FLUO-OIL 135:
AN ALTERNATIVE TO NOVEC™ 7500 FLUORINATED OIL

Discover Emulseo's novel fluorinated oil that can be used as a replacement for Novec™ 7500 in microfluidic experiments.

INTRODUCTION

Fluorinated oils have received increasing interest over the last few years, as applications in the field of microfluidics have largely expanded. Especially, droplet-based microfluidic platforms have successfully been developed for a wide range of applications such as macromolecular engineering, drug screening, digital PCR or cell encapsulation and sorting, to name a few.
Droplets are also widely used as microreactors for chemical or biological reactions as they allow running thousands of experiments in parallel while minimizing costs and volumes in a time-effective manner.

For all these applications, the compartmentalization into droplets requires a fluid that is immiscible with water and a surfactant to prevent droplets from merging.[1] By far, the preferred fluids for such applications are fluorocarbon oils.
They are inodorous, transparent, and they exhibit great retention of reagents in droplets since most organic compounds are insoluble in fluorinated oils. Additionally, they are biocompatible and permeable to gases allowing passive diffusion of oxygen and carbon dioxide, which is critical for cell viability. Novec 7500™ fluorinated oil from 3M is one of the most widely used and formulated by Emulseo as Fluo-Oil™ 7500 for specific usage with FluoSurf™ and the other Emulseo's surfactants.

In this rapidly evolving market, as more and more research teams and companies in the world rely on the use of that specific fluorinated oil, produced and sold by a single manufacturer, Emulseo's teams have worked on finding an alternative replacement solution to anticipate any supply disruption.

We hereby present a comparative report on physico-chemical properties and microfluidic performance of Fluo-Oil™ 135 versus Novec™ 7500 fluorinated oil.

I) Chemical properties

In the table below are summarized the main properties of both oils.

	Novec™ 7500	Fluo-Oil 135
Appearance	Transparent	Transparent
Boiling point (°C)	129	135
Viscosity (mPa.s)	1.24	1.72
Density	1.61	1.72

Table 1: Appearance and chemical properties of Novec™ 7500 and Fluo-Oil™ 135.



Appearance and boiling points are similar. Density and viscosity are a bit higher for Fluo-Oil™ 135 and thus switching from Novec™ 7500 to Fluo-Oil™ 135 could cause a small variation in experimental results for applications where these parameters are critical. However, for most applications, a simple tuning of specific parameters (such as flow rates) could solve the issue. For instance, water-in-oil droplets generated with Fluo-Oil™ 135 could slightly differ in size as compared to droplets produced in Novec™ 7500 and thus flow rates would have to be adjusted.

II) Microfluidic performance

In order to evaluate the impact of switching from Novec™ 7500 to Fluo-Oil™ 135, water-in-oil droplets were generated using both oils. Droplet size distribution, stability through thermocycling and incubation as well as molecular retention were evaluated and compared for both droplet populations.

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1. Material and methods

A microfluidic polydimethylsiloxane (PDMS) flow-focusing device was fabricated according to standard protocols using soft lithography. The device was then permanently bonded to a microscope glass side after activation with plasma treatment. Surface coating of the microfluidic walls was performed with Fluo-ST1™ (Emulseo) to ensure surface hydrophobicity.

The oil continuous phase was prepared by dissolving 4wt/wt% neat FluoSurf™ surfactant in either Novec™ 7500 or Fluo-Oil™ 135. The dispersed aqueous phase used for these experiments was either PBS or 100µM fluorescein in PBS.

Syringe pumps were used to control the flow rates of the different phases in the microfluidic device:
- Oil continuous phase: 300 µL/hr
- Aqueous dispersed phase: 100 µL/hr

Droplet generation was monitored using an inverted microscope. The ImageJ software was used for data analysis.

2. Results

- **Droplet size and size distribution**

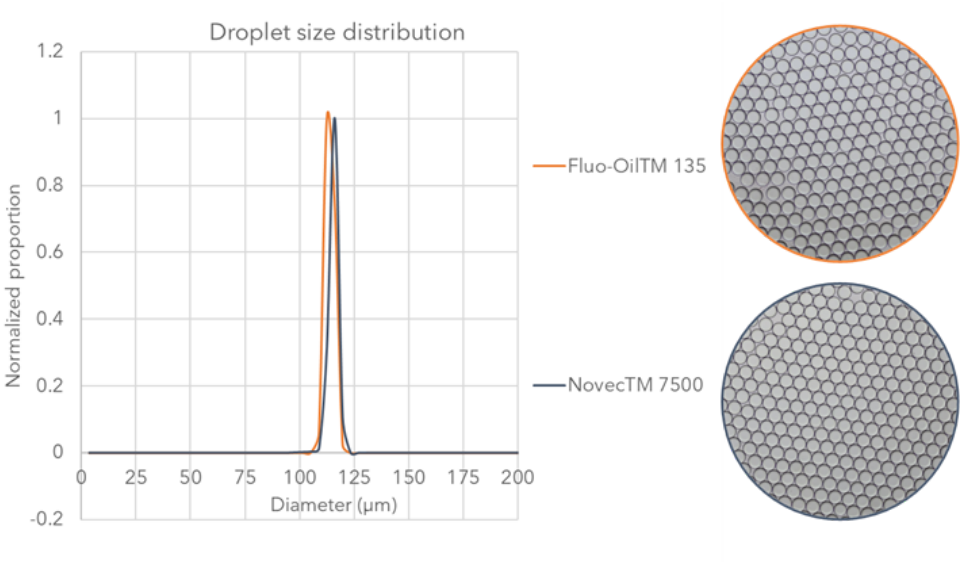

Figure 1: Droplet size distribution and associated pictures of water-in-oil droplets generated in Fluo-OilTM 135 and NovecTM 7500

Droplet generation using both oils resulted in similar droplet sizes. With the chosen flow rate parameters, droplet average diameter was 110µm (Figure 1).

Monodisperse populations were obtained using both oils and image statistical analysis showed size coefficients of variation to be respectively 2.3% and 2.9% for FluoSurf™-stabilized droplets generated in Fluo-Oil™ 135 and Novec™ 7500.

- **Droplet stability**

A majority of research teams use droplet microfluidics for cell encapsulation or for PCR applications. Therefore, there is a need to produce droplets that are stable through heating cycles from PCR, as well as through long-term incubation at 37°C. The choice of surfactant is crucial but choosing the oil is equivalently important, to ensure reproducible experiments and the generation and conservation of monodisperse droplet populations.

To evaluate the impact of switching oils on droplet stability, water-in-oil droplets were generated, and three different emulsion samples were collected for both oil types. The first one was directly re-injected into a glass observation chamber. The second one was submitted to a classical PCR thermocycling process with 30 heating cycles [98°C 10s, 50°C 5s, 72°C 10s] to evaluate droplet thermo-stability. The third one was placed in a 37°C incubator for 3 days. For each sample, a population of about 10000 droplets was statistically analysed to determine the average droplet size and size coefficient of variation.

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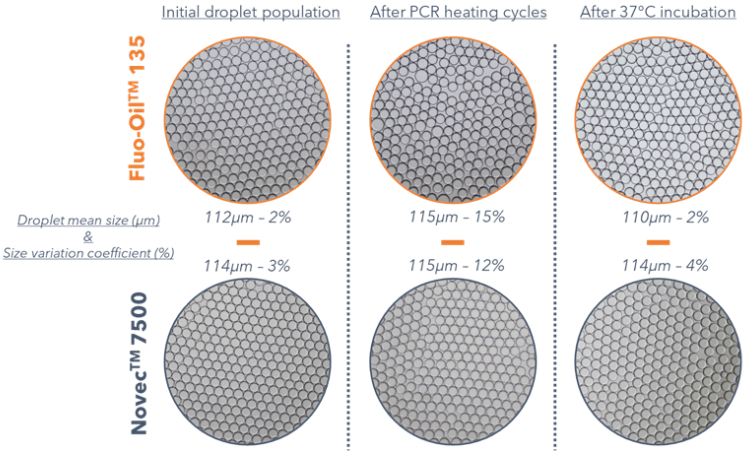

Figure 2: Pictures and associated average sizes and size coefficient of variation of water-in-oil droplets generated in Fluo-Oil™ 135 and Novec™ 7500 after generation, after 30 PCR heating cycles and after 3 day-incubation at 37°C.

Whether FluoSurf™-stabilized droplets are formed in Novec™ 7500 or Fluo-Oil™ 135, the average size remains constant [around 110µm (0.7nL)] at generation, after heating cycles from PCR or after 3-day incubation at 37°C, as can be seen in the pictures from Figure 2. Additionally, droplet populations remain monodisperse. From the statistical analysis, it was calculated that after heating cycles, only 5% of the overall droplet population had a size that differed by more than 10% of the average initial droplet diameter.

Overall, switching the oil from Novec™ 7500 to Fluo-Oil™ 135 has no impact on droplet stability.

- **Molecular retention inside droplets**

Droplet-based chemical or biochemical assays rely on the reagents to remain isolated in individual droplets. Leakage to the continuous phase and exchange between droplets, even low, could impact the integrity of the results and therefore, molecular retention is an important parameter to consider when choosing the oil. Fluorinated oils are known to limit molecular exchange between droplets and thus the impact of switching oils should be verified.

Two water-in-oil emulsions ["empty" (PBS-loaded) and "full" (fluorescein-loaded) droplets] were generated simultaneously, once using Novec™ 7500 and then using Fluo-Oil™ 135 as the continuous phase. Both mixes, each containing the two populations, were incubated at 37°C and pictures were taken at different timepoints. A statistical quantitative analysis of the evolution of fluorescence intensity inside droplets was performed using the ImageJ software.

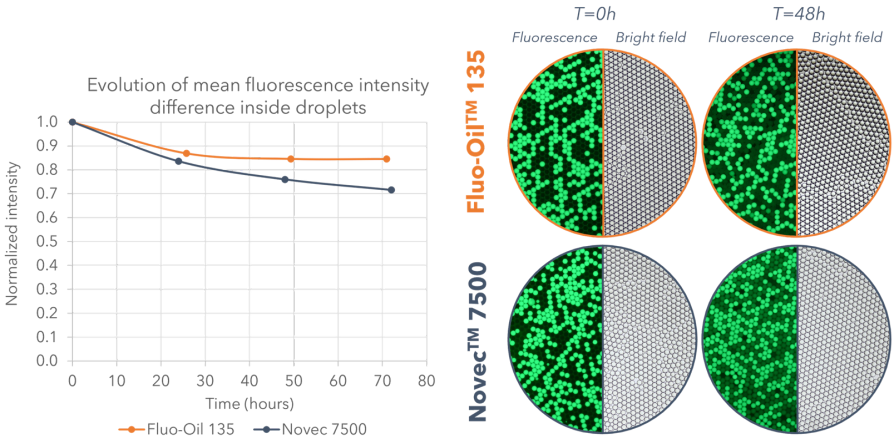

Figure 3: Evolution of mean fluorescence intensity inside water-in-oil droplets generated in Fluo-Oil™ 135 and Novec™ 7500. Droplet mean size=50µm, 37°C incubation

Figure 3 shows the evolution of the mean fluorescence intensity difference between the two populations (dye-loaded and empty droplets) and associated fluorescence and bright field pictures at initial timepoint and after 48 hours.

Over the course of 24 hours, leakage rate of fluorescein from droplets is similar for both oils (about 15% leakage) and stays constant for 3 days in the case of droplets generated in Fluo-Oil™ 135. On the contrary, after 24hrs, leakage from droplets in Novec™ 7500 continues to decrease and reaches around 30%. This poorer retention performance for longer incubation times could be attributed to the slightly lower viscosity of the latter oil which contributes to a faster molecular exchange between droplets.

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CONCLUSION

Switching from one fluorinated oil type to another could have a significant impact on the course of a microfluidic experiment and especially on the efficient generation of droplets, for droplet-based microfluidic applications. In the case of Novec™ 7500 and Fluo-Oil™ 135, it was verified that changing oils had no impact on experimental conditions and results. A better retention of fluorescein inside droplets was even observed when using Fluo-Oil™ 135 as the continuous phase.
Fluo-Oil™ 135 constitutes an efficient alternative to Novec™ 7500 for microfluidic applications.

REFERENCES

[1] Jean-Christophe Baret, Surfactants in droplet-based microfluidics. Lab on a chip, 12 (3), pp.422-433 (2012).



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