

Electronic Supplementary Information

A large-scale on-chip droplet incubation chamber enables equal microbial culture time

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Materials and Methods

1. Device fabrication and droplet generation

The master mold for the incubation chamber was fabricated from a photopolymer (LS600, EnvisionTEC) using a 3D printer (Perfactory Ultra, EnvisionTEC). Prior to PDMS replication, the master was exposed to UV light (Omnicure S1000, Lumen Dynamics) for 5 mins and baked overnight at 50 °C, followed by (tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (TFS) (T2492, United Chemicals) treatment for 30 mins. Polydimethylsiloxane (PDMS) (Sylgard 184,

Dow Corning) (elastomer:crosslinker =10:1) was poured onto the master and baked for 2 h at 85 °C. The chamber was released from the master and a hole was punched at the top as an inlet. A thick PDMS slab with a straight channel (250 µm high and 250 µm wide) was fabricated by pouring PDMS (elastomer:crosslinker =15:1) onto a 3D printed master mold and baked for 1 h at 85 °C. After punching holes in this PDMS slab, it was placed on a glass slide and baked for 1 h at 85 °C. Then, the incubator was bonded onto the slab and baked overnight at 85 °C. The diameter of the incubation chamber is 1 cm and the height is 4.3 cm, having a volume of 2.4 ml.

Fluorinated oil (Novec 7500, 3M) and 2 % (wt/wt) fluorosurfactant (PFPE-PEG-PFPE, RAN Biotechnologies) was used to generate water-in-oil droplets using a T-junction droplet generator. Surfactant was used to stabilize the droplets and prevent droplet coalescence. Syringe pumps (Fusion 400, Chemyx Inc.) were used to control volumetric flow rates of all input streams. The 121 µm droplets were generated at 200 µl/h for the oil phase and 180 µl/h for the aqueous phase. The droplet generation speed was determined by recording droplet generation using a CMOS camera (C11440, Hamamatsu) at 150 frames per second with a 6.67 ms exposure time.

2. Fungal spore encapsulation and culture in droplets

Wild-type spores of *Fusarium verticillioides* were suspended in deionized water at concentration of 5×10^7 spores/ml. The fungal spore concentration was adjusted to 5.5×10^5 spores/ml in YEPD medium. The fungal spores were encapsulated in droplets at a generation speed of 48 Hz, where about 10 % of the droplets contained a single fungal spore. After 12.5 h of droplet generation, the chamber accommodated 2.14×10^6 droplets. The fungal spores in droplets were incubated at 23 °C during culture. To prevent undesired growth of fungal samples in the syringe prior to droplet

generation, ice was used to maintain cold temperature and to minimize cell growth inside the syringe.

3. Growth quantification and statistical analysis

An inverted microscope (Eclipse TS100F, Nikon) equipped with a CCD camera (DS-2MV, Nikon) was used to capture bright-field images of droplets at 40× magnification. The growth of fungi was quantified by measuring the area of fungal cells on images using the ImageJ software (NIH). ANOVA test was used to determine the statistical significance of growth of fungual cells encapsulated at different time points (Fig. 3c). Normality and equal variance assumption were checked by using Anderson-Darling and Bartlett's tests, respectively (MINITAB 14, Minitab Inc.).

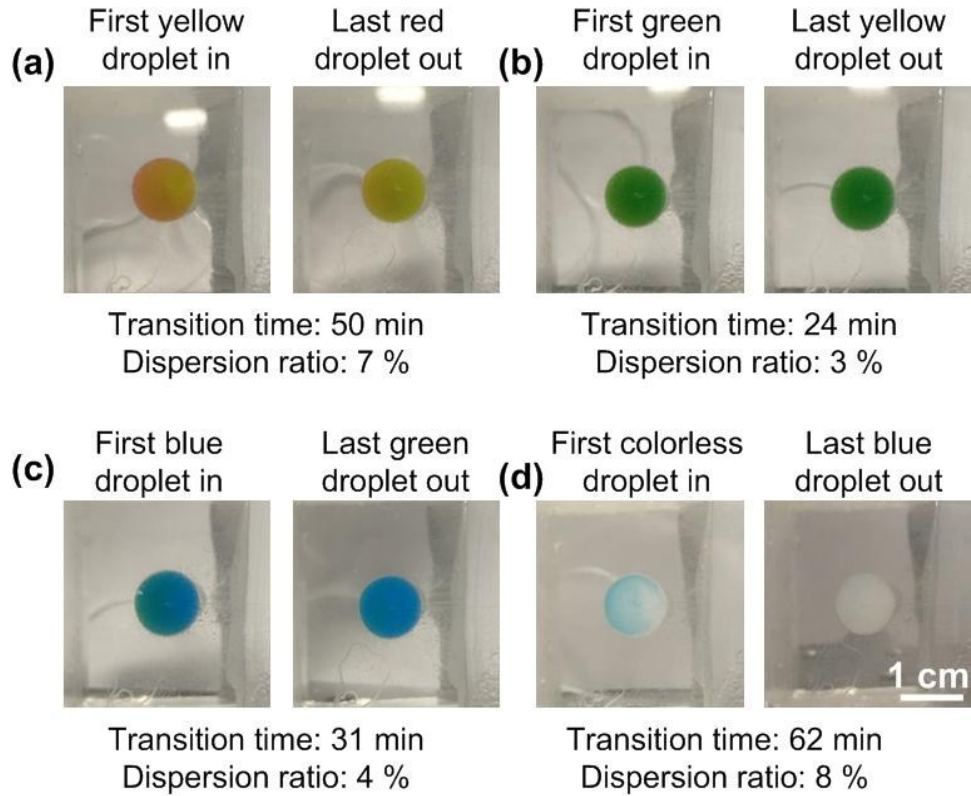


Fig. S1 Transition of color dye droplets at the bottom outlet of the incubation chamber that was initially filled with layers of red, yellow, green, and blue droplets. (a) Transition of red droplets to yellow droplets. (b) Transition of yellow droplets to green droplets. (c) Transition of green droplets to blue droplets. (d) Transition of blue droplets to colorless droplets.