MICROSCOPY AND ANALYSIS

M&A 30

Pioneers and leaders in microscopy

by Dr Rebecca Pool

WILEY

INTRODUCTION

Passionate people are one of the principle driving forces in science, this has always been the case and advances in 21st century microscopy continue this trend around the world.

As a celebration of the 30th anniversary of *Microscopy and Analysis*, we have assembled a collection of profiles of noted microscopists from around the globe who have shaped the way we see and use microscopy in all its forms. In compiling this book, we have tried to reflect the diversity of microscopies available, covering optical, electron and scanning forms, whilst casting a spotlight on individuals from a range of geographical and scientific areas.

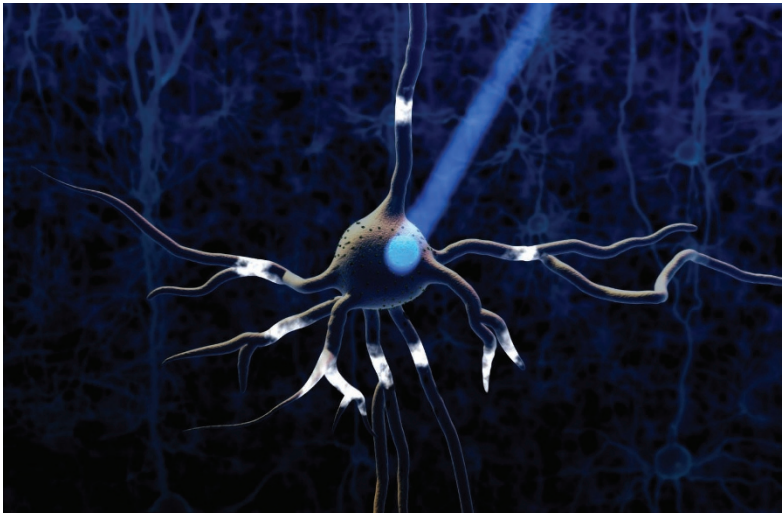
In each case, you will discover their origins, what motivated them, how their careers have been shaped by their decisions and their mentors but also sometimes by factors beyond their control. Time and time again, I was left captivated at the dedication, passion and belief of the interviewees who often faced scepticism, ridicule, dead-ends, financial hardships and other less than perfect conditions to make their dreams reality.

I'd also like to take a second to comment on the behind the scenes work that often isn't acknowledged, by which I mean the people who supported each of our profiled scientists. Science is typically a collaborative endeavour and often it can be difficult to recognise the contributions made by members of a team when papers get published or prizes are awarded. I know that for each of our featured scientist and engineers there will have been a collection of people who gave time and energy as a contribution to the stories that we're celebrating here. Every story has had a multitude of silent authors and so I'd like to thank them all for doing their part for our community.

Whatever your interests in microscopy, I really hope that by understanding a little more from the lives of people and the technologies that you know today, you will draw inspiration for your own microscopy journey!

Dr Chris Parmenter, PhD FRMS,
Editor, *Microscopy & Analysis*

CONTENTS



ERIC BETZIG
BEYOND THE DIFFRACTION LIMIT

Ask Professor Eric Betzig what he feels about his Nobel Prize in Chemistry and you might be surprised. "It's been very weird to see people's reactions as you know you're the same guy, but you're not treated as if you are."

"Still it settles down and I'm getting to the point now where I can go at least a day maybe without thinking about it," he adds. "In terms of drive, I don't see much to be gained by having it, so I hope soon I will rarely think about it."

But luckily for the world of microscopy, the reluctant Nobel Laureate has never been a reluctant scientist. His passion for science started with space, and growing up in the 1960s in the college town of Ann Arbour, the Apollo programme fuelled astronaut aspirations while friends and teachers linked to the University of Michigan stoked scientific curiosity.

"In Fourth grade, my teacher's husband was an assistant professor just when quarks were discovered," he says. "I remember writing letters to him, asking questions like 'What's the charge of a quark?', so there was always an outlet for my interest."

Come the end of school and with astronaut ambitions put aside, Betzig wanted to be a theoretical astrophysicist, so headed out to the California Institute of Technology (Caltech) to study physics. However, a couple of summer placements at Caltech, setting up a system to explore instability modes in gas jets, would change his mind.

"This was my first real taste of doing experimental research

and it was going to be very important for me," he says. "Before I'd thought 'well I'm going to be an astrophysicist' but by the time I was doing this, I was thinking 'Gee I really prefer doing stuff with my hands'."

ACADEMIC ANGUISH

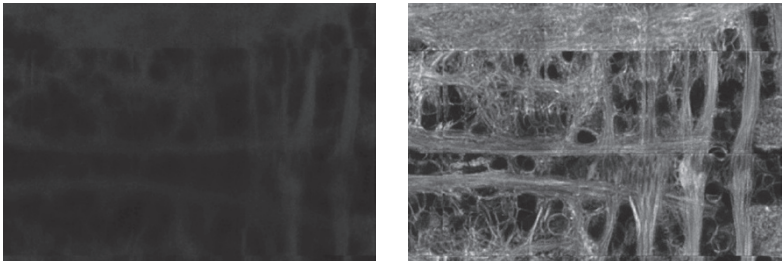
Having spent the best part of his career in research, Nobel Laureate, Professor Eric Betzig, clearly has mixed feelings about working in academia.

"You train someone through their postdoc to excel at bench science and then you make them an assistant professor, rip all that away and make an administrator out of them," he says. "And then you have green as grass graduates trying to do the research."

After pioneering near-field microscopy, Betzig left research for several years to work in industry before returning and delivering microscopy method after microscopy method.

As he puts it: "Too many scientists working in microscopy and technology sweep problems under the rug. Overhyping or a need for funding requires them to focus on what's good about something."

"I prefer to focus on what's bad and that's where the creativity begins," he adds. And this is where he believes organisations such as Bell Labs and Janelia, with a strong research-ethic, work. "Unlike a standard university I've been able to focus 100% of my attention on research," he says. "I don't need to hype to get a job and I don't need to publish if I don't want to."



NEURONS *in the hindbrain of a developing zebrafish: left, without adaptive optics and right, with adaptive optics showing an increase in signal and recovery of resolution*

Kai Wang, Eric Betzig, Janelia Research Campus

So with his degree also in his hands, Betzig set out to study applied physics. It was the mid-1980s and only two universities offered the right Masters programmes; Cornell and Stanford. Betzig chose Cornell – he'd had enough of California – and plumped for a near-field microscopy project. The aim was to develop an optical microscope that could image living cells with the resolution of an electron microscope, in Betzig's words: a 'really big deal'.

His supervisors, Professors Mike Issacson and Aaron Lewis, had been using electron beams to drill 30 nm holes in opaque membranes to form apertures that could create a sub-wavelength light-source for scanning samples to generate super-resolution images. Betzig soon discovered the apertures were too fragile, so pioneered a scheme based on glass micropipettes previously developed by Erwin Neher and Bert Sakmann for use as recording electrodes to study single ion channels in cells.

Betzig and colleagues would pull glass micropipettes and then coat the structures with aluminium to create an opaque tapered probe with a sub-wavelength aperture. Neher and Sakmann would later win a Nobel Prize in Physiology or Medicine

for their micropipette-aided research, while Betzig would build a near-field scanning optical microscope, break the diffraction limit and take the technology to Bell Labs.

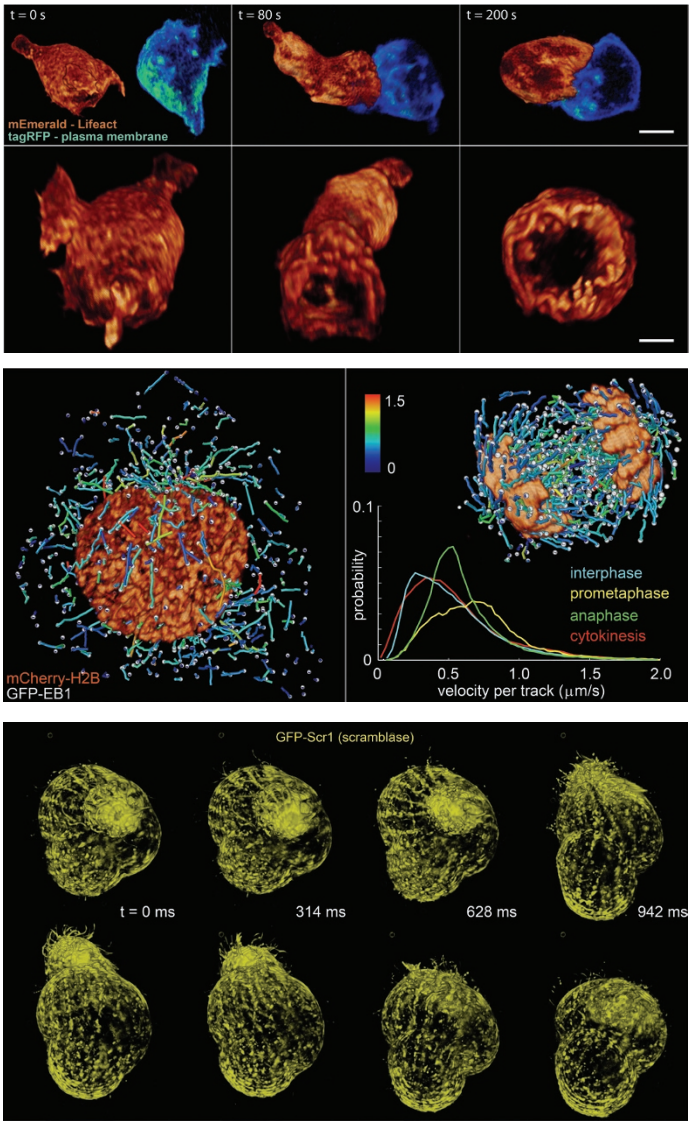
"The microscope was a pain in the neck to work with and the resolution gain over the diffraction limit wasn't huge, but it was enough to get me into the door of Bell Labs," he says. "So here I developed the technology to actually work routinely and with better resolution."

At Bell Labs, he switched the micropipette probe for an adiabatic tapered optical fibre that guided light more efficiently to the tip region and also invented a dithering technique to oscillate the probe and regulate its contact with a sample surface. The probe was some ten thousand times brighter than past probes and could reach 15 nm resolution.

Come the early 1990s, Betzig had demonstrated super-resolution photo-lithography, nanoscale spectroscopy, and crucially, super-resolution fluorescent imaging of actin filaments in fibroblast cells.

"There were a couple of significant innovations to make the technology work at a better resolution and routinely as opposed to the instrument I had at Cornell," he says. "But I was the first to see super resolution fluorescence imaging of cells and from these images it was clear that the signal and noise levels were good enough to see single fluorescent molecules from the near-field."

So he re-arranged his experimental set-up, and in just an afternoon, was able to repeatedly detect and determine molecule position at room temperature. What's more, he could also use the method to map the electric field distribution in his near-field aperture with molecular spatial resolution. He had achieved single



DRIVING *light-sheet microscopy forward [Betzig Lab], from top:*
1 Cells in prophase (left) and anaphase (right) with growing microtuble ends
2 T cell approaching target cell
3 Protozoan T Thermophila at eight consecutive time points

"This is probably one of the experiments I am most proud of," he says. "We could see the molecules, determine their orientations... So we wrote up a paper in a week, it was accepted by *Science* in two weeks, and two weeks after that was in print."

At the same time, Betzig was also working with his now lifelong friend, Harald Hess. Hess was pioneering low temperature STEM, so the pair combined Betzig's near-field probe with Hess's STEM to study luminesence centres in quantum wells.

"One of the reasons my boss at Bell Labs hired me was to find a way of looking at the energy levels in semiconductor structures at very high spatial resolution," explains Betzig. "After five or six years, Harald and I bowed to this idea, and we were surprised at the results."

The pair discovered the luminescence glowed from discreet points and in different wavelengths according to quantum well width. And as Betzig highlights: "Even though there might have been many of these structures under our probe at any one time, we could still study them individually as they glowed in different colours."

So now having the ability to see single molecules and isolate discreet luminescent emitters, the seeds of Betzig's Nobel Prize winning breakthrough – super-resolution photoactivated localization microscope PALM – were sown.

CHANGE OF HEART

But despite progress Betzig was tired of research. He'd realised near-field microscopy, given its short depth of focus, would never image live cells with the resolution of an electron microscope. So come 1994, he left Bell Labs to work for his father, at the Ann

Arbor Machines company, which manufactured parts for the automobile industry (see 'Academic anguish').

"I was full of myself from my success with near-field and thought I could change this [industry]," he adds. "My Dad's company was making enough money to support an idiot like me to mess around with ideas so he humoured me and I went there."

Yet Betzig still had research on his mind and within a year had published a short paper in Optics Letters ideas outlining a method for molecular optical imaging based on his Bell Labs' single molecule localisation research and luminescence results.

"This was the idea for PALM but I knew it would be a very challenging experiment," he says. "It would have been very difficult for biology at the time."

Betzig spent six years at his father's company, after which time the automobile industry has dwindled, but his scientific aspirations hadn't. Crucially, during his time in industry, Betzig had also learned, in his words, a huge lesson.

"The most painful and important lesson, and this is for business or science, if you want to make a significant impact you have to listen to the customer," he says. "Now, open any issue of Optics Express, Optics Letters and you see a tonne of microscopy stuff, but most of it, even if it's successful is not anything anybody really cares about. "

So with the customer in mind, Betzig left his father's company, started reading scientific literature, and learned green fluorescent protein had been discovered. He quickly realised the potential of photoactivated fluorescent proteins in super-resolution microscopy, the impact this could have on biologists and reconnected with Hess. Based on their work at Bell Labs and

Betzig's most recent paper - now nearly a decade old - the pair built the first super-resolution photoactivated localization microscope (PALM) prototype in Hess's home.

The method relies on the ability to turn the fluorescence of molecules on and off. Molecules are imaged many times, with a small subset of fluorescent tags switched on each time. These images can then be superimposed to yield a single picture crammed full of glowing molecules.

Eventually, Betzig and colleagues would compile thousands of images to generate a super-resolution image, but as the Nobel Laureate points out: "We had no cell culture facilities, molecular biology or cloning capabilities in Harald's living room." So they tracked down Jennifer Lippincott-Schwarz and fellow biologists working with photoactivated GFP, at Florida State University, and persuaded them of their microscope's potential.

"Many people would have blown off these guys who were ten years from having written a paper and talking crazy talk," laughs Betzig. "But Jennifer said the microscope sounded great."

In 2005, Betzig and Hess moved to the National Institutes of Health campus and would soon start long, prosperous research careers at Janelia Farm Research Campus, Howard Hughes Medical Institute. Working in Lippincott-Schwarz's laboratory, the researchers set to work developing PALM and within months were getting results, creating, for example, images of organelles at the molecular level.

MOVING ON

But come 2008 Betzig was, as he puts it, 'bored and frustrated' to the same extent as he had been with his near-field microscopy in

1994. As he points out: "I found PALM too slow, too damaging to look at much in the way of living things and it required ridiculously high labelling densities to get high resolution."

"The technologies are now only just developing to get to the kind of density that you really want to work with on a routine basis," he adds.

However, at around this time, microscopy pioneer, Ernst Stelzer, was fast developing light sheet microscopy to image fixed and live embryos. Here, a laser light sheet is repeatedly swept across the field of view of a sample to build a 3D image.

Betzig had heard about this technique but quickly noted that the Gaussian beams being used were too thick to look inside a single cell. Drawing from the near-field microscopy research of his early days, he realised he could switch the thick Gaussian beam for a non-diffracting narrow Bessel beam to create a thin virtual light sheet to image inside single cells.

By 2011, Betzig had perfected Bessel beam plane illuminating microscopy for live cell imaging, yet still felt he could achieve more. To reduce the time taken to scan the section, he decided to divide the Bessel beam into parallel parts, pioneering a new class of light beams, the 2D optical lattice.

As Betzig explains, the lattices are created by modulating laser light with a fast-switching spatial light modulator, which are then filtered and focused together to form the light sheet. Crucially, these ultra-thin lattice light sheets can non-invasively image live specimens at higher spatial and temporal resolutions.

Late last year, Betzig's team revealed videos of a host of processes from embryonic development in nematodes and fruit flies down to chromosomes moving in dividing cells, publishing

results in Science. And now the researcher is looking to integrate adaptive optics he has been working on for several years to the lattice light sheet process.

"This is only applicable to transparent organisms, but that's ok as there's still plenty we can learn biologically from those types of specimens," he says.

So with Nobel Prize in tow, research clearly continues for Betzig. But what, does he believe, is the secret of success? His answer is simple. "Talk to people to find out what makes a difference,"he says. "And once you've convinced yourself it can make a difference, be passionate about it and work ridiculously hard."


ED BOYDEN
BRAVE NEW BRAIN SCIENCE

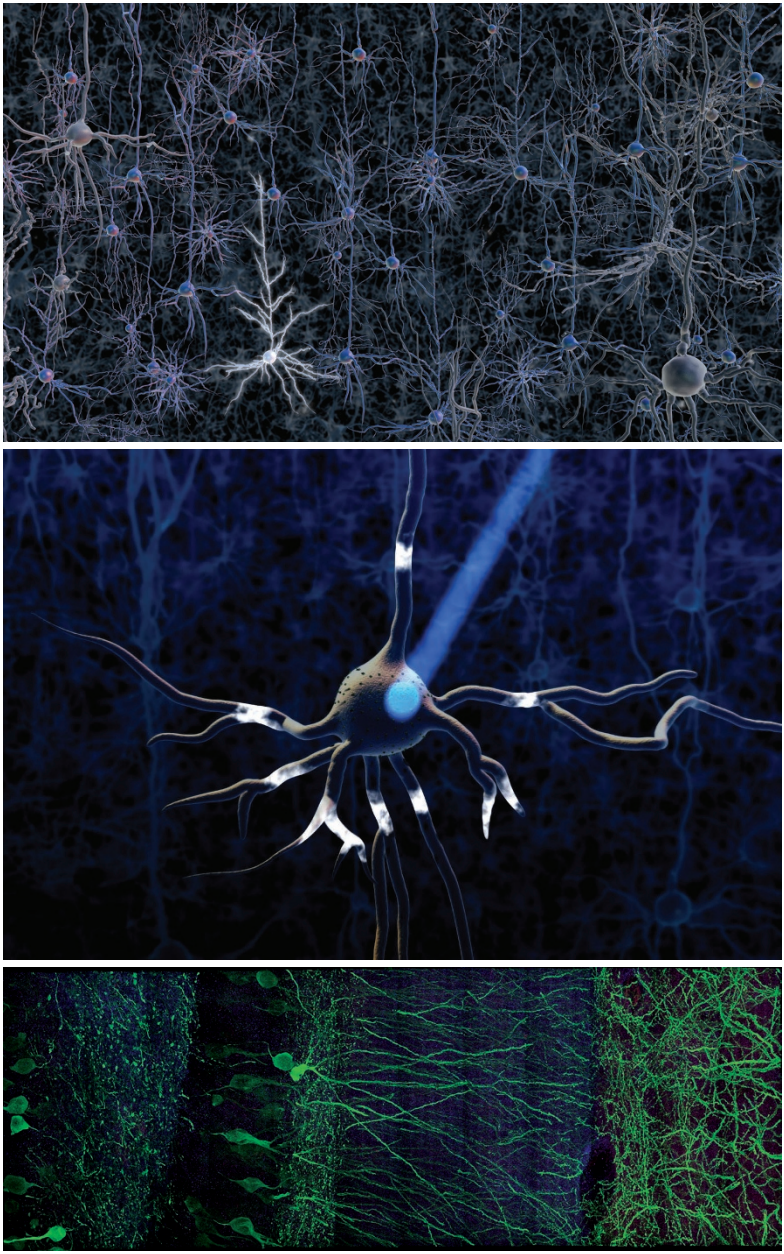
From switching neurons on and off with light to swelling brains for imaging with nanoscale precision, Professor Ed Boyden's unconventional breakthroughs have changed the face of neuroscience.

Now heading up the Synthetic Neurobiology Group at the MIT Media Lab, the researcher is applying his eclectic brand of brain science to build tools to revolutionise biology and medicine. His methods are already being used worldwide to repair retinas, develop neural-control prosthetics and map parts of the human brain. But the road to radical research has been far from straightforward.

Like his research, Boyden's early education didn't follow convention. At age 14, the young student went to the University of North Texas to study chemistry. "I was a philosophical student and was working with a research group that was trying to create the building blocks of life from scratch."

"Our goal was to create molecules such as DNA," he says. "It was fun, I learned a lot but of course it didn't work or you'd have heard about it by now."

Two years later, in 1995, Boyden transferred to MIT to study Physics as well as Electrical Engineering and Computer Science. By 1999, he had graduated with degrees in both, as well as a Masters in Electrical Engineering and Computer Science working on quantum computation, and crucially had also discovered a passion for building tools.



OPTOGENETICS *(Top and middle) 3D super-resolution microscopy of mouse brain tissue: volume rendering of a portion of hippocampus showing neurons (green) and synapses*

Fei Chen, Paul Tillberg, and Ed Boyden, MIT, Research published in Science January 2015, Vol 347, Issue 6221

EXPANSION MICROSCOPY *(Bottom) 3D super-resolution microscopy of mouse brain tissue*

Boyden et al, Science

As part of his graduate research, the young Boyden had worked with Professor Neil Gershenfeld from MIT, as well as Professors Michale Fee and Sebastian Seung from Bell Labs, all renowned for a 'free-range' approach to research. As such, Boyden developed computational models of how birds sing, engineered control software for an autonomous submarine, programmed machine-learning tools for reconstructing digital violin dynamics, and more.

Given this, as well as his interdisciplinary degrees, Boyden figured he was ready to study the brain. "I felt perfectly poised to bring these points of view into neuroscience and to really think like an inventor," he says. So he did.

ROAD TO OPTOGENETICS

In 1999, Boyden embarked on a PhD at Stanford University. Working for neurobiologists, Professors Jennifer Raymond and Richard Tsien, he studied how neural circuits use plasticity to store memories.

However, at the same time, he also started to wonder how technologies could be used to control the electrical activity of different neurons in the brain. A few late night conversations with his colleague, Dr Karl Deisseroth, led to the idea that light

could be harnessed to control a single brain cell.

"We thought about magnetic fields, mechanical force, light and all sorts of ways to deliver energy into the brain," he explains. "But in the end we picked light as it is fast and can be focused."

At the time, other researchers were experimenting with light, using laser pulses for example, to activate many neurons. However, Boyden and Deisseroth wanted to switch specific neurons on or off as part of a grand plan to determine which type of neuron was responsible for a certain character trait, brain disorder or even disease. But of course, the first, critical question was how?

Help first came in 2002, when Professor Gero Miesenbock and colleagues from Sloan-Kettering Cancer Center in New York City, showed that genetically-sensitised Drosophila neurons could be driven by light.

Then, in 2003, Professor Georg Nagel, University of Würzburg, Germany, discovered a light-sensitive protein from green algae – Channelrhodopsin-2 – and showed that the protein could be used to depolarise mammal cells.

Excited by these developments, Deisseroth and Boyden obtained a clone of the protein from Nagel, and engineered cultured neurons that could express Channelrhodopsin-2. Then, late one night in 2004, Boyden placed a dish of the cultured neurons under an epifluorescent microscope, patch clamped one of the glowing neurons and pulsed blue light at the samples. Immediately the patched neuron fired electrical signals, Boyden had demonstrated neurons could be activated with light, and suddenly, this fledgling field of neuroscience – now known as

optogenetics – was ready to explode.

Since this time, Boyden and colleagues have developed strategies to 'silence' the cells and also used different proteins to control neurons in live mammal models. Key breakthroughs, primarily achieved working alongside experts on specific diseases, have included restoring vision in mice and suppressing beta amyloid plaque production – the hallmark of Alzheimer's disease – in mice brains.

Crucially, along the way, Boyden has also shared his methods with thousands of other research groups in academia and industry, and today, several start-ups are also pursuing optogenetics research in humans.

"The field is now mainstream, and there must be hundreds of papers published in which, say, researchers turn-off certain neurons in a mouse brain to try to shut down epileptic seizures or work out how emotional feelings arise," he says.

"A Caltech group used our tools to photo-activate neurons deep in the brains of mice, so the mice would become aggressive," he adds. "Such research is helping people realise the power of these tools in finding brain circuits that implement complex functions that you probably couldn't probe in any other way."

But while optogenetics progress has been rapid, for Boyden, it is just one pioneering method in his growing neuroscience toolbox. Completing his PhD in late 2005, the researcher moved to MIT Media Lab in 2006, to set up his own neuroengineering research laboratory, the Synthetic Neurobiology Group.

As he puts it: "The MIT Media Lab is MIT's home for misfits and has given us time to grow on our own outside the confines of traditional disciplines."

And unconventional imaging tools have followed. For example, in the Summer of 2014, Boyden and collaborator Alipasha Vaziri revealed a system based on light-field microscopy to generate 3D videos of the entire brains of C. Elegans nematodes and zebrafish larva at millisecond timescales. A microlens array is inserted into the optical train of an epifluorescence microscope so that sensor pixels capture and recombine the 2D location and 2D angle of incident light to recreate the 3D structure.

However, in January 2015, Boyden revealed what is arguably his biggest breakthrough since Channelrhodopsin-2-based optogenetics. So-called expansion microscopy uses an expandable polymer to swell tissue to around four and a half times its usual size, so that nanoscale structures appear within focus on an ordinary confocal microscope.

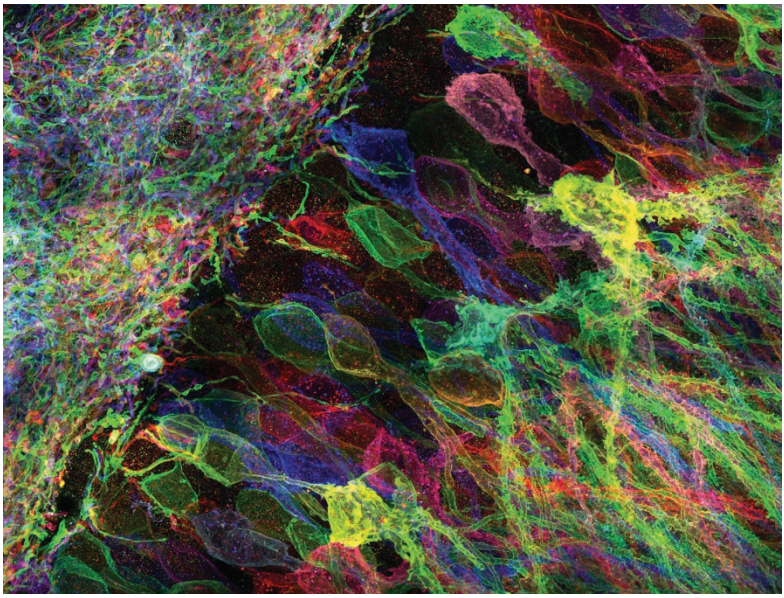
According to Boyden, his team first explored concepts similar to expansion microscopy in 2007 as a means to separate and more easily tag proteins in cells. However, they quickly shelved the idea as PALM, STORM and other super-resolution microscopy methods were emerging around the same time.

Still, come 2012, Boyden's graduate students Fei Chen and Paul Tillberg were struggling to use STORM as well as electron microscopy to map the brain. The old idea was unearthed.

"Expansion microscopy in 2012 emerged as a bit of a joke borne out of frustration as we asked ourselves, 'why can't we just make the darn thing bigger?'," explains Boyden. "Performing multiplexed biomolecular mapping to look at how biomolecules are organized was appearing really difficult using STORM and electron microscopy, so we thought, why don't we just expand these samples."

They went onto infuse sodium acrylate into chemically fixed brain tissue, adding polymerisation agents to form a tissue-polymer network composite within the sample. Fluorescent labels were covalently anchored to the polymer at biomolecular sites within the tissue-polymer sample, which was then treated with protease to homogenise its mechanical properties and immersed in water to trigger a 4.5 times linear expansion.

According to Boyden, labels spaced closer than the optical diffraction limit were separated and he and colleagues used the method to image human embryonic kidney cells and mouse brain tissue slices with 70 nm lateral effective resolution.



Brainbow hippocampus; nanoscale resolution imaging on diffraction-limited microscopes

Boyden et al, Nature Biotechnology

"Embedding tissues in polymers has a very long history... but with our expansion method we can begin to image in 3D with nanoscale precision across large areas," says Boyden. "My hope is that we can use this tool to get full maps of the brain with molecular information, and maybe someday be able to simulate what is happening when a brain circuit generates a thought or a feeling."

Since its development, the researchers have honed the method to rely on off-the-shelf chemicals, rather than the original custom-designed chemical tags. What's more, in the last year, and in a similar vein to their optogenetics tools, they have shared the method with more than 100 research groups.

"Researchers are using this tool to figure out where pathogens are hiding in tissues, as well as how cancers differ from normal tissues," highlights Boyden.

"We have no idea what's going on in so many diseases," he adds. "But if we can use tools such as optogenetics and expansion microscopy to figure out what's important, perhaps we can develop better therapies." Boyden and colleagues now intend to combine optogenetic tools with expansion microscopy; the results look set to be mind-boggling.

As Boyden points out, he and colleagues could use optogenetics to activate certain neurons and, in turn, observe how these neurons control other neurons, then map the brain circuit using expansion microscopy. "We could create computational models of brain circuit," he says. "We might be able to use these to predict what a brain circuit will do during a certain behaviour or disease."

So as research continues apace, what advice would Boyden

give to the aspiring researchers of the future? In his words: "Every time somebody gives you advice, consider doing the exact opposite."

PRATIBHA GAI
AN EYE FOR ATOMS

In March 2013, Pratibha Gai, Professor of Chemistry and Physics at the University of York, UK, won the L'Oréal-UNESCO For Women in Science Award for her pioneering work in electron microscopy. In her quest to understand atoms, she drilled a hole in her multi-million pound electron microscope to image dynamic chemical reactions on an atomic scale.

Her breakthrough followed more than a decade of determined research and marked the beginning of many discoveries. And it all started with Marie Curie and an interest in logic.

"Reading about Marie Curie when I was in school in India inspired me to become a physical scientist, but I was always fascinated by the logic behind science and how everyday things could be explained rationally," she says.

Gai's interests saw her selected as a National Science Talent scholar in the physical sciences, and she later won a scholarship to the University of Cambridge, UK.

Gai admits being so far from home was daunting, but adds: "I had this over-riding desire to do something new in the physical sciences, and at this age you have tremendous confidence that if you want to do something, you can."

Completing her PhD in physics at Cambridge, she moved to the University of Oxford, where she established, and led, the surface reactions group. Soon, she realised she wanted to scrutinise chemical reactions, at atomic resolution, for the very first time.

"To study reactions, researchers would insert an in situ re-

action chamber into the electron microscope," she explains. "We needed to frequently open up the electron microscope column to the air, put the jig in and re-pump the instrument, which also led to difficulties in the alignment of the electron beam as well as mechanical stability and performance problems."

Still, using this clumsy methodology Gai reached nanoscale resolution. But this wasn't enough. "I wanted to understand what to do to get to atomic resolution. Conventional wisdom dictated it impossible to do this under high gas pressures and temperatures, but my work at Oxford had given me the foundations to advance further," she says.

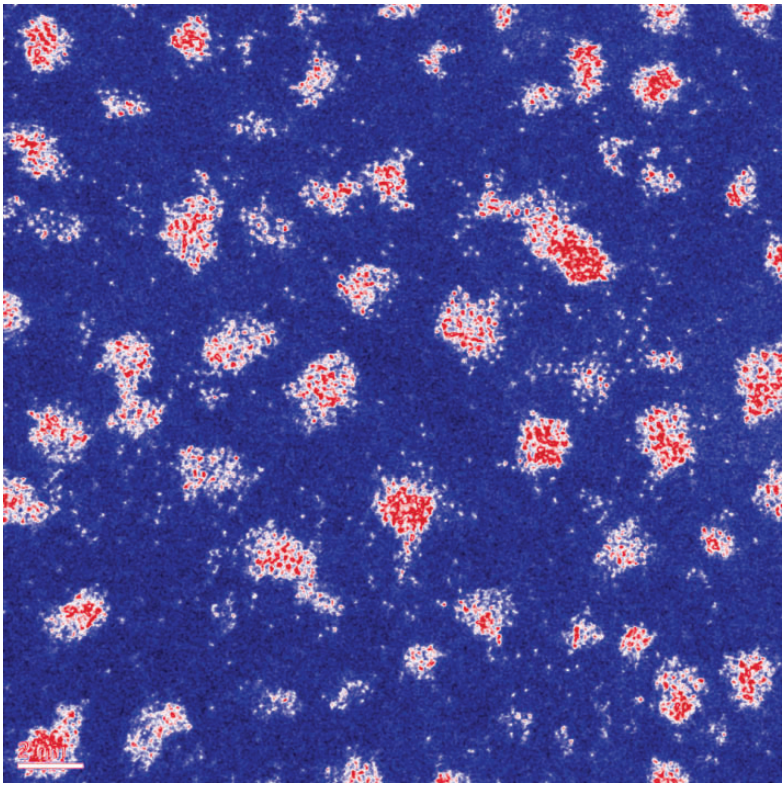
At the same time Gai was unsatisfied with Oxford life. In her words, she "missed not having female senior scientist role models in the physical sciences", but knew in the US, women of all ages were working in this field.

So she accepted a senior scientist position leading to DuPont Research Fellow at DuPont Central Research Laboratory in Wilmington, Delaware, and also served as adjunct Professor of Materials Science at Delaware University. Crucially, DuPont dedicated a modern electron microscope to her research, which led to the world's first atomic resolution environmental TEM.

Armed with her initial calculations from Oxford, Gai teamed up with Professor Ed Boyes to put theory into practice. "We worked every hour of every day; nobody knew what we were doing, but with just Ed Boyes and our technician, we made it possible," she says.

Gai's calculations indicated that if she drilled a hole through the instrument's imaging lens, gas could be pumped through, and into, the electron microscope column to create a reaction

environment where the sample was. In this way, the electron microscope chamber would be the chemical reactor, eliminating the need to insert a reactor jig into the column.



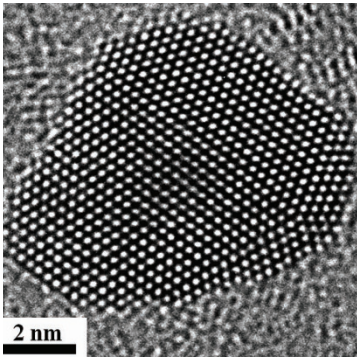
ESTEM *of single atoms and clusters of platinum*

ETEM of a gold decahedral particle at the atomic level, important in understanding the stability of active reaction sites at the atomic level in catalysis.

"It took nearly two years to finish calculations, build a mock-up, test everything from how large the hole should be to

pumping speed," she says. "And because the lenses are electromagnetic we knew that even a tiny mistake would ruin the machine. We knew it was risky but you need to take some intelligent risks to advance science."

And they did. In 1993, Gai used the ETEM, to watch for the first time ever, atoms working in chemical reactions.



ETEM *of a gold decahedral particle at the atomic level, important in understanding the stability of active reaction sites at the atomic level in catalysis*

"I could see how a substrate interacted with a noble metal nanoparticle catalyst and watch the changing structure at atomic level," she says. "It was thrilling."

Crucially, Gai didn't patent the atomic resolution ETEM design, instead wanting researchers to access her development. "It has since been commercialised by leading manufacturers, and replicated by researchers worldwide," she says. "This shows that you can have a simple idea and just do it with the right background and calculations."

Gai spent eighteen years at DuPont, studying reactions with her ETEM. During this time she discovered an atomic scale reaction site mechanism in catalysts, since called the Gai glide shear mechanism, key to efficiently producing biofuels and polymers.

She also developed antibiotic nanoparticles for healthcare applications and a green nanocoating process for strong polymers and coatings. Her ETEM research led to significant technological and economic benefits, but still she wanted to push the boundaries of microscopy further.

Come 2007, she moved back to the UK with Boyes, to set up the York JEOL Nanocentre at the University of York and develop an electron microscope to study single atoms. Together, Gai, Boyes and their team developed atomic resolution in-situ aberration corrected ESEM. By enhancing the atomic resolution of ETEM, they combined ETEM and ESTEM in a single dual aberration corrected instrument, and imaged, for the first time, individual atoms in a chemical reaction environment.

"With the ETEM we were looking at columns of atoms, but with AC ESTEM we see single atoms scurrying around in a chemical reaction, trying to find other atoms and making dimers, trimers and clusters," says Gai. "It's amazing; the whole living atomic world opens up in front of you."

Gai also devotes time to teaching and supervising students, studying materials systems crucial for applications from medicine development to water purification. "With environmental electron microscopy students can watch reactions happening and this is really fantastic for them," she says.

In 2010 Gai won the Institute of Physics Gabor medal for her pioneering development of atomic resolution ETEM and has now scooped the L'Oréal-UNESCO For Women in Science Award as the 2013 Laureate for Europe. What next?

Research continues and with her students Gai intends to help develop novel energy sources and infection medicines.

"The L'Oréal-UNESCO award puts women scientists' research on the world stage, and these senior scientists are role models for the next generation of women to pursue scientific careers," she says. "In my own class many women PhD students are interested in continuing. Now that has to be a good sign."

LEO GROSS
PLAYING WITH ATOMS

In 2009, Leo Gross revealed the first ever images of individual bonds between the atoms of a molecule that he had taken using a modified atomic force microscope (AFM). At the same time, he used this technique to measure the charge state of single atoms, again, a world first.

And then, in 2013, the IBM Research-Zurich scientist imaged the different chemical bonds between carbon molecules. Yet again, this marked a first as scientists had never before seen the physical differences between these different bond types.

Each of these breakthroughs has delivered a deeper understanding of chemical reactions, but ask Gross about his success and you'll receive a modest answer. "I think good teamwork is important, or at least for me it has always been very important," he says. "If you see things, you can discuss these with your colleagues so it's very good to have close collaborations with colleagues and friends. You discuss your results and this generates new ideas."

Today, and since 2005, Gross has been working alongside Gerard Meyer, who heads up IBM's scanning tunnelling microscopy-related research. Meyer himself pioneered low temperature scanning tunnelling microscopy (STM), which is now used worldwide, and Gross describes the researcher as a 'great role model'.

"He's also responsible for most of the instrumental achievements that we have," explains Gross. "As well as having knowl-

edge on so many different fields, in addition to physics, he has all the skills you need to build such machines. He's constantly improving the low temperature AFM electronic software and hardware."

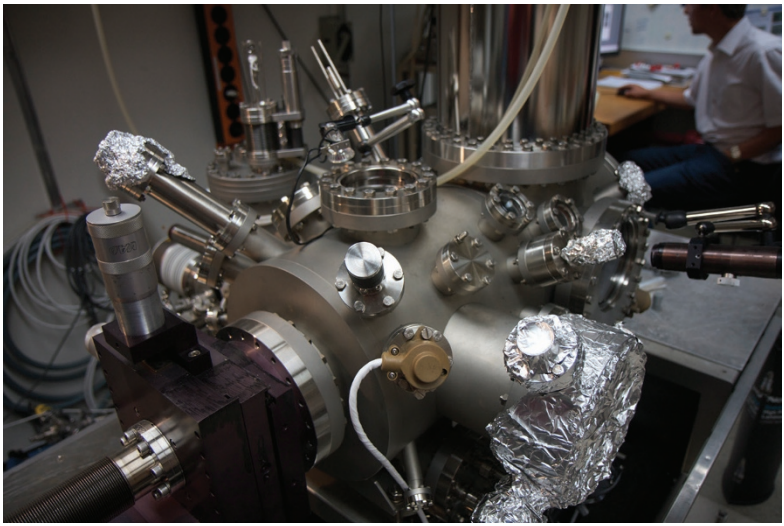
It is this passion for instrumentation that has also driven Gross. As part of his degree in physics from the Free University of Berlin, Germany, the young scientist spent a year working in Tulane University, New Orleans, with Professor Ulrike Diebold. Diebold, now at the Vienna University of Technology, is renowned for her STM studies of atoms, notably titanium dioxide.

During his time with Diebold, Gross also studied titanium dioxide, encountering STM for the first time, an experience that stirred initial curiosity in these microscopes. "Titanium dioxide is now one of the most studied materials, especially for catalysis, but it was really the techniques we used that caught my interest," he says.

"I liked that you get these visual images directly and that you can really optimise images while you work," he explains. "So I enjoyed the whole process of measuring and analysing the data, and it is at this time that I decided I wanted to work with scanning probe microscopy."

With this in mind, and having started out with Diebold, Gross continued to gain experience with the great and the good from the world of scanning probe microscopy (SPM). From Berlin and New Orleans, he moved to the University of Muenster, Germany, to join an STM group led by Professor Harald Fuchs, a leading light in SPM and nanotechnology. And after completing his Master's Diploma here, he then returned to the Free University of Berlin in 2001, to join STM and nanofabrication pioneer,

Professor Karl-Heinz Rieder. Here, using low temperature STM, he explored the behaviour of organic molecules on metallic surfaces, a fundamental step to building molecular electronics devices, and was hooked.



ABOVE *The actual scanning tunnelling/atomic force microscope used to image the pentacene molecule with atomic resolution*

IBM Research-Zurich

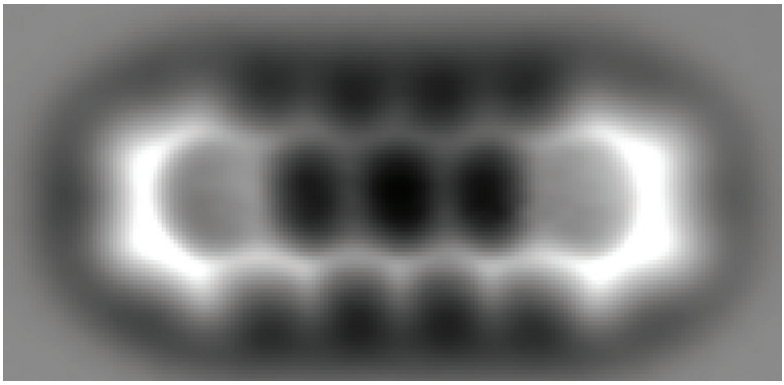
As he explains, low temperature STM – close to absolute zero – brings the possibility of manipulating atoms. "You pull and you push atoms with the apex of your tip to build up an experiment, and that's really nice," he says.

As part of his thesis, he took a so-called six-leg benzene-based molecule, designed by collaborating with chemists from CEMES-CNRS, Toulouse, France, and found that by moving it along a copper surface with an STM tip, he could collect and carry up to six copper adatoms. These adatoms could then be further manipulated to construct molecular-metal atomic-scale

structures.

The technique as since described as a “molecular hoover”, and as Gross says: “You just took this molecule and used it to collect single atoms and then make a small cluster out of these atoms. This was really fun.”

Come the end of his PhD, this research had hit the cover page of prestigious journal, Nature Materials, an achievement many fresh post-graduates only dream of. But by this time, Gross had moved to IBM Zurich – where STM has been invented in 1981 – and was already focusing on new research.



THE DELICATE *inner structure of a pentacene molecule imaged with an atomic force microscope*

IBM Research-Zurich

FROM STM TO AFM

During his first years at IBM, Gross looked at nanostencil lithography, a tool for the fabrication and in-situ characterization of ultraclean nanostructures for fundamental surface science research. However, he hadn’t forgotten his low temperature STM research, and come 2007, was investigating how to apply these techniques to AFM.

While STM had been used to image atomic-scale features on surfaces, resolving single atoms within molecules remained a challenge as the tunnelling current was sensitive to the electron density of states near to the Fermi energy level. Using non-contact AFM would alleviate this problem but moving from low temperature STM to low temperature AFM also had issues.

"AFM is a little bit more challenging than STM. You have more feedback loops and technically [low temperature AFM] is more challenging," says Gross.

A first step was to implement the so-called qPlus sensor – a quartz tuning fork with a much higher stiffness than standard silicon cantilevers – with a metal tip into a low temperature STM/AFM to ensure stable operation at 5K and in a ultrahigh vacuum.

Sensor success in hand, Gross and colleagues then stumbled across something that would lead to the AFM images of molecules that stunned scientists worldwide. As Gross explains, during STM, carbon monoxide molecules are a well known surface contaminant that can be inadvertently picked up by a cantilever tip.

"In our first [non-contact AFM] images of molecules, we started seeing some strange contrasts that we just didn't understand," he says. "We tried to reproduce them and realised that these contrasts were actually due to a carbon monoxide molecule that was accidentally on the tip."

Gross and colleagues repeated imaging experiments with a single carbon monoxide attached to the tip, now described as a CO-modified tip. And as the researcher says, the images just got 'clearer and clearer', boosting AFM resolution to resolve the

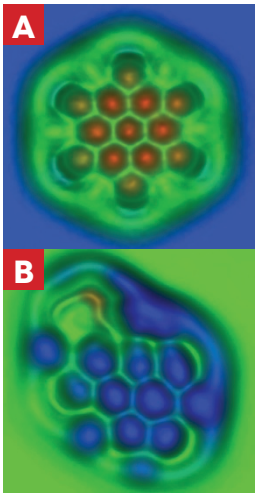
atomic positions and bonds inside a planar pentacene molecule, precisely revealing the atomic structure.

Further studies at IBM Zurich confirmed Pauli repulsion forces were the source of the unprecedented atomic resolution, and Gross and colleagues went onto publish their results and stunning images in Science, in 2009.

The breakthroughs that followed have built on Gross's modified AFM and a growing understanding of the imaging mechanism. For example, further imaging of pentacene revealed differences in the brightness its bonds.

DIFFERENT CHEMICAL *bonds in nanographene molecules, imaged by non contact atomic force microscopy using a carbon monoxide functionalised tip. The carbon-carbon bonds in the imaged molecule appear with different contrast and apparent lengths*

IBM Research-Zurich



Later electron density calculations indicated these could be due to bond order, so the researchers repeated imaging on molecules, such as C60 fullerene, with more distinct differences in bond order. They were able to map subtle differences in charge density and bond length in molecular systems and correlate them with bond order. Results were published, again in Science, and in short, the researchers had captured the first images of different chemical bonds.

And at the same time, the team was also working on other SPM developments, including Kelvin probe force microscopy to measure the charge distribution within a single molecule. As Gross says: "For me, it's been very important that this work is fun, and at the moment

I'm still having fun working with these machines."

Gross and colleagues are currently experimenting with different tip terminations, in a bid to find out how resolution changes with different molecules at the tip and also to gauge which termination best suits certain materials. "Xenon works very well and gives you a realistic topography of the electron density of a molecule," he says. "We've also tried different halogens such as iodine, bromine and chlorine, as well as krypton, nitrogen oxide, copper and gold."

Alongside his research, Gross has scooped award after award, including the first Gerhard Ertl Young Investigator Award, for outstanding research in surface science, and the Feynman Prize for experiment in Nanotechnology, which he won with collaborators, Meyer and Professor Jascha Repp.

And for Gross, collaboration remains key. "I have always had a good working atmosphere with my colleagues, and this, I think it very important to successful research," he says. "When it comes to research, you cannot do it entirely on your own."


MIN GU
NEW DIMENSIONS

From a young age, Professor Min Gu was always interested in lasers. Studying physics in communist China in the early 1980s, he was drawn to optics and embarked on his PhD in laser fusion, studying the optical properties of plasma, in 1984.

"After the cultural revolution in China, there was a national campaign to encourage the smarter kids from the young generation to do physics," he says. "For me, physics was a very logical move and I was very interested in optics, particularly lasers."

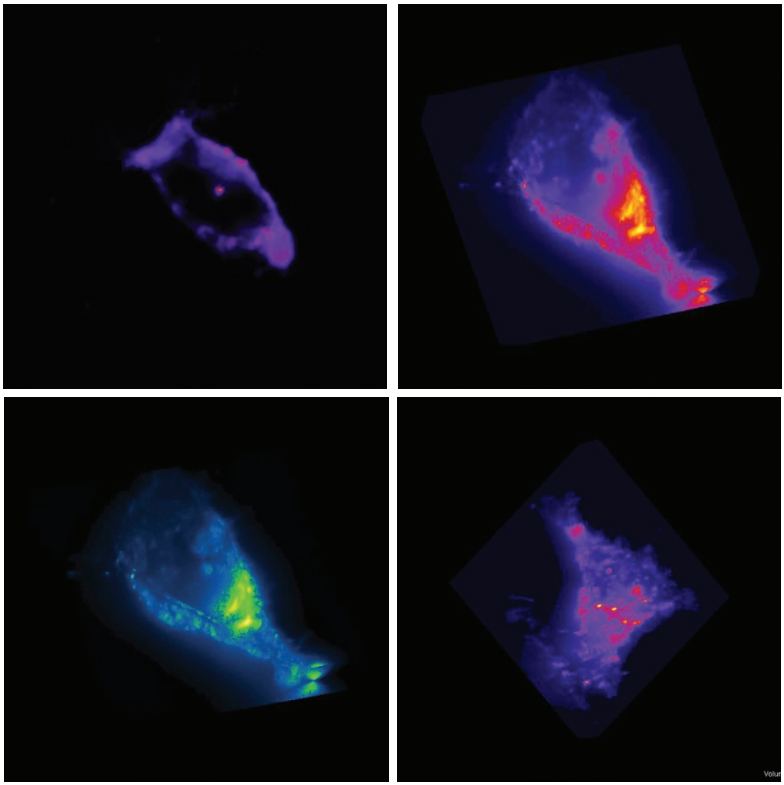
Come 1987, Gu had published his thesis, which to his surprise, was picked up by the then head of theoretical physics at the University of New South Wales, Professor Heinrich Hora. The renowned laser physicist reckoned Gu's experimental results supported his predictions, which at the time no other researcher believed, and urged the young researcher to join him in Australia.

"I joined him as a visiting scholar, solved his problem on numerical saturation and then he helped me to find a postdoctoral position," explains Gu. "He didn't have the money to fund my research at the time... it was and still is very difficult to do plasma physics research."

Within months, Gu had moved across to UNSW's engineering faculty, studying a topic completely unrelated to laser fusion, and any of his yet-to-be determined research: Global Positioning Systems. With an emphasis on numerical simulation, rather than the aerospace engineering, Gu drew up simulations for sat-

ellite signal transmission from the satellite to the ground.

"I was excited to get this position and my contributions to the field were important," says Gu. "But I didn't finish the post-doctorate as I wanted to get back into physics."



LEFT *New depths: Volumetric optical image of Hela cells labelled with a fluorescent dye DiD to mark the membrane; just one example of Gu's research*

Swinburne

Instead, Gu applied, after the application deadline, for a postdoctoral position with Professor Colin Sheppard. Now known around the world for his breakthroughs in confocal and

multi-photon microscopy, Sheppard had recently joined the University of Sydney to set up his Department of Physical Optics.

"It was New Year [1990] and I didn't get a reply, so I gave him a call," laughs Gu. "He said he had received my email and asked if I'd come over for a chat. I did and got the position."

And so started Gu's illustrious research career in microscopy. Primarily focusing on 3D confocal microscopy, Gu experimented with using ultrashort pulsed laser beams in bright-field confocal microscopy as well as two-photon and single-photon fluorescence microscopy to better image thick samples in turbid media; the clear application being cancer research.

In the next five years he would receive an Australian Research Fellowship, publish 79 papers and introduce the first femtosecond pulsed laser to Australia.

As Gu puts it: "Before Colin, I had no idea about confocal microscopy, and he was a great scientist to work with and had many, many ideas, every day; this was a very special time."

But as he adds: "If I had the chance to re-do my life, I would not publish 79 papers. I would not advise young researchers to publish so many papers, we have to balance quality and quantity."

BEYOND CONFOCAL MICROSCOPY

Come 1995, Gu was offered a permanent lecturing position at the University of Victoria, which he accepted. With his first book 'Principles of Three-Dimensional Imaging in Confocal Microscopes' soon to be published, he was beginning to wonder what exactly could be done with confocal microscopy.

"When I left Sydney, I had this idea to use a microscope

rather than study it," he says. "I wanted to explore what impact confocal microscopy could have beyond biology, and had this vision of modern optical microscopy being the combination of the microscope and laser."

On joining Victoria University, he set-up the optoelectronic imaging group. Research into imaging turbid media continued but at the same time the team spent what Gu calls 'three very productive years' developing key applications that formed the pillars of future research.

Laser tweezers came first with the team experimenting with pulsed lasers to optically confine and manipulate microscopic particles. A lot of time was spent characterising trapping forces in terms of the numerical apertures of the objective lenses used in the laser set-up. And this research ultimately led to Gu's breakthrough near-field, single-beam laser tweezers used to manipulate nanometre-sized biological samples with relative ease and little damage.

Gu's ground-breaking research into 3D optical data storage also has its roots at Victoria University, with researchers using infrared lasers to record and write data in photorefractive polymers. These studies went onto form the basis of Gu's five-dimensional data storage, a system of two overlapping laser beams to read and write data at 9 nm resolution that could lead to 1 petabyte DVDs.

As the researcher simply says: "If you understand optical data storage, then basically it's just a confocal microscope."

Having spent a little more than three and a half years at Victoria University, Gu took his research team over to Swinburne University of Technology, and set up the Centre for Mi-

cro-Photonics.

"Swinburne wanted to develop its research, so they approached me and asked if I could join, with my entire group," he explains. "Eventually I thought, yes this is good, as they had offered me significant funding to start up a more comprehensive lab than I had at Victoria, with more students."

And it was at this point that Gu's research really exploded. While his team had made great strides in using two-photon fluorescence microscopy to image in turbid media, such as tissue, Gu knew clinical settings demanded an endoscope. After more than a decade of research he unveiled the first fibre-optic STED two-photon fluorescence endoscope, in 2014, breaking the diffraction-limited resolution of previous two-photon fibre-optic endoscopes by a factor of three.

Similarly, he and his researchers started using multi-photon microscopy to fabricate more and more complex photonic crystals. As Gu explains: "We understood, for example, aberration correction, and knew microscopes so well, so were one of the first groups to make photonics crystals in this way."

Indeed, Gu's photonics crystals are now finding use as chiral beamsplitters in integrated photonic circuits and have paved the way to sub-diffraction optical beam lithography and, of course, better optical data storage. As the researcher adds: "I wanted to do more research into photonics but keep it related to microscopes. That's been my vision and why [my Centre] uses the word 'Micro-Photonics'."

But Gu's thirst for applications hasn't stopped here. In recent years, his team has fabricated photonic bio-chips to 'cage' single T-cells while developing photo-activated luminescence

microscope (PALM) to image these and individual protein molecules.

Meanwhile, the researchers have also developed thin film solar cells that use nanoparticles to scatter light into broadband wavelength ranges, leading to greater photon absorption and a more efficient solar cell. Gu currently heads up the Victoria-Suntech Advanced Solar Facility, a joint venture between the university and solar panel manufacturer, SunTech, to develop the technology for commercial applications.

In the researcher's words: "We have developed a high-efficiency solar cell, but this is also based on using the microscope to understand the solar cell at the light conversion level."

But does Gu's success in such diverse research areas all come back to confocal microscopy? Yes, and no.

As he explains: "Confocal microscopy has been very important. Without my knowledge on this, I would not have reached where I am today, but I have always liked to try new things." And this is a strategy he is keen to impress on his researchers. "When my students come to my office, I usually do very little talking about their projects at a technical level, there's always another time for that," he says. "Instead I tell them that the research they are doing always links to something bigger and ask them what are these bigger things that they can produce?"

STEFAN HELL

THE NOBEL LAUREATE NOBODY BELIEVED

Professor Stefan Hell's Nobel Prize in Chemistry was a long time coming. Rewarded in 2014 for the development of super-resolved fluorescence microscopy, Hell had worked out how to break the diffraction barrier in far-field microscopy as early as 1993 but at the time his approach challenged convention and was dismissed.

Seven tortuous years later with rejection from journals far and wide, he finally published his seminal paper in the *Proceedings of the National Academy of Sciences.* The results stunned the skeptics, sparked interest in the overlooked topic of far-field light microscopy and helped to save his career.

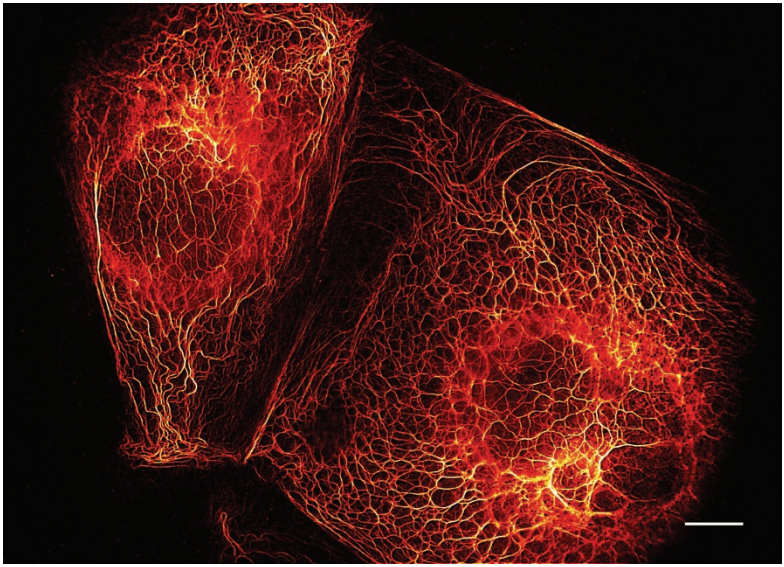
"We were able to show that we could overcome the diffraction barrier which made a huge difference," he says.

"A couple of years earlier I had to struggle to find money to basically live and now I was known within the microscopy community and was finally getting job offers," he adds. "It changed everything."

Hell's journey to acceptance and recognition in the world of science began in communist Romania. His parents were passionate about education and filled their family home with as many books as they could get their hands on. "In those days, information was scarce but my parents tried to buy books that were not biased by communist ideology," he says. "We listened to Western Radio, watched, for example, the moon landings – probably not the live event – and I soon became fascinated by

scientific progress as well as nature."

Still, stifled by the communist regime, the family emigrated to Ludwigshafen, West Germany in 1978, when Hell was fifteen years old. As his parents adapted to their new lives, Hell thrived at secondary school, inspired by a physics teacher and new found freedom. "In Romania, I had already realised that what was said publicly was not necessarily the truth, so in the more liberal West I was prepared to be skeptical at times," he says.



LIVE-CELL IMAGING ***with parallelized RESOLFT nanoscopy. The image shows a RESOLFT recording of PtK2 cells expressing the fusion protein keratin 19–rsEGFP(N205S). The image is based on recording 144 frames, the total image acquisition time was on the order of a second. Scale bar: 10 µm***

Andriy Chmyrov, Stefan Hell, Max Planck Institute for Biophysical Chemistry

"And I now realise that teachers can play a crucial role; at school I was the best in physics and my teacher really encouraged me to study the subject," he adds.

Hell's education went so well that come 1981, the enthu-

siastic 18 year old left school a year early to attend the nearby University of Heidelberg to, quite naturally, study physics. Feeling a little daunted, Hell immersed himself in studies and university life.

The university itself had always housed a liberal and free-minded spirit, and exams were kept to a minimum. Hell loved it. "The atmosphere allowed a lot of freedom of thinking and creativity, and I could basically go to any lecture I wanted to," he recalls.

"I was fascinated by physics and wanted to understand things at a deep level, so spent hours pouring over text books so I really understood the basic points," he adds. "I just wanted to know the essential phenomenon behind the greater picture."

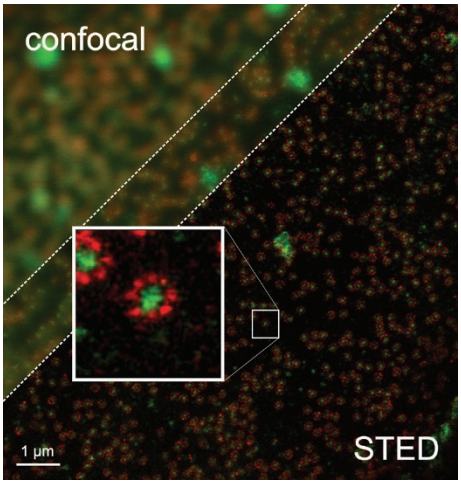
Hell's obsession with understanding the essence of any subject would one day prove crucial to the world of microscopy but at the time and like many other young physicists, he was drawn to theoretical particle physics. However, without firm financial backing and given Germany's surplus of physicists, Hell followed advice from an older colleague and opted for a diploma thesis in the very practical microlithography.

Low temperature solid-state physicist, Professor Siegfried Hunklinger, had just moved to Heidelberg and wanted to produce piezoelectric surface-wave transducers lithographically using laser scanning optical systems. Hell, against his better judgment, concurred and several years later found himself still working with Hunklinger on his PhD.

By now, Hunklinger had launched a start-up company to develop laser-scanning systems for myriad applications including confocal microscopy and microlithography inspection. Hell

was tasked with finding out if this up and coming microscopy method could be used to accurately measure transparent 3D photoresist microstructures.

"This is how I, very reluctantly, got acquainted with optical microscopy, and I found it so very, very boring," he laughs. "At the time, I thought there was nothing interesting you could do with optical microscopy so I started trying to work out if there was actually something interesting I could do with this subject of 19th century physics."



RESOLUTION OF CONFOCAL *versus STED microscopy: proteins of the nucleus, labelled with fluorescent dyes*

"And that's when I thought wouldn't it be cool to break the diffraction barrier?" he says. But the year was 1988, breaking the diffraction barrier was deemed crazy, Hunklinger's start-up was about to go bankrupt and Hell had a PhD to complete.

While finishing his thesis, he ruminated over the diffraction barrier, mulling over the Stark and Zeeman effects, and the like, and even hit on the notion of molecule localisation. But in his words: "I couldn't work out how to separate the molecules... and just couldn't find a concept that would give me an image."

Instead, he developed a two-lens method to drastically boost

the axial resolution of confocal microscopy and on completing his doctorate in 1990 headed to the European Molecular Biology Laboratory, Heidelberg, to demonstrate the new concept. Working under the head of microscopy at EMBL, Dr Ernst Stelzer, he proved what would come to be known as the 4Pi microscope, a laser scanning microscopy breakthrough that would later be commercialised by Leica Microsystems.

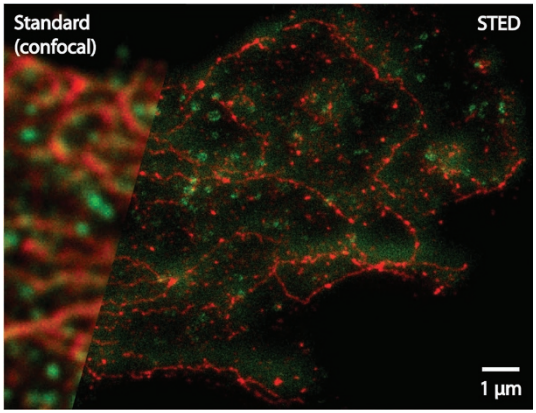
Crucially, the microscope also showed the world that the resolution of far-field microscopy was ripe for change. And as Hell says: "I had to offer EMBL something concrete so for me, [4Pi] was just a foot in the door. I couldn't have gone to EMBL to speculate overcoming the diffraction barrier, I would have been labelled 'crazy'."

Come 1993, EMBL funding ran out and Hell headed out to University of Turku, Finland, to set-up a small optics laboratory largely based on 4Pi microscopy with Professor Erkki Soini and former EMBL colleague Pekka Hänninen. Soini had brought Hell in for his fluorescence microscopy expertise but was also willing to give him 'a little freedom' to think about the resolution barrier. By this time, Hell had well and truly realised the importance of, as he says, 'following your passion', so he made the most of this freedom.

Hell's hunch was that merely changing the way light is focused or re-arranging lenses, as in 4Pi microscopy, would not bring a fundamental leap forward in resolution. Instead, he figured changing the states of the molecules being imaged would deliver a bigger breakthrough.

Quickly his thoughts focused on fluorescent molecules, whose states could be most easily altered, and within months

he had conceived stimulation emission depletion – STED- microscopy.



TWO-COLOUR STED, *left, image of a glioblastoma, the most frequent malignant brain tumor in adults. Clathrin protein is green; ß-tubulin protein is stained red. In contrast to the blurred classical image (left), the STED image (right) shows finer structures*

J. Bückers, D. Wildanger, L. Kastrup, R. Medda; Max Planck Institute for Biophysical Chemistry

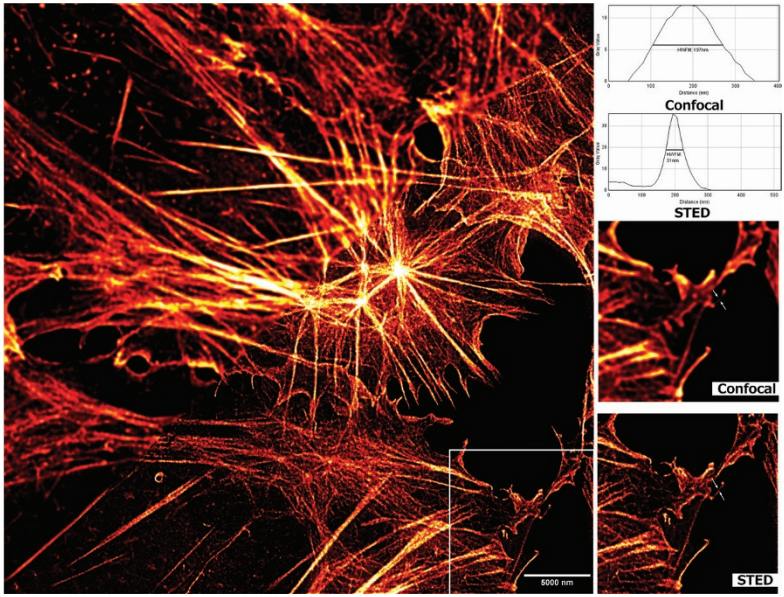
"I had been thinking about this for years and was so excited as I felt STED could work," says Hell. "At that very moment I wasn't one hundred percent sure it would definitely work in practice but I couldn't find a flaw in my thinking and preliminary calculations."

Come 1994, Hell had published his approach for a 'new type of scanning fluorescence microscope capable of resolving 35 nm in the far-field' in Optics Letters. The method was based on stimulated emission to inhibit the fluorescence of molecules and, critically, overcame the diffraction limit.

Hell was pleased but also frustrated. His second paper on a related idea submitted in the same year had been previously rejected by more popular journals as referees with expertise in near-field rather than far-field optical microscopy had requested experimental data.

"Of course, I hadn't got my my own laboratory and didn't have the means to do this. So the theory was now in Optics Let-

ters and Applied Physics B, but I was still a kind of nobody," he says. "Laboratories across the US and Europe had funds to build near-field optical microscopes but I was seen as this funny guy claiming these efforts were ill-fated for the life sciences as super-resolution microscopy should be done in the far-field instead. It was a problem."



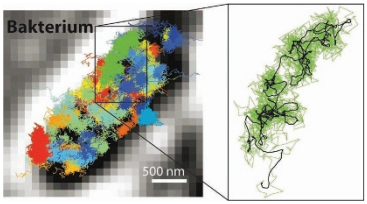
STIMULATED EMISSION DEPLETION, *below, (STED) microscopy provides significant resolution improvements over those possible with confocal microscopy. Cells were stained with 647-phalloidin to show actin filaments and the images were taken on a Leica SP5 2P STED microscope*

Howard Vindin

Still development continued and come late 1996, Hell received his habilitation from the University of Heidelberg – crucial to access a professorship – and moved over to the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany.

Until now, Hell hadn't written up his experimental devel-

opments on STED for submission to journals for fear of rejection, but swiftly submitted a grant for the method to the Germany Federal Ministry of Research. It was promptly rejected but granted on appeal against referee opinion. Within months, Hell had produced what he describes as his first 'compelling images' while working alongside his doctoral student Thomas Klar.



WITH MINFLUX, *users can follow faster movements than is possible with STED or PALM/STORM microscopy. Left: Movement pattern of 30S ribosomes (parts of protein factories, coloured) in an E. coli bacterium (black-white). Right: Movement pattern of a single 30S ribosome (green) shown enlarged*

MPI f. Biophysical Chemistry/ Y. Eilers

"You could clearly see the resolution was better and we showed details that would not have been attainable in a diffraction-limited system," he says. "It was now clear to people that this was going to work."

Come 1999, Hell's research group was expanding and he was ready to publish unequivocal proof that the resolution of far-field fluorescence microscopy could be radically improved via STED. Following rejection from Nature and then Science, he finally published results in PNAS that spelt out that the diffraction barrier limiting resolution in far-field fluorescence microscopy had been fundamentally broken.

"STED had put super-resolution on the map and was the first concept that showed you could overcome the diffraction barrier by briefly switching fluorophores off," explains Hell. "I was now getting job offers for chaired positions from several places, including Kings College London, but accepted a counter offer from Max Planck. That paper saved my career."

Post-PNAS, far-field super-resolution imaging progress was rapid. Hell and colleagues continued to develop STED, synthesizing photoswitchable fluorophores and reporting the first nanoscale far-field immunofluorescence images using STED.

Around the same time, the critical pieces of the super-resolution microscopy puzzle were falling into place. In 2006, former advocate of near-field microscopy, Eric Betzig, had joined the far-field, with photo-activated localization microscopy, PALM, and Professor William E Moerner, had honed single molecule localisation. At the same time, the development of the first commercial STED microscope was underway.

Eight years later, Hell, Betzig and Moerner, were awarded the 2014 Nobel Prize in Chemistry for the development of super-resolved fluorescence microscopy. But for Hell, super-resolution microscopy had already moved on.

In 2011, Hell came up with a new approach that he reckoned combined the advantages of STED with PALM as well as STORM, pioneered by Professor Xiaowei Zhuang, Harvard University. In his never-ending eagerness to get to the bottom of any phenomenon, the researcher had asked himself 'what is it that really makes these super-resolution methods tick'?

His answer was 'MINFLUX' - MINimal emission FLUXes - a concept that combines the power of the two different superresolution families. In MINFLUX, as in PALM/STORM, individual molecules are switched randomly on and off but at the same time, a doughnut-shaped laser beam - as used in STED - excites the fluorescence so the user can locate molecule position.

Using the method, Hell has since resolved molecules only 6 nm apart, surpassing the best super-resolution fluorescence mi-

croscopy methods by up to twenty times. Results were published results in Science in late 2016, and Hell and his team will now expand the method's field of view and introduce a multi-colour scheme, ready for commercial exploitation.

Hell believes that breakthrough concepts, such as STED and now MINFLUX emerge around every decade, highlighting: "We saw STED emerge in 1994, PALM in 2006 and now we have MINFLUX."

And for him, the secret of success will always lie in tracing anything back to first principles. "My inclination of really trying to boil down everything to basics, as well as coming up with explanations that are different from the scientitic mainstream, helped me to develop STED and now MINFLUX," he says. "You have to be able to break it down to very simple language, if you can't do that then you just haven't understood it."

LENA KOURKOUTIS
RESOLUTION REVOLUTION

Late last year, Lena Kourkoutis, Assistant Professor of Applied and Engineering Physics at Cornell University, won a multi-million dollar grant from the US National Science Foundation for a revolutionary new cryogenic, aberration-corrected STEM.

Designed for both life sciences and physical sciences applications, Koutkoutis is leading a team of physicists, chemists and materials scientists at Cornell that will develop the instrument further.

"We're going to combine low-vibration cryo-stages adapted from biology with the resolution-enhancing aberration-correctors used in the physical sciences," she says. "Our aberration-corrected cryo-STEM is going to be unique in its set-up"

Crucially, users, will for the first time, be able to study chemical and physical processes at liquid-solid and at soft-hard matter interfaces, at high spatial resolution. Kourkoutis is excited.

Once instrument installation is complete, the young researcher intends to first scrutinise the electrode-electrolyte boundaries in batteries. These interfaces have yet to be imaged at high spatial resolution and the results will be instrumental to the future development of catalysts, fuel cells and next-generation energy storage.

The STEM will also be used to study the many soft-hard interfaces of materials and tissue systems that exist in materials

science, medical, geology research and more. And in the long-term, Kourkoutis intends to map the chemistry and bonding of materials at atomic scale, while the sample is cooled close to the temperature of liquid nitrogen.

"Atomic resolution mapping at low temperatures is important in many systems from batteries to complex electronic materials," she says. "We will tackle all of these problems with this microscope."

Kourkoutis's leadership of this NSF-funded project follows years of electron microscopy-related interdisciplinary research, and is, in many ways, very apt. As a child, her first interests lay in mathematics and science.

"My father is a physicist, in fact he's an electron microscopist," she laughs. "When I started as a graduate student at Cornell I didn't quite realize that I would end up in the same field, visiting the same conferences."

But joking aside, she adds: "I have always liked to build things, explain things, understand why things function, and how to make them better. And that's what I still do today."

On leaving school, Kourkoutis opted to study physics in her home-town of Rostock, Germany, studying in the same university department as her father. During her time here, she also spent a year, as an exchange student, working with Professor Robert Nemanich in his research laboratory at the North Carolina State University.

Nemanich, now at Arizona State University, was developing microscopy and spectroscopy to characterise thin film interfaces and nanostructures, and at the time, the young Kourkoutis was tasked with fabricating nanowires with very high aspect ratios.

"I was trying to understand how to make small nanowires grow with very high aspect ratios, and used microscopy to image the growth processes in real-time," she says. "Nemanich was a very supportive advisor and it was during my time at NC State that I realised I wanted to continue my studies in the US. I then applied to a PhD program at Cornell University."

Kourkoutis returned to Rostock to complete her 'Diplom' in physics, and come 2003, with degree in hand, plus two papers in the pipeline from her short time with Nemanich, she moved over to Cornell.

Here, she studied for her Master's and PhD in applied and engineering physics.

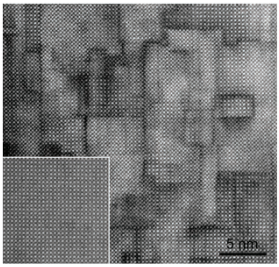
"On leaving Rostock, I could have ended up in a lot of different fields, but I knew there were areas that I didn't want to go into," she says. "For example, I didn't want to depend on beam time on a synchrotron, I wanted to be able to do an experiment and then go back and repeat it to check the answers, I wanted the freedom to go back and explore."

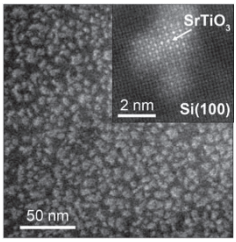
Cornell had a long history of electron microscopy, a number of electron microscopes on site, and importantly for Kourkoutis, ex-Bell Labs physicist, and then new Cornell professor, David Muller, had just set-up a laboratory with a heavy emphasis on high resolution imaging of electronics materials with electron microscopy and spectroscopy. She joined.

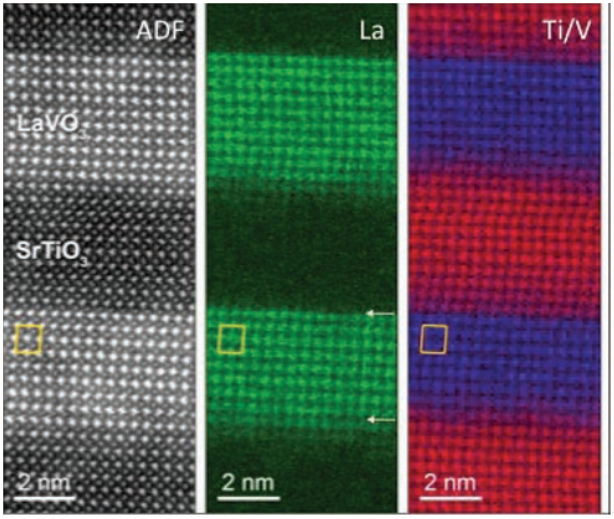
"I started to focus on this interesting class of materials – perovskite oxides – and studied the phases that emerge at the interfaces between two oxides," she explains. "I tried to understand the underlying physics using microscopy and spectroscopy as the primary tools. This was fundamental research but at the

same time we were also thinking about how this could be applied to future electronics devices."

According to Kourkoutis, Muller's research group was 'wonderfully collaborative', and from word go, she was sent to a wealth of conferences and met a thriving electron microscopy community. "I really enjoyed this and it was especially helpful at the beginning of my career," she highlights. "The electron microscopy community is relatively small and it is nice to be a part of it."







Also, unlike many young US-based researchers, Kourkoutis started her PhD in the lab, rather than in lecture room, and quickly garnered a lot of experimental experience. She completed her PhD in 2011 and shortly after was encouraged to apply for a faculty position, which she received.

As she says: "At the time I was still very young and had little experience outside of Cornell, so my department offered me two years to develop further and do research in my area of choice and place of choice."

Given her family roots, Kourkoutis opted for the Germany-based Max Planck Institute of Biochemistry. But while Germany wasn't new, her choice of research field was.

"I wanted to leave what I was doing at the time, explore a completely new area, and come up with new ideas," she says. "So I chose a biology lab that did some of the best cryo-electron microscopy."

She contacted Professor Wolfgang Baumeister, head of the department and world-leading biophysicist, and as she recalls: "He said, 'you work in a very different area, but come for an interview'. I did, got the position and it was great."

Kourkoutis spent the next two years using cryo-EM to study the proteins on chromatophore vesicles of purple bacteria that are used to very efficiently harvest and convert light to energy. Clearly a world away from her electronic materials research, Kourkoutis had to quickly learn everything from sample preparation, to how to record images of sensitive materials using a new electron microscopy method. She loved it.

"We [physical scientists] can learn so much from microscopists in the life sciences," she says. "For example, they've fig-

ured out how to deal with radiation sensitive specimens and this [method] is becoming much more important in the physical sciences now."

"We shouldn't reinvent the wheel, we should learn from what has been done before, and that's what I did in Germany. I learned a lot," she adds.

Come 2013, Kourkoutis returned to Cornell to finish her postdoctoral research, set up her research laboratory and start the application process for her NSF-funded instrumentation grant.The researcher had already published myriad papers in Nature, Science and more, and given her interdisciplinary experiences, was ready to lead a laboratory. More importantly, she was eager to explore the uncharted territory between the soft and hard interfaces of materials so important to next generation batteries and more.

Two years on, Kourkoutis has the NSF grant and has already probed materials interfaces using non-aberration corrected, standard cryo-electron microscopy.

"We've been able to image charge density waves in materials directly using electron microscopy," she says. "We've also made a lot of progress in accessing the internal interfaces between hard and soft material."

And without a doubt, Kourkoutis's short time in life science research has proven key to the NSF-funded instrumentation grant and her future research path.

"Some of the ideas for the NSF grant nucleated at the Max Planck Institute, as this is where I actually learned what's do-able with cryo-electron microscopy," she says. "We can't apply all the techniques life scientists use but we can apply some of them and

combine these with what we do in the physical science to solve problems."

Looking back on her, so far, short yet very successful career, Kourkoutis asserts results come from taking some risks, learning something new and developing expertise in different disciplines to make a difference and solve problems.

"I have been trained to really push electron microscopy to its limits and that is still what I do now," she says. "We spend a lot of time developing new techniques but always with the ultimate goal to actually solve the scientific problem we are interested in."

PAUL MUNROE

THE ART OF MICROSCOPY

In September 2014, Australia-based microscopist Professor Paul Munroe unveiled striking images of dead insects that he had 'decorated' using his FIB-SEM system.

Working with artist Stephanie Valentin, the pair created a series of extraordinary images, now exhibiting at STILLS gallery in Sydney, symbolising the natural world's attempts to adapt to climate change. From milled moth wings and ants' thoraxes to platinum deposits on insect eyes, the results are compelling, and highlight Munroe's unusual skills as a microscopist.

"I know it sounds obvious, but we quickly discovered that all insects are different," he says. "We found that the focused ion beam more or less instantly destroyed the moth wings, but cockroaches were almost ceramically hard and very difficult to melt."

"For every insect we had to really think about its structure and what beam conditions would work best - this was challenging," he adds.

Former director of Electron Microscopy and now head of the School of Materials Science and Engineering at the University of New South Wales (UNSW), Australia, Munroe has intermittently worked with Valentin for more than a decade. They first made headlines in 2002 after etching words such as 'enchant' and 'endure' in single grains of pollen. Their work featured in Nature, and several 'science meets art' projects have followed.

As Munroe puts it: "Stephanie appears in my doorway every few years... I like being a scientist, but I like the idea of working

on artistic projects too - it takes you away from a lot of the rigour of science, peer review, and so on."

But unorthodox art aside, conventional science is where Munroe first discovered his passion for electron microscopy. Following a degree in metallurgy and materials science at the UK-based University of Birmingham, the young student remained at the institution to complete his PhD, exploring titanium alloys, supervised by renowned electron microscopist Professor Mike Loretto.

INSECT ***Professor Paul Munroe and Stephanie Valentin used an FIB-SEM to mill holes into the thorax of an ant***

Valentin, Stills

"My research was about 90% TEM work, looking at secondary phase erbium oxide nanoparticles within the alloy," he says. "Obviously the only thing you could resolve them with was a TEM."

With PhD in hand, Munroe crossed the Atlantic to join Dartmouth College, US, in 1987, where he continued his TEM analysis on different alloy systems. As he jokes: "There was almost an underground railroad of Loretto's PhD students going to America to work, because at the time, they were fabulously well paid compared to researchers in Britain."

But New Hampshire weather got the better of Munroe, and following what he describes as three 'fiercely cold winters', he swore his next would be in hotter climes. So come 1990, he had taken up his first post at the UNSW's Electron Microscope Unit, as Director in Physical Sciences. The position wasn't huge. Munroe was in charge of one SEM and one TEM, but as the researcher states, the hands-on role suited him and he split his time between supervising students and helping them solve problems on the electron microscopes.

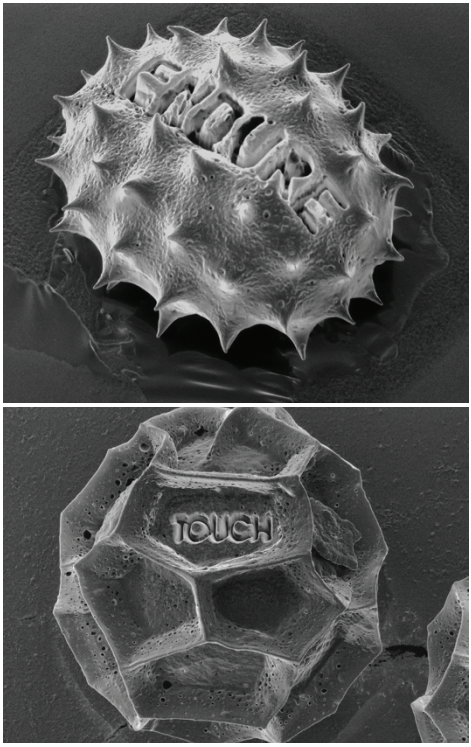
Come the mid-1990s though, change was afoot. As Munroe puts it: "Probably by luck, or otherwise, the university started to realise that electron microscopy was important."

UNSW's physics, chemistry, mechanical engineering and chemical engineering departments were expanding. Critically, researchers from these disciplines were becoming interested in microscopy, as well as materials scientists and biologists, and so the university built a large microscopy centre for all disciplines.

By 2005, the Electron Microscope Unit housed 17 microscopes, including AFMs, SEMs and TEMs, and Munroe contin-

ued as centre director. During this time, the researcher had also realised the potential of focused ion beam microscopes.

"I was at a conference and saw a paper on galvanised steel from a group in Japan," he says. "The researchers had produced beautiful images of the steels, while we were still struggling to make TEM specimens. I asked them 'how did you do that, because we can't?' They said 'we made this with a focused ion beam'."



Munroe and Valentin used FIB-SEM to etch words onto pollen grains

Valentin, Stills

According to Munroe, UNSW's Electron Microscope Unit actually installed its first focused ion beam microscope in the late 1990s, at a time when most instruments resided in semiconductor companies, being used to etch chips.

These microscopes were far from user-friendly but the researcher asserts: "We did a lot of fundamental work on what happens when your beam hits a specimen, and went on to do a lot of research on thin films, photovoltaics as well as semiconductors that just hadn't been done before."

Critically, with the microscope's potential proven – Munroe

and Valentin's amazing etched pollen grains were testament to this – other researchers started looking at it. "There was a lot of development work to improve the optics and make them user-friendly, but it was one of those things that just kind of exploded around ten years ago, when people saw what these instruments could do."

And FIB microscopy has proven crucial to Munroe's research. His team was amongst the first to study thermal spray-coated materials, such as yttria-stabilised zirconium-coated nickel alloys.

"Using FIB techniques we were able to look beneath the particles and get rich microstructural information on the [droplet-surface] interactions that had taken place," explains Munroe. "People would say to us, 'wow we've never seen that before'."

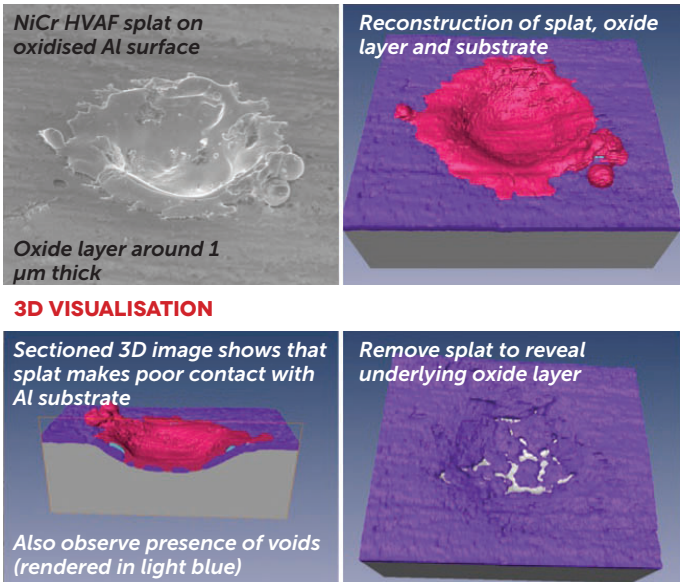
But while such microscopy techniques have always been key to Munroe's work, from word-go, the researcher has also focused on teaching. From the early days of running a two-instrument strong electron microscope unit to his current position of Head of the School of Materials Engineering, training up and coming researchers has always been a passion.

According to the researcher, he enjoys taking the students on a journey from knowing very little about microscopy to having all the knowledge they need to know. And hands-on experience is a crucial part of this.

"Students put a lump of metal under a microscope and get to see it at very high magnifications," he says. "This is the first opportunity they have to resolve structures, and then they start to relate that to, for example, the material's mechanical properties."

So given his experience with myriad students what would Munroe's advice be to the aspiring microscopist? Quite simply; get involved.

"I often put my 'head of school' hat on now and talk to first year undergraduates and new postgraduates," he says. "I always say, get involved and be active. Employers like people with good degrees and good marks, but they really like people that get up and do things."



Above: Vivid 3D FIB reconstructions of NiCr alloy particles thermally sprayed onto aluminium

Journal of Thermal Spray Technology, volume 19, Issue 5, pp 1024-1031/Springer
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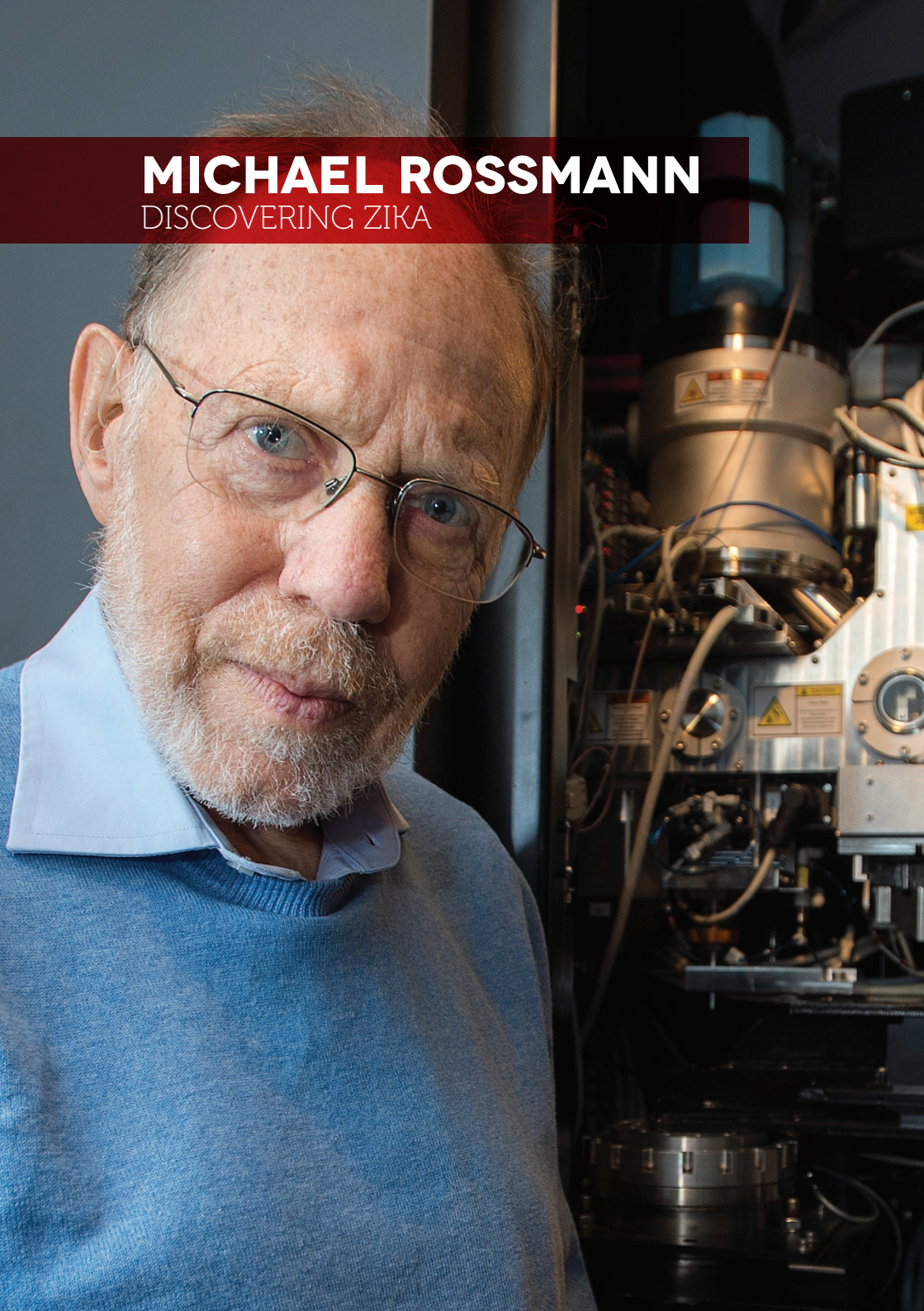


MICHAEL ROSSMANN
DISCOVERING ZIKA

In March 2017, researchers from US-based Purdue University, led by physicist Professor Michael Rossmann and virologist Professor Richard Kuhn, were the first to reveal the structure of the Zika virus.

Using an FEI Titan cryo-electron microscope with Gatan K2 detector, Rossmann, Kuhn and colleagues mapped the virus atomic structures to 3.8 Å resolution, pinpointing regions that differed from the related dengue, West Nile and yellow fever viruses, they had previously determined.

The world is now closer to an effective vaccine, but for Rossmann, the breakthrough underlines his persistent passion for structure, symmetry and puzzles.

"I started working with plant viruses around 1971, and this eventually led me to Zika," he says. "Curiosity pushed me on through this and I've always found it's fun to solve problems; solving one problem usually gives you ideas for ten other problems."

Born in Frankfurt, Germany, Rossmann studied Physics and Mathematics at the University of London where he received BSc and MSc degrees. Come the early 1950s, he moved to the Royal Technical College in Glasgow, to lecture electricity and magnetism to physics undergraduates, but in his words: 'I really wasn't very satisfied here'.

Keen to find something he actually wanted to do, he contacted Professor John Monteath Robertson, an early researcher

into the structure of organic molecules using crystallography, at nearby University of Glasgow. He was admitted as a graduate student into Robertson's laboratory, and as he puts it: "I've enjoyed myself ever since."

With his doctorate on 'A Study of Some Organic Crystal Structures' in hand, Rossmann left for the University of Minnesota in 1956, where he worked for two years as a postdoctoral fellow with Professor William Lipscomb. Here, Rossmann spent a lot of time writing computing programs to analyse crystal structures.

"I stayed with Lipscomb for two years and learnt how to use what was one of the first commercially available electronic computers," he says. "Here, I wrote most of the essential programmes for doing crystallography."

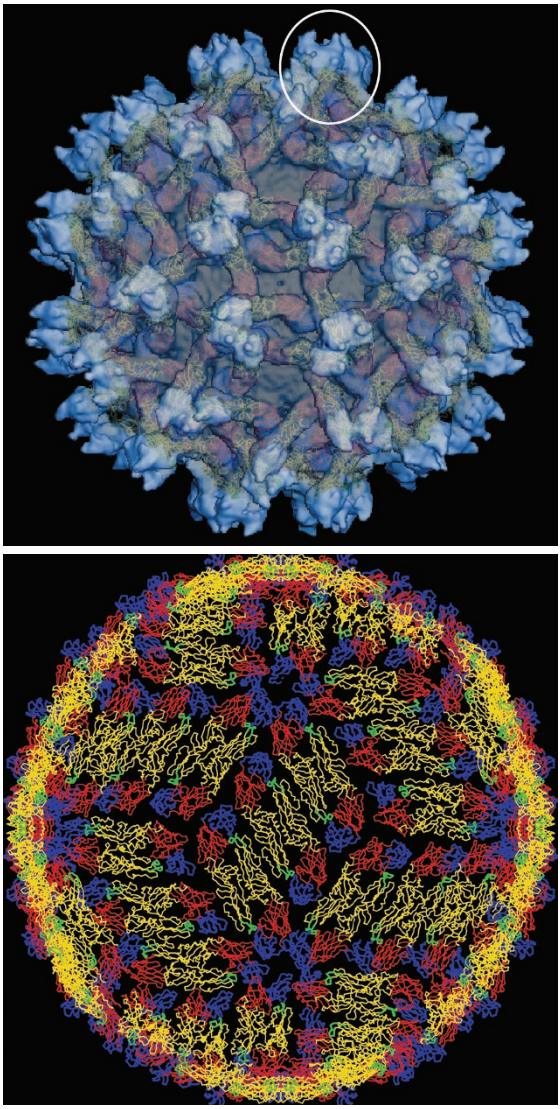
"In Glasgow we'd had to do all our enormous calculations by hand, and now having a computer to do this was a new, wonderful world," he adds. "This was a place where you could be very creative in how you approached things."

Lipscomb would later win a Nobel Prize in Chemistry for studying borane structures, but come 1958, Rossmann's US visa had expired so he travelled back to the UK. This time he went to work as a research associate at the University of Cambridge with Max Perutz, studying the structure of haemoglobin.

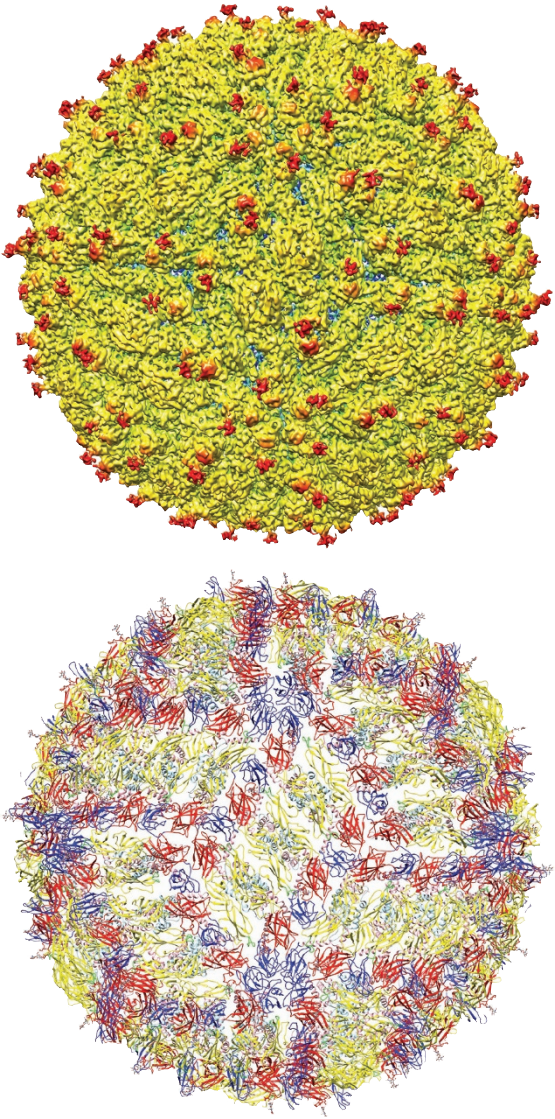
Perutz had set up the Molecular Biology Unit at the Cavendish Laboratory, which had already attracted Francis Crick and and James Watson, soon to win Nobel Prizes for their discoveries on the molecular structure of nucleic acids.

As Rossmann highlights: "Crick was in the office opposite and Watson was a frequent visitor; so with Perutz, we had many

coffee conversations and this was such a highly stimulating environment with all these future Nobel Prize winners."



DENGUE VIRUS
computer illustrations of the immature, top, and mature, below, dengue virus; this was the first flavivirus structure to be determined
Purdue



ZIKA VIRUS *The surface of the Zika virus provides insights critical to the development of an effective vaccine and antiviral treatments. The outside surface (also on right) shows the classic "herringbone" arrangement of the major glycoprotein (yellow), that allows the virus into a cell*

Purdue

Whilst here, Rossmann spent a lot of his time writing programs for the custom-built EDSAC 2 computer. This was the suc-

cessor to early British computer, the Electronic Delay Storage Automatic Calculator.

"I had a wonderful time programming this computer and developing methods to solve the structure of haemoglobin," he says. But crucially, his patience and painstaking analyses had also sowed the seeds for a new method to solve protein crystal structures that would eventually prove instrumental to future crystallographers.

While, Rossmann had helped to develop 'multiple isomorphous replacement', a sometimes difficult method widely used to recover missing phase information from protein crystals, he now realised the non-crystallographic symmetry that existed within and between crystal structures could also be exploited.

From here, he went on to develop the "Molecular Replacement", a relatively straightforward method to map the structures of protein complexes, now used to determine the majority of the hundreds of thousands of structures deposited with the Protein Data Bank.

ENZYMES FIRST

Come 1964, Rossmann joined the Department of Biological Sciences at Purdue University, as associate professor, where he still resides today as Hanley Distinguished Professor of Biological Sciences.

On leaving Cambridge, Rossmann had wanted to determine the structure of viruses, but explains: "No atomic resolution virus structures were known at the time, and the idea of working on a structure of a virus was mostly considered to be ridiculous."

So instead, the up and coming researcher looked at small-

er protein complexes, choosing lactate dehydrogenase, found in nearly all living cells and vital to metabolism.

At the time, researchers around the world were discovering enzyme structures, such as alcohol dehydrogenase, adenylate kinase and hexokinase. And as Rossmann explains, he had started to recognise certain properties in these structures that were repeated over and over again.

This insight led to his discovery of the 'Rossmann fold', a nucleotide binding motif associated with enzymes that is now described in most biology textbooks.

"We worked so hard to get these results but as soon as we had discovered and realised the significance of this common protein structure I thought, 'well maybe I can get some money to do some virus work'," says Rossmann. He did.

By now it was the early 1970s, and while computers were rapidly advancing, the relatively slow processing powers of the day meant determining the structure of human viruses was still unthinkable. So instead, Rossmann focused on the southern bean mosaic virus. In his words: "Purdue University had lots of greenhouses so we could go and infect bean plants with the virus and then collect grams of the virus very easily."

Come the end of this decade, the researcher and colleagues had constructed a 3D model of the southern bean mosaic virus, making them the second research team in the world to map a virus.

Now keen to move onto human viruses, Rossmann contacted Professor Roland Rueckert from the University of Wisconsin, who was making great strides towards determining the structures of small RNA viruses, picornaviruses.

Rueckert persuaded Rossmann to take a look at the structure of a rhinovirus, the predominant cause of the common cold. And with funding from Purdue, Rossmann set up a tissue culture laboratory to propagate the virus.

Rossmann and colleagues team went onto produce crystal after crystal after crystal of the human rhino-virus-14, one of about 100 known common cold virus strains. Then, using the Cornell University High Energy Synchrotron Source, they bombarded their delicate samples with short, intense X-ray bursts, recording the virus structure across hundreds of diffraction patterns at various angles.

With patterns in hand, they reconstructed an atomic map of the virus using a then, state-of-the-art, Cyber 205 super computer. It was 1985, and at the time, the rhinovirus narrowly beat the polio virus as the first animal virus to have its structure published.

CLOSER TO ZIKA

While Rossmann had relied on X-ray diffraction to determine past viruses, he was well aware that cryo-electron microscopy was on the rise. Having taken a short sabbatical with molecular biologist Richard Henderson at the Laboratory of Molecular Biology in Cambridge, he began to combine cryo-electron microscopy with X-ray crystallography on his return to Purdue.

In the Spring of 1998, Rossmann's group had analysed in atomic detail the part of a cell's receptor that binds to a cold virus. He and his colleagues used the structure of the common cold virus to develop an anti-common cold agent, Pleconaril. Then in 1999, the researchers won the first of a series of multi-million

dollar grants from the US National Institutes of Health to study a series of viral pathogens belonging to the flavivirus family.

ZIKA VIRUS

The virus, a mosquito-borne disease, has been associated with the birth defect microcephaly which causes brain damage and abnormally small heads in babies born to mothers infected during pregnancy. It has also been associated with the auto-immune disease, Guillain-Barré syndrome, which can lead to temporary paralysis.

Zika virus transmission has been reported in some 33 countries and had been declared by the World Health Organisation as a 'public health emergency of international concern'.

The structure of the Zika virus is described in Science: http://science.sciencemag.org/content/352/6284/467

By now, Rossmann had joined forces with Purdue biologist, Professor Richard Kuhn, and over at least the next decade, the researchers used X-ray diffraction and cryo-electron microscopy to determine the dengue and West Nile virus structures, and more. However, Zika would be different; this time, the researchers relied on cryo-electron microscopy alone to determine its structure.

According to Rossmann, past flavivirus structure discoveries had been instrumental to Zika success but recent advances in cryo-electron microscopy and detector technology were imperative to further progress.

"Once Kuhn had produced a pure sample, it didn't take long [only months] to determine the structure," he says. "The Gatan

K2 detector was critical. I knew we needed this, so I begged the Purdue University's president for the money; I guess I begged hard enough."

And at a time when the virus is still sweeping across South America, parts of Asia and Africa, resolving its structure has never been more important. Researchers worldwide now have a map of the virus, revealing regions that could be vulnerable to treatments. And as such, many are trying to develop effective vaccines.

Rossmann doesn't believe he will be the first to develop such an anti-viral compound, but he reckons a successful vaccine will be developed within three years. And as he points out: "While studying ways to develop a vaccine we will also learn a lot more about the virus; the same happens with all studies - you make discoveries and they become useful in unexpected ways."

So what advice would the man, who has solved what can only be described as some of the world's greatest structural biology puzzles, give to the researchers of tomorrow? "Just be yourself and be interested," he says. "If you're not interested, forget it."

TONY WILSON
HIDDEN DEPTHS

Long before Professor Tony Wilson had discovered microscopy, his passions were mathematics and the arts. Torn between which of the two disciplines to focus on, he opted for the former, concluding you could always read a book at home.

"Chemistry books were too thick and seemed to contain too much to remember," he adds. "But I realised mathematics was a beautiful language which you could use to express concepts that were terribly difficult to express in words."

Still, it wasn't just about the language. Wilson also wanted to apply mathematics to real world problems, so on completing A' Levels in pure and applied mathematics, and physics, he decided to study engineering science at the University of Oxford.

"The advantage of this course was it included bits of everything. We were using maths to describe the real world and were applying science to engineering," he recalls.

Wilson completed his degree in 1976, and by this time, had discovered optics. He started a Doctorate based on integrated optics, including waveguides and using optical fibres, at the University of Oxford but limited funding thwarted experimental work.

However, at around the same time, he met Austrian-born physicist Rudolf Kompfner. Known for inventing the travelling wave tube, Kompfner was exploring scanning acoustic microscopy, prompting Wilson to ditch integrated optics and pursue scanning optical microscopy.

With a fresh focus, Wilson teamed up with several Oxford University researchers, including Colin Sheppard, a fellow optics pioneer now at the Italian Institute of Technology, Genoa, and set forth establishing the theory and practicalities of scanning optical microscopy including the confocal optical arrangement.

"It was a great time, we didn't have a formal education in optics so didn't know what you could do and what you couldn't do," says Wilson. "Looking back we were pretty green, not that we thought that at the time."

The principle of confocal microscopy had been established by American cognitive scientist Marvin Minsky as early as 1957. The MIT Professor had suggested confocal imaging to overcome the limitations of the traditional wide-field fluorescence microscope when imaging deep inside the brain but crucial components, including the laser, had not yet been developed.

However, in the years to follow, researchers around the world would build on the concept. And come 1978 Wilson, Sheppard and colleagues at Oxford University had developed a confocal set-up with laser illumination, stage scanning and photomultiplier tubes as detectors.

"People had tried to build scanning microscopes in the past but didn't have sufficiently bright light sources," points out Wilson. "We were lucky, the commercially affordable laser had arrived and this was one of its early applications; without this we wouldn't have been able to get sufficient signal [to generate an image]."

Crucially, the young researchers had also designed a system which allowed an optically-sectioned image to be obtained at a particular depth. According to Wilson, it was then straightfor-

ward to record a through-focus series of thin, high resolution, images from which a 3D image of the specimen could be created.

"Our whole idea had been to simply get better resolution but this set-up also gave us the optical sectioning. And that eventually led to 3D imaging," he adds.

COMMERCIALISING CONFOCAL IMAGING

Wilson completed his doctorate in 1979 and moved to Brasenose College, University of Oxford, as a junior research fellow, while also launching Oxford Optoelectronics with Colin Sheppard.

The researchers had won funding from several sources, including the Prince of Wales Innovation Award. And as Wilson says: "We thought enough people were interested in confocal scanning microscopy by now, so why not set up a company to manufacture the thing?"

As Wilson points out, this was an unusual time to spin out a company from the University, but the venture worked, and come 1982 Oxford Optoelectronics had sold the first commercial laser scanning microscope, the stage-scanner SOM-100.

However by this time, Wilson was keen to explore new avenues. An opportunity to work at Bell Labs came up, and the researcher accepted.

"This wasn't scanning optical microscopy but I wanted to see this research lab," he says. "It was an amazing place and was the only place in the world that had the mantra, 'spend money to save time'."

Still two years later, the future of Bell Labs had become uncertain and Wilson returned to the University of Oxford and the world of microscopy, taking the position of tutorial fellow at

Hertford College in 1984.

Wilson had realised, as had the rest of the world he jokes, that biology was critically important. Prior to heading out to Bell Labs, Wilson and Colin Sheppard, had developed the theory for a direct-view confocal microscope for optical sectioning. This, they believed, combined the resolution and depth discrimination of confocal microscopy with the ease of operation of a conventional microscope.

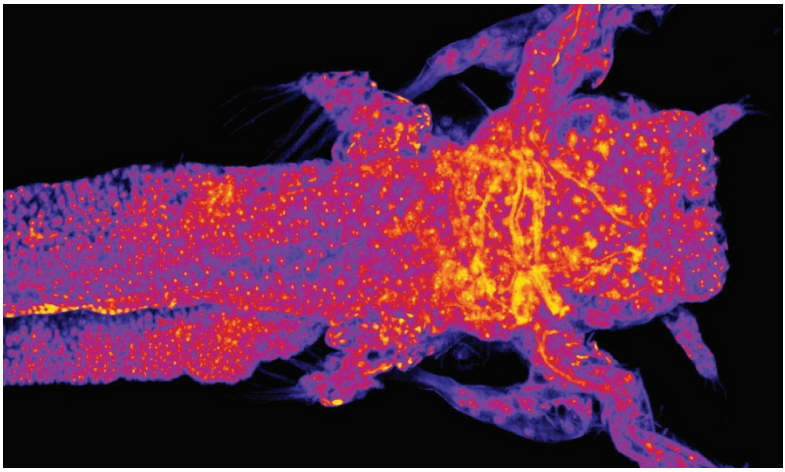
"We wrote a paper about this and our referees told us to take a look at a similar concept that we hadn't heard of then, tandem scanning microscopy," he says.

IN SHORT TONY WILSON

Professor Wilson graduated from the University of Oxford in 1976 and completed his DPhil in 1979. He was appointed as a Tutorial Fellow in Engineering Science at Hertford in 1984.

His work has led to the formation of Oxford Optoelectronics and Aurox, and resulted in many awards including the 2012 Institute of Physics innovation Award and a Queen's Award for Enterprise.

He was President of the Royal Microscopical Society from 2010 to 2013, Master of the Worshipful Company of Scientific Instrument Makers from 2014 to 2015 and is also Thousand Talents Professor at the Harbin institute of Technology, China. Wilson is also editor of the *Journal of Microscopy*, which celebrated its 175th year in 2016.



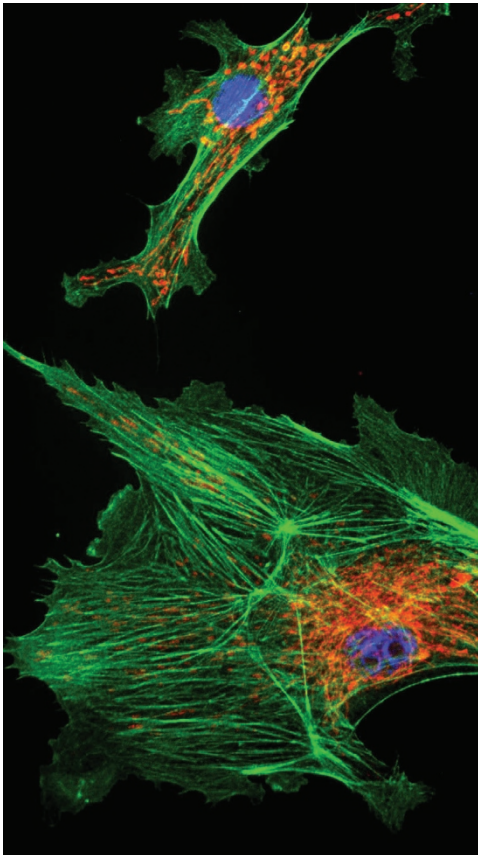
LABELLED FLUORESCENT *image of brine shrimp captured using an Aurox structured illumination/detection system*

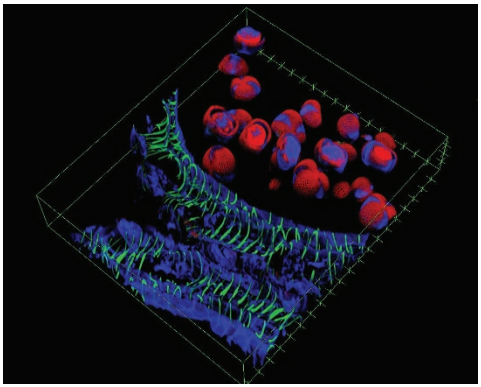
Aurox Ltd

Years later, Wilson and his colleagues realised that they could build on this. Tandem scanning microscopy designed by researchers at Charles University in Prague and Yale, used a perforated spinning disk placed in front of the microscope aperture, to generate multiple emission pin-holes and obtain real-time confocal images.

But as Wilson highlights: "This method was very light inefficient as it threw away almost 99% of the light available for imaging. So we had this idea to modify the conventional microscope while providing optical sectioning, and this was the birth of structured illumination."

As part of this development, Wilson had been keen to dispense with laser light and reverted to using a simple white light excitation source with a patterned spinning disc for optical sectioning.





LABELLED FLUORESCENT *images of brassica flower bud, left, and fibroblasts in muntjac skin, below*

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"We patterned the disc in a defined geometrical grid and projected the light pattern onto the specimen," explains Wilson. "By processing a series of images, we achieved optical sectioning."

Concept to practical instrument took years of development. But by the late-1990s, Wilson and colleagues had published several high profile papers on how to use structured illumination to obtain optical sectioning and 3D imaging in a wide-field microscope.

They went onto develop light demodulation algorithms to extract optical sections from raw data images. Meanwhile other key developments included the developments in CCD camera technology along with new disc designs and optical architectures to optimise optical sectioning.

Then, in 2004, Wilson, alongside fellow researchers, Rimas Juskaitis, Mark Neil and Martin Booth, launched 'Aurox', to market spinning disk confocal microscopy system, SD62.

In the years that followed, Carl Zeiss and Andor Technology would come on board to distribute the systems, the company would win the Queen's award for Enterprise and Innovation, and the company would earn more than £1m in revenue thanks to the system's development.

BEYOND SPINNING OPTICAL MICROSCOPY

But while Wilson is perhaps most well known for his pioneering development of confocal imaging and widefield sectioning microscopy, today he heads up the Scanning Optical Microscopy group within the Department of Engineering Science at the University of Oxford.

Here, he and colleagues also develop multi-photon and non-linear microscopy methods, as well as use adaptive optics to overcome the unavoidable aberrations associated with focusing deep within a specimen. And, importantly, the team has developed a remote focusing technique to allow the user to image along planes, curbed surfaces and arbitrary 3D trajectories.

More than ever, Wilson is collaborating closely with biologists from Oxford University, Guy's Hospital, London, and elsewhere to develop techniques to image cardiac tissue, mouse embryos and more.

Looking to the future, Wilson will continue to develop instruments and believes the next big imaging breakthrough could centre around axial resolution. While lateral resolution in confocal microscopy can reach around 180 nm, thanks to spherical aberration, axial resolution is limited to around 500 nm.

"I have this gut feeling that if we could improve axial resolution of both brightfield and fluorscence microscopes in an elegantly simple manner, that would be a really good thing to do," he says. "I don't yet know how to do this, and perhaps you can't, but if we could crack this, well that would be very neat."

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