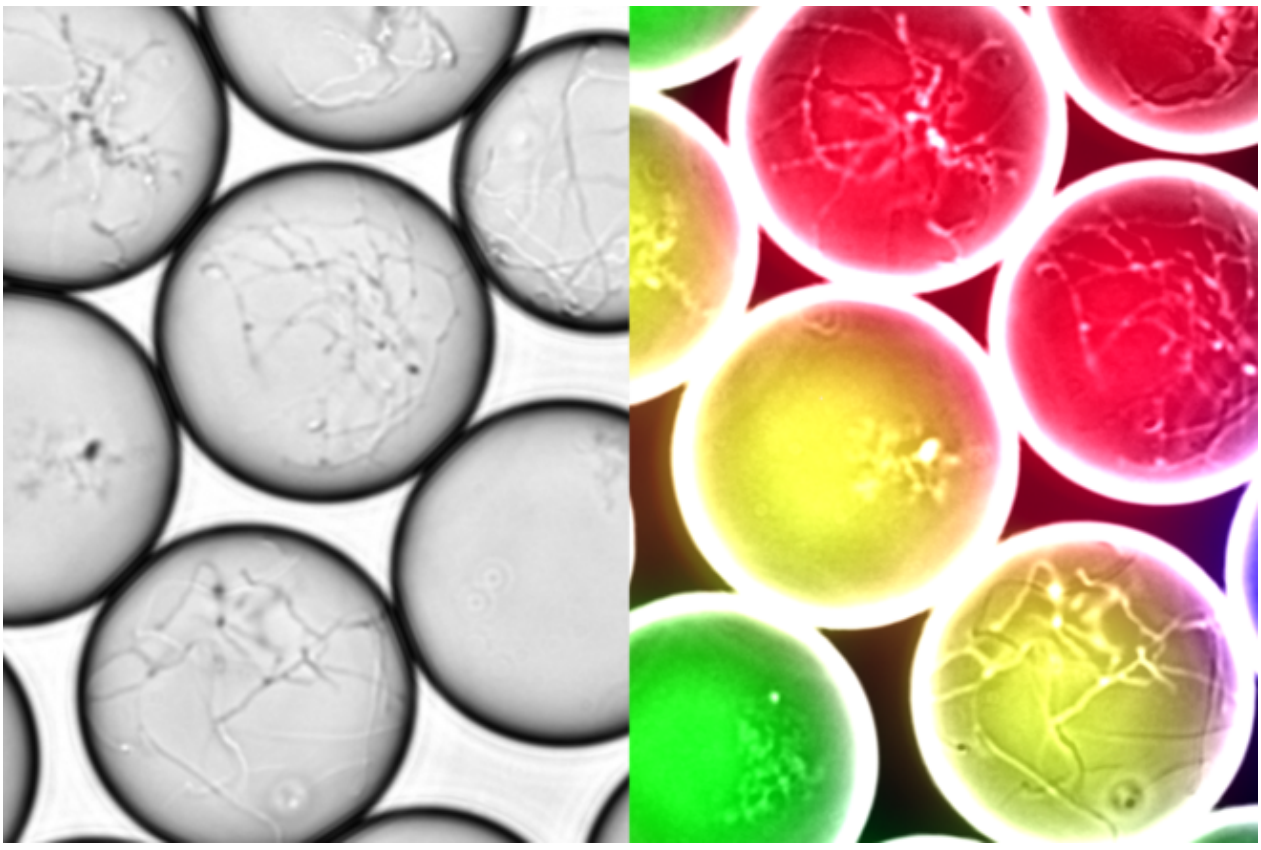
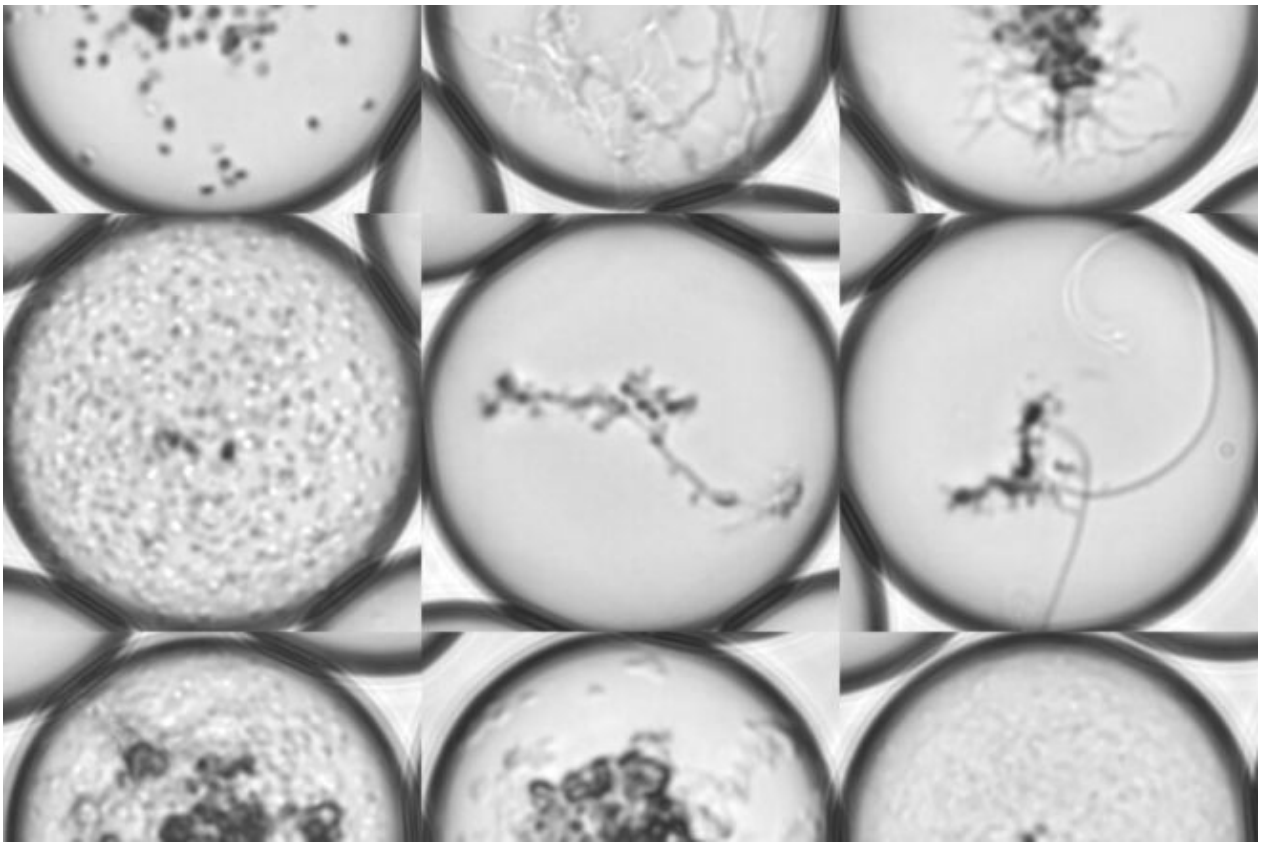
Tropfenbasierte Mikrofluidik

Die in der Natur vorhandene, nur in sehr geringem Umfang erschlossene mikrobielle Diversität ist eine praktisch unerschöpfliche Quelle in der Evolution selektierter Naturstoffe. Es sind jedoch neue Ultrahochdurchsatz-Technologien erforderlich, um die Vielfalt der Mikroorganismen besser zu erschließen und bestehende Hürden bei der Suche nach neuen Antibiotika zu überwinden, wie stille Sekundärmetabolit-Gencluster, langsam wachsende oder bisher nicht kultivierbare Mikroorganismen. Wir nutzen dafür die tropfenbasierte Mikrofluidik, die eine Ultrahochdurchsatz-Kultivierung von Mikroorganismen in Picoliter-Tropfen und eine Prüfung der Mikrokulturen auf Produktion neuer Antibiotika ermöglicht.

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_ Fig. 1. pL-droplets as microcultivation vessels for various streptomycetes

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_ Fig. 2. Droplets with unknown microbes from a soil sample

We use aqueous droplets in oil as miniaturized cultivation vessels for a variety of bacteria. Here we inoculated droplets with spores of different streptomycetes. After droplet incubation for several days the typical mycelium of Streptomyces strains is visible. To distinguish which strains grew under the applied conditions we employed fluorescent markers. (Fig. 1)

We not only cultivate lab strains in droplets but use them also for the discovery of new bacterial strains from natural samples. The chances to find new species in droplets is higher compared to standard isolation techniques, because we can singularize and compartmentalize within one hour up to 9 million single cells derived from natural habitats. Thereby we prevent competition for nutrients or space among the bacteria allowing also slow growing species to grow. Here examples for growing bacteria extracted from a soil sample are shown. Pictures were obtained after one month of droplet incubation. (Fig. 2)

Videos

Generation of aqueous, surfactant stabilized droplets in oil; volume 50 – 200 pL, frequency 1500 Hz

The aqueous phase is guided by microfluidic channels to a flow focusing unit, in which two channels with oil are directed orthogonally to the water channel. The flowing oil is pinching of little water droplets that are immediately stabilized by a surfactant that is dissolved in the oil.

Dynamic droplet incubation (Mahler et al., 2015)

To supply large droplet collections with oxygen in order to promote bacterial growth inside droplets we developed a droplet incubation unit. During incubation the oil, that is anyway surrounding the droplets, is pumped from top to bottom through the droplet population (in the video from left to right). Thereby the droplets are mixed as can be seen here with 3 million red and 3 million blue droplets. Furthermore the inter-droplet distance is increased, which results in a higher and homogeneous oxygen supply during long-term incubation.

Re-injection of droplets into a chip for further analysis

After incubation droplets have to be introduced into a chip again in order to monitor and manipulate single droplets. Since droplets are stabilized by a surfactant this is not a problem.

Picoinjection of additional fluids into preformed droplets; frequency 500 Hz

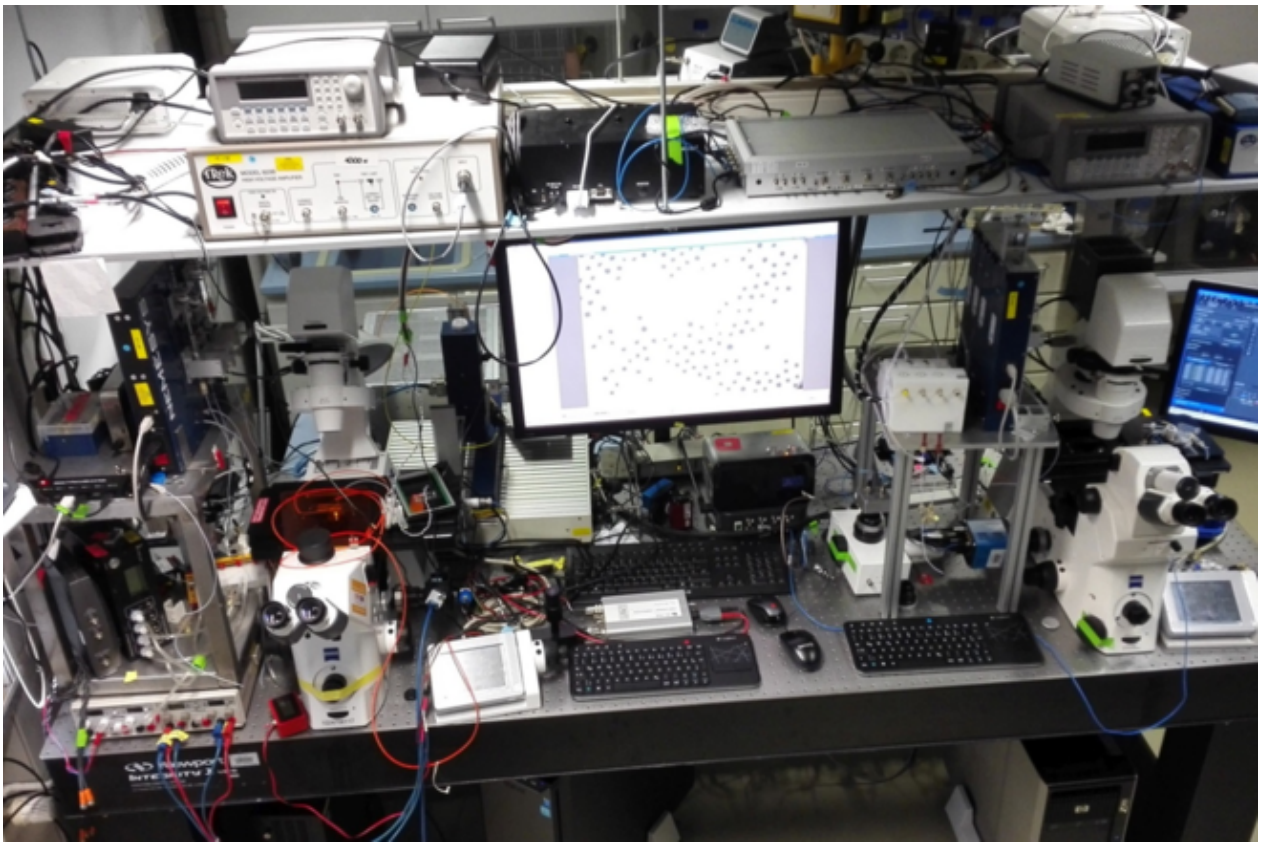
In case assay reagents have to be added after droplet generation, droplets are led through an electrical field that is destabilizing the water/oil interphase. In this moment an additional fluid can be inserted into the droplets, that carries an enzyme substrate or even bacterial cells.

Droplets that possess a desired trait can be separated from the rest of the droplets by this unit operation. To achieve this, the droplets have to be analyzed right before the sorting and then only a few milliseconds are left until the real-time sorting decision has to be made. In case the droplet should be sorted an electrical field is applied for a short moment that is pulling the wanted droplet into the lower channel. The remaining droplets go on their own into the upper channel and can be discarded.

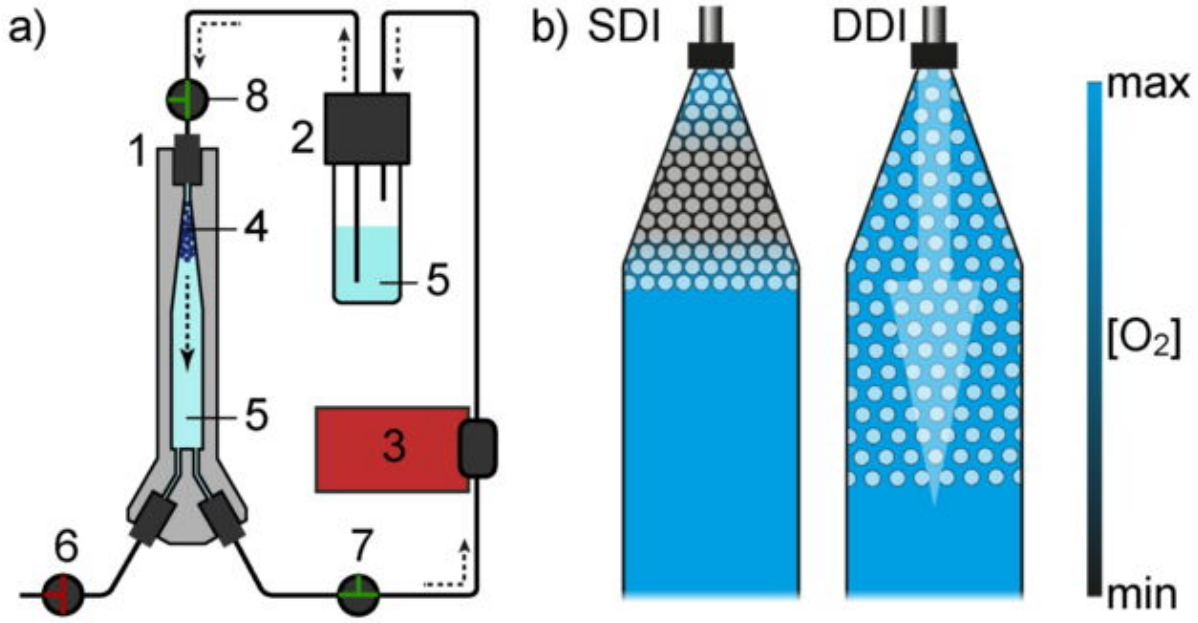
Sorting of droplets based on online monitored properties; frequency 500 Hz

Droplets that possess a desired trait can be separated from the rest of the droplets by this unit operation. To achieve this, the droplets have to be analyzed right before the sorting and then only a few milliseconds are left until the real-time sorting decision has to be made. In case the droplet should be sorted an electrical field is applied for a short moment that is pulling the wanted droplet into the lower channel. The remaining droplets go into the upper channel and can be discarded.

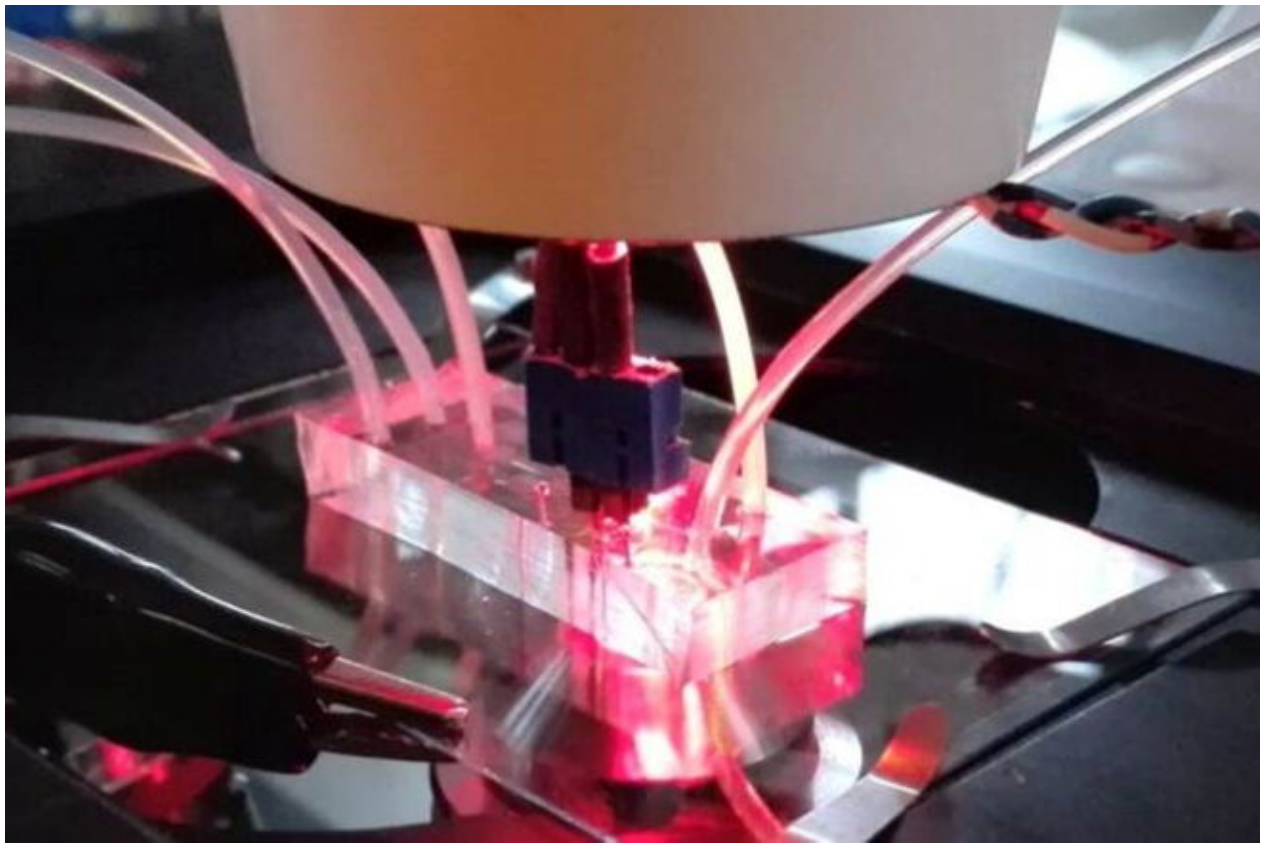
Bildergalerie

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  _ Microfluidics laboratory
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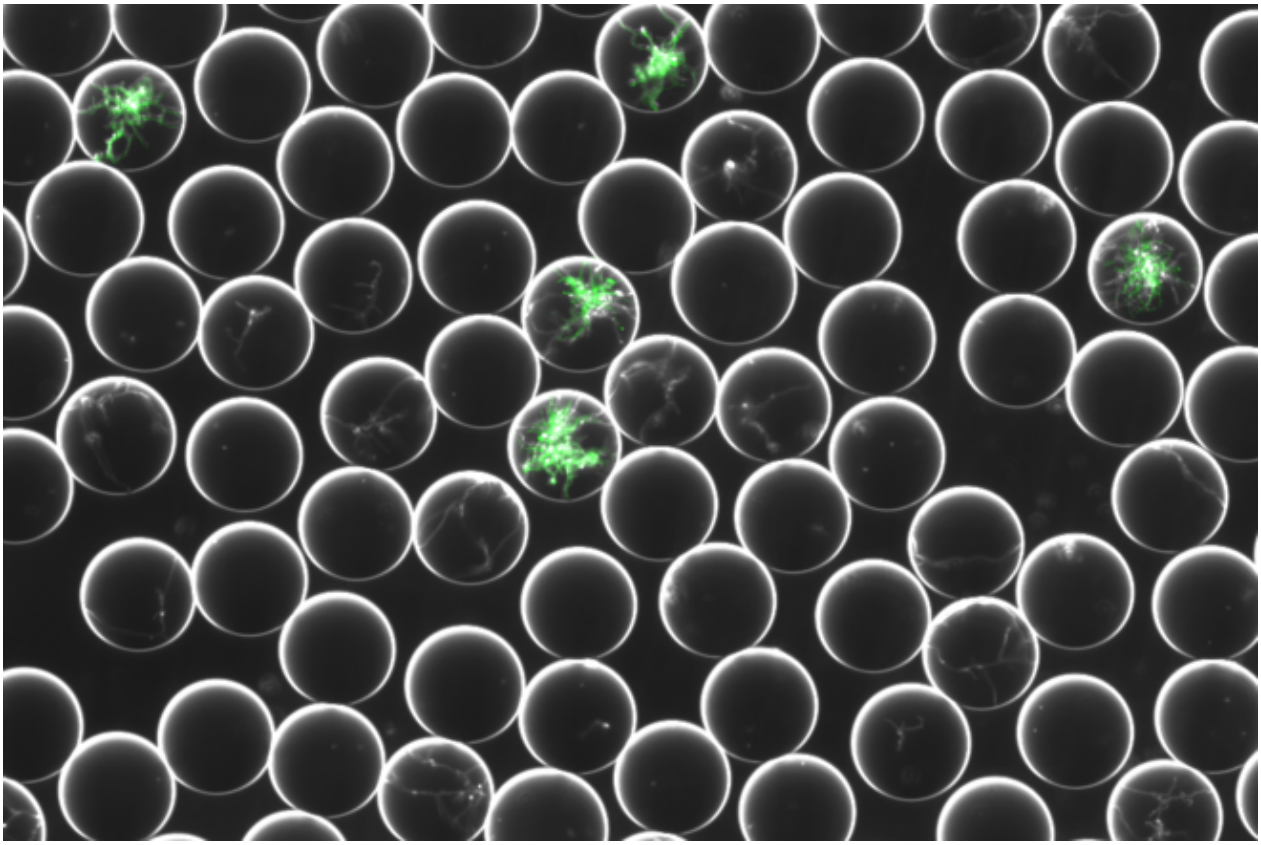


_ Dynamic droplet incubation (Mahler et al., 2015)

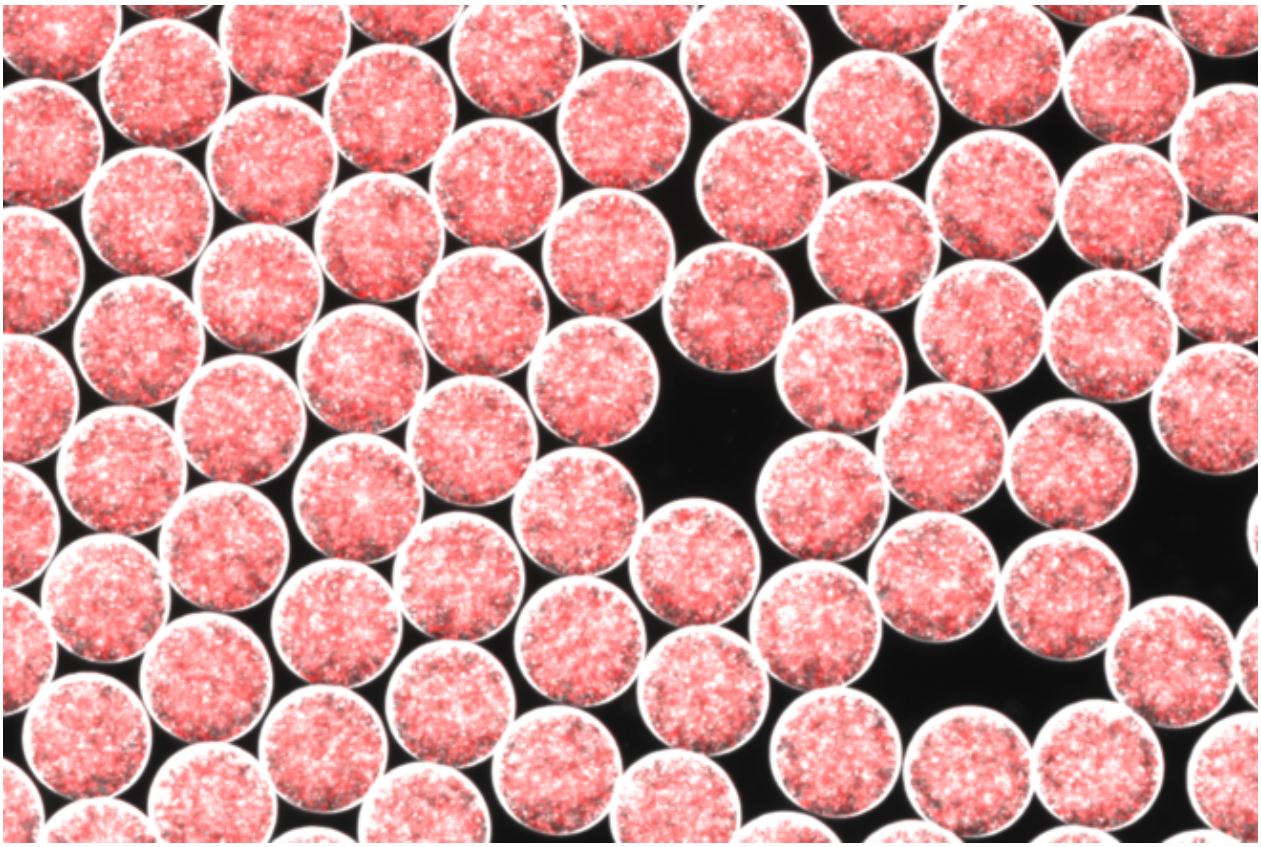
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_ Microfluidic Chip

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_ *S. aureofaciens* und *S. lividans* in 170 pl Tropfen

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_ Rot fluoreszierende *E. coli* Zellen in 170 pl Tropfen