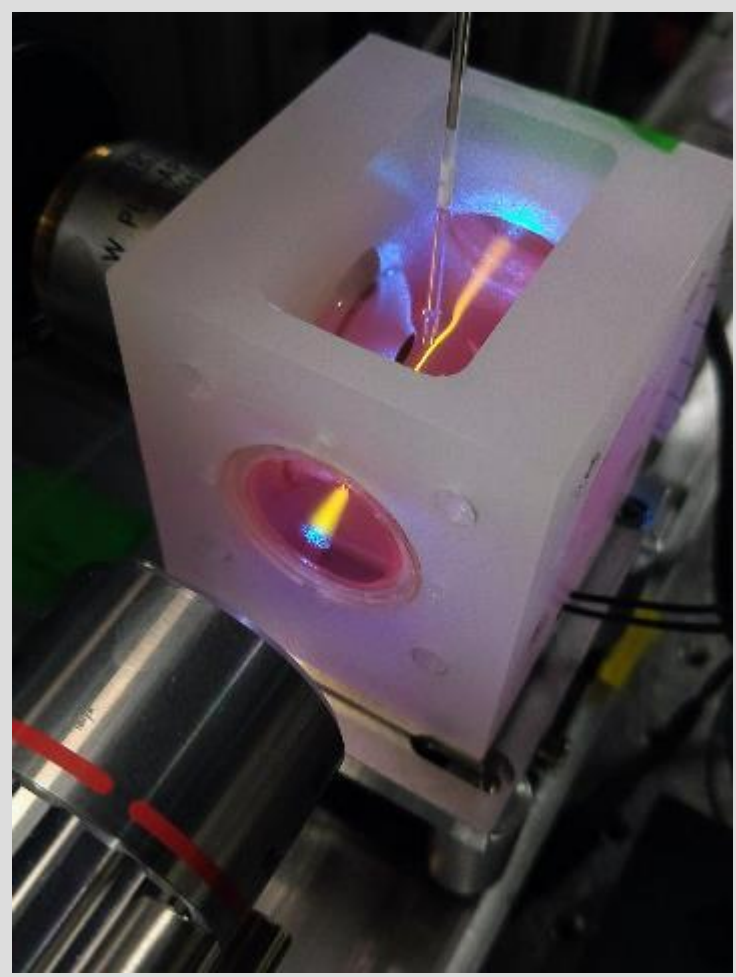
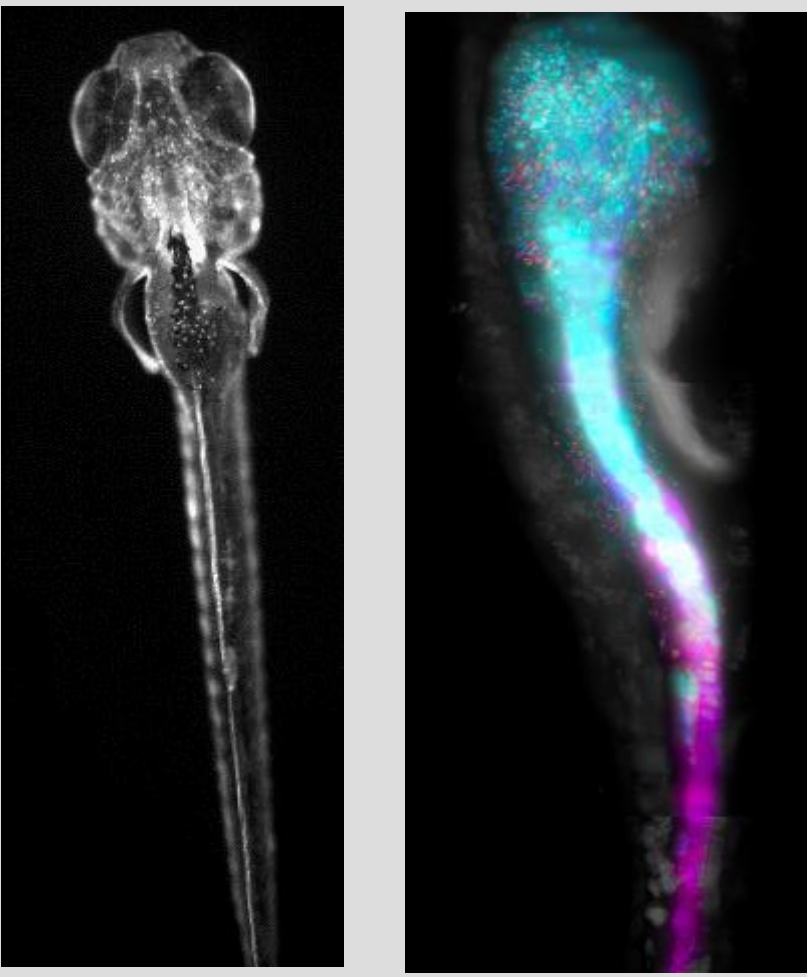




Microbes, Microscopes, and More

An overview of the *Parthasarathy Lab*

Department of Physics, The University of Oregon



Biophysics

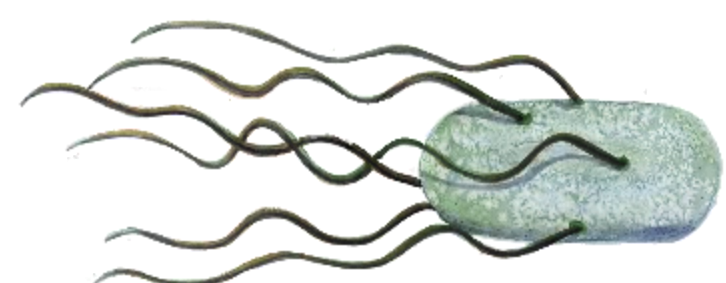
Our lab explores **biophysics** – we ask how physical laws are manifested in the living world and how the physical features of biological materials guide and constrain life.

We primarily investigate **bacterial communities**, especially the mysterious ecosystems contained inside each of us (and all other animals).

We develop new **experimental tools**, such as 3D microscopes, and **computational tools**, such as cutting-edge image analysis methods, to make these studies possible. Many of our experiments are unique, revealing phenomena that have never before been seen!

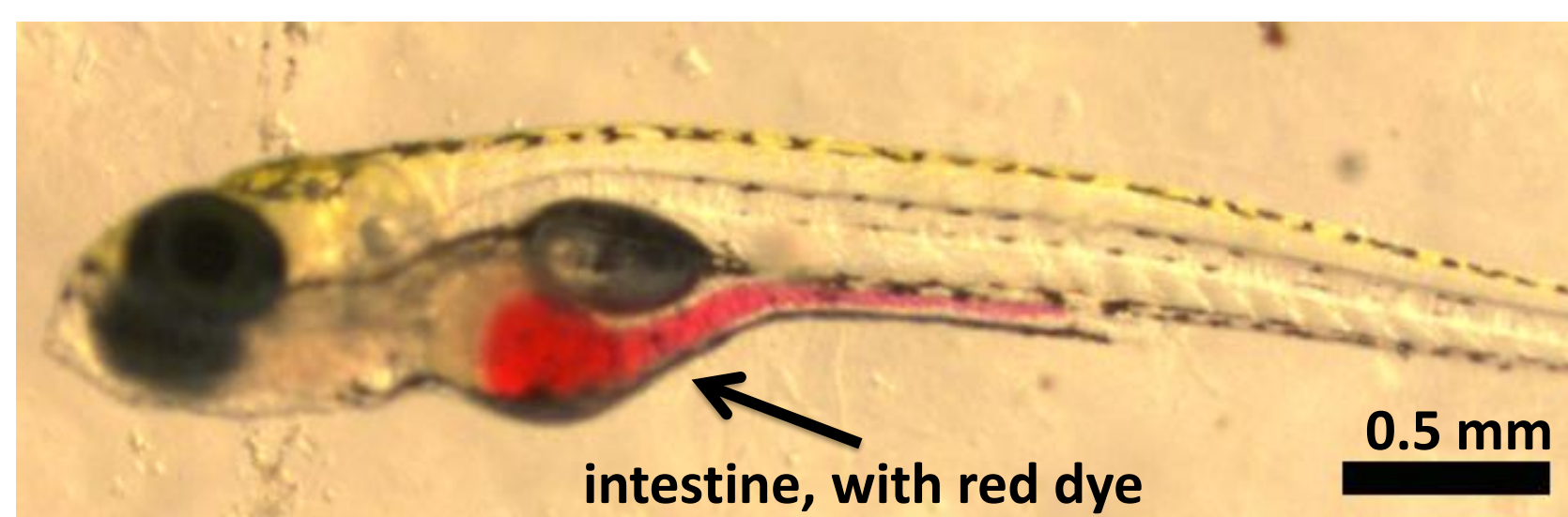
Physics and the Gut Microbiome

Bacterial communities are perhaps the quintessential **active matter** system. Individual microbes move, grow, divide, forage, share material with their neighbors, kill their neighbors, etc. Somehow, robust characteristics emerge from the interactions of individual cells. We seek to understand how this occurs. We focus most of our efforts on a particularly important class of bacterial communities: the gut microbiome.



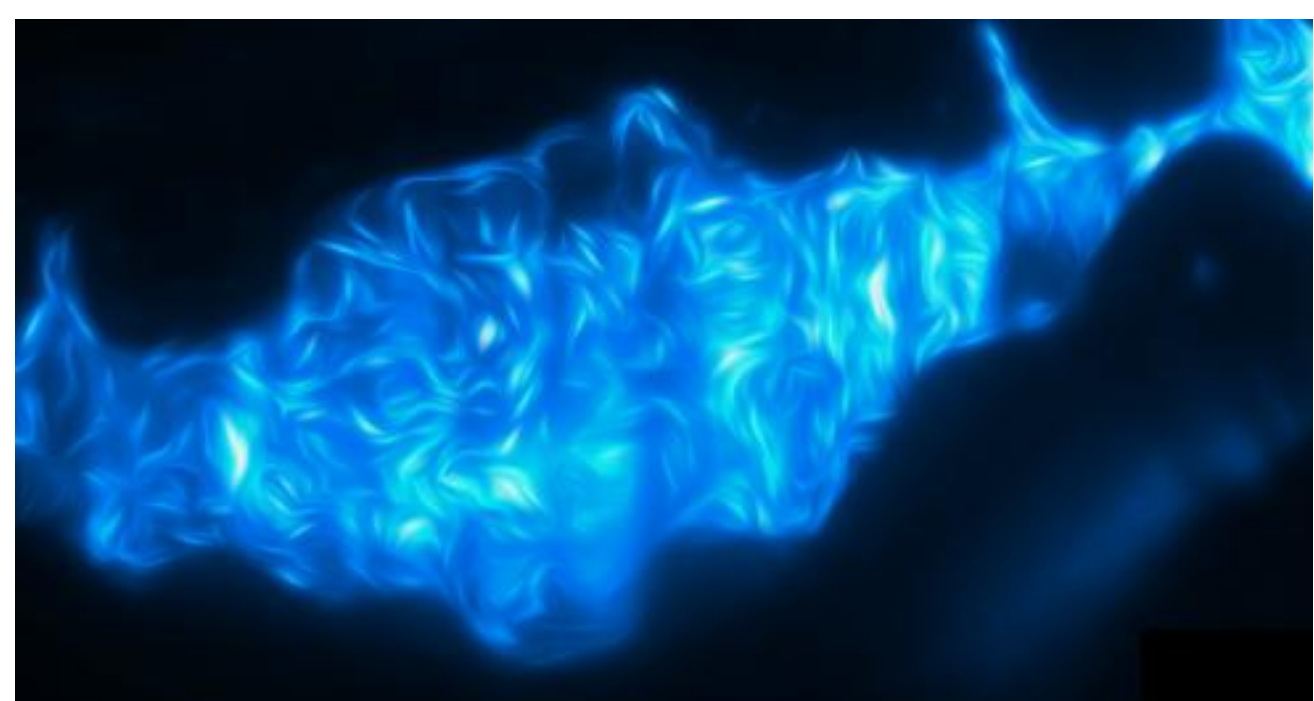
You are not (only) human. Each of us is host to trillions of microbes, mostly resident in the intestines, whose major roles in health and disease are just beginning to be grasped. Conventional methods provide little insight into the **physical structure and temporal dynamics of the gut microbiome**, properties that must influence the functioning of this multispecies ecosystem. Can we discover and make sense of these properties?

To study the gut microbiome we use a powerful model system: **zebrafish**. Larval zebrafish (shown) are transparent, physiologically similar to humans and other vertebrates, and amenable to techniques that let us **control the gut microbiome**, for example by creating fish devoid of all microbes and then introducing specific bacterial species.



Microbial Communities

What do gut bacterial communities look like? Using the zebrafish model system and three-dimensional imaging methods, our lab obtained the first cell-resolved images of bacteria inside a live, vertebrate gut! Different species show different characteristics of motility and aggregation, which can be influenced by other species.



This *Vibrio* species lives as highly motile individuals. Shown: a composite of several movie frames, with motile bacteria appearing as extended tracks. Image height: $\approx 200 \mu\text{m}$.

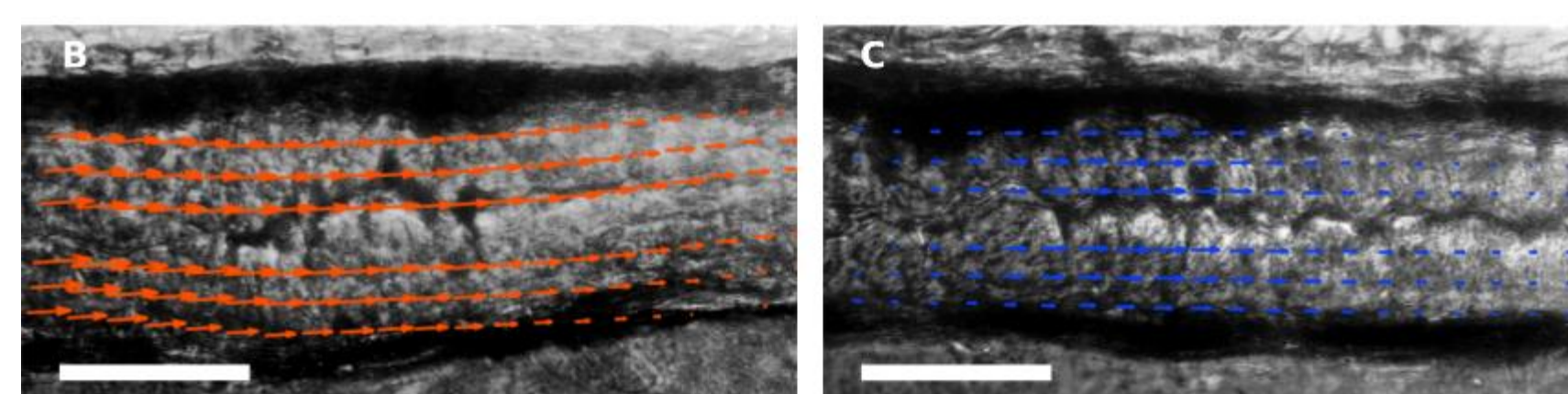
Questions

How can bacteria invade the gut and displace resident microbes?

Vibrio cholerae, the pathogen that causes cholera, as well as zebrafish-native *Vibrio* species, possess a “Type VI Secretion System” (T6SS): a syringe with which the bacterium **stabs adjacent cells** and delivers toxins.



Image velocimetry of intestines invaded by *Vibrio* with (left) and without (right) the T6SS. Bar: $50 \mu\text{m}$

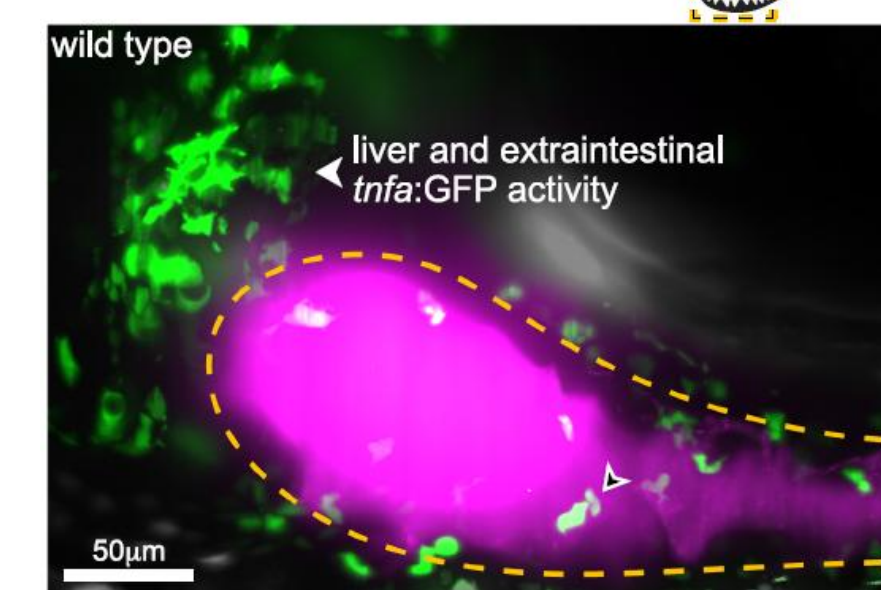


We discovered that the T6SS can alter the **mechanical contractions of the gut** – a previously unknown ability of bacteria to manipulate animal physiology! The resulting forces can drive the expulsion of resident microbes. What links bacterial stabbing and gut motility? ...

How does the immune system respond to gut microbes?

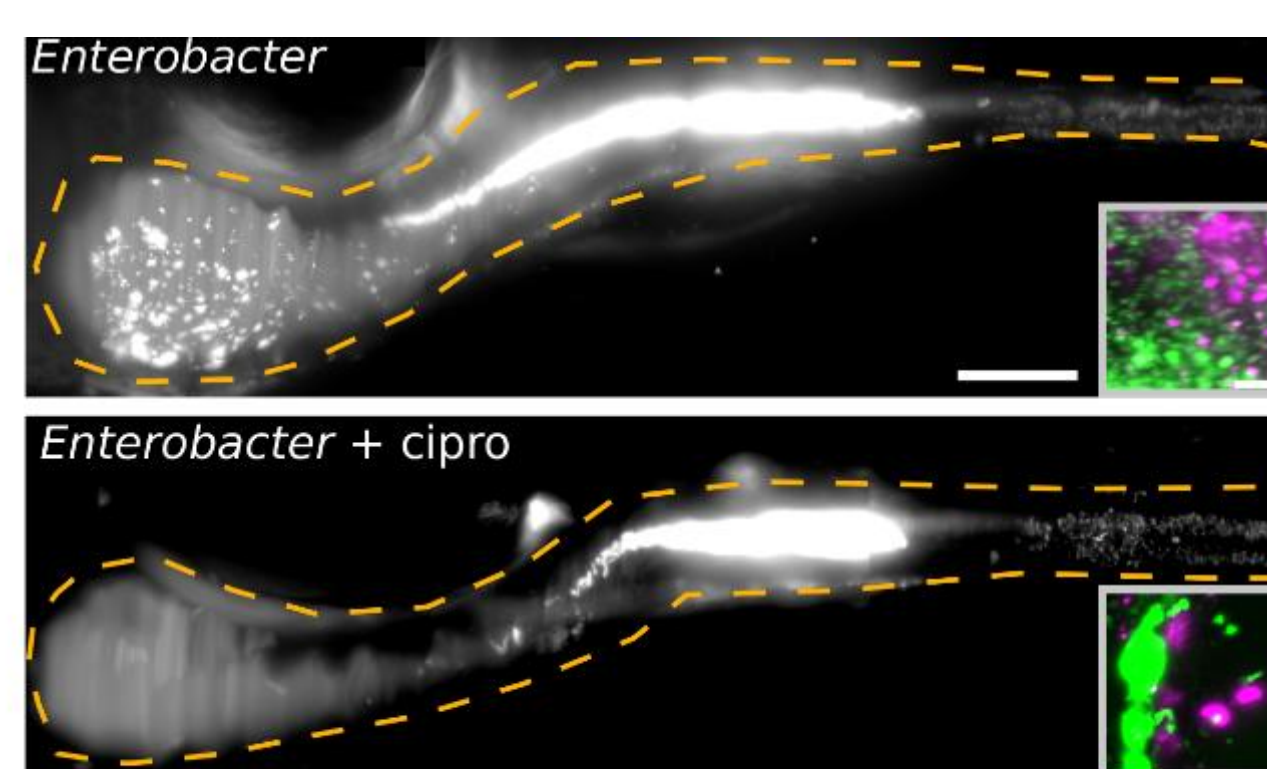
... The immune system! Using fluorescent reporters of immune cell types and signaling behaviors, we found that **bacterial motion** can stimulate immune activity. Moreover, we discovered that bacteria-induced spatial reorganization of immune cells can modulate the peristaltic contractions of the gut.

Shown: Dense, motile gut bacteria (magenta) and a reporter of immune cell activity (green) in a live zebrafish.



How stable are gut bacterial communities?

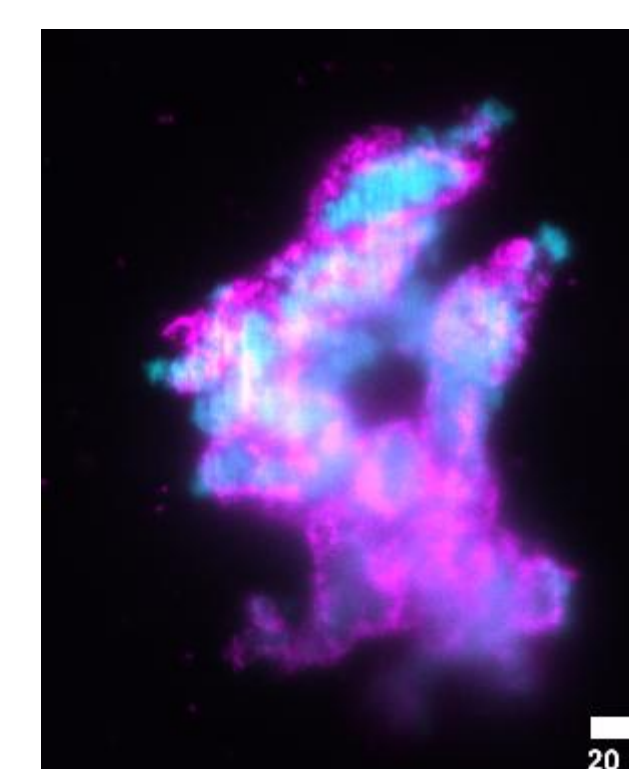
We study microbiome stability, especially in response to perturbations like **antibiotics**. We discovered that even weak antibiotics can have strong effects on gut bacterial populations, due to changes in bacterial morphology, motility, and transport. Shown: an *Enterobacter* species, with and without weak antibiotics; the antibiotic suppresses the formation of new bacterial clusters, leading to a population collapse. Bar: $200 \mu\text{m}$.



We’re also developing new methods that, for example, allow feeding of prey such as rotifers with minimal introduction of unknown microbes. Shown: A rotifer amid fluorescent bacteria. (Merged frames from a movie; time=color.)

Can we study bacterial aggregation in vitro?

Aggregation, we’ve found, is central to the population dynamics of gut microbial communities. Can we recapitulate 3D aggregation behavior *in vitro*? We’ve developed methods to do so, allowing questions about depth-dependent growth, geometric signatures of competition, and more to be investigated. Shown: one optical slice of an *in vitro* aggregate of two gut-native bacterial strains.



How are social interactions and gut bacterial communities linked?

How are gut microbes transmitted among a community of interacting animals? Do social interactions shape the composition of the gut microbiome? Does the gut microbiome affect social interactions? We’re exploring these questions through imaging-based tracking and behavioral analysis.



Optics and Microscopy

Our investigations are based on optical microscopy, often pushing the boundaries of what can be imaged. We extensively use **light sheet fluorescence microscopy**, in which a thin sheet of fluorescence excitation light optically sections a specimen. This enables fast, high resolution, **three-dimensional imaging** with low photodamage. Our **home-built** light sheet microscopes are ideal for studies of the gut microbiome, combining performance and customizability.

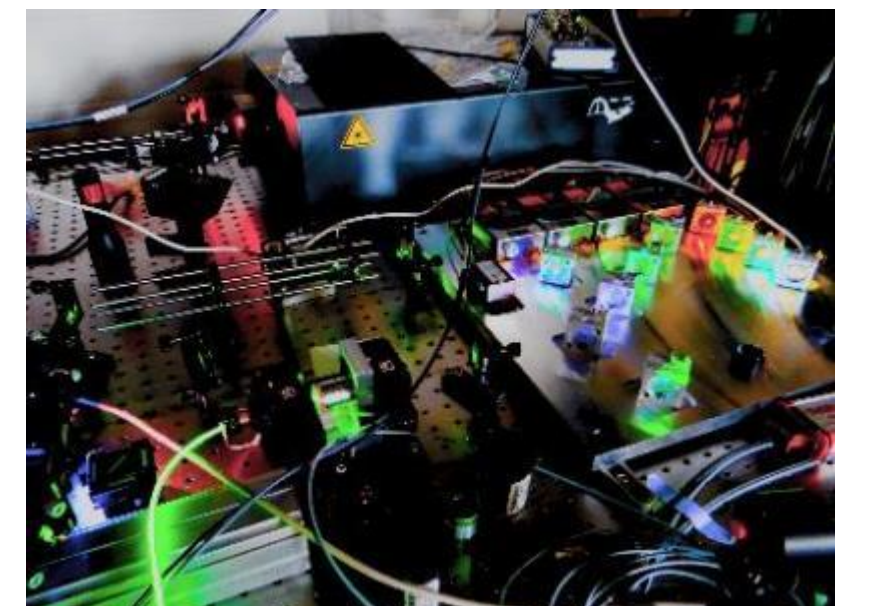
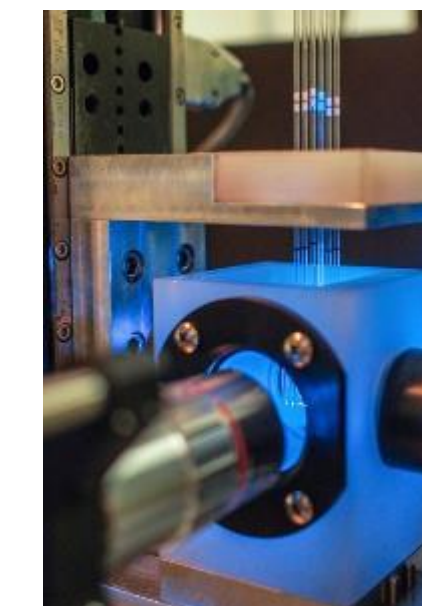
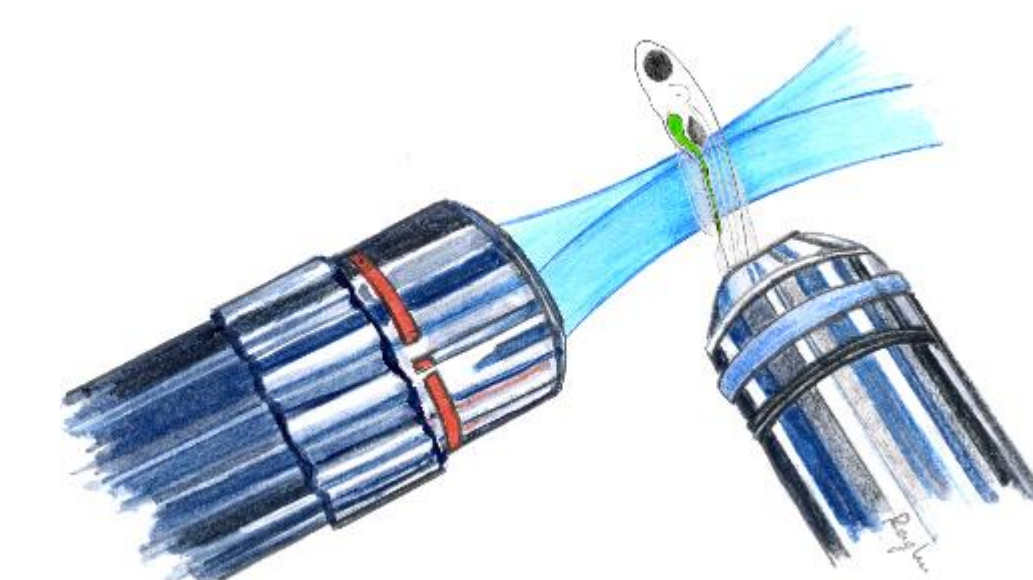
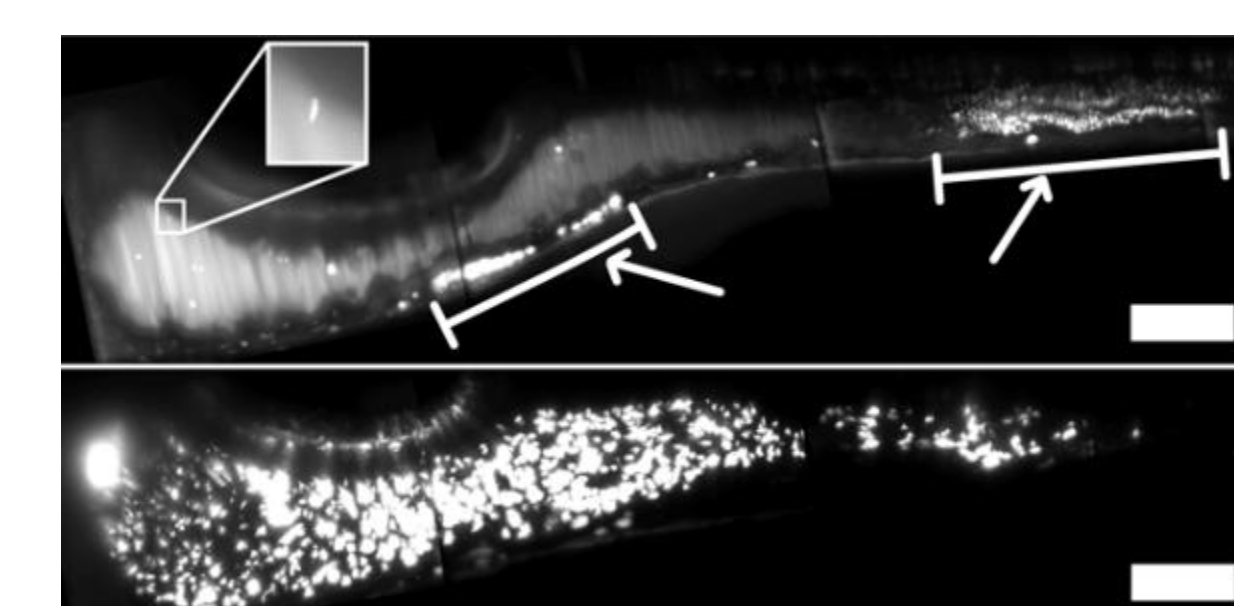
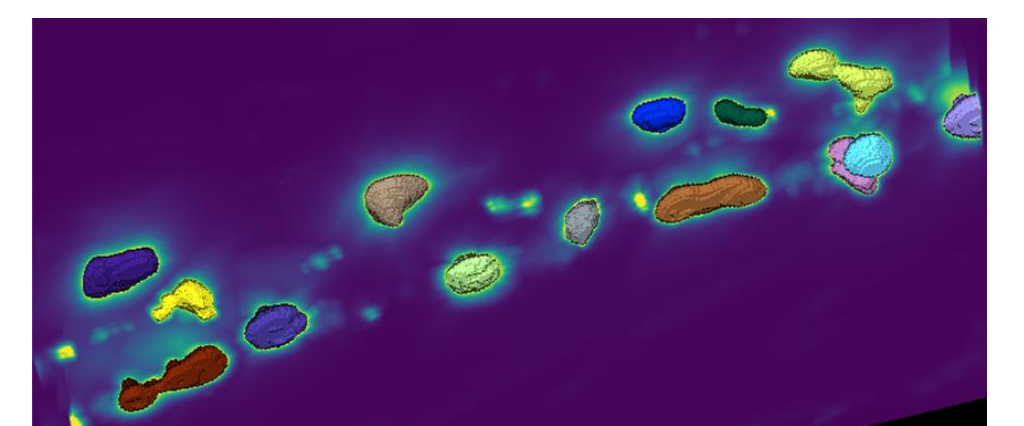


Image Analysis

We seek **quantitative insights** from vast amounts of image data. We often identify bacteria in complex, 3D images in which the population can span five orders of magnitude, or track immune cell trajectories over several hours. We therefore develop and implement advanced computational approaches, including **machine learning methods** such as neural networks.



Top: Gut bacteria imaged at 1.6 hrs. post-inoculation. Few bacteria are present. **Inset:** a single bacterium. White bars: autofluorescent zebrafish calls. **Bottom:** The same fish 7.5 hrs. later has a large bacterial population. Bars: $100 \mu\text{m}$.



Immune cells identified and tracked from a time-series of 3D images.

People, etc.

Our group consists of a wonderful team of graduate and undergraduate students who have gone on to exciting positions in academia and industry.



Left: Susana Marquez Rosales at Cookie Meeting; Right: one our high school outreach activities.

We have an excellent set of collaborators, especially other **U. of Oregon groups** interested in host-microbe interactions.

We’ve been funded by the NSF, the NIH, RCSA, the Gordon and Betty Moore Foundation, the Simons Foundation, and others.

For more information: Talk to us! Also check out the group web page: <https://pages.uoregon.edu/raghu/>, especially our list of published papers.

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