# PRODUCTION OF WATER-IN-OIL EMULSIONS USING A DROPLET GENERATOR CHIP

## INTRODUCTION

Microfluidic generation of droplets has attracted a lot of interest due to the ability of this method to produce highly monodispersed droplets with high frequency (up to hundreds of kHz). Interest in droplet-based microfluidic systems has grown substantially in the past decade[1,2] as they offer the ability to handle very small volumes (µl to fl) of fluids conveniently, providing better mixing, encapsulation, sorting, sensing as well as suitability for high throughput experiments. Microfluidic-based droplets have many diverse and varied applications such as particle synthesis[3] and chemical analysis[4]. Highly controlled droplet production also makes single cell analysis, or drug testing possible[5,6].

Droplet microfluidic concepts, components and processes are now being adopted and leveraged by end-users to engender completely new science and innovation. Real-world success is now evidenced through a range of mainstream commercial products that are being aggressively applied to key biological and healthcare related problems (e.g., *10X Genomics*, *Drop-seq* and nucleic acid quantification via *Droplet Digital™ PCR systems*)[7]. It is fair to say that droplets have now become an indispensable tool in chemical and biological research.

Droplet generation in microfluidics is based on the use of two immiscible phases that are referred to as the continuous phase (medium in which droplets flow) and dispersed phase (the droplet). For generating droplets, microfluidic systems generally include a microfluidic chip, a fluid handling system and tubing. This system is usually connected to a computer and a microscope to visualize droplet formation.

Fluigent develops, manufactures and supports innovative fluid handling solutions for a variety of applications using microfluidic droplet generation.

The objective of this applicaion note is to demonstrate droplet generation using the Fluigent microfluidic system including pressure pumps, chemicals, tubing and a Droplet Generator Chip obtained from our partner *microfluidic ChipShop*.

## Materials and Method

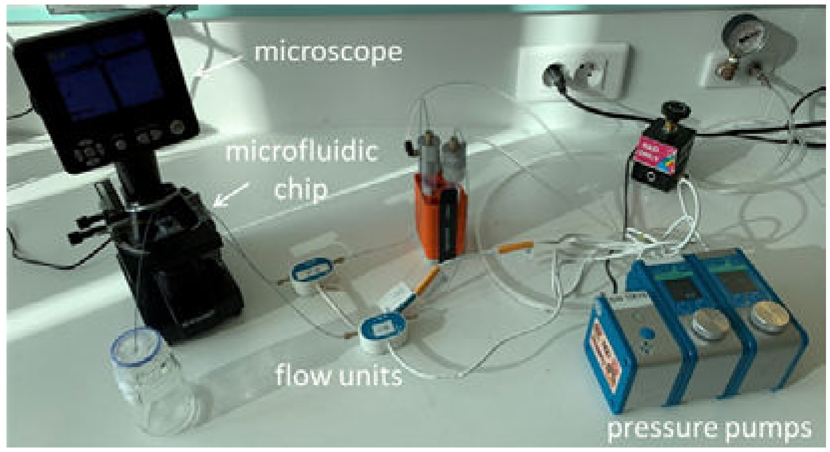


***Figure 1:*** *System setup. Two Flow EZ pressure pumps are connected to the droplet generator from microfluidic ChipShop. The tubing passes through Flow units to control pressure and flow. Visualization of the chip channels is performed* using an optical microscope.

An external pressure source is connected to two Flow EZ pressure pumps that are connected to the *microfluidic ChipShop* chip via tubing. The tubing passes through Flow units to measure pressure and flow rate. Visualization of the chip channels is performed using an optical microscope. A LINK can connect the Flow EZ module to a PC for monitoring pressure and flow rate in real time.

## Microfluidic flow controller

The Flow EZ is the most advanced flow controller for pressure-based fluid control. It can be combined with a Flow Unit to control pressure and flow. It can be used without a PC. A flow EZ 1 bar was used here

## Reagents

**Continuous phase:** dSurf 2% diluted in 3M™ Novec™ 7500: dSURF is a biocompatible fluorosurfactant providing highly reliable droplet production and stability even under PCR amplification conditions. Fluorinated oil is used instead of mineral oil as it has overall better properties (highly biocompatible, immiscible, low viscosity ...).

**Dispersed phase:** distilled water

## Flow Unit

The flow unit is a sensor included in the fluidic system and allows real-time flow rate measurements. By combining a Flow Unit with Flow EZ, it is possible to switch from pressure control to flow rate control, allowing enhanced droplet monodispersity over a long period of time.

## Microfluidic chip

The microfluidic chip used here is the Droplet Generator Chip Fluidic 440, obtained from our partner *microfluidic ChipShop*. The chip is made of polycarbonate (PC) and features eight individual droplet generator units and Mini Luer interfaces. Fluidic 440 is designed to generate droplets with channel dimensions at the droplet formation region of 80 µm (Design 1 and 2), 70 µm (Design 3 and 4), 60 µm (Design 5 and 6) and 50 µm (Design 7 and 8) channel width and height. Droplets are generated using a flow focusing method: the dispersed phase is introduced directly into the main channel while the continuous phase is injected by two perpendicular channels. The dispersed phase is then pinched on both sides by the continuous phase, a droplet thus is formed due to the viscous force and chip geometry, as shown in Figure 2. Accessories to connect the chip to tubing, as well as a variety of different droplet generator chips are available from *microfluidic ChipShop*.

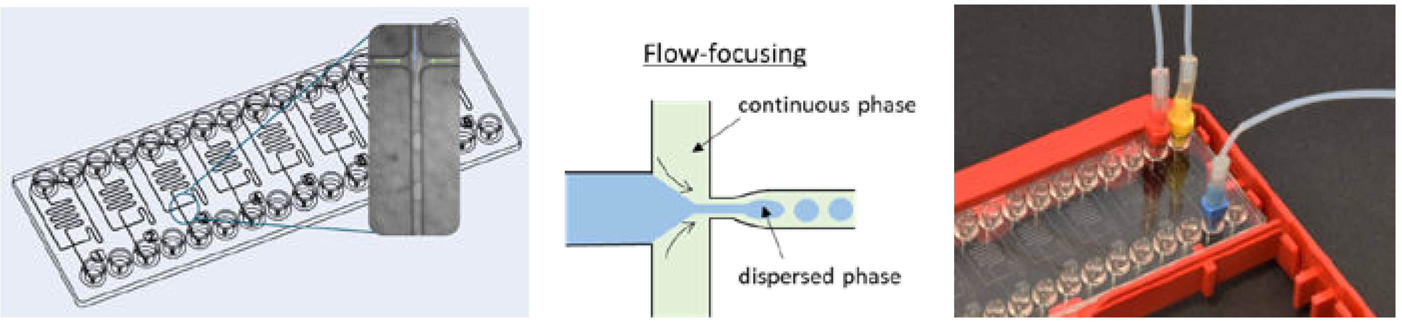


***Figure 2:*** *Droplet generator Fluidic 440. General layout of the chip (left). Scheme illustrating droplet generation using flow focusing geometry (middle) and a droplet generator setup with appropriate accessories, such as connectors and tubing (right)*

## Droplet generation

A scheme of the microfluidic setup is presented in Figure 3. The design 4 of the Droplet Generator Chip is used. This consists of 70 µm width and height channel dimensions at the droplet formation region. Two 15 mL reservoirs containing water and 2% dSurf are connected to the two inlets of the microfluidic chip via 1/32 in. PEEK tubing of 254 µm inner diameter. The tubing passes through flow units allowing flow rate measurement and control. The tubing length from the reservoir to the microfluidic chip is 80 cm. Pressure is applied using the Flow EZs on the reservoirs containing distilled water and dSurf. Water is injected in the inner channel and dSurf is injected in the surrounding channel of the microfluidic device. The pressure applied ranges from 100 to 300 mbar depending on the droplet size and frequency required. Visualization of the chip channels is performed using an optical microscope. A LINK can be connected to the Flow EZ module and a PC for monitoring pressure and flow-rate in real time.

To calculate the diameter of the generated droplets, we measured the droplet length within the microfluidic channel using the software ImageJ and calculated the droplet volume using the following equation proposed by *Musterd* et *al*[8]:

$$V = \left[HW - (4-\pi)\left(\frac{2}{H}+\frac{2}{W}\right)^{-2}\right]\left(L-\frac{W}{3}\right)$$

With *H* and *W* the channel height and width, and *L* the droplet length. We next equaled this volume to the volume of a sphere, and the droplet diameter can be derived.

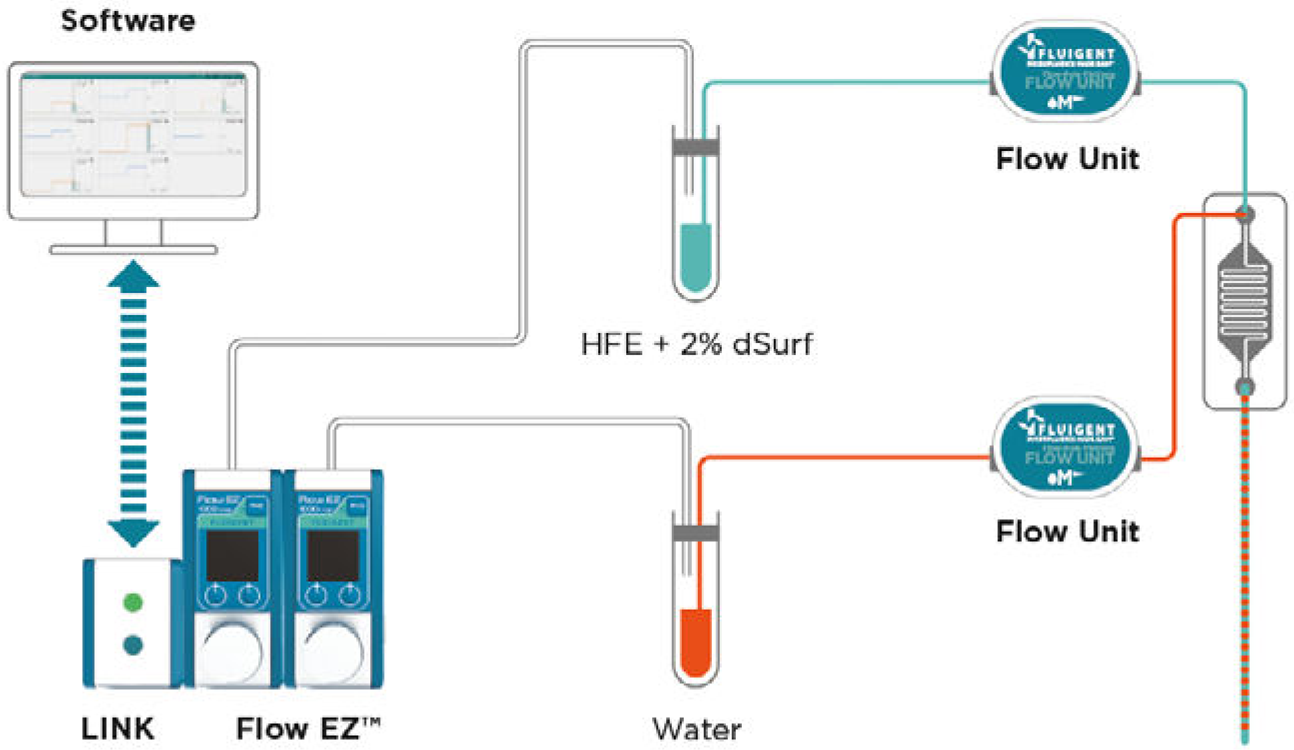


***Figure 3:*** *Schematic of the microfluidic system used for droplet generation*

## Results

Using the microfluidic system presented in the previous part, droplets were generated. Table 1 shows water-in-oil droplet generation as a function of the flow rate and the pressure. The oil flow rate remains constant at 5 µL/min while the water flow rate ranges from 2.5 µL/min to 10 µL/min. The droplet diameter, generation rate and its related picture are also presented in the same table.

We observe that by increasing the water flow-rate and keeping the oil flow rate constant, the droplet diameter increases.

***Table 1:*** *Droplet diameter and generation rate as a function of water flow-rate*

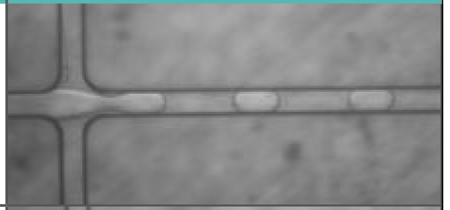
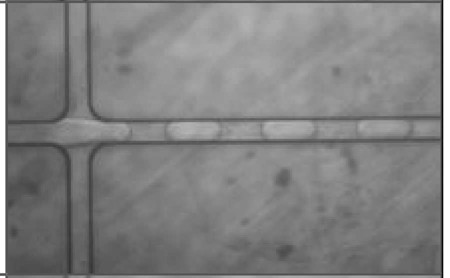
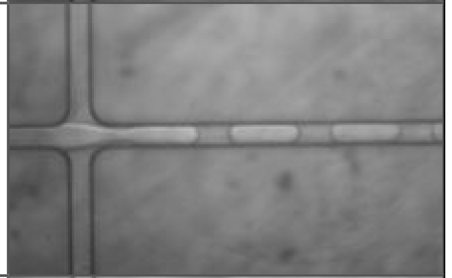
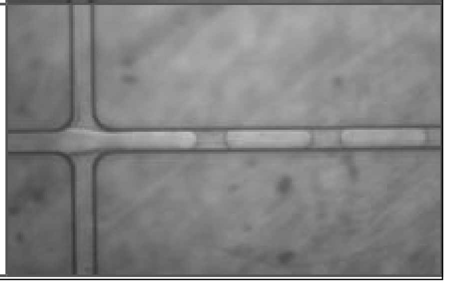
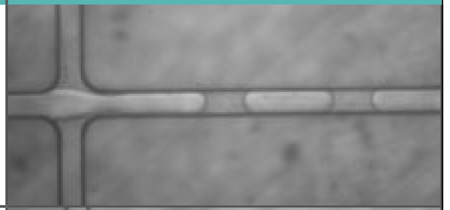
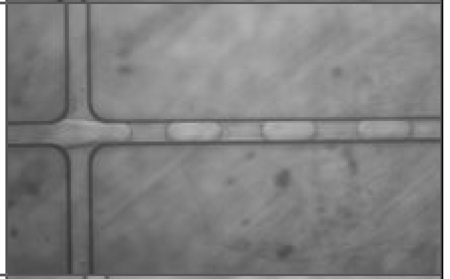
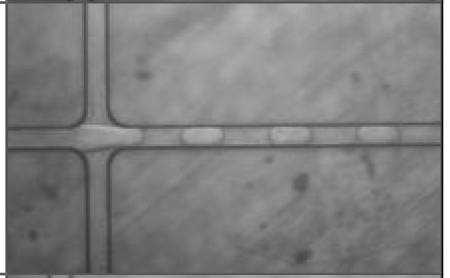
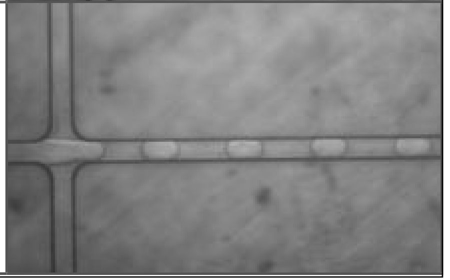
| Water | | HFE with 2% dSURF | | Droplets | | Picture |
|---|---|---|---|---|---|---|
| Q [µL/min] | P [mbar] | Q [µL/min] | P [mbar] | Diameter [µL] | Rate [Hz] | |
| 2,5 | 152 | 5 | 160 | 98 | 84 |  |
| 5 | 170 | 5 | 152 | 111 | 117 |  |
| 7,5 | 252 | 5 | 207 | 120 | 139 |  |
| 10 | 342 | 5 | 270 | 133 | 135 |  |

Table 2 shows water-in-oil droplet generation as a function of the flow rate and the pressure. The water flow rate remains constant at 5 µL/min while the oil flow rate is ranging from 2.5 µL/min to 10 µL/min. The droplet diameter, generation rate and its related picture are also presented in the same table.

We observe that by increasing the oil flow-rate and keeping the water flow rate constant, the droplet diameter decreases.

**Table 2:** *Droplet diameter and generation rate as a function of oil flow-rate*

| Water | | HFE with 2% dSURF | | Droplets | | Picture |
|---|---|---|---|---|---|---|
| Q [µL/min] | P [mbar] | Q [µL/min] | P [mbar] | Diameter [µL] | Rate [Hz] | |
| 5 | 168 | 2,5 | 136 | 129 | 74 |  |
| 5 | 170 | 5 | 152 | 111 | 117 |  |
| 5 | 171 | 7,5 | 167 | 100 | 158 |  |
| 5 | 177 | 10 | 186 | 94 | 190 |  |

The droplets generated and placed on a glass slide are shown in Figure 4. Figure 5 summarizes droplet diameter and generation rate as a function of the oil and water flow rates, using flow rates ranging from 2,5 to 10 µL for each solution. This graph demonstrates that the droplet diameter increases when the water flow rate is increased, and the droplet diameter decreases when the oil flow rate increases. We can also note that by increasing both water and oil flow rates, the droplet generation frequency increases. Using this range of flow rates controlled by Flow EZs with the droplet generator from *microfluidic ChipShop*, it is possible to obtain droplets with a diameter ranging from 80 µm to 160 µm, and with a generation rate ranging from 50 Hz to 250 Hz.

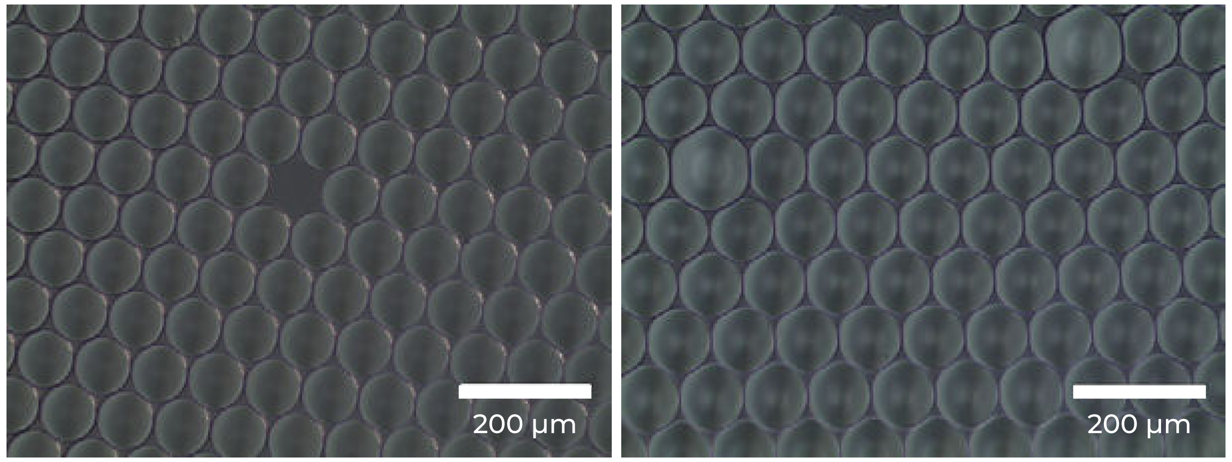


**Figure 4:** *Droplets on a glass slides observed with an optical microscope*

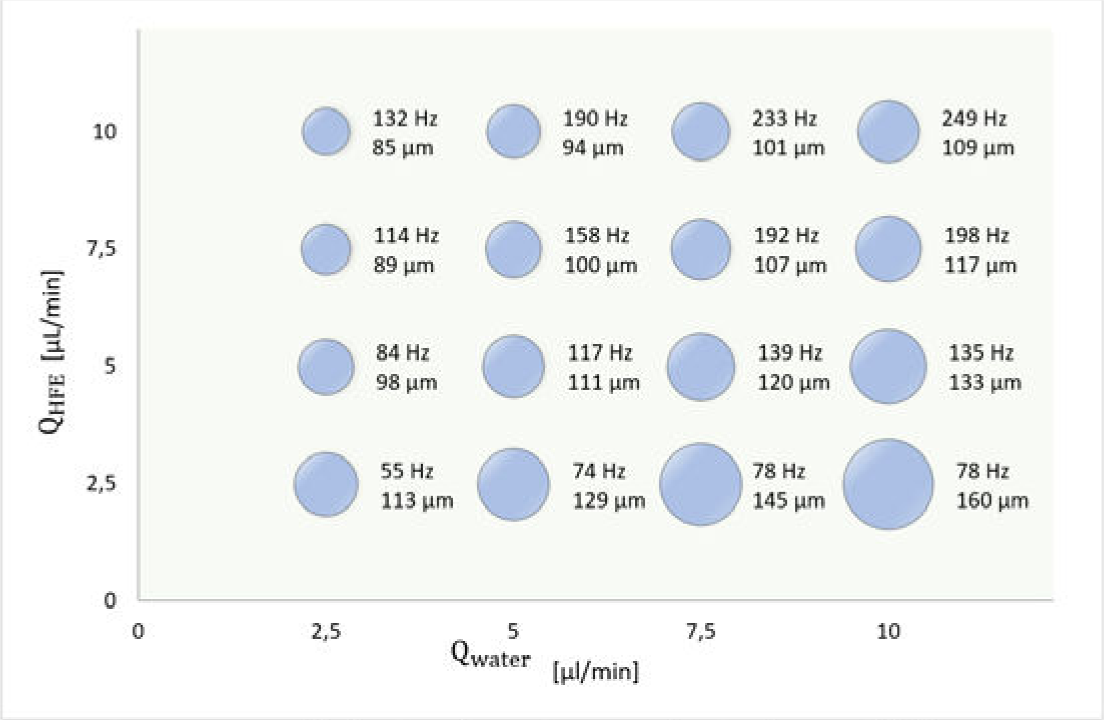


**Figure 5:** *Summary of droplet size and generation rate as a function of water and oil flow rates*

## Droplet Generator Chip for cells encapsulation

We have demonstrated in the previous section that it is possible to generate droplets with a diameter ranging from 80 µm to 160 µm, and with a generation rate ranging from 50 Hz to 250 Hz. In these ranges, one can encapsulate particles, solutes or cells within a droplet. Indeed, *Langer* and *al*. demonstrated E. Coli encapsulation using the Droplet Generator Chip from *microfluidic ChipShop*. We can observe that about ten bacteria were encapsulated within a droplet.

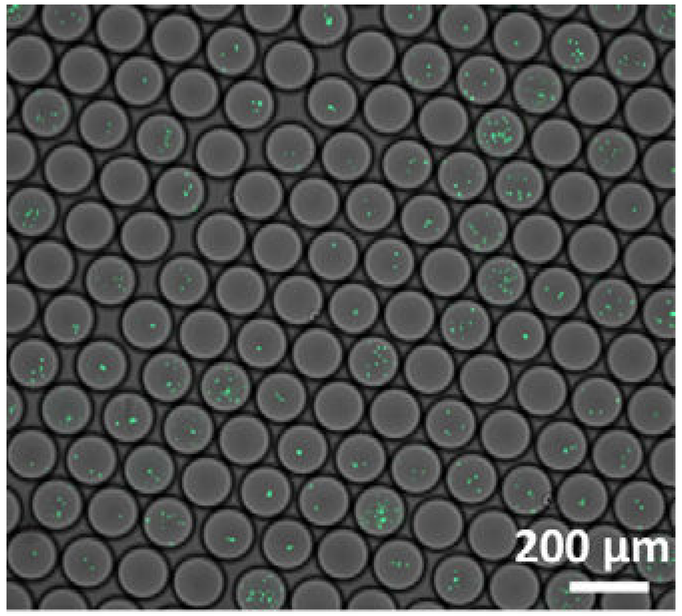


***Figure 6:*** *Example of E. coli encapsulation in droplets*

Hence, using the microfluidic setup presented in the technical note, it is possible to generate monodispersed droplets that are suitable for the encapsulation of particles or cells.

## CONCLUSION

**We have demonstrated the use of Fluigent pressure pumps combined with *microfluidic ChipShop*'s chip for the generation of monodispersed water-in-oil droplets. The versatility of the system allows one to easily produce droplets of different diameters and at several generation rates. Droplets fabricated from this system are suitable for many applications, including those that require biocompatibility.**

## References

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This application note was developed using dSurf, a discontinued Fluigent product. Discover the 008-Fluorosurfactant, offering similar performance and enhanced usability.