**Title:** A microfluidic cell-trapping device for single-cell tracking of host-microbe interactions

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Movie Legends

**Movie 1.** Representative time-lapse microscopy experiment using the presented device. 52 chambers starting each with a single *D. discoideum*were imaged for 27 hours. A lot of heterogeneity can be seen in this movie in terms of cell size, time to division and motility.

**Movie 2.** Co-culture of *D. discoideum*and *K. pneumoniae* (expressing a cytosolic GFP). As *D. discoideum*moves around the chambers and encounters bacteria, it phagocytoses them and subsequently kills them.

**Movie 3.**  Co-culture of *D. discoideum*and *M. marinum* (expressing a cytosolic mCherry). After a long co-culturing time at 21 hours the bacteria are taken up, replicate and eventually lyse their host.

**Movie 4.** Co-culture of *D. discoideum*and *M. marinum* (expressing a cytosolic mCherry). Following phagocytosis the bacteria will remain inside its host several hours before being released.

**Movie 5.** Co-culture of *D. discoideum*and *M. marinum* (expressing a cytosolic mCherry). Following phagocytosis the bacterium will be killed by the host cell.

**Movie 6.** *M. marinum* can be killed by *D. discoideum*. Following phagocytosis the bacterium will be killed by the host cell (disappearance of fluorescence and phase contrast signal).

Supplementary Figures



**Supplementary Fig S1.** **(A)** Three independent distribution of the number of *D. discoideum* per chamber (circles) with fitted Poisson distributions (lines with corresponding color. Only *D. discoideum* was present in these experiments. Mean = 1.16 for red; 0.66 for green; 0.49 for blue and n = 260; 170 and 275 chambers respectively.

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**Supplementary Fig S2.** This graph shows the total fluorescence per frame overtime in one chamber. The medium was repeatedly changed over time switching to medium containing or not FITC. Syringe changed every 10 min.



**Supplementary Fig S3. (A)** Cross-sectional representation of a cell inside a trapping chamber within the InfectChip device. The dimensions of the different parts are indicated but are not to scale. **(B)** Confocal image of an ABD-GFP *D. discoideum* in a trapping chamber. This confocal image illustrates the flattening of cells within the trapping chambers. Scale bar is 4 µm.  **(C)** Total fluorescence of a cell along the z dimension. The two peaks represent the fluorescence from the bottom and top membranes of the cell. The cell thickness is defined as the difference of the two peaks. **(D)** Cell thickness in the trapping chamber as measured in (C) for three different InfectChip devices and n = 70, 16, and 100 cells, respectively.



**Supplementary Fig S4. Bacteria co-localizing with *D. discoideum* are intracellular.** **(A)** cross-sectional view an of single *M. marinum* (mCherry) in a *D. discoideum* (ABD-GFP). **(B)** Corresponding total fluorescence per section in the region of interest, green line is GFP signal and red is mCherry signal (e.g. within blue line in A). **(C-J)** These are all the cases where the intracellular localization could not be demonstrated using confocal microscopy.



**Supplementary Fig S5.** Single-cell analysis of *M. marinum* grown in 7H9 at 30 °C of one experiment. **(A)** Interdivision time distribution of the bacteria measured as the time at division minus time at birth (mean 6.65; std 2 h, n = 112). **(B)** Distribution of bacterial length at birth (green bars, mean 2.8 h; stdev 0.56 h, n=136) and division (red bars, mean 4.5 h; stdev 0.76 h, n=137).