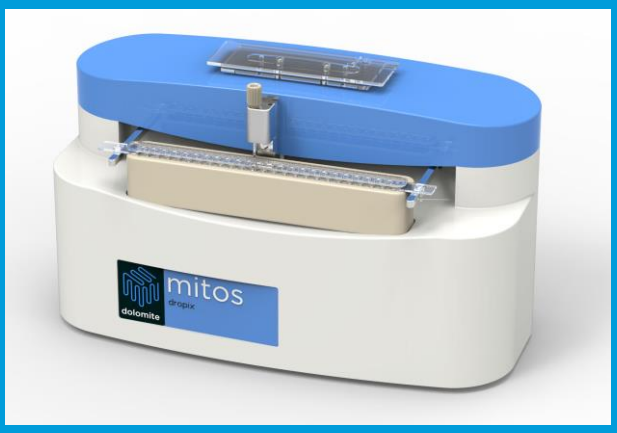
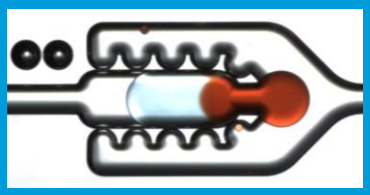
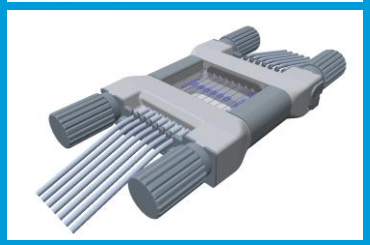
Droplet on Demand Generation and Pairwise Merging

Mitos Dropix® Droplet Merging System







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Summary

This application note demonstrates the ability of the Mitos Dropix® Droplet Merging System to reproducibly generate and subsequently merge droplets from tens to hundreds of nanolitres with a wide range of volume ratios. The amount of one component of a mixture relative to that of all other components $V_r/(V_r+V_b)$ $V_b + V_r = V_r+V_b$, is controlled by generating a pre-merger droplet sequence of suitably selected volumes. The novelty lies in the ability to generate and merge droplet pairs of typically tens of nanoliters in volume. There exists no such comparable commercially available automated continuous flow fluid handling systems. In tests, we show:

- Merging phase diagram – Droplet merging is parametrically investigated to establish effects of flow rate, and droplet volumes on the success of merging. Higher flow rates (10 – 20µL/min) mean a narrower size range of merged droplets (50 – 125 nL), and vice versa (40 – 200 nL for 5 – 10µL/min).
- Production of a concentration gradient – A sequence of 100 droplet pairs is generated, where the volume ratio of $V_r/(V_r+V_b)$ is 25% at the front of the sequence, and 75% at the rear of the sequence. The total volume of V_r+V_b is held constant at 200nL. Merging pairwise leads to a sequence of 100 individual droplets displaying a continuous color transition from blue to red.

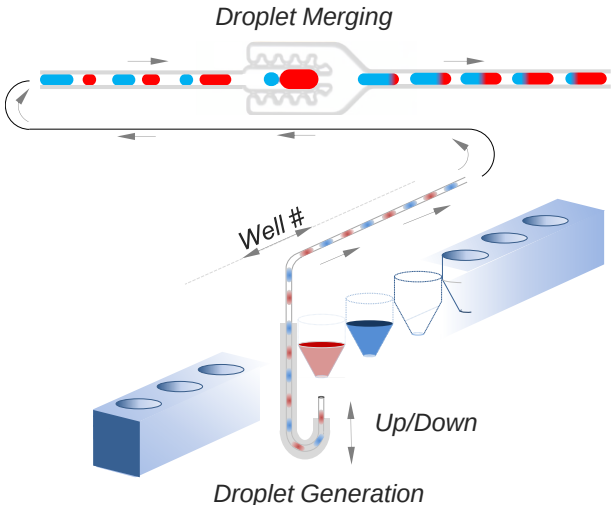


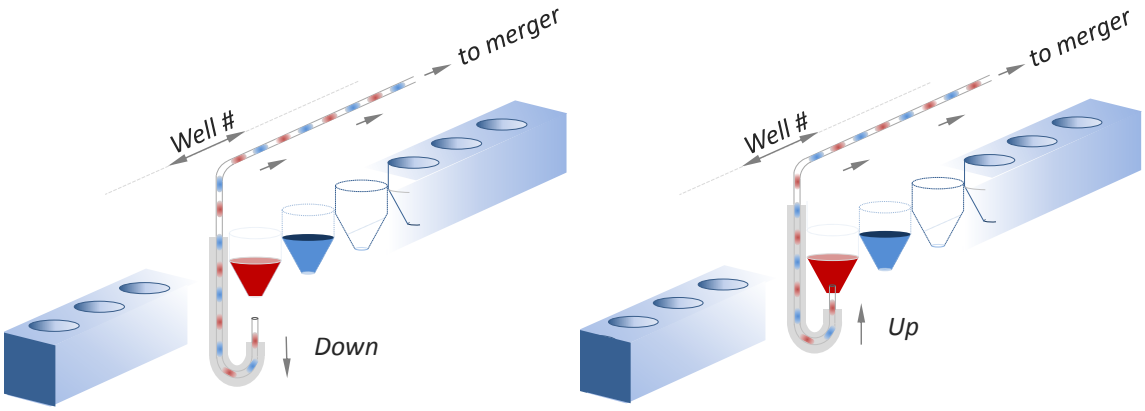
Illustration of droplet-on-demand and droplet merging of red and blue colored aqueous droplets using a Mitos Dropix® System.

The concept can be extended more generally for example: a sample and a diluent could be combined in varying proportions to create a concentration gradient for dose-response testing; a sample droplet and a test droplet could be combined to generate a data point for a screening program; or reagents A_1 to A_n could be combined with B_1 to B_n in different ways to create a combinatorial library. When used in this way, the Mitos Dropix® system can perform a wide range of user applications at nanoliter volumes, including:

- Automated screening experiments (sample/screen pairing)
- Dose-response testing (sample/diluent pairing to deliver to screen)
- Concentration/Stoichiometric testing (variation of mix ratio)
- Combinatorial chemistry (A_nB_n reagent pairings)

Mitos Dropix Technology

The Mitos Dropix® enables droplet-on-demand fluid handling. It is useful to create user-defined droplet libraries on demand, using a rack of up to 24 liquids, all loaded onto the Mitos Dropix®. Any combination of droplet count, sequence, and droplet volume (ranging from 10nl to 1µl) can be generated and delivered at up to 5 droplets per second.



Droplet on demand – a constant suction driven flow results in segmented flow depending on whether the J-hook is in 'up' or 'down' position and on the transverse position of the hook.

Surface tension and buoyancy are key physical properties made use of in the design of the fluid sample wells. The aqueous sample fluid (shown in the above schematic as red and blue) is contained in a polymeric sample strip with a hole at the bottom. The sample is prevented from falling out of the bottow by the presence of a denser heavier fluorocarbon oil. The buoyancy and the surface tension keep the aqueous sample afloat in the sample well.

A constant flow in withdrawl mode – using a syringe pump – is set up. The J-hook moves up and down, as well as from one well to another in the transverse direction. Depending upon the position of the hood, and given the fact that it is also aspirating fluid, a segmented flow is generated. When the hook is positioned in the sample well, red colored water is aspirtated into the tubing, and as the hook moves down, the fluorocarbon oil is aspirated. Therefore, when creating a droplet sequence, the hook samples the sample wells, and in moving between wells aspirates the separating oil in the down positions. The timing of the rise and fall of the hook characterizes the size of the droplets and the spacing between.

In addition to droplet production, merging (also called Droplet fusion) is a critical operation for droplet manipulation. It allows the combination of different reagents at a controlled time and location. This pairwise merging capability allows controlled initiation of a wide range of processes in microfluidic devices, ranging from simple mixing or dilution to more advanced chemical reactions or biological interactions. Many applications would benefit from a more advanced capability: the ability to combine a sequence of droplets in a controlled manner. This application note demonstrates the ability of the Mitos Dropix® system to carry out these operations, by creating a droplet sequence and merging successive pairs of droplets.

How can droplets be merged?

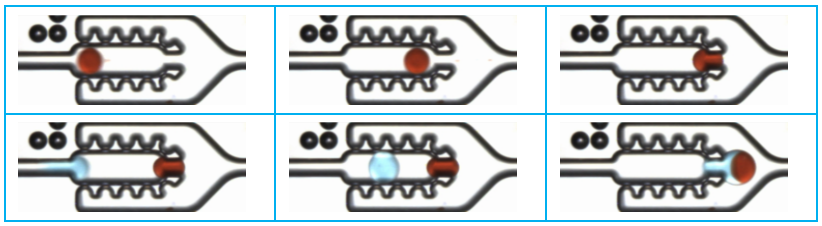
Active Merging

One option is to use *active* merging techniques such as electrocoalescence, which cause merging by disrupting the droplet surface with an electric field. These methods have some benefits, but are also complex and costly, or sometimes harsh and intrusive for the constituents contained inside the droplet.

Passive Merging

Passive merging with the Mitos Dropix®, using just the motion of the droplets within the channel to induce merging, offers a low cost, simple, and compact approach. However, simply initiating droplet collisions is usually insufficient to cause fusion between droplets. Efficient merging of surfactant-stabilized droplets presents a further challenge, as the surfactant is designed to prevent unwanted merging. (Unwanted droplet merging is often called coalescence). Careful control of the approach and interaction of the droplets is therefore required to destabilize the interface and give reliable merging. The Droplet Merger Chip is based around a proven concept where a channel expansion depletes the continuous phase between adjacent droplets.

The Droplet Merger Chip is a glass microfluidic device designed for the continuous merging of droplets. Fabricated by a wet-etch lithography method at Dolomite's micro-precision glass foundry utility, it provides micrometer resolution features limited to nanometer scale surface smoothness.



Time lapse sequence of images representative of droplet merging.

The leading red colored droplet enters merging section and slows to a halt due to the design. A trailing blue colored droplet enters merging section, impacts the red droplet and merges. The resultant significantly larger droplet is then 'pushed' by the clear separating carrier fluid out of the merger section, downstream. A detailed description of the underlying physics and operating principle is described in a paper (X. Niu, S. Gulati, J. B. Edel and A. J. deMello, 2008) authored by the scientific collaborative partners*.

* Mitos Dropix was developed by Dolomite under exclusive sub-licence with Drop-Tech Ltd. having won Dolomite's 2012 Productizing Science® competition. Drop-Tech was formed from an academic collaboration between Cambridge University and Imperial College London and is the exclusive licensee of their patented droplet generation technology used in Mitos Dropix **(Patent Pending: PCT/GB2013/051668).**

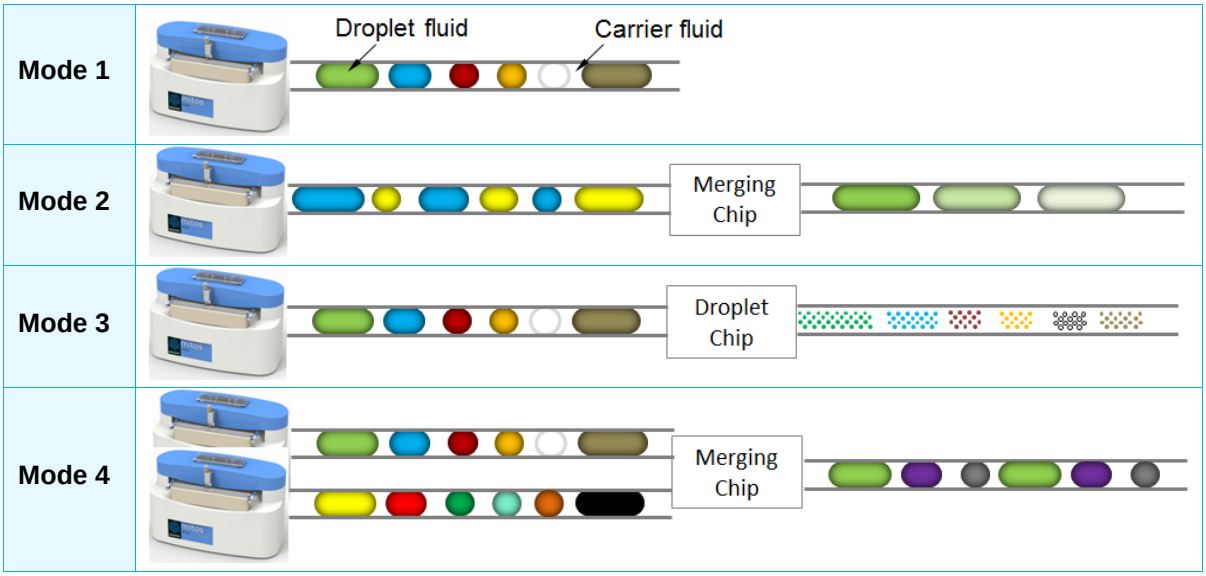
Modes of Operation

The ability to create such a droplet sequence is termed **Mode 1** operation of the Mitos Dropix®. Droplet libraries are used for a wide range of applications, including high throughput screening in synthetic chemistry, fundamental biology and pharmacology. This is demonstrated in the application note on droplet-on-demand sequencing.

Sequence generation and pairwise merging is termed **Mode 2** operation of the Mitos Dropix® system. This application note focusses on Mode 2, and presents experimental results in greater detail.

Mode 3 of operation involves sequence generation and "explosion" into picoliter daughter droplets. This extends the working volume per droplet from the nanoliter to the picoliter range using the Mitos Dropix® in combination with flow focussing geometry.

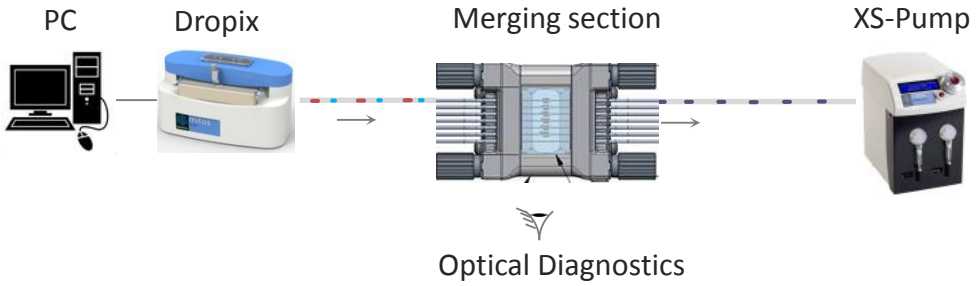
Mode 4 involves advanced sequence generation and combination using multiple Mitos Dropix® useful for situations where more than 24 different reagents are required to be handled simultaneously.



The 4 suggested modes of Mitos Dropix® operation.

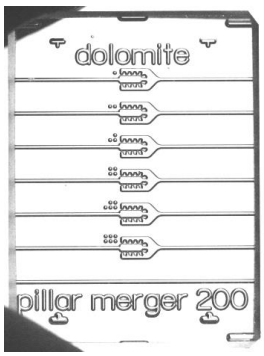
Test Setup

The system setup is shown in the figure below. Fluidic connections between the Mitos Dropix® (Part No. 3200350), Droplet Pillar Merger Chip (200µm etch depth), fluorophilic (Part No. 3200378), the Dropix® Sample Hook (Part No. 3200353, 3200355) and the Mitos Duo XS-Pump (Part No. 3200057) are made using FEP tubing of OD 0.8mm and ID 0.25 mm (Part No. 3200302). Flangeless Ferrule 0.8mm, ETFE (Part No. 3200306) and End Fittings and Ferrules for 0.8mm Tubing (Part No. 3200307) are useful for making connections with the pump. Visualization was achieved using a High Speed Camera and Microscope System (Part Number: 3200050).



Schematic showing assembly of droplet merger setup. A total length of 1500mm of 0.25mm ID FEP tubing connects the Mitos Dropix ® to the interface, and to the interface to the pump.

Two sample wells on the Sample Strip (3200351) are loaded with 50µL aqueous samples made by diluting red and blue food coloring respectively. The Dropix® Fluid Reservoir - PMMA (Part No. 3200414) was filled with fluorinated oil. The Dropix® was connected to a Mitos Duo-XS Pump which aspirated the droplets and carrier fluid into the Droplet Merger Chip (Part No. 3200378). The XS pump, due to its dual syringe function, has the added benefit of constantly refilling the carrier fluid bath during operation, thereby maintaining a steady fluid level.



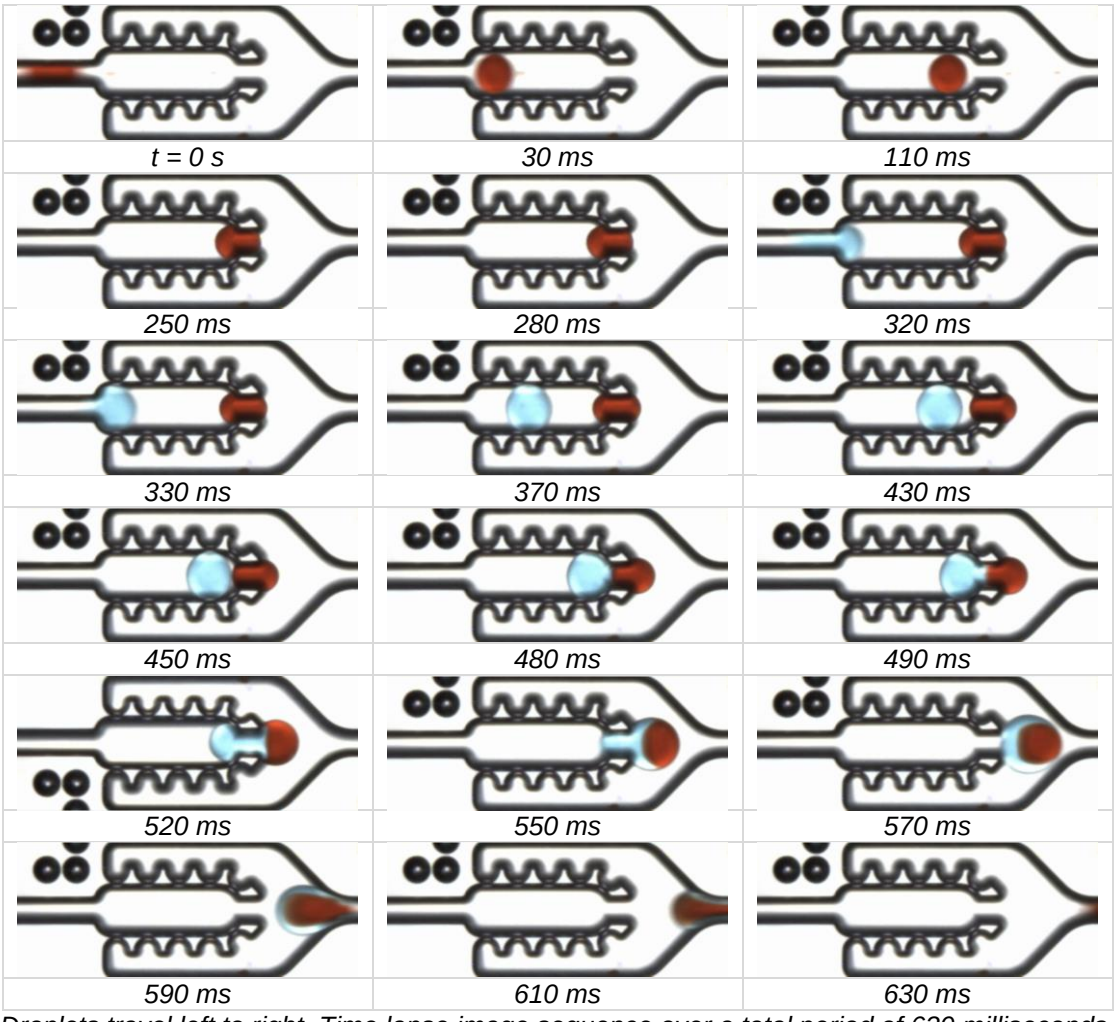
Detailed image of droplet merger chip showing 6 numbered merging sections, and 1 pass-through observation channel. Channel depth is 200 µm, fluid flow is from left to right.

The Droplet Pillar Merger Chip (200µm) is a glass microfluidic device designed for the continuous pairwise merging of droplets. The Droplet Pillar Merger Chip is designed to fit into the H Interface 7-way (11.25mm) (Part No. 3200379) with two Linear Connectors 7-way microchannel (Part No. 3200290). This connector uses micro alignment pins to ensure accurate alignment of tubing to channel. Six independent merging junctions on the chip are provided to allow long life or use of multiple parallel merging processes.

FC-40 oil is a highly non-polar perfluoro-tri-*n*-butylamine having average molecular weight MW 650 g mol^{-1}, kinematic viscosity ν 1.8 cSt and density ρ 1850 kg m^{-3}. The FC-40 oil was supplemented with surfactant in varying percentage. DuPont™ Krytox® 157, with a polar fluorinated molecular structure, was added in varying concentrations (0.0001% (v/v), 0.001%, 0.01%, 0.1%, 1%) to the carrier FC-40 as a dynamic lubricating agent to further reduce contact angle.

Results

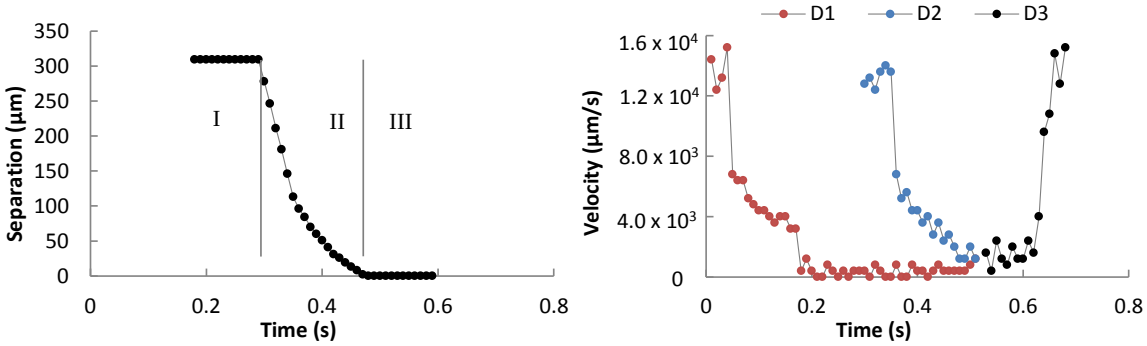
A sequence of droplets of alternating blues and reds was generated, separated by the clear carrier oil. These travel in tubing from the Dropix® unit towards the merger chip, aspirating in the direction of the syringe pump. On arrival at the merger chip, they merge pairwise, and are transported further downstream. During the merging process, a video recording of the merging provides spatial and timescale information. This information is used to investigate the actual merging process between and red and a blue colored droplet.



Droplets travel left to right. Time lapse image sequence over a total period of 630 milliseconds, showing two consecutive droplets merging. A 30nL sized red leading droplet mixes with a 40nL sized trailing blue droplet resulting in a 70nL sized purple droplet. Time stamps are not equally spaced, but instead capture significant instances during merging.

As the leading droplet enters the expansion volume, it is squeezed between the sets of pillars. Once the droplet has completely entered the expansion volume, continuous phase behind the droplet is allowed to drain around the rear of the droplet into the widened channel via the pillars. The droplet is stopped in the pillar array due to the increase in surface tension it experiences. As the pillars narrow, the radius at the front of the droplet becomes smaller than the radius at the back of the droplet, which produces a net surface

tension pressure that counters the hydrodynamic pressure induced at the other end of the droplet by the continuous phase. The droplet is now held indefinitely until the next droplet in the line approaches – this feature decouples droplet fusion from inter-droplet spacing dependency. The leading droplet remains in the trap until a second trailing droplet arrives. As the continuous phase drains from between them, the two droplets contact, and under optimum conditions fuse. The reduced drainage area for the carrier means an increased hydrodynamic pressure. When a sufficiently large number of pillars are blocked off, the increased hydrodynamic pressure competes with the retarding net surface tension pressure. When this balance of pressures changes, the merged droplet is pushed out of the merger section and flows downstream. A more detailed description of the underlying physics is presented in the Appendix.



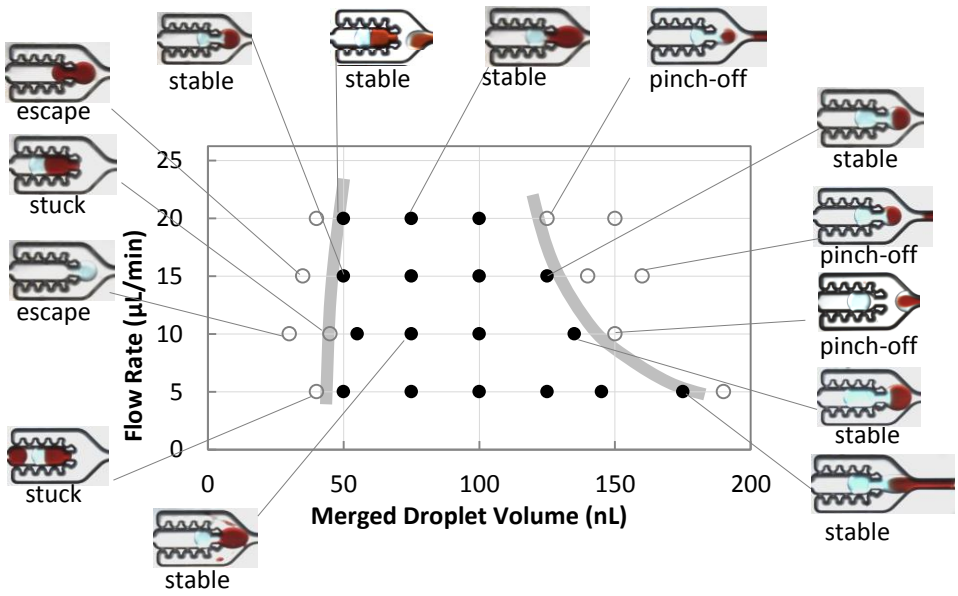
Left: Progressively decreasing spacing between two droplets, leading to merge. Right: Velocities of two approaching droplets as well as the merged droplet tracked over the merging period.

The above figure attempts to capture the dynamics of the merging process by tracking the leading interfaces of all droplets involved. The leading drop (D1) enters the merging chamber, decelerates during expansion, travels till the end of the merger section and finally comes to rest. It then effectively 'waits' for a subsequent trailing droplet (D2) to enter the expansion zone. The leading edge of D2 then pushes at the tail of D1 (at ~ 510 ms). During this interaction, the interfaces between the two droplets are disturbed via drop deformation, and merging occurs.

The figure plots the inter-droplet separation as a function of time. The transport dynamics has three distinct stages. Stage I is indicative of droplets travelling in tubing, where the inter-droplet spacing is constant. Stage II indicates the point at which the leading droplet enters the merging zone. It slows down on entering the expansion zone, travels at a lower constant speed to the end of the merging zone, and stops. Subsequently, the trailing droplet catches up, reducing the inter-droplet separation to zero. The merged droplet continues past the chip, and into the outlet tubing indicated by Stage III.

Merging Phase Diagram

A parametric study was performed by varying the mass flow rate *Q* against the droplet volumes of the leading and the trailing droplet. The parametric space is divided into three areas according to the droplet merging observed. It can be seen that as *Q* increases more side channels are needed to decrease release pressure and therefore the total volume of droplets that can be merged in the chamber reduces.



Various outcomes of the merger chip depending on flow conditions – Surfactant concentration 0.01% (v/v) *Krytox 157 in FC-40. Open circles represent failed merges, closed circles represent successful merges.*

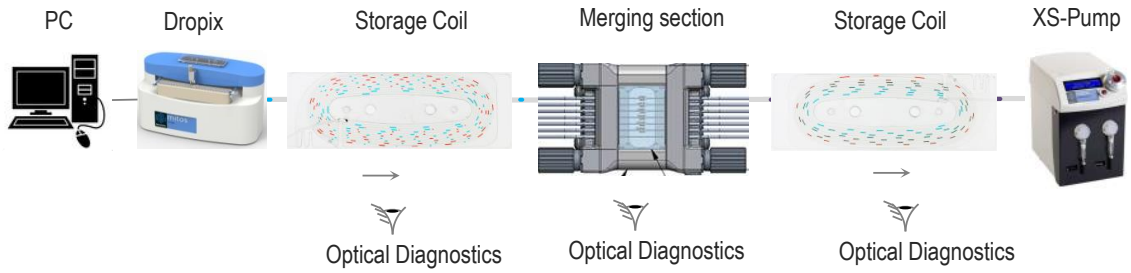
The grey lines in the above graph represent the change in operating regime into three zones – escape or stuck zone, stable merging zone, and pinch-off zone. These are described below. These bounding lines are dependent on fluid properties as well as merging chip geometry.

- Stable merging – This is the desirable performance zone. The leading droplet is temporarily stopped in the merging section. As the trailing droplet impacts, the interface merges. The combined volume of the merged droplet offers increased hydrodynamic pressure, overcoming the holding pressure due interfacial tension, thereby releasing the merged pair.
- Escape or Stuck – When droplets are too small, or if the flow is too high, the surface tension effect is too feeble compared with the inertial effects, resulting in loss of holding force. The leading droplet escapes before merging. At very low flow rates, interfacial tension effects dominate. Inertial effects which are responsible for the release of the merged droplet are too small to overcome the interfacial tension. Therefore, all droplets continue to merge, while simultaneously, flow focussing based pinch-off occurs downstream of the merging section.
- Pinch-off – The merged droplet is liable to split by pinch-off effects due to flow-focussing effect of the carrier fluid, especially for large droplets.

The overall results show that merging can be achieved over a useful working range, from approximately 50nl to 125nl total merged volume. If we assume droplets are spaced with an equal amount of the carrier phase, then this corresponds to a total fluid volume of 100nl to 250nl. At the maximum flow rate of 20ul/min, the peak volume of 0.25ul therefore corresponds to a sample throughput rate of 20/0.25 = 80 merging operations per minute, and the minimum volume corresponds to 20/0.1 = 200 merges per minute. The merging chip is therefore well matched to the Dropix® operating speed.

Concentration Gradients

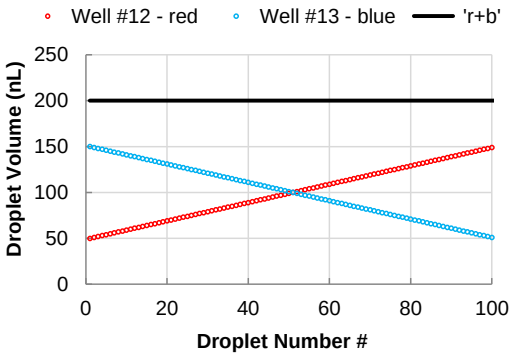
In a different experiment, it was sought to generate concentration gradients by merging a red droplet with a blue droplet in stoichiometrically varying proportions. The total merged droplet volume was sought to be held constant. The setup was slightly modified with the only change being the addition of two Droplet Storage Coils (3200349). As shown below, these were positioned between the Dropix® and the merger chip, and between the merger chip and the pump respectively. The objective was to be able to view droplet sequences before and after merging. The storage coil uses FEP tubing of OD 0.25mm which being semi transparent allows optical detection.



Setup for generation and imaging of concentration gradients.

The flow rate was set to withdrawl mode at 5 µL/min. Krytox® 157 at a concentration 0.0001% (*v*/*v*) was added to the FC-40 used in the Dropix® bath. A 200 droplet sequence is requested. These are merged pairwise to obtain 100 merged droplets. Spreadsheet software was used to create the table, which was then saved in 'csv' format and imported as a datafile into the PC control software.

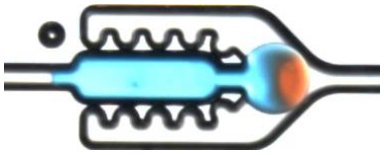
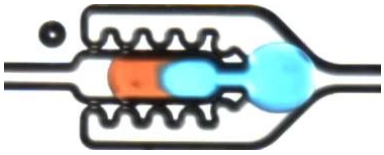
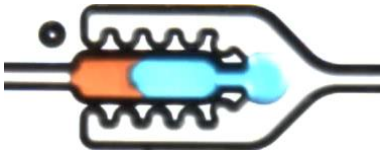
Step #	Well #	Aq. vol. (nL)	Oil vol. (nL)	Quantity
1	13	150	50	1
2	12	50	500	1
3	13	149	50	1
4	12	51	500	1
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
198	12	149	50	1
199	13	51	500	1
200	12	150	50	1
201	13	50	500	1



Left: Droplet sequence requested in the PC-control program. Right Graphical representation of evolution of droplet volume over droplet number. The blue and red datapoints indicate the volume of the blue and red droplets pair repsetively.

While the volume ratio of the red and blue change over time, the total volume remains constant.

Images recorded at select time stamps are shown in the below table. Initially while the ratio between red and blue in the merged droplet is small, it progressively increases with time.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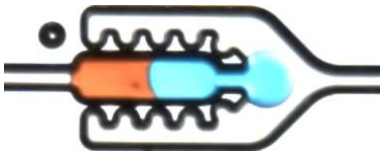	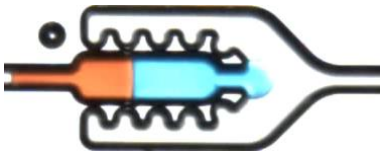	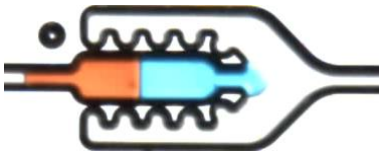
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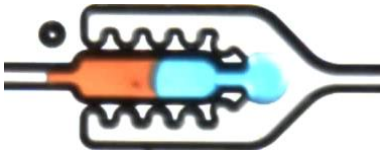	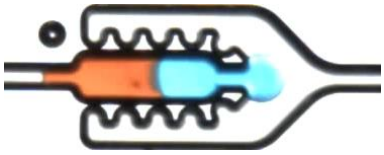	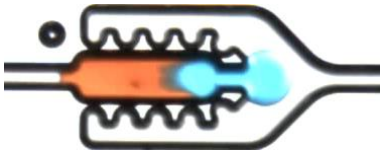
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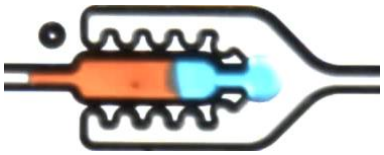	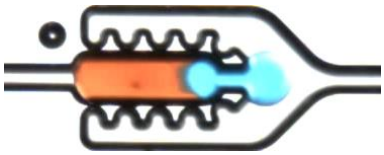	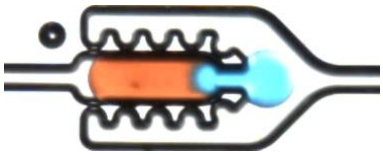
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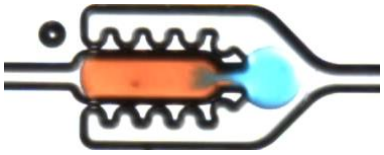	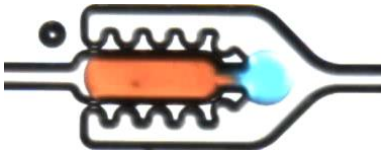	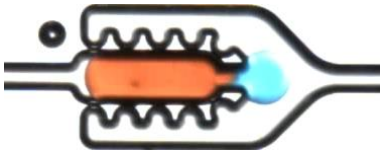
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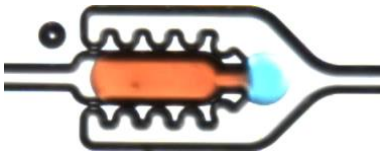
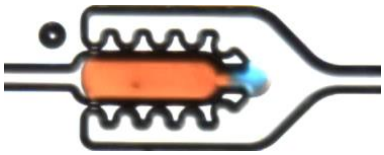
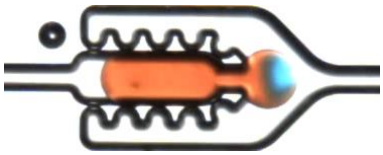
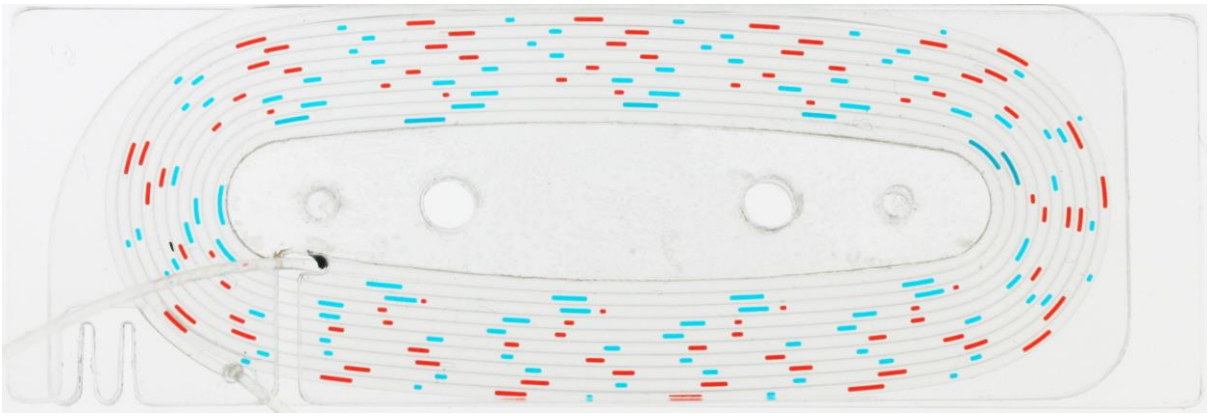
		
08:21.200	08:39.799	08:45.799

Time stamp – mm:ss.millisecond.

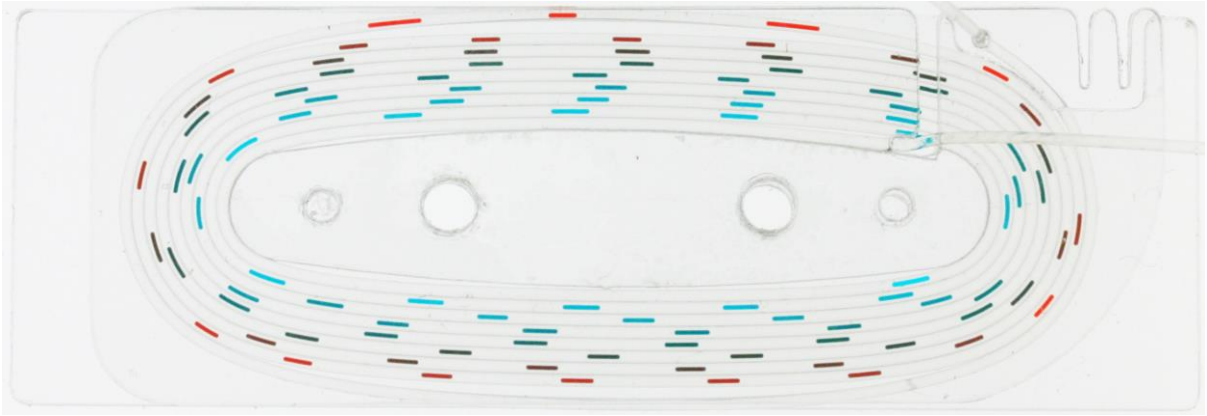
Merged droplets are imaged with direct brightfield imaging. In both storage coils shown below, the fluid inlet is on the inside of the spiral, and the outlet towards the outside. Droplets travel counter-clockwise and outwards.

The innermost droplets of the spiral lead the sequence whereas the outermost droplets are the trailing end of the sequence. The sequence consists of 200 droplets with relatively larger blues and smaller reds at the start, and smaller blues and larger reds towards the tail end.



Storage coil with 100 pairs of red and blue droplets.

The innermost droplets of the spiral lead the sequence whereas the outermost droplets are the trailing end of the sequence. The sequence consists of 200 droplets with relatively larger blues and smaller reds at the start, and smaller blues and larger reds towards the tail end.



Storage coil with 100 merged droplets. Colors range from red to blue with a total of volume 200 nL, generated out of two sample wells.

Conclusion

The Mitos Dropix® system is tested in Mode 2 operation. A sequence of droplets in generated, and then merged pairwise. The system is found to be easy to setup and reliable to use over a range of droplet volumes.

Pairwise merging is seen to occur within a time frame of approximately 0.5 seconds or less. With consideration to the merging time-frame, the frequency therefore with which droplets enter the merging section and end in a merged state, is up to 1-3 Hz, compatible with the Dropix® droplet generation rate of 1 to 5Hz per droplet, or 0.5 to 2.5Hz per pair of droplets.

The success rate of merging is found to be slightly dependent on the flow rate, and more significantly dependent on the surfactant concentration. Only the final volume of the merged droplet appears significant in assessing merging efficiency. At low flow rates of 5 µL/min, the total volume of droplet pairs that can be merged varies from 50 to 175nL. At higher flow rates, the range of droplet pair volumes that can be efficiently merged varies from 50 to 125nL.

The minimum volume appears to be largely independent of the flow rate. The maximum volume has a strong inverse relationship, whereby higher flow rates are detrimental to merging. This is expected and in line with the physical mechanism of merging in the Appendix. Increasing flow rate increases inertial effects. Interfacial effects are dependent on the chip geometry and the surfactant concentration.

The surfactant concentration in FC-40 was varied between 0.0001% and 1.0%(v/v). Higher surfactant concentration was found to over-stabilize the emulsion and supress merging. Lower surfactant levels were found to interfere with the hydrophobicity of the chip. It is likely that the surfactant concentration will depend upon additional reagents in the aqueous phase.

Based on the results observed in parametric tests, the operating space was mapped, and three distinct zones were observed. Stable merging was observed for a set of conditions, and bounding limits were recorded, outside which droplets failed to merge. Thereafter, the effect of surfactant was tested, by adding in varying concentrations. Droplets are shown to contact and merge within a period of milliseconds. The range of droplet sizes that the merging chip can successfully accommodate varies from a minimum of about 40nL, and up to a maximum of 175nL. The expanse of this limit reduces are higher flow rates, and increases at lower flow rates.

In another test, starting with a sequence of 200 droplets, pairwise merging was executed to end with 100 merged droplets. The initial sequence was produced from two samples wells, each loaded with 50µL of water distinguished by the addition of red and blue colored food dye. The initial sequence further consisted of varying sizes of droplets. Towards the start of the sequence were pairs of blue and red droplets of volumes 150nL and 50 nL. Towards the end of the sequence, the droplets transitioned to 50nL and 150nL respectively. When these merged, the final sequence consisted of droplets 200nL in volume with a continuous color transition from blue to red, thereby demonstrating the production of a concentration gradient.

The merging test demonstrated with simple fluids can be extended to more complex multi-component fluids. The fluids' interfacial tension remains as the most critical factor that dictates the probability of success of the system.

Appendix A: System Component List

Part No.	Part Description	#
3200350	Mitos Dropix®	1
3200360	Dropix® Software Memory Stick	1
3200197	RS232 to USB Cable	1
3200349	Droplet Storage Coil – 0.25mm	2
3200414	Dropix® Fluid Reservoir – PMMA	1
3200351	Dropix® Sample Strip (Pack of 8)	1
3200356	Dropix® Sample Strip Holder	1
3200353	Dropix® Sample Hook – 0.8mm	1
3200355	Dropix® Sample Hook Fitting – 0.8mm	1
3200378	Droplet Pillar Merger Chip (200 µm), fluorophilic	1
3200302	FEP Tubing, 0.8mm x 0.25mm, 10 metres	1
3200306	Flangeless Ferrule 0.8mm, ETFE (pack of 10)	1
3200307	End Fittings and Ferrules for 0.8mm Tubing (pack of 10)	1
3200057	Mitos Duo XS-Pump	1
3000252	Syringe for Mitos Duo XS-Pump, 1ml	2
3000245	Valve for Mitos Duo XS-Pump (3 Port)	2
3200050	High Speed Camera and Microscope System	1

Appendix B: Physical Mechanism of Merging

The physical processes underlying merging is dominated by the interplay between

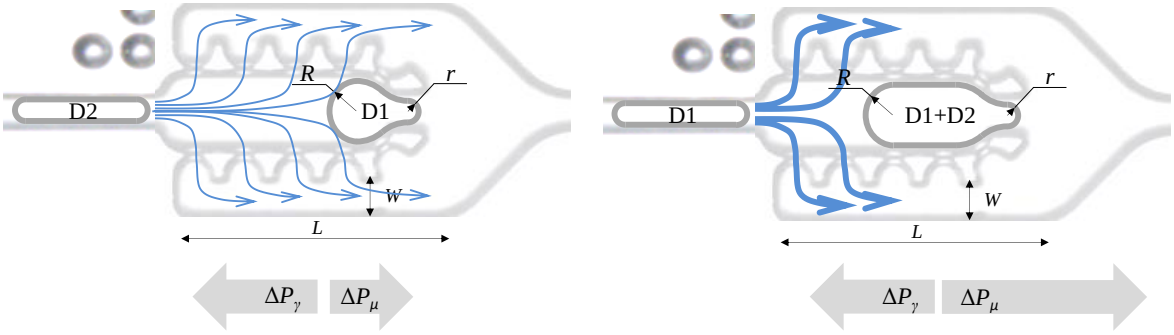
- Hydrodynamic effect $\Delta P_{\mu} \propto \frac{\mu L Q}{H W^3}$.

  The dominant parameters are viscosity μ and the flow rate, Q. H & W define the geometry of the chip, and are fixed.

- Surface tension effect $\Delta P_{\gamma} \propto \gamma . L . \left(\frac{1}{r} - \frac{1}{R}\right)$.

  The dominant parameters are interfacial tension γ and the downstream radius of curvature r of the lodged droplet.

During the separation phase an initially stable film forms a dimple that reduces film thickness between two droplets and increases the negative (sucking) pressure leading to the deformation of the interface and coalescence.



Pressure balance on droplet at the end of the merging section. The droplet remains confined to the merging section held by interfacial tension. Blue lines indicate streamlines of the carrier fluid, draining via the space between the pillars. Bolder streamlines are indicative of higher flow velocities due to reduced cross sectional area. H represents the channel depth (into the image plane).

As D1 merges with D2, the number of side exits in the merging sections get progressively blocked. The flow therefore increases in the remaining unblocked exits, increasing inertial effects at the droplet/carrier interface.

The deformation of the droplet generates a differential Laplace pressure gradient between the head and the tail of the droplet and causes the droplet to be quasi-stationary. At the same time, the oil phase flows continuously through available side channels to the side branches. Accordingly, the hydrodynamically induced total pressure drop between the tail and the head of a droplet in the middle branch is a combination of the pressure drop in the side channels (near the tail) and in the side branches.

$$F = \nabla(\Delta P_{\gamma} + \Delta P_{\mu})$$

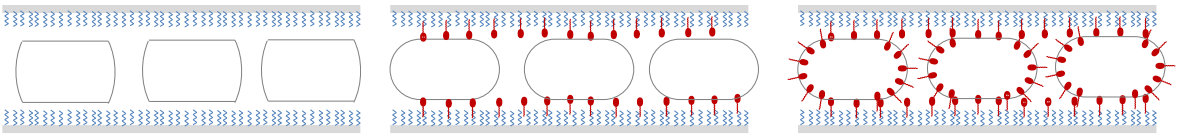
- *F>0* implies that the merged droplet will be released.
- *F=0* indicates the droplet will stay in the merging section. *F* is never negative since the interfacial effects are always balancing, and not absolute.

Appendix C: Lubricating Effect of Surfactant

Fluorocarbons are a chemical family of all organic compounds consisting of a carbon backbone fully surrounded by fluorine and represent a large and complex group of organic substances with unique characteristics. They are used in several industrial branches, but they also occur in a large range of consumer products due to their extraordinary properties such as chemically inert, non-wetting, very slippery, nontoxic, nonstick, highly fire resistant, very high temperature ratings, highly weather resistant). Here, FC-40, a 3M fluid was used. The FC-40 is highly non-polar having extremely low intermolecular attractive force.

Practice shows that it is not sufficient to have only terminal CF_3 groups in a fluorinated chemical. Optimum reduction of the surface energy γ_c is achieved by dynamic lubrication using a small amout of surfacant in the solution. Perfluorinated polyethers (PFPE) offer long-term stabilization. They have ionic head groups such as poly(perfluoropropylene glycol)-carboxylates sold as “Krytox” by DuPont. DuPont™ Krytox® 157 was used as a lubricating agent. Krytox itself has a fluorinated structure, is a polar molecule, and mixes easily with FC-40.

The lubrication content in fluorocarbon carrier phase is recommended regardless of the hydrophobic coating on the glass chip surface. The delicate balance between surface tension and hydrodynamci pressure, which is critical to merging operation of the chip, is promoted by a more slippery surface. Moreover, the lubricating action ensures that any leeched biomolecules have a low likelihood of adsorbtion to the surface.



Under lubricated | Optimally lubricated | Over lubricated/Over stabilized emulsion

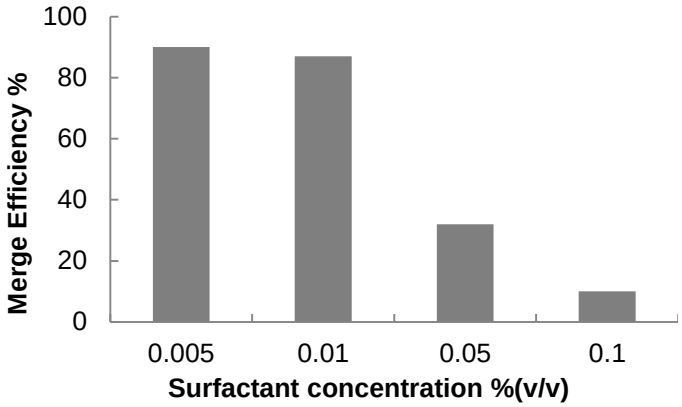
Substrate, Surface fluorocarbon end groups, Krytox® 157 molecules. The contact angle appears to vary with level of lubrication. Inadequate lubrication fails to provide the near-frictionless conditions conducive for droplet release. Excessive lubrication also works as surfactant thereby over-stabilizing the emulsion, and retarding droplet merging.

While primarily lubricating the solid/liquid interfaces, the Krytox molecules can be suspended in the bulk fluorocarbon oil and function as a surfactant when used at higher concentrations, thereby making droplet fusion more challenging.

Out of a total of 100 passing droplets, the number of droplet merging versus the number of droplets missed is recorded. This highlights the dependence on the surfactant levels, as well as quantifies the efficiency of merging. It can be seen that at low surfactant levels, a larger number of droplets merge.

Surfactant concentrations greater than 0.1% caused the interfacial tension of the system to become undesirably low. In these cases, the sample fluids, which are otherwise stably contained in the sample strip, fall out. Surface tension, which aids in holding the fluids in

the sample wells, is significantly reduced, thereby reducing the ability to load reagents. This makes the Dropix® challenging to operate and droplet production difficult.



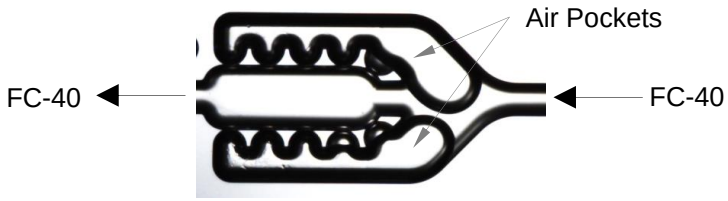
Merging efficiency (percentage of droplets that merge from a sample size of 100) at various concentrations of surfactant (v/v) Krytox 157 in FC-40. Note: Semi-log data is presented on a linear axis and may falsely appear equally spaced.

The graphic above qualitatively depicts the effect of surfactant on droplet merging. It was found that 0.01% (v/v) or lower Krytox 157 in FC-40 created an adequate trade-off between emulsion stability and ease of merging.

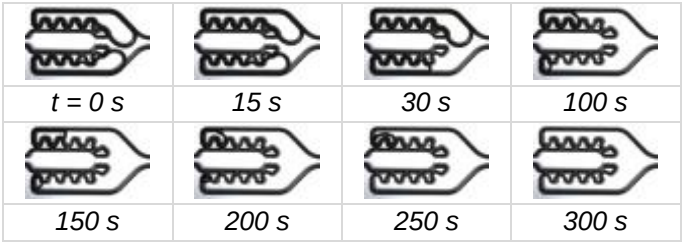
Appendix D: FAQs & Helpful Tips

Q: What is the priming method for the chip?

A: Priming the chip requires special attention. It must be primed in reverse flow to purge the air pockets on either side. In forward flow, the air pockets will NOT purge, causing failure to merge.



To prime, flush with FC-40 at high flow rate (100 µL/min) in reverse direction. The air pockets will dissolve into the FC-40, progressively reducing in size until they finally disappear. A time sequence of this is shown below.



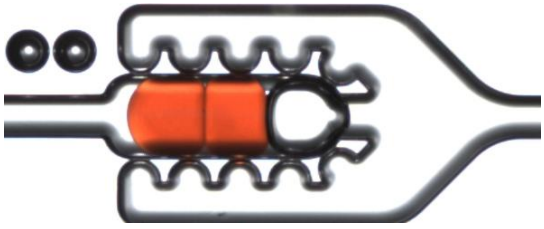
Chip priming to purge air pockets by dissolving into FC-40.

Q: Is it possible to store the merged droplets?

A: Yes, the storage coil (Part No. 3200349) may be used in conjunction with the mode 2 setup to store droplets.

Q: Is it possible to space droplet sequences with air bubbles?

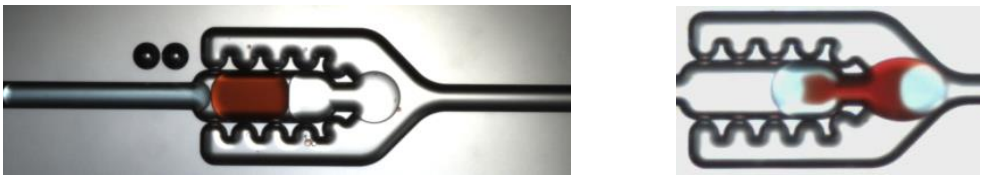
A: Yes, an empty well on the sample strip will produce air bubbles. These can be useful when working especially challenging fluids, whose large difference in properties can cause catch up in tubing. In such adverse situations, air bubble can prove as convenient spacers utilizing their significantly higher interfacial tension with most liquids.



An air bubble spacer with it's higher interfacial tension allows additional control on merging.

Q: Is it possible to merge more than droplets at a time?

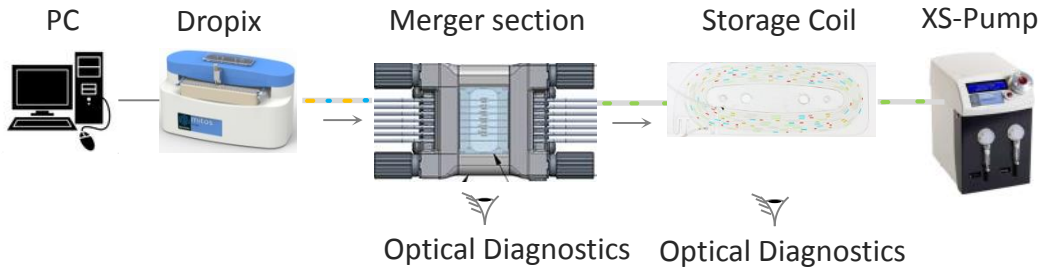
A: Yes, this may be possible at low flow rates, when the hydrodynamic forces are insufficient in comparison with interfacial forces, especially when low levels of stabilizer/surfactant is used. The stuck zone to the left of the stable merging regime in the working envelope shown earlier is the best operating space to attempt this.



Merging 3 droplets. Left: Clear + red + blue. Right: Red + blue + red.

Q: Can the merged droplets be stored in the storage coil?

A: Yes, the storage coil can be added in-line. When added downstream of the merging chip as shown below, it becomes the features of Mode 1 and Mode 2, creating the capability of storing merged droplets. This can be used for incubation, visualization, storage and recall.



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