CAREERS

TURNING POINT Former athlete's solar-telescope designs lead to award **p.141**

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Individual cells, such as this immortal human skin cell, can now be studied in unprecedented detail.

SINGLE-CELL ANALYSIS

Imaging is everything

Advances in single-cell imaging bring opportunities for physicists, biologists and chemists alike.

BY AMY MAXMEN

Enrico Gratton gave his research centre an aspirational and prescient name: the Laboratory for Fluorescence Dynamics. When the lab was established at the University of Illinois at Urbana-Champaign in 1986, Gratton and his colleagues could make lipids and proteins in clumps of cells glimmer using fluorescent probes, but imaging moving molecules in individual cells was impossible. Yet Gratton had hope: “We put ‘dynamics’ in the

centre’s name because we knew we wanted to eventually follow the behaviour of molecules inside individual cells,” he says. With technological advances, Gratton can now do just that at the lab, which moved to the University of California, Irvine, in 2006. He can watch lipids aggregate and proteins exit membranes. His dream has been realized.

Gratton and his colleagues are at the centre of a growing field focused on single cells. Improvements in microscopes, lasers, cameras, computers and fluorescent labels let researchers

directly observe and measure dynamic cellular processes (see page 133). Scientists can look at how tumour cells change over time, measure how long it takes for DNA to bind to a transcription factor or trace the route of RNA as it exits the nucleus, for example.

Since Gratton’s lab became the first single-cell-imaging centre to receive US federal funding, many facilities have opened around the world — including at the German Research Center for Environmental Health in Munich and the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. “Institutions are quite eager to set up these cores because there’s a huge pay-off,” says Robert Singer, co-director of the Gruss-Lipper Biophotonics Center at the Albert Einstein College of Medicine in New York. “With the right set-up, their faculty can write more-competitive grants and get their studies into better journals.” Investigators can reveal previously unknown biology, and contribute to medical research. A single-cell specialist might reveal, for example, how stem cells differentiate as wounds heal.

The field is inherently interdisciplinary. Microscope developers usually hail from biophysics, which is rich in optics research, but cell biologists and chemists also have key roles in the field, with their expertise in handling living material and labelling it non-invasively. Single-cell-imaging labs are found in physics, chemistry and cell-biology departments at universities. Others are affiliated with medical schools, at which imaging experts aid in disease research, diagnostics and drug discovery. Singer and Gratton say that their students and postdocs find careers in both academia and industry.

A single-cell researcher’s academic background isn’t as important as their ability to integrate approaches from various fields. They must apply a quantitative approach to all things biological — rather than just observing cells under the microscope, they must measure how the contents of cells change, for example, or quantify how cellular contents, properties and molecular dynamics vary between cells. And it helps to enjoy tinkering with optical devices.

HIGH-TECH TOYS

High-end fluorescence microscopes cost between US\$300,000 and \$1.5 million, and US federal funding agencies are getting plenty of requests for them. Last year, proposals for microscopes accounted for about half of the grant applications submitted to the biology arm of the Major Research Instrumentation Program at the US National Science Foundation. ►

► The US National Institutes of Health (NIH) Shared Instrumentation Grant Program supports the purchase of research equipment that costs \$100,000–600,000; requests for high-resolution, high-contrast confocal microscopes doubled between 2008 and 2010. And the NIH's High End Instrumentation Program, which supports instruments in the range of \$750,000 to \$2 million, has seen applications for confocal microscopes roughly double every two years since 2005. Price tags are increasing as instruments become more versatile: the average cost of microscopes funded by the German Research Foundation was €300,000 (US\$403,000) five years ago, but is €480,000 now.

Advances in the past decade have brought better probes and microscopes, and improved tools for analysing gene expression. To capitalize on this, the NIH has launched a \$90-million, five-year single-cell-analysis programme to support single-cell imaging and studies on the genomes and other aspects of individual cells. It will award its first grants in 2012 — as long as funds from NIH budgets permit. Other microscope funding programmes include the multi-user equipment grants from the Wellcome Trust in London, which fund microscopes costing up to £1 million (US\$1.6 million), and the Netherlands Organisation for Scientific Research's Investment Subsidy NWO Large programme. Finding out how cells differ is important in disease, says Richard Conroy, programme director at the National Institute of Biomedical Imaging and Bioengineering in Bethesda, Maryland. Not all cancer cells are the same, for example, and it is important to learn which pose a risk to health and which are resistant to treatment.

FINE DETAIL

Gratton spends most of his day doling out advice on imaging experiments to the 25 students, technicians and postdocs who work on the 11 custom-made microscopes at his lab. On one occasion, graduate student Chi-Li Chiu told him that an artificial collagen matrix she was handling seemed to be densest around certain breast-cancer cells embedded in it — something that would have been hard to detect before single-cell imaging. To verify the observation, Gratton asked Chiu exactly how dense the matrix became. Chiu used a confocal imaging microscope to take hundreds of snapshots of matrices at different depths. With a computer programme called SimFCS, she analysed the intensity of light reflecting off fibres

"You want to apply your skills and toolbox to a research problem that will have a future."

Rick Horwitz

Researchers can zoom in on single cells, such as a primary rat hippocampal neuron expressing green fluorescent protein (left), or a connective-tissue cell called a fibroblast (right).

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in the matrix in different places, related those measurements to density and calculated how the living cells deformed the tissue-like matrix.

Chiu's brief task demonstrates the technical and scientific acumen demanded by single-cell imaging — and how it can open up new avenues of research. Gratton now wants to learn how some cancer cells can directly alter the density of tissue. "Is the cell producing more collagen around itself, or is it extending protrusions to grab the collagen and bring it close to the cell?" he asks. "And as these cells are cancerous, does altering the surrounding tissue allow them to migrate and spread?"

Biophysicists often find work in microscope development, which calls for knowledge of the physical properties of lasers. However, they may need contact with a biologist to help them to stay attuned to biological questions and keep cells alive, says Rick Horwitz, a cell biologist at the University of Virginia in Charlottesville, who has a PhD in biophysics. Biophysicists don't necessarily need to move to a cell-biology department, he says, but they should collaborate closely with biologists working in cancer biology, neurobiology, cardiovascular biology or another well-funded field that can be advanced by single-cell imaging. "You want to apply your skills and toolbox to a research problem that will have a future," says Horwitz.

Cell biologists can also become leaders in single-cell imaging — especially now that microscopes and fluorescent probes to tag molecules need not be made from scratch. Manufacturers, including German companies Carl Zeiss in Jena and Leica Microsystems in Wetzlar, and Japanese firms Olympus and Nikon in Tokyo, offer microscope systems built for single-cell and single-molecule imaging, along with the software for analysing the data. Likewise, fluorescent probes can now be purchased from biotechnology companies such as Life Technologies in Carlsbad and BioSearch Technologies in Novato, both in California. With these tools and reagents for sale,

faculty members working in single-cell imaging no longer need the chemical or physical expertise to make their supplies themselves.

Biologists who wish to specialize in single-cell imaging must learn to think quantitatively to design successful experiments, says Paul Selvin, a biophysicist at the University of Illinois at Urbana-Champaign. In his imaging workshops, he has seen biologists who have not grasped this principle. For example, they will predict that a protein will diffuse along a brain-cell membrane and into a synapse, but forget to account for the drag from the fluorescent label that they have attached to the protein, which can slow down or halt diffusion. "It's not very hard to do the calculations to account for drag, but you need to think about the problem in the right terms," says Selvin.

Chemists can contribute to single-cell imaging by developing innovative ways to label molecules. Roger Tsien, a biochemist at the University of California, San Diego, received a share in the 2008 Nobel Prize in Chemistry for his part in discovering the green fluorescent protein, which transformed single-cell analysis by allowing scientists to image the components of living cells non-invasively. And imaging labs often welcome scientists familiar with computer programming, who can develop software to analyse the terabytes of uncompressed image data collected from every experiment.

FINDING A NICHE

Single-cell imaging draws on so many fields that it can be the ideal niche for technology-minded scientists who have struggled to find out where they belong. "There were times when I thought I studied the wrong thing, because I realized I needed to know more biology or more about software development, and I had to learn it on my own," says Christian Hellriegel, an application specialist for Zeiss. "But in the end, I found I had a much wider scope than many people, and that turned out to be a great plus." That scope comes in handy in his job, where

Hellriegel eases communication between university biologists and company physicists who design microscopes. Zeiss and its competitors have been racing to produce high-end microscopes, meaning that there is ever more potential for positions like Hellriegel's.

Hellriegel moved to Zeiss from academia. He has lost the freedom to run his own experiments, but says that not having to write grant applications is well worth any downside. And he is still engaged in his favourite aspect of single-cell imaging — tinkering with microscopes. "One of the things that dawned on me is that there's a lot of thorough science that occurs in industry," he says. "I'm still helping to design equipment for biologists, only now I really have to make sure the equipment works well and is as user-friendly as possible."

Most jobs in single-cell imaging are in university departments and imaging centres. Eric Potma, a physical chemist at the Beckman Laser Institute at the University of California, Irvine, says that researchers should apply to universities at which there are already regular collaborations between imaging centres and medical schools or cell-biology departments. He adds that if the university is not equipped with the necessary high-end instruments and enough space, a large start-up package is essential. "I needed a package of about \$1 million for the kind of work I do, in addition to plenty of space," he explains. His laser system, microscope, reagents, biosafety hood and incubator cost about \$700,000. Another \$300,000 went to support two graduate students and a postdoc for two years. "If you accept lower than what you really need," warns Potma, "your research won't be successful and you may not get tenure." A generous start-up is crucial, Gratton agrees, because it is nearly impossible for an investigator to get a federal grant to purchase high-end imaging equipment early in their career.

Graduate students and postdocs who are interested in single-cell imaging should seek out interdisciplinary labs, advises Singer. "It's great when the people who build the microscopes, make the probes and understand the biology get together in one place," he says. "It's really a great synergy and everything progresses quickly because you can bring a lot more breadth to the problem."

Despite the daunting amount of knowledge required to become an expert in single-cell imaging, those who succeed will be gainfully employed, says Singer. Potma sees the field as part of the progression towards better understanding of how cells contribute to health and disease. "There's still so much left to visualize," he says, "that I don't see an end to this field any time soon." ■

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TURNING POINT

Jonathan Cirtain

On 26 September, Jonathan Cirtain, a solar physicist at NASA's Marshall Space Flight Center in Huntsville, Alabama, received a Presidential Early Career Award for Scientists and Engineers.

What made you pursue a scientific career?

When I started college, I went to play American football at the University of Alabama in Tuscaloosa, but an accident in 1993 ended my football career. Lacking motivation, I didn't start college again until 1999 — after my dad made me promise to get a degree and stop wasting my life. He died two weeks later and I kept my promise. I started an undergraduate degree in mathematics and physics, subjects I'd always enjoyed, at the University of Memphis, Tennessee. I wanted to work for NASA or an outfit that supplied NASA with instrumentation. I thought that the best way to achieve that was to get a PhD in physics.

How did you get interested in solar physics?

At a conference of the American Astronomical Society I met Piet Martens, an astrophysicist at Montana State University (MSU) in Bozeman, who told me that his team would be opening a slot to work on NASA's Transition Region and Coronal Explorer (TRACE), which launched in 1998. The telescope took high-resolution images of the Sun's atmosphere, and MSU was involved in mission operations. As a graduate student, I got to operate the scientific payload. I was dropped into the middle of the action.

How did you earn a predoctoral fellowship?

The Sun's atmosphere had always looked fuzzy and out of focus in observations. It turns out that the community was not achieving the best resolution for engineering reasons. During my first year at MSU, I demonstrated a physical model for the Sun's atmosphere using observations from different instruments in space, and showed how imaging instrumentation should be altered to bring the Sun's atmosphere into focus. That caught the attention of Leon Golub at the Harvard-Smithsonian Center for Astrophysics in Cambridge, Massachusetts, and led to a predoctoral fellowship in 2004. Since then, my work has included helping to build the next-generation telescope for TRACE.

How did you accelerate your academic career so quickly?

While calibrating the Hinode X-ray telescope in 2005 at the Marshall Space Flight Center, I got to analyse the data and demonstrate performance. I became one of the few people who

really understood the ins and outs of how the instrument works. I worked on my dissertation and defended it in March 2005. So, in 5 years and 10 months, I went from no undergraduate degree to a PhD.

What are your next big launches?

In June next year, as head of the solar-physics team, I'm going to launch a telescope that will offer a tenfold improvement over any previous extreme ultraviolet instrument. It is called the High Resolution Coronal Imager (Hi-C) and it will have 150-kilometre spatial resolution. A week before I launch that, I will launch the Solar Ultraviolet Magnetograph Instrument (SUMI), which will be used to infer the magnitude and direction of the Sun's magnetic field.

Despite the economic challenges facing NASA, is now a good time to seek a space-related career in the United States?

Perhaps I'm still young enough that I'm naive. The challenges that NASA faces are the ones the nation faces. This is a real economic crisis in the government; I don't think NASA is particularly different from any other agency. However, there is a shortage of US citizens pursuing careers in scientific instrumentation. Most of the graduate students who are interested in this area are foreign nationals. I think that's bad for the nation's scientific competitiveness. Now is as good a time as any to go into space-based astrophysics.

To what do you attribute your career success?

I don't think I'm a genius; I work hard and make the most of opportunities I'm given. I don't know how I would have got this far without my wife, whose support and willingness to move around with me have been extremely important. ■

INTERVIEW BY VIRGINIA GEWIN