REAL-TIME IMAGE-BASED SORTING OF PICOLITER DROPLETS
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ABSTRACT
Picoliter droplets containing Actinobacteria are sorted using real-time image analysis, which in contrast to most available sorting approaches is independent of fluorescent labels. Images are triggered by changes in brightness due to total internal light reflection at the interface of each droplet, which is detected by a photodiode with a reduced field of view. The time interval between triggered imaging of single droplets and sorting is in average 0.5 ms for 5-fold magnification (72 × 72 px) and 1.6 ms for 10-fold (144 × 144 px), enabling sorting rates higher than 100 Hz.

KEYWORDS: droplet microfluidics, sorting, real-time, image analysis, Actinobacteria

INTRODUCTION
Over the past years, droplet-based microfluidics evolved into a powerful high-throughput screening platform [1,2,3]. However, droplet interrogation is limited to fluorescence-based readouts, which is hitherto the only available technique providing satisfying signals at minimal integration times. Hence, feasibility of ultra-high-throughput screening in droplets is strictly dependent on a suitable fluorescent marker [4]. To extend the range of methods for data assessment in pL-droplets, we developed a novel approach for triggered imaging of single pL-droplets, which allows for fast, marker-free droplet analysis.

FUNCTIONAL PRINCIPLE
In droplet-based microfluidics, image analysis provides an easy and fast way for determination of droplet size and velocity [5], as well as experimental validation. However, processing and data extraction is performed only after experimentation is completed. Here, we developed real-time image processing (RTIP) to detect bacterial structures within pL-droplets and establish image-based droplet sorting. Actinobacteria, a bacterial phylum with an extensive potential for secondary metabolite production, was selected in order to test the applicability of the image analysis algorithm for the recognition of complex biological structures. Nevertheless, recognition of different structures is also plausible with minor algorithm modifications. The core of the image processing function consists of a Difference of Gaussians (DoG) algorithm tailored for simultaneous detection of droplets and confined mycelial structures (Fig. 1).

EXPERIMENTAL
To enable triggered imaging of single droplets in transmissive mode, changes in brightness caused by total light reflection at the droplet interface are detected by a photodiode (Fig. 2). This signal triggers a high-speed camera to generate images of single droplets. Frame grabbing and automated real-time image processing are implemented in a callback function (C++) using the computer vision library opencv [6]. Depending on the image analysis result, an AC sorting pulse is generated via an A/D-converter, function generator and high-voltage amplifier. The microfluidic sorting chip (Fig. 4) was fabricated using DRI etching on a fused silica wafer. Platinum electrodes were sputtered in a second wafer, which was bonded to the first one using an insulating silicon-dioxy nitride layer.

Fig. 1: Image processing steps for droplet segmentation and growth classification. First and second rows show empty and colonized droplets, respectively. The blue and red circles represent classification as empty and occupied droplets, respectively. [7]†
RESULTS AND DISCUSSION

By combination of triggered imaging with RTIP-based on-chip droplet sorting, we were able to separate pL-droplets containing microcultures of mycelial growing Actinobacteria from empty droplets at frequencies of 100 Hz. Reliable triggering of the camera is enabled by the photodiode with a signal-to-noise ratio of 230:1 (Fig. 3). Frame grabbing and analysis lasts 1.61±0.13 ms for 144x144 px images at 10x magnification (Fig. 5). Video validation confirmed a sorting accuracy of 90%, diminished only due to some bacterial structures hiding in a different focal plane upon imaging. The sorting rate is primarily limited by computational power.

CONCLUSION

The combination of triggered-imaging with RTIP provides a generally applicable alternative to fluorescence-based pL-droplet analysis and sorting. Moreover, detection and evaluation can be adapted to assess any droplet-confined structures. The performance of this approach is mainly determined by computational power and the employed sorting structures, which may be easily improved to achieve even higher sorting rates. This technique expands the possibilities for droplet-based microfluidics by enabling label-free, real-time analysis and discrimination of droplets and their contents.
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REFERENCES
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