People & Ideas

Hari Shroff: Taking a closer look

Shroff works on developing new super-resolution imaging tools and using them to study cell biology.

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Hari Shroff

Hari Shroff was born in India, but grew up in England and the US. He became interested in animals and plants at an early age—his parents would take Shroff and his brother out to local parks, fields, and woods—and he has vivid memories of trying to raise tadpoles in jars and being fascinated by reptiles and amphibians. In one of his earliest scientific experiments, for a 5th grade science fair, Shroff went to the local dentist to obtain teeth extracted from patients and attempted to test the effect of different sodas and juices on the teeth.

Shroff's interests have since gone well beyond reptiles and tooth decay. Now with his own group at the National Institute of Biomedical Imaging and Bioengineering, Shroff is developing novel imaging tools to more closely study cell biological processes, including neurodevelopment in the nematode worm. We contacted him to learn more.

Where did you study before starting your own lab?

As an undergraduate I studied bioengineering at University of Washington. This was an extremely formative time for me, as I approached a professor of Chemistry, James Callis, and he took me into his lab. Callis had invented a pressure sensitive paint (possibly still) used in wind tunnel tests when designing airfoils. He wanted to use the same paint—or a variant—in order to measure the lift pressure generated by a bee in hovering flight. The project ultimately failed because the paint was too insensitive to monitor the minute changes in air pressure generated by the insects, but I learned a ton about chemistry and engineering during the experience, and even more importantly, that real research is messy and the answers not always straightforward or easy to obtain.

> "Real research is messy and the answers not always straightforward."

As a graduate student I worked for Jan Liphardt at UC Berkeley, obtaining a PhD in biophysics. I was one of Jan's first graduate students and I experienced firsthand the challenges (and joys) of starting a lab. I built my first microscope during this time, using it to calibrate tiny sensors of mechanical force. The goal was ultimately to use these sensors to measure forces inside living cells, for example on molecular machines. The sensors I built were based on DNA: I attached a FRET pair to a single-stranded DNA molecule and pulled on them using magnetic tweezers. When the DNA extended due to an increasing force, the FRET efficiency dropped, and I could measure the changes by observing the very faint single molecule fluorescence with a home-built TIRF microscope (1). Although the sensors I built during my PhD would have been chewed up inside a cell, the overall concept proved powerful and now there are several groups who are using the same idea to measure forces in vivo.

For my postdoc, I worked for Eric Betzig at HHMI Janelia Farm on early applications of photoactivated localization microscopy (PALM). These included some of the first live-cell PALM experiments, dual-color PALM, and single-molecule tracking with PALM; technical applications that have now been used and improved by many others. We also applied PALM to the study of chemoreceptors in bacteria and actin in dendritic spines. I learned a ton about light microscopy during this time, and about how fast a field can develop around a single transformative technology.

What was it that first drew your interest to super-resolution microscopy?

I've always been interested in microscopy, but in graduate school I really began to appreciate the difference between what we can easily see in a light microscope (e.g., cells and organelles) and what a cell biologist might *really* want to see (e.g., interacting protein distributions). When Eric Betzig rolled through Berkeley on a recruiting trip, before he started the lab that I eventually joined, he mentioned that he had a method for doing super-resolution microscopy that was incredibly simple (this turned out to be photoactivated localization microscopy, or PALM) and his talk reinforced my growing sense that advanced microscopy was essential to cell biology. I'm lucky that he took me into his lab and lucky that I was able to work on PALM during this early developmental period. It became clear quickly that any kind of microscopy—including super-resolution—involves tradeoffs. Thinking about the tradeoffs in PALM (e.g., a temporal resolution that is terrible relative to conventional microcopy) eventually led me to work on other forms of microscopy—like structured illumination microscopy (SIM).

What is your lab actively working on?

I'm very interested in improving microscopy techniques that are well suited to imaging live samples. To that end, I am continuing to work on improving SIM and light sheet fluorescence microscopy (LSFM). On the SIM front, we've improved the speed of the technique far beyond other implementations (2) and have also contributed to improving the depth penetrance of the technique (3). I'm currently working on further improving the depth penetrance, by combining SIM with adaptive optics, and we are also working on a high speed TIRF implementation.

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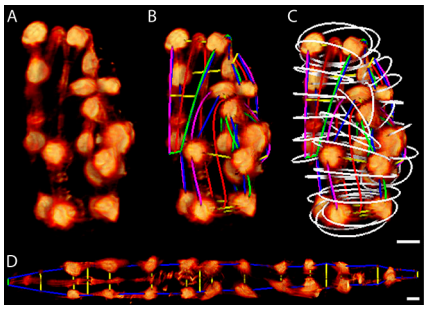

IMAGE COURTESY OF RYAN CHRISTENSEN

Stages involved in "untwisting" a late-stage *C. elegans* embryo. Panels show the embryo (A) twisted-up, (B) after a lattice has been built, (C) after the worm model has been generated, and (D) after untwisting. Scale bar, 5 um.

On the light sheet front, we have invented an implementation, dual-view selective plane illumination microscopy (diSPIM) (4), that is well suited to imaging single cells or small (e.g., worm) embryos. We are further improving the spatial resolution of this technique.

The original motivation for developing our light sheet microscope was to image neurodevelopment in *C. elegans* embryos, in order to construct a 4D atlas of neurodevelopment. This is a multi-PI effort, and we are collaborating with Daniel Colón-Ramos (Yale), Zhirong Bao (Sloan Kettering), and Bill Mohler (University of Connecticut). We have recently built a computational tool that allows us to "untwist" images of late-stage embryos (after the muscles have started to twitch) in order to track neuronal morphology during this somewhat understudied period of development (5). We are currently working on studying calcium flux in the embryo, as well as continuing our efforts to build the neurodevelopmental atlas.

What kind of approach do you bring to your work?

We are an interdisciplinary team composed of physicists, engineers, and, more recently, developmental biologists. When we started the lab, we needed to build tools; now, although tool development will always be a focus of my lab, we're interested in using them too, and so the focus has shifted more towards biology. We try not to place too much importance on the boundary between fields, though, as frequently we find that bringing a more physical/microscopy-based approach to a biological problem provides novel insight that might otherwise be missed.

We also try to work on problems that are a bit outside the mainstream: we are a relatively small lab and cannot compete directly with the "gorilla" in the field. We try to make lateral moves and hunt for problems and applications that otherwise might be missed.

What did you learn during your PhD and postdoc that helped prepare you for being a group leader?

Working hard, learning how to effectively manage my time, and trying to understand the problems that come up all the time during experimental work were key lessons from my PhD. Understanding the problem is usually half the battle! The largest challenge was learning that the people in my group that I supervise are not me! Learning that everyone has different strengths and weaknesses and how to deal effectively with people are not necessarily topics that are emphasized in either a PhD or postdoc—or even if they are, I think one really has to experience this aspect of management and make mistakes in order to learn from them.

> "It is only after failing... that we have the best chance of learning."

What has been the biggest accomplishment in your career so far?

I'm very proud of the diSPIM system I mentioned above—many others (including biologists) are now building their own, and it is gratifying to see them use the microscope to obtain data. I'm also happy that we've been able to make real progress in imaging neurodevelopment in late embryos using the system—something that wouldn't have been possible with conventional microscope techniques.

What has been the biggest challenge in your career so far?

See people, above. Also, the nematode embryo is constantly challenging, as the images we obtain are never *quite* good enough to satisfy me. I have learned a lot from the embryo, and it has pushed me (and continues to push me) to develop better microscopes.

What hobbies do you have?

I enjoy reading, traveling, and spending time outside, particularly hiking and climbing. I also like to cook with my wife, although I'm no expert at cooking.

What is the best advice you have been given?

"Head for the murky water"—I can't remember where I read this, but the key point is take a deep breath and try and pursue areas that are underexplored. This goes along with, "Forge your own path," something that Betzig emphasized. The other key piece of advice that I have heard from many people—and it really is true—is that failure should be the model, not success. Too often we are afraid to fail, but it is only after failing—and failing—and failing again, that we have the best chance of learning.

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4. Wu, Y., et al. 2013. *Nat. Biotechnol.* 31:1032–1038.
5. Christensen, R.P., et al. 2015. *eLife*. 4:e10070.

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Shroff and a friend