

[0244] Drops were generated and diluted on-chip by bringing together two channels containing the cell suspension and sterile media respectively and varying the relative flow rates while keeping the overall aqueous flow rate constant at 2000 microliters/h using two syringe pumps. The number of cells per drop was determined by evaluating movies taken with a high speed camera (Phantom V4.2) mounted on a microscope. For each dilution, 120 drops were analyzed to determine the number of cells per drop. Subsequently the data was fitted to a Poisson distribution ($p(x=k)=e^{-\lambda}\lambda^k/k!$) using XmGrace (<http://plasma-gate.weitzmann.ac.il/grace>).

[0245] The emulsions were collected in open syringes (without the plunger being inserted) and incubated within a water-saturated atmosphere (37 degrees C., 5% CO₂). During the encapsulation step, a laser beam (488 nm wavelength) was focused onto the channel using an objective with a 40-fold magnification (FIG. 15D, downstream of the nozzle) to excite the fluorophore. Emitted light was diverted by a dichroic mirror (488 nm notch filter), filtered (510 nm+10 nm) and collected in a photomultiplier to record the first fluorescence measurement (t_0). After the desired incubation time mineral oil was added to fill the syringe completely before inserting the plunger and re-injecting the emulsion together with 0.5% w/w DMP-PFPE surfactant in FC40 (injected into the oil inlet to space out the drops) into a chip with the same design as for the encapsulation step. To avoid fragmentation of the drops before the second fluorescence measurement (at t_1) the flow direction was reversed compared to the encapsulation step (the emulsion was injected into the outlet (FIG. 10A) to avoid branching channels). All signals from the photomultiplier were recorded using Labview (National Instruments) running an in-house program for the data analysis.

[0246] To prepare the plugs 5×10^6 cells/ml (determined with a Neubauer counting chamber) were stirred at 510 rpm within a 1.8 ml cryotube (Nunc) using an 8 mm magnetic stir-bar (Roth) and kept at 4 degrees C. Subsequently 660 nl plugs of this cell suspension and perfluorinated oil (FC40, 3M) were aspirated (at 500 microliters/h) into PTFE tubing (0.56 $\mu\text{m} \times 1.07$ mm internal/external diameter, Fisher Bioblock) in an alternating fashion using a syringe pump (PhD 2000, Harvard Apparatus). For each sample, 30 plugs were loaded before the tubing was sealed (by clamping microtubes to both ends) and incubated at 37 degrees C. within a CO₂ incubator (5% CO₂, saturated with H₂O). After incubation, the plugs were infused into a 25 cm² tissue culture flask. Subsequently 4 ml of live/dead staining solution (LIVE/DEAD Viability/Cytotoxicity Kit for animal cells, Invitrogen Kit L-3224) were added and the samples were incubated for one hour at room temperature. When using adherent cells, the staining solution was additionally supplemented with 0.25 g/l trypsin (Gibco) to break up cell clumps.

[0247] After staining, live and dead cells were counted manually using a microscope (Leica DMIRB) with a UV-lightsource (LEJ ebq 100). For each sample within a 25 cm² tissue culture flask 30 fields of view (corresponding to ~ 4.2 mm²) were evaluated to calculate the total number of living (green stain) and dead (red stain) cells.

[0248] Eggs were resuspended in M9 minimal media (Sigma) supplemented with *E. coli* OP50 (10% w/v of pelleted bacteria). Plugs of the resulting suspension were aspirated into PTFE tubing and incubated at room temperature.

[0249] For recultivation of cells recovered from drops or plugs, semi-conditioned media supplemented with 30% fetal bovine serum (Gibco) was added to the cells instead of the

staining solution. Cells were then incubated for two days at 37 degrees C. within a CO₂ incubator (5% CO₂, saturated with H₂O) before imaging using bright-field microscopy.

[0250] While several embodiments of the present invention have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the functions and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the present invention. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the teachings of the present invention is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, the invention may be practiced otherwise than as specifically described and claimed. The present invention is directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the scope of the present invention.

[0251] All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

[0252] The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

[0253] The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0254] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of ele-