Evaluation and comparison of the performance of FluoSurf-C™ and FluoSurf-O™ for droplet generation, droplet stability along thermocycling and oil phase fluorescence

PROTOCOL

- Droplet generation & thermocycling

For each surfactant, droplet generation was performed under the following conditions:

- Oil phase: 4w/w% surfactant in HFE-7500
- Aqueous phase: 100 µM rhodamine 6G in PBS
- Chip: PDMS/glass chip treated with Fluo-ST2B

After collection, the droplets were submitted to thermocycling (30 cycles):

Step	Amplification program	Number of cycles
1	**30 s at 98°C**	1
2 3 4	**10 s at 98°C** **5 s at 50°C** **10 s at 72°C**	30
5	**∞ at 10°C**	1

Table 1 : Standard amplification program

Statistical analysis of droplet size was performed by image analysis (ImageJ).

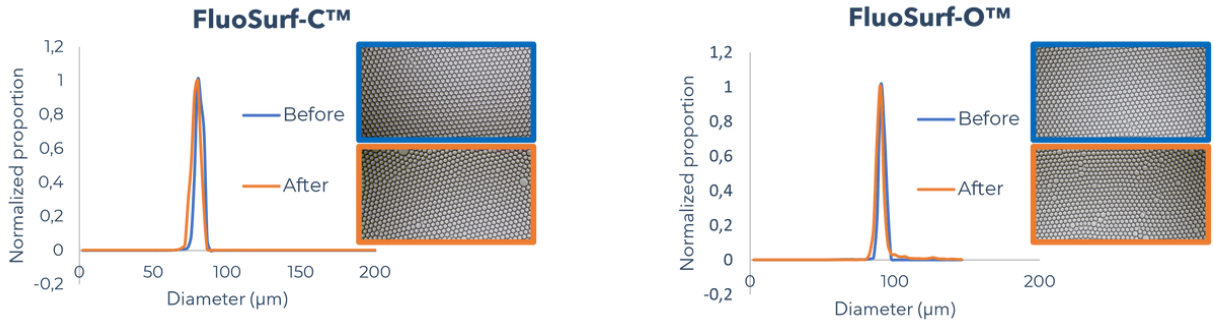
- Fluorescence

For each surfactant, samples diluted to 4w/w% in HFE-7500 were prepared.
Fluorescence spectra at different wavelengths of excitation (405 nm, 488 nm, 561 nm and 647 nm) were obtained using a plate reader.

RESULTS

- Droplet stability before and after thermocycling

The statistical droplet population size analysis before and after 30 PCR heating cycles is reported in the figure below.



	FluoSurf-C™ 4w/w%	**FluoSurf-O™ 4w/w%**
Size variation coefficient before heating cycles	3.3%	3.6%
Size variation coefficient after heating cycles	12.1%	10.1%
Difference	**8.8%**	**6.5%**

Figure 1: Statistical droplet population size determined through image analysis before and after thermocycling

Monodisperse populations are obtained before and after thermocycling using both surfactants.
The image statistical analysis showed size coefficients of variation to be respectively 12.1% and 10.1% for FluoSurf-C™ and FluoSurf-O™ after thermocycling.

- Oil phase fluorescence

The main difference between the surfactants lies on their fluorescence when diluted in fluorinated oil. The fluorescence of both diluted surfactants is presented in the figure below.

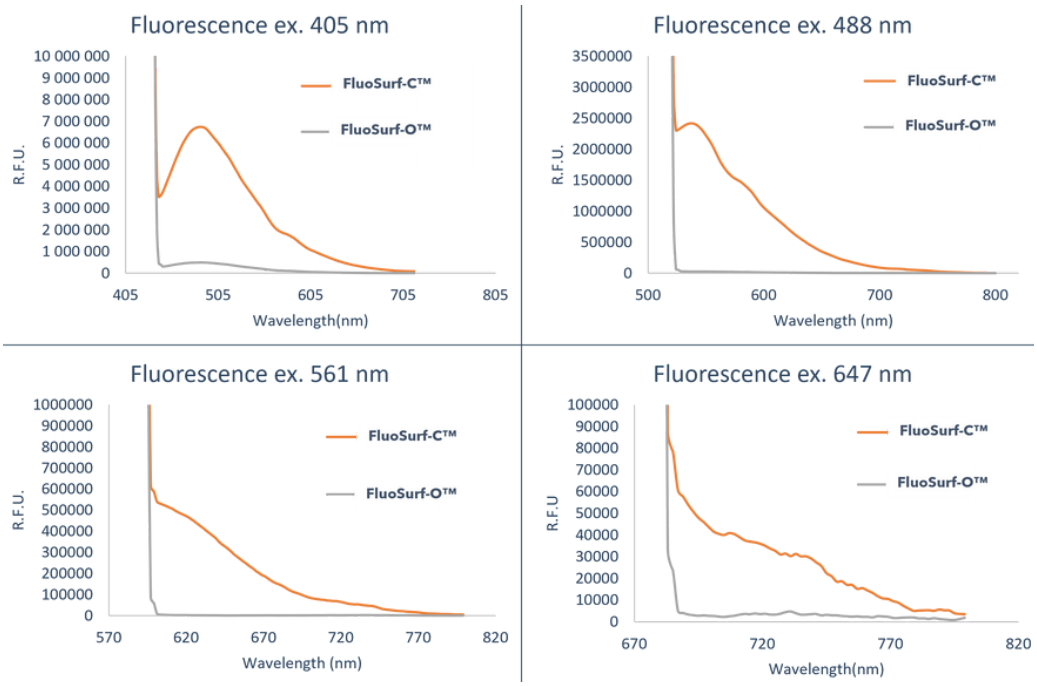


Figure 2: Fluorescence spectra of FluoSurf-O™ and FluoSurf-C™ at several wavelengths of excitation.

At every wavelength of excitation, the FluoSurf-O™ presents a fluorescence considerably lower than the fluorescence of the FluoSurf-C™.

CONCLUSIONS

FluoSurf-C™ and FluoSurf-O™ show a good control on the generation and the stability of the droplets along thermocycling.
FluoSurf-O™ exhibits an ultra-low autofluorescence compared to FluoSurf-C™.
This property can allow to increase the signal to noise ratio and give access to high resolution results for ddPCR application for instance.

Notes:
1. The FluoSurf-C™ and the FluoSurf-O™ show the same dye retention
2. All the experiments were also realized in Fluo-Oil 7500 and show the same results

To learn more about surfactants and other formulation products for droplet microfluidics, please visit www.emulseo.com

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