

## EVALUATION AND COMPARISON OF EMULSEO'S FORMULATIONS PERFORMANCE FOR FLUOROPHORE RETENTION IN DROPLETS

Our flagship products, FluoSurf™-S and FluoSurf™-O, are high-performance fluorinated surfactants designed and optimized to stabilize aqueous droplets in fluorinated oils, such as Fluo-Oil™ 7500 and Fluo-Oil™ 135, for a wide range of biotechnological applications. These products are especially adapted for droplet-based microfluidic experiments such as single cell analysis, often involving the use of fluorophores. Leakage of these fluorophores from one droplet to another can distort the analysis and lead to misinterpretation of the results. Good retention of fluorophores during experiments is therefore essential. This document presents the results of an experimental study comparing the performance of FluoSurf™-S and FluoSurf™-O surfactants and Fluo-Oil™ 7500 and Fluo-Oil™ 135 fluorinated oils for fluorophore retention in droplets.

### EXPERIMENTAL CONDITIONS

For each surfactant, water-in-oil emulsions were generated alternately in Fluo-Oil™ 7500 and in Fluo-Oil™ 135 with 4 w/w% FluoSurf™-O or 4 w/w% FluoSurf™-S. The mixes contained two populations of droplets, "empty" (PBS) and "full" (20  $\mu$ M fluorescein in PBS), that were incubated at 37°C. After incubation, droplets were injected into a microfluidic chamber for observation and pictures were taken at different timepoints (0h, 24h, 48h and 72h). Each experiment was repeated 3 times and ImageJ software was used to determine the fluorescence intensity of the droplets.

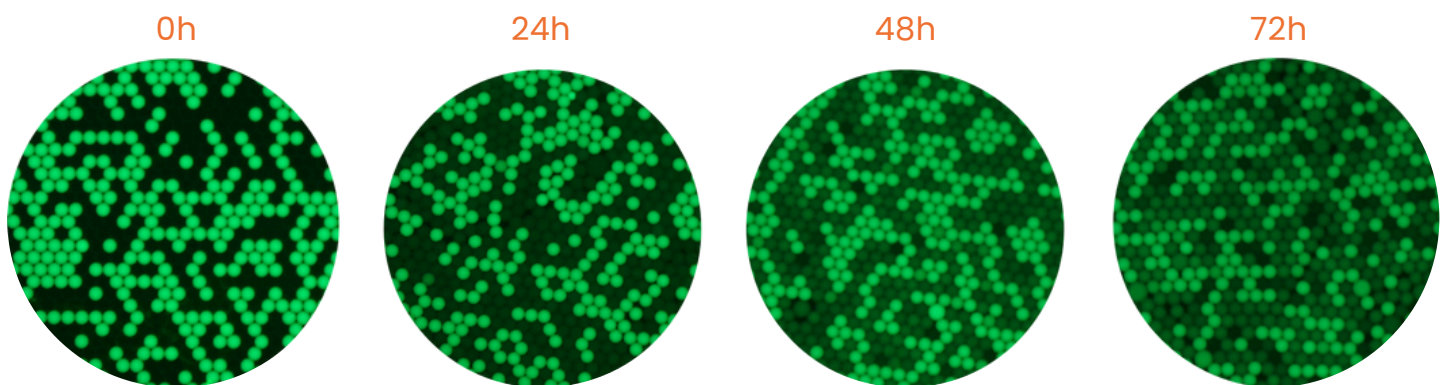


Figure 1: Time evolution of full and empty droplets in fluorescence

The fluorescence intensity difference between the two droplet populations decays exponentially as shown in Figure 2. The time scale  $\tau$  of the exchange is an indicator of the effectiveness of the formulation tested in retaining the fluorophore in droplets. The longer the time scale  $\tau$ , the slower the exchange of fluorophore between full and empty droplets, and the more effective the formulation is at limiting fluorophore leakage.

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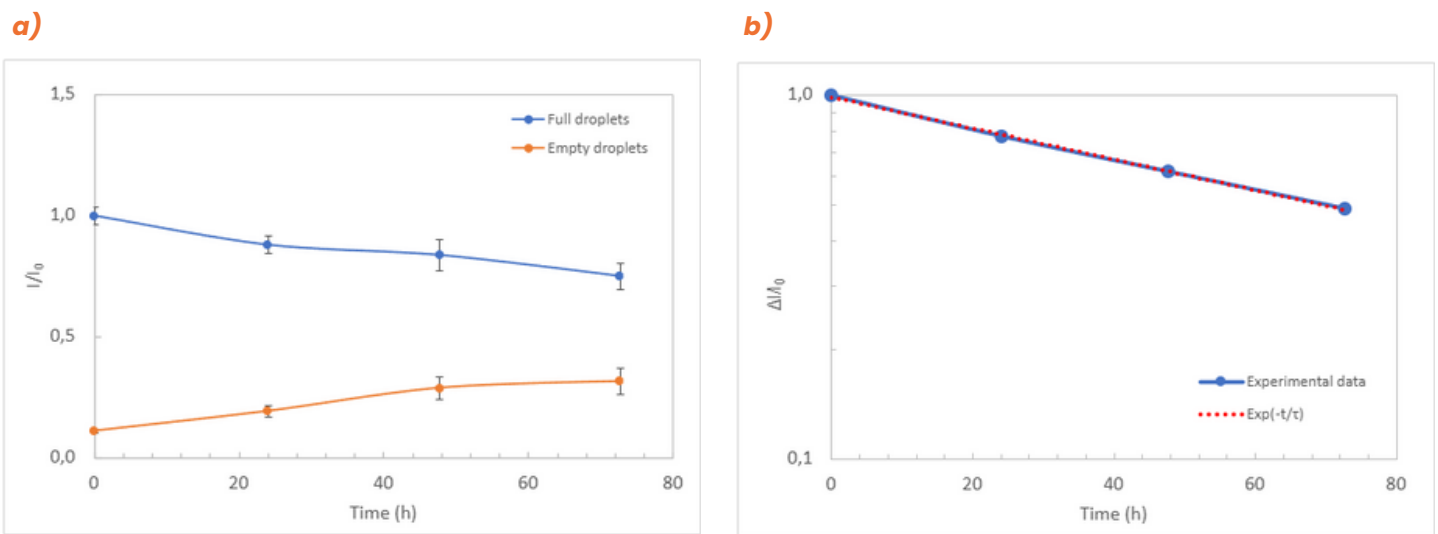


Figure 2:

a) Evolution of the average fluorescence intensities of full (blue circles) and empty (orange circles) droplets  
 b) The intensity difference between the two droplet populations decays exponentially

## RESULTS

The time scales  $\tau$  obtained for droplets generated with 4w/w% FluoSurf™-O or 4w/w% FluoSurf™-S diluted in Fluo-Oil™ 7500 or Fluo-Oil™ 135 are shown in the figure below:

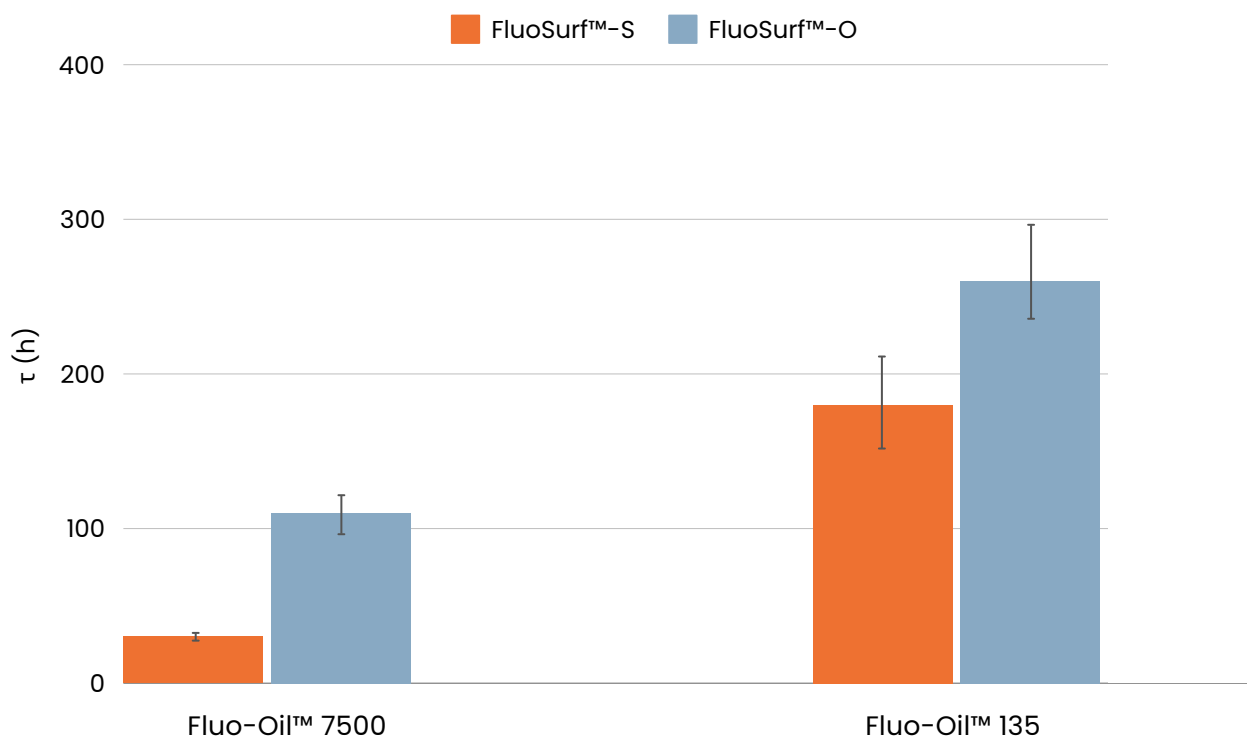


Figure 3: Time scales  $\tau$  determined through image analysis

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Varying the fluorinated oil only, the results show for the two tested surfactants that the time scales obtained for droplets generated in Fluo-Oil™ 135 are higher than those obtained for droplets generated in Fluo-Oil™ 7500. Fluo-Oil™ 135 is thus more effective at retaining fluorophores in droplets than Fluo-Oil™ 7500.

Varying the surfactant only, the results demonstrate, for both tested oils, that the time scales obtained for droplets generated with FluoSurf™-O are higher than those obtained for droplets generated with FluoSurf™-S. FluoSurf™-O is thereby more effective at retaining fluorophores in droplets than FluoSurf™-S.

### CONCLUSION

In this study, the two best performing products for retaining fluorophores in droplets are the surfactant FluoSurf™-O and the fluorinated oil Fluo-Oil™ 135. FluoSurf™-O + Fluo-Oil™ 135 formulation is therefore particularly well suited to microfluidic applications for single cell analysis.

To learn more about surfactants and other formulation products for droplet-based microfluidics, please visit [www.emulseo.com](http://www.emulseo.com)

The logo for emulseo, featuring the word "emulseo" in a dark blue, lowercase sans-serif font, followed by a stylized orange circle containing a white letter 'e'.

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