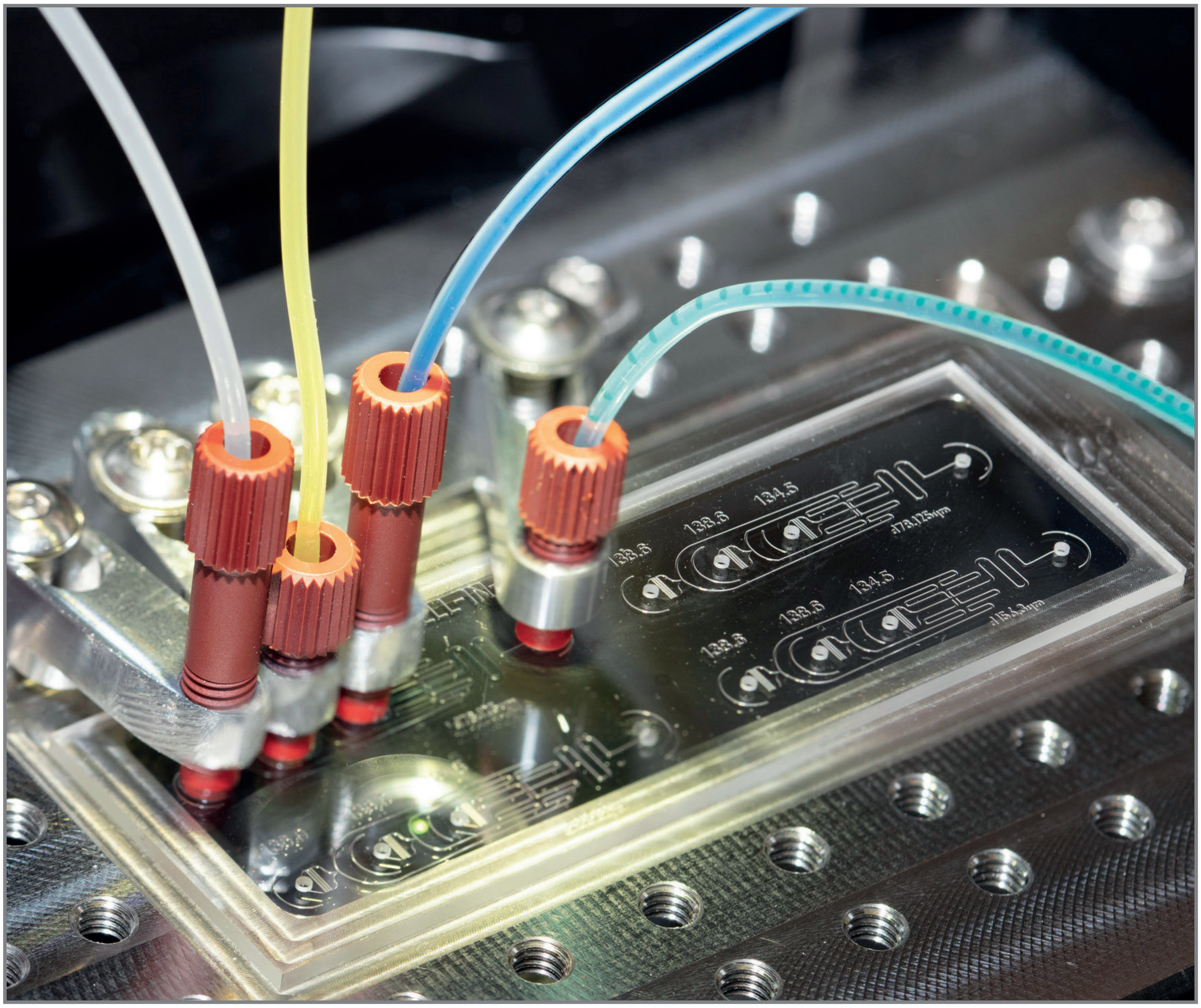
Application Note



Microfluidic glass devices for flexible droplet-based single-cell sample preparation

HEIDENHAIN | IMT

Introduction

Microfluidic-based droplet generators are used to create highly reproducible and monodisperse droplets. Generating droplets by microfluidic technologies is very common and useful to the life science research and the clinical diagnostics market. Application examples in life science research include single cell expression profiling, where droplet-based microfluidic devices facilitate high-throughput single cell analysis. Cells can directly be sorted, separated and subsequently encapsulated into nano-liter sized droplets, which serve as downstream reaction chambers. Additionally, droplet generators enable single cell analysis through the simultaneous encapsulation of a single cell together with a primer-coated micro-bead.

Droplet generators also have several applications in the pharmaceutical industry (e.g. controlled release of sensitive biomolecules in pharma for drug discovery and therapeutic drug delivery), cosmetic industry (e.g. encapsulated active components for conservation), and food industry (e.g. conservation of flavors).

Results

HEIDENHAIN has utilized an all-glass microfluidic device for droplet generation for encapsulating single cells, for e.g. single cell profiling. This device can either be used to produce oil-in-water droplets or water-in-oil droplets. To produce the former, the surface of the microfluidic channels does not have to be modified, since one of the superior characteristics of glass is that it generally provides a hydrophilic surface without further pre-treatment. However, to produce the latter, the surface of the channels has to be modified by applying a hydrophobic surface coating to the microfluidic channel design of the device. This coating modifies the surface energy of the glass substrate and attracts organic solvents like oil to the surface and repels water, resulting in water-in-oil droplets.

Modifying the properties of a droplet generator by surface coating

In order to generate oil-in-water emulsions, a microfluidic glass device with a cross-channel configuration and with hydrophilic surface properties is used (see Figure 1A). A hydrophobic surface is needed, in order to produce water-in-oil droplets. Therefore a silane is used to apply a hydrophobic coating to the channel walls inside the glass device. The microfluidic devices can be mounted on standard laboratory equipment (see Figure 3). By using a commercial pump system, an aqueous solution containing a dye and an oil without dye were injected into the uncoated glass device and oil droplets in water were formed. When the same solutions were introduced into the surface-treated glass device, water droplets in oil were produced (see Figure 1B). QX200 oil (Bio-Rad Laboratories, Inc., USA) was used for the organic phase and PBS / 0.01 % BSA mixed with a blue dye (Pelikan Holding AG, Switzerland) was used for the aqueous phase. Droplet size and droplet generation frequency were controlled by varying the flow rate of the various phases. For glass microfluidic devices with a hydrophobic coating, an increase in the oil phase flow rate (while the aqueous flow rate is constant) enables the creation of smaller droplets and leads to an increased production frequency for the droplets (see Figure 1C). Conversely, a decrease of the oil phase flow rate results in an increase in droplet size and decrease in the droplet production rate. Additionally, using this microfluidic device provides stable droplet formation with a high degree of monodispersity and accurately known droplet size.

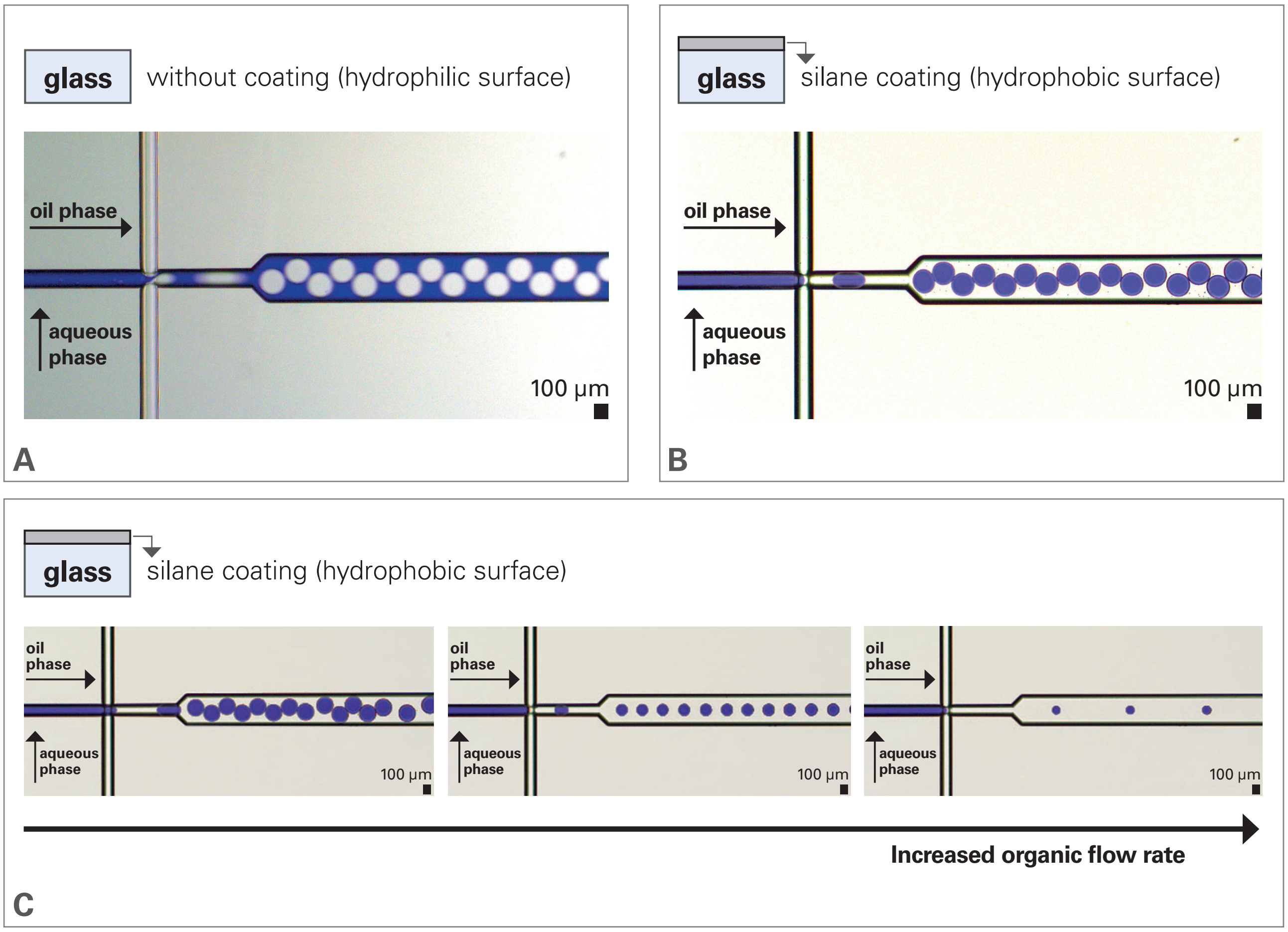


Figure 1. Surface coatings modify the properties of the droplet generator. **A.** Oil-based droplets surrounded by water can be generated using uncoated glass microfluidic devices, as glass surfaces by nature provide hydrophilic properties (contact angle: 15-25°) without further treatments. The aqueous colored solution wets the channel surface in contrast to the organic oil, therefore oil droplets are produced within the device. **B.** Applying a hydrophobic surface treatment to the device channels (silane) changes the properties (contact angle: ~105°) of the microfluidic device, resulting in the generation of an emulsion with uniform water droplets in an organic phase. **C.** An all-glass hydrophobic-coated microfluidic device is used to generate water-in-oil droplets of different sizes and generation frequencies. By maintaining a constant aqueous flow rate, increasing the organic flow rate increases the droplet generation frequency while simultaneously decreasing the droplet diameter. Pictures are imaged with 5x magnification on an inverted microscope.

Microfluidic co-encapsulation of cells and micro particles

Furthermore, we demonstrated the co-encapsulation of two discrete particles (such as cells or micro particles) in droplets, using a microfluidic glass droplet generator with double cross-junction designed by HEIDENHAIN. A hydrophobic coating was applied to the channel surface, allowing aqueous droplets in an organic oil phase. Two aqueous solutions, one containing cells (HL-60 leukemia cell line, Sigma-Aldrich, USA) and another one containing micro particles (based on polystyrene, Sigma-Aldrich, USA), were applied into the microfluidic device under flow control at both ends of the cross-junction. An image of the droplet generator is shown in Figure 2A.

Cells as well as micro particles tend to adhere to each other. To avoid aggregation of the material to be encapsulated, different filter structure pore sizes were used in this particular design. A dedicated filter structure was designed and integrated following the access ports to separate the cells prior to encapsulation. Here a cell strainer with 40 µm mesh width was used. For all other access ports the pore size was 20 µm mesh width. Additional to enhancing the separation of the cells and particles, each filter operates as a filter unit to retain unintended particles, such as dust and dirt. A microscopic picture (magnified 5-fold) of the microfluidic filter system is enlarged in Figure 2B displaying the 20 µm mesh width.

The cells and the micro particles were simultaneously encapsulated in water-in-oil droplets (see Figure 2C). One input channel of the cross-junction contained an HL-60 cell suspension in 0.01 % PBS/BSA merged with blue aqueous-based color, and the other input channel contained micro particles merged with yellow aqueous-based color. When both liquids were encapsulated by the oil phase, a homogenous green-colored water-in-oil droplet containing a single cell and a micro particle was formed. Thus, a homogenization of two reactive liquids is ensured by the microfluidic device designed by HEIDENHAIN (see Figure 2D). This demonstrates that it is possible to design microfluidic droplet generator devices that can be used to create upstream reaction chambers for individual single cell experiments, thus enabling high-throughput single cell downstream applications.

The co-encapsulation of HL-60 cells and micro particles in water droplets in an organic oil phase utilizing a glass microfluidic droplet generator device is shown in Figure 2.

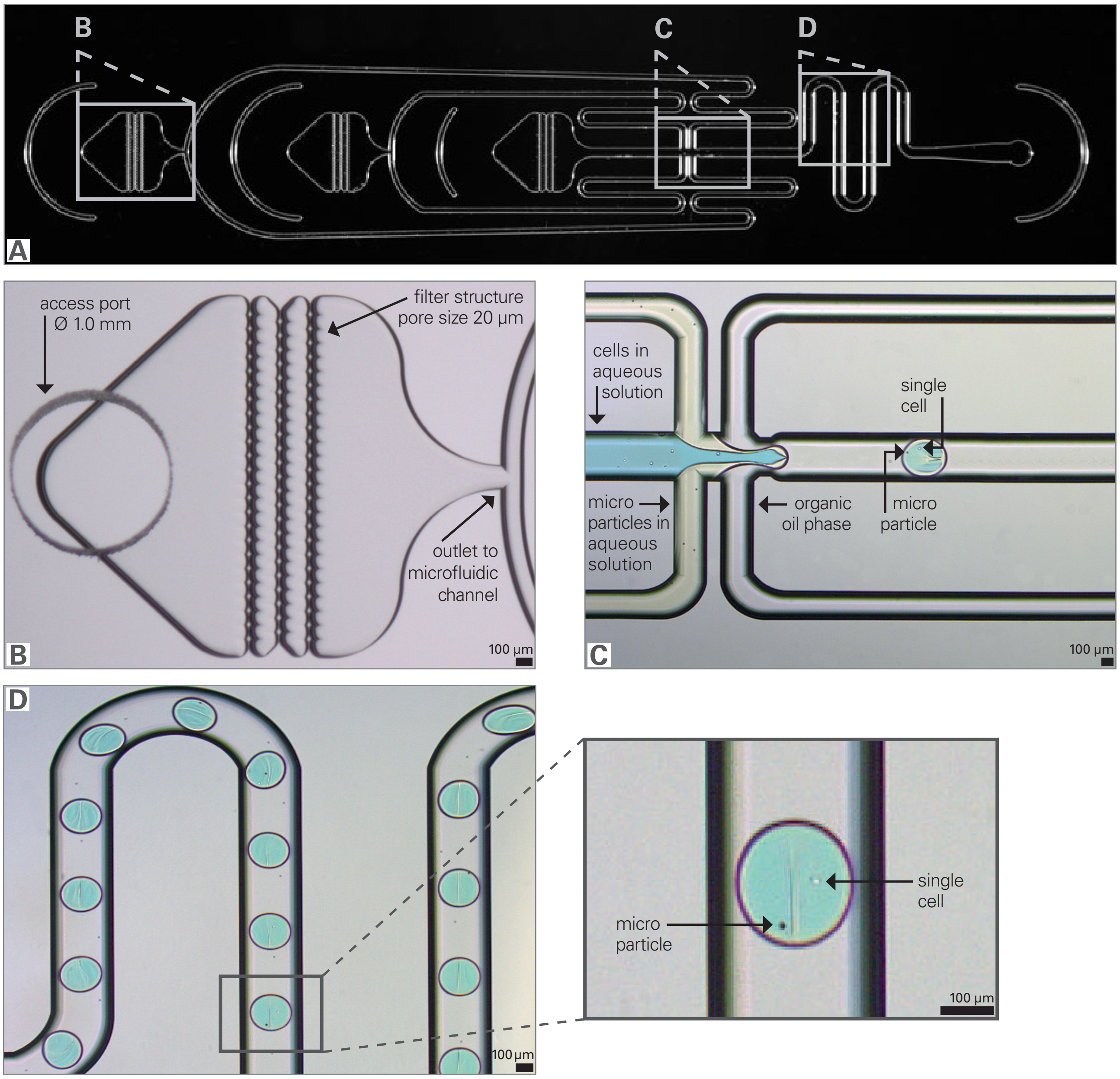


Figure 2. A. An image of a glass droplet generator with total dimensions of 37.5 mm x 12.5 mm x 1.4 mm, designed by HEIDENHAIN. Sections of interest (B. filter, C. cross-junction, D. microfluidic channel system) are highlighted in the respective boxes. **B.** Cells often have the property to stick to each other, therefore a cell strainer (40 µm mesh width) is included in the design in order to pass the cells through and singulate them prior to encapsulation. All other access ports, each with through glass via (TGV) diameter of 1.0 mm, are provided with filters (20 µm mesh width) to restrain unintended large particles and protect the channel from dust and dirt. **C.** The two aqueous solutions (in this demonstartion, blue solution containing HL-60 cells and yellow solution containing micro particles) are combined with equal flow rates at the first cross-junction of the microfluidic device. The two liquids are joined in a laminar flow until they are encapsulated simultaneously in an organic oil phase at the second cross-junction. Within this microfluidic device, a water-in-oil droplet is generated, containing a single cell (white spot) and a micro particle (black spot). **D.** A microfluidic serpentine is integrated into the design to induce a homogeneous mixing (green) of the two aqueous solutions (blue and yellow) within the produced droplets. Microscopic pictures (2B-D) are imaged with 5x magnification.

The whole microfluidic device is depicted in Figure 3, showing the droplet generator with the set-up including all three inlets (oil colorless, micro particles yellow, HL-60 cells blue) and the outlet tube, where droplets (green) can be collected for downstream applications.

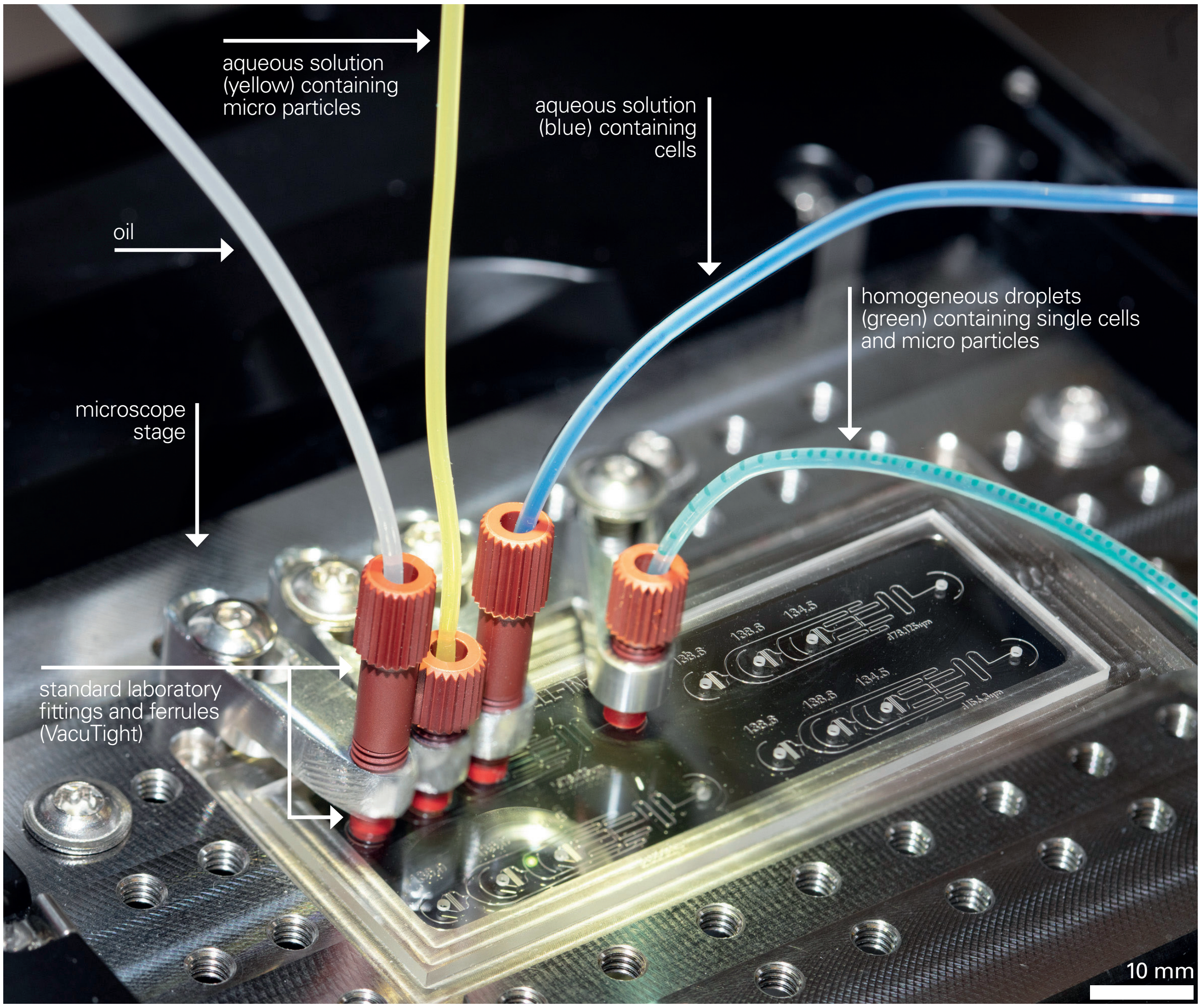


Figure 3. Cell and micro particle co-encapsulation with a droplet generator. A self-designed and hydrophobic-coated glass microfluidic device containing four microfluidic droplet generators was used to enable the formation of uniform aqueous droplets in an organic carrier phase. One generator at a time was connected for each experiment. The precisely defined microfluidic geometrics allows the efficient homogenization of two aqueous fluids in highly monodispersed droplets. Within this application, a simultaneous co-encapsulation of an HL-60 single cell together with a micro particle was successfully demonstrated and may be used for molecular downstream applications, including digital droplet PCR (for gene expression profiling) and further biological droplet-based assays.

Conclusion

HEIDENHAIN provides superior microfluidic glass devices with various design options for generating stable droplets for single cell profiling and other droplet-based applications. Custom-designed microfluidic devices give the flexible opportunity to produce monodisperse droplets with high repeatability and a real-time modification of droplet size and generation frequency. In this application note we demonstrated a microfluidic glass device with a co-encapsulation of different biological cargo to demonstrate this highly precise and accurate way of producing droplets. HEIDENHAIN microfluidic devices are offered with a variety of surface coatings, even beyond hydrophilic or hydrophobic functionalities. Different options are available and can be fully customized, according to individual technological needs and requirements.

The microfluidics product line includes customized micro- and nano-patterns and structures in glass, integration of electrodes, waveguides, and structured functionalization for life science applications. We provide flexible offerings from design consultancy and prototyping to scalable manufacturing.

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