FLUIGENT O-DE-STD-PCK Double Emulsion Production Station Instructions

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OBJECTIVE OF THE PROTOCOL

The following document presents all the basic steps to follow to start and stop your experiments cleanly with the RayDrop.

MATERIALS

To realize this protocol you need the following material:

Product	Reference	Content description
Double emulsion production station	O-DE-STD-PCK	3 *FlowEZ 2 bar
		1 *Link
		1 *Pcap 15 ml
		2 *Pcap 50ml
		2 *Flow unit M
		1 *Flow unit L
		2*2-switch
		Switch EZ
		Raydrop double emulsion
		Flow EZ supply kit
		2 *Pcap kit 50ml
		1 *Pcap kit 15ml
		2 *Low flow rate kit
		High flow rate kit
		2*2-switch kit
		Raydrop double emulsion connector and tubing kit
Optional product	IMCA001	Digital high speed microscope
	DR-RE-SU-12	12mL dSURF
	FLPG+	Pressure source

Setup description

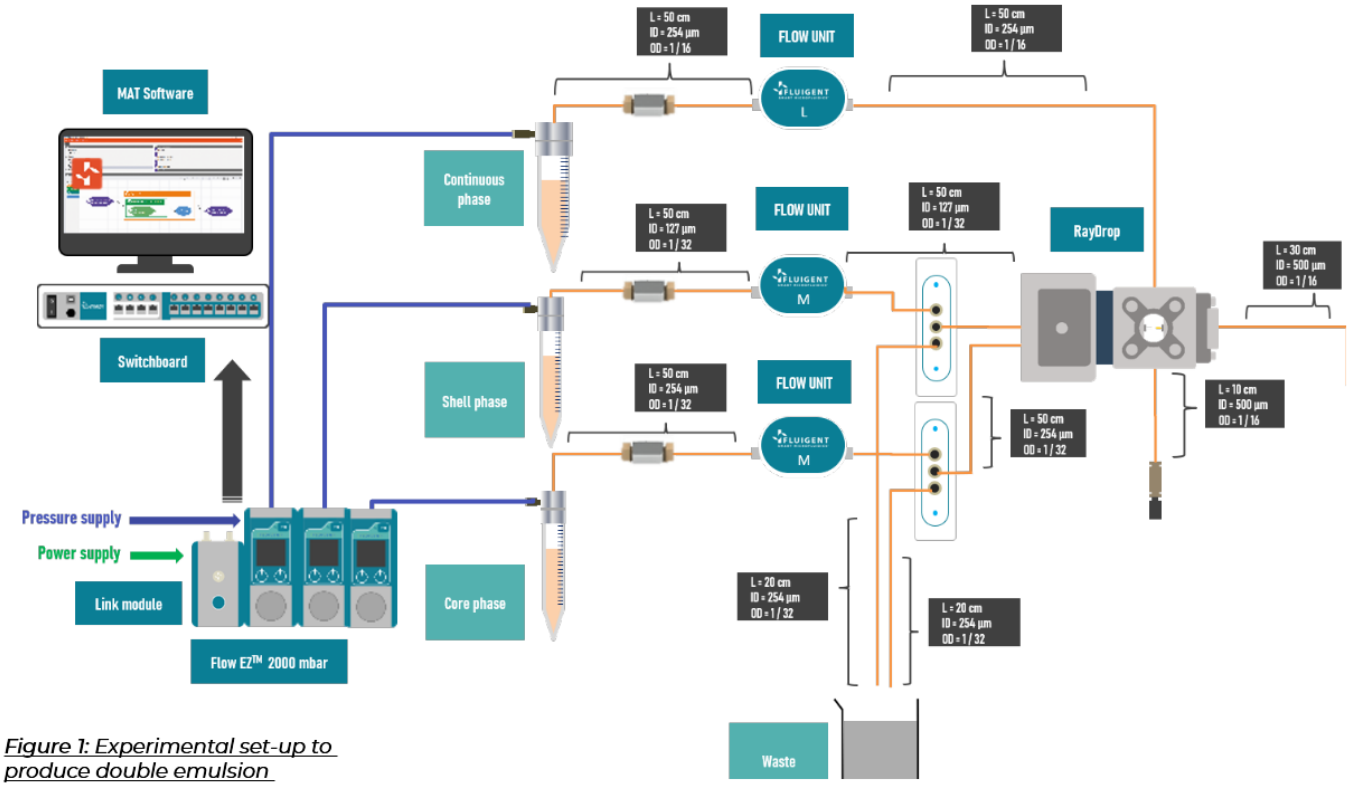


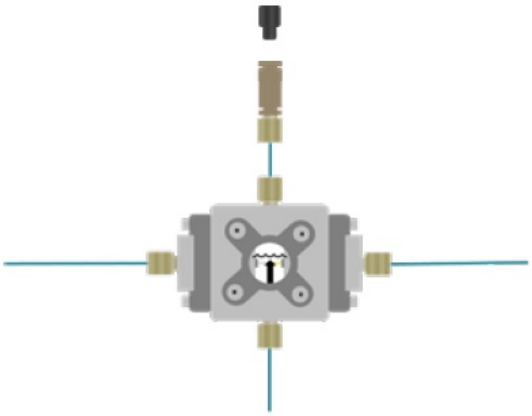
Figure 1: Experimental set-up to produce double emulsion

The inline filter minimizes the clogging of the double nozzle which is of the utmost important for the Shell channel. The length and ID diameter of the tubing proposed here are meant for water in dSurf in ware double emulsion. They have to be adapted to the fluid in use in order to have a pressure drop that is compatible with your pressure controller and that the pressure drop on the continuous phase is superior to the pressure drop on the shell phase which is superior to the pressure drop of the core phase.

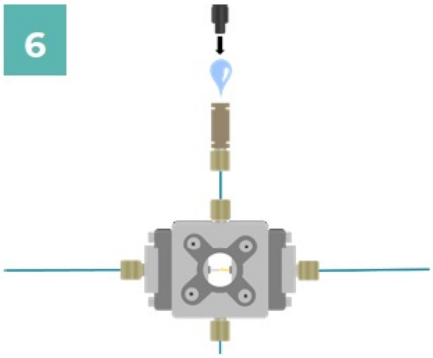
PROTOCOL

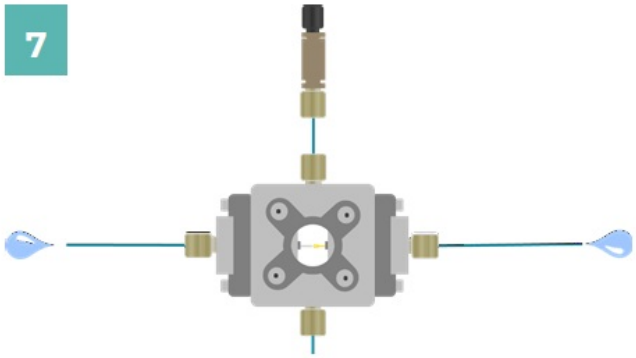
Priming the system

1. Filter the three solutions with a 0.2µm or 0.45µm syringe filter and degas them using, for example, an ultrasonic bath for 30 min
2. Fill in the different reservoirs with the filtered solutions (Continuous, Shell and Core phase)
3. Set both valves on inlet 2, corresponding to the waste, and place both waste tubing in the waste collector
4. Before connecting the Shell phase and Continuous phase to the 3-way valves, the user must flush the capillaries between the reservoir and the valve with the corresponding liquid. After a couple of minutes of flushing, the user can connect the tubing to the inlet 1 of each 3-way valves. After that the user can set the pressure at 0 mBar for the Shell phase and the Core phase.
5. Place the Raydrop double emulsion vertically in the Raydrop holder with the purge at the top. Open the purge and set a pressure of 1 Bar for the continuous flow rate.



6. Once the Raydrop double emulsion is filled, close the purge and wait for the continuous phase to exit both waste capillaries that are connected to the valves.
7. Once the liquid exits the waste capillaries, close them off and reduce the continuous pressure to 200 mBar



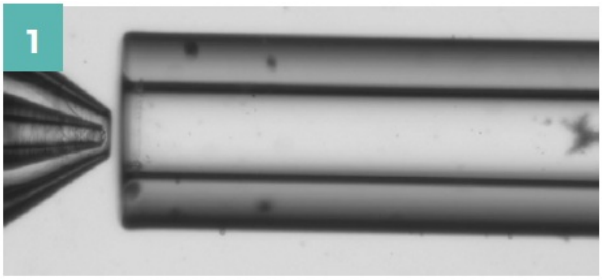


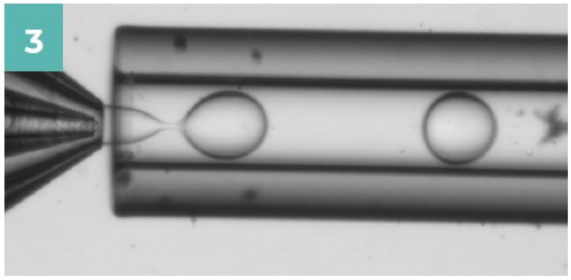
8. Place the Raydrop under your microscope. You should see a clean double nozzle fill with the continuous phase.

Double emulsion production

Shell droplet generation

1. Set the pressure for the continuous flow rate to get 250 µl/min.
2. Set the pressure of the Shell phase at 1.5 bar of pressure and switch the Shell valve to position 1; the flowrate of the shell phase should be around 30 µl/min.
3. After a few minutes, the shell phase should fill in the nozzle and oil droplet should be formed at the exit of the nozzle.
4. The goal to use a very high flowrate is to purge all the remaining continuous phase inside the nozzle. However, if a bubble or droplet is still trap inside the shell part of the double nozzle, increase the Shell flow rate to get rid of it. Sometimes you must do cycles of increased/decreased flow rate of the shell phase. But be careful to not use a too high flow rate that will result in oil going inside the chamber.
5. Once there is shell phase inside the whole shell channel, decrease the shell phase pressure to around 900 mBar in order to get 15 µl/min of shell phase.



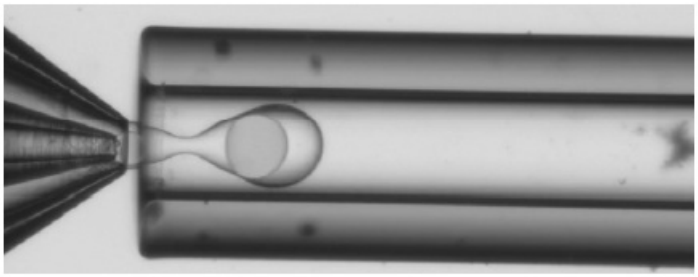


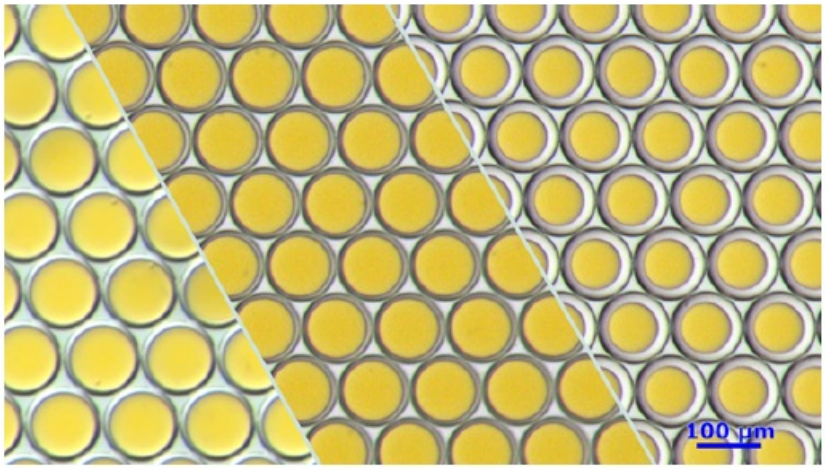
Core-Shell droplet generation

1. 1. Set the pressure of the Core phase at 250 mBar and set the Core valve to position 1. The core phase flow rate should be around 7µl/min

Getting the desired double emulsion sizes

1. The continuous flow rate variation will lead to variation of the external diameter of the double emulsion. Increasing the continuous flow rate will decrease the double emulsion external diameter while decreasing the continuous flow rate will increase its external diameter.
2. The size of the core can be adjusted by varying both shell phase flow rate and core phase flow rate.





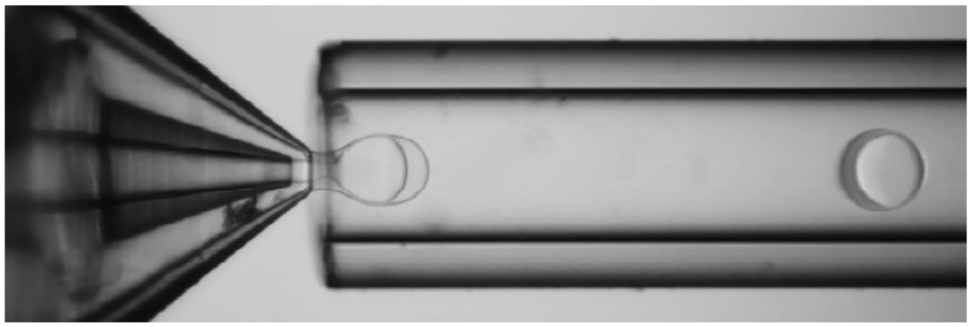
Stop properly an experiment

To stop an experiment, follow these steps

1. Switch the Core valve to position 2 and set the pressure for the core phase to 0 mBar
2. Switch the Shell valve to position 2 and set the pressure for the shell phase to 0 mBar
3. Open the two waste capillaries and let flow the continuous phase for 30” to avoid the core phase or the shell phase to pollute the chamber.

4. Close the waste capillaries
5. Slowly decrease the pressure for the continuous flow rate until reaching 0 mBar.

How to clean the Raydrop properly



Removing particles stuck in the nozzle
When dealing with microfluidics experiment, it is common to have a small particle that leads its way into the fluids despite best efforts to use filtered solution. If these particles are large enough, they will be stucked in the nozzle more often at the end of the nozzleas you can see in the picture below. However, in most case it will not prevent the formation of double emulsion. Several procedures exist to remove such particles from the nozzle is quite easy and most of the time successful.

Soft cleaning
Let us consider the case where particles, like fiber or dust, are stuck in the shell phase.

1. Switch both valves to position 2.
2. Place a plug on the exit of raydrop (cf double emulsion kit)
3. Open the waste capillary for the shell phase and place it in the waste container
4. Ramp up the continuous flow rate until the particle detached
5. Let the flow running for a couple of minutes to be sure that the particle has been removed from the system
6. Reduce the continuous phase flow rate to zero
7. Close the waste capillary of the shell phase
8. Remove the plug on the outlet and place back your original collection capillary
9. You are ready to start again your process

Medium cleaning
If you have trouble to remove your particles or if they come back, here is a few tips to increase the cleaning success.

1. You can directly disconnect the shell capillary from the raydrop after step 4. The pressure drop will decrease and therefore the flowrate will increase.
2. After the particle has been removed, wait a minute before continuing
3. Before reconnecting the shell capillary drop a few droplets of acetone from a wash bottle on top of the upchurch connection; it should prevent any remaining dust to reenter the raydrop. Continue at step 6 of the soft cleaning procedure

Medium cleaning+
If the particles are still stuck, the next procedure should help.

1. Disconnect all the capillary of the raydrop except the purge and place it in the Raydrop holder with the purge capillary at the top.
2. Open the purge and let the chamber of the raydrop purge
3. Close the purge of the raydrop
4. Fill the chamber of the raydrop with isopropanol, but be sure that this solvent is compatible with your solvent. To ease the process, you can use a 10ml plastic syringe with a setup like in the picture below.
5. Close the output and the core inlet of the raydorp with a plug (IDEX p-309)
6. Apply a pressure on the syringe to remove the particle
7. Once the particle is gone, continue to apply the pressure for 30”
8. Disconnect the syringe tubing
9. Purge the raydrop by opening the purge capillary

To start again your experiment, and depending on your continuous phase, you can either dry completely the raydrop by applying a small gas flow rate inside the chamber or start again the priming of the system with you continuous flow rate. You simply need to flush the chamber with your continuous phase for a few minutes to remove any remains of isopropanol.

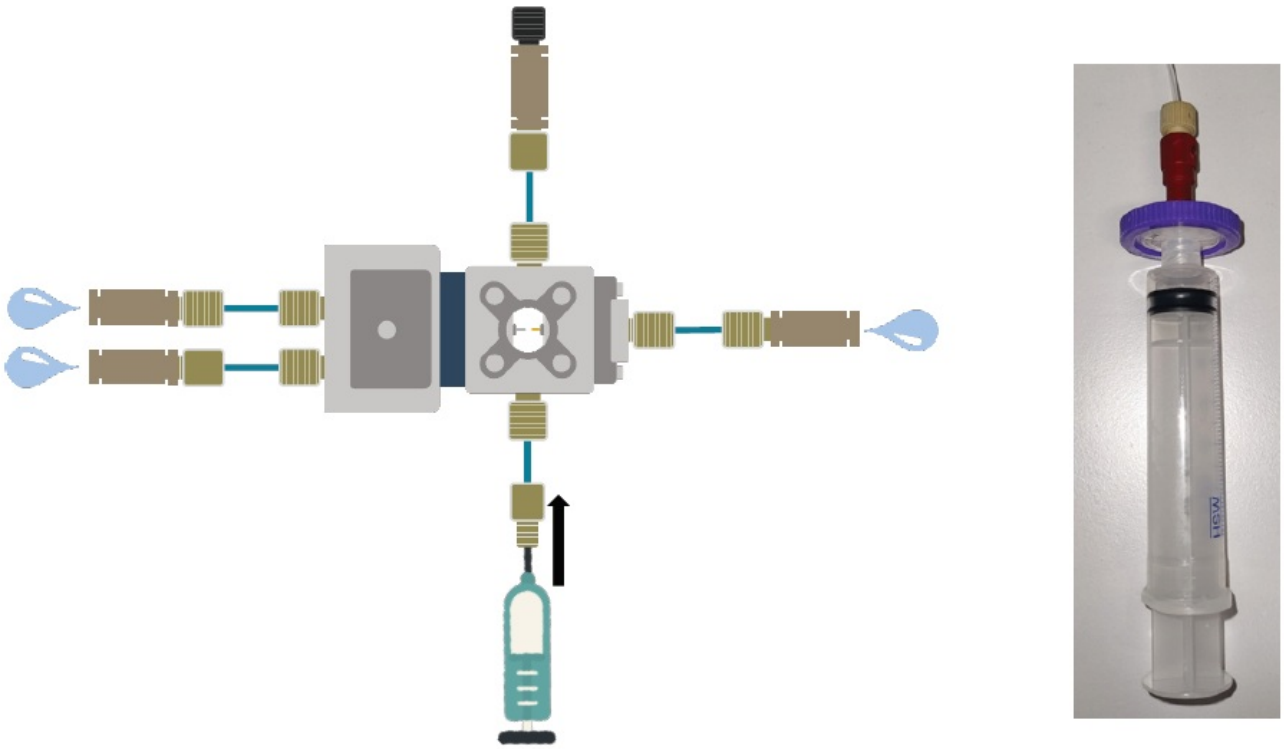


Figure 2: 10 ml syringe with 0.2µm syringe filter with p-658and capillary with ID > 0.5mm; the syringe filter has to be forced a bit inside the p-658 to maintain sealing

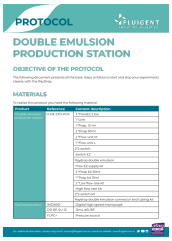
Strong cleaning

If after step 6 of the medium cleaning+, the particles do not go away, you can use an ultrasonic bath to enhance the cleaning.

1. After step 6, place the Raydrop in the basket of an ultrasonic bath
2. While applying a pressure on the liquid syringe start the ultrasonic bath
3. Continue this cleaning procedure for 30”
4. Stop the ultrasonic bath and remove the raydrop from it

5. Check if the particles are gone
6. If not, you can start again step 1-2-3 with a higher pressure on the syringe
7. But in most case, it should be sufficient

Documents / Resources

	FLUIGENT O-DE-STD-PCK Double Emulsion Production Station [pdf] Instructions O-DE-STD-PCK, Double Emulsion Production Station, Emulsion Production Station, O-DE-STD-PCK, Production Station