

PARALLEL BACTERIAL ESTERASE ASSAY IN TRAPPED 35-nL-DROPLETS USING pL-EMULSION TRANSPORT

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Task

Bacterial Esterase activity in Soil Microbiom

Proteolytic enzymes play an essential role in the soil microbiome [1]. Soil bacteria require on the one hand an efficient strategy to liberate small molecular nutrients from soil based resources and a defending strategy for competing microorganisms on the other. In both cases secreted esterases and lipases play an essential role as exoenzymes working in the surrounding space of the liberating organisms. In complex soil communities a cocktail of proteolytic enzymes may act as indicator for the vitality and diversity of the soil microbiome. For the investigation of cooperative microbial soil communities on the level of proteolytic enzyme activities a suited research approach is required. Here, the essential step is the compartmentalization of the complex bacterial mixtures in sub-populations and the investigation of their proteolytic activity in dependence on different stimuli.

Challenge

The application of arrays of trapped droplets for screening purposes is difficult because the addition of assay substrates or other components into the droplets is a challenging task and cannot be done by direct injection or dosing. To overcome this problem, we made use of an emulsion transport principle to add an esterase assay substrate MUB (see Scheme 1) into trapped 35-nL-droplets.

Technique

Therefore we developed a droplet based microfluidic chip which allows the compartmentalization of complex soil communities into 4.5-nL-droplets. These compartments are generated within a flow focusing unit as disk shaped droplets and trapped by from relaxation into spherical droplets in appropriate cavities. In Figure 1 the chip, flow-focusing unit and trap geometry are shown.

Experiment

Therefore, the assay substrate solution was emulsified in fluorinated oil by the help of a surfactant and ultrasound. The analysis of the received micro-emulsion by zeta-sizer analysis reveals a mean droplet size of about 700 nm and a size distribution of about 550 nm (FWMH) (see Fig. 3A). The received emulsion was flushed into the droplet array (see Fig. 3B) which contained different proteolytic bacteria. As readout, the fluorescence development at 445 nm caused by the cleaved substrate was monitored by the help of a microscope setup.

Results

In Fig. 4A a set of 10 selected droplets is shown. Fig. 4A shows the fluorescence image ~20 min after flushing with the micro-emulsion. The development of the fluorescence indicates clearly the transport of the assay substrate into the trapped droplets and subsequent cleavage by the proteolytic enzymes. The image based fluorescence intensity analysis reveals highly comparable enzyme kinetics for all droplets.

In a second experiment, three different droplet populations were trapped in the array chip. First fraction of droplets contained *Bacillus subtilis* (1×10^8 /ml, PBS, pH=7.40), second *E. Coli* (1×10^8 /ml, AM, pH=7.40) as negative control and the third fraction plain PBS buffer as media control. The two bacterial fractions were color coded to distinguish the different species. After flushing with the MUB micro emulsion the fluorescence development kinetic was monitored for about 2 h. As a result, the three different droplet population were clearly distinguishable one by another. Whereas all the *B. Subtilis* containing droplets had a similar kinetic as shown in Fig. 4B, the *E. coli* have shown a much slower kinetic. The PBS containing droplets had a negligible fluorescence development.

Conclusion

With our experiments we proved the principle of emulsion transport for the duration of esterase assays in trapped 4.5 nL droplets. Therefore we developed a Chip with 2592 individual droplet traps. The assay substrate was added by emulsion based transport.

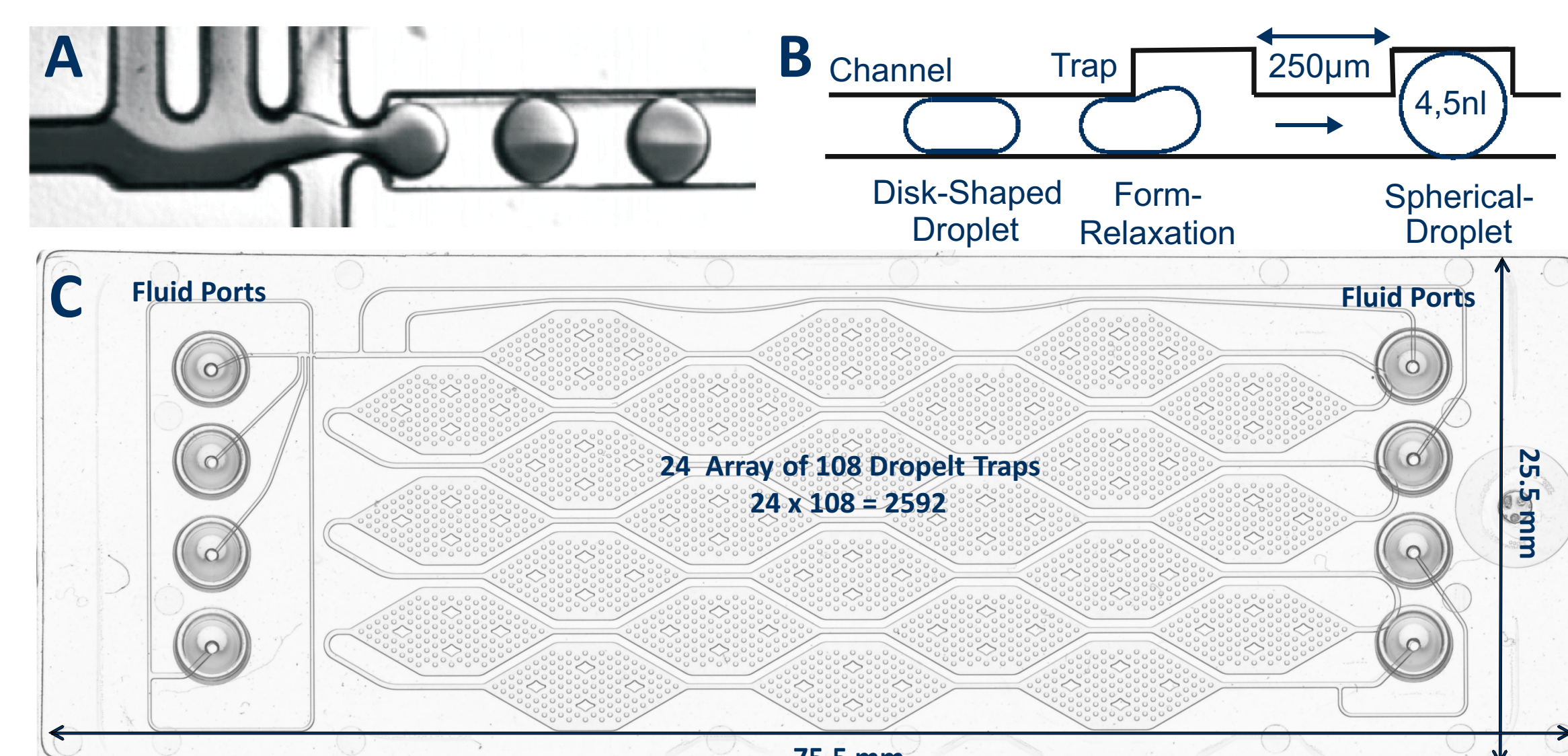


Figure 1: A) Image of the droplet forming unit B) Droplet trapping process steps C) Image on the complete chip comprising 2592 individual traps, fluid ports and channels. [2]

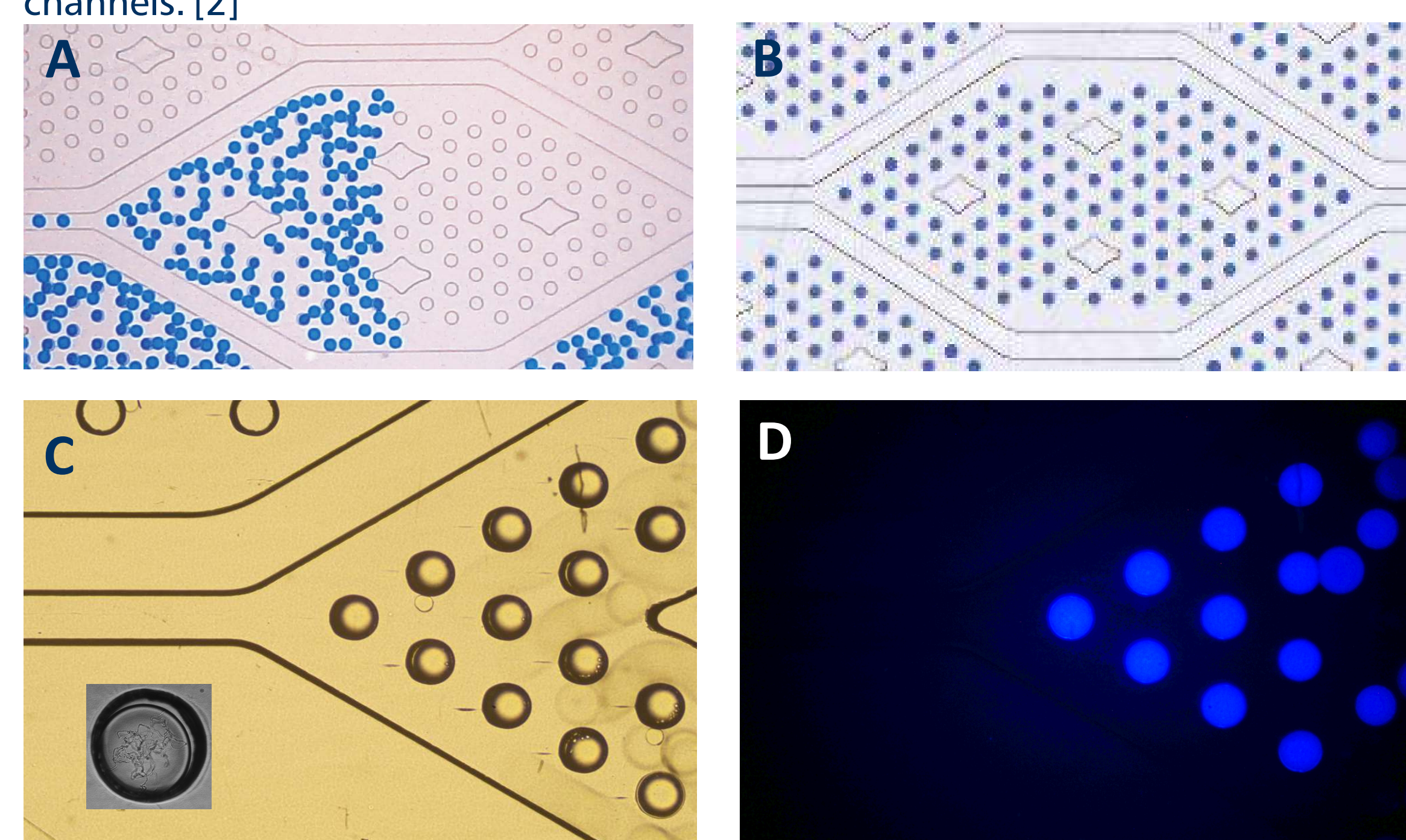
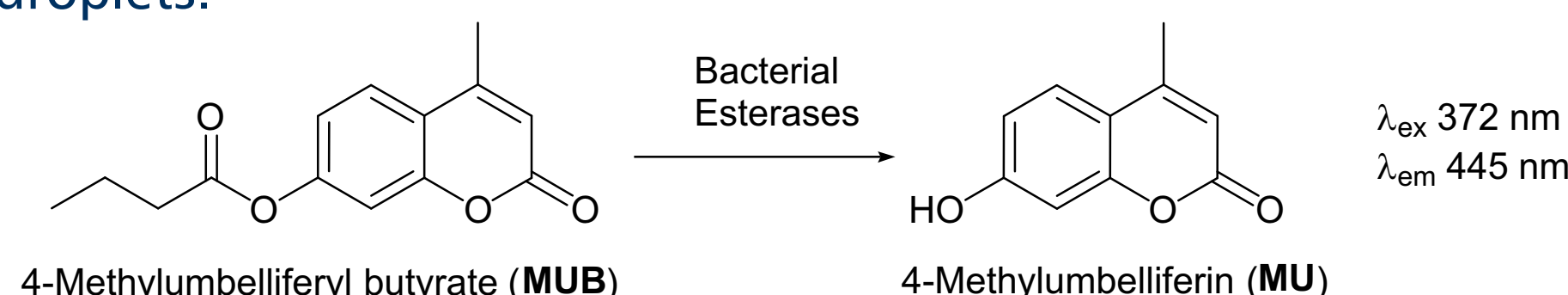


Figure 2: A) Image during the droplet generation and trapping process. B) Array filled with droplets after rinsing of non-trapped droplets. C) Microscope image of droplets with soil bacteria (Inlay Streptomycetes Stain) D) Fluorescence image of trapped droplets.



Scheme 1: Fluorimetric assay reaction

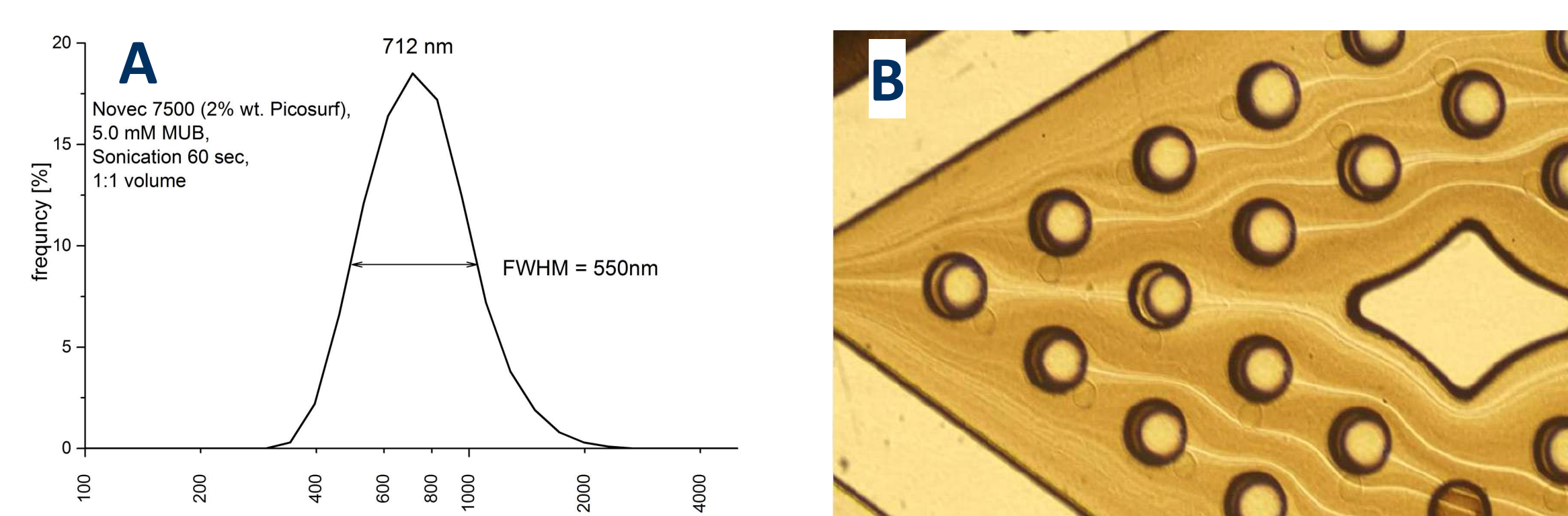


Figure 3: A) Droplet size distribution of the feeding emulsion. B) Image of the chip section during flushing the trapped droplets with the feeding emulsion under dark field excitation.

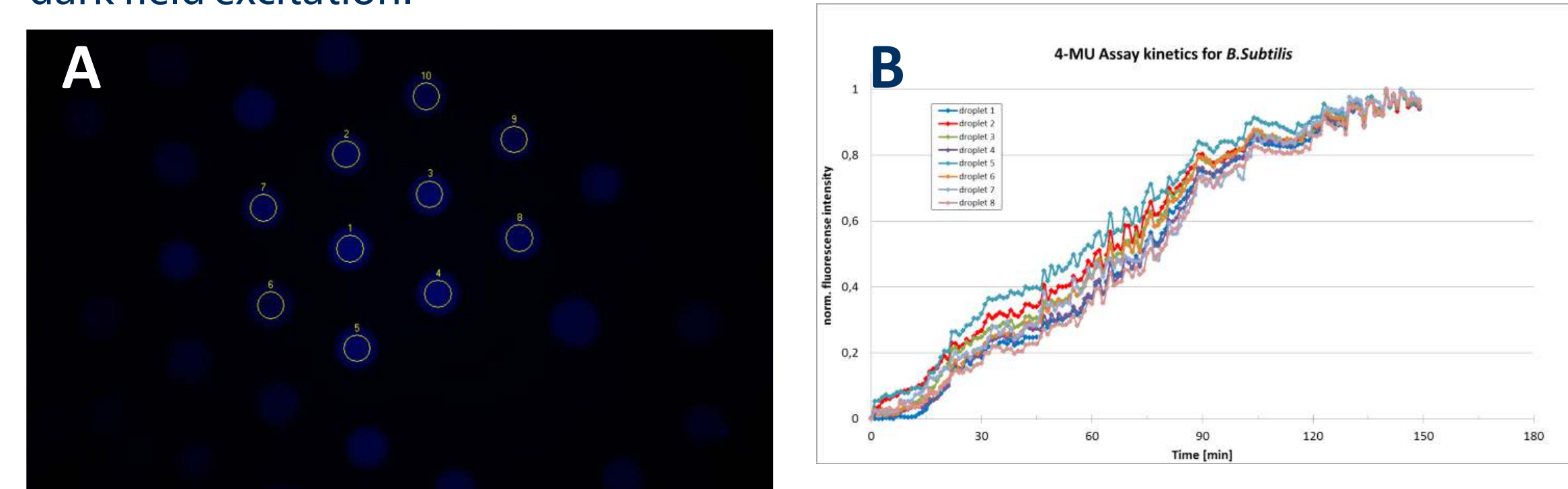


Figure 4: A) Picture analysis for automated droplet recognition and fluorescence intensity detection B) Exemplaric kinetic result for different droplets.

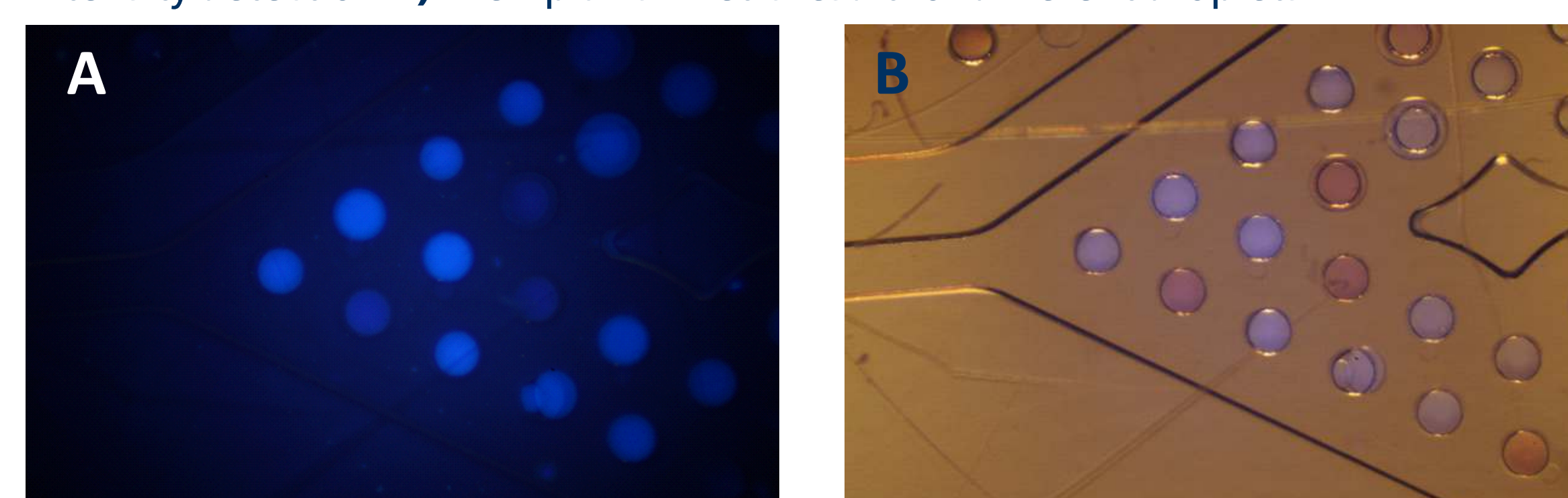


Figure 5: A) Three different bacteria types compartmentalized and trapped in different droplet populations. B) Overlay image. The three different bacteria can be determined easily.