Telos®

High-Throughput Parallelized Droplet Production

dolomite

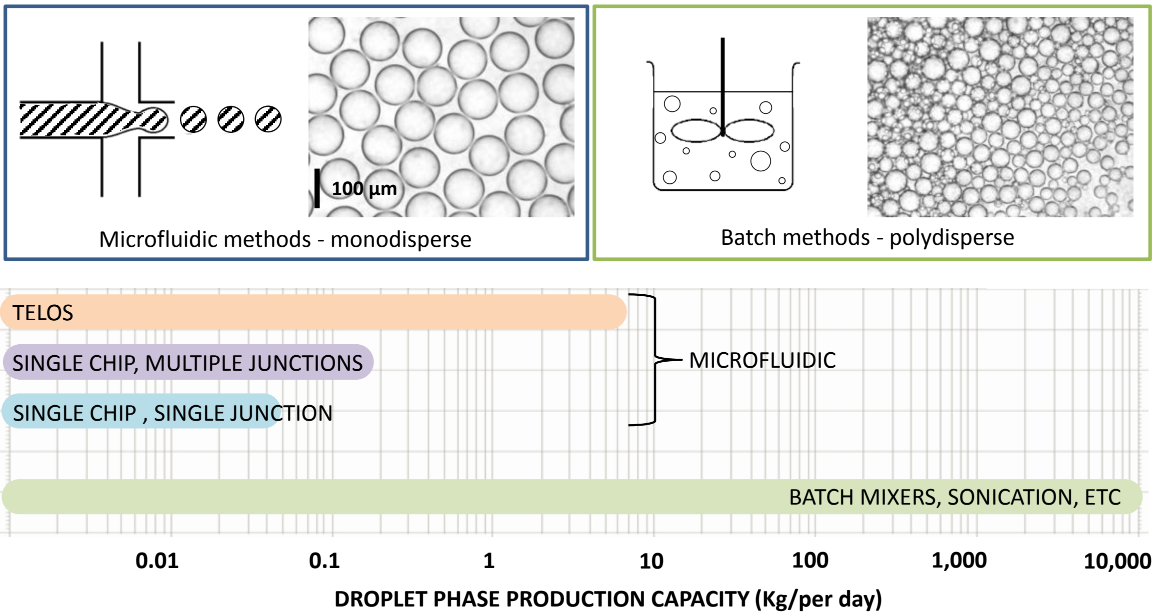
Introduction

This application note describes the operating procedure and performance of the Telos® system for the production of monodisperse water-in-oil emulsions using 35 droplet junctions in parallel. This ground-breaking, modular concept combines the unrivalled size distribution control of microfluidics with production rates comparable to those achieved by traditional batch methods.

The test work was carried out with a system consisting of 5 Telos® modules, each loaded with a 7-junction, 2-reagent droplet chip. Droplet size measurements were made by imaging droplet formation at each junction using Dolomite's image analysis software. The size measurements were repeated over a period of 48 hours to evaluate droplet size consistency.

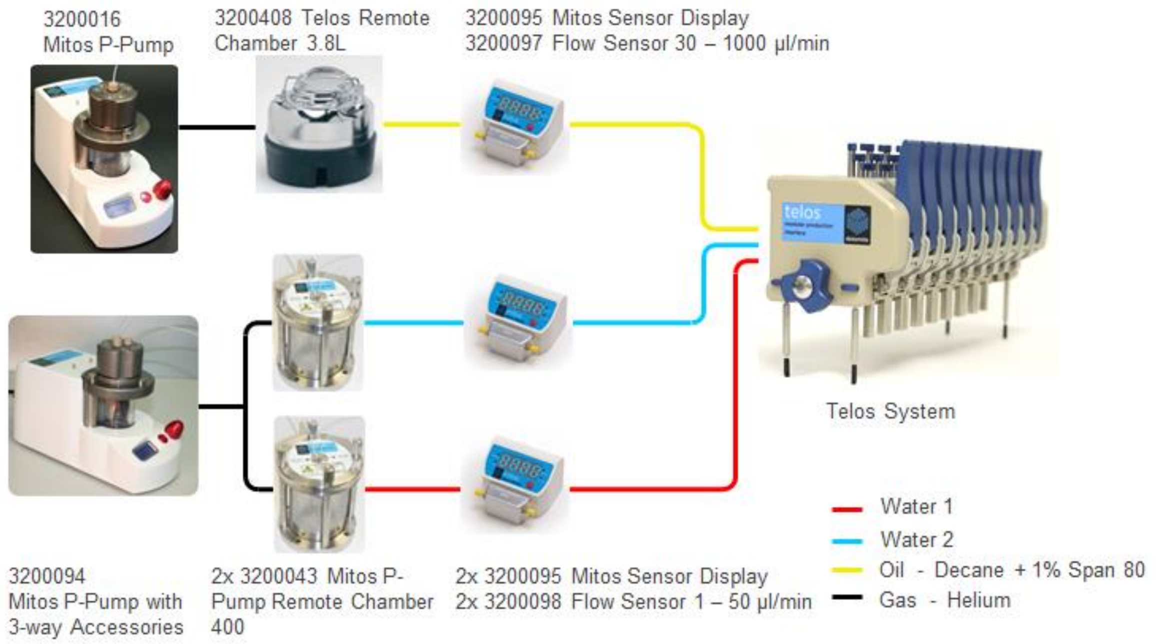
Size distribution was shown to be consistent over time and across 35 droplet junctions. The maximum droplet rate achieved was 156 kHz, corresponding to an emulsion flow rate of 20 litres per day. These rates could be further doubled by using the maximum number of modules.

The Telos® system can be run with up to ten modules in parallel, each supplying a chip from common fluid inputs. Each module has independent valving, allowing control of input streams, chip replacement and inspection without interrupting droplet production. A wide variety of channel geometries may be implemented, allowing the system to be customised for many different applications.

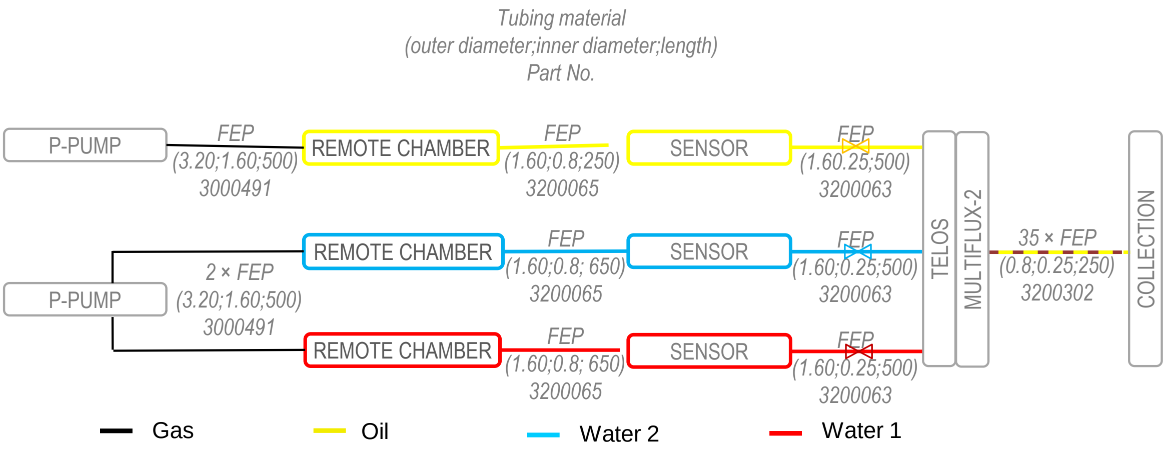


System Configuration

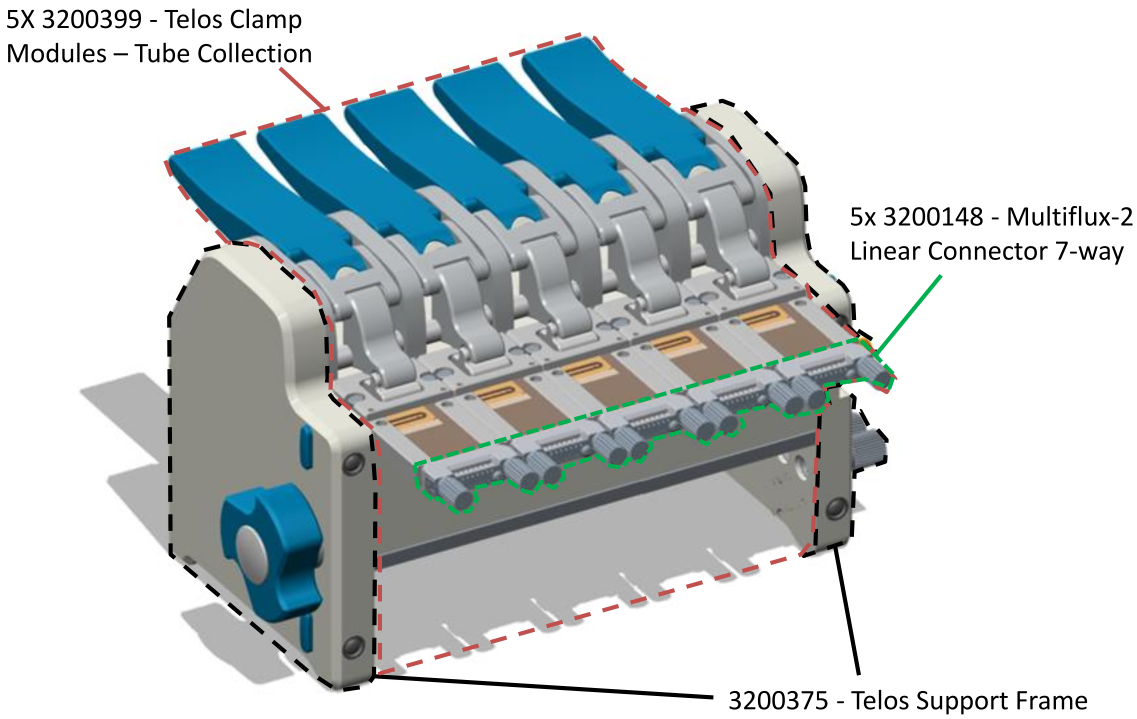
The system was set up using 5 Telos® Clamp Modules (Part No. 3200399), each supplying a 7-junction, hydrophobic Telos® 2 Reagent Chip (Part No. 3200358). Hydrophobic chips were used in order to generate water-in-oil emulsions. The carrier phase was Decane with 1% Span 80 (surfactant) and the droplet (dispersed) phase was water. The water phase was held in two 400 ml Mitos P-Pump Remote Chambers (Part No. 3200043) and the oil phase was held in a 3.8 litre Telos® Remote Chamber (Part No. 3200408). A 10 µm pore size PEEK Bottom-of-the-Bottle Filter (Part No. 3200409) was used in each remote chamber. The 3.8 litre remote chamber and the two 400 ml remote chambers were pressurised using a Mitos P-Pump (Part No. 3200016) and a Mitos P-Pump with 3-way accessories (Part No. 3200094) respectively, as shown in the diagram below. Helium was supplied from a cylinder via a two-stage regulator and Pneumatic Connector Kit (Part No. 3200034) to the P-Pumps at a pressure of 8 bar (helium was used to eliminate the absorption of gas into the water and oil phases, which can be a problem for long duration tests). Flow rates were measured using three flow sensors, with a range of 1-50 µl/min (Part No. 3200098) for the water phase and a range of 30-1000 µl/min (Part No. 3200097) for the oil phase. Each sensor was coupled to a Mitos Sensor Display (Part No. 3200095).



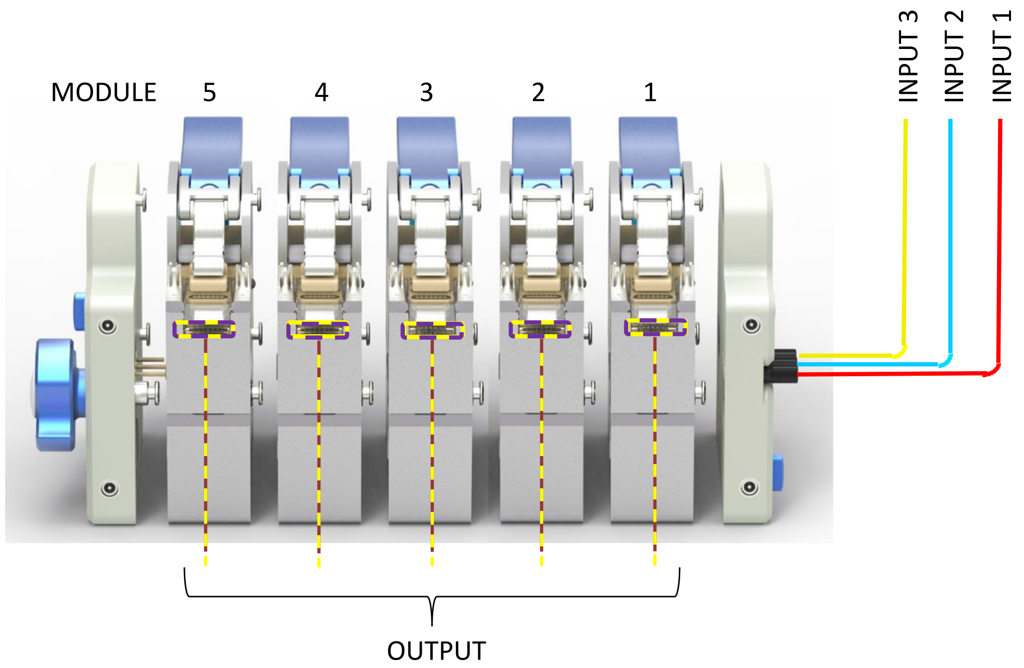
Pressurising helium was supplied from the P-Pumps to the remote chambers via Pneumatic Connectors (included with Part No. 3200043 and 3200408). 1.6mm O.D. x 0.8mm I.D. FEP tubing (Part No. 3200065) was used to connect each chamber to its flow sensor, while the final connections to the Telos® were made using 1.6mm O.D. x 0.25mm I.D. FEP tubing (Part No. 3200063). End Fittings and Ferrules were used with the tubing (Part No. 3000477). Output from each chip was routed to collection via a Multiflux-2 Linear Connector 7-way (Part No. 3200148) and 0.8mm O.D. x 0.25mm I.D. FEP tubing (Part No. 3200302). In this arrangement, the output flow of each individual junction could be collected separately and analysed.



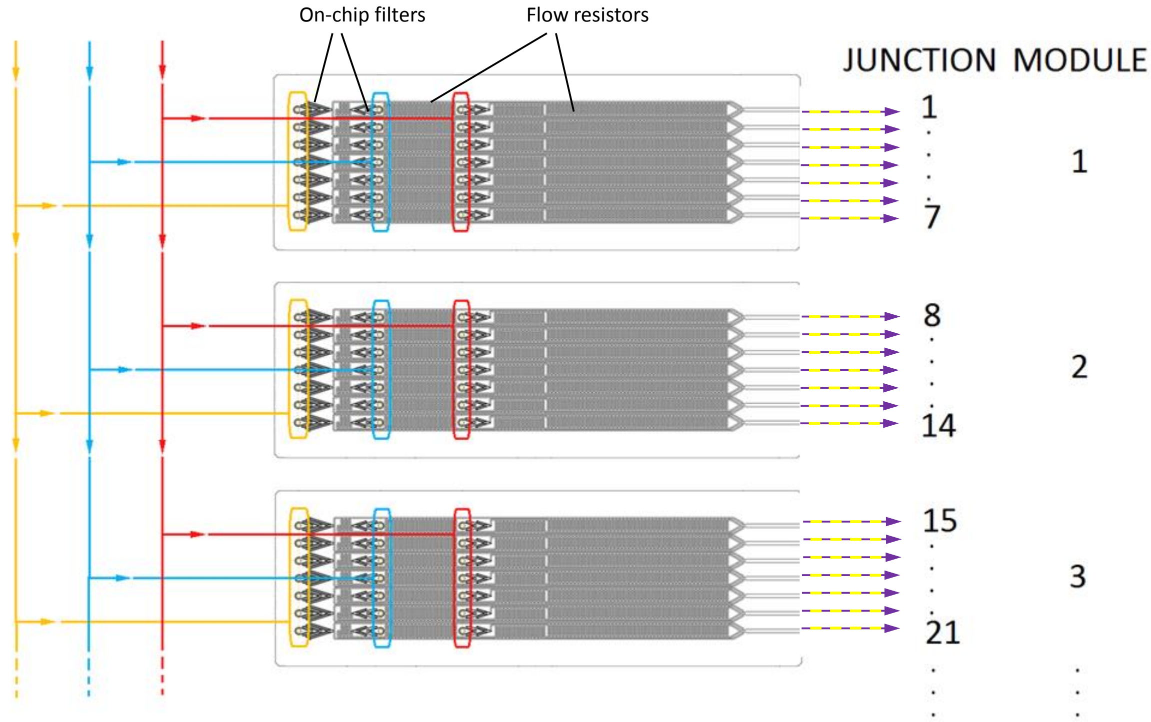
The Telos® system was set up using 5 Telos® Clamp Modules with tube collection interface (Part No. 3200399), each supplying a Telos® 2 Reagent Chip (Part No. 3200358). The modules were stacked within the Telos® Support Frame (Part No. 3200375).



The three input fluids were introduced to the Telos® system via ports in the end clamp, while output emulsion exited from the chips to collection via Multiflux-2 connectors. Modules were numbered from 1 to 5 starting at the input end of the system as shown below. Similarly, junctions on each chip are numbered from 1 to 7 in the same direction, so in this test we refer to junction numbers from 1 to 35.



The 7-junction, 2-reagent chip allows two dispersed phase inputs (in this case water) and one carrier phase input (in this case oil + surfactant). This chip can accommodate two miscible dispersed phases which combine at a Y-junction prior to forming droplets at a cross-junction. On-chip filters prevent junction blockage, however it is also important to prevent accumulation of debris in the on-chip filters as this can reduce flow rate, by filtering the input streams before they reach the chip (see Appendix C). The output droplet flow stream from each junction exits via the edge of the chip into Multiflux-2 connectors as described above.

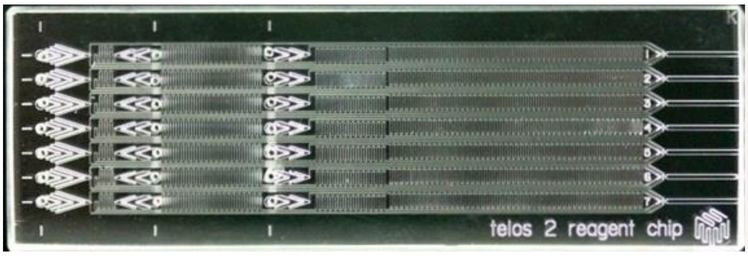


Boxes are illustrative and indicate fluid entry ports on chip. On-chip flow resistors are visible as serpentine pathways. Fluid flow is from left to right exiting from the right edge of the chip. Junctions are visible as 'Y' geometry towards the right edge of the chip.

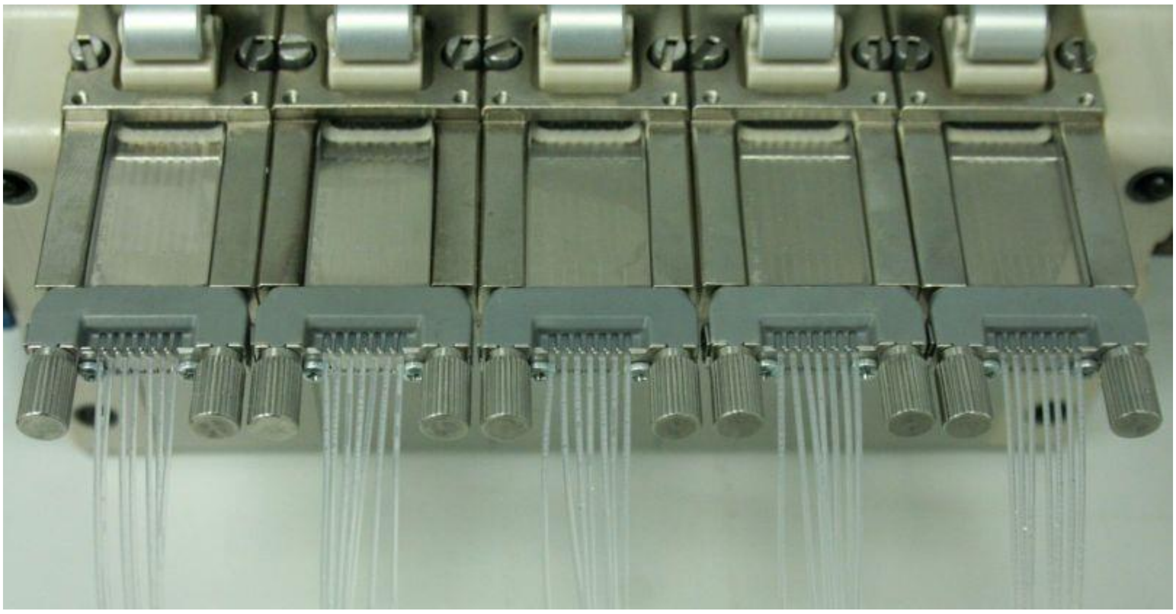
On-chip flow resistors are single-etched (semicircular cross-section) with a width of 105 µm and a depth of 50 µm. Approximations for back pressure were calculated based on fluid viscosities at 300K. Calculated back pressures resulting from on-chip resistances and off-chip tubing etc are given in the table below.

	Oil	Water	Emulsion
Flow resistor length (mm)	36	103	-
Flow rate per 35 junctions (µl/min)	1000	100	1100
On-chip back pressure (mbar)	135	40	1.3
Off-chip back pressure (mbar)	707	36.5	14.5
Pumping pressure required (mbar)	857.8	92.3	-

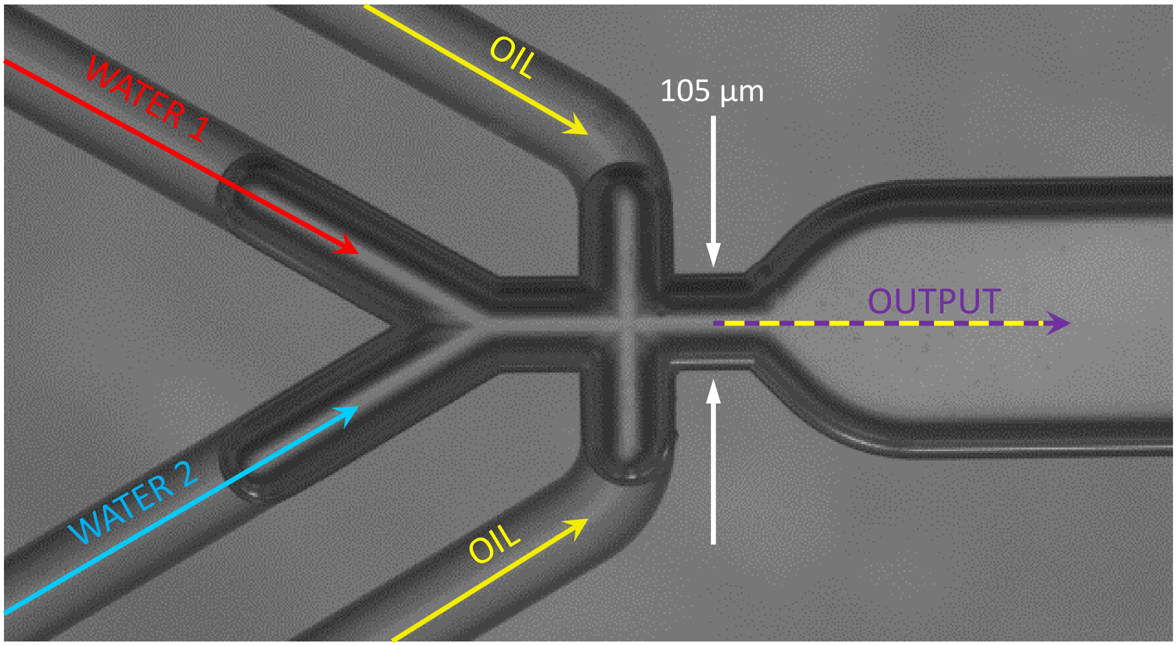
In practice, the pressure required to produce a given flow rate is strongly affected by viscosity, with more viscous fluids resulting in a higher pressure value. In the case of certain fluids including decane, viscosity is strongly affected by temperature. Further information on flow rate calculation is given in Appendix A.



Telos® 2-reagent chip *Multiflux-2 Linear Connector 7-way*



Multiflux-2 connectors deliver emulsion from each junction to collection vessel separately.

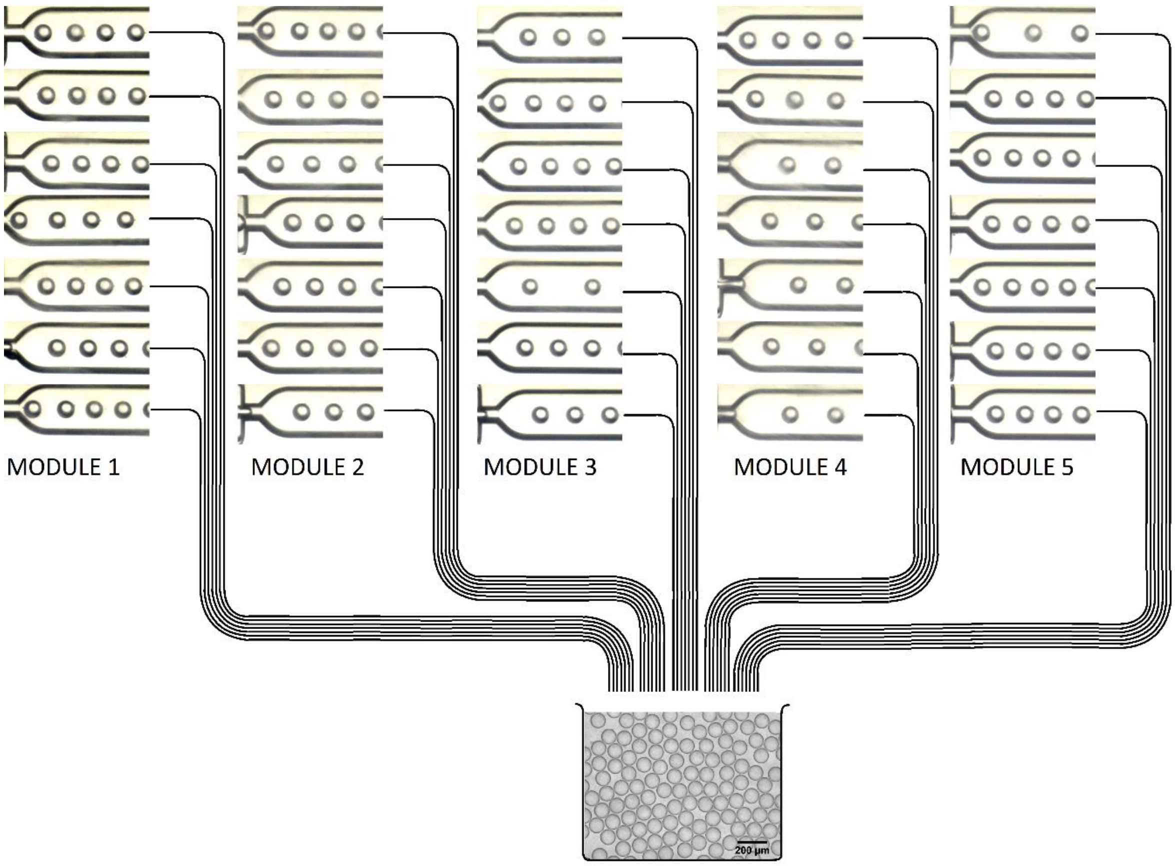


Junction geometry and flow paths. Transition from single-etched to double-etched channels takes place where channels appear darker.

Imaging of on-chip droplet formation was accomplished via a High Speed Camera and Microscope System (Part No. 3200050). This consists of a light source, fibre optic cable, microscope stage, microscope head, camera and software interface. The microscope focus was moved across the 35 junctions to monitor and track droplets across the entire system. Images were processed using Dolomite Droplet Monitor Software (Part No. 3600037) to extract droplet diameter data for each junction.

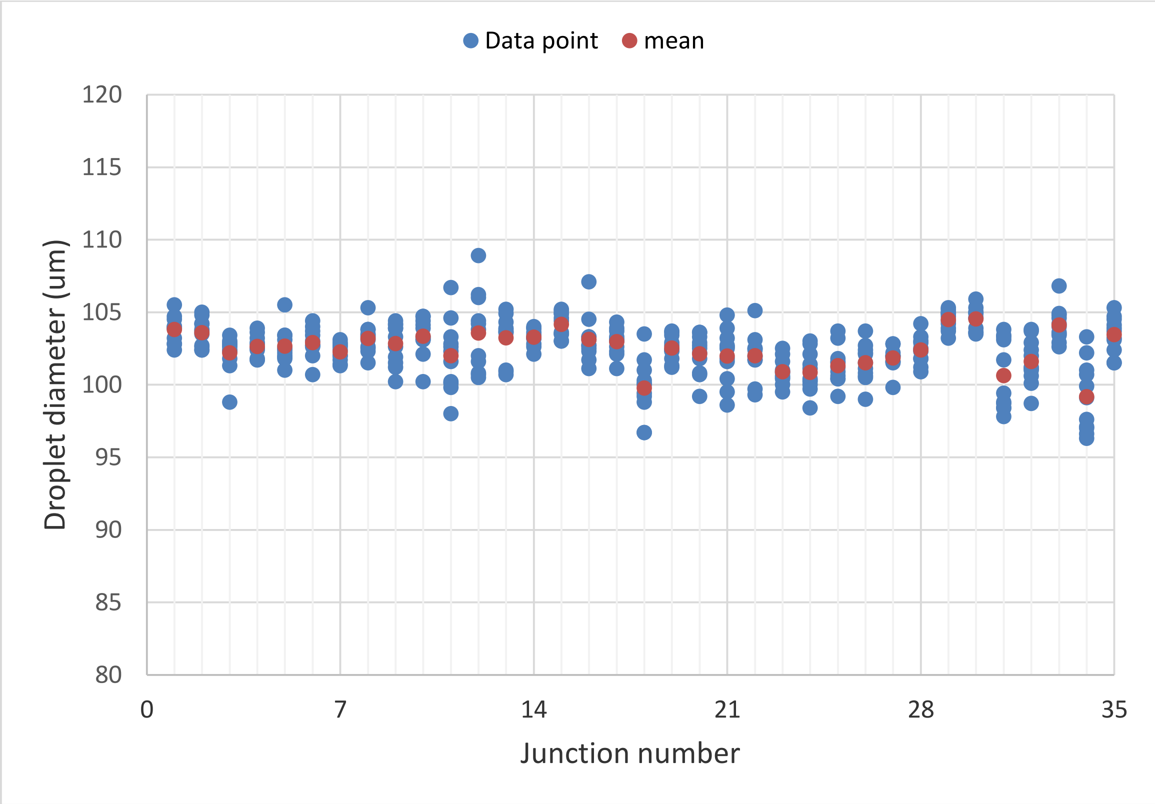
Test Performance

The system was run for 48 hours and droplet size measurements taken at each junction at intervals. The P-Pumps applied pressures of 1200 mbar (Oil) and 200 mbar (Water). Resulting flow rates were initially 1049 µl/min and 101 µl/min for the oil and the water inputs respectively.

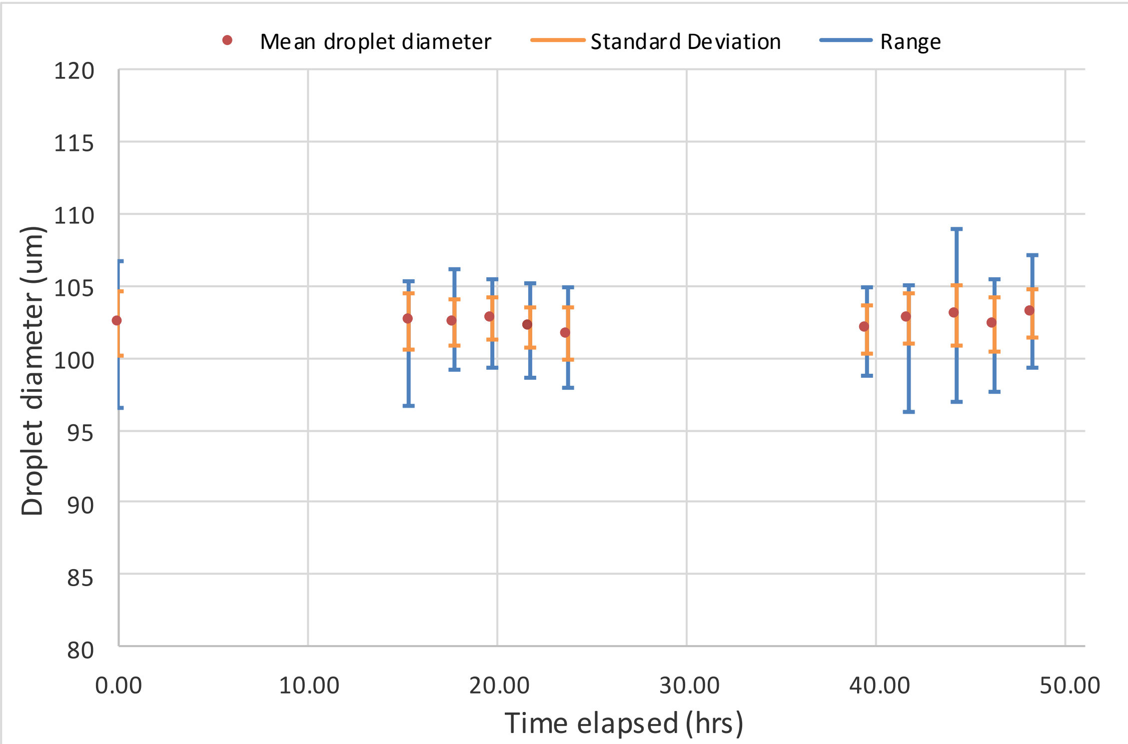


The diagram above shows the images that were captured during droplet generation for all 35 junctions

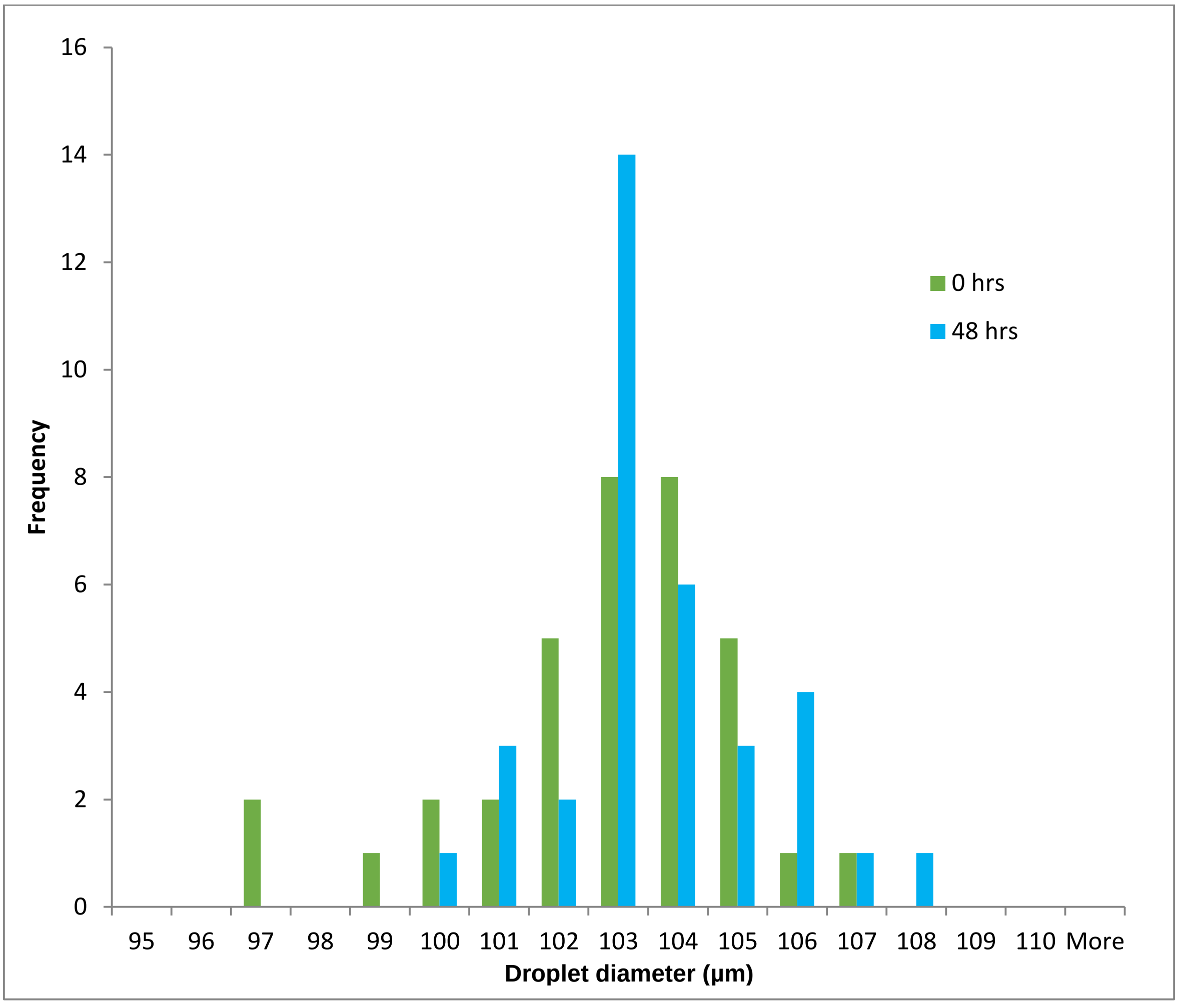
Initial mean diameter of the samples measured at all 35 junctions was 102.4 µm with standard deviation 2.2 µm (2.2%). The range of diameters measured at this time step was 96.6 to 106.7 µm with 80% of junctions producing droplets between 99.4 and 104.9 µm. Size measurements over 48h are summarised below.



Droplet diameters across 35 junctions



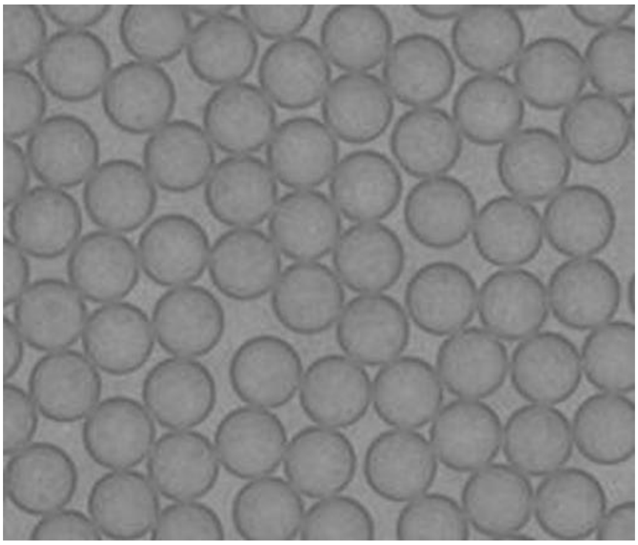
Droplet diameter variation over 48 hours across 35 droplet junctions



Histogram showing droplet size variability across 35 junctions at 0 and 48 hours

A further test was carried out to determine the maximum droplet rate achievable by the Telos® system. Droplet rate in microfluidic junctions is limited by the transition of flow behaviour to “jetting” at high flow rates. Jetting describes behaviour where the droplet phase continues unbroken downstream of the junction before breaking up into very large and inconsistent droplets.

The maximum droplet rate achieved using 5 modules without jetting occurring at any junction was 156 kHz, or 4.46 kHz per junction. This occurred at flow rates of 12600 µl/min for the oil phase and 2400 µl/min for the water phase. This equates to an emulsion production rate of 15 ml/min or 21.6 litres per day with a droplet phase volume fraction of 16%. Mean droplet diameter under these conditions was 79.2 µm with standard deviation 0.9 µm (1.1%). The rates achieved here could be further doubled by operating with the maximum 10 modules.



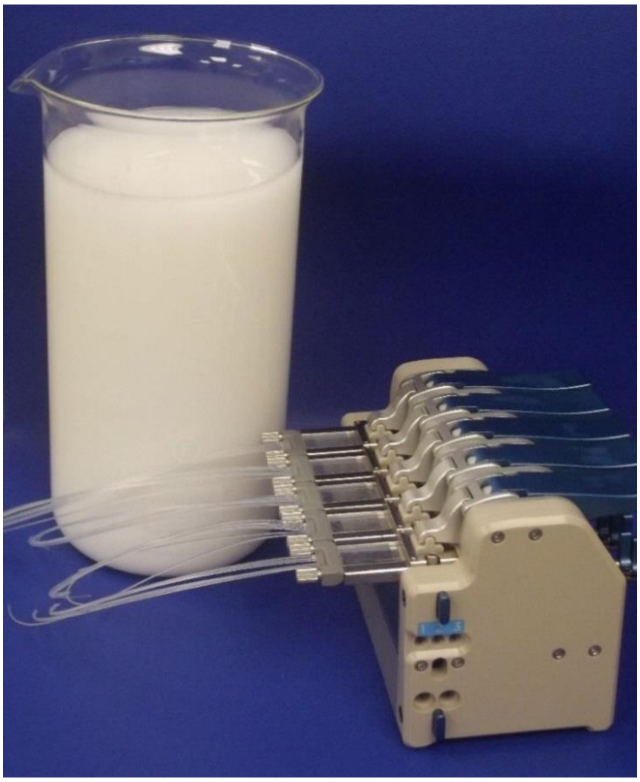
Droplets generated by the Telos® system during high-speed test at 156 kHz

Analysis

There was no overall trend observed in droplet diameter with respect to time over 48 hours. Mean diameter across 35 junctions varied over time between 101.7 and 103.2 µm with an overall average across the 48 hours of 102.3 µm. Minor variations are attributed to changes in room temperature since decane viscosity is significantly affected by temperature. Ambient temperature was found to vary by ± 2.5˚C over a 24 hour period while oil flow rates varied correspondingly between 970 and 1237 µl/min at constant pumping pressure. Measurement uncertainty is also a significant source of error (pixel resolution of images was 1.42 µm).

There was also no overall trend in droplet diameter by junction number across 35 junctions. Standard deviation between junction samples at each time step was between 1.4 and 2.3 µm, comparable to the measurement uncertainty.

The maximum droplet rate achieved using the 5-module setup was 156 kHz. Total emulsion production rate was the equivalent of over 20 litres per day. A ten-module system would be capable of running at 300 kHz or 40 litres per day. This delivers the capacity to scale up the precision offered by droplet microfluidics to commercial production rates.



Emulsion generated by the Telos® system over 2 hours.

Summary

- The Telos® system is capable of producing large volumes of droplets with narrow size distribution.
- 40 litres of water-in-oil emulsion can be produced in 24 hours using 10 Telos® modules.
- Mean droplet diameter varied between 101.7 and 103.2 µm across all 35 junctions over 48 hours. This variation is most likely due to changes in ambient temperature – more consistent results may be achievable in a controlled temperature environment.
- The standard deviation of droplet diameter across the 35 junctions was 1.4 at best and 2.3 µm at worst during the 48 hour run.
- See Appendices for helpful operating guidelines.

IP License

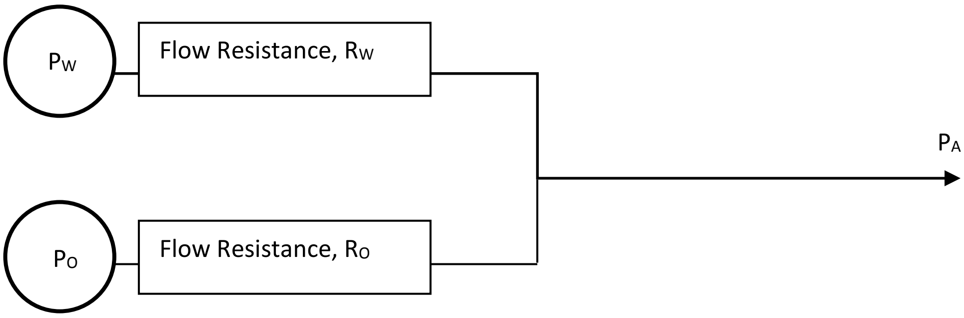
Dolomite is a licensee of Japan Science and Technology Agency (“JST”) under JST’s microdroplet generation technology.

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: 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.



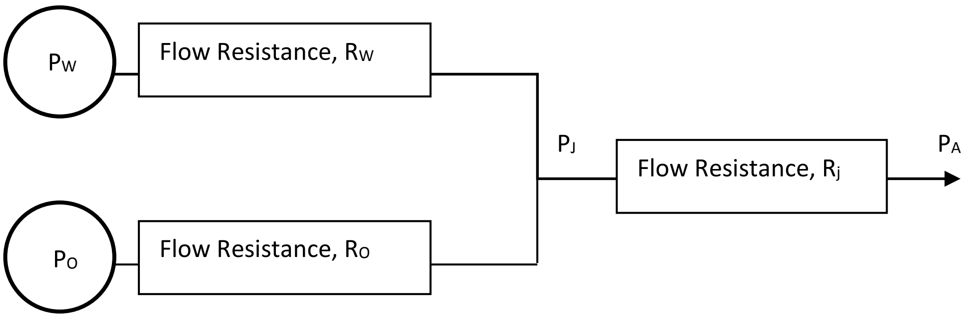
The flow rate in each feed stream can be estimated using the following two equations:

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

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 B: Helpful guidelines

System setup

- 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 (See Appendix C).
- For the same reason, it is recommended that the system is used in a clean air environment such as a laminar flow chamber, particularly during assembly and disassembly.
- Working fluids should be filtered before pumping onto chip. In-line filters are always recommended.
- When cutting tubing, the use of a tube cutter is recommended as this reduces the 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.
- Multiflux connectors are designed to be thumb-tight. Over-tightening may cause undesirable deformation of linear connector seal and deviation from designed flow.

Initialising

- When starting up or changing fluids, use low-resistance Telos® Purging Chips (Part No. 3200370) to prime the system with fluids.
- An additional module installed with purging chip is a useful option to drain the Telos® system without removing chips from other modules. This draining module is a useful option during start-up, shutdown or fluid changeover.
- Once the function chips are connected, ensure that outlet tubing is filled with fluid before logging data. The flow sensor will stabilize after the entire system is well wetted.

During production

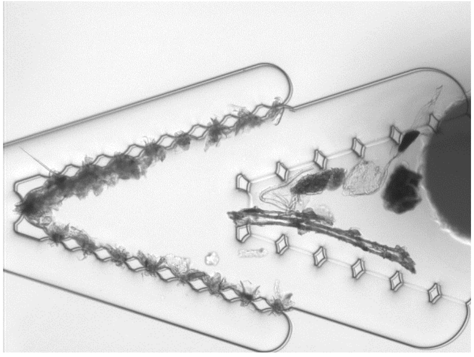
- Fluid supply to each module can be independently switched, enabling chips to be added or removed without interrupting production.
- In case of blockage, sequential flushing from inlet with acetone, water and air may purge contaminants. Plug unaffected junctions to direct contaminant to outlet. It is not recommended to flush from outlet end due to presence of on-chip filters.

Shutting down

- When shutting down, fluid changeover to simple fluids (pure water, or pure oil) is recommended. Subsequently, the water and oil should be purged with pressurized gas.
- The chips should be removed, cleaned independently and stored in a cool dry place.

APPENDIX C: Filtering working fluids to avoid blockage

The Telos® 2-reagent chip features on-chip filters to avoid blocking of the junction by any debris in the input streams. However it remains important to use clean fluids, as a build-up of debris in the filters can increase flow resistance significantly, leading to a reduction in flow rate for that fluid. Fluids containing solid particulate matter can cause blockage of the on-chip filters as shown below. A severe blockage such as this in the carrier phase filter, for example, will lead to variations in flow rates and as a result droplet size in that junction.



It is recommended that all fluids are pre-filtered in addition to using several stages of filtration in the system itself. 10 µm PEEK Bottom-of-the-Bottle Filters (Part No. 3200409) are recommended for use in remote chambers to prevent influx of particulate matter to the system. A second stage of filtration is included in each Telos® module through the use of PEEK frits with pore size 10 µm (Part No. 3200372). Finally, the on-chip filters are manufactured with pore sizes of 85 and 50 µm in order to catch any debris introduced during system assembly, however upstream filtration is vital to prevent clogging of on-chip filters as described above. It may be necessary to use different pore sizes in the case of fluids containing significant particulate matter or with high viscosity, or with chips having smaller channel width.

Filtration Stage	**Pore Size**
In-chamber filter	10 µm
In-module filter	10 µm
On-chip filter 1	85 µm
On-chip filter 2	50 µm

APPENDIX D: System Description

Part No.	Part Description	#
3000477	End Fittings and Ferrules for 1.6mm Tubing (pack of 10)	1
3200016	Mitos P-Pump	1
3200034	Pneumatic Connector Kit	2
3200043	Mitos P-Pump Remote Chamber 400	2
3200050	High Speed Camera and Microscope System	1
3200063	FEP Tubing, 1.6 x 0.25mm, 10 metres	1
3200065	FEP Tubing, 1.6 x 0.8mm, 10 metres	1
3200094	Mitos P-Pump with 3-way Accessories	1
3200095	Mitos Sensor Display	3
3200097	Flow Sensor (30-1000 µL/min measurement range)	1
3200098	Flow Sensor (1-50 µL/min measurement range)	2
3200148	Multiflux-2 Linear Connector 7-way	5
3200302	FEP Tubing, 0.8 x 0.25mm, 10 metres	2
3200358	Telos® 2 Reagent Chip (100µm), hydrophobic	5
3200370	Telos® Purging Chip (Pack of 5)	1
3200372	10 µm PEEK Filter, FFKM (Pack of 10)	2
3200375	Telos® Support Frame	1
3200399	Telos® Clamp Module – Tube Collection	5
3200408	Telos® Remote Chamber 3.8L	1
3200409	PEEK B_o_B Filter 10um	3
3600037	Dolomite Droplet Monitor Software	1

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