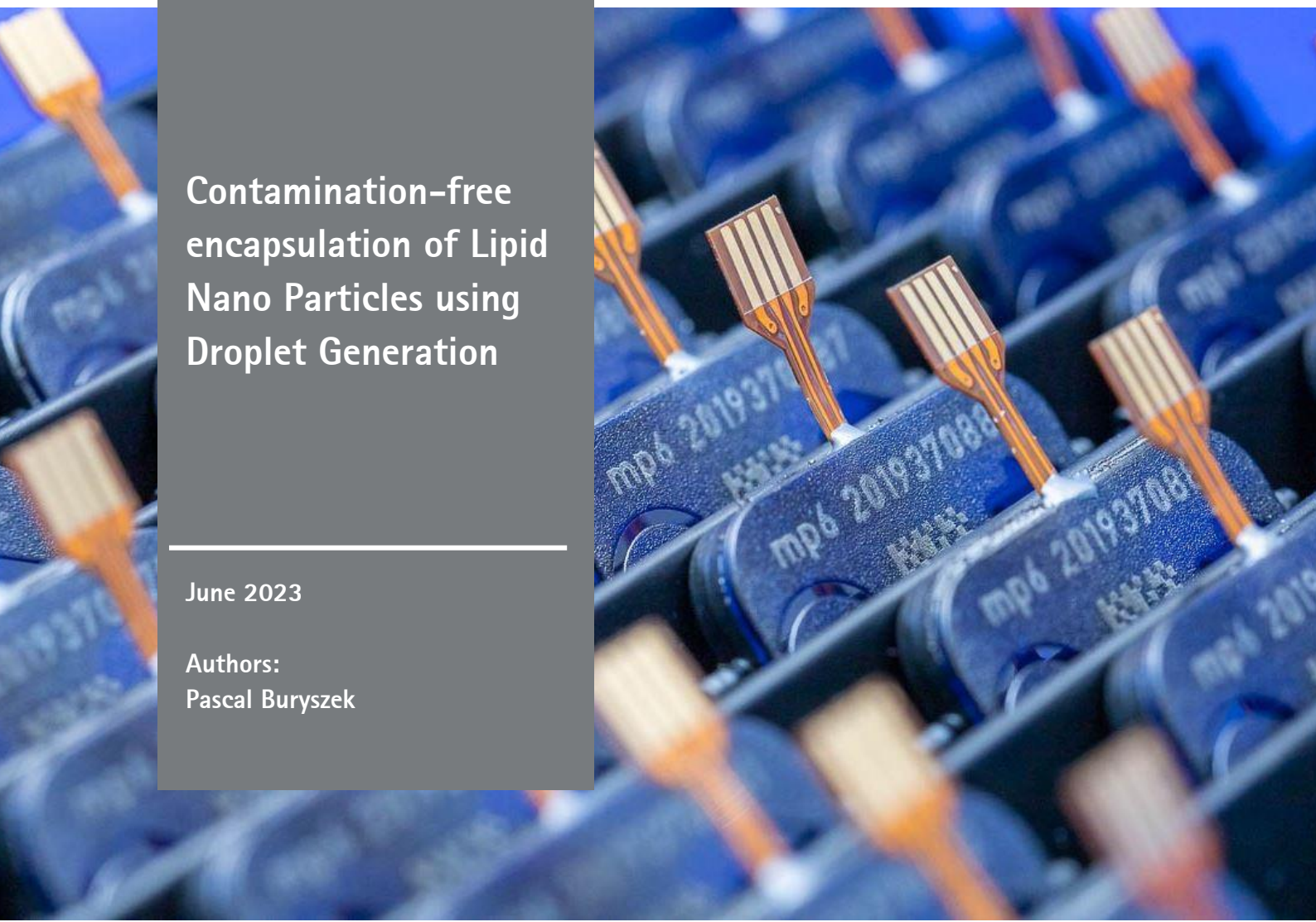
CASE STUDY

Bartels mikrotechnik

with passion for microfluidics

Contamination-free encapsulation of Lipid Nano Particles using Droplet Generation



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Authors:
Pascal Buryszek

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Single cell analysis & sorting

Ultra small reaction volume

Lipid nanoparticles (LNPs) are spherical particles with an outer shell made of biocompatible polymers encapsulating drugs in nanoscale. The physiochemical diversity and biocompatibility of lipids enhance oral bioavailability of drugs and positively influence drug absorption. Other than drug delivery are more applications for LNPs, like vaccines by encapsulating antigens adjuvants, as well as gene therapy for DNA or RNA delivery. Compared to conventional pharmaceutical manufacturing this technology is less solvent intensive and generates a way smaller waste-to-product ratio. This in conjunction with lower energy requirements make pharmaceuticals with LNPs make them a green & sustainable chemistry approach.

Droplet generation in microfluidics is based on the use of two immiscible phases that are referred to as the continuous phase (oil + surfactants, medium in which droplets flow) and dispersed phase (water, the droplet). For generating droplets, microfluidic systems generally include a microfluidic chip, a fluid handling system and tubing. This system is usually connected to a computer and a microscope to visualize droplet formation.

The objective of this case study is to demonstrate contamination-free droplet generation using the mp6 micropump with pressure sensors, fluidic accessories, reagents like the surfactants provided by Emulseo and a Droplet Generator Chip obtained from our partner *microfluidic ChipShop*.

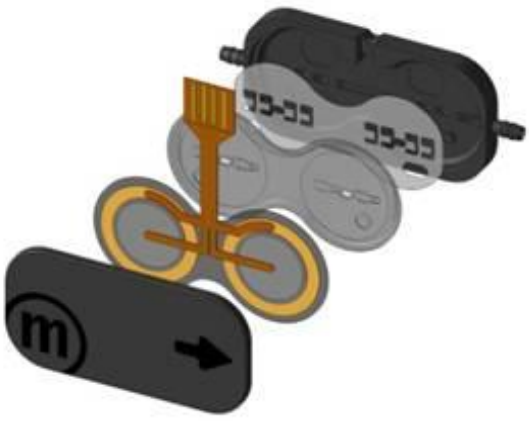
What is microfluidics?

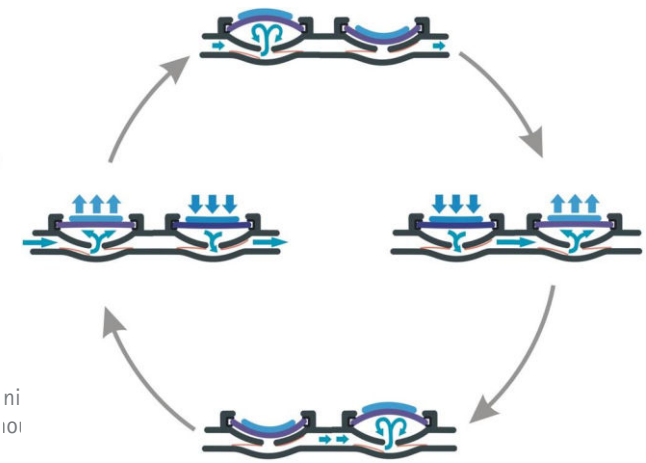
Microfluidics is the fine art of creation and manipulation of small portions of fluids, often realized by flow within small, sub-millimeter-scale channels. These small dimensions allow the fluid flow to be controlled with exquisite precision (Seifert, Thiele; 2020).

About the mp6 micropump

The available, industrialized and commercialized example is the mp6 micropump by Bartels Mikrotechnik GmbH. This micro pump is a positive displacement membrane pump utilizing piezo buzzers. The alternating displacement of the piezo acutators lead to the following typical fluidic values of the pump:

- Liquids (eta = 1 mPas): q = 5 - 8000 µl/min in free flow and p > 600 mbar
- Gas: q > 25 ml/min in free flow and p > 150 mbar





Lipid Nanoparticles

Lipid nanoparticles are small particles composed of lipids, which are naturally occurring molecules found in fats and oils. They have a spherical structure with a lipid bilayer surrounding an aqueous core. The size of lipid nanoparticles typically ranges from tens to hundreds of nanometers. They are used to deliver drugs, vaccines, and gene therapies to specific targets in the body. LNPs offer advantages such as stability, compatibility with the body, and the ability to carry both water-soluble and fat-soluble substances. They can protect drugs from degradation, improve solubility, and enhance drug effectiveness while reducing side effects. Lipid nanoparticles are also utilized in research, diagnostics, cosmetics, and personal care products. Characterization techniques such as dynamic light scattering (DLS) and electron microscopy are commonly used to measure their size and morphology

Droplet Generator Chips

To generate droplets many different chips can be used, each with its advantages. For this case study two chips are selected to show droplet generation in an easy way.

Fluidic 719 – Droplet generation and storage chip

The droplet generator chip Fluidic 719 combines the generation of the droplets with the storage and capture of single droplets for optical analysis (see Figure 1). The continuous phase is introduced through one Mini Luer inlet, which separates into two channels. Operation of one unit of Fluidic 719 therefore requires a control unit with the ability to control two individual flows, one for the continuous and one for the disperse phase. This can be realized with the mp-Multiboard2 as explained in the experimental setup.

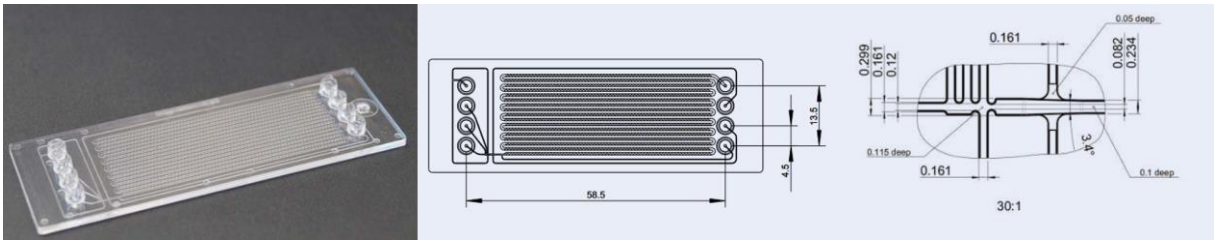


Figure 1: Microfluidic chip 719 – Droplet generator and storage chip from our partner microfluidic ChipShop with Mini Luer interfaces for W/O droplet generation (Left), schematic drawing (middle) and flow focusing junction in detail (right)

Surfactants for droplet generation

Surfactants are compounds that lower the surface tension between two substances, such as a liquid and a gas or a liquid and a solid. They have both hydrophilic (water-loving) and hydrophobic (water-repelling) regions in their molecular structure. In microfluidic droplet generation, surfactants are used to stabilize and control the formation of droplets by reducing the interfacial tension between the dispersed and continuous phases. This allows for the production of uniform and well-defined droplets with precise control over size, shape, and monodispersity, which is crucial for various applications such as drug delivery, chemical synthesis, and biological assays.

FluoSurf-O™ Surfactant

FluoSurf-O™ is a high-performance fluorinated surfactant designed and optimized to stabilize aqueous droplets in fluorinated oils (proposed by Emulseo) for chemical or biotechnological applications. FluoSurf-O™ is an inert block copolymer designed to stabilize droplets containing biological compounds. It is suitable for droplet-based microfluidic experiment such as droplet digital polymerase chain reaction (ddPCR) or single cell analysis. Thanks to its low autofluorescence, FluoSurf-O™ is particularly efficient for fluorescent dyes detection even at low concentration.

Experimental Setup – Overview

For this case study, the following components are used to generate droplets:

1. **Microfluidic droplet generation and storage chip Fluidic 719**

2. **Fluidic accessories like**
   a. Fluidic interfaces: e.g. Male Mini Luer Fluid Connectors – Fluidic 331
   b. mp-t (1,3 mm) Tubing
   c. Handling frame for convenient handling

3. **Reagents in a reservoir**
   a. Fluo oil 7500 containing FluoSurf-O™ Surfactant (continous phase)
   b. Water (dispersed phase)

4. **Pump setup including driver unit**
   a. mp-Multiboard2 with mp-Highdriver4 and mp-Highdriver
   b. 2-4 mp6 micropumps

5. **Sensors**
   a. 2 pressure sensors (Honey ABP-series)

For this application a steril and contamination free pumping approach is chosen. Both phases are put inside pressurizable reservoirs and can conveyed via pressure-over-liquid. The Pressure in each reservoir is monitored via pressure sensors and is controlled with their set of mp6 micropumps. A scheme of the microfluidic setup is presented in Figure 2.

The mp6 micropumps are controlled by the mp-Highdriver4, which means that four pumps can be controlled at same time. The set frequency is the same for all pumps, but it is possible to set the voltage individually for every pump. The resulting pressures can be set individually for the continuous and dispersed phase.

To adjust droplet size and droplet generation rate the pressure was varied and ranges from 100 to 160 mbar for DI-water and 135 to 210 mbar for oil. The generated droplets are visualized with an microscope in Table 1 for microfluidic chip 719. Meassurements of droplet size were taken from pictures of storage segments of the microfluidic chip Figure 3.

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It can be observed that with increasing water pressure and oil pressure, the droplet diameter decreases and the generation rate increase significantly. Video footage will be shared as well on Bartels Mikrotechniks social Media paltforms.

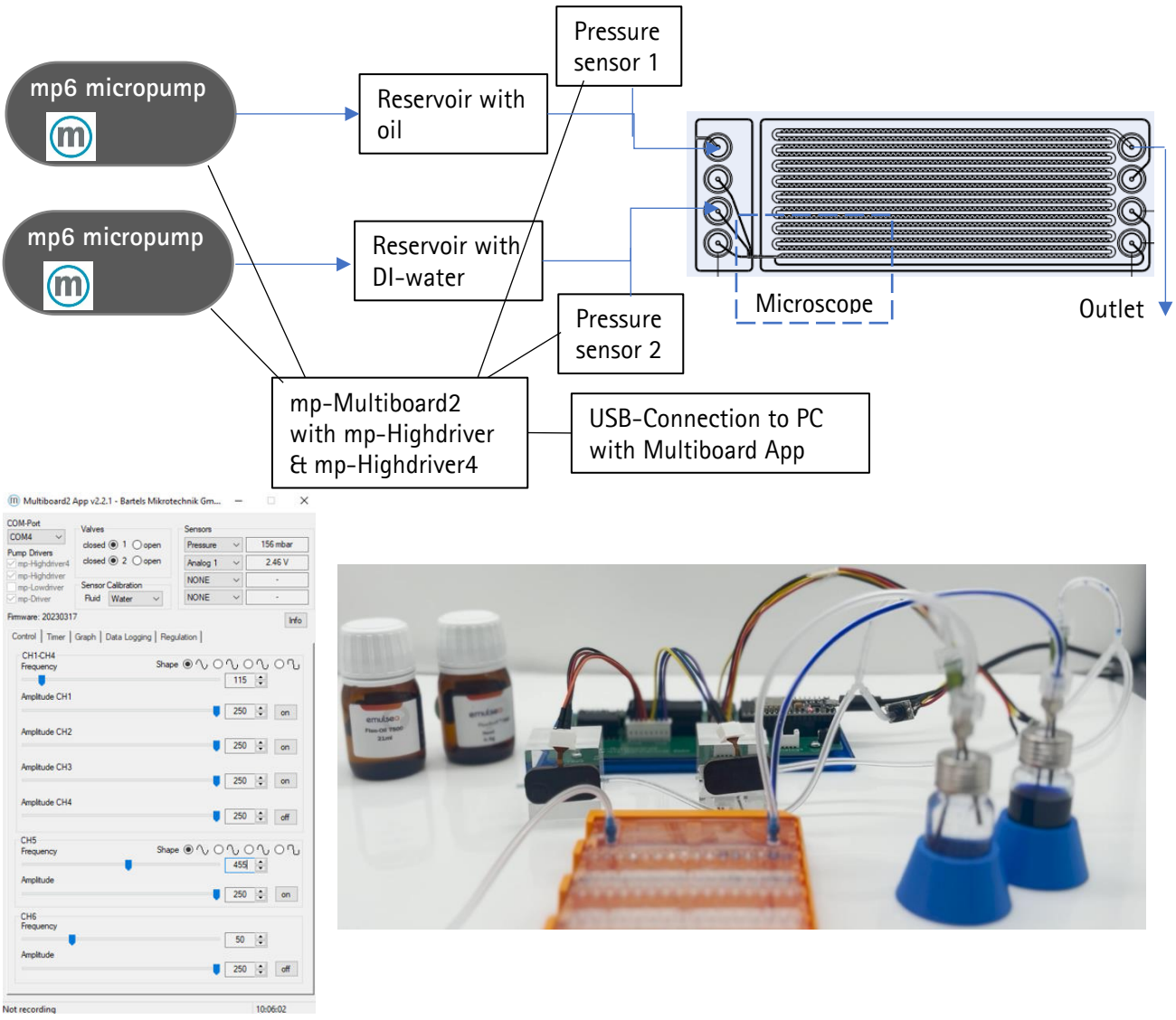

Figure 2 Setup of the microfluidic system used for droplet generation with microfluidic Chip 719. Above: Schematic, Bottom: In real with control via Multiboard2 APP on PC

Experimental Results

Table 1: water-in-oil droplet generation with microfluidic chip 719 as a function of the pressure & pressure ratio of continuous and dispersed phase

No. [#]	Droplet size [µm]	Droplets per minute	Pressure continous phase [mbar]	Pressure dispersed phase [mbar]	Pressure ratio continous : dispersed phase	Picture
1	239,5	102	109	135,34	1:1,24	
2	219,5	60	121	150,86	1:1,25	
3	257,1	252	130	168,97	1:1,3	
4	66,51	>2000 (Not countable)	160	185,34	1:1,16	
5	93,12	>2000 (Not countable)	129	147,41 – 175	1:1,14 1:1,36	
6	106,42	>2000 (Not countable)	156	168,97 – 208,62	1:1,08 1:1,34	

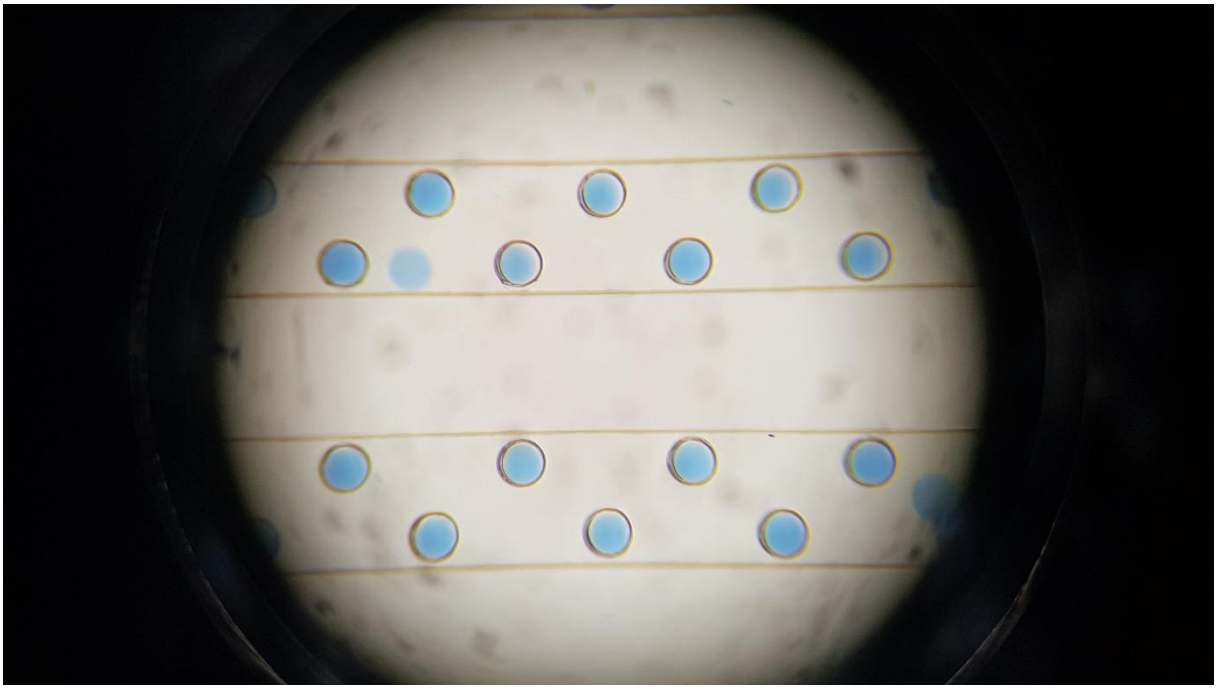

Figure 3 Picture of storage section of microfluidic chipshop Fluidic 719 chip (Test No.6)

It is possible to generate droplets with pressure over liquid technique in different droplet sizes and droplet generation rates. Additionally, due to the oil and surfactant used, higher droplet generation rates at even lower applied pressures were achieved compared to previous droplet generation case study.

In general it can be shown that it is possible to generate monodisperse droplets at high throughput rates with option of storage and optical analysis.

Components and systems used:

- mp6 micropump by Bartels Mikrotechnik
- mp-Multiboard2 incl. Mp-Highdriver4 by Bartels Mikrotechnik
- ABP pressure sensor by Honeywell
- Fluidic 719 by microfluidic ChipShop
- Biocompatible Fluo-oil 7500 & FluoSurf-O™ surfactant by Emulseo

Acknowledgements:

Our Partner, *microfluidic ChipShop* from Jena, Germany, was instrumental in defining our research path, whereby we were able to develop great solution for generating droplets. For that, we are extremely grateful and we are looking forward to our close collaboration. In case you are interested in the above-described microfluidic components or if you are interested in getting in touch with either one of us, *microfluidic ChipShop* or Bartels Mikrotechnik, please feel free to contact us. You can find the contact details below. Also, check out the microfluidic ChipShop website to learn more about their whole portfolio: https://www.microfluidic-chipshop.com/

We also like to thank our new partner, *emulseo* from Pessac, France. Their expertise and collaboration have been significant in providing insightful guidance and access to their cutting-edge microfluidic technologies. The company's commitment to innovation and dedication to advancing droplet generation techniques have greatly enhanced the quality and relevance of this research. In case you are interested in the above-mentioned oils and surfactants or if you are interested in getting in touch with either one of us, *emulseo* or Bartels Mikrotechnik, please feel free to contact us. You can find the contact details below. Also, check out the emulseo website to learn more about their whole portfolio: https://www.emulseo.com/

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Bartels Mikrotechnik is a globally active manufacturer and development service provider in the field of microfluidics. In the microEngineering division, the company supports industrial customers in the modification, adaptation and new development of high-performance and market-oriented product solutions through the innovative means of microsystems technology. The second division, microComponents, produces and distributes microfluidic products and systems, especially for miniaturized and portable applications. Our key products are micropumps that convey smallest quantities of gases or liquids and are used in a variety of ways in biotechnology, pharmaceuticals, medical technology and numerous other applications.

Bartels Mikrotechnik with passion for microfluidics!

Contact us:

Bartels Mikrotechnik GmbH
Konrad-Adenauer-Allee 11
44263 Dortmund Germany

www.bartels-mikrotechnik.de
info@bartels-mikrotechnik.de
Tel: +49-231-47730-500

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