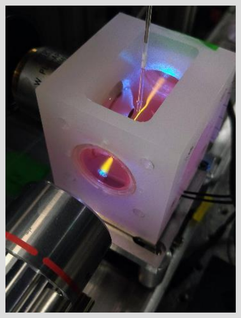
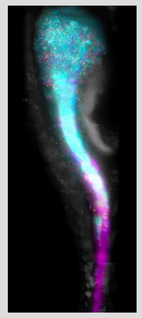


Microbes, Microscopes, and More

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).

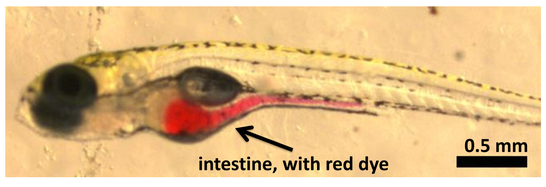
We develop new experimental tools, such as 3D microscopes, and computational tools, such as cutting-edge image analysis methods, to make these studies possible. Many of our experiments are unique, revealing phenomena that have never before been seen!

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.

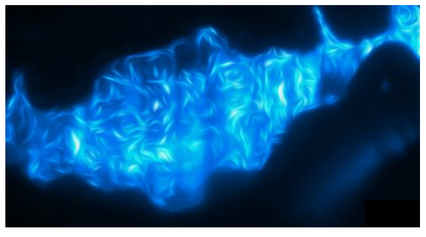
You are not (only) human. Each of us is host to trillions of microbes, mostly resident in the intestines, whose major roles in health and disease are just beginning to be grasped. Conventional methods provide little insight into the physical structure and temporal dynamics of the gut microbiome, properties 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: ≈ 200 µm.

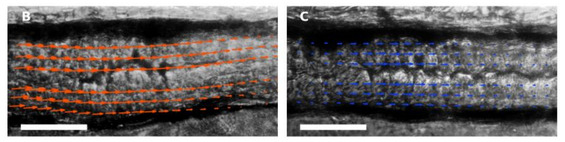
Questions

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.

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. We suspect that the host immune system mediates this interaction, which we are investigating through a combination of live imaging and genetic manipulation.

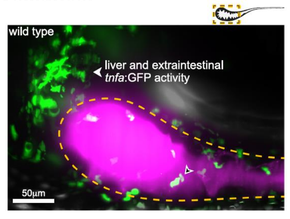
Image velocimetry: of intestines invaded by *Vibrio* with (left) and without (right) the T6SS. Bar: 50 µm



How does the immune system respond to gut microbes?

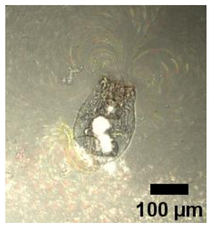
The immune system is aware of resident microbes, though the nature of this communication is unclear. Using fluorescent reporters of immune cell types and signaling behaviors, we found that bacterial motion can stimulate immune activity. Conversely, we are studying immune cell motion, characterizing patterns of cellular surveillance.

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 stability using time-series analyses, modeling, and new experimental 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.)

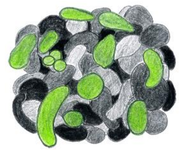


We also examine how intestinal communities respond to perturbations including antibiotics, which has revealed surprising connections between bacterial aggregation and population collapses.

How do material properties influence bacterial groups?

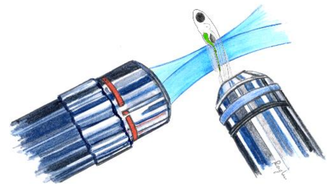
Aggregation, we've found, is central to the population dynamics of gut microbial communities. How do physical mechanisms such as adhesion create bacterial structures, and how are these mechanisms influenced by inter-species competition? "In vivo" live imaging and "in vitro" assays reveal surprising abilities of bacteria to influence each other, and to be influenced by the physical and chemical environment of the gut.

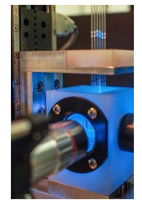
Relatedly, we examine the complex fluid environment of the intestinal space, for example by using bacteria themselves as probes.



Optics and Microscopy

Our investigations are all 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 microbiome, combining performance and customizability.





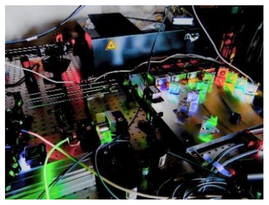
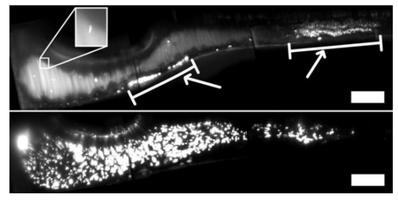
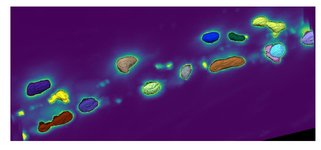


Image Analysis

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 µm.



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

People, etc.

Our research group consists of a wonderful team of graduate students, undergraduates, and a lab technician.

Group photo, August 2023. Left to right: Julia Ngo, Emily Avey, Jonah Sokoloff, Susana Marquez Rosales, Piyush Amitabh, Peter Bouchard, Fulton McKinney, Hailey Currie, Raghu Parthasarathy

Left: Susana 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 the Kavli Foundation.

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

Email Prof. Parthasarathy at: raghu@uoregon.edu

Poster: September, 2023