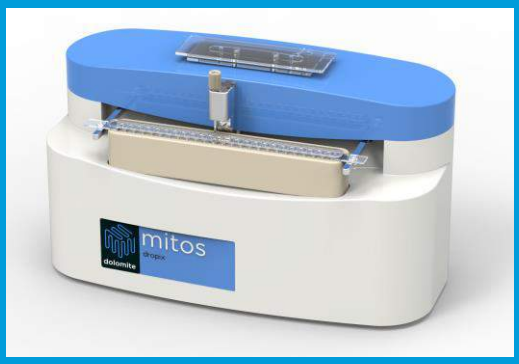
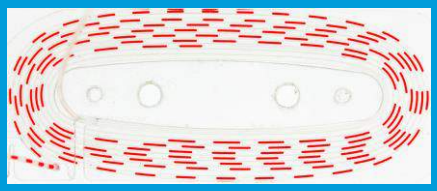
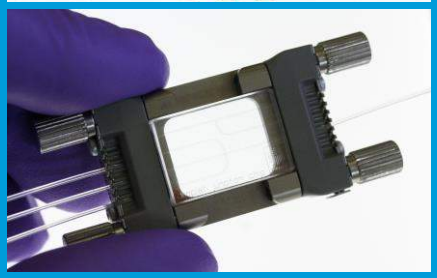
Droplet on Demand Generation & Droplet Splitting

Mitos Dropix Droplet Splitting System





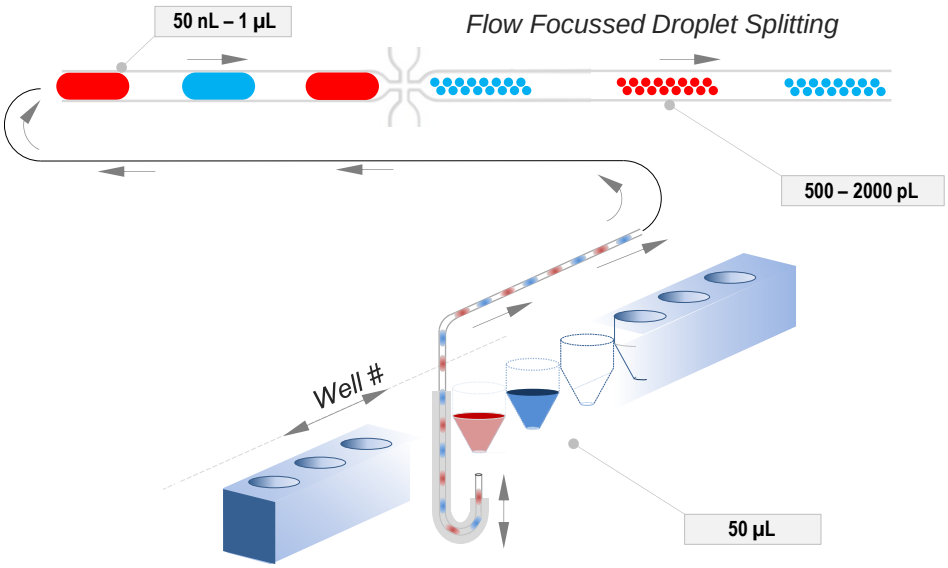


dolomite

Summary of Droplet Splitting

Dropix delivers a Droplet-on-demand capability by creating programmable sequences of nanoliter and picoliter volume droplets. This application note demonstrates the Mitos Dropix® Splitting System to reproducibly generate nanoliter volume droplets, and subsequently split them into picoliter droplets of varying volumes. In tests, we show:

- Setup of a differential flow between a positive displacement XS-Pump (in withdrawal mode with suction flow rate 5 – 15 µL/min) and a pressure driven P-Pump (pressure driven flow 5 – 15 µL/min).
- Creation of a sequence of 200 nanoliter (nL) aqueous plugs, each separated by 200 nL of fluorocarbon oil.
- Transport of the 200 nL plugs from Dropix to the flow focussing microfluidic device, and subsequent splitting of the plugs into many picoliter (pL) droplets. Depending on the split volume, the number of resulting droplets ranged from 100 up to 450.
- The droplet size (90-180µm) and droplet production rate (0-75 Hz) range is parametrically characterized as a function of fluid flow rates.
- The picoliter droplets are stored in a Storage Coil made up of a length of FEP tubing.



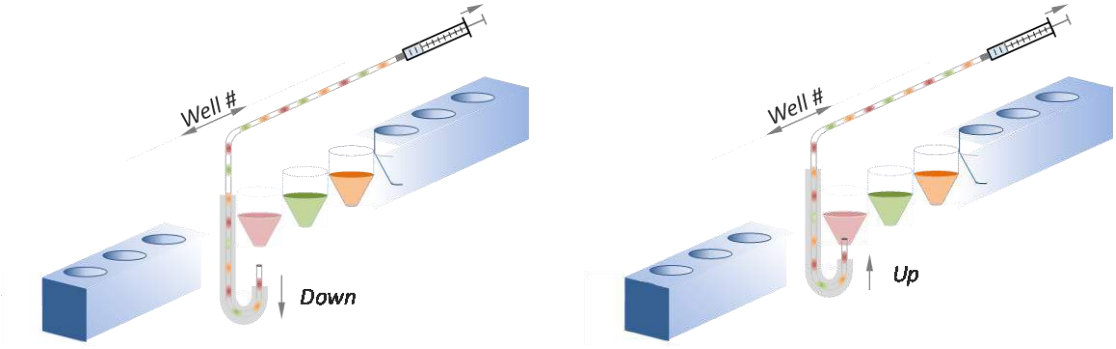
Droplet Sequence Generation

Schematic showing droplet on demand production and subsequent splitting. Typical aqueous volumes are shown at various stages of the process.

Mitos Dropix Technology

Droplet Production

Droplet-based microfluidic systems enable bio-chemical reactions on a miniaturized scale. The technological challenge of interfacing the benchtop world with μsystems is solved by the capabilities offered by Dropix. Its droplet-on-demand approach enables automated creation of a sequence of chemically distinct plugs from microtiter well plate formats. The droplet libraries can have variable composition well as size. The Dropix can store up to 24 different liquids in fluid reservoirs of 50 µL volume. Any combination of droplet count, sequence, and droplet volume (ranging from 10nL to 1µL) can be generated and delivered at rates of up to 5 droplets per second.



Droplet on demand – a constant suction driven flow results in the creation of a segmented flow. The timing of the 'up' or 'down' position of the sampling hook dictates the droplet volume and spacing volume respectively. The transverse position of the hook dictates the selection of the sampling well.

Surface tension and buoyancy are the key fluid properties exploited in the design of the fluid sample wells. The aqueous sample fluid (shown in the above schematic as colored fluids) is contained in a polymeric sample strip that has an orifice at the bottom. The aqueous sample is prevented from falling out of the bottom by the buoyant effect due to a denser heavier fluorocarbon oil underneath. Further, the interfacial surface tension pins the interface to the rim of the orifice, thereby assisting in keeping the aqueous sample afloat in the sample well.

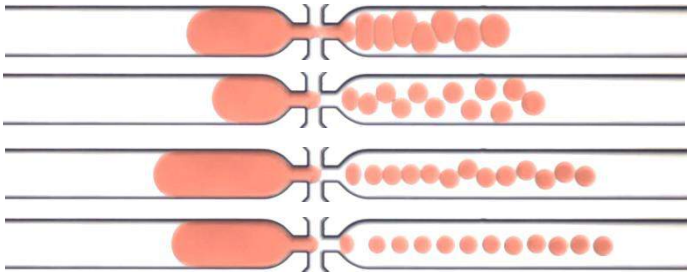
A constant withdrawal flow setup using a syringe pump and a pressure pump aspirates fluid from the Dropix. The vertical and transverse motion of the Sample hook dictates the time spent by the tubing tip in the wells containing aqueous samples. When 'up', aqueous fluid is aspirated, and when 'down', fluorocarbon carrier fluid is aspirated. The transverse motion of the hook controls the well number being aspirated. The constant fluid flow along with the timed vertical and transverse J-hook motion creates a segmented plug flow in the tubing. The timing of the rise and fall of the hook controls the size of the droplets as well as the spacing in-between.

The segmented flow of plugs spaced apart by fluorocarbon oil when split into still smaller droplets extends the working volume the picoliter range. These split droplets are stored in optically clear tubing in a Storage Coil.

Droplet Splitting

The ability to divide droplets is a necessary operation for the execution of assays and the production of sample replicates. The ability to create user-defined droplet libraries on demand is therefore useful. Droplet splitting is a significant feature of the Dropix, and allows aliquoting picoliter volumes from a nanoliter plug. The Dropix produces nanoliter volume droplets and when coupled with flow focussing technology, works as a droplet splitter (named Mode 3).

Droplet splitting further increases throughput of droplet production. Like droplet merging, both passive (geometry mediated), and active (electromagnetic) splitting methods are available. A passive method presented here exploits Plateau–Rayleigh instability which is related to fluidic interfacial tension and viscosity. A stream of fluid breaks up into smaller droplets with the same volume but less surface area. The breakup volume is uniform due to the geometry of the flow focussing junction, and due to the symmetry of the surrounding flow.



Nanoliter plugs are broken into picoliter droplets at the flow focussing junction as a result of flow focussing mediated pinch-off at 4 different flow ratios.

Operating in this mode, each nanoliter droplet is broken into many small droplets each of picoliter volume. The split picoliter droplets can be collected as a library and are kept in sequence by storing in tubing. This technology eliminates several bottlenecks of current droplet-based microfluidic systems and opens the way for picoliter range bio-chemical and cell-based screens.

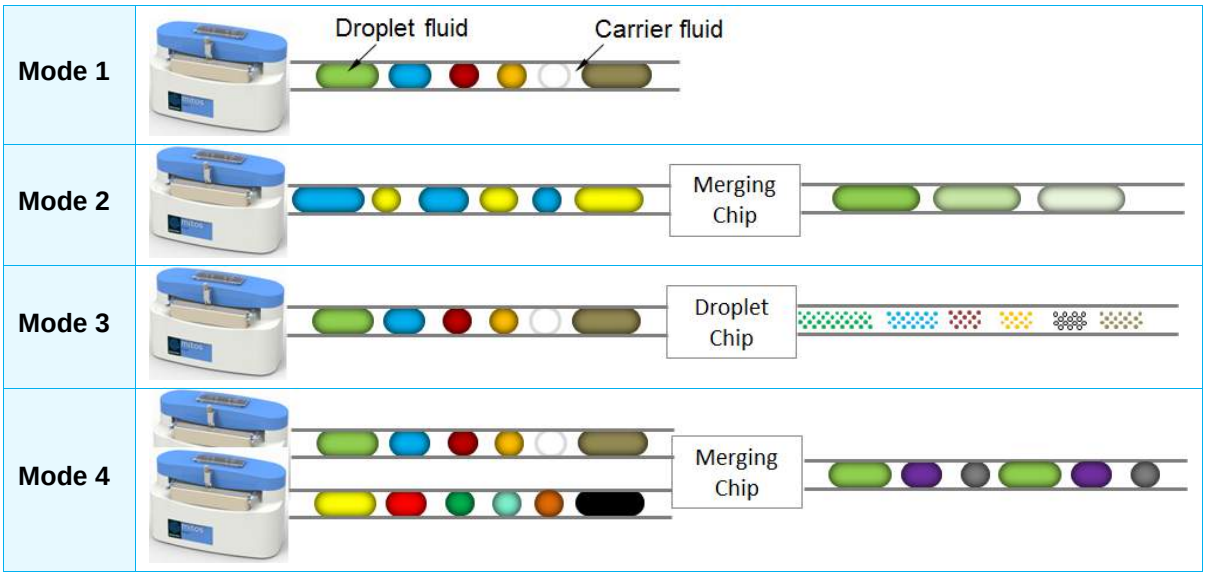
Modes of Operation

Mode 1: Production of a nanoliter droplet sequence is termed Mode 1 operation of the 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.

Mode 2: Sequence generation and pairwise merging is termed Mode 2 operation of the Dropix system.

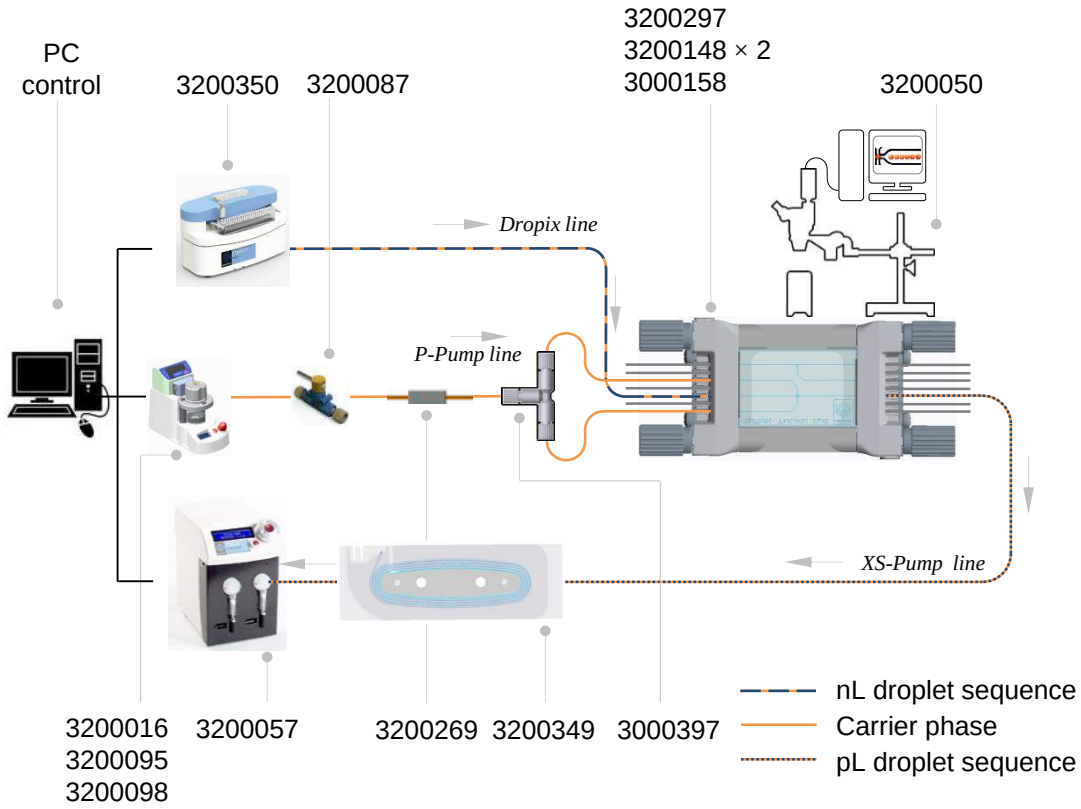
Mode 3 is the focus of this application note. It involves nanoliter droplet sequence generation and "explosion" into picoliter daughter droplets. This extends the working volume per droplet from the nanoliter to the picoliter range using the Dropix in combination with flow focussing geometry.

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



The 4 suggested modes of Dropix operation.

Test Setup



Setup schematic. Yellow lines represent fluorocarbon carrier with 0.1 % surfactant, blue/yellow segmented lines represent nanoliter plugs made from Dropix. PC control is achieved via 'Flow Control Center' software. Image not to scale.

Detail of tubing used to connect the individual components is listed in the table below. The triplet numbers indicate Outer Diameter (mm), Inner Diameter (mm), and length (mm).

	Dropix line	P-Pump line	XS-Pump line
P-Pump to Remote 30		pneumatic	
Remote 30 to Flow Sensor		0.8, 0.25, 300	
Flow Sensor to 2-way In-line Valve		0.8, 0.25, 200	
In-line valve to T-connector		0.8, 0.25, 200 + F10 + 0.8, 0.25, 200	
T-connector to chip		0.8, 0.25, 300	
Dropix to Chip (Storage coil)	0.8, 0.25, 200		
Chip to XS-Pump (Storage coil)			0.8, 0.25, 1000

Details of tubing used to make connections. All tubing is FEP with triplet set representing OD (mm), ID (mm), and L (mm). An F10 resistor is equivalent to approximately 1500 mm of FEP tubing with ID 100 µm and OD 1/16".

The Dropix is controlled with the Dropix Control Software. The P-Pump and XS-Pump are controlled with the Flow Control Center software in this test, but may also be controlled manually. The system setup is shown in the figure above. Fluidic connections between

the Mitos Dropix (Part No. 3200350), 100 µm Droplet Junction Chip (Part No. 3000158), the Dropix Sample Hook (Part No. 3200353, 3200355), P-Pump (3200016), Storage Coil (Part No. 3200349) and the XS-Pump (Part No. 3200057) are made using FEP tubing of OD 0.8 mm and ID 0.25 mm (Part No. 3200302). The chip is assembled with 2 Linear Connector 7-way (Part No. 3200148) and H Interface 7-way (22.5mm) (Part No. 3200297).

Flangeless Ferrule 0.8mm, ETFE (Part No. 3200306) and End Fittings and Ferrules for 0.8mm Tubing (Part No. 3200307) are used for completing the tubing connections. Additional fluidic accessories used are 2-way In-line Valve (Part No. 3200087), T-Connector ETFE (Part No. 3000397), PTFE Plug 0.8mm (Part No. 3200305), and PTFE Tube Cutter (Part No. 3000398). An F10 flow resistor (Part No. 3200269) is used between the P-Pump and the chip. Visualization was achieved using a High Speed Camera and Microscope System (Part Number: 3200050). Videos of droplet production recorded at frame rates of about 500 Hz are processed using Droplet Monitor Software to yield droplet size and production rate.

Image of droplet junction chip with H-interface, connectors, and tubing assembled. Channel depth is 100 µm; fluid flow is from left to right.

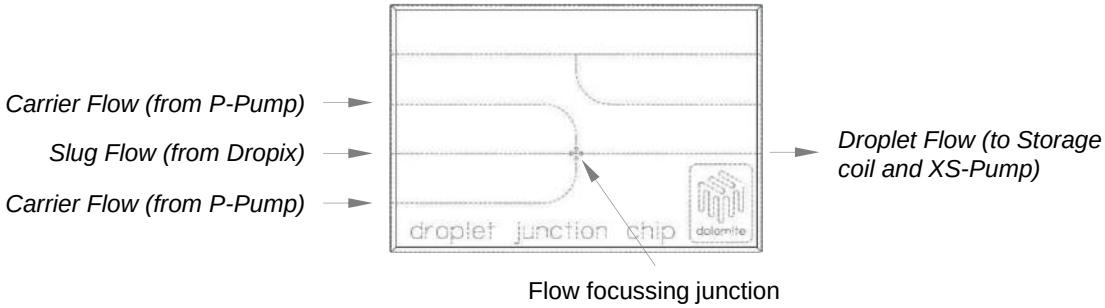
It is important to use the green integrated ferrule filters so that the flow resistor or the chip is protected from any particulate matter that may be carried with fluids. As good lab practice, it is highly recommended to filter fluids prior to using in the system.

Chip Surface Conditioning

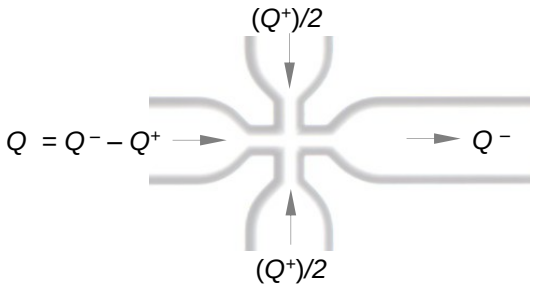
The microfluidic device used was a Dolomite hydrophilic chip (3000158), which underwent surface conditioning[*] as reported in a peer reviewed journal. The reagents were purchased from Sigma-Aldrich and from Aquapel® respectively as described in the paper. This surface conditioning step allows the use of Dolomite's hydrophilic chip with Fluoridrop (fluorinated oils) following the two separate protocols in separate tests, both with successful outcomes.

[*] *Single-cell analysis and sorting using droplet-based microfluidics. Linas Mazutis, John Gilbert, W Lloyd Ung, David A Weitz, Andrew D Griffiths & John A Heyman.* ***Nature Protocols 8, 870–891 (2013) doi:10.1038/nprot.2013.046***

Fluid Flow Setup



Droplet Junction Chip.



Detailed view of the flow focussing junction illustrating the flow distribution. (Net flow rate at Dropix) = (Suction from Syringe pump) – (Infusion rate of P-Pump)

The fluid flow in the system is created by a combination of two pumping elements. Of particular importance is the flow rate at three locations. These are:

- XS-Pump (Q^-) – Represents syringe pump flow rate in withdrawal mode. This flow assists with loading of the product Storage Coil. The syringe pump uses the 3-way valve and a 500 µL† syringe.
- Pressure pump (Q^+) – Pressure driven infusion flow rate from P-Pump. This flow rate directly affects the flow ratio between droplets and carrier fluid at the flow focussing junction. The P-Pump is run in flow control mode.
- Differential flow rate between XS-Pump and P-Pump (Q) – Net flow rate being the difference between the suction flow rate and the infusion flow rate. For positive flow in the flow focussing junction on the droplet chip, the criteria $|Q^-| > |Q^+|$ must hold at all times. The differential flow rate is the suction flow rate experienced at the Dropix Sample Hook. This value is required to be re-calculated whenever either the XS-Pump or the P-Pump flows are changed, and manually entered into the Dropix software.

In order to maintain net positive flow, the constraint on the P-Pump flow rate is that it may never exceed the syringe pump flow rate. The flow ratio F_R (= Q^+/Q^-) is therefore positive,

† *Large volume syringes pump for longer period before needing a refill, but result in a less smooth flow. Eg. A 500 µL syringe operating at 10 µL/min will need a refill every 50 minutes. The range of syringe volumes available is 50, 100, 250, 500, 1000, 2500 and 5000 µL. As reference, the storage coil has a volume of 50 µL.*

but always less than 1. In this regard, the setup differs from conventional droplet systems, where the flow ratio can be lesser as well as greater than 1. This constraint on the flow ratio limits the droplet sizes available. For $F_R < 1$, there is relatively more droplet fluid available compared with carrier fluid, and thus large droplet sizes are expected. For $F_R = 1$, equal droplet and carrier flow rates indicate droplet sizes comparable to channel etch depth. $F_R < 1$ is indicative of a situation where there would be excess of carrier fluid relative to droplet fluid, and the expectation would be droplets much smaller than channel etch depth. This is however not allowed as $F_R \leq 1$, and therefore droplets smaller than channel etch depth are expected to be difficult to achieve.

Reagent Preparation

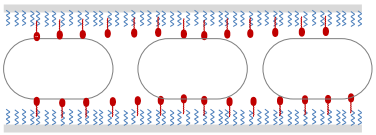
As the carrier fluid consists of fluorocarbon oil, Helium is the gas of choice used to pressurize the P-Pump. This supresses gas dissolution into the fluid, which otherwise is likely to degas downstream at lower pressure condition. In a steady state fully primed flow situation, the fluids in different sections of the setup are:

	Carrier fluid	Droplet fluid	Droplet Size	Casual Naming
Dropix line	Fluoridrop 40 + 0.0001% Krytox 157 FS	Dilute food color	Nanoliter	Plug flow in carrier
P-Pump line	Fluoridrop 40 + 0. 1% Krytox 157 FS	-	-	Carrier Flow
XS-Pump line	Fluoridrop 40 + Variable‡ % Krytox 157 FS	Dilute food color	Picoliter	Droplet Flow in carrier

Fluoridrop 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}. It was supplemented with surfactant in various percentages as shown in the accompanying table. DuPont™ Krytox® 157 was used for this. and acts as a dynamic lubricating. All fluids were degassed prior to use.

Surfactant Optimization

- Lubrication in Dropix line – Optimum reduction of the surface energy γ_c is achieved by dynamic lubrication using a small amout of lubricant in the solution. Practice shows that it helps to have terminal CF_3 groups in a fluorinated reagent (such as Fluoridrop-40), and in addition, a lubricating agent. DuPont™ Krytox® 157 was used as a lubricating agent. Krytox itself has a fluorinated structure, is a polar molecule, and mixes easily with Fluoridrop-40.



Lubricating effect of 0.0001% (v/v) Krytox in Fluoridrop-40.

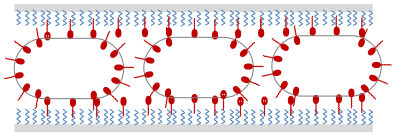
Substrate, Surface fluorocarbon end groups, Krytox® 157 molecules.

The lubrication in fluorocarbon carrier phase is highly recommended. It additionally reduces the adsorbtion of any leeched biomolecules to the glass surface.

- Emulsion stabilization post droplet splitting – While primarily lubricating the solid/liquid interfaces, the Krytox molecules are suspended in the bulk fluorocarbon oil and function as a surfactant when added at higher concentration.

‡ *The percentage of surfactant in the mixture depends on the flow rate ratio, and follows mixture laws.*

The surfactant then works towards ensuring the stability of emulsions to supress coalescence.



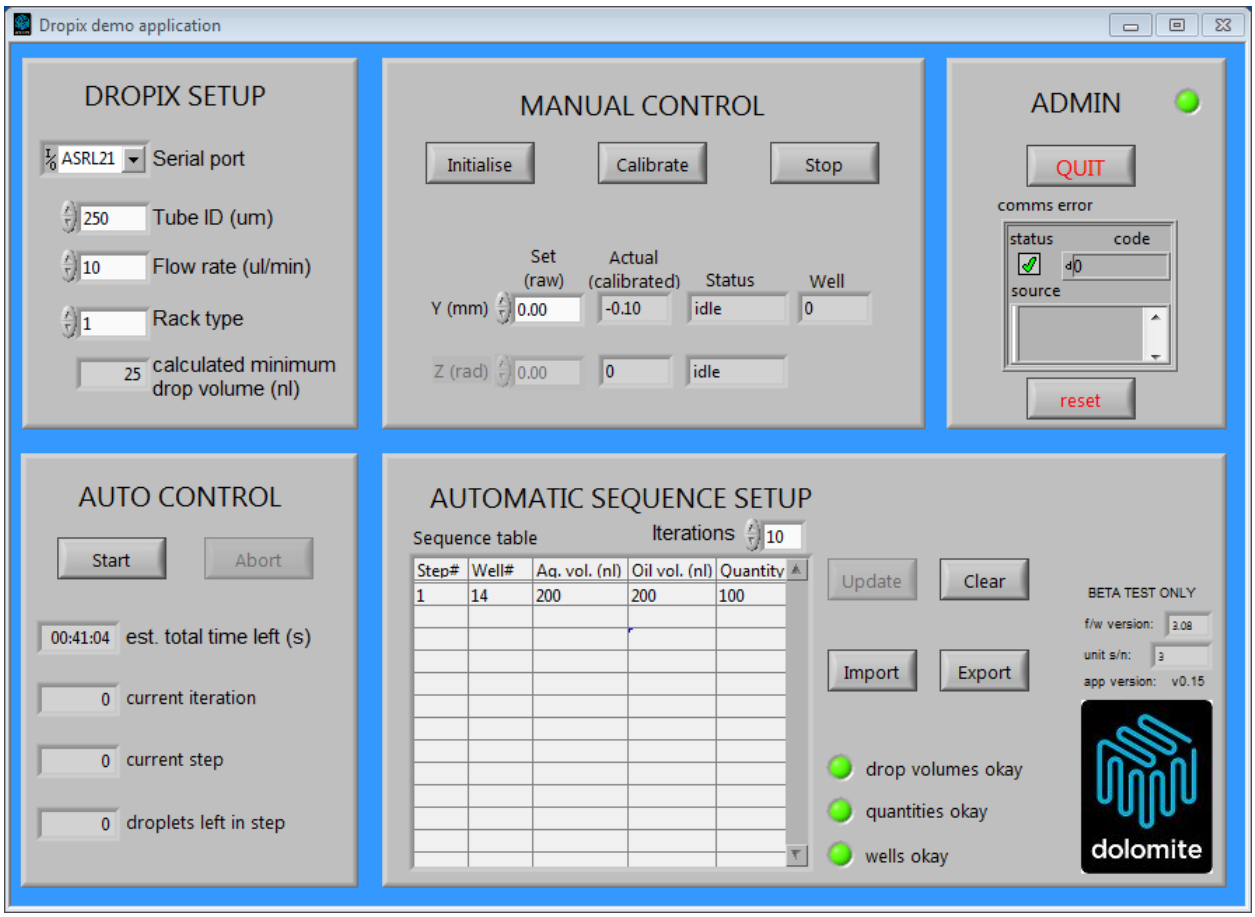
Emulsion stabilization effect of 0.1% *Krytox in Fluoridrop-40.*

Substrate, Surface fluorocarbon end groups, Krytox® 157 molecules.

Surfactant concentrations greater than 0.1% lower the interfacial tension of the system excessively. This is of significance in the Dropix line, where surface tension aids in holding the fluids in the sample wells. If the surface tension is significantly reduced, the ability to load reagents is affected. In such a case, the sample fluid contained in the sample strip is destabilized, and falls out. Therefore, low surfactant is necessary on the Dropix line.

Conversely, a low surfactant concentration at the flow focussing junction may not adequately stabilize the emulsion, and result in coalescence. Therefore, downstream of the droplet splitting junction, a higher surfactant concentration is preferable.

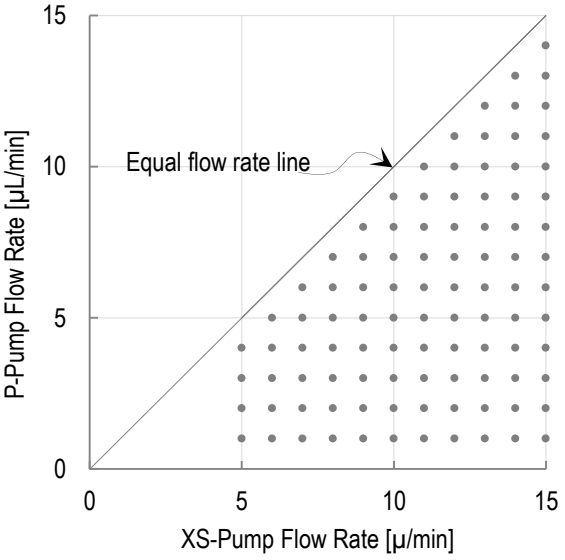
Software Setup



Screenshot of Dropix Software setup. Well # 14 of the Sample strip is loaded with dilute food coloring. The Flow Rate option is the DROPIX SETUP section of the software is updated manually to match the flow rate set on the XS-Pump.

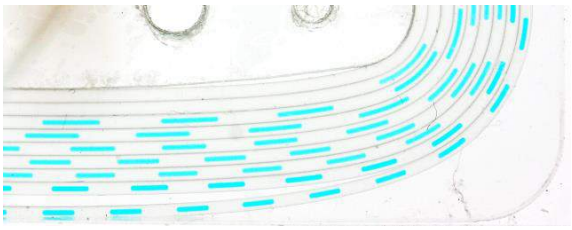
Results & Analysis

The method of testing involves setting the XS-Pump flow rate, and then sequentially raising the P-Pump flow rate from low to high. Two limiting conditions are encountered while varying the P-Pump flow rate. The lower limit is encountered when the suction rate of the syringe pump creates a baseline flow is faster than the lowest pumping rate of the P-Pump (the P-Pump was not set up to work in vacuum mode). The upper limit is encountered when the P-pump flow rate approaches the syringe pump flow rate, creating a near stagnant flow at the Dropix. The optimum range for Mode 3 operation is in between these two limits.

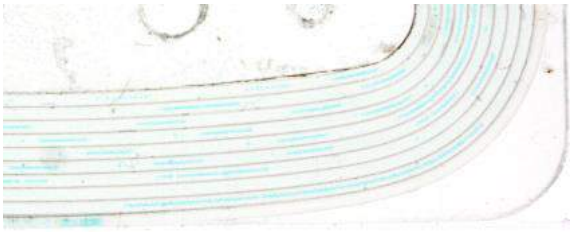


Flow rate matrix for tests. Each data point represents a test condition. All data points necessarily lie under the 'equal flow rate' line.

A sequence of aqueous plugs of volume 200 nL is produced. The plugs are formed in tubing (ID 250 µm, OD 800 µm and length 1 m) and are segmented by a fluorocarbon carrier fluid. In this case, the carrier fluid spacing was approximately 200 nL. Each plug is approximately 4 mm long.



A portion of the storage coil with nanoliter plugs. When P-Pump flow rate = 0, the plugs go through the droplet junction chip without break-up. The Storage Coil is then loaded with nanoliter sized plugs.



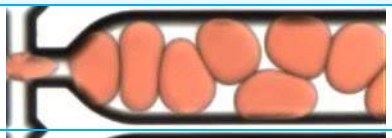
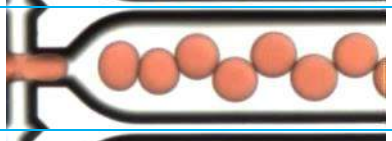
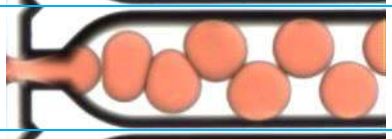
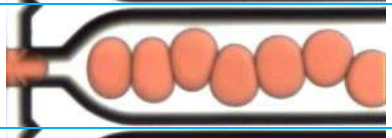
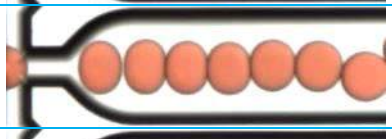
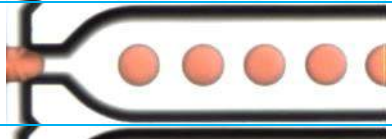
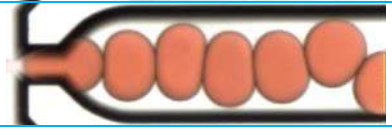
A portion of the storage coil with picoliter droplets. Individual droplets are not easily visible, however clusters can be seen. Each of the droplets in the cluster is about 100 µm in diameter.

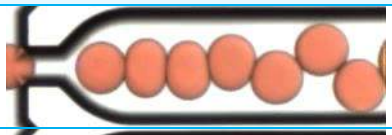
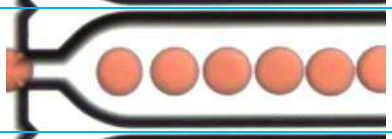
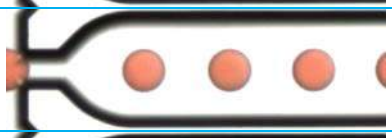
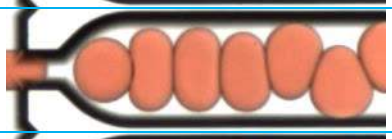
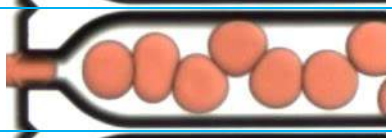
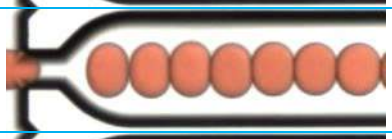
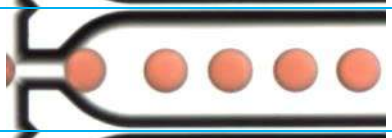
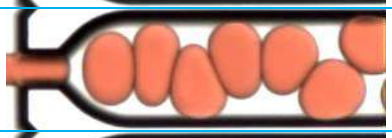
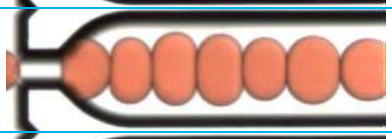
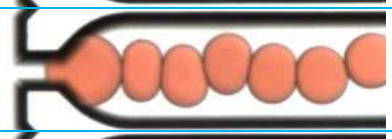
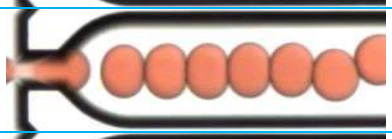
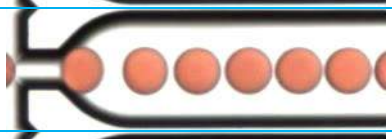
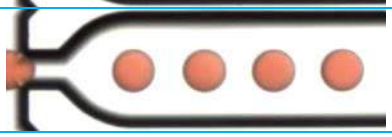
The volume of droplets V_D (nL) = $\{(4/3)\cdot\pi\cdot\varphi^3\} \times 10^{-6}$, where φ, the droplet diameter is measured in µm. Droplet diameter φ is measured using the Droplet Monitor software. The plug volume (V_S) is known *a priori* as it is an input to the Dropix software. The number of droplets N is (V_S/ V_D). V_S = 200 nL for all cases reported in this application note.

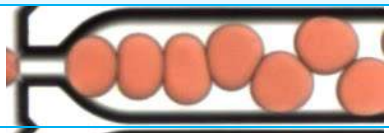
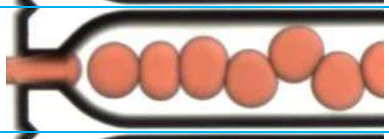
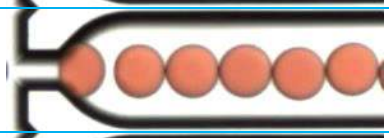
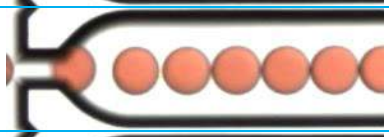
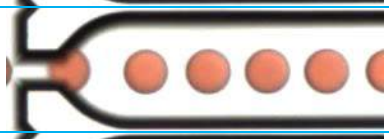
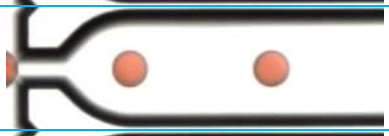
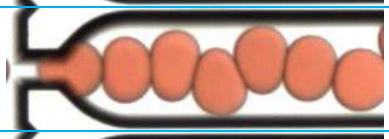
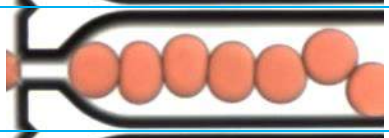
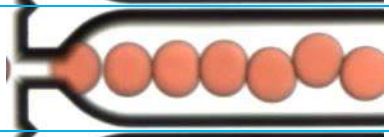
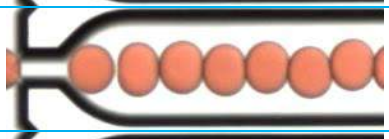
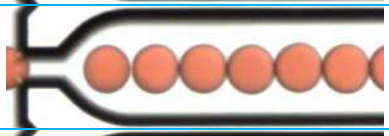
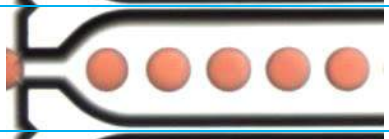
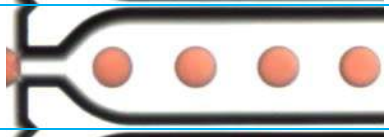
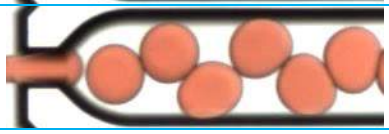
Droplet size and rate dependence on flow rates

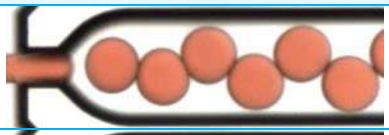
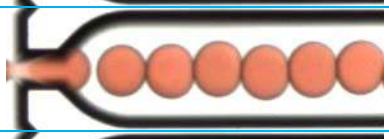
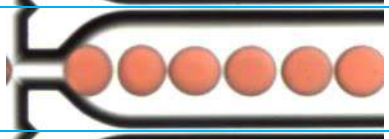
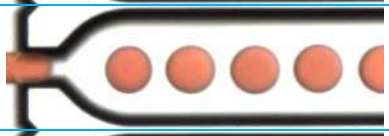
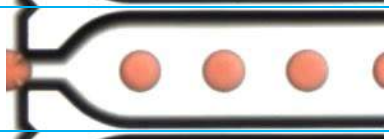
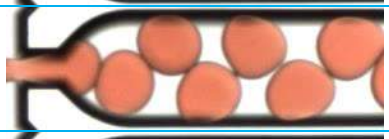
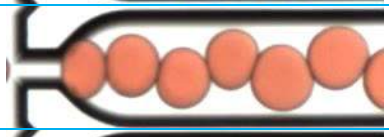
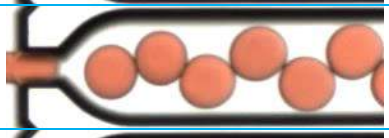
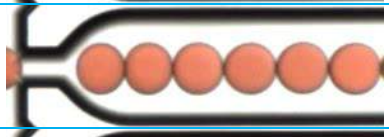
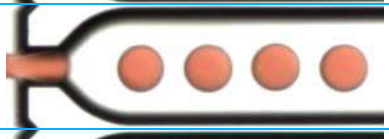
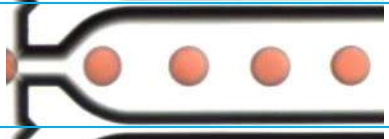
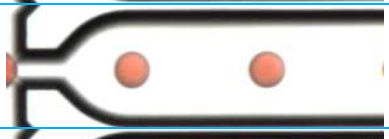
The droplets travel in tubing from the Dropix to the flow focussing junction on a droplet chip. Additional carrier fluid is pumped on to the chip with the help of which the 200 nL droplet is broken into many smaller droplets. The droplet breakup is a direct function of the flow ratio between the plug flow and the spacer/carrier flow. Depending upon flow ratio, the split droplets arrange in single file, or in zig-zag formation, the difference being the size and therefore the pinchoff rate.

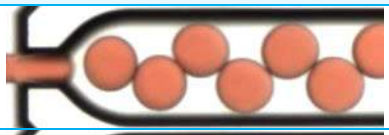
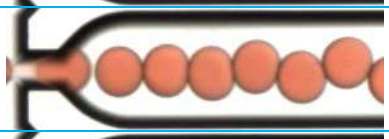
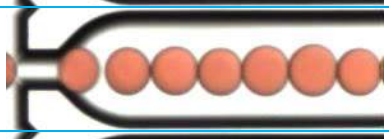
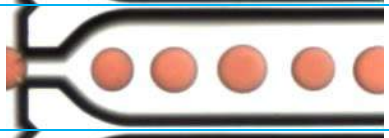
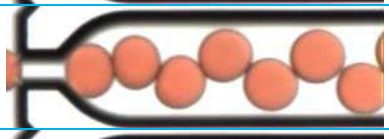
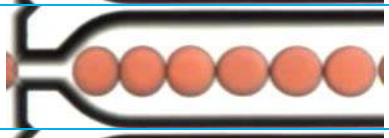
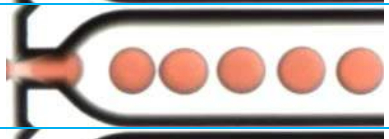
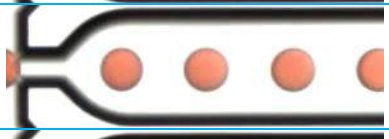
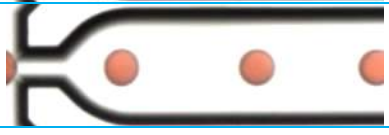
Short videos (100 frames) of droplet production are recorded at relatively high frame rates (500 fps). These videos are later analysed using the Droplet Monitor Software. The process is described in detail in the Appendix. The resulting droplet size and frequency is listed in the table below.

XS-Pump Q^- (µL/min)	P-Pump Q^+ (µL/min)	Dropix Q = (Q^--Q^+) (µL/min)	Junction Image	Size D (µm)	Rate f (Hz)	Number of Droplets
5	2	3		151.6	23	109
5	3	2		125.1	23	195
5	4	1		112.3	18	271
6	2	4		158.2	32	96
6	3	3		135.2	33	154
6	4	2		123.0	29	205
6	5	1		110.9	20	279
7	3	4		146.1	36	122

XS-Pump Q^- (µL/min)	P-Pump Q^+ (µL/min)	Dropix Q = (Q^--Q^+) (µL/min)	Junction Image	Size D (µm)	Rate f (Hz)	Number of Droplets
7	4	3		130.5	33	173
7	5	2		120.7	28	221
7	6	1		111.3	22	286
8	3	5		155.7	41	102
8	4	4		138.3	43	145
8	5	3		125.1	40	195
8	6	2		116.8	35	244
8	7	1		106.2	23	320
9	3	6		161.0	44	93
9	4	5		142.7	46	133
9	5	4		128.9	46	182
9	6	3		118.1	42	232
9	7	2		108.9	35	294
9	8	1		103.5	25	349

XS-Pump Q^- (µL/min)	P-Pump Q^+ (µL/min)	Dropix Q = (Q^--Q^+) (µL/min)	Junction Image	Size D (µm)	Rate f (Hz)	Number of Droplets
10	4	6		151.0	50	110
10	5	5		140.1	51	139
10	6	4		129.6	59	177
10	7	3		120.8	43	221
10	8	2		109.7	36	286
10	9	1		102.0	25	381
11	4	7		154.8	53	102
11	5	6		145.3	54	125
11	6	5		135.0	55	155
11	7	4		126.1	52	195
11	8	3		115.5	46	251
11	9	2		103.2	37	349
11	10	1		97.2	26	431
12	5	7		151.6	56	109

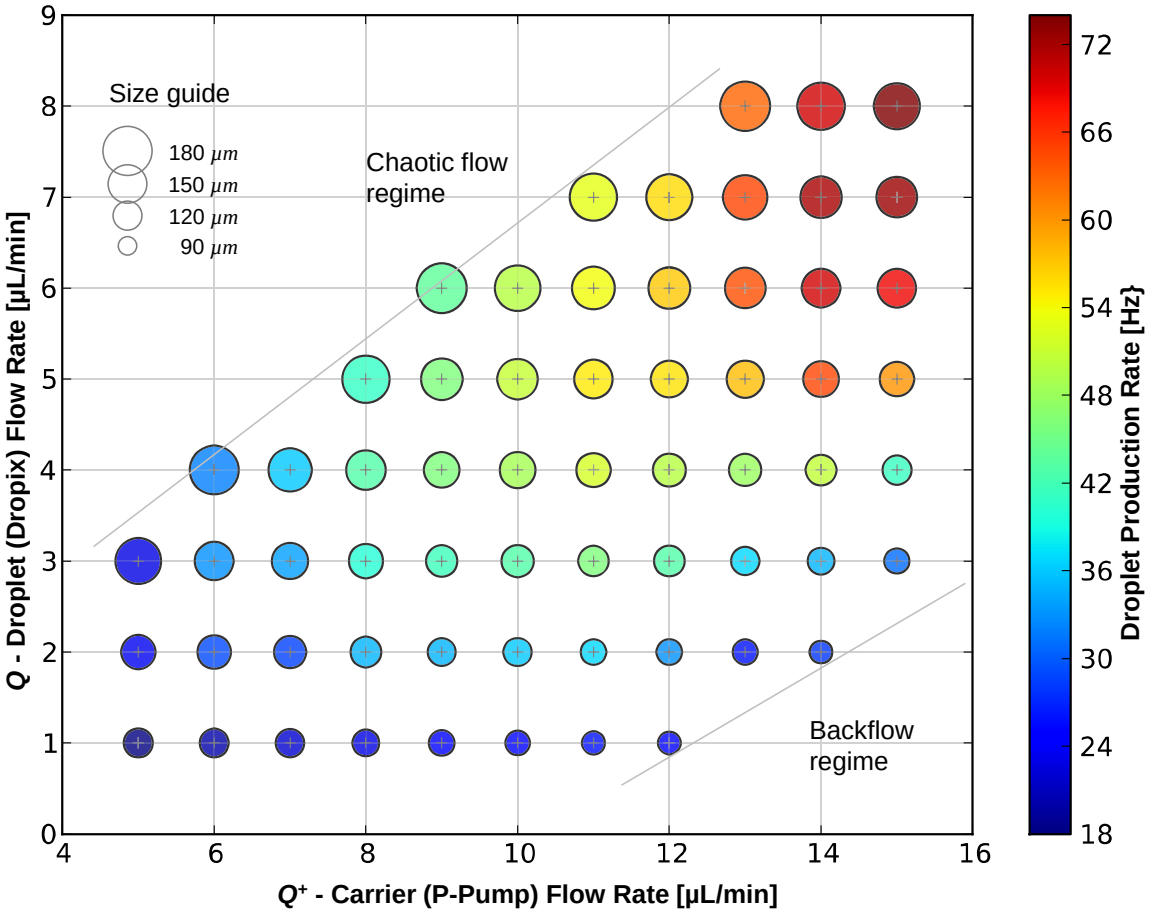
XS-Pump Q^- (µL/min)	P-Pump Q^+ (µL/min)	Dropix Q = (Q^--Q^+) (µL/min)	Junction Image	Size D (µm)	Rate f (Hz)	Number of Droplets
12	6	6		142.0	57	133
12	7	5		131.2	53	169
12	8	4		122.7	50	210
12	9	3		116.8	43	244
12	10	2		103.2	33	349
12	11	1		95.0	25	445
13	5	8		160.1	63	93
13	6	7		148.4	65	116
13	7	6		140.0	64	139
13	8	5		132.1	58	165
13	9	4		120.5	48	221
13	10	3		110.6	37	286
13	11	2		101.4	26	366
14	6	8		155.6	70	102

XS-Pump Q^- (µL/min)	P-Pump Q^+ (µL/min)	Dropix Q = (Q^--Q^+) (µL/min)	Junction Image	Size D (µm)	Rate f (Hz)	Number of Droplets
14	7	7		141.8	72	134
14	8	6		135.4	70	155
14	9	5		128.5	65	180
14	10	4		116.3	51	244
14	11	3		106.2	35	320
14	12	2		96	28	445
15	7	8		152.1	74	108
15	8	7		141.3	72	139
15	9	6		135.6	68	155
15	10	5		124.7	60	194
15	11	4		112.3	41	271
15	12	3		102.7	31	359

Net flow is from left to right with all droplets travelling downstream of the junction and off the chip. At the outlet, the Storage Coil receiving the droplet sequence is about 1 meter long. Therefore a large number of droplets can be stored on this storage coil. Once this coil is filled, the test is complete and all flows are stopped.

Droplet Splitting Phase Chart

The data from the above table is presented in a graphical format below. The size of the circles indicates the diameter of the droplets using the size guide. The color of the circles indicates the production rate from the color bar shown to the right.



Phase Diagram: Droplet size and production rate dependence on flow rates.

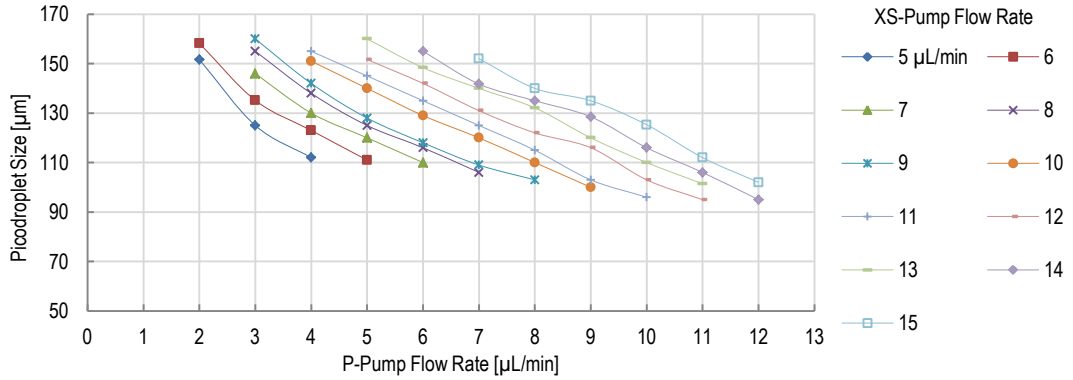
The phase diagram depicts the relationship between droplet size and production rate with flow rates. The two flow rates that can be directly controlled are XS pump suction flow rate Q^-, and P-Pump infusion flow rate Q^+. Q is an outcome of the difference between the two, and is not directly set anywhere.

An example of droplet production in the region marked 'Chaotic Flow'. These droplets are not monodisperse and occur at excessively high droplet to carrier flow ratio.

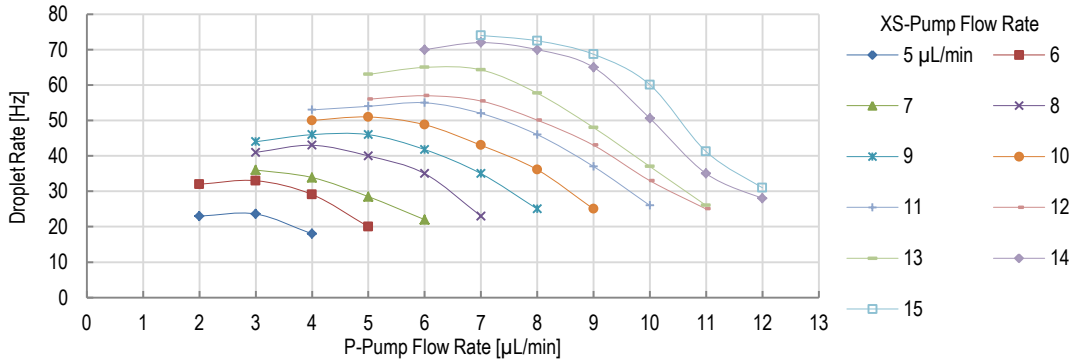
For a given droplet flow rate, increasing carrier phase causes smaller droplets to be produced with increased spacing. Initially the frequency rises, but then falls. While the initial rise is intuitive, the latter fall in frequency is due to near stalling of the dripping. Any further increase would tip the phase diagram to the region marked 'Backflow' where the

droplet phase flows backwards, and is undesirable. Reducing the carrier flow too much drives the phase diagram into the chaotic flow regime – here very large plugs are produced and are considered non-monodisperse – an undesirable situation.

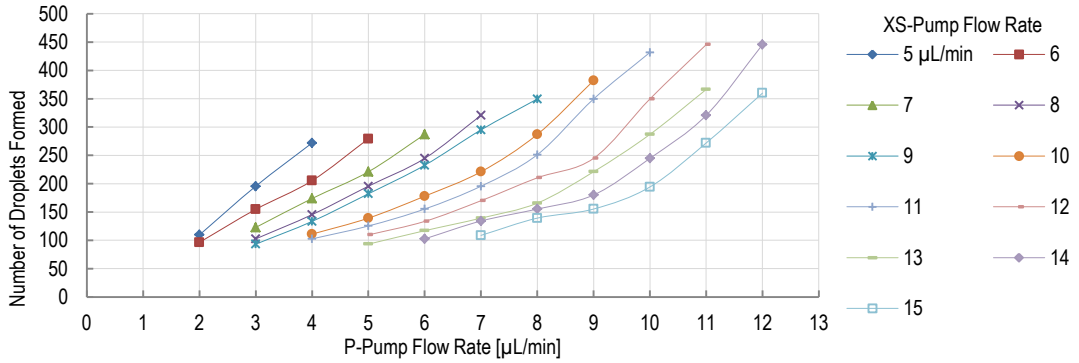
Droplet Splitting Characterization



Size dependence on flow rate. XS-Pump flow rate Q^- range shown in legend.



Droplet breakup rate dependence on flow rate. XS-Pump flow rate Q^- range shown in legend.



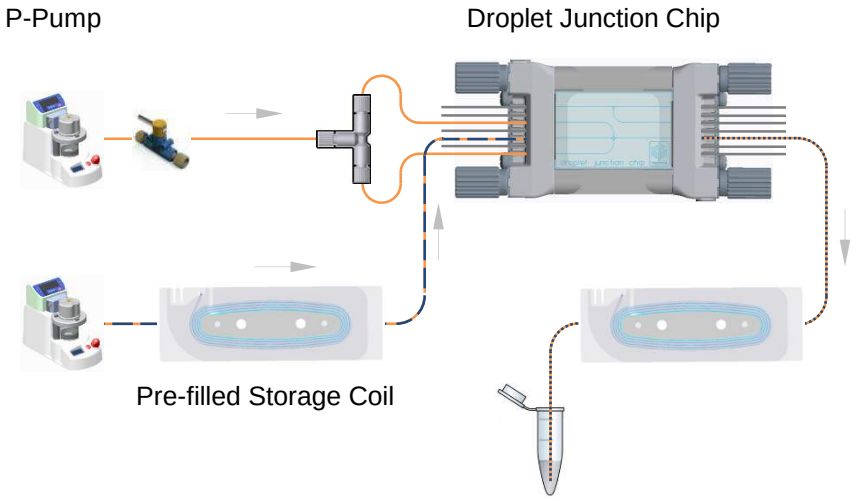
Number of split droplets generated.

2-Step Alternate Method for Converting nL plugs into pL Droplets.

Droplet production tests warrant the use of P-Pumps which provide ultra smooth pulseless flow. These use pressure to displace fluids in contrast with positive displacement pumps. In this application note however, the XS-Pump used to produce the base flow rate is a syringe pump. While it houses precision stepper motor based mechanisms, it is inherently not as smooth as a P-Pump. For this reason, the picoliter droplets produced in this application note are found to have size variation outside of +/- 10% of droplet diameter. An alternate setup is presented below using a 2-step process.

- Step 1: The first step involves a simpler setup where the XS-Pump aspirates fluid via the Storage coil from the Dropix (similar to Mode 1 operation). This fills the storage coil with nanoliter plugs of fluid.

- Step 2: In the second step, a more involved setup is used, much like a general droplet production setup. The storage coil from the first step is placed inline between a P-Pump and the droplet junction chip.



Conclusion

Droplet-on-demand technology is used to create a segmented flow of aqueous plugs spaced apart by fluorocarbon oil. These plugs are split into still smaller droplets which are stored in optically clear tubing in a Storage Coil. A fluorinated surfactant is used in small amounts to stabilize the emulsion as well as to lubricate the surface for controlling surface wettability. The system is found to be easy to use and reliable over a wide range of test conditions.

The droplet splitting test demonstrates the ability of the droplet-on-demand feature of the Dropix. The Dropix system is tested with this additional feature of droplet splitting (named Mode 3 operation). A sequence of 200 nL aqueous slugs in generated, and then split into many smaller droplets. Droplet splitting converts 200 nanoliter plugs into picoliter droplets. The size of the picoliter droplet depends on the flow ratio between the XS-Pump flow rate and the P-Pump flow rate. The number of split droplets per plug is dependent on the ration between the nanoliter plug and the picoliter droplet volume. Although the tests show the splitting of a 200 nL aqueous plug, the system is capable of splitting plugs of volumes ranging from as low as 50 nL to as high as 1 µL.

The XS-Pump flow rate was varied between 5 and 15 µL/min. The P-Pump flow rate was varied from 1 to 15 µL/min. Droplet sizes of between 90 µm and 180 µm were produced at production rates of 10 Hz to 70 Hz respectively. Droplet production rate increases with the total amount of fluid throughput, and is highest at peak pumping rates.

The fluid plugs, once split on the flow focussing chip are stored in a Storage coil. The Storage Coil made of optically clear polymer, has wetted parts made of FEP. FEP is chemically resistant to most fluids, and allows optical diagnostics to be performed on the encapsulated fluidic content within.

The droplet splitting mode of Dropix extends the working volume to picoliters. Sample replication and library generation are core applications dependant on this feature coupled with the advantage of droplet on demand.

Appendix A: System Component List

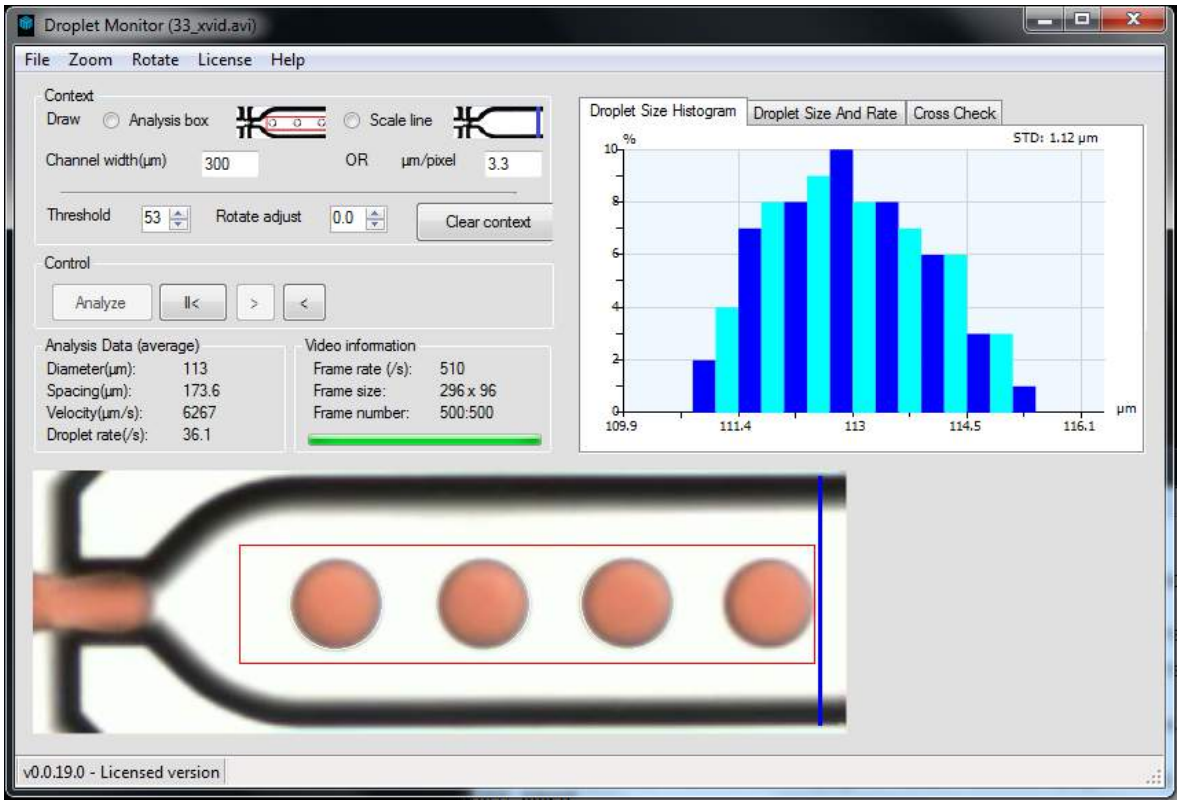
Part No.	Part Description	#
3200350	Mitos Dropix ®	1
3200197	USB to RS232 Adaptor Cable	1
3200349	Droplet Storage Coil – 0.25mm	1
3200414	Dropix® Fluid Reservoir - PMMA	1
3200351	Dropix® Sample Strip (Pack of 8)	1
3200356	Dropix® Sample Strip Holder	1
3200353	Dropix® Sample Hook – 0.8mm	1
3200355	Dropix® Sample Hook Fitting – 0.8mm	1
3200302	FEP Tubing, 0.8mm x 0.25mm, 10 metres	1
3200057	Mitos Duo XS-Pump	1
3000245	Valve for Mitos Duo XS-Pump (3 Port)	1
3000251	Syringe for Mitos Duo XS-Pump, 500µl	1
3200016	Mitos P-Pump	1
3200095	Mitos Sensor Display	2
3200098	Mitos Flow Rate Sensor (1-50 µl/min)	2
3200297	H Interface 7-way (22.5mm)	1
3200148	Linear Connector 7-way	2
3000158	Droplet Junction Chip (100µm etch depth)	1
3200307	End Fittings and Ferrules for 0.8mm Tubing (pack of 10)	1
3000477	End Fittings and Ferrules for 1.6mm Tubing (pack of 10)	1
3000397	T-Connector ETFE	1
3200305	PTFE Plug 0.8mm (pack of 10)	1

3200087	2-way In-line Valve	1
3200302	FEP Tubing, 0.8 x 0.25mm, 10 metres	1
3200063	FEP Tubing, 1/16" x 0.25mm, 10 metres	1
3200300	FEP Tubing, 1/16" x 0.1mm, 10 metres	1
3200269	F10 Flow Resistor	1
3000398	PTFE Tube Cutter	1
3200245	Ferrule with Integrated Filter (pack of 10)	1
3200050	High Speed Camera and Microscope System	1
3200422	Fluoridrop 500 ml	1
	Droplet Monitor Software	1
	Flow Control Center Software	1

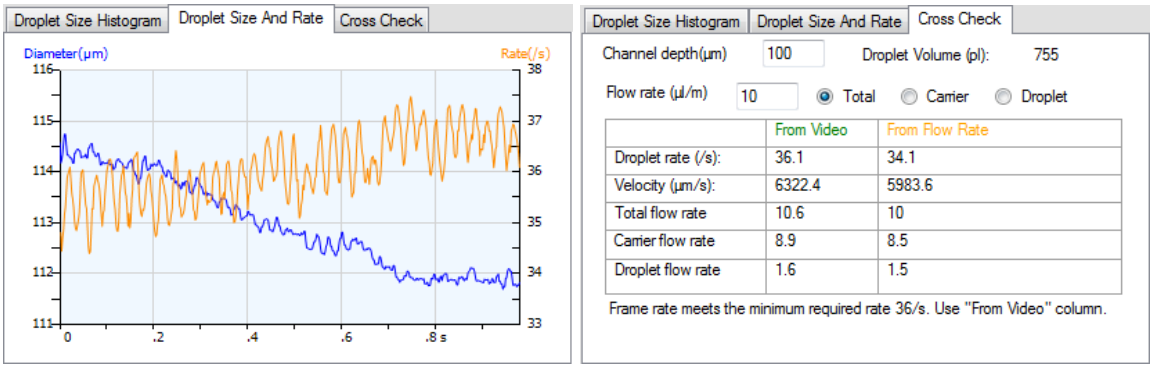
Appendix B: Droplet Monitor Software

Droplet Size Characterization

Videos of droplet production are analyzed using Dolomite's Droplet Monitor Software. A screenshot is shown below. The average Diameter and Droplet Rate are recorded for each video and are presented in the following results table.



Screenshot of Droplet monitor software. An imported video file is analyzed to give the droplet size and drpolet production rate variation over the duration of the video.



Left: 'Droplet Size and Rate' tab. Right: 'Cross Check' tab.

A check is done to ensure that the droplet production rate is always slower than the video capture rate. This ensures that each droplet is imaged, and that the production rate is accurate. The Cross Check involves comparing software determined Droplet Rate with the quantity (Droplet Flow rate/Volume of droplet).

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)**.

Cover Images:

- Top left: CAD Render of Dropix.
- Lower left: Microphotograph of a nanoliter plug breakup into picoliter droplets in the droplet junction chip.
- Mid right: Photograph of storage coil filled with nanoliter plugs.
- Lower right: Image of flow focussing droplet chip assembled with multiflux 2 connectors, interface, and tubing.

ДИА•М
современная лаборатория

Москва ■ ул. Космонавта Волкова, 10 ■ тел./факс: (495) 745-0508 ■ sales@dia-m.ru

Новосибирск
пр. Акад. Лаврентьева, 6/1
тел./факс: (383) 328-0048
nsk@dia-m.ru

Санкт-Петербург
ул. Профессора Попова, 23
тел./факс: (812) 372-6040
spb@dia-m.ru

Пермь
Представитель в УФО
тел./факс: (342) 202-2239
perm@dia-m.ru

Казань
Оренбургский тракт, 20
тел/факс: (843) 277-6040
kazan@dia-m.ru

Ростов-на-Дону
пер. Семашко, 114
тел/факс: (863) 250-0006
rnd@dia-m.ru

Воронеж
тел./факс: (473) 232-4412
voronezh@dia-m.ru