

E. COLI CULTURE IN DROPLETS USING FLUIGENT FLUOROSURFACTANT

INTRODUCTION

In vitro cell culture is a fundamental component of biological production systems and biotechnological research. The ability to grow cells outside of their natural environment offers many possibilities ranging from high quantity production of enzymes to cell toxicity studies.

Traditionally, *in vitro* cell culture methods use petri dishes or culture flasks for laboratory cell culture. In culture flasks, cells are grown in a homogenous liquid medium, while petri dishes allow the growth of colonies on a solid or semi-solid substrate surface. Nevertheless, these two methods have limitations including the difficulty to compartmentalize clones and single cells. Compartmentalization allow single cells or clones to be individually assayed or exposed to different culture conditions or stimuli for versatile (or multiparametric) analysis of cells heterogeneity.

To tackle this issue droplet based microfluidics appears as an emerging cell culture method enabling compartmentalized culture of single cells and clones in droplets.

Using **droplet based microfluidics** allows to encapsulate single or multiple cells into tiny droplet of pL volume which are generated at a rate of thousand per seconds. This method is based on two different non-miscible solutions, mostly aqueous solution and oil solution (as carrier phase) which are used to generate stable droplet and encapsulate cells in them

Perfluorinated oils have shown several advantages compare to other carrier fluids such as mineral oils. Their low viscosity allows easy handling in microfluidic systems without need of high-pressure pumps.

Long term cell culture experiments have been successfully performed with water-in-perfluorinated oils^[1] droplets. Moreover, fluorinated oils show lower organic molecule transfer drop to drop^[2]. Finally, while mineral oils swell PDMS chips, fluorinated oils show a better compatibility^[3]. Also, oxygen is well soluble in the fluorinated oils which ensures sufficient gas diffusion between oil and droplet content. Though perfluorinated oils offer benefits for applications such as cell cultivation or dPCR, to find an adequate and **efficient surfactant** for droplet stabilization in this oil remains a challenge. To date, most commercially available surfactants present limitations.

The objective of this study is to highlight the biocompatibility of the new surfactant **dSURF** by culturing microorganisms in picoliter droplets.

For this purpose, a model microorganism that is commonly used in droplet based microfluidic for the development and testing of new methods has been employed: *Escherichia coli ECJW922*

MATERIALS AND METHODS

Reagents:

Continuous phase Reagents:

Novec HFE-7500(Sigma Aldrich) containing 0.5% or 3% dSURF (Fluigent, P/N DR-RE-SU-A1)

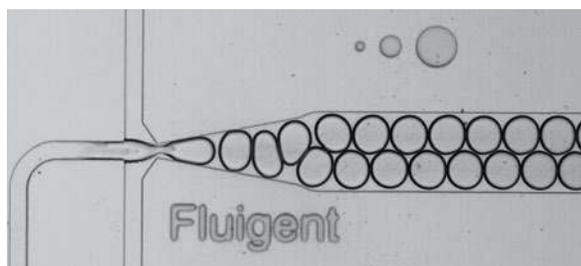
Dispersed phase Reagents:

500 μ l suspension of *E. coli ECJW922* in TB (Terrific broth) medium with OD600 (optical density) of 0.005 (5×10^6 : CFU*/ml) or 0.01 (10×10^7 CFU/ml), , which means cell concentration in the first suspension was 5×10^6 CFU/ml and in second suspension 10×10^7 CFU/ml. CFU .

The idea is to obtain cell concentrations in the suspension such that after their encapsulation into droplets approximately 50% of droplets will be occupied mainly with one or two cells and in the second solution approximately 90% of droplets will be occupied with single, two, three or more cells according to the Poisson distribution.

*CFU: colony forming unit

Droplet Generation:



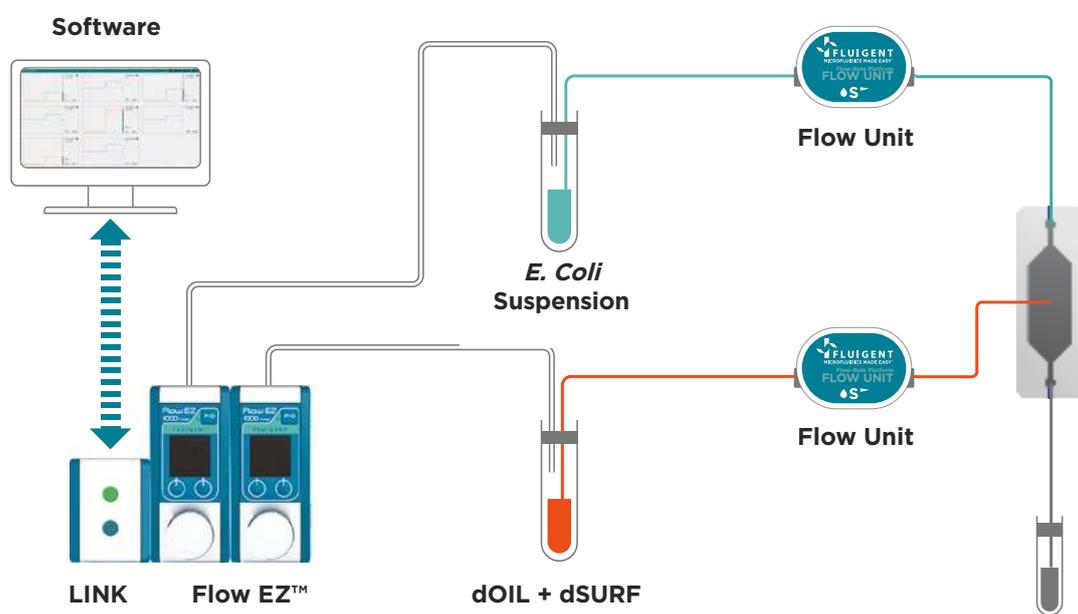
The Fluigent Droplet Starter Pack

Droplet generation was performed with customer designed PDMS chip but we would recommend to use the EZ drop chip (Fluigent, P/N DropKit01)



To generate droplets, fluid handling devices such as Pressure pump, Syringe pump or peristaltic pump could be used with a flow focusing PDMS chip^[4]. In this case Syringes pump have been used.. Syringes that are loaded into the syringe pump are connected to the chip with 1/32 OD 250 µm ID PTFE tubing in order to inject continuous and dispersed phases at determined flow rates into the chip to achieve desired droplet size and droplet generation frequency.

Flow EZ™ flow controller can be also used to increase droplet generation performances such as Flow rate stability and droplet monodispersity. In this case, *E. Coli* suspension and **dOIL with dSURF** are loaded into vials. A pressure is applied to the reservoir to ensure a continuous and pulseless injection of both phases into the chip. The determined flow rate is monitored and controled by using the A-i-O software or local control on the Flow EZ™ to achieve the desired droplet size and frequency.



Experimental Setup for Cell Encapsulation

Droplet incubation and Monitoring:

Droplets were incubated at 28 or 37 degree Celsius on an Eppendorf shaker (but could be incubated in any incubation devices or chip^[5]).

To monitor droplet stability and bacterial growth, droplets were transferred into the observation chambers of another microfluidic chip. This was placed on an inverted microscope (Axio Observer Z1, Zeiss). The standard halogen lamp of the microscope was used as light source for bright and dark field illumination images. For image acquisition a PCO camera and 10x magnification objective were used.

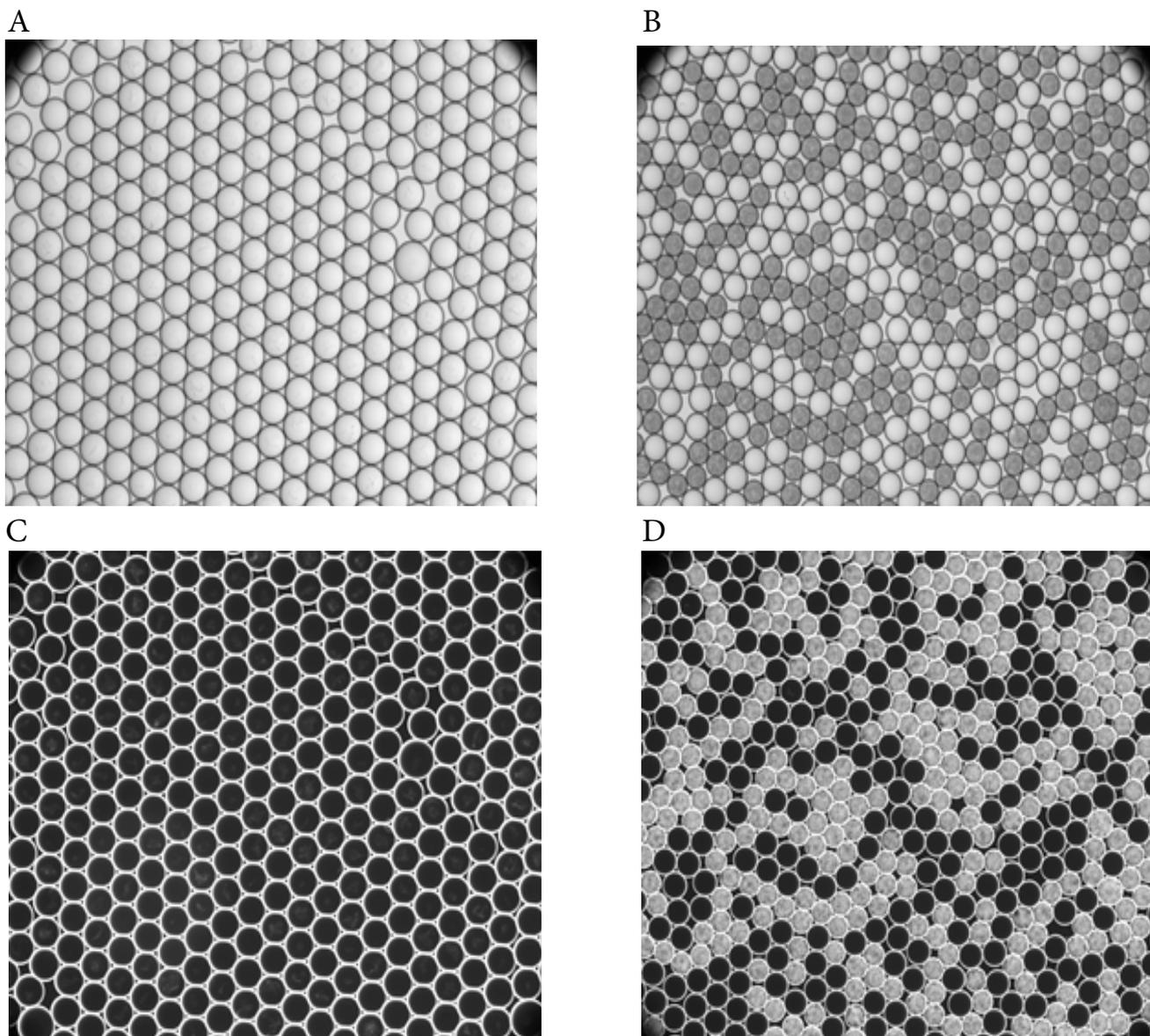
Droplet stability and presence/absence of microbial growth were checked after 2 or 4h and after 20h. Time points of 2 or 4 h were necessary, because after prolonged incubation time, the quality and stability of droplet emulsion could be affected by the growth of bacteria.

Therefore, droplets have been checked after 2 or 4h of incubation when some growth of bacteria could be observed and later after 20h.

RESULTS

Test with *E.Coli* at OD=0.005 and 0.5% dSURF, incubation at 37°C:

Stable and monodisperse droplet emulsion and *E. coli* in some droplets were observed.



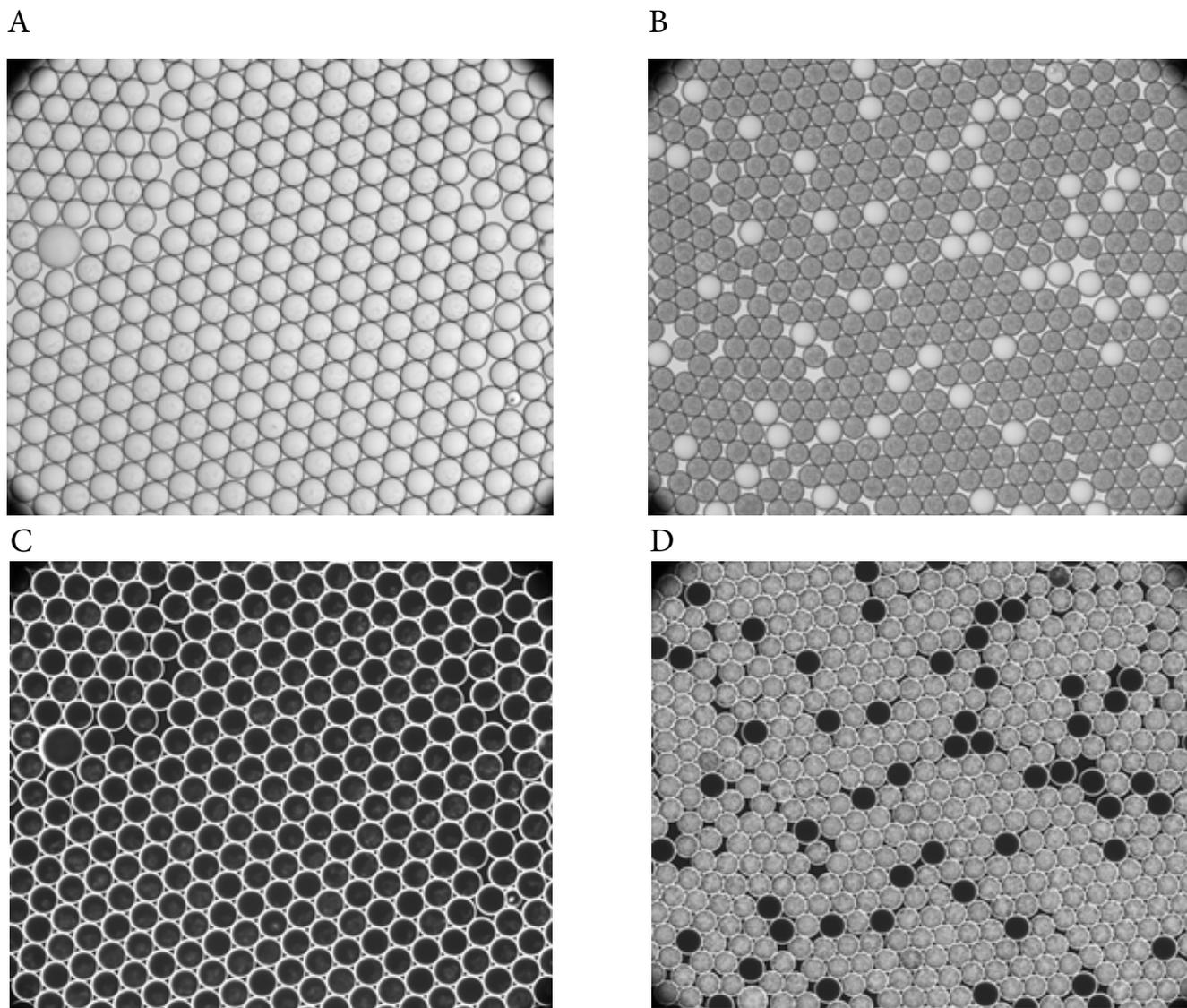
Legend:

(A) Image of droplets under bright-field after 4h incubation (B) Image of droplets under bright-field after 24h incubation (C) Images of droplets under dark-field after 4h incubation (D) Images of droplets under dark-field after 24h incubation.

Stable, monodispersed droplet emulsions were observed on both images. High numbers of *E. coli* cells were observed in almost half of the droplets, particularly in $46 \pm 0.8\%$ of droplets, which is in a good agreement with the theoretical number of 54% calculated from the Poisson distribution of cells over $67.2\mu\text{m}$ droplets (160pL) at a given concentration of 5×10^6 CFU/ml. The variability between theoretical and experimental numbers could be explained by the choice to use a low *E.coli* concentration and fractional droplet occupation with cells as well as by small variation in the experimental procedure, such as general pipetting imprecision

Test with *E.Coli* at OD=0.01 and 0.5% dSURF, incubation at 37°C:

Stable and monodisperse droplet emulsion and *E. coli* in some droplets were observed



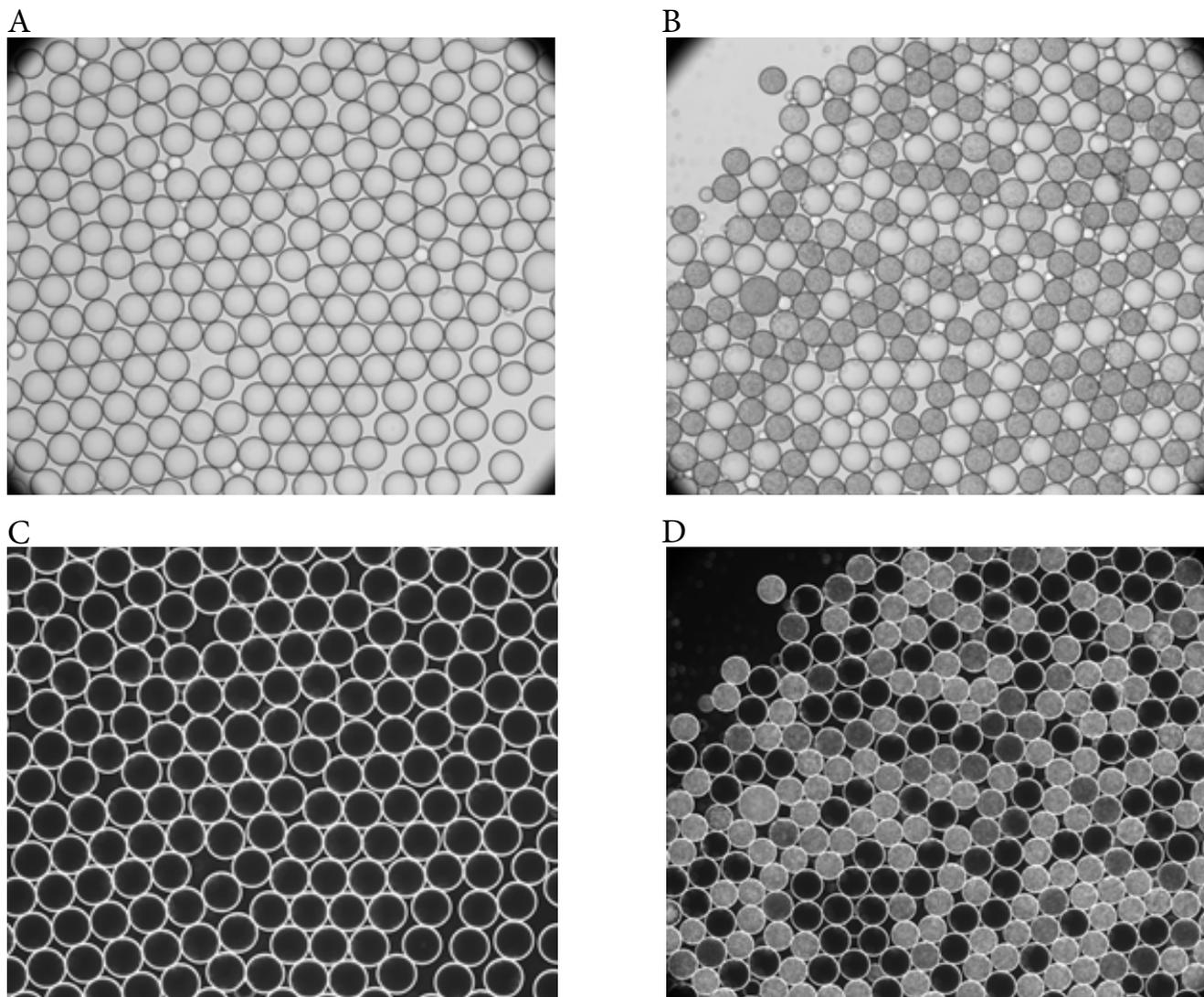
Legend:

(A) Images of droplets under bright-field after 4h incubation (B) Image of droplets under bright-field after 20h incubation (C) Images of droplets under dark-field after 4h incubation (D) Images of droplets under dark-field after 20h incubation

Stable, monodispersed droplet emulsion could be observed on both images. High numbers of *E.coli* cells were observed in most droplets, particularly in $89 \pm 1\%$ of droplets, which is in a good agreement with the theoretical number of 86% calculated from the Poisson distribution of cells over $72.4\mu\text{m}$ droplets (200pL) at a given concentration of 10×10^7 CFU/ml.

Test with *E.Coli* at OD=0.005 and 3% dSURF, incubation at 28°C:

Stable and monodisperse droplet emulsion and *E. coli* in some droplets were observed



Legend:

(A) Images of droplets under bright-field after 4h incubation (B) Image of droplets under bright-field after 20h incubation (C) Images of droplets under dark-field after 4h incubation (D) Images of droplets under dark-field after 20h incubation

Stable, monodispersed droplet emulsions could be observed on both images. High numbers of *E. coli* cells were observed in many droplets, particularly in $63 \pm 1.1\%$ of droplets, which is in a perfect agreement with the theoretical number of 63% calculated from the Poisson distribution of cells over $72.4\mu\text{m}$ droplets (200pL) at a given concentration of 5×10^6 CFU/ml

CONCLUSION

Growth of *E. coli* after 20 h of incubation in all above described cases has highlighted that dSURF is biocompatible at concentrations ranging from 0.5% to 3%. For routine applications and cost reduction, 0.5% concentration of dSURF can be used. It is also suitable for experiments starting with single and multiple microbial cells per droplet.

The emulsion stability after 20 h shows the good droplet stabilization which will be of use for longer duration experiments.

REFERENCES

- [1] C. H. J. Schmitz, A. C. Rowat, S. Koester and D. A. Weitz, Dropspots: a picoliter array in a microfluidic device, *Lab Chip*, 2009, 9, 44-49.
- [2] Bai Yunpeng, He Ximin, Liu Dingsheng, Patil Santoshkumar N, Bratton Dan, Huebner Ansgar, Hollfelder Florian, Abell Chris, Huck Wilhelm TS. A double droplet trap system for studying mass transport across a droplet-droplet interface. *Lab Chip* May 2010;10(10):1281-5.
- [3] Lee JN, Park C, Whitesides GM. Solvent compatibility of poly(dimethylsiloxane)-based microfluidic devices. *Anal Chem* 2003, 75:6544-54.
- [4] Svensson C-M*, Shydkevich O*, Dietrich S, Mahler L, Weber T, Choudhary M, Tovar M+, Figge MT+**, Roth M**. Coding of experimental conditions in microfluidic droplet assays using colored beads and machine learning supported image analysis 2018
- [5] Mahler L, Tovar M, Weber T, Brandes S, Rudolph MM, Ehgartner J, Mayr T, Figge MT, Roth M, Zang E (2015) Enhanced and homogeneous oxygen availability during incubation of microfluidic droplets. *RSC Advances* 5, 101871-101878.