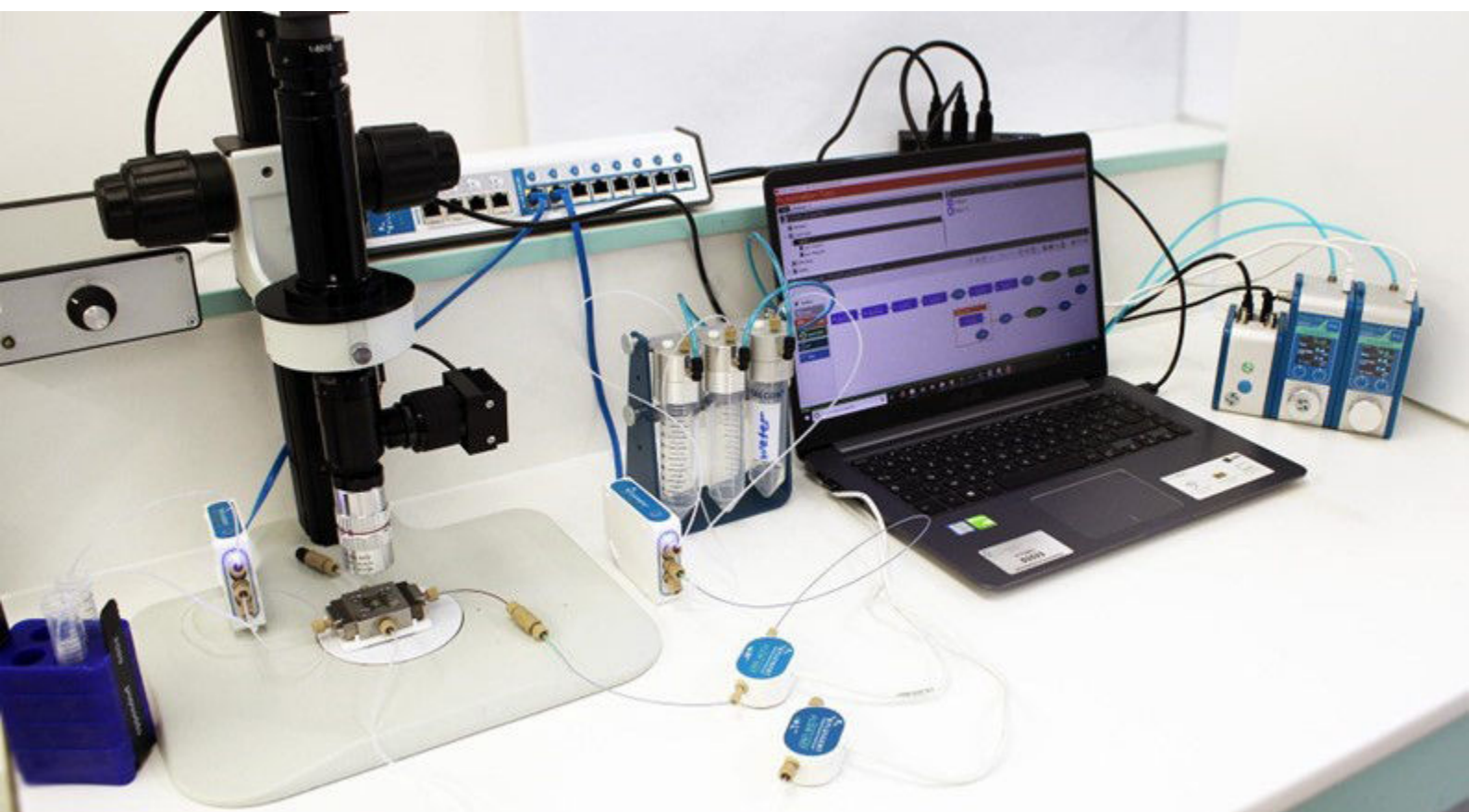


LIPOSOME NANOPARTICLES PRODUCTION STATION

PRODUCT DESCRIPTION

P/N: O-MIX-LIPO-PCK

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



CONTENT

Starting an experiment	p2
Liposome nanoparticles production	p4
Stopping Experiments	p6
Possible issues and cleaning procedure	p7

STARTING AN EXPERIMENT

As a reminder, The different inlets and outlets are shown on the following scheme:

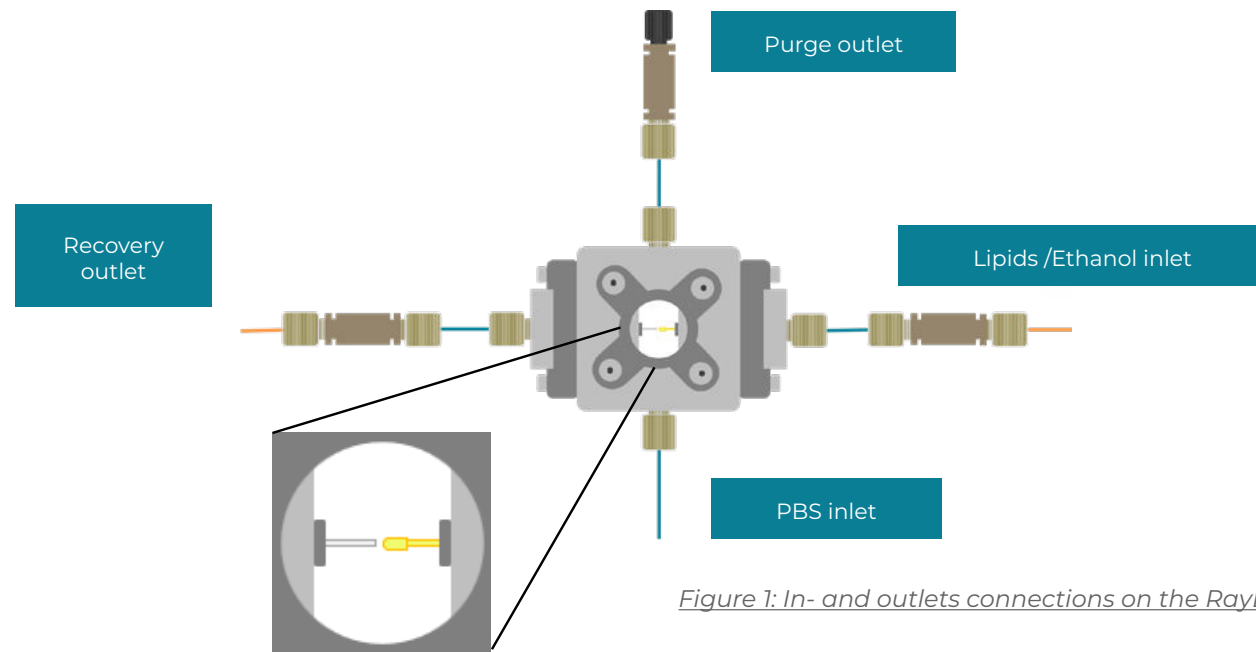


Figure 1: In- and outlets connections on the RayDrop

1 Assemble the entire set-up as shown below depending on which pack you use (the use of filtered solution is highly recommended). Depending on your application, installing inline filters (included in the kit and their fittings) between the RayDrop and the Flow Units is highly recommended.

Example: Liposome nanoparticles production station package*

* Same procedure for PLGA nanoparticles production station package

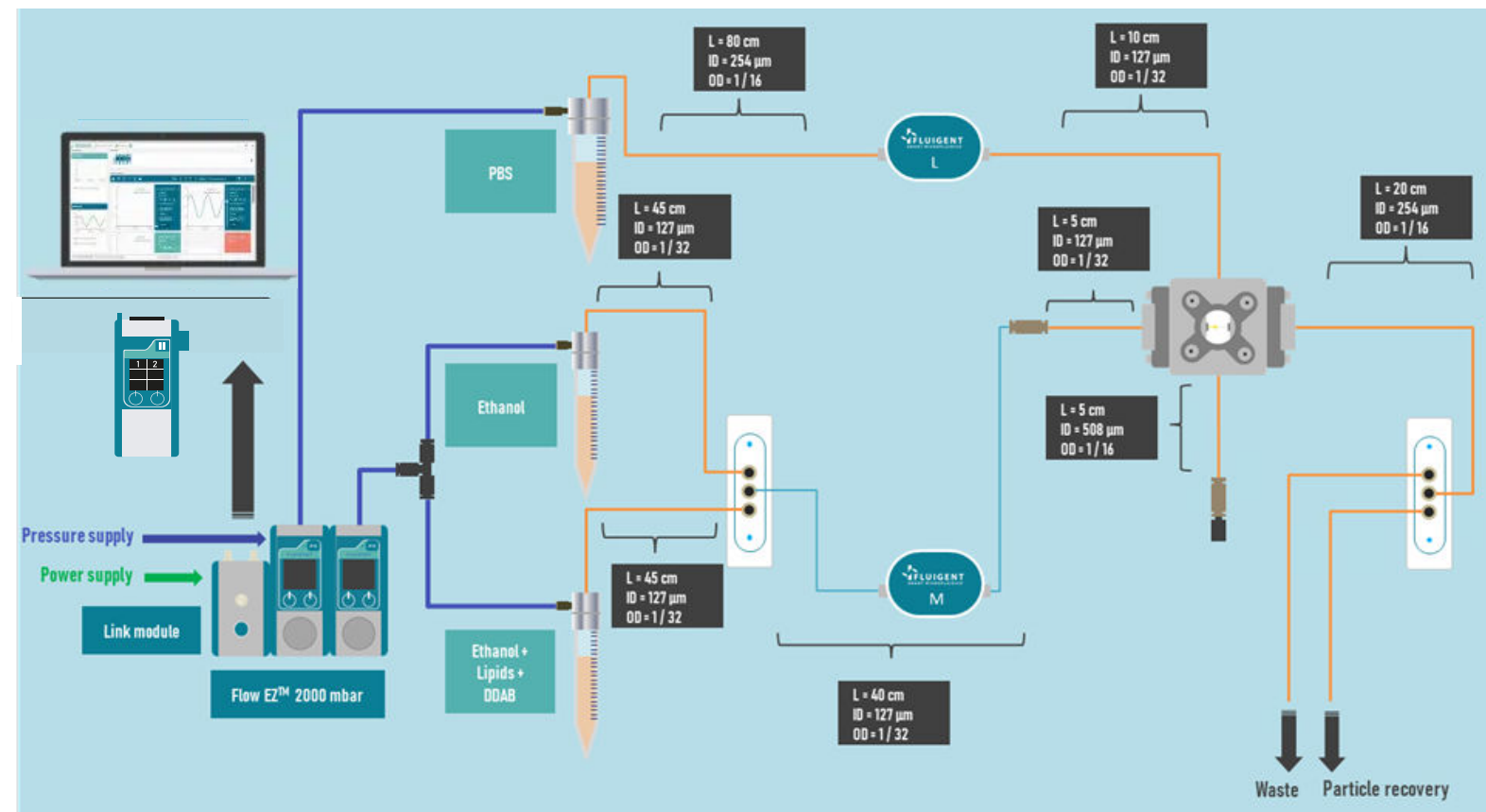


Figure 2: Set-up of Alginate microbeads production station (full package)

2 Disconnect all channels except the PBS inlet. Set a pressure of approximately 1bar on the PBS inlet to start filling the RayDrop chamber (It could be more than 1 bar to have a faster filling)

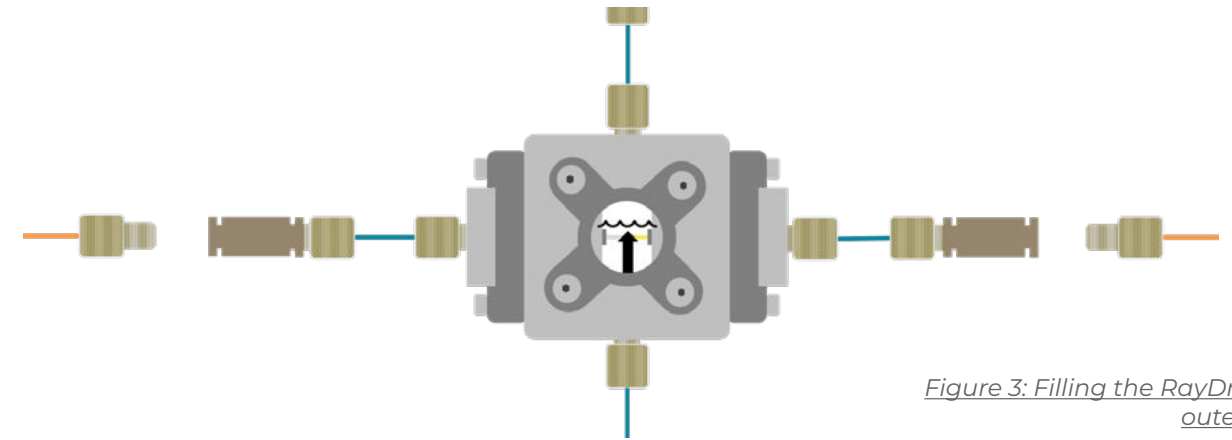


Figure 3: Filling the RayDrop with outer phase

3 Hold the RayDrop vertically to evacuate the air through the purge outlet



Figure 4: Holding the RayDROP to evacuate the air

4 When outer phase is coming out of the purge outlet, use a plug to close the purge outlet. Continuous phase will flow out of the other in-outlets.

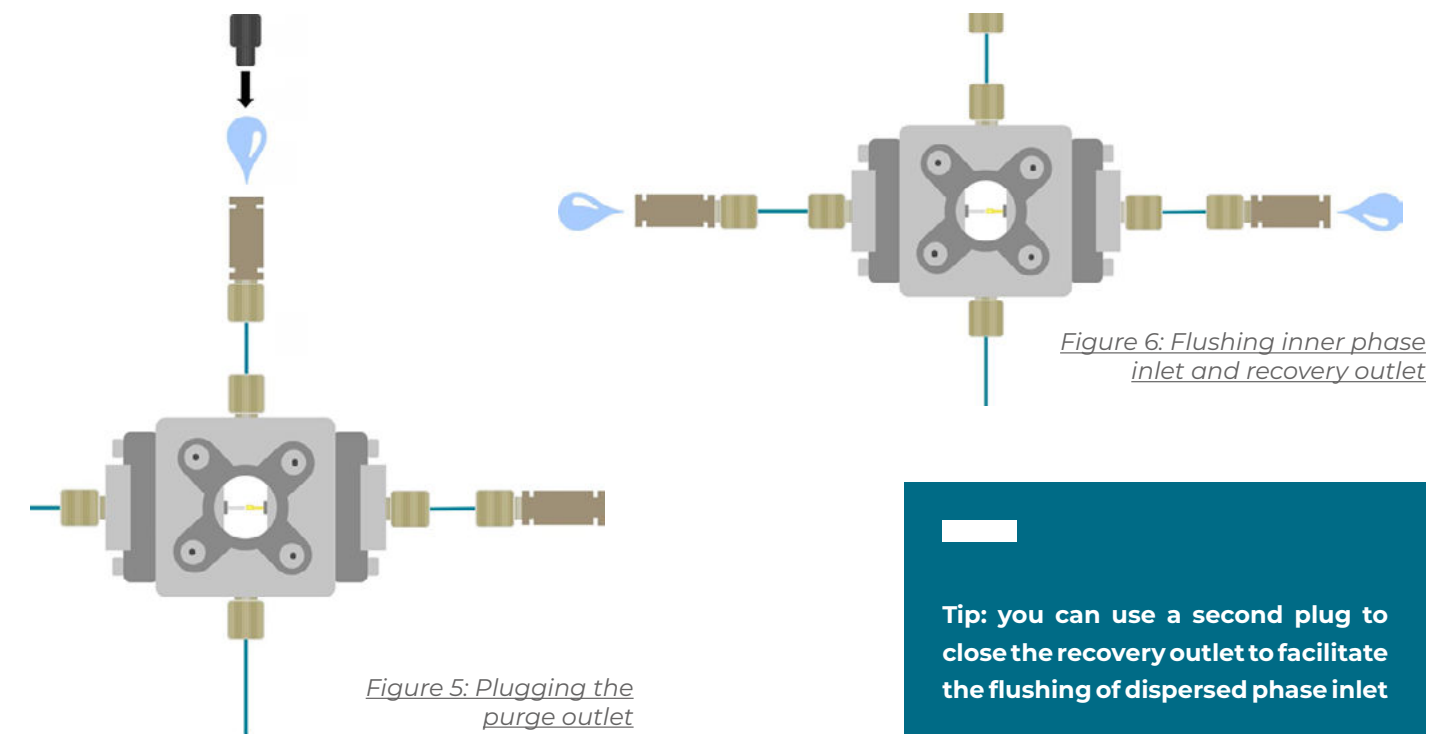


Figure 6: Flushing inner phase inlet and recovery outlet

Tip: you can use a second plug to close the recovery outlet to facilitate the flushing of dispersed phase inlet

At this point, the RayDrop is ready to start the Lipids microbeads production

LIPOSOME NANOPARTICLES PRODUCTION

With a filled RayDrop, follow the next steps to produce lipids microbeads

5 Set the PBS pressure to 900 mbar. Start applying pressure (~100 mbar) on the Ethanol inlet. Ethanol should flow into the Lipids/Ethanol inlet which is still disconnected from the RayDrop.

5A – Note the correct position of the valves to direct ethanol through the system and to waste.

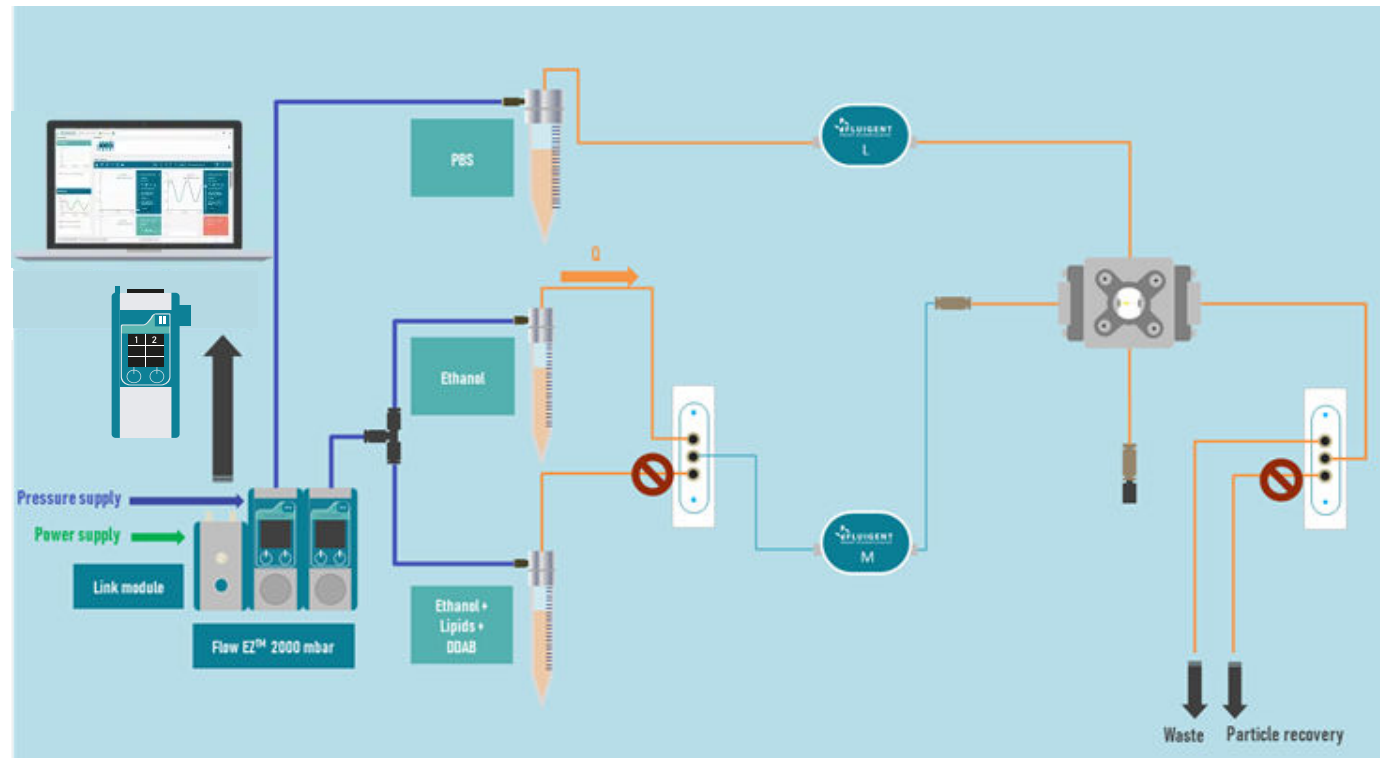


Figure 7: 2-SWITCH™ redirecting Ethanol in waste

6 Once Ethanol is coming out of the tubing at the end of the FLOW UNIT M, connect it to the Ethanol inlet

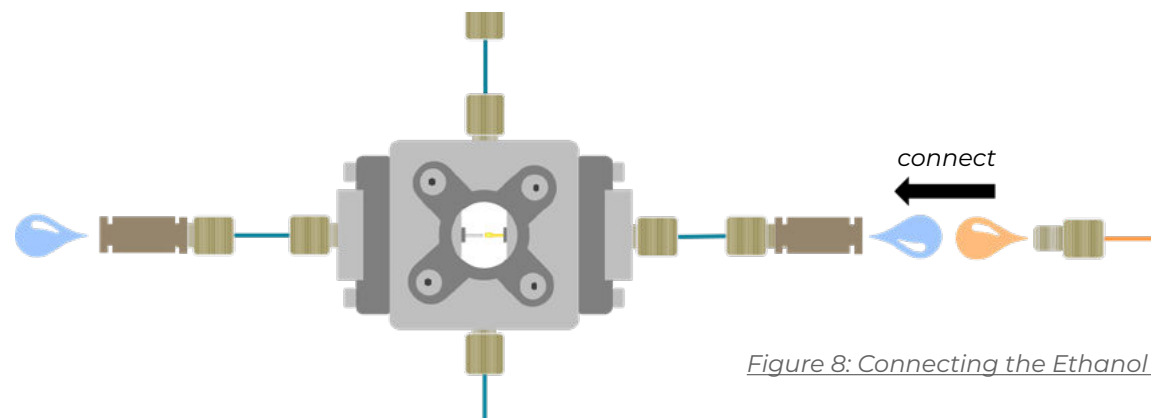


Figure 8: Connecting the Ethanol inlet

At this point, a backflow may occur in the Ethanol channel. This is expected.

Ethanol is then injected as inner phase, and PBS as surrounding outer phase to prime the system and generate a co-flow of ethanol and PBS. This step allows to avoid contact between the inner phase and water in the injection capillary (uncontrolled precipitation of lipids and formation of aggregates, potentially clogging the nozzle).

7 Set the same pressure for both phases. Example:

PBS: 500 mbar | Ethanol: 500 mbar

You should obtain a co flow regime quite quickly (Figure 9)

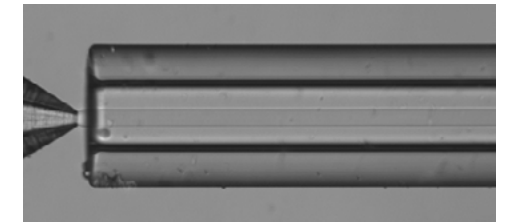


Figure 9: Co flow regime

8 Switch the 2-SWITCH™ to the Ethanol + Lipids reservoir. Liposome should be produced in less than 1 min (time for the liquid to switch)

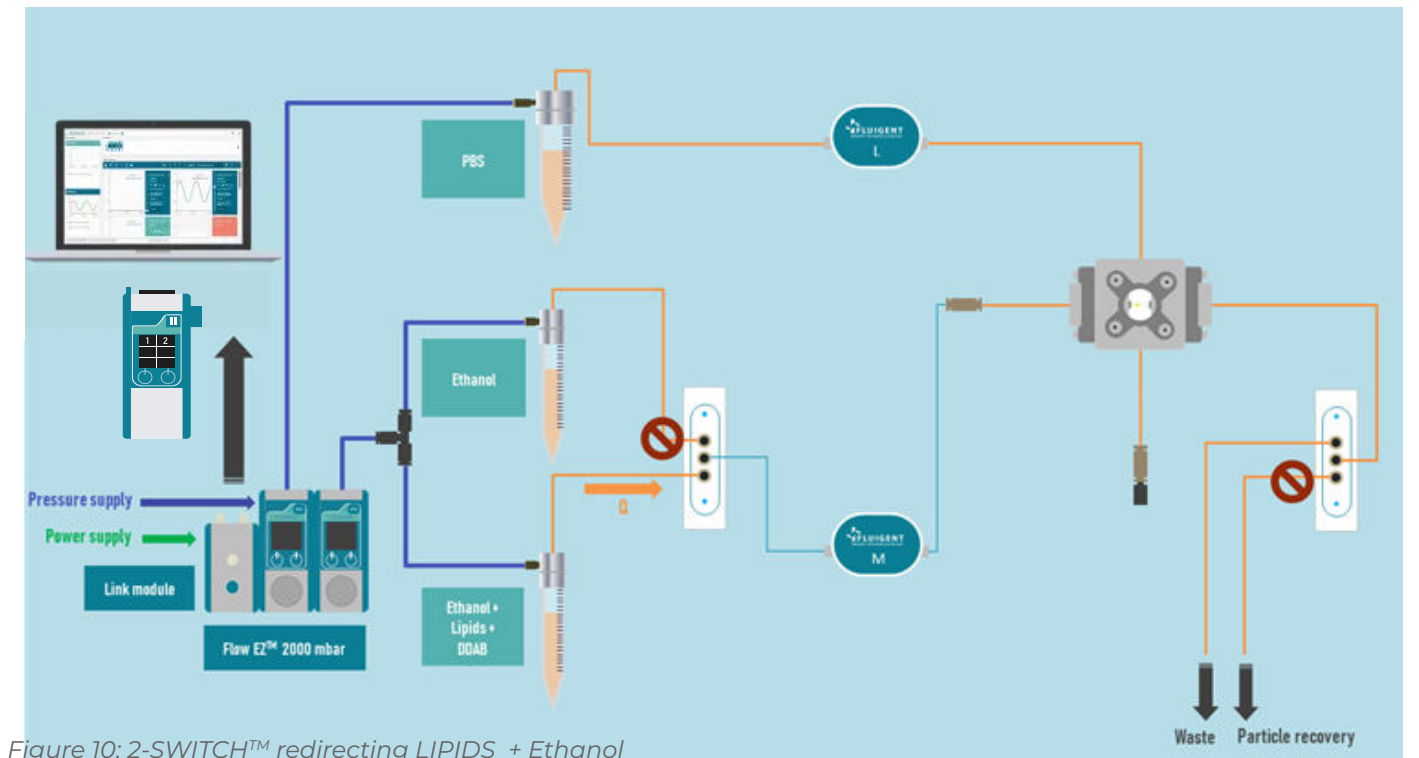


Figure 10: 2-SWITCH™ redirecting LIPIDS + Ethanol

9 Switch After about a minute, switch the second 2-SWITCH™ on the right to your recovery reservoir to collect the Liposomes.

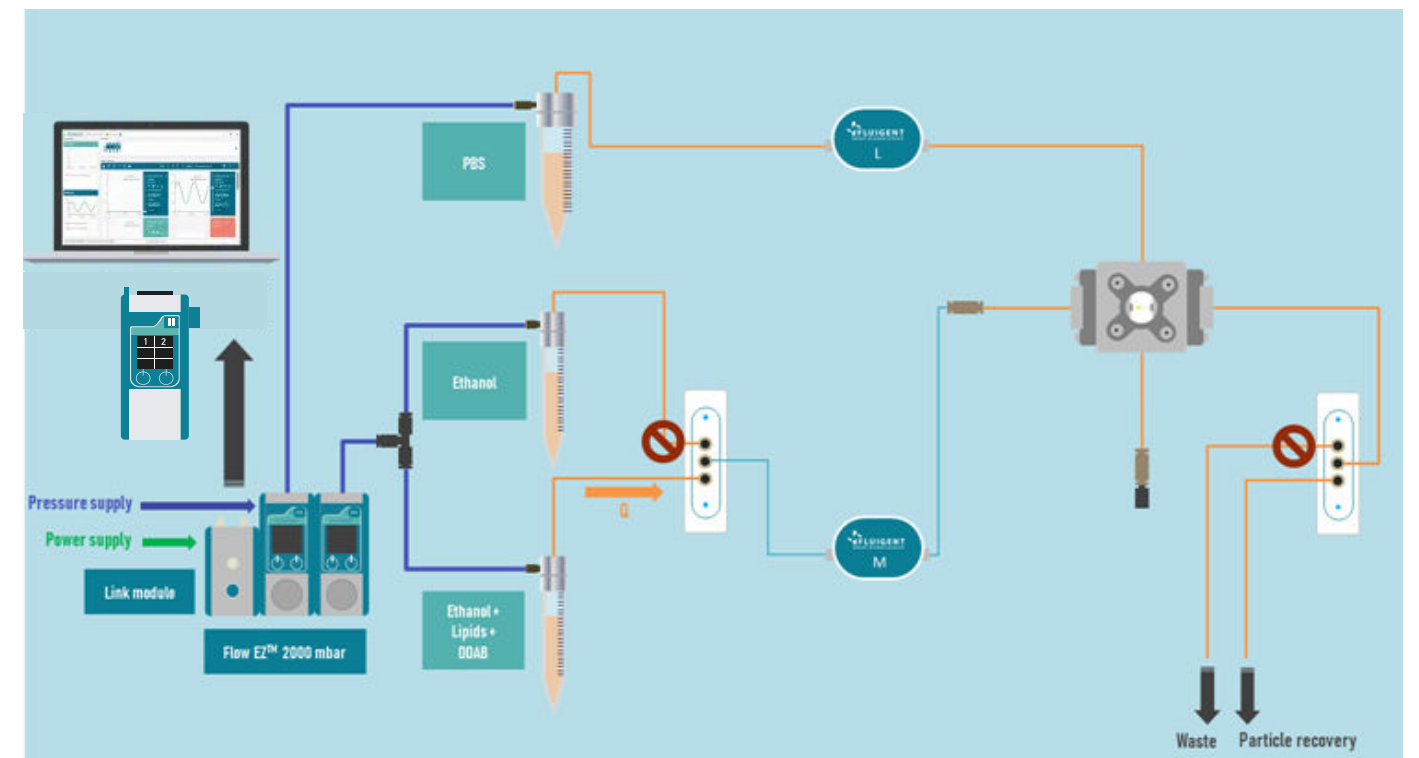


Figure 11: 2nd 2-SWITCH™ in recovering position

10 Pressure and/or flow rate can be then adjusted to target the stream diameter desired TFR and FRR, and the related liposome size (for more information regarding TFR , FRR and relation with liposome size check the liposome nanoparticle production application note)

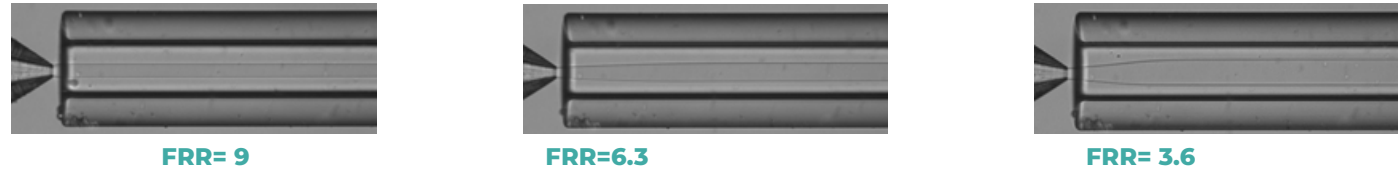


Figure 12: Adjusting pressure and flow rate to change stream diameter.

STOPPING THE EXPERIMENT

The following steps should be performed **after each experiment** in order to prevent any clogging of the RayDrop.

Perform the following steps to stop the experiment.

- 1 Switch the 2nd 2-SWITCH™ (on the right) to the waste reservoir
- 2 Switch the first 2-SWITCH™ (on the left) to the Ethanol reservoir for at least a minute to flush the lipid solution from the system..

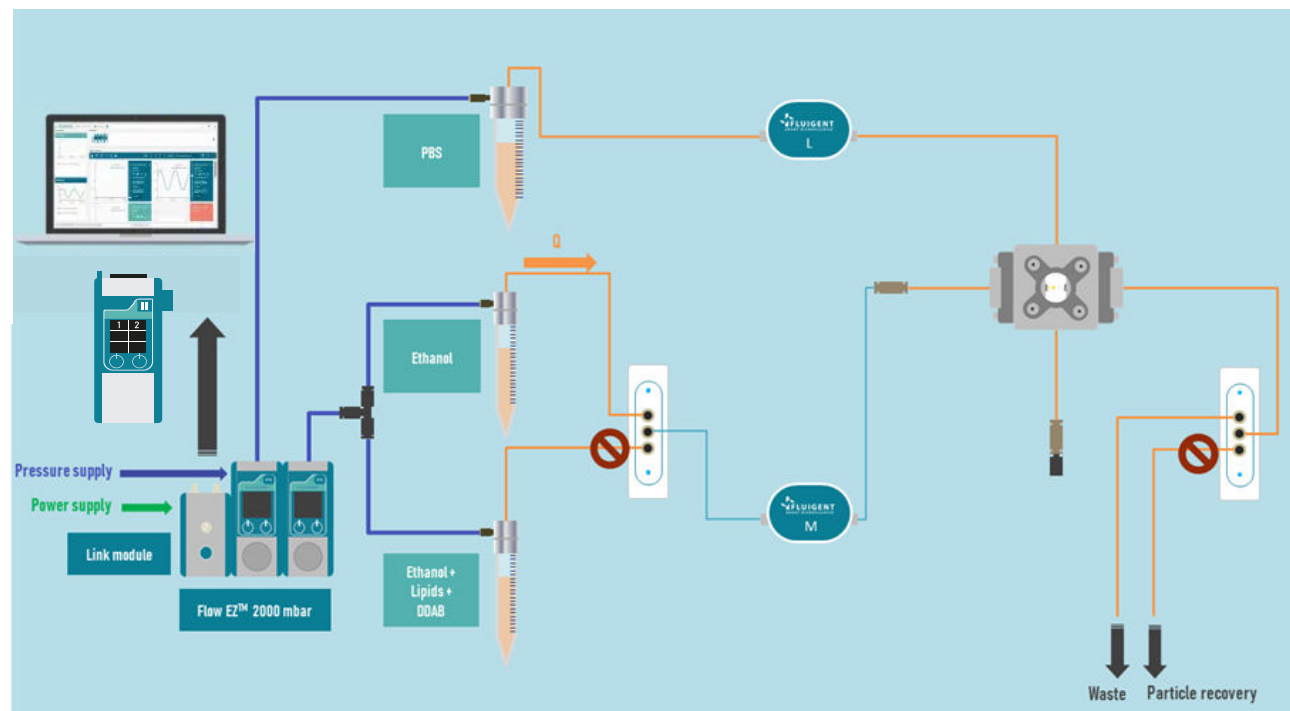


Figure 13: 2-SWITCH™ redirecting Ethanol

- 3 Lower the pressure on the Ethanol until you reach 0 mbar.
- 4 Disconnect the Lipids/Ethanol inlet.

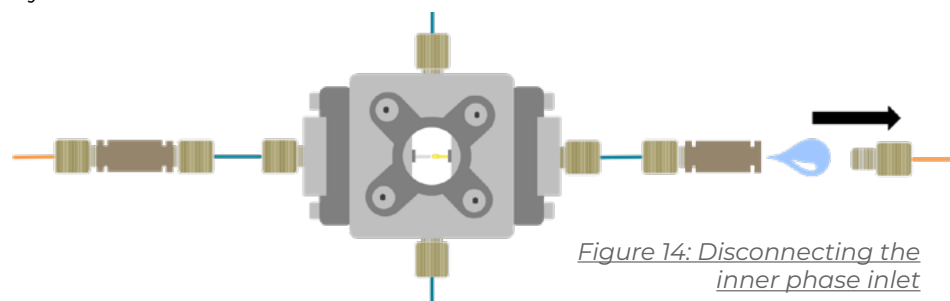


Figure 14: Disconnecting the inner phase inlet

- 5 Keep some pressure on the PBS inlet and flush for about a minute

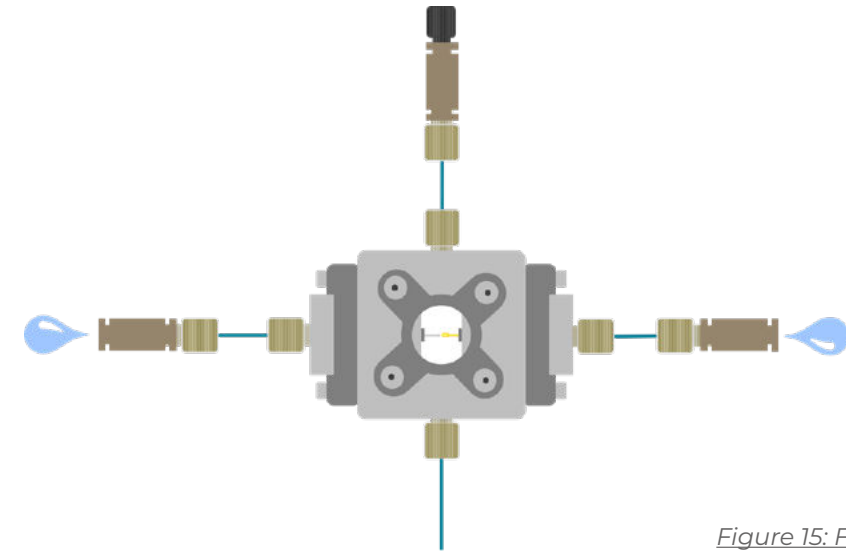
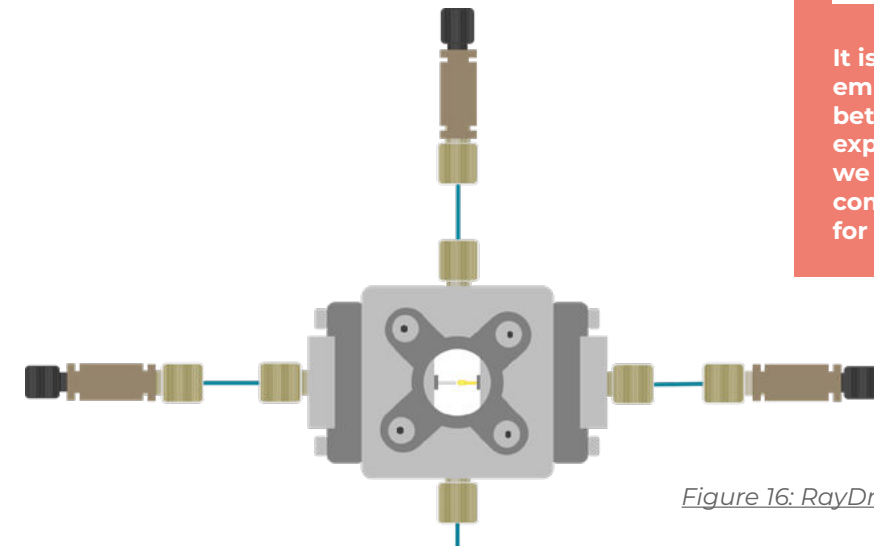


Figure 15: Flushing the RayDrop

- 6 Plug all outlets with plugs until the next experiment



It is not mandatory to empty the RayDrop between each experiments. Nevertheless we recommend emptying completely if it is unused for long periods (> 3 days)

Figure 16: RayDrop with plugged in-outlets

POSSIBLE ISSUES AND CLEANING PROCEDURE

Some of the Ethanol has flown into the outer phase chamber or has aggregated on the outside of the nozzle

If some Ethanol (without Lipids) flows into the chamber you can continue your experiment if it is only a small quantity that does not affect your experiment.

If there is a larger quantity that disturb visualization of droplet formation, perform the following steps:

- 1 Stop the flow of Water
- 2 Disconnect the Lipids/Ethanol inlet

The same procedure can be used if some Ethanol is fixed on the outside of the nozzle.

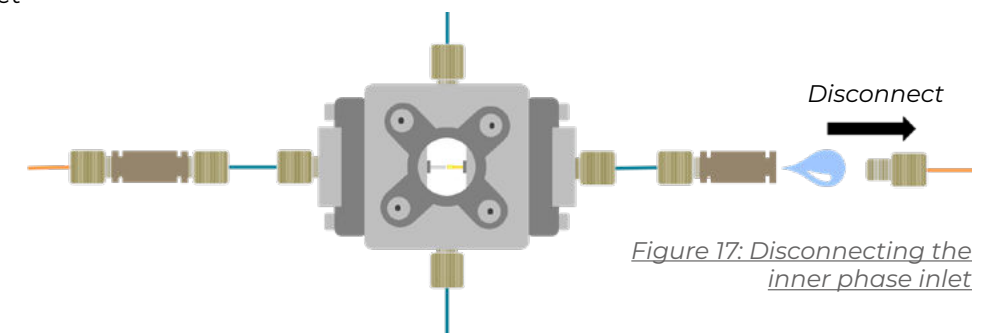


Figure 17: Disconnecting the inner phase inlet

3 Open the top plug

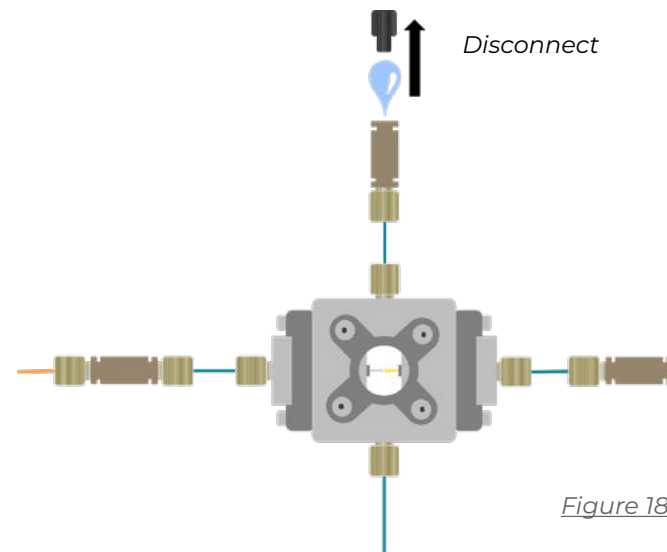


Figure 18: Unplugging the purge outlet

- 4 Flush with the PBS solution approximately for a minute or until you see all the Ethanol has disappeared.
- 5 Restart your experiment as described previously in the «Starting an experiment» section.

Some Ethanol solution has flown into the chamber or is fixed on the outside of the nozzle

If some LIPIDS solution has flown into the chamber perform the following steps quickly:

- 1 Switch back your 2-SWITCHTM or manual valves to the Ethanol solution and let flow for 30 seconds
- 2 Disconnect the Lipids/Ethanol inlet
- 3 Open the top plug

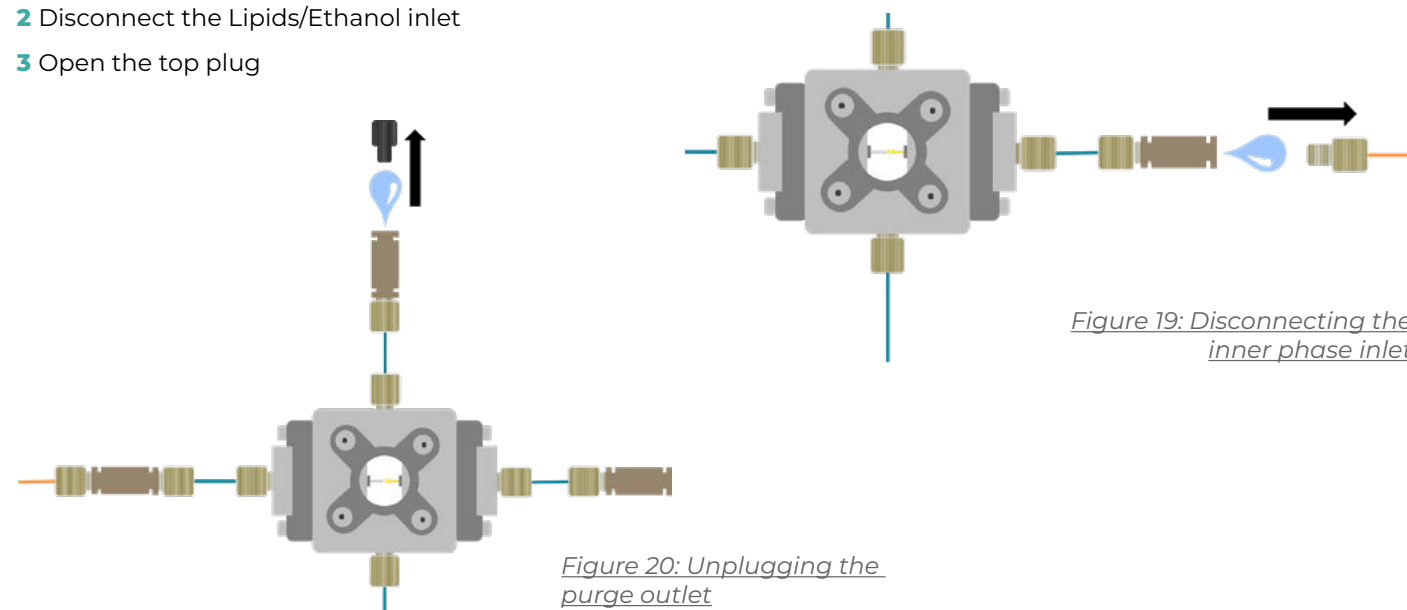


Figure 19: Disconnecting the inner phase inlet

Figure 20: Unplugging the purge outlet

- 4 Flush with the PBS solution approximately for a minute or until you see all the Ethanol has disappeared

Some LIPIDS has flown into the chamber

If the Lipids solution has flown in the chamber you can repeat the previous procedure and use the purge to eliminate the Lipids stuck in the chamber.

If this procedure is still not working, you can try to do a complete washing of the RayDrop using the procedure described in the next section.

CLOGGED NOZZLE OR CAPILLARY

In case of a clogged nozzle or capillary you have different process to follow:

Soft cleaning

Let us consider the case where particles, like fiber or dust, are stuck in the nozzle.

- 1 Switch both valves to waste position and ethanol position (Fig 7).
- 2 Place a plug on the exit of RayDrop

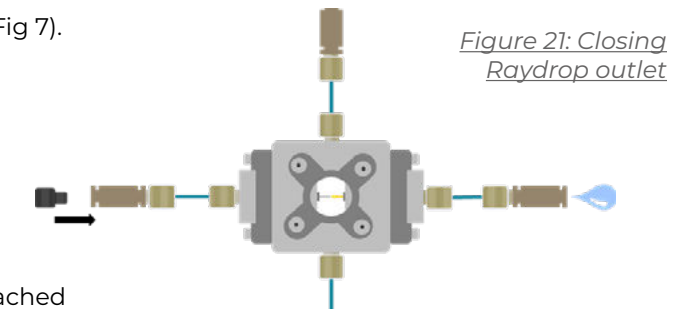


Figure 21: Closing Raydrop outlet

- 3 Ramp up the outer phase flow rate until the particle(s) detached

- 4 Let the flow running for a couple of minutes to be sure that the particle has been removed from the system

- 5 Reduce the outer phase flow rate to zero
- 6 Remove the plug on the outlet

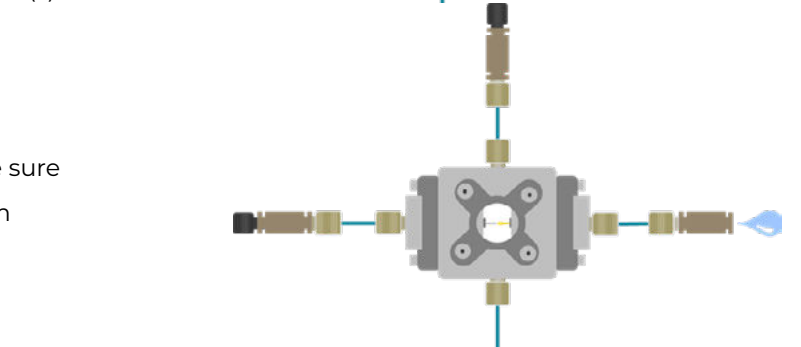


Figure 22: Nozzle flushing

- 7 You are ready to start again your process

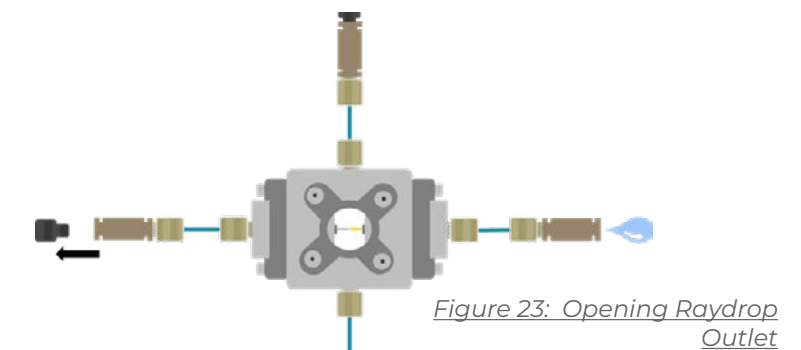


Figure 23: Opening Raydrop Outlet

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 Reproduced previous procedure
- 2 You can directly disconnect the inner phase tubing from the RayDrop after step 4. The pressure drop will decrease and therefore the flowrate will increase.
- 3 After the particle has been removed, wait a minute before continuing
- 4 Before reconnecting the inner phase tubing, drop a few droplets of acetone (or other solvent such as IPA, ethanol...) from a wash bottle on top of the connection; it should prevent any remaining dust to reenter the RayDrop. Continue at step 6 of the soft cleaning procedure

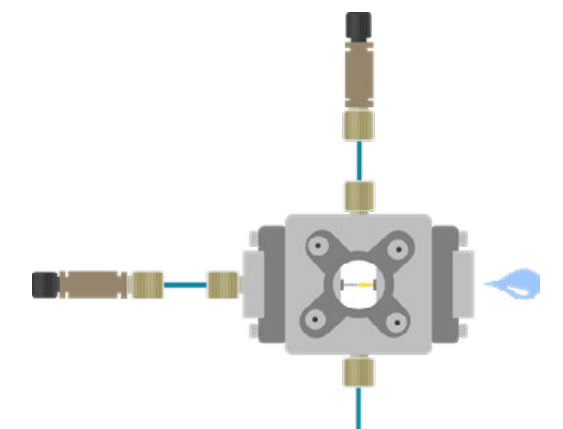


Figure 24: Removing Tubing at Raydrop inlet

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 vertically with the purge capillary at the top (Fig 25).
- 2 Open the purge and let the chamber of the RayDrop purge (Fig 26).
- 3 Fill the chamber of the RayDrop with ethanol or other solvent which could dissolve the inner phase stuck in the nozzle (IPA, Acetone...) (Fig 27).

To ease the process, you can use a 10ml plastic syringe as shown in the picture below.

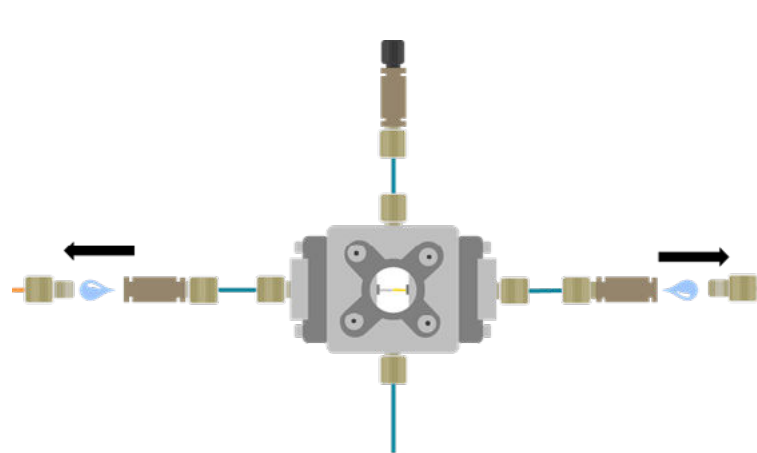


Figure 25: Disconnecting the Raydrop from the fluidic setup

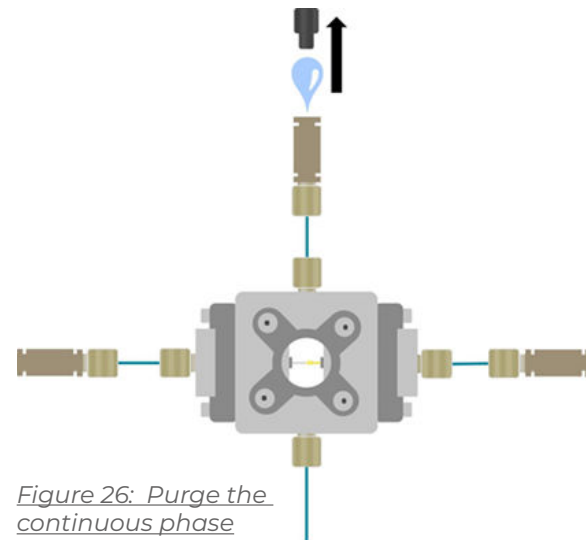


Figure 26: Purge the continuous phase

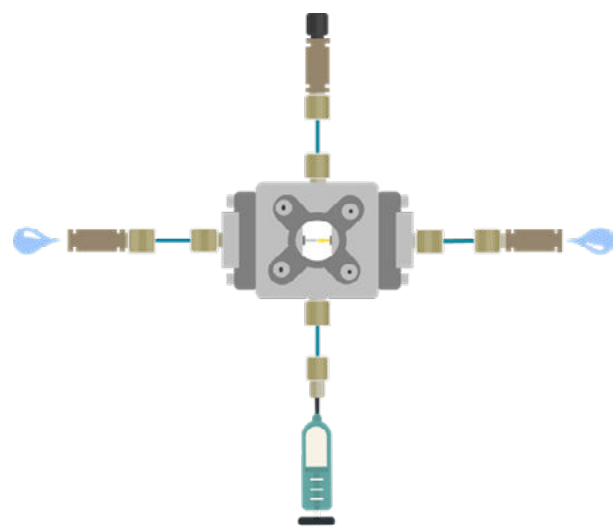


Figure 27: Filling the Raydrop with specific solvent



If you are using other solvents make sure they are compatible with the RayDrop. For more information download the datasheet.

- 4 Close the purge of the RayDrop (Fig 28).
- 5 Apply pressure on the syringe to remove the particle
- 6 Once the particle is gone, continue to apply the pressure for 30 seconds

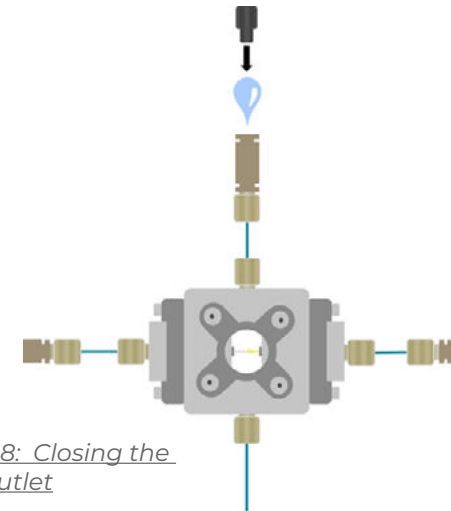


Figure 28: Closing the purge outlet

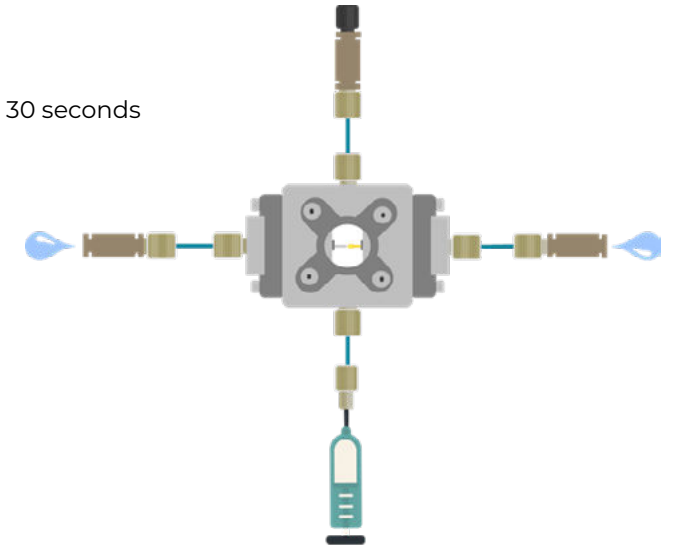


Figure 29: Flushing capillaries with .2µm syringe filter with p-658 and capillary with ID > 0.5mm; the syringe filter has to be forced a bit inside the p-658 to maintain sealing

- 7 Disconnect the syringe tubing (Fig 30).
- 8 Reconnect the RayDrop to your fluidic system (Pressure controller, Flow Unit...)
- 9 Purge the RayDrop by opening the purge capillary by applying pressure (Fig 31).

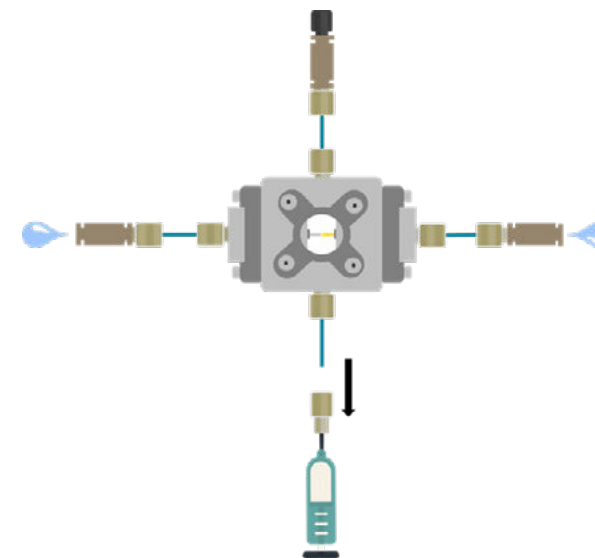


Figure 30: Disconnecting the Syringe with solvent

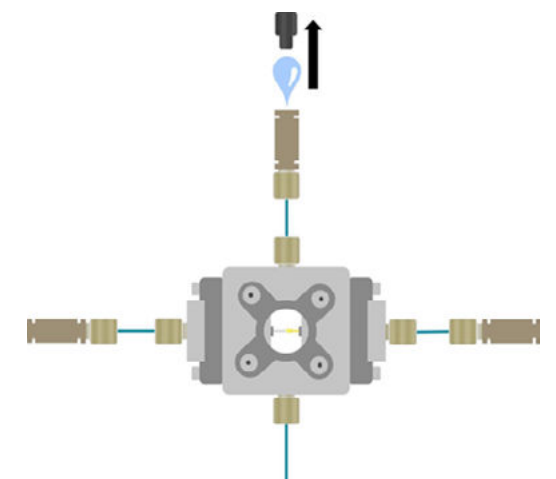


Figure 31: Purging the Raydrop from Solvent solution

To restart the experiment, depending on your continuous phase, you can either completely dry the RayDrop by applying a small gas flow rate inside the chamber, or prime the system with the continuous phase.

Flush the chamber with the continuous phase for a two minutes to remove any remains of solvent (ethanol, IPA...).

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 pressure on the liquid syringe start the ultrasonic bath
- 3 Continue this cleaning procedure for 30 seconds
- 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

In case of a completely clogged nozzle or capillary, contact customer support for assistance.

COMPLETE CLEANING OF THE RAYDROP CHAMBER

In some cases, you might want to refresh the phases inside the chamber of the RayDrop or wish to try other solutions. In this case, the RayDrop chamber should be completely cleaned.

Be careful when performing the following cleaning procedures as the Ray-Drop nozzle and capillary are fragile.

Cavity Emptying

- 1 Hold the RayDrop vertically, with the closed purge outlet to the top.
- 2 Loosen the inlet of the outer phase (the PBS inlet).
- 3 Open the purge outlet at the top. The outer phase should flow out of the bottom outlet (the PBS inlet).

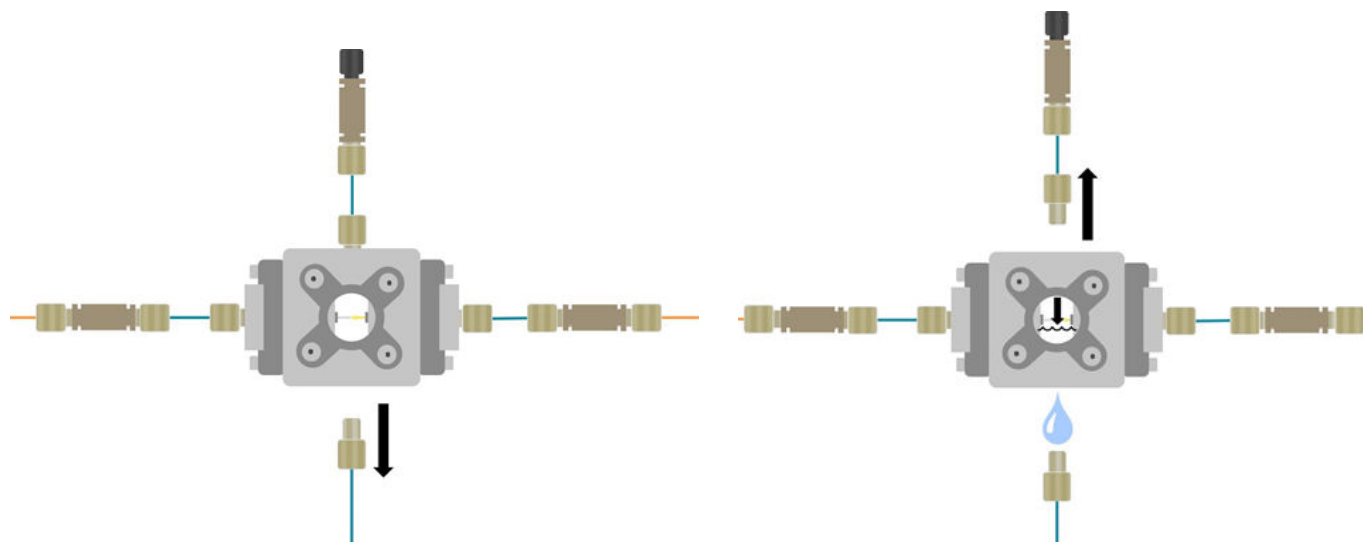


Figure 32: Disconnecting the outer phase inlet

Figure 33: Emptying the RayDrop

Soft cleaning

This operation should be performed if a dust, a particle, or a small amount of the dispersed phase has been accidentally introduced in the cavity and jeopardizes the droplet generation process, or its visualization.

Two cases are considered below:

- » **Case 1:** The contaminant is located on a glass window.
- » **Case 2:** The contaminant is located on the nozzle or glass capillary.

Case 1: The contaminant is located on a glass window.

- 1 Empty the cavity following the instructions for emptying the cavity. Total emptying is not necessary. Just remove enough liquid to avoid leakage when the window will be opened.

- 2 Place the RayDrop on a hard stable surface with the glass window to be cleaned on the top.

- 3 Unscrew the four screws on the X-shaped cover using an Allen key number 3. Use tweezers or forceps to remove the metallic X-shaped cover.

- 4 Use tweezers/forceps to remove the glass window and O-ring

DO NOT disconnect the RayDrop from the fluidic circuit during these operations.

Tip: the glass window and O-ring can stick to the X-shaped cover. In this case separate them carefully following the cavity emptying steps

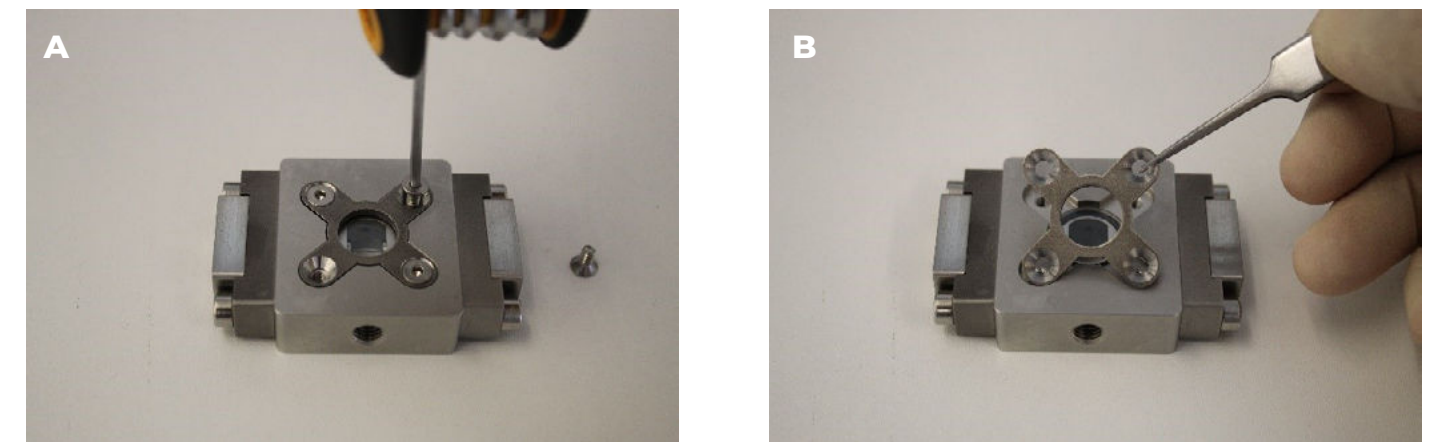


Figure 34: Dismantling the RayDrop A) Removing the screws B) Removing the X-shaped metallic

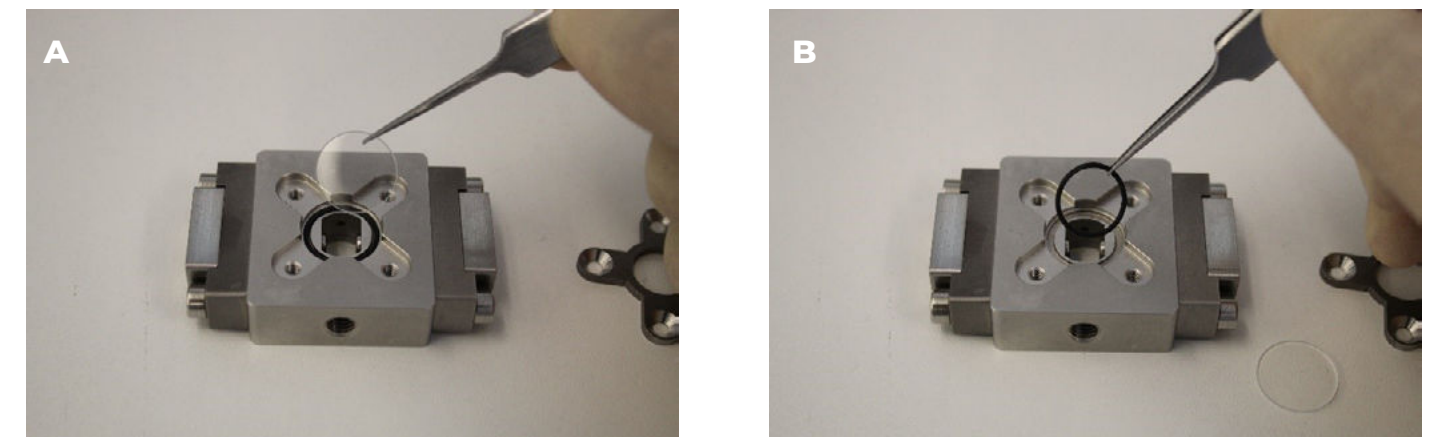


Figure 35: Dismantling the RayDrop A) Removing the glass window B) Removing the O-ring

5 Wash the glass window, O-ring and X-shaped cover using glassware detergent or iso-propanol and dry it carefully. The best results are obtained using glassware detergent and an ultrasonic bath (1 minute). The parts need to be carefully rinsed with water before drying.

6 Install in order the o-ring, glass window and x-shaped cover on the RayDrop body using tweezers.

7 Using the Allen key number 3, tighten the four screws by alternatively giving a screw turn on each in a star shaped pattern. (how tight is there a point where it can break)

Case 2: contaminant on the nozzle and/or the capillaries

1 Completely empty the cavity following the instructions for emptying the cavity

2 Inspect carefully where the contaminant is located: If it is swept away by the continuous phase when emptying the cavity, the following steps are not necessary.

3 Place the RayDrop on a hard stable surface.

4 Completely remove the 4 screws using an Allen key number 3. Use tweezers to remove the metallic X-shaped part.

5 Use tweezers to remove the glass window and O-ring.

6 Using a syringe filled with continuous phase, clear out the contaminant by gently flushing it away.

7 Drain the cavity again to remove the continuous phase added by the rinse as well as the contaminant.

8 Install in order the o-ring, glass window an x-shaped cover on the RayDrop body using the tweezers.

9 Using the Allen key number 3, evenly tighten the four screws by alternatively giving a screw turn on each.

The glass window and o-ring can stick to the x-shaped meta piece. In this case the three parts are removed together so be careful when handling.

COMPLETEY CLOGGED NOZZLE OR CAPILLARY

In case of a completely clogged nozzle or capillary, contact customer support for help.

Tip: the glass window and O-ring can stick to the X-shaped cover. In this case separate them carefully following the cavity emptying steps