringe Pump Starter Kit (Part No. 3000335) is used to deliver the fluids across the system.



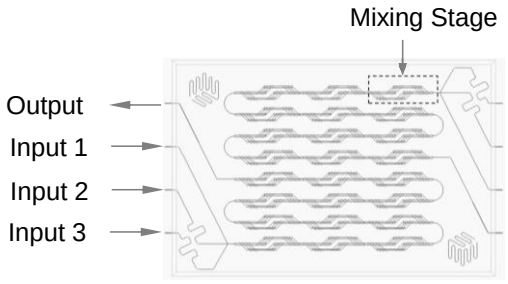
Schematic showing test system setup. Bright field imaging using a high speed magnification system was used to capture images. Numbers in parenthesis indicate FEP tubing (from Part No. 3000335) dimensions (ID in mm, Length in mm). OD is 1.6 mm.

A T-Connector ETFE (Part No. 3000397) splits the single FEP tube with the sodium hydroxide into two separate FEP tubes leading to the Linear Connector 4-way (Part No. 3000024). The reaction products exit the chip on the same edge as the input. A High Speed Camera and Microscope System (Part No. 3200050) was used for visualization of the Micromixer Chip.

Hotplate Adaptor – Chip Holder H (Part No. 3200111) with Chip Interface H (Part No. 3000155) and 2 Linear Connector 4-way (Part No. 3000024).

The chip interface is placed on a Hotplate Adaptor (Part No. 3200111) as shown above. The temperature was set to 25°C to supress any fluctuations in ambient temperature from causing transient effects in the mixing. The Hotplate is a digitally controlled hotplate with external Pt100 (thermocouple), which locates in hotplate adaptor for optimal control of reaction temperature. A maximum temperature setting ensures safe unattended operation. Temperature range is room temperature to +300°C.

Sodium hydroxide (aqueous) solution and phenolphthalein (organic) are used as the two fluidic components to be mixed. 0.1M NaOH (aq) at a pH of approximately 13, and 0.5% w/v phenolphthalein (60% methanol, 40% H_2O) are freshly prepared before the test. Their viscosities are similar to water (~1mPa·s). A further test involved varying the viscosity of one or both of the two phases by addition of glyrcerol. The viscosity of the glycerol solution was measured by comparing with pressure gradient driven pumping of pure water, and assuming Newtonian fluid flow ($\Delta P = 32\mu L\bar{u}/d^2$) with the change in flow rate (gravimetric test) arising solely out of change in viscosity.



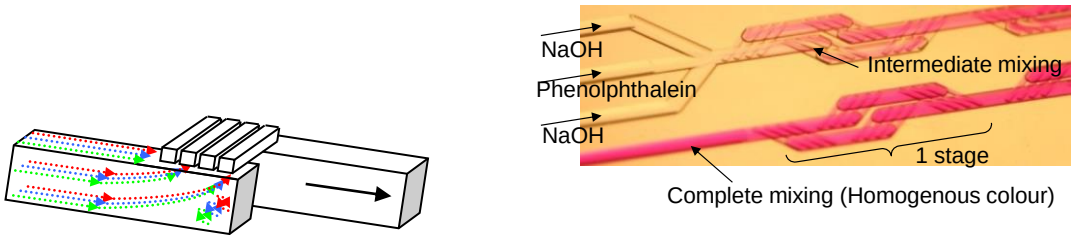
Chip gemoetry showing connections for using 1 mixing path.

The image above shows microfluidic device (chip) detail. The large channel is etched onto the lower piece of glass (125µm deep, 350µm wide), and the smaller ‘herring bone’ channels are etched onto the upper piece (50µm deep, 125µm wide). The device is fabricated using two pieces of glass. Dimensions: large channel 125µm deep and 350µm wide, small channel 50µm deep and 125µm wide.

With a low dead volume, maximum fluid recovery is ensured and accurate data is easily obtained. Furthermore, this micromixer chip provides high levels of visibility, which allows excellent access for optical inspection systems. A wide temperature and pressure range along with excellent chemical compatibility makes this microfluidic micromixer chip ideal for a wide range of microfluidic applications.

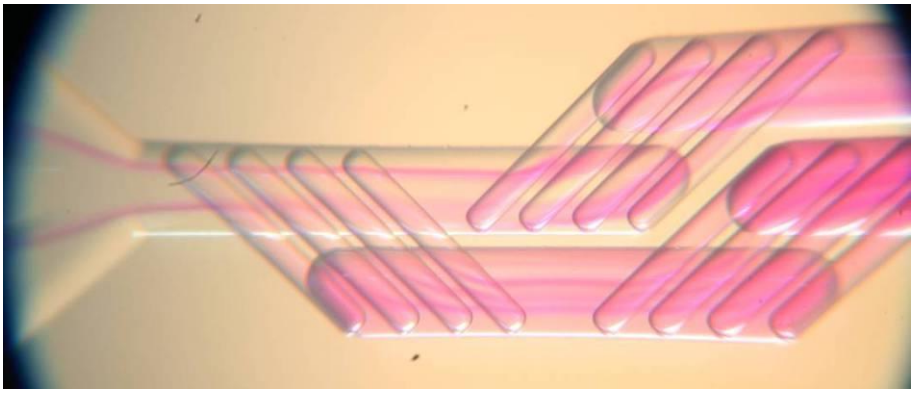
Test Results

The Mitos Micromixer Chip is tested by mixing phenolphthalein with sodium hydroxide (NaOH). The reaction causes the solution to change color to bright pink. The homogeneity in colour of the solution is used as indication of the level of mixing, with greater than 90% mixing indicated by completely uniform colour. A high concentration of NaOH was used to obtain a large colour change in the phenolphthalein. Despite the high pH value, no reactions were observed between the sodium hydroxide stream and the substrate.

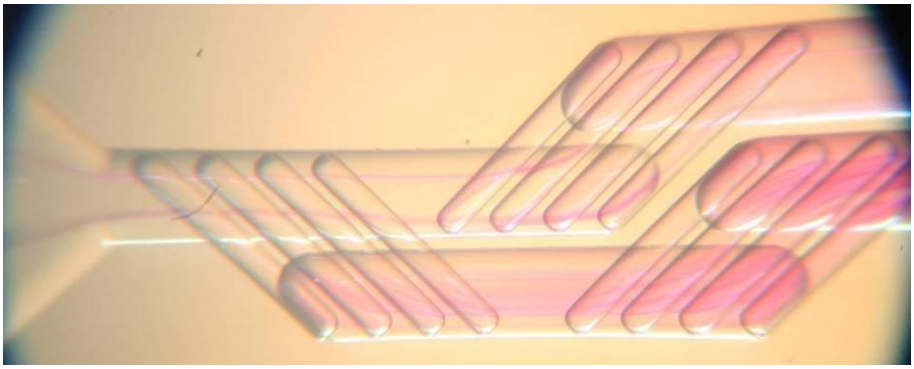


Left: A simplified schematic of the fluid flow path in the micromixer stage. Right: High magnification image at the junction and in the mixing stages of the Micromixer Chip. The image shows the different flows entering as clear fluids, and gradually changing colour to dark pink. This colour change represents mixing, and progresses at each subsequent mixing stage.

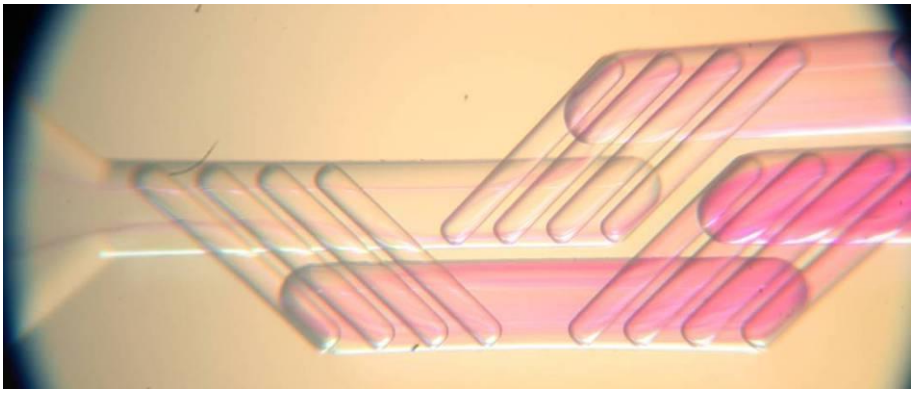
As the two colourless streams come in contact, both sodium hydroxide and phenolphthalein begin to inter-diffuse. As the mixing is exothermic, bubble formation is supressed by diluting the organic phase with water pre-use at 60% methanol and 40% water. The mixing ability of the device is quantified by optically determining how much of the phenolphthalein changes color during the mixing process.



The first stage of the 40µl/min flow with two low viscosity fluids. Note that the pink streak, which shows the reaction, quickly increases in width because of mass diffusion. Flow direction is from left to right.



The first stage of the 320µl/min flow with two low viscosity fluids. The reaction streak has less width than at 40µl/min because of less time for mass diffusion. Flow direction is from left to right.



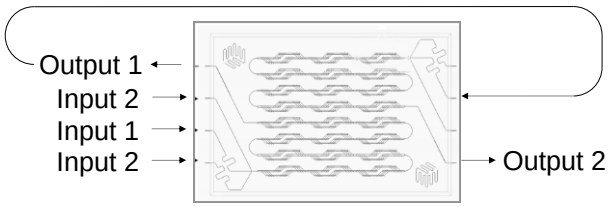
640µl/min: The first stage of the 640µl/min flow with two low viscosity fluids. The reaction streak has a smaller width than seen at lower flow rates but the amount of lamination has increased. Note the dead zone caused by the increased flow rate. Flow direction is from left to right.

At low flow rates, more mixing stages are required as diffusional mixing dominates. This effect increases as viscosity increases. The number of stages (and therefore the time) required for perfect mixing was counted at a variety of flow rates and viscosities, and the mixing time was calculated using this information.

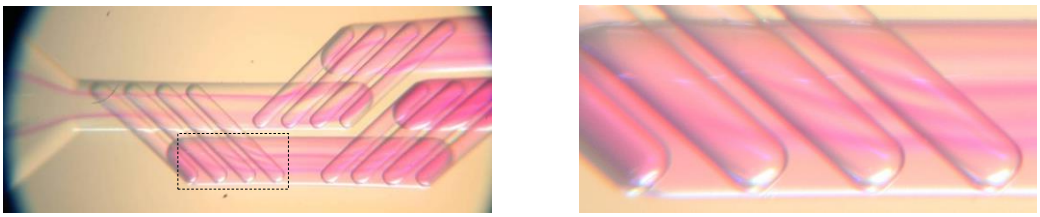
Mixing can also be evaluated by considering the uniformity of the intensity in the imaged fluid volume. The uniformity can be quantified by calculating the deviation of the pixel intensity values D_i in a given image from the maximum intensity value.

$$D_I = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(I_i - I_{max})^2}$$

A fully mixed channel will have $D_i = 0$. Photographs taken during the data collection process allow analysis of the mixing. Graphs where 12 or more stages are required for complete mixing indicate that the output from the 12 stage mixing chip would not be completely mixed. Options are to use a second mixer chip in-line, or re-direct the output back as input into the second mixer on the same chip as shown below. The number of stages required was calculated based on image processing data obtained from stage #12, and extrapolated.



Flowpath schematic for double mixing option – extending the mixing stages from 12 to 24.



Left: Low magnification view of the junction. Right: Magnified view of the region marked in the left image. Streaks are visible as having doubled in number from 2 to 4. 4 streaks are visible towards the right end of the image.

Note that the pink streak, which shows the reaction, quickly increases in width because of mass diffusion. The width of the pink streaks decreases with higher flow rates due to suppressed lateral diffusion.

Mixing occurs through the cumulative action of two phenomena.

- Mass diffusion – Mixing on molecular level

  This takes place whenever there is a concentration gradient. The rate of mass diffusion depends only on material properties, and temperature, not on flow conditions. Diffusive mixing is the dominant mixing method at lower flow rates, and is directly proportional to the surface to volume ratio.

- Advection – Generation of heterogeneous mixture

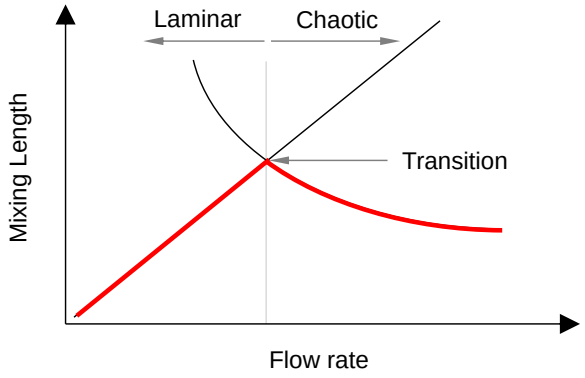
  Chaotic mixing is a type of advective mixing in which the fluid is displaced by intermixing. The rate of chaotic mixing is highly dependent on the flow patterns, and in general increases in effective as the flow swirls and moves about, and increases with the flow rate. Advective mixing greatly exceeds diffusive mixing as the flow rate increases.

An inlet feed of two adjacent concentrations enters. Left: Laminar flow and diffusive mixing – At low flow rates, the flow is split horizontally into 4 lamellae. The split streams are re-joined side by side. The widths of the lamellae decrease as they reshape to the minimum channel cross section. Right: Chaotic flow and advective mixing – at higher flow rates, swirling and turning of the flows result in marble-like mixing.

The compact mixing chip, uses splitting, stretching, and recombining flow to give an effective combination of diffusive and advective mixing. Above figures illustrate how the

Micromixer takes different layers of the fluid, separates them, and then merges them back together. A single mixing stage has a volume of approximately 0.35µL.

Most microfluidic chips have low flow rate, and therefore are dominated by laminar flow. In such cases, effects of advection on mixing are small in comparison to the effects of diffusion on mixing. However, in the case of the Micromixer Chip, the design incorporates features that reduce the laminarity of the flow. Chaotic advection takes over, the mixing length significantly reduces, and continues to reduce with increasing flow rates. This is marked as the point of transition, and is the basis for the reduction in the number of mixing stages required at higher flow rates.



Transition from laminar mixing to chaotic mixing causes a reduction in the mixing length, which is seen in the lower number of mixing stages required for complete mixing. The red line indicates the mixing strategy followed.

In the schematic illustrated above, the mixing length is plotted as a function of the total flow rate of the fluids in the mixer chip. This is a sum of the flow rate of the two fluid components. The minimum length to complete diffusion of a simple microfluidic reactor assuming laminar flow is directly proportional to the flow rate, and inversely proportional to the diffusivity.

Tests Conducted

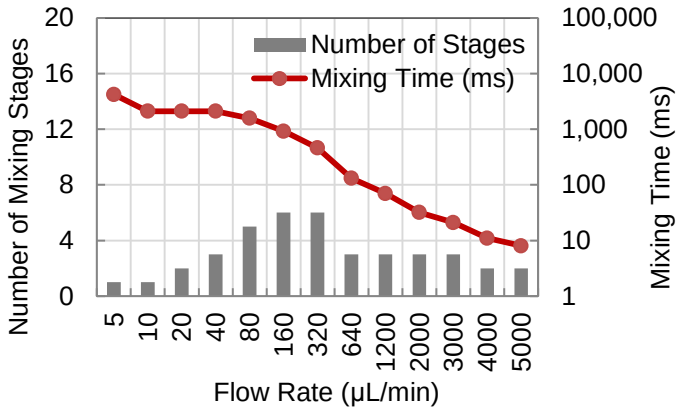
Test matrix to study the effect of changing viscosity on mixing time. NaOH and Phenolphthalein are the two fluids to be mixed. Their viscosities are changed by addition of glycerol.

	μ_{NaOH}	$\mu_{Phenolphthalein}$
Test 1	1 ×	1 ×
Test 2	5 ×	5 ×
Test 3	5 ×	1 ×
Test 4	1 ×	5 ×

The table shows the viscosities of the two fluids relative to that of water (μ_{Water} = 1 cP).

Test 1: Low viscosity NaOH, low viscosity Phenolphthalein

The above graph represents mixing of the two fluids NaOH and Phenolphthalein at equal flow rates. The graph shows the total flow rate and its effect on the mixing time. The mixing time is observed to reduce exponentially – the secondary axis on the right is logarithmic.

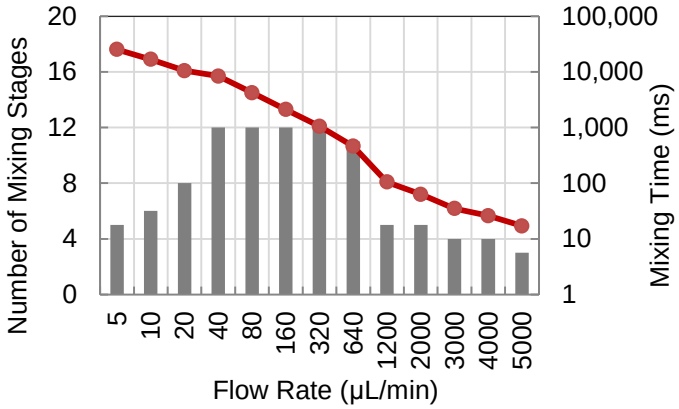


$Q_{NaOH} = Q_{Phenolphthalein}$. $\mu_{NaOH} = \mu_{Phenolphthalein} = \mu_{water}$.

The bars in the graph indicate the number of stages of mixing required to fully mix. For example, at 320 µL/min, 6 mixing stages are required. As the chip has 12 mixing stages, the fluid that exits the chip is fully mixed. The flow rate of 320 µL/min also represents the inflection point in the trend of the phenomenon, beyond which chaotic mixing dominates, and prior to which diffusional mixing dominates. It should be noted that microfluidic devices are typically confined to diffusional mixing.

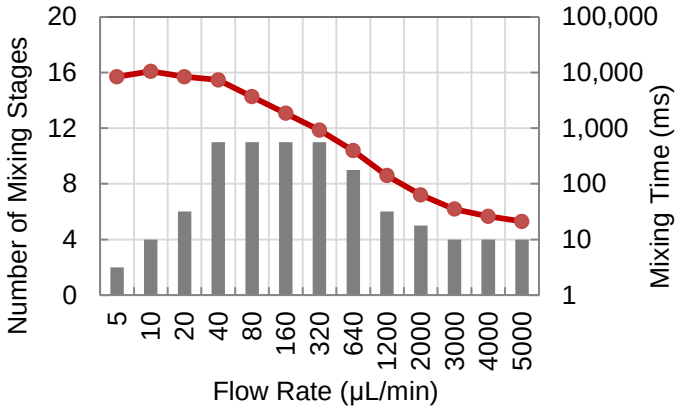
Test 2: High viscosity NaOH, high viscosity Phenolphthalein

The viscosities of both phases were then raised by adding glycerol. The outcome of added glycerol is an increased viscosity. Since the flow is driven by a positive displacement XS-Pump, the increased viscosity has little effect on flow rates. The larger impact is on the mixing, which greatly reduces. The bars representing flow rates of 40, 80, 160 and 320 indicate that the fluid exiting the chip is not fully mixed. In such a situation, it would be beneficial to redirect the output into the second on-chip mixing path, and utilize the additional 12 mixing stages, effectively treating the fluids to a total of 24 mixing stages.



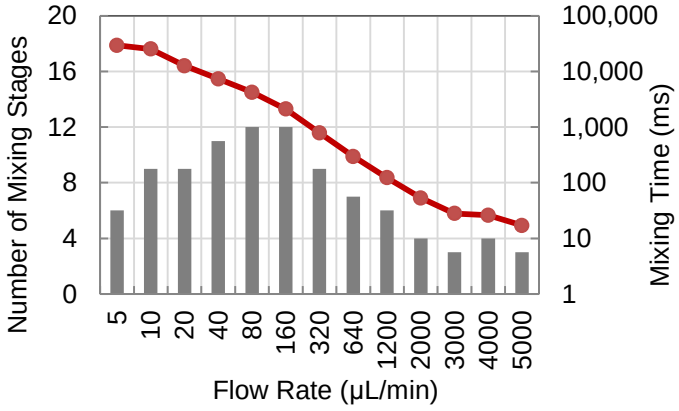
$Q_{NaOH} = Q_{Phenolphthalein}$. $\mu_{NaOH} = \mu_{Phenolphthalein} = 5 \times (\mu_{water})$.

Test 3: High viscosity NaOH, low viscosity Phenolphthalein



$Q_{NaOH} = Q_{Phenolphthalein}$. $\mu_{NaOH} = 5 \times (\mu_{water})$; $\mu_{Phenolphthalein} = \mu_{water}$

Test 4: Low viscosity NaOH, high viscosity Phenolphthalein



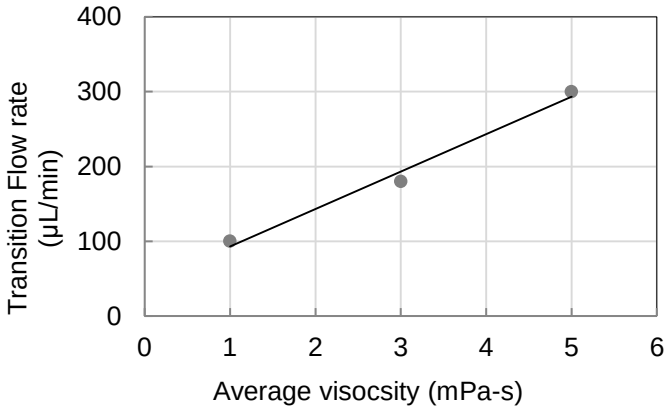
$Q_{NaOH} = Q_{Phenolphthalein}$. $\mu_{NaOH} = \mu_{water}$; $\mu_{Phenolphthalein} = 5 \times (\mu_{water})$

In Tests 3 and 4, the number of stages of mixing required is intermediate to Tests 1 and 2. This is expected since the fluid flow rate has a linear dependence on the fluid viscosity.

Transition from Laminar to Chaotic Flow

The transition point is identified by a situation wherein mixing transits from diffusion dominated to advection dominated. This transition is dependent on geometric as well as material properties.

When testing the Micromixer Chip, while the geometry is unchanged, the dependence of the transition point on the fluid viscosity is observed to be linear in the range of flow rates studied. This is depicted in the graph below.



Average viscosity dependence on transition of flow from laminar to chaotic.

Viscosity of fluid mixtures is usually calculated in logarithmic relation to constituent molar ratios. Here, for simplicity, a mathematical average is used. As the viscosity of the fluids increases, the laminar flow persists at higher flow rates. At very high viscosities, the larminar flow regime would be expected to dominate, and diffusion would be negligible, with folded but unmixed fluids exiting the chip.

The lower transition flow rates imply a more favourable mixing outcome. At low viscosities, the transition is at much lower flow rates. This has implications on mixing of gas flows, which are equally relevant for the Micromixer Chip.

Conclusion

The Mitos Micromixer System is shown to be effective at mixing fluids at a wide range of flow rates (50 - 1000µl/min), and at viscosities which reasonably span fluids for most practical applications. The microfluidic mixing strategy used here exploits chaotic advection. Laminar flows that exhibit seemingly random and chaotic particle trajectories are used to mix multiple fluid streams relative to regular laminar flow, in which fluid particles largely move in a single direction, these chaotic flows exhibit more three-dimensional particle motion. As a result, the flow field being sufficiently three-dimensional, with secondary flows stretching and folding the fluid, greatly increasing the interfacial area across which diffusion occurs.

Flow visualization experiments confirm that the three-dimensional serpentine channel mixes significantly better than a similar straight channel for the Reynolds numbers studied here from 1 to 500 (relating to velocities range of 20 mm/s – 10m/s or flow rates of 7.5 – 3750 µL/min). The experimental results confirm the benefit of chaotic advection in the serpentine micromixer.

The Na0H and phenolphthalein with similar viscosities to that of water at an average flow rate of 110µl/min, perfect mixing was achieved in about 2 ms (12 stages). Testing with fluid viscosities 5x that of water (~5mPas) at an average flow rate of 640 µl/min achieved perfect mixing in 0.459 seconds. Equal flows of Na0H and phenolphthalein at a normal viscosity (0.6 mPa·s methanol, 0.9 mPa·s water) were always perfectly mixed before reaching the end of the chip.

Increasing the viscosity reduces the effectiveness of each mixing stage, so that more stages are required to achieve perfect mixing. When the viscosity of NaOH, phenolphthalein or both was increased to approximately 5 mPas by adding glycerol, the number of stages increased and at the mid-range flow rates incomplete mixing was observed. Higher viscosities also required a higher flow rate to enable mixing.

The results shown here were conducted using steady flow, which is a conservative case when compared with unsteady flows.

The system was found to be easy to use, with each component easily accessible for inspection. Further, the accessibility enables integration of process as well as additional diagnostic elements across the system.

The Mitos Micromixer System is ideal for applications such as reaction kinetics, sample dilution, improved reaction selectivity, rapid crystallisation and nanoparticle synthesis, as well as biological procedures such as cell activation, enzyme reactions, immunoassays, DNA hybridization and protein folding.

APPENDIX A: System Component List

Part No.	Part Description	#
3200401	Micromixer Chip	1
3000024	Linear Connector 4-way	2
3000155	H Interface	1
3200057	Mitos Duo XS Pump	2
3000252	Syringe for Mitos Duo XS Pump, 1ml	2
3200050	High Speed Camera and Microscope System	1
3000056	Plug FEP (Pack of 10)	1
3000397	T-Connector ETFE	1
3200111	Hotplate Adaptor - Chip Holder H	1
3000335	Syringe Pump Starter Kit	1

Optional Extras:

Part No.	Part Description	#
3000222	Hotplate 110	1
3000223	Hotplate 230	1

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