Electronic Supplementary Information (ESI)

Supplementary Materials and Methods

Polydimethylsiloxane stamp fabrication

The stamp consists of a 10 x 10 mm glass (Borofloat Schott) back plate with polydimethylsiloxane (PDMS) (Sylgard184 Dow Corning) pillars. These pillars were made by casting PDMS using a silicon wafer as a mold. The silicon wafer was patterned by deep reactive ion etching (DRIE) in an Adixen AMS100 equipment using a OiR907/17 (Fujifilm) photoresist pattern as a mask.[1] The depth of the holes is 15 μm. After removing the photoresist, a fluorocarbon layer was deposited by plasma deposition in an Elektrotech Reactive Ion Etcher to prevent sticking of the PDMS to the mould. [2] Next a layer of 20 μm PDMS was spin-coated on the mould, which was degassed under vacuum. *In situ*, a cleaned glass wafer was glued on top of it, forming the back plate. After a 1 h PDMS cure at 80 ºC the wafer stack was diced into the 10 x10 mm stamps by a Disco dicing machine. Prior to use, the silicon part is removed from the stamp by insertion with a surgery knife. Stamps fabricated are described in Table 1 of the Main Text.

Stamp holder fabrication

Holders for the stamp and the PAO (porous aluminum oxide) strip or culture chip were created that allowed both precise positioning and culture with imaging *in situ*. This was a two layer construction of etched silicon on top of a Borofloat glass base. The 50 μm deep grooves for the PAO chip and the stamp tool area and the 200 μm deep grid pattern were created on one side of a double polished silicon wafer by DRIE

in an Adixen AMS100 etcher, using a buried mask process, i.e. the etched mask for the grooves was fabricated from silicon dioxide, grown by thermal oxidation and patterned by subsequent photolithography followed by buffered HF etching. The mesh that supports the chip was created by DRIE with photoresist as a mask. After photoresist stripping, the revealed oxide pattern was used as mask for DRIE of the 50 μm grooves. Silicon nitride was deposited by low pressure chemical vapor deposition and patterned on the backside of the wafer by photolithography and DRIE. The silicon was removed from the backside of the wafer up to the grid by KOH etching to create the nutrient chamber. After stripping the residual nitride and oxide layers in HF, anodic bonding was used to attach the upper silicon wafer to the lower Borofloat wafer. The 100 mm silicon wafer containing three stamp holders was then diced with the area of a microscope slide (20 mm x 70 mm). The stamp holder was highly robust, and could be sterilized by autoclaving for 15 min at 121 ºC.

Culture and handling of microorganisms

The Gram-positive bacterium *Lactobacillus plantarum* strain WCFS 1 was grown in liquid MRS medium or on MRS agar at 37 ºC under aerobic conditions.[3] The Gram-negative bacterium *Escherichia coli* expressing green fluorescent protein (GFP) was grown in Mueller Hinton broth or Mueller Hinton agar, both supplemented with 100 μg ml^{-1} ampicillin. For printing experiments the strain was labeled by binding quantum dots (QDOTS) to the exterior of the cell wall by means of wheat germ agglutinin. Fluorescence labeling was performed by incubating 10^9 bacteria in 100 μl with 10 nM QDOTS (WGA conjugated with 655 nm emission, from Invitrogen, NL) for 2 h at 20 ºC. Prior to incubation, aggregated QDOTS were removed by centrifugation and as described by the manufacturer. Separation of the labeled

bacteria from unadsorbed QDOTS by was by two gentle centrifugation steps (3000 rpm for 4 min, Eppendorf centrifuge) and resuspending the labeled bacterial pellet in fresh medium. From 2 to 8 QDOTS were conjugated to each cell. Spores of the filamentous fungus *Aspergillus fumigatus* JBZ1 were harvested from Sabouraud agar plates after 4 days culture at 37 ºC. Where it was necessary to label spores before printing this was done by incubating c. 10^9 spores with NanoOrange dye (NanoOrange Protein Quantification Kit, from Invitrogen NL) diluted 1/200 from the manufacturer's stock for 20 min at 20 ºC. Spores for printing were resuspended in phosphate-buffered saline (PBS) with 5% (w/v) glycerol at a density of 10^9 cfu ml^{-1}. *Candida albicans* JBZ32 was cultured on Sabouraud medium or broth. Microorganisms used for printing were resuspended in PBS supplemented with 5% (w/v) Ficoll (Sigma) and 5% (w/v) glycerol (Sigma). Where outgrowth or viability testing of organisms was needed, the PAO or PAO chip was transferred to the appropriate nutrient agar. For *L. plantarum* incubation was on MRS agar under aerobic conditions for 5 to 12 h at 30 ºC. For *E. coli* incubation was on Mueller Hinton agar containing 100 µg ml^{-1} ampicillin with direct observation of GFP. For *A. fumigatus* or *C. albicans* incubation was on Sabouraud agar for 10 h at 37 ºC under aerobic conditions. When it was necessary to stain microorganisms on PAO after growth, this was done by transfer of the PAO or culture chip to an agar slide covered with a thin film of low-melting-point agarose containing the dye, staining the microorganisms on the upper surface through the pores of the PAO. For *L. plantarum* and *C. albicans* staining was with Syto 9 (Invitrogen) as previously described.[4] Where spores or mycelia of *A. fumigatus* were labeled after printing on PAO this was done by transfer of the PAO to a slide covered with low melting point agarose containing

NanoOrange at 1/100 dilution from the concentration supplied in a NanoOrange protein quantification kit (Invitrogen, NL).

Alignment and printing protocols

Before printing, the set up was aligned. First, the microscope XY table was leveled. Next the rotation of the microscope table was adjusted and fixed, by moving along the edge of the chip holder until it was in line with the X-direction. A PDMS stamp was cleaned by reactive oxygen plasma and then was placed and aligned in the lower right corner of the stamp area on the substrate holder. This was the upper left corner in microscope view. The crosshair of the eyepiece was set to the upper left PDMS pillar of the pillar array. This location was set to the zero reference point of the Corvus controller. Next a PAO chip was put on the substrate holder. Alignment to the stamp was accomplished by using the *3-point alignment* facility of the Corvus software. After this alignment procedure, the stamp was picked up using the adaptor by moving to the zero reference position, insertion of the lens and moving the adaptor down. Before the adaptor was fixed again, the PDMS pillars were brought into focus again. By turning on the vacuum, the stamp was clamped to the adaptor. The *set zero reference point* of the control software was then applied again, to have a reference point for the Z-direction also. This was also the ultimate Z-position to prevent damaging by pushing the microscope table to the objective. The printing was now started by picking up the microorganisms from one region of the PAO chip. After the stamp was released from its base position by moving in the minus Z-direction, it was brought to the microorganisms supplied to the PAO chip, by means of the *position* facility of the control software. The convex lens was pulled out, and the table moved up in the plus Z-direction until the grid of the PAO chip was in focus with a 50 μm

gap between the stamp and the PAO chip. The crosshair was now in the centre of a cavity of the grid pattern. After insertion of the additional lens the pillars became visible again and the grid was brought into focus by moving the table up again. At 5 μm above the surface the stamp was dropped by releasing the vacuum. After several seconds the stamp was clamped again by the vacuum. The pillars were then loaded with microorganisms, which were printed to a new section of the PAO culture chip. The procedure was the same as described above, when the stamp was picked up for the first time. Printing on unstructured PAO was performed in a similar fashion, but omitting the alignment process.

Supplementary References

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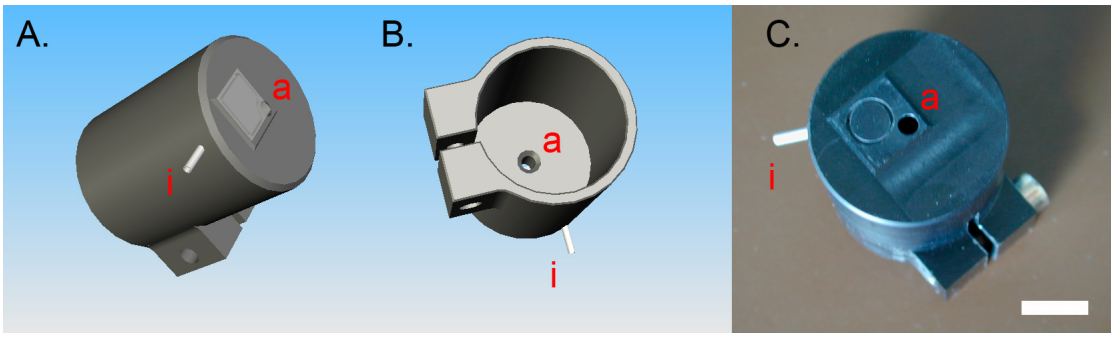
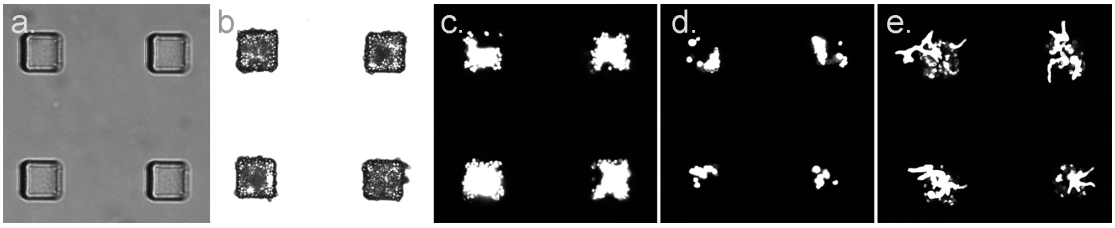


Supplementary Figure 1 Adaptor Design. **(A and B)** 3D CAD image viewed from two angles. The inlet used to apply the vacuum is shown (i). **(C)** Photograph of adaptor from below. The 10 x 10 mm square area is used to hold the stamp with a vacuum using the ring channel. The smaller aperture within this square area is for observation through the stamp during printing. Scale bar indicates 1 cm.



Supplementary Figure 2 Example of printing fungal spores. **(A)** Four pins (20 x 20 μm cross-section) viewed from below by transmission light microscopy. **(B)** Four pins with spores of *A. fumigatus*, viewpoint as A. **(C)** As B, but spores labeled with NanoOrange before printing and viewed by fluorescence microscopy. **(D)** Printed spores on PAO, the result of contacts by 4 pins with staining after printing. **(E)** Germination and outgrowth of printed spores. As panel D, but after printing PAO was transferred to Sabouraud agar and incubated for 10 h at 37 ºC then stained and imaged.