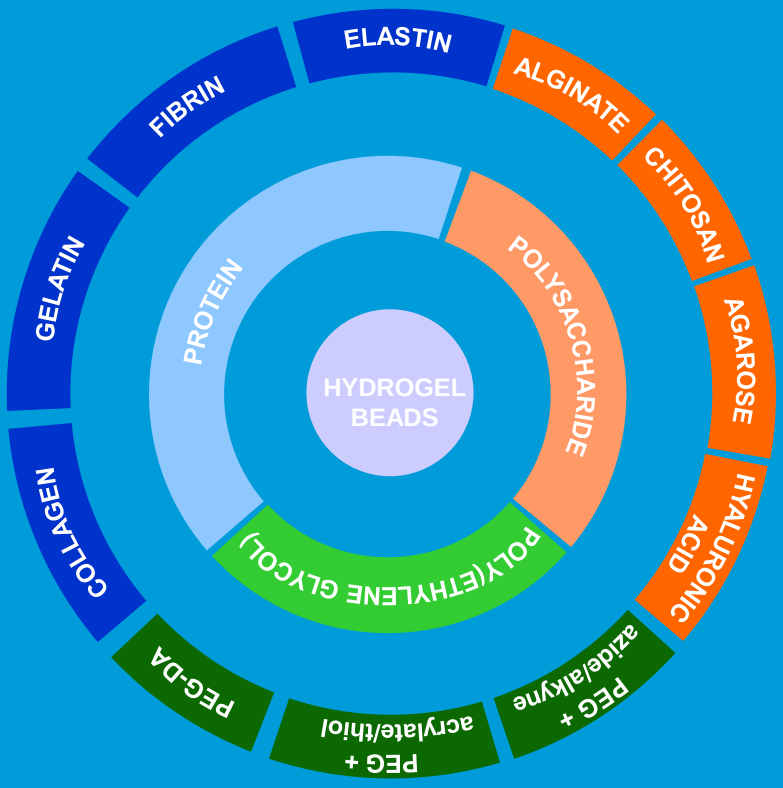
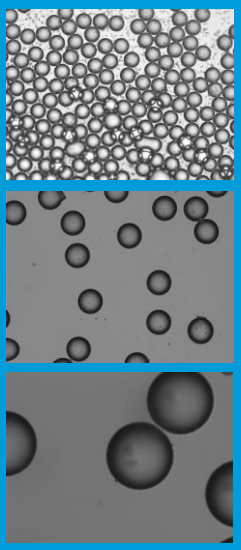
Agarose Bead Synthesis

A microfluidic route for the production of micron sized agarose hydrogel beads





dolomite

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Introduction

This application note describes the generation of agarose droplets at elevated temperature and subsequent cooling to form solid microparticles. The highest droplet generate rate is 2800 droplets per second corresponding to a droplet diameter of ~ 60 µm.

The system is based around a standard hydrophobic droplet junction chip. Flows rates are controlled via Dolomite P-Pumps providing smooth pulseless flow. Droplet size and droplet generation frequency are inter-related and used to define the operating limits of the system. Temperature and concentration variations shift the operating limits as demonstrated in the following note.

Particle production frequencies (in Hz) achieved in test conditions are shown in the table below:

		Temperature T (°C)			
Concentration c (% g/ml)		45	55	65	75
	0.5	**1800**	**2200**	**2500**	**2775**
	2.0	**-**	**-**	**1150**	**-**
	3.3	**-**	**-**	**800**	**-**

Agarose Hydrogel beads

Hydrogels are hydrophilic polymeric materials that can absorb water without dissolving. They are composed of naturally occurring materials such as proteins (Collagen, gelatine, fibrin, elastin) or polysaccharides (alginates, chitosans, agarose hyaluronic acid) or artificial materials (PEG and its derivatives). Hydrogels are used in a wide range of biomedical applications

Agarose (made from seaweed (Agar)) has applications that include:

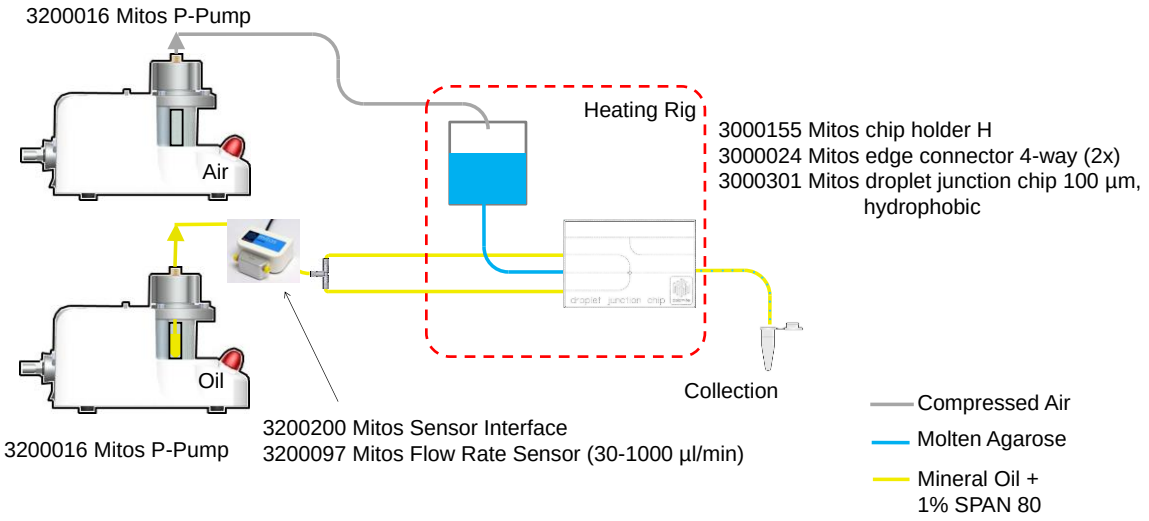
- Separation of biomolecules for analysis
- Scaffolds for tissue engineering
- Vehicle for drug delivery (in microparticle form)
- Actuators for optics and fluidics
- Model extracellular matrices for biological studies

Droplet based microfluidic systems have shown unparalleled advantages for the synthesis of polymer particles and been utilized to produce hydrogel particles with a well-defined size, shape and morphology.

Agarose exhibits a high difference between melting and gelling (solidification) temperatures (high melt / freeze hysteresis). The gelling temperature ranges from 32 - 45°C, and the melting temperature range is normally 80 - 95°C. Agarose is dissolved in water at around 90-100° C and the solution is used at between 45-75° C in the tests.

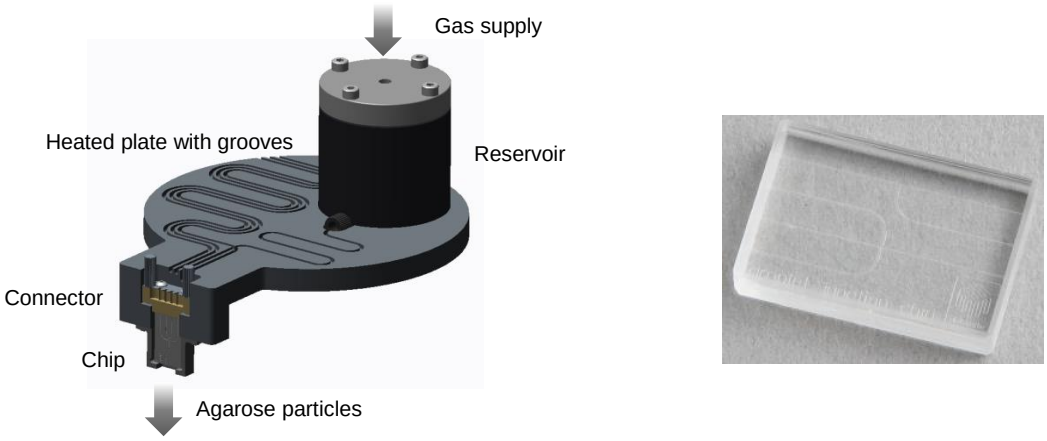
Demonstration Setup

A standard Droplet Junction Chip (100µm etch depth), hydrophobic (Part No. 3000301) is used with a Linear Connector 4-way (Part No. 3000024) and an chip interface H (Part No. 3000155) to interface the fluidic connection between tubing and chip. Plug FEP (Part No. 3000056) block unused ports on the Multiflux Connector. Two Mitos P-Pumps (Part No. 3200016) deliver the droplet and carrier fluids.



Dolomite standard products assembled to show fluid pathways

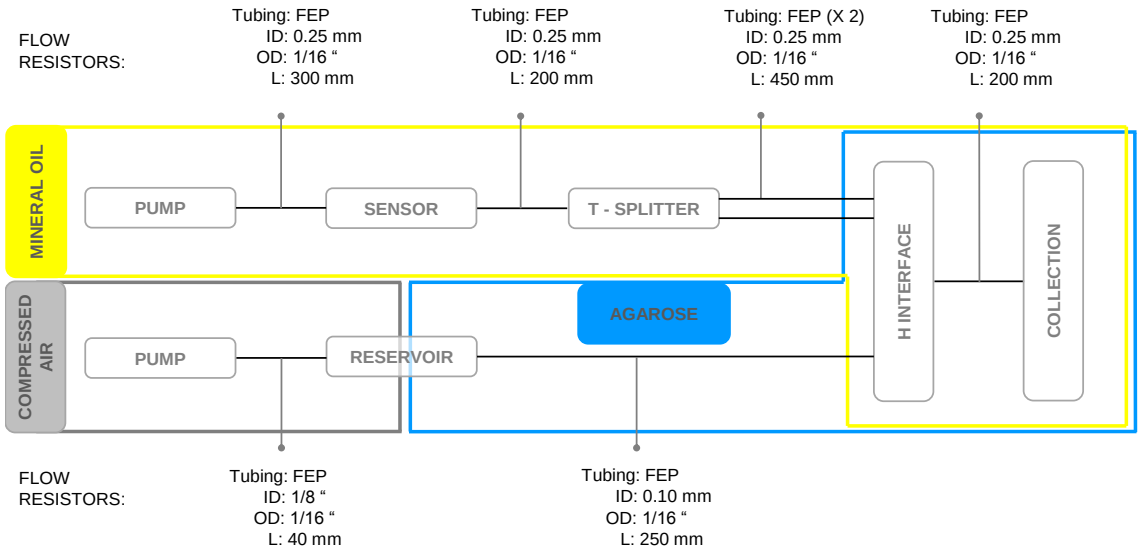
Mitos sensor interface (Part No. 3200200) equipped with a Mitos Flow Rate Sensor capable of 30-1000 µL/min measurement range (Part No. 3200097) records the mineral oil flow rate. FEP tubing is used to deliver fluids across the system.



Left: Custom heating Rig showing agarose reservoir, tube heating pathways and chip connector and chip placement. Right: Standard droplet junction hydrophobic chip

The heating rig consists of a custom designed aluminium block which is placed on top of a hot plate. The block contains a reservoir where molten agarose is introduced. The reservoir is connected to a P-pump by which the pressure in the reservoir is controlled. Grooves in the rig surface are designed to comfortably hold the fluid tubing (OD 1/16th inch). As a result flowing mineral oil heats up prior to entering the chip ensuring consistent temperature when the two fluids contact at the chip junction.

In order to form agarose (aqueous phase) droplets in mineral oil with 1% SPAN 80 (organic phase with surfactant), surface functionalization renders the channel surface hydrophobic. The agarose forms the droplet phase, approaching the junction along the central channel. Mineral oil is the continuous outer carrier phase.

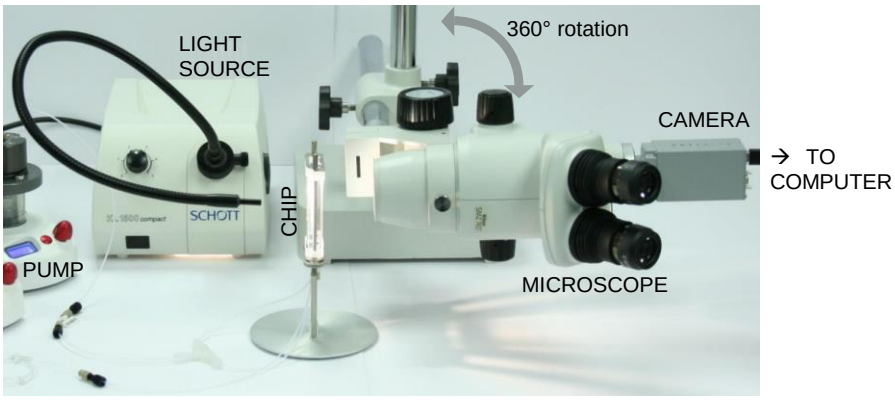


Details of tubing used for setting up flow network. Colors indicate the fluid contacting wetted components Chip is placed inside the 'H Interface'

Flow resistors enable the system to be used in an exploratory mode. By selecting flow resistors, a droplet system may accommodate fluids with a wide range of viscosities. The resistances are selected based on calculations performed using fluid properties of Mineral Oil (with 1% SPAN 80) and Agarose solution.

The use of flow sensors enables flow-control mode of operation. This eliminates the possibility of fluctuation in flow despite changes in ambient temperatures and is particularly useful for long run times. For the purpose of this test study, pressure-control mode is used.

FEP tubing used is Part Number: 3200300 (1/16" OD x 0.1mm ID, 10 metres) and Part Number: 3200063 (FEP Tubing, 1/16" OD x 0.25mm ID, 10 metres). Depending on whether more viscous or less viscous fluids are used, the tubing can be changed accordingly to smaller or larger bore. Tuning the system flow resistance thus allows for higher resolution in fluid control.



Imaging system

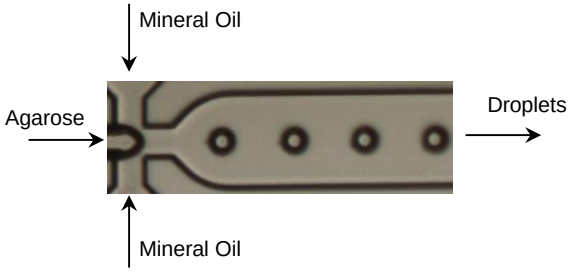
Imaging and acquisition is accomplished via a High Speed Camera and Microscope System (Part No. 3200050). This is a high quality and flexible solution for general microscopy and high speed image capture in microfluidic applications. The system comprising a light source, fibre optic cable, microscope head, camera and software to interface is a standard Dolomite product featured in the product catalogue. Shown above is the horizontal viewing ability of the imaging system for a vertically mounted chip.

Results and Analysis

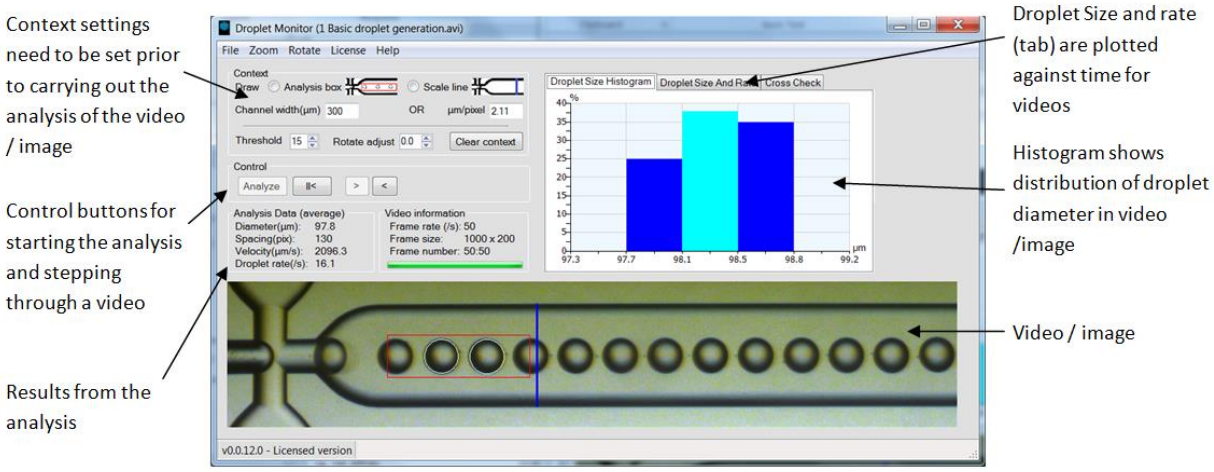
The pulseless flow generated by the P-Pumps ensures production of controlled droplet sizes with a high degree of monodispersity.

Agarose particle production is demonstrated with the described setup. Fluid pressures are shown to accurately control droplet size and frequency. The size and frequency are directly related to production capacity.

A sample image shown below shows the mechanism of droplet breakup. These two phases meet at the junction where the local flow field determined by the geometry of the junction and the flow rates of the two fluids deforms the interface. Droplets pinch off from the agarose phase by a free surface instability.



Sample image of droplet generation on chip



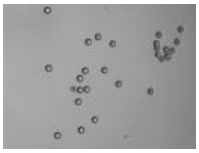
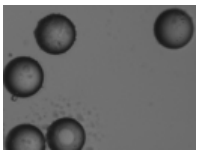
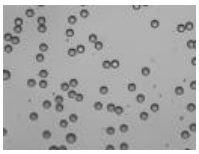
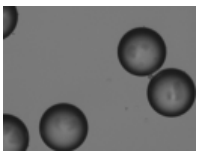
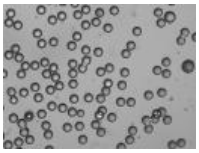
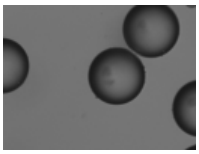
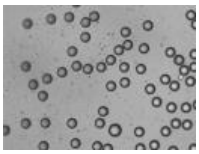
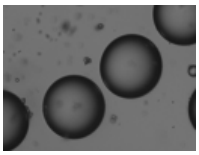
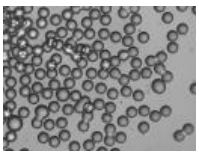
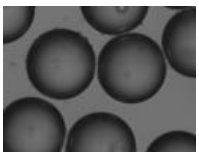
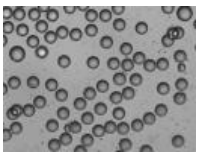
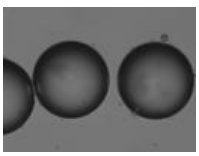
Screenshot of 'Droplet Monitoring Software'

In order to estimate droplet size and frequency, image analysis is performed on the captured videos. Droplet size and frequency are obtained by processing 1 sec duration video clips. These are processed by Dolomite's 'Droplet Monitoring Software'.

After droplet generation the droplets flow out of the chip via tubing to a collection vessel which holds mineral oil at 25° C. The drop in temperature causes the droplets solidify into

microparticles. A small sample of particles is then removed from the collection vessel and placed on a glass slide and viewed under high magnification. Some sample images are presented in the below table for a case of T = 65° C and c = 3.33 %. T is the temperature of the hot plate as well as the temperature of the agarose melt and c is the concentration of the agarose solution (mass of agarose[g] ×100 /volume of water [ml]).

The table demonstrates the effect of increasing agarose flow while keeping the carrier oil flow rate constant.

Mineral Oil		Agarose		Droplet		Particle		
P	Q	P	Q	Image	Size	Image		Size*
[bar]	[µL/min]	[bar]	[µL/min]	-	[µm]	Low Magnification	High Magnification	[µm]
1	37	0.075	0.15		47			46
1	37	0.15	0.29		55			54
1	37	0.30	0.53		65			64
1	37	0.60	1.13		69			69
1	37	1.20	2.19		78			76
1	37	2.40	3.93		89			86

* Droplet size is reported as directly measured from image. Since channel depth is 100 µm, a droplet diameter measured larger than 100 µm diameter refer to a flattened droplet.

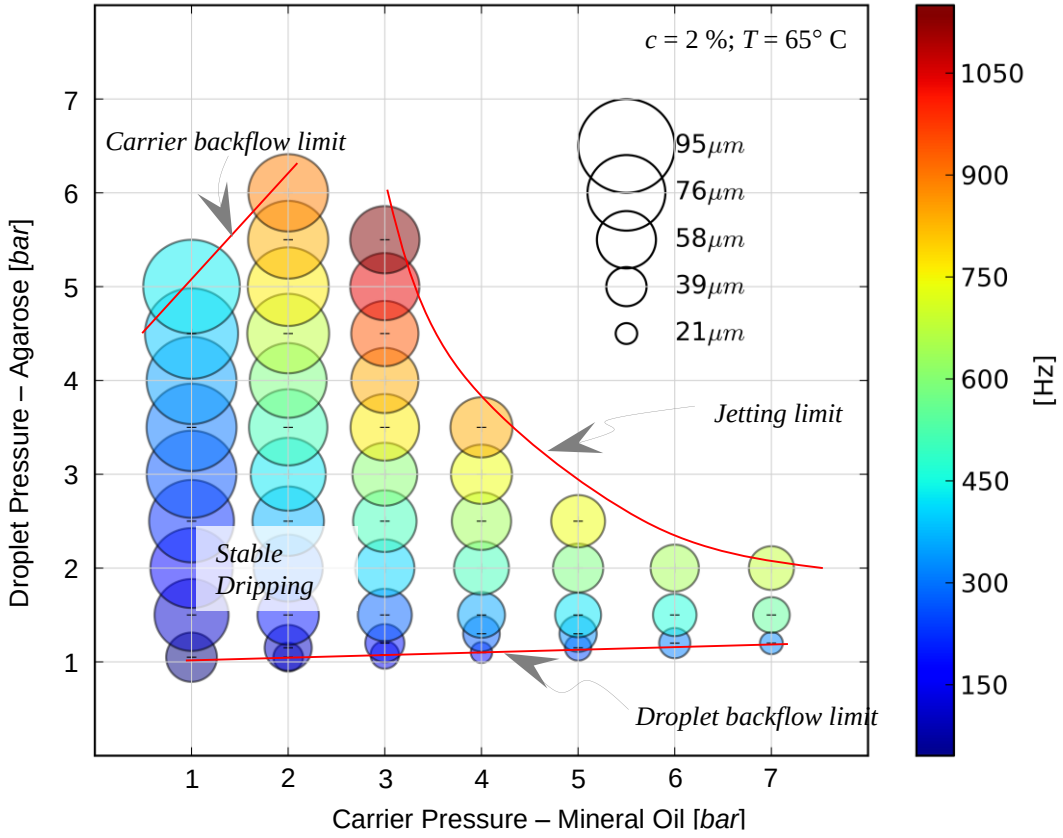
For the purpose of demonstration, the following temperatures and Agarose concentrations were used in the experiments:

		Temperature T (°C)			
Concentration c (% g/ml)		45	55	65	75
	0.5	✔	✔	✔	✔
	2.0			✔	
	3.3			✔	

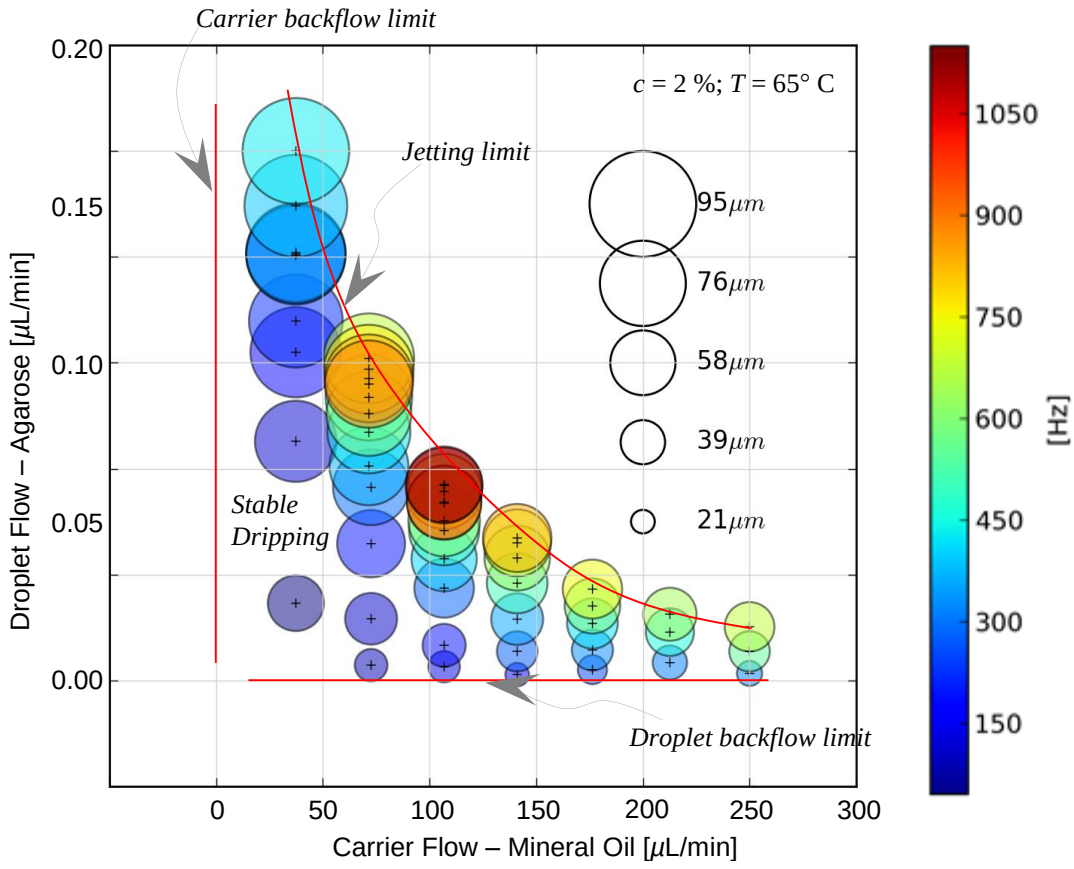
The graph on the next page illustrates droplet diameter variation over a pressure range of 0-7 bar on both carrier and droplet phase. Droplet size is directly proportional to agarose pressure, and inversely proportional to mineral oil pressure. Production frequency increases towards the upper right corner and is lowest at the bottom left corner of the pressure spectrum.

High frequency corresponds to small droplet size and vice versa. The operating space is bounded by three regimes beyond which droplet generation is inhibited. This occurs via droplet coalescence, jetting or backflow. Backflow is a situation where the pressure of either phase becomes negative at the junction, resulting in the fluid flowing backwards. For stable production, operating conditions are recommended to be well within the three limiting bounds.

The same information as a function of flow rates is presented in the figure on the following page. Flow rates for the carrier phase are recorded directly with a flow sensor. The flow sensor has pre-set modes calibrated for a number of fluids such as Mineral Oil. Flow rates for the droplet phase are indirectly obtained by calculating frequency and droplet size.



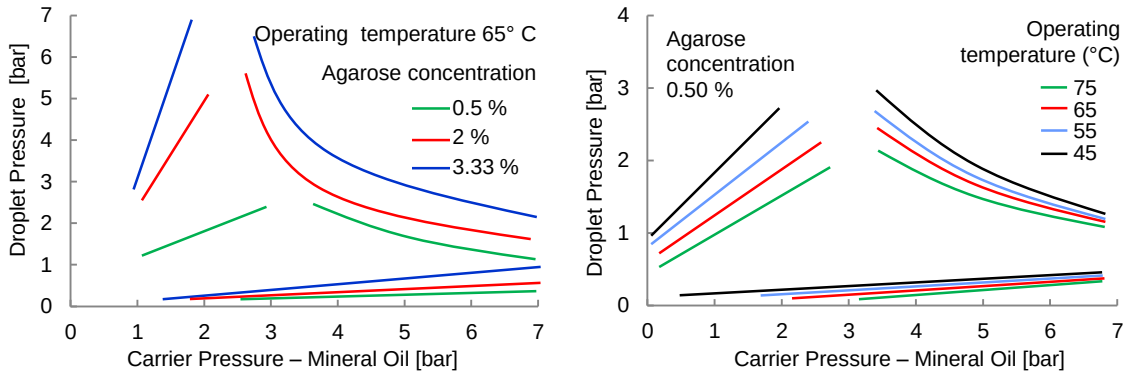
Variation of droplet size and frequency by changing droplet and carrier pressures



Variation of droplet size and frequency by changing droplet and carrier pressures displayed as a function of flow rates

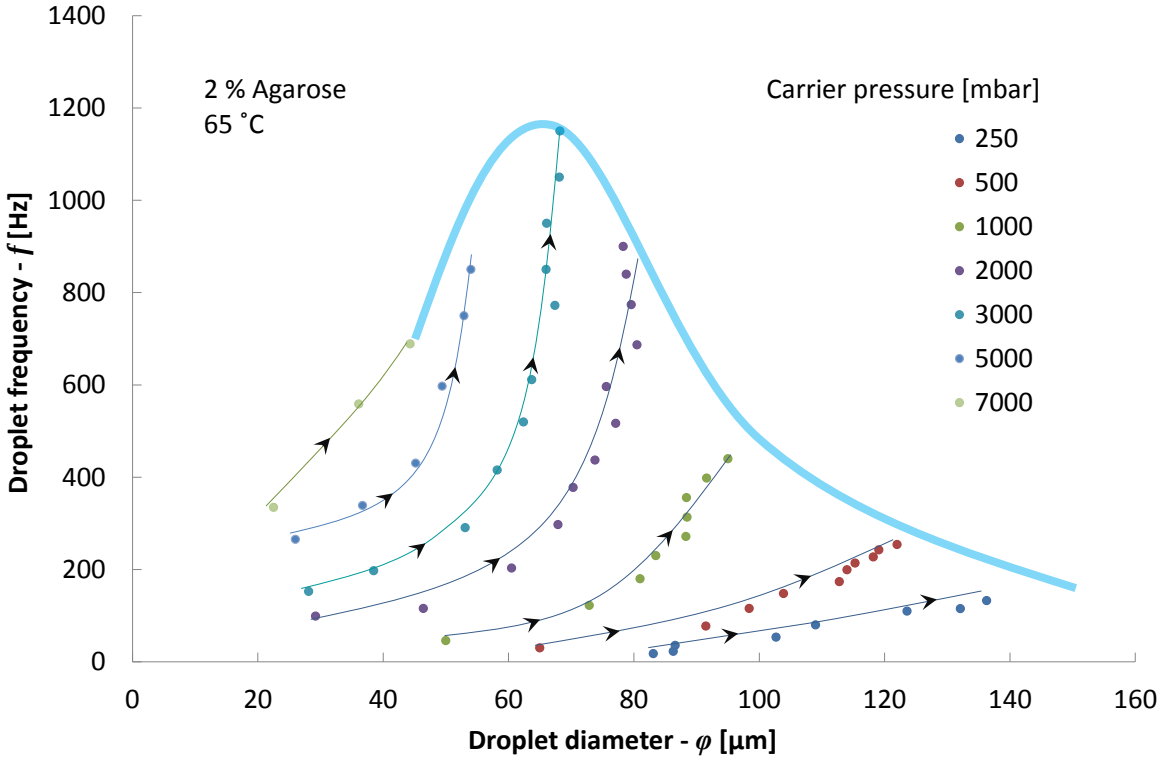
Effects of Varying Operating Temperature and Agarose Concentration

The effect of temperature change and concentration change is observed by plotting the movement of the bounding lines. Decreasing agarose concentrations results in decreased viscosities and thereby increased frequencies. Likewise, increasing temperatures result in decreased viscosities, and therefore increased droplet generation frequency.



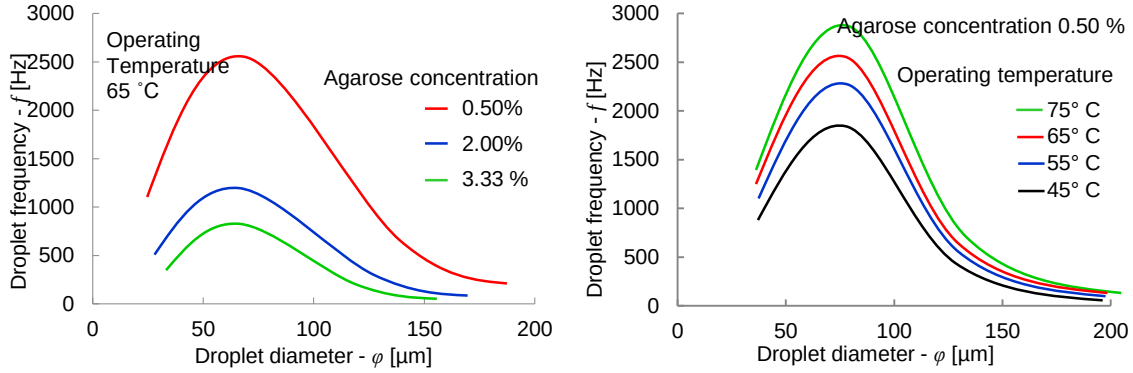
Effect of change in melt temperature and agarose concentration on bounds of operating space

The viscosity of the agarose increases with concentration. Greater pressure is therefore required to pump the solution. Similarly, high temperatures lower the viscosity, thereby reducing the pumping effort required.

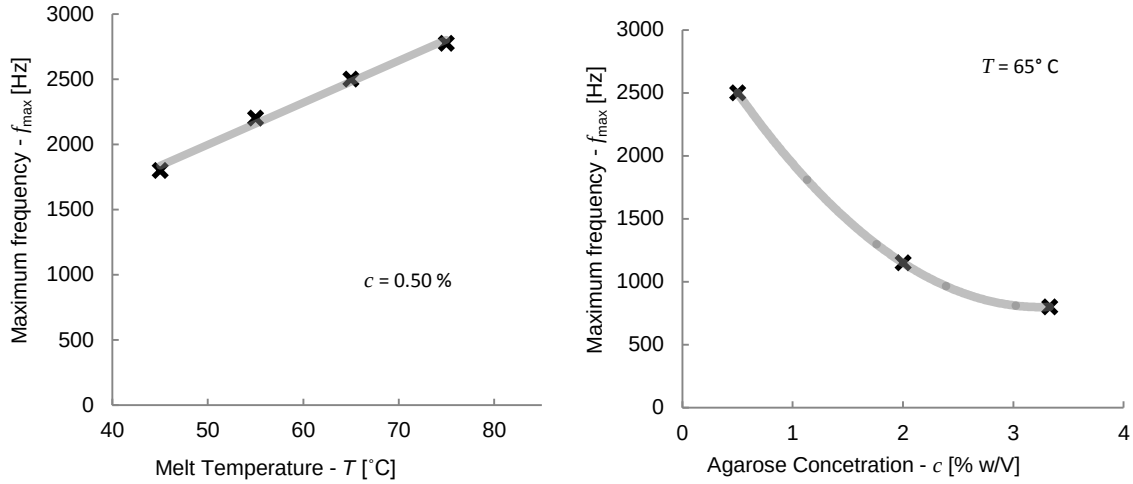


Detail of droplet generation frequency as a function of droplet diameter

For a given pressure on the carrier (Mineral Oil), droplet frequency increases with increasing pressure of the dispersed phase (Agarose). This feature is illustrated in the graphic above 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 limit the maximum achievable frequency. The band of achievable droplet sizes also depends strongly on the operating frequency. This band narrows if higher generation rates are required. The legend indicates carrier pressure. For each set of data points of fixed carrier pressure, droplet frequency and size increases with increasing droplet pressure as indicated by the direction arrows. The line defined by the locus of points connecting the maximum achievable frequencies bounds the stable operating space.



Effect of change in melt temperature and agarose concentration on production output



Maximum frequency achieved in test conditions as a function of temperature and agarose concentration

Finally, the maximum frequency possible from the earlier bell curves is plotted as a function of temperature and concentration. This shows the production capacity increases linearly with temperature, while concentration has a much stronger effect. It is therefore important for users to determine the free variable in their systems to be able to maximize output.

Summary

Agarose particle production, a key step in many biochemical processes, is demonstrated. Solid agarose is melted into a solution at elevated temperature. The solution is then used in the Dolomite droplet chip to generate droplets. The chip and the setup are heated to ensure a liquid state of the agarose solution. Droplets are transferred to a lower temperature zone where the phase change to solid occurs, thereby forming microparticles. The size of the microparticles obtained is controlled and can be varied between 20µm to 130µm.

The highest droplet generate rate is 2800 droplets per second and correspond to a droplet size of ~ 60 µm produced with agarose concentration of 0.5% and operating temperature of 75° C. Lower temperature or higher concentration results in an increase in fluid viscosity which lowers the limit of droplet production.

Generation of hydrogel beads using microfluidics opens up possibilities for more complex particles. One common example is the encapsulation of biological cells, DNA or nanoparticles inside each hydrogel bead. Other examples include double emulsions for generating shelled particles and multicomponent droplet generation for Janus particles. There is also the possibility to form nanoparticles from hydrogel microparticles by evaporating the water from the particles resulting in significant shrinkage.

APPENDIX A: System Component List

Part No.	Part Description	#
3200016	Mitos P-Pump	2
Custom part	Heating Rig	1
3000301	Standard droplet junction hydrophobic chip	1
3000024	Multiflux Linear Connector 4-way	2
3000155	H-type chip interface	1
3200200	Flow sensor interface	1
3200097	Mitos Flow Rate Sensor (30-1000µl/min)	1
3200095	Mitos Sensor Display	1
3200050	High speed camera and microscope system	1
3200063	FEP Tubing, 1/16" x 0.25mm, 10 metres	1
3200300	FEP Tubing, 1/16" x 0.10mm, 10 metres	1
3000397	T- Connector ETFE	1
3000056	FEP plugs	2
3000477	End Fittings and Ferrules for 1.6mm Tubing (pack of 10)	1
3000398	PTFE Tube Cutter	1

The dominant forces at the microscale are interfacial forces and viscous forces. The relative strength of these two is represented by the dimensionless Capillary number Ca, expressed by $Ca = \mu v/\sigma$. μ is the viscosity of the agarose solution, v is the velocity, and σ is the interfacial tension between agarose and mineral oil. The interfacial tension always acts to reduce the interfacial area. This is crucial to the formation of droplets and also for their subsequent stability. Viscous forces on the contrary extend and stretch the interface. Without a change of fluids, Ca changes by varying flow velocity v. At limiting velocities (P-Pump pressures), the flow regime transitions from droplet to chaotic and finally a jetting regime indicating too high a Ca (>>1).

Production capacity of agarose droplets is governed by fluid properties of the system. Agarose viscosity is varied by either changing temperature or concentration. Generally, higher temperatures as well as lower concentrations yield high productions rates. Likewise, lower temperatures or high concentrations yield relatively lower production rates.

Satellite droplets are observed occasionally and vary between 1 – 5 µm in diameter. These are very small particles relative to the target particle size in the final sample. Satellite particles are generated when the droplet breaks off at the droplet junction and can sometimes be seen ‘orbiting’ the droplet. These small particles may be removed by batch filtration.

Partially coalesced (joined) droplets were observed occasionally in the collected samples. Coalescence typically occurs when droplets which are not fully solidified come into contact. Further accurate control of temperature is required to eliminate the possibility of coalescence, whether on-chip or in collection vessel.

Sometimes samples of agarose particles in an oil carrier phase are seen to shrink in volume, in particular around the edge of the sample. This shrinkage occurs as a result of evaporation of water out of the particle into the air (drying). To avoid this issue the particles should be kept in a good depth of oil.

The application note demonstrates Dolomites enhanced capability in providing solutions for production of mobile biochemical substrates. Novel additional active means of control via hydrodynamic manipulation are easily adapted to the described setup. We add onto the flexibility of developing multifunctional microfluidic devices substantially. Innovative microfabrication technologies are harnessed to optimize droplet manipulations with Dolomite standard products.

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Dolomite has worldwide rights to sublicense this IP. 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 sub-licensing this IP for your custom application or chip design.

Further information is available at the Dolomite Website under IP Solutions.

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