Coding of experimental conditions in microfluidic droplet assays using colored beads and machine learning supported image analysis.

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*equal contribution

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Abstract

To efficiently exploit the potential of several millions of droplets that can be considered as individual bioreactors in microfluidic experiments, methods to encode different experimental conditions in droplets are needed. The approach presented here is based on co-encapsulation of colored polystyrene beads with biological samples. The decoding of the droplets, as well as content quantification, are performed by automated analysis of triggered images of individual droplets in-flow using bright-field microscopy. The decoding strategy combines bead classification using a random forest classifier and Bayesian inference to identify different codes and thus experimental conditions. Antibiotic susceptibility testing of nine different antibiotics and the

determination of the minimal inhibitory concentration of a specific antibiotic against a laboratory strain of *Escherichia coli* are presented as a proof-of-principle. It is demonstrated that this method allows successful encoding and decoding of 20 different experimental conditions within a large droplet population of more than 10^5 droplets per condition. The decoding strategy correctly assigns 99.6% of droplets to the correct condition and a method for the determination of minimal inhibitory concentration using droplet microfluidics is established. The current encoding and decoding pipeline can readily be extended to more codes by adding more bead colors or color combinations.

Beteiligte Forschungseinheiten

Angewandte Systembiologie Marc Thilo Figge Mehr erfahren

Biotechnikum Miriam Agler-Rosenbaum Mehr erfahren

Leibniz-HKI-Autor*innen



Mahipal Choudhary

Details



Stefanie Dietrich

Details



Marc Thilo Figge

Details



Lisa Mahler

Details



Martin Roth

Details



Oksana Shvydkiv

Details



Carl-Magnus Svensson

Details



Miguel Angel Tovar Ballen

Details



Thomas Weber

Details

Themenfelder

Tropfenbasierte Mikrofluidik

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