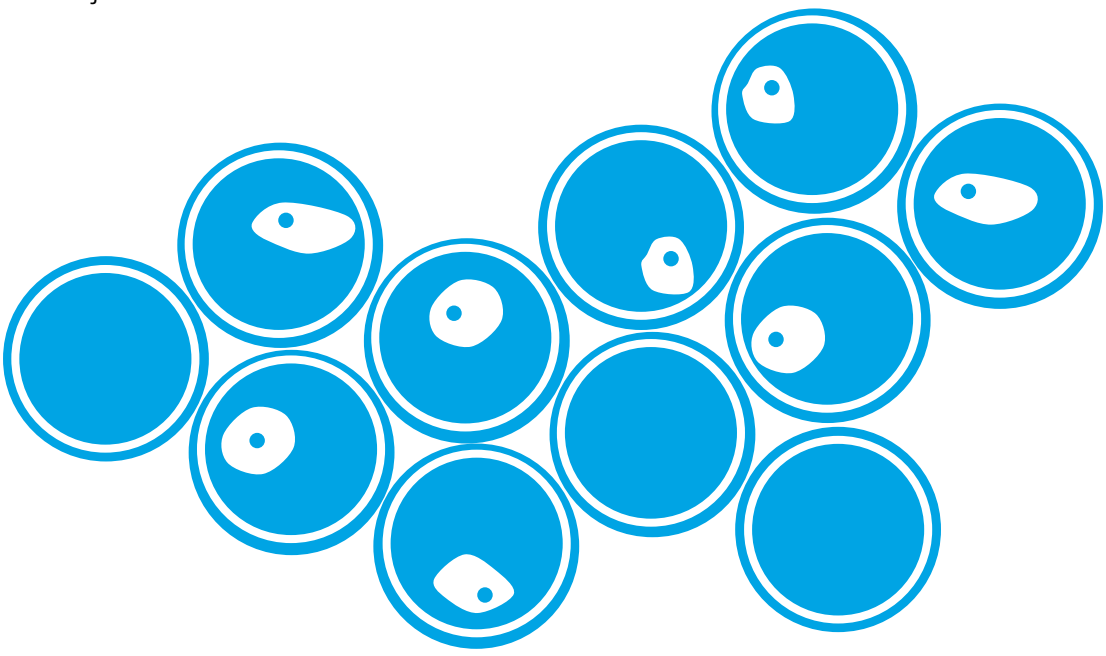
µEncapsulator Application Note

For Double Emulsions

Version: 3.0

Issue Date: 27 March 2019

Author: JF2



Dolomite Microfluidics is a brand of Blacktrace
Registered office: The Dolomite Centre Ltd. 3, Anglian Business Park, Royston, SG8 5TW, UK
Reg in England No. 04257809

1 Disclaimer

This product is for research use only and is not to be used for any other purposes, including, but not limited to, use in drugs, in vitro diagnostics, or use in humans. The document and its content are proprietary to Dolomite Microfluidics and is intended only in connection with the products described herein and for no other purposes.

The document and its contents shall not be reproduced, disclosed, or used for any other purpose without written consent of Dolomite Microfluidics. Dolomite Microfluidics does not convey any license under its trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

Dolomite Microfluidics makes no warranty of any kind, either expressed or implied. This includes merchantability for this product, and the fitness of the product for any purpose.

µEncapsulator is a trademark of Dolomite Microfluidics. All other brands and names mentioned within this document are the property of their respective owners.

2 Introduction

This application note summarises the results of the production of a double emulsion on the µEncapsulator system using a 30µm µEncapsulator 1- 2 Reagent Droplet Chip. It details parameters such as flow rates and pressures.

Screening of individual cells for an enzymatic activity or antibodies using FACS is an efficient approach for library screening, however there are restrictions to this method. The reaction product must be retained by the cell or at least be displayed on the cell surface and at the same time the reaction substrate must be able to diffuse into the cells to label the product. Most library screens do not meet those criteria and therefore alternative approaches such as the encapsulation of cells and their reaction substrate into enclosed compartments like droplets are highly valuable. When encapsulating cells into droplets with the reaction substrate, the product secreted by the cell can be detected and be traced back to the cell of origin as the product won't be able to leave the droplet. Furthermore, this method also reduces the assay volume leading to cost savings and allows for the screening of hundreds of thousands of cells at the same time. Aqueous droplets are commonly surrounded by an oil carrier phase which does not serve as a suitable medium for FACS. Therefore, water in oil droplets are typically re-encapsulated using a water-based carrier phase. Thereby water in oil in water emulsions are produced that can readily be FACS sorted. The following application note describes results obtained when creating double emulsion on the µEncapsulator system, literature on this topic is listed below.[1]

[1] Anastasia Zinchenko et al., 2014, One in a Million: Flow Cytometric Sorting of Single Cell-Lysate Assays in Monodisperse Picolitre Double Emulsion Droplets for Directed Evolution

Andrew C. Larsen et. al., 2016, A General Strategy for Expanding Polymerase Function by Droplet Microfluidics

3 Materials and Methods

Droplet system.

The µEncapsulator system (Figure 1 A) uses pulseless Dolomite Microfluidics P-Pumps and can be driven from a PC via Dolomite Microfluidics's Flow Control Centre (FCC) software. There are three highly precise pressure pumps driving the cell, reaction substrate and oil lines (Figure 1 B). They are connected to a sample reservoir where cells/ reaction product and reaction substrate are stored which sits directly on top of the temperature control unit keeping the whole microfluidic path at a set temperature.

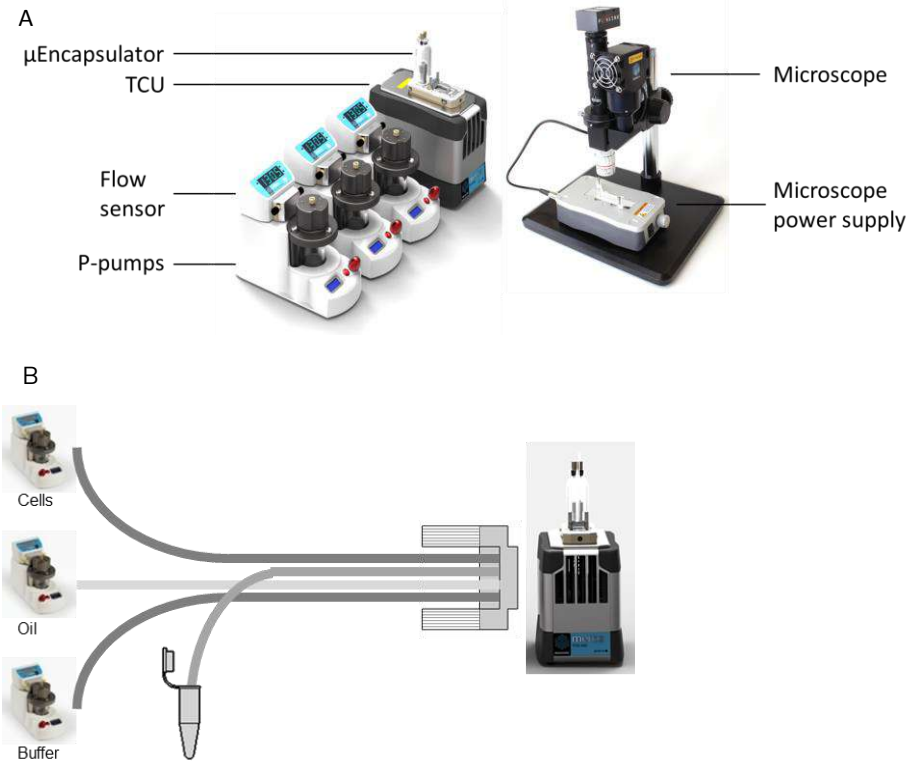


Figure 1 µEncapsulator system set up. (A) Image of the system set up depicting all the components of a µEncapsulator. (B) The schematic of the individual fluidic lines driving cells, reaction substrate, and oil

High Speed Microscope.

The Dolomite High Speed Microscope (Figure 1 A) is a simple compact microscope, with a convenient long working distance lens. There are no eye-pieces, partly to protect users from the very bright light source. The microscope allows short exposure times (50 µs), which is useful in monitoring high speed droplet production.

Glass µEncapsulator Chip.

The current literature describes prototype PDMS Chips for droplet formation. For standard products, glass is preferred, as the glass chips are robust, achieve highly reproducible performance, and are chemically inert. Dolomite Microfluidics therefore produced a glass µEncapsulator droplet chip (Figure 3, see appendix for a full list of available chips) for the easy flow of reagents. The channels allow the use of robust, standard, leak-free connectors, while allowing the junction to be readily imaged. The chips used in this application note had a 30µm microfluidic junction and fluorophilic as well as hydrophilic coating.

1st step: Primary water in oil emulsion.

All the liquids used (oil, reaction substrate, cell suspension buffer) were pre-filtered using 0.2 µm syringe filter prior to loading onto the system. The oil pump was loaded with 2 % FluoSurf, an oil commercialised by Dolomite Microfluidics and containing a proprietary surfactant, and the two pumps driving the aqueous lines with Novec 7500. Novec 7500 loaded into the 'aqueous' pumps functioned as a 'driving liquid', to push the cell suspension buffer and reaction substrate out of the sample reservoir chip. The system was primed by running all three lines at 2 bar until drops appeared at the linear connector (this will happen within seconds). The pumps were then stopped, and all three valves closed. The cell suspension buffer and reaction substrate were loaded into the sample reservoir chip (Figure 5 in the Protocol document) and the linear connector fixed to the µEncapsulator module. All three p-pumps were pressurised to 200 mbar, firstly the cell suspension buffer line valve was opened and switched to flow mode (flow rates listed in Table 1). Next the same was done by opening the valve for the reaction substrate line and switching to flow mode to set the flow rate. Finally, the oil valve was opened and switched to flow mode.The flows were started in the order cell suspension buffer>reaction substrate>oil, to avoid backflow of reaction substrate solution into the cell line. Droplet production was stable and easily initiated at flow rates listed in Table 1. At these flow rates about 11,800 droplets per second are generated. This means that a full run for encapsulating 2 x 100 µl will take about 20 min. The collected emulsion was transferred to room temperature to allow for complete separation of oil and emulsion.

Pump	Flow rate [µl/ min]
Oil	35
Reaction substrate	5
Cells	5

Table 1

Preparing the primary water in oil emulsion for encapsulation.

Before starting to encapsulate the water in oil emulsion, we allowed the emulsion to sit for approximately 10 minutes in the micro-centrifuge tube, resulting in a close packing of the droplets. Slightly more than 100 µl were directly pipetted from the emulsion floating on the top. The emulsion in the pipette tip was observed against a dark background to check if any oil was carried over. If no oil was found, the emulsion was directly loaded into the sample reservoir chip.

2nd step: Secondary water in oil in water emulsion.

All the liquids used (oil and TBS-T) were pre-filtered using 0.2 µm syringe filter prior to loading onto the system. All pump reservoirs were loaded with TBS-T. TBS-T loaded into the 'aqueous' pumps functioned as a 'driving liquid', to push the sample (2 % FluoSurf and the primary emulsion) out of the sample reservoir chip. The system was primed by running all three lines at 2 bar until liquid appeared at the linear connector. The primary emulsion and 2 % FluoSurf were loaded into the sample reservoir chip and the linear connector fixed to the µEncapsulator module. The flows were started in the order primary emulsion>TBT-T >2 % FluoSurf (Please refer to the double emulsion protocol for further details). Droplet production was initiated at pressures listed in Table 2. At those pressure the collection of 20 µl emulsion will take about 1h.

Pump	Pressure [mbar]
TBS-T	150
Emulsion	350
2 % FluoSurf	200

Table 2

4 Results

The objectives of these tests were 1) to define optimal flow rates for the first stage primary emulsion and 2) to identify pressures that lead to a stable production of a water in oil in water secondary emulsion. Flow rates suggested for standard water in oil emulsion on a 30 µm fluorophilic chip are 5 µl/min for aqueous samples and 30 µl/min for oil. As droplets produced in the 1st stage primary emulsion needed re-encapsulation into a water-based carrier phase for the 2nd step we aimed to produce slightly smaller droplets in the 1st stage emulsion. The flow rate for the oil line was therefore increased to 35 µl/min to achieve a more frequent pinching of droplets and thereby a reduction of the overall droplet size (Figure 2).

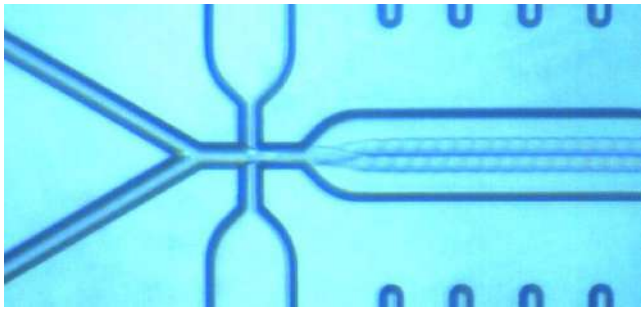


Figure 2 Formation of water in oil emulsion for the 1st stage

5 Conclusion

This application note demonstrated the successful formation of double emulsion droplets. This method will be of high value for the target selection of producer strains even if the product is secreted. This has proven valuable for a lot of application such as the directed evolution of industrial enzymes. This is the only commercially available system that allows for the formation of double emulsions on a high scale.

6 Product Information

6.1 µEncapsulator System

Orders from	Instrument/Consumables	Order Number
US and Canada	µEncapsulator System with Enhanced Control (110v, 60Hz, US) - Excludes Applications Pack	3200554
	High Speed Digital Microscope and Camera	3200531
UK	µEncapsulator System with Enhanced Control (230V, 50Hz, UK) - Excludes Applications Pack	3200556
	High Speed Digital Microscope and Camera	3200531
Europe	µEncapsulator System with Enhanced Control (230V, 50Hz, EU) - Excludes Applications Pack	3200558
	High Speed Digital Microscope and Camera	3200531
Japan	µEncapsulator System with Enhanced Control (100V, 50-60Hz, JP) - Excludes Applications Pack	3200560
	High Speed Digital Microscope and Camera	3200531
Rest of the world	µEncapsulator System with Enhanced Control (230V, 50Hz, UK) - Excludes Applications Pack	3200556
	High Speed Digital Microscope and Camera	3200531
	Installation and Basic Training (supplement for 2 days, on site RoW)	3200571

6.2 µEncapsulator Chips

Orders from Instrument/Consumables	Order Number
µEncapsulator 1 Sample Reservoir Chip (2x 100µl) Pack of 3	3200562
µEncapsulator 1 - 2 Reagent Droplet Chip (50µm etch depth), fluorophilic, Pack of 3	3200563
µEncapsulator 1 - 2 Reagent Droplet Chip (50µm etch depth), hydrophilic, Pack of 3	3200564
µEncapsulator 1 - 2 Reagent Droplet Chip (30µm etch depth), fluorophilic, Pack of 3	3200567
µEncapsulator 1 - 2 Reagent Droplet Chip (30µm etch depth), hydrophilic, Pack of 3	3200568
µEncapsulator 1 - 2 Reagent Droplet Chip (15µm etch depth), hydrophilic, Pack of 3	3200565
µEncapsulator 1 - 2 Reagent Droplet Chip (15µm etch depth), fluorophilic, Pack of 3	3200566