Devices for Biological Systems: On-Chip Horizontal Gene Transfer and 3D-Printed Microfluidic Applications

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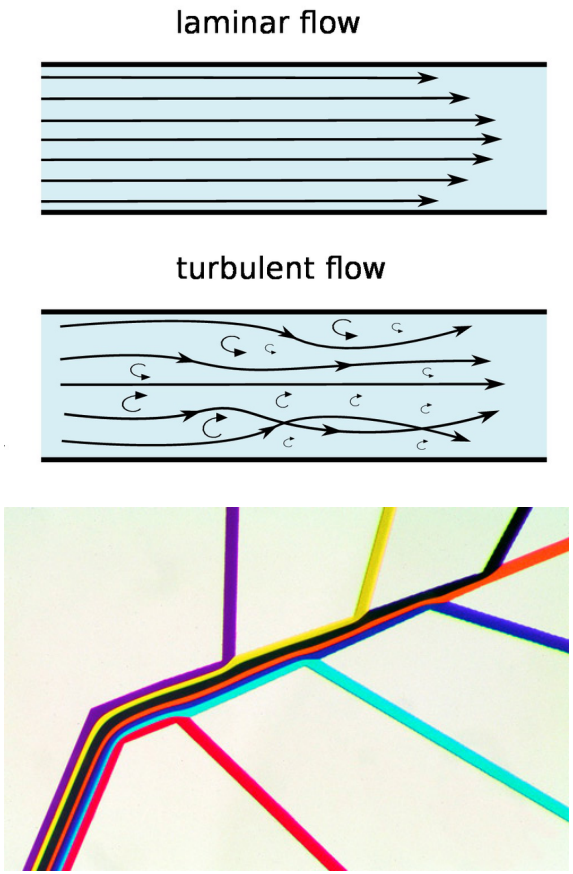
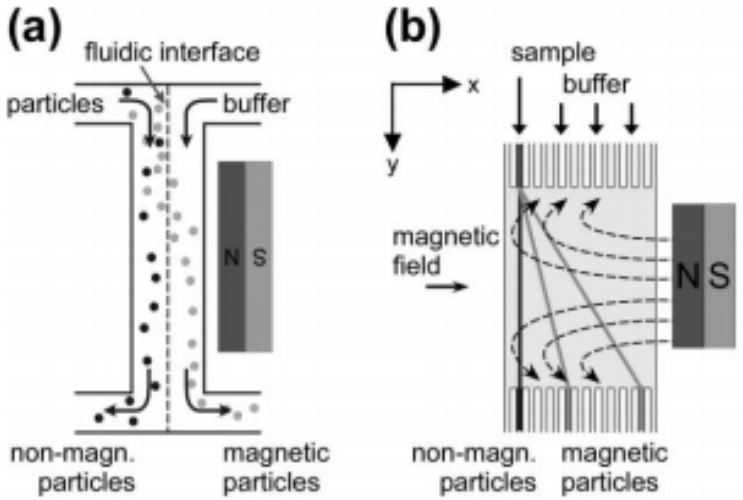
Overview

1. Introduction of Microfluidics and applications to biological research
2. Specific Aim 1 : Demonstrate a microfluidic approach for co-encapsulation of two strains of strep to observe single gene transfer events
3. Specific Aim 2: Develop an easy to use and fabricate oxygen control insert for a 24-well plate
4. Specific Aim 3: Develop a 3D printable micropipette and compare its performance to commercial versions
5. Conclusion and Thanks

Microfluidics

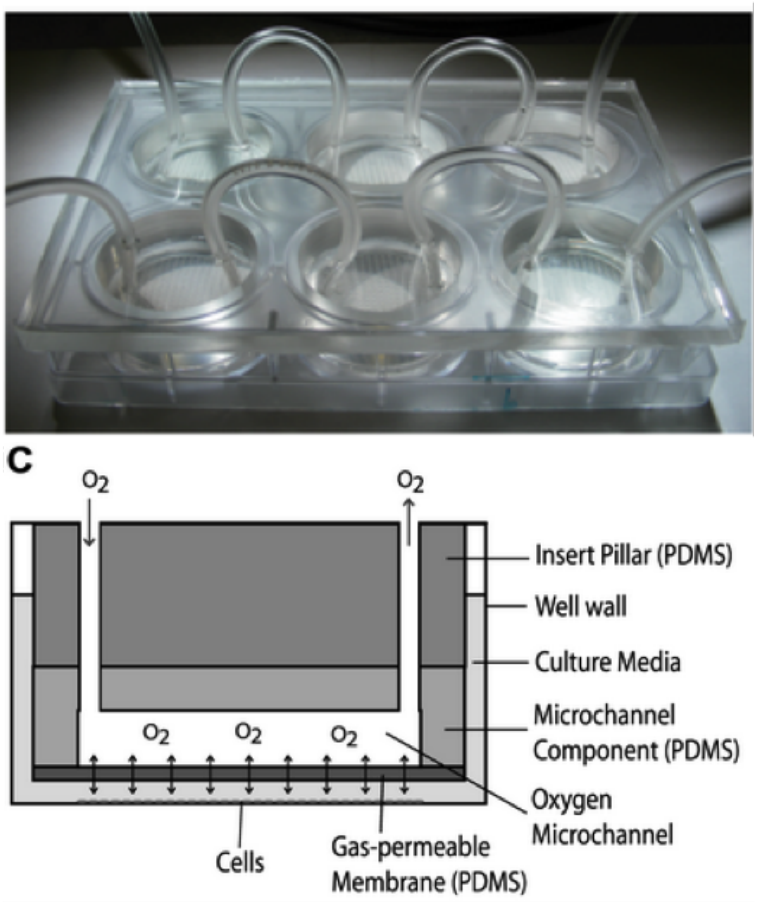


P.J.A. Kenis et al. Science 285 (1999): 83-85.

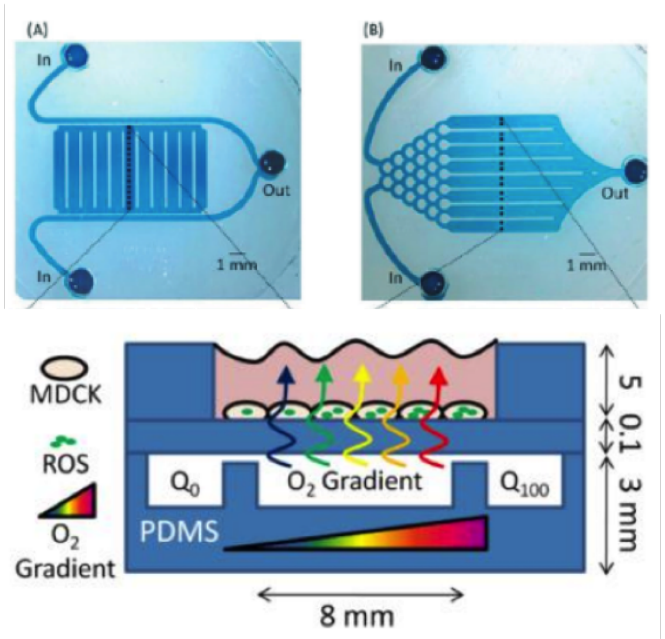


Pamme, Lab Chip. 2007

Rapid Diffusion Enables Oxygen Control

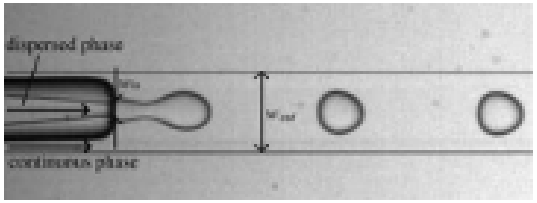


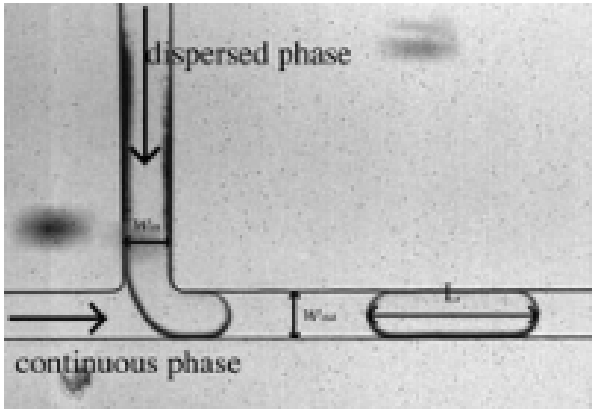
Oppegard et al. Plos One 2009

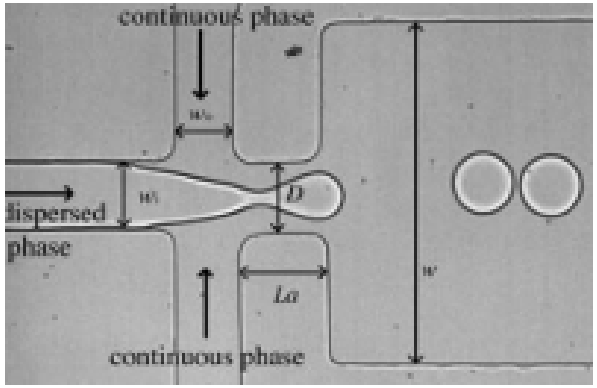


Lo et al. Lab Chip, 2010 10(18): 23942401

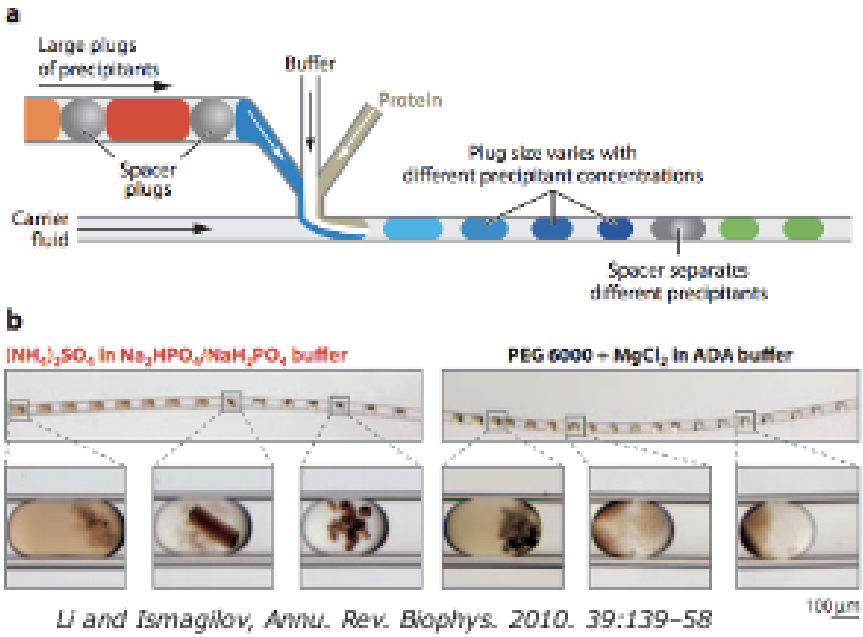
Droplet Microfluidics



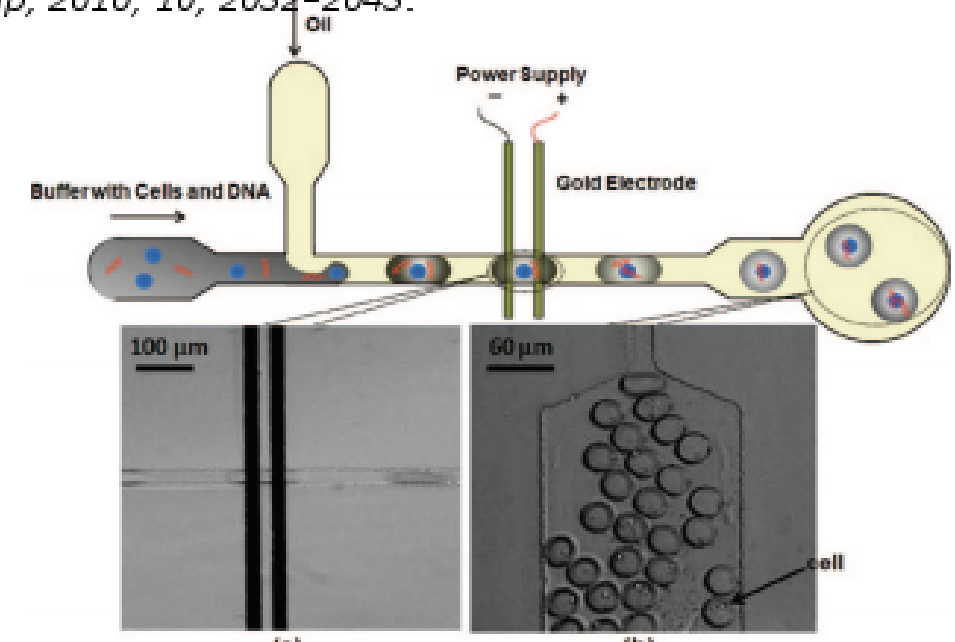




Baroud et al. Lab Chip, 2010, 10, 2032–2045.

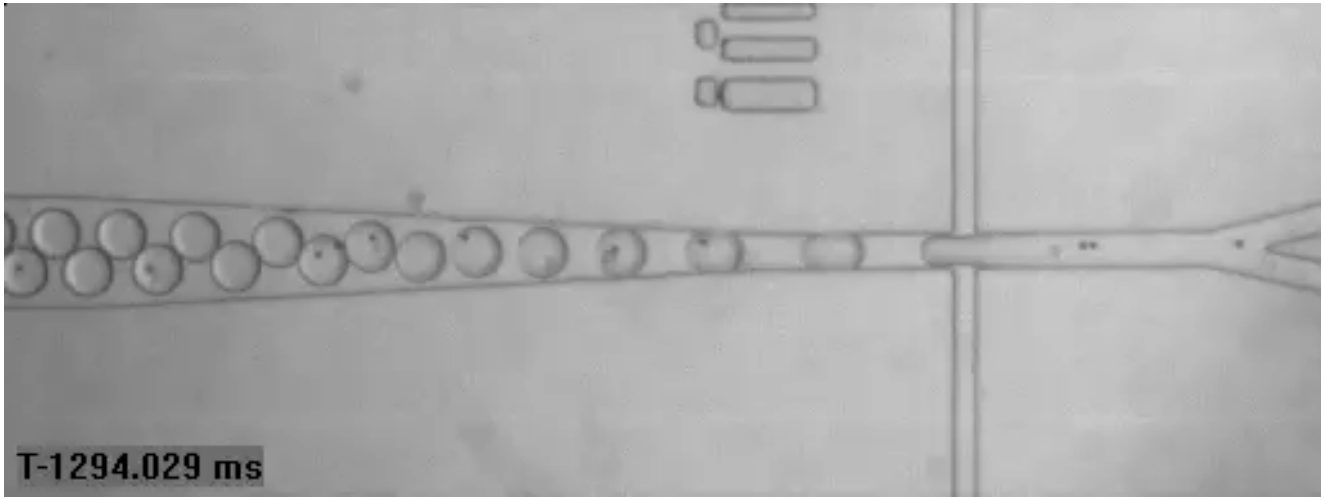


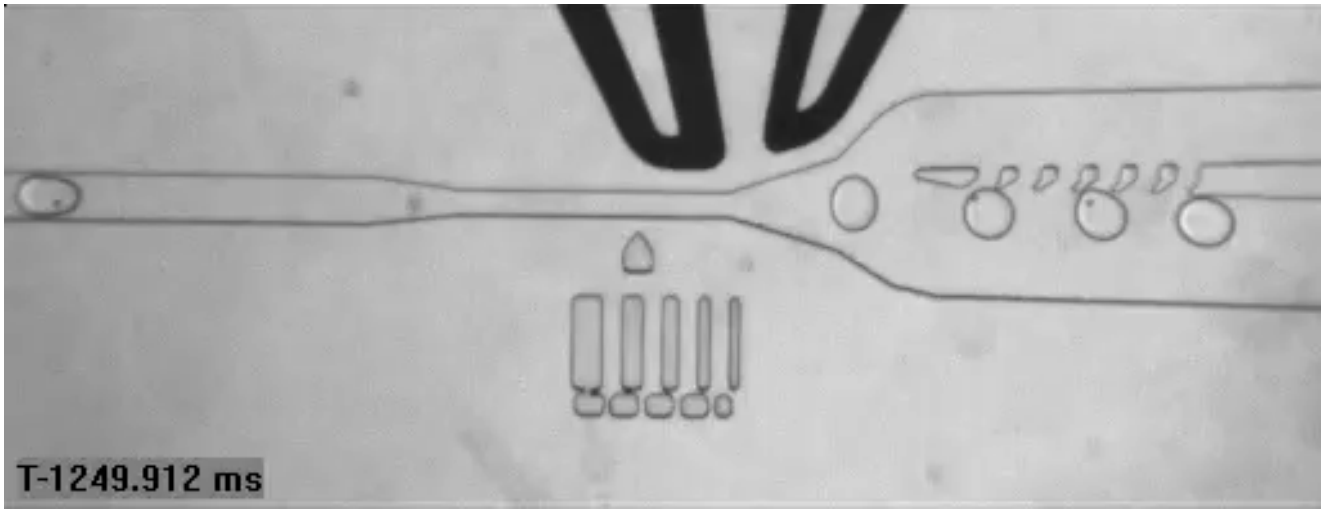
Li and Ismagilov, Annu. Rev. Biophys. 2010. 39:139–58



Zhan et al. Anal. Chem. 2009, 81, 2027–2031.

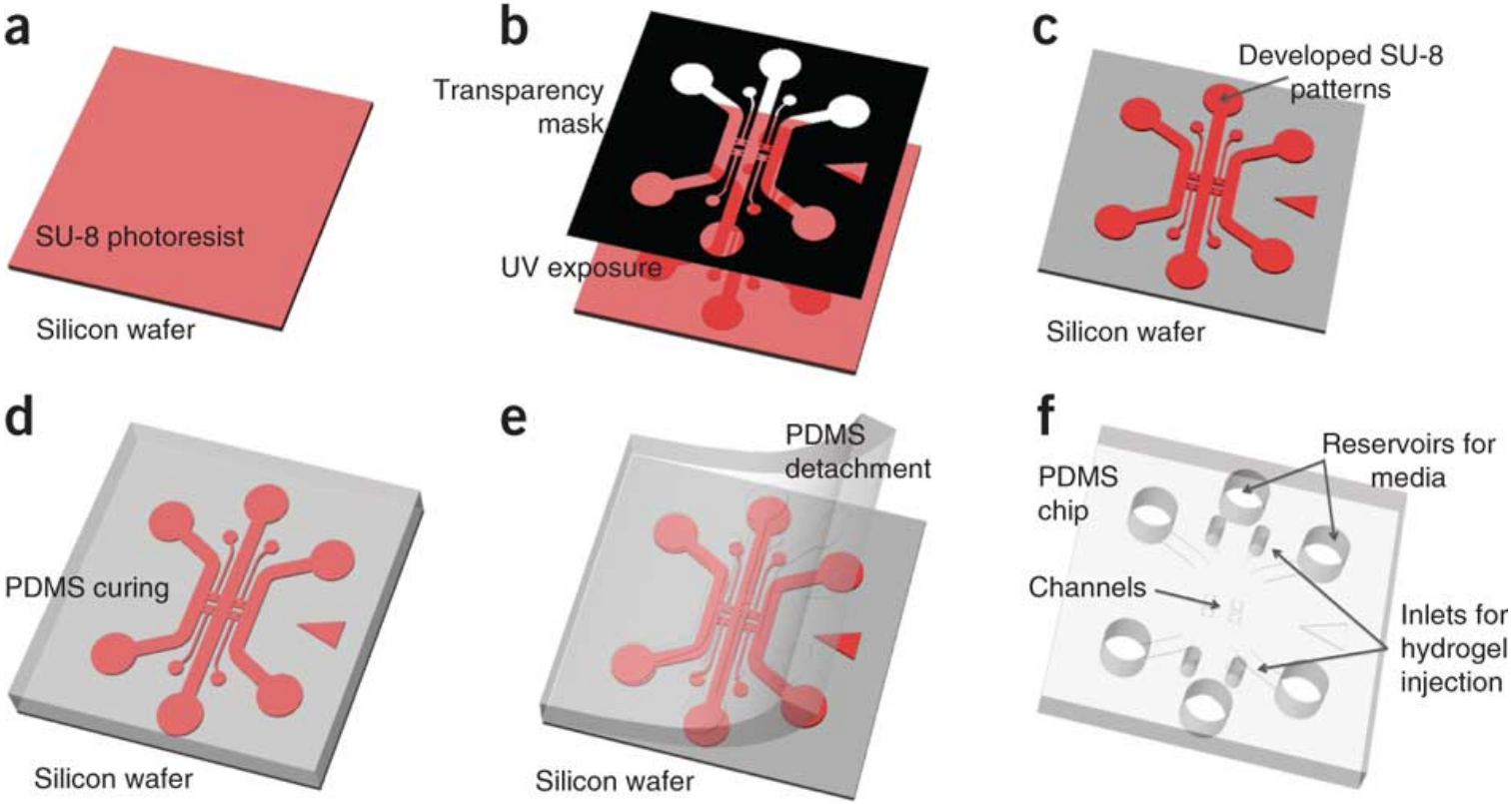
Droplet Microfluidics with cells





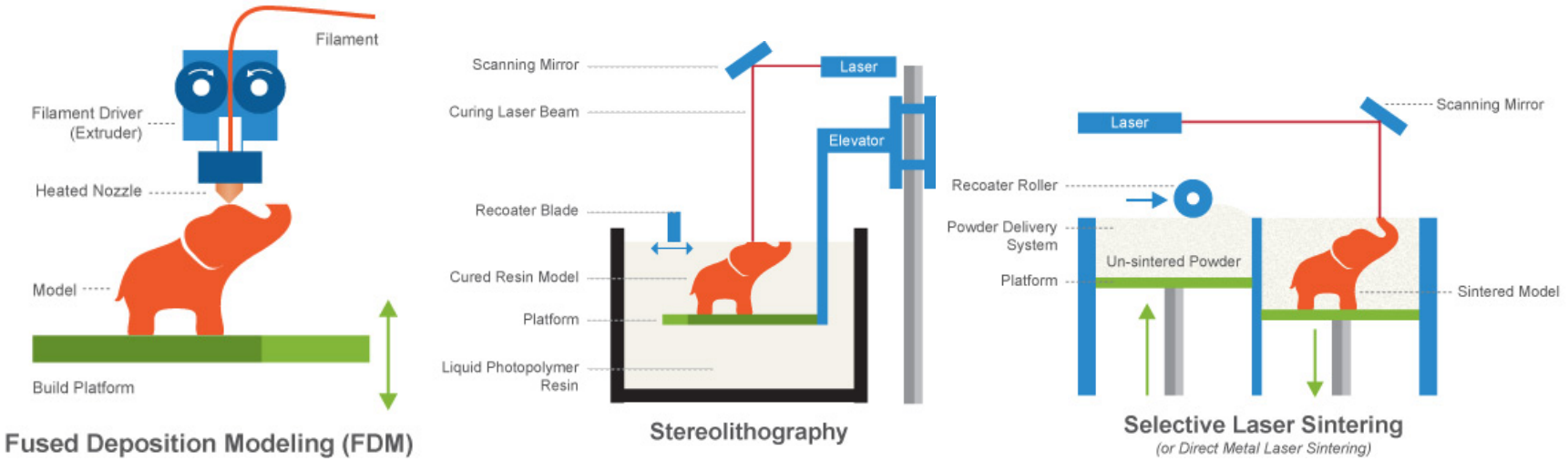
Mazutis Nature Protocols 8, 870891 (2013).

Fabrication of Microfluidic devices - Soft-Photolithography



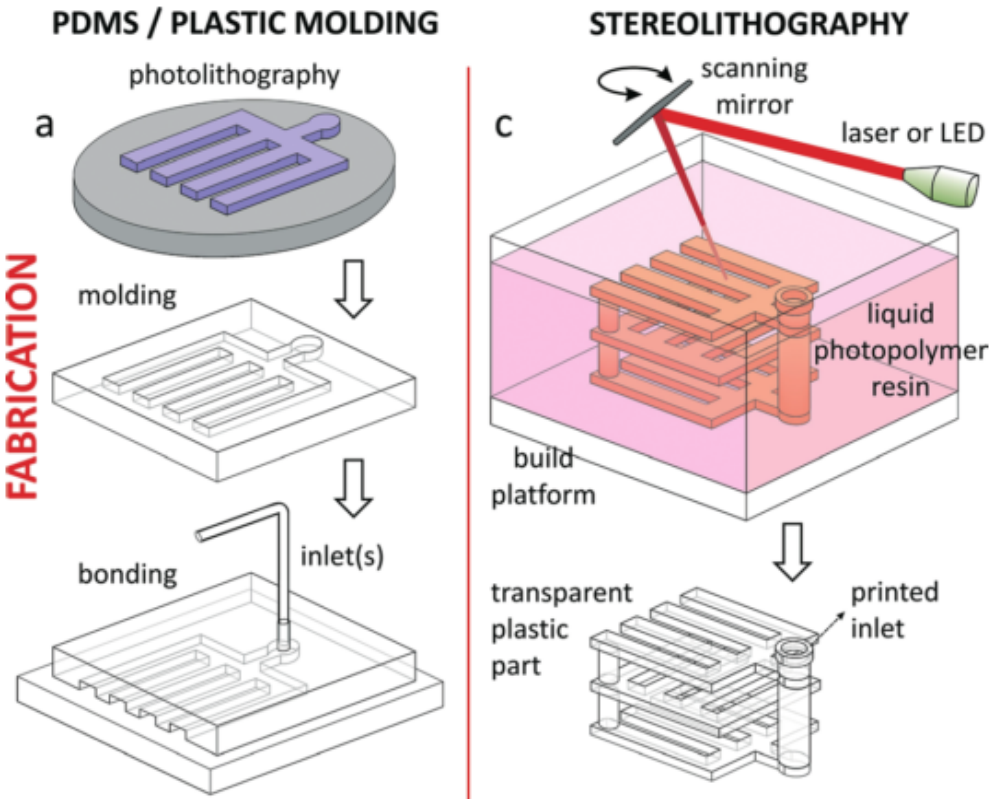
Shin et al. Nature Protocols 7, 12471259 (2012).

3D-Printing - Additive Manufacturing

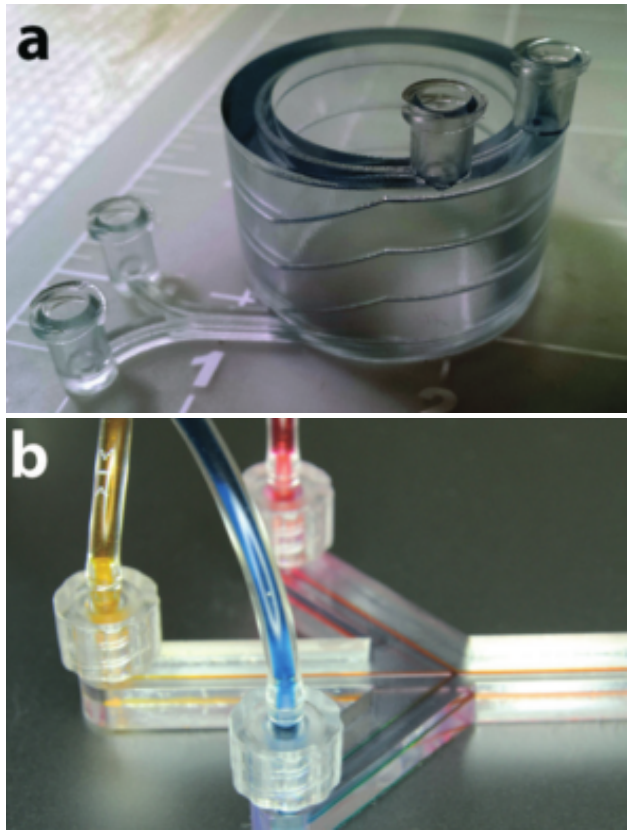


PrintSpace.com

3D-Printing as Alternative to Microfabrication

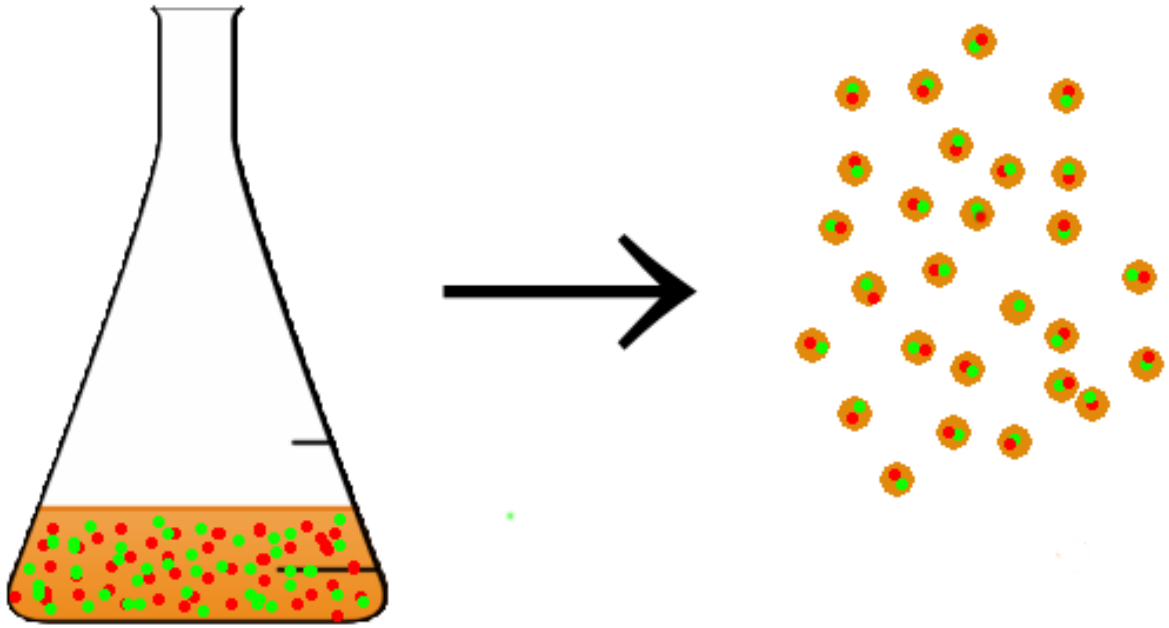


Au et al. Lab Chip. 2014

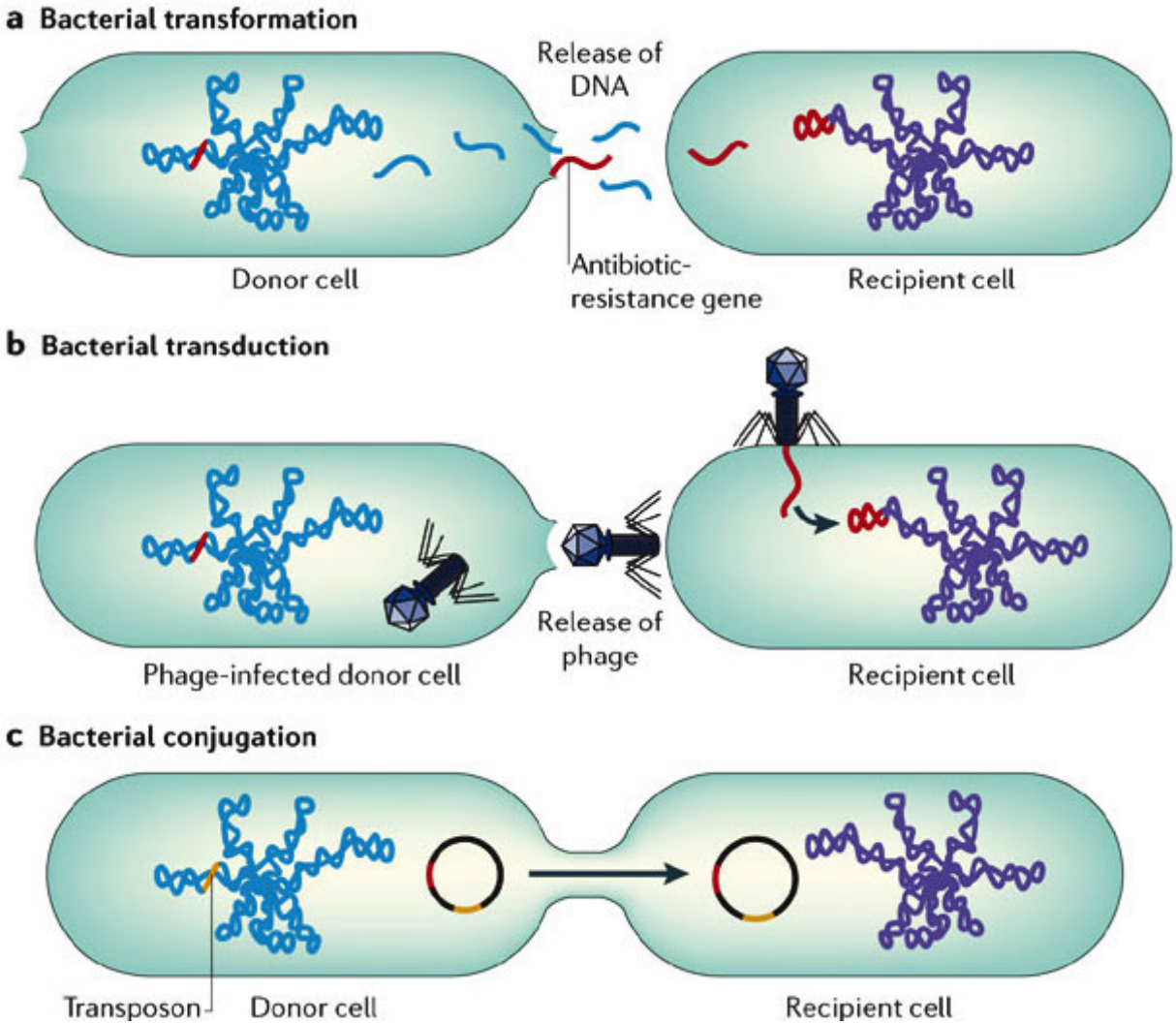


Au et al. Lab Chip. 2014

Tango Device - Co-Encapsulation and Observation of Gene Transfer

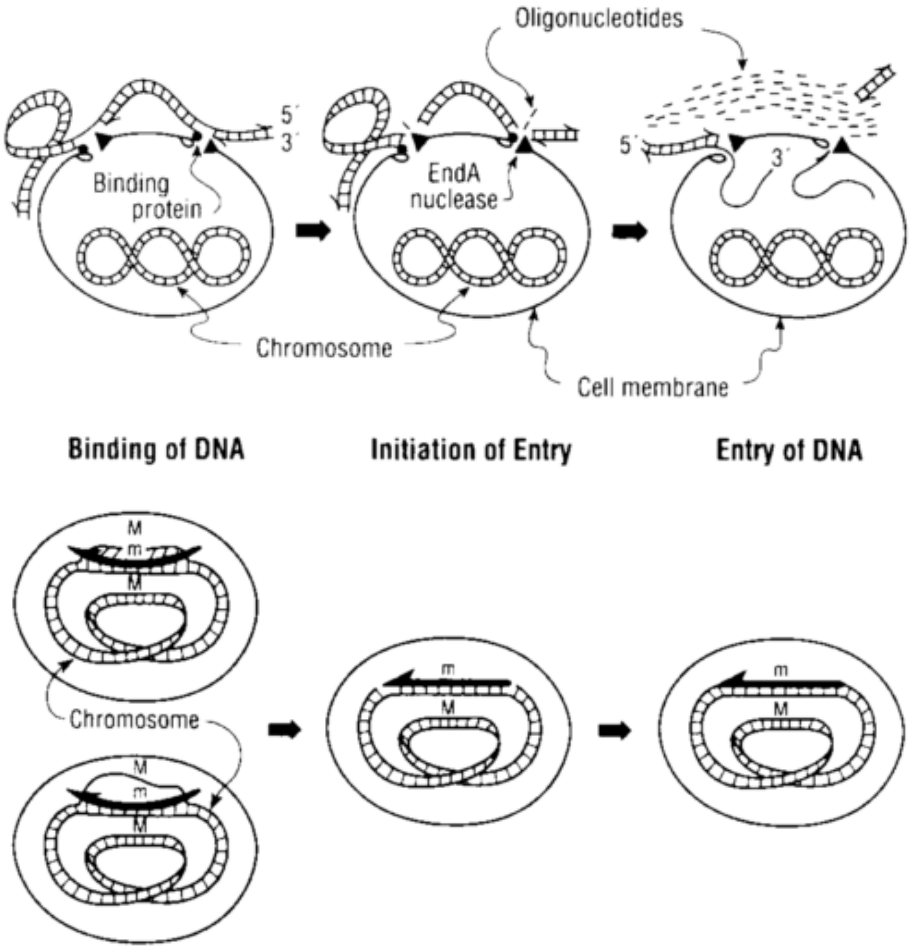


Horizontal Gene Transfer



Furuya and Lowy. Nature Reviews Microbiology 4, 36-45, 2006

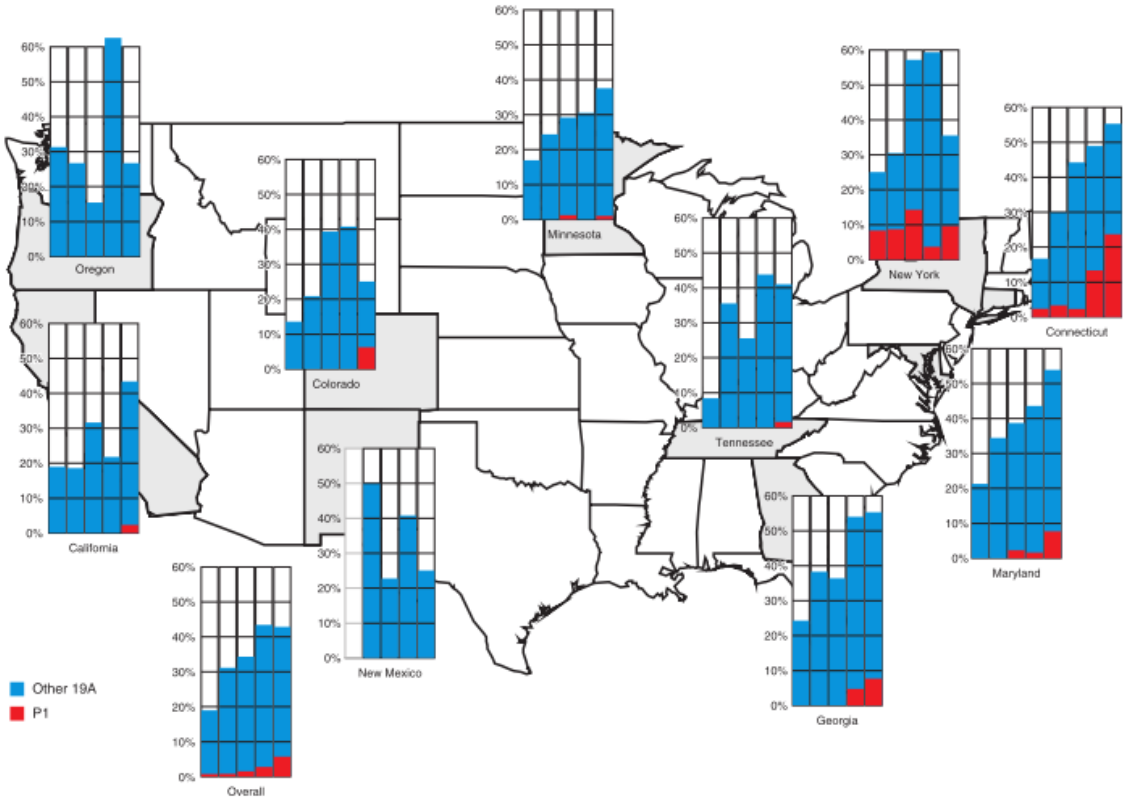
Uptake of Free DNA by a Competent Cell



Lacks. MICROBIAL DRUG RESISTANCE Volume 3, Number 4, 1997.

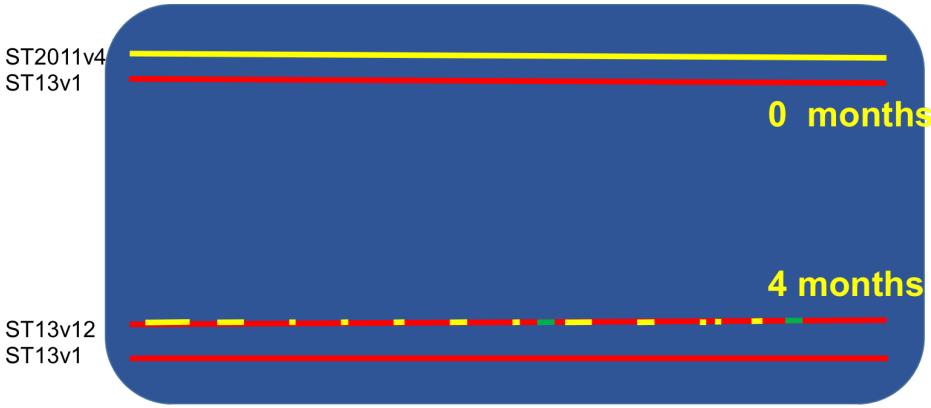
Impact of *S. pneumoniae*

- 14.5 million cases of serious disease, 826,000 deaths, in children less than 5 years
- pneumonia, meningitis and sepsis

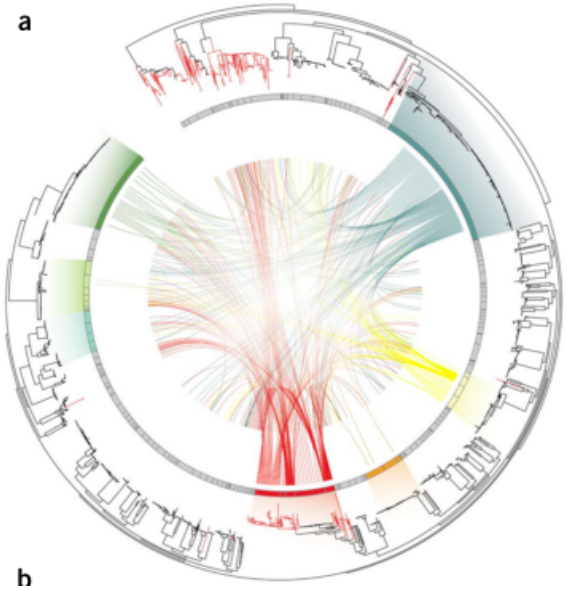


Golubchik. Nature Genetics. March 2012, Vol 44, Num 3.

In vivo



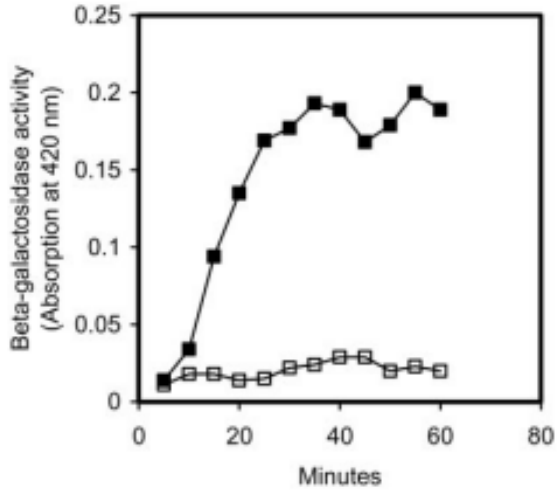
Hiller 2010 (Reproduced by Morrison)



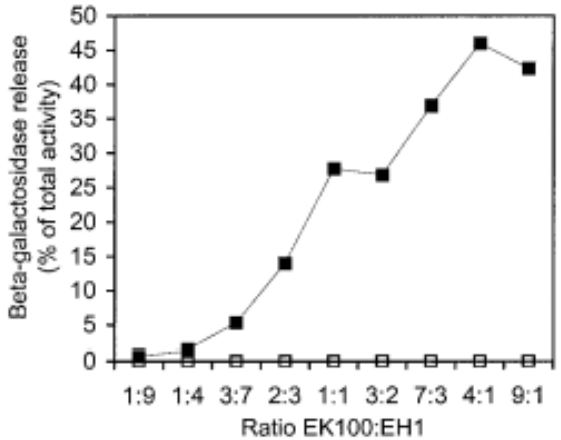
Chewapreecha. Nature Genetics. 2014, Vol 46, Num 3.

Competence is induced and involves attack on donor cell

- Competence is CSP concentration dependent, 1-10 ng, 10^7 cells/mL
- Fraction of cells lyse at critical CSP concentration
- Competence-induced cells lyse competence-deficient cells
- Lysis is dependent on activity of CbpD protein



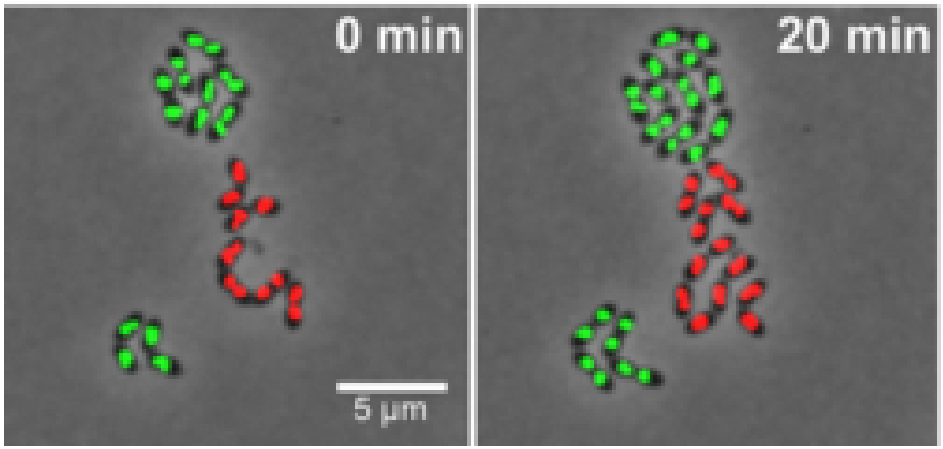
Steinmoen et al. PNAS 2002, Vol. 99, no. 11, 7681-7686



Steinmoen et al. J. Bacteriology, Dec. 2003, p. 71767183

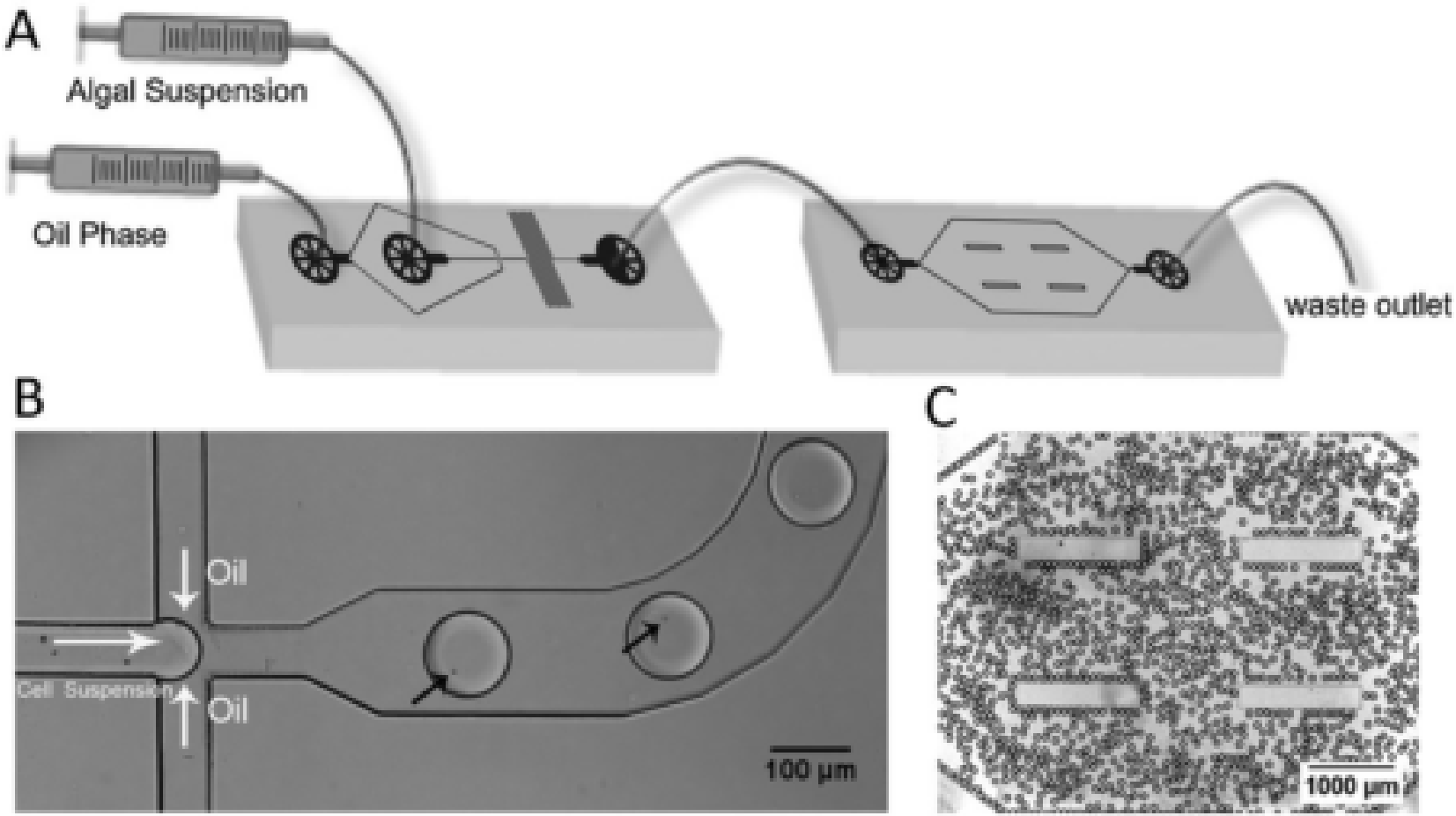
Aim: Co-encapsulation of two strains of *S. pneumoniae* to observe single gene transfer events

- Isolate two cells in a confined area to determine what happens in a single gene transfer
- Attack strain with inducible competence (Does not produce CSP), RFP labeled
- Non-competent victim strain (cannot sense CSP), GFP expressing
- Encapsulate attacker-victim pairs in Droplets



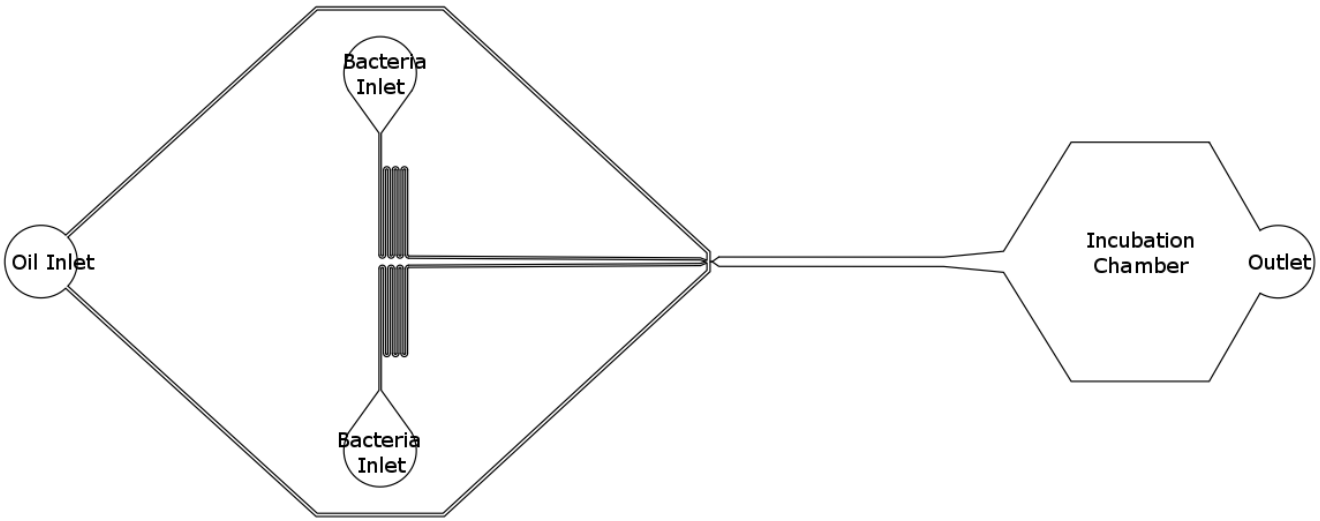
Kjos et al. Journal of Bacteriology 2015 Vol 197 No 5.

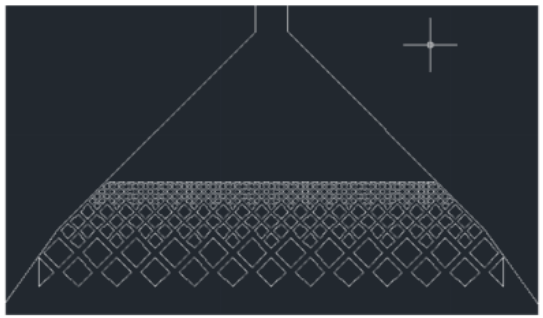
Inspiration - Algae Encapsulation Device



Pan et al. Integr. Biol., 2011, 3, 10431051

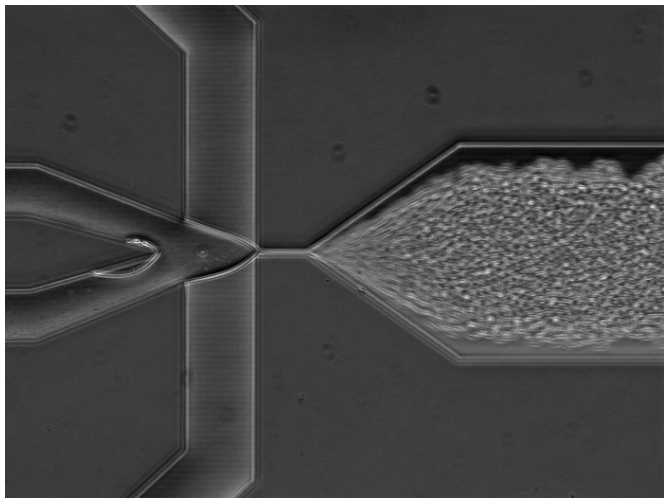
Device Design





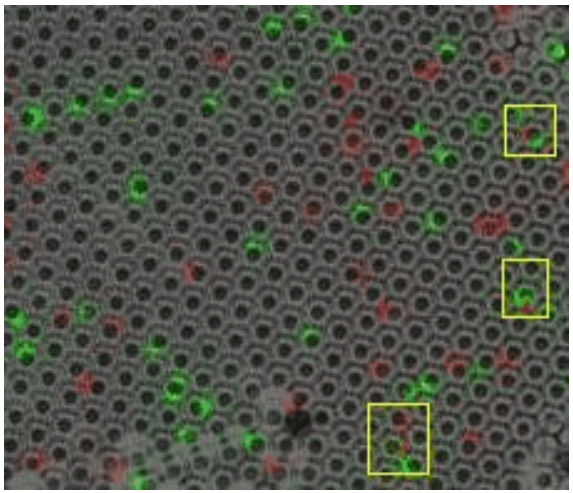
Droplet Generation

- Oil flowrate of 150 μmL/h, aqueous 50 μmL/h
- Neck width of 5 μm
- Droplets of 5-10 μm are generated
- 5 femtoliter droplets



Beads in Droplets

- Green and red florescent beads of 1 μm diameter
- 4x10^9 beads/ml
- 20% occupancy, 5% R&G

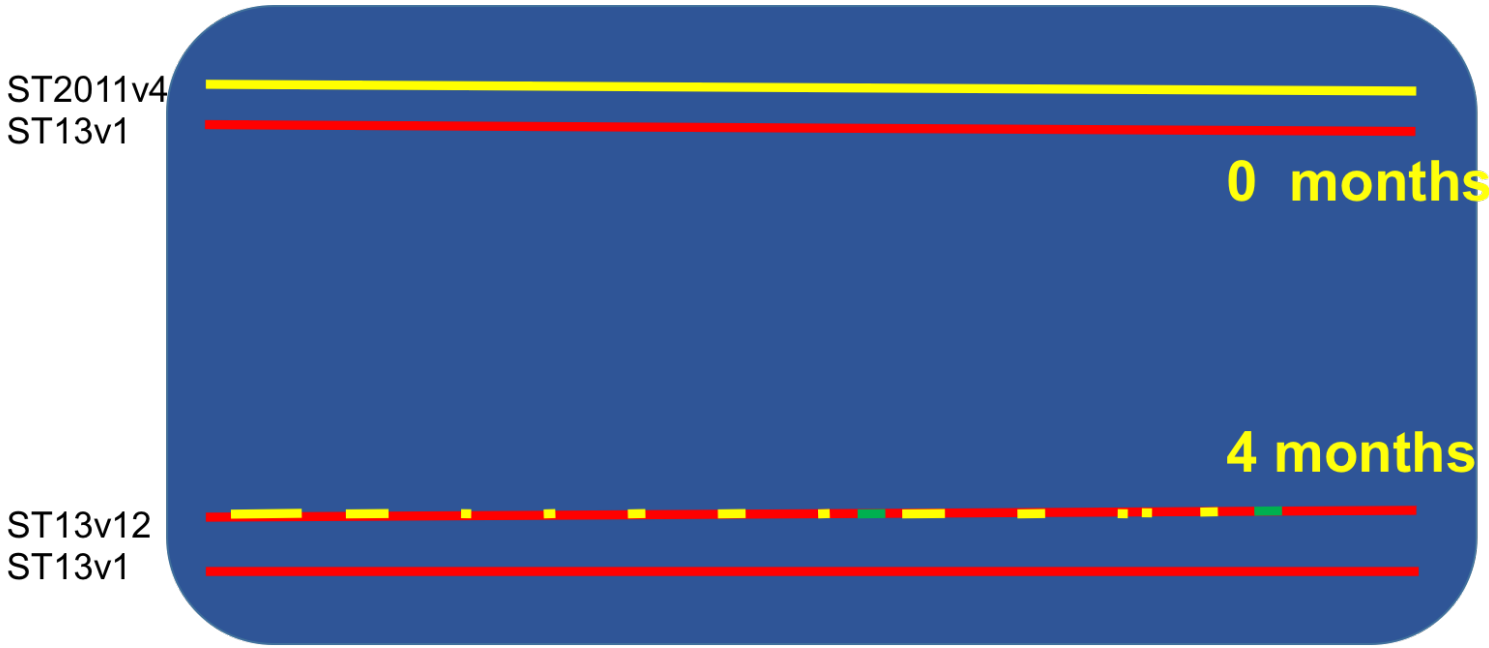


Full Experiment Procedure

1. Encapsulate attacker and victim strains in droplets
2. Incubate droplets in the device and observe attack
3. Break the emulsion, dilute and plate
4. Assess gene transfer with whole genome sequencing of remaining cultures

Expected Results

- Encapsulate cells and determine occupancy
- Image cells during incubation to observe attack
- Ultimately determine what can be expected from a single transfer event

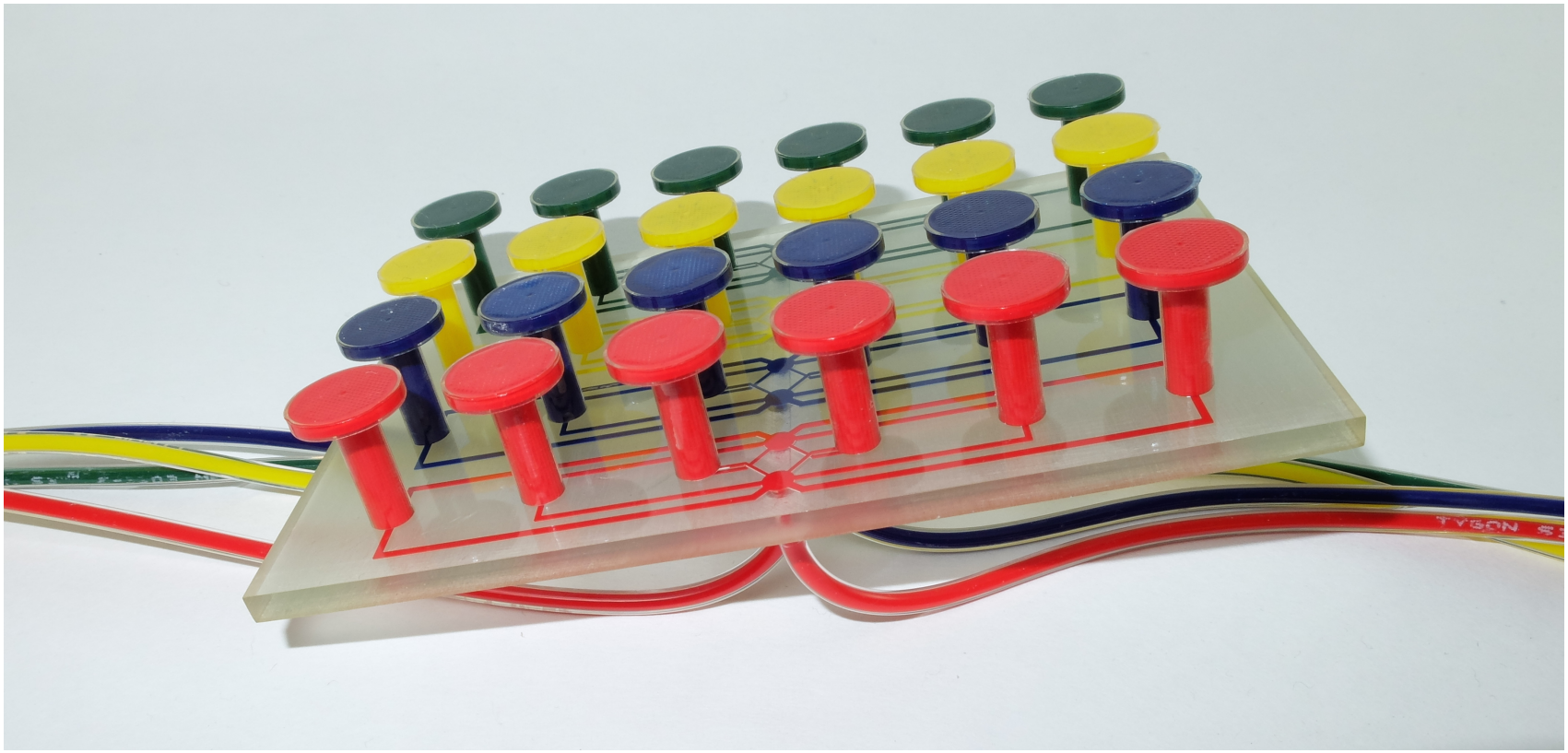


Hiller 2010 (Reproduced by Morrison)

Potential Difficulties

- Cells may not become competent due to encapsulation
- Cells may not physically meet
- Attack or transformation may require more than 2 cells
- Confinement may cause lysis of both cells

3D-Printed Oxygen Control Insert



Oxygen in Vivo Versus Oxygen in Research

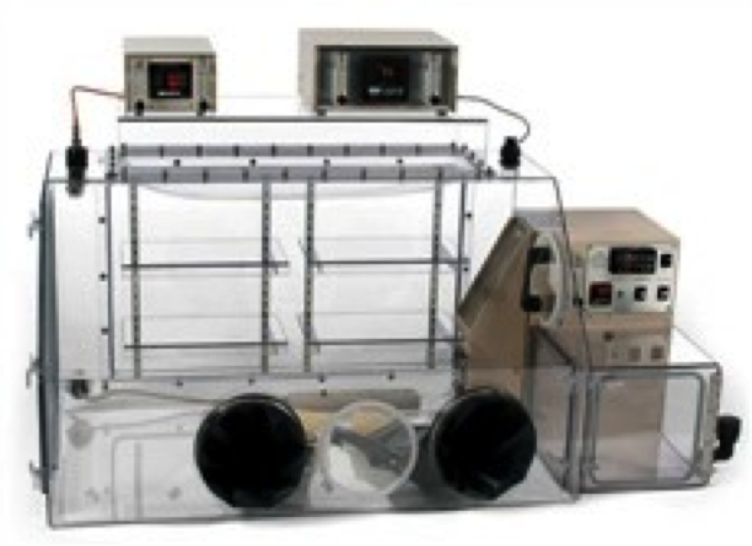
- Typically ignored, culture studied at 21%
- Equipment is inconvenient for oxygenating to multiple levels and is expensive
- Microfluidic oxygen devices must be expertly made and operated

Tissue	Physiologic oxygen (%)
Lung alveoli	13
Liver	10–13
Arterial blood	10–13
Venous blood	5
Bone marrow	0.5–7
Brain	0.5–7
Cartilage	1

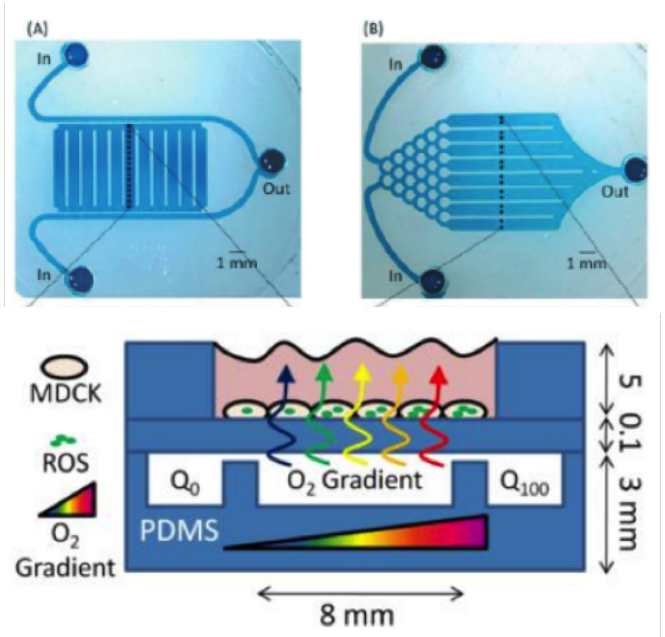
Brennan et al. Lab Chip, 2014, 14, 43054318

Methods for Oxygen Control

Hypoxic Workstations



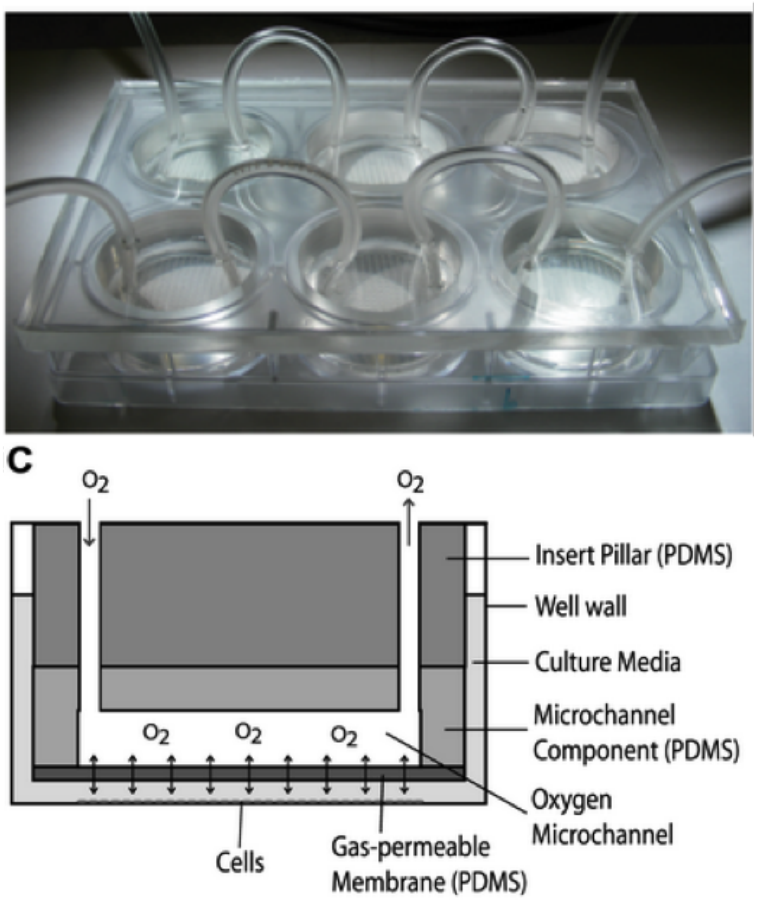
Microfluidic Devices



Lo et al. Lab Chip, 2010 10(18): 23942401

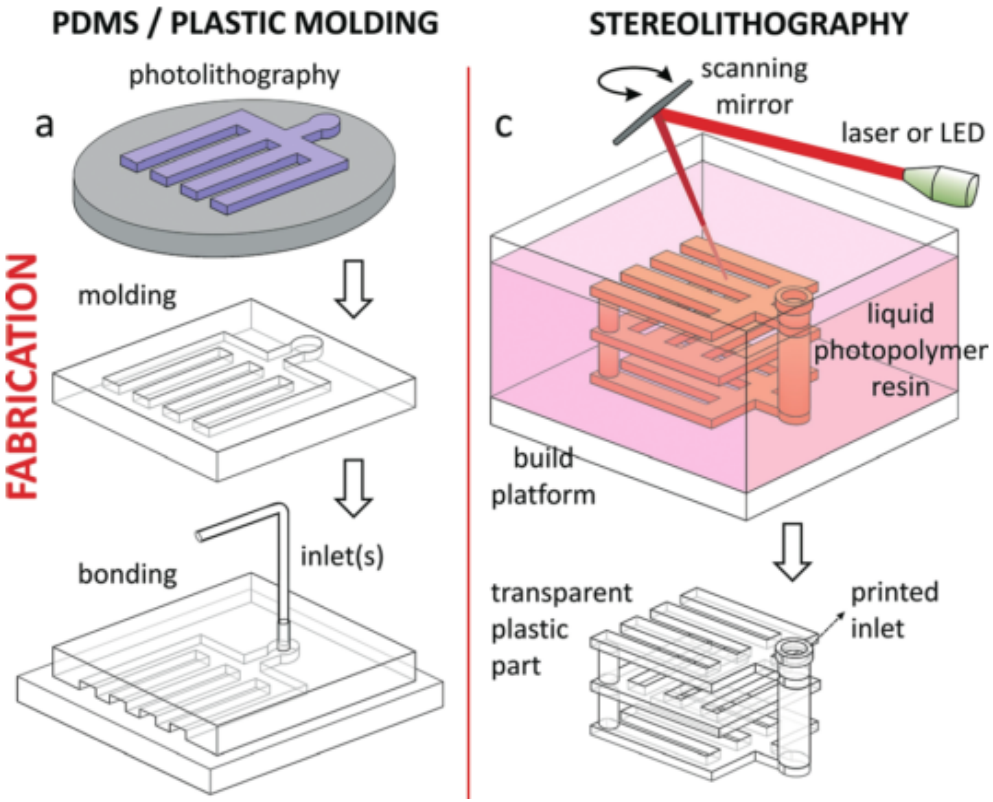
Aim: Develop an easy to use and fabricate oxygen control system for researchers

- Use Multi-well plate platform
- 3D-printing - outsource fabrication
- Integrate functionality

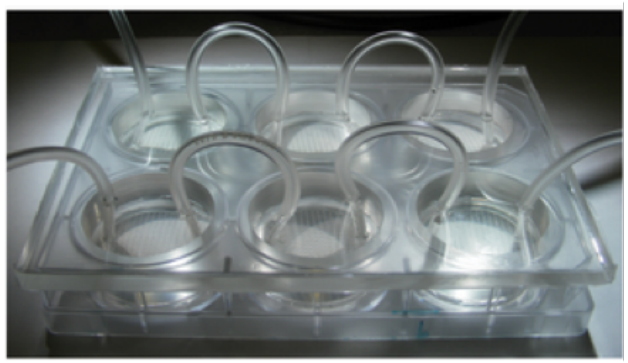


Oppegard et al. Plos One 2009

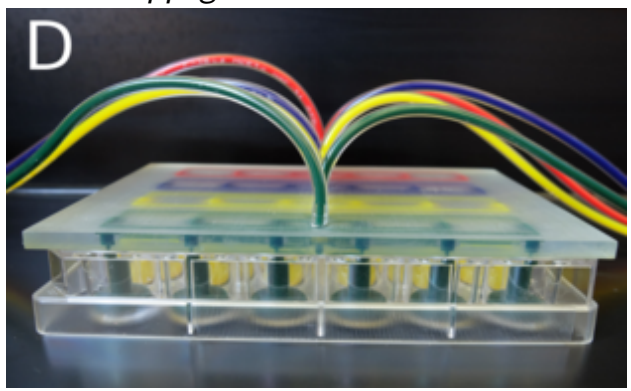
3D-Printing



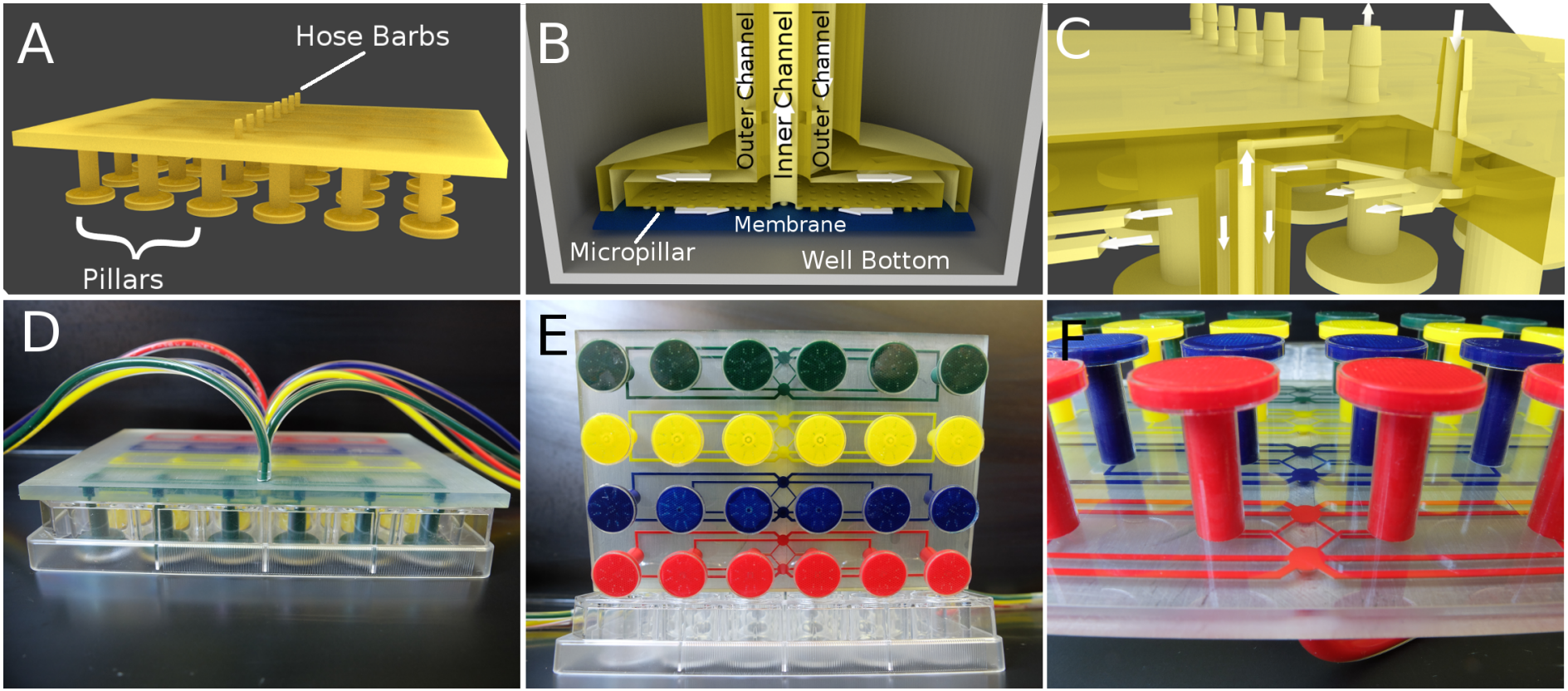
Au et al. Lab Chip. 2014



Oppegard et al. Plos One 2009



Insert Design

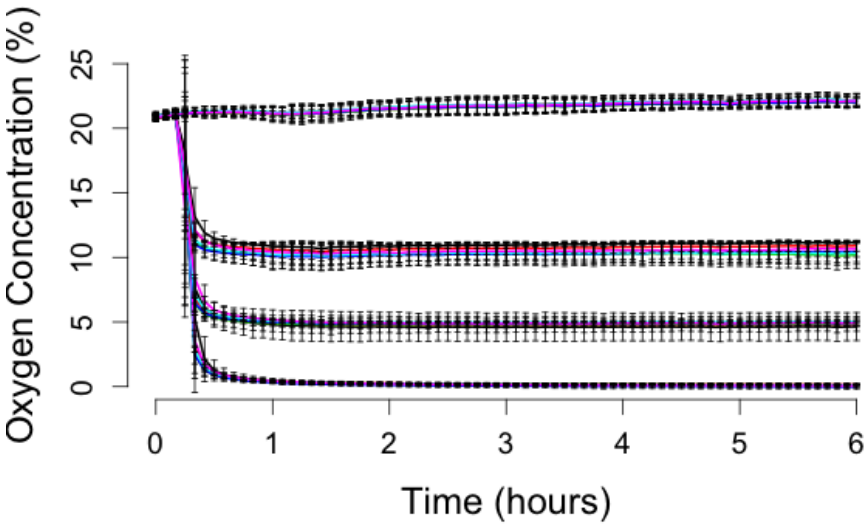


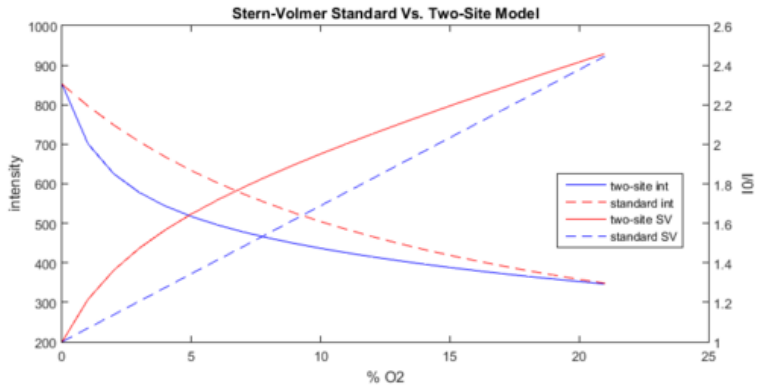
Brennan et al. Plos One 2015

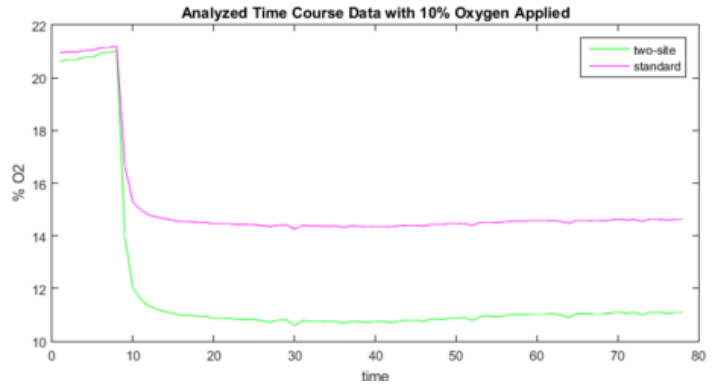
Oxygen Performance - Stern Volmer Analysis

$$I_0/I = 1 + Ksv \cdot [Q]$$

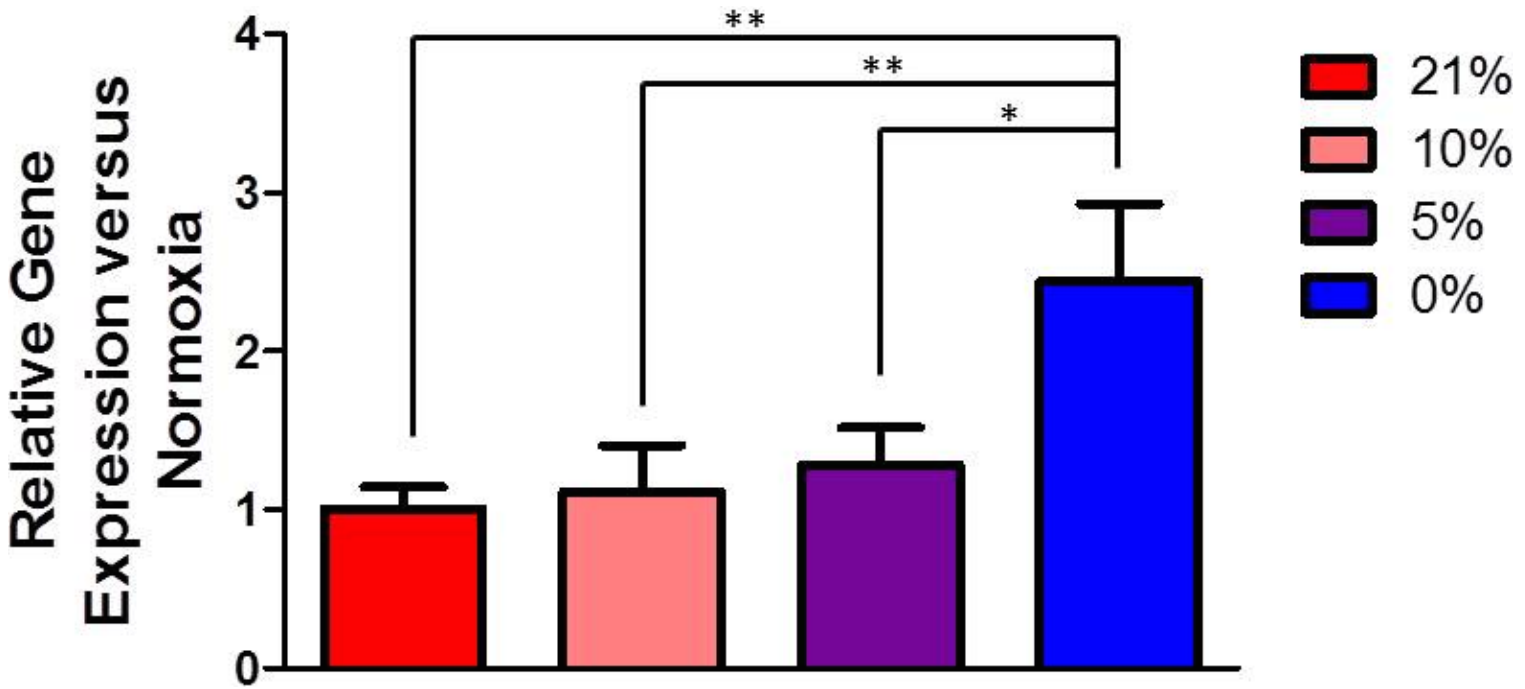
$$I/I_0 = \frac{f_1}{1 + Ksv_1 \cdot [Q]} + \frac{f_2}{1 + Ksv_2 \cdot [Q]}$$







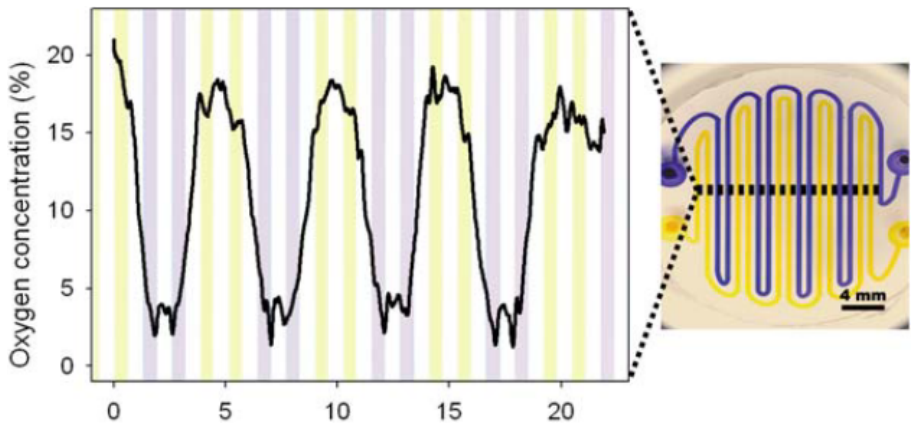
Bioverification



Brennan et al. Plos One 2015

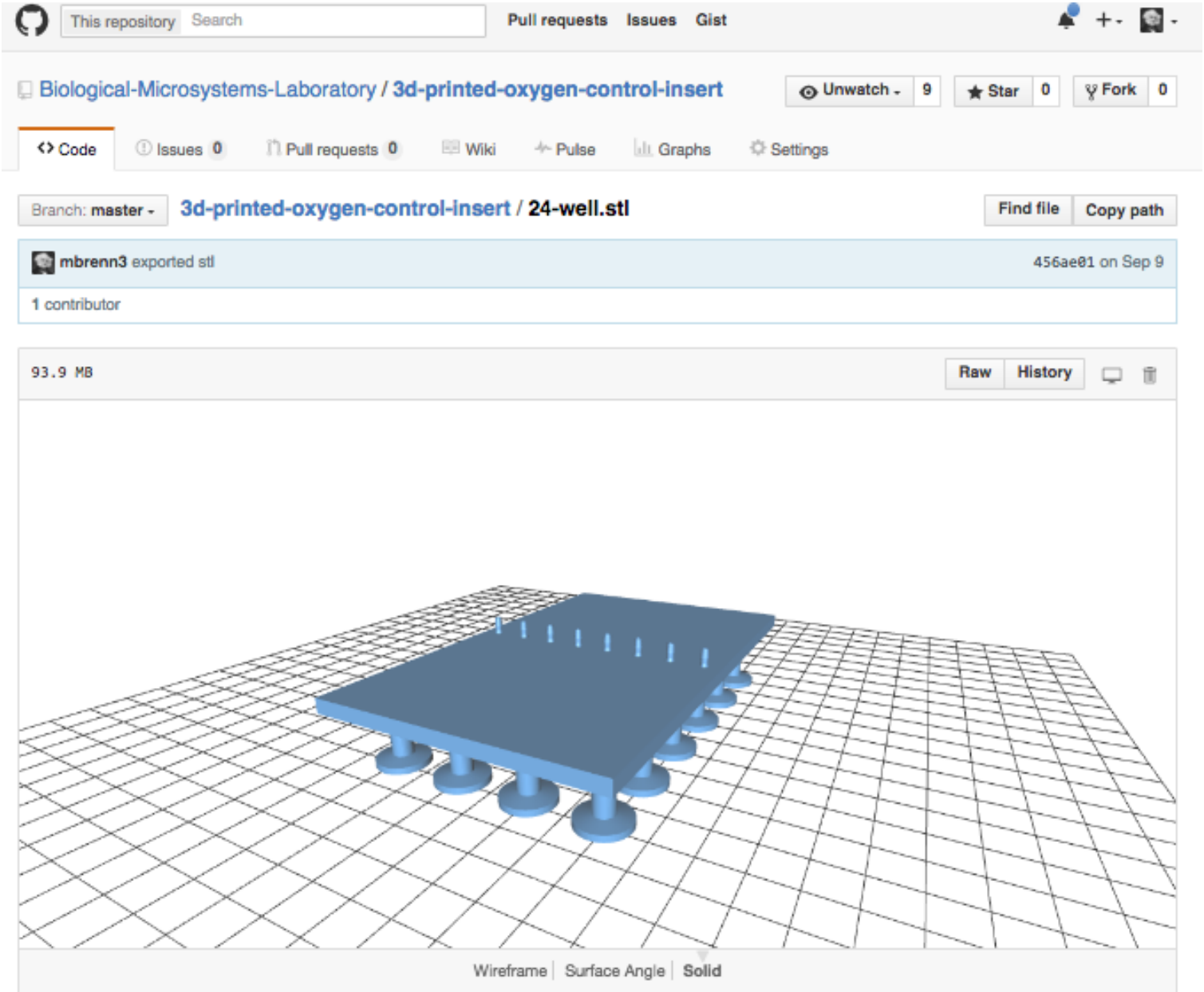
Expanding and improving the device

- Membrane material
- Media exchange
- Pattering of oxygen in wells

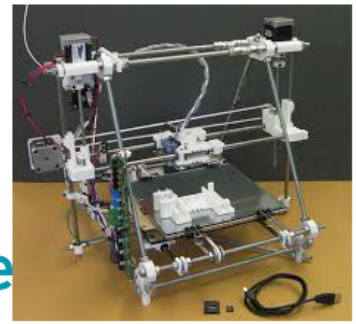


Oppegard et al. Plos One 2009

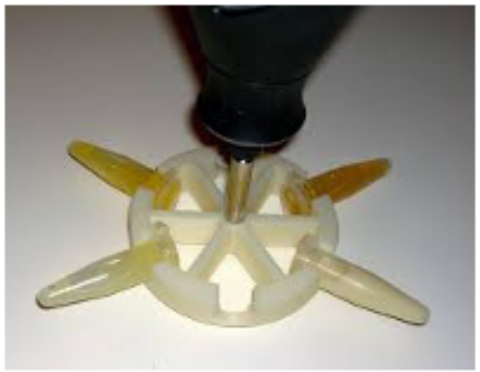
GitHub

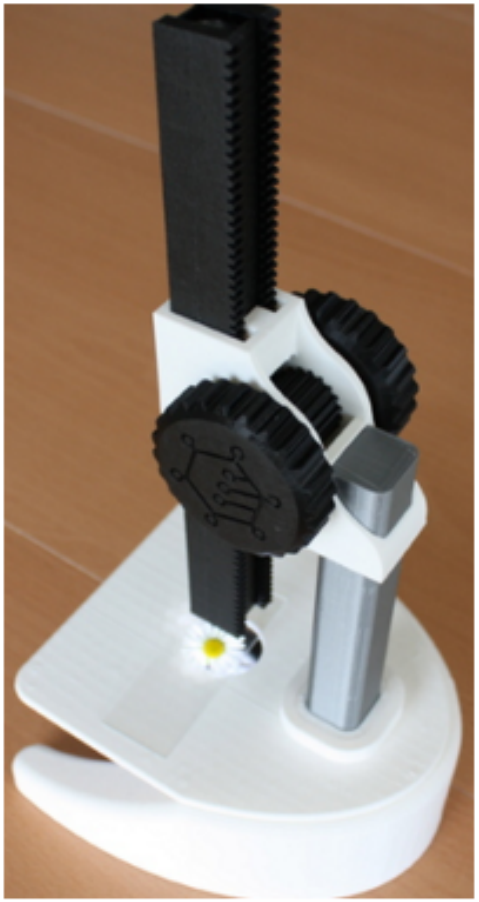
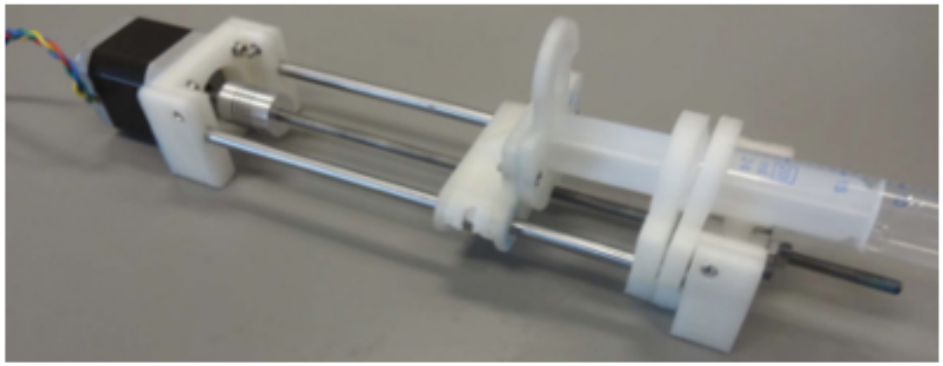

This repository
Search
Pull requests
Issues
Gist
Biological-Microsystems-Laboratory / 3d-printed-oxygen-control-insert
Unwatch
9
Star
0
Fork
0
Code
Issues 0
Pull requests 0
Wiki
Pulse
Graphs
Settings
Branch: master
3d-printed-oxygen-control-insert / 24-well.stl
Find file
Copy path
mbrenn3 exported stl
456ae01 on Sep 9
1 contributor
93.9 MB
Raw
History
Wireframe
Surface Angle
Solid

Open Design Scientific Tools



3D-Printed Scientific Tools

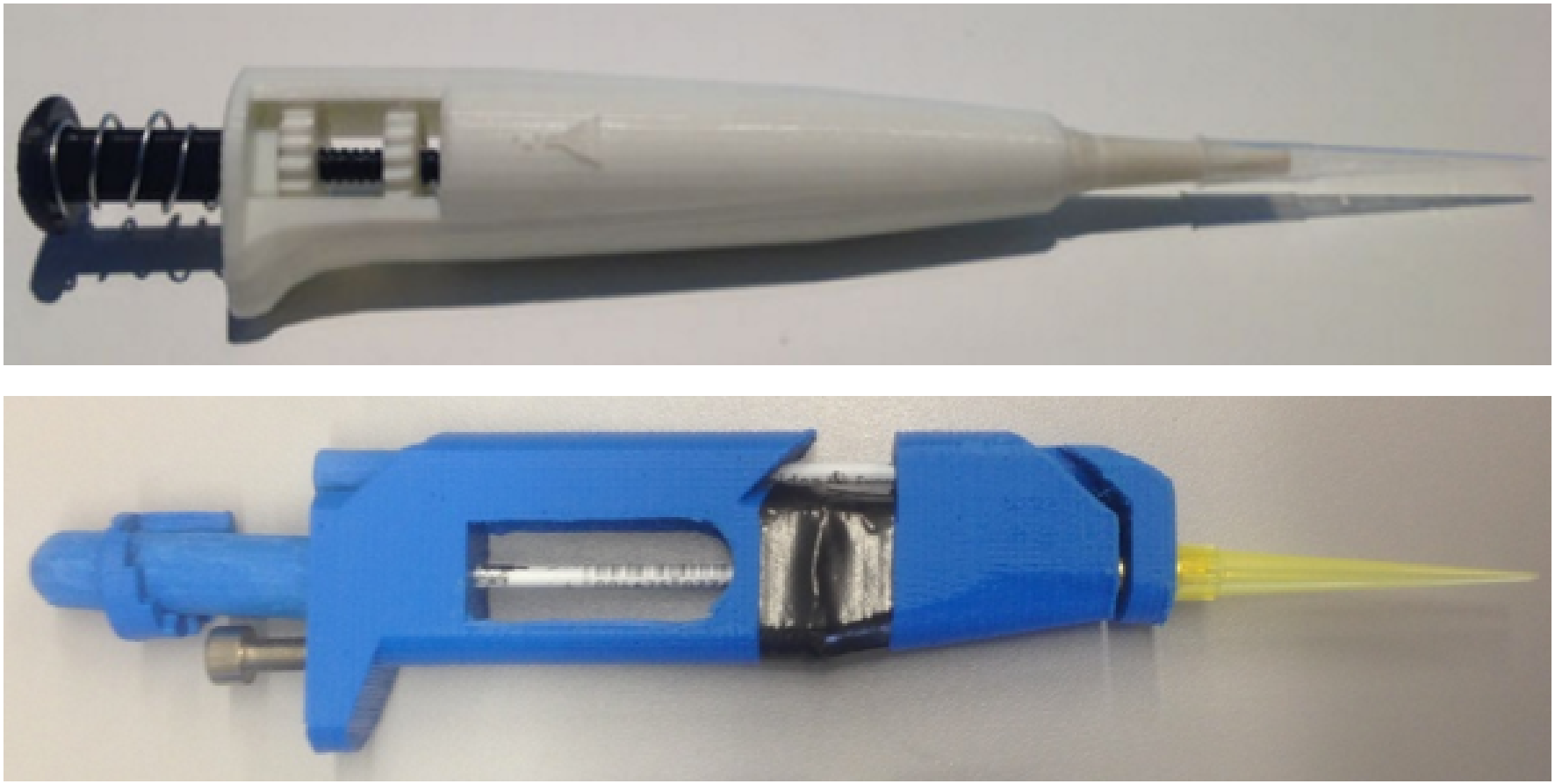


Thing:1483 Dremelfuge, Thing:26553 Filterwheel, Thing:77450 Microscope

Wijnen et al. 2014, Volume 9, Issue 9, e107216.

3D-Printed Pipettes

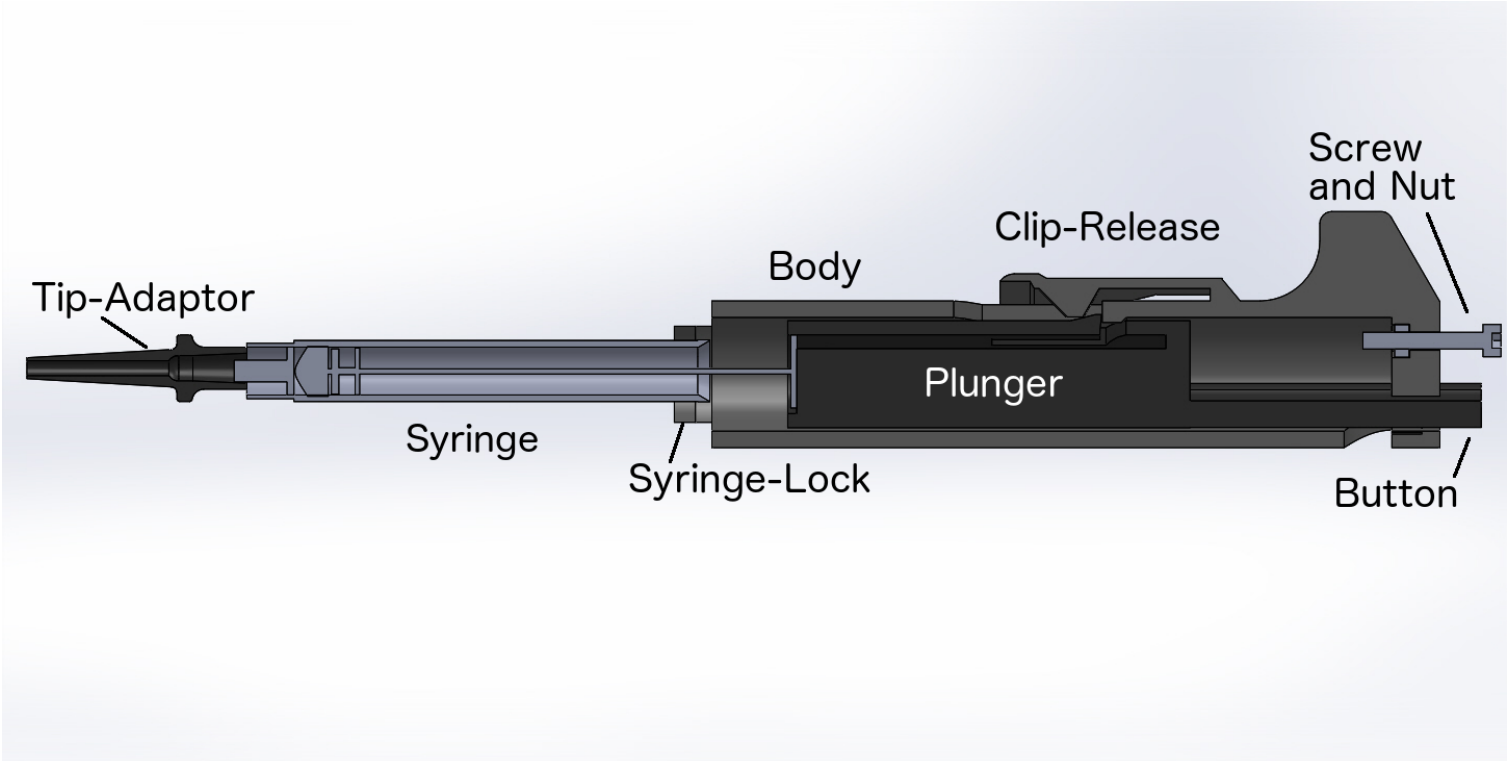


Thing:159052 Laboratory pipette, Thing:25519 Briopipette

Aim: Develop a 3D printable micropipette and compare to commercial versions

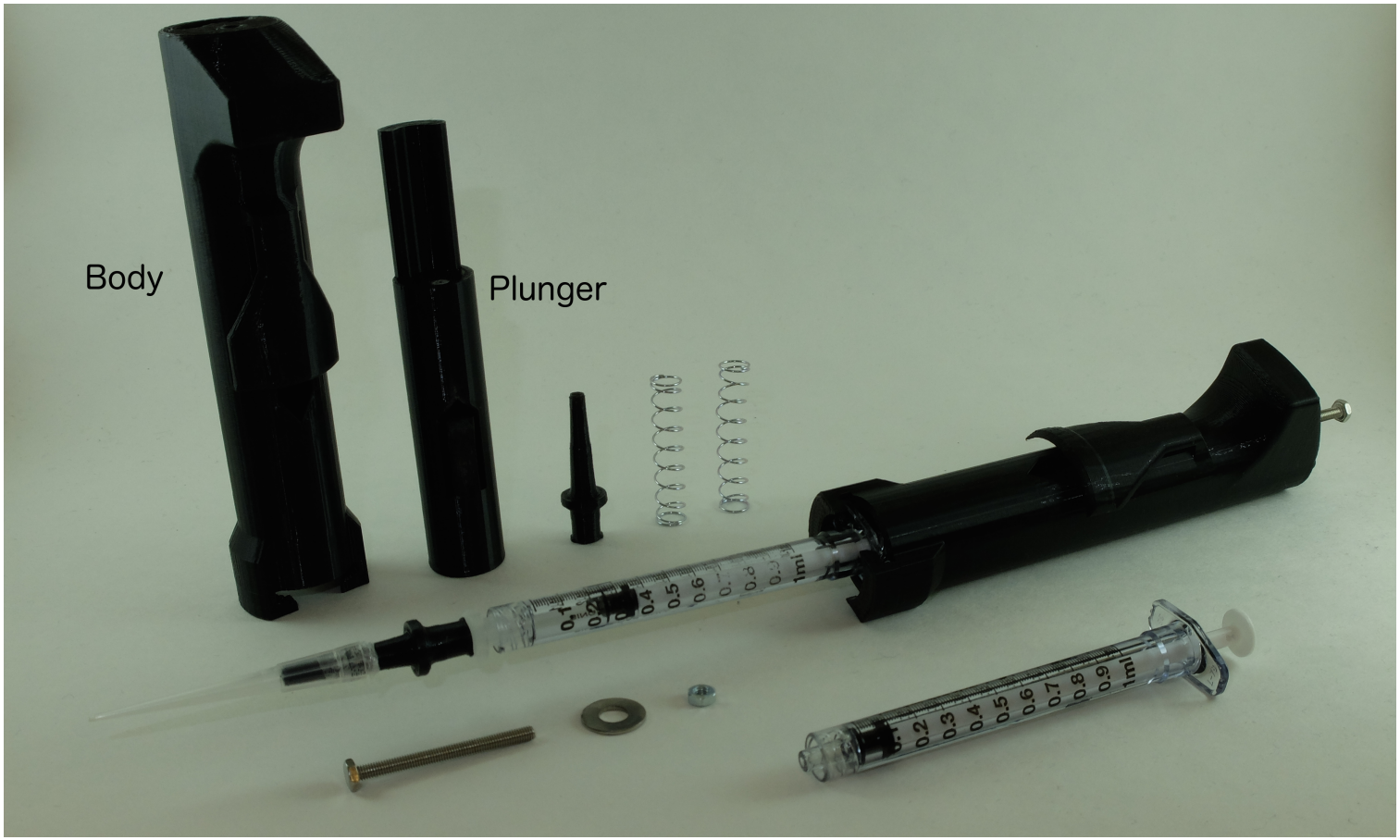
- Use a consumer grade printer
- Simple hardware
- Adjustable to desired volume a priori
- Comparable accuracy to a commercial pipette

Design of Pipette

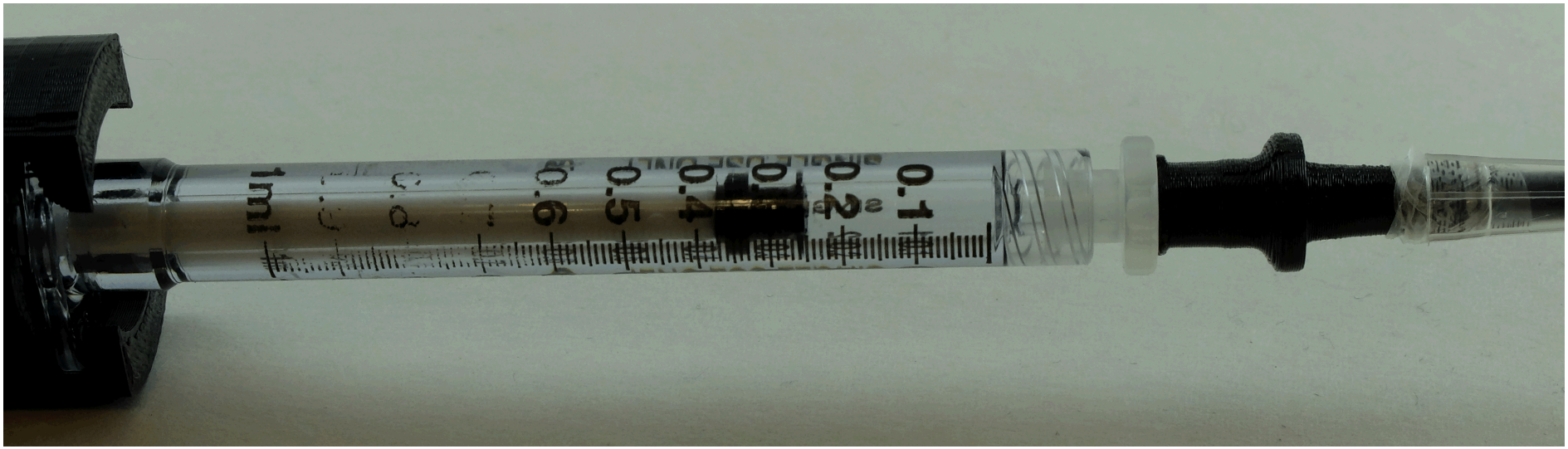


Current Prototype

- Makerbot printable, $5 USD including parts
- Uses syringe graduations to set volume
- Competitive with commercial pipettes in accuracy



Operating Procedure



- Compare displacement from bottom and top stop with graduations
- Adjust top stop with screw
- Re-check displacement
- Pipette

Preliminary Results

Table 1. Comparison of error between the printed and commerically produced pipette

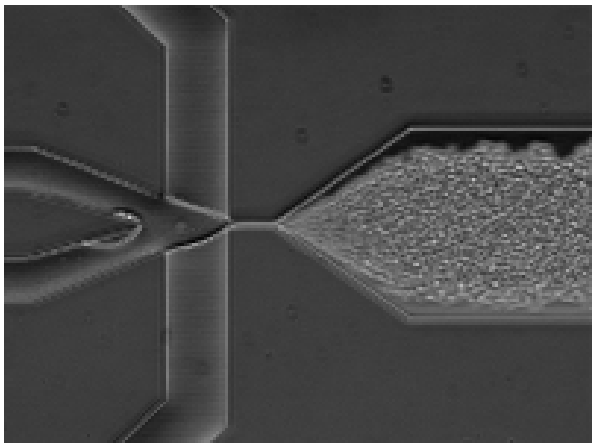
Volume		Systematic error		Random error	
10 μL	printed	2.17 %	$\pm 0.217\mu$L	1.96%	$\pm 0.196\mu$L
	commercial	3%	$\pm 0.3\mu$L	1%	$\pm 0.1\mu$L
20 μL	printed	0.33%	$\pm 0.066\mu$L	1.18 %	$\pm 0.236\mu$L
	commercial	2.50%	$\pm 0.5\mu$L	0.70%	$\pm 0.14\mu$L
50 μL	printed	1.17%	$\pm 0.585\mu$L	0.50%	$\pm 0.25\mu$L
	commercial	1%	$\pm 0.5\mu$L	0.30%	$\pm 0.15\mu$L
200 μL	printed	2.52%	$\pm 5.04\mu$L	0.69%	$\pm 1.38\mu$L
	commercial	0.6%	$\pm 1.2\mu$L	0.20%	$\pm 0.4\mu$L

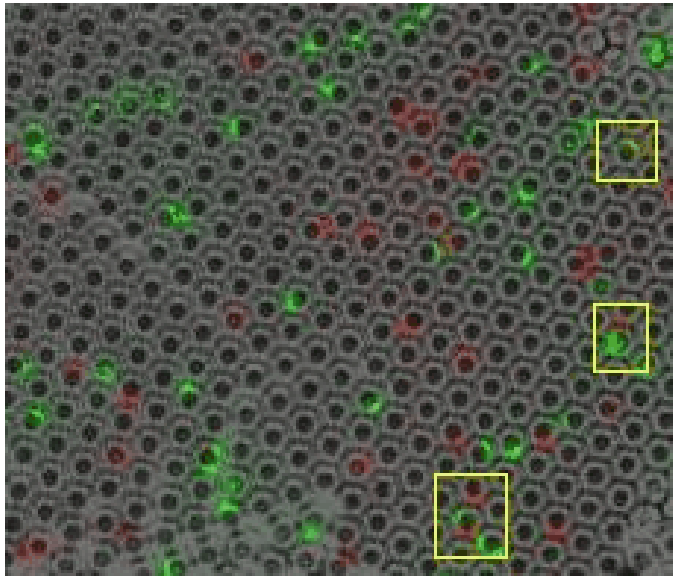
Error assessed for average measured volume by weight of water compared with published specifications of commercial pipette.

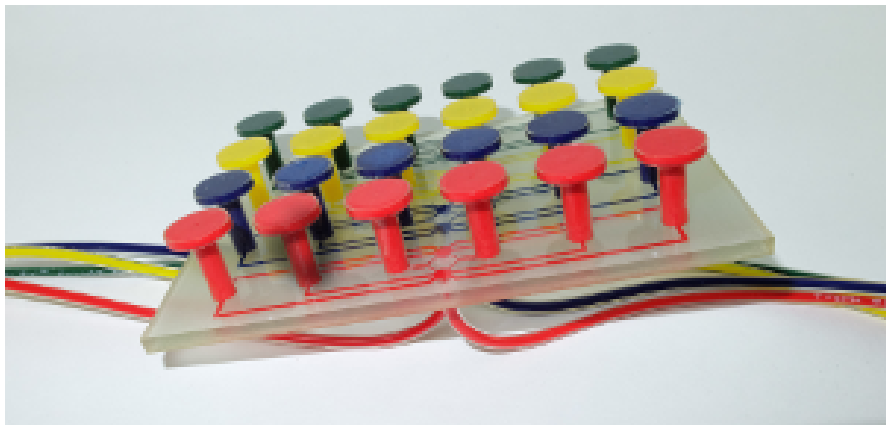
Future Approach

- Add features such as ejector for tips
- Push-push operation
- improve user friendliness

Wrap-Up







Acknowledgments

Thank You

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Thank You