Application note
Emulseo x LiveDrop
Droplet stability, monodispersity & leakage control

INTRODUCTION

FluoSurf™ and Fluo-Oil™ by Emulseo are advanced solutions designed for droplet-based microfluidic applications. FluoSurf™ is a biocompatible fluorinated surfactant optimized for stabilizing monodisperse aqueous droplets in fluorinated oils, ensuring long-term stability even under extreme conditions like thermocycling. It is ideal for applications such as droplet digital PCR (ddPCR) and single-cell analysis (SCA), offering optimal stability, experiments reproducibility and excellent leakage control. Fluo-Oil™ 135, a fluorinated oil, serves as an alternative to Novec™ 7500 with similar chemical properties and microfluidic performances. It is specifically formulated to solubilize surfactants like FluoSurf™ and is widely used in encapsulating cells, proteins and nucleic acids for applications like genome sequencing and high-throughput screening. Together, these products enable reliable and precise microfluidic workflows.

Figure 1: FluoSurf™ surfactants by Emulseo

Figure 2: ModaFlow™ by LiveDrop

The ModaFlow™ by LiveDrop excels in gentle, high-throughput droplet biology, boasting advanced detection and single droplet sorting. With a modular design for cost-effectiveness and flexibility, it facilitates efficient functional response bioassays for both soluble and insoluble antigens (peptides, proteins, GPCR, etc.), including agonist/activation of reporter cells, target-binding, secretion, FRET, and enzyme activity. Technically, the system features 4 lasers and 5 highly sensitive photodetectors, characterized by minimal sample requirements, zero dead volume, and gentle cell handling. It processes up to 14,000 droplets per second, encapsulating millions of cells daily, supports droplet sizes from 15 µm (~2 pL) to over 250 µm (~8 nL), and can sort more than 3,000 droplets per second. Patented SMART connectics, SMART optimized chips, real-time visual monitoring, and an intuitive user interface enable performant gating and quality control. The ModaFlow™ offers a leading combination of advanced features and cost-effectiveness in its class.

This application note presents the use of Emulseo's surfactant/oil chemical formulations for high-performance droplet generation within the ModaFlow™ instrument. The study first emphasizes the excellent stability and monodispersity of droplets produced using the FluoSurf™-O / Fluo-Oil™-135 formulation in ModaFlow™. It also addresses the common issue of incomplete sealing in microfluidic emulsion droplets, which can lead to dye leakage or cross-contamination, significantly impacting the reliability and accuracy of droplet-based microfluidic experiments.
It underscores the importance of selecting appropriate surfactant and oil to minimize or even eliminate this problem. In the second part of the study, the diffusion of two different dyes, dye 405 and dye 647P, is evaluated. The assessment evaluates dyes diffusion through droplets, into the oil, and into adjacent droplets. The results demonstrate that the FluoSurf™-O / Fluo-Oil™-135 formulation used within ModaFlow™ is highly effective in retaining dyes inside droplets, thus preventing leakage issues.

EXPERIMENTAL SETUP AND MATERIALS

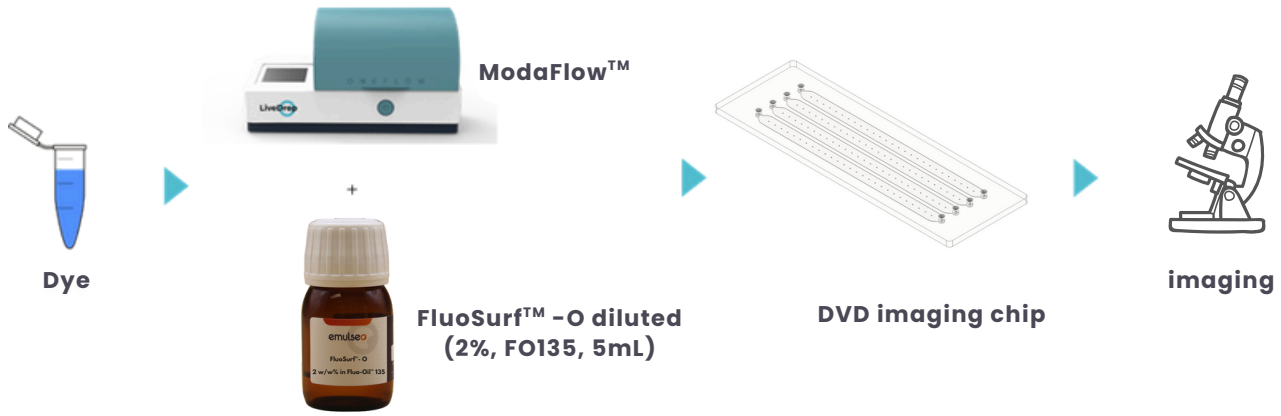


Figure 3: Experimental setup

Material:

- Droplet generation chip: Drop-100
- Surfactant/oil formulation: FluoSurf™ -O diluted at 2w/w% in Fluo-Oil™ -135
- Aqueous samples: Dye 405 (DY405) at 1 µM and dye 647P (DY647P) at 1 µM
- Pressure driven droplet generation: $P_{formulation}$ = 1850 mBar ; P_{sample} = 1350 mBar
- Imaging chamber: DVD (5 µL - 30 µm thick)

EXPERIMENTAL PROTOCOL

1

In the initial phase of the experiment, two distinct emulsions were produced using different dyes:

- A blue-emitting, fluorogenic DNA-binding dye. It can be excited with a 405 nm blue laser, and its emission is captured using a 450/50 nm bandpass filter. It is equivalent to DAPI.
- A fluorophore with an excitation peak at 654 nm and an emission peak at 672 nm. It is commonly used in flow cytometry and fluorescence microscopy. It is equivalent to Cy5.

2

The second stage of the experiment involved imaging each droplet population separately to assess droplet stability and monodispersity. Droplets were imaged at T=0h, T=24h and T=48h.

3

In the final phase, a mixture containing 10% of blue droplets population and 90% of red droplets population was prepared and imaged at T=0h, T=24h and T=48h. This step was designed to evaluate the diffusion of the two different dyes between droplets and into the surrounding oil phase over time.

RESULTS

A) Droplets generation, stability and monodispersity

Two emulsions containing respectively 1 µM of DY405 dye and 1 µM of DY647P dye were generated using the Drop-100 chip. The droplets were successfully produced, and Figure 4 showcases the high-quality droplet generation by ModaFlow™ using Emulseo's FluoSurf™-O / Fluo-Oil™-135 formulation. Droplet volume and diameter can be estimated during droplet formation with the ModaFlow's™ WebApp.

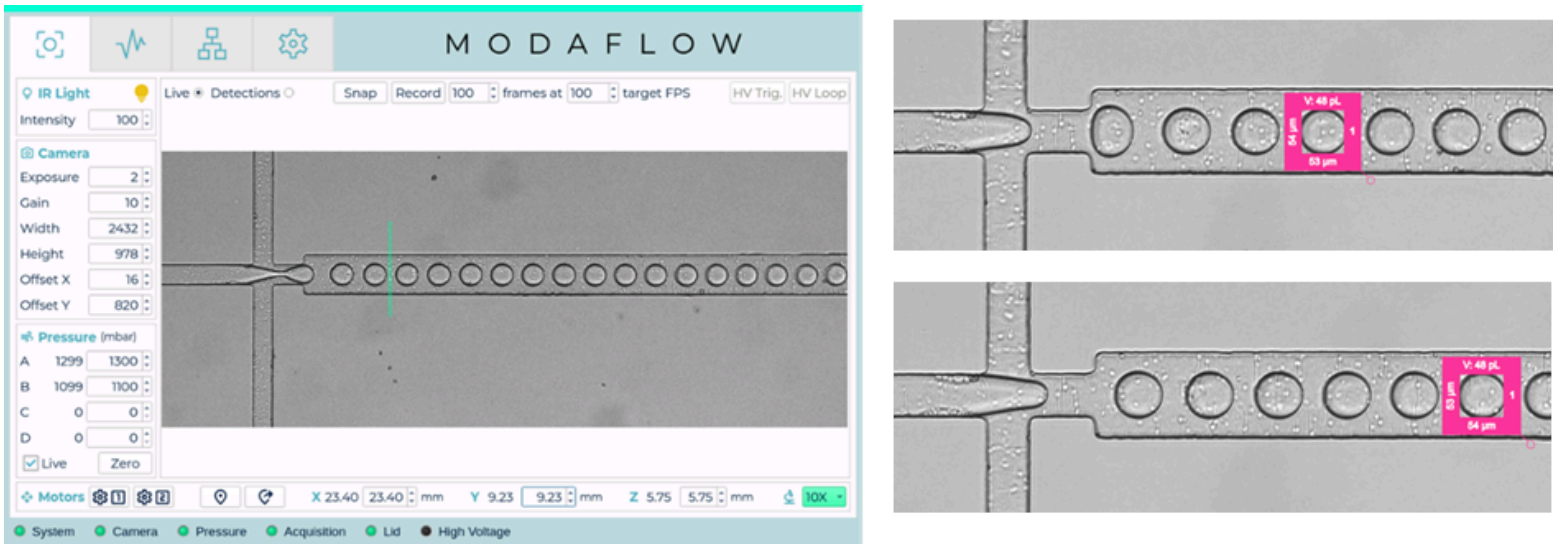
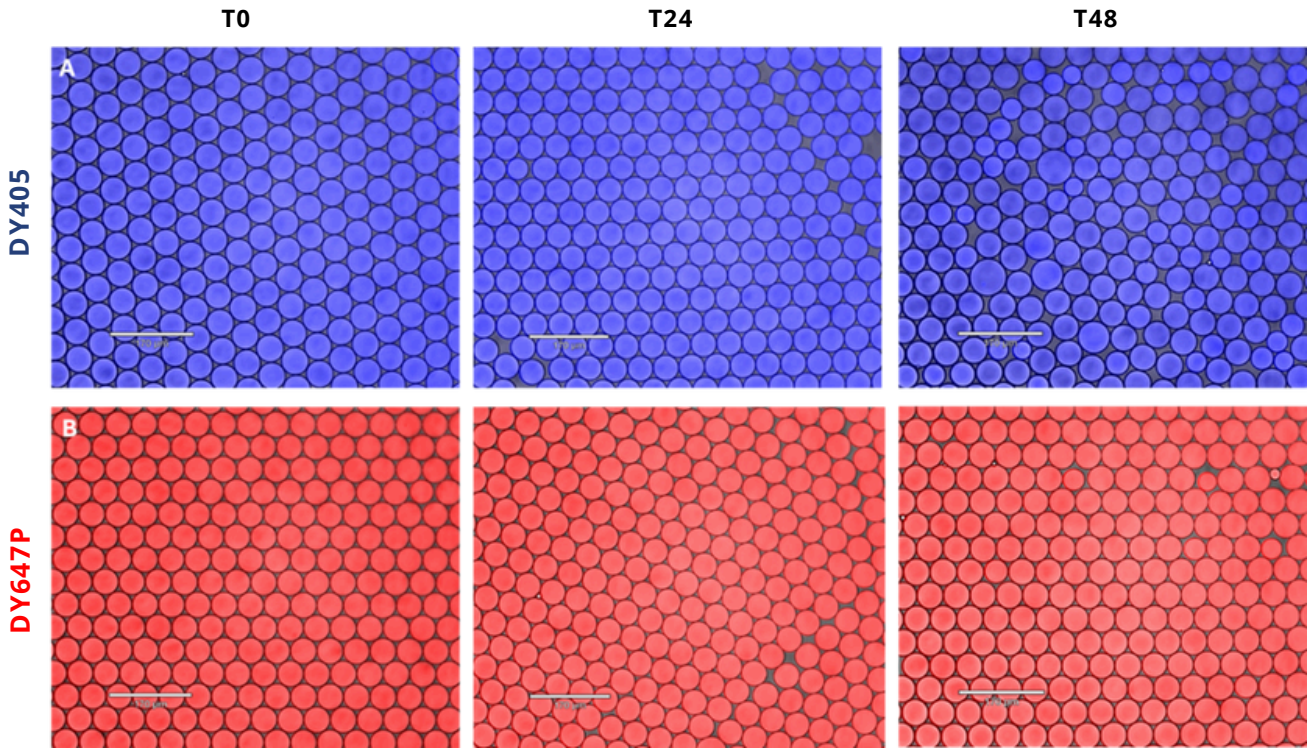


Figure 4: Production of an emulsion of 48 pL droplets - with 1 µM DY405 in a Drop-100.

After generation, droplets were injected in the DVD imaging chip for observation. Figure 5 shows the images taken at T0, T24h and T48h of the droplets containing the blue dye and the red dye, respectively.



Scale : 170 µm

Figure 5: Images acquired under an Echo Revolve microscope with transmission and (A) DAPI or (B) Cy-5 filters to visualize the DY405 and DY647P used to produce the emulsions. Images are overlays taken at T=0h, T=24h and T=48h under all filters at 10X magnification, showing the uniform distribution of the dye within the droplets.

The tables below and Figure 6 show the mean droplet volume and standard deviation automatically calculated for both emulsions over time. For both dyes used, the droplets maintain the same volume over time. This observation validates the stability and homogeneity of the emulsions obtained, essential for the targeted applications.

DYE COLOR	Timing	Volume (pL)	SD (pL)
BLUE	T0	46.4	1.8
BLUE	T24	46.2	2.2
BLUE	T48	43.5	10.4

DYE COLOR	Timing	Volume (pL)	SD (pL)
RED	T0	44.4	2.2
RED	T24	44.1	3.8
RED	T48	42.1	3.3

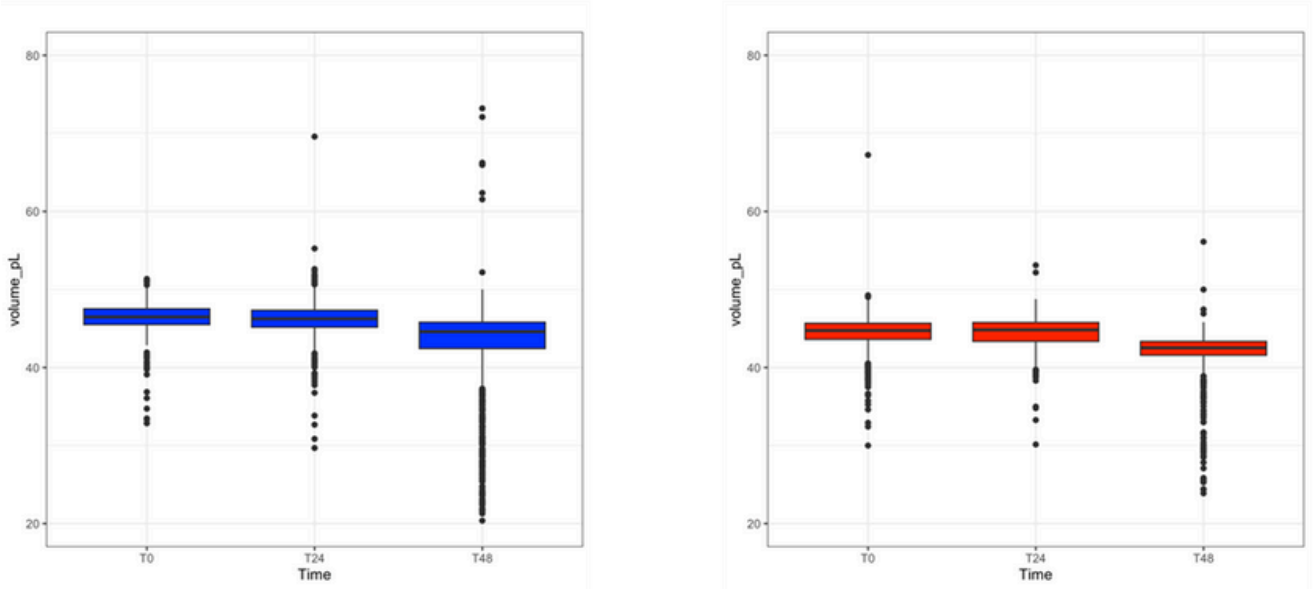


Figure 6: Evolution of droplet volumes at different times (0, 24h, 48h) for emulsions containing dye DY405 (blue) and dye DY647P (red).

B) Study of dye retention

A mixture consisting of 10% by volume of blue emulsion (DY405) and 90% by volume of red emulsion (DY647P) was produced and subsequently introduced at different times into a DVD imaging chip for visualization purposes (Figure 7).

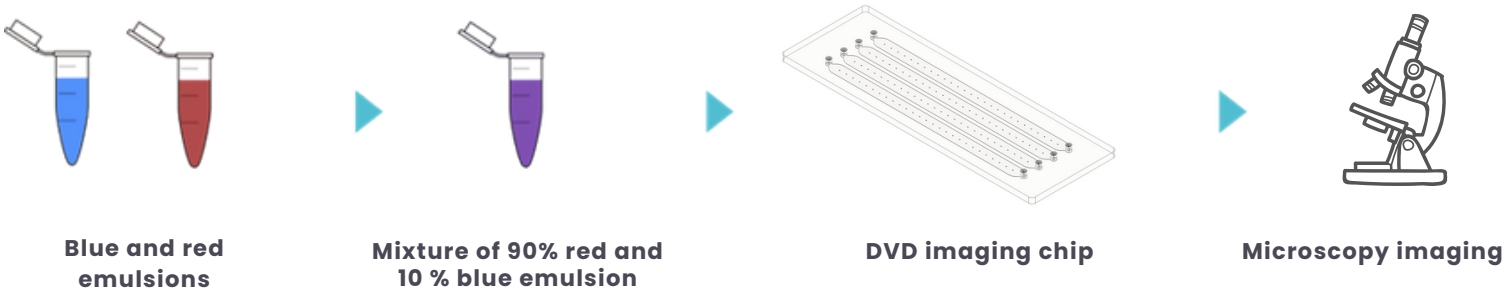


Figure 7: Workflow used to evaluate dye retention inside droplets.

Figure 8 illustrates the retention of the dyes within droplets.

Images A, B, and C demonstrate that the dyes remain confined within their respective droplets, with no evidence of leakage or cross-contamination over time. This workflow also confirms the stability of the droplets throughout the entire process, including generation, collection, mixing, and injection into the imaging chip.

Note: at T0 and T24h, the percentages of red droplets and blue droplets are not visually observed because the emulsion has not yet homogenised.

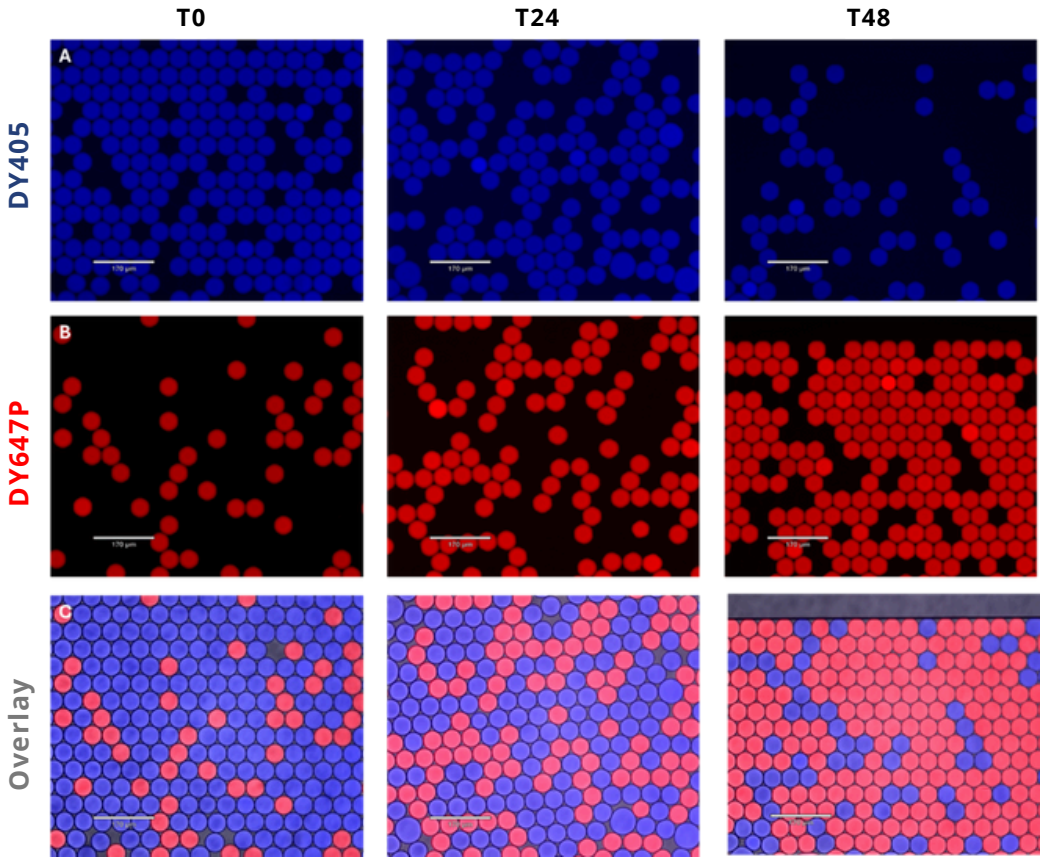


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Figure 8: Images acquired over time under Echo Revolve microscope with transmission, with DAPI and CY-5 filters to visualize both DY405 and DY647P used to produce the emulsion.
(A) CY-5 filter (B) DAPI filter (C) Overlay at 10X magnification.

CONCLUSION

The compatibility of Emulseo's FluoSurf™-O / Fluo-Oil™ -135 formulation with LiveDrop's ModaFlow™ and chips has been confirmed. The formulation demonstrated excellent droplet stability and monodispersity during production, as well as after 24 and 48 hours. Furthermore, the combination of Emulseo's and Livedrop's products showed superior dye retention within droplets, ensuring excellent leakage control. The next step of this ongoing collaboration will involve more complex experiments, including reinjection and sorting of the produced emulsion, to further highlight the performance of Emulseo's and Livedrop's products and their synergy.

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