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A researcher uses a confocal microscope equipped for super-resolution and fluorescence imaging at the University of Bristol, UK.

MEET THE MICROSCOPES

Advances in imaging have paved the way for new careers in cell biology. **By Nic Fleming**

Scientists are understandably wary of the word 'revolution'. Major breakthroughs that shake the foundations of established thinking are rare compared with the small, additive steps by which science tends to progress. Yet when Werner Kühlbrandt at the Max Planck Institute of Biophysics in Frankfurt, Germany, wrote about the promise of a microscopic technique that could reveal the structure of large biomolecules at near-atomic resolution in 2014, he chose as his headline 'The Resolution Revolution'¹.

Kühlbrandt was referring to the potential of

electron cryo-microscopy (cryo-EM), a technique that fires beams of electrons at proteins frozen in solution to reveal their structures. Cryo-EM is just one of many imaging tools driving rapid advances in cell biology and related fields. "New techniques have come on board over the last ten years that allow us to see things inside cells that we couldn't resolve before," says Anne Ridley, a cell biologist at the University of Bristol, UK, and president of the British Society for Cell Biology. "We can see whether two proteins are located in the same place, doing the same things, coming together

or apart in real time, understand how enzymes work and obtain structures of complexes of proteins in ways we couldn't have dreamed of doing before."

Scientists have been improving microscopes ever since the devices were invented in the late sixteenth century, and this process has accelerated markedly over the past decade. The 2014 Nobel Prize in Chemistry was awarded to the developers of super-resolution fluorescence microscopy, and the 2017 prize recognized the development of cryo-EM. The specifics of each microscopy technique vary hugely, as do

Meetings of minds

Symposia and conferences are good for getting updates and overviews of a field.

Researchers often have to choose between going to broad or specialized meetings. For those seeking an overview of the state of the field, the joint meeting of the American Society for Cell Biology and the European Molecular Biology Organization is by far the largest annual gathering of cell biologists in the world. Around 6,000 people are expected to attend this year's, in Washington DC on 7–11 December. Subjects to be covered will be wide-ranging, including emerging topics such as non-conventional model organisms, computational modelling and synthetic biology.

Bruce Stillman, president and chief executive of Cold Spring Harbor Laboratory in New York, will give the keynote lecture on his work on chromosome duplication in cells. There will be a variety of symposia, workshops, poster sessions and special-interest sessions. On the day before the

main meeting, there will be a full day of session on careers and professional development for academics, and a one-day mini biotech course, at which attendees can learn how scientific discoveries are turned into bioscience ventures. Other sessions will cover careers in non-profit science advocacy, science policy, outreach, scientific infrastructure management and bench-based research in industry.

There are many other options for researchers wanting to dig deeper into a particular branch of the discipline. A symposium called Seeing is Believing, for example, brings together the developers of cutting-edge imaging techniques with those applying them in the lab. This meeting attracted some 400 participants when it was last held, at the European Molecular Biology Lab in Heidelberg, Germany, in October this year. It featured sessions on the latest tools and methods transforming researchers' abilities to visualize proteins, protein complexes, organelles, cells, tissues, organs and whole organisms.



the subsets of scientists that favour them. But, fundamentally, all are ways of seeing the cell in greater detail than is possible by eye.

Each microscope technique involves a compromise. Fluorescence microscopy, in which fluorescent molecules are used to light up target proteins, cells or cellular components, allows biologists to observe live samples in real time. But because visible light cannot distinguish between objects closer than 200 nanometres to each other, it is not, on its own, enough to reveal the detailed structures of tiny functional components in cells called organelles. Electron microscopes can achieve much higher resolutions, but require a vacuum and so cannot be used on live samples. In modern science, cell biologists have access to an ever-growing armoury of microscopy tools, which can be used either alone or in combination and offer many improvements over the much older technique of crystallography (see 'Tools of the trade').

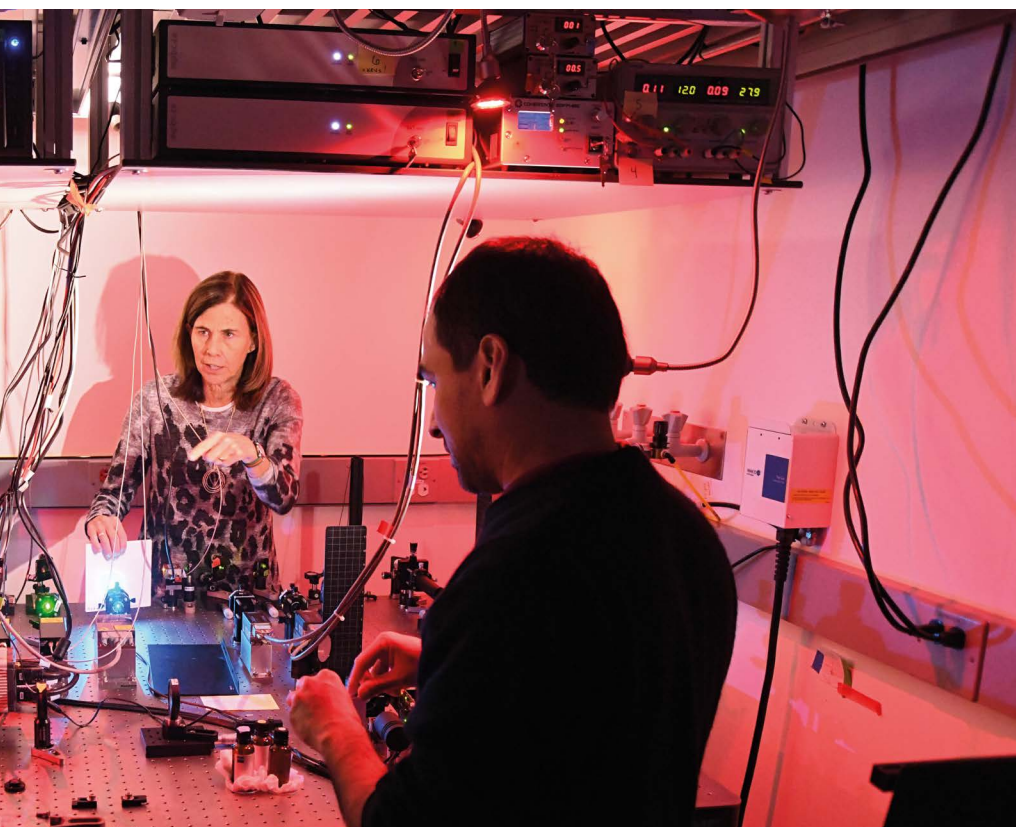
Cross-disciplinary approach

These advances have been the result of contributions from scientists in many different fields. Physicists have provided much of the technology, such as the advanced electron detectors that increased the speed and sensitivity of modern cryo-EM devices. Chemists

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Delegates at the joint meeting of the American Society for Cell Biology and the European Molecular Biology Organization meeting in 2018.



Jennifer Lippincott-Schwartz demonstrates a microscope capable of super-resolution imaging.

have developed brighter fluorescent probes that illuminate targets for longer. Statisticians and computer scientists have improved image processing and analysis techniques. “The acceleration in imaging has come about through this incredible synergy,” says Jennifer Lippincott-Schwartz, a cell biologist at the Howard Hughes Medical Institute’s Janelia

“These techniques open up tremendous vistas for the types of questions we can answer.”

Research Campus in Ashburn, Virginia, who helped to lay the foundations for the development of super-resolution microscopy with work during the 1990s on the use of green fluorescent proteins to visualize cellular trafficking pathways in living cells².

Many advances have been made using these microscopy tools. Lippincott-Schwartz and her colleagues, for example, used a form of light-sheet fluorescence microscopy with confocal microscopy to capture 3D colour footage of the interactions between different types of organelle. “We were able to map out the relationships between six types of

organelles, how fast they were moving and the contacts they made with each other,” says Lippincott-Schwartz, whose paper³ was published in *Nature* in 2017. “That’s important if you want to understand the cross-communication between organelles, which is one of the big interests among cell biologists right now.”

The growing availability of these advanced techniques presents opportunities for early-career cell biologists. Most obviously, it increases the number of processes cell biologists can probe. “These techniques open up tremendous vistas for the types of questions we can answer,” says Lippincott-Schwartz. Structural biologist David Barford at the MRC Laboratory of Molecular Biology in Cambridge, UK, has used cryo-EM to advance understanding of some of the cellular mechanisms involved in mitosis⁴, a type of cell division that results in the formation of two daughter cells with the same chromosomes as the parent cell. “For academic scientists, the ability to determine structures at atomic resolution with electron cryo-microscopy can be very important in the design of new experiments and testing of biological hypotheses,” he says.

Barford adds that the potential benefits to early-career researchers of acquiring an in-depth understanding of the latest imaging techniques could extend beyond the

Tools of the trade

A wide variety of techniques open up multiple lines of investigation.

1

Widefield microscopes use intense light sources to illuminate entire specimens, and are less complex than other technologies.

2

Confocal microscopes use pinholes to illuminate points of interest. That cuts down on out-of-focus or background fluorescence and means they can achieve higher resolution and greater contrast than widefield microscopes.

3

Light-sheet microscopy scans samples using a very thin plane of laser light rather than a point. It has transformed developmental biology by allowing the tracking of cells and tissues in living organisms.

4

Structured illumination microscopy is a variant of super-resolution microscopy (SRM) that is useful for live cells and produces images of higher contrast than do widefield microscopes.

5

Stochastic optical reconstruction microscopy, another variant of SRM, records the positions of sets of fluorescing chemicals that are switched on and off sequentially to produce images with very high resolutions.

6

Electron cryo-microscopy can reveal the atomic structures of biomolecules such as large proteins and dynamic protein complexes. The technique’s resolutions could previously be achieved only by X-ray crystallography, which requires samples to be in crystal form.



Q&A

Cell biology outside the lab

Erika Shugart had wanted to be a scientist from childhood. So she found it tough to turn her back on research in 1997, after completing a molecular biology PhD at the University of Virginia in Charlottesville. But Shugart stayed close to science, and went on to carve out a successful career in science communications and policy. She has been chief executive of the American Society for Cell Biology (ASCB) since 2016.

Why did you leave research behind?

It was a difficult choice, but once I was actually doing science, I realized that although I was fascinated by it, what I really wanted was to connect science with the public and policymakers, to work in teams and to help people understand science. I also realized that I liked projects with a defined ending, which research doesn't normally provide.

What was your path out of academia?

I put together what would now be called an out-of-academia blog. I interviewed science journalists, patent lawyers and people working in communications, technology transfer and policy, and put the results up on a website. I also learnt some web skills, which probably got me my first internship at the National Research Council of the US National Academy of Sciences, because they needed a website for a meeting.

Why have non-academic careers been such a focus at the ASCB in recent years?

A 2012 international survey, with participants mostly in the United States and mostly from the life sciences and medical sciences, showed that unemployment after a postdoc had risen from 4% in 2008 to 10% in 2012 (see go.nature.com/37eug8f). Employment in what are known as alternative careers doubled between 2010 and 2012, to 16%. Around this time, it was beginning to really hit home that most people who get a cell-biology PhD will not become a professor or stay at a university. So the society created a Committee for Postdocs and Students, whose members took matters into their own hands and started to provide help, advice and support for those moving out of academia.

How does the ASCB help those looking at careers outside academia?

We offer a wide range of talks on careers and professional development at our annual meeting (see 'Meetings of Minds'). They're generated by our committees and members, and offer people a taste of the many options out there. One of our most successful programmes is a week-long course for those considering leaving academia, with interactive sessions and speakers from biotech and pharma, and also entrepreneurs. Some 67% of attendees have gone on to jobs in industry, regulatory affairs or tech transfer. We also run careers webinars.

What trends should early-career cell biologists be aware of when thinking about their futures?

The other side of the decline in available academic positions is the uptick in jobs in industry, as well as in other sectors, such as patent law, communications and policy. There are growing opportunities to collaborate with those in other fields. We continue to see more men, white men in particular, than women and people of colour staying in academia. That's a concern for academia because diversity of backgrounds brings diversity of thought.

What careers advice do you offer early-career researchers?

I've seen a lot of CVs of people looking to make the shift out of academia. What I look for is those who go out to get extra experience and skills, whether taking a course, writing articles or volunteering at a tech-transfer office. It shows commitment to a path and initiative. There are so many enriching careers for people with advanced training in science. If people think about what it is about science that makes them passionate — maybe the writing, communicating or project management — and pursue that, they'll be able to find a satisfying career.

Interview by Nic Fleming

This interview has been edited for length and clarity.

immediate research questions they are seeking to answer. "Drug companies are becoming very keen on electron cryo-microscopy as a means to determine the structures of proteins and drug targets, so moving into it could be a very good career choice," he says. Barford also thinks these techniques will grow more important and overtake older techniques used by biologists. "It will probably supersede crystallography in the job market."

It is impossible to become proficient in the use of all or even many of the latest imaging tools. Early-career cell biologists seeking to use them need to decide whether to specialise in a particular technique, or to identify

"If you become just a user rather than a developer, it limits your future potential to contribute to the field."

collaborators who can do it for them. Ridley, who studies the role of cell migration in cancer progression, advises those doing PhDs to take up any opportunities available to them to get a flavour of the different techniques. "I would recommend that anyone doing a PhD programme with the option to do rotations in different labs and gain experience of different imaging areas to do so," she says. "Even if you don't become an expert in electron microscopy, for example, working in that area for a couple of months will give you an understanding of what it can and can't do." Barford adds that researchers who leave it to collaborators to do their imaging for them risk falling behind in other ways. "If you become just a user rather than a developer, it limits your future potential to contribute to the field through developing and advancing the technology."

One of the draws of imaging for Lippincott-Schwartz is its purity as an empirical method for acquiring knowledge. "When you are imaging, you are first observing, then generating hypotheses and then designing approaches for testing your hypotheses. It's the perfect avenue for fulfilling the scientific method." She adds that the proliferation of advanced tools has made microscopy all the more attractive as a focus for cell biologists. "It can make imaging a very creative direction to take," she says.

Nic Fleming is a science writer based in Bristol, UK.

1. Kühlbrandt, W. *Science* **343**, 1443–1444 (2014).
2. Priestly, J. F. et al. *Nature* **389**, 81–85 (1997).
3. Valm, A. M. et al. *Nature* **546**, 39–40 (2017).
4. Alfieri, C. et al. *Nature* **536**, 431–436 (2016).