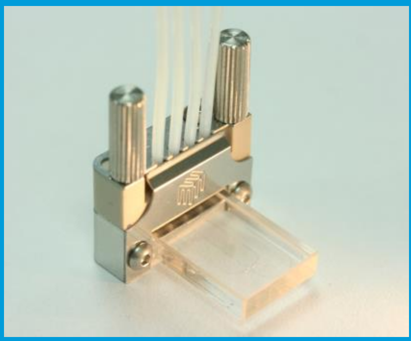
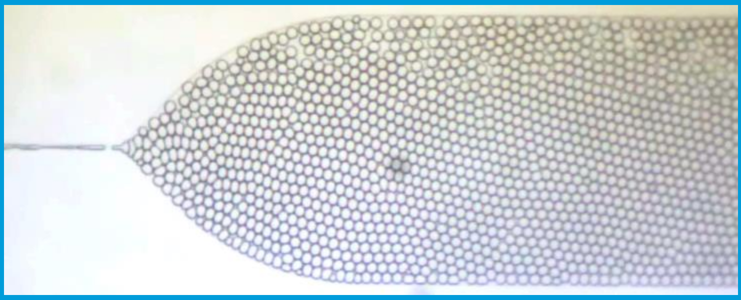
2 – 10 µm Diameter Water Droplets in Mineral Oil Emulsion Production

Small Droplet System (5 µm etch depth and 8 µm junction width)





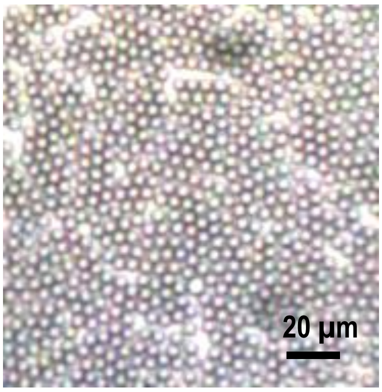
Application Note	Page

Summary

The Small Droplet System setup is described in detail to illustrate the assembly of components along with the fluidic setup. The system is used in a test case to generate water droplets in mineral oil, an organic fluid.

By setting the pressures on the P-pumps, stable and monodisperse droplets are found to be produced over a wide range of sizes.

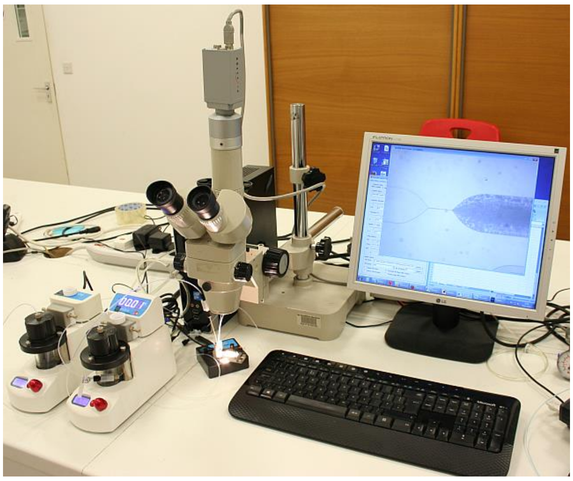
The experiment showed successful generation of droplets with the following specification.



A microscopic image of approximatley 4 µm diameter droplets produced in tests.

- Small droplet chip used with etch depth of 5 µm and junction width of 8 µm.
- Water-in-mineral oil emulsion generated.
- Droplet sizes achieved.
    - Smallest monodisperse droplet size 2.7 µm diameter
    - Largest monodisperse droplet size 10.7 µm diameter.
    - Largest non-monodisperse droplet size 18 µm (cover photo) diameter.
- SPAN-80 surfactant used to stabilize the emulsion.
- Test setup utilizes 2 P-Pumps, Flow sensors, Optical system, and Flow accessories such as tubing, connectors and flow resistors.
- System tested in ‘Pressure Control Mode’ varied between 0-5 bar each.
- The flow rates observed were 0 – 300 nL/min and 0 – 60 nL/min for the mineral oil and water respectively.
- Production rates varied between 26 and 1600 Hz.
- In general, larger droplets imply lower production rate and vice versa.

Flow Focussing Based Droplet Production

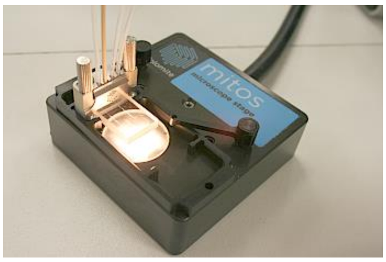


Small droplet system. A live video feed is available for capture on the PC monitor. The high speed camera is mounted on the top viewing port of the stereo microscope and connects to the PC via firewire cable.

An emulsion is a fluid consisting of two immiscible liquids, one dispersed as droplets surrounded by the other as carrier. Emulsions are used in a wide range of industries including life sciences, pharmaceutical, food, materials science, petrochemical, agricultural and others. Very small sized emulsions in the sub-10 µm range are particularly interesting due to their relatively higher interfacial area which facilitates highly efficient reactions and diffusive release in drug delivery applications. Also, single cell encapsulation is relevant for droplets that are just slightly larger than the cells themselves, falling in the 5-20 microns in size. Cell encapsulation is generally used for exploratory R&D such as allergic response studies or cancer screening. Indeed some researchers have attempted to use droplets as cell surrogates to model interfacial diffusion as cross-membrane ion transport mechanisms.

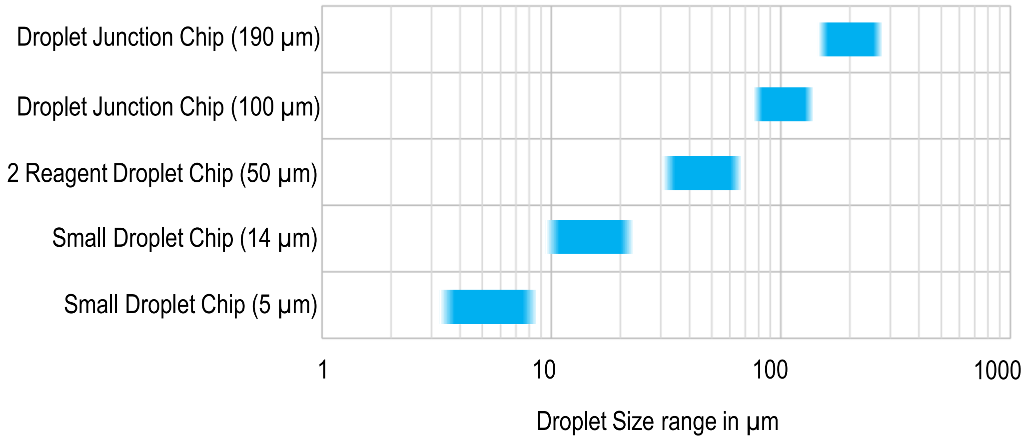
When considering bulk emulsification, gentle methods of production result in relatively large emulsion size (30-100 µm). To access smaller sizes, greater mechanical power input is necessary, via impellers, or chemical breakdown of the fluidic interface achieved with surfactants. Such approaches do not provide acceptable quality in the production of robust emulsions, or of solid particle production. The resulting droplets have wide size distributions requiring time-consuming and often wasteful purification.

There is however a class of applications utilizing biomolecules and cells, where a limiting shear force precludes production methods using high power mechanical input. Especially in such applications, the small droplet system is particularly useful as with no external energy input (other than the pressure energy of the fluid), droplet sizes in the 2-30 µm sizes are readily produced in one step. More so, achieving the size range is enabled by the system geometry and not surfactant dependence. The Dolomite Small Droplet System comprises pumping, valving, the microfluidic device, and control software. The two key elements of the system are the Mitos P-Pump which delivers precise flow to ensure droplet monodispersity, and the precision fabricated microfluidic device which offers excellent dimensional tolerance, ease of setup and use, and on-chip filtration to maximize life.



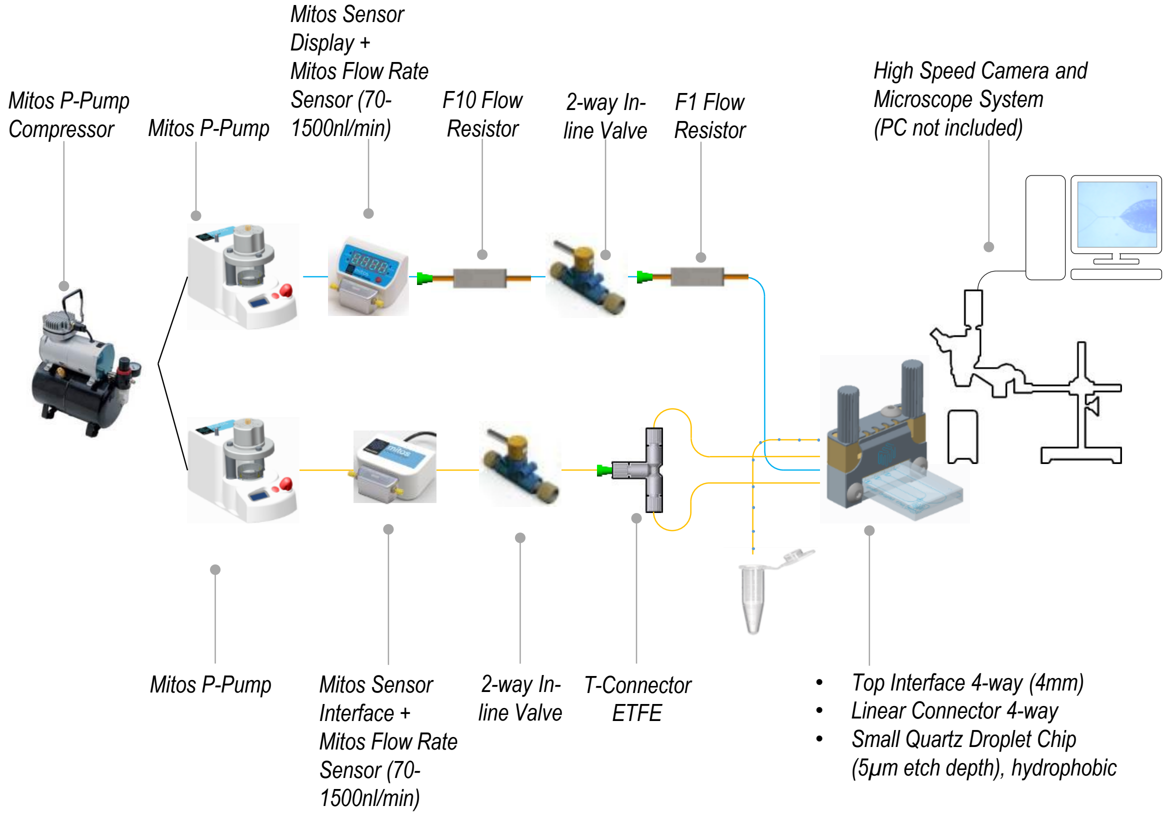
Microscope Stage Adaptor for Top Interface with chip, connector and interface, all mounted on a light stage.

Dolomite's droplet systems utilize microfluidic methods to directly generate monodisperse emulsions, eliminating the need for further processing. The Small Droplet System generates monodisperse droplets in the sub-30 µm range, and is available to create oil-in-water or water-in-oil emulsions. This application note describes the setup and methods used to generate water-in-mineral oil.



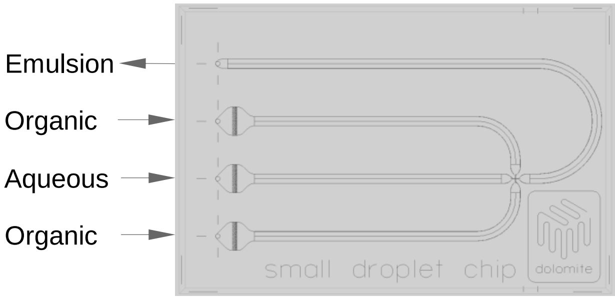
Some standard chips (etch depth listed) listed along with range of droplet sizes achievable respectively. Guideline for droplet size range is 0.75 to 1.25 × etch depth.

Setup



Sketch of the test setup. Ferrules with Integrated Filters are shown in green color, other ferrules not shown here to keep schematic clear. Sketch not to scale. Part numbers available in Appendix.

The Small Droplet System is based around Dolomite's Small Droplet Chips. These are available with 5 µm or 14 µm channel depth, with hydrophobic coating or hydrophilic plain glass. This test was carried out using a hydrophobic, 5 µm Small Droplet Chip (Part No. 3200153).



*Schematic of chip layout showing inlets and outlets. Detailed chip information is available on the datasheet**.

* http://www.dolomite-microfluidics.com/images/stories/PDFs/datasheets/small_droplet_chips_product_datasheet.pdf

Fluids were supplied via two P-Pumps, tubing, connectors and flow resistors as shown below. The droplet phase consisted of water and the carrier phase Mineral Oil + 1% (v/v) Span 80 (Span is a surfactant used to increase droplet stability).

In-line filters are included in addition to the filters included on-chip, to reduce the likelihood of particulate blockage. Each flow resistor kit also contains 5 sets of flow accessories to enable the flow resistors to be connected in-line and the fluids to be filtered before entry into the flow resistor.

Aqueous droplet line

Section	FEP Tubing OD(mm), ID(mm); L(mm)
Compressor to P-Pump	Pneumatic (provided with P-Pump)
Pump to Sensor	Flow adaptor (provided with sensor)
Sensor to F10 Flow Resistor (with green filter ferrule)	1.60, 0.25, 200
F10 Flow Resistor to 2-way in line valve	1.60, 0.25, 200
2-way in line valve to F1 Flow Resistor (with green filter ferrule)	1.60, 0.25, 200
F1 Flow Resistor to Linear Connector 4-way	1.60, 0.10, 200

Organic Carrier Line

Section	FEP Tubing OD(mm), ID(mm); L(mm)
Compressor to P-Pump	Pneumatic (provided with P-Pump)
Pump to Sensor	Flow adaptor (provided with sensor)
Sensor to 2-way in line valve	1.60, 0.25, 200
2-way in line valve to T-connector ETFE (with green filter ferrule)	1.60, 0.25, 300
T-connector ETFE to Linear Connector 4-way	2 × (1.60, 0.10, 300)

Collection line

Section	FEP Tubing OD(mm), ID(mm); L(mm)
Chip to collection	1.60, 0.10, 500

Pumping pressures and thus flow rates were varied and high-speed images of droplet formation were captured using a High Speed Camera and Microscope (Part No. 3200050).

The flow resistance of the system was kept constant and the pressure varied. By estimating the flow resistance and recording the two pressures the flow rates for the water and oil were calculated.

Results & Analysis

For given droplet phase flow rate, increasing the carrier phase flow rate resulted in smaller droplets. Pressures also had an influence on the spacing between droplets, with close proximity of droplets leading to relatively higher incidents of coalescence.

Stable droplet production occurs within a range of droplet phase and carrier phase flow rates, as indicated by the graph below. Outside the stable range, chaotic flow or backflow occur. An example of chaotic flow at high pressures shown below.

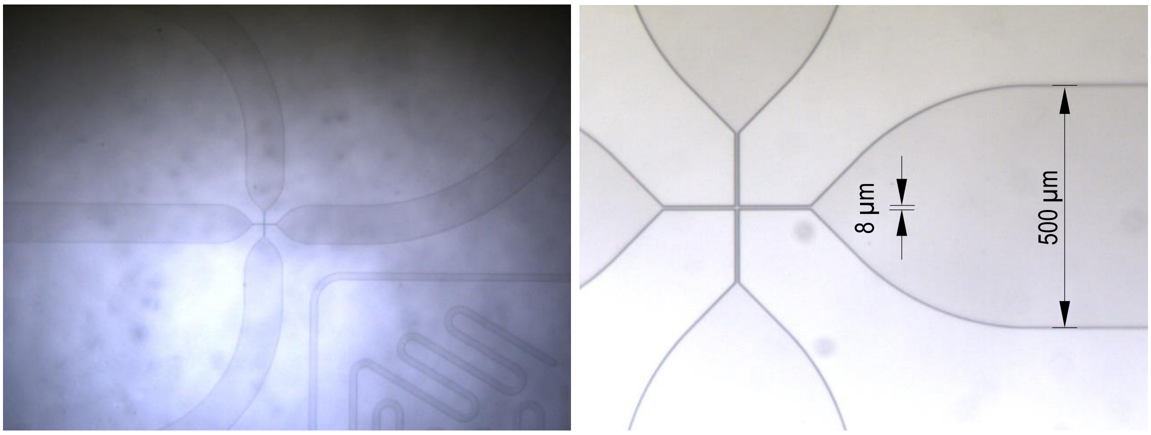
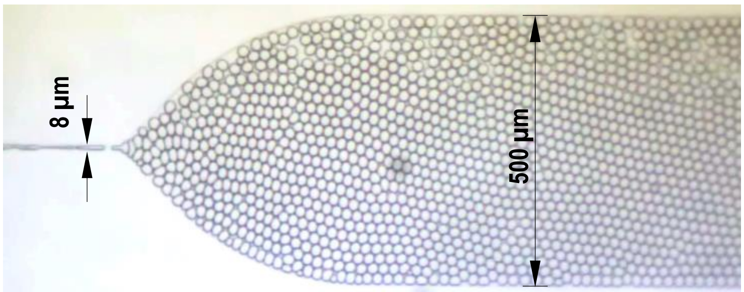
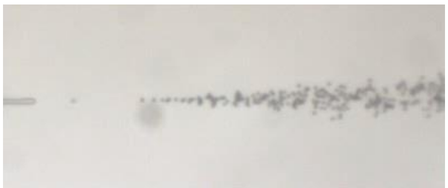
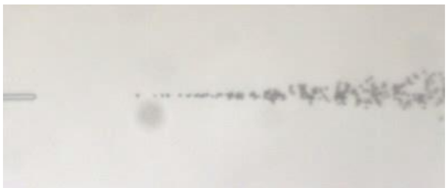
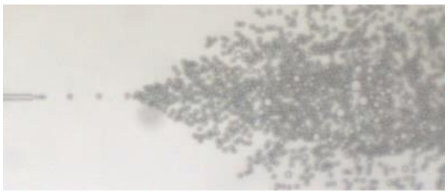
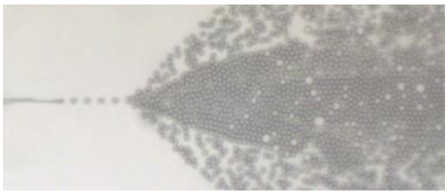
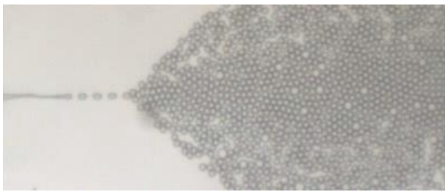
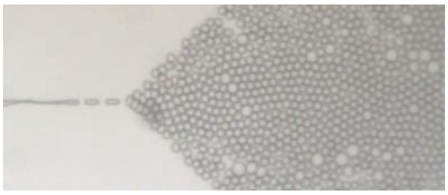
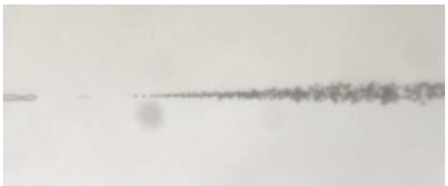


Image of chip junction at minimum (left) and maximum magnification (right) available using the High Speed Imaging System. The smallest feature size on the chip is the channel junction with a depth of 5 µm and a width of 8 µm.

From captured images, the droplet size is estimated by comparing the pixel size of the droplet versus a known reference length which in this case is the channel width. Once the droplet size is known, the volume can be calculated. Because of the small size of the droplet despite the high magnification, the pixel/µm ratio is as low as 1, and therefore there is some uncertainty in the droplet size estimation. The frequency is then calculated as volumetric flow rate of droplet fluid/volume of a single droplet.

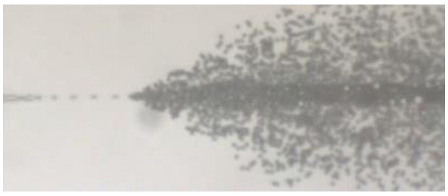
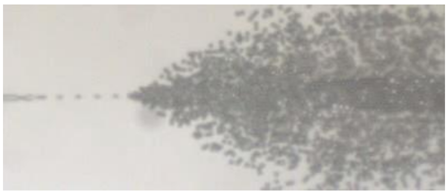
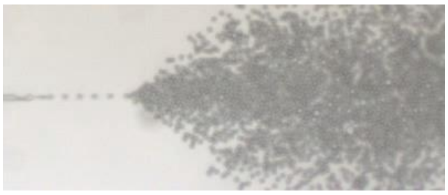
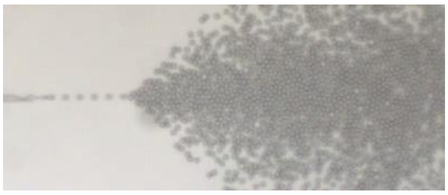
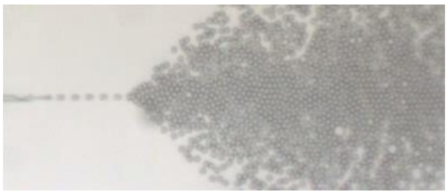
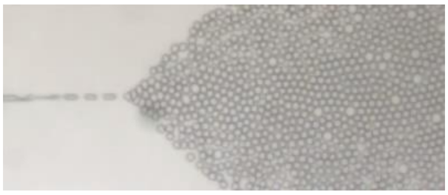


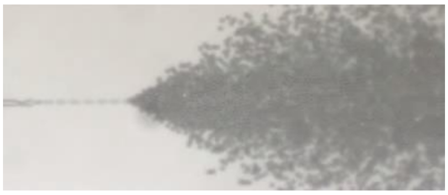
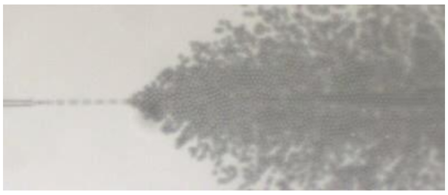
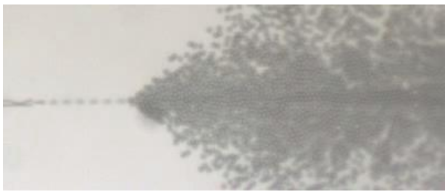
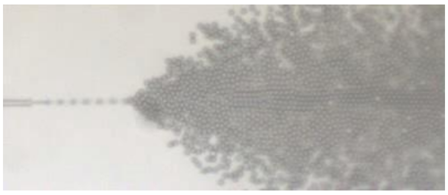
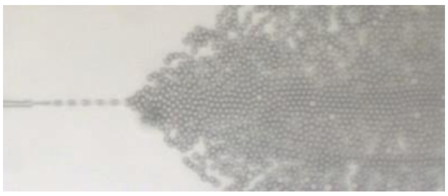
Droplets are sized by comparing their pixel size along with the pixel width of a known chip dimension. This could be either the wide part (0.5 mm) or with the junction width (8 µm).

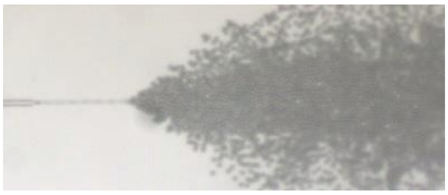
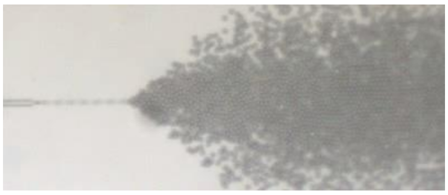
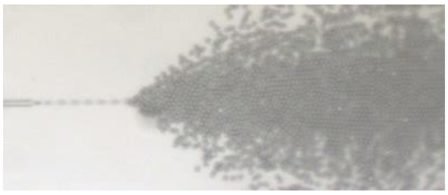
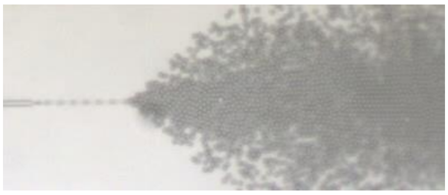
Organic Carrier		Aqueous Droplet		Junction Image	Droplet Size†	Rate
P	Q	P	Q		*D*	*f*
bar	nL/ min	bar	nL‡/ min		µm	Hz
1	20	0.77	1		5.3	210
1	18	0.8	3		5.3	210
1	13	0.85	5		6.7	107
1	10	0.9	9		6.7	107
1	8	0.95	12		8.0	62
1	6	1	14		10.7	26
2	95	1.47	3		4.0	497

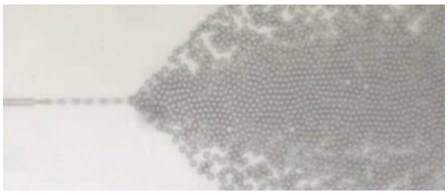
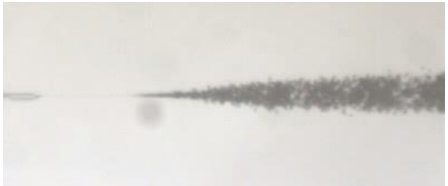
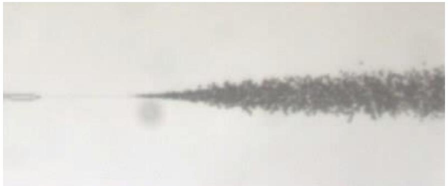
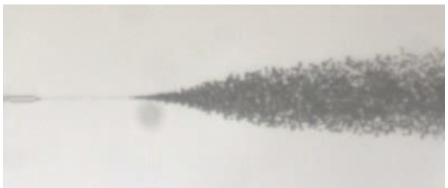
† *Droplet sizes larger than 5 µm channel etch depth are squashed and appear larger due to the deformation. Volume correction is suggested on Page 6 of http://www.dolomite-microfluidics.com/images/stories/PDFs/application_notes/Droplet_Junction_Chip_characterisation_-_application_note.pdf*

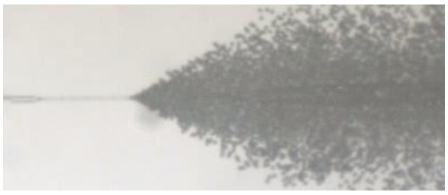
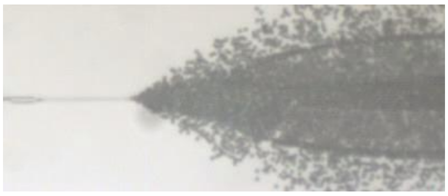
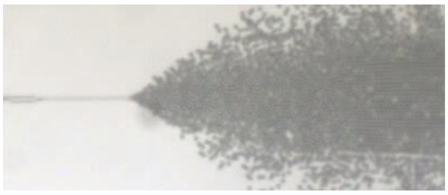
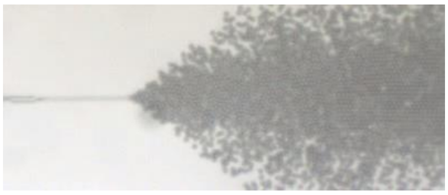
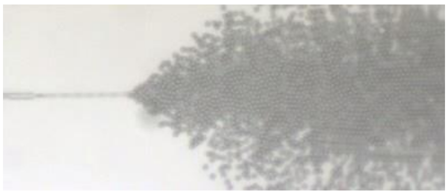
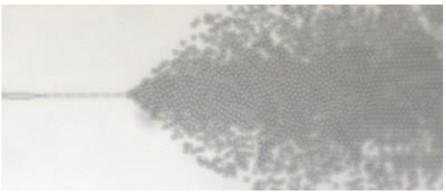
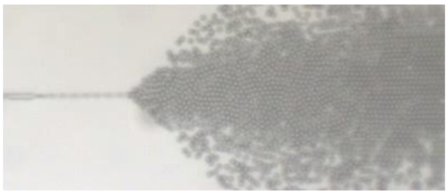
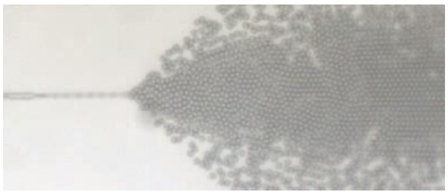
‡ *The flow sensor is recommended for flow rates in the range of 70-1500 nL/min. Some flow rates reported here were out of range. The error expected could therefore be more than the rated 5%.*

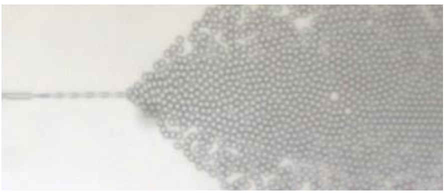
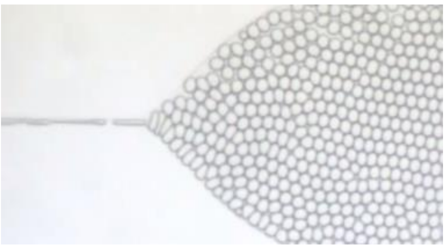
Organic Carrier		Aqueous Droplet		Junction Image	Droplet Size†	Rate
P	Q	P	Q		*D*	*f*
bar	nL/ min	bar	nL‡/ min		µm	Hz
2	68	1.5	10		4.0	497
2	47	1.55	16		5.3	210
2	38	1.6	21		5.3	210
2	30	1.65	23		6.7	107
2	25	1.7	26		6.7	107
2	20	1.75	28		8.0	62
2	18	1.8	30		8.0	62
2	10	1.9	32		9.3	39

Organic Carrier		Aqueous Droplet		Junction Image	Droplet Size†	Rate
P	Q	P	Q		*D*	*f*
bar	nL/ min	bar	nL‡/ min		µm	Hz
2	8	2	35			
3	180	2.15	1		2.7	1679
3	165	2.25	4		2.7	1679
3	130	2.35	10		5.3	210
3	120	2.45	13		5.3	210
3	92	2.5	23		6.7	107
3	67	2.6	30		6.7	107
3	46	2.7	40		8.0	62
3	37	2.75	45	streaming flow		

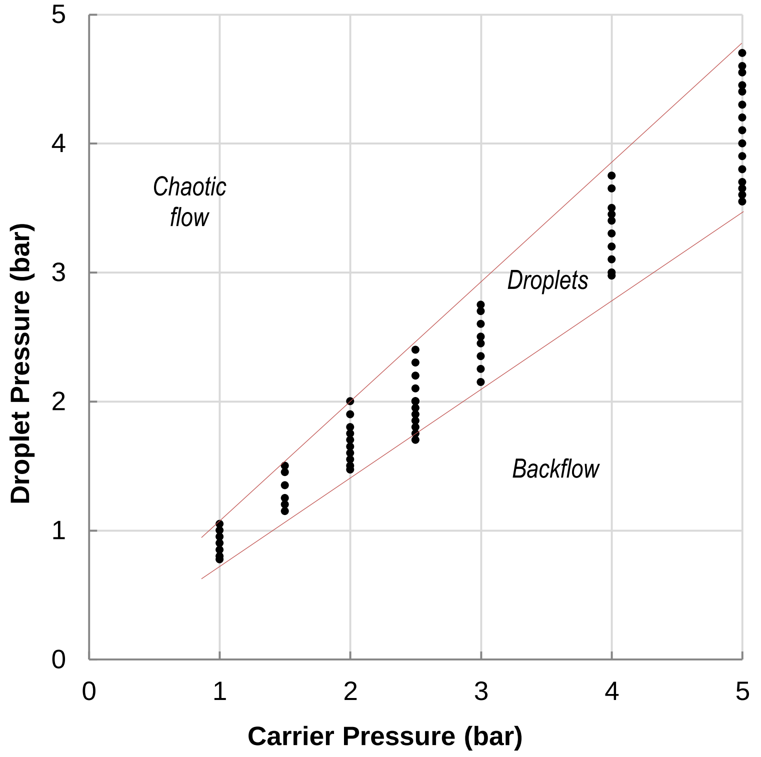
Organic Carrier		Aqueous Droplet		Junction Image	Droplet Size†	Rate
P	Q	P	Q		*D*	*f*
bar	nL/ min	bar	nL‡/ min		µm	Hz
4	260	2.97	1		4.0	497
4	240	3	3		4.0	497
4	205	3.1	6		4.0	497
4	180	3.2	10		5.3	210
4	155	3.3	15		6.7	107
4	140	3.4	18		6.7	107
4	135	3.45	21		6.7	107
4	100	3.5	35		8.0	62

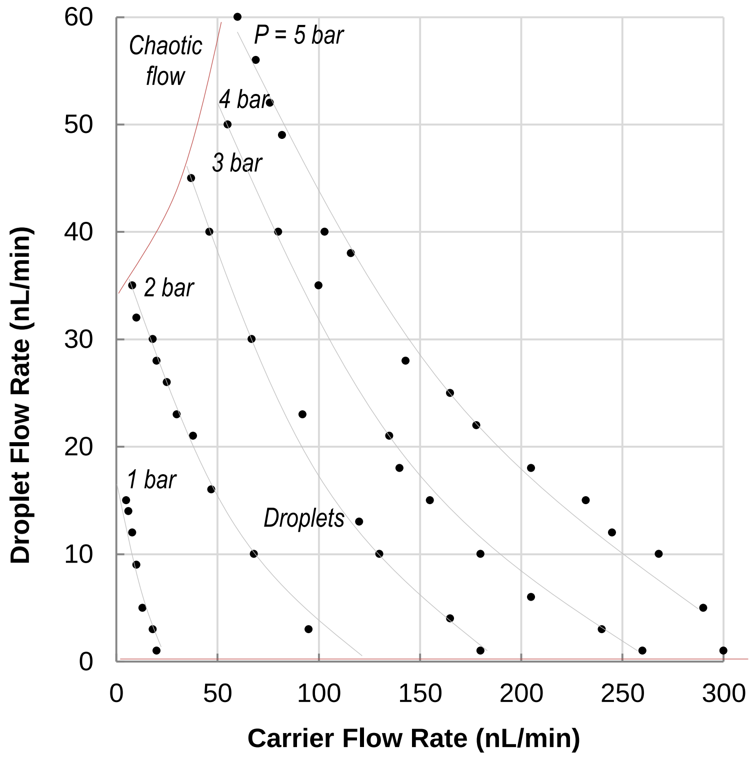
Organic Carrier		Aqueous Droplet		Junction Image	Droplet Size†	Rate
P	Q	P	Q		*D*	*f*
bar	nL/ min	bar	nL‡/ min		µm	Hz
4	80	3.65	40		8.0	62
4	55	3.75	50			
5	300	3.55	1		2.7	1679
5	290	3.6	5		2.7	1679
5	268	3.65	10		2.7	1679
5	245	3.7	12		2.7	1679
5	232	3.8	15		4.0	497
5	205	3.9	18		4.0	497

Organic Carrier		Aqueous Droplet		Junction Image	Droplet Size†	Rate
P	Q	P	Q		*D*	*f*
bar	nL/ min	bar	nL‡/ min		µm	Hz
5	178	4	22		5.3	210
5	165	4.1	25		5.3	210
5	143	4.2	28		6.7	107
5	116	4.3	38		6.7	107
5	103	4.4	40		6.7	107
5	82	4.45	49		6.7	107
5	76	4.55	52		6.7	107
5	69	4.6	56		8.0	62

Organic Carrier		Aqueous Droplet		Junction Image	Droplet Size[†]	Rate
P	Q	P	Q		*D*	*f*
bar	nL/min	bar	nL[‡]/min		µm	Hz
5	60	4.7	60		9.3	39
6	50	5.5	32		18.7	5

The datapoints from the previous table are plotted on two different axes, first the pressure axes (plotting carrier pressure vs. droplet pressure), and then on the flow axes (plotting carrier flow rate vs. droplet flow rate).





Operating space tested. Stable droplets are obtained for the conditions marked by the region 'Stable'. Outside of this, either backflow, or chaotic polydisperse droplet production occurs.

The objective is to map the operating space and identify where stable droplet generation is expected. In both grpahs, the limits of stable droplet production are identified by the red lines beyond which the zone is marked as *Chaotic Flow* and *Backflow* respectively. On the flow rates axes, *Backflow* regime is when a datapoint lies below the x-axis.

Three zones are observed. These are:

- Stable droplet zone – Monodisperse droplets are achieved with diameters varying between 2 and 10 µm. The monodispersity can be quantified in terms of coefficient of variation, which is expected to be between 20%. The appearance of the CV to be large is an artefact arising from the very small droplet sizes produced. This is a desirable operating zone.
- Chaotic flow zone – In this zone, the cumulative flow rates are excessively high. Varying the flow ratios fails to cause droplet pinchoff and as a result, the droplet formation fails. This is undesirable.
- Back flow – This occurs when the pressure ratio is excessive. The droplet fluid forces the carrier fluid to flow backwards, or vice versa. This too is undesirable and should be avoided. Monitoring flow rates with flow sensors will help avoid this situation.

The shape and extent of the operating space depends strongly on the flow resistances selected for the setup. If the flow resistances are reduced on both droplet and carrier lines, the operating space (on the pressure axes for example) would shift to the lower left hand corner, and vice versa. Very small changes flow rate show up as large fluctuations due to the very small cross sectional area of the channel. If excessive fluctuation is observed in the flow rates, then addition of flow resistance will help resolve the problem. This is usually added on to the less viscous fluid path.

Flow conditions were varied by controlling the droplet system in flow control mode. In this mode, the target pressures are set and the flow rate is an outcome of the flow resistance. When working in flow control (not demonstrated in this application note), a target flow rate is set, and the appropriate pressure is found to achieve this target flow rate. Flow control is recomended for advanced users who have done some preliminary characterization of their droplet system. The pressure on the P-pumps are sequnetially changed. Effectively the pressure ratio changes

It is important to note that impurity or particulate matter in the fluids may cause chip/tubing clogging. For this reason, care must be taken to ensure that all hardware and reagents are free from foreign particulate matter and all fluids filtered prior to use. Care should be taken to ensure that the working area is generally dust free. When cutting tubing, the use of a tube cutter is recommended as this maximizes the likelihood of consistency in connections.

Conclusion

The Small Droplet System is setup and used to produce an emulsion. The emulsion consists of water droplets suspended in mineral oil. There is SPAN-80 surfactant added in a volumetric ratio of 1% to the mineral oil, and this stabilizes the suspension.

The system setup is depicted along with some detail about tubing used as well as flow resistances used in the system. The system is run in pressure control mode using 2 P-Pumps. The pressures on the pumps are sequentially varied so as to explore a 2 dimensional operating space over the working pressure range of the pumps.

For a given carrier pressure (flow), changing the droplet pressure affects the droplet size and production frequency as follows. Droplet fluid moves backwards (backflow) at low droplet pressures. Droplet fluids moves forwards (desirable) and pinches off at the flow focussing junction forming monodisperse droplets. Droplet fluid moves forwards excessively fast and fails the droplet pinchoff forming a streaming flow.

The pressures on the two P-Pumps were varied between 0-5 bar each. Setting optimal flow resistances ensures that the entire range of pressures is usable on each of the droplet and carrier line. The flow rates observed were 0 – 300 nL/min and 0 – 60 nL/min on the droplet line. The resulting monodisperse droplet sizes ranged from 2.7 µm – 10.7 µm diameter range and production rates varied between 26 and 1600 Hz. Larger droplets could be made at higher flow rates however these were not monodisperse.

Appendix A: System Component List

Part No.	Part Description	#
3200016	Mitos P-Pump	2
3200095	Mitos Sensor Display	1
3200200	Mitos Sensor Interface	1
3200100	Mitos Flow Rate Sensor (70-1500nl/min)	2
3000024	Linear Connector 4-way	1
3000109	Top Interface 4-way (4mm)	1
3200153	Small Quartz Droplet Chip (5µm etch depth), hydrophobic	1
3200073	Pressure-based Droplet Starter Kit	1
3200300	FEP Tubing, 1/16" x 0.1mm, 10 metres	1
3200063	FEP Tubing, 1/16" x 0.25mm, 10 metres	1
3200245	Ferrule with Integrated Filter (pack of 10)	1
3200272	Flow Resistor Kit	1
3000397	T-Connector ETFE	1
3200087	2-way In-line Valve	2
3200314	Microscope Stage Adaptor for Top Interfaces	1
3200128	Mitos P-Pump Compressor 6bar EU (230V/50Hz) (3200128)	1
3200050	High Speed Camera and Microscope System	1
	Pixelink OEM Capture Software	1
	Flow Control Center Software	1
	Droplet Monitor Software	

IP License

Dolomite is a licensee of Japan Science and Technology Agency ("JST") under JST's microdroplet generation technology.

This enables our customers to purchase and use our droplet chips for R&D purposes without any restriction from this comprehensive IP family.

Contact us for more information about licensing this IP for your custom application or chip design.

Cover Images:

- Top left: Photograph of a Small Droplet Chip assembled in a Top Interface along with fluid supply tubing.

- Top right: Micrograph of droplets produced using the Small Droplet System.

- Bottom: Microscopic image of the flow focussing junction on a Small Droplet Chip showing production of ~18 µm diameter water droplets in Mineral Oil (with 15 v/v SPAN-80 as surfactant).

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