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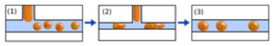
1. Introduction and Experimental Setup

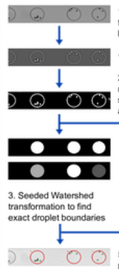
Secondary metabolites of *Actinobacteria* and other microbes are valuable natural substances for the development of new antibiotics. As most of these species are still unknown and conventional cultivation methods have their limits, the development of new and innovative cultivation methods is essential.
We are developing an ultra-high-throughput screening method based on a microfluidic system on a chip to discover a large amount of new species and their metabolites [1].



(1) Droplets with bacterial spores are automatically generated and incubated. Coloured beads inside the droplet are used as a barcoding system. (2) Droplets can be dosed with different substances. (3) Droplet contents are analysed after a second incubation phase.

Automated image analysis is used to analyse and assess picolitre droplets and their content. The determination of the volume of the added substance by comparison of the droplet volume before and after the dosing is the subject of this work.

2. Automated Image Analysis



1. Application of Laplacian of Gaussian filter to detect edges of droplets and beads [2]:

$$LoG(x,y) = -\frac{1}{\pi\sigma^4}\left[1-\frac{x^2+y^2}{2\sigma^2}\right]e^{-\frac{x^2+y^2}{2\sigma^2}}$$

2. Intensitiy thresholding, noise elimination and separation of droplets and beads

3. Seeded Watershed transformation to find exact droplet boundaries

4. Correlation with bead model to seperate bead clusters

5. Detection of droplet area, radius, position and number of beads

3. Volume Analysis

Comparison of Volume Distribution before and after Dosing (Population-based Approach)

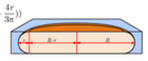
Direct computation (individual-based approach) of the added volume in images of the dosing process is not possible due to the asymetric form of the droplets in the narrow dosing channel. Therefore the distributions of volumes of whole droplet populations before and after the dosing are compared in a population-based approach using images from step (1) and (3) in the experiment.

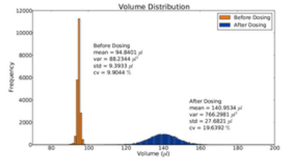
Computation of Droplet Volume

Droplets are round and flat due to the channel height. Therefore the "pancake formula" can be used to calculate the volume [3]:

$$V(R) = 2\pi r(R-r)^2 + \pi^2 r^2 (R - r(1-\frac{4r}{3\pi}))$$

R... Droplet radius
r... Half of channel height





The diagram shows volume distributions of droplets before and after the dosing for more than 18000 droplets in each data set.

4. Outlook

Using the two calculated distributions, it is possible to determine the distribution of the added volume. This information can be exploited to compute the volume difference in the images of the dosing process.

Barcoding through coloured beads will be promising in this context by allowing to link identical droplets in the three different image stages.

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