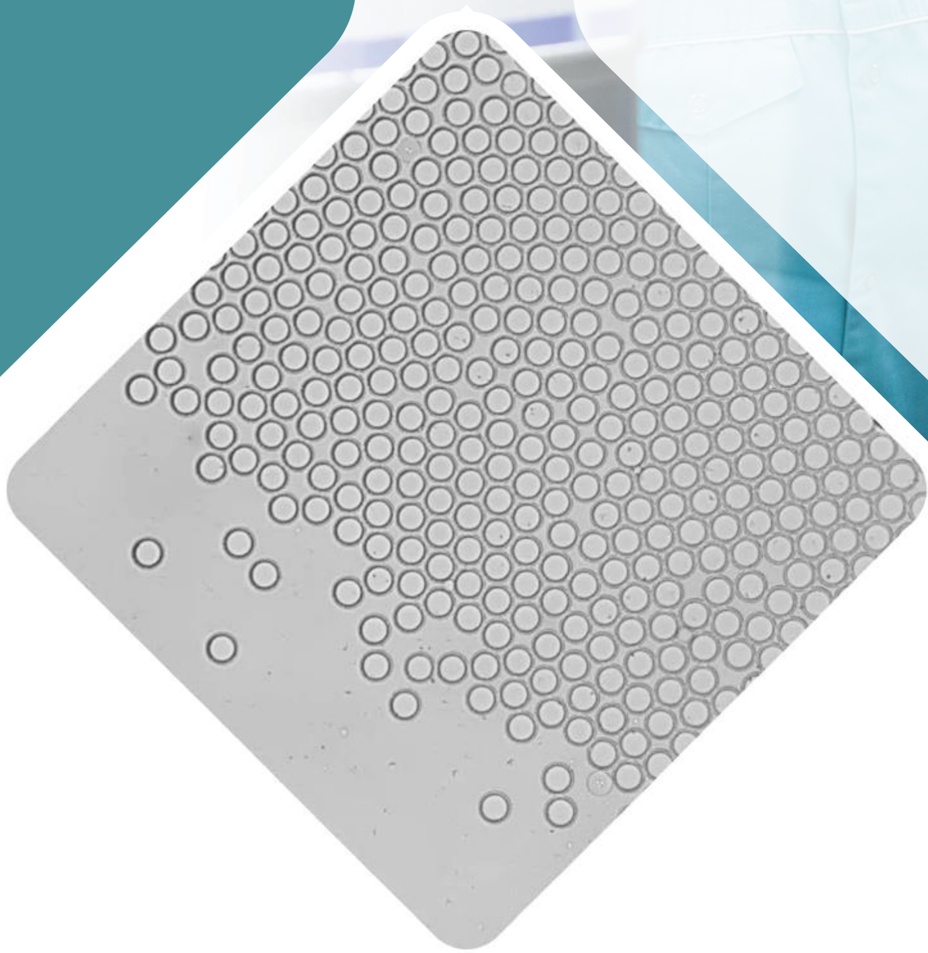
RayDrop Platform for cell encapsulation **adapted to FACS**



Ref: FACS0823/2

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1 Safety precautions

Only trained personnel should be allowed to use this equipment.

The safety information in all sections of the RayDrop Platform's manual must be complied with.

The operating manual must be read and fully understood by the specialist personnel/ operators responsible prior to use.

The contents of the operating manual must be always available to the specialist personnel at the site.

The operator is responsible for ensuring compliance with all local regulations not taken into account in this operating manual.

Unattended operation of the RayDrop Platform should only be undergone after thorough risk assessment and after adequate safety and monitoring measures have been put in place.

Do not exceed maximum operating parameters.

Always wear appropriate personal protective equipment:

safety glasses   adequate gloves   lab coat

When using organic solvent, the RayDrop Platform must be used under a fume hood.

2 Abstract

This document is intended for people who want to produce water/oil/water double emulsions with a diameter of between 25 and 55 µm.
The goal of this document is to guide you step by step from the filling of the droplet generator (RayDrop®) to the generation of your small double emulsion by using the RayDrop Platform for cell encapsulation adapted to FACS. You will use a Raydrop with a counter nozzle to produce your double emulsion.

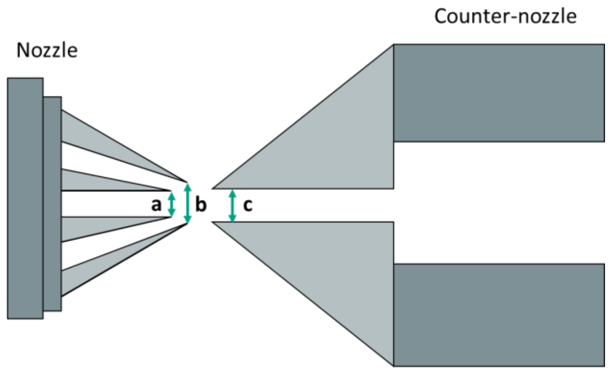


Figure 1: Configuration of the Raydrop with an injection nozzle and a counter-nozzle.

Depending on the size of your counter nozzle, you will be able to produce a specific range of droplet size. Two different counter nozzles are available:

- with the 60µm counter nozzle and a 60-120µm injection nozzle, the size of the double emulsion produced will be between 55µm and 40µm.
- with the 45µm counter nozzle and a 60-120µm injection nozzle, the size of the double emulsion produced will be between 40µm and 25µm.

To follow the instructions of this guide, you will need approximatively 4 hours.

3 Overview

Figure 2: Front view of the RayDrop Platform for cell encapsulation adapted to FACS

This RayDrop Platform is a fast and easy screening tool to setup double emulsion production processes using Secoya's emulsification technology: the Raydrop. It includes a comprehensive flow path developed by Fluigent, with high pressure controllers (LineUp FlowEZ), filters, flowmeters (Flow Units), and valves to ease the start-up, shutdown and cleaning of the system. An injection loop is connected to the core phase to produce numerous samples of double emulsions containing the compound to encapsulate. Thanks to the Raydrop specific configuration including a counter nozzle, double emulsions that are produced are small enough for high-throughput screening and cell sorting. A suitable optical system guarantees the optimum visualisation of the emulsification process inside the Raydrop. The open design of the platform allows its adaptation to your needs and facilitate its maintenance. All tubing and connectors are standard, commonly used references that you can adapt to your own application. Yet, we recommend using the references provided by Secoya.

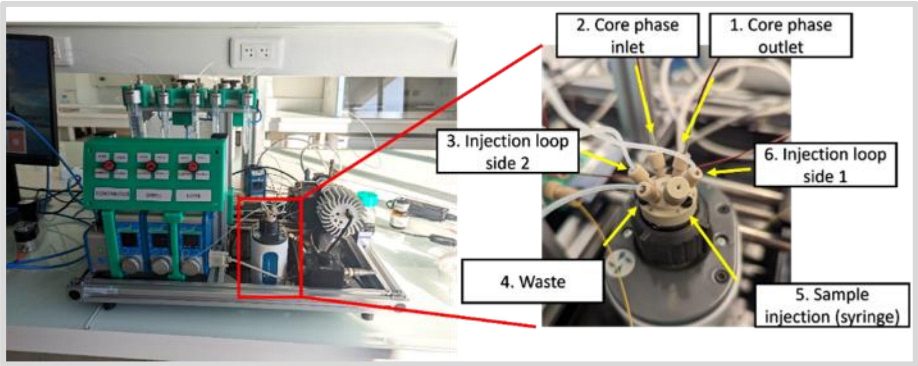


Figure 3: Injection loop included in the platform

The left-hand side of the RayDrop Platform comprises the equipment needed to control the flows that will produce an emulsion. The RayDrop Platform use pressure controllers that apply pressure within the tightly closed reservoirs to push the fluid into the fluidic system. It encompasses the pressure controllers (FlowEZ), flowmeters (Flow Unit), feeding vessels, online filters and switch valves. The complete composition of the equipment will be described later. On the right side of the RayDrop Platform, the RayDrop holder is surrounded by an optical setup that will provide control on the emulsification process inside the RayDrop.

The complete flowpath of the RayDrop Platform is presented in Figure 4.

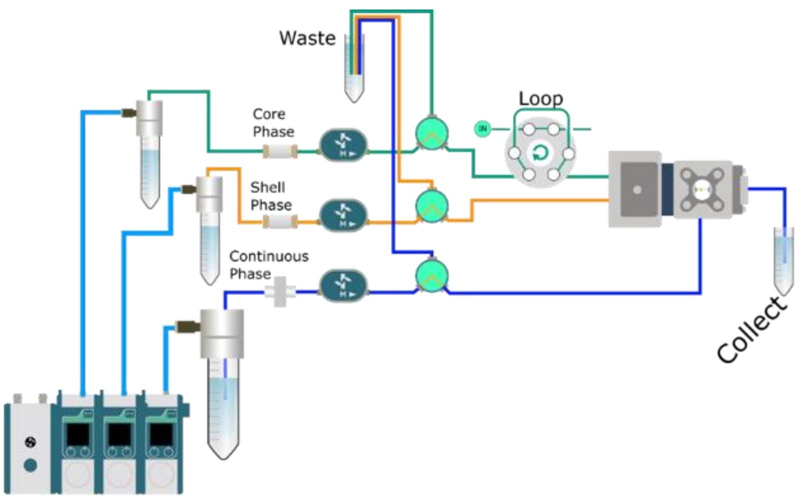
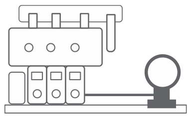
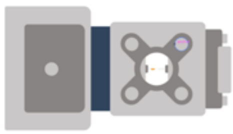


Figure 4: Flowpath of the RayDrop Platform

4 Related products

Product	Description	Reference
	An easy-to-use equipment allowing to produce emulsions using Fluigent's pressure controllers (FlowEZ) and Secoya's microfluidic droplet generator (the Raydrop).	RayDrop Platform
	A droplet generator that produces simple and emulsions with a precise control of droplet size.	Double emulsion with counter nozzle

5 Using the RayDrop Platform to produce double emulsion

In this document, the use of the RayDrop Platform for FACS application is described.

The Raydrop used is the double emulsion version with a 60-120 nozzle (60µm inside diameter (ID) for the Core phase, 120µm ID for the Shell phase) and a 45µm ID counter nozzle.

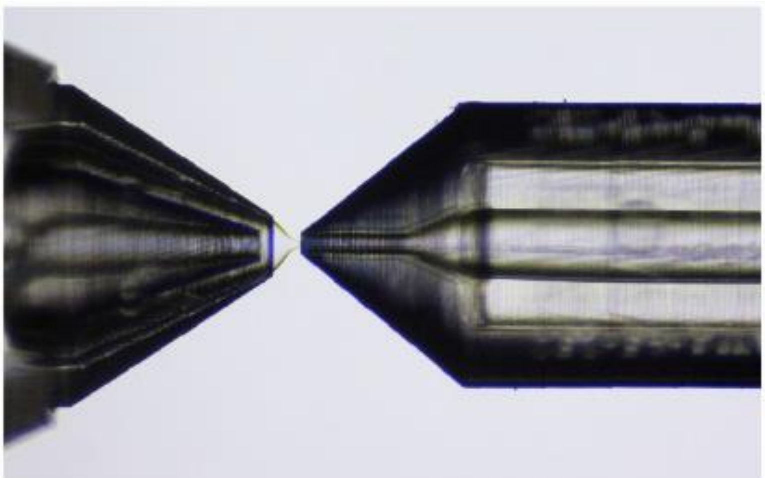
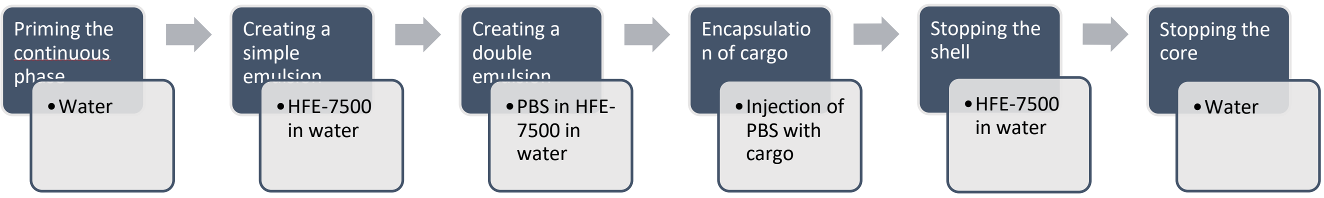


Figure 5: Nozzle (on the left) and counter nozzle (on the right)

The fluids used for the double emulsion production are:

- Continuous phase: Filtered water + 1% surfactant (PVA, Tween 20, …)
- Shell phase: HFE-7500 with surfactant (dSurf, RAN Biotech, …)
- Core phase: Filtered distilled water
- Injected core: PBS with the substance to be encapsulated (cargo in the following)
- Collection bath: identical to the continuous phase

The following diagram describes the steps to follow to produce FACS-compatible double emulsions.

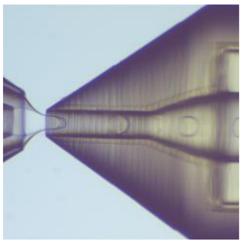


<u>Note:</u> Even if the platform integrates filters for each phase, it is a good practice to filter the solution before pouring them in the Test tube to increase the life of the online filters. Of course, the online filters are easily exchangeable for new one.

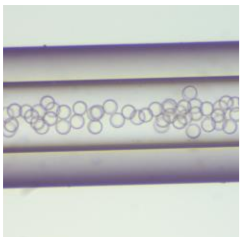
<u>Note:</u> Pressure and flowrate mentioned below are indicative. Every fluid system will need adjustment.

Raydrop Platform

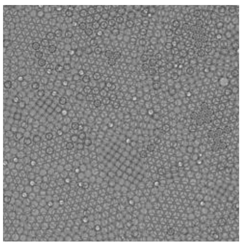
Nozzle



Capillary



Sample



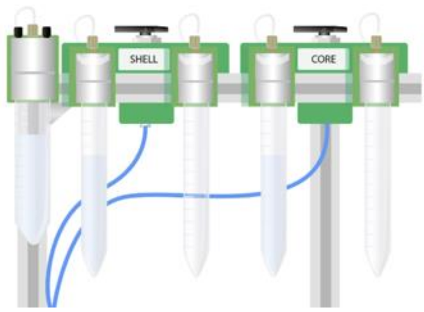
5.1 Filling the Raydrop

5.1.1 Fill reservoirs

F.01

To produce a double emulsion suitable for FACS, 3 phases are needed. For the shell and core phases, 2 test tubes are available per phase. However, to produce a water in oil in water double emulsion compatible with FACS only one test tube per phase is necessary. In total, 3 test tubes are needed: the continuous phase test tube, one of both shell test tubes and one of both core test tubes. Fill each test tube (here those on the left) with the respective **0.22 µm filtered** solution:

- Continuous phase: 45 mL of water + 1% w/w surfactant (PVA, Tween 20, ...)
- Shell phase (left Test tube of the shell): 13mL of HFE-7500 with surfactant (dSurf, RAN Biotech, ...)
- Core phase (left Test tube of the core): 10mL of distilled water



Place the test tube back in the platform.

Note: When filling the test tubes, always leave at least 1cm between the maximum level of the test tube and the liquid level. This prevents liquid from getting into the gas lines and damaging the pressure controllers.

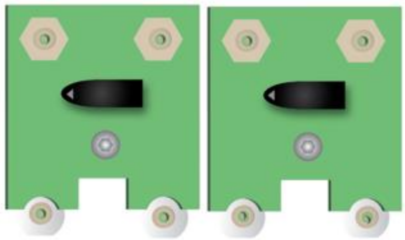
Note: Even if one test tube of the shell and one test tube of the core are empty, all test tubes must be mounted on the platform (whether for the shell or the core). ***If a test tube is not mounted, the pressure will not increase and there will be no fluid flow.***

F.02

Place every waste tubing inside a waster container; here we use a GL45 bottle with the supplied GL45 pierced cap.

F.03
Switch all 4-way valves to the position Reservoir-Waste.

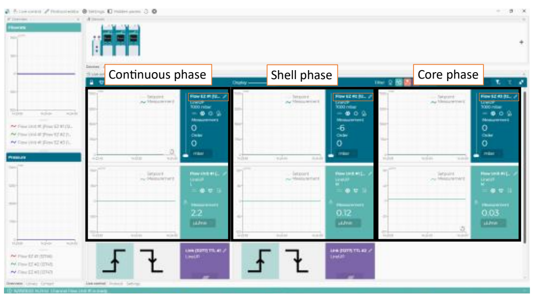
F.04
Switch all black 3-way valves to the position Left Test tube
For the shell and core phases, only one test tube per phase is necessary. Therefore, all black 3-way valves will always stay in the left test tube position.



F.05
Press the blue button of pressure controllers to start them.

F.06
Start the Fluigent OxyGEN software on your computer.
This software allows to control the pressure and the liquid flowrates.

When opening the software, a landing page opens. Choose the Live control to close this window and to be able to control the flow of the fluids.



On the software, 6 small windows are available.
For each phase (continuous, shell and core), there are 2 windows (one for the pressure and one for the liquid flow):
Flow #1 ⇔ Continuous phase
Flow #2 ⇔ Shell phase
Flow #3 ⇔ Core phase

Advice:
For more convenience, to easily change the flow rates and observe the inside of the RayDrop at the same time, split the computer screen in two with one part displaying the camera and a second part displaying the Fluigent software. Moreover, it is possible to rename each of the 6 small windows with the corresponding phase to clarify the use of the software.

F.07
Change the type of fluid measured by the Flow Unit for the Shell phase to HFE. To do that, click on the wheel of the shell flowrate small window to set the channel parameters.

Once that the parameters of the shell are open, change the fluid of the calibration table to HFE.

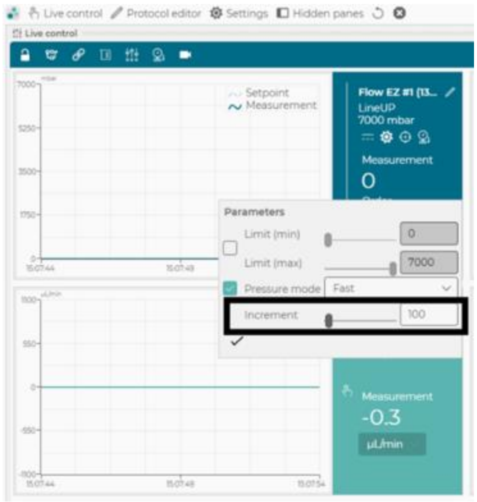
For this application, the reference of the shell fluid in Fluigent software is HFE.
For the continuous phase and the core phase, the fluid reference in Fluigent software is Water.

Note: Please refer to Fluigent Oxygen user manual to have more information about the pressure controller software.

F.08

In this RayDrop configuration, pressures and flow rates must be increased and decreased slowly to avoid creating a backflow in the injection nozzle and to reduce the risk of oil spreading in the continuous phase and on the nozzles. To simplify the modification of values (for pressures and flowrates), change increments in the parameters:

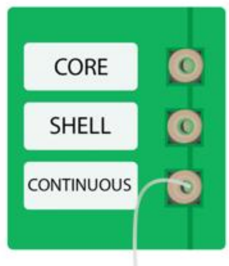
- Pressure increment of 100 for the continuous phase ;
- Pressure increment of 10 for shell and core phases ;
- Flowrate increment of 20 for the continuous phase ;
- Flowrate increment of 10 for shell and core phases.



5.1.2 Purge the gas present in all tubing

F.09

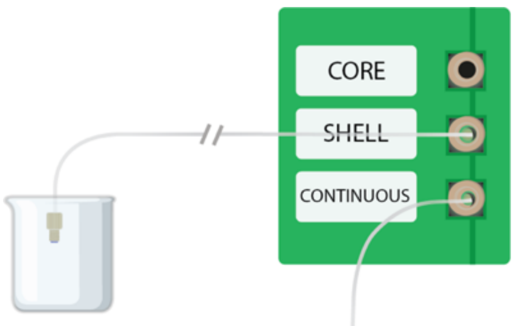
Connect the continuous phase tubing to the platform and put the unconnected end in a beaker.



F.10

Connect the shell phase tubing (30 cm, 125 µm ID, red PEEK, blue+sleeve and green fittings) to the platform and put the unconnected end in a beaker. The **green fitting** will be connected to the platform and the blue one to the Raydrop.

Screw the green fitting to the platform and place the blue fitting in a beaker.

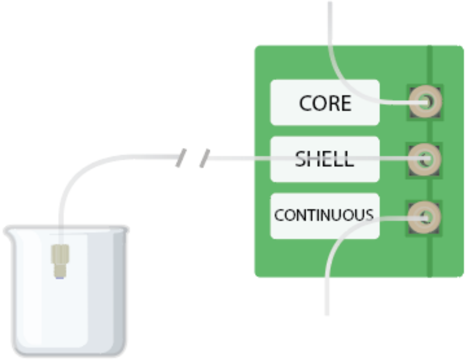


Note: the tubing is modified from the initial configuration. The aim is to adjust the pressure drop into the ideal operating range.

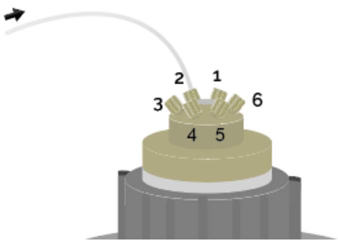
Note: When using 1/16" OD fittings with smaller tubing, a sleeve adaptor (green piece of tubing) must be added to ensure tight fitting.

F.11

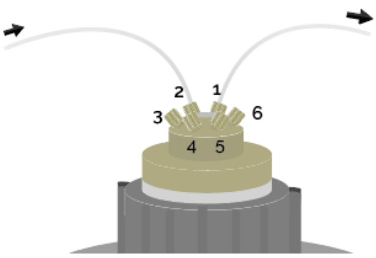
To plug in the injection loop on the core phase, connect the first core phase (15 cm, 125 µm ID, red PEEK) tubing to the platform.



F.12
Connect the other extremity of the core phase tubing to the injection loop, entry number 2.



F.13
Connect the second core phase tubing (20 cm, 125 µm ID, red PEEK) to the injection loop, entry number 1 and put the unconnected end in a beaker.

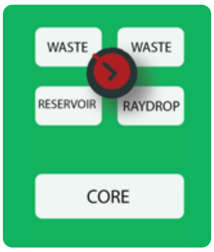


F.14
Switch the Continuous 4-way valve to Reservoir-Waste.

F.15
Switch the Shell 4-way valve to Reservoir-Waste.

F.16

Switch the Core 4-way valve to Reservoir-Waste.



F.17

Increase gradually the pressure for the Continuous phase up to 500 mbar on the software. This corresponds to about 235 µL/min.

After about 1 minute, you should see a flowrates measurement.
Wait until the flow measurement is stable (i.e. the value varies by maximum 5 µL/min over 5 seconds).

As soon as the liquid flowrate is stable and once that the liquid flows out the waste tubing, you can set the Continuous pressure to 0 mbar.

F.18

Increase gradually the pressure for the Shell phase up to 500 mbar. This corresponds to about 20 µL/min.

After 3 minutes, you should see a flowrates measurement.
Wait until the flow measurement is stable (i.e. the value varies by maximum 5µL/min over 5 seconds).

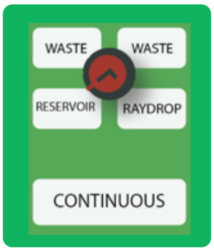
As soon as the liquid flowrate is stable and once that the liquid flows out the waste tubing, you can set the Shell pressure to 0 mbar.

F.19
Increase gradually the pressure for the Core phase up to 500 mbar. This corresponds to about 20 µL/min.

After 3 minutes, you should see a flowrates measurement. Wait until the flow measurement is stable (i.e. the value varies by maximum 5 µL/min over 5 seconds).

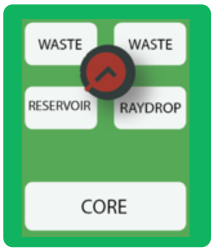
As soon as the liquid flowrate is stable and once that the liquid flows out the waste tubing, you can set the Core pressure to 0 mbar.

F.20
Switch the Continuous 4-way valve to Reservoir-RayDrop.



F.21
Switch the Shell 4-way valve to Reservoir- RayDrop.

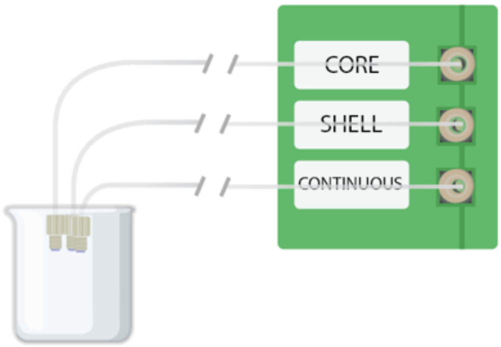
F.22
Switch the Core 4-way valve to Reservoir- RayDrop.



F.23

Increase gradually the pressure for the Continuous phase up to 2000 mbar on the software.

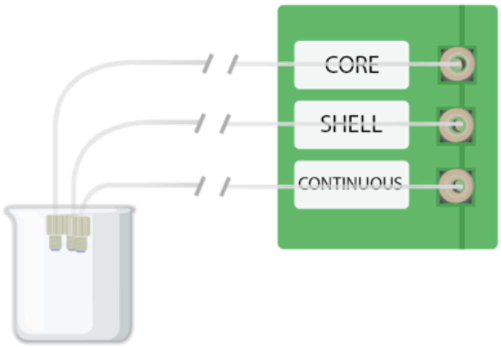
Wait until the liquid flowrate is stable (i.e. the value varies by maximum 5 µL/min over 5 seconds) and let the fluid flow outside of the tubing during 30min at a flowrate of minimum 150 µL/min to remove any dust from the tubing. After the 30 minutes, set the Continuous pressure to 0 mbar.



F.24

Increase gradually the pressure for the Shell phase up to 3000 mbar on the software.

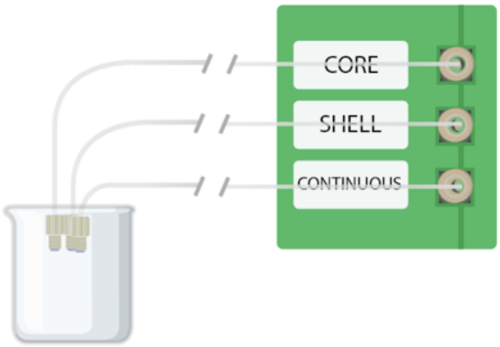
Wait until the liquid flowrate is stable (i.e. the value varies by maximum 5 µL/min over 5 seconds) and let the fluid flow outside of the tubing during 30min at a flowrate of minimum 50 µL/min to remove any dust from the tubing. After the 30 minutes, set the Shell pressure to 0 mbar.



F.25

Increase gradually the pressure for the Core phase up to 3000 mbar on the software.

Wait until the liquid flowrate is stable (i.e. the value varies by maximum 5 µL/min over 5 seconds) and let the fluid flow outside of the tubing during 30min at a flowrate of minimum 50 µL/min to remove any dust from the tubing. After the 30 minutes, set the Shell pressure to 0 mbar.



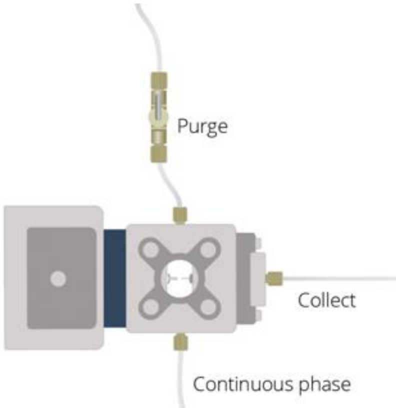
F.26

At this point, for each phase, liquids are present in the tubing from the Reservoirs to the 4-way valves and from the 4-way valves to the waste container and to the tubing that are going to be connected to the RayDrop.

To prevent liquids to flow naturally from the Test tube to the waste container, switch all the 4-way valves to the position Waste-Waste. This position corresponds to complete closure of the valve, with no passage for either liquid or air.

F.27

Connect to the RayDrop:



- the purge line with on/off valve;
- the collect tubing: yellow 185 µm ID tubing, one end with the blue ferrule fitting;
- Continuous phase tubing in two parts:
    - Part connected to the flowmeter: 80 cm yellow tubing DI 180 µm
      UNION
    - Part connected to the RayDrop: 25 cm transparent tubing DI 500 µm.

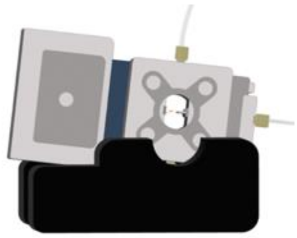
Note: to tighten tubing to equipment, several types of fitting (nut+ferrule) exist. In the platform two types of fitting are mostly used:

- *Flatbottom flangeless fitting (two parts, nut, and blue ferrule)*
- *Flatbottom super flangeless fitting (three parts, nut, ring, and yellow ferrule).*

The super flangeless fittings offer a great benefit compared to the flangeless fittings: you don't twist the tubing while screwing the nut. However, this advantage comes with one major drawback: the tubing is not perfectly aligned for the thread. As the inlet diameter of the core and shell phases tubing is only 150µm, it is mandatory to connect these two inlets with flangeless fittings (the blue one).

F.28

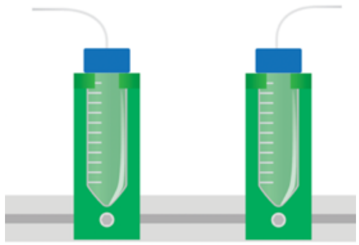
Place the Raydrop in its sample holder; use the groove to guide the continuous phase tubing.



F.29

Attach to the right side of the platform both Test tube holder.

Place the purge tubing in one test tube and the collect tubing in the other Test tube.

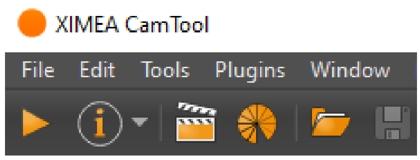


F.30

Turn on the platform lamp by turning the dimmer switch.

F.31

Start the camera software xiCamTool on your computer.
Launch the live video by pressing the triangle start button.
The view of the inside of the Raydrop should appear on the screen.
For the FACS application, some settings should be adapted to see clear drops.
In the software, go to Tools → Settings and uncheck:



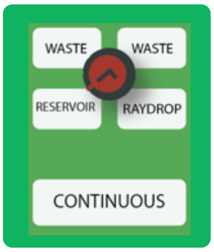
- Autoexposure
- Autowhite balance

Then set:

- Exposure at 2 µs
- Gain at 9 or 12 dB (for light intensity boost)

5.1.3 Fill the metalic chamber of the Raydrop

F.32
Switch the Continuous 4-way valve to Reservoir-RayDrop.



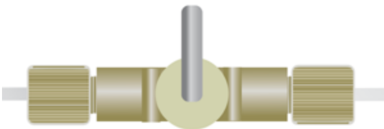
F.33
Open the purge valve.

Note: the open position is where the metal bar is aligned with the tubing (see scheme on the right)

F.34
Increase gradually the pressure for the Continuous phase until you reach a flowrate of 400 μL/min.

F.35
After less than a minute, the continuous phase exits the purge tubing.

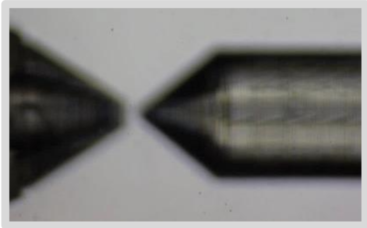
Let the continuous phase flows in the purge waste Test tube for 5 minutes.



Close the purge valve.

Set the pressure to 0 mbar.

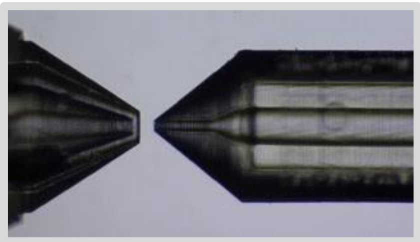
5.2 Adjusting the optical setup

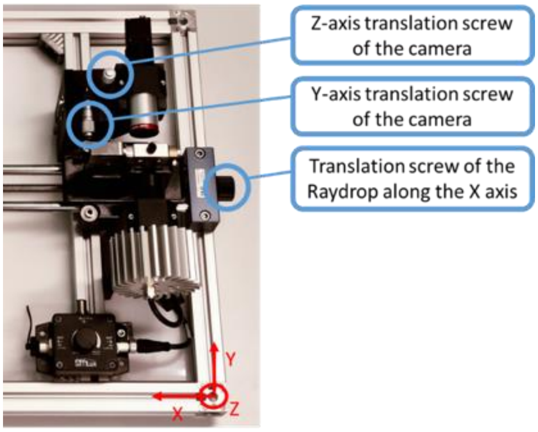


O.01

Most of the time, the glass capillary and nozzle are already visible in the camera view and only a slight adjustment is needed.

- Adjust the focus of the camera by moving the camera Y-axis translation screw ;
- Adjust the high-low position of the nozzle by moving the camera Z-axis translation screw ;
- Adjust the left-right position of the nozzle by moving the RayDrop X-axis translation screw.

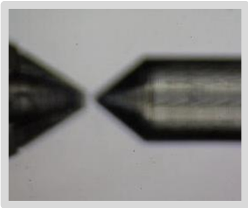




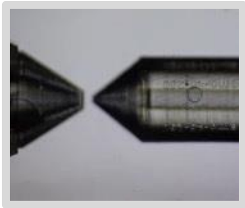
O.02

In some case, due to poor transportation condition or manutention, the focus of the RayDrop might be not good at all.

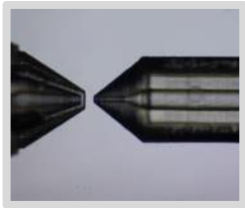
1) Check that the RayDrop is properly installed in its holder. Don't hesitate to move a bit the RayDrop in its sample holder to find the sweet spot;
2) Slowly move the camera along the Z-axis to find the Nozzle and output capillary in the camera view. If the nozzle is out of focus, you will only see a shadow;
3) Move the RayDrop along the X-axis to adjust the observation windows to your needs;
4) Move the camera on the Y-axis to adjust the focus.



Out of focus Nozzle

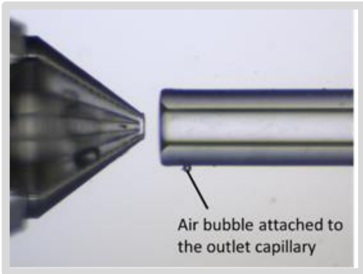


Almost in focus nozzle



In focus nozzle

O.03
Sometimes, when filling the RayDrop, a large bubble remains attached to the outlet capillary. To remove this bubble, apply a small mechanical vibration by gently tapping the RayDrop.



In the picture on the right, you can see a very small air bubble, but it does not interfere with the formation of emulsions.

5.3 Priming the shell and the core phases

5.3.1 Connect the shell to the RayDrop

P.01
The procedure to connect the Shell and Core phases tubing is slightly different from the Continuous phase tubing as we want to avoid introducing too much air in the RayDrop.

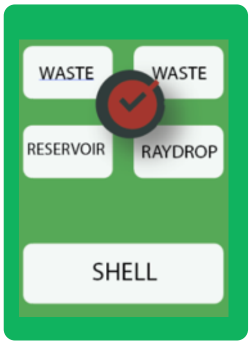
P.02

Switch the Shell 4-way valve to Reservoir-RayDrop.

Increase gradually the pressure for the Shell priming phase up to 1000 mbar.
After a couple of minutes, you should see the liquid flowing out to the beaker. As soon as the liquid flows out the tubing, you can set the Shell phase pressure to 0 mbar.

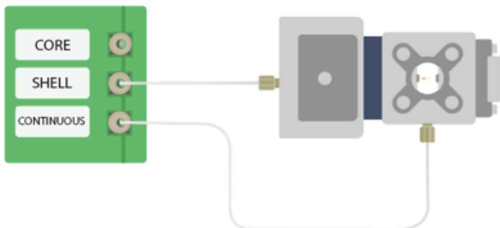
P.03

Switch the Shell 4-way valve to Waste-Waste.



P.04

Connect the Shell phase tubing to the RayDrop:



- To avoid any twisting of the tubing, loosen the Shell blue ferrule to be connected to the RayDrop;
- Rinse the blue ferrule with an acetone wash bottle to prevent dust from entering the RayDrop when screwing;
- Screw and tighten the fitting to the Shell inlet of the RayDrop, the one with a circle.

5.3.2 Connect the core to the Raydrop

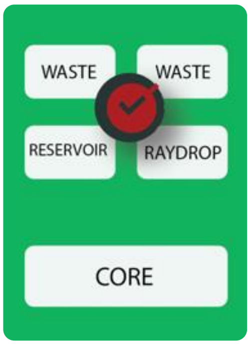
P.05

Switch the Core 4-way valve to Reservoir-RayDrop.

Increase gradually the pressure for the Core priming phase up to 1000 mbar.
After a couple of minutes, you should see the liquid flowing out to the beaker. As soon as the liquid flows out the tubing, you can set the Core phase pressure to 0 mbar.

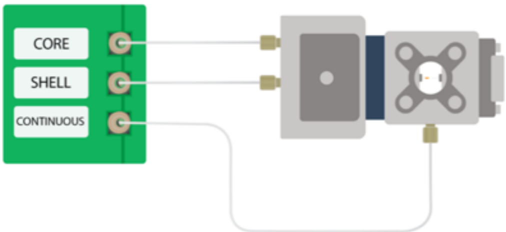
P.06

Switch the Core 4-way valve to Waste-Waste.



P.07

Connect the Core phase tubing to the RayDrop:



- To avoid any twisting of the tubing, loosen the Core blue ferrule to be connected to the RayDrop;
- Rinse the blue ferrule with an acetone wash bottle to prevent dust from entering the RayDrop when screwing;
- Screw and tighten the fitting to the Core inlet of the RayDrop, the one with a dot.

5.4 Creating a simple emulsion of shell phase

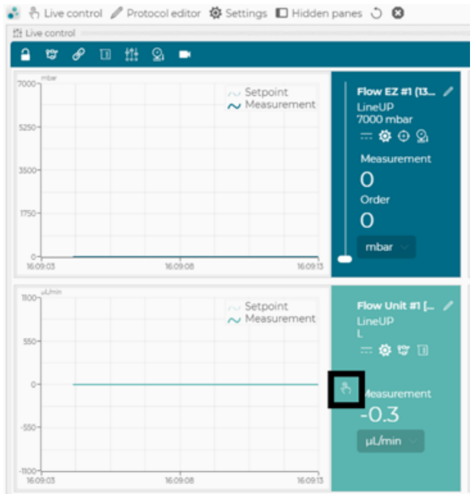
It is best to work in pressure regulation mode with the pressure controller instead of flowrate regulation mode when starting the RayDrop system. The pressure controllers' algorithm is not fast enough to compensate the large variation in pressure and flowrate. However, once the system is stable, you can switch to flowrate regulation mode as it is more convenient to use.

However, the continuous phase has to be used in flowrate regulation mode for the use with a RayDrop with a counter nozzle. Moreover, pressures and flow rates must be increased and decreased slowly (do not go from 50 µL/min to 5 µL/min all at once) to avoid creating a backflow in the injection nozzle.

SE.01
Set the Continuous 4-way valve to Reservoir-RayDrop and both Shell and Core 4-way valves to Reservoir-Waste.

SE.03
Increase gradually the flowrate of the continuous phase to 200 µL/min in flowrate regulation mode.

Note: the switch to flowrate regulation mode, just click on the button framed on the image on the right.

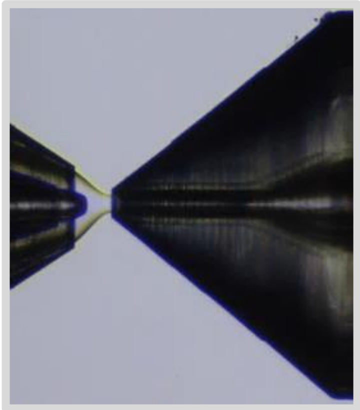


SE.04
Increase the pressure of the Shell phase to obtain a flowrate of 40 µL/min.

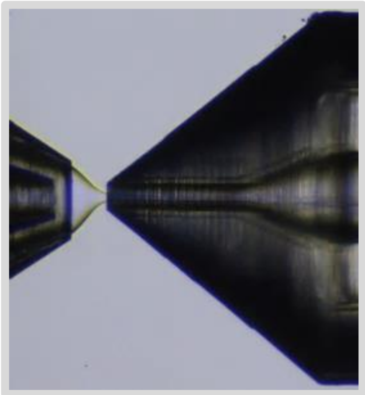
SE.05
Switch the Shell 4-way valve to Reservoir-RayDrop. Due to the difference in pressure drop between the two flow paths, this will result in a significant diminution of the flowrate.

SE.06
Adjust the Shell pressure to obtain a 15 µL/min Shell flowrate. This will help flushing the nozzle from the remaining air (see picture on the left) and water.

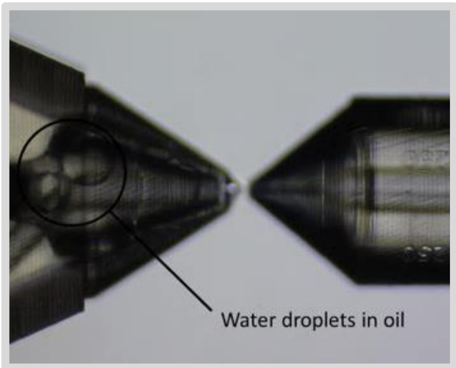
Note: the Shell flowrate might become negative when switching the valve. It is due to the pressure balance between Continuous and Shell phases. Simply increase the Shell pressure until having a positive flowrate.



SE.07
After approximatively 30 seconds, a clean jet of Shell phase flows out of the nozzle.
If drops are formed instead of a jet, increase the flow rate slightly until a jet is obtained.

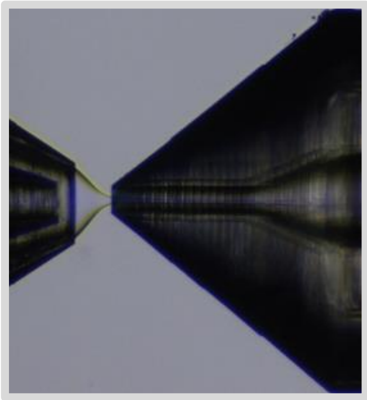


Frequently, water droplets are stuck in the oil of the shell nozzle. To eliminate these water droplets, slowly increase the pressure of the shell phase to get the drops out of the shell channel. If the pressure is not sufficient (pressure controllers are limited to 7 bar) to get the drops out, be careful during the future production of double emulsion. Once that droplets are out of the shell channel, reduce slowly the pressure of the shell, avoiding a backflow of the continuous phase in the shell.



SE.08
When the jet is stable and regular, decrease the Shell pressure to reach a Shell flowrate of ~15 µL/min.
Shell phase should still be in jetting mode from the RayDrop.

Note: dripping and jetting regimes are described in the glossary

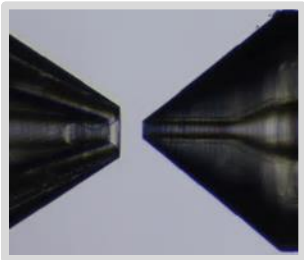


5.5 Creating a double emulsion

DE.01
Adjust again the Continuous pressure to reach a Continuous flowrate of ~150 µL/min and the Shell pressure to obtain a Shell flowrate of ~15 µL/min.

DE.02
Increase the pressure of the Core phase to obtain a flowrate of 15 µL/min.

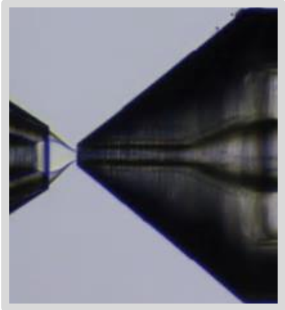
DE.03
Switch the Core 4-way valve to Reservoir-RayDrop. If the indicated Core flow rate is negative and you observe that the Shell goes inside the Core in the nozzle (see picture), simply increase the pressure of the core phase until it becomes positive again and drops come out of the core.

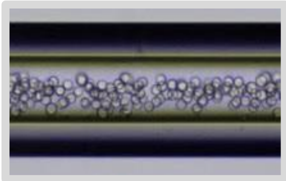


Note: the Core flowrate might become negative when switching the valve. It is due to the pressure balance between the three phases. Simply increase the Core pressure until having a positive flowrate.

DE.04

Adjust the Core pressure to obtain a 15 µL/min Core priming flowrate. First, the remaining mixture of air/continuous phase/shell phase must be flowed out of the RayDrop.

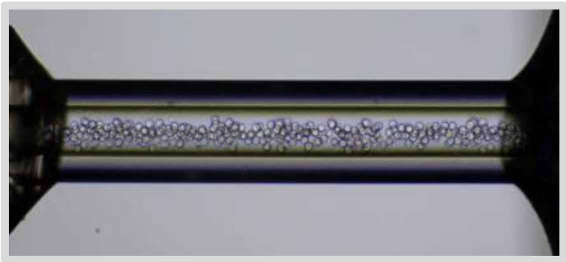




Note: if an air bubble, coming from the shell or the core, breaks the double emulsion generation, stop the Core flowrate by switching the Core 4-way valve to RayDrop-Waste and repeat steps DE3 and DE4.

DE.05

To observe the double emulsion formed, use the X translation stage to see beyond the counter nozzle.



If the size of the double emulsion is polydisperse, first try to slowly increase the core flowrate. You can also adjust the core flowrate until a better monodispersity is reached.

DE.06

Here, you can play with the system to understand its basic principle:

- When you increase the Continuous flowrate (by increasing the Continuous pressure) while keeping constant Shell and Core flowrates: the droplet size decreases.

- When you decrease the Continuous flowrate (by decreasing the Continuous pressure) while keeping constant Shell and Core flowrates: the droplet size increases.

- When you increase the Shell flowrate (by increasing the Shell pressure) while keeping constant Continuous and Core flowrates: the shell thickness of the double emulsion increases.

- When you decrease the Shell flowrate while keeping constant Continuous and Core flowrates: the shell thickness of the double emulsion decreases.

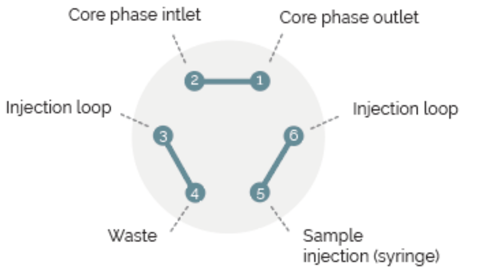
- When you increase the Core flowrate (by increasing the Core pressure) while keeping constant Continuous and Shell flowrates: the core of the double emulsion increases and so the size of droplets increases.

- When you decrease the Core flowrate while keeping constant Continuous and Shell flowrates: the core of the double emulsion decreases and so the size of droplets decreases.

5.6 From a double emulsion to the encapsulation of the cargo with the use of the injection loop

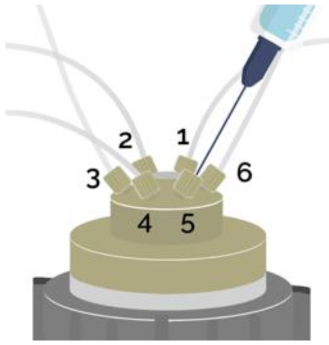
ENCAPS.01

Once that a stable double emulsion is generated, it is time to encapsulate the cargo with the use of an injection loop. To do that, verify on the Oxygen software that the injection loop is in position 2. If not, switch from position 1 to position 2. The position 2 is used to load the cargo in the sample loop.



ENCAPS.02

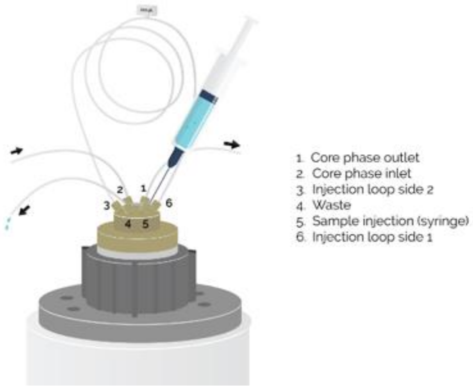
In position 2, insert the flat bottom needle with the syringe full of cargo in the diaphragm on the entry number 5.



ENCAPS.03

Inject the cargo in the sample loop until liquid flows out of the tubing connected to the entry number 4.

The cargo is now ready to be injected and encapsulated in the double emulsion.



ENCAPS.04

Switch the position of the injection loop to the position 1 and collect your sample in a vial.

ENCAPS.05

To collect another sample with a different cargo, first put the injection loop in position 2.

Note: it is possible to flush the sample loop in position 2 by injecting ethanol. If you make this flush, also inject air after in order not to mix ethanol and the cargo solution.

ENCAPS.06
For the loading, the injection and the collect, repeat steps ENCAPS.02, ENCAPS.03 and ENCAPS.04.

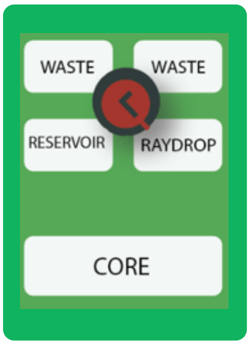
ENCAPS.07
Once you collected all your samples, empty the sample loop by setting the injection loop in position 2 and injecting firstly ethanol and secondly air.

5.7 Stopping the core of the double emulsion

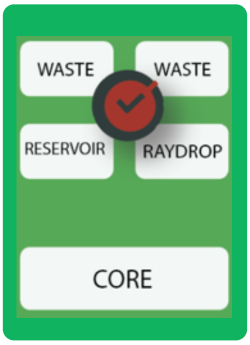
Once that all samples have been collected, the double emulsion production can be shut down.

S.01
First, decrease slowly the pressure of the core up to 0 bar.

S.02
The core of the double emulsion is now stopped, and you can see a jetting of oil in water.



Switch the Core 4-way valve to RayDrop-Waste for 30 seconds. This will restore the equilibrium pressure in the RayDrop.

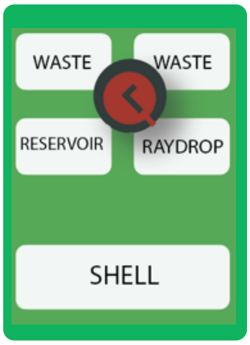


S.03
Put the Core 4-way valve to Waste-Waste.

5.8 Stopping the shell and the continuous phase

C.01
Now, decrease slowly the pressure of the Shell up to 0 bar.

C.02
The shell is now stopped; however you can see some droplets of oil flowing in the counter nozzle. This is not disturbing, and even if some oil wet the injection nozzle this oil will be eliminated at the next use by the flow of the continuous phase.
Switch the Shell 4-way valve to RayDrop-Waste for 30 seconds. This will restore the equilibrium pressure in the RayDrop.



C.03
Put the Shell 4-way valve to Waste-Waste.

C.04

Let the continuous phase flowing for 5 minutes at 100 µL/min to evacuate emulsions present in the collect tubing.

C.05
Reduce slowly the Continuous pressure to reach around 20 µL/min for the Continuous phase.

C.06
To avoid any pollution of the chamber, it is a good practice to let the pressure inside the system decreased for a couple of minutes.
For this reason, set the pressure of the Continuous phase to 0 mbar and let the 4-way valve in position Reservoir-RayDrop for 10 minutes.

C.07
After these 10 minutes, switch the Continuous phase 4-way valve in position Waste-Waste.

Note: we recommend keeping the tip of the collect tubing in a liquid (water for instance). If the liquid contained in the collect tubing dry, it could plug it.

5.9 Putting the platform in standby mode

SM.01
Turn off the platform lamp by turning the dimmer switch.

SM.02
Exit software used for the camera and pressure controllers.

SM.03
Press the blue button of pressure controllers for 4 seconds to turn them off.

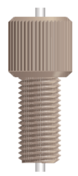
The solutions used for the continuous, shell and core phases can be used up to 1 week after preparation. If the platform is not used for more than one week, it is necessary to clean and empty the RayDrop and all tubing on the platform.

6 Glossary

Test tube
Plastic tube for centrifugal process that are used, in combination of a Fluigent's P- CAP, as a pressurised reservoir. The P-CAP does not accept every commercially test tubes. We recommend the use of Falcon test tubes ref. 352097 (15 mL) and 352070 (50 mL).

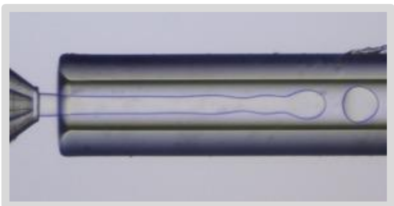
Fluigent's P-CAP
Air-tight metal cap that allows to pressurize a Test tube.

Fitting (Flatbottom Super flangeless)
Ensemble of nut, ring and ferrule.

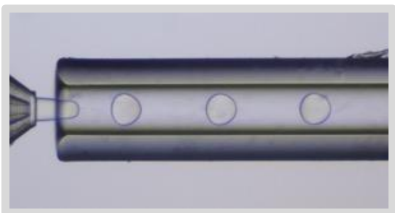


Nut
Hard material screwed to apply a pressure on the ferrule.

Ferrule (yellow part) with ring (metallic piece)
Soft material used to seal the tubing thanks to the ring action.



Jetting regime
Droplets are generated through the destabilization of a jet far from the nozzle tip. This regime, while giving access to higher flow rates, is also less stable than the dripping regime.



Dripping regime
Droplets are generated at the outlet of the nozzle, in a stable manner.