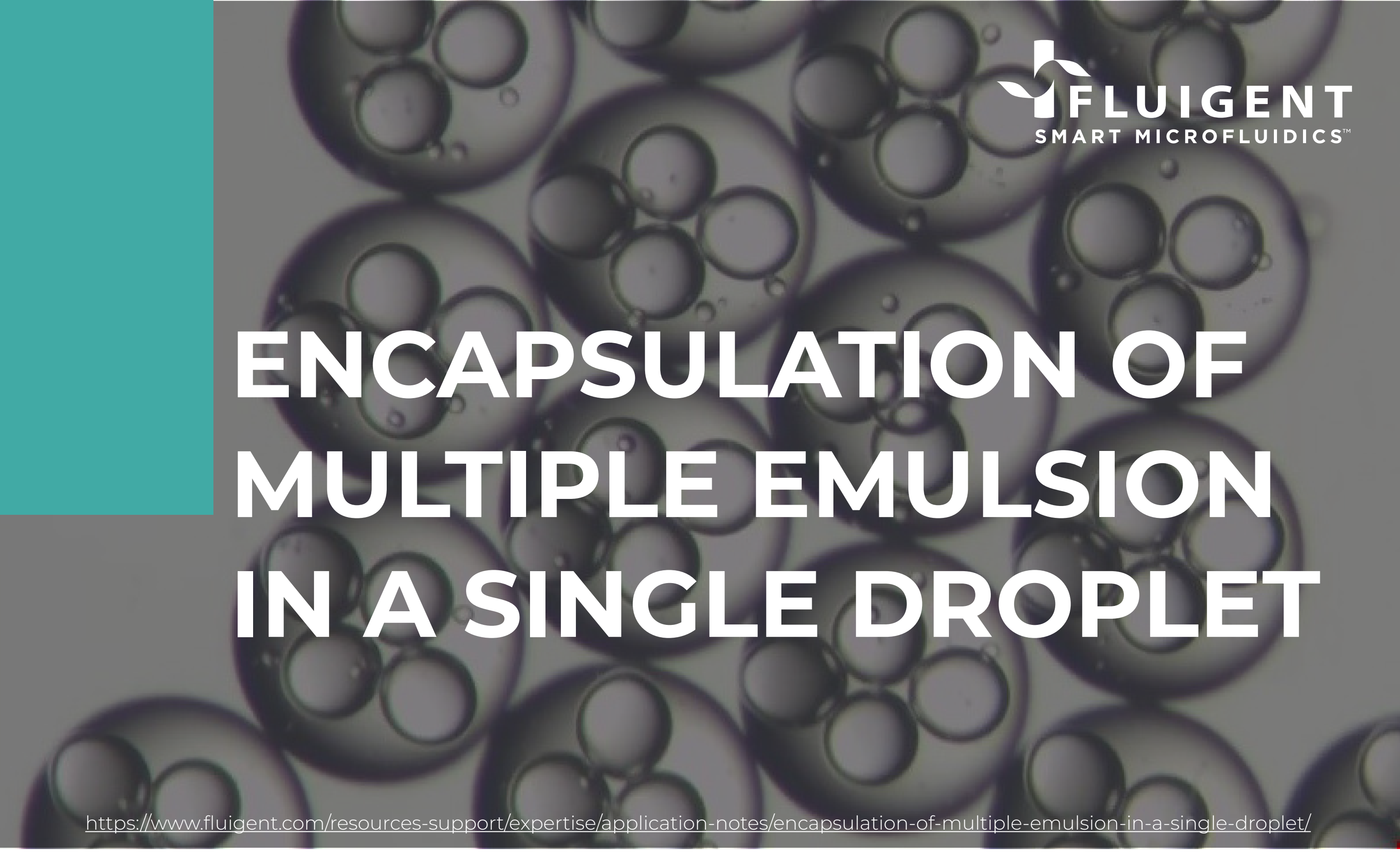
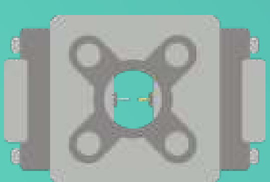


ENCAPSULATION OF MULTIPLE EMULSION IN A SINGLE DROPLET

https://www.fluigent.com/resources-support/expertise/application-notes/encapsulation-of-multiple-emulsion-in-a-single-droplet/

CONTEXT

Traditionally, batch methods are used to produce emulsions in industry. The use of bulk mixing allows one to produce large quantities of emulsions but to the detriment of quality. The particle size distribution in a bulk mixer is large leading to numerous particles sizes and a low encapsulation rate of Active Pharmaceutical Ingredient (API) or double emulsion production (core-shell particles)[1] . Batch methods make the control of multiple emulsions more complex, i.e. the encapsulation of a precise number of droplets of liquid A in a droplet of liquid B.



The use of microfluidics provides monodispersed emulsions with high control over both the size and structure can be obtained[2]. Microfluidic tools are also used to create emulsions of varying compositions. It is possible to produce water–in-oil–in-water (W/O/W) emulsions or oil–in-water–in-oil (O/W/O) emulsions. A microfluidic device developed by Secoya Technologies - called the RayDrop – allows one to easily produce highly controlled emulsions. Examples of applications in single and double emulsion can be found on our website.

Following the work of Li, E. Q. et al [3], the experts of Secoya Technologies demonstrate the ability to produce multiple emulsions using the RayDrop. These multiple emulsions are precursors in the creation of solid microcapsules used for triggered release1. These multicompartmental microspheres are also of interest to co-encapsulate incompatible solutions (which would react if they were in contact)[4].

In this Application Note, aqueous droplets are obtained using the combination of two RayDrop devices placed in series. The influence of the fluidic parameters on the number of cores contained in the oil shell is underlined in this application note.

MATERIAL & METHODS

MATERIALS

Core phase solution:

Water containing 2% Tween 20 (viscous liquid, Sigma-Aldrich)

Shell phase solution:

Mineral oil light (Sigma-Aldrich) containing 2% ABIL EM 90 (Evonik) and 0.08% Bromocresol Purple (Sigma-Aldrich).

Here, bromocresol is used as a dye.

Continuous phase solution:

Solution of 70% wt glycerol (>99.5%, Sigma-Aldrich) in water with 2% Tween20 (Sigma-Aldrich)

Glycerol is added in the continuous phase to increase its viscosity. The viscosity of the continuous phase influences droplet formation in the microfluidic device and as a consequence the number of cores into shell droplets. It is also possible to work with pure water at low viscosity but the reachable number of cores would be decreased.

PLATFORM DEVICE

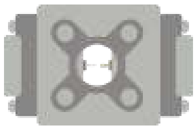
The production of droplets is performed with the RayDrop platform, a fully integrated system comprising all the components needed to produce simple and double emulsions. This platform is divided into three parts: mechanics, fluidics and optics (see Figure 1).

More information about this platform can be found here:

https://www.fluigent.com/research/instruments/packages/complex-emulsion-production-platform/.

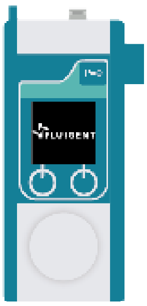
Figure 1: Complex Emulsion Production Platform

Mechanics:



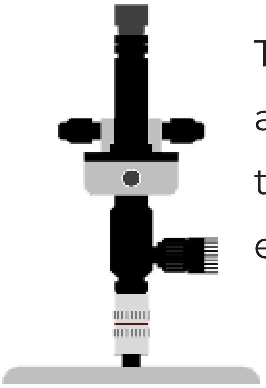
The mechanical part includes x-y-z displacement stage that enable to adjust the focus and the observation window of the RayDrop.

Fluidics:



The fluidic part consists of three Flow EZ™ pressure-based flow controllers with the required tubing and valves, allowing for automated fluidic injection. A pressure is set on each reservoir, and fluids are delivered to the microfluidic chip. It also includes Falcon reservoirs and the RayDrop, in which emulsions are generated. After each reservoir, a filter prevents impurities from reaching the RayDrop.

Optics:



The optical part of the platform contains a LED light source and a color USB 3.0 camera. This camera is connected to a computer to observe the droplet formation in live, control the stability of the emulsion and measure the size of droplets core and shell.

A schematic representation of the setup used for this work is depicted in Figure 2. Please note the particular configuration in which a second RayDrop (R2) is placed next to the first RayDrop (R1).

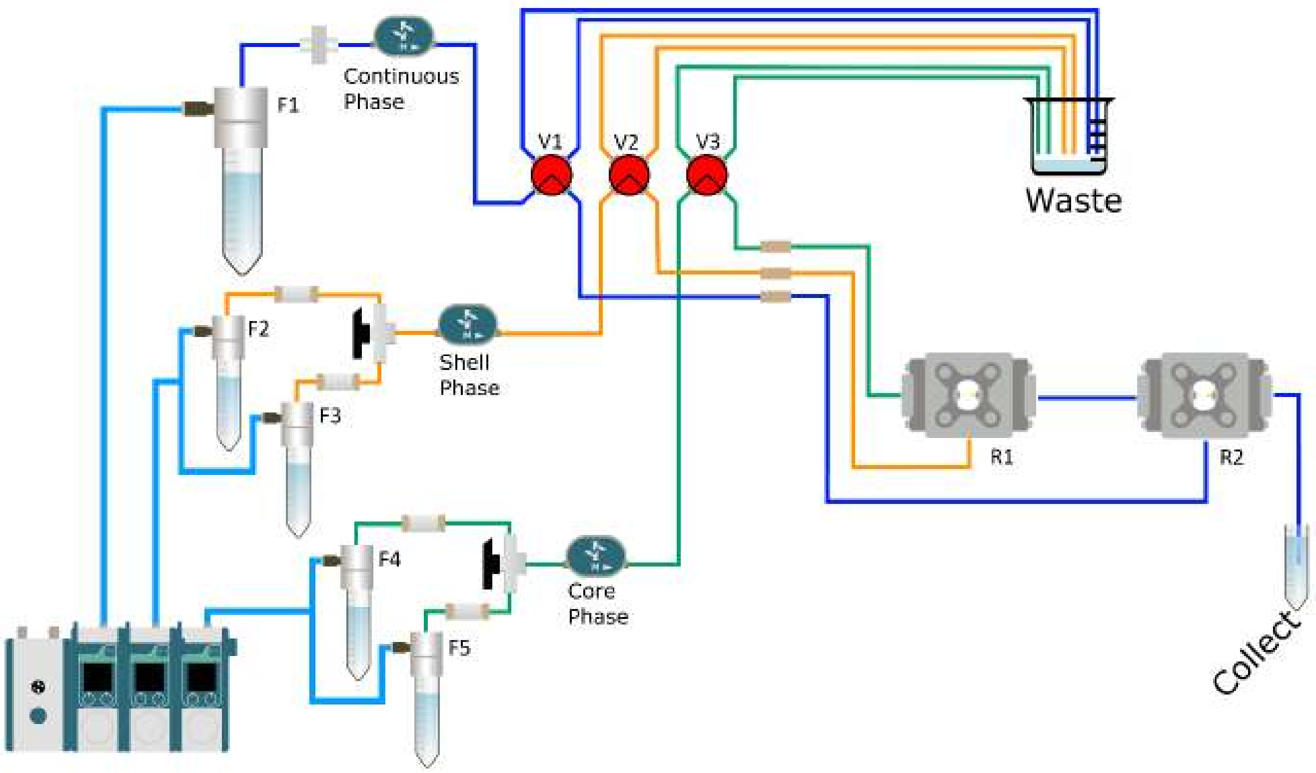


Figure 2: Experimental set-up to produce double emulsion with multiple cores inside a single shell

Fluid reservoirs

Reservoir	Volume (mL)	Phase[1]	Composition
F1	50	Continuous	70% glycerol + 30 water + 2% Tween 20
F2	25	Shell (priming & cleaning)	Not used in this application note
F3	25	Shell	Mineral oil light + 2% ABIL 90 + 0.08% Bromocresol
F4	25	Core (priming & cleaning)	Not used in this application note
F5	25	Core	Water + 2% Tween 20

Table 1: Reservoirs phase and composition

Each phase is filtered to avoid clogging the tubing or the RayDrop nozzle. There is an integrated filter after each reservoir on the platform. In this case, the continuous phase filter has a 10µm pore size and the shell and core filters have a 2µm filter pore size.

RayDrop configuration

The RayDrop is a microfluidic droplet generator. This emulsification device is based on the alignment in a metallic cavity of two glass capillaries: the first one is terminated by a 3D printed nozzle and injects the droplet phase in the second one (see Figure 3). At the junction of both capillaries, the continuous phase filling the cavity pinches the jet of droplet phase, leading to the formation of droplets with a high monodispersity. More information on the RayDrop can be found in the publication of Dewandre A., et al. [5].

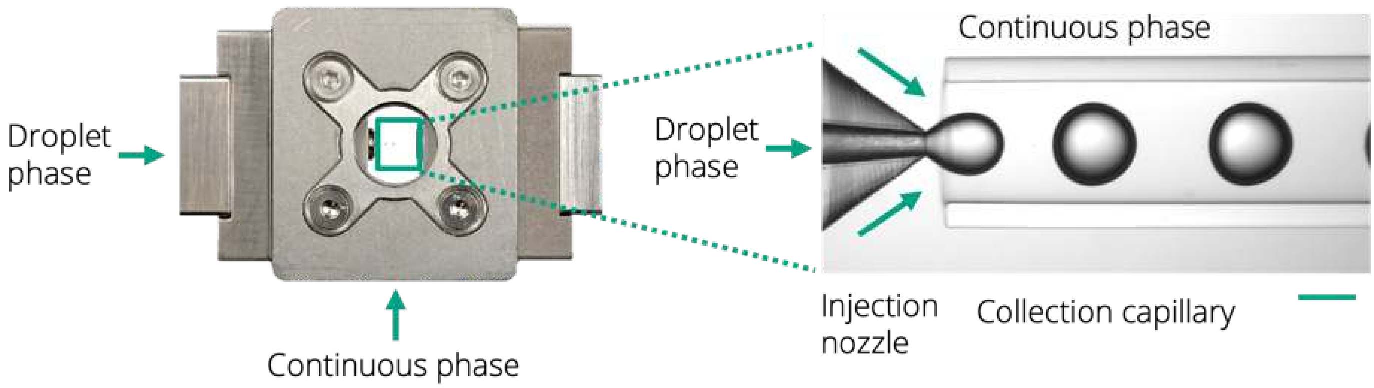


Figure 3: Working principle of the RayDrop single emulsion device

In this case, two RayDrop are placed in series. The first is placed on the platform as usual and the second is added after, as shown in Figure 2. More details can be seen in Figure 3. To control the formation of droplets inside the second RayDrop, an additional camera and an additional LED light source are used.

Droplets formed in RayDrop 1 (R1) are transported as the "shell" phase (see Figure 4) in RayDrop 2 (R2). Here, the "shell" phase is pinched by the continuous phase, leading to the formation of a shell droplet encapsulating multiple core droplets.

The range of droplet sizes formed depends on the dimension of both the injection nozzle and collection capillary. The dimensions used for this application note are reported in Table 2.

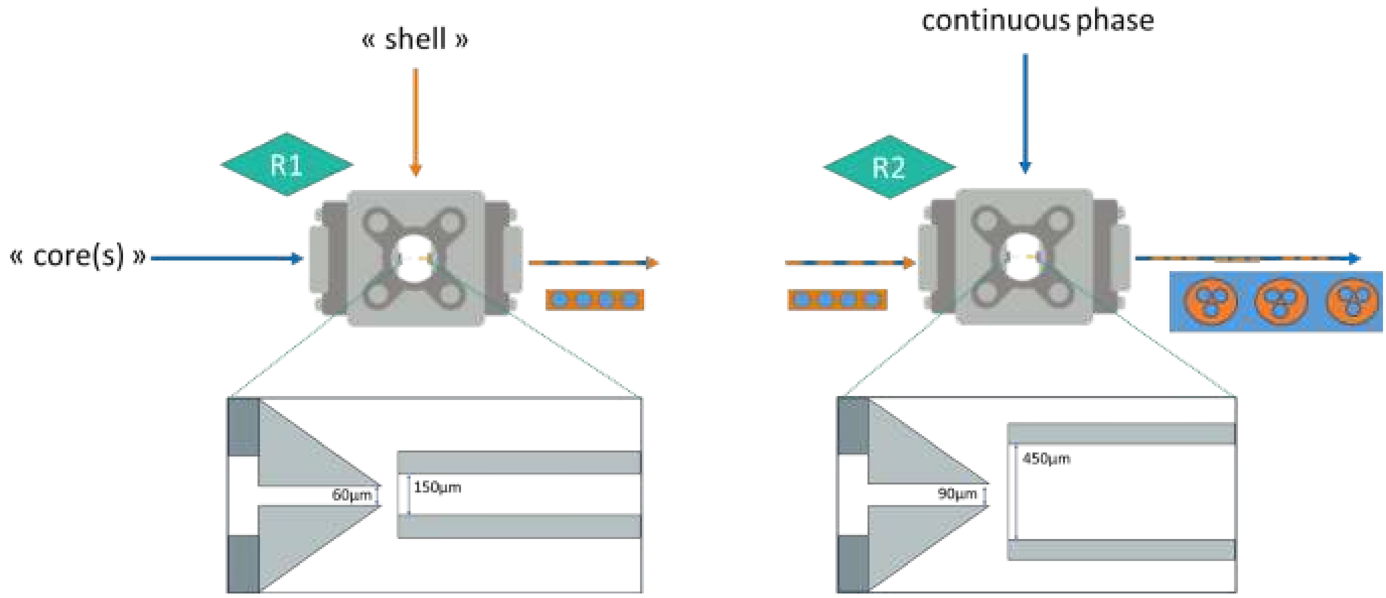
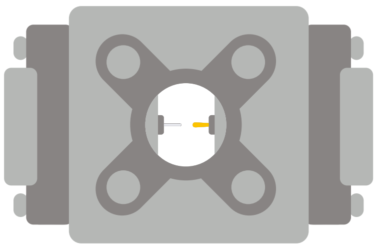


Figure 4: Two RayDrop placed in series (their nozzle dimensions are displayed)

Nozzle information

Part	RayDrop	Nozzle size (µm)	Collection capillary size (µm)
Inner diameter	1	60	150
	2	90	450

Table 2: Nozzle and collection capillary dimensions



EMULSION GENERATION

Oily double emulsions with multiple cores are formed. To generate droplets, the protocol below can be followed. Let the two RayDrop separated, which means that there is no tubing between the outlet of the first RayDrop and the second RayDrop, to fill them in one by one.

1. Set the valve V2 on the reservoir F3 containing the shell phase
2. Set the valve V3 on the reservoir F5 containing the core phase
3. Fill RayDrop R1 with the shell phase solution (refer to the user guide for more details about how to fill the RayDrop)
4. Fill RayDrop R2 with the continuous phase solution
5. Connect the two filled RayDrop together with a tubing
6. Carefully set the continuous phase to a low flow rate (for example, Qconti-nuous= 40µl/min) and check that there is no backflow in the first RayDrop.
7. Set the shell phase to a low flow rate (for example Qshell= 7µl/min with Qshell<Qcontinuous) to create a simple emulsion oil in water visible in the se-cond RayDrop.
8. Set the core phase to a low flow rate (for example Qcore= 1µl/min) to generate simple emulsions in R1 which leads to the generation of multiple emulsions in R2.

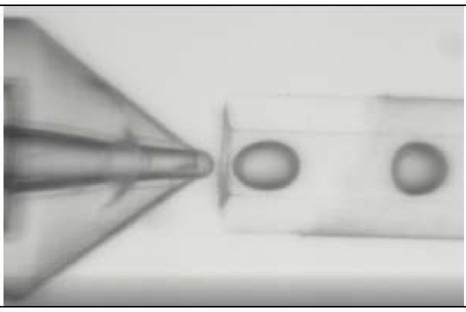
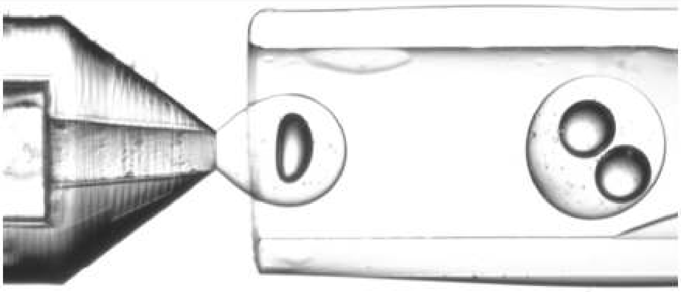
	
Water in oil droplet generated in R1	Water in oil droplet generated in water in R2

Figure 5: Inserts and extraction capillaries to produce multiple emulsions

9. The single encapsulated multiple emulsions are now produced. The dripping mode provides a high stability for droplet formation. Droplets show high mo-nodispersity. It is possible to vary the flow rates in order to change the size of the emulsion as well as the number of cores (see the following section for more details).

RESULTS

OVERVIEW: VARIOUS NUMBER OF CORES IN A SINGLE DROPLET

By changing the flow rate values of the different phases, it is possible to tune the number of cores inside one droplet. Figure 6 shows an overview of the achievable configurations: from a single core to six cores.

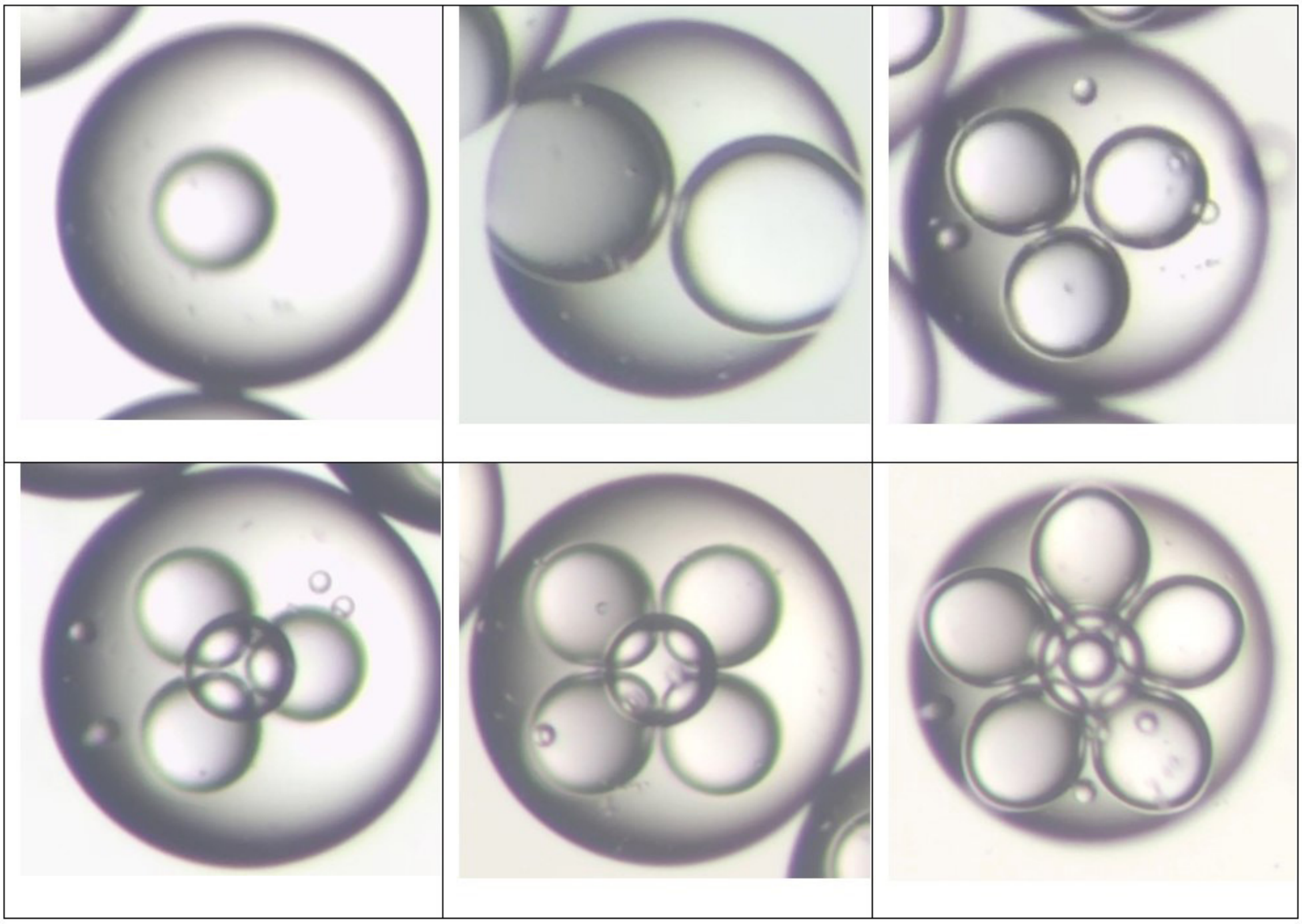


Figure 6: Images of multiple emulsions obtained at different flow rates to vary the number of cores in a single droplet

The number of cores in a single droplet depends on the flow rate of the core phase

During the formation of the multiple emulsions in RayDrop R2, the flow rates of the shell and of the continuous phases are kept fixed at values reported in Table 3.

Phase	Continuous	Shell	Core
Composition	Glycerol 70% in water + 2% Tween 20	Mineral oil + 2% ABIL 90 + 0.08% Bromocresol	2% Tween 20 in water
Pressure (mbar)	6666	800	Varied
Flowrate (µL/min)	38.6	Shell	From 0.5 to 2.3

Table 3: Experimental conditions for the evaluation of the number of cores in single droplets

Thus, only the flow rate of the core phase is changed. For each flow rate, an analysis of the number of cores in 50 droplets in average is performed. The results are depicted in Figure 7.

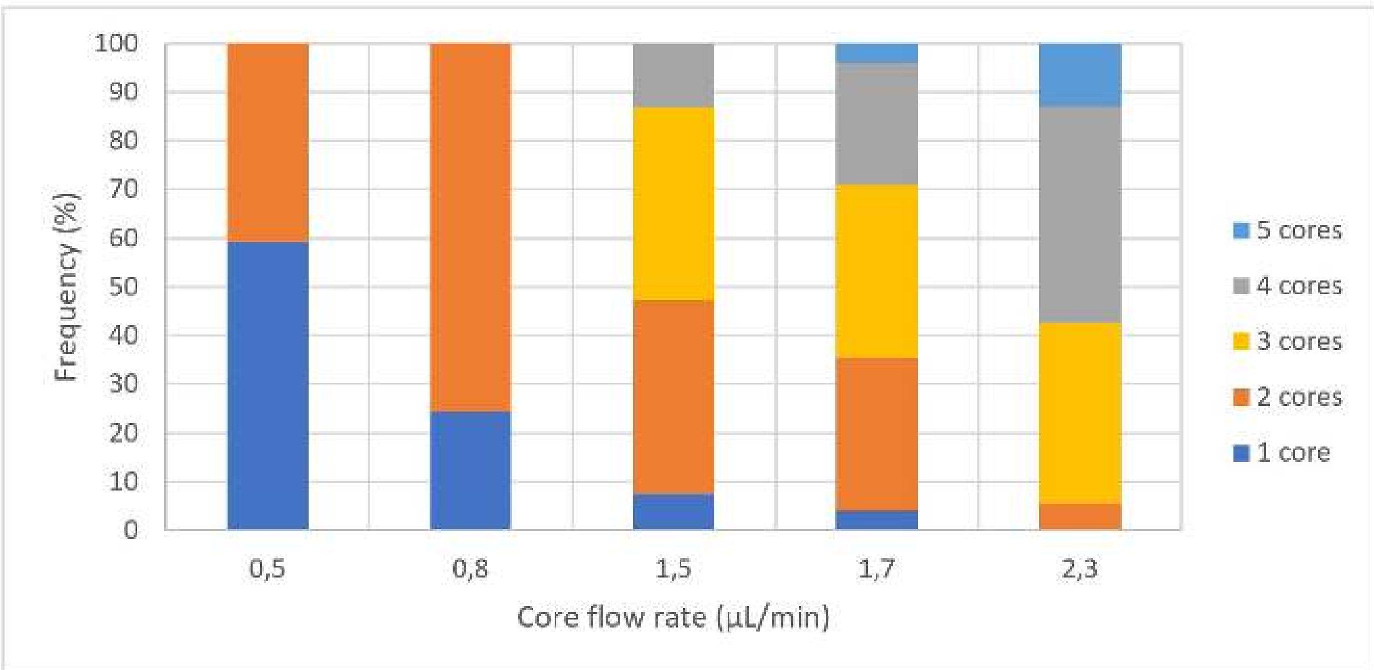


Figure 7: Frequency distribution of the number of cores within a single droplet as a function of the core flow rate

By increasing the core flow rate, the number of encapsulated core droplets increases (Figure 7). For a core flow rate of 0.5µl/min, most droplets contain one core while for a core flow rate of 2.3µl/min, most droplets contain four cores (Table 4).

Core flow rate (mL/min)	Number of cores
0.5	1
0.8	2
1.5	2 - 3
1.7	3
2.3	4

Table 4: Experimental conditions for the evaluation of the number of cores in single droplets

Conclusion & Perspectives

We have shown the feasibility of producing multiple emulsions in a single shell using two RayDrop devices placed in series and integrated onto the RayDrop platform. We further demonstrate how to tune the number of cores in a droplet by varying the flow rate of the core phase.

Using a Double Emulsion RayDrop, several type of emulsions containing multiple cores could be produced. For example, using a Double emulsion RayDrop as RayDrop R1 would allow one to obtain multiple double emulsions inside a single droplet, as shown in Figure 8.

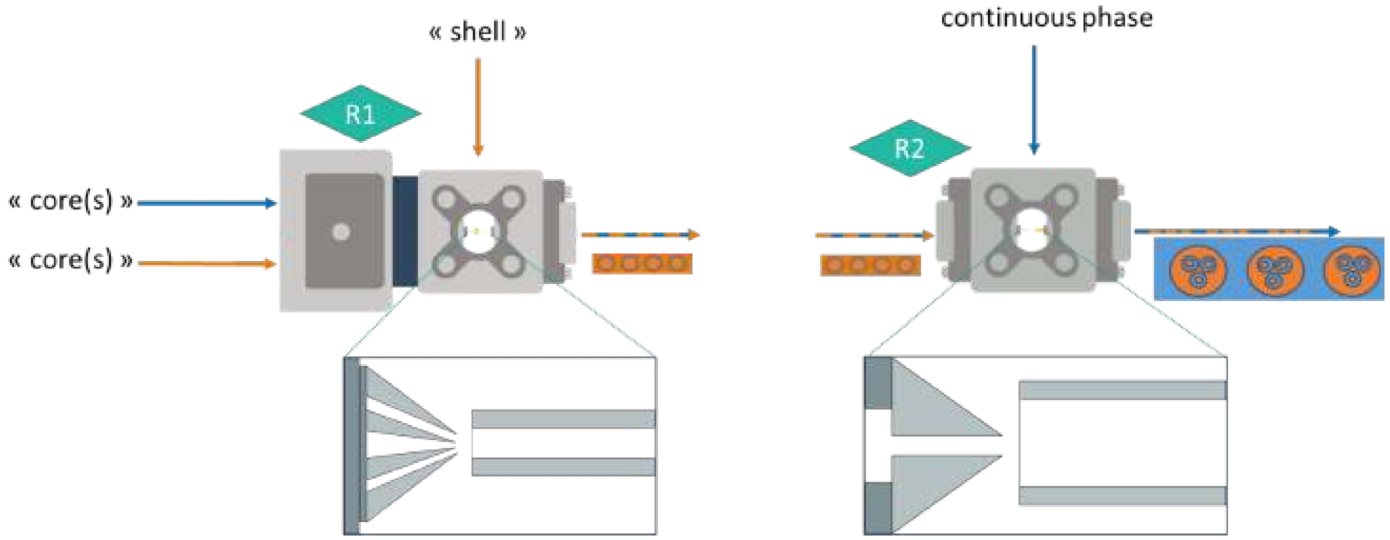


Figure 8: A RayDrop Double emulsion followed by a RayDrop single emulsion

Alternatively, a setup composed of a single emulsion RayDrop as R1 and a Double emulsion RayDrop as R2 would produce multiple droplets in one double emulsion droplet as schematically shown in Figure 9.

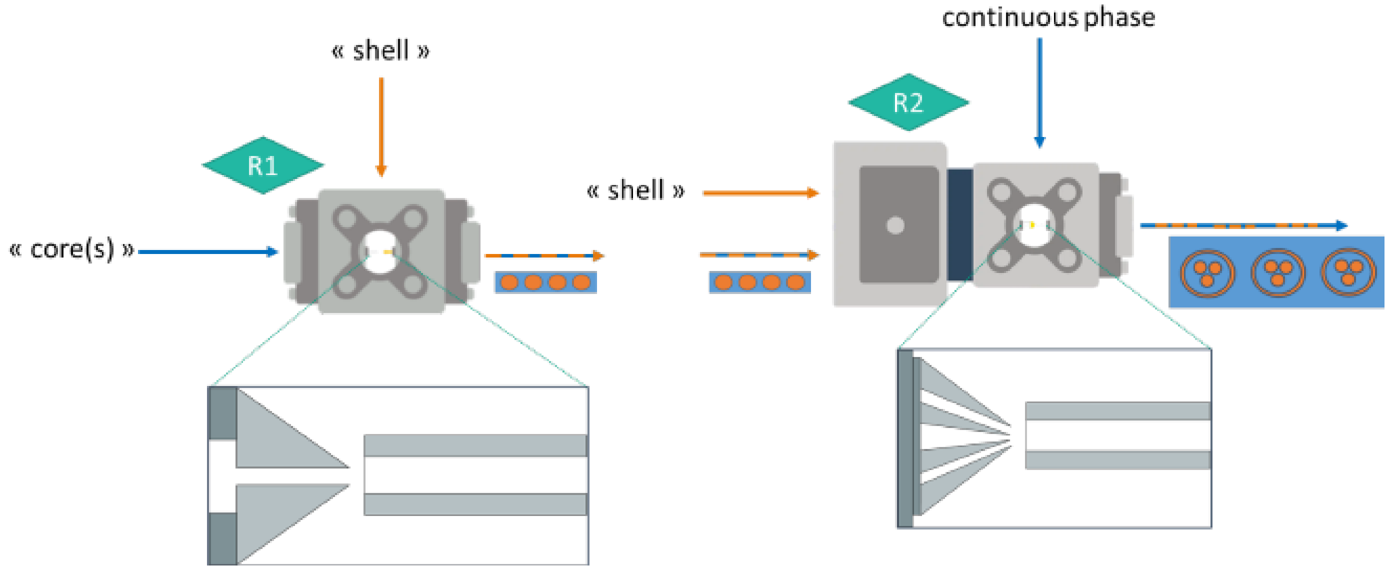


Figure 9: A RayDrop single emulsion followed by a RayDrop double emulsion

Another application of this microfluidic setup would be the co-encapsulation, i.e. the encapsulation inside the core of different fluids in a shell droplet. It could serve as a synergistic delivery system or as a chemical microreactor for incompatible API's or chemicals4.

The configuration shown in Figure 10 would produce multiple emulsions containing different core compositions in the same shell with a microfluidic setup ensuring a high control over the number of cores.

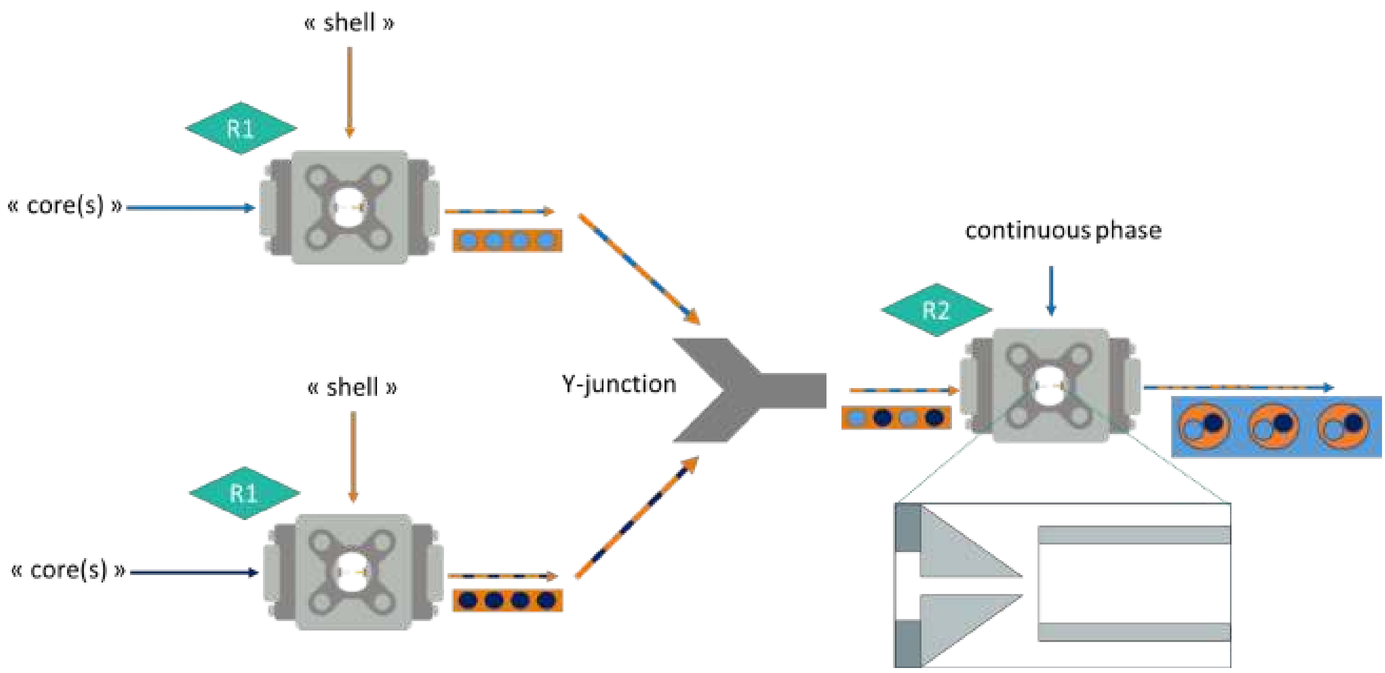


Figure 10: Set-up for the co-encapsulation of two liquids

REFERENCES

[1] Vladisavljević, G., al Nuumani, R. & Nabavi, S. Microfluidic Production of Multiple Emulsions. Micromachines (Basel) 8, 75 (2017).

[2] Lee, T. Y., Choi, T. M., Shim, T. S., Frijns, R. A. M. & Kim, S.-H. Microfluidic production of multiple emulsions and functional microcapsules. Lab on a Chip 16, 3415–3440 (2016).

[3] Li, E. Q., Zhang, J. M. & Thoroddsen, S. T. Simple and inexpensive microfluidic devices for the generation of monodisperse multiple emulsions. Journal of Micromechanics and Microengineering 24, 015019 (2014).

[4] Wang, W. et al. Controllable microfluidic production of multicomponent multiple emulsions. Lab on a Chip 11, 1587 (2011).

[5] Dewandre, A., Rivero-Rodriguez, J., Vitry, Y., Sobac, B. & Scheid, B. Microfluidic droplet generation based on non-embedded co-flow-focusing using 3D printed nozzle. Scientific Reports 10, 21616 (2020).