ELVEFLOW
PLUG & PLAY MICROFLUIDICS

Elveflow user guide

DROPLET GENERATION PACK

DOCUMENT REF: UG_DROPLETPACK_20243101

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SYMBOLS USED IN THIS DOCUMENT

IMPORTANT INFORMATION. Disregarding this information could increase the risk of damage to the equipment, the risk of personal injuries, or degrade your user experience.

HELPFUL INFORMATION. This information facilitates the use of the instrument and contributes to its optimal performance.

ADDITIONAL INFORMATION is available on the internet or from your Elveflow representative.

INTRODUCTION

The droplet starter kit offered by Elveflow doesn’t need prior knowledge in microfluidics. Simple and intuitive instructions are provided to quickly and easily make droplets and control droplet generation parameters.

When starting out, the user can follow step by step the provided protocol to obtain droplets of the specific size. In a second stage, the user can rely on the numerous tips provided and explore the "going further" section to complete its training in droplet generation and microfluidic flow control.

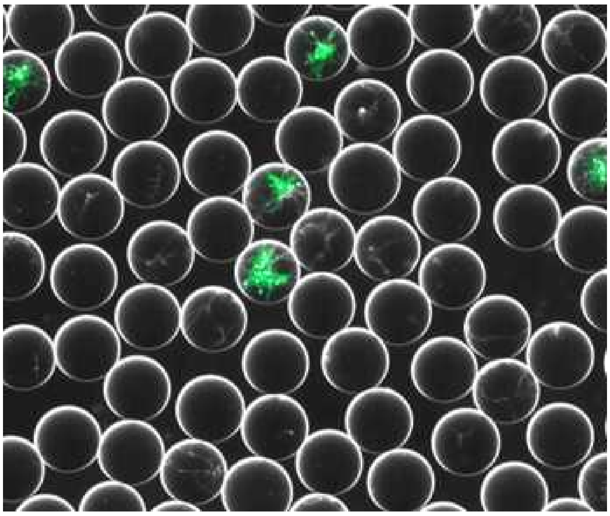


Fig.1 Example of droplets

Two chips are provided with this pack allowing you to create droplets within a diameter range from 10 to 80 µm. The dimensions can be adapted to reach bigger or smaller sizes of droplets through various commercially available chips. In this droplet kit, the provided materials and experimental protocol are fully versatile. Thereby, it can be used with other types of microfluidic chips.

Droplet generation in a microfluidic chip

The two chips provided with the pack are based on flow focusing droplet generator geometry. Each chip is composed of four different nozzles (the nozzle is the part of the fluids merged see figure2.) providing a broad range of droplet sizes. There are 8 devices in total, 2 devices per design.

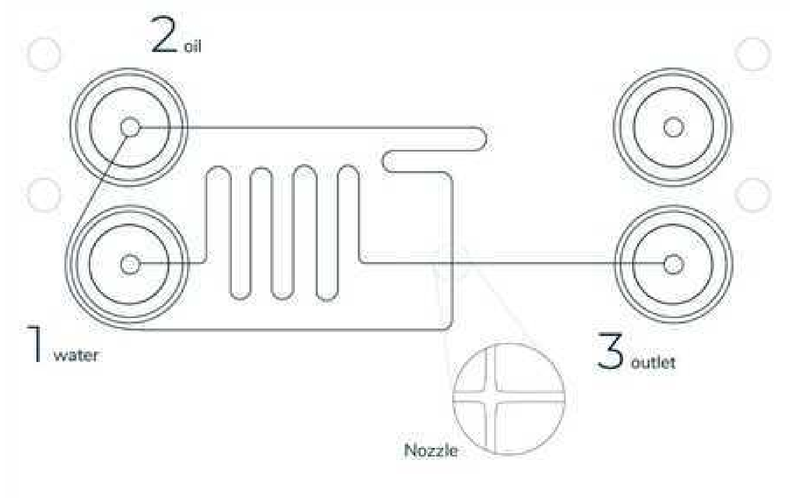


Fig.2. Schematic of a flow focusing design zoom on the nozzle

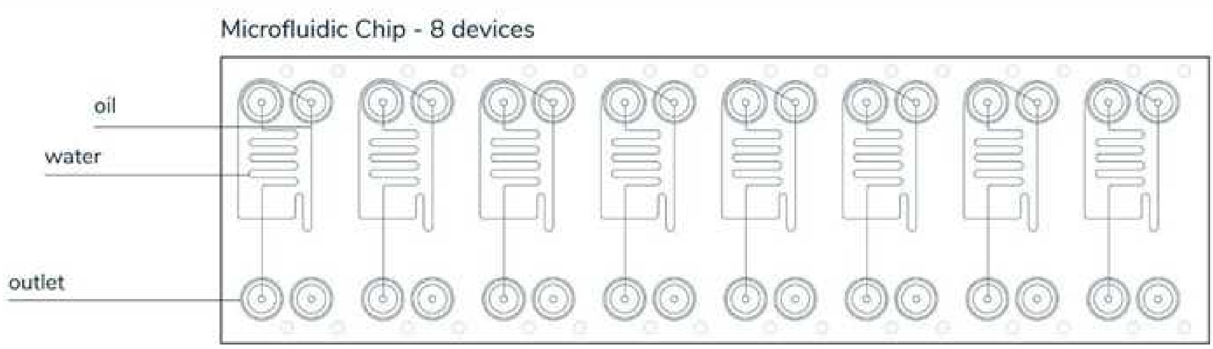


Fig.3 Schematic of a chip with 8 devices (4 times 2 nozzle sizes)

Through this geometry, monodisperse droplets are formed by the combined action of the continuous and dispersed phases. The dispersed phase (the phase that will become the inner phase of the droplets, here, the water) is squeezed between two flows of the continuous phase (the phase that will carry the droplets, here, the oil and surfactant), which leads to the formation of droplets(see figure 4). The surfactant contained within the continuous phase (e.g. the oil) stabilizes the oil/water interface, allowing the droplets to be stable in time and preventing droplet coalescence when in contact with each other. A surfactant is an amphiphilic molecule that adsorbs at the oil-water interface, reduces the surface tension at the curved interface and stabilizes the resulting oil-water two-phase mixture. Thus, it allows the suspension of water droplets in oil (or Water-in-Oil emulsion) to be stable over time. Both chips are made by injection molding in Topas polymer (COC) and are hydrophobic. A hydrophobic surface ensures an effective water-in-oil droplet generation as the water droplet won't adhere to the channel walls.

The final features, properties and characteristics of the generated droplets (size, frequency) depend on the chip geometry (shape and dimensions of the channels and the nozzle), the physical parameters of the liquid (surface tension, viscosities) and the channel wall surface treatment.

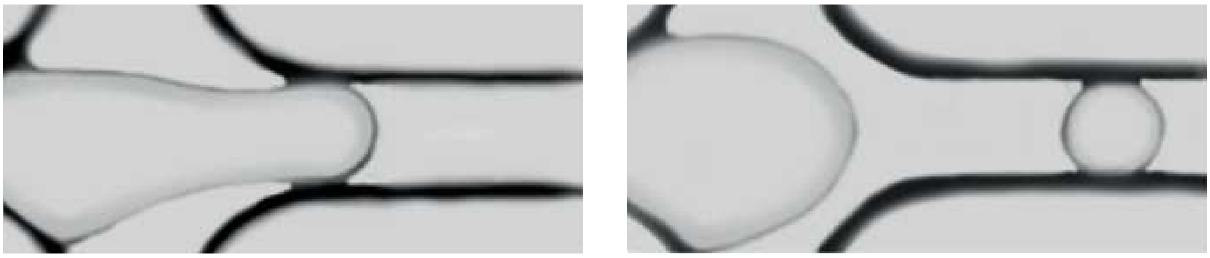
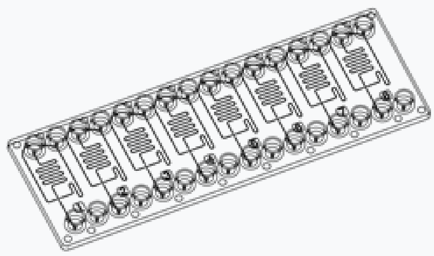


Fig.4 Droplet forming inside the microfluidic chip

Design and characteristics of the chips

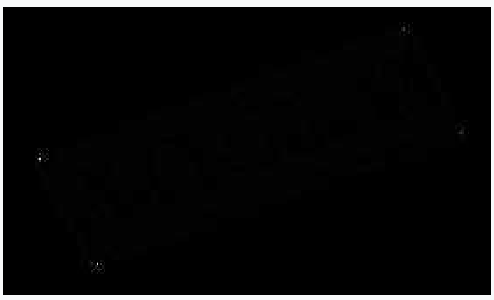
Droplet size between [10 - 40] µm: Fluidic 947 from Microfluidic Chipshop

Interface type	Mini Luer
Nozzle sizes	10 – 15 – 20 – 30 µm
Lid thickness	140 µm
Material	Topas



Droplet size between [50 - 80] µm: Fluidic 440 from Microfluidic Chipshop

Interface type	Mini Luer
Nozzle sizes	50 – 60 – 70 – 80 µm
Lid thickness	140 µm
Material	Topas



For a given system (a microfluidic chip with defined continuous and dispersed phases), the characteristics of the droplets generated will depend on the flow rates of the two immiscible phases. The setup and experimental protocol given within this droplet kit will allow you to finely tune the flow rate of the two liquids to obtain the desired droplet properties.

Contents of the droplet starter kit

2 Topas (COC) microfluidic chips

The two microfluidic chips are made of Topas. Topas is a cyclic olefin copolymer (COC) resin which is a chemical relative of polyethylene and other polyolefin plastics. Both microfluidic chips contain 8 independent fluidic systems, and 4 different nozzles.

1 bottle of surfactant in oil

(FluoSurf 2% in HFE-7500, 10 mL)

FluoSurf (Emulseo) is a fluorinated surfactant specially designed and optimised to stabilise aqueous (water-in-oil) droplets in fluorinated oil (HFE 7500) and thus, prevent droplet coalescence.

1 reservoir holder

with 2 push-in connectors

A specific reservoir holder has been designed to securely hold your reservoirs and solutions. It can hold up to two reservoirs with an easy-to-plug push-in connector ensuring a secure connection.

2 fluidic resistances

Resistances are used to increase the resistivity of the microfluidic system to improve the stability and control of the flow rate in the system.

22-H (60cm - 100µm inner diameter peek tubing) for the dispersed phase. It enables stable control of flow rates ranging from 0,42 µL/min to 7 µL/min using water or liquids with water-like viscosity (close to 1 mPa.s).

22-I (20cm - 100µm inner diameter peek tubing) for the continuous phase. It enables stable control of flow rates ranging from 5 µL/min to 70 µL/min for the case of oil (HFE-7500) or liquids with viscosities close to 1.24 mPa.s.

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1 tubing 1/32" OD (30 m)

The tubing is used to connect all the elements together: the reservoirs, the flow sensors, the chip... Its external diameter is 1/32 of inches and its internal diameter is 300 µm.
It's made of PTFE.

10 sleeves to adjust 1/32" OD tubing to 1/16" OD connectors are included.

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10 Mini Luer connectors

The Mini Luer fluid connectors are designed to connect 1/32" OD tubing to the Chipshop mini Luer compatible microfluidic chips.

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2 Union for ¼-28 connectors

The union connectors allow you to connect your resistances into your fluidic set-up.

In addition to the starter kit

Not included into this kit, your microfluidic setup must include the following items to be able to generate droplets:

- A pressure controller with at least 2 channels
- At least two flow sensors (MFS or BFS range)
- At least two reservoirs to hold your solutions

This starter kit has been designed to be used with at least an OB1 2 channels. Depending on your specific needs, your OB1 may have more than 2 channels. The following experimental protocol has been made using a 2-channel OB1 with 0-2 bar pressure channels. If you have an OB1 with different pressure channels, the principle of the protocol remains unchanged.

OB1 Pressure-driven flow controller

At least 2 channels

The pressure controller is the centerpiece of the setup, enabling accurate control over the pressure difference across the microfluidic system and thus, a fine control over the liquid flow in the microfluidic device.

Flow sensors

At least 2

A flow sensor measures the flow rate of the liquid going through the sensor. Combined with the pressure controller, it allows the user to monitor and precisely control the flow rate.

NB: This user guide has been prepared using 2 x MFS 2 for the main core and 1x MFS 2 and 1x MFS 3 in the section "going further".

Reservoirs

At least 2 with pressurized reservoir caps

Reservoirs are used to hold your liquids. They will be connected to your reservoir holder. Three sizes are compatible with the holder 1,5 mL, 15 mL and 50 mL. We recommend choosing the closest size to your volume solution.

Before starting the experiment

1. To begin with, make sure you have installed the software. Launch it.

You can refer to [this video](#).

Before manipulating any instrument, please read its specific user guide.

The user guides can be downloaded from the support section on our website.

Each user guide describes the good practices to handle Elveflow's equipment.

2. Add the OB1 to the software and calibrate it

Connect your OB1 pressure controller to an external pressure source using pneumatic tubing, to a computer using a USB cable and plug it to the power source.
Once the OB1 is correctly connected, switch it on and close all the channels using the plug fittings.
Add the OB1 to your ESI software.

Before using it you should launch a calibration. Make sure the OB1 is connected to an external pressure source and that it is properly supplied with air. Check that all the channels are plugged. Wait 30min for warming.

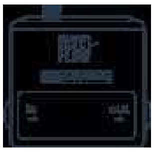
3. Add the flow sensors on the software

If you have MFSs:
Connect the two flow sensors to the OB1.
"Add sensors" the flow sensors to your ESI software.

Fig.5 How to connect a MFS to the OB1

In this user guide, the MFS-2-D measuring the oil flow rate will be called **Oil**, and the MFS-2-D measuring the water flow rate will be called **Water**.

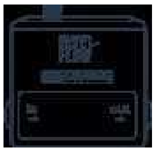
The digital MFS has two available calibrations: Water and Isopropyl alcohol.



Oil | MFS-2-D

Measures the oil flow rate.

Calibration **Isopropyl**



Water | MFS-2-D

Measure the aqueous buffer flow rate.

Calibration **Water**

Why is it necessary to calibrate the MFS?

The flow sensors measure the flow rates of a liquid by locally warming it and by measuring the temperature differences in different locations of the sensor capillary. The relation between temperature measurement and the flow rate highly depends on the physical properties of the liquid passing through the MFS. That is why a calibration is required. Two calibrations are implemented directly in the MFS : Water and Isopropyl. The Water calibration is suited for all aqueous solutions. The calibration Isopropyl is appropriate for all the carbon chains (fluorinated oil), with an additional linear adjustment.

With the **MFS-2-D Oil**: it is necessary to use a scale factor and an offset after setting the calibration to **Isopropyl**.

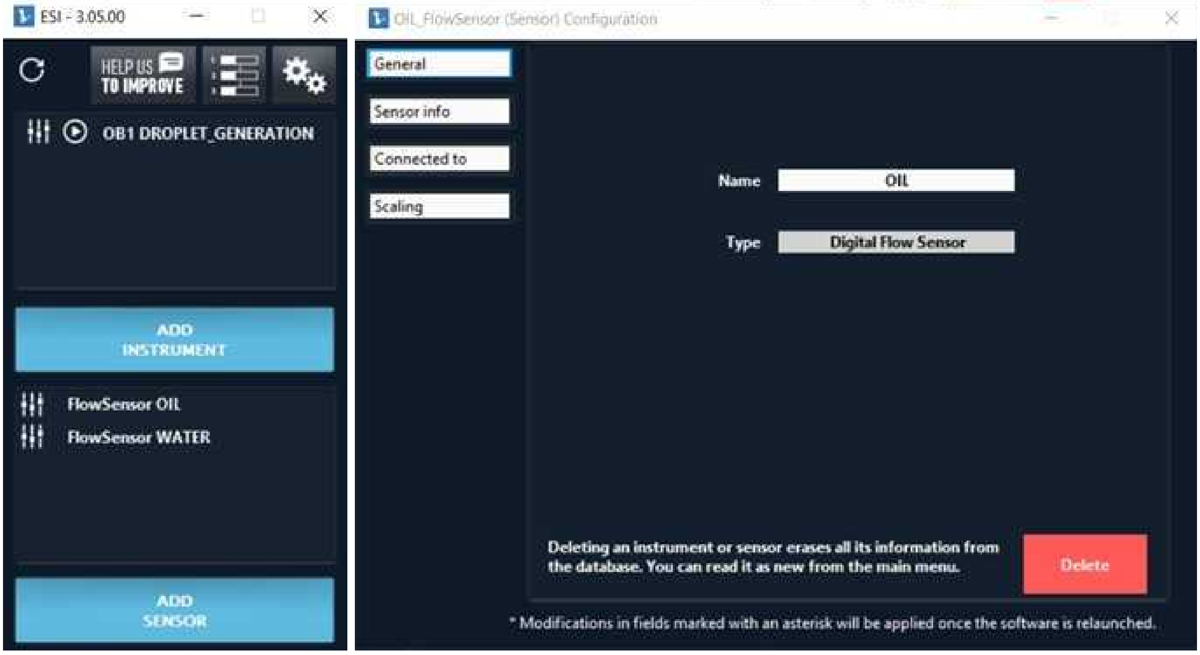


Fig.6 : How to add a Flow sensor and set the appropriate calibration in the ESI. Add a Flow Sensor, access the calibration parameters, choose the calibration type and modify the scale factor and offset.

Based on our experience with HFE7500, we advise you to use the following values of offset and scale factor for the MFS2 measuring oil flow rates:

SCALE FACTOR	OFFSET
7,24	0

For other oils, please follow the calibration procedure following the user guide of the MFS

If you have BFSs:
Connect the two BFS to the power and to your computer.
“Add instruments" to add the BFS to your ESI software.

In the settings of the BFS visualize each one on your OB1 channels.

The BFS is calibration free so no need to change the settings more.

Let’s start making droplets!

1. Set up the reagents

Water phase: Attach the reservoir (1.5, 15 or 50 ml) filled with water to the pressurized reservoir cap and connect it to the supplied 1/32” OD tubing and fittings.

Oil phase: Attach the reservoir (1.5, 15 or 50 ml) filled with HFE-7500 oil + 2% Fluosurf surfactant to the pressurized cap and connect it to the supplied 1/32” OD tubing and fittings.

Plug both reservoirs to the reservoir holder and to the corresponding OB1 pressure controller outlet.

Fig. 7: Elveflow's reservoir holder

TIP

The percentage of surfactant required will depend on your experiment (stability and integrity of your droplets). For typical water-in-oil droplets, 0.5% to 2% of Fluosurf surfactant is enough to generate, collect and reinject the droplets.

TIP

All the liquid must be preferably filtered and not exposed to the environment, as dust will settle in it. It is recommended to manipulate and put the caps inside a fume hood that avoids airborne particles settlement.







Fig.8 How to connect the reservoir to the reservoir holder and the reservoir holder to the OB1

2. Set up the microfluidic chip

Add Mini Luer connectors to the inlets and outlets of the microfluidic chips for the fluidic system chosen.

Fig.9 How to connect 1/32" OD PTFE tubing to the Mini-luer connectors and to the Chipshop device.

3. Set up the microfluidic system

Set up the following system.

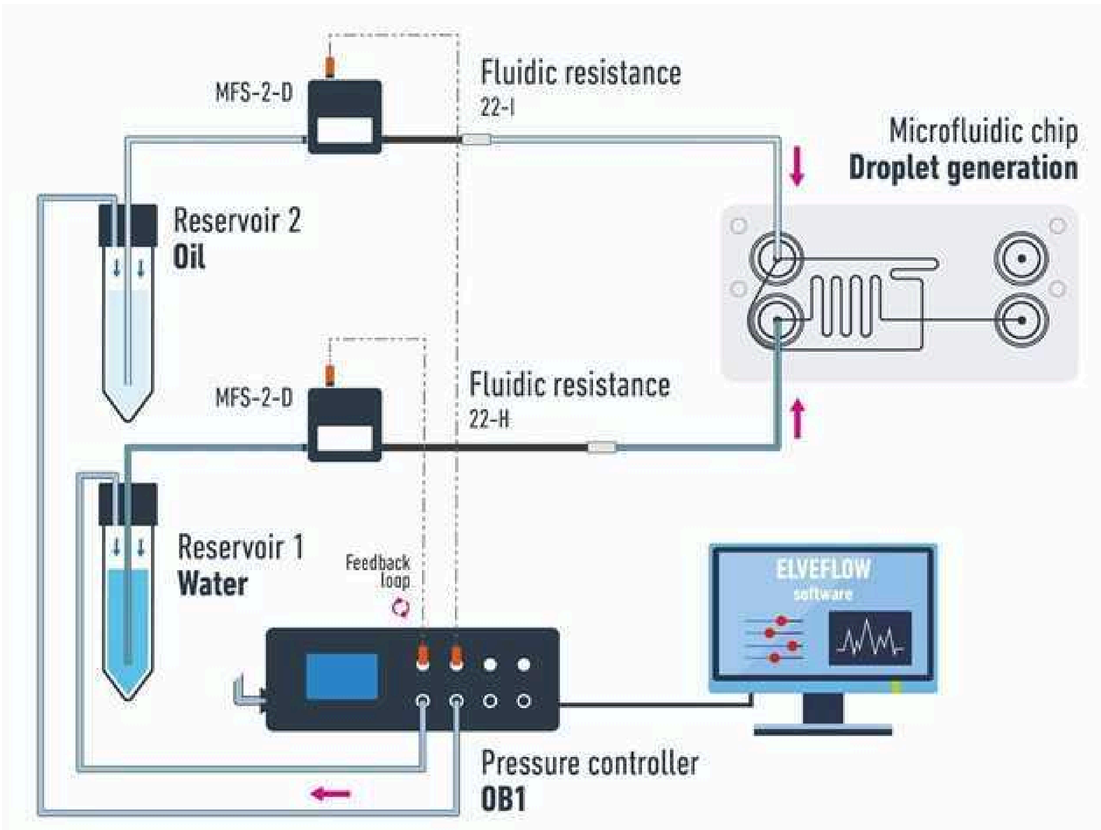


Fig. 10 Sketch of the droplet generation setup

Channel 1 - 2000 mbar

The MFS-2-D Water is connected between the water reservoir and the resistance **22-H** using the supplied 1/32” OD tubing and fittings. The resistance is connected to an union before using the 1/32" OD tubing to connect the microfluidic chip inlet for the dispersed phase (first inlet).

Channel 2 - 2000 mbar

The MFS-2-D Oil is connected between the oil reservoir and the resistance **22-I** using the supplied 1/32” OD tubing and fittings. The resistance is connected to an union before using the 1/32" OD tubing to connect the microfluidic chip inlet for the continuous phase (second inlet).

Add sleeve on the tubing

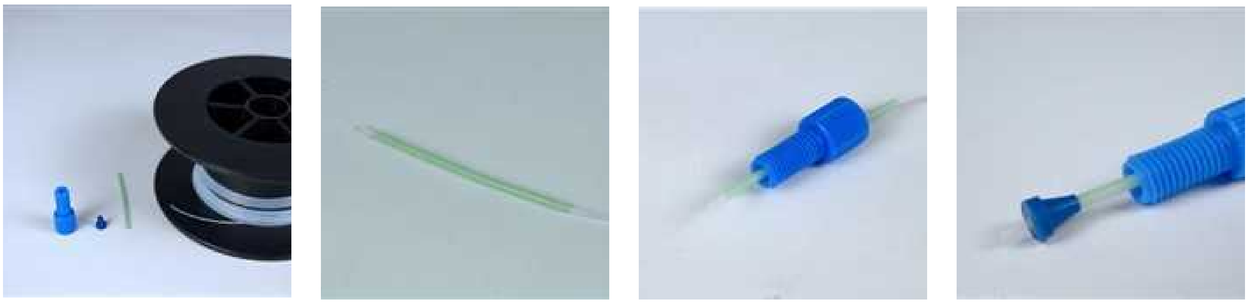


Fig.11 How to add sleeves to 1/32”OD tubing to be compatible with 1/4-28UNF + ferrule connectors fit for 1/16”OD tubing.

Prepare the resistance and connect it to the flow sensor

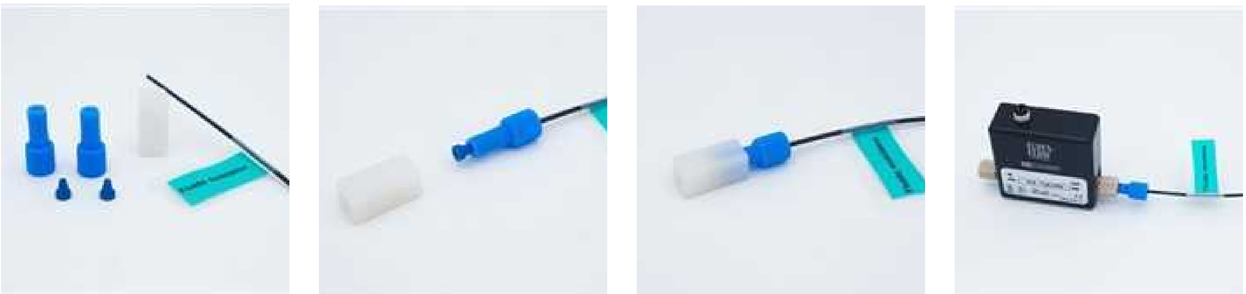


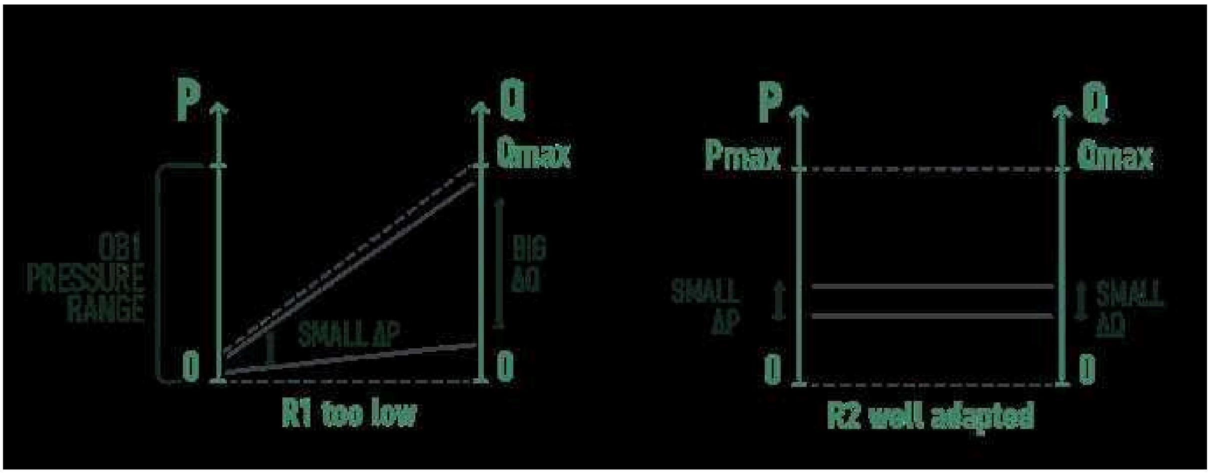
Fig.12 How to add a resistance and how to use an union connector to connect to regular tubing.

TIP

The resistance should always be placed downstream of the MFS (between the MFS and the chip) to get more stable flow rate control.

Why is it important to work with resistances?

Adding the right amount of fluidic resistance in the fluidic path is important as it is the way to finetune the system's whole resistance R to obtain the best performance in terms of flow rate control. It allows the adjustment of R so that the range of accessible ΔP (given by the range of the OB1 channel output) matches the range of the flow rate sensor used. Doing so, the system uses the whole dynamic range of both the sensor and the regulator to control the flow rate. For instance in a system (with a 2 bar OB1 channel) where R=R1 is too low, few mbar of ΔP generate a high flow rate that can saturate the flow rate sensor.



To learn more: https://www.elveflow.com/microfluidic-applications/setup-microfluidic-flow-control/microfluidic-flow-restrictors/

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4. Fill the microfluidic chip

Set the oil channel (here, channel 2) pressure to 100 mbar until the chip is filled. Once the microfluidic chip is filled, decrease the pressure of the oil channel to 50 mbar. You can see that the chip is filled when the oil is going through the outlet after having passed through the chip (a difference of color within the channel of the microfluidic chip can be observed when the oil flows through it).

Then, set the water channel (here, channel 1) pressure to 100 mbar and slowly increase the pressure until you see both phases in the microfluidic chip and droplets are starting to be generated (aqueous phase overcoming the back pressure of the oil phase). You might see air bubbles after having set the water channel pressure, before the water has reached the chip. Wait for the air in the system to be completely replaced by water. Air bubbles tend to be more contrasted than water droplets.

*Refer to the **Troubleshooting section** if this does not work.*

TIP

To reduce the formation of bubbles and shorten the initial filling time, it can be useful to fill the tubing from the reservoir to the chip with liquid before connecting the chip. Set a low mbar command on the OB1 and watch the liquid interface move through the tubing. Set the pressure to zero when the interface reaches the tubing extremity then connect the extremity to the chip.

TIP

Make sure that the outlet reservoir is not totally empty, and that the outlet tubing plunges into the solution, otherwise there will be dripping, which will perturb the stability of the flow rate.

Congratulations, you now control the pressure.

This is enough to make droplets! But if you want to control the flow rate and thus have more precise control over the size and frequency of the droplets, there are a few last things to set up.

Why control the flow rate rather than the pressure?

The characteristics of your droplets are determined by the flow rates of both dispersed and continuous phases. Requesting a flow rate instead of imposing a pressure has mainly two advantages:

- The flow rate stays constant throughout time, even though the experimental conditions slightly change (if a bubble or clogging occurs, the system compensates and maintains a constant flow rate.)

- It compensates for the hydrostatic pressure changes. The inlet pressure of the fluidic system is equal to the atmospheric pressure, plus the pressure imposed by the OB1 on the air above the liquid in the seal reservoir, plus the pressure exerted by the water column (height of liquid between the water/air interface in the reservoir and the tube inlet at the bottom of the reservoir) around 1 mbar per cm of water. So when the liquid level decreases, the ΔP decreases over time so will Q in pressure control. In Flow rate control, the ΔP is adjusted to maintain a constant flow rate.

- Even if your setup changes from one experiment to another, the same flow rates will lead to the same droplets (whereas when changing your setup, the fluidic resistances will change too and the same pressure will lead to a different flow rate).

5. Control of the flow rate

The last step is to set a flow rate feedback loop.

The feedback loop consists of a constant adaptation of the OB1 pressure output to impose a flow rate in the system as close as possible to the flow rate targeted by the user. The feedback loop relies on an algorithm (PI

Basic) which depends on two parameters : P and I. The user can set these parameters to match the requirements in terms of responsiveness and stability.

To have very monodisperse droplets, Elveflow advises to set low values of P and I, which will decrease the responsiveness of the system but guarantee its stability. Elveflow advises you to begin with the following feedback parameter values:

SENSOR TYPE	P	I
MFS-2-D measuring oil flow rate	**0,01**	**0,005**
MFS-2-D measuring water flow rate	**0,01**	**0,005**

These values are appropriate for this setup and for all pairs of water and oil flow rates, and give very stable flow rates.
However, be aware that the responsiveness of the system with these parameters is quite low: up to several minutes may be needed before the flow rates stabilize (especially when working with low flow rates such as 2 µl/min for the water). If you have another setup (especially, if you use different flow resistances) and/or depending on your needs (if you want to increase the responsiveness for instance), you can fine-tune the values of the feedback parameters.

To learn more about the tuning of the P and I parameters, please refer to the flow sensors user guide. ([MFS](MFS) or [BFS](BFS))

To set the feedback parameters, follow these instructions:

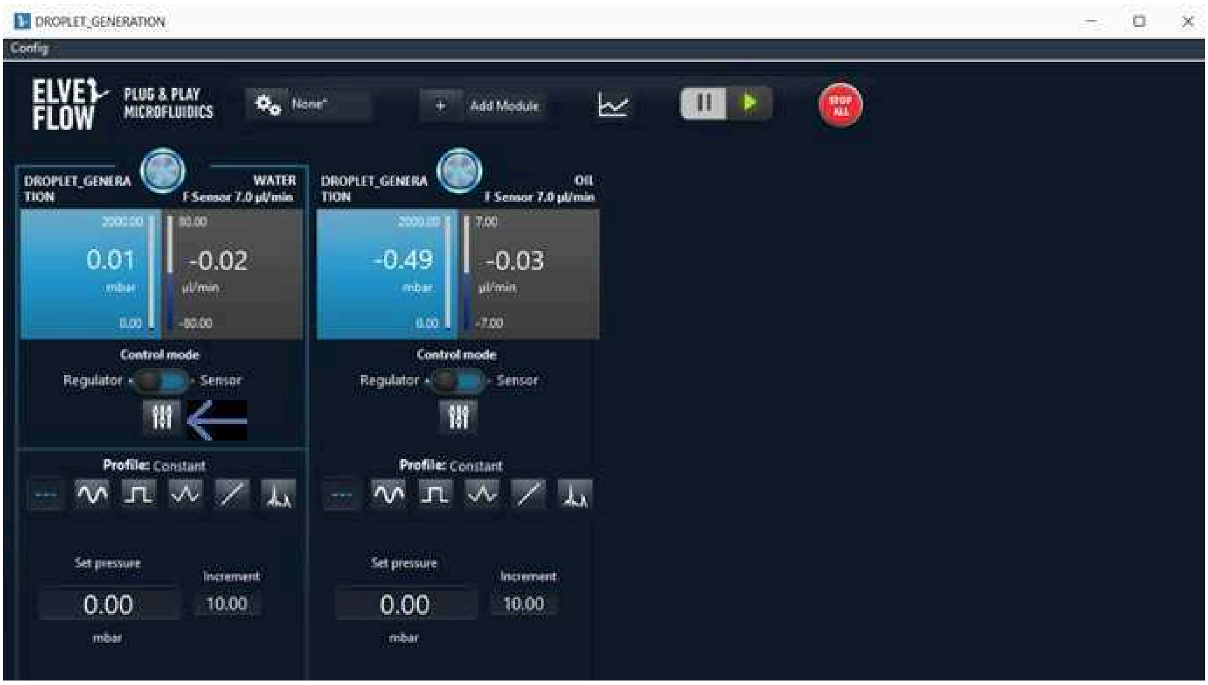


Fig.13 How to set the P & I feedback loop parameters in the ESI, Feedback loop parameters access from the main window, Parameters window

Several algorithms are available. Elveflow advises to use the **"PI Basic" algorithm.**

Now, you can start the feedback loop of flow rate control by **choosing the sensor control mode** (flow rate control instead of pressure control), and setting the flow rate you want to have (you are now controlling the flow rate instead of the pressure) (Figure 8).

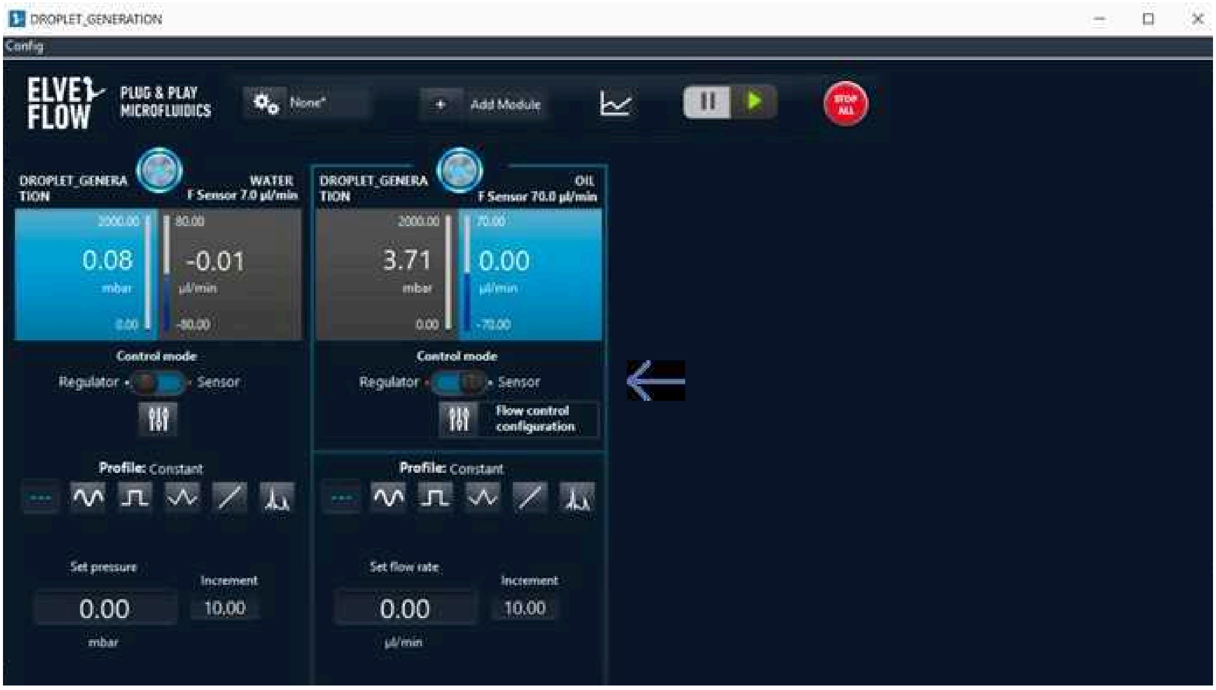


Fig. 14 How to switch from pressure control to flow rate control in the ESI software

For example, you can set the water flow rate to 1 µL/min and the oil flow rate to 50 µL/min to make your first droplets. The dependency of droplet size and frequency on oil and water flow rates is given in the following section.

Don't forget to make sure that the whole fluidic system is filled with liquid before switching to flow rate control mode.

TIP

While the tubing is filled with air, you should not control the flow rate but the pressure. Indeed, the MFS filled with air will not measure any flow rate, and the OB1 will keep increasing the pressure until the fluid finally reaches the MFS or the pressure reaches its maximum, which should be avoided. Once the fluid has reached the MFS, it is safe to switch to flow rate control.

TIP

Increasing the value of the parameter I in the PI Basic algorithm while controlling the flow rate ("sensor" mode) will induce an overflow. If you want to prevent this overflow, only change the values of the parameter I while controlling the pressure ("Regulator" mode) then switch back to flow rate control.

6. Characterisation of the droplets

Generally, increasing the oil flow rate will result in increasing the frequency of production of the droplets. Droplet size and production frequency can be fine tuned depending on the flow rates of the dispersed and continuous phases and the size of nozzle used, as shown in the tables below.

Fluidic 440 _ Droplet Generator Chips _ Multi Channel Design

Design # 1 and # 2: 80 µm nozzle (CV < 2%)

		OIL (µL/min)										
		50	52	54	56	58	60	62	64	66	68	70
WATER (µL/min)	0.4	83 µm (13 Hz)	83 µm (14 Hz)	83 µm (14 Hz)	82 µm (15 Hz)	82 µm (15 Hz)	82 µm (16 Hz)	81 µm (18 Hz)	80 µm (19 Hz)	80 µm (19 Hz)	79 µm (20 Hz)	79 µm (20 Hz)
	0.6	> 80 µm	83 µm (26 Hz)	82 µm (26 Hz)	82 µm (29 Hz)	82 µm (30 Hz)	81 µm (31 Hz)	81 µm (32 Hz)	81 µm (34 Hz)	81 µm (34 Hz)	80 µm (36 Hz)	80 µm (36 Hz)
	0.8	> 80 µm	> 80 µm	> 80 µm	> 80 µm	83 µm (45 Hz)	83 µm (45 Hz)	82 µm (46 Hz)	82 µm (49 Hz)	82 µm (49 Hz)	81 µm (51 Hz)	81 µm (51 Hz)
	1	> 80 µm	> 80 µm	> 80 µm	> 80 µm	> 80 µm	83 µm (47 Hz)	83 µm (49 Hz)	83 µm (52 Hz)	83 µm (54 Hz)	82 µm (57 Hz)	82 µm (57 Hz)

Design # 3 and # 4: 70 µm nozzle (CV < 2%)

		OIL (µL/min)									
		52	**54**	**56**	**58**	**60**	**62**	**64**	**66**	**68**	**70**
WATER (µL/min)	**0.6**	73 µm (43 Hz)	72 µm (45 Hz)	71 µm 47 Hz)	70 µm (48 Hz)	70 µm (48 Hz)	70 µm (50 Hz)	69 µm (51 Hz)	69 µm (51 Hz)	69 µm (52 Hz)	68 µm (53 Hz)
	0.8	> 70 µm	> 70 µm	73 µm (56 Hz)	72 µm (58 Hz)	71 µm (59 Hz)	71 µm (61 Hz)	70 µm (62 Hz)	70 µm (63 Hz)	69 µm (64 Hz)	69 µm (67 Hz)
	1	> 70 µm	> 70 µm	> 70 µm	73 µm (68 Hz)	72 µm (69 Hz)	72 µm (71 Hz)	71 µm (72 Hz)	70 µm (74 Hz)	70 µm (76 Hz)	70 µm (77 Hz)
	1.2	> 70 µm	> 70 µm	> 70 µm	73 µm (79 Hz)	72 µm (81 Hz)	72 µm (83 Hz)	71 µm (85 Hz)	71 µm (88 Hz)	71 µm (90 Hz)	70 µm (93 Hz)
	1.4	> 70 µm	> 70 µm	> 70 µm	73 µm (79 Hz)	73 µm (95 Hz)	72 µm (81 Hz)	72 µm (101 Hz)	72 µm (101 Hz)	71 µm (104 Hz)	71 µm (107 Hz)
	1.6	> 70 µm	> 70 µm	> 70 µm	> 70 µm	73 µm (107 Hz)	73 µm (111 Hz)	72 µm (115 Hz)	72 µm (119 Hz)	72 µm (123 Hz)	71 µm (128 Hz)
	1.8	> 70 µm	> 70 µm	> 70 µm	> 70 µm	73 µm (123 Hz)	73 µm (49 Hz)	73 µm (52 Hz)	72 µm (54 Hz)	72 µm (57 Hz)	71 µm (145 Hz)

Design # 5 and # 6: 60 µm nozzle (CV < 2%)

		OIL (µL/min)										
		50	**52**	**54**	**56**	**58**	**60**	**62**	**64**	**66**	**68**	**70**
WATER (µL/min)	**0.5**	62 µm (69 Hz)	61 µm (71 Hz)	61 µm (72 Hz)	60 µm (74 Hz)	59 µm (83 Hz)	57 µm (85 Hz)	58 µm (88 Hz)	58 µm 90 Hz)	57 µm (95 Hz)	57 µm (98 Hz)	56 µm (101 Hz)
	1	> 60 µm	62 µm (95 Hz)	62 µm (98 Hz)	61 µm (101 Hz)	61 µm (104 Hz)	60 µm (107 Hz)	60 µm (111 Hz)	59 µm (115 Hz)	59 µm (119 Hz)	58 µm (123 Hz)	57 µm (128 Hz)
	1.5	> 60 µm	> 60 µm	62 µm (133 Hz)	61 µm (139 Hz)	61 µm (145 Hz)	60 µm (151 Hz)	60 µm (159 Hz)	59 µm (167 Hz)	58 µm (175 Hz)	58 µm (185 Hz)	58 µm (196 Hz)
	2	> 60 µm	> 60 µm	> 60 µm	62 µm (151 Hz)	62 µm (159 Hz)	61 µm (167 Hz)	60 µm (175 Hz)	60 µm (185 Hz)	59 µm (196 Hz)	59 µm (208 Hz)	59 µm (222 Hz)
	2.5	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 µm (167 Hz)	62 µm (175 Hz)	61 µm (185 Hz)	61 µm (196 Hz)	60 µm (222 Hz)	60 µm (238 Hz)	59 µm (256 Hz)
	3	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 µm (208 Hz)	62 µm (217 Hz)	61 µm (238 Hz)	61 µm (250 Hz)	60 µm (263 Hz)	60 µ (278 Hz)
	3.5	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 µm (250 Hz)	61 µm (263 Hz)	61 µm (278 Hz)	60 µm (294 Hz)	60 µm (312 Hz)
	4	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 µm (278 Hz)	61 µm (294 Hz)	60 µm (312 Hz)	60 µm (333 Hz)

Design # 7 and # 8: 50 µm nozzle (CV < 2%)

WATER (µL/min)	OIL (µL/min)										
	50	**52**	**54**	**56**	**58**	**60**	**62**	**64**	**66**	**68**	**70**
0.6	> 50 µm)	> 50 µm	> 50 µm	> 50 µm	50 µm (75 Hz)	49 µm (77 Hz)	49 µm (79 Hz)	48 µm (80 Hz)	48 µm (82 Hz)	47 µm (84 Hz)	47 µm (85 Hz)
1	52 µm (81 Hz)	51 µm (82 Hz)	51 µm (85 Hz)	50 µm (86 Hz)	50 µm (87 Hz)	49 µm (90 Hz)	49 µm (92 Hz)	49 µm (93 Hz)	48 µm (97 Hz)	48 µm (98 Hz)	47 µm (102 Hz)
1.4	52 µm (93 Hz)	52 µm (95 Hz)	51 µm (98 Hz)	51 µm (100 Hz)	50 µm (102 Hz)	50 µm (106 Hz)	49 µm (108 Hz)	49 µm (112 Hz)	49 µm (117 Hz)	48 µm (120 Hz)	48 µm (122 Hz)
1.8	> 50 µm	52 µm (108 Hz)	52 µm (110 Hz)	51 µm (115 Hz)	51 µm (117 Hz)	50 µm (120 Hz)	50 µm (122 Hz)	49 µm (128 Hz)	49 µm (131 Hz)	48 µm (134 Hz)	48 µm (138 Hz)
2.2	> 50 µm	> 50 µm	52 µm (125 Hz)	52 µm (128 Hz)	51 µm (131 Hz)	51 µm (138 Hz)	50 µm (141 Hz)	50 µm (149 Hz)	49 µm (153 Hz)	49 µm (157 Hz)	48 µm (162 Hz)
2.6	> 50 µm	> 50 µm	> 50 µm	52 µm (145 Hz)	51 µm (153 Hz)	51 µm (157 Hz)	50 µm (162 Hz)	50 µm (167 Hz)	49 µm (172 Hz)	49 µm (184 Hz)	49 µm (190 Hz)
3	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (178 Hz)	51 µm (184 Hz)	51 µm (190 Hz)	50 µm (197 Hz)	50 µm (204 Hz)	49 µm (212 Hz)	49 µm (220 Hz)
3.4	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm	51 µm	51 µm	50 µm	50 µm	49 µm

							(197 Hz)	(204 Hz)	(212 Hz)	(220 Hz)	(230 Hz)	(240 Hz)
	3.8	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (220 Hz)	51 µm (230 Hz)	51 µm (240 Hz)	50 µm (251 Hz)	50 µm (262 Hz)
	4.2	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (251 Hz)	51 µm (262 Hz)	51 µm (276 Hz)	50 µm (290 Hz)
	4.6	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (290 Hz)	51 µm (306 Hz)	51 µm (324 Hz)
	5	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (344 Hz)	51 µm (367 Hz)

Fluidic 947 _ Droplet Generator Chips _ Multi Channel Design

Design # 7 and # 8: 30 µm nozzle (CV < 1.5 %)

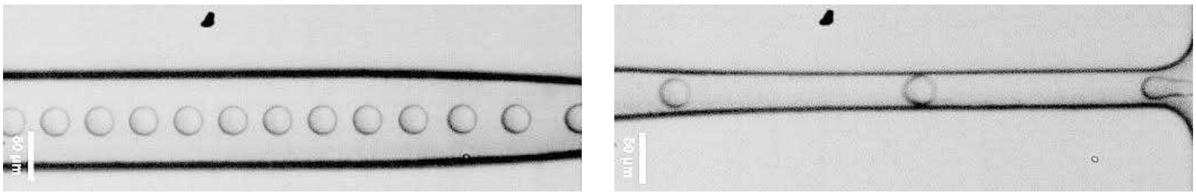


Fig.15 Droplet generation in design Fluidic 947 nozzle 30µm

		OIL (µL/min)					
		50	**54**	**60**	**63**	**67**	**70**
WATER (µL/min)	**0.5**	30 µm (109 Hz)	< 30 µm	< 30 µm	< 30 µm	< 30 µm	< 30 µm
	1	< 30 µm	30 µm (163 Hz)	< 30 µm	< 30 µm	< 30 µm	< 30 µm
	1.5	< 30 µm	< 30 µm	30 µm (235 Hz)	< 30 µm	< 30 µm	< 30 µm
	2	< 30 µm	< 30 µm	< 30 µm	30 µm (294 Hz)	< 30 µm	< 30 µm
	2.5	< 30 µm	< 30 µm	< 30 µm	< 30 µm	30 µm (385 Hz)	< 30 µm
	3	< 30 µm	< 30 µm	< 30 µm	< 30 µm	< 30 µm	30 µm (417 Hz)

Design # 5 and # 6: 20 µm nozzle (CV < 1.5 %)

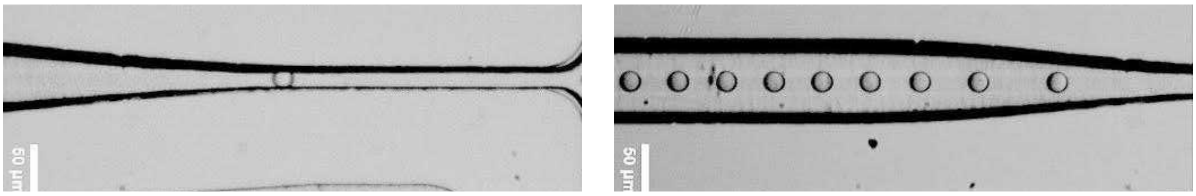


Fig.16 Droplet generation in design Fluidic 947 nozzle 20µm

		OIL (µL/min)			
		60	**64**	**67**	**70**
WATER (µL/min)	**0.5**	20 µm (385 Hz)	> 20 µm	> 20 µm	> 20 µm
	1	> 20 µm	20 µm (426 Hz)	> 20 µm	> 20 µm
	1.5	> 20 µm	> 20 µm	20 µm (465 Hz)	> 20 µm
	2	> 20 µm	> 20 µm	> 20 µm	20 µm (512 Hz)

Design # 3 and # 4: 15 µm nozzle (CV < 2 %)

		OIL (µL/min)	
		60	**70**
WATER (µL/min)	**0.5**	14.5 µm (590 Hz)	polydisperse
	0.8	polydisperse	14.9 µm (752 Hz)

Design # 1 and # 2: 10 µm nozzle (CV < 3%)

		OIL (µL/min)	
		5060	70
WATER (µL/min)	0.5	9.5 µm (910 Hz)	polydisperse
	1	polydisperse	9.7 µm (1270 Hz)

7. After generating your droplets

Once you are done making your droplets, we recommend that you clean your device to be able to reuse it later. The cleaning protocol may be different and adapted to the solution used to make your droplet but we advise to use IPA then to dry the device pushing air.

Make sure to empty the tubings and to properly clean the flow rate sensors according to the cleaning procedures described in the Flow Rate Sensor user guides and in the support section of the Elveflow website.

Switch off the OB1 pressure controller, remove the tubing from the OB1 channel and put back the plugs. Also you can stop pressurizing your OB1.

TROUBLESHOOTING

The flow rate measured by my MFS suddenly drops down for a short duration

The reason is probably that you have air bubbles in your system. When an air bubble passes through the MFS, the sensor measures a zero flow rate while the bubble hasn't left. To get rid of air bubbles, just wait for it to get out by maintaining a constant pressure or flow rate in both channels. The initial filling of the system is critical to avoid bubbles.

The flow rate doesn't stabilize

If the flow rate can't stabilize itself on the fixed value, first check that you haven't forgotten to put the fluidic resistance in the fluidic system.
If it's not the cause of the problem, you should consider changing the values of P and I.
The lower the values of P, the more stable the flow rate will be. The **"going further"** section details this solution.

Another way of solving this problem is to increase the microfluidic resistance. The **"going further"** section details this solution.

I see only one phase (water or oil) in my chip

Check both your MFS. The MFS should be connected in the right direction (indication of inlet/outlet are depicted on top of the sensor).
If both MFS are connected in the right direction and are measuring a positive flow rate, and if the situation is lasting too long, check all your connections, there must be a leak.
If one of the values measured by the MFS is negative:

- if you are controlling the pressure, increase the pressure of the channel flowing in the wrong direction

- if you are controlling the flow rate, the flow rate should gradually increase to the fixed value. If it takes too much time, you can open the flow control configuration of the channel flowing in the wrong way, and gradually increase the value of I in the PI Basic algorithm (refer to the control of the flow rate section)

I have dust in my chip/my chip is clogged

In case your microfluidic chip starts to clog (dust, particles, etc.), try to increase the pressure (or flow rate) of both phases to expel the dust from the chip. If it doesn't work, you should change the device you are working with. The presence of dust in the chip should not be ignored. The monodispersity of the droplets can't be ensured in this case, and the size of the droplets could significantly change from the expectations of the user. Working with "clean" solutions and reservoirs is essential to prevent chip clogging. We highly advise to filter solutions before using them in a microfluidic chip.

My OB1 is very noisy

If your OB1 starts making loud noise, it's probably because of a leak in your system: the pressure source tries to permanently compensate for the lack of pressure due to the leak. Check all the connections of the system (using teflon tape is often useful to avoid leaks on fluidic and pneumatic connections).

This PDF is dedicated to the problem

I don't have the same results as those provided in the diagram

If your droplets don't have the size you expected, the reason could be a light systematic error of the MFS or the chip dimensions. You should consider calibrating the MFS once again or adjusting the flow rate values to get the target droplet size.

Be careful when controlling very low oil flow rate (close to 5 µl/min) with the MFS-2-D, even though the measurements of the MFS are highly repeatable, they could lack accuracy in this range of flow rates.

Refer to the MFS user guide for more details

I would like to produce droplets with a lower frequency, is it possible with this chip?

It is possible, but you will have to reach lower flow rates than the one presented here. Doing so you reach the limit of the flow sensors used here and so the flow rate control will be less precise and stable.

My aqueous phase sticks to the wall downstream of the generation region

As the hydrophobicity from the surface can sometimes wear off during the experiment, the aqueous phase can create a fluid path and prevent droplet generation. If that happens, it is best to reduce the water flow rate so that the aqueous phase recedes to a position upstream of the droplet generation region. From there, slowly increase the flow rate to the desired value again to continue producing droplets.

TIP

If water is attaching to the walls of your channel, treat the microfluidic chip with a hydrophobic treatment, (Aquapel from Autoserv, or Rain X (ITW Glodal Brands). Treatment should preferably be done inside a fume hood to prevent dust, particles or fibers from entering in the microfluidic chip. The chip must be dry while inserting the liquid, left to rest 3-5 min minutes, and rinsed with water or oil. We recommend repeating the process 3 times. After rinsing it is important to dry the chip by air injection (unless you are using Aquapel).

I would like to make oil in water droplets, can I do it just by switching the water and the oil inlet?

To generate these droplets, a hydrophilic chip should be used (e.g a glass chip).

Please feel free to reach out to our technical sales team **contact@elveflow.com** for more information about it.

GOING FURTHER

The droplet starter kit is very flexible. Depending on your needs, you could find it beneficial to change elements of the setup. For example, working with another chip, fluidic resistances, or even other liquids. You must be aware that, even though it is not hard to adapt your setup to new experimental conditions, there always are a few elements to adjust.

This section is here to introduce further upgrades and improvements to the system to perfectly tailor it to your specific experiment!

TIP

How to make other droplets than those given in the diagram:

Diagrams show you the monodisperse droplets you can produce with the microfluidic chips supplied in the droplet starter kit. If your application requires droplets with other characteristics (e.g. size, throughput) than the one provided in this kit, other commercial microfluidic chips are available. If you want to make oil in water droplets, you will need a chip made of hydrophilic material (such as glass or with appropriate treatment).

Calibrate the flow rate sensor if you are using a different liquid

If you plan to use an alternative oil you will have to calibrate the flow rate sensor, following the calibration procedure.

Adapt the fluidic system

By changing your microfluidic setup (e.g. microfluidic chips, liquid viscosity), the total fluidic resistance of the setup will be different. Therefore, an adaptation of the fluidic resistance and potentially the flow sensor is needed to allow the range of the pressure controller to match the range of the flow rate used.

To learn more about resistances

Change the values of P and I in the PI Basic algorithm

Whenever your setup changes (new liquids, new chip…), you will have to check that your feedback parameters P and I are still appropriate. Even if you keep the same setup, you could find a benefit in changing the values of P and I. If you want to make droplets of a specific size and you know the flow rates you need, you can gradually change the values of P and I until you find more optimized ones for these flow rates (more responsiveness or more stability).

As it can be seen on the diagrams in the "*Characterisation of the droplets*" part, the parameters of the oil-to-water flow rate ratios and throughput to generate droplets between 10 and 15 µm are not optimal using the Fluidic 947 microfluidic chip. You get polydisperse droplets and the generation can not be stabilized. A simple change of configuration within the microfluidic setup can be done to improve those actions, by a change of the flow sensor and therefore a change of the fluidic resistance. By changing the MFS2 for a MFS3 flow sensor for the oil phase, a higher flow range of up to 500 µL/min can be achieved and therefore, a higher versatility of oil-to-water flow rate ratios can be reached.

Alternative set-up

From the start we have explained that this kit is quite versatile and that it can be adapted depending on each experiment. To show you how, we also did more tests working with a flow sensor MFS-3 for the oil phase.

1 Flow rate sensor #2 MFS-2-D
1 Flow rate sensor #3 MFS-3-D

2 fluidic resistances

As the flow sensors are different, the fluidic resistance has to be adjusted too. If you opt for this solution, please use the following resistances:

- **22-H for the dispersed phase**. (60cm - 100µm inner diameter peek tubing) It enables stable control of flow rates ranging from 0,42 µL/min to 7 µL/min using water or liquids with water-like viscosity (close to 1 mPa.s).

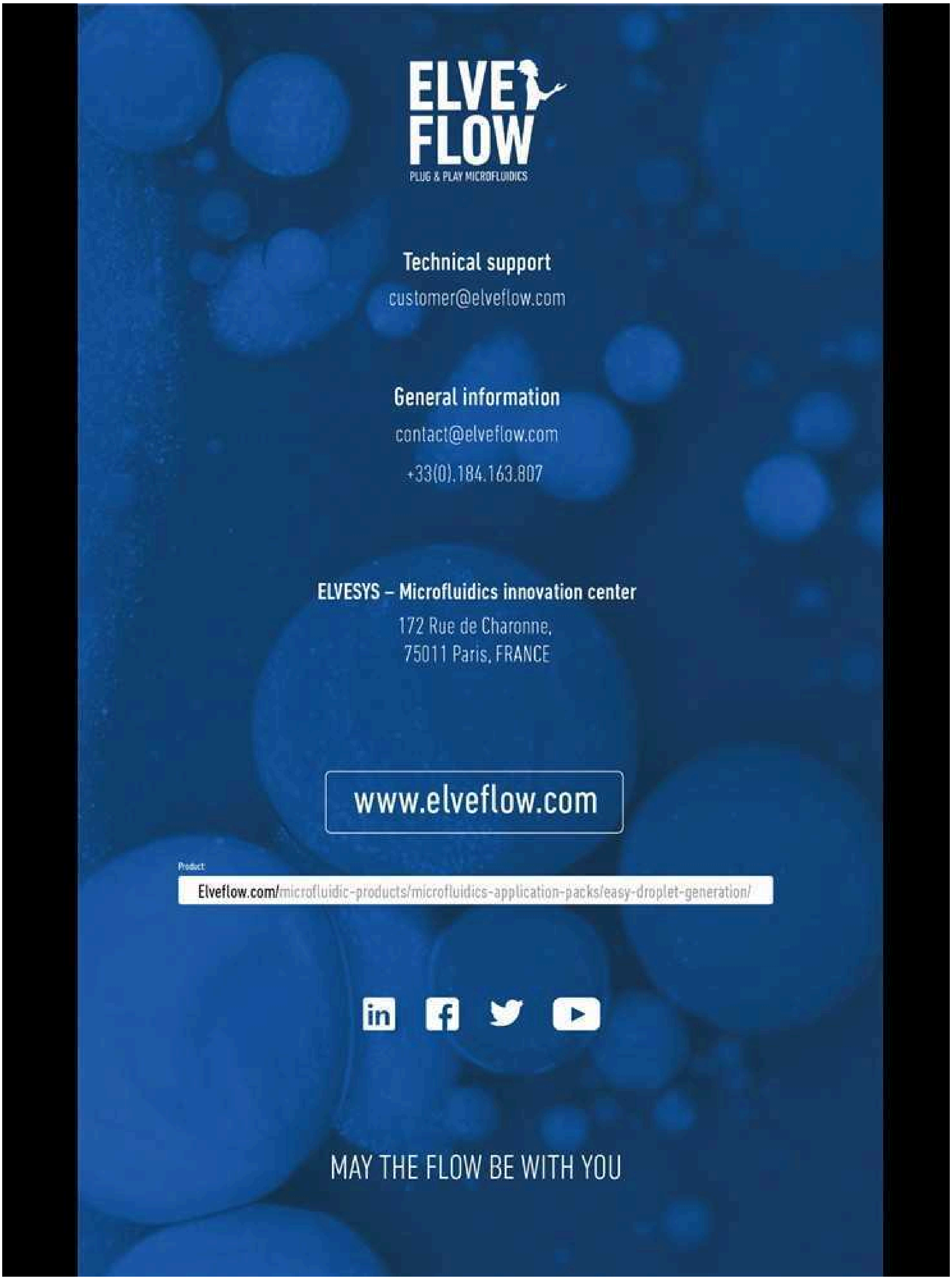
- **23-I for the continuous phase**. (41cm - 175µm inner diameter peek tubing) It enables stable control of flow rates ranging from 25 µL/min to 500 µL/min for the case of oil (HFE-7500).

FLUIDIC 947 _ Droplet Generator Chips: 10 µm nozzle (CV < 3%)

		OIL (µL/min)				
		65	70	80	85	90
WATER (µL/min)	0.5	10 µm (910 Hz)	< 10 µm	< 10 µm	< 10 µm	< 10 µm
	1	< 10 µm	10 µm (1270 Hz)	< 10 µm	< 10 µm	< 10 µm
	1.5	< 10 µm	< 10 µm	10 µm (1830 Hz)	< 10 µm	< 10 µm
	2	< 10 µm	< 10 µm	< 10 µm	10 µm (2120 Hz)	< 10 µm
	2,5	< 10 µm	< 10 µm	< 10 µm	< 10 µm	10 µm (2520 Hz)

FLUIDIC 947 _ Droplet Generator Chips: 15 µm nozzle (CV < 2%)

		OIL (µL/min)			
		60	70	90	105
WATER (µL/min)	0.5	15 µm (590 Hz)	< 15 µm	< 15 µm	< 15 µm
	1	< 15 µm	15 µm (830 Hz)	< 15 µm	< 15 µm
	1.5	< 15 µm	< 15 µm	15 µm (1310 Hz)	< 15 µm
	2	< 15 µm	< 15 µm	< 15 µm	15 µm (1970 Hz)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