PROFILE

Profile of Xiaowei Zhuang, winner of the 2020 Vilcek Prize in Biomedical Science

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In 2006, the New York City-based Vilcek Foundation created an annual prize program for foreign-born biomedical scientists who have made major contributions to their fields while living and working in the United States. The founders, themselves immigrants from Czechoslovakia, established the program to raise public awareness of the indispensable role of immigrant scientists in ensuring the United States' leadership on the world science stage. Recipients currently receive a cash award and commemorative sculpture designed by New York-based graphic designer Stefan Sagmeister. Over the past 15 years, scientists born in 12 different countries have been selected by a jury of peers to receive Vilcek Prizes, illustrating the diversity of backgrounds of immigrant scientists in the United States who become leaders in their fields of study. In 2009 the prize program was expanded to include a second tier of prizes, Vilcek Prizes for Creative Promise, for a younger generation of foreign-born biomedical scientists who have risen to prominence among peers through noteworthy early-career accomplishments. Vilcek Prizes for Creative Promise include a cash award and commemorative plaque. Currently, three recipients are chosen each year to receive Vilcek Prizes for Creative Promise (The Foundation also honors outstanding immigrants working in the arts and humanities through a parallel track of the prize program).

The 2020 Vilcek Prize in biomedical science has been awarded to Chinese-born Xiaowei Zhuang, a biophysicist at Harvard University, for her work in super-resolution and genome-scale imaging.

Fig. 1. Xiaowei Zhuang receiving the 2019 Breakthrough Prize in Life Sciences. Image credit: Breakthrough Prize Foundation and Breakthrough Prize in Life Sciences Foundation.

Visualizing the Hidden World of Cells: Xiaowei Zhuang

In his masterwork *On the Origin of Species*, Charles Darwin marveled at the diversity of life on Earth, musing at the common origins and quiet grandeur of "endless forms most beautiful" (1). Life's true majesty, however, may lie in the countless molecules swirling inside cells, shielded from human view. With the invention of the first microscope in the late 16th century, the drive to bring life's majesty to light was sparked. And when the English microbiologist Robert Hooke published his treatise *Micrographia* in 1665 (2), attempts at biological imaging, however crude, took off in earnest. Over the centuries, increasingly sophisticated methods of imaging have revolutionized the field. Harvard University biophysicist Xiaowei Zhuang, winner of the 2020 Vilcek Prize in Biomedical Science, has spent decades assiduously observing the secret lives of cells. Her efforts have led to strikingly clear views of the workings of biomolecules in cells, unearthed novel insights into the biology of behavior, and vaulted her to the forefront of a fast-growing field. Along the way, Zhuang has earned coveted honors, including memberships in the National Academy of Sciences, the American Philosophical Society, and the American Academy of Arts and Science; foreign membership in the Chinese Academy of Sciences and the European Molecular Biology Organization; a Howard Hughes Medical Institute Investigator award; a 2019 National Academy of Sciences award for

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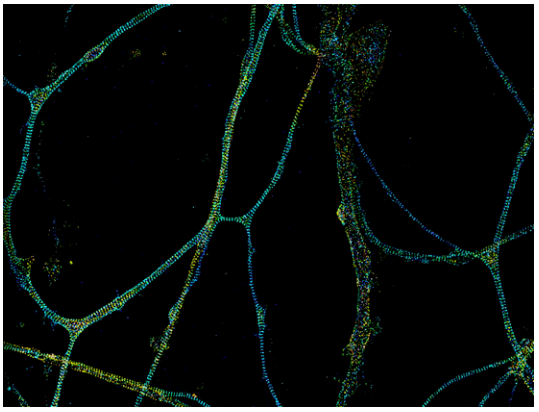

Fig. 2. MPS in neurons revealed by STORM. Image credit: the Xiaowei Zhuang Laboratory.

scientific discovery, and a 2019 Breakthrough Prize, among others.

Born in the town of Rugao in China's Jiangsu province, Zhuang became enamored with science at an early age. Her father, a physicist, and her mother, a mechanical engineer, are both professors at the University of Science and Technology of China. As a young girl, Zhuang surprised her father with her scientific imagination when he posed a physics question about the kinds of forces acting on a cup of water resting on a table. Zhuang recalls, "My brother, who is older, quickly said gravity and the opposing support from the table. My dad asked if we could imagine any other forces." Whereupon, to her father's delight, Zhuang ventured, "Maybe the air?" betraying a precocious grasp of atmospheric pressure even before she entered elementary school.

Zhuang's love of physics led her to major in the subject at college. When she graduated with a bachelor's degree in physics from the University of Science and Technology of China in 1991, her pursuit of higher education in the United States seemed a foregone conclusion, and she chose the University of California at Berkeley from a handpicked list of top graduate schools.

At Berkeley, Zhuang enrolled for a doctorate with physicist Yuen-Ron Shen, a prominent figure in the field of nonlinear optics. "Ron was a great mentor because he encouraged his students to think independently, deeply, and critically and arrive at logical conclusions, rather than simply telling us what to do," she says. In Shen's laboratory, Zhuang probed interfaces between materials, including liquid crystals and glass, work with implications for improving displays in electronic devices. Although Zhuang veered away from pure physics upon graduation, her apprenticeship with Shen shaped her approach to science, instilling in her both the courage to question conventional wisdom and a lifelong mistrust of dogma.

World in a Grain of Sand

In 1997, armed with a doctorate and a postdoctoral fellowship, Zhuang joined the laboratory of physicist Steven Chu at Stanford University, shortly before Chu won a Nobel Prize for his groundbreaking work on using lasers to trap atoms. As Zhuang angled for a suitable research topic, Chu exhorted her to follow her passion but focus on subjects with far-reaching impacts. "Steve is one of the most creative, visionary, and inspiring people I know," says Zhuang. "When I told him that I wanted to do something new and different from what I had done before, he urged me to think about biology."

At Chu's instigation, Zhuang trained her sights on the physics of molecules found in cells, marking a career-defining departure. "I was a pure physics major and had not taken biology since high school; at the time, I didn't even know the difference between DNA and RNA," says Zhuang.

Single-molecule studies can unveil an otherworldly landscape in cells, allowing researchers to acquire snapshots, if not entire sequences, of changes occurring in the hordes of molecules that keep cells humming. As a prelude to studying individual molecules in cells, Zhuang used a method called single-molecule fluorescence resonance energy transfer, or smFRET, to unravel the structure and dynamics of RNA enzymes in solution. The work resulted in a pair of reports in *Science* (3, 4), marking Zhuang's debut on the world biophysics stage. On the strength of those reports, Zhuang received coveted job offers, and in 2001, she accepted an assistant professorship at Harvard University, where she began to focus in earnest on biological imaging, driven by a passion to render the abstract concrete through stunningly realized visualizations of biomolecules in their native habitats: cells.

Ever since the 17th century Dutch cloth merchant Antonie van Leeuwenhoek used custom-built

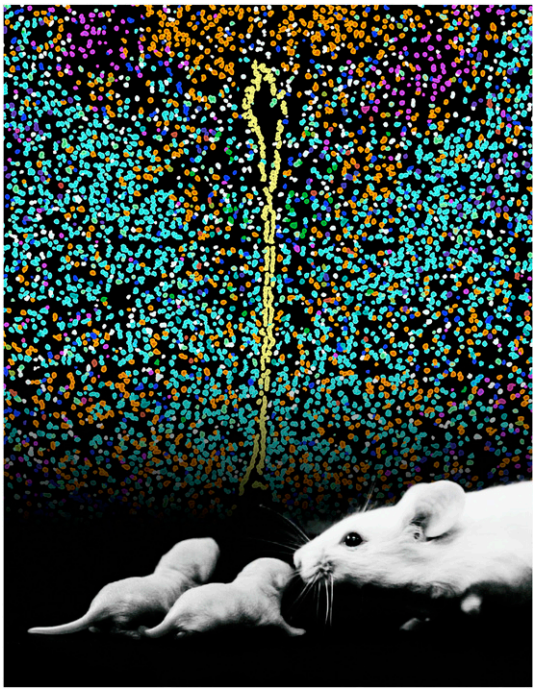

Fig. 3. Cell atlas of the preoptic region in mouse brain generated by MERFISH. Image credit: the Xiaowei Zhuang and Catherine Dulac Laboratories.

microscopes to peer into living cells, light microscopy has been a method of choice for visualizing the hidden workings of cells. Yet a barrier called the diffraction limit makes it difficult to focus light on a spot small enough to tease apart densely packed structures. As a result, the resolution of conventional light microscopes is limited to around 200 nm, meaning that the instruments can only resolve structures at least that far apart. In contrast, most biomolecules are no more than a few nanometers in size, making conventional light microscopy an imperfect tool to resolve individual biomolecules, let alone observe their interplay, inside cells. Enter superresolution fluorescence microscopy, an approach to boost image resolution by surpassing the diffraction limit. Effectively, superresolution imaging helps realize what the English poet William Blake once epigrammatically rendered as seeing "a world in a grain of sand."

Light Fantastic

In 2006, Zhuang reported one such superresolution imaging approach to breach the seemingly impregnable diffraction barrier. Dubbed stochastic optical reconstruction microscopy, or STORM, the technique, published in *Nature Methods* (5), uses fluorescent molecules that can be switched on and off using light, and Zhuang's laboratory discovered many such light-switchable fluorescent dyes (6). By using low levels of light to activate a fraction of the dyes within the field of view, Zhuang and her team limited the number of dye-labeled target molecules activated at any given moment within cells. This trick of sparse sampling allowed the team to resolve only a subset of labeled molecules in the teeming throng and to pinpoint their positions. Repeating the process revealed the positions of all target molecules, resulting in a superresolution image.

In her initial report on STORM (5), Zhuang showed that fine structures spaced merely 20 nm apart on linear strands of DNA could be clearly resolved, affording crystalline views. By this time, researchers had demonstrated other types of superresolution imaging methods, including stimulated emission depletion microscopy (STED) (7) and saturated structured illumination microscopy (SSIM) (8), which are based on a different principle (both STED and SSIM use patterned illumination to achieve superresolution imaging). Against this backdrop, STORM burst onto the scene, and along with another technique developed in parallel, photo-activated localization microscopy (PALM) (9, 10), ushered in a new class of superresolution imaging based on single-molecule switching and imaging.

"Being able to overcome the diffraction limit by one order-of-magnitude got us close to the molecular scale inside cells, which is why the field was so excited about this development," says Zhuang.

A year after unveiling STORM, Zhuang redoubled the excitement, extending the method to image biomolecules in three dimensions (11). Despite the massive gain in lateral resolution afforded by 2D STORM, the ability to distinguish molecules within cells depends on improved resolution in the axial, or third, dimension; after all, most molecules in cells are not neatly anchored or affixed to surfaces in pristine rows but instead float in a maddening pell-mell. Once again, Zhuang's team thwarted the limit decreed by diffraction, this time by using a cylindrical lens to focus light, a trick that allowed the molecules' axial positions to be determined from the elliptical shapes of their images. The maneuver yielded images of cellular structures at 50-nm resolution—an order-of-magnitude improvement over conventional light microscopes—in the axial dimension. Reported in *Science* (11), Zhuang's 3D STORM images of microtubules—slender tubes that serve as cellular highways—and clathrin-coated pits—membrane invaginations that ferry cargo into cells—created waves that continue to ripple in the cell biology community. To this day, the images are held up as exemplars of superresolution imaging, their sheer pulchritude attesting to the method's power in bringing cellular secrets to light.

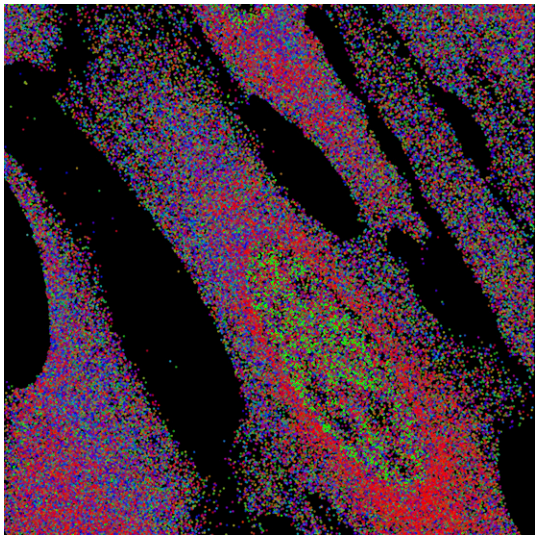

Fig. 4. MERFISH images of RNAs from 10,050 genes in individual cells. Image credit: the Xiaowei Zhuang Laboratory.

Hidden Scaffolds

Before long, 3D STORM led to unexpected insights, not to mention entirely novel structures that had eluded even the most powerful visualization methods. Chief among these insights was Zhuang's discovery of a backbone in axons (12), the slender stems of neurons that serve as information highways for electrical signals coursing through the brain. The axonal backbone is primarily composed of the proteins actin and spectrin. Zhuang and colleagues used 3D STORM to produce pin-sharp images of actin and spectrin in axons (12). Clear as brookwater, these images revealed a periodic structure in axons that had never been observed previously.

Rings of actin filaments capped by the protein adducin were wrapped around and evenly spaced on the axonal shaft, each ring set 180 to 190 nm away from the next, just shy of the notorious diffraction limit. Zhuang and colleagues (12) found that the sartorially

precise spacing of the rings on the shaft was achieved using spacers made of proteins called spectrins, which form rod-shaped tetramers that are a seemingly tailor-made 180 to 190 nm in length. Subsequently, Zhuang and others showed that this structure also exists in parts of dendrites, which are finger-like projections of neurons that reach out to other neurons (13–15).

"This was a compelling example of the power of superresolution microscopy in making not only beautiful images but also fundamental discoveries," says Zhuang. Lending substance to the sentiment, the findings have major functional implications. Zhuang and colleagues showed that the backbone, dubbed membrane-associated periodic skeleton (MPS), serves as a platform to assemble key molecules to enable signal transduction in neurons (16), and that the MPS can anchor ion channels along axonal membranes (12), potentially shaping how electrical signals travel. This backbone can also bolster the axon membrane, providing both the elasticity and stability needed for axons to remain stable under stress, as suggested by Zhuang and colleagues (12); supporting this idea, axons lacking spectrin tend to break when animals move (17). Zhuang's recent work, in collaboration with neuroscientist Marc Tessier-Lavigne and colleagues, suggests that the MPS is important for the early stages of axon degeneration (18).

Carta Magna

If Zhuang's articles on STORM were a high-water mark in a rising career, her recent work extending imaging to the genome scale made resonant waves. Analyzing gene expression patterns in individual cells allows researchers to probe cell function and characterize cells. Such characterization can help create catalogs of cell types that make up entire organisms. Conventional methods to image and count RNA molecules—signatures of gene expression—suffer from limitations, not the least of which is the number of RNA species that can be simultaneously visualized and identified. Zhuang invented a method to exponentially increase this number and enable genome-scale imaging. "Scale was a critical feature of this development," notes Zhuang. "We gave each RNA species a distinct barcode, and the barcodes are not detected one by one but in parallel in a combinatorial manner over multiple rounds of imaging, which allows a large number of RNA species to be imaged and identified with a small number of imaging rounds," she explains. To minimize the concomitant rise in detection errors from multiplexing, Zhuang and her team developed barcoding schemes that enabled the detection and correction of errors.

"A great idea is just the beginning," says Zhuang. "After conceiving the idea, I set an ambitious goal for our first paper on this method, simultaneous imaging of 1,000 genes, experimentally demonstrating genome-scale imaging. It took us multiple years to achieve this goal, overcoming many challenges in labeling and imaging." Reported in *Science* in 2015 (19), the method, dubbed multiplexed error-robust fluorescence in situ hybridization, or MERFISH, allowed Zhuang and colleagues to image as many as 1,000 distinct RNA species, and hence 1,000 genes, in individual mammalian cells, and this spatially resolved profile of gene expression helped them identify networks of coregulated genes, ascribe roles to genes of unknown function, and map the spatial distribution of RNA molecules in cells. More recently, Zhuang's team further boosted the scale by an order of magnitude, imaging RNAs from 10,050 genes in individual cells (20).

MERFISH plays a major role in a much-publicized international effort named The Human Cell Atlas project, a global initiative that is nothing if not ambitious. One of the project's main goals is to map all of the major cell types that make up an entire human being, not to mention their 3D arrangement and functional connections. Such an atlas could help limn brain circuits, unravel organ development, and uncover the interplay of cancer cells and immune cells, among other applications.

Zhuang's work in the project has led to fundamental insights on brain function. Together with Harvard University neuroscientist Catherine Dulac and others, Zhuang focused on a region of the mouse hypothalamus, dubbed the preoptic region, implicated in an array of visceral traits, such as thirst and sleep, as well as social behaviors. Through MERFISH analysis of individual cells in intact brain tissues, Zhuang and colleagues (21) identified more than 70 distinct neuronal types in this region, many novel to science, and mapped their spatial arrangement and functional ties.

The experiments revealed plenty of intriguing insights: Distinct groups of neurons fired during mating and parenting in mice; mothers and fathers appeared to activate common as well as sex-specific groups of neurons during parenting; a different subset of neurons lit up when male mice engaged in aggression toward infants and other males (21). Together, these insights underscore how animals' brain physiology affects behavior. Zhuang puts it eloquently: "What we reported is a molecularly defined, functionally annotated, and spatially resolved cell atlas of an important brain region. We envision using MERFISH to generate similar cell atlases of many other tissue types in different organisms, including humans, to advance understanding of fundamental biology and disease."

Recently, Zhuang and her colleagues have applied multiplexed FISH imaging to unravel the 3D organization of genetic material in chromosomes. Through high-resolution imaging of targeted regions of the genome in single mammalian cells, Zhuang's work has provided a physical view of genome organization and paved the way toward understanding how genome structure affects gene expression and cell function (22, 23).

For techniques that have allowed a clear-eyed glimpse into a hidden world, Zhuang has earned a wealth of accolades, including a 2019 Breakthrough Prize in Life Sciences, a top honor bestowed on scientists by titans of the technology industry at a glittering ceremony in Silicon Valley, touched by the glamor and brassy lights of tinseltown. At the ceremony in November 2018, as her mentor Chu watched

contentedly from the audience, Zhuang, resplendent in an evening gown, accepted the prize from Hollywood star Lupita Nyong'o and Facebook cofounder Mark Zuckerberg. With a modesty that masked the depth of her accomplishment, Zhuang explained her approach to science: "I have loved science since I was a school kid, amazed by the simple and elegant physics laws that explain the universe. As I grew older, I got more and more attracted by the unsolved mysteries in life sciences. . . So, I decided to merge these interests and devote my research to solving biological problems using multidisciplinary approaches." Zhuang's continuing efforts to transcend long-established limits through leaps of scientific imagination are a solid testament to her embrace of cross-disciplinary science.

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