

WATER IN FLUOROCARBON OIL EMULSIONS

INTRODUCTION

Water in fluorocarbon oil emulsions (W/FO) are widely used in R&D environments primarily to achieve compartmentalisation of biological samples within droplets and prevent egress of the material to the surrounding media such as Drop-Seq and RNA-Seq. Of particular importance, is the ability to produce high-quality, monodisperse droplets and the ability to do so reproducibly and at a high production rate.

The combination of Fluigent pressure pump units and Raydrop microfluidic devices enable smooth fluid delivery, precision flowrate control, automation and reproducibility necessary to generate high quality water in fluorocarbon oil emulsions.

In this application note, we present droplet generation data obtained using Fluigent's own fluorocarbon oil formulation – dSURF to demonstrate the ability of Fluigent equipment coupled with Raydrop microfluidic devices to generate high quality emulsions with controlled droplet sizes and with high throughput.

MATERIALS & METHODS

1. Reagents

Droplet Phase: Water (Mili Q)

Continuous phase: dSURF

REAGENT	SUPPLIER	CATALOGUE NUMBER	CAS NUMBER
WATER	ULTRAPURE 18.2 MΩ – CM	-	7732-18-5
dSURF	FLUIGENT	DR-RE-SU-12	-

2. Microfluidic setup

The microfluidic setup was comprised of:

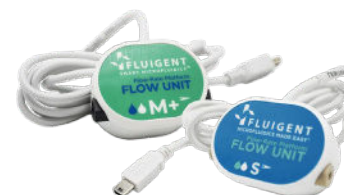
- 2x FlowEZ (2000 mbar) pressure pumps - Microfluidic flow controller

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 or flow rate. It can be used without a PC. Two Flow EZ with 2 bar of full-scale pressure are used in the setup presented here.



- 2x Flow units enabling flowrate control - Flow sensor

The Flow Unit is a flow sensor that allows real time flow rate measurement. By combining a Flow Unit with the Flow EZ, it is possible to switch from pressure control to flow rate control, allowing for the generation of highly monodispersed droplets over a long period of time. Two Flow Units M are used here to monitor and control the flow rates of the dispersed and continuous phase during the run.



- Droplet generator:

The RayDrop droplet and emulsion chip is used to control the generation of alginate droplet. The RayDrop is based on the alignment of two capillaries immersed in a pressurized chamber containing the continuous phase. The dispersed phase exits one of the capillaries through a 3D-printed nozzle, placed in front of the extraction capillary for collecting the droplets. This non-embedded implementation of an axisymmetric flow-focusing is referred to co-flow-focusing.



- Optical microscope:

Fluigent high-speed camera is a package which contain all necessary instrument to have good optical instrument.

It contains:

- Microscope
- High speed camera
- Light controller

This package is connected with an USB 3.0 wire to a PC where the user can visualize its microfluidic device with the Pixelink Capture Software together with other Fluigent Software (OxyGEN). The camera has been selected to reach rates up to 7028 fps which is ideal for droplet experiments.



3. Fluidic path

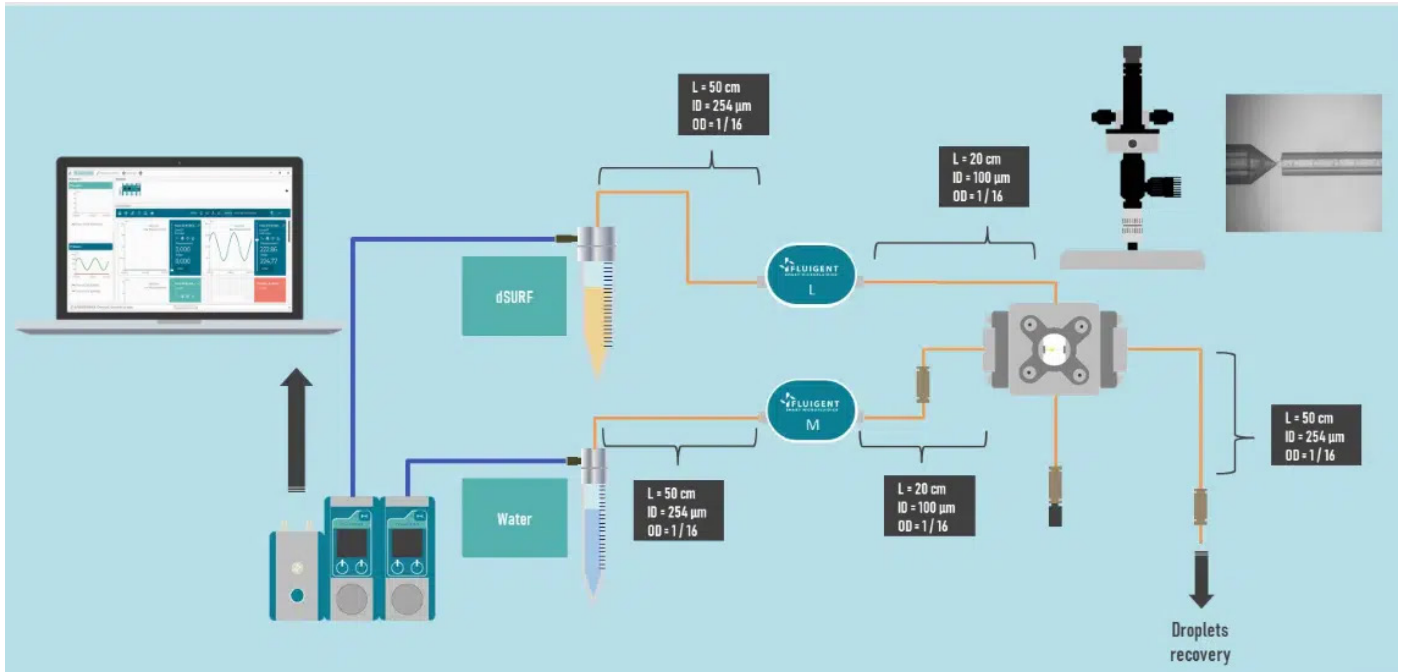


Figure 1: Scheme of the fluidic setup.

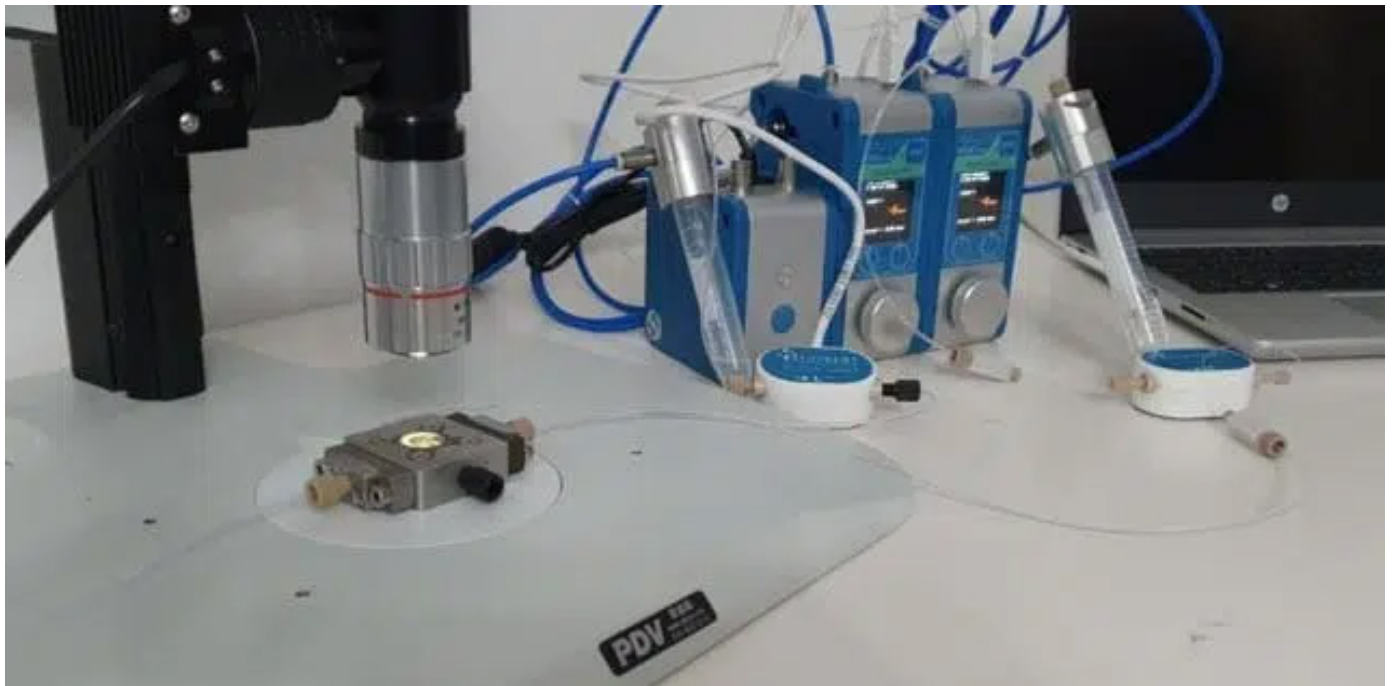


Figure 2: Pictures of the Fluigent equipment.

RESULTS

CONTINUOUS PHASE FLOWRATE (mL/min)	DROPLET PHASE FLOWRATE (μl/min)	DROPLET DIAMETER (μM)	PRODUCTION RATE (Hz)
100	5	63	636
100	10	67	1058
100	15	71	1334
50	5	68	506
50	10	73	818
50	15	76	1087
25	5	72	426
25	10	75	754
25	15	81	898
15	15	85	777



Figure 3: Droplet phase diagram.

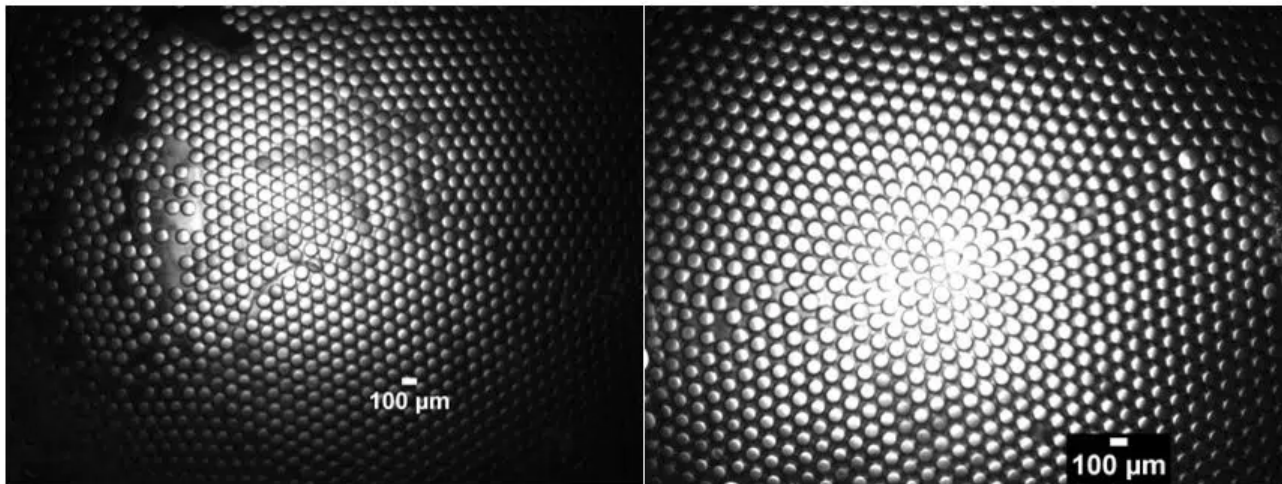


Figure 4: Images of water droplets in dSURF generated using Fluigent equipment and Raydrop microfluidic device

CONCLUSION

Fluigent pressure-based flow controller units and Raydrop microfluidic device were successfully used to generate high-quality, monodisperse droplets of water in fluorocarbon oil. The droplet size was controlled in the range of 63 – 85 μm by adjusting the continuous and dispersed phase flowrates. Peak stable droplet production rate was recorded for 71 μm droplets at 1334 Hz. The production techniques developed here can be used in a wide range of compartmentalisation applications such as Drop-Seq and RNA-Seq.