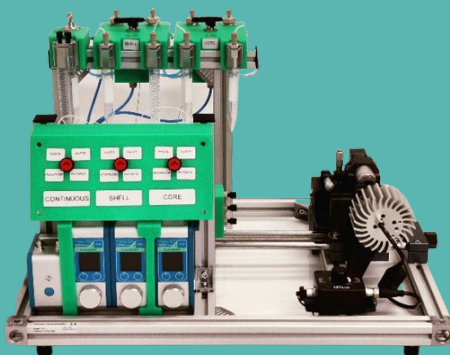


USER'S
MANUAL

CELL ENCAPSULATION PLATFORM



USER'S
MANUAL

CELL ENCAPSULATION PLATFORM

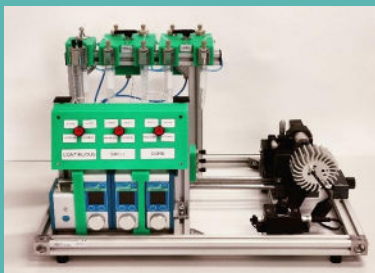


Figure 1: Face view of the RayDrop platform

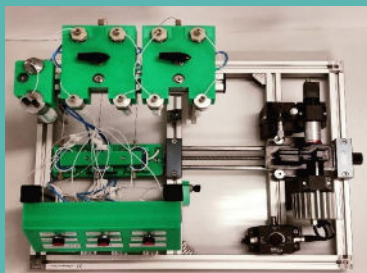


Figure 2: Top view of the RayDrop platform

CELL ENCAPSULATION PLATFORM USER'S MANUAL

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SAFETY PRECAUTIONS

- Wear of normal laboratory protection gear is mandatory: safety glasses, adequate gloves and lab coats.
- Pressure applied to the 50 mL Falcon must not go above 4 bar.
- When using solvent, the Raydrop platform must be placed under a chemical hood.

OVERVIEW

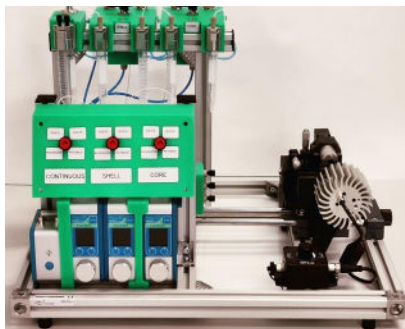


Figure 3: Face view of the RayDrop platform

The Raydrop Platform is a fast and easy screening tool to setup double emulsion production process using Secoya's emulsification technology: the Raydrop. It includes a comprehensive flow path with pressure controllers, filters, flowmeters, and valves to ease the start-up, shutdown and cleaning of the system in between tests. 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.

On the left side of the Raydrop platform is the equipment needed to control the flows that will produce an emulsion. The composition of the fluidic 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.

This optical setup will be detailed later.

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

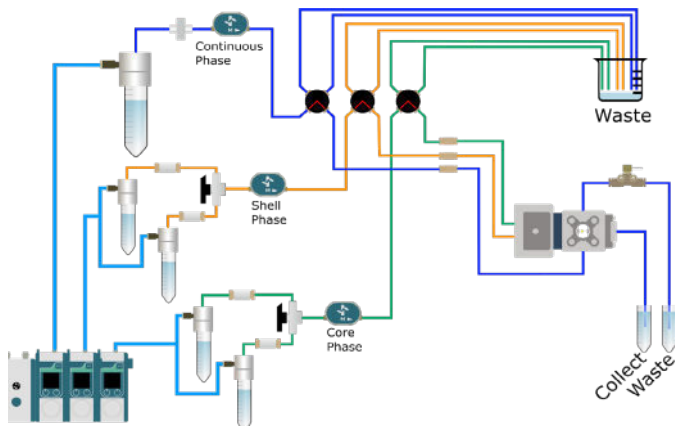


Figure 4: Flowpath of the RayDrop Platform

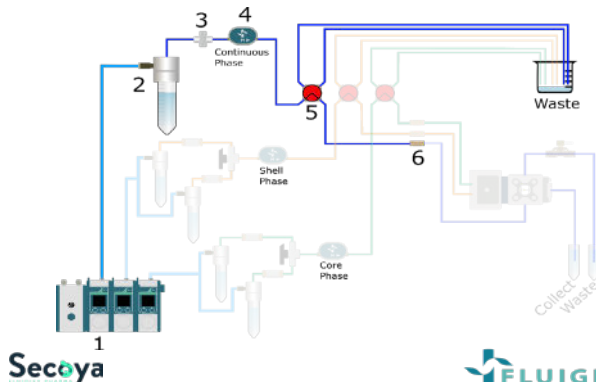
DESCRIPTION OF THE FLUIDIC FLOWPATH

CONTINUOUS PHASE

O.01

The continuous phase flowpath is mostly located on the left side of the platform and is composed of:

- 1) Fluidigent pressure controller
- 2) Reservoir: 50 mL Pcap and 50 mL Falcon
- 3) 10 μm stainless steel filter
- 4) Flowmeter: Fluidigent L Flow Unit (not visible on the picture)
- 5) 4-way valve
- 6) Outlet to the Raydrop on the right side



OVERVIEW

O.02

The 4-way valve allows to easily direct the Continuous phase in the system between the 50 mL Reservoir, the Raydrop and the Waste. The black angle on the red rotary indicates the two inlets that are connected. For instance, on the picture on the right, Reservoir is connected to the Raydrop meaning that the Continuous phase can go from the Reservoir to the Raydrop and viceversa. The other two inlets are closed. The Waste-Waste position is used as a stop flow position for the system.

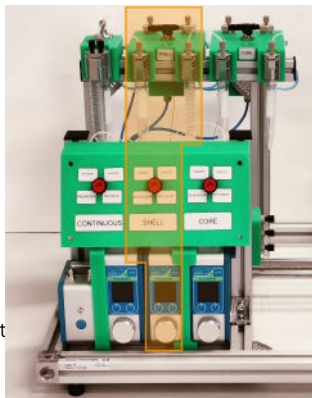


SHELL PHASE

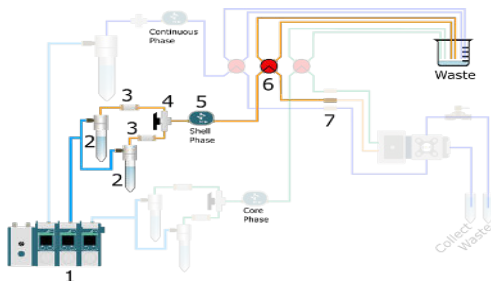
O.03

The Shell phase flowpath is mostly located in the middle of the platform and is composed of:

- 1) Fluigent pressure controller
- 2) Reservoir: Two 15 mL Pcap and Two 15 mL Falcon
- 3) Two bio-compatible 2 μ m Peek filters
- 4) 3-way valve
- 5) Flowmeter: Fluigent M Flow Unit (not visible on the picture)
- 6) 4-way valve
- 7) Outlet to the Raydrop on the right side



OVERVIEW



O.04

The 3-way valve at the top of the system allows to choose which of the 15 mL Falcon is connected to the Shell flowpath. On the picture on the right, the liquid contained in the left Falcon will flow in the system while the liquid on the right Falcon will remain still. This valve allows to easily switch from one liquid to another mainly for priming or cleaning procedure.

Note: both Falcons are always pressurized at the same pressure meaning that both Falcon must be fixed to the Pcap for the system to run.



O.05

The 4-way valve allows to easily direct the Shell phase in the system between the 15 mL Reservoir, the Raydrop and the Waste. The black angle on the red rotary indicates which inlets are connected. For instance, on the picture on the right, Reservoir is connected to the Waste meaning that the Shell phase can go from the Reservoir to the Waste and vice-versa. The other two inlets are closed. The Waste-Waste position is used as a stop flow position for the system to run.

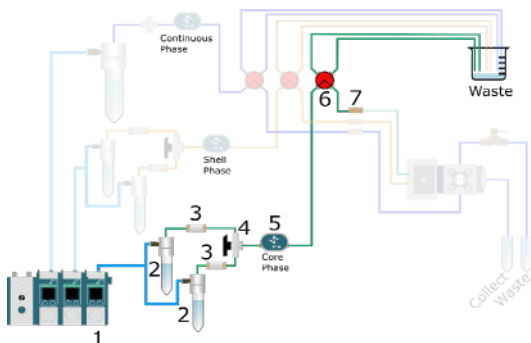


CORE PHASE

O.06

The Core phase flowpath is mostly located on the right of the platform and is composed of:

- 1) Fluigent pressure controller
- 2) Reservoir: Two 15 mL Pcap and two 15 mL Falcon
- 3) Two bio-compatible 2 μ m Peek filters
- 4) 3-way valve
- 5) Flowmeter: Fluigent M Flow Unit (not visible on the picture)
- 6) 4-way valve
- 7) Outlet on the right side



OVERVIEW

O.07

The 3-way valve at the top of the system allows to choose which of the 15 mL Falcon is connected to the rest of the Core Flow path. On the picture on the right, the liquid contained in the right Falcon will flow in the system while the liquid on the left Falcon will remain still. This valve allows to easily switch from one liquid to another mainly for priming or cleaning procedure.

Note: both Falcons are always pressurized at the same pressure meaning that both Falcon must be fixed to the P-cap for the system to run.



O.08

The 4-way valve allows to easily direct the Shell phase in the system between the 15 mL Reservoir, the Raydrop and the Waste. The black angle on the red rotary indicates which inlets are connected. For instance, on the picture on the right, Reservoir is connected to the Waste meaning that the Core phase can go from the Reservoir to the Waste and vice-versa. The other two inlets are closed. The Waste-Waste position is used as a stop flow position for the system.

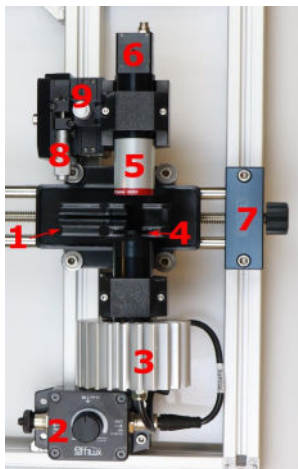


OPTICAL SYSTEM

O.09

The optical system is composed of:

1. Raydrop holder
2. Dimmer
3. LED Light source
4. Optical diffuser
5. Microscope objective with 10X magnification
6. USB3 Colour camera
7. X translation stage
8. Y translation stage
9. Z translation stage



O.10

The DIMMER allows to adjust the light power of the LED

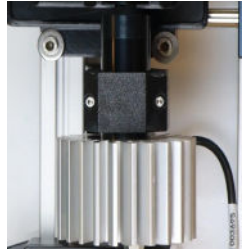
Note 1: On some DIMMER, the sticker showing the power is wrongly placed and the low power of the DIMMER corresponds to the high power of the LED while the high power of the DIMMER corresponds to the low power of the LED

Note 2: Depending on the power supply used, the DIMMER might not work as intended and the light power is always be at its maximum. This behaviour does not affect the effectiveness of the platform



O.11

The LED light source has a huge heat-sink that will heat during operation. It is best to not touch it to avoid any skin burn.



O.12

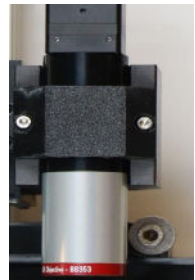
The optical diffuser enhances the observation of the emulsion produced by the Raydrop.



O.13

5X magnification microscope objective and USB3 color camera.

Note: in some case, the parallelism of the camera with respect to the collect capillary of the Raydrop might not be optimal due to transportation. It can easily be adjusted by untightening the two screws, rotating the camera while observing the Raydrop and then tightening the screws.



O.14

The X translation stage allows to move the Raydrop along the collect capillary axis.



O.15

The Y translation stage allows to adjust the focus of the camera for a clear observation of the emulsion process.

Note: Y position has been adjusted during manufacturing process with a Raydrop filled with water. Depending on your continuous phase fluids and transportation, you might need to adjust the focus



O.16

The Z translation stage allows to move the camera along the vertical direction



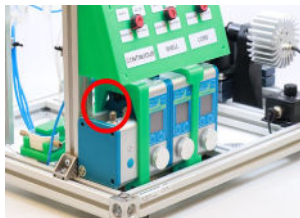
INSTALLATION

After placing the Raydrop platform on your lab bench or under your chemical hood, you can start setting it up.

PRESSURE CONTROLLER

I.01

All the input required for the pressure controller to work are located on the left of the platform



I.02

Connect the Power cable to the Fluidgent Link



I.03

Connect the USB2 cable to the Fluidgent Link



I.04

Connect the gas supply to the
Fluigent Link Plug the USB 2 cable
to the computer
Plug the power supply to the
power socket

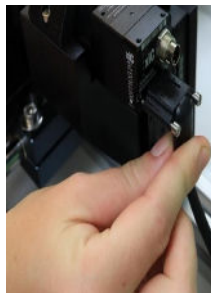


OPTICAL SYSTEM

I.05

While maintaining the camera,
connect the Usb 3 cable to the came-
ra and tighten the two screws.

Plug the USB3 cable to a USB3 port
on the computer.



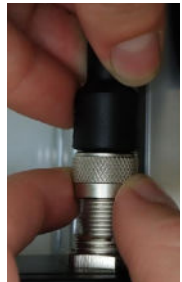
I.06

Connect the M12 power supply to the dimmer and screw it. You do not have to push strongly, but simply tighten the screw mechanism

Plug the power supply to the power socket.

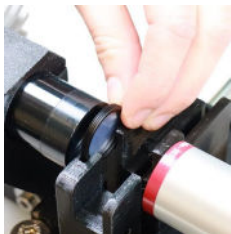
Note: If light comes out of the LED you can turn the dimmer off. In some version of the platform, the sticker is misleading, and you have to turn the knob the other way to turn off the light

If the dimmer does not dim the LED light, it may be because the power supply switch (not shown) needs to be changed.



I.07

Place the optical diffuser into its dedicated socket



SOFTWARE INSTALLATION

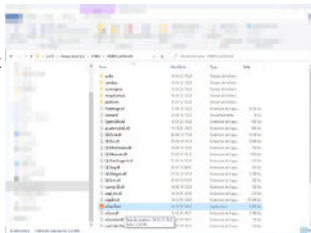
- Install Fluigent's software provided with the USB stick
- Download and install the Ximea software from Ximea's website
https://www.ximea.com/support/wiki/apis/XIMEA_Windows_Software_Package
- As their software is continuously updated, it is good to check from time to time for updates

CAMERA BASIC USAGE

I.08

The Ximea software package does not install any shortcuts on the desktop, therefore you must find the software XiCamtool.exe manually in the file explorer. On Windows and with a default installation, it is located here:

C:\XIMEA\XIMEACamTool64



I.09

Click on the play icon at the top left to launch the camera view

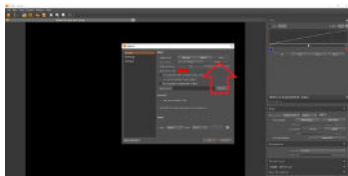


I.10

To enhance the observation of the emulsion formation, several things must be setup.

Go to Edit->option

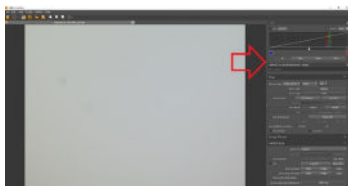
In the display option, switch to Guru mode to have access to important parameter



I.11

Start the LED light. You can use it to its highest power.

To optimise the visualisation of the parameters on the right panel, drag the menu up to the bottom of LUT window



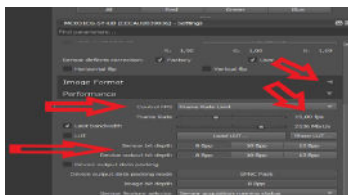
I.12

The automatic control of the software can interact poorly with the lighting system.

Therefore, you must:

- uncheck Auto exposure
- uncheck Auto white balance
- set the Exposure manually to the minimal value that provide a good exposure

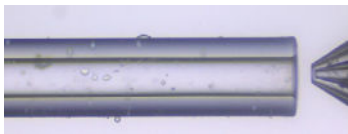
Note: The emulsion produced inside the Raydrop flows at a high speed. Therefore, a good practice is to set the light to its maximum power and set the exposure to the minimum value.



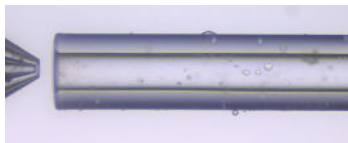
I.13

Depending on your visualisation preference, you can check or uncheck the Horizontal flip option.

Horizontal flip uncheck



Horizontal flip check

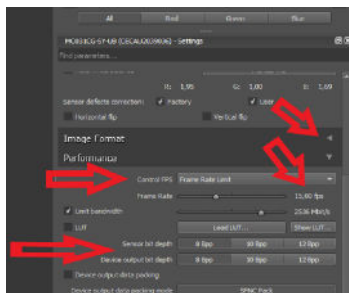


I.14

Hide the Image Format menu

In the Performance menu:

- Select Frame Rate Limit in the Control FPS
- Set a Frame Rate of 15fps or 30fps
- Set the Sensor bit depth and Device output bit depth to 8 Bpp



FLUIGENT PRESSURE CONTROLLER BASIC USAGE

For the use of the Fluigent pressure controller, please refer to [Fluigent's manual](#).

USING THE RAYDROP PLATFORM TO PRODUCE DOUBLE EMULSION

Prior to use the platform with expensive and/or hazardous material it is strongly advised to experience the production of emulsion with safe and inexpensive material like the one presented below.

Here, we will cover the use of the Raydrop Platform to produce water in oil in water emulsion (w/o/w). The Raydrop used is the standard version with a 30-70 DE nozzle (30µm inside diameter (ID) for the Core phase, 70µm ID for the Shell phase) and a 150µm ID collection's capillary. The three phases used to produce the double emulsion are:

- Continuous phase: Water with 2% of Tween20 (surfactant), filtered with a 0.2µm filter
- Shell phase: Mineral oil with 2% of Abil90 (surfactant), filtered with a 0.2µm filter
- Core phase: Water with 2% of Tween20 and a blue dye, filtered with a 0.2µm filter

Note: The dye used in the core phase helps to see the formation of the emulsion; it is a good help when using the Raydrop Platform for the first time. Here we use water soluble food colorant that is available in any supermarket.

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

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

PRIMING THE SYSTEM

R.01

Fill Falcon with respective solution and place them back in the platform:

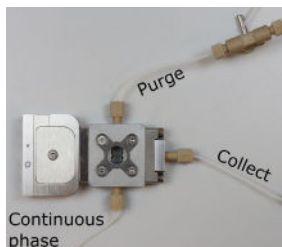
- Continuous phase: Water with 2% of Tween20
- Shell phase: Mineral oil with 2% of Abil90
- Core phase: Water with 2% of Tween20 and a couple of drop-lets of blue dye



R.02

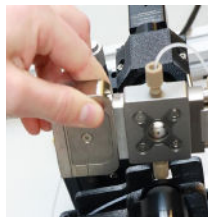
Connect to the Raydrop:

- the purge: tubing with on/off valve
- the collect tubing: 50 cm 250µm ID, one end blue fitting
- Continuous phase tubing: 25 cm 250 µm ID both end yellow fitting



R.03

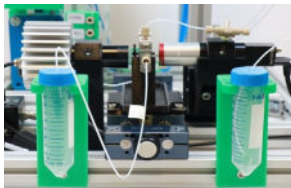
Place the Raydrop in its sample holder; use the groove to guide the continuous phase tubing



R.04

Attach to the right side of the platform both Falcon holder.

Place the purge tubing in one falcon and the collect tubing in the other falcon.



R.05

Connect the continuous phase tubing to the platform



R.06

Switch all 4-way valves to the position Reservoir-Waste



R.07

Place all the waste tubing inside a container; Here we use a GL45 bottle with the supplied GL45 pierced cap



R.08

Start the pressure controller

Start the camera software

Start Fluigent software

Change the type of fluid measured by the Flow Unit for the Shell phase to Oil (in Fluigent software)

Increase gradually the pressure for the Continuous phase up to 500 mbar.

After a couple of minutes, you should see a flowrates measurement.

As soon as the liquid flows out the waste tubing, you can set the Continuous pressure to 0 mbar



R.09

Increase gradually the pressure for the Shell phase up to 500 mbar.

After a couple of minutes, you should see a flowrates measurement.

As soon as the liquid flows out the waste tubing, you can set the Shell pressure to 0 mbar



R.10

Increase gradually the pressure for the Core phase up to 500 mbar.

After a couple of minutes, you should see a flowrates measurement.

As soon as the liquid flows out the waste tubing, you can set the Core pressure to 0 mbar



R.11

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.



To prevent liquids to flow naturally from the Falcon to the waste container, switch all the

4-way valves to the position Waste-Waste.

R.12

Switch the Continuous 4-way valve to Reservoir-Raydrop



R.13

Open The purge valve



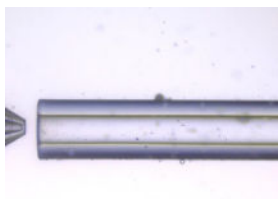
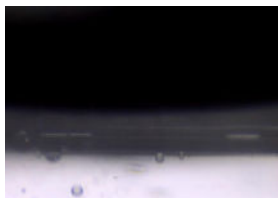
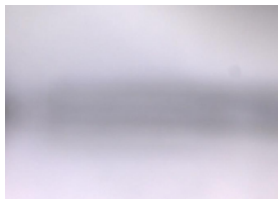
R.14

Increase gradually the pressure for the Continuous phase until you reach a flowrate of 400 $\mu\text{L}/\text{min}$.

After less than a minute, you will see the continuous phase filling the Raydrop chamber in the camera view, revealing the Raydrop nozzle.

The focus of the Raydrop, will be adjusted later, once that every tubing is connected to the Raydrop.

Note: in some rare case, the Raydrop nozzle might be not visible in the camera view due to poor transportation condition. A procedure to find the nozzle and adjust the focus will be described later in this manual.



R.15

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

Let the continuous phase flows in the purge waste Falcon for 30 seconds.

Close the purge valve.

Set the pressure to 0 mbar.



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

- flangeless fitting (two parts, like the blue one)
- super flangeless fitting (three parts, like the yellow one).

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 with respect to 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).

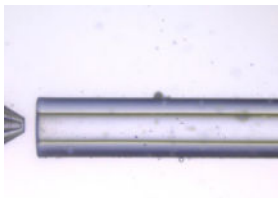
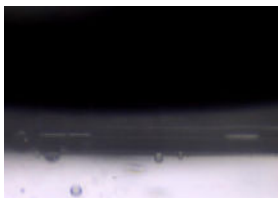
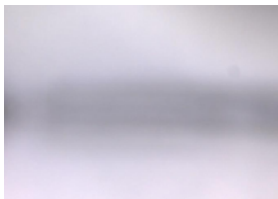
R.14

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Note: in some rare case, the Raydrop nozzle might be not visible in the camera view due to poor transportation condition. A procedure to find the nozzle and adjust the focus will be described later in this manual.



R.15

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Let the continuous phase flows in the purge waste Falcon for 30 seconds.

Close the purge valve.

Set the pressure to 0 mbar.



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

- flangeless fitting (two parts, like the blue one)
- super flangeless fitting (three parts, like the yellow one).

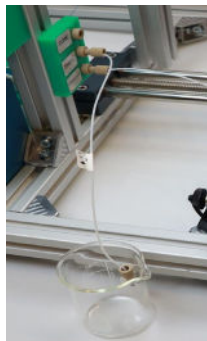
The super flangeless fittings offer a great benefit compared to the flangeless fittings: you don't twist the tubing while screwing the nut. However, this advantage comes with one major drawback: the tubing is not perfectly aligned with respect to the thread. As the inlet diameter of the core and shell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

R.16

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.

First connect the Shell tubing (20cm, 250 μ m ID, blue and yellow fittings) to the platform. As explained earlier, the yellow fitting will be connected to the platform and the blue one to the Raydrop.

Place the blue fitting in a beaker



R.17

Switch the Shell 4-way valve to Reservoir- Raydrop

Increase gradually the pressure for the Shell phase up to 500 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, set the Shell phase pressure to 0 mbar



R.18

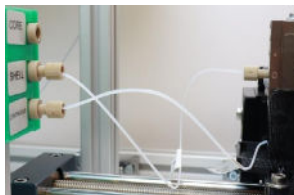
Switch the Shell 4-way valve to Reservoir- Waste



R.19

Connect the Shell phase tubing to the Raydrop:

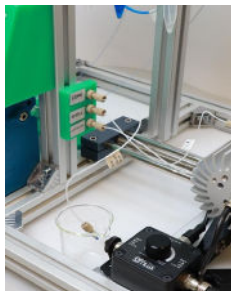
- To avoid any twisting of the tubing, loosen the Shell fitting on the platform side
- Flush the blue fitting with acetone or air to remove any dust
- Tighten the blue fitting to the Shell inlet of the Raydrop. The Shell inlet is the one with the large circle mark
- Tighten the yellow fitting on the platform side



R.20

Connect the Core tubing (20cm, 250µm ID, blue and yellow fittings) to the platform. As explained earlier, the yellow fitting will be connected to the platform and the blue one to the Raydrop.

Place the blue fittings in a beaker.



R.21

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

Increase gradually the pressure for the Core phase up to 500 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.



R.22

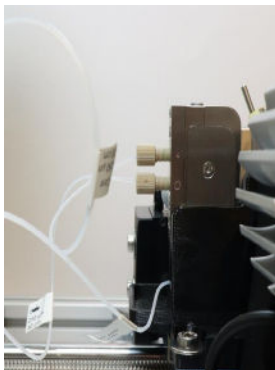
Switch the Core 4-way valve to Reservoir- Waste.



R.23

Connect the Core phase tubing to the Raydrop:

- To avoid any twisting of the tubing, loosen the Shell fitting on the platform
- Flush the blue fitting with acetone or air to remove any dust
- Tighten the blue fitting to the Core inlet of the Raydrop, the one with a dot
- Tighten the yellow fitting on the platform side



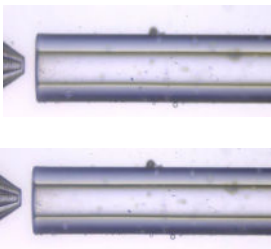
ADJUSTING THE OPTICAL SETUP

R.24

You can now adjust the optical setup to fit your needs.

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
- Move the camera along the Z axis or the Raydrop along the X axis to have a good overview



R.25

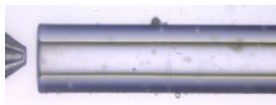
In some case, due to poor transportation condition or manutention, the focus of the Raydrop might be not good at all.

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. Move slowly 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

Out of focus Nozzle



Almost in focus Nozzle



In focus nozzle



CREATING DOUBLE EMULSION

Note: It is best to work in pressure mode with the pressure controller instead of flowrate mode when starting the Raydrop system. Fluigent's 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 mode as it is more convenience to use.

R.26

Check that the Continuous 4-way valve is set to Reservoir-Raydrop and both Shell and Core 4-way valves are set to Reservoir- Waste



R.27

Increase gradually the pressure in the continuous phase to obtain a flowrate ~200 $\mu\text{L}/\text{min}$

R.28

Increase the pressure of the Shell phase to obtain a flowrate of 20 $\mu\text{L}/\text{min}$

R.29

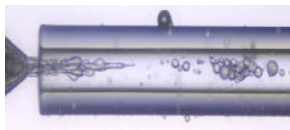
Switch the Shell 4-way valve to Reservoir- Raydrop

R.30



Adjust the Shell pressure to obtain a 20 $\mu\text{L}/\text{min}$ Shell flowrate. This will help flushing the nozzle from the remaining water and air. First, a mixture of oil, water and air flows through the nozzle.

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.



R.31

After approximatively 30 seconds, a clean jet of Shell phase flows out of the nozzle.

R.32

It is a good idea to look at the nozzle and check if there is no bubble that are trapped inside of it

To remove it, increase the Shell phase flowrate.

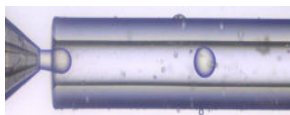
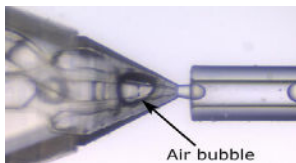
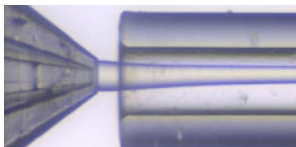
R.33

When everything is clean, decrease the Shell pressure to reach a Shell flowrate of $\sim 8 \mu\text{L}/\text{min}$.

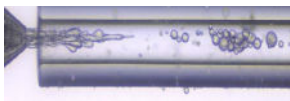
R.34

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

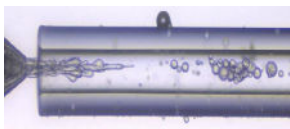
- When you increase the Continuous flowrate (by increasing the Continuous pressure) while keeping a constant Shell flowrate: Shell droplet size decreases until reaching a plateau.
- The minimum droplet diameter will depend on the fluid system and the geometry
- When you decrease the Continuous flowrate (by decreasing the Continuous pressure) while keeping a constant Shell flowrate: Shell droplet size increases.



Increase of Continuous phase flowrate, decrease of Shell droplet;



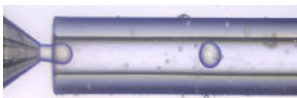
Decrease of Continuous phase flowrate, increase of Shell droplet;



- When the Shell flowrate is too large compared to the Continuous phase flowrate, a jetting regime will be obtained; in such a case, decreasing the Shell flowrate will revert to a dripping regime.

R.35

Adjust again the Continuous pressure to reach a Continuous flowrate of $\sim 200 \mu\text{L}/\text{min}$ and the Shell pressure to obtain a Shell flowrate of $\sim 8 \mu\text{L}/\text{min}$



R.36

Increase the pressure of the Core phase to obtain a flowrate of $40 \mu\text{L}/\text{min}$

R.37

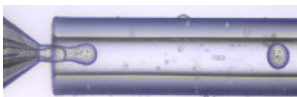
Switch the Core 4-way valve to Reservoir- Raydrop

R.38

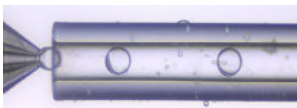
Adjust the Core pressure to obtain a $10 \mu\text{L}/\text{min}$ Core flowrate.

First, the remaining mixture of air/continuous phase/shell phase must be flowed out of the Raydrop

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.

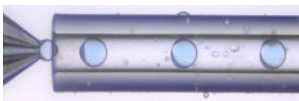


Air bubble in Shell phase droplet



R.39

Quickly after that, the double emulsion formed with your mixtures appears, as seen on the right with the blue dye.



Congratulation, you have formed your first double emulsion with the Raydrop!

R.40

You can now play with all flowrates to adjust your double emulsion

STOPPING THE SYSTEM

R.41

Stopping the system is much easier than starting the emulsification process.

First, switch the Core 4-way valve to Reservoir-Waste and set the Core pressure to 0 mbar.



R.42

Switch the Shell 4-way valve to Reservoir- Waste and set the Shell pressure to 0 mbar

R.43

Reduce slowly the Continuous pressure to reach around 50 $\mu\text{L}/\text{min}$ for the Continuous phase

R.44

To avoid any pollution of the chamber from the Shell or Core phase, it is a good practice to let the pressure inside the system



R.45

Switch the Shell and Core 4-way valves to Raydrop-Waste to completely decrease the pressure inside the Core



R.46

After approximately 15 seconds, switch the Core and Shell 4-way valves to the position Waste-Waste to completely stop the flow for these two phases



R.47

Switch the Continuous phase 4-way valve to Reservoir-Waste and set the Continuous pressure to 0 mbar.

R.48

Switch the Continuous phase 4-way valve to Waste-Waste to completely stop the flow for the Continuous phase.



GLOSSARY

- Falcon: plastic tube for centrifugal process that are used, in combination of a PCap, as a pressurised reservoir
- PCap: air-tight metal cap that allows to pressurize a Falcon



Figure 5: Falcons with PCap

- Fitting: ensemble of nut and ferrule



Figure 6: fitting (Super flangeless)

- Nut: hard material screwed to apply a pressure on the ferrule



Figure 7: nut

- Ferrule: soft material used to seal the tubing

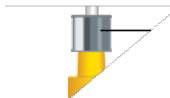


Figure 8: ferrule (yellow part)

- ID: internal diameter