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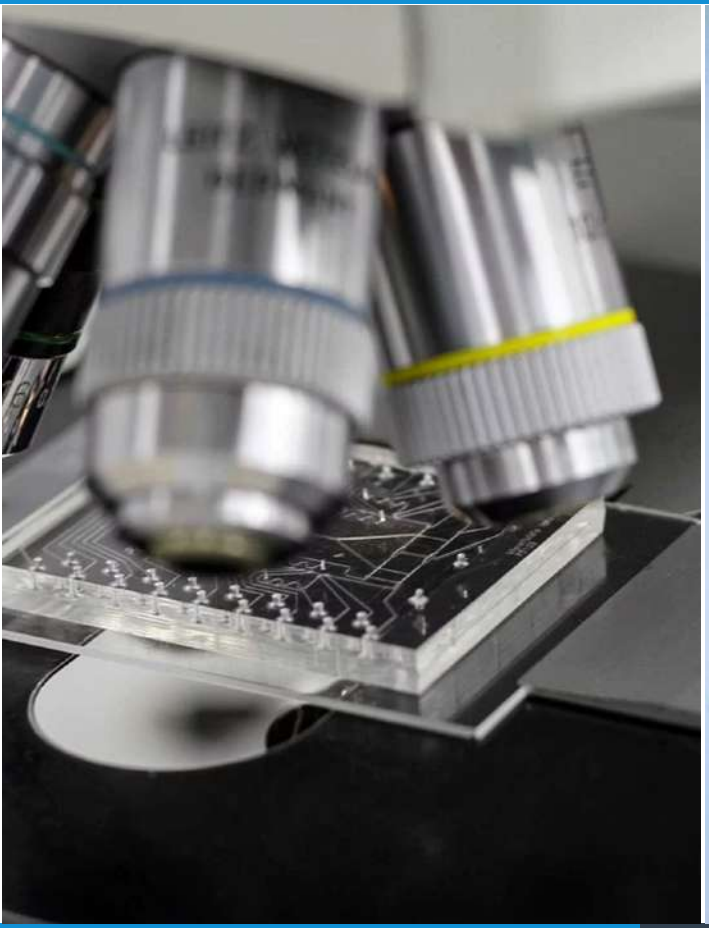
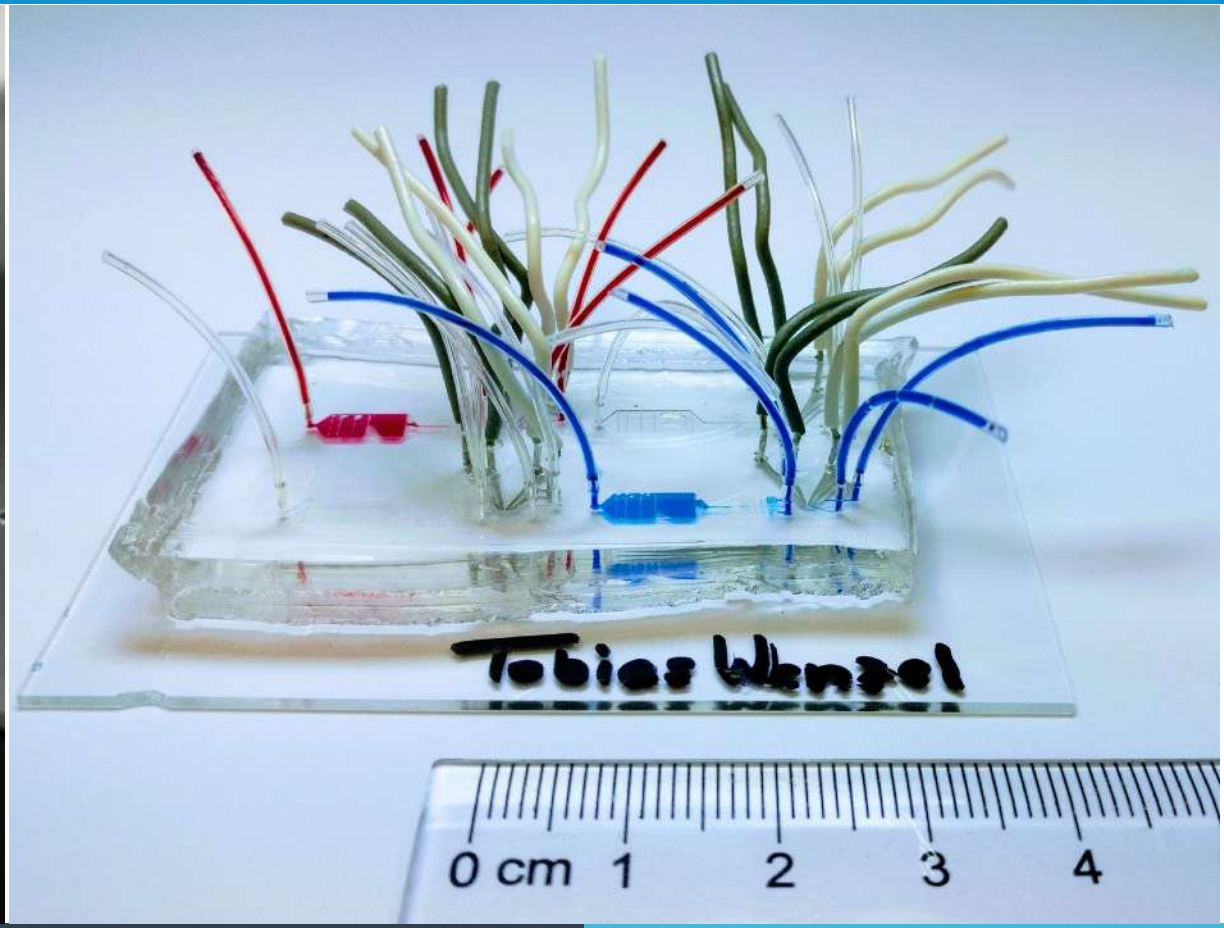
Scaling Up Bioimaging with Microfluidic Chips

Asst. Prof. Dr. Tobias Wenzel

Microfluidic chip - What does ‘chip' mean here?





Making Chips: Photolithography and soft lithography

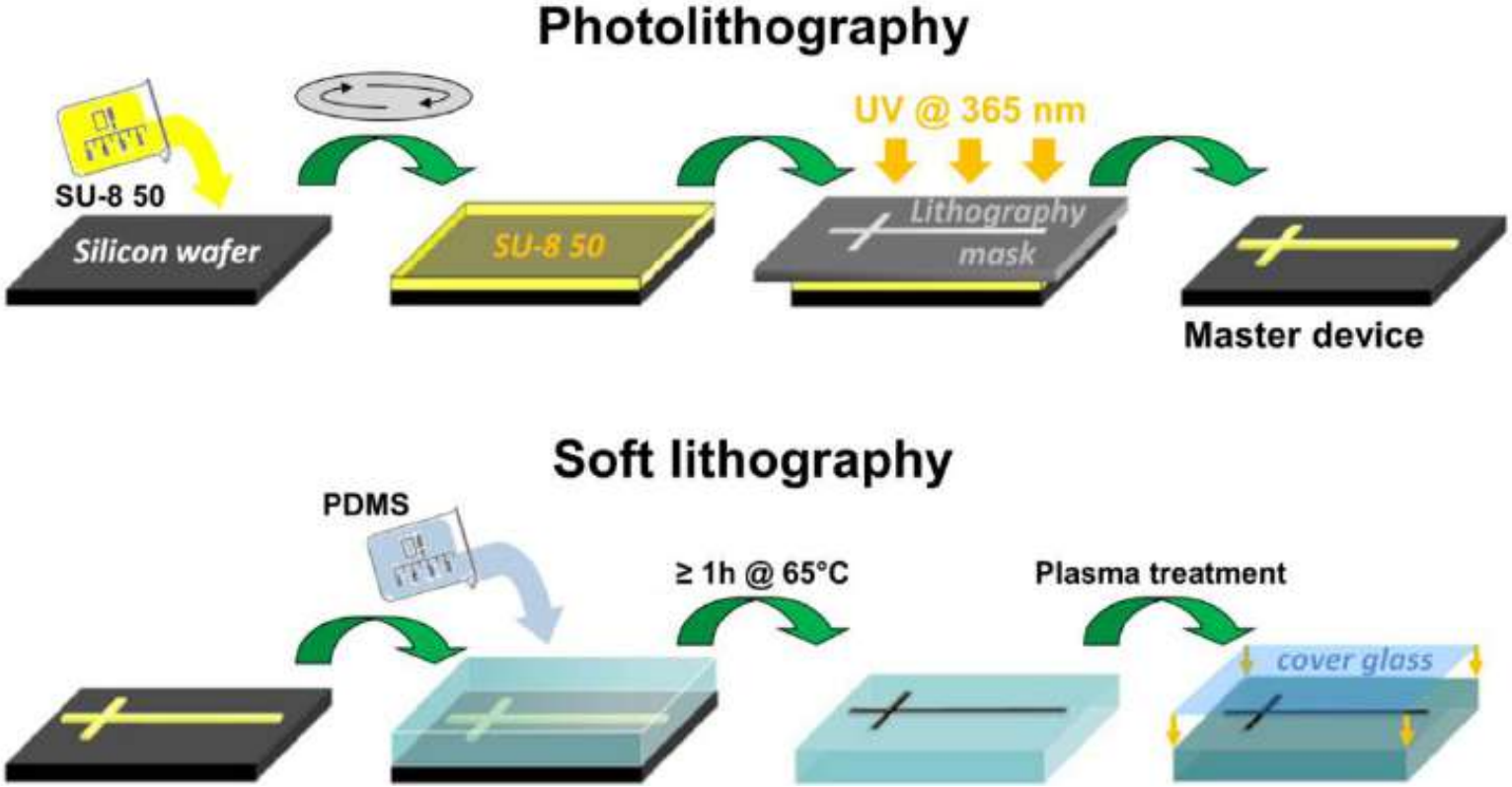
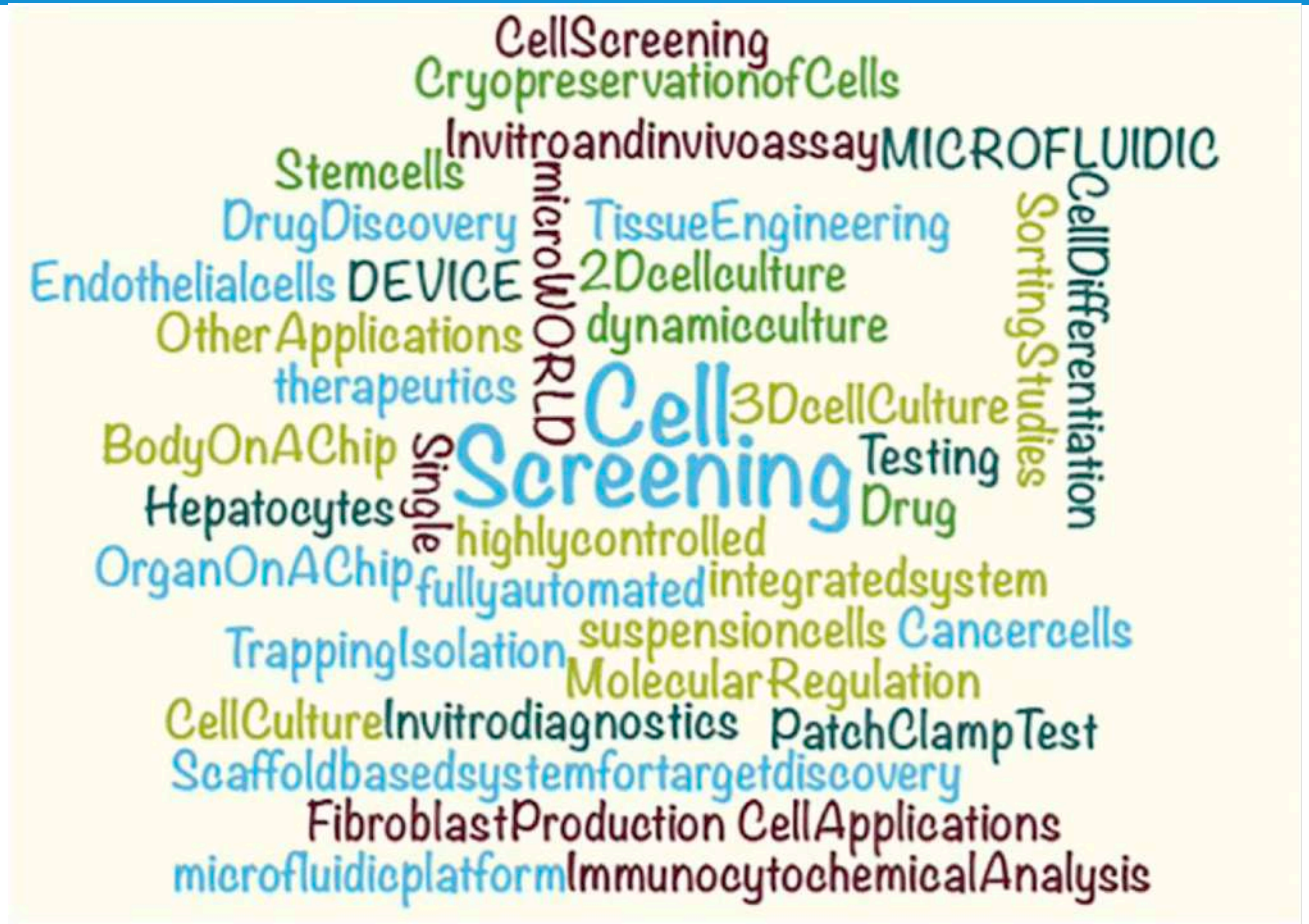


Image credit: Ma, Yujie, et al. "Biocompatible macro-initiators controlling radical retention in microfluidic on-chip photo-polymerization of water-in-oil emulsions." *Chemical Communications* 50.1 (2014): 112-114.

Microfluidics is a broad field of method development 6k/y



What Can You Do With a Chip on a Microscope?

Basic Applications

Gravity-powered systems for straightforward biological observations.

Intermediate Systems

Traps, gradients, and interface control for complex experimental conditions.

Advanced Platforms

Droplets, microgels, and organ-on-chip for cutting-edge research.

Flow chambers

Simple chambers to be mounted on advanced microscopes, and added controls

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Flow Chambers for Microscopy

Simple Yet Powerful

- Gravity-driven flow is an option
- Ideal for observing biofilms, plant roots, and phase transitions over time
- Perfect for live cell imaging applications

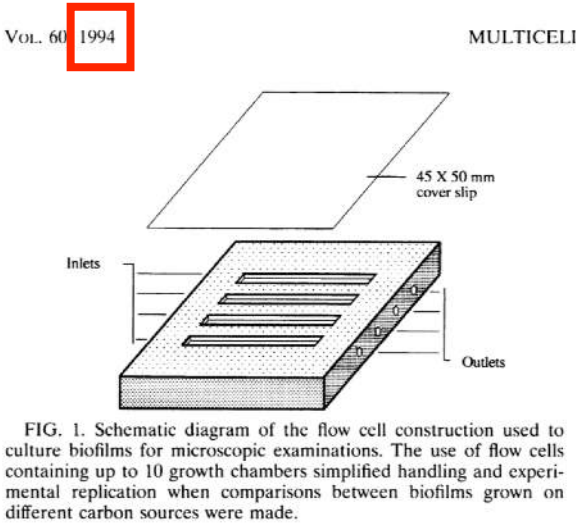
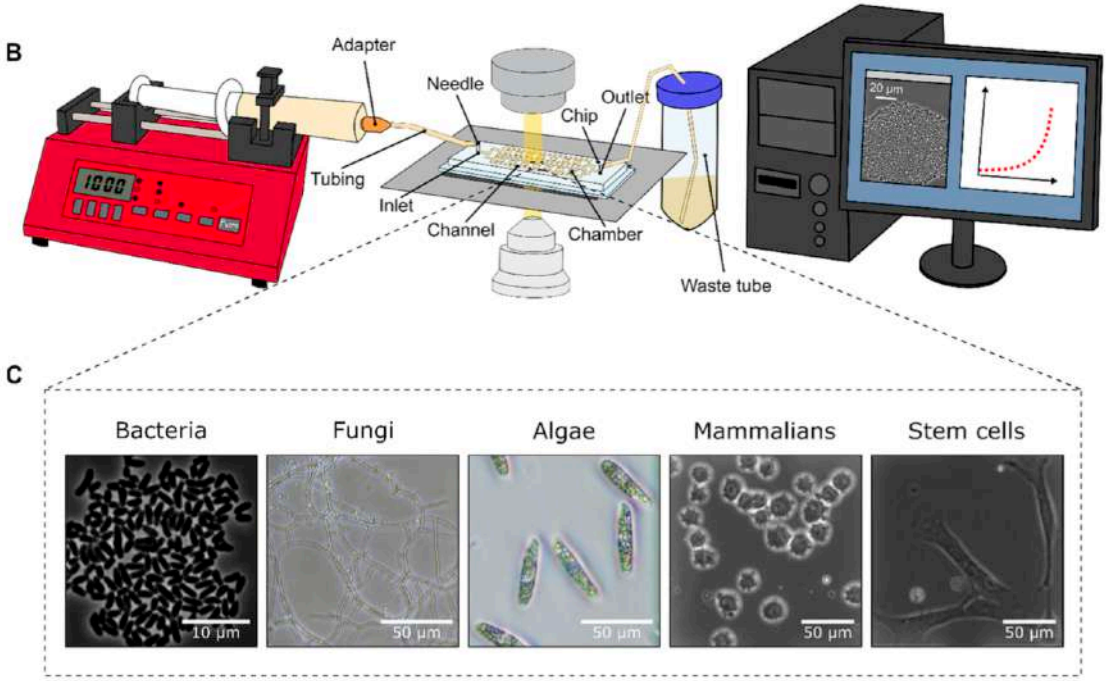
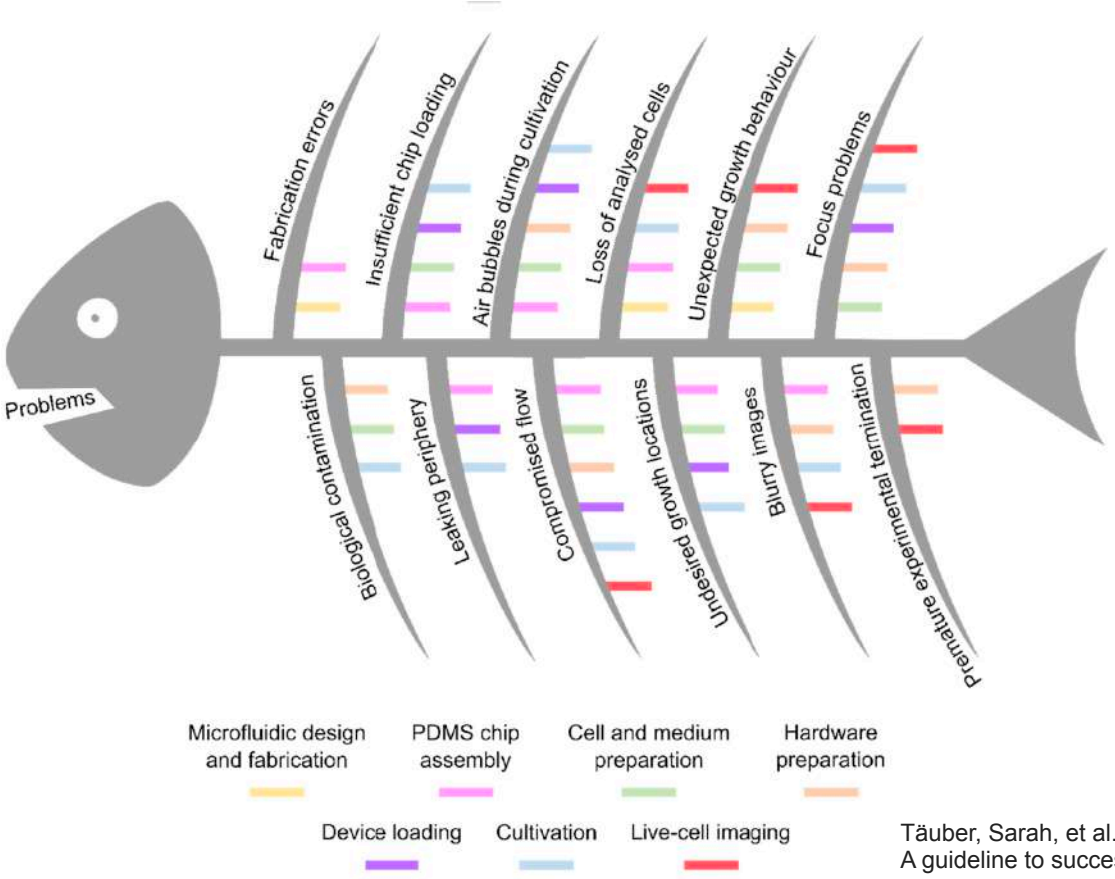


FIG. 1. Schematic diagram of the flow cell construction used to culture biofilms for microscopic examinations. The use of flow cells containing up to 10 growth chambers simplified handling and experimental replication when comparisons between biofilms grown on different carbon sources were made.



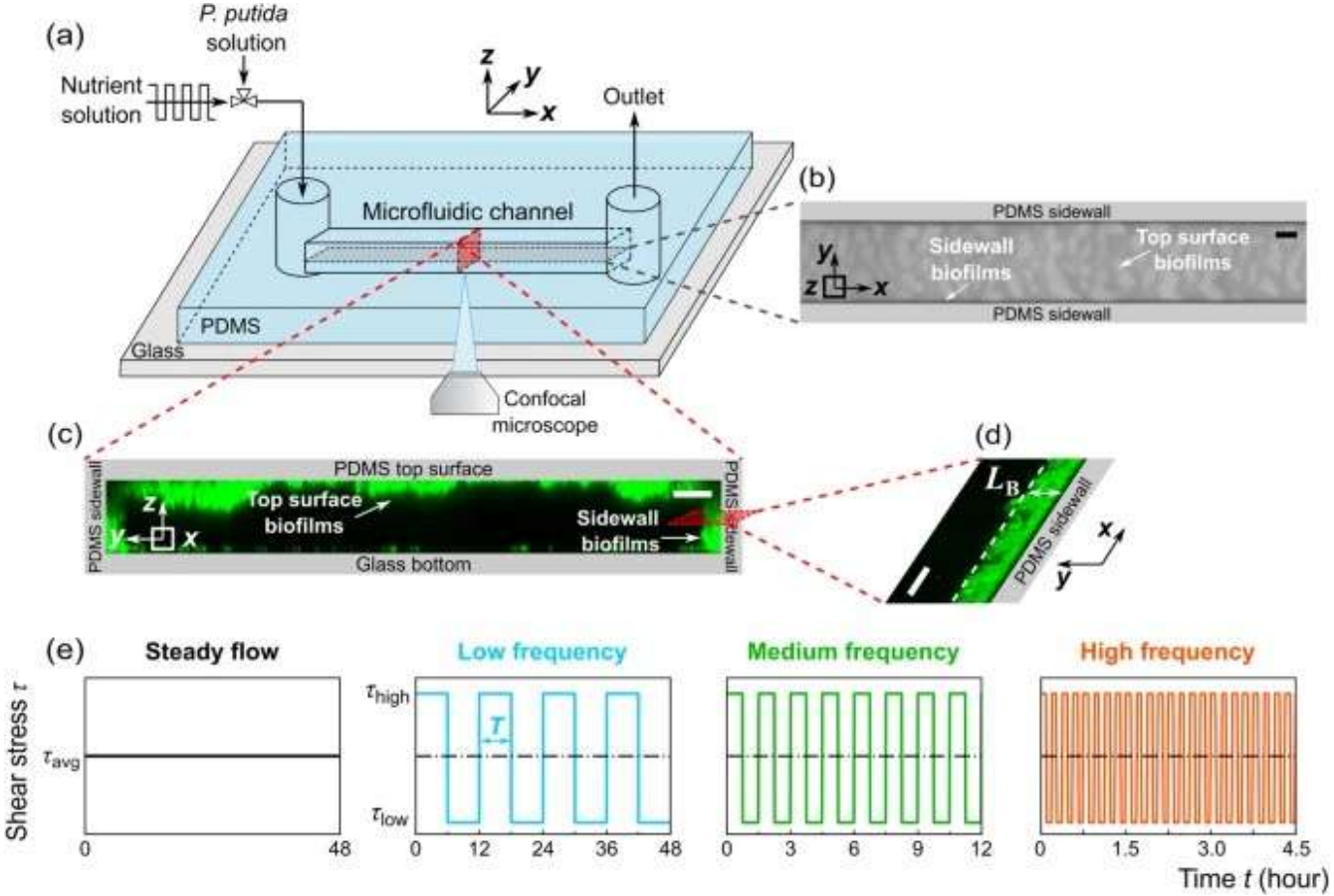
Täuber, Sarah, et al. "How to perform a microfluidic cultivation experiment—A guideline to success." *Biosensors* 11.12 (2021): 485.

Microfluidics cultivation chambers - trouble shooting



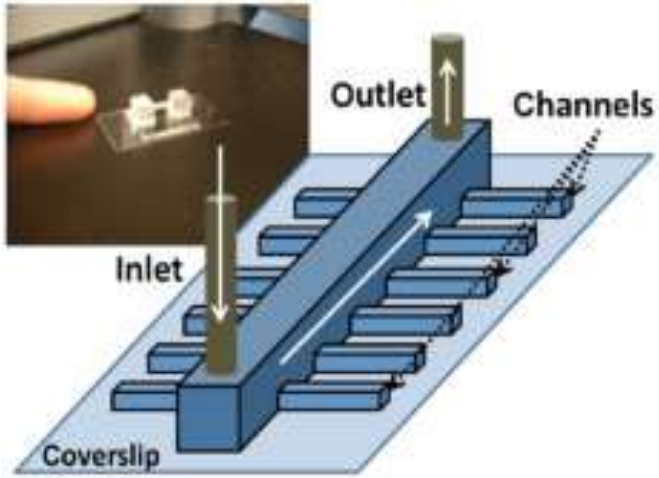
Täuber, Sarah, et al. "How to perform a microfluidic cultivation experiment—A guideline to success." *Biosensors* 11.12 (2021): 485.

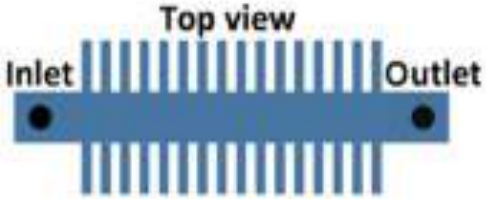
Cell adhesion studies with flow control

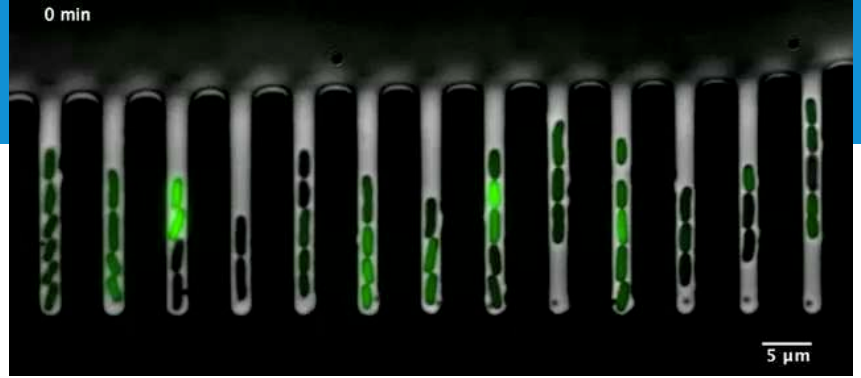


Wei, Guanju, and Judy Q. Yang. "Microfluidic investigation of the impacts of flow fluctuations on the development of Pseudomonas putida biofilms." *npj Biofilms and Microbiomes* 9.1 (2023): 73.

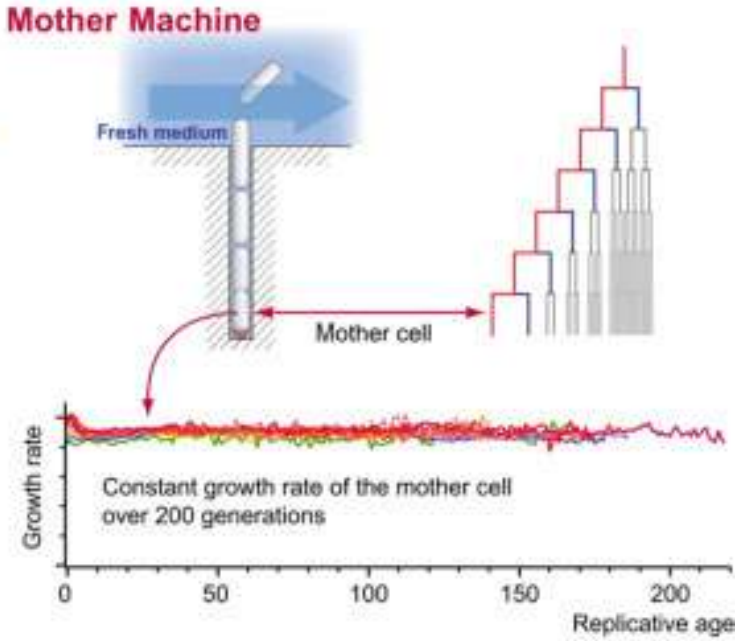
The mother machine







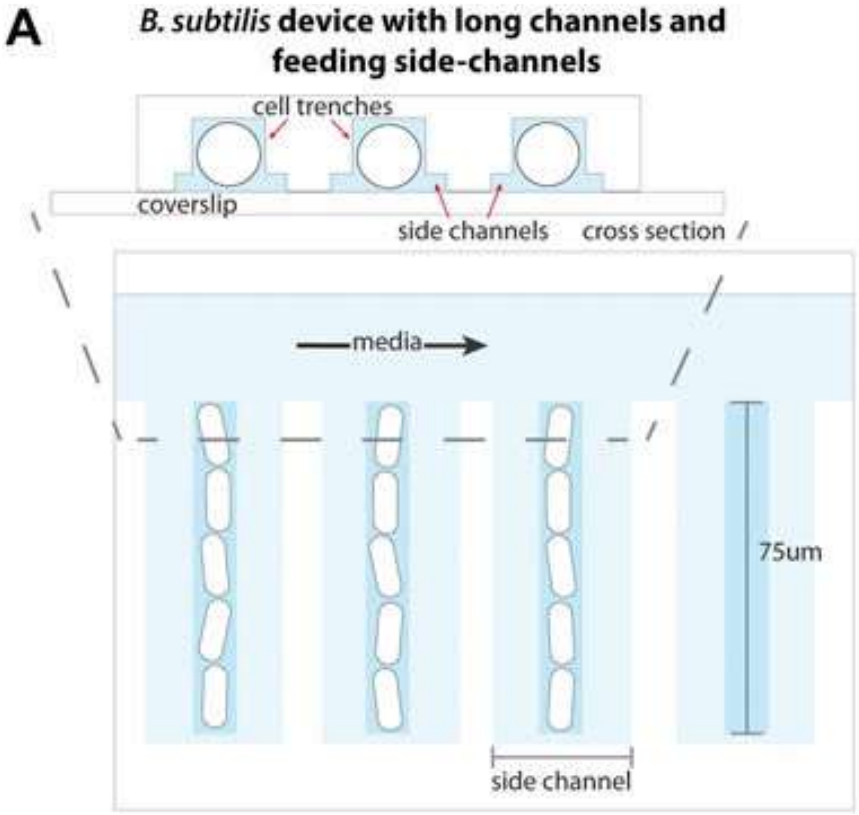
Niederholtmeyer, H. et al. Rapid cell-free forward engineering of novel genetic ring oscillators. Elife 4, e09771 (2015).

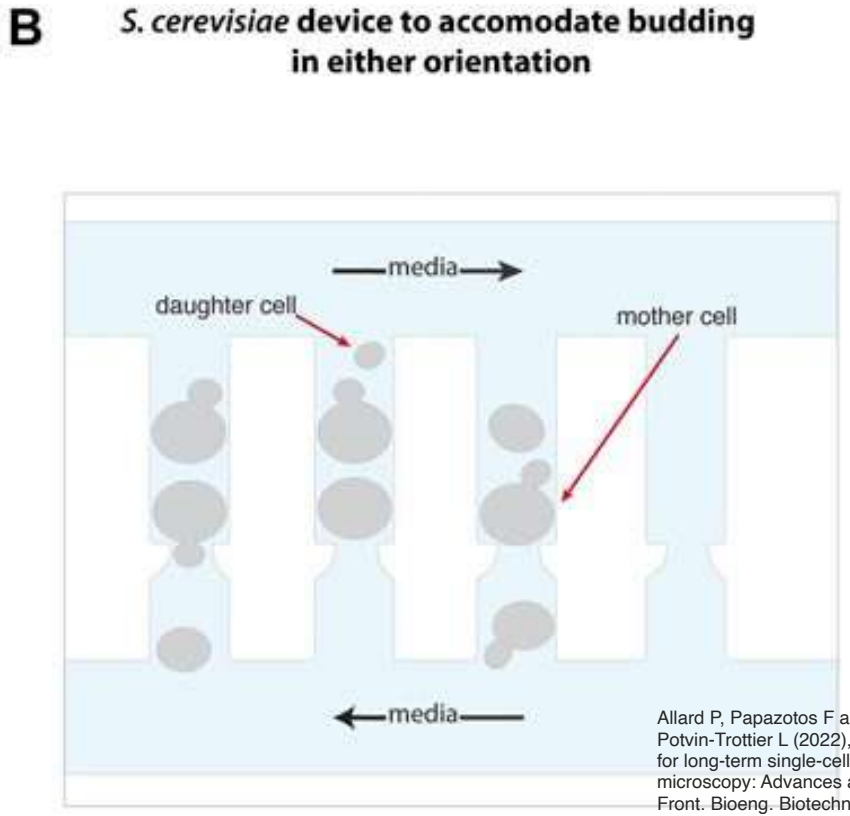


Wang, Ping, et al. "Robust growth of Escherichia coli." *Current biology* 20.12 (2010): 1099-1103.

1.Eland, L. E., Wipat, A., Lee, S., Park, S. & Wu, L. J. Chapter 3 Microfluidics for bacterial imaging. Methods Microbiol. 43, 69–111 (2016).

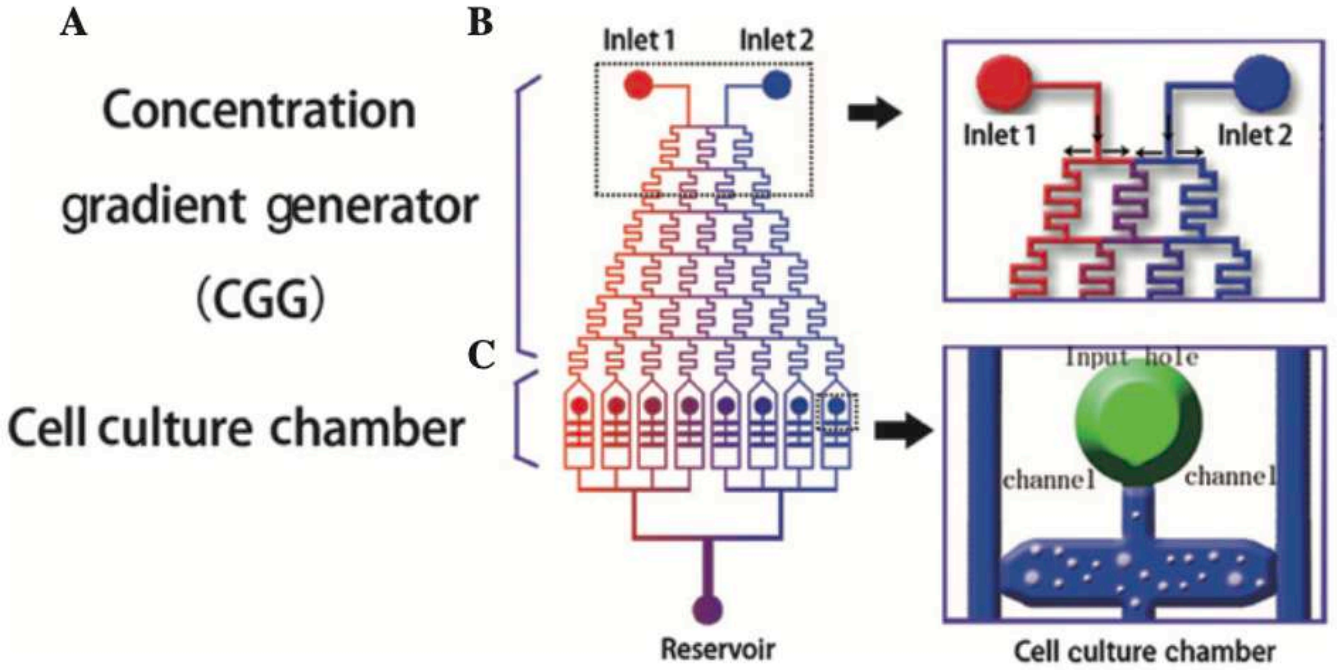
Long-term single-cell time-lapse microscopy - by type





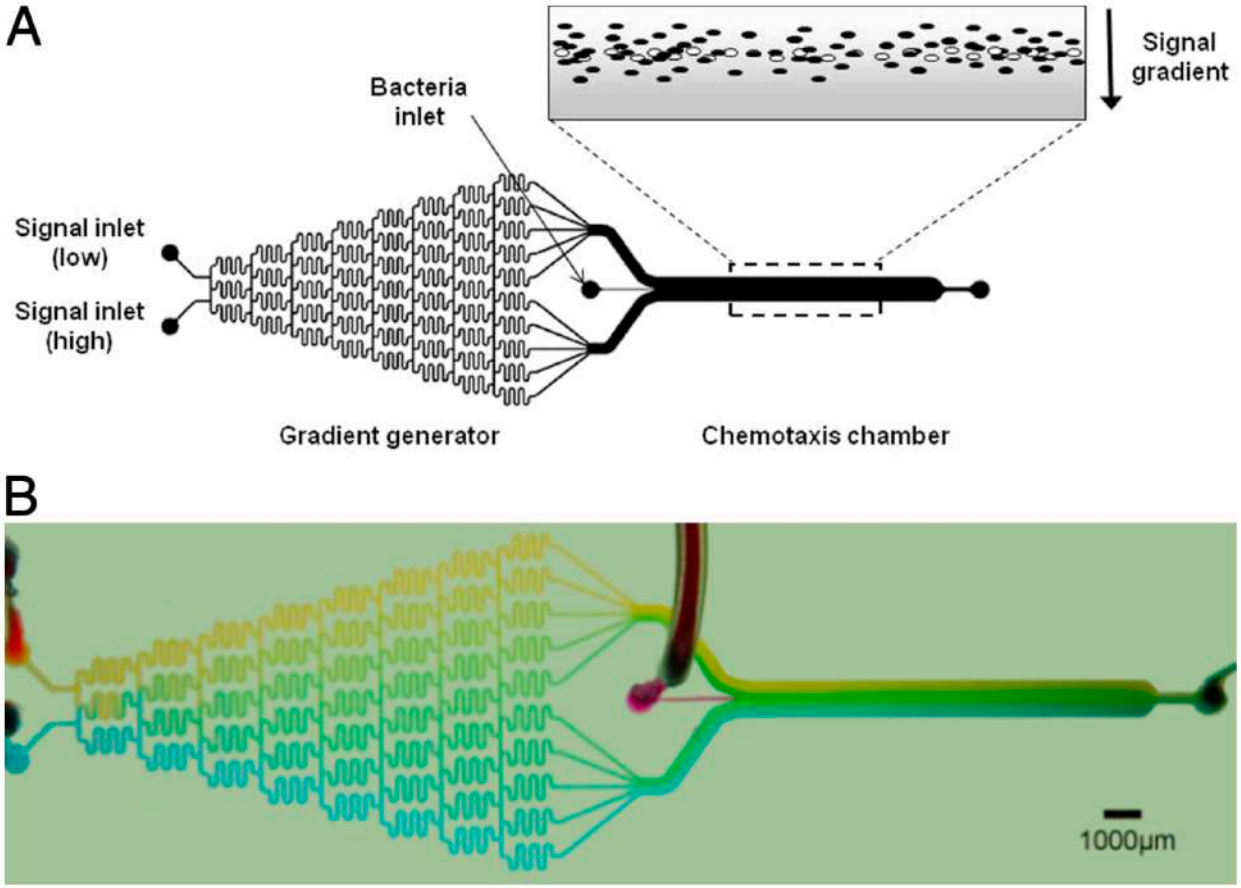
Allard P, Papazotos F and Potvin-Trottier L (2022), Micro uidics for long-term single-cell time-lapse microscopy: Advances and applications. Front. Bioeng. Biotechnol. 10:968342. doi: 10.3389/fbioe.2022.968342

Gradients: Concentration testing in growth chambers



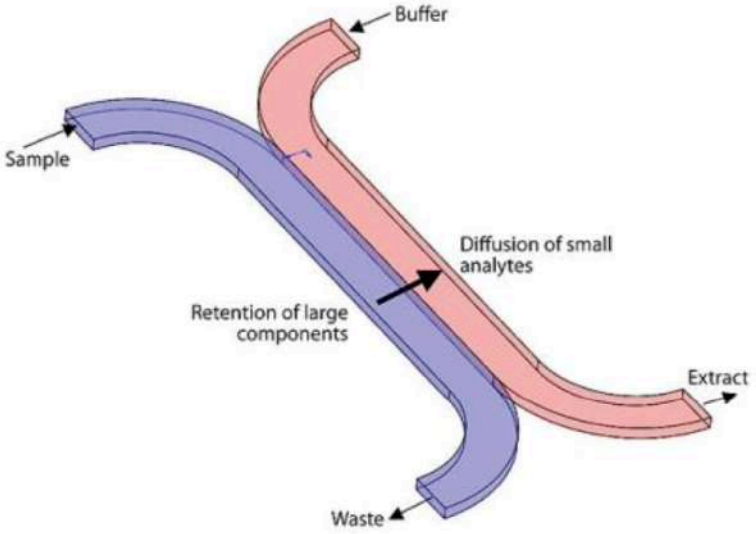
Yin, Bao-Sheng, et al. "An integrated microfluidic device for screening the effective concentration of locally applied tacrolimus for peripheral nerve regeneration." *Experimental and therapeutic medicine* 9.1 (2015): 154-158.

Gradients: Quantifying Bacterial Chemotaxis in Competing Gradients



Englert DL, Manson MD, Jayaraman A. Flow-based microfluidic device for quantifying bacterial chemotaxis in stable, competing gradients. Appl Environ Microbiol. 2009 Jul;75(13):4557-64. doi: 10.1128/AEM.02952-08.

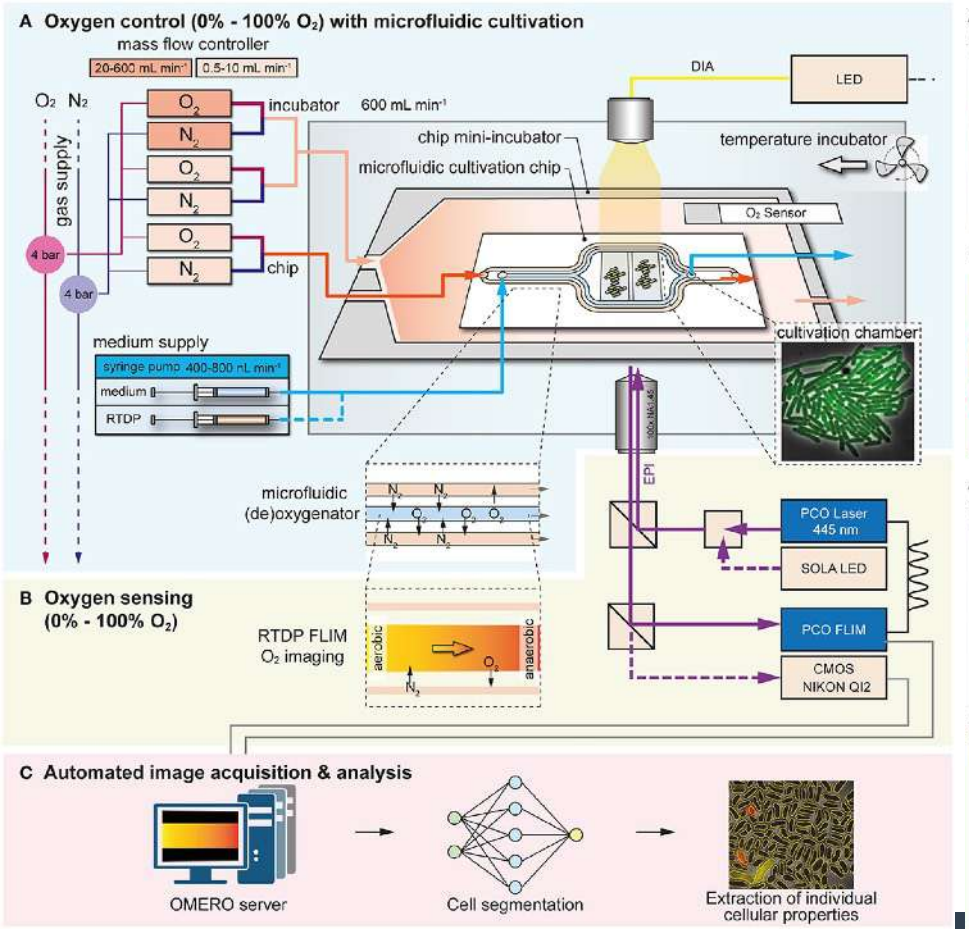
Diffusion between laminar flows - organs on a chip

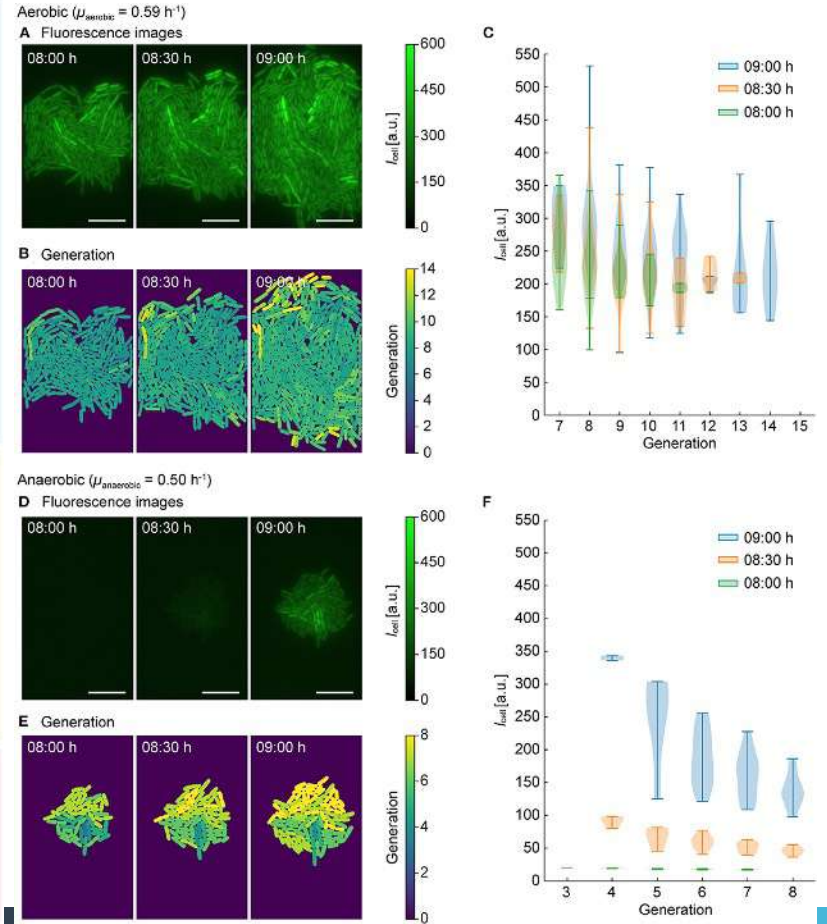


Right: P. Yager et al. *(2006). Nature 442: 412-18.*

Left: P.J.A. Kenis et al. *(1999). Science 285: 83-5.*

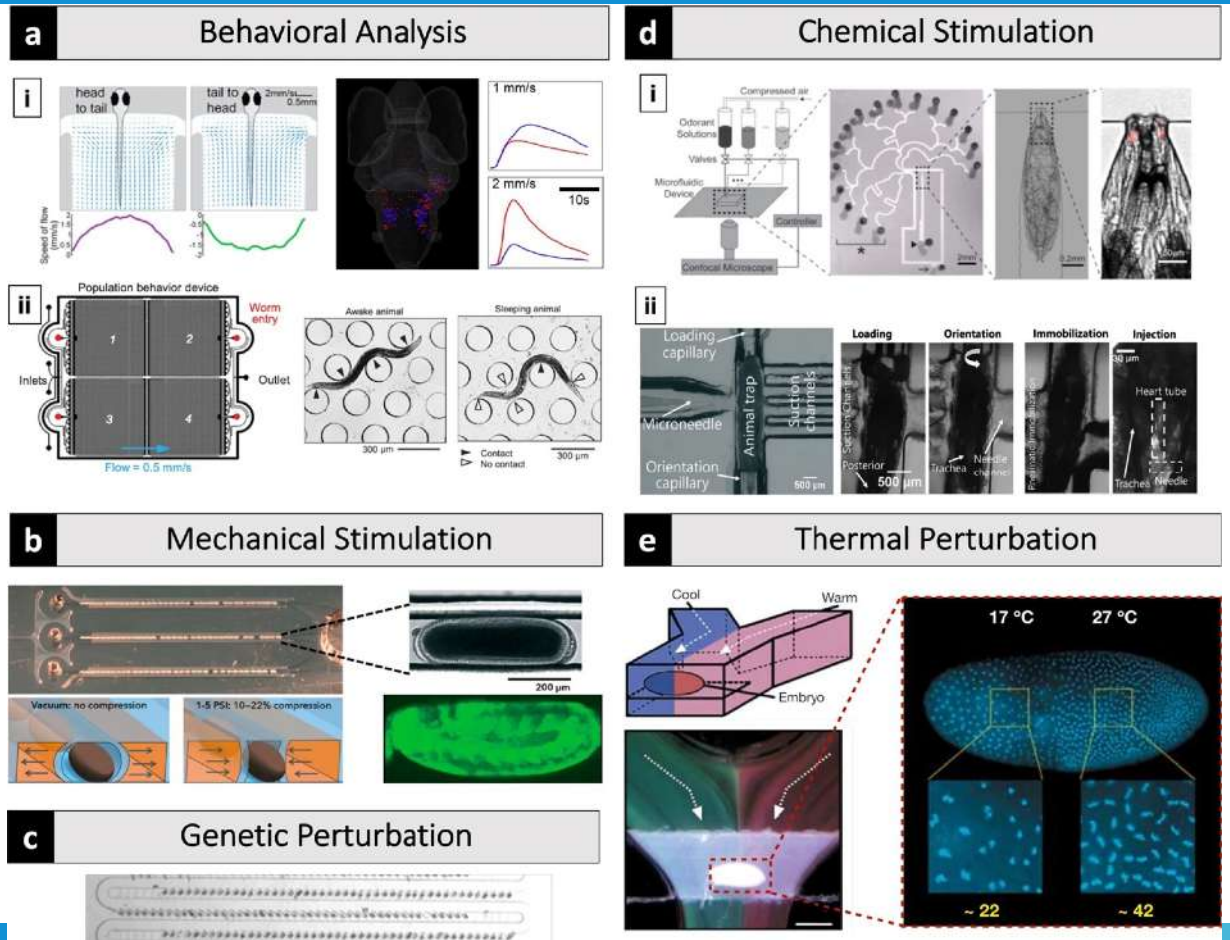
Gas control





Kasahara, Keitaro, et al. "Enabling oxygen-controlled microfluidic cultures for spatiotemporal microbial single-cell analysis." *Frontiers in microbiology* 14 (2023): 1198170.

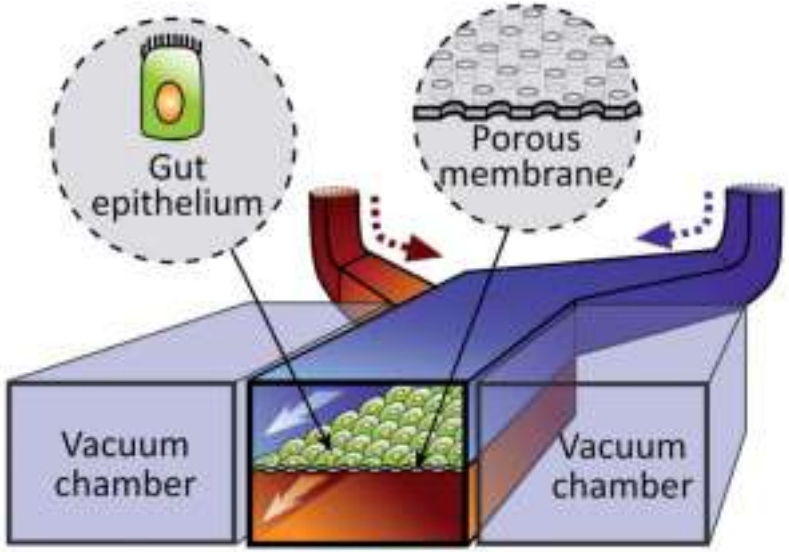
Microfluidics for precise manipulation of model organisms



Frey, N., Sönmez, U.M., Minden, J. *et al.* Microfluidics for understanding model organisms. *Nat Commun* **13**, 3195 (2022). https://doi.org/10.1038/s41467-022-30814-6

Gut-on-a-Chip

- Studies of microbiome-host interactions without mice
- Greater control over sample space, time, and flexibility



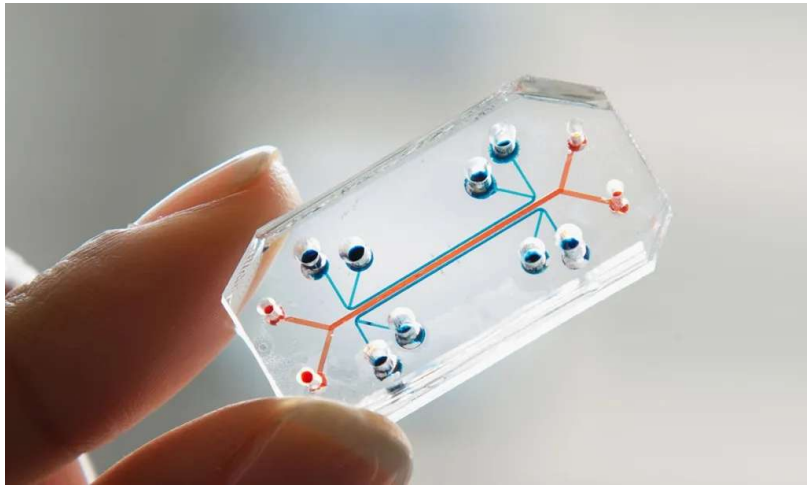
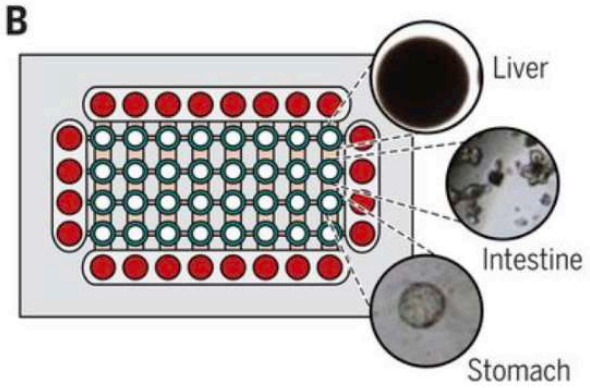
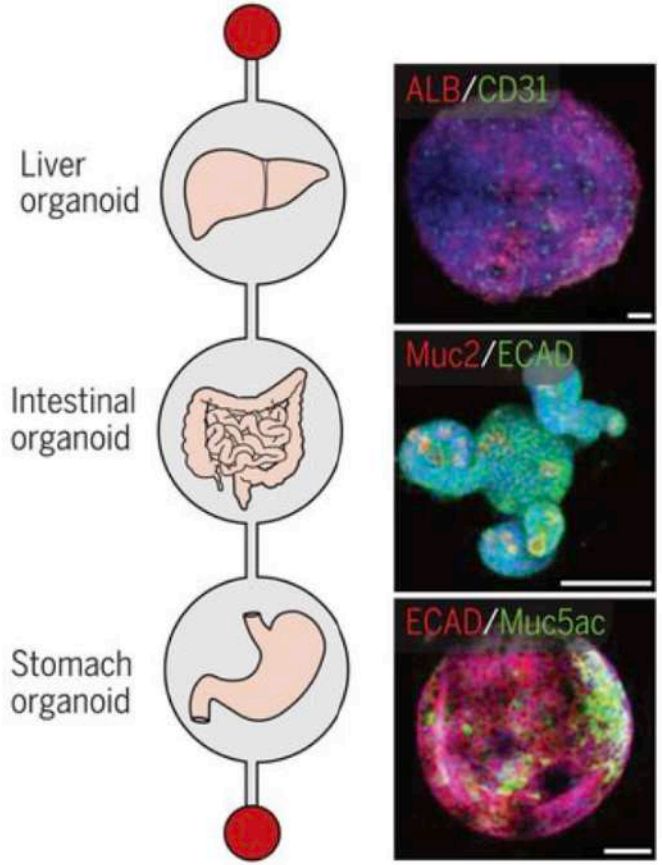


Image credit: Hyun Jung Kim, Dongeun Huh, Geraldine Hamilton and Donald E. Ingber, human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow, 2012

Organoids-on-a-chip: Modeling organ–organ interactions





Jin, Yoonhee, et al. "Vascularized liver organoids generated using induced hepatic tissue and dynamic liver-specific microenvironment as a drug testing platform." *Advanced Functional Materials* 28.37 (2018): 1801954

Trap chips

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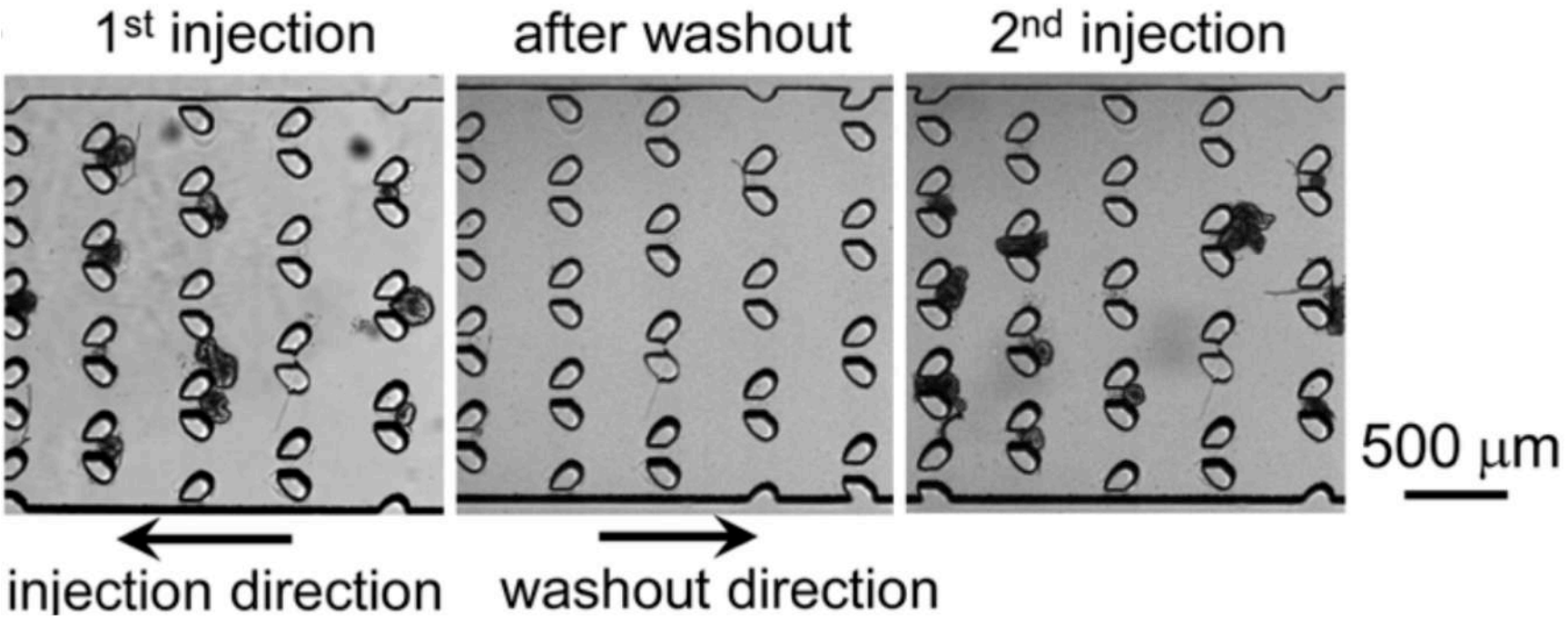
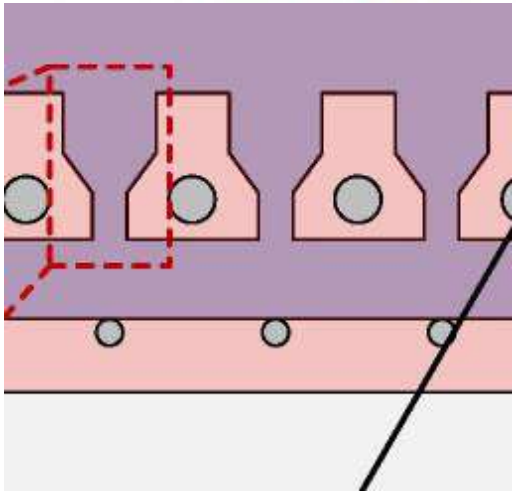
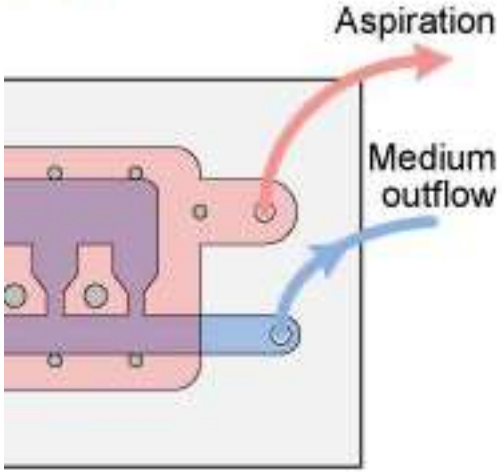
Cultivation control in chips with traps and flow



Jin, Byung-Ju, et al. "Microfluidics platform for measurement of volume changes in immobilized intestinal enteroids." *Biomicrofluidics* 8.2 (2014)

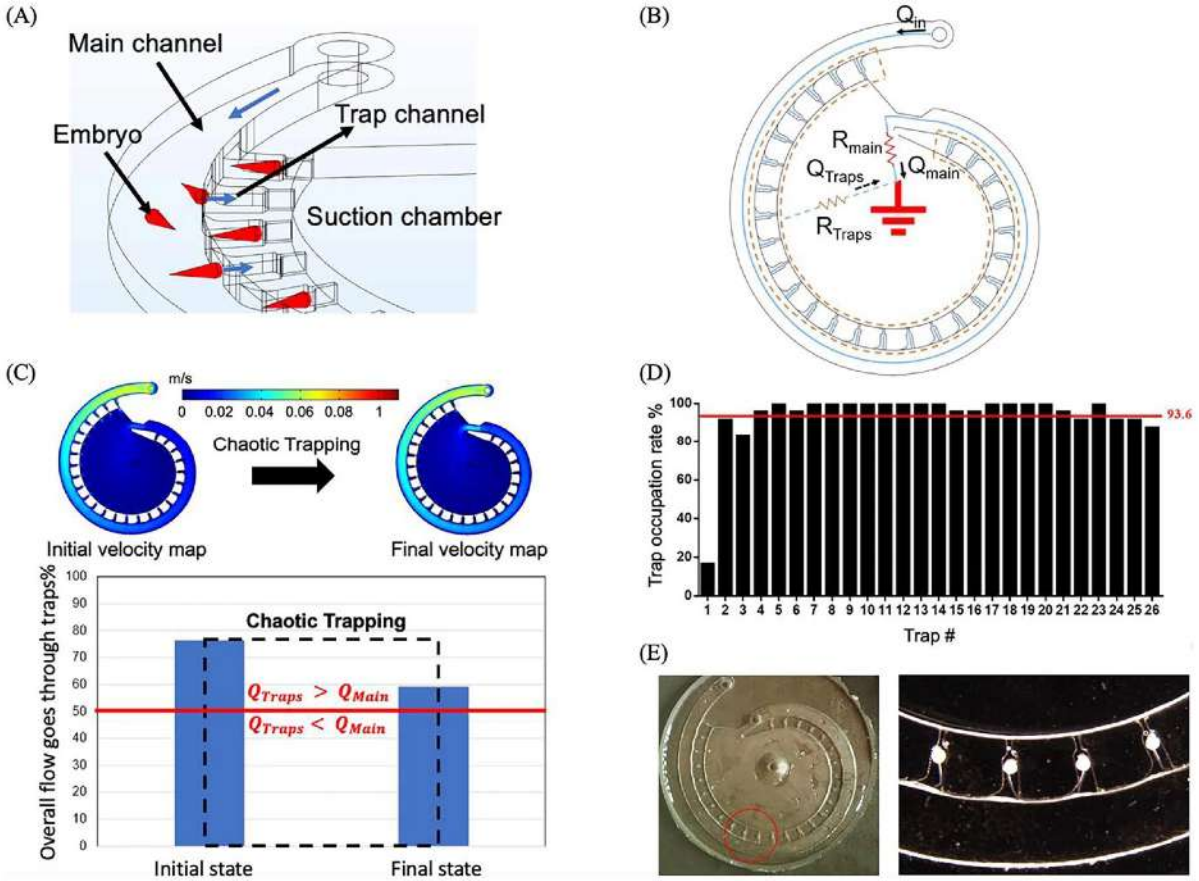
A second layer for bubble trapping (zebrafish embryos)





Zhu, Z., et al. "A Bubble-Free Microfluidic Device for Easy-to-Operate Immobilization, Culturing and Monitoring of Zebrafish Embryos. Micromachines 10 (3), 168 (2019)." 2019,

Zebrafish trapping for antibody staining cycles



Ye, Songtao, Wei-Chun Chin, and Chih-Wen Ni. "A multi-depth spiral milli fluidic device for whole mount zebrafish antibody staining." *Biomedical Microdevices* 25.3 (2023): 30.

Plant root interface imaging

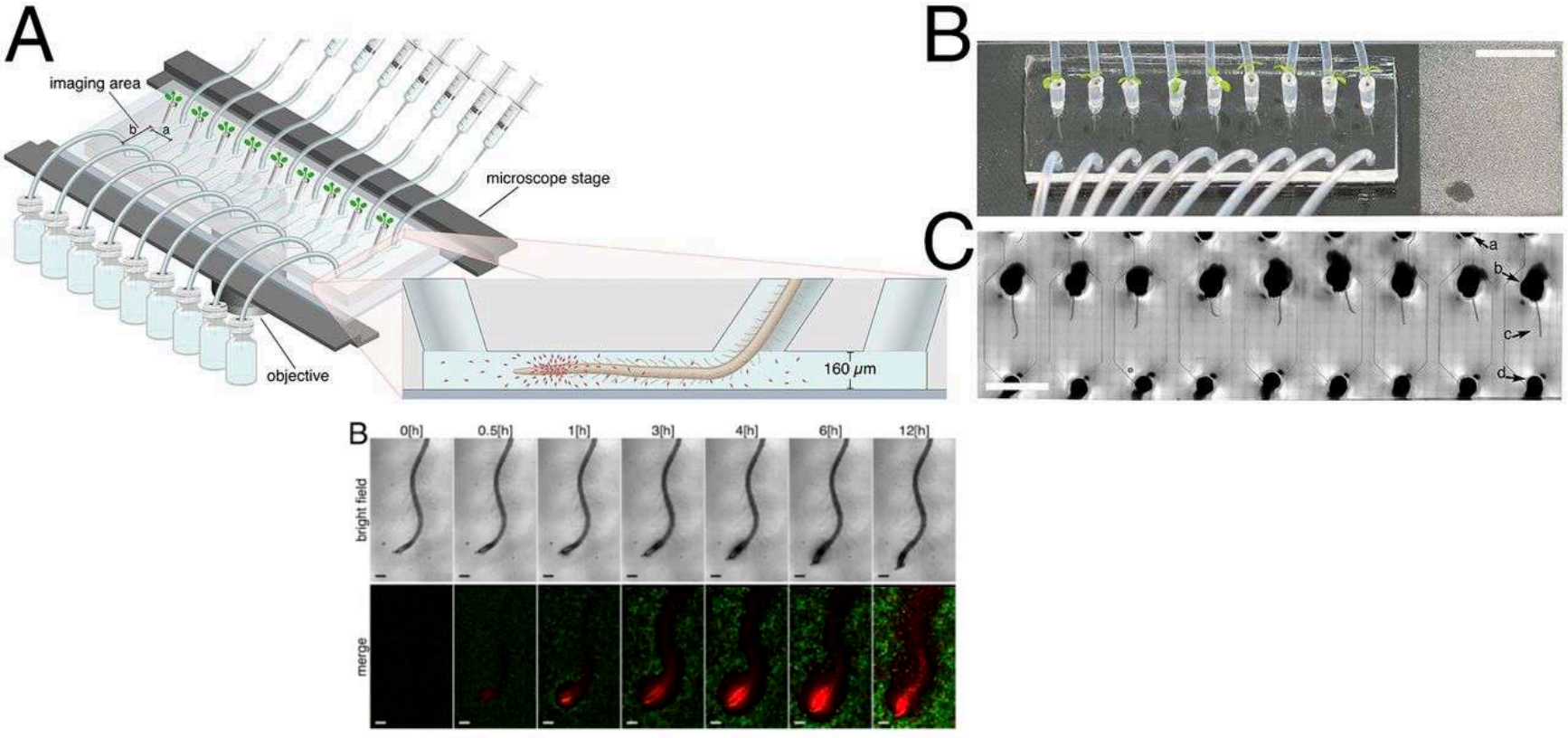
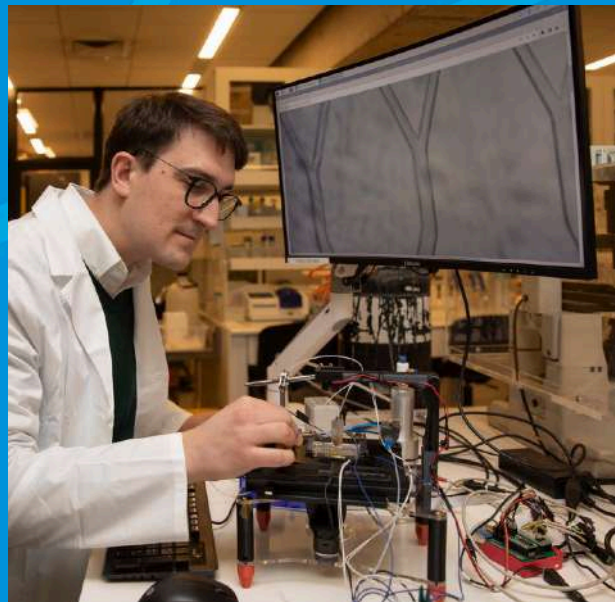


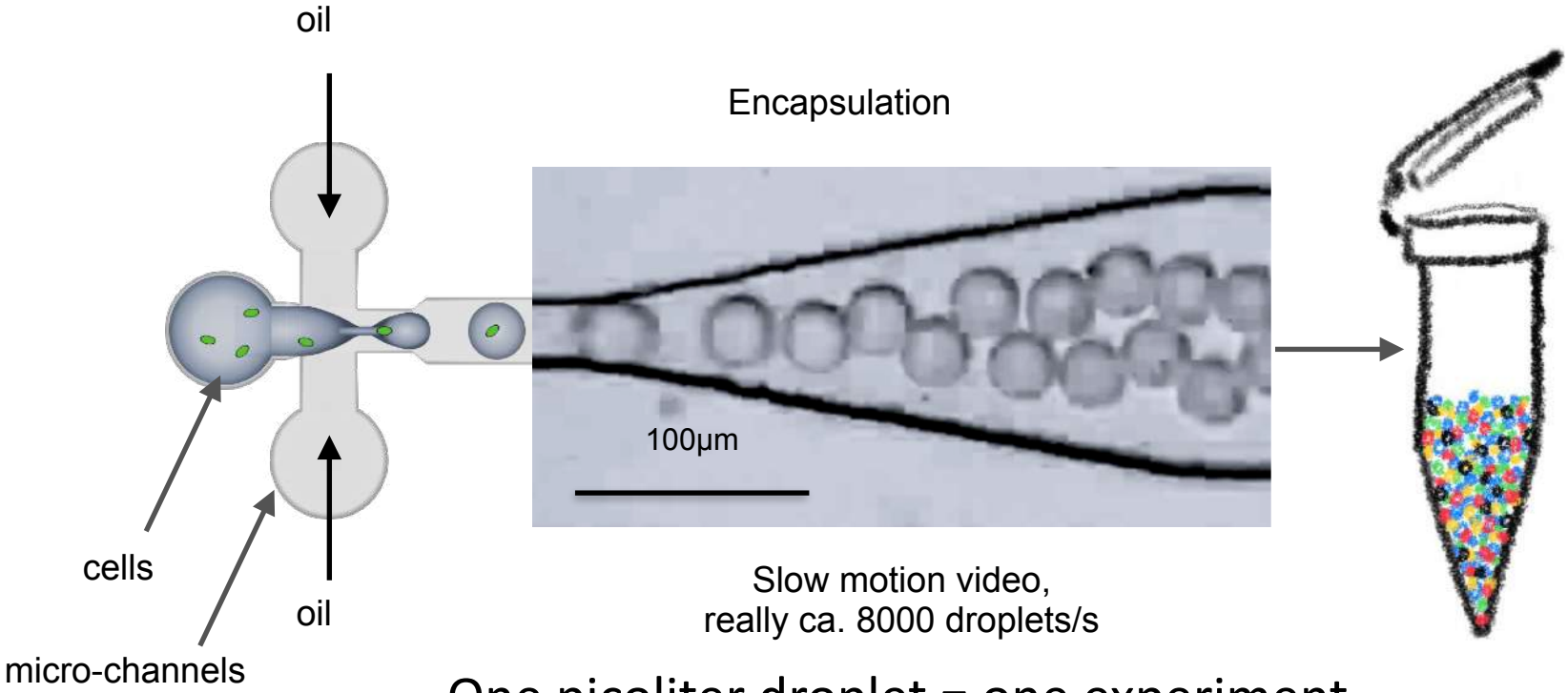
Fig. 4. Bacterial competition for root surface colonization. Real-time im-

Massalha, Hassan, et al. "Live imaging of root–bacteria interactions in a microfluidics setup." *Proceedings of the National Academy of Sciences* 114.17 (2017): 4549-4554.

Droplet-Based High-Throughput Assays



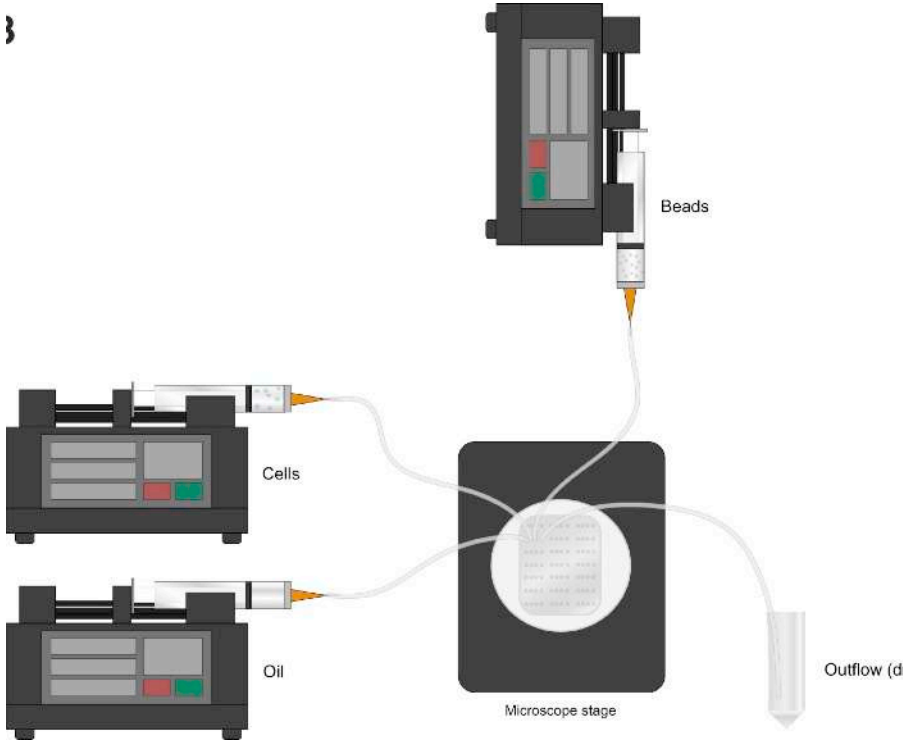
Microfluidic droplets - record-keeping high-throughput

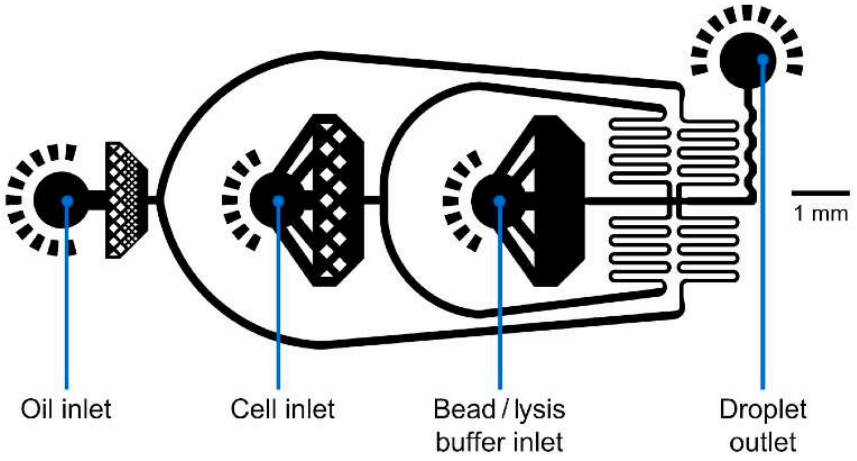


One picoliter droplet = one experiment

millions per hour

Running a microfluidic experiment (DropSeq)





Cell 2015 1611202-1214DOI: (10.1016/j.cell.2015.05.002)

Droplet Microfluidics

- Ultra-high throughput
- Single cell control
- Less contamination
- Versatile
- Reagent efficient

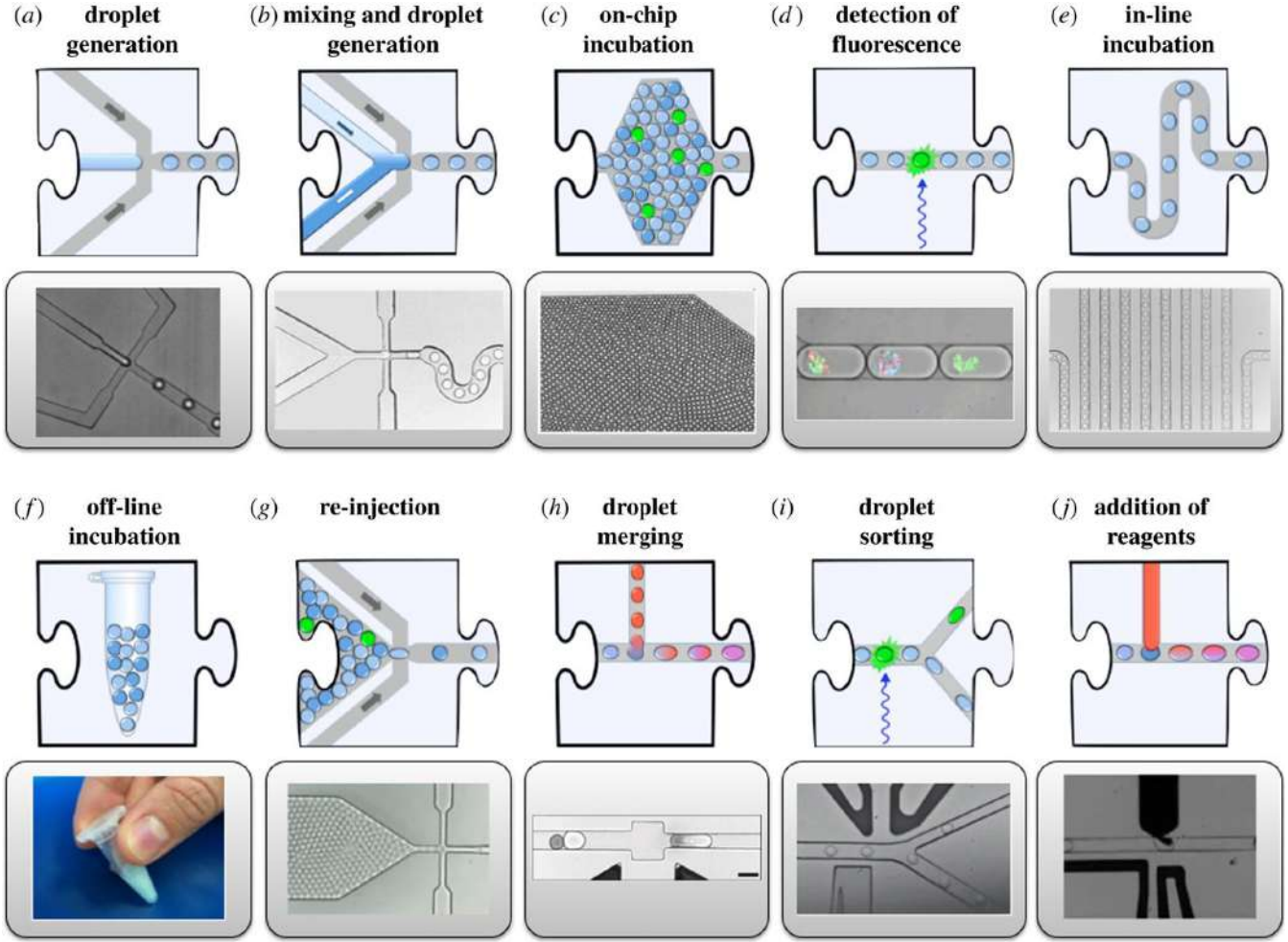


Image source: Liisa D. van Vliet et al. Interface Focus 2015

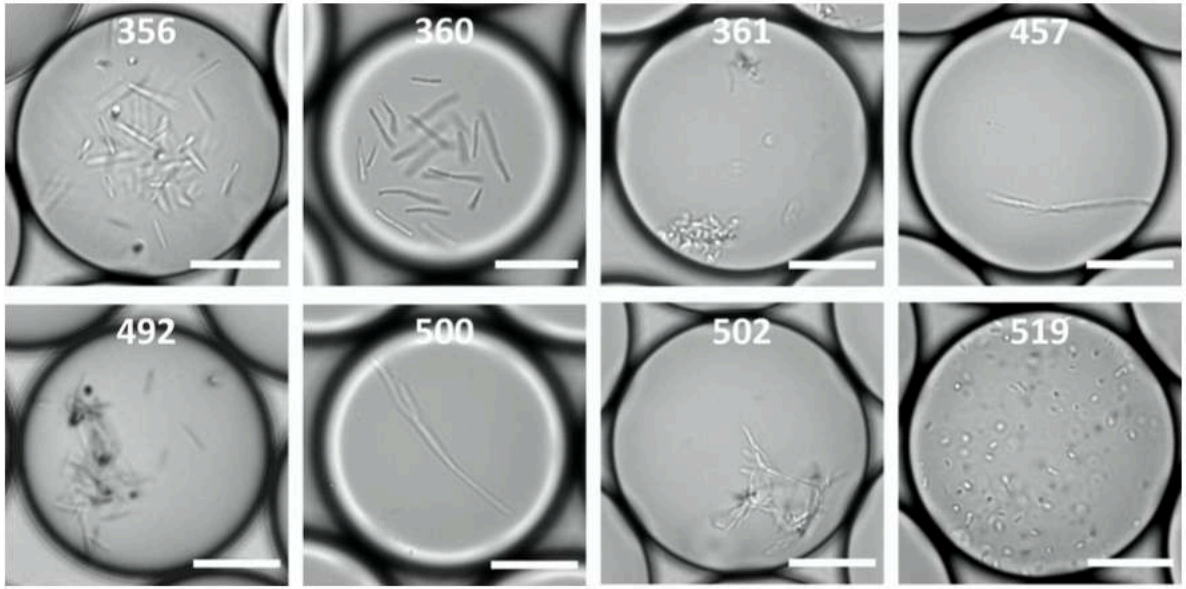
Microbial culture-omics

Droplet-based high-throughput cultivation for accurate screening of antibiotic resistant gut microbes

William J Watterson[1,2*], Melikhan Tanyeri[1,2,3], Andrea R Watson[4], Candace M Cham[4], Yue Shan[4], Eugene B Chang[4], A Murat Eren[4,5,6*], Savas Tay[1,2*]

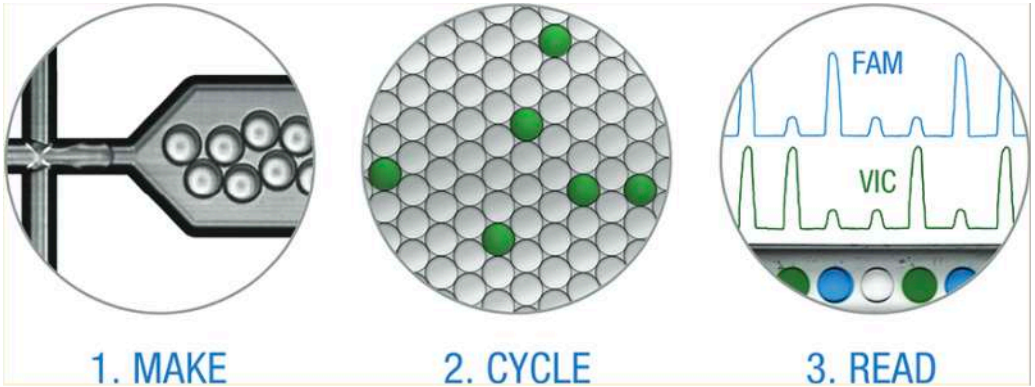
eLife

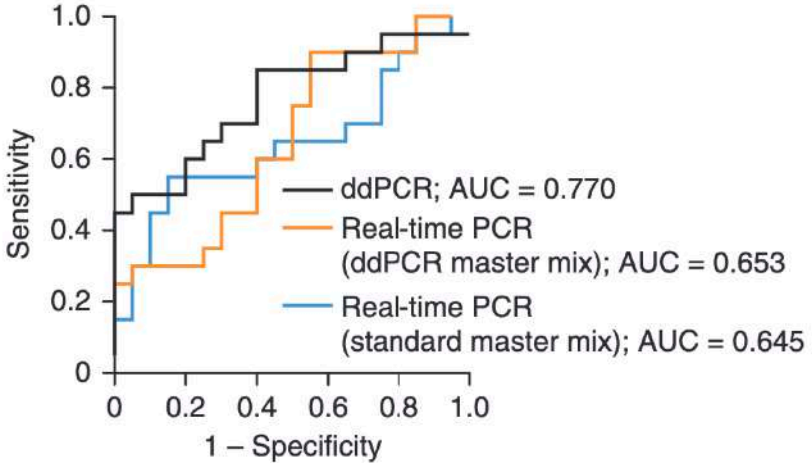
2020



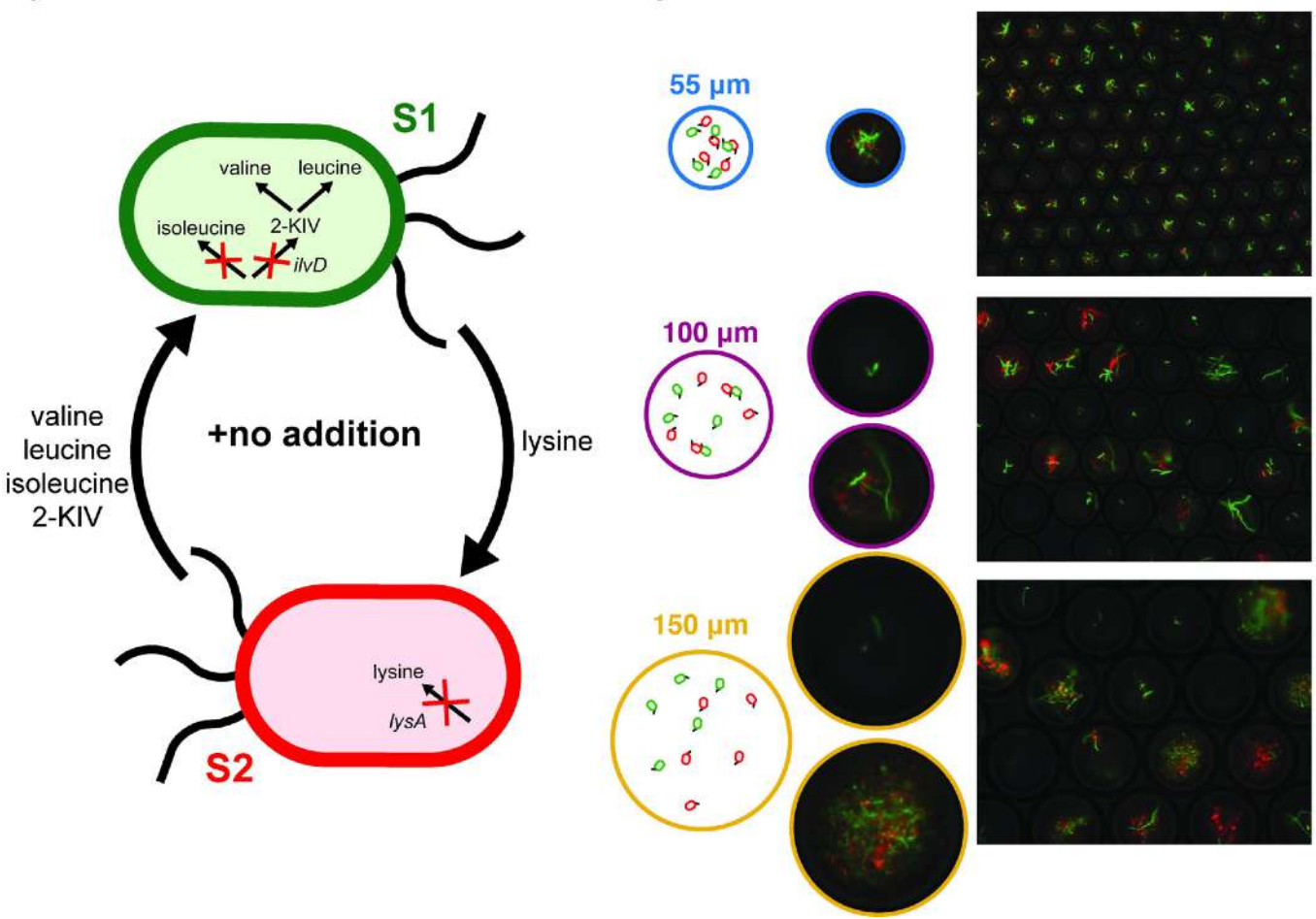
Droplet digital PCR (ddPCR)

Applications: absolute abundance, viral load, genome copy number, etc.





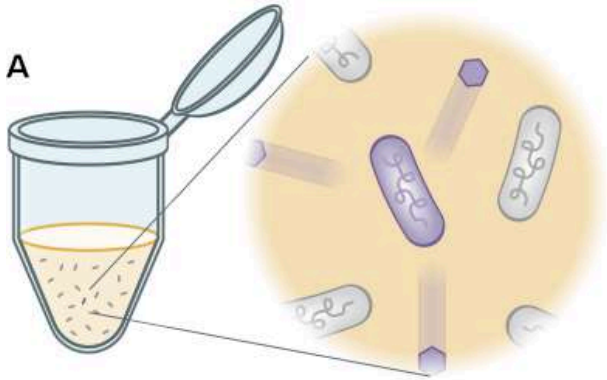
- Li, H. *et al.* Application of droplet digital PCR to detect the pathogens of infectious diseases. *Bioscience Rep* **38**, BSR20181170 (2018).
- Hindson, B. J. *et al.* High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number. *Anal Chem* **83**, 8604–8610 (2011).
- Martinez-Hernandez, F. *et al.* Droplet Digital PCR for Estimating Absolute Abundances of Widespread Pelagibacter Viruses. *Front Microbiol* **10**, 1226 (2019).
- Hindson, C. M. *et al.* Absolute quantification by droplet digital PCR versus analog real-time PCR. *Nat Methods* **10**, 1003–1005 (2013).
- Yang, R., Paparini, A., Monis, P. & Ryan, U. Comparison of next-generation droplet digital PCR (ddPCR) with quantitative PCR (qPCR) for enumeration of Cryptosporidium oocysts in faecal samples. *Int J Parasitol* **44**, 1105–1113 (2014)

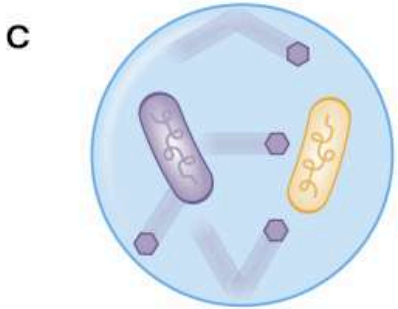


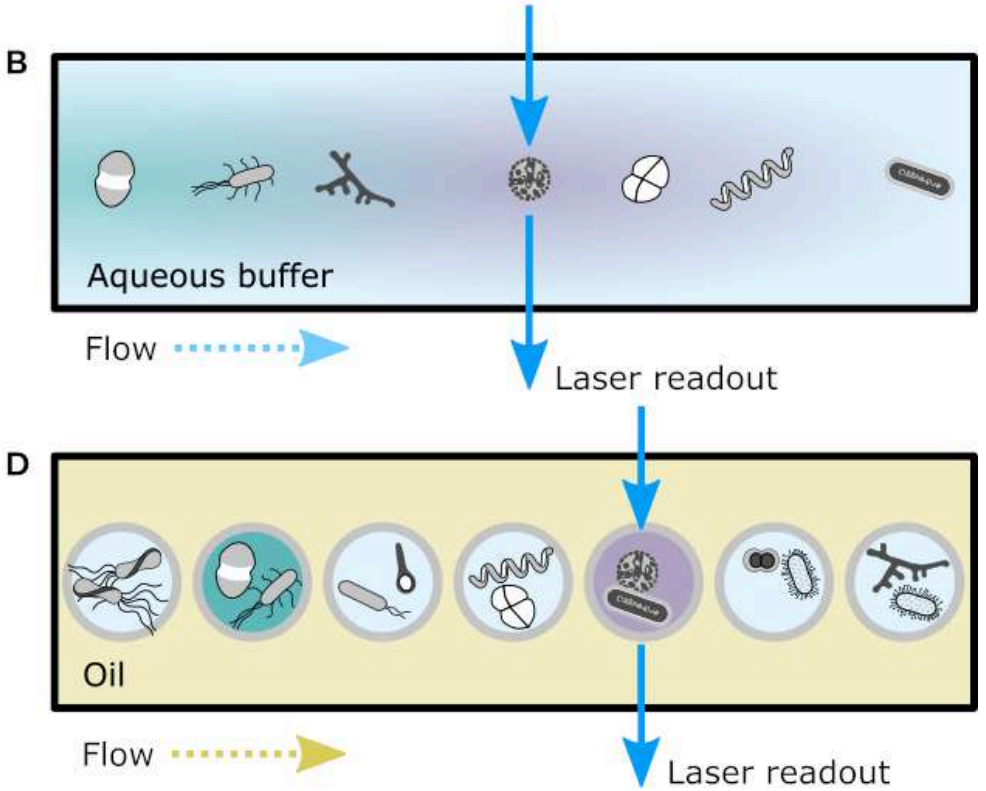
With the highest degree of interaction, syntrophic growth can be substantially hindered in larger droplets to the extent where a significant subpopulation fails to establish co-growth .

Tan JY, Saleski TE, Lin XN. The effect of droplet size on syntrophic dynamics in droplet-enabled microbial co-cultivation. PLoS One. 2022 Mar 31;17(3):e0266282. doi: 10.1371/journal.pone.0266282. PMID: 35358282; PMCID: PMC8970485.

Interactions are best studied in droplets!

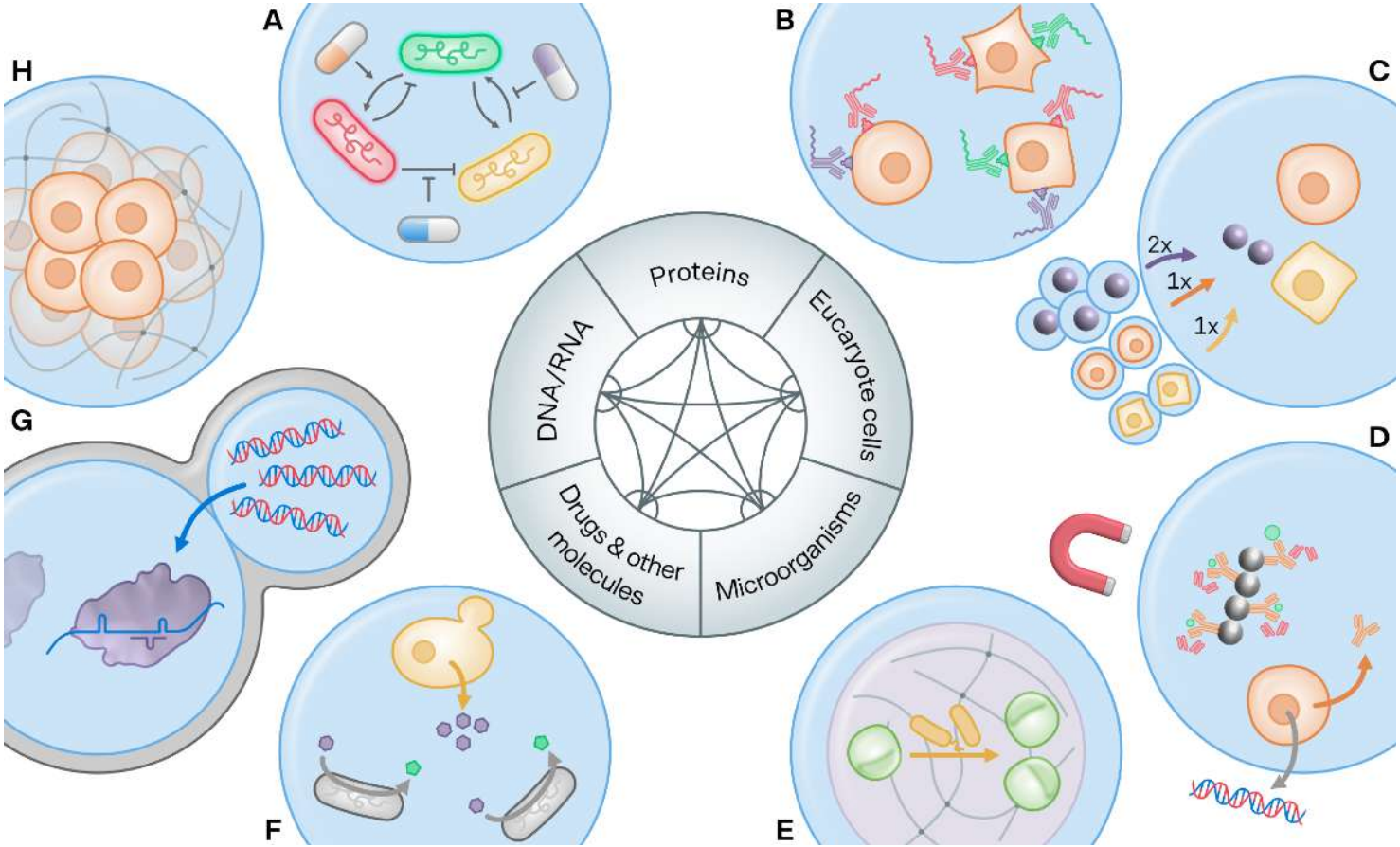






Variation of: Leveraging interactions in microfluidic droplets for enhanced biotechnology screens
C. Vitalis & T. Wenzel; Current Opinion in Biotechnology, 2023/08

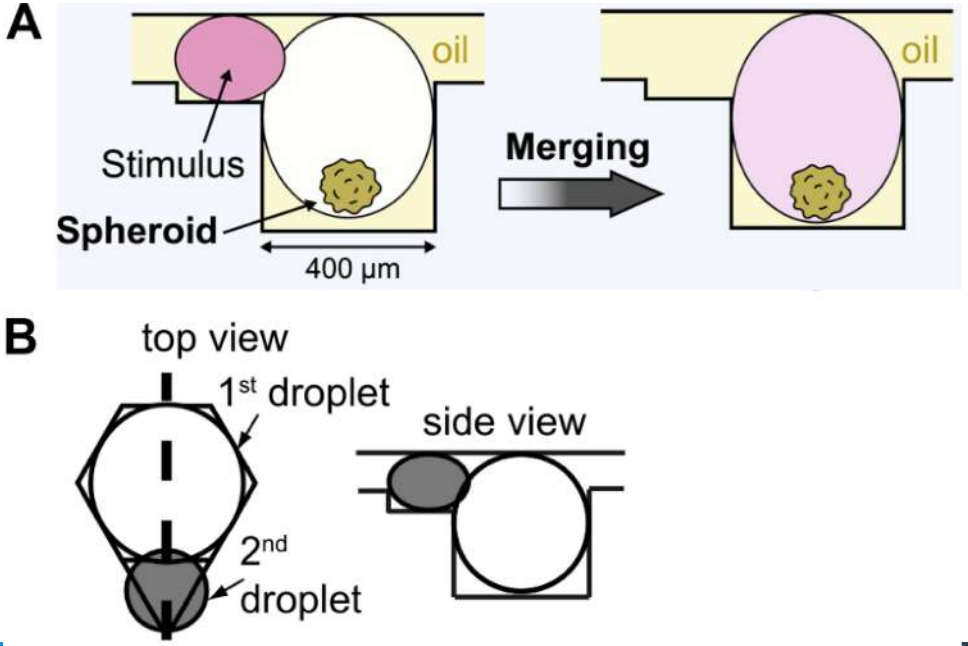
Many interactions can be studied in droplets!

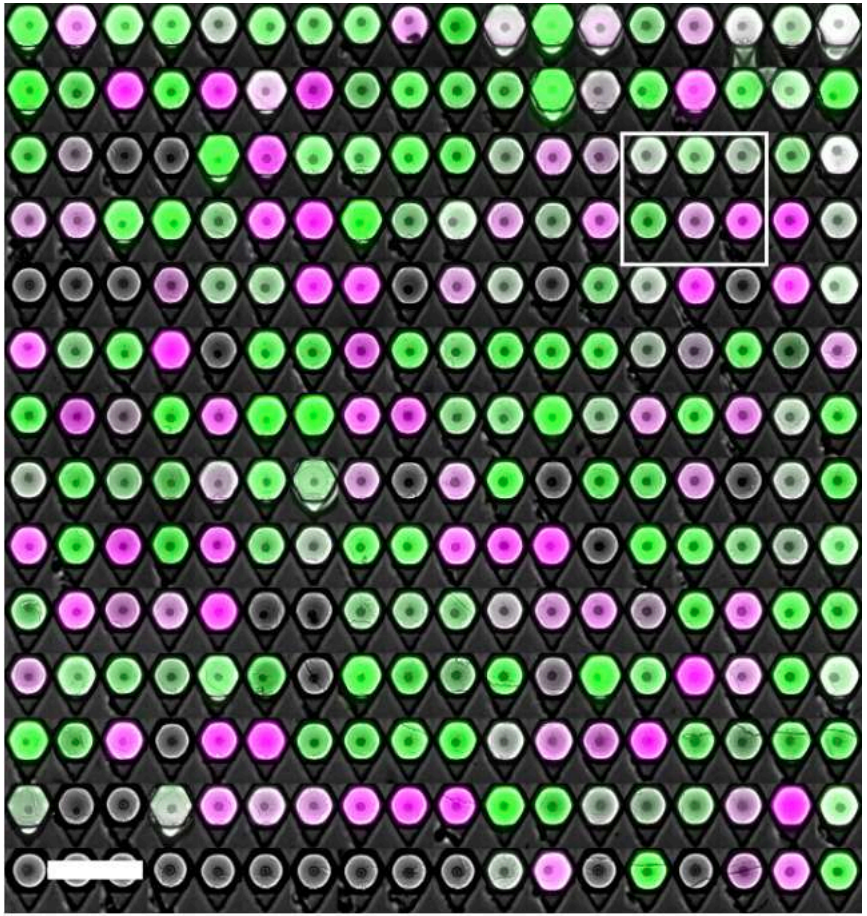


Leveraging interactions in microfluidic droplets for enhanced biotechnology screens
C. Vitalis & T. Wenzel
Current Opinion in Biotechnology, 2023/08

Bioimaging in droplets for organoids and interactions

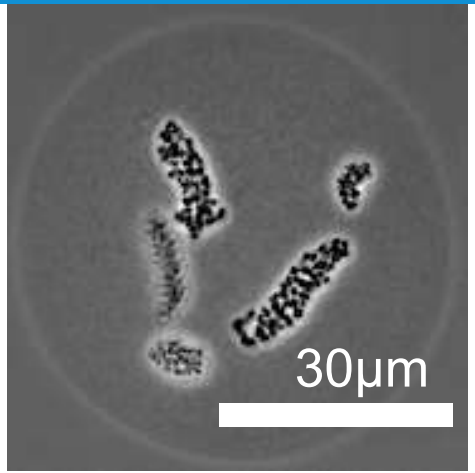
- **High throughput analysis of host-pathogen interactions in droplets**

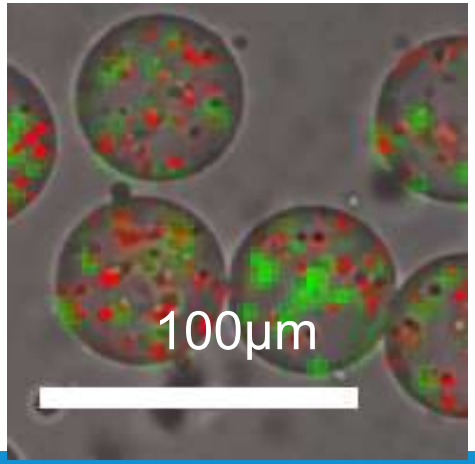


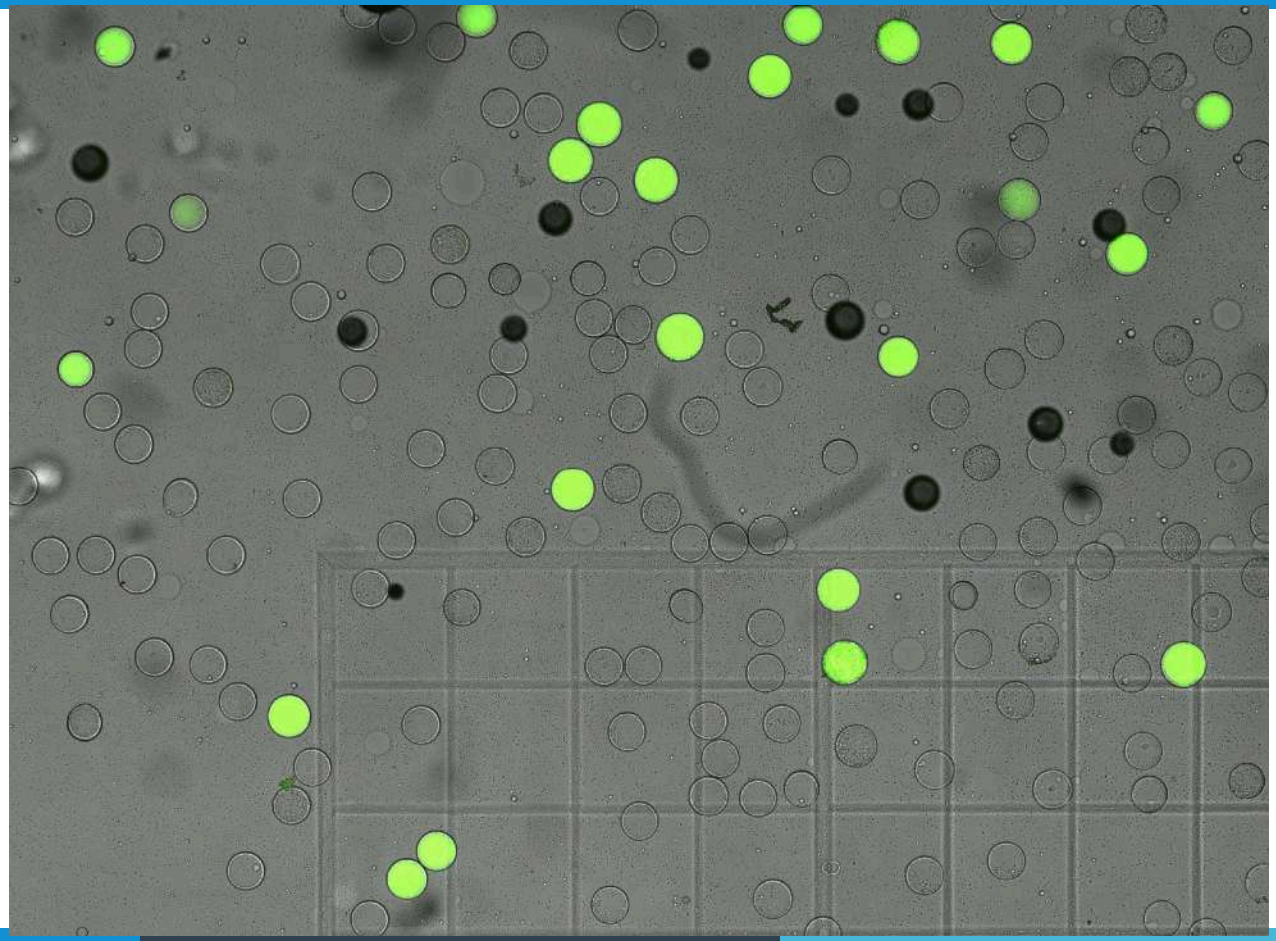


Tomasi et al. (Cell Reports. 2020)

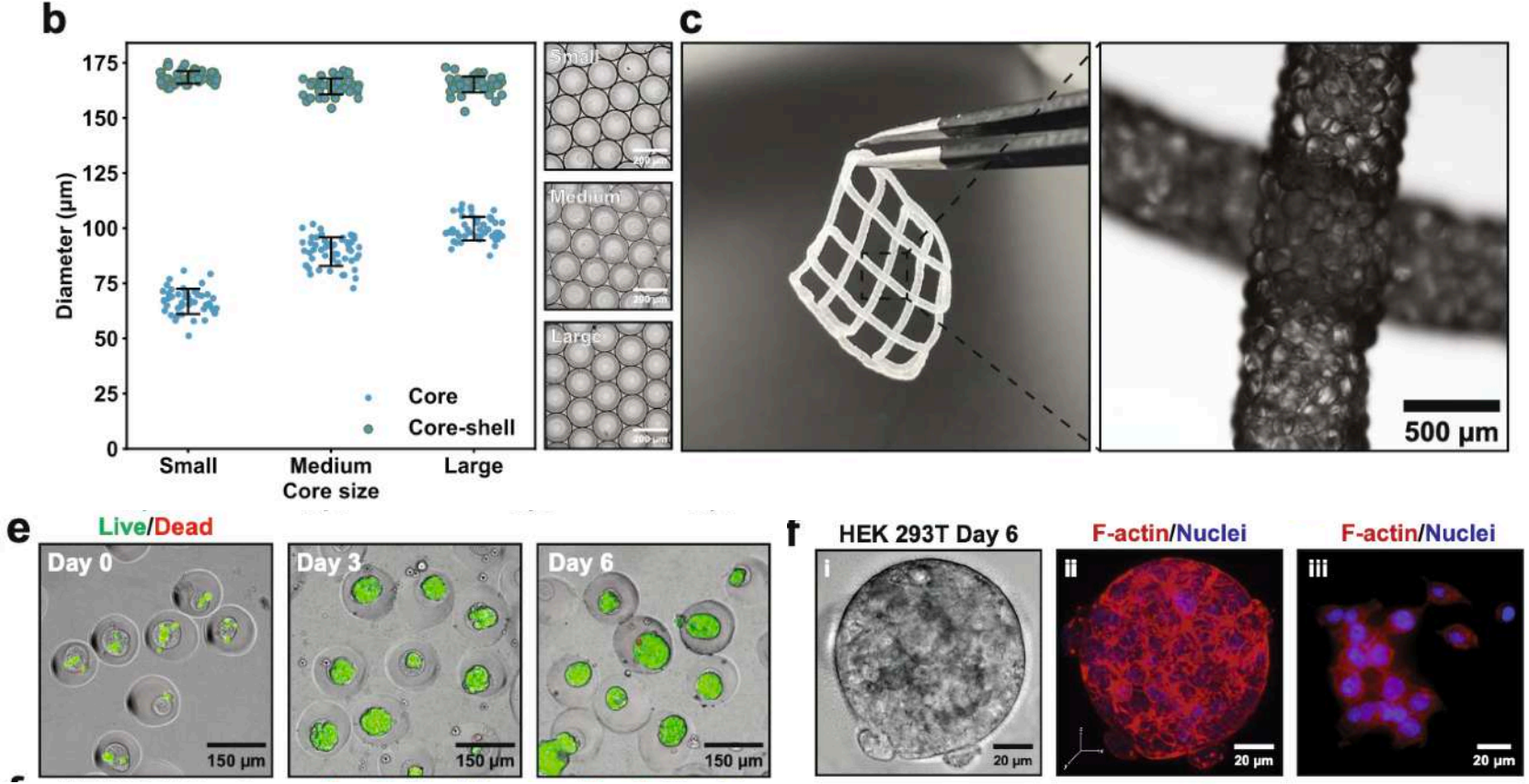
Gel-Microdroplets and Semi-Permeable Capsules







3d-printed cellular interactions

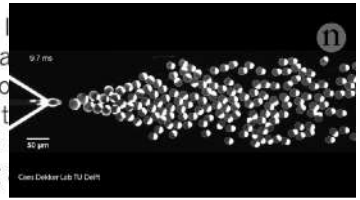


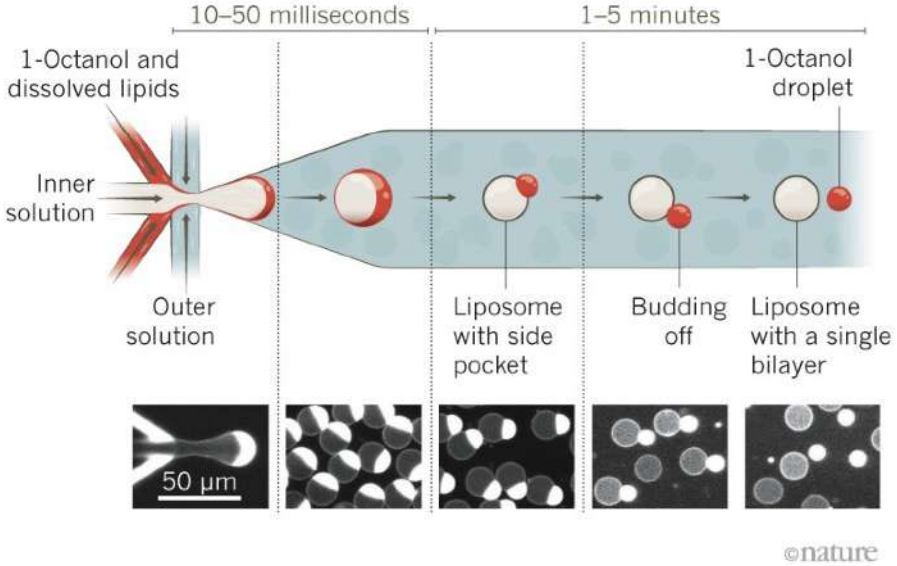
Ou, Y. *et al*. Bioprinting microporous functional living materials from protein-based core-shell microgels. *Nat Commun***14**, 322 (2023).

Assembling liposomes and beyond

THE BUBBLE MACHINES

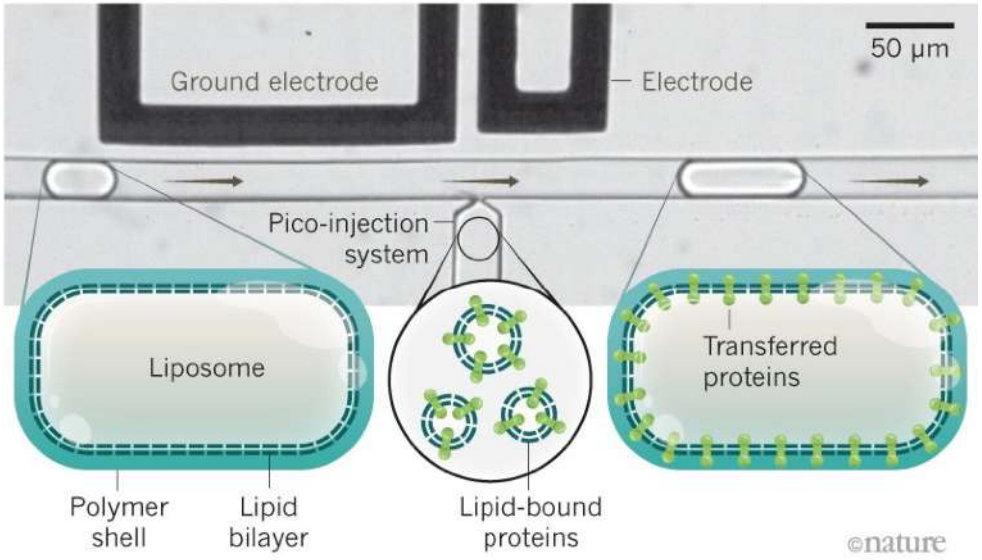
Researchers use microfluidic chips to make l
which are similar to the envelopes that conta
features a six-way junction that can fill liposo
them off. With the fatty alcohol 1-octanol in t
around the inner solution. Over time, excess
one end and spontaneously split off, leaving





THE ASSEMBLY LINES

A pico-injection system allows researchers to load cell-membrane-like compartments called liposomes with functional proteins. Liposomes are stabilized by a polymer coating and pushed through a microfluidic channel. As they pass over a pico-injection site, an electrical pulse can trigger the incorporation of internal proteins or membrane-bound proteins (as shown) into the liposomes.



Weiss, M., Frohnmayer, J., Benk, L. *et al.* Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics. *Nature Mater* **17**, 89–96 (2018). https://doi.org/10.1038/nmat5005

https://doi.org/10.1038/d41586-018-07289-x

Open source tools from our lab

Making microfluidics accessible across Latin America

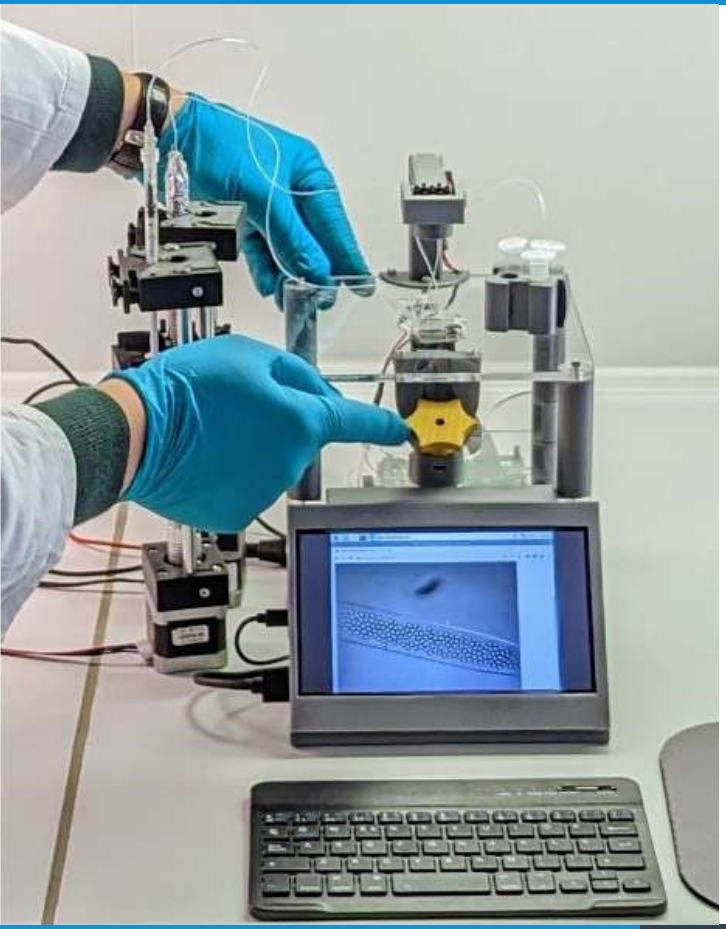
iibm
Instituto de Ingeniería Biológica y Médica

Open Source Syringe Pumps and Controller

- Low-cost
- Precise low-flows (with gearbox)
- Allows manual intervention
- And remote automation

Design files, documentation and acknowledgements: https://wenzel-lab.github.io/syringe-pumps-and-controller/2_syringe-pump.html

Low-Cost Open Hardware Droplet Workstation

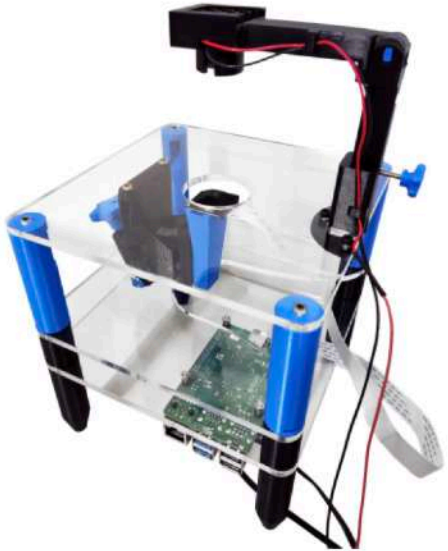


Detailed documentation and open license

Strobe-enhanced microscopy stage

by Pierre Padilla-Huamantinco, Matías Hurtado-Labarca, and Tobias Wenzel
Latin American Hub for Bioimaging Through Open Hardware (LIBRE hub)

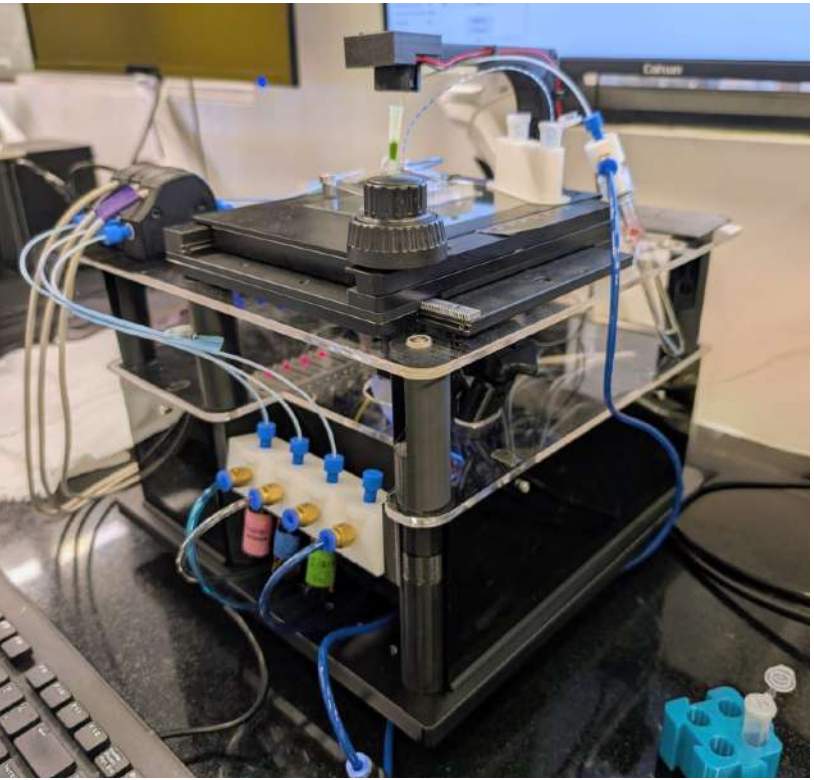
3-level microscopy stage

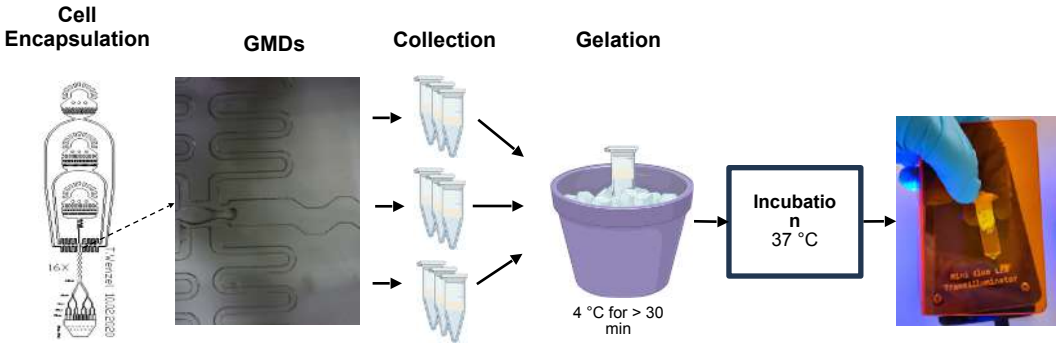


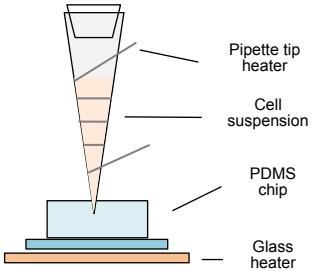
Before you start building the station, you will need to source all the components listed in our bill of materials (HTML, CSV), which is given on the next page.

Instructions

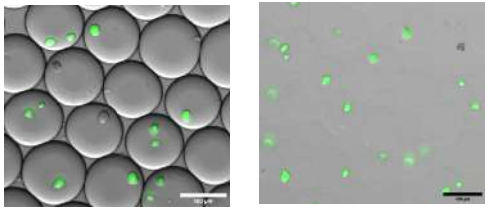
Flow Platform for Advanced Microfluidics and Gel-Microdroplets







sfGFP expressing *E. coli* colonies inside of gel microdroplets (Scaler bar: 100 µm)

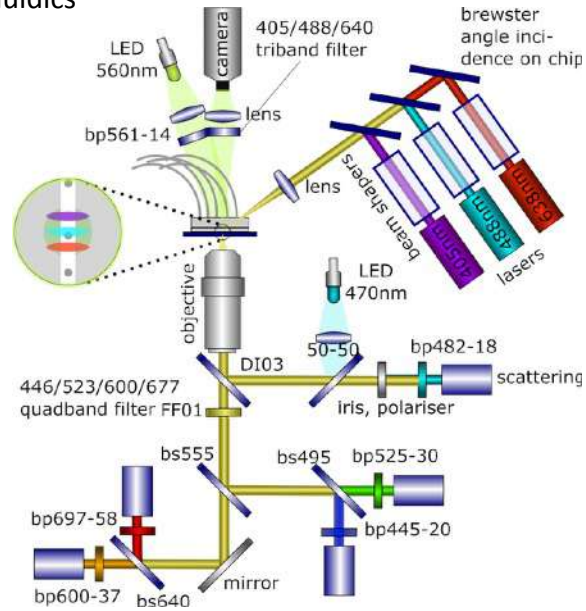


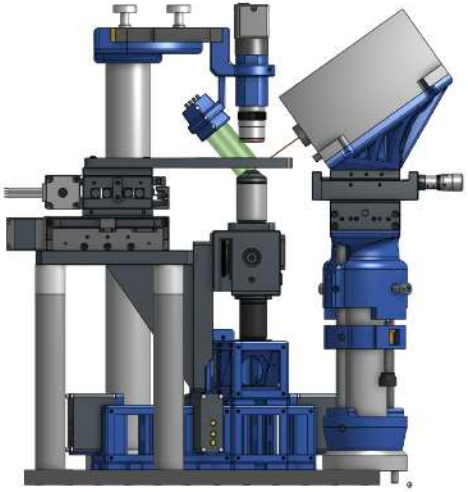
in oil | in buffer

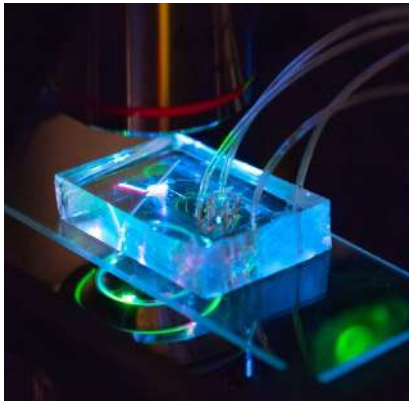
Reference: Padilla-Huamantinco, P., Durán, E., Wenzel, T. Plasmid Stability Analysis with Open-Source Droplet Microfluidics. J. Vis. Exp. (214), e67659, doi:10.3791/67659 (2024).

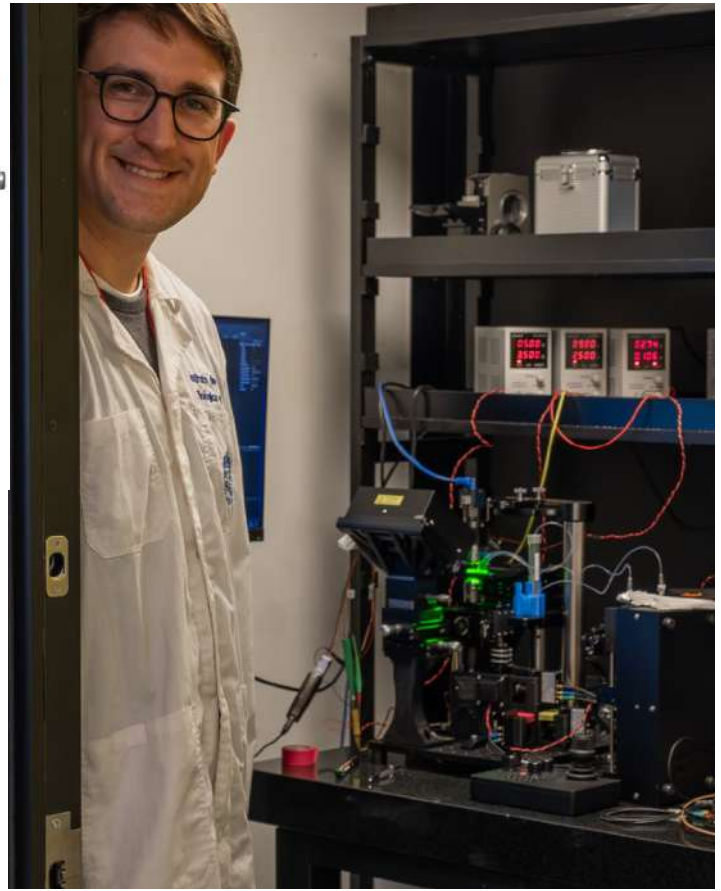
Open source cytometry & droplet sorting (FADS)

- Open microscope alignment stage(s)
- High-speed imaging with strobe
- Realtime FPGA electronics
- High voltage amp and electrodes on-chip
- Optomechanics
- Fluidics









Wenzel Lab

Wenzel Lab at IIBM, Universidad Católica de Chile, check also: https://github.com/LIBREhub

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Popular repositories

droplet-sorter-master — Public

Master repository that documents how the different parts come together of the open source hardware Fluorescence Activated (Microfluidic) Droplet Sorter (FADS).

☆ 14

modular-microfluidics-workstation-controller — Public

Repository for the development of the modular parts of a free and open source microfluidic workstation.

G-code ☆ 9 2

droplet-sorting-FPGA-controller — Public

IT setup for realtime analysis of microfluidic droplets with FPGA board (RedPitaya) based on python pyprl library

Python ☆ 8

syringe-pumps-and-controller — Public

Design of 3d printable syringe pumps and dual controller.

JavaScript ☆ 5 2

high-voltage-pulse-generator — Public

Custom electronics solution for microfluidic droplet sorting pulses

flow-microscopy-platform — Public

Our open hardware flow microscopy platform is a more comprehensive version of our strobe-enhanced microscopy stage design, including the

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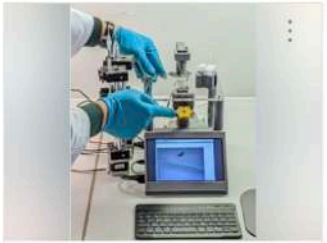
Adaptable Pipette Holders

62 5 261

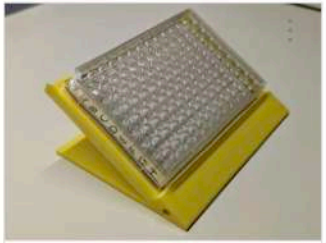


Strobe-enhanced microscopy stage

18 5 41

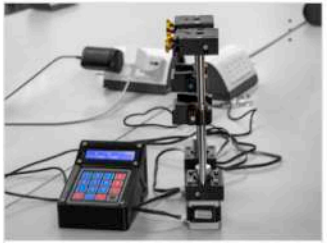
Moldular tube holders

35 5 147



96 well-plate locator stand

62 4.5 351



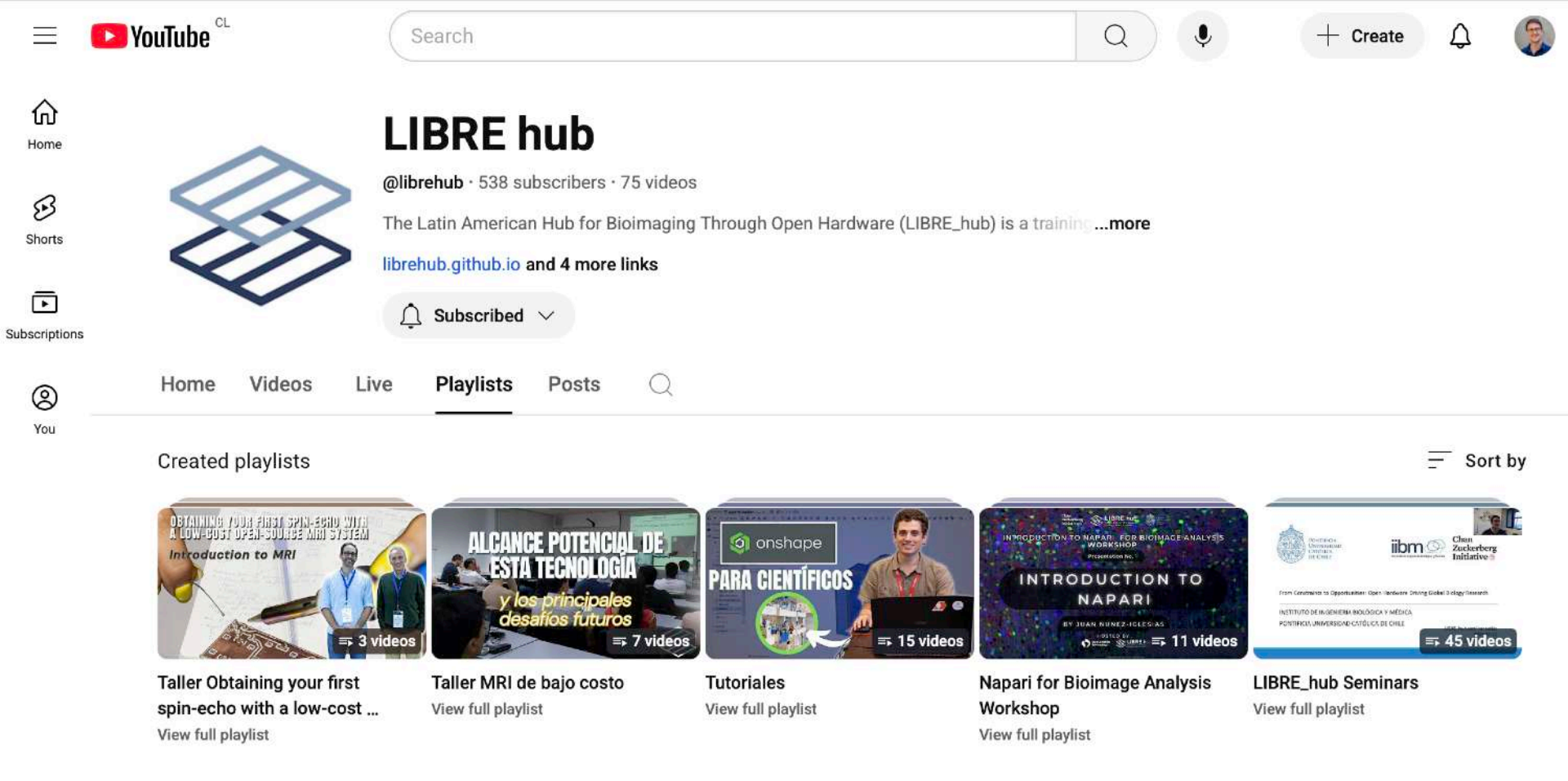
Open-Source Syringe Pumps

71 5 142

Optical filter cubes - openUC2 (improved)

28 0 95

Building a LatAm science maker community



Chan Zuckerberg Initiative

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Instituto de Ingeniería Biológica y Médica

- Tobias Wenzel #OpenHardware
- Pablo Irarrázaval #openMRI
- Vicente Parot #compMicroscopy

Latin American Hub for Bioimaging through Open Hardware (LIBRE hub)