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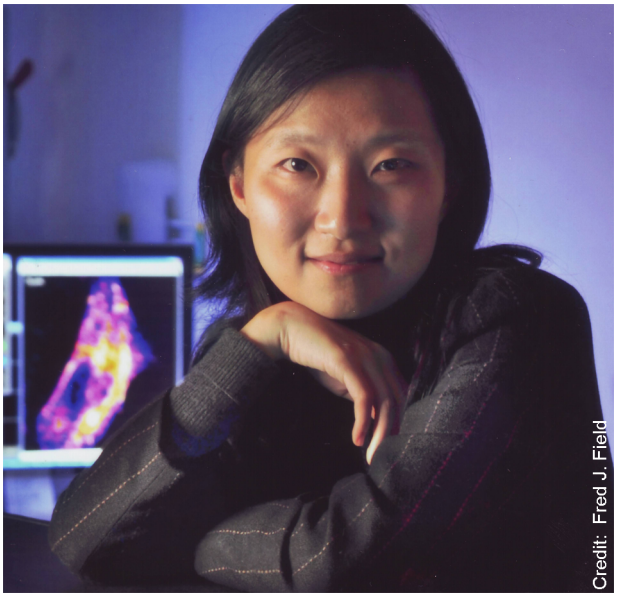
A Conversation with Xiaowei Zhuang

Katherine Bourzac

The biophysicist discusses the prospect of using super-resolution microscopy to image every molecule in a cell.



In 2006, Harvard University biophysicist Xiaowei Zhuang developed one of the first imaging methods to break the diffraction limit—an optical effect that limits the resolution of conventional light microscopes to a few hundred nanometers. Zhang's method, known as stochastic optical reconstruction microscopy (STORM), combines many different images of fluorescently tagged single molecules to resolve features smaller than 10 nm. Zhuang sat down with Katherine Bourzac to talk about how neuroscientists are using super-resolution imaging, and her ultimate goal of imaging every molecule in a cell.

What can you see with STORM that wasn't visible before with standard microscopy?

After we reported STORM, neuroscientists began contacting us about collaborating. Even with the most advanced imaging approaches, they were still noticing a lot of limitations, things they could not see in the brain. For example, subsynaptic structures were still hard to resolve because the synapse—the place where neurons connect and communicate—is a few hundred nanometers wide and is blurry under a conventional microscope. We're using STORM to look at subsynaptic structures to know precisely where individual molecules are inside them.

A few years ago we discovered a new structure in neurons. We saw a periodic membrane skeleton structure made of actin, spectrin, and other associated molecules that spanned the entire length of the axon. Prior to us showing this in 2012, people didn't even know such a structure existed. We continue to study this structure in terms of its molecular components, its functions, and how it goes wrong in diseases.

How would you like to improve on STORM's capabilities?

One of the goals we have is to further improve the resolution. Imagine you could take an image of a cell, and then get to see every molecule and which ones they're directly interacting with. That kind of structural information would really transform our understanding of a cell. I think that requires a resolution that is close to 1 nm.

The temporal resolution, meaning how fast you can image, is also important, because a cell is a living system, so there are dynamics that you want to catch, changes that you would want to monitor in real time. I also like to say that it's not just the temporal resolution, it's also how many images you can take of a living system before all your fluorophores are exhausted, and then you can no longer collect information on the cell. These are all fronts of improvement that we're interested in.

How are you working toward that huge goal of seeing every molecule in a cell?

Looking at all RNAs in a cell simultaneously turns out to be a substantially easier goal than looking at all proteins. The way we solve this problem is through a multiplexed approach where we image a specifically selected subset of RNA species at a time, so that in relatively few rounds we can assess up to thousands of RNA species, if not all of them eventually.

Single-cell RNA sequencing has really transformed many areas of biology, but it also has its limitations. It's a method

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that requires you to dissociate the cell from the tissue and extract the RNA from the cell. You've lost the spatial context, so you don't know where your cell is located in the tissue that you extract it from. That's a particular problem in the brain, where cell location and connections are important, but it's true of all tissues. By imaging, we can see gene expression without removing a cell from the tissue, and without removing the RNAs from the cell.

Is it truly possible to image every molecule in a cell?

Whether it's possible or not is something that I don't worry too much about, because even if we accomplish only 50% of the goal, that's already extremely powerful.

If you could get all this information simultaneously in a cell with very, very high resolution, it would revolutionize our knowledge of the cell. Look at how much structural biology has taught us about how molecules function. There, you know every single atom and where it is within a molecule. From that, we now know so much more about how molecules function.

This picture that I painted might seem far-fetched, but a lot of things at one point seemed impossible, but were not truly impossible. Before the diffraction limit was broken, it was considered an unbreakable law of physics. Now it's been completely circumvented.

Katherine Bourzac is a freelance contributor to Chemical & Engineering News, the weekly newsmagazine of the American Chemical Society. Center Stage interviews are edited for length and clarity.

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