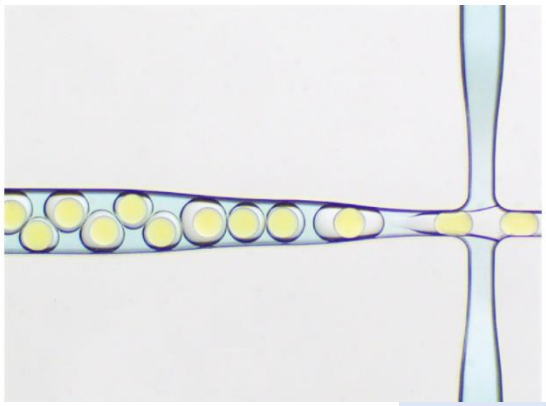
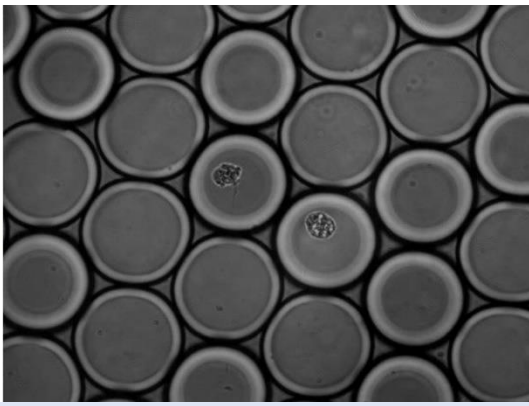




Application Note – Double Emulsion Generation

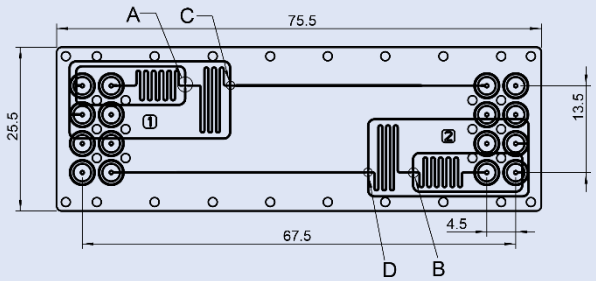
Using Fluidic 1480 and Specific Surfactants

Introduction

The realm of microfluidics has witnessed an increasing demand for precise control in the generation of double emulsion droplets. These droplets, characterized by their multi-layered encapsulation, have paved the way for a multitude of applications across diverse industries, redefining possibilities in pharmaceuticals, biotechnology, cosmetics, and materials science.

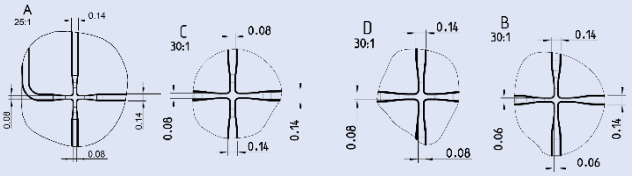
Fluidic 1480, a double emulsion droplet chip has two droplet generator units, each housing a double-cross geometry with variable channel and nozzle sizes at the second cross. Its specialized surface coating facilitates the generation of double emulsions, enabling the encapsulation of droplets, particles, or cells from the initial channel intersection within an additional droplet shell at the second channel cross.

The chips can be provided with a partially hydrophilic coating at the second nozzle to generate water-in-oil droplets in an aqueous continuous liquid. The chip can also be used untreated to generate water-water-oil double emulsions using different aqueous viscosities or to confine an aqueous phase in two different oil phases for the production of w/o/o droplets. In addition, Fluidic 1480 can also be used without pretreatment to mix two aqueous phases and to generate water-in-oil emulsions. For double emulsion experiments, at least three individual pump channels are required.



Chip Description – Fluidic 1480

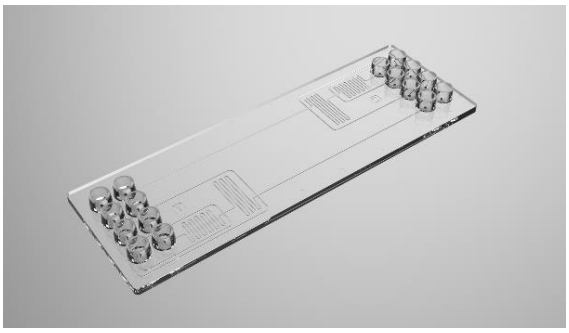
The Fluidic 1480 includes two functional droplet generator units with varying channel sizes at the second cross (unit 2, detail B). Each unique unit consists of a flow-focusing cross for a dispersive aqueous core phase, a shell phase (oil + surfactant) and a continuous phase (water+ surfactant) input and an output. The sizes of the two nozzles on unit 1 are 80x80 μm^2 and on unit 2 60x60 μm^2 and 80x80 μm^2 to generate double emulsion droplets of different sizes.

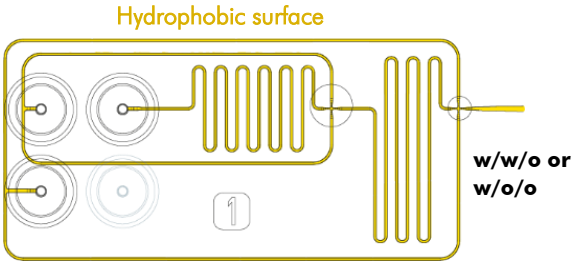


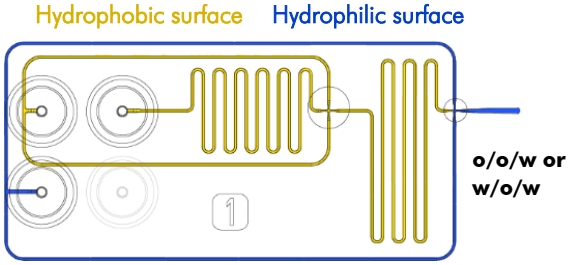
Parameter Fluidic 1480	Description
Chip format	75.5 x 25.5 mm
Interface type	Mini Luer
Droplet generator units	2 x 2
Nozzle type	Flow-focusing
Nozzle size, unit 1	80x80 μm^2
Nozzle size, unit 2	60x60 and 80x80 μm^2
Surface treatment	Untreated (w/w/o or w/o/o), Partially treated (o/o/w or w/o/w)
Lid thickness	140 μm (Topas), 175 μm (PC)

Droplet generation based on surface treatment

For droplet generation, a specific microfluidic channel design (nozzle) is essential. Here, two immiscible phases—the dispersed and continuous phases—meet at an angle within the nozzle, resulting in droplet formation. Typically, the continuous phase flow rate surpasses that of the dispersed phase. The droplet size is defined by the nozzle size and the ratio of sample (disperse phase) to oil (continuous phase), while system throughput is determined by the flow rates of these phases. Different types of droplets can be produced based on surface properties (hydrophobic or hydrophilic) and the phases involved.







Top: Image and schematic drawing of droplet generation chip Fluidic 1480. Bottom: Droplet generation: Left: Untreated microchannels utilized for generating water-in-water-in-oil (w/w/o) droplets or water in two different oil phase (w/o/o). Right: Partially treated channels enable the generation of either two different oil phases in water (o/o/w) or water-in-oil-in-water (W/O/W) droplets.

Materials

Hard and software

- Fluigent system (MFCS-EZ, Flowboard, Flow-Units, Fluiwell with reservoirs)
- External light source and camera (Basler acA800) equipped with 4x objective
- PC-Monitoring with Pylon Viewer Software (Basler) and A-i-O 2019 Software (Fluigent)
- Fluidic 1480 double emulsion generation chip (*microfluidic ChipShop*)
- Capillary PEEK tubing (ID: 0,02", OD: 1/32") from *microfluidic ChipShop* (Article 10002000)
- Male Mini Luer tube tuck connectors from *microfluidic ChipShop* (Article 10001764)
- Pneumatic tubings

Reagents

emulseo

Core Phase

- Millipore H2O or cells in adequate culture medium

Shell Phase

- FluoSurf™ (emulseo) 2% w/w in HFE 7500 (3M)

Continuous phase

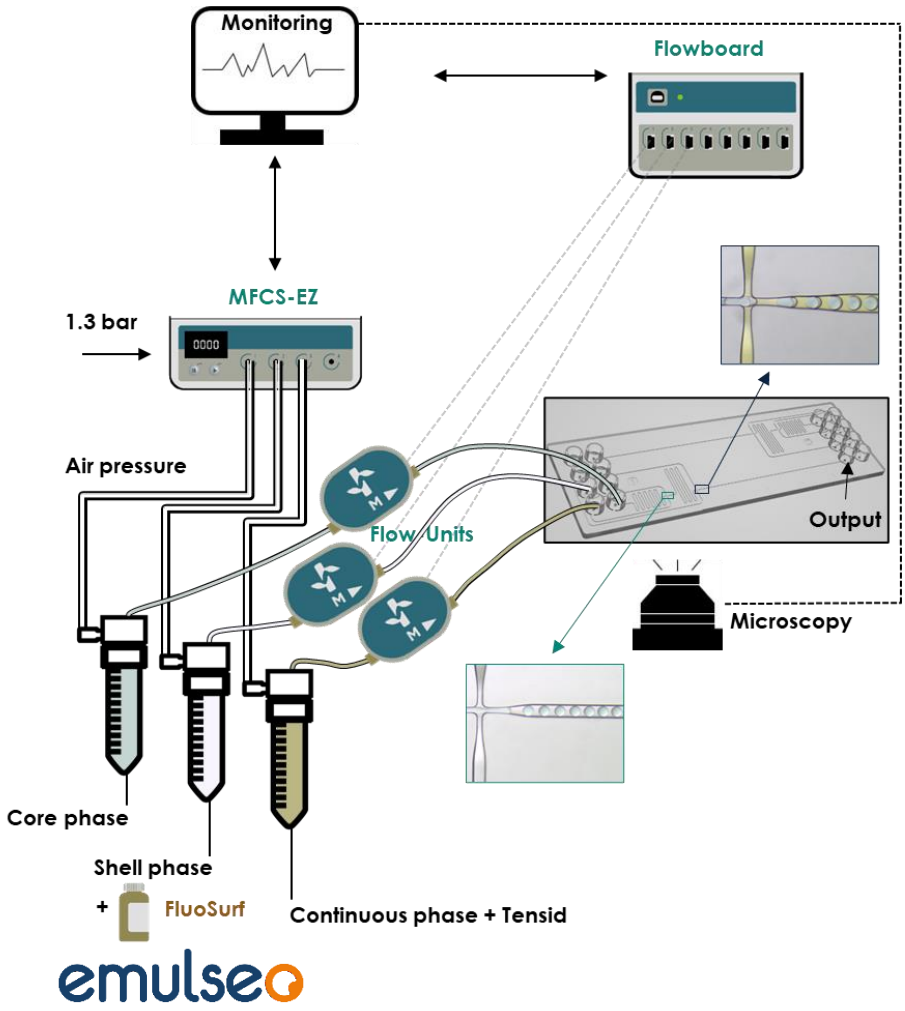
- Pluronic F-68 (5-10% w/w) in Millipore H2O

Expert Tip: For silicone-based oils, Topas chips are preferred, whereas mineral oils necessitate PC chips. Fluorinated oils are best used with PC as well.

Expert Tip - Surfactants: Other suitable surfactants such as PVA or Triton X-100 in concentrations of 5 % w/w may also be suitable for generating double emulsions. The production of double emulsions is even possible when using lower flow rates with 2 % Tween 20, if the second junction and the channel to the outlet have been previously primed with 5 % Pluronic F68 or 5 % PVA. Note: If the surfactants are not suitable or the concentrations are too low, the hydrophilic channel wall will be wetted with the oil phase.

Expert Tip – Sensitivity of organisms and cells to surfactant:
The compatibility of organisms and cells with surfactants must be considered when conducting experiments. In our experiments, for instance, *E. coli* cells remained stable with up to 9% surfactants like Pluronic F68, PVA 4-88, and PVP. However, it's important to note that different cell types may react sensitively to these surfactants[1,2]. When in doubt, preliminary tests assessing cytotoxicity should be conducted using the specific cell types and surfactants involved. Alternatively, referring to relevant literature on this subject is advisable.

Experimental Setup



[1] Tharmalingam T, Goudar CT. Evaluating the impact of high Pluronic® F68 concentrations on antibody producing CHO cell lines. *Biotechnol Bioeng* 2015 Apr;112(4):832-7. doi: 10.1002/bit.25491. Epub 2014 Dec 18. PMID: 25384465.
[2] Huang, CY., Hu, KH. & Wei, ZH. Comparison of cell behavior on pva/pva-gelatin electrospun nanofibers with random and aligned configuration. *Sci Rep* 6, 37960 (2016). https://doi.org/10.1038/srep37960

Quick Start Guide

Setup preparation

1. Connect the MFCS-EZ pressure controller input with pneumatic tubings to an external pressure supply that provides ideally ~1.3 bar and the outputs with the Fluiwell air pressure inputs for two reservoirs Connect the Flowboard to the Flow-Units and to a computer using USB cables. Fill the three reservoirs with water or cells in adequate medium (core phase), FluoSurf 2% (emulseo) (shell phase) and Pluronic F-68 5% (continuous phase) and place them in the Fluiwell.
2. Connect the Flow-Units with PEEK capillaries on both sides, and with the Fluiwell liquid output of the three reservoirs.
3. One side of the tubing is located at the bottom of a reservoir (volume 2 ml) which is used as a liquid reservoir whereas the other side interfaces with the inlet of the droplet generator.
4. Turn on all necessary electronics and devices as light source, camera, computer, and MFCS-EZ.
5. Launch the pylon viewer imaging software (Basler) and the A-i-O 2019 software (Fluigent). With the easy handling A-i-O software air pressure and/or the flowrate can be controlled and monitored for each Flow-Unit.
6. Connect the chip outlet with tubing to a collection container.

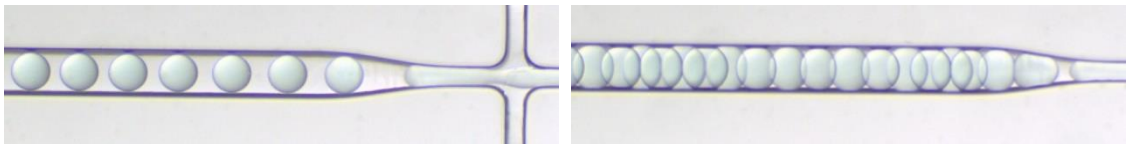
Expert Tip: In replacement of the described Fluigent flow system, other highly accurate and pulsation-free pressure or syringe pump system can be applied to generate monodispersed droplets via a continuous flow and determined flow rates to increase droplet generation performance. In this experiment, the droplet generation is performed with pressure-driven flow control using a Fluigent-MFCSTM-EZ pressure control system and A-i-O 2019 – Software.

Droplet generation

1. Remove air from the tubes of all three liquid phases and then keep the pressure constant at 30-45 mbar or use a flowrate of ~0.1-0.2 µl/min.
2. Connect the inlets on chip, firstly for the continuous phase, secondly for the shell phase, and lastly for the core phase, using Male Mini Luer tube tuck connectors.
3. Increase the pressure of the continuous phase stepwise (max. 10 mbar) to 120 mbar (~4 µl/min) to remove air. (Note: Sometimes higher pressures or flow rates are required to completely remove the air from the channel, especially at the nozzle constrictions).
4. Increase the pressure of the shell phase to 60 mbar (~0.5 µl/min), then gradually increase the pressure for the shell phase in steps of 10 mbar to max. 100 mbar (~ 2 µl/min) to remove the air

Expert Tip: Importantly, the second nozzle (= shell phase-in-continuous phase) (oil-in-water emulsion) should be observed and the flowrate of the shell phase should be adjusted to avoid jetting of the oily phase on the hydrophilic channel walls.

5. Increase the pressure of the dispersive phase to 60 mbar and then stepwise (5 mbar) to 85 mbar (~ 1.0 µl/min).
6. Set the parameters of core and shell phase so that a compact and continuous droplet generation takes place at the first junction. Ideally, the core and shell phase fluids should be operated at identical or closely related pressures or flow rates.



Generation of sequential water-in-oil droplets (left) and (right) closely spaced w/o droplets at the first nozzle.
Expert Tip: The frequency of generation of w/o droplets influences whether monodisperse w/o/w emulsion is successfully formed at the 2nd junction.

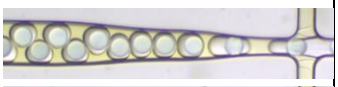
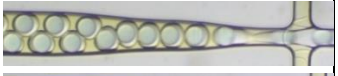
7. Adjust the pressure or flow rate of the continuous aqueous phase for the required shape of the double emulsions.

8. Run the droplet generation for at least 5 min to guarantee stable double emulsion from the dispersed inlet, and to ensure stable droplet formation.

Expert Tip: The flow rates of core and shell phase are freely adjustable, but should be lower than the continuous phase and at a minimum as given values to achieve the desired droplet size and droplet generation frequency. By increasing the continuous flow rate and keeping core and shell constant, the shell diameter decreases. Importantly, the system is very sensitive, and changes to the applied pressures or flow rates should be made in small steps coordinated with all three phases.

Results

Stable double emulsions have been generated using different pressure settings, respectively flow rates. The size of the droplets, the ratio of core and shell droplets and the droplet generation frequency can be regulated using the fluidic settings.

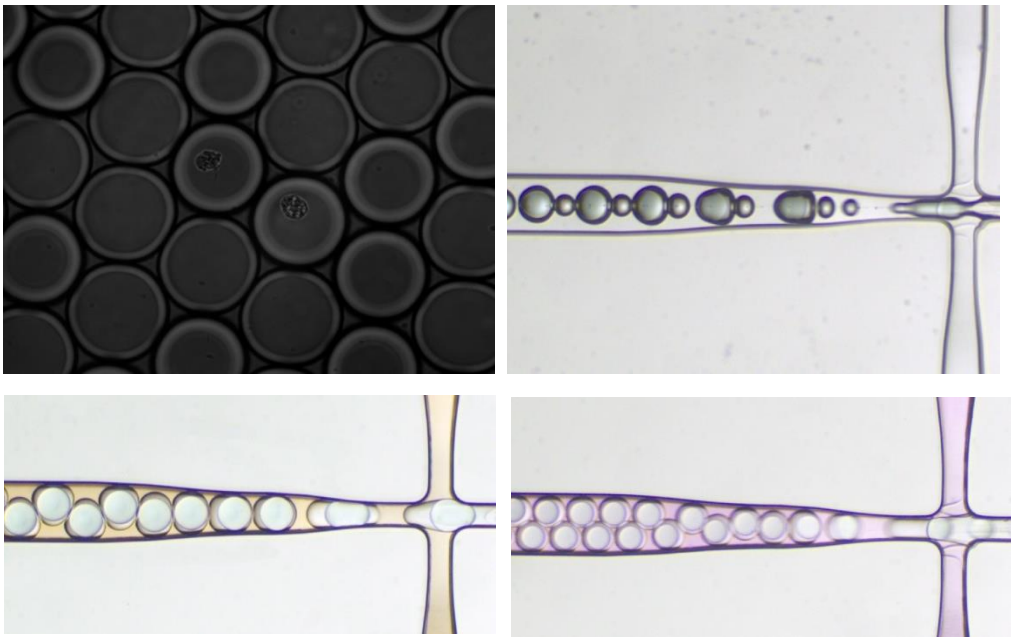
Figure	Shell droplet size	Core droplet size	Core phase		Shell phase		Continuous phase	
Monodispersed w/o/w double emulsion droplets using 5% w/w Pluronic F-68 (continuous phase) and FluoSurf 2% (shell phase)	[µm]	[µm]	Pressure [mbar]	Flow rate [µl/min]	Pressure [mbar]	Flow rate [µl/min]	Pressure [mbar]	Flow rate [µl/min]
1 	150	130	50	0.1	50	0.2	30	0.6
2 	120	80	50	0.1	50	0.4	50	3.0
3 	100	78	53	0.2	44	0.2	35	1.2
4 	112	78	80	1.0	80	1.2	80	6.3
5 	110	80	85	1.0	80	1.0	62	2.0
6 	114	78	90	1.2	90	1.6	90	7.8
7 	104	79	115	2.0	118	2.0	75	4. 0
8 	135	105	73	1.2	73	1.2	118	7.5
9 	100	96	160	4.0	145	3.1	120	10.0
10 	~200	2x110	78	1.2	78	1.2	70	2.0

Conclusion

The main advantage of the system is that only one easy single chip is needed for double emulsion generation. The chip handling ensures w/o/w-droplet formation with high monodispersity (CV < 2%) and stability. However, to ensure emulsification at the second nozzle of each unit a relatively high surfactant concentration is needed.

Further Examples

- Single cell encapsulation, cultivation and observation (e.g., eukaryotic or microbial cultivation)
- Generation of water-in-oil-in-oil droplets (w/o/o) or production of various water-in-water-in-oil droplets (w/w/o, e.g., hydrogel particles)



Bright field microscopy images showing (top, left) single HeLa cells in DMEM medium; (top, right) Generation of w/o/o droplets with blue inked H_20 (core) in 2% FluoSurf (emulseo) (shell) in mineral oil with 4% Span 80 (outer continuous phase). Generation of w/o/w droplets with 5 % (bottom, left) and 2% (bottom, right) Tween 20 as surfactant, after continuous phase channel priming with 5 min flow-through (1 μl/min) with PVA 5% wt.

Other Applications

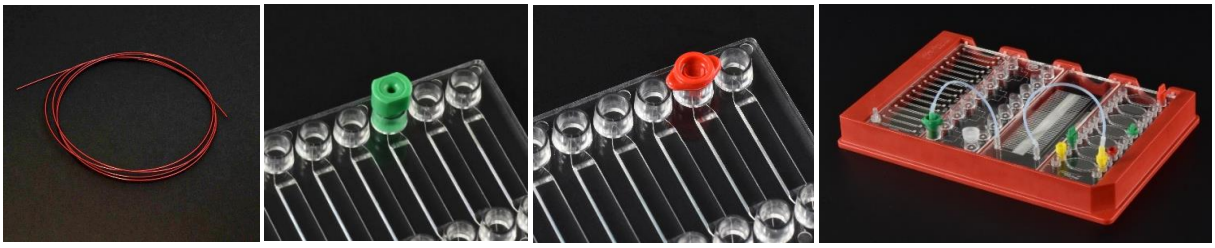
- Antimicrobial or metabolically screening (e.g., AST)
- Chemical-cell interaction testing (e.g., tumor cell treatment)
- Cosmetic studies (e.g., encapsulation and controlled release of substances)
- Crystallography in droplets (e.g., growth of protein crystals)
- Drug delivery systems (e.g., controlled release of active compounds)
- Emulsion stability studies (e.g., long-term applicability of w/o/w, w/w/o or o/o/w droplets)
- Food applications (e.g., supplement encapsulation)

Related Products

To facilitate droplet generation, a range of products is essential, including polymer chips, fluid connectors, tubing, pumps and oil with surfactant. Additional products such as plugs for sealing or handling frames for enhanced experimentation can be utilized. For further technical inquiries, please reach out to us at inquiries@microfluidic-ChipShop.com.

Product Code Fl. 1480	Surface Treatment	Droplet Generation	Material	Lid Thickness [µm]
10002061	Untreated – hydrophobic surface	w/w/o	Topas	140
10002062	Untreated – hydrophobic surface	w/w/o	PC	175
10002106	Treated – Partial surface treatment	w/o/w or w/w/o	Topas	140
10002107	Treated – Partial surface treatment	w/o/w or w/w/o	PC	175

Tubing and accessories



Product Code	Description	Material	Quantity
10002009	Capillary PEEK tubing, orange, ID: 0.508 mm (0.02“), OD: 0.794mm (1/32“)	PEEK	3.049 m

Product Code	Fluidic	Description	Type	Material	Color
10001764	997	Mini Luer tube tuck connector for 1/32“ tubing	Single	TPE	Green
10000205	438	Male Mini Luer plugs – Low volume displacement	10 pc/pack	TPE	Opaque
10000042	352	Handling frame with high skirt	1 pc	PC	Orange

Droplet oil and surfactant

Explore our selection of oils and surfactants available in diverse formats. For expert guidance, feel free to reach out to us at inquiries@microfluidic-ChipShop.com.

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