

Microfabricated Platforms for Microbiome Culture: From Artificial Mouths to Guts

Dr. Todd Thorsen
MIT Lincoln Laboratory
13 September, 2018

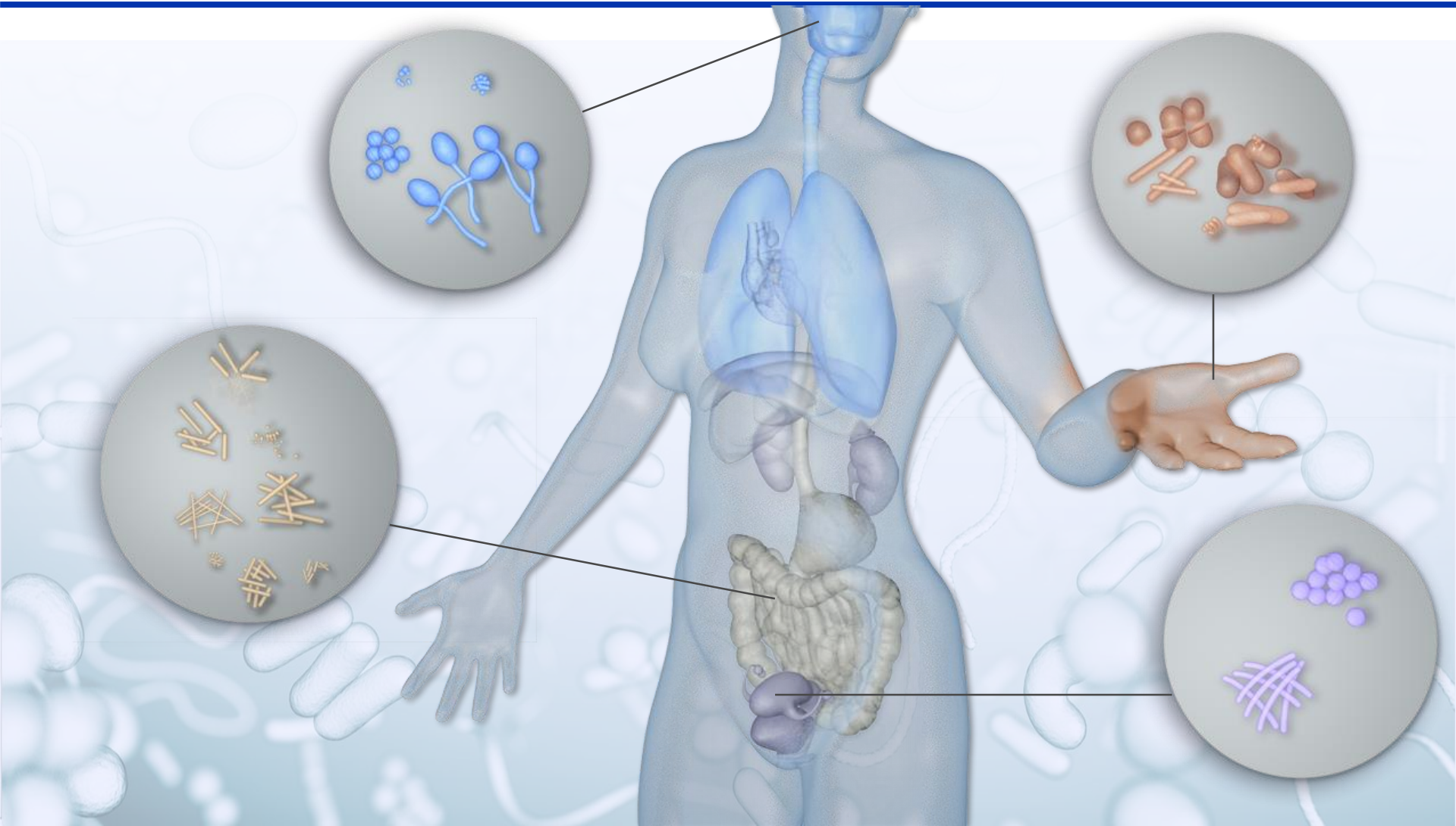
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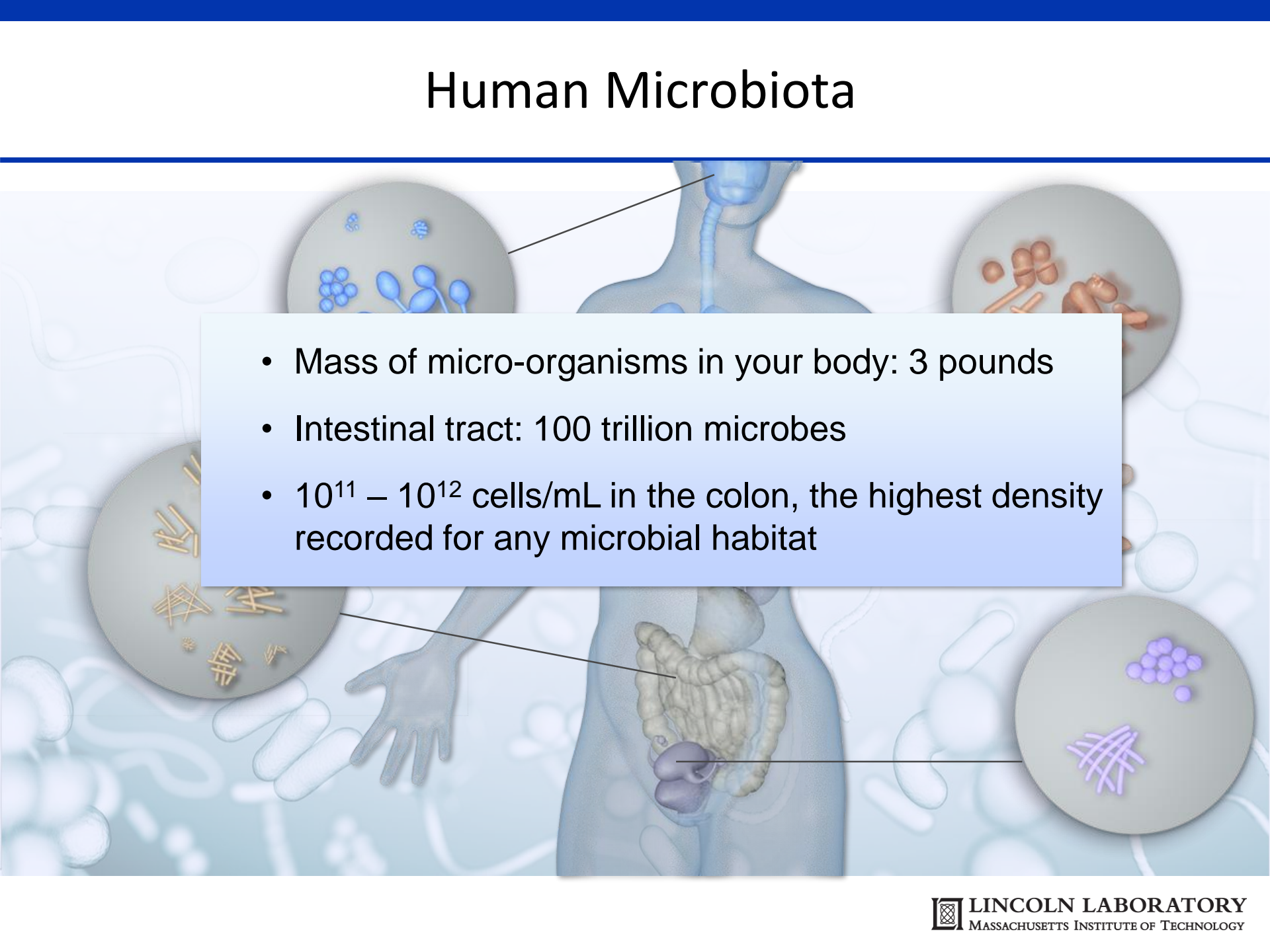
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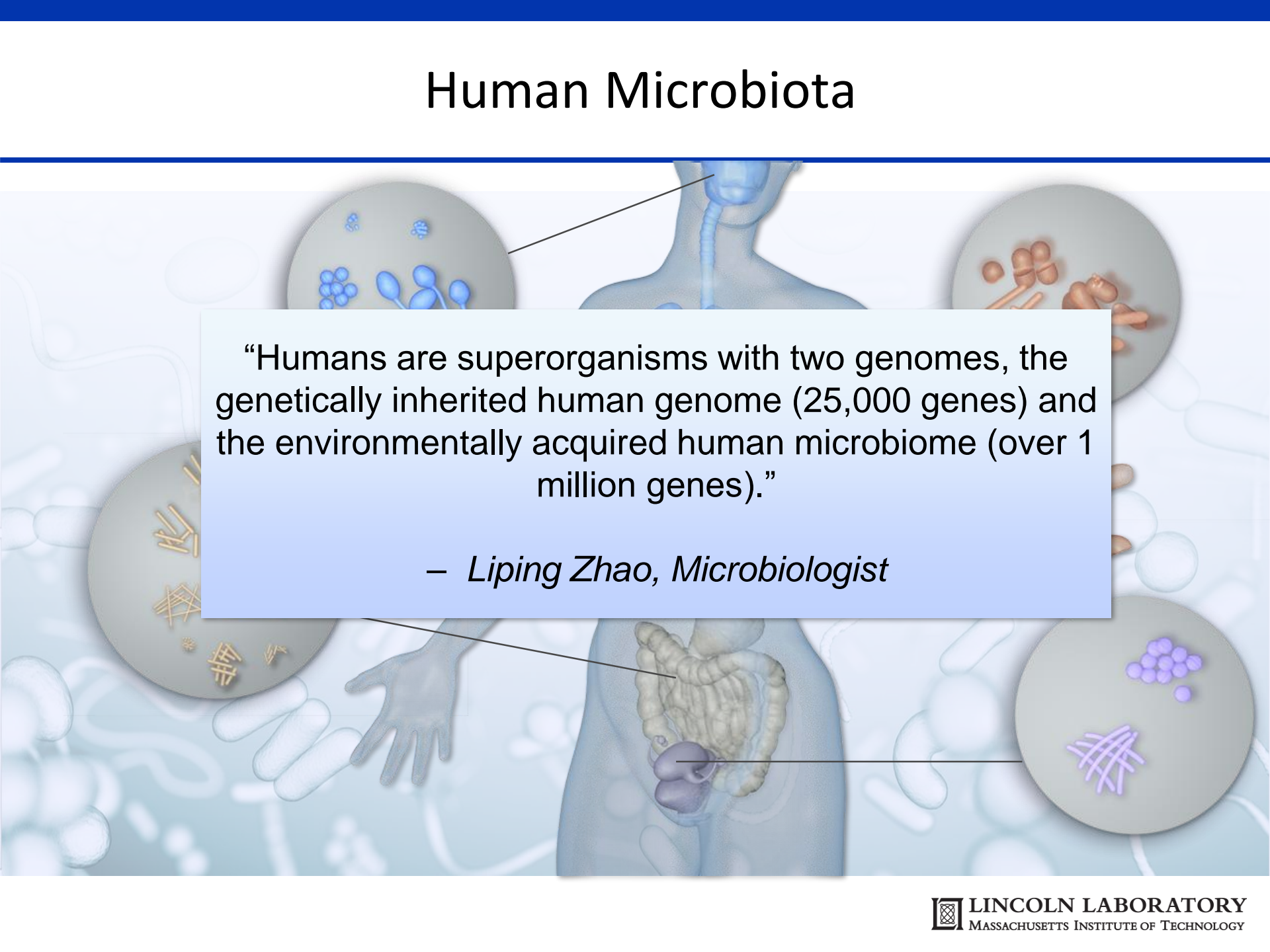
Human Microbiota



Human Microbiota

- 
- A diagram illustrating the human microbiota. It features a central human silhouette with lines pointing to four circular insets showing different microbial communities: the mouth (top left, blue cocci and bacilli), the gut (bottom center, purple cocci and bacilli), the skin (bottom left, yellow rod-like structures), and the hands (bottom right, purple cocci and bacilli). The background is a light blue gradient with faint, larger-scale microbial shapes.
- Mass of micro-organisms in your body: 3 pounds
 - Intestinal tract: 100 trillion microbes
 - $10^{11} - 10^{12}$ cells/mL in the colon, the highest density recorded for any microbial habitat

Human Microbiota

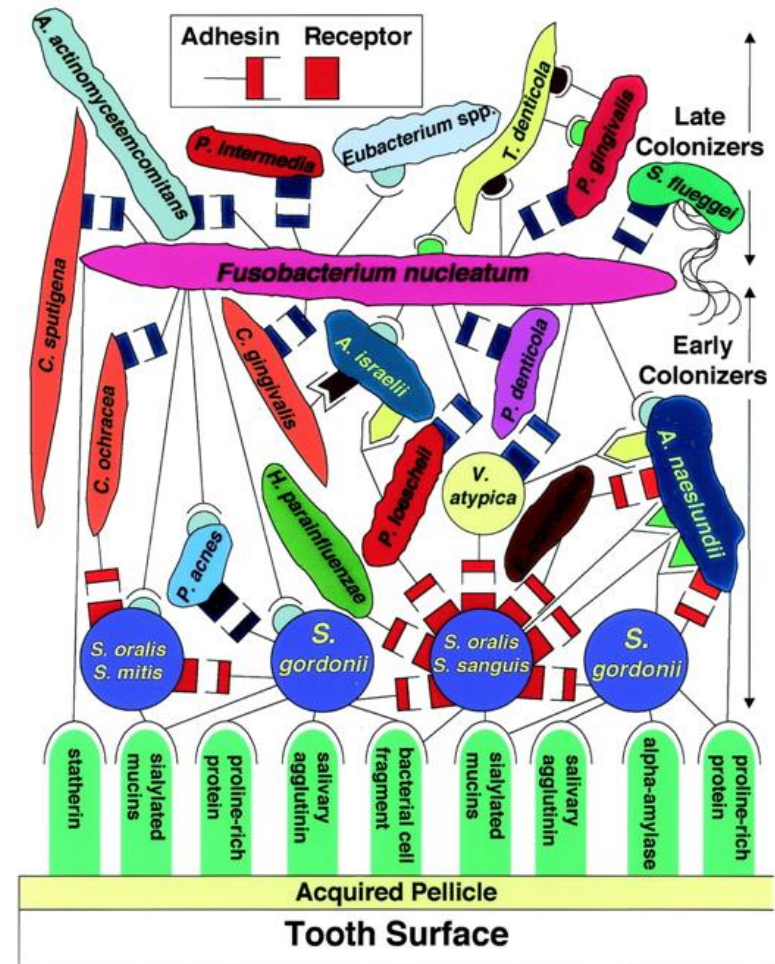
A background illustration showing a human silhouette with lines connecting various body parts to circular insets of microorganisms. The mouth is connected to blue, rod-shaped bacteria. The hands are connected to yellow, rod-shaped bacteria. The gut is connected to purple, rod-shaped bacteria. The skin is connected to orange, rod-shaped bacteria. The background is filled with various shapes representing different types of microorganisms.

“Humans are superorganisms with two genomes, the genetically inherited human genome (25,000 genes) and the environmentally acquired human microbiome (over 1 million genes).”

– *Liping Zhao, Microbiologist*

Oral Microbiome

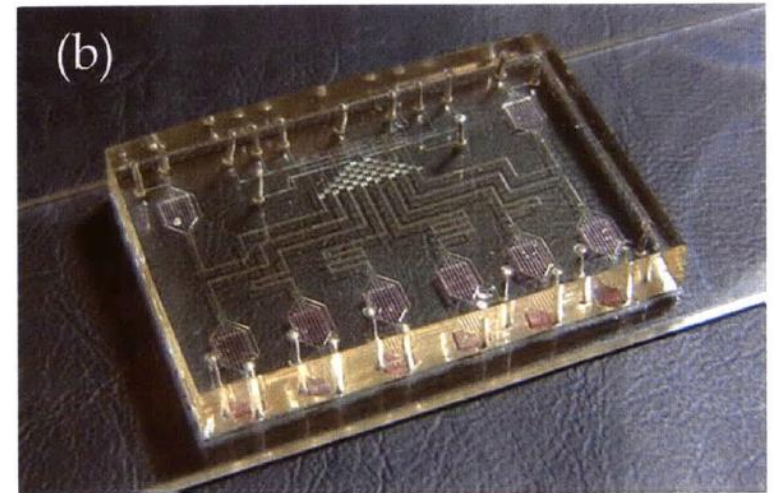
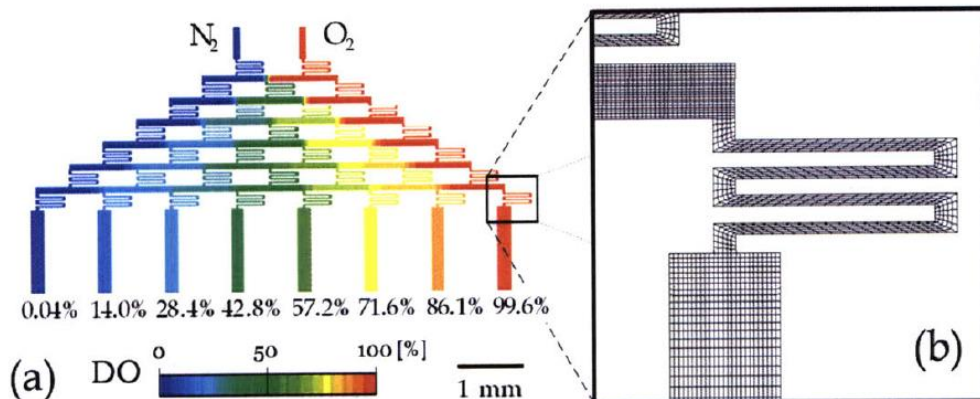
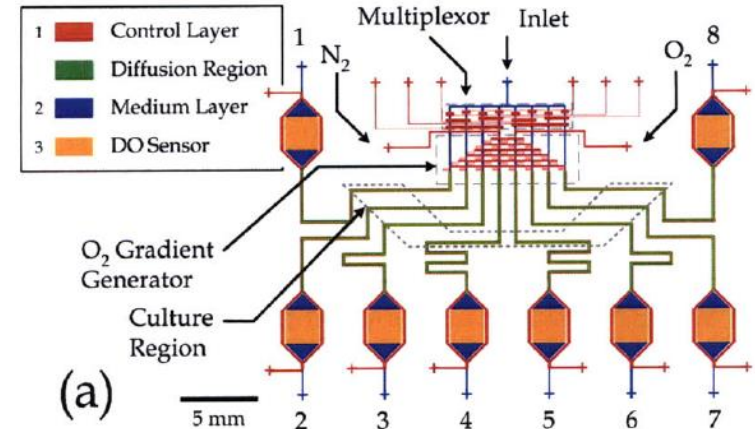
- Over 600 species of bacteria found in the mouth
- Complex biofilms are formed on the teeth consisting of a symbiotic network of aerobic and anaerobic bacteria
- Oral dysbiosis
 - Gum disease
 - Cancer
 - Heart disease



Kolenbrander et al. Microbiol. Mol. Biol. Rev. 2002;66:486-505

Emulating the Mouth to Study Dental Biofilms

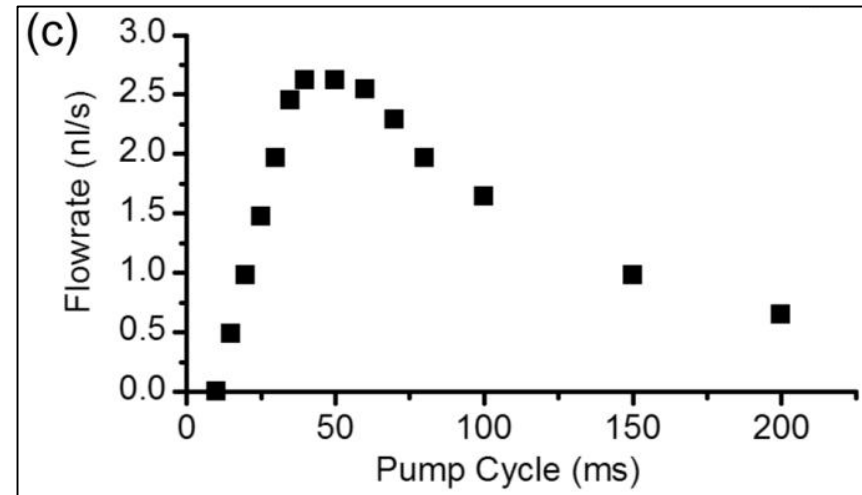
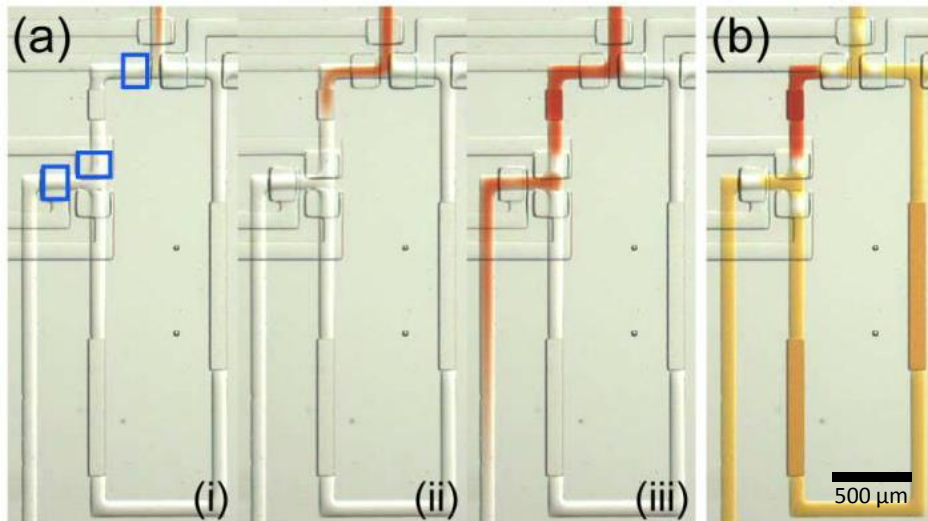
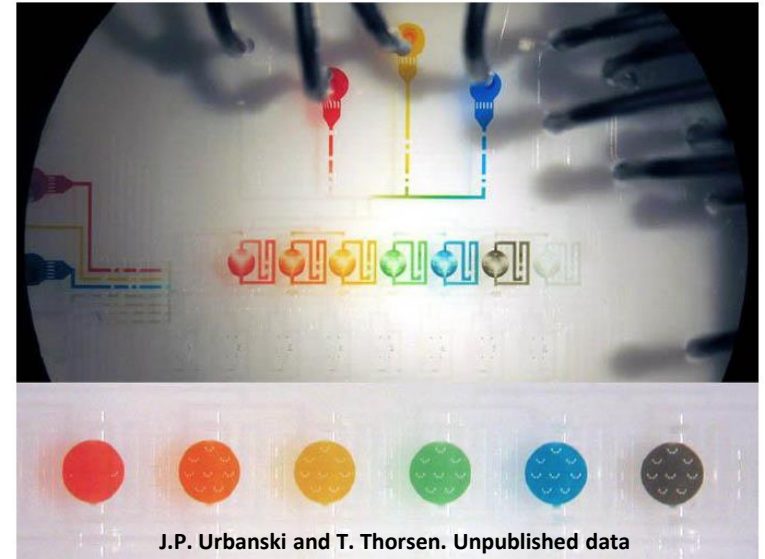
- Multilayer elastomeric PDMS microfluidic chips are an excellent platform for the growth and characterization of dental biofilms
 - Excellent gas permeability



R.H.W. Lam, M.-C. Kim and T. Thorsen. Anal. Chem. 2009, 81:5918-5924

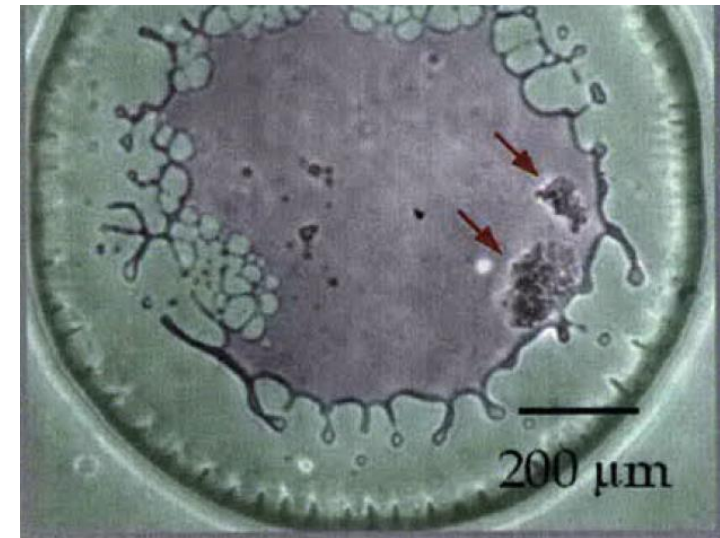
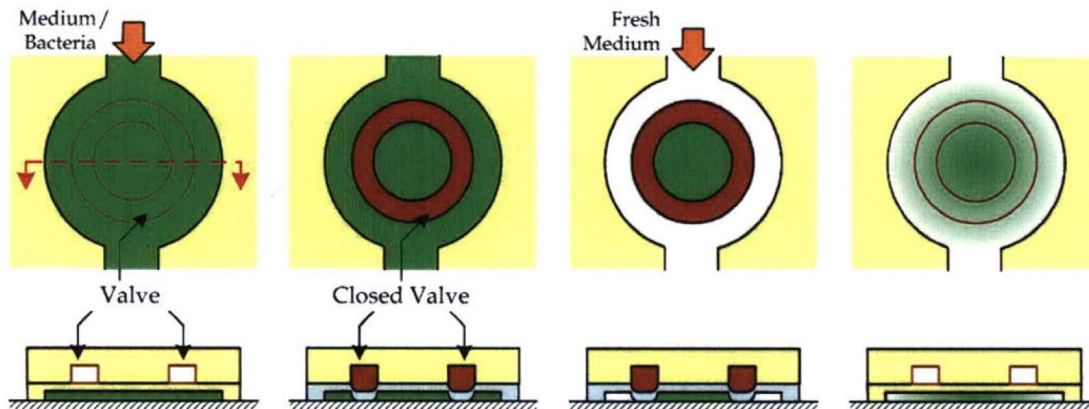
Emulating the Mouth to Study Dental Biofilms

- Multilayer elastomeric PDMS microfluidic chips are an excellent platform for the growth and characterization of dental biofilms
 - Excellent gas permeability
 - Programmable fluidic routing and mixing

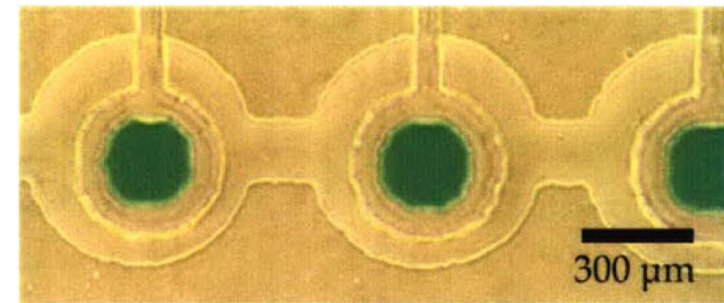


Emulating the Mouth to Study Dental Biofilms

- Multilayer elastomeric PDMS microfluidic chips are an excellent platform for the growth and characterization of dental biofilms
 - Excellent gas permeability
 - Programmable fluidic routing and mixing
 - Parallel seeding of bacteria cultures for testing under multiple environmental conditions

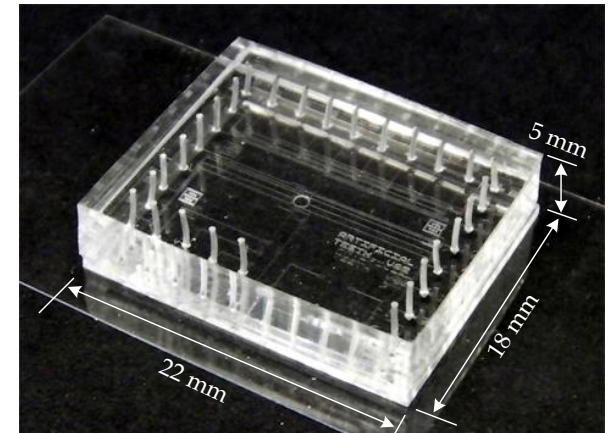
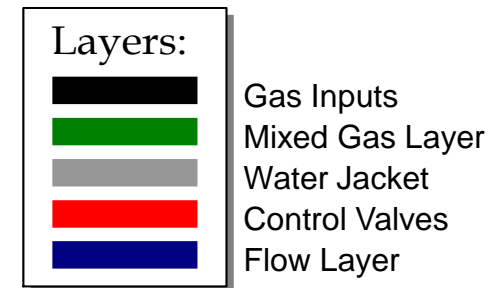
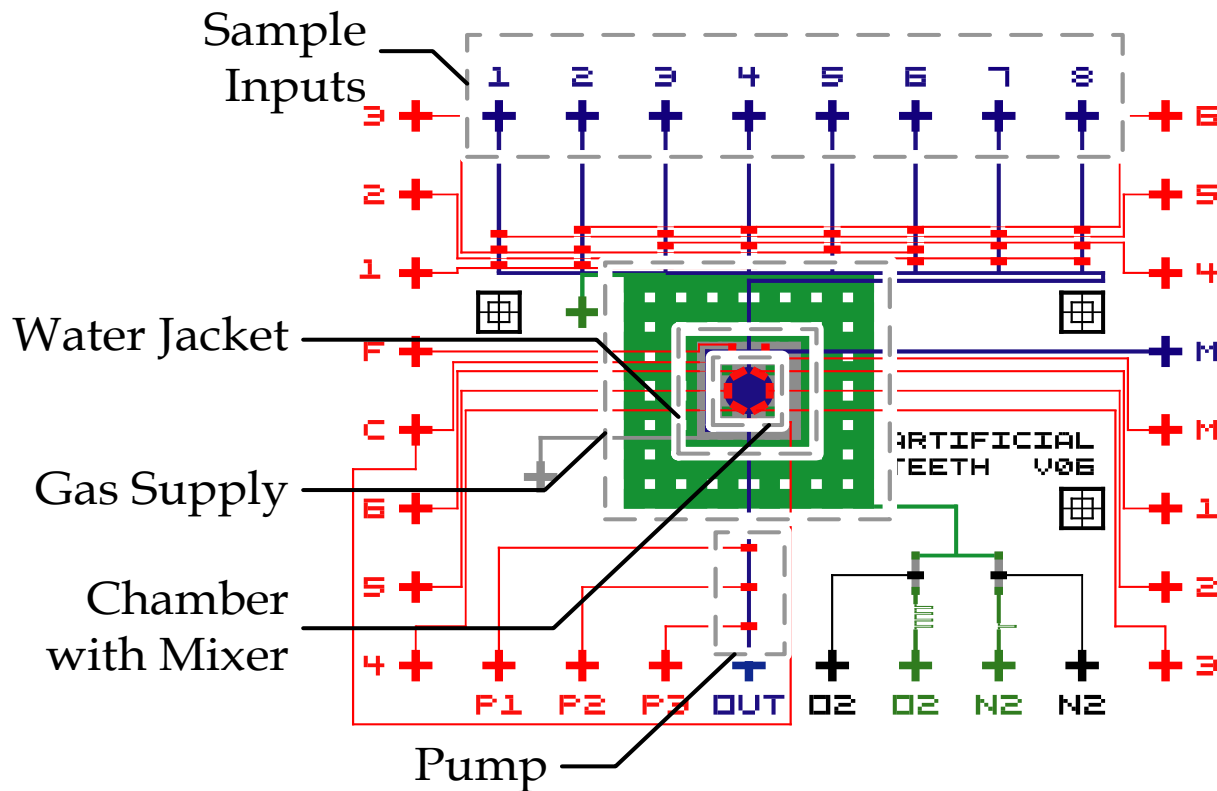


S. sanguis culture on polystyrene-coated PDMS

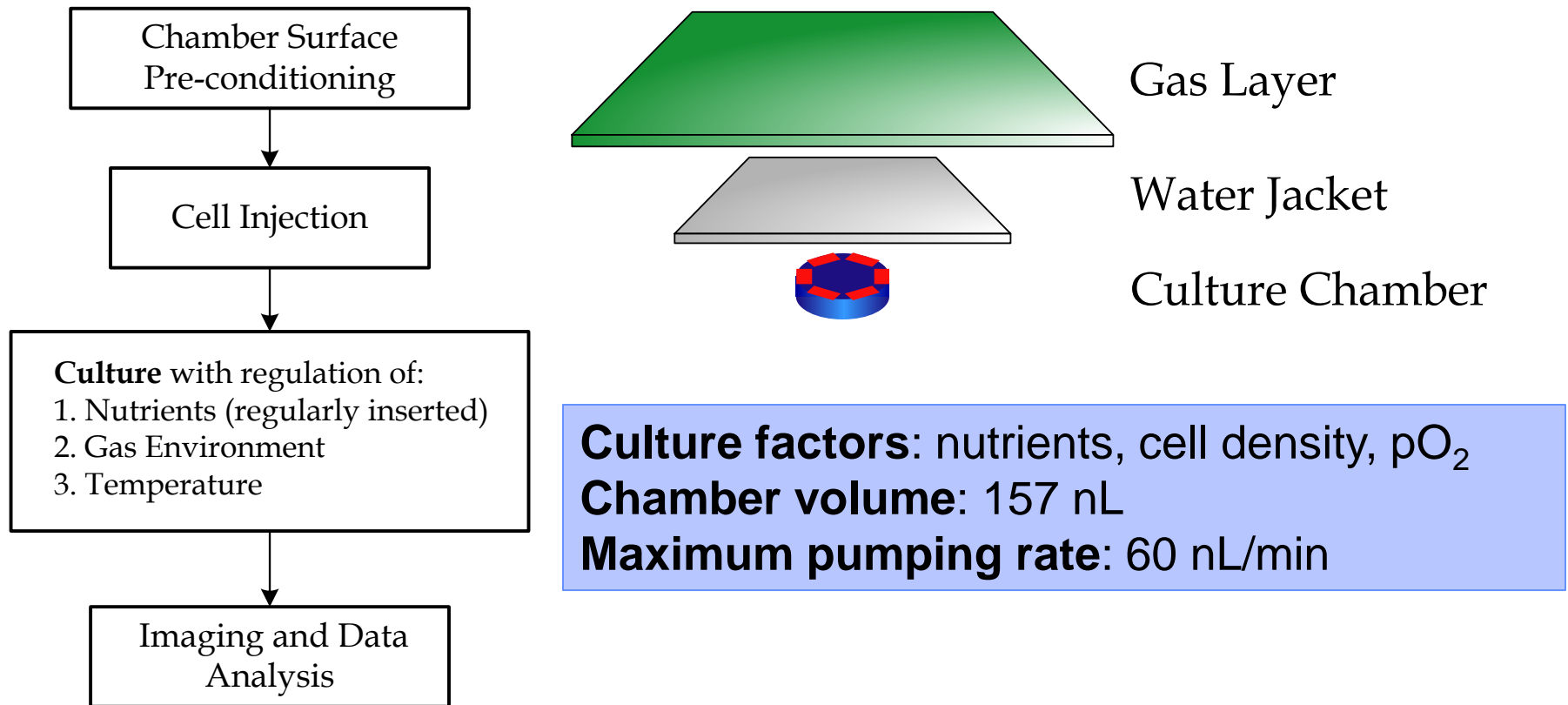


R.H.W. Lam and T. Thorsen. Unpublished data

Dental Biofilm Chip: Single Chamber Design



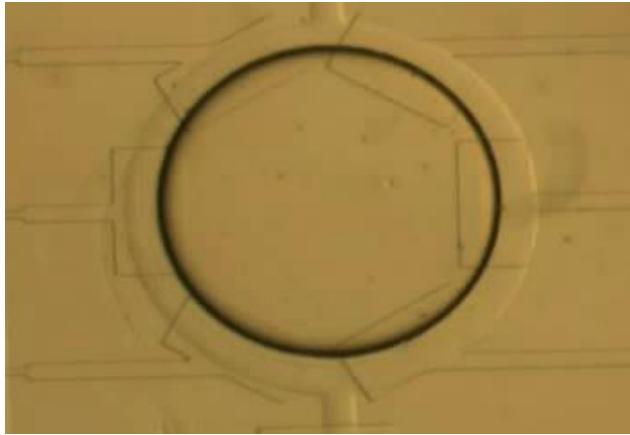
Single Chamber Chip Operation



Culture Chamber Mixer Design

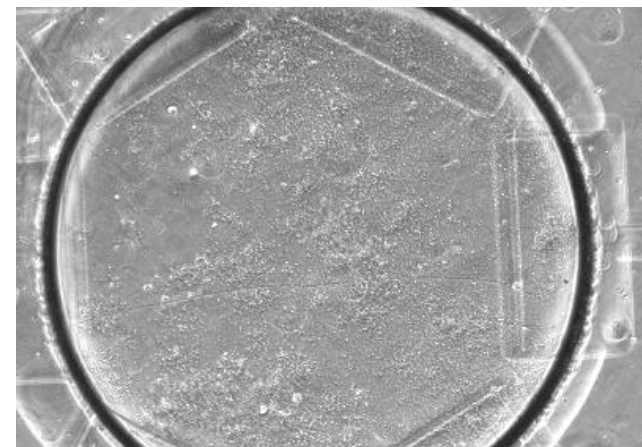
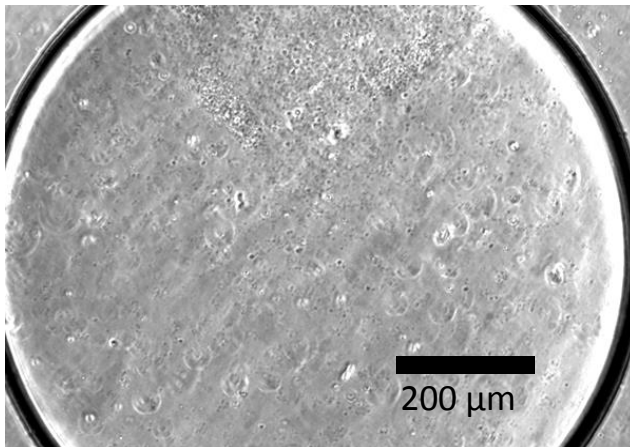
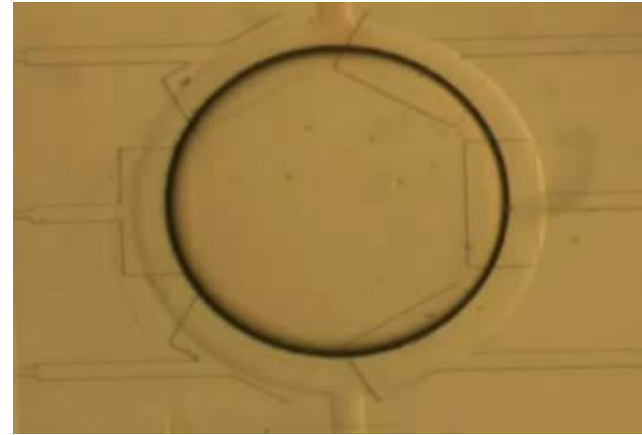
Without Mixing

Mixing time: ~5-8 min Speed: 8x

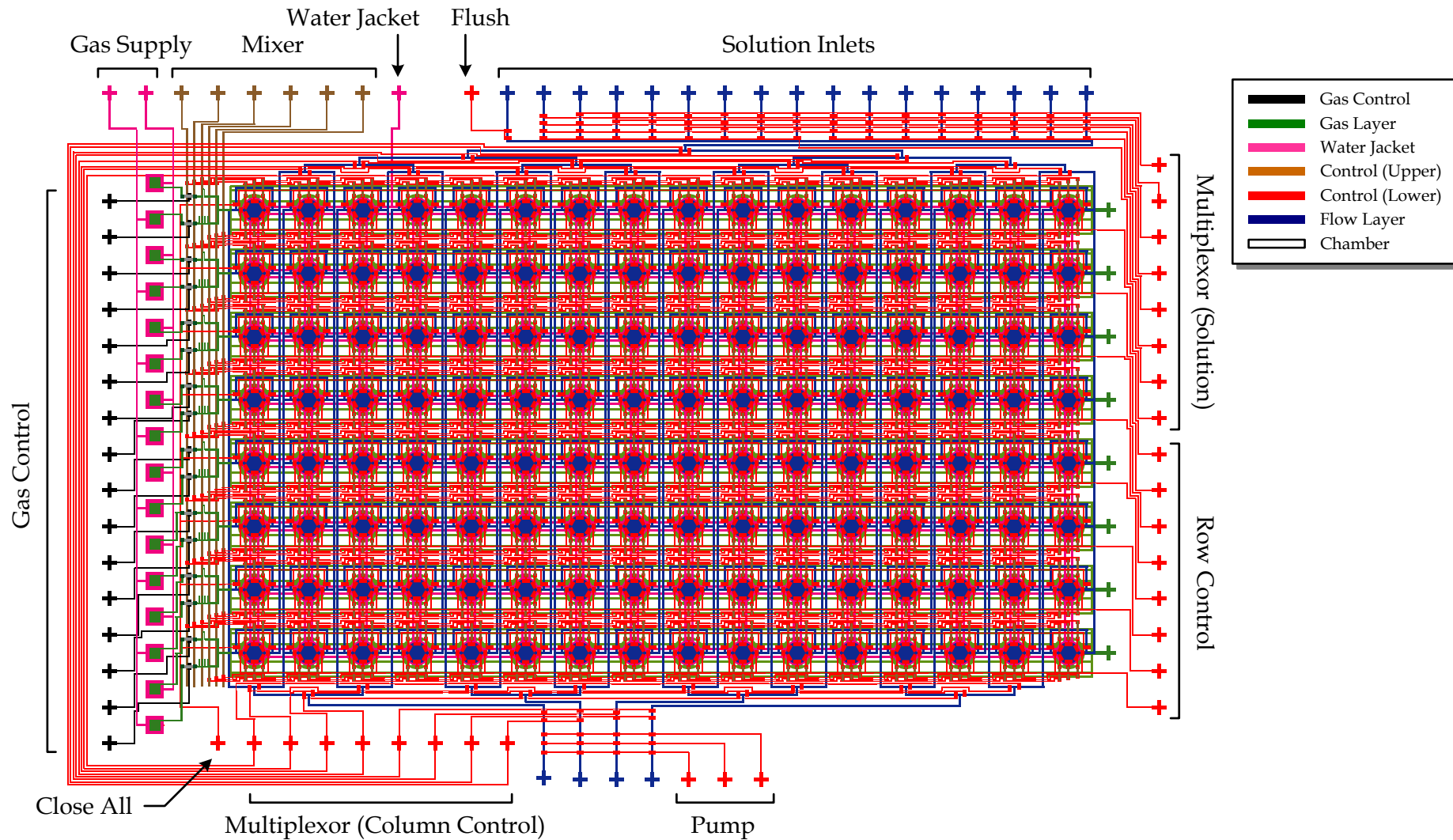


With Mixing

Mixing time: ~1 min Speed: 8x

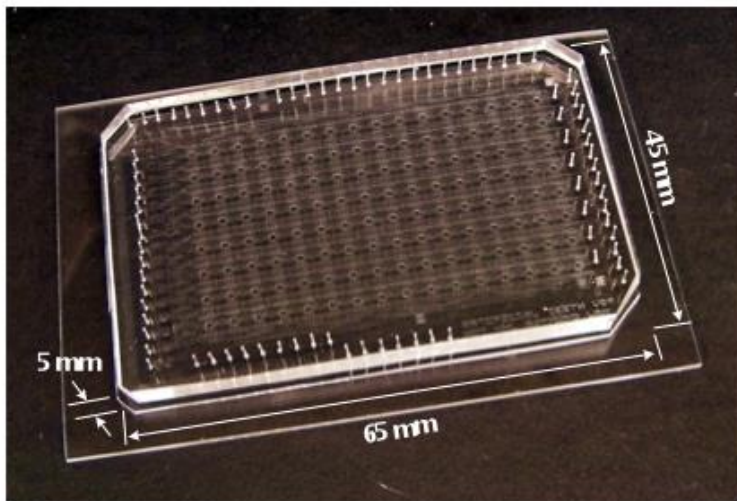


Chip Layout: 128-Chamber Design

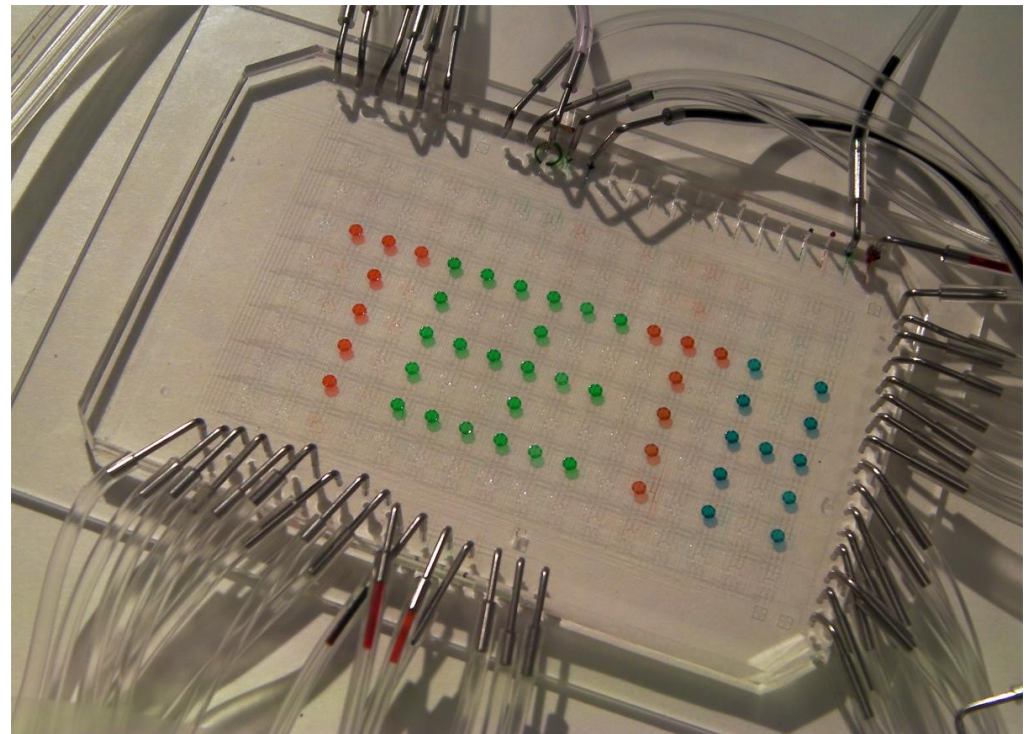


Chip Layout: 128-Chamber Design

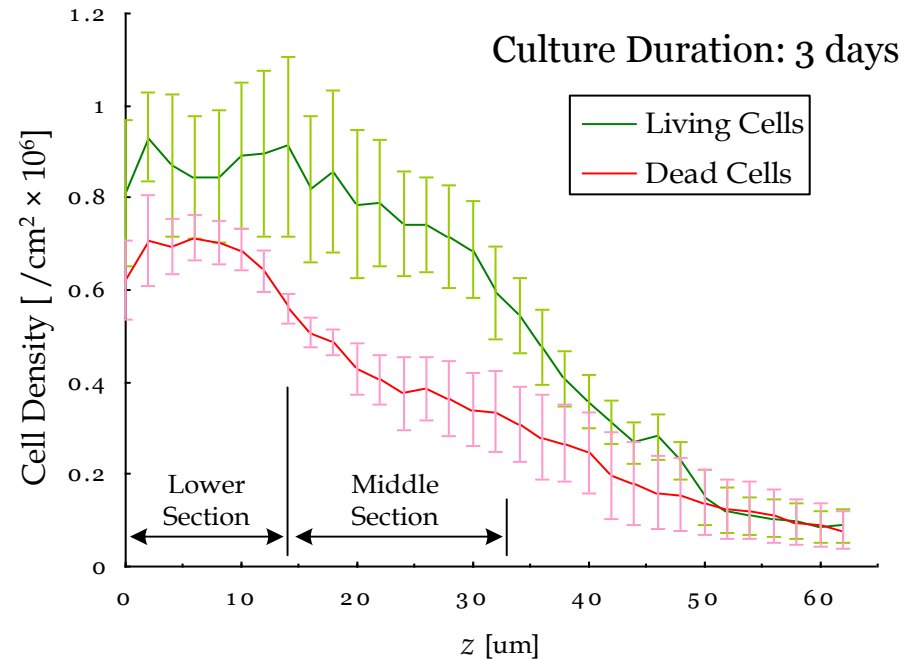
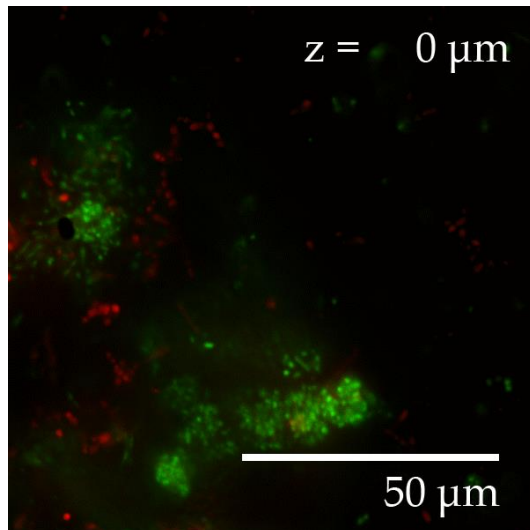
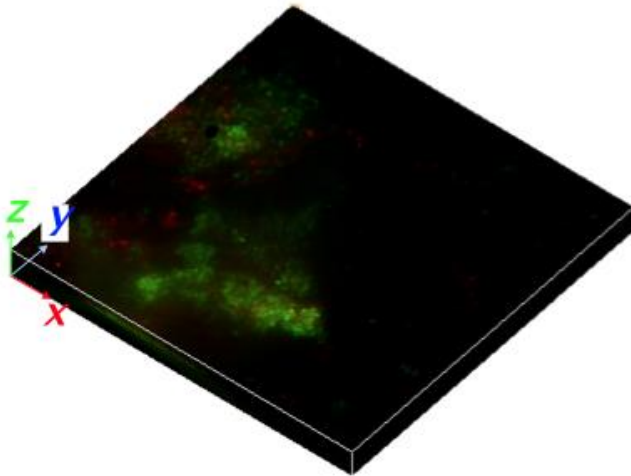
Fabricated Device



Sample Operation: Solution Injection



Microfluidic Biofilm Culture: Growth Characteristics

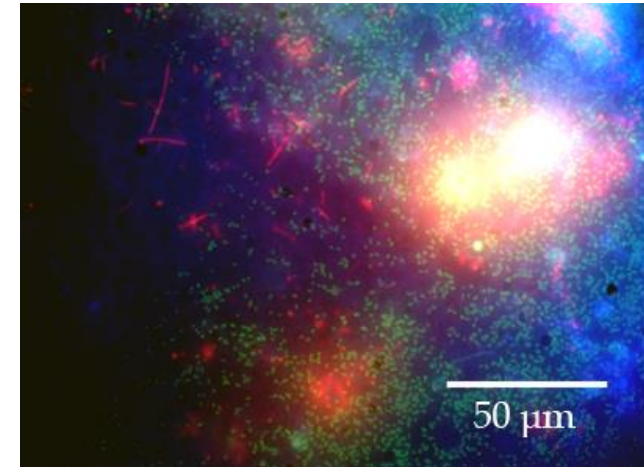


- Live/dead stain using BacLight kit
- Biofilm composition at different heights measured by inverted fluorescence microscopy (60x objective) and a z-axis motorized stage


Living Cells Dead Cells

Microfluidic Biofilm Culture ID: Fluorescence In-Situ Hybridization


- Fluorescence *In-Situ* Hybridization (FISH) for pathogen identification: Cells are fixed (paraformaldehyde) followed by hybridization-based labeling with a fluorescently-tagged oligonucleotides

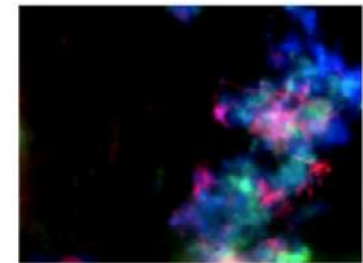
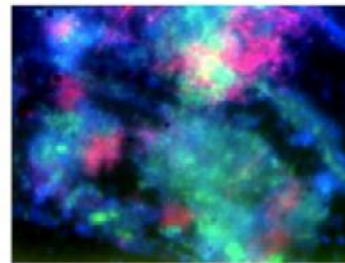
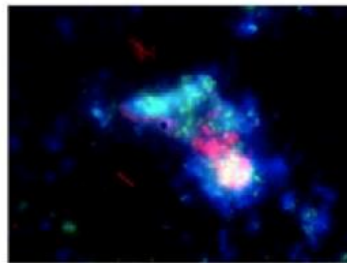
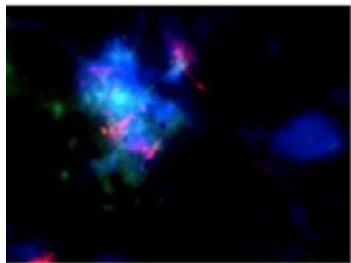


 All Bacteria

 *Streptococci*

 *F. Nucleatum*

 100 μm



The Gut Microbiome and Human Health

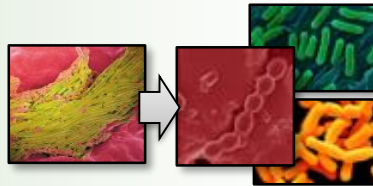
Predicting/Understanding Responses
(food, drugs, environmental insults, stress)



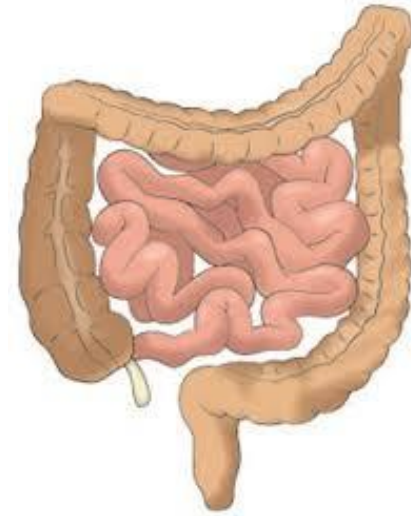
Sensing
(trauma, disease, exposures)



Responding
(mitigate, heal)



Enhancing
(endurance, resilience, immunity, mood)



Gut microbiota associated with physiological and psychological disorders

- Vaccine efficacy
- Inflammation
- Gastroenteritis
- Parkinson's
- Arthritis
- Immune system function
- Cancer treatment response

Platforms for Gut Microbiome Studies

In vivo

Gnotobiotic (Germ-free) Mice



- Ideal physiology
- Poor temporal and spatial resolution

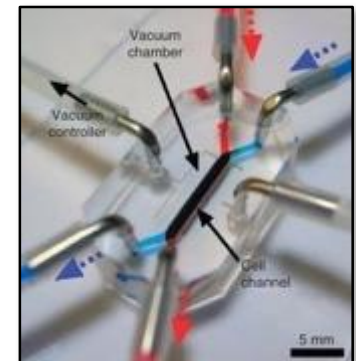
In vitro

Simulator of the Human Intestinal Microbial Ecosystem (SHIME)



- Improved temporal and spatial resolution
- Poor physiology

“Gut-on-a-Chip”



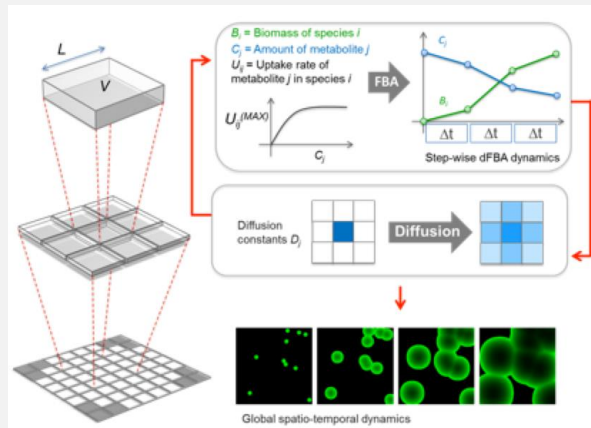
Huh et al., *Nature Protocols*, 2013

- Improved resolution and physiology
- High complexity

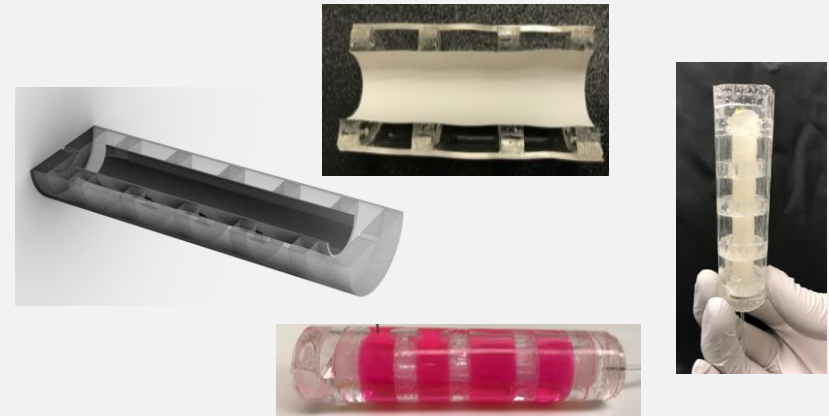
- No adequate platform exists to study the microbiome
- MIT LL is tackling the issue through development of new platforms

MIT Lincoln Laboratory Gut Microbiome R&D

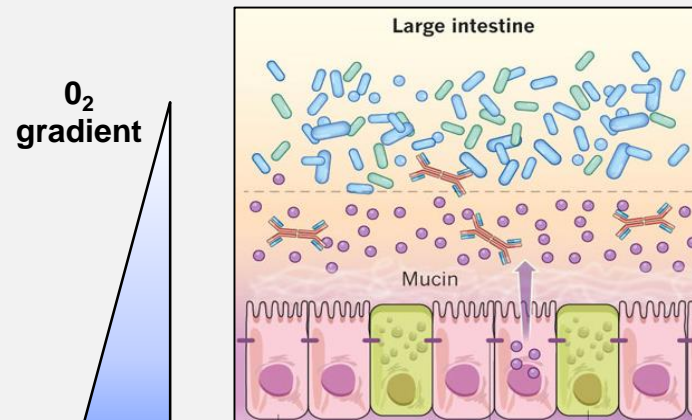
In Silico Modeling of Communities



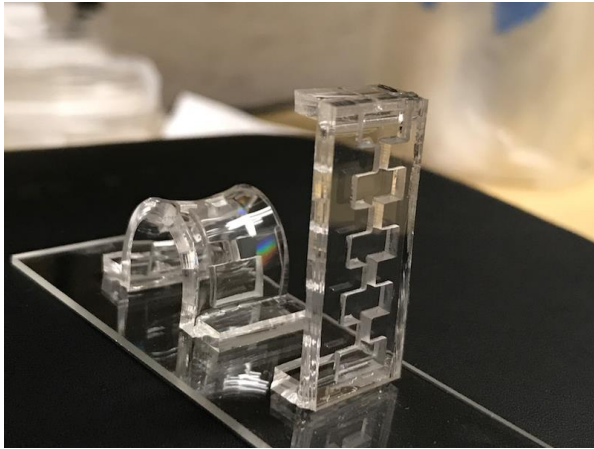
Morphology



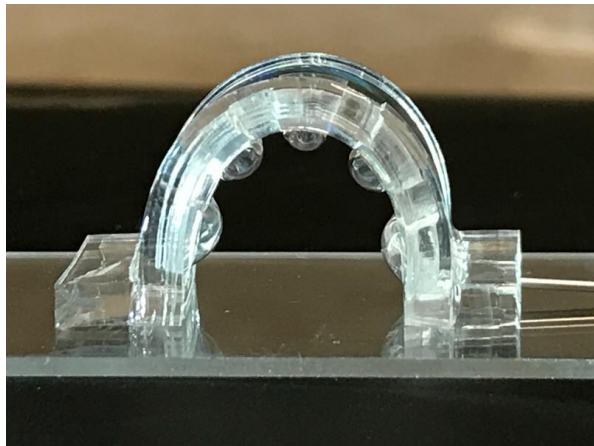
Human-Microbe Interface



Mimicking Gut Physiology In Microfabricated Devices



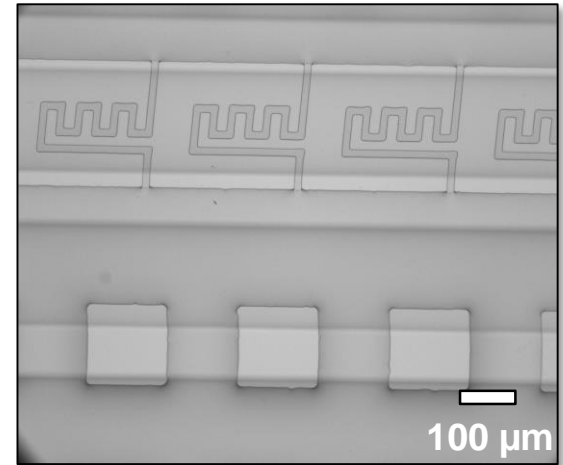
Bonded and Unfolded Valve



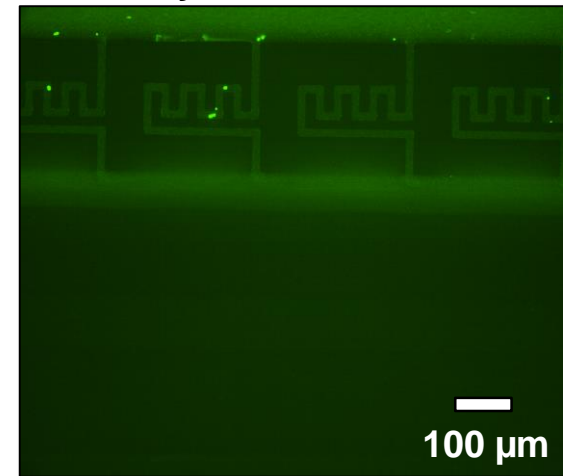
Pneumatic Actuation



**Integrated Microchannels
for Gas Control**

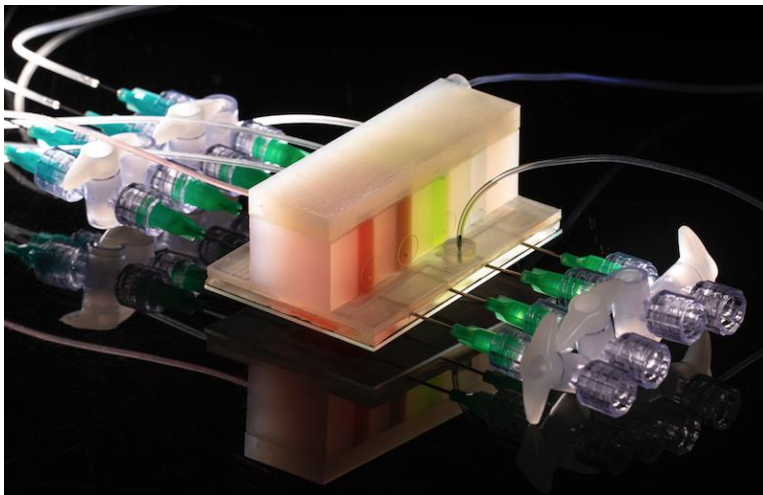
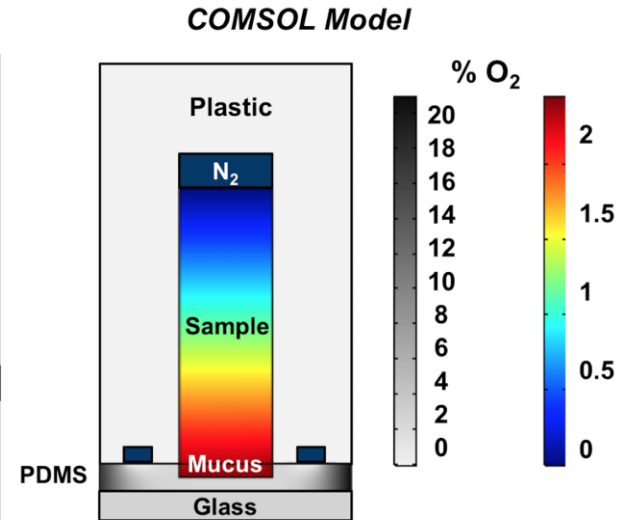
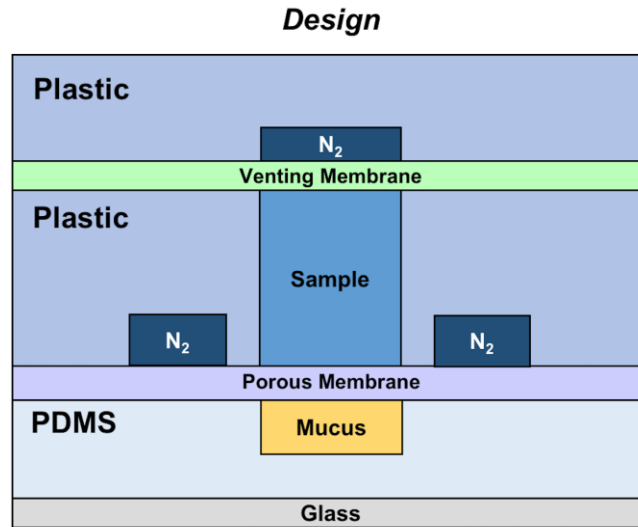
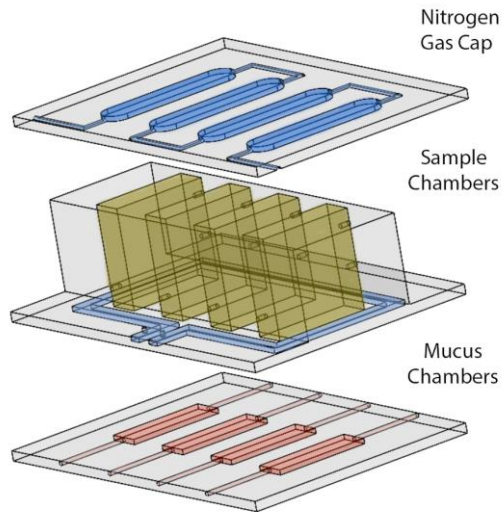


Dry, air-filled device



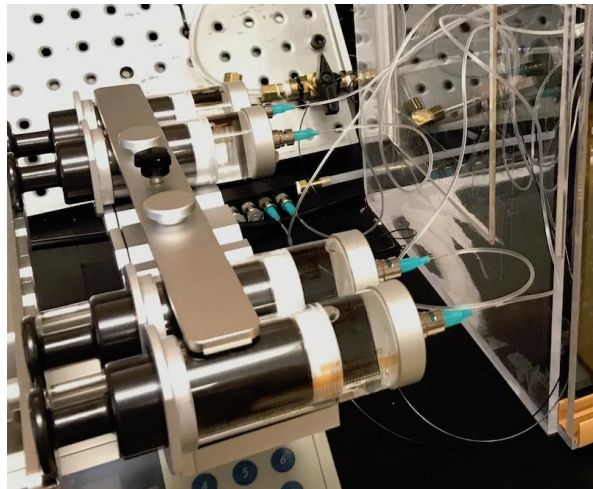
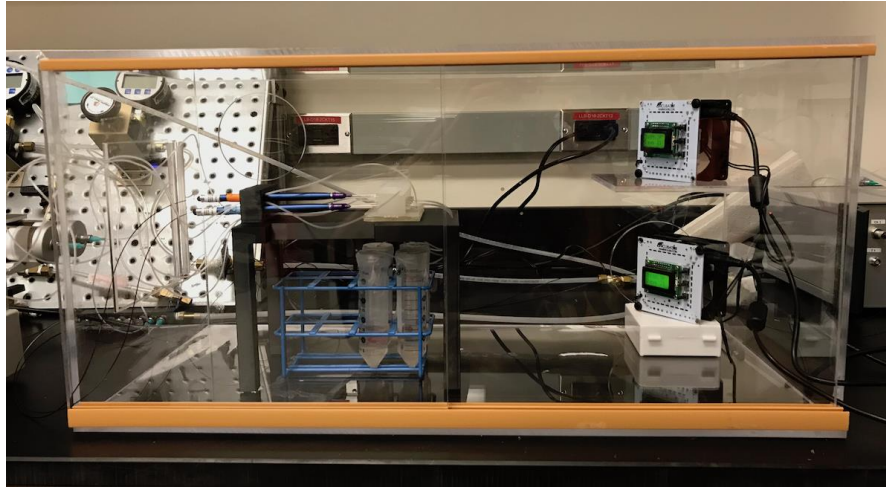
~100 μm thick mucin secretion

Milliliter Scale Artificial Gut Modules

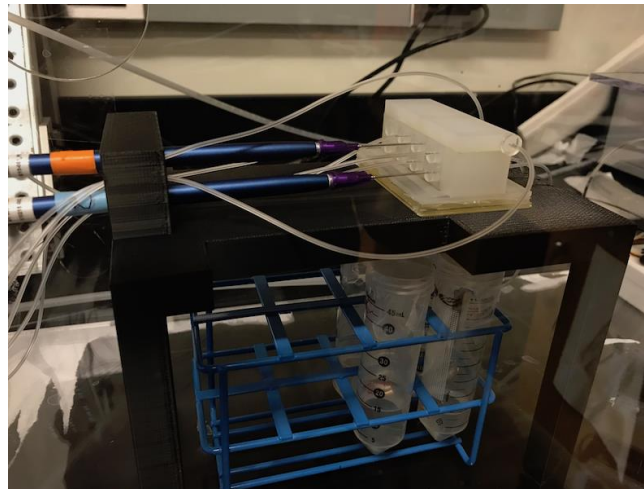


- Multimaterial device designed to mimic the microbial environment of the colon
- Oxygen gradient supports the co-culture of strict and facultative anaerobes found in the gut
- Mucus layer supports the colonization of bacteria that thrive close to the colon wall

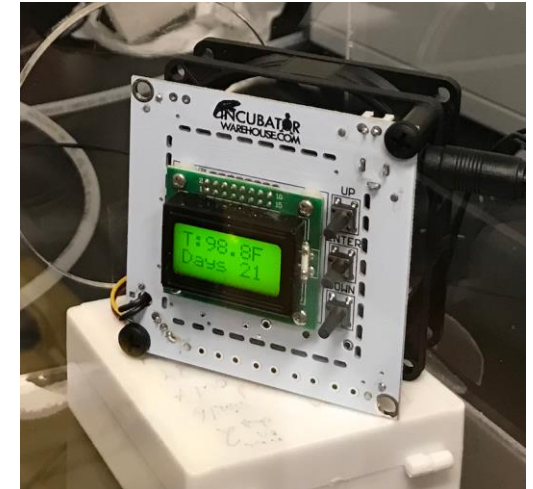
Integrated MIT LL Benchtop Artificial Gut



Programmable Media Feeds



Dynamic Device O₂ Monitoring



Convective Thermal Regulation

Acknowledgements

MIT Lincoln Laboratory

Dr. Catherine Cabrera	Dr. Peter Carr	Dr. David Walsh
Dr. James Comolli	Johanna Bobrow	Eric Hollihan
Catherine Van Praagh	Dr. Mordechai Rothschild	

MIT

J.P. Urbanski (ME Doctoral Student)
Min-Cheol Kim (Post-Doc)

City University Hong Kong

Prof. Raymond Lam

Funding Sources

MIT Lincoln Laboratory Biomedical Line
NIDCR (5R21017412)