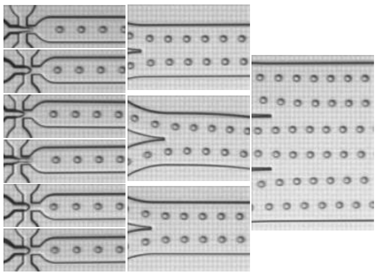
Parallel Droplet Generation

Demonstration of a Parallel Droplet Generation setup for producing water droplets in hexadecane

dolomite

Introduction

This application note describes the performance of a high throughput droplet generation system. Dolomite's advanced fabrication processes and high density microfluidic connections allow the design of a chip with 6 flow-focussing junctions in parallel that can achieve generation rates of up to 30 kHz in test conditions.



The system is based around a standard Dolomite chip with a series of parallel droplet junctions producing water-in-hexadecane droplets. Flows rates of both fluids are controlled via Dolomite P-Pumps providing pulseless flow. The resulting droplet size and frequency is varied to explore the operating boundary of the system.

Applications of droplet generation systems include but are not limited to drug and food manufacturing. Production volumes of emulsions are attainable via 'numbering up' strategies which allow monodisperse droplet production at uncompromised rates.

The method demonstrated here utilizes 6 junctions in parallel, all etched on a single chip. The concept which is successfully demonstrated here holds for an even higher junction density* per chip, limited only by the physical layout requirement of the microfluidic architecture. Increasing junction density* linearly increases production capacity.

Salient features are:

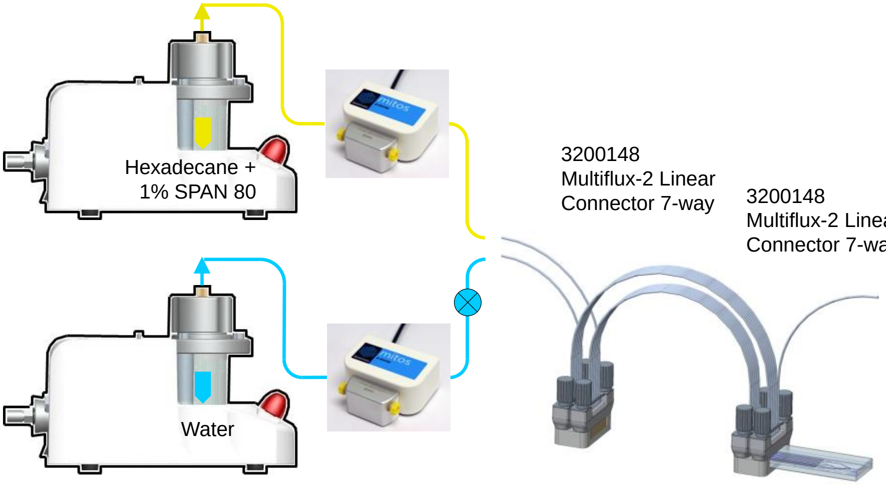
- **Monodisperse droplets.**
- **Up to 30 kHz droplet generation frequency.**
- **Droplet diameters in the range of 20 µm to 60 µm.**
- **Over 600 ml emulsion production capacity per 24 h period.**

System Configuration

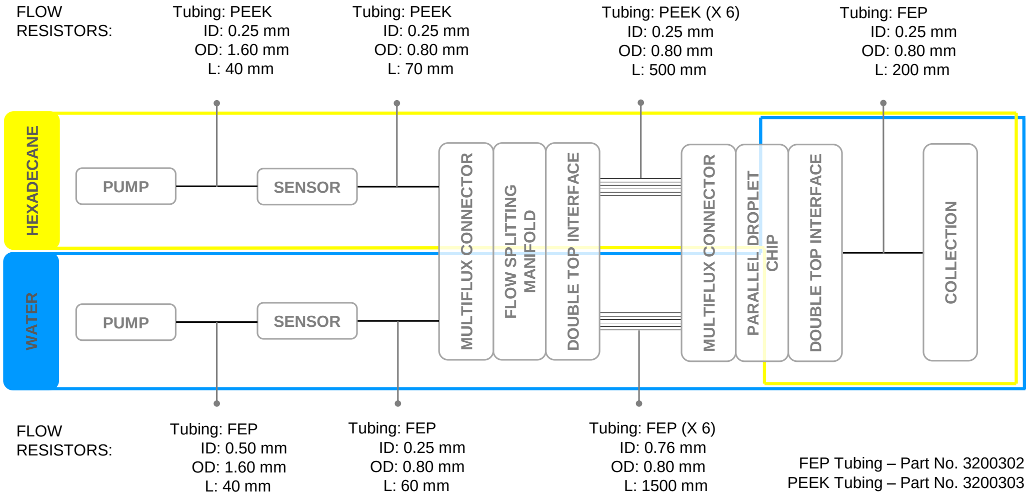
The 6 Junction hydrophobic droplet chip (Part No. 3200289) is used with a Linear Connector 7-way (Part No. 3200148) and a Double Top Interface (Part No. 3200296) to interface the fluidic connection between tubing and chip. PTFE Plug 0.8 mm (Part No. 3200305) is used to block unused ports on the Connector. A double Flow Splitting Manifold 7-way (Part No. 3200308) distributes fluid equally into all 6 inlet ports from a single pump source.

* Junction density refers to the number of parallel junctions per chip, in this case 6

In the experimental set-up shown the two Mitos P-Pumps (Part No. 3200016) deliver hexadecane and water streams to the parallel droplet chip. Flow sensor interface (Part No. 3200200) equipped with a sensor capable of 30-1000 µL/min measurement range (Part No. 3200097) records the hexadecane flow rate. Flow sensor interface (Part No. 3200200) equipped with a sensor capable of 1-50 µL measurement range (Part No. 3200098) records the water flow rate. FEP tubing (Part No. 3200302) and PEEK tubing (Part No. 3200303) are used to deliver fluids across the system.



Flow resistors enable the system to be used in an exploratory mode. By suitably selecting flow resistors, a droplet system may accommodate fluids with a wide range of viscosity. Suitable lengths of tubing are cut as flow resistors and connected in the setup as shown in the block diagram below. The resistances are selected based on calculations performed using fluid properties of Hexadecane (with 1% SPAN 80) and Water.



With a change of fluids, the tubing is easily modified to change the flow resistance, thereby enabling quick parametric variation. Additionally, the ability to tune resistances in the system allows for higher resolution in fluid control. 12 resistors interface between the connectors to enable desired flow rates. A brief description of leveraging resistors to tune droplet setups is presented in Appendix C.

‘On-chip resistors’ on the other hand are recommended for production oriented applications where the fluid properties are known beforehand and unlikely to change.

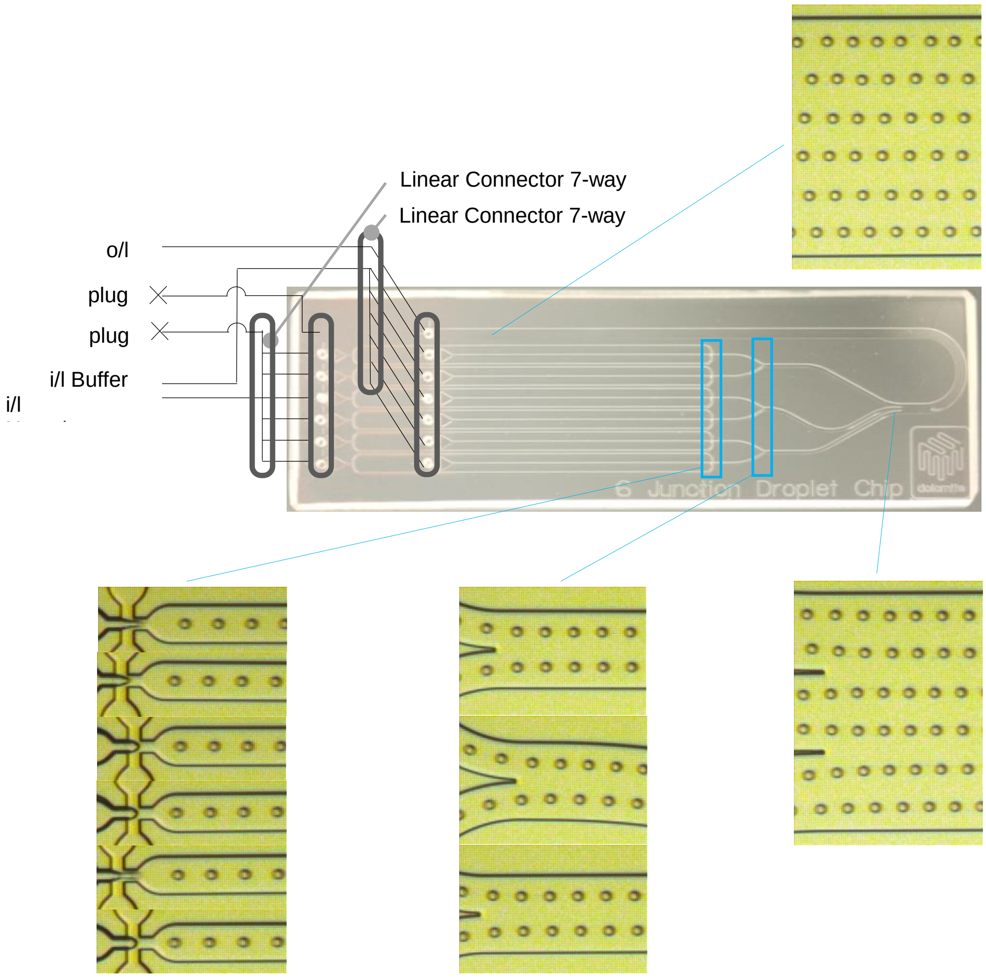
Imaging and acquisition is accomplished via a high speed camera and microscope system (Part No. 3200050). This is a high quality and flexible solution for general microscopy and high speed image capture in microfluidic applications. The system comprising a light source, fibre optic cable, microscope head, camera and software to interface is a standard Dolomite product featured in the product catalogue.

Test Performance

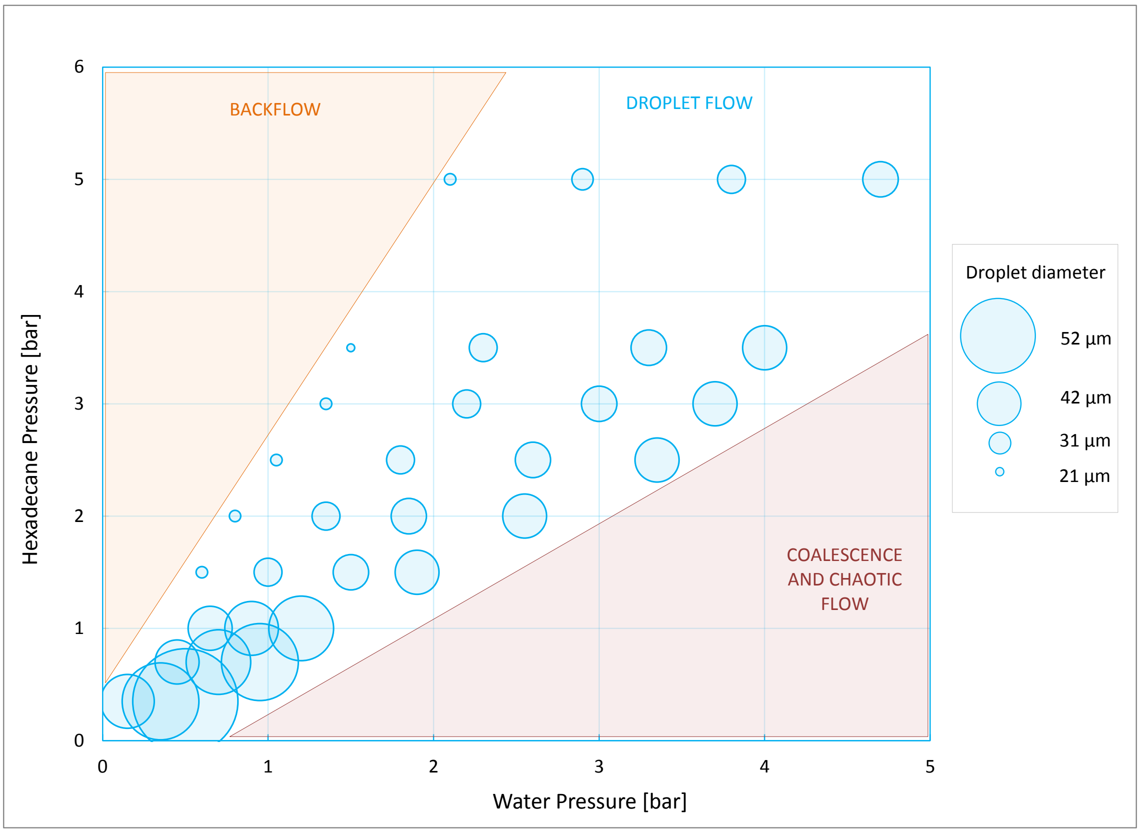
A wide range of droplet sizes can be generated at frequencies up to 30 kHz. The flow ratio which dictates the performance is controlled with P-Pumps. As the relative pressures are changed, the relative flows change, consequently yielding larger or smaller droplets.

In general, higher total flows generate higher droplet generation rates. Increasing the pressure on the hexadecane pump causes backflow. The other extreme is marked by droplet coalescence. Beyond droplet coalescence, chaotic flow ultimately dominates, marked by highly irregular sized droplet emulsion.

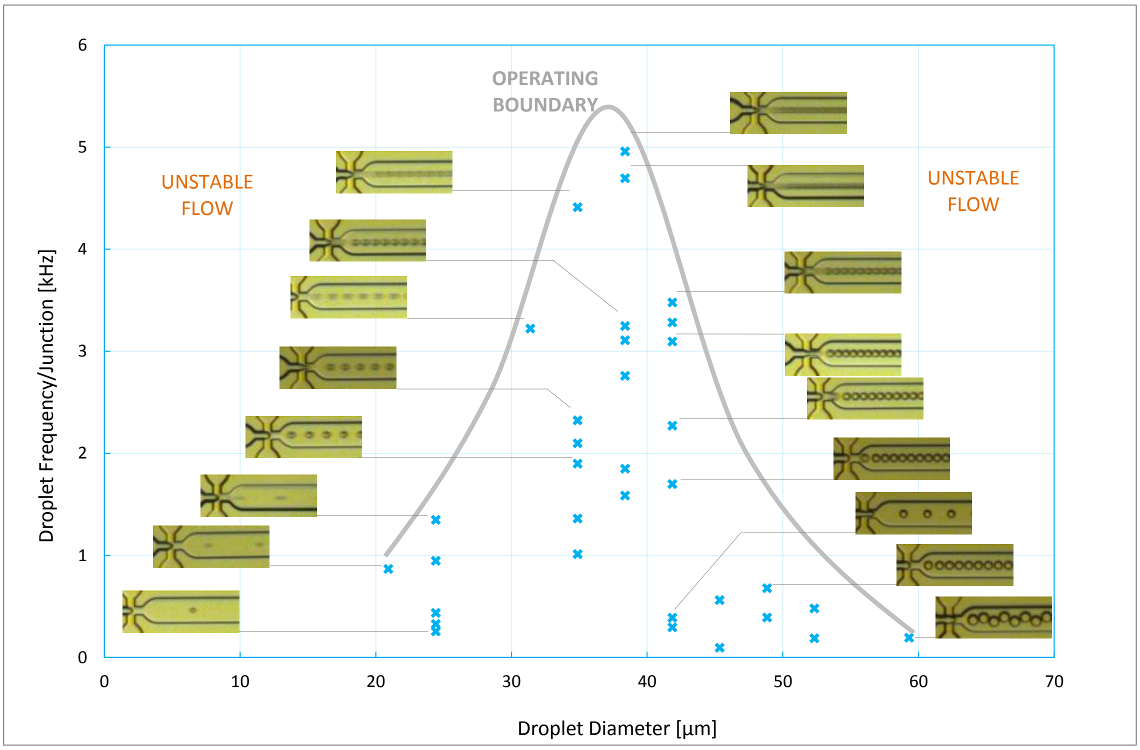
Six separate junctions on the chip generate droplets simultaneously. The flow splitting manifolds ensure consistent fluid pressure and flow rates across all six junctions. The droplet streams from each junction is pairwise merged to form three streams, which in turn are all merged towards one single chip outlet. This outlet is directed off-chip via tubing to a collection vial. A typical image of droplets generated is shown. The focus is moved between 11 locations on the chip to monitor and track droplets across the chip. Droplets larger than the channel depth of 50 µm are flattened in the channel. These same droplets are however collected off-chip in spherical shape.



The graph illustrates the size variation over a pressure range of 0-5 bar on both carrier and droplet phase. Very large droplets are possible at very low pressures. At higher pressures the generation frequency increases, however this comes at the cost of reduced range of droplet sizes. Maintaining a fixed Hexadecane carrier pressure, the Water pressure can be varied from very low (near backflow) to very high (near chaotic). A similar graph based on flow rates is presented in Appendix A.



For a given Hexadecane pressure condition, the frequency increases with increasing Water pressure. This feature is illustrated in the following graphic showing the relationship between droplet diameter and generation frequency. The highest frequencies are found to correlate with a medium droplet size. Larger or smaller droplets are limited to lower frequency. The band of achievable droplet sizes also depends strongly on the operating frequency. This band narrows with progressively higher generation rates. Unstable droplets or even jetting phenomena occur at conditions outside of the operating boundary depicted by the grey curve.



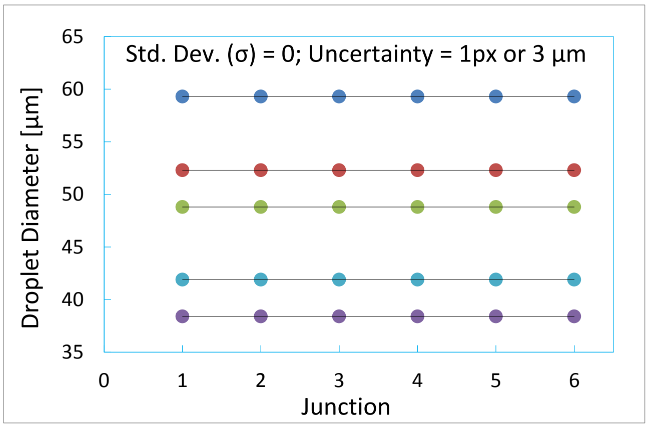
Single junction images are presented in the same graphic to illustrate the quality of droplet formation at select data points. Droplet spacing is seen to be the most prominent change across data points. In test conditions, the highest frequency was found to be approximately 5 kHz per junction. The total droplet rate across the entire chip comprising 6 junctions amounts to 30 kHz. The test system is capable of producing a total emulsion volume of 688 ml (droplet phase = 76 ml, carrier phase = 612 ml) over a 24 hour period at stable peak performance.

Droplet Size and Rate Estimation

In order to estimate droplet size and frequency, pixel analysis is performed on the captured images. The droplet size relative to the absolute size of the 150 µm wide channel provides the droplet volume. The droplet generation rate is calculated by dividing the flow rates by the droplet volume. Flow rates are directly recorded from the flow sensor reading.

Consistency across the multiple junctions on the chip is critical to relevant applications. Image analysis characterizes droplet size variation across all six junctions where droplet generation occurs. A very high level of consistency is obtained as depicted in the inset below. All six junctions generated exactly the same droplet size at the limiting resolution

(Uncertainly due to pixel resolution limitation - minimum pixel resolution limited to 1 px = 3 µm).



Analysis

Test images illustrate at least three different flow regimes dynamically and accurately controlled with changing fluid supply pressures.

- Droplet regime – This occurs at lower flow rates and results in monodisperse droplet formation.
- Chaotic – This occurs when the water flow rate is significantly higher than the hexadecane flow rate. Droplet size is generally polydisperse.
- Backflow – This is characterised by the hexadecane stream flowing back into the water feed channel. This occurs when the backpressure generated in the output channel and output pipe is greater than the pressure set on the water Mitos P-Pump. To avoid backflow the resistance of the flow resistor on the water input stream should be increased.

Summary

- The parallel droplet generation system is shown to be a versatile and powerful system to generate monodisperse droplets at an extremely high rate.
- Test conditions generating Water droplets in Hexadecane were able to produce droplet sizes varying from 20 µm to 60 µm diameter and frequency ranges of up to 30 kHz.
- Capability to produce a total emulsion volume of approximately 688 ml (droplet phase = 76 ml, carrier phase = 612 ml) over a 24 h period at stable peak performance.

- Operating characteristics of the setup are presented employing P-Pumps and flow sensors.
- The P-Pump pressure is used to tune droplet size.

- Monodisperse droplet generation occurs over a wide flow rate range of approximately 20 to 450ul/min, with a range of flow rate ratio of 2 to 200 (continuous phase flow/droplet phase flow).
- Operating characteristics presented are dependent on fluid properties. A change in fluid viscosity directly affects generation frequency. Viscous fluids such as alginates, polymers and hydrogels may result in comparatively lower production rates.

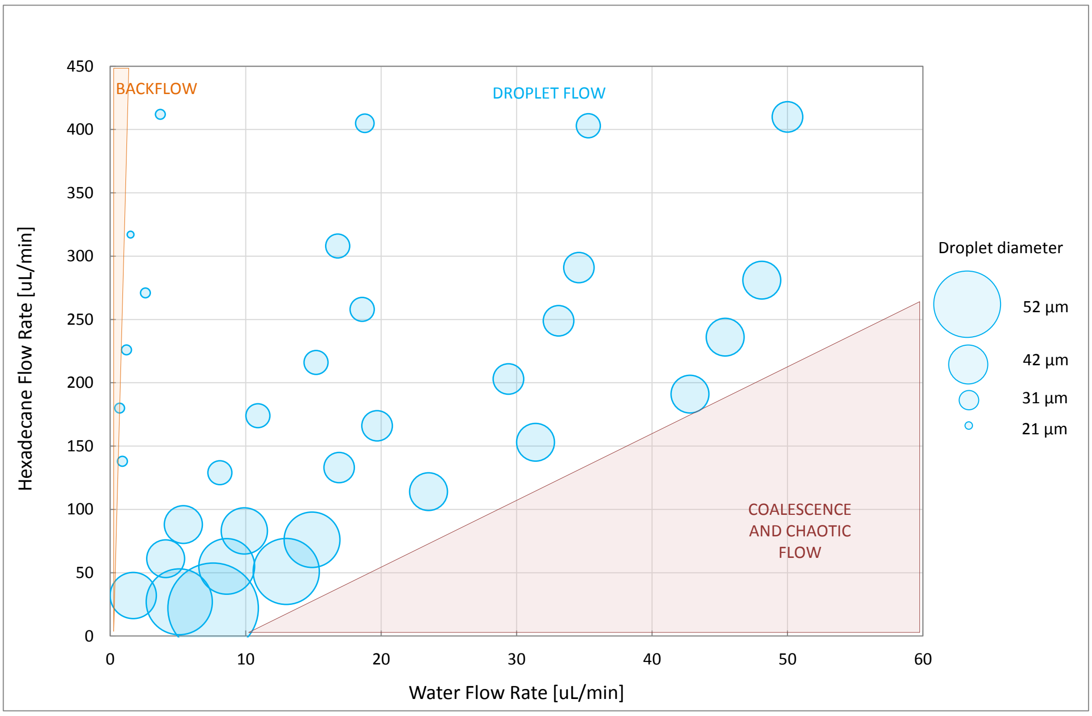
IP License

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.

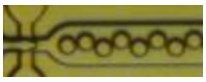
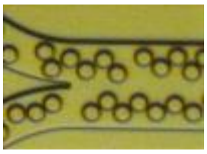
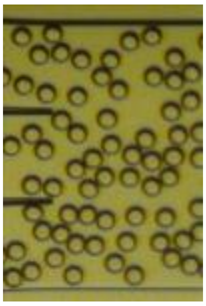
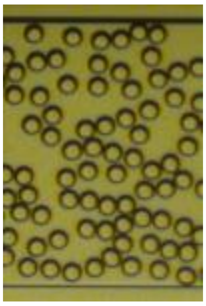
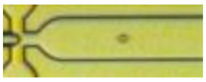
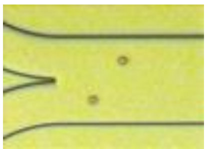
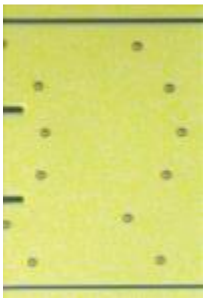
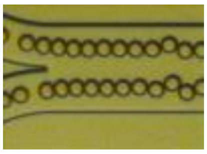
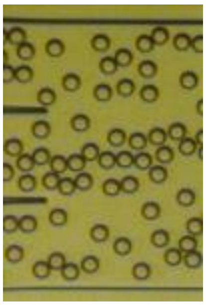
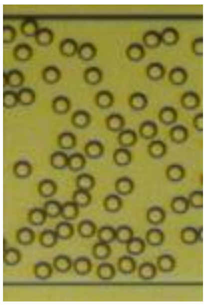
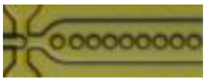
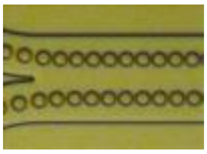
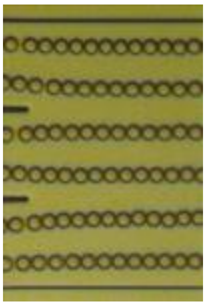
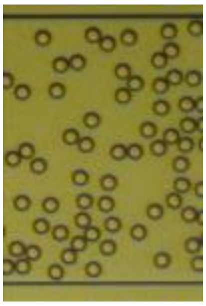
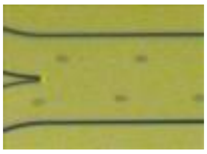
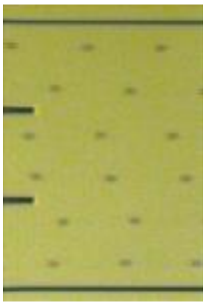
Contact us for more information about licensing this IP for your custom application or chip design.

APPENDIX A: Dependence of droplet size on flow rates

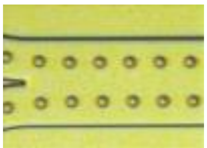
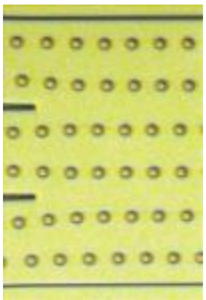
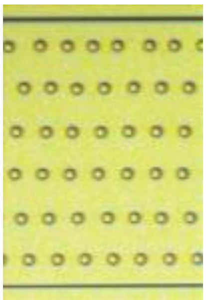
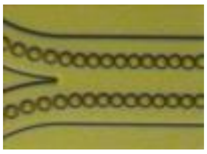
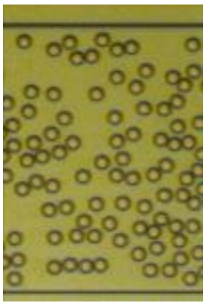
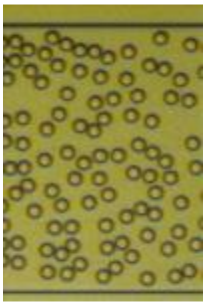
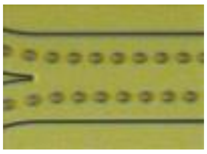
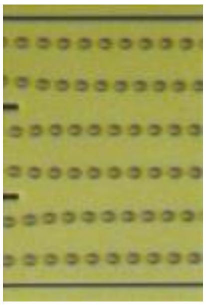
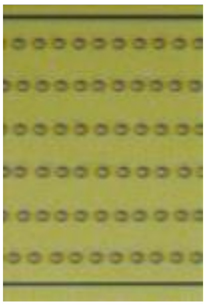
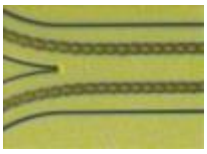
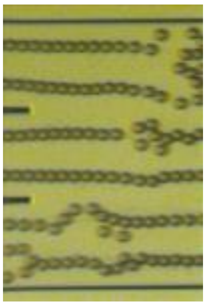
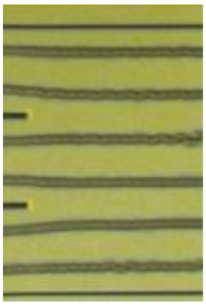
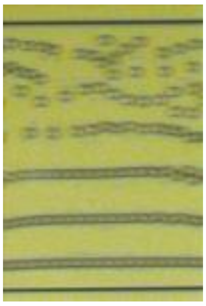


Shown above is information contained in an earlier graphic (relating pressures and droplet size) presented here as a function of fluid flow. This is useful in situations where flow sensors are used in testing setups. Use of in-line flow sensors are always recommended in order to better characterize and thereby optimize test conditions.

APPENDIX B: Illustrative test cases (aggregate over all 6 junctions)

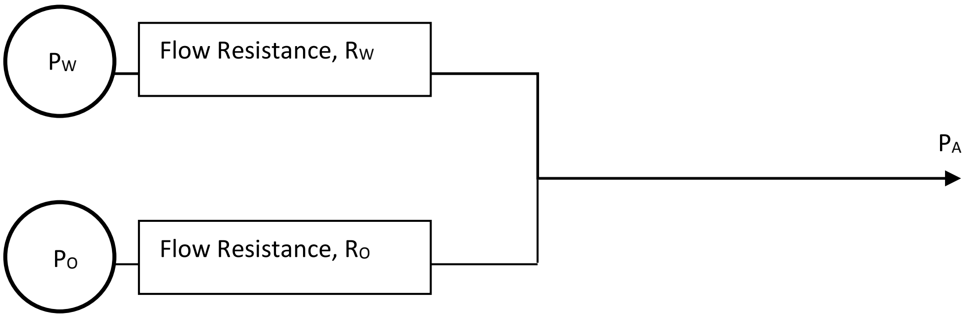
f ↓ (kHz)	Flow Ratio†	(×6)	(×3)	(×1)	(×1)
1.16	2.89				
1.96	153				
2.88	3.92				
4.07	5.10				
5.20	211				

† Flow Ratio = Flow rate of Hexadecane(continuous phase) / Flow rate of Water (droplet phase)

$f\downarrow$ (kHz)	Flow Ratio	(×6)	(×3)	(×1)	(×1)
8.17	15.96				
10.19	4.85				
13.94	13.87				
20.87	5.84				
29.74	8.05				

APPENDIX C: Flow Rate Calculation

The fluidic layout can normally be represented schematically as shown in the diagram below where W is the water droplet stream and O is the oil carrier fluid. This assumes that the flow resistance after the droplet junction, R_J, is low relative to the flow resistance of the two input streams R_W and R_O.



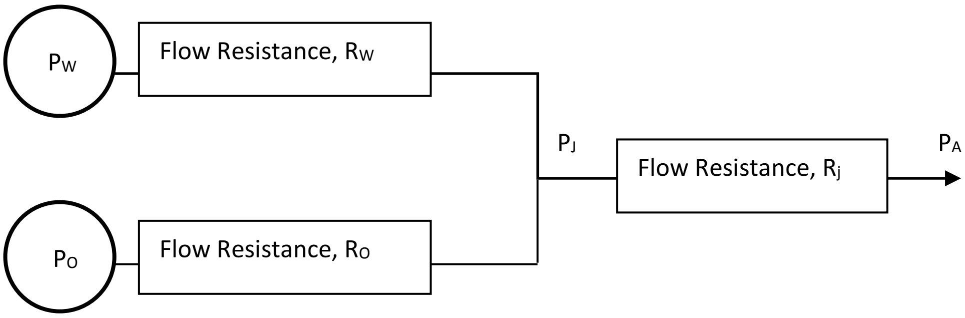
The flow rate in each feed stream can be estimated using the following two equations:

$$Q_W = \frac{P_W}{R_W \times \mu_W}, \qquad Q_O = \frac{P_O}{R_O \times \mu_O}$$

Q = Flow rate
P = Pressure in P-Pump
µ = viscosity
R = flow resistance

The Microfluidic Calculator on www.dolomite-microfluidics.com can be used to estimate flow rates using the equation shown above.

If R_j is high relative to R_W and R_O then it is necessary to first calculate the pressure at the droplet junction to get an accurate estimate of all the flow rates in the system. The schematic below shows R_J and the equation can be used to estimate the pressure at the junction, P_J. The equation assumes that the viscosity of the output stream is equal to the viscosity of the carrier fluid. This is generally a good approximation if the carrier flow rate is higher than the droplet flow rate.



$$P_J = \frac{P_W.W + P_O.O}{J + W + O}$$

Where:

$$W = \frac{1}{R_W \times \mu_W}, \qquad O = \frac{1}{R_O \times \mu_O}, \qquad J = \frac{1}{R_J \times \mu_O}$$

R_W = flow resistance of the water input channel

R_O = flow resistance of the oil input channel

R_J = flow resistance of the channel after the junction

μ_W = viscosity of water

μ_O = viscosity of oil

P_J = pressure at junction

P_W = Mitos P-Pump pressure on water

P_O = Mitos P-Pump pressure on oil

The flow rates can then be calculated as follows:

$$Q_W = (P_W - P_J).W, \qquad Q_O = (P_O - P_J).O$$

These equations are useful in avoiding situations of backflow for a Mitos P-Pump set-up.

APPENDIX D: Helpful guidelines

Care must be taken to ensure that all hardware and reagents are free from foreign particulate matter. Any dirt may irreversibly block chip or impair system performance.

- In case of blockage, sequential flushing from inlet with acetone, water and air may purge contaminants. Plug unnecessary junctions to direct contaminant to outlet. It is not recommended to flush from outlet end due to presence of on-chip filters.
- Working fluids should be filtered before pumping onto chip. In-line filters are always recommended.
- When cutting tubes, the use of a tube cutter is recommended as this reduces possibility of inconsistency.
- Avoid sharp bends in tubing. Large lengths of tubing designed to act as flow resistors may bend during testing if not monitored. This will cause deviation from designed flow resistance.
- Ensure that all tubing is filled with fluid before connecting to chip. All fluid pathways must be primed with test fluids for at least 5 minutes before start of test.
- Once chip is connected, ensure that outlet tubing is filled with fluid before logging data. The flow sensor will stabilize after the entire system is well wetted.
- Connectors are designed to be thumb-tight. Over-tightening may cause undesirable deformation of linear connector seal and deviation from designed flow.

APPENDIX E: System Description

Add-on module – To run a Parallel Droplet System with existing P-Pumps and flow accessories. A quick and easy option for those already running Dolomite P-pump setups in their labs.

Part No.	Part Description	#
3200289	6-Junction Droplet Chip (50um etch depth), hydrophobic	1
3200288	6-Junction Droplet Chip (50um etch depth), hydrophilic	1
3200148	Linear Connector 7-way	4
3200296	Double Top Interface	2
3200308	Double Flow Splitting Manifold 7-way	1

Comprehensive System – To get started with droplet generation from scratch. The best option for new customers. Use the above 'Add-on' module with the below list to create a comprehensive Parallel Droplet System with components designed to work together.

Part No.	Part Description	#
3200016	Mitos P-Pump	2
3200302	FEP Tubing, 0.8 x 0.25mm, 10 metres	1
3200303	PEEK Tubing, 0.8 x 0.25mm, 10 metres	1
3200309	Custom Flow Resistor Kit 0.8 mm	1
3200307	End Fittings and Ferrules for 0.8mm Tubing (pack of 10)	1
3200305	PTFE Plug 0.8 mm (pack of 10)	1

Flow Sensor Upgrade – Better characterization of droplet systems is achieved with quantifying flow parameters using flow sensors.

Part No.	Part Description	#
3200200	Mitos Sensor Interface	2
3200097	Mitos Flow Sensor (30-1000 µL/min)	1
3200098	Mitos Flow Sensor (1-50 µL)	1

Accessories – To enable more testing and less troubleshooting.

Part No.	Part Description	#
3200050	High Speed Camera and Microscope System	1
3200087	2-way In-line Valve	2
3200245	Ferrule with Integrated Filter (pack of 10)	1
3200043	Mitos P-Pump Remote Chamber 400	2
3200095	Mitos Sensor Display	2

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