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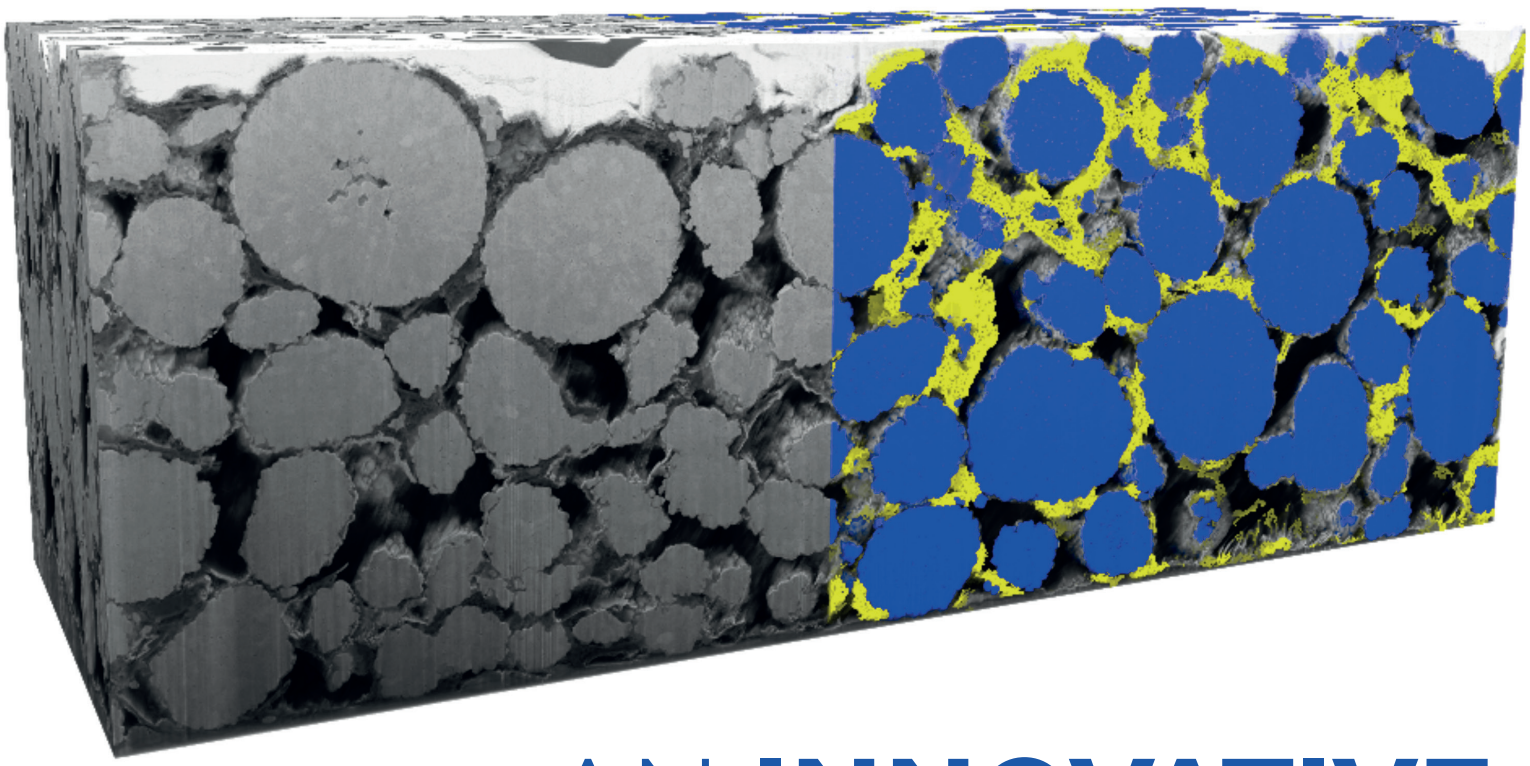
MICROSCOPY AND ANALYSIS



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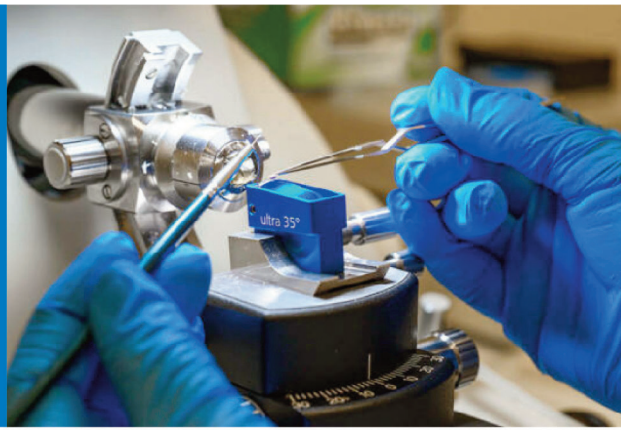

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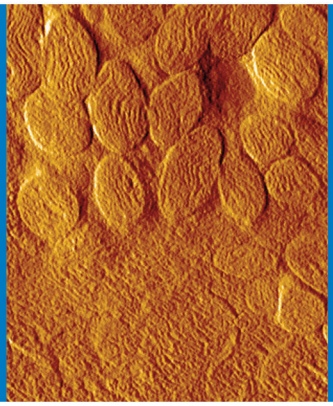

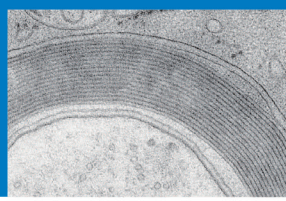

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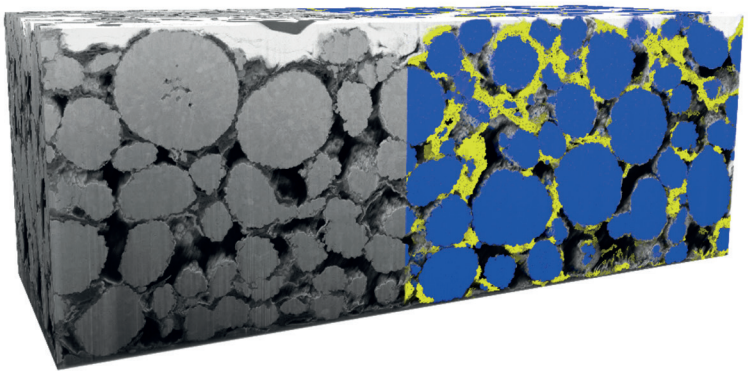
Cover story

Investigating Large Volumes of Li-ion Batteries in 3D: An Innovative Workflow for Battery Design



FAST CHARGING of electric vehicles is a significant challenge in the automotive industry. One solution to this problem is optimizing the electrode's microstructure through a thorough investigation of the 3D micro- and nanostructure features enabled by a comprehensive characterization of features e.g. type and size of particles or packing density of active particles and non-active components. Tomography, either using a non-destructive XRM (X-ray microscope) or destructive FIB-SEM (focused ion beam scanning electron microscope), is the most relevant technique for 3D characterization as it allows for the electrode's components segmentation and quantitative data interpretation.

FIB-SEM tomography uses e.g. the gallium beam of a FIB-SEM to remove a slice from the sample surface, the electron beam of the SEM acquires images of this freshly cut surface, followed again by the FIB beam removing the next slice and so on. The result is a z-stack of images of the removed material that can be reconstructed into a virtual 3D volume which can be subject of further analyses or post-processing. Material removal and image acquisition both take time in which the sample might drift, or the FIB beam stability might cause uneven slice thicknesses. This might result in losing the region of interest or in difficulties to correctly align the images in z-direction and correctly reconstruct the volume in 3D. Therefore typically, only volumes of some tens of cubic micrometers are milled with a gallium FIB.

But what if you want to investigate larger volumes in a decent time frame? You would need a FIB-SEM with high stability of the FIB beam over long runs, plus a procedure to detect, track and correct sample drift, plus image acquisition delivering the information you need, e.g. surface topography and material composition done fast enough so you don't lose too much time.

In this experiment, a large volume of 136 µm length, 52 µm width, and 50 µm depth of an NMC (nickel manganese cobalt) cathode of a lithium-ion battery was investigated using a ZEISS Crossbeam combined with Atlas 5, an engine tailored for fast and precise large area milling and drift correction providing great stability of the run and precise control of the slice thickness via special 3D tracking marks. SEM secondary and backscatter electron images demonstrated cracks in the active NMC particles, some pores between the particle grains, and particle fragments.

After material removal and imaging, computational software (u-net with the single input from Dragonfly) was engaged to analyze performance-defining properties and simulate dynamic processes such as battery charge/discharge cycles. Segmentation showed that the binder takes up 8.9% of the volume, and the NMC takes up 55%. NMC and the binder were segmented as two separate structures using deep learning models. NMC segmentation properly reflected cracks, internal particle porosity, and porosity in the binder. With the known importance of the binder's nanoporosity at higher binder loading, it becomes obvious how important battery performance simulation is for digital battery design.

This innovative workflow of large volume 3D tomography has the potential to streamline and advance battery design. The use of a ZEISS Crossbeam and Atlas 5, both tailored for fast and precise large area milling and drift correction, provides a comprehensive understanding of the electrode's microstructure and performance. This has significant implications for the future of battery design and the optimization of fast-charging electric vehicles.

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INSIDE

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INTRO

EDITOR'S LETTER
DR CHRIS PARMENTER

HELLO FRIENDS, we're flying through the year and conference season is underway. I hope that your plans are coming together and that you'll get a chance to get out, meet colleagues and see what's happening in your field. In recognition of the conference season we preview this year's forthcoming meetings, conferences and courses. I've also written a reflection on how meetings and conferences have changed in the past four years and whether we need to consider embracing some of what we learned during the Covid years.

Our profile this issue features Erin Tranfield, Director of the VIB Bioimaging Core in Ghent. Erin talks with Rebecca Pool about her experiences across the world, working with scientists in a wide range or institutions with varying access to cutting edge facilities. She talks about how her attitude to problem solving has changed and why representation for core facilities is so important to her.

Rebecca also talks with Nigel Browning about the recently announced Relativistic Ultrafast Electron Diffraction and Imaging facility (RUEDI) that seeks to be a first of its kind facility anywhere in the world. It is the hope that ultra-high speed and high energy microscopes will enable breakthroughs in life and materials sciences.

We have a special feature covering some recent scientific image competitions from all over the globe that showcase some truly amazing images. Another exciting feature focuses on the recently launched open-source platform DL4MicEverywhere which seeks to offer cutting edge deep learning techniques to those with no coding experience to enable dataset analysis in the area of bioimaging.

Our scientific editorials come from Robert Sturm who reports on a way to interrogate the magmatic crystal destruction using SEM and from Vahid Sandoghdar and colleagues who present a technique enabling label free imaging of organelles.

Of course, it goes without saying that we have the latest news and 'what's new' from the commercial sector so I hope there's plenty to keep you interested. Have a great summer (or winter) and I hope to see some of you out and about!

Until next time

CHRIS

Enzyme for neuron communication uncovered

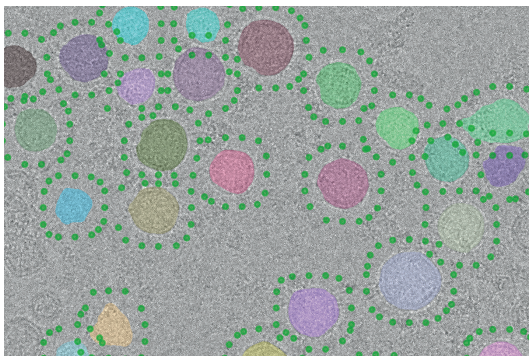
Using single particle electron cryomicroscopy Canada-based researchers have determined the atomic structure of an enzyme used in neuron communication. Dr John Rubinstein, Senior Scientist in Molecular Medicine at The Hospital for Sick Children, and colleagues, captured hundreds of thousands of high-resolution images of the vesicular-type ATPase (V-ATPase) enzyme to reveal synaptic function with new clarity. The enzyme acts as a pump to drive neurotransmitters into synaptic vesicles and also regulates neurotransmitter release from the vesicles.

At the 'SickKids' Nanoscale Biomedical Imaging Facility, Rubinstein and colleagues isolated synaptic vesicles and and then obtained images using a Titan Krios G3 electron microscope, operating at 300 kV and equipped with a Falcon 4i camera. They then developed computational approaches to analyze the images and create high resolution 3D models of the V-ATPase – something that they say has not been done before. As part of this, a newly-developed computer program was used to detect vesicles in images with the Segment Anything model, a tool for image segmentation.

Analyses indicated that the V-ATPase interacts with several components of the synaptic vesicle, which contains many proteins and lipids that are involved in neurotransmitter release. "Most surprisingly, we learned that the V-ATPase interacts with a protein called synaptophysin... What we found shows that synaptophysin could be helping to recruit V-ATPase to synaptic vesicles when they initially form," highlights Rubinstein. "Until now, [synaptophysin's] function in neurons was not understood."

The researchers hope their work will point to a therapeutic target for many health conditions, including epilepsy.

Research is published in *Science*.



PURIFIED SYNAPTIC VESICLES *(upper): The researchers used the newly-developed computer program to detect vesicles in images. Synaptic vesicles are highlighted with different colours and candidate positions for V-ATPase are indicated with green dots.[The Hospital for Sick Children (Sickkids)]*

New atomic detail on how catalysts work

US-based researchers have developed an electrochemical polymer liquid cell for TEM, to study the atom dynamics that take place as a copper-based catalyst reduces carbon dioxide.

According to Lawrence Berkeley National Laboratory researchers, and colleagues, the platinum, aluminum oxide cell, coated with a polymer film, can be frozen to stop the reaction at specific timepoints and so they can characterize composition changes at each stage of a reaction.

"[PLC-enabled TEM] allows us to see what's going on at the solid-liquid interface during reactions in real time, which are very complex phenomena," highlights Haimei Zheng, senior scientist in Berkeley Lab's Materials Science Division. "We can see how the catalyst surface atoms move and transform into different transient structures when interacting with the liquid electrolyte during electrocatalytic reactions."

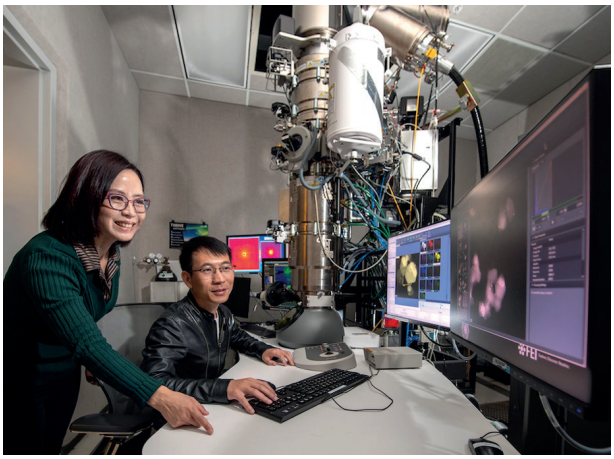
Zheng and colleagues tested their PLC approach on a copper-based CO_2 reducing catalyst that transforms atmospheric CO_2 molecules into carbon-based chemicals such as methanol, ethanol, and acetone. Using TEM, electron energy loss spectroscopy, and energy-dispersive X-ray spectroscopy, the researchers studied reactions and atomic dynamics at the electrified solid-liquid interface of the catalyst and an electrolyte.

Data revealed copper atoms leaving the solid, crystalline metal phase and mingling with carbon, hydrogen, and oxygen atoms from the electrolyte and CO_2 to form a fluctuating, amorphous state between the surface and the electrolyte. Dubbed an "amorphous interphase" - neither solid nor liquid - this phase disappeared once the current stopped flowing, with most of the copper atoms returning to the solid lattice.

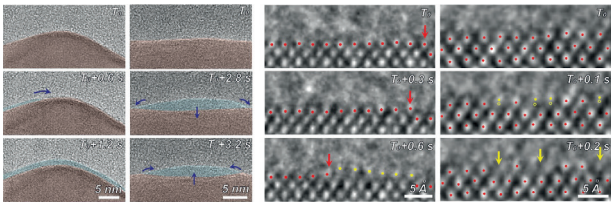
According to Zheng, the dynamics of the amorphous interphase could be leveraged to make the catalyst more selective for specific carbon products. Additionally, understanding the interphase will help researchers better combat surface degradation of catalysts and develop systems with longer operational lifetimes.

Zheng and her colleagues are now using PLC-enabled TEM to study other electrocatalytic materials, and have begun investigations into problems in lithium and zinc batteries.

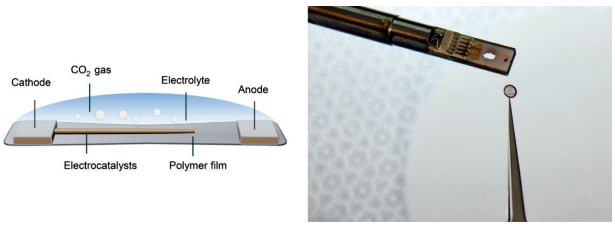
Research is published in *Nature*.



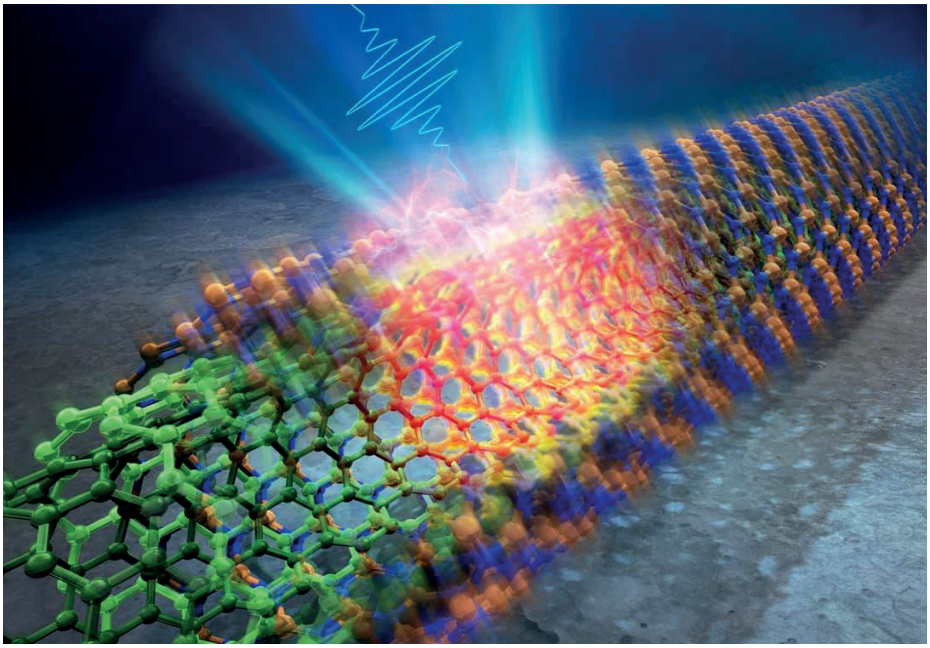
HAIMEI ZHENG, *left, and colleague, Qiubo Zhang, at Berkeley Lab's National Center for Electron Microscopy, where they studied electrified solid-liquid interfaces in liquid-cell TEM. [Thor Swift/ Berkeley Lab]*



(LEFT) *In-situ TEM images showing the amorphous interphase (light blue) between the solid copper catalyst (brown) and liquid electrolyte (grey) at different timepoints.* **(RIGHT)** *High-resolution TEM images showing catalyst atomic dynamics mediated by the amorphous interphase during the carbon dioxide electroreduction reaction. Red dots highlight copper atoms on solid copper catalyst surface. Yellow dots highlight copper atoms with displacements while transforming into the amorphous phase. [Zheng et al/Berkeley Lab]*



LEFT: *Polymer liquid cell components.* **RIGHT:** *The PLC (in tweezers) ready for insertion to TEM. [Thor Swift/Berkeley Lab]*



UNCOVERING *photo-induced dynamics in one-dimensional heterostructured materials. [University of Tsukuba]*

Electron dynamics discovery in 1D materials

A Japan-France team of researchers from the Universities of Tsukuba and Rennes has discovered new properties in carbon nanotubes. Professor Masaki Hada, Institute of Pure and Applied Science and Tsukuba Research Center for Energy Materials Science (TREMS), and colleagues, wrapped carbon nanotubes in boron nitride nanotubes and then examined how ultrashort light pulses induced electron and atom motion in the material.

Electron motion was monitored using broadband ultrafast optical spectroscopy while atomic motion was observed via ultrafast time-resolved electron diffraction. Analyses revealed a novel electron escape route in which a channel forms, allowing electrons to escape from specific subparts of the material.

The research team discovered that electrons excited in the carbon nanotubes by light exposure could transfer into the boron nitride nanotubes via these electronic channels, where their energy would be rapidly converted into thermal energy, facilitating extremely fast thermal conversion. This ultrafast thermal energy transport has potential applications in the development of ultrafast optical devices, rapid manipulation of electrons and holes generated by light and and efficient heat dissipation from devices.

Research is published in *Nature Communications*.

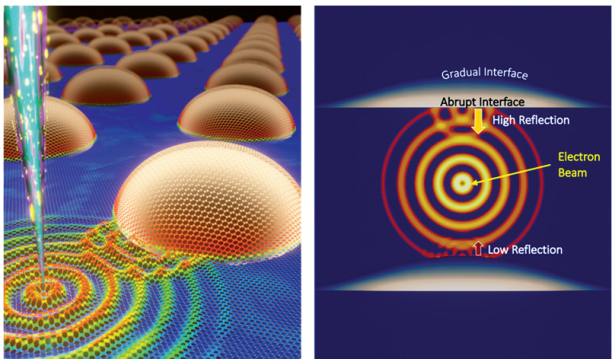
How heat moves through nanostructures

US-based University of California Irvine researchers have developed cutting-edge monochromated electron energy-loss spectroscopic methods in a transmission electron microscope to map lattice vibrations - phonons - at atomic resolution. The spatially-resolved vibrational mapping method from Professor Xiaoqing Pan and colleagues provides a deeper understanding of the way heat travels through nanostructured quantum dot superlattices.

Investigations on stacked silicon germanium alloy quantum dots separated by silicon, via spatially-resolved vibrational mapping, showed that vibrations were being softened in the quantum dot due to the strain built within the nanostructure. The softening of vibrations is behind one of the many mechanisms of how thermoelectrics impede the flow of heat.

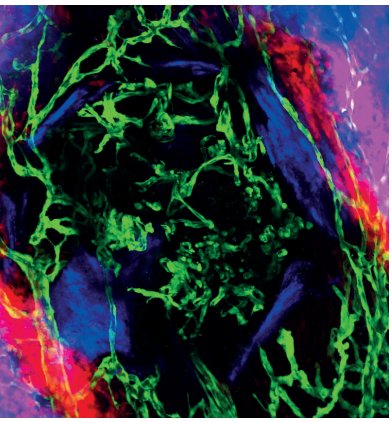
More notably, the researchers were able to map the direction the thermal carriers, phonons, travel. They found that the more atomically sharp the interface between the quantum dot and the surrounding silicon is, the more effectively the phonons are reflected. Conversely, if the composition change from quantum dot to silicon is more gradual, far fewer phonons are reflected. This work not only demonstrates the viability of vibrational spectroscopy in the electron microscope for the field of thermoelectrics, but also provides a physical foundation for their targeting engineering.

Research is published in *Nature*.



LEFT: *A beam of electrons generates vibrational waves in a crystal lattice that are then reflected by quantum dots.* **RIGHT:** *Generated vibrations are more easily reflected by abrupt, sharp interfaces of materials than by diffuse ones. [Xiaoqing Pan, University of California Irvine]*

Multi-photon microscope upends bone-healing wisdom



BLOOD VESSELS *(green) have completely vascularized the bone injury (blue), while bone cells (red) are just beginning to form new bone. [MPI for Molecular Biomedicine/ Gabriele Bixel]*

Using multi-photon microscopy, researchers at the Max Planck Institute for Molecular Biomedicine, Germany, have imaged the healing process of skull bones and the growth of blood vessels without the typical migration of bone precursor cells. Their findings reveal that the healing process in flat bones is fundamentally different from that in tubular bones.

Project leader, Gabriele Bixel, and colleagues tracked the growth of new blood vessels in the healing skull bone for over a month. Their multiphoton microscope - a TriM Scope II multi photon system from LaVision BioTec - allowed deep penetration into regenerating tissue, providing high-resolution images of vascular and bone cells, as well as bone matrix collagen fibers.

Analyses revealed during cranial bone healing, sprouting vessels initially grow alone, unlike in long bones where vessels and bone precursor cells grow in close proximity. These regenerating vessels establish a primitive blood supply before bone cells migrate to the injury site and begin ossification.

"A broken femur heals by first forming a soft callus, a cartilage cuff, around the fracture site. This callus of cartilage cells forms a temporary stabilizing structure around the broken bone," explains Bixel. "As the bone heals, this soft callus is gradually transformed into bone tissue on both sides, starting from the outer ends, by ingrowing vessels with co-migrating bone progenitor cells. The bone progenitor cells follow the course of the newly formed vessels in immediate vicinity."

The study focused on small skull injuries, leaving open questions about the role of regenerating blood vessels in healing large bone defects or deep skull fractures. "We cannot yet conclude what role regenerating blood vessels play in the healing of large bone defects or deep skull fractures, such as a skull base fracture," says Bixel.

""Another exciting question for us is how vascular and bone cells communicate with each other and grow together into the injured bone, and how and why this angiogenic-osteogenic coupling is abolished during the healing of small injuries to the skull bone," she adds.

Research is published in *Nature Communications*.

Sandstone fragment comes from Stone of Destiny

A piece of sandstone is a missing part of the 'Stone of Destiny', claims a research team from the Historic Environment Scotland (HES). Using X-ray, scanning electron microcopy and other non-destructive scientific analyses, Dr Aurélie Turmel, Science Manager at HES, and colleagues, have concluded 'to a high degree of certainty', that the fragment was taken from the Stone, used in the coronation of Scottish kings until the 13th Century and now considered to be a powerful symbol of Scotland.

"The new scientific analysis that we carried out on the Stone of Destiny ahead of the Coronation last year effectively enabled us to 'fingerprint' the Stone, and by performing the same techniques on this fragment we have been able to identify matches in terms of the chemical elements and minerals it comprises, the grain characteristics, and its colour," says Turmel.

X-ray fluorescence analysis, with a handheld Bruker Tracer 5g, was used to examine the chemical element composition of the fragment. SEM, using a JEOL JSM IT200 equipped with a backscatter electron detector, secondary electron detector and integrated JEOL energy dispersive spectroscopy detector, provided high-resolution analysis of the fragment's mineralogy and chemical composition.

Meanwhile, stereo microscopy, with an Olympus SZ61 binocular microscope provided detail on grain structure, and USB microscope analysis, with a Dino-Lite Edge AM7915MZT, provided data on the fragment mineralogy and texture, particularly grain size, shape and compaction. Colour analysis using a Konica Minolta chroma meter CR-400, compared the fragment's colour to that of the Stone of Destiny.

According to the researchers, the same methodology was used in an overall analysis of the Stone of Destiny in 2023 – at the time, a 3D digital model of the stone was created. In their latest study, all results were consistent with the sandstone fragment being derived from the Stone. The fragment had been stored by the Scottish National Party after being gifted to a former First Minister in 2008.

"This has been an exciting piece of scientific detective work for our team to undertake," says Turmel. "We're pleased to have had the opportunity to demonstrate the importance and potential of [our] world-leading heritage science technology... in particular how we are now able to combine these different techniques and datasets to strengthen certainty in our findings and how this can help enrich understanding of our past."

The Stone of Destiny - also known as the Stone of Scone - is still used to crown kings and queens of the United Kingdom today, including the coronation of King Charles III in May 2023.

THE STONE OF DESTINY: *Historic Environment Scotland (HES) care for the Stone of Destiny on behalf of the Commissioners for the Safeguarding of the Regalia. [HES]*

Scanning synapses at high resolution

MIT researchers, US, have developed a microscopy system to enhance the study of brain plasticity by capturing this constant editing and remodeling of synapses at high resolution. Multiline orthogonal scanning temporal focusing (mosTF) scans brain tissue using perpendicular lines of light, improving on two-photon microscopy methods that excite photon emission from brain cells engineered to fluoresce.

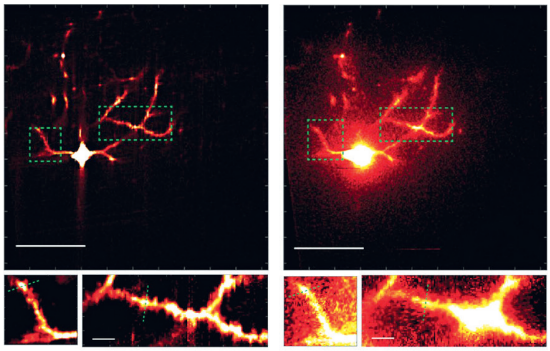
According to Professor Elly Nedivi, Picower Institute, MIT, and colleagues, mosTF demonstrated an eightfold increase in speed and a fourfold improvement in signal-to-background ratio compared to conventional two-photon systems. "While two-photon microscopy is the only method that allows high-resolution visualization of synapses deep in scattering tissue, such as the brain, the required point-by-point scanning is mechanically slow," highlights Nedivi. "The mosTF system significantly reduces scan time without sacrificing resolution."

Scanning an entire line of a sample is faster than scanning point-by-point, but also increases light scattering. While some systems discard scattered photons as noise, the mosTF system reassigns scattered photons back to their origin, producing a stronger signal. This process is more effective with the 2D perpendicular-direction orthogonal scanning of mosTF than with single-direction systems.

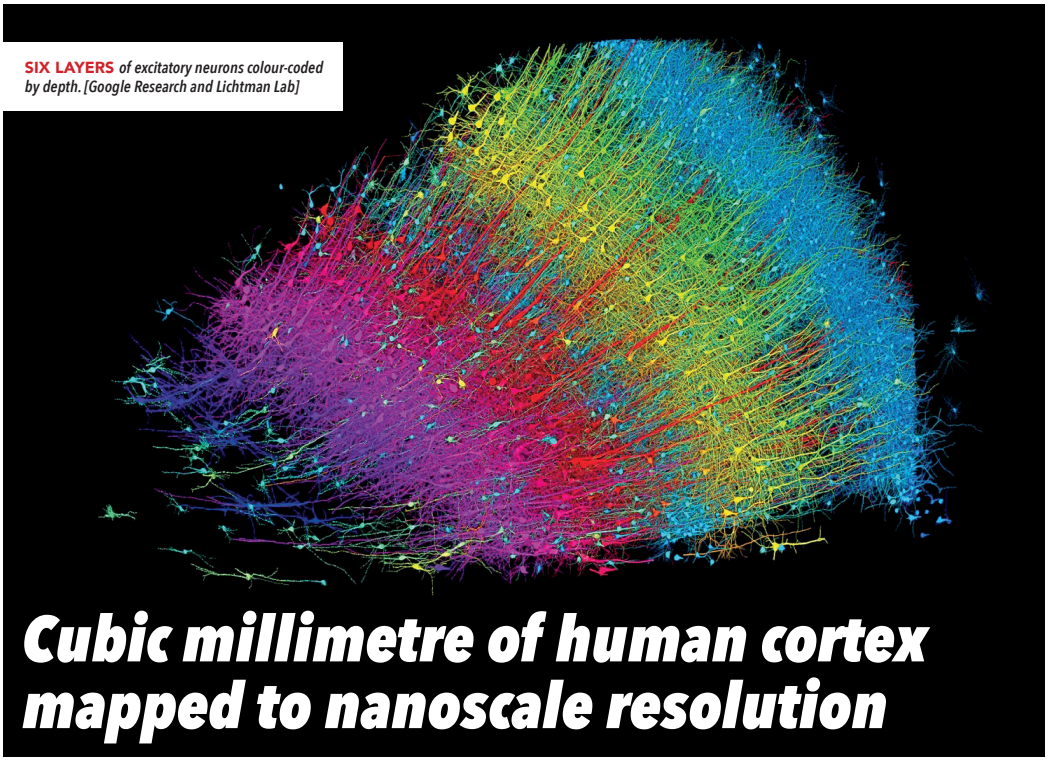
The researchers tested their system against a point-by-point two-photon laser scanning microscope and a line-scanning temporal focusing microscope (lineTF). The mosTF system achieved a 36-fold better signal-to-background ratio than lineTF when imaging fluorescent beads through water and a lipid-infused solution simulating biological tissue scattering. Furthermore, when imaging neurons in the brain of a live, anesthetized mouse, mosTF maintained a four-fold better signal-to-background ratio, clearly revealing dendritic spines where many synapses reside.

"The speed of mosTF is still limited by needing to use high sensitivity, low noise cameras that are often slow," says research partner, Professor Peter T C So, MIT. "We are now working on a next-generation system with new types of detectors such as hybrid photomultiplier or avalanche photodiode arrays that are both sensitive and fast."

Research is published in *Scientific Reports*.



LEFT: *A neuron imaged with multiline orthogonal scanning temporal focusing (mosTF).* **RIGHT:** *The same neuron imaged with line-scanning temporal focusing microscope (lineTF). Below are magnifications of the dotted areas. In the mosTF images dendritic spines are clearly visible in the magnified images, while they are obscured by noise in the lineTF image. [Yi Xue, et. al.]*



SIX LAYERS *of excitatory neurons colour-coded by depth. [Google Research and Lichtman Lab]*

Cubic millimetre of human cortex mapped to nanoscale resolution

Using more than 1.4 petabytes of electron microscopy imaging data, Google Research and Harvard University researchers have generated a nanoscale-resolution reconstruction of a millimetre-scale fragment of human cerebral cortex. The latest results from Jeff Lichtman, Harvard, Viren Jain, Google Research, and colleagues, provide an unprecedented view into the structural organization of brain tissue at the supracellular, cellular, and subcellular levels.

"The word 'fragment' is ironic," says Lichtman. "A terabyte is, for most people, gigantic, yet a fragment of a human brain - just a minuscule, teeny-weeny little bit of human brain - is still thousands of terabytes."

The 3D reconstruction, put together using Google's AI algorithms, reveals in vivid detail each cell and its web of connections in the cubic millimetre piece of temporal cortex. According to the researchers, the reconstruction contains roughly 57,000 cells, about 230 millimetres of blood vessels, and nearly 150 million synapses. They have developed a set of freely available tools for visualizing and analyzing the vast dataset.

"Given the enormous investment put into this project, it was important to present the results in a way that anybody else can now go and benefit from them," says Jain.

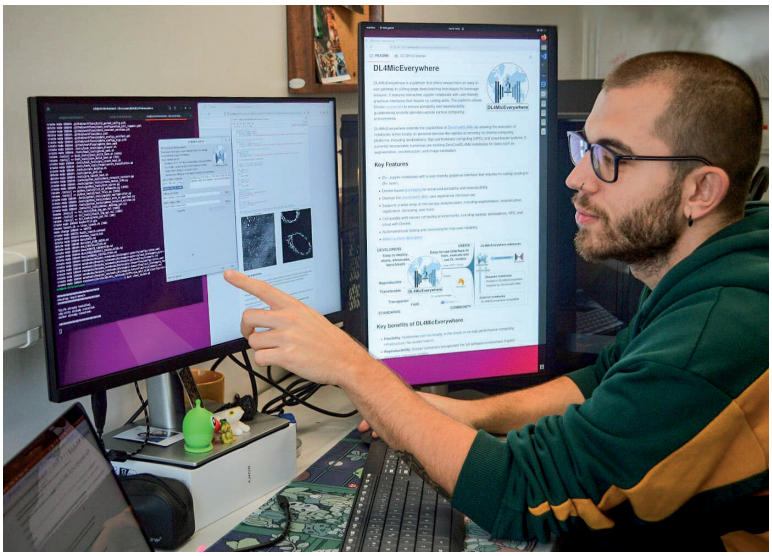
Using the data, the researchers discovered previously underappreciated aspects of the human temporal cortex, including the disproportionate number of glia over neurons and the existence of rare yet powerful axonal inputs that contain up to ~50 synapses. They also noted oddities in the tissue, such as a small number of axons that formed extensive whorls. Since their sample was taken from a patient with epilepsy, they're unsure if such unusual formations are pathological or simply rare.

The researchers now plan to tackle the mouse hippocampal formation, which is important to neuroscience for its role in memory and neurological disease.

Research is published in *Science*.

Advanced image analysis for all

Open source AI platform, DL4MicEverywhere, is set to help life scientists harness cutting-edge, deep learning techniques for biomedical research. Rebecca Pool reports.



IVAN HIDALGO-CENAMOR, *IGC, works with the DL4MicEverywhere platform. [Instituto Gulbenkian de Ciência]*

An international team of researchers has released ' DL4MicEverywhere', an open-source platform that aims to make deep learning more accessible for bioimage analysis. With development co-led by Professor Ricardo Henriques's laboratory at Instituto Gulbenkian de Ciência, Portugal, and Professor Guillaume Jacquemet's laboratory, Åbo Akademi University, Finland, and building on 'ZeroCostDL4Mic', the latest framework is designed to help researchers train and deploy deep learning models on a wide range of experiments and computing infrastructures, from basic laptops to sophisticated high-performance clusters. The platform features a user-friendly interface and broadens the scope of methodologies available for common microscopy image analysis tasks.

"DL4MicEverywhere establishes a bridge between AI technological advances and biomedical research," highlights Ivan Hidalgo-Cenamor from the Optical Cell Biology Group, IGC. "With it, researchers gain access to cutting-edge methods, enabling them to automatically analyze their microscopy data and potentially discover new biological insights."

DATASET ANALYSIS

Deep learning has transformed the analysis of large microscopy datasets but many researchers have not had access to annotated data, high performance computing resources and the necessary expertise to develop and run deep learning models. Tools such as the BioImage Model Zoo have helped researchers to share and reuse of pre-trained models, yet often, deep learning models still need to be trained or fine-tuned on the end-user dataset to perform well.

With this in mind, a huge team of researchers from IGC, Åbo Akademi University, MRC-Laboratory for Molecular Cell Biology, The Francis Crick Institute, University of Turku, Max Planck Institute for Molecular Cell Biology and Genetics, Chan Zuckerberg Biohub and other organisations, initially developed ZeroCostDL4Mic. This entry-level platform was designed to simplify deep learning access by leveraging the free, cloud-based computational resources of Google Colab.

As the team reported in *Nature Communications* just after ZeroCostDL4Mic development: "ZeroCostDL4Mic allows researchers with no coding expertise to train and apply key DL networks to perform tasks including segmentation (using U-Net and StarDist), object detection (using YOLOv2), denoising (using CARE and Noise2Void), super-resolution microscopy (using Deep-STORM), and image-to-image translation (using Label-free prediction - fnet, pix2pix and CycleGAN)."

DL4MicEverywhere now takes this a step further, and promises to make deep learning even more accessible, doubling the number of deep learning approaches compared to those available in ZeroCostDL4Mic. This time around, development came from international collaborators including IGC, Åbo Akademi University, European Molecular Biology Laboratory - European Bioinformatics Institute, Israel Institute of Technology, University of the Basque Country and Israel Institute of Technology. The EU-funded AI4Life consortium, which aims to facilitate life scientists' access to FAIR (findability, accessibility, interoperability and reusability) and open data, also contributed, and the platform adheres to FAIR principles to enhance data-driven scientific discoverability.

USING THE PLATFORM

As described on the DL4MicEverywhere github page of the Ricardo Henriques lab, deep learning techniques can be applied to image analysis using the web-based application, Jupyter notebook, for tasks such as segmentation, reconstruction and image translation. These notebooks are user-friendly graphical interfaces that require no coding skills, and use Docker containers - lightweight, portable, and self-sufficient packages to run the necessary software. The notebooks can be run on on personal devices, say, laptops, or remotely on diverse computing platforms, including workstations, high-performance computing (HPC), and cloud-based systems.

Pre-trained models are also shared to allow researchers to quickly get started analyzing their data. The platform lowers barriers to entry by abstracting away unnecessary complexity in model development. Other key features include transparency, with notebooks and models readily shareable to enable replication of analyses, and accessibility - interactive widgets and automated build pipelines promise to lower barriers for non-experts. The platform is also interoperable, adhering to standards such as BioImage Model Zoo for model sharing.

"DL4MicEverywhere aims to democratize AI for microscopy by promoting community contributions and adhering to FAIR principles," explains IGC's Dr Estibaliz Gómez-de-Mariscal.

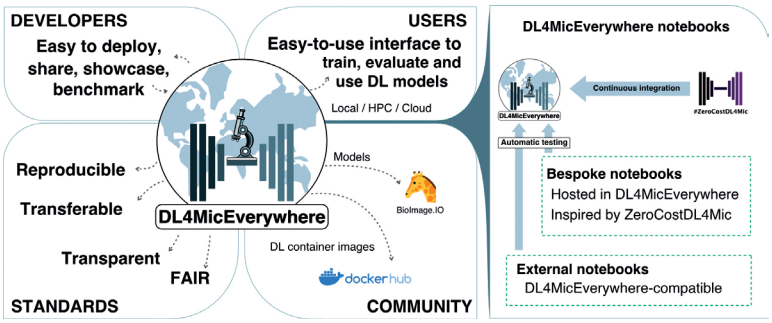
"We hope this platform will empower researchers worldwide to harness these powerful techniques in their work, regardless of their resources or expertise," adds Dr Joanna Pylvänäinen, Åbo Akademi University. "It will allow life scientists without coding experience to use deep learning on large numbers of microscopy images and videos to make discoveries. This will revolutionize how researchers plan their experiments and extract new information from microscopy datasets."

The DL4MicEverywhere platform is freely available as an open-source resource, reflecting the collaborators' commitment to open science and reproducibility. The researchers believe that by lowering the barriers to advanced microscopy image analysis, DL4MicEverywhere will enable breakthrough discoveries in fields ranging from basic cell biology to drug discovery and personalized medicine. Source code, documentation and tutorials for DL4MicEverywhere are available at https://github.com/HenriquesLab/DL4MicEverywhere under a Creative Commons CC-BY-4.0 license.

As Jacquemet points out: "This work represents an important milestone in making AI more accessible and reusable for the microscopy community. By enabling researchers to share their models and analysis pipelines easily, we can accelerate discoveries and enhance reproducibility in biomedical research."

"DL4MicEverywhere has the potential to be transformative for the life sciences. It aligns with our vision in AI4Life to develop sustainable AI solutions that empower researchers and drive innovation in healthcare and beyond," concludes Henriques.

Research is published in *Nature Methods*.



DL4MICEVERYWHERE *is a platform that offers researchers an easy-to-use gateway to cutting-edge deep learning techniques for bioimage analysis. [HenriquesLab/DL4MicEverywhere]*

Schottky SEM with large specimen chamber expands application capabilities

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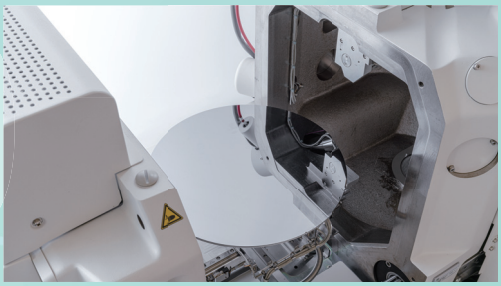
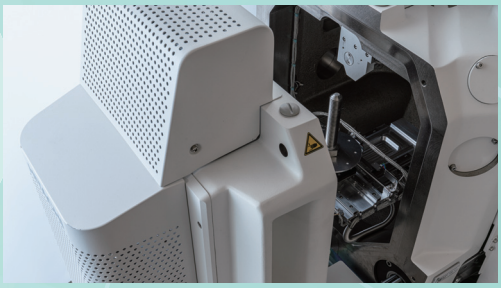
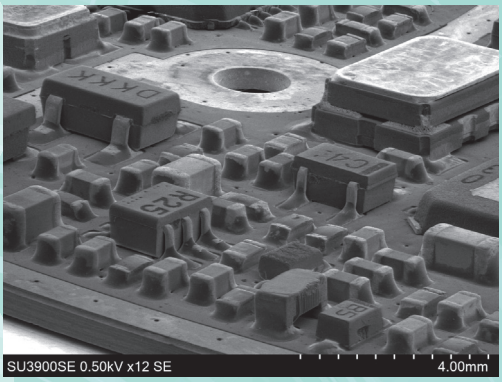


Example with 300 mm diameter wafer loaded



Example with 130 mm height bolt loaded



High-tilt observation of printed circuit board

	SU3900SE
Maximum Specimen size (diameter)	Φ300 mm
Maximum observable range (diameter)	Φ229 mm*
Maximum loadable weight*1*2	5 kg
Maximum loadable height*2	130 mm

*1 Using the heavy weight holder (optional). *Optional
*2 The weight and height that can be loaded differ depending on the dimensions of the specimen being loaded.

The SU3900SE/SEPlus series is equipped with a stage that supports large/heavy specimens. A maximum weight of 5 kg can be driven on 5-axis stage.

- Supports up to 300 mm diameter/130 mm height specimens
- A maximum weight of 5 kg using 5-axis stage
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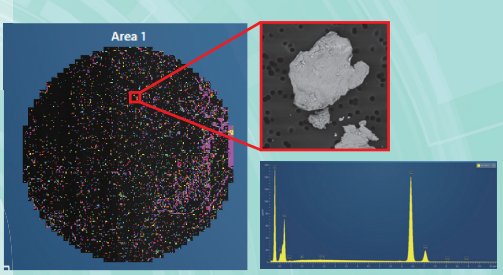

Automatic Particle Analysis of Metallic Foreign Matter Captured by a filter

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Reaching the heart of research

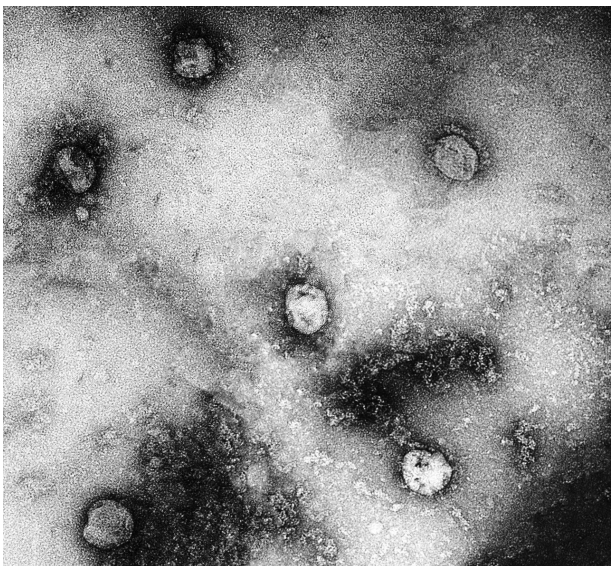
DR ERIN TRANFIELD *Head at the VIB BioImaging Core Ghent. [Sandra Miller Photography and Films]*

Erin Tranfield joined a core facility just as the world was well and truly waking up to this new way to navigate research. A decade-and-a-half later, she is Head of VIB BioImaging Core Ghent in Belgium. Here's her journey from PhD to Core leader, and a glimpse of what lies beyond. By Rebecca Pool

As a self-confessed nerd, Erin Tranfield's passion for tech has taken her from a PhD in dust toxicology and human health at the University of British Columbia, Canada, to postdoctoral research at the NASA Ames Research Center in California and then into the wonderful, and rapidly evolving, world of core facilities. Just a few months ago, the biologist and electron microscopist took on the role of Head at the VIB BioImaging Core Ghent, Belgium, which provides light and electron microscopy services and image analysis to VIB and non-VIB academics, and industry. The move follows 15 years at both a world-renowned laboratory and a resource-stretched facility, and Tranfield hopes her contrasting experiences will now stand her in good stead for what lies ahead.

"I hope I've taken away the perfect combination of the two – at one there was often the option to solve a problem with investments and at the other, there were limited opportunities for investments," she says. "But either way, I've always liked the challenge of coming up with a technical solution to a research question, and working out how to study that with the most accurate method possible. I find it fun – some might find that crazy, but yes, I use the word fun."

Tranfield's first experience of a core facility was at the European Molecular Biology Laboratory (EMBL)



FIRST ELECTRON *microscopy images to emerge in Portugal of SARS-CoV-2 came from the Institute of Molecular Medicine (IMM) and the Gulbenkian Institute of Science (IGC), whilst Tranfield was heading up the Electron Microscopy Centre there. Here, colleagues at IMM used clinical swab samples to isolate the virus, propagate it and infect cells in culture. [João Lobo Antunes Institute of Molecular Medicine (Tânia Carvalho/Pedro Pereira) and sample imaging; Gulbenkian Institute of Science (Erin Tranfield).]*

in Heidelberg, Germany – it was 2009, and such facilities were not yet commonplace. "I was really seeing, for my first time, a big European core facility in action," she says.

At the time, Tranfield was attempting to reconstruct the ultrastructure of the meiotic spindle of the clawed frog egg at 2 nm resolution. Sample preparation was laborious and experiments were lengthy. Yet, the daily research grind instilled persistence and resilience to Tranfield's scientific approach and she appreciated the many scientists she met. And while, as a postdoctoral researcher, she wasn't part of the core facility team here, she says: "By osmosis and proximity, you see and learn a lot... I feel very lucky that I happened to come onto this scene around the time the core facility opportunities were really growing."

Tranfield remained at EMBL for nearly four years, and in 2013, moved to the Instituto Gulbenkian de Ciência (IGC) Electron Microscopy facility, in Portugal. Heading up the IGC electron microscopy facility, she recalls arriving with visions of purchasing new equipment, but after learning about the financial and research landscape, it became clear a new approach was needed.

"While there was limited money in Portugal, there was this great opportunity to learn and grow, and challenge how to approach things," she recalls. "For example, we'd ask ourselves, do we have to use the expensive sapphire discs for high pressure freezing or can we get away with the cheaper plastic film? You have more sample loss with the plastic, but at least you'll get something into the microscope for half the price. This was always the challenge."

Along the way, Tranfield coined the term 'MacGyvering' – a nod to the TV show, MacGyver, in which the problem-solving hero applies his scientific knowledge - and a Swiss army knife – to always save the day. "Very often it really did feel like this is what we were doing in Portugal," she says. "There wasn't the money so we'd throw creativity at the problem – we were always just trying to be as creative as possible."

During her time at IGC, Tranfield also recalls calling on her European connections for help. "Just as we got results, we'd then have to go to collaborators with more advanced technology to continue the work," she says. "You'd then see their data coming in and you'd think, 'we could have also done this' but we just didn't have the instruments."

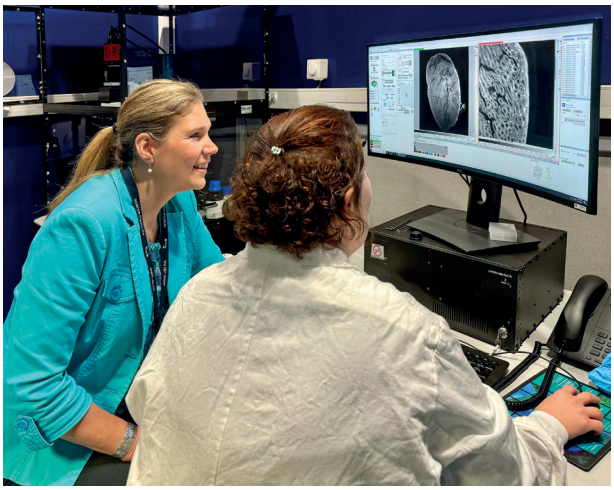
FROM PORTUGAL TO BELGIUM

Just over a decade after joining IGC, and as the research institute merges with the Institute of Molecular Medicine in Lisbon to become the Gulbenkian Institute of Molecular Medicine, Tranfield has moved on. Now heading up the VIB BioImaging Core Ghent, Belgium, she is excited. "In terms of experience, knowledge and community network, I have a lot to bring to this position – but there's always much to learn," she says. "I will grow as a leader, a scientist and hopefully a mentor to my team."

In terms of resources, the VIB BioImaging is more akin to EMBL than IGC – a recent demo of Leica's latest ultramicrotome also prompts her to recall how much easier it is to collaborate with other organisations when based in northern Europe. Still, Tranfield is hopeful that "the juxtaposition" of the two core facilities she has already spent time at will help her enormously in this new role. Portugal has equipped her with a real understanding of the need to create inexpensive, effective solutions, whilst imparting a willingness to invest. Yet, as she also highlights, investments in infrastructure at EMBL were always careful and considered.

"I hope I take away the perfect combination of the two," she says. "No-one has enough money in research and no-one has endless time and an endless budget. We all have to make decisions – where do we benefit from strategic investment and where can we be creative? Where can we merge or repurpose systems?"

Indeed, at VIB BioImaging Core Ghent, she is already asking herself these very questions. Right now, she and colleagues are working out how best to set up a workflow for cryo-volume electron microscopy/cryo-FIB scanning electron microscopy for the Ghent and Leuven VIB BioImaging Cores while also investing in new light microscopes – repurposing the bodies of the existing light



AMANDA GONÇALVES *and Erin Tranfield looking at data on the MACS Miltenyi Ultraview II - Light Sheet Microscope. [Femke Baeke/VIB BioImaging Core Ghent.]*

Meet the Core

In 2012, the VIB Bioimaging Core was established as a two-sited core with platforms in Ghent and Leuven, in Flanders, Belgium, with each core supporting imaging from in vitro to in vivo, from the meso to the Ångström scale, and from morphology to mechanism.

The Bioimaging Core that Erin Tranfield currently leads evolved from the VIB Inflammation Research Center to a VIB wide facility. Here, light and electron microscopy services and image analysis were integrated into one unit and made accessible beyond the institute, for academics and industry.

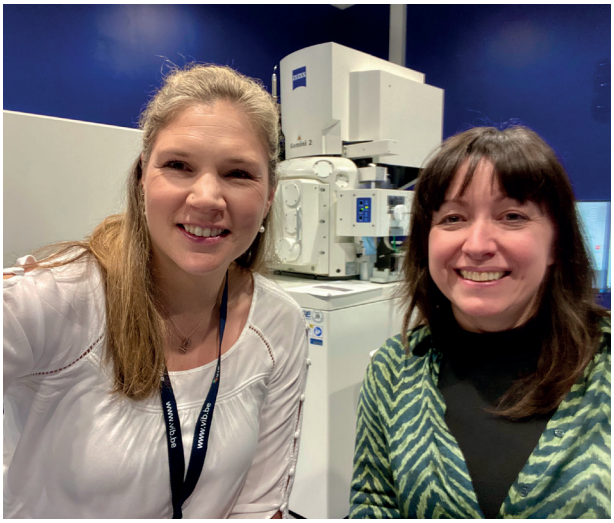
Today, the core at Ghent provides consultancy on complete workflows, and has a broad range of techniques, covering macroscopic imaging, confocal techniques, super resolution and electron microscopy. Applications include live cell and functional imaging techniques (FRET, FLIM, FRAP). The Core is home to several CLEM workflows, and is also equipped with volume EM techniques, including Serial Block-face SEM and Focussed Ion Beam SEM - so 3D CLEM can also be performed. The Core also has a strong focus on image analysis.

Light microscopy includes a Nikon TI-E inverted microscope, a Leica Stellaris 8, a MACS Miltenyi Ultraview II light Sheet microscope, a range of Zeiss light sheet and spinning disk microscopes, Zeiss Axioscans, a Zeiss super-resolution TIRF3. The Core also has three electron microscopes - a Jeol JEM 1400 Plus TEM, a Zeiss Merlin SEM equipped with an Gatan 3View 2XP and a Zeiss CB540 Focussed Ion Beam SEM.

The VIB Bio Imaging Core-Leuven includes confocal microscopes, slide scanning, high content screening, and 3D whole organ imaging, and like Ghent, offers support for image analysis both with infrastructure and expertise.

THE VIB BIOIMAGING CORE *- Ghent Team [VIB BioImaging Core Ghent]*

PABLO HERNÁNDEZ VARAS, *Axelle Kerstens, Eef Parthoens, Erin Tranfield and Amanda Gonçalves at the ELMI Meeting in Liverpool June 2024.*



SASKIA LIPPENS *and Erin Tranfield from the VIB Technologies with the Zeiss Crossbeam volume electron microscope in the background. [Erin Tranfield]*

microscopes to address their cryo-EM and cryo-FIB needs is a real option. "In our new world of sustainability, we should reuse and recycle – do we need to buy new all of the time?" she asks.

Across the next year or so, Tranfield will be optimising existing workflows while getting to grips with the needs of the scientific communities at VIB. Possible long-term investment in light and electron microscopes will follow, depending on where portfolios need to grow.

"Our wishes for 2024 are to upgrade the camera on our JEOL TEM so we can better support cryo-EM workflows for structural biology," she says. "Funding for an upgrade on our cryo-FIB is already in place, so we're optimising workflows around this."

"Three months in, I'm learning where the urgent gaps in the workflows are, and once these are addressed, we're going to look at the shopping list – you know; 'Dear Santa we would like'," she laughs. "I really do want to lead a core facility that is there to serve the needs of the community."

As part of this, Tranfield is keen to introduce the concept of expert-redundancy at VIB Ghent. Whilst working as a postdoctoral researcher at the NASA Ames Research Center, she saw the clear benefits of always having two people within any team that could work on the same method. "In a dynamic core there is always growth of infrastructure and it is a challenge to keep redundant technical expertise. In Ghent redundant expertise doesn't exist for all our techniques so we're looking to ensure we always have a second member of a team that can, say, keep collecting data and using equipment, when the key expert isn't around," she says. "I want this to be clear for the team, but also the users of the instruments."

Tranfield is also working with a new colleague, Pablo Hernández-Varas, who recently joined VIB-KU Leuven as Head of this neighbouring BioImaging Core. Each shares the vision of building a core facility unified across their two sites. "If you go to Ghent or you go to Leuven, you'll get the same sort of experience – we're really going to streamline how researchers contact the core and make sure there's no barriers to [accessing] equipment," she says.

This move towards facility unification is a topic that Tranfield wrote about in her recent paper, 'Future proofing core facilities with a seven-pillar model', alongside VIB Technologies colleague Saskia Lippens, in the *Journal of Microscopy*. Here, Tranfield and Lippens outline the vital role of centralised core facilities in life science research, detailing a model to ensure research success. In addition to training, technical support, transparency and other pillars, community - in terms of researchers and support services as well as the global core facility community - was highlighted as being instrumental to innovation.

As Tranfield explains, the global community can provide the opportunity to share experiences and knowledge between core facilities that can be essential in challenging projects or implementing innovative technologies. "Inter-core collaboration – where you have the technology experts saying, 'yes, we can mix these methods or instruments and come up with a new adaptation' – I think that's going to be the future," she says.

"As the Cores, our focus is to bring our resources to the researchers," she adds. "In answer to [researchers'] questions, we can say, 'you need this technology, that instrument, or how about combining these workflows, and I also know someone in another Core who can help'. This way we are building bridges [between facilities]."

Core collaborations aside, Tranfield also believes the future needs to be more multi-modal - and not just in imaging. "At times we can get very fixated in, I am an electron microscopy person, I am a light microscopy person, or I do proteomics – we need to be more transdisciplinary across the cores to build more sophisticated and robust workflows."

THE VIB BIOIMAGING CORE *- Ghent Team at a chocolate making workshop at La Maison 12 in Ghent. [Riet De Rycke]*

But whilst putting together the workflows of the future, the VIB Ghent BioImaging Core Head is also keen to remember the all-important fundamental technology that can be taken for granted. "Who wants a vibratome? Well actually everyone does at some point but you don't think about it until you can't access it," she points out. "We need to build these complete portfolios that include the advanced, new, fun and interesting technologies as well as the foundational technologies – so we can really support whoever comes through the door."

CHANGING TIMES

With an eye on tech-developments, Tranfield has also been keenly watching the rise of volume electron microscopy, and the very many ways in which the various techniques are used to approach three-dimensional analysis. Like many she appreciates how advances in artificial intelligence and machine learning are fuelling the advance of the method and analysis of the vast swathes of data it generates. But looking to the future, she's also wondering how X-ray imaging will develop and be used. "This really is bringing something new to research and will become part of a bigger portfolio," she says. "And this is an example of why centralised core facilities are so important – portfolio items such as this can range in cost from half-a-million to well over one-and-a-half million dollars. You can't expect every research institute to build a facility for a portfolio item – we should have [such instruments] being used across a city or a district."

Over the years, Tranfield has also been carefully observing core facilities and her take on the role of these ever-changing labs has shifted somewhat. Once upon a time she believed that a core facility should be invisible – if all is working as it should, then you simply won't hear about it. But now she believes that instead, the core facility should be ever-present.

"The core facility has always been a place you go to, to answer your questions, be it about the technology, the technique, the sample preparation or the application," she says. "But now we don't want to be invisible – if the core works well, users don't realise the work that went into it. We should be a known resource for the community – if you're stuck, come to us for help, to get to your answer faster."

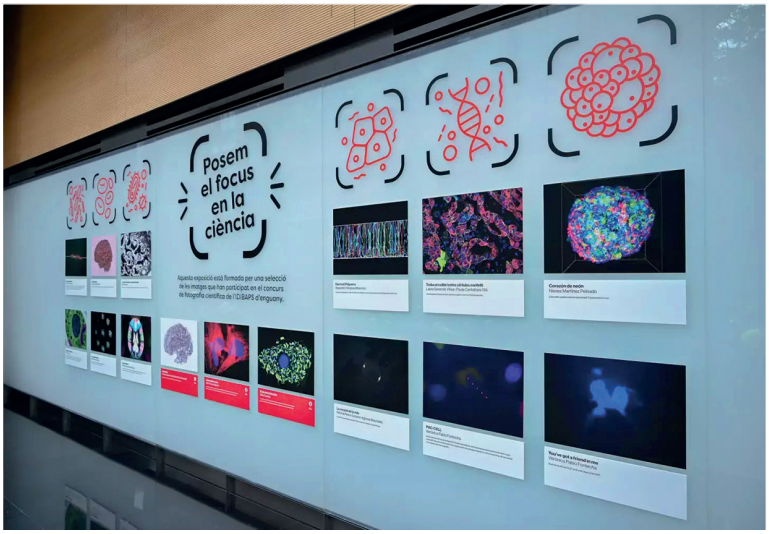
Tranfield is also looking forward to a time when the 'just a core facility person mentality' has well and truly disappeared. As she highlights, the core facility field is evolving to a place that experts choose to join, rather than opting to be in as being a PI didn't quite happen. "To the imaging scientists in the core facilities – do not negate that you are a scientist, you are a different kind of scientist," she says. "I think we're moving beyond the negative mentality around core facility staff and we now have this calling for nerdy people like me who just really like the technology, helping others and being very resourceful in the process."

Brains, bacteria and beauty: the images that light up our lives

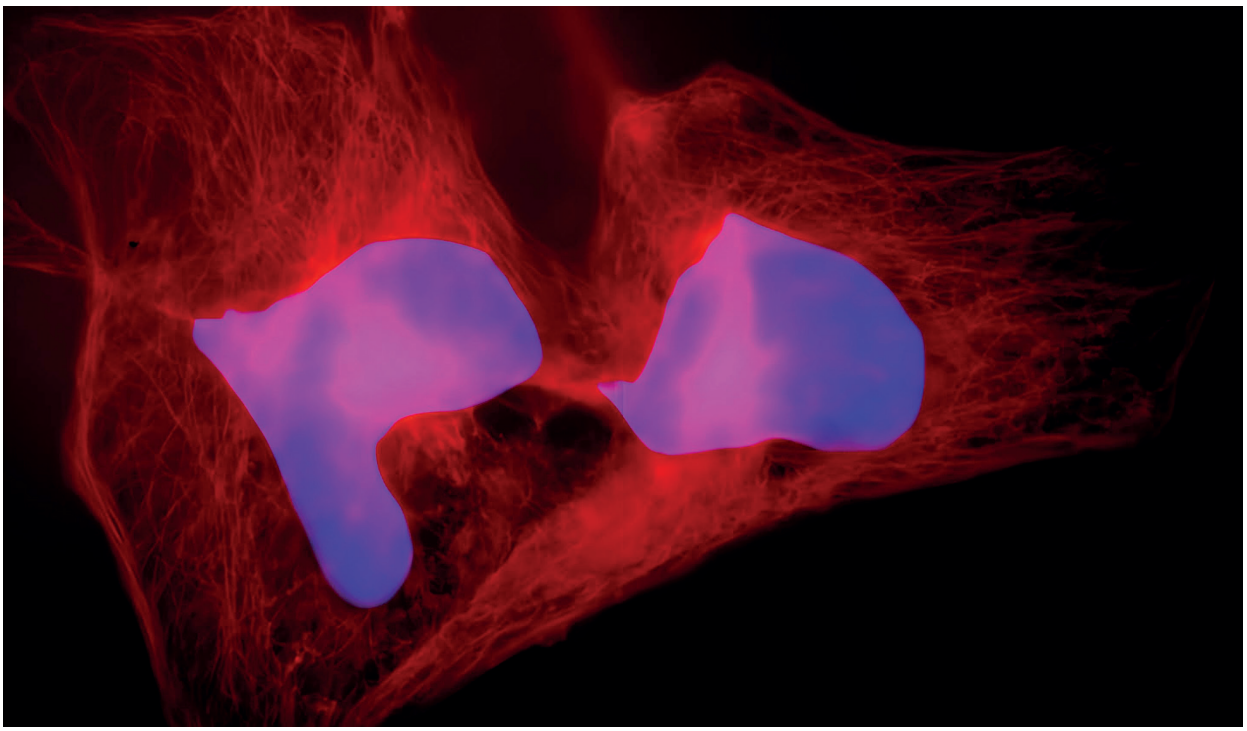
By Rebecca Pool

Every year, R&D consortium, Institut d'Investigacions Biomèdiques August Pi i Sunyer - IDIBPAS - in Spain, organizes a scientific photography contest in which its employees from the Catalan Government, the Hospital Clínic of Barcelona, the University of Barcelona's School of Medicine and the CSIC Biomedical Research Institute in Barcelona can take part. Submitted images are posted online, anyone can 'like' their favourite image and a panel of judges chooses three winners from the top 15 entries. This year, an image called "Jelly Brain" by Cerebrovascular Diseases technicians, Andrea Cabero Arnold and Marta Peña González, won, and then 'Knowing how to let go' showing cell division, and 'The eye of infection', an image of a parasite infected cell, came second and third.

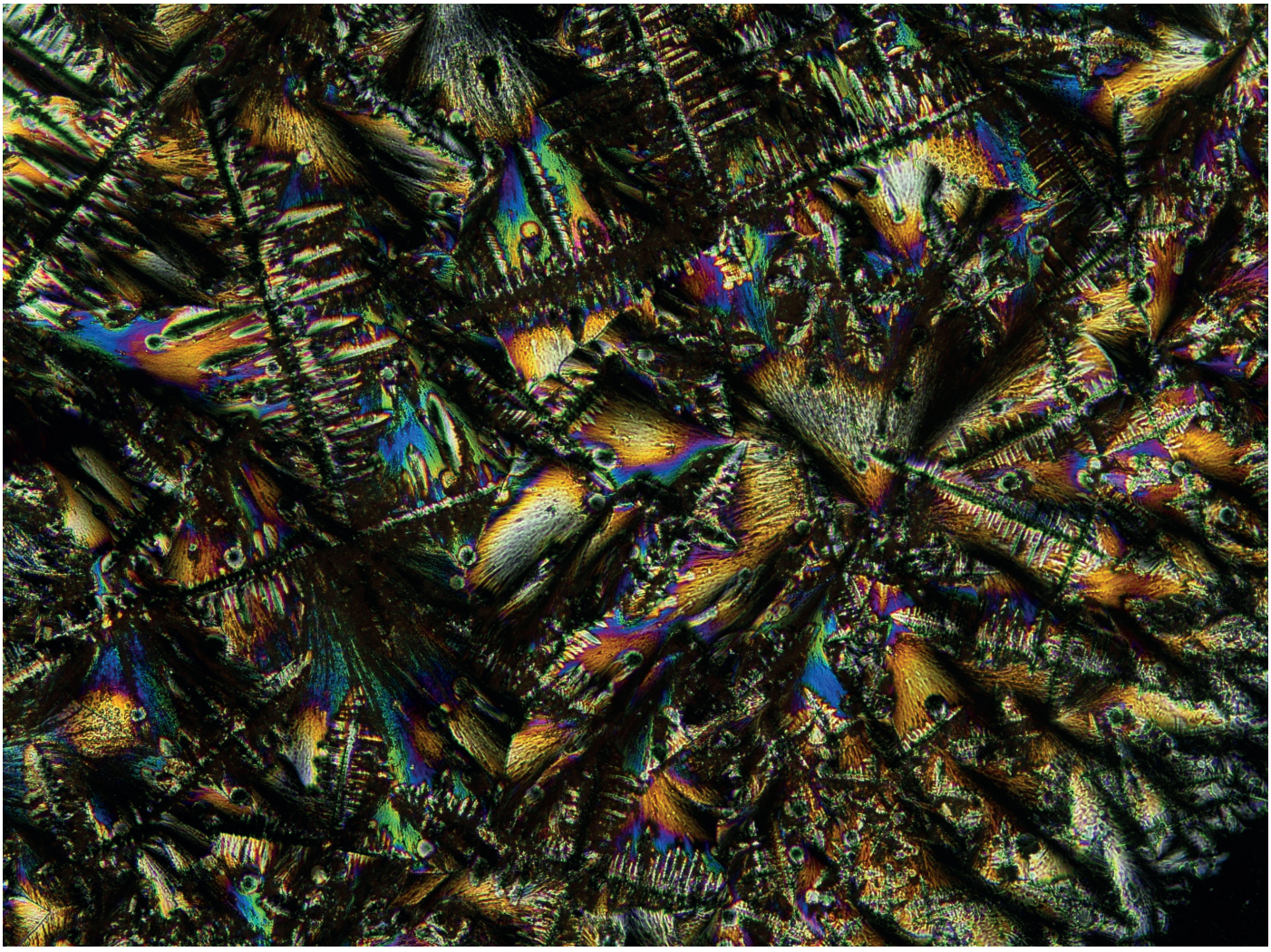
This 7th IDIBAPS scientific photography competition isn't perhaps one of the better known contests and prizes are relatively modest - book vouchers from La Impossible, a book shop in Barcelona. But like many contests, its images celebrate the beauty of science and the microscopic world while applauding the work of those from science-related fields – in this case biomedicine. Today, tens and tens of competitions from tech-giants, charitable foundations, non-profits, publishers and more, provide avenues for researchers to gain recognition for their work and promote a wider appreciation for science. Here's a look at some recent winning images and a glimpse of what is to come.



THE 15 *most-voted images from the 7th edition of the IDIBAPS Scientific Photography Contest. [IDIBAPS]*



'KNOWING HOW TO LET GO' *by Martí Torres-Marcén from Translational Research in Hepatic Oncology, Barcelona Clinic: The image shows two cells that are dividing, and are stained with DAPI (double-stained) and anti-Tubulin. As Torres-Marcén says: "From this moment each will follow its own path." [Martí Torres Marcén/ IDIBAPS]*



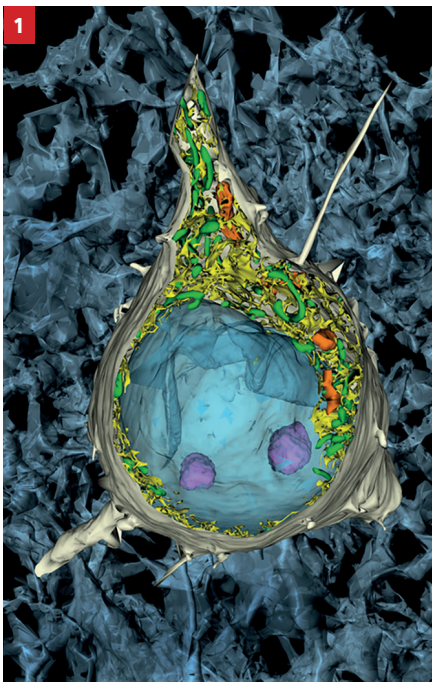
HIDDEN CITY, *Kaylie Scorza [University of Chicago Science as Art]*

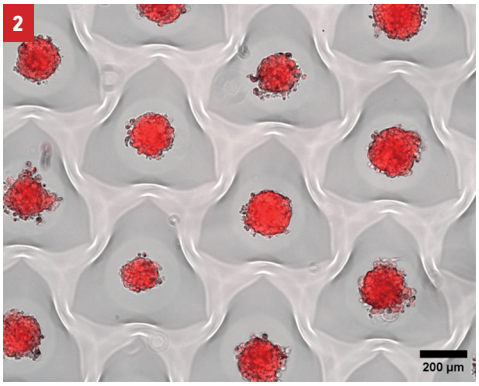
Science as Art in Chicago

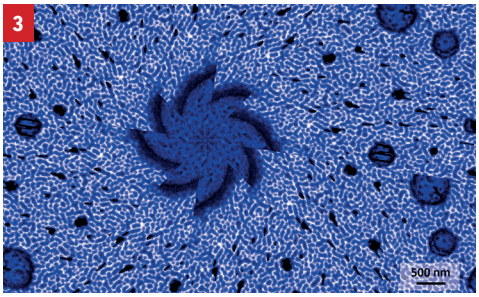
"Hidden City" by research assistant, Kaylie Scorza, was awarded the audience favourite in the 2024 University of Chicago Science as Art competition. The image was taken during research on gut bacteria - Scorza grew gut bacteria in broth; as the culture dried, the bacteria metabolites formed structures that react with polarized light to create vivid, kaleidoscopic patterns. This year, the contest drew more than 60 entries from undergraduates, graduate students, staff, alumni, postdoctoral researchers and faculty members, showcasing everything from fossils to fly anatomy.

Mighty microscopists

Every two years, the UK-based Royal Microscopical Society holds its Scientific Imaging Competition in which microscopists from around the world show exactly what they can do – the results are always magnificent. In last year's competition, Josef Spacek from Charles University

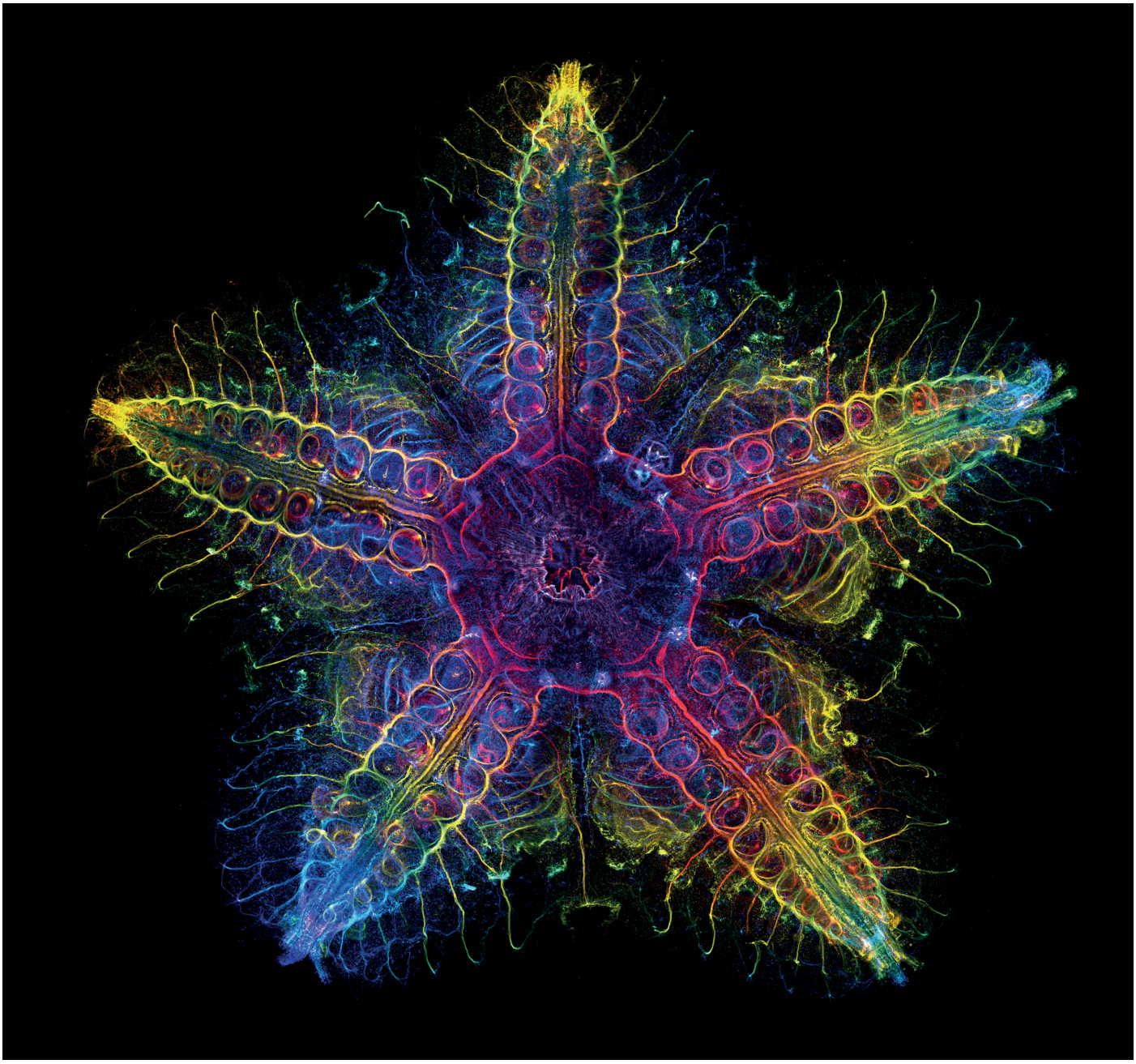






1: *Pyramidal cell of the cerebral cortex from Josef Spacek [RMS/Charles University Hosptial]*
2: *Dimensionality in ovarian cancer from Dan Marks [RMS/ Imperial College London]*
3: *Polymer brush PS-PMMA from Lars Mester [RMS/attocube systems]*

Hosptial, Czechia, took a look inside the pyramidal cell of the cerebral cortex with his 3D serial electron microscopy reconstruction. With an original magnification of X 6000, the nucleus is pseudocolored in blue, nucleoli in pink, endoplasmic reticulum in yellow, mitochondria in green and Golgo apparatus in orange. His image won first place in Electron Microscopy – Life Sciences. Meanwhile, Dan Marks, Imperial College London, won second place in Light Microscopy – Life Sciences with 'Dimensionality in ovarian cancer', showing an array of spheroids grown from cells derived from a patient. A polymer brush PS-PMMA, imaged with tapping AFM-IR at 8.7 µm wavelength by Lars Mester, attocube systems, Germany, came second in AFM and Scanning Probe

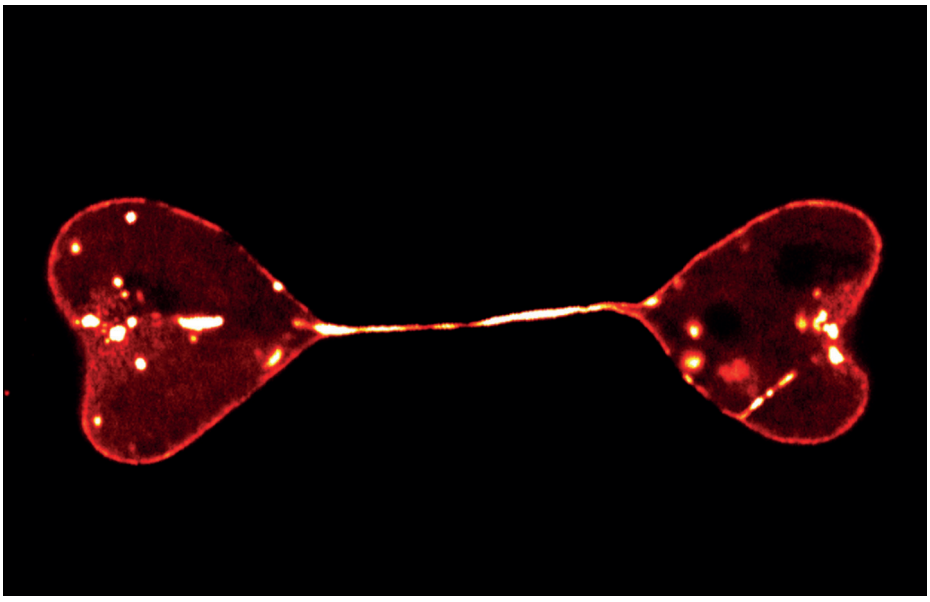


Microscopies. The original 5x5 µm image was cut at a 45° angle and is shown 8 times, with different rotations, and no further post-processing.

SEA STAR *from Laurent Formery [Laurent Formery, Evident 2022, Image of the Year (IOTY)]*

Global light

In 2022, Laurent Formery, Postdoctoral Researcher, Hopkins Marine Station, Stanford, US, won Evident's Image of the Year, Global Scientific Light Microscopy Award, with his image of a juvenile sea star's nervous system. The 1 cm sample was optically cleared, stained using an anti-acetylated-tubulin antibody and imaged at 5X magnification with tile-scanning on a confocal microscope. The image shows five nerve cords running along the sea star's five arms – these are linked together by a nerve ring around the mouth. Regularly spaced lateral nerves branch from the nerve cords toward the edges of each arm.

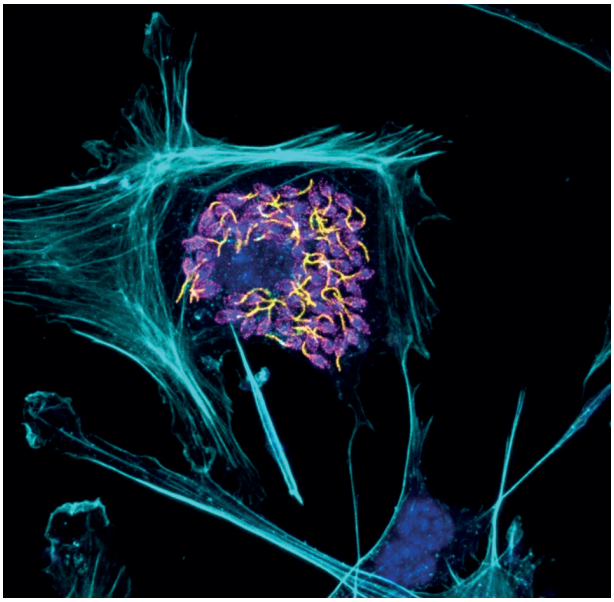


A HEART-TO-HEART *of semi-separated nuclei from Di Lu. [Di lu, Evident 2021, Image of the Year (IOTY)]*

In 2021, Di Lu, China, captured the semi-separated nuclei of two cells forming a heart-to-heart shape on an Olympus FV3000 confocal microscope. The nuclei were labelled by lamin, the fibrous protein of the nucleus. Evident will announce the winners of fifth Image of the Year competition in the Autumn of this year. [Di Lu, Evident 2021 Image of the Year (IOTY) - honorable mention]

Modern BioArt

For several years, FASEB - the Federation of American Societies for Experimental Biology - has held its BioArt Awards to, as it says, "honour original, visually stunning photographs, illustrations, data visualizations, or videos that effectively communicate an important aspect of 21st century biological research". As well as axons and sea urchins, last year's winners included "A Cardiac Cell's Final Moments" by Leroy Versteeg, Research Associate at Baylor College of Medicine. Here, Versteeg had captured a mouse cardiac fibroblast (cyan) infected with T. cruzi parasites (magenta and yellow). As he explained, once these intracellular parasites are fully grown, they swim within the cardiac fibroblast's cytoplasm until they die and rupture. The deadline for this year's competition is September 30.



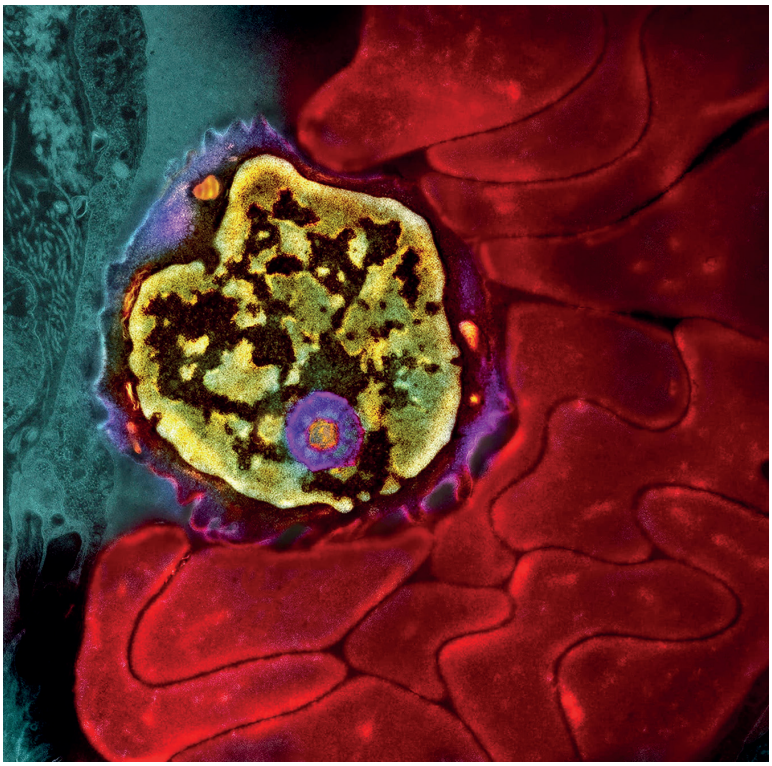
CARDIAC CELL *from Leroy Versteeg. [Leroy Versteeg/BioArt/FASEB]*

Light and electrons

This year's competition from FocalPlane/elmi (European Light Microscopy Initiative) showcased myriad stunning microscopy images - with the winning image from Rossana Melo, Professor of Cell and Molecular Biology at the Institute of Biological Sciences, Federal University of Juiz de Fora (UFJF), Brazil, actually being an electron micrograph. Melo imaged a leukocyte (lymphocyte) trapped among aggregates of red blood cells in a small lung vessel of a patient with asthma. She used a Tecnai

BELOW: *Glorious Lymphocyte from Rossana Melo. [FocalPlane/elmi]*

BOTTOM: *Beetle from Jiří Černý. [FocalPlane/elmi]*

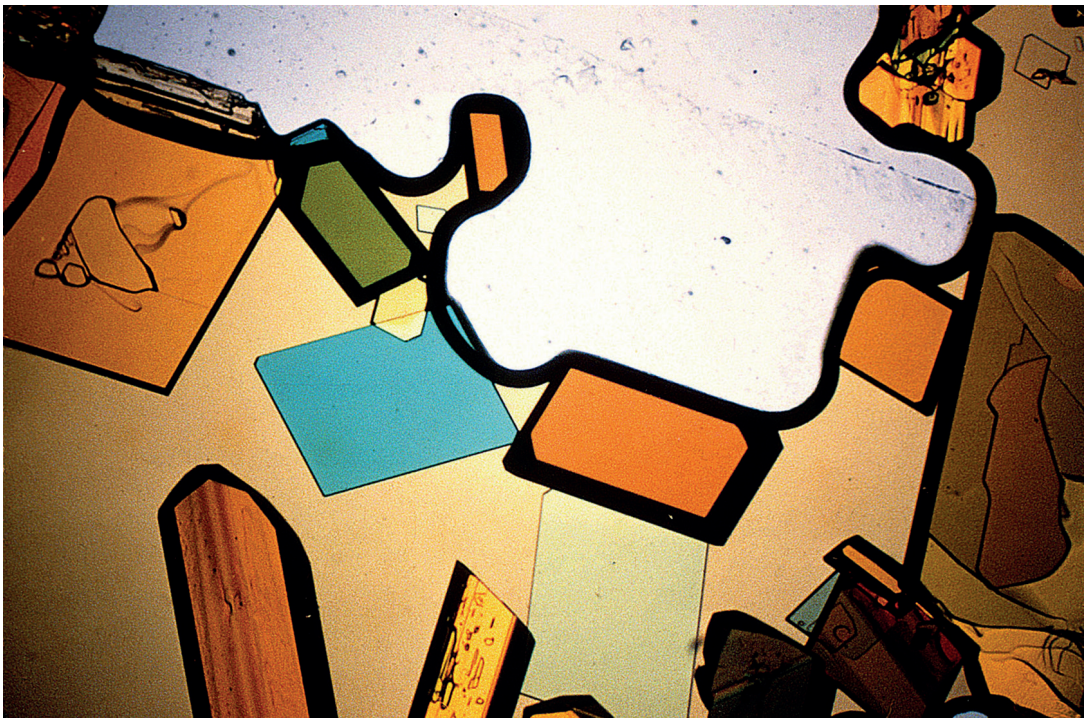


G2 Spirit Bio Twin ThermoFischer Scientific TEM at 80 KV and colorized the images using Photoshop. So-called Glorious Lymphocyte will feature on the cover of the *Journal of Cell Science*. Shortlisted winner, Jiří Černý captured a stunning image of a beetle using light-sheet fluorescence microscopy and Imaris and HuygensPro softwares for postprocessing and reconstruction.

Smallest worlds

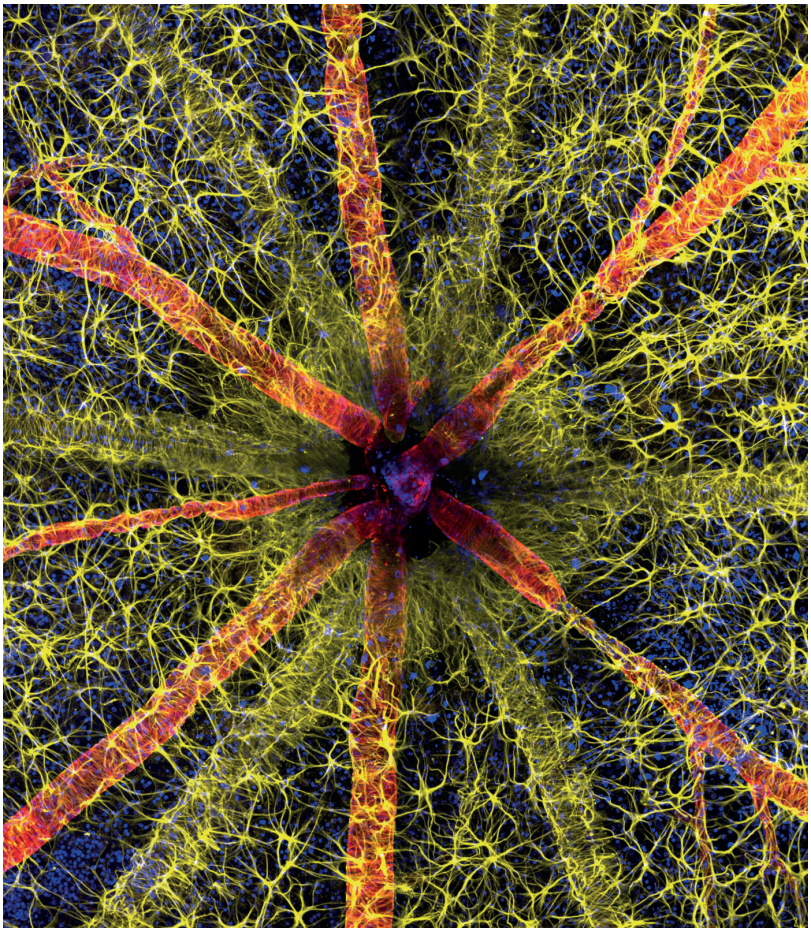
Running since 1975, Nikon's Photomicrography Competition – now Nikon Small World – is one of the oldest and most renowned microscopy competitions. The 1975 winner was James Dvorak with a transmitted polarized light image of oxalic acid crystals during precipitation, at X 100 magnification. Fast forward five decades, and the latest competitions have embraced the numerous microscopy developments that have followed. First place, 2023, went to Hassanain Qambari, assisted by Jayden Dickson, both from the Lions Eye Institute, Department of Physiology & Pharmacology, Western Australia, for their X20 fluorescence image of a rodent optic nerve head showing astrocytes (yellow), contractile proteins (red) and retinal vasculature (green).

Capturing the image was no easy task - after locating the fine vessels near 110 microns in diameter, Qambari established a protocol for labeling different cell types. As he said: "The visual system is a complex and highly specialized organ, with even relatively minor perturbations to the retinal circulation able to cause devastating vision loss. I entered the competition as a way to showcase the complexity of retinal microcirculation." Taking a very different tack, Ángel Navarro Gómez, Madrid, Spain, captured a remarkably striking 20X image of a Carpenter bee head and antenna, via image stacking. The results of Nikon's 2024 Small World and Small World in Motion competitions will be released in Autumn, this year.



OXALIC ACID CRYSTALS *from James Dvorak, [Nikon Small World/Dvorak]*

CARPENTER BEE HEAD *and antenna from Ángel Navarro Gómez [Nikon Small World/ Navarro Gómez]*



RODENT OPTIC NERVE *head from Hassanain Qambari and Jayden Dickson [Nikon Small World/Qambari and Dickson]*

Confocal Interferometric Scattering Microscopy Label-Free Imaging of Intracellular Organelles in Live Cells

MICHELLE KÜPPERS[1,2,3] DAVID ALBRECHT[1,2] VAHID SANDOGHDAR[1,2,3]

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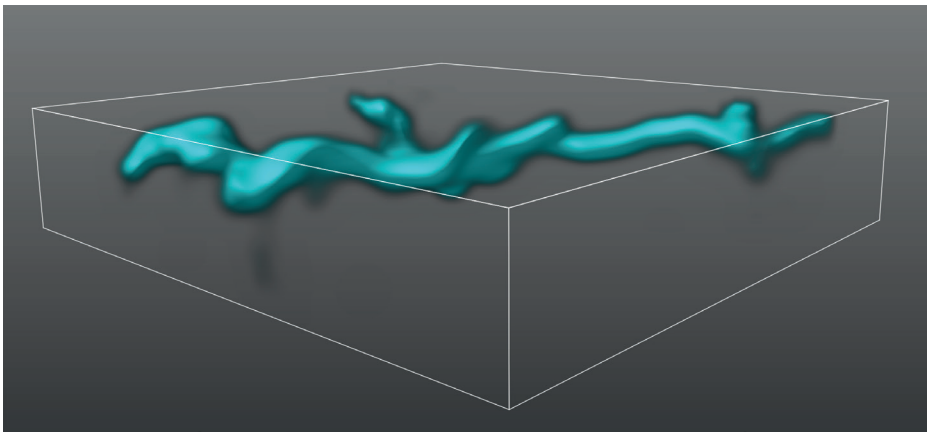
2. Max-Planck-Center for Physics and Medicine, Erlangen, Germany
3. Friedrich-Alexander-University Erlangen-Nuremberg, Erlangen, Germany



INTRODUCTION

A quantitative understanding of the complex and dynamic molecular processes in cells is essential for comprehending life. Fluorescence microscopy has helped scientists visualize cellular and subcellular processes in three dimensions (3D) over a century, also leading to single-molecule sensitivity and super-resolution microscopy. However, this approach is fundamentally limited by the photophysical properties of fluorophores and the need for labeling. Over the past two decades, the interferometric detection of scattering (iSCAT) has offered a label-free alternative imaging modality. Recently, we extended iSCAT to operation in the confocal mode (C-iSCAT), achieving high-quality 3D images of nanoscopic features of live cells. C-iSCAT can be simply performed on a commercial laser scanning microscope and yields label-free images simultaneously with its fluorescence counterpart.

METHODOLOGY

Interferometric Scattering Microscopy (iSCAT) is a homodyne technique based on the interference of the light scattered from a nano-object with reference light, e.g., reflected from the sample substrate. Over the past 2 decades, the high sensitivity of iSCAT has enabled the detection and tracking of small gold nanoparticles, viruses, and proteins [1-5]. Similar to fluorescence microscopy, iSCAT microscopy can be performed in different illumination and detection modes [6]. The sensitivity of iSCAT makes it also capable of detecting any small scattering object, thus, causing a complex speckle-like background. In particular, the wealth of macromolecular structures, vesicles, proteins, and nucleic acids in the complex environment of a live cell provides a challenge for the identification of specific nano-objects. In previous works, we applied advanced image analysis to track gold-labeled transmembrane proteins in wide-field iSCAT [7]. Recently, we demonstrated that confocal iSCAT not only addresses the background challenge in live cell imaging but also offers label-free 3D images of intracellular organelles at the nanoscale [8]. The ease of implementation and its compatibility with conventional fluorescence measurements make C-iSCAT highly desirable.

IMAGE FORMATION IN C-ISCAT AND EXPERIMENTAL 3D IPSF

The interaction of light with macroscopic objects is usually described by transmission, reflection, and absorption coefficients. To treat sub-wavelength objects, however, one needs to resort to the formalism of scattering [6]. In the simplest case, an incident electric field $\vec{E}_{inc}$ illuminates a spherical object much smaller than the wavelength of light λ, causing Rayleigh scattering [9]. The resulting scattering is governed by the nano-object's polarizability α, which in turn depends on the particle volume as well as the complex dielectric functions of the particle and its surrounding medium [10]. The more commonly known scattering and absorption cross-sections can be derived from a knowledge of α and λ.

The iSCAT signal on the detector I_{det} in reflection geometry can be formulated as:

$$I_{det} \propto \left|\vec{E}_{ref} + \vec{E}_{sca}\right|^2 = \left|\vec{E}_{inc}\right|^2 (r^2 + |s|^2 + 2r|s|sin\Delta\varphi) \quad (1)$$

where $I_{ref} = \left|r\vec{E}_{inc}\right|^2$ denotes the intensity of the reflected electric field, $I_{sca} = \left|s\vec{E}_{inc}\right|^2$ stands for the intensity of the scattered electric field, r is the reflectivity at the substrate interface, and $s \propto \alpha$ signifies the scattering strength of the nano-object. In cases where a nanoparticle is very small, $|s|^2 \ll 2r|s|sin\Delta\varphi$ so that the iSCAT signal is dominated by the cross term in Eq. (1). It follows that the iSCAT signal encodes the traveling phase difference between the scattered and reflected electric field in φ, allowing one to determine the axial position of a nano-object. The quantity φ also includes other phase components originating from the Gouy phase and material-dependent dielectric functions.

The coherent nature of iSCAT imaging renders the resulting interferometric point-spread function (iPSF) much richer than a conventional fluorescence PSF [11]. As illustrated in Figure 1a, in reflection-

BIOGRAPHY

Michelle Küppers received her Ph.D. in 2023 from the Friedrich-Alexander-University Erlangen-Nuremberg, Germany, on the development of confocal iSCAT in the Nano-Optics Division at the Max Planck Institute for the Science of Light, Erlangen, Germany. She received her Bachelor's and Master's degrees in Physics from the Elite Graduate Program at the Friedrich-Alexander-University Erlangen-Nuremberg in 2018. She is currently a postdoctoral fellow in W.E. Moerners group at Stanford University.

David Albrecht studied biochemistry and went to ETH Zurich where he received his Ph.D. in 2016, supervised by Dr. Ewers. He continued at University College London, England, on a Marie Sklodowska-Curie fellowship to image viruses with Dr. Mercer and Dr. Henriques. David joined the group of Dr. Sandoghdar at MPL in Erlangen in 2019 to develop microscopy methods for imaging virus-host interactions.

Vahid Sandoghdar received his Ph.D. in physics from Yale University, US, in 1993. After his postdoctoral studies at the École Normale Supérieure in Paris, France, and his habilitation in Physics and leadership of the Nano-Optics group at the University of Konstanz, Germany, he was a full professor at the Swiss Federal Institute of Technology (ETH) in Zurich, Switzerland, from 2001 to 2011. Since 2011, he has held an Alexander von Humboldt Professorship at the Friedrich-Alexander University Erlangen-Nuremberg, Germany, and is the Director at the Max Planck Institute for the Science of Light, Erlangen, Germany. Vahid Sandoghdar is the founder of the Max-Planck-Zentrum für Physik und Medizin in Erlangen.

ABSTRACT

Label-free microscopy methods are highly desirable and have been a subject of study for several decades. Several approaches have been successfully employed to examine cellular and subcellular features. However, sensitivity to smaller nanostructures such as vesicles and microtubules has been elusive. By employing Interferometric Scattering (iSCAT) Microscopy in scanning confocal mode, we have now opened the door to label-free live imaging of subcellular features and organelles.

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mode C-iSCAT the interference takes place between two quasi-spherical waves. Analogous to conventional confocal microscopy, out-of-focus contributions are spatially filtered via a pinhole in the conjugate focal plane of the objective, giving access to ultra-thin optical sections. While in confocal fluorescence microscopy the pinhole is typically set to 1 Airy unit (AU) due to a finite photon budget, the high shot-noise limited signal-to-noise ratio (SNR) in iSCAT allows one to close the pinhole further, providing a better background suppression and higher spatial resolution. In order to reach the highest possible sensitivity and resolution, however, the alignment of the pinhole in the detection path requires more care than in conventional instruments.

Figure 1b-h presents a quantitative characterization of the iPSF in C-iSCAT. Here, we measured two sets of fluorescence-labeled polystyrene nanoparticles, which were immobilized on the cover glass (marked in green) and embedded in a gel volume (marked in magenta). By scanning the focal plane in the axial direction, we determined the precise location of each nanoparticle with respect to the surface. Figure 1c depicts the fluorescence of a focal plane position located slightly above the cover glass surface, and Figure 1d shows the simultaneously acquired C-iSCAT optical section. The extracted 3D PSFs are presented in Figure 1e-h. It is important to note that the axial contrast of the iPSF experiences an inversion due to the variation of the Gouy phase through the focus.

ORGANELLE IDENTIFICATION

Both light and electron microscopy techniques have been steadily improved to investigate cellular organelles and their interactions. Larger organelles such as the nucleus and mitochondria have been accessible to several label-free light microscopy methods such as phase contrast and quantitative phase imaging. Light microscopy on smaller organelles such as the microtubules, endoplasmic reticulum (ER), or lysosomes, however, has been mostly restricted to fluorescence studies. In Figure 2, we present C-iSCAT (gray) images of intracellular organelles which we identified based on simultaneously recorded confocal fluorescence (cyan) data.

The mitochondria in Figure 2a are double-membrane structures with cross-sectional areas from 0.75 μm^2 to 3 μm^2. These cellular powerhouses change their morphology through processes like membrane fusion and fission [12]. Figure 2b shows actin bundles and focal adhesions at the basal surface of a cell. The range of positive to negative contrast of individual bundles indicates variations in their axial position, information that is entirely missed by the corresponding fluorescence image. The darker confined areas at the edge of the actin bundles are focal adhesions, which form mechanical links to the extracellular environment. In Figure 2c, the endoplasmic reticulum is prominent at the cellular periphery. The reticular network is constantly remodeled and exhibits various interactions with many of the other organelles. Figure 2d shows lipid droplets, which play a role in regulating lipid metabolism and storage. These densely packed entities span sizes from approximately 20 nm to 1 μm [13], yielding substantial C-iSCAT contrasts. Figure 2e presents images of lysosomes, which are membrane-bound organelles taking the form of quasi-spherical vesicles. In addition to their role in polymer degradation, lysosomes are involved in processes such as secretion, cell signaling, energy metabolism, and other cellular functions [14]. With sizes ranging from 50 nm to 500 nm [15] and substantial protein content, lysosomes are easily detectable in C-iSCAT.

In the studies shown in Figure 2, we verified the assignment of C-iSCAT images of various organelles through specific fluorescence labeling. However, in our first study, we have shown that molecular specificity in fluorescence labeling can be substituted with computational specificity if one trains a neural network on C-iSCAT and fluorescence image pairs [8]. In the future, the generalization of this approach promises to usher in truly label-free organelle-specific investigations of subcellular dynamics and interactions.

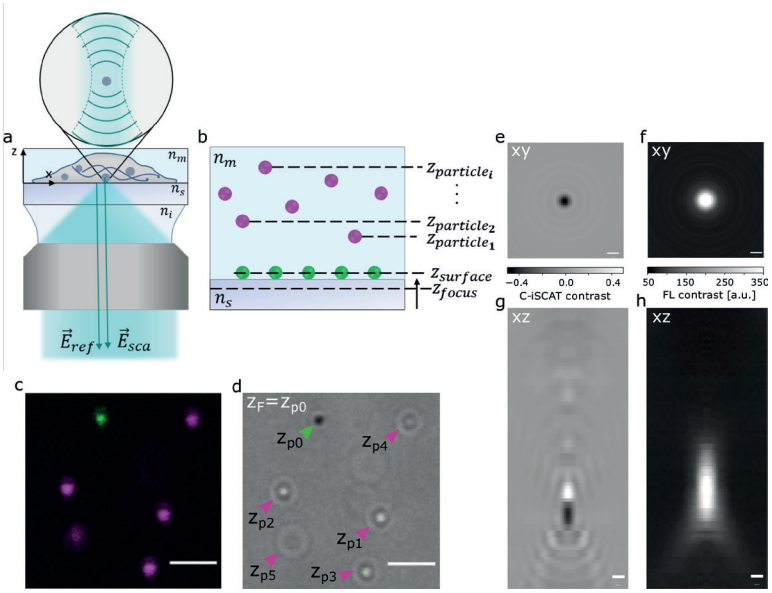

FIGURE 1: *Experimental iPSF characterization above the cover glass. (a) Schematic illustration of confocal iSCAT illumination and detection in a heterogeneous 3D sample, such as a cell. (b) Fluorescence polymer beads are located in a 3D matrix of glycergel. The focus was scanned over 3 μm in steps of 30 nm. (c) Merged confocal fluorescence image of 100 nm beads (green, 505/515 nm) and (magenta, 560/580 nm). (d) Background corrected C-iSCAT image of (c) with green and magenta arrows indicating the corresponding particle positions. Focal plane axial position is close to the surface of the cover glass. Scale bar (c, d) is 1 μm. (e) Close-up of the lateral C-iSCAT iPSF and (f) the corresponding fluorescence. (g) Axial C-iSCAT iPSF and (h) the corresponding fluorescence. Scale bars (e-h) are 200 nm.*

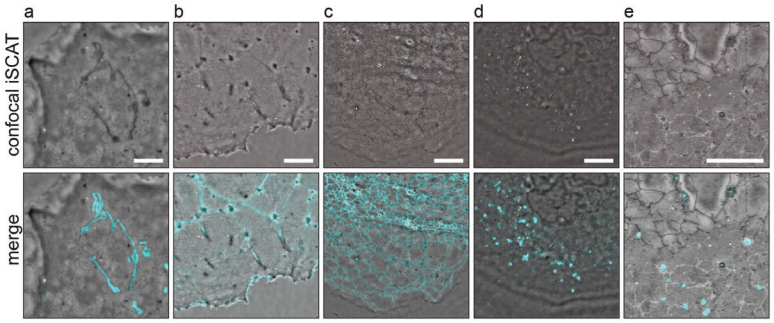

FIGURE 2: *Simultaneous confocal iSCAT and fluorescence imaging of live cells. Two rows present C-iSCAT (top) and their fluorescence-merged images (bottom) for five different organelles: (a) Mitochondria; (b) Actin filaments connected to focal adhesions on the basal membrane; (c) Intricate network of the endoplasmic reticulum; (d) Lipid droplets; (e) Lysosomes in contact with the endoplasmic reticulum. Scale bars are 2 μm.*

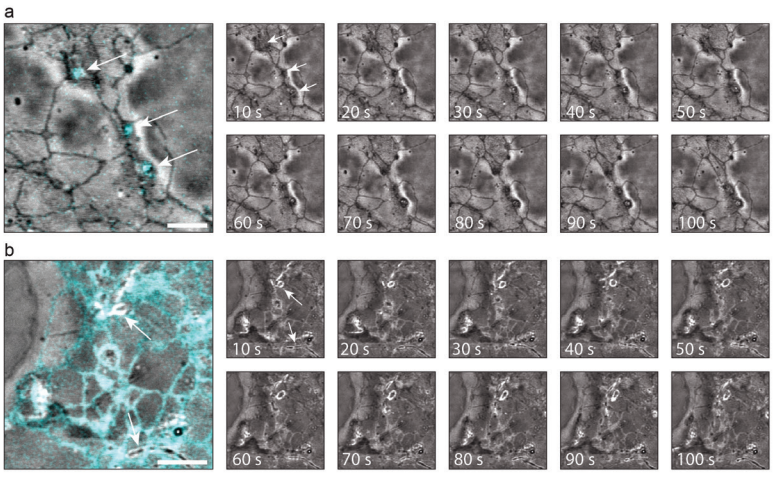

FIGURE 3: *Confocal iSCAT imaging of dynamic organelle interactions. Impartial to the contributing signals, C- iSCAT is ideally suited to address interactions at the organelle level. (a) Lysosomes (cyan) in contact with the ER move in a concerted fashion in the periphery of the cell. (b) Interaction between the ER (cyan) and mitochondria (arrows). Typically, in close proximity, the organelles appear to interact on the time scales of seconds in the sequence of images.*

DYNAMICS OF INTRACELLULAR ORGANELLES

Although C-iSCAT can be performed in a 3D volume, ultra-thin C-iSCAT optical sections combined with its unprecedented sensitivity for the detection of small nanoparticles give access to the dynamics of organelles even at a constant focal plane because the signal contains information on the composition and axial position of structures. In Figure 3, we demonstrate the advantage of confocal iSCAT by following the interactions of various organelles in a crowded cellular environment. In Figure 3a, we identify lysosomes (cyan, arrows) juxtaposed to the endoplasmic reticulum (ER). Their concerted motion over time (see panels) indicates mutual contact, critical for reshaping the reticular network [16]. Mitochondria also interact with the ER (Fig. 3b). The various biological processes regulated by these dynamic contact sites form an emerging field of research [17].

CONCLUSION

Confocal iSCAT imaging exploits information about the material, size, shape, and axial position of a nano-object. The technique presents a new powerful addition to the microscopy toolbox, can be easily implemented on existing commercial instruments, and be carried out simultaneously with fluorescence microscopy. Future works hold great promise in the combination of the C-iSCAT contrast with machine learning to achieve label-free specificity.

Article, and references available online at: analyticalscience.wiley.com/publication/microscopy-and-analysis

Analysis of Magmatic Crystal Destruction by Stereoscopic SEM

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INTRODUCTION

The mineral zircon ($ZrSiO_4$) represents an important minor constituent of plutonic rocks with granitic or granite-like composition. Beyond that, this accessory can be also found in sedimentary rocks and metamorphites that have been formed from acidic rocks by different processes of alteration [1-4]. In connexion with the research of crystal deformation (destruction) zircon plays a superior role because of its high resistance to most types of metamorphic rock transformation and processes of rock weathering. Due to this specific feature the mineral could establish as essential indicator phase for the clarification of various petrogenetic issues (e.g., change of magma chemistry during cooling, order of mineral formation during magma petrification, estimation of magma temperature) [5, 6].

Even though zircon turns out to be highly resistant to metamorphic events overprinting its host rock, the accessory may still undergo physical and/or chemical damages that may remarkably change its crystal habit [7-9]. Those host rocks coming under the influence of intense shearing (dislocation metamorphosis) commonly include zircon crystals with significant traces of destruction. The degree of crystal impairment is additionally increased if metamorphosis takes place at temperatures >500°C and pressures <0.5 GPa. The destructive effect of rock deformation on zircon is once more enhanced if the respective lithology is infiltrated by fluid phases (e.g., H2O, HF). Crystal damage resulting from high-grade and fluid-involved shearing mainly includes superficial dissolution with the formation of local »corrosion pits« and overgrowths [7-9]. Rock deformation taking place at temperatures <500°C and pressures >0.5 GPa chiefly forces crystal fracture with a significant increase of crystal fragments occurring within a given zircon population. Finally, combination of high temperatures with high pressures entails the simultaneous occurrence of dissolution and fracture which, in extreme cases, causes the complete vanishing of the crystal population (Fig. 1) [7-9].

In the present contribution main focus is set on the various types of damages which may occur on zircon crystals during a metamorphic event. For a detailed documentation of any physical and/or chemical impairments of single grains stereoscopic scanning electron microscopy was applied. This three-dimensional visualization technique turned out to be useful in the past, because it enables a very punctual study of small superficial structures.

MATERIALS AND METHODS

SCANNING ELECTRON MICROSCOPY

Zircon crystals separated from their host rocks [7-9] and pre-selected under the stereomicroscope were mounted on a specific sample holder with electrically conducting surface. After fixation of single grains the specimen was vaporized with carbon and sputtered with gold in order to obtain optimal electric conductivity. Documentation of deformation-induced zircon damage was carried out with an electron microprobe (JEOL JXA-8600) which was set up to the SEM mode. For the receipt of best possible visual results the acceleration voltage of the apparatus was adjusted to 15 kV, whereas the current of the electron beam was turned to 3 nA. Photographs of the crystals were produced by using a fully integrated digital camera system and associated computer equipment [7-9].

STEREOSCOPIC VISUALIZATION

For the production of stereoscopic photographs consisting of two semi-images the tilt or rotation technique introduced in previous studies [10, 11] was made use of. With this method the object of interest is first photographed in its initial position. After this step the sample holder is tilted or rotated by 2° to 10°, and the object is recorded again (Fig. 2). The two semi-images resulting from the two-step process and containing the three-dimensional information of the studied structure are either combined to a classical stereogram (stereopair) or overlapped to a red-cyan anaglyph by application of specific computer software (e.g., PICOLAY). For an appropriate inspection of the stereopair either stereoglasses or autostereoscopic viewing techniques have to be used, whereas inspection of the anaglyph requires the use of red-cyan glasses.

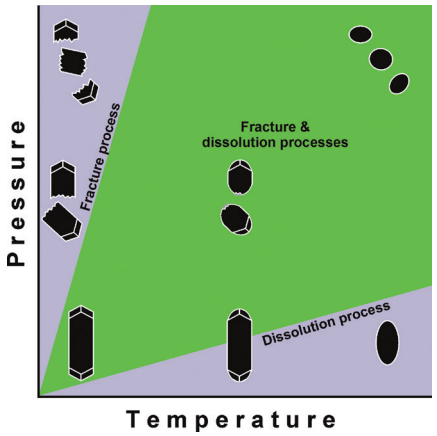


FIGURE 1 *Diagram illustrating the relationship between temperature, pressure and zircon damage taking place in the host rock. With the presence of fluid phases the dissolution process is further amplified.*

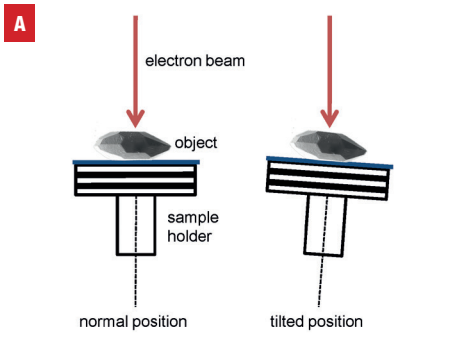
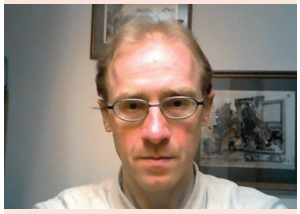


FIGURE 2 *(A) Sketch depicting the rotation (tilting) method for the production of stereoscopic images in electron microscopy. (B) Red-cyan anaglyph of a zircon crystal resulting from the rotation (tilting) method.*

RESULTS / DISCUSSION

UNDAMAGED CRYSTALS

In granitic or granite-like rocks with no or weak metamorphic overprint zircon crystals commonly adopt an ideal shape with flat faces and linear separating edges between prisms and pyramids (Fig. 3A, B). Such ideal grains are among other used for a systematic classification of the so-called zircon typology which is closely connected with the chemical composition of the host rock and the velocity of magma crystallization [7-9]. Unscathed crystals



BIOGRAPHY
Dr Robert Sturm works as author and freelance researcher at the University of Salzburg. He has a PhD in physics and is author of about 300 refereed articles in international journals. Robert looks back on 30 years of experience regarding the use of electron microscopy and particularly of BSEI systems. His scientific focus is among other set on the imaging of internal crystal structures and related growth phenomena.

ABSTRACT
Using the example of accessory zircon the phenomenon of magmatic crystal destruction with all its impressive facets is presented in more detail. For this purpose the innovative visualization technique of stereoscopic SEM is brought to application. Based upon the results of electron microscopy it can be stated that enhanced lithological pressures and moderate temperatures mainly force crystal fracture, whereas moderate pressures but higher temperatures preferably result in a continuous dissolution of the grains. The latter process is significantly amplified by the presence of fluid phases (e.g., H_2O, HF) which are produced as by-products of metamorphic mineral alterations.

ACKNOWLEDGEMENTS
Dr Hans-Peter Steyrer is thanked for comprehensive discussions with regard to the classification of destruction phenomena on magmatic crystals.

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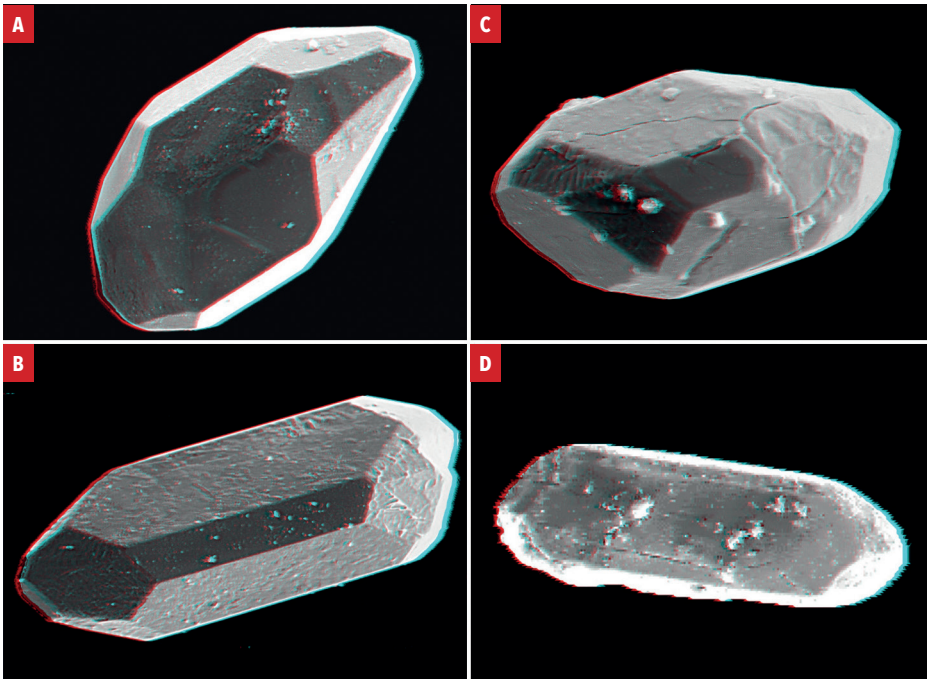


FIGURE 3 *(A) and (B) Stereoscopic images of undamaged zircon crystals with flat crystal faces and linear separating edges between prisms and pyramids. (C) and (D) Zircon grains affected by weak to moderate physical (mechanical) damage. In the concrete case crystal faces are characterized by fissures or fissure bands. The length of all crystals shown above ranges from 100 to 150 µm.*

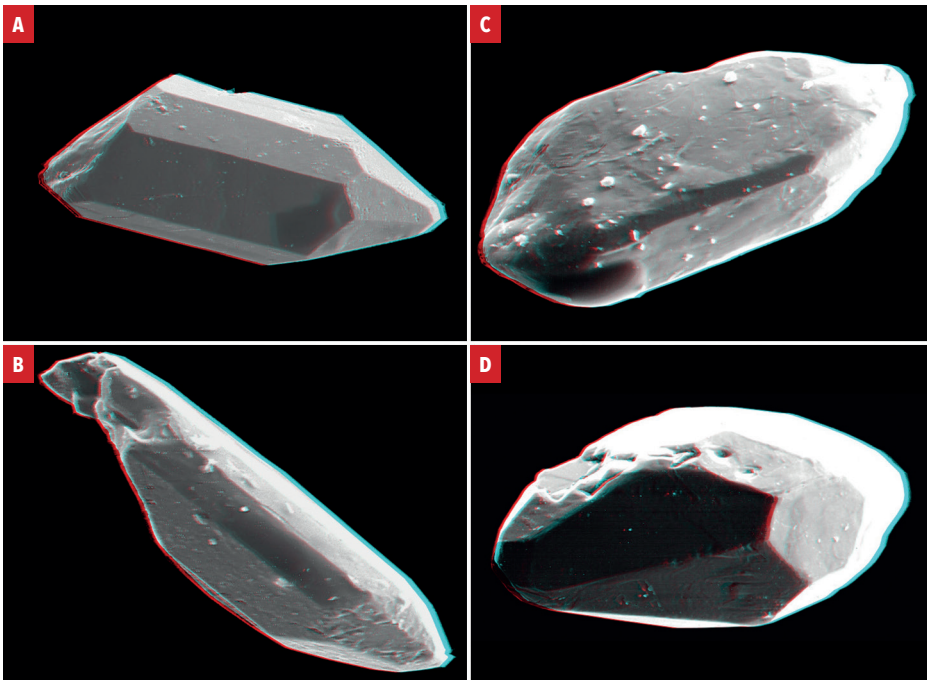


FIGURE 4 *(A) and (B) Stereoscopic images of zircon crystals marked by fractures. The process of fragmentation affected by dissolution mainly concerns elongated grains which come into the sphere of highly increased shear forces. (C) and (D) Zircon grains affected by local dissolution with the formation of corrosion (dissolution) pits and the successive rounding of edges. The length of all crystals shown above ranges from 100 to 150 µm.*

can be also subjected to intense morphological investigations, whereby lengths and widths of the grains enable additional statements on the crystallization process of the host rock.

MECHANICAL IMPACT ON ZIRCON CRYSTALS

If zircon grains run into the reach of intense pressure-induced shear forces, they commonly sustain mechanical (physical) damages of variable extent. In the case of weak or intermediate forces scattered fissures or fissure bands are formed on single crystal faces. These microstructures are often oriented parallel or in sharp angles to the principal crystallographic axis (Fig. 3C, D). With increasing pressure formation of fissures undergoes a continuous intensification which finally results in the generation of one or more fractures and the decay of a crystal into several fragments (Fig. 4A, B). The tendency of a crystal towards the formation of fractures is primarily determined by grain length. Whilst elongated crystals with reduced width have a high fracture probability, short and compact grains are mostly able to escape from the sphere of shear forces [5-9].

CHEMICAL IMPACT ON ZIRCON CRYSTALS

In the case of rock metamorphosis taking place at high temperature and moderate pressures fluid-induced processes of dissolution are frequently activated. These phenomena may result in a complete alteration of the main mineral ensemble on the one hand and a significant impairment of the accessory minerals on the other. Low fluid infiltration of the host rock may cause the formation of small dissolution or corrosion pits on single crystal faces. In addition, crystal edges experience enhanced rounding

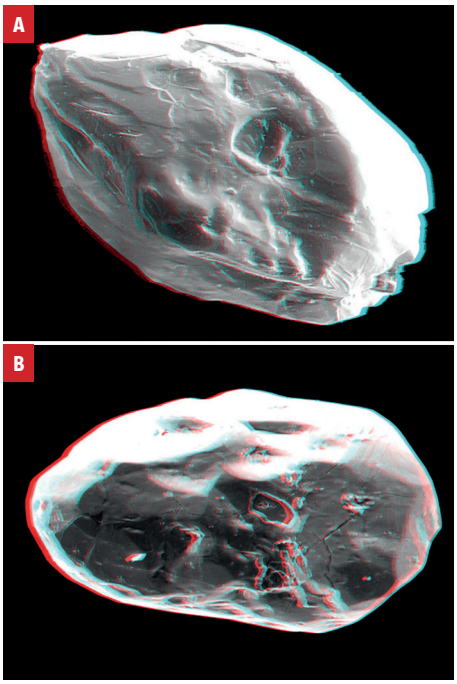


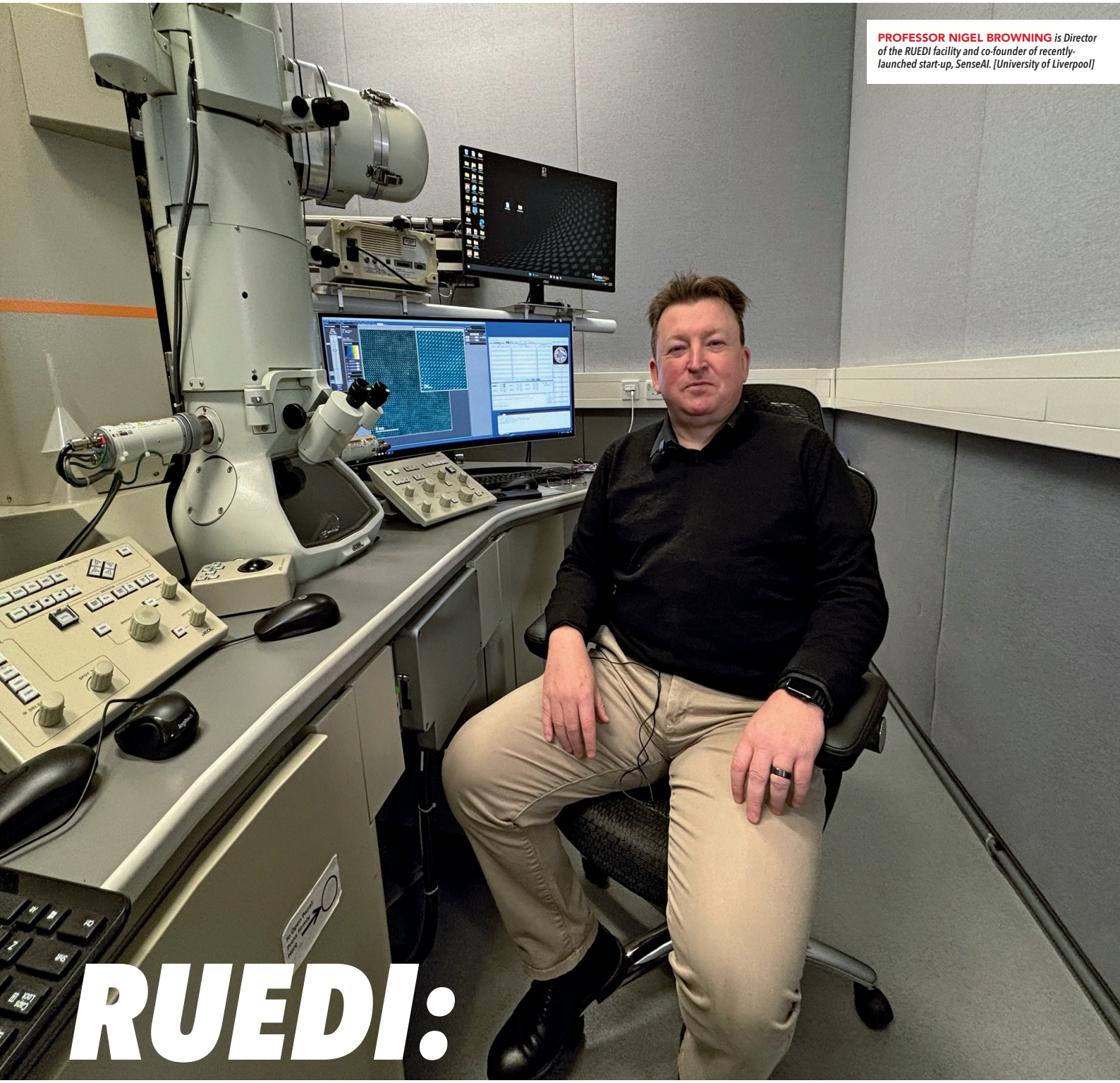
FIGURE 5 *(A) and (B) Stereoscopic images of zircon crystals from a host rock affected by high-grade deformation. In both cases a combination of physical and chemical crystal damage can be recognized, whereby the destructive process is further amplified by the presence of fluid phases. The length of all crystals shown above ranges from 100 to 150 µm.*

(Fig. 4C, D). High fluid infiltration of the host rock results in a successive intensification of dissolution processes up to the complete unrecognizability of the initial crystal shape (Fig. 5) [5-9]. Under extreme conditions of rock metamorphosis zircon may be affected by a partial or complete elimination from the mineral ensemble. This may for instance take place in the case of anatexis, where the host rock is partially transformed into a magma due to extreme temperatures. As a result of the process the old zircon population may be replaced by a new one which is formed during the further cooling of the magma [1, 2].

SUMMARY AND CONCLUSIONS

The study of zircon crystals damaged by metamorphic events represents a large field of activity for microscopists and is able to come up with partly spectacular results. Thereby, stereoscopic visualization offers a remarkable support, because damage-associated structures such as fissures, fractures or traces of dissolution can be exhibited with higher plasticity. In general, pressure-dominated rock metamorphosis may cause other patterns of damage than temperature-dominated metamorphosis. In deformation zones affected by increased pressures and temperatures, symptoms of mechanical and chemical crystal degradation are commonly superimposed with their efficiency being additionally enhanced by the presence of fluid phases. In future crystal impairment has to be subjected to further investigations in order to answer remaining open questions.

Article, and references available online at: analyticalscience.wiley.com/publication/microscopy-and-analysis



PROFESSOR NIGEL BROWNING *is Director of the RUEDI facility and co-founder of recently-launched start-up, SenseAI. [University of Liverpool]*

RUEDI: Extreme electron microscopy

The world's 'most powerful' microscope, is about to be built in the UK. Rebecca Pool talks to Professor Nigel Browning to find out how the Relativistic Ultrafast Electron Diffraction and Imaging facility is set to change science.

In 2027, a £125 million national user facility, that will use relativistic bunches of electrons to image biological and chemical reactions to the atomic scale and in real-time at the femtosecond time-scale, will begin to be built. Designed to be the world's most powerful electron microscope and the fastest electron diffraction instrument, RUEDI - Relativistic Ultrafast Electron Diffraction and Imaging - is claimed to be unique in the UK and the rest of the world.

"Nobody else in the world has tried to make an imaging system that can go to these kinds of temporal and spatial resolutions," highlights Professor Nigel Browning, Director of the RUEDI facility and Chair of Electron Microscopy in the School of Engineering at the University of Liverpool, UK. "It's taken a significant amount of work over the last five to six years, to get to this point, and the technology is still moving forward – the specification we have right now is what the technology can do today... this is likely to get even better in terms of the combined spatial and temporal resolution that we'll get of of the system."

The system is set to use a high energy, 4 MeV RF source to generate its relativistic electron bunches - groups of electrons that travel at velocities close to the speed of light, that will then be directed through diffraction and imaging beamlines. Using high energy electron bunches instead of lower energy electrons provides key advantages; the electrons can penetrate greater depths, meaning thicker samples can be studied, while the coherence and minimal space-charge effects of electron bunches contributes to high spatial resolution.

RUEDI's electron bunches will contain up to 10^7 electrons, with the length of each bunch being either 10 or 100 femtoseconds – the 10 fs length will allow the system to resolve structural changes on the timescale of biochemical processes. And alongside its electron bunches, RUEDI will also have advanced detection systems and integrated AI, delivering single-shot imaging and diffraction, and stroboscopic pump-probe methods, essential for capturing fast, transient processes. For example, biologists will be able to study interactions within a cell at molecular resolution as they happen, opening the door to the discovery of new medicines. Materials scientists will also be able to scrutinise how structural changes in materials evolve through time-resolved experiments, rather than relying on static studies.

"RUEDI is going to have such an impact on major areas of science that are important to society, including medical research and climate change research," says Browning. "The combination of spatial and temporal resolution in diffraction or imaging will provide new insights into complex dynamic phenomena, and allow us to develop new technologies from these results."

One case in point is batteries. With RUEDI, electron diffraction will be fast, but the system will also provide the necessary spatial resolution to image irreversible processes. "You might want to see a reaction at an interface – say, how a catalyst works in a particular environment," says Browning. "For energy storage, transfer and conversion, electrons are being moved around a device on the ultrafast timescale – with RUEDI, we can actually look inside these devices instead of cutting them up to get results."

Indeed, as part of catalysis and electrochemistry studies, the system could be used to directly observe the molecular origins of the all-important electrical double layer in electrochemical systems. This structure forms at the interface between a conducting electrode and an electrolyte solution and is critical to determining battery, sensor or capacitor performance, and developing efficient energy storage.

At the same time, experiments could also help determine fundamental mechanisms in catalysis, opening the door to new catalysts and photocatalysts for renewable energy sources. The interplay between phonon and electron oscillations will also be directly observable and controllable, allowing RUEDI to probe new concepts in plasmonics and strongly coupled systems for electronic devices and sensors, which could transform the use of quantum materials in nanotechnology.

Experiments at the frontiers of spatial and temporal resolution using in-situ cells are also set to deliver new insights to how materials and devices perform under extremes of temperature, pressure, field and environment. And from a biosciences perspective, RUEDI's high electron voltages mean researchers can study thicker, whole cells instead of purifying proteins to study dynamics. "We can start thinking about drug interactions in an entire cell, and [speed up] drug development and personalised medicine," says Browning.

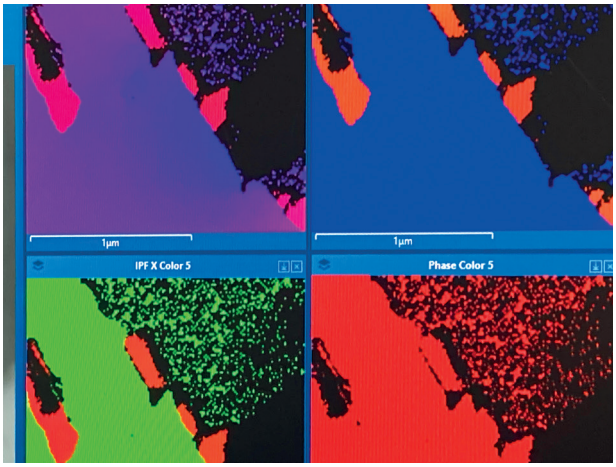
Critically, for the UK, Browning also reckons that right now, RUEDI is the only system of it kind currently being built in the world. "Researchers in the UK could get the first experimental results [in such fields], leading to new IP in many applications," he says.

AS NIGEL BROWNING *says, RUEDI allows the evolution of structural changes in materials to be observed and determined through time-resolved experiments, rather than by static structure. [Liverpool University]*

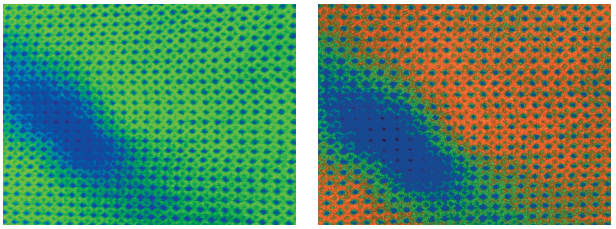
What makes RUEDI unique?

Many MeV-ultrafast electron diffraction facilities, employing high-energy electron beams to investigate ultrafast dynamics in materials and processes at atomic and molecular levels, exist around the world. Key examples in the US include the SLAC National Accelerator Laboratory, Brookhaven National Laboratory's Accelerator Test Facility and the University of California Los Angeles Pegasus Laboratory. Other facilities include the Korea Atomic Energy Research Institute, Shanghai Tech University, the accelerator Lab at Tsinghua University - all in Asia - and the European XFEL, the Laboratory for Ultrafast Electron and X-ray Science (LUXS) at EPFL and Deutsches Elektronen-Synchrotron (DESY), in Europe.

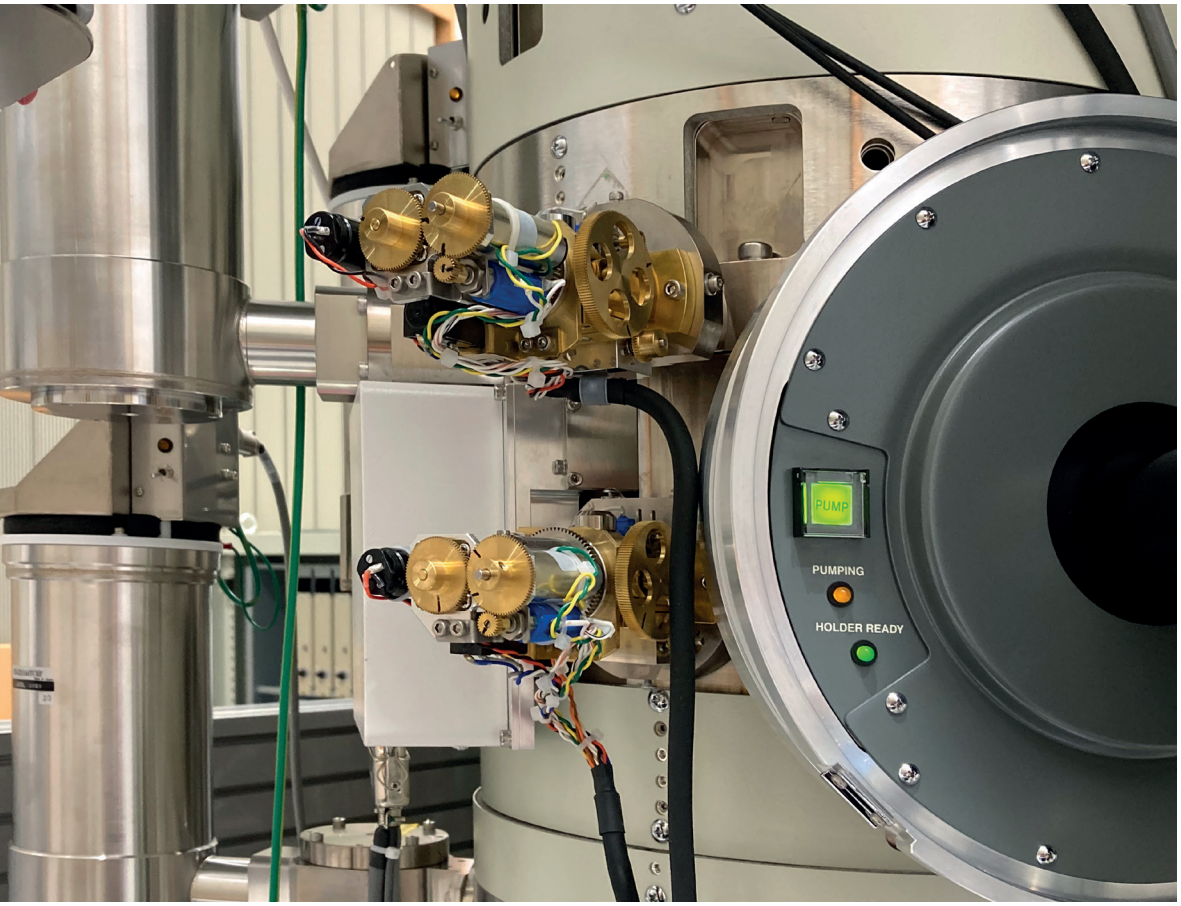
But what will set RUEDI apart from these existing systems? As RUEDI partners have highlighted, the facility will combine MeV-ultrafast electron diffraction and MeV-ultrafast electron microscopy, and image dynamics with single electron precision. Molecular-scale dynamics will be investigated in-vivo, in-site and under operando conditions, and artificial intelligence will be integrated for low-signal image analytics.

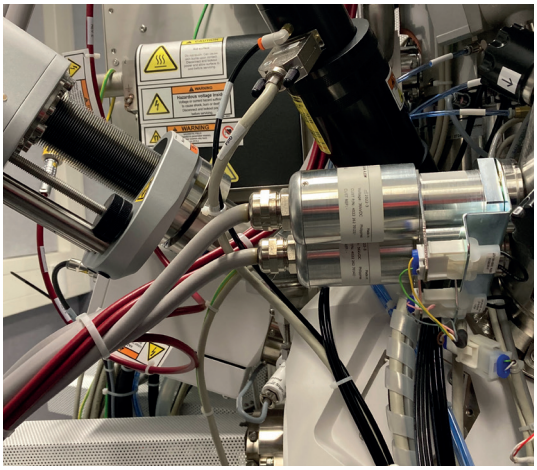


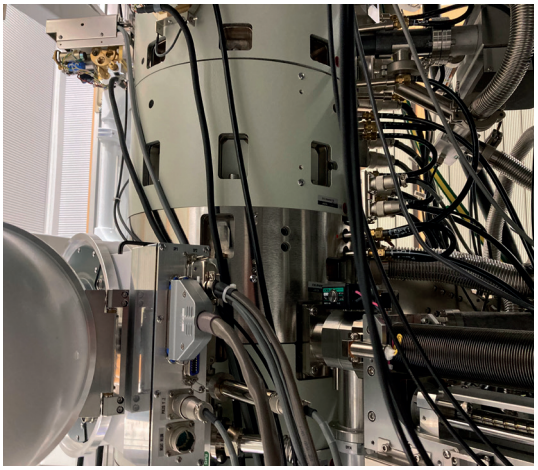
EBSD MAPS *of Aluminium Foil. [Browning]*



HIGH RESOLUTION *STEM images of Strontium Titanate crystal [Browning]*







BUILDING RUEDI

So what happens now? Once the business plan is finalised and construction commences, component development and integration will take place. Higher electron voltages not only deliver faster time-resolutions but also simplify stage design. Browning highlights how, at electron voltages greater than 1 MeV, the pole piece gap in the electron microscope is around 1 cm, accommodating thicker samples and easing in-situ stage design and integration.

"We're going to use a lot of existing microscopy technologies, but rather than scale these down, we'll scale them up so we can look at much larger samples," he says. "We'll be using a different type of gun and will need to integrate this with the sets of lenses, stages and then detectors at the back-end – this is going to be a hardware opportunity."

Crucially, from the outset, RUEDI will use artificial intelligence in myriad ways, including maximising the effectiveness of data analytics. "We're going to build artificial intelligence into all parts of the facility - this is a first," highlights Browning.

As part of his research at Liverpool and recently-launched start-up, SenseAI, Browning has shown that AI can be used to generate high-quality images with less data. "It's still a challenge to see how many electrons you can get into a short pulse, but by using artificial intelligence to help reconstruct images, we can get away with fewer electrons – this also makes it easier to optimize our hardware for higher resolutions," he says.

AI will also be used to help to develop a digital twin - virtual copy - of RUEDI that will support facility users. Browning and his SenseAI colleagues are currently developing a digital twin of a STEM, in which components are simulated in a 3D environment and the system simulates real-time hardware behaviour and sensor performance. This twin is set to be up-and-running in the next year, and the more complex RUEDI twin will follow.

As Browning explains, the terminology of the twins is the same, but the RUEDI digital twin is more complex due to its relativistic electron bunches and complex electron to electron interactions. Still, he is confident that given today's rapid computational and algorithm developments, the RUEDI virtual copy will be ready at around the same time as its hardware is fully-developed.

ELECTRON MICROSCOPY
in Professor Nigel Browning's lab at the University of Liverpool. [University of Liverpool]

With the RUEDI digital twin in place, potential facility users can then gather the data they need from the digital twin to optimise later experiments on the actual RUEDI system. "This all fits in with the sustainability of the project – fewer researchers will use the hardware if they can test it on a digital twin – and we'll have less people taking trips to Daresbury to use [RUEDI]," says Browning.

"It makes a difference when you're building an imaging systems and you know you're going to use artificial intelligence," he adds. "We can look at much slower signal levels, we can use data science to help us identify RUEDI users and the digital twin will help design the experiments for RUEDI."

UNITING FACILITIES

RUEDI is being built in collaboration with the Rosalind Franklin Institute at Harwell, and will be sited at the Science and Technology Facilities Council's (STFC) Daresbury Laboratory to exploit the infrastructure already here for other similar-sized electron accelerators. Key facilities include the Accelerator Science and Technology Centre (ASTeC), dedicated to research in charged particle accelerators, the Versatile Electron Linear Accelerator (VELA) for research in accelerator science, including applications requiring high-quality electron beams, and ALICE (Accelerators and Lasers in Combined Experiments), which combines accelerators and lasers for innovative experiments. The cutting-edge computational, data analytics and AI facility, the Hartree Centre, is also at Daresbury, which according to Browning, will be instrumental to helping to handle the huge datasets that the RUEDI will inevitably generate.

"We don't reproduce everything from scratch with a new facility, but find an integrated program to deal with the, say, the data," he says. "RUEDI isn't a separate infrastructure, and this has been implicit in its design... [The facility] will coordinate with Diamond Light Source, neutron facilities – so we're not trying to do the same experiments at the same time but are working together to do the best experiments with the capabilities that each facility has."

Once up and running, the first experiments will likely centre around energy storage and drug interactions with cells. For his part, Browning is excited to find out exactly what they can explore with RUEDI, given that researchers will be able to see individual atom movements in extended functional materials, biological cells and mechanical deformations, and glean the data they need to design next-generation devices.

"We literally can't see these [atom movements] at the moment, but we will with RUEDI," he says. "I develop microscopes so am interested in finding out where are the information limits in physical science – and it is the images that follow, that excite me."

Moving on, but not forgetting

Four years after Covid, Chris Parmenter explores whether we should reexamine how we meet for the benefit of everyone and future generations.

Think back four years to July 2020. We were facing a once in a lifetime global pandemic that has changed the way we live our lives forever. As scientists and engineers, we did what we do best, we innovated and applied our skills to solve many issues in daily life to carry on as best we could to live the 'new normal'. Technology was at the heart of so many of these changes, and the world experienced a change in how we communicated. Whether it was MS Teams, Zoom or other platforms we used them to share information and collaborate at a distance, when 'in person' was no longer an option.

I was impressed at how despite the cancellation of scientific meetings in person in March and April of 2020, by May meeting organisers and attendees had adapted to online presentations and forums. By the summer of 2020 the Microscopy Society of America's annual meeting (M&M) had adopted a platform that hosted most of the talks that had been pre-recorded and were available on demand. They even had poster presenters narrate their posters for the attendees, all of whom they would never meet face to face. I know of many smaller meetings that used Zoom or other platforms to host their gatherings and who were successful in bringing in new attendees, who were eager to participate and only had to travel to their computer to be connected. Some of these may never have attended if travelling to a venue would not have been practical.

THE ETERNAL PROBLEM, OF TOO MUCH

In the design of a globally engaging congress there are many challenges. Reflecting the diversity of fields of study and applications that microscopy lends itself to naturally means that there will be a lot of parallel sessions. There are generally no shortage of people wanting to contribute both oral and poster presentations, as well as invited speakers for each session. The volume of talks and sessions means that there will always be clashes, leading to frustrations and the inevitable inability to be in multiple sessions simultaneously. I often joke that I need a clone at such meetings, but perhaps what can be a frustrating problem could be solved by technology.

During the enforced lockdowns and online only meetings, this age-old problem had been addressed as we learned how to present at a scientific conference without being in the same room. One of the huge benefits of this was access to presentations that could be viewed after they had been given (either live or pre-recorded), as well as the technology that allowed speakers who couldn't be there in person to present and take questions, which was a decent substitute in many cases.

I've found events such as training courses organised fully online or at least partly online (hybrid). They offer the participants flexibility in their day and reduce concerns about travelling. This balances the benefits of 'in person' meetings such as a more familiar 'getting to know you' session. This can be followed by sharing of online content while enabling small group discussions remotely which can be

achieved via breakout rooms. Let's not forget that at many universities around the world other content is being shared and online assessments that are still happening. Remote medical assessment by physicians and engineers are often still performed, via connected instruments and interactive services. In the field of microscopy this is particularly true, and I can recall training and troubleshooting happening remotely during lockdown. Some of these approaches have been retained as customers and staff realised their benefits.

BACK TO NORMAL?

As we slowly emerged from the Covid bubble and life got back to 'normal' some of the 'new normal' stuck around. This ranged from online shopping for anything and everything and online grocery orders. Having discovered the convenience of it all many have not switched back. The use of on demand content was increasingly popular pre-Covid, but the enforced lockdowns and convenience of streaming services mean that now many people are abandoning viewing in real-time altogether. The workplace was changed with homeworking now embedded in some companies as a functioning concept, albeit not for all job roles.

I suppose the question I'm really asking it 'Can this apply to larger meetings and conferences?'

Of course, it was inevitable that once countries fully lifted travel restrictions and in person events were permitted that we would come back together, why wouldn't we? I can understand the tendency to revert to the former way of doing things. It is more familiar and one could argue the way that human being like to interact, we also know how things work and how to organise them – but we did also learn a lot during the pandemic. I'm sure you all know the thill of knowing you have a few days away from your normal place of work to meet friends, exchange ideas and see what others are doing. I've written a number of meeting reports in 2022 and 2023 where I reported the mood of the meetings was jubilant and celebratory that attendees could meet again, exchange handshakes and interact freely.

Since we've returned to in person meetings, we seem to have dropped the option of remote presentation, recording of live content or pre-recorded content - rather reverting to in-person only. I have occasionally been to a meeting where a speaker was unable to attend and gave the talk remotely, but in other meetings, the audience was informed that that the talk was cancelled, with no such offer or embracing the remote delivery option. I can't help but think that this is a great loss where the technology does exist.

Travel has always been expensive, however, for those who have been back to in person events since the pandemic you'll know that prices are higher than ever and travel routes are still restricted. The reduction in number of flights and certain routes means that international travel can be prohibitively expensive. For example, the cost of an economy flight is roughly what a premium economy flight would have been in 2019. Hotel costs are higher and so the question of traveling and staying somewhere is not a trivial one. With this in mind, the days of taking a small group on a conference trip may be numbered, and so remote participation options would be of benefit. This is particularly important so that students don't miss out on the experience of presenting a talk or poster.

NOT JUST FOR SHARING

Besides the enduring technological legacy of Covid there was a new appreciation of other concerns that colleagues may be experiencing. Many of us became more sensitive to issues such as taking care of coworkers' (and our own) mental health. Flexible work schedules and colleagues with caring responsibilities working a non-traditional work pattern became normal.

While travel was not a concern, those with limited travel funds benefitted from no longer being excluded. This applied equally to those who found it hard to travel (such as those with physical disabilities or those with caring responsibilities) meaning they could be involved as fully as possible. Many of these changes had the effect of widening participation for as many as possible to attend conferences.

Another aspect that we became aware of was that while nobody travelled our carbon footprints were lowered. For those concerned with the environmental impact of travel to conferences and meetings there was a realisation that it was possible to participate without taking a flight, train or bus to get there. As global warming continues to be an issue, it would be good to see the remote options being embraced again, so that people have a choice to participate without the need to travel.

IN PERSON BENEFITS

As much as I have advocated for not forgetting the on demand and hybrid aspects of conferencing, I am also aware that people like meeting in person - I certainly do. I've definitely heard people say they are less than enthusiastic about expanding online participation. One big attraction is the chance to have demonstrations of equipment, and some exhibitors do still bring microscopes to the show. Others have opted for remote demonstration or a virtual reality / augmented reality approach, while others have booths where they present the latest workflows in private cinema style booths. A number of these options could clearly be adapted to suit hybrid participants.

In contrast to the scientific part of a conference which we know can be done remotely or hybrid, the exhibition hall is a different story. How to present the booth and the feel of the experience of interacting with the company representatives presents some issues. In 2020 and 2021 the large international meetings had exhibitors available via the chosen meeting platform to the online attendees. Clearly there was no real choice, but in many cases the materials that were presented were far from adequate and I'm sure both attendees and exhibitors were longing to get back to 'in-person'. That being said, I think that if the exhibitors were able to dedicate resources to doing a hybrid style presentation I really think it could work well.

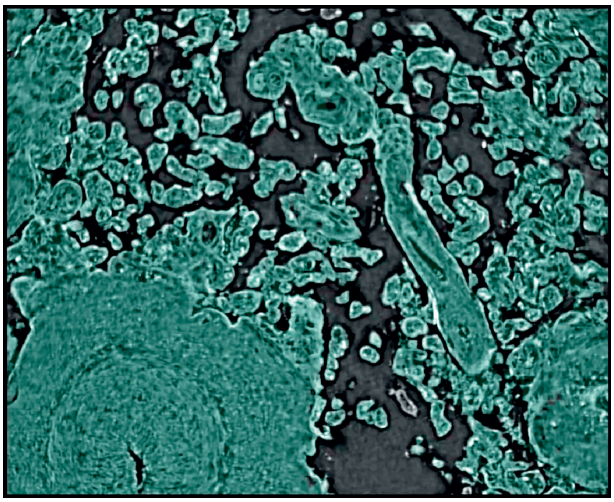
Perhaps a significant concern about increasing the online participation comes from the exhibitors, who have to pay a lot to have a stand at the exhibition to present their latest technologies. I know they enjoy engaging in demonstrations and discussions and this is undoubtedly easier in person. I understand that more online content and virtual attendance carries the potential for a reduction in footfall. The same concern applies to conference organisers, event budgets are calculated on predicted attendance and a reduced attendance could have a severe impact on the running of the event.

THE FUTURE

I would suggest that in the majority of cases people will decide with their feet whether it is worth attending in person for the social and networking aspects of a conference that are hard to replicate online. In either case, the scientific presentation of content shouldn't suffer at the expense of the in-person aspects – we just need to find a balance and a way for everyone to feel they're getting the ability to participate. Options include access to the presented content that could be viewed for a certain period after the event. I'm not claiming to have all the answers here and I agree that where remote options and recorded content is available there is a risk that people choose to stay away from in person attendance, but I'm urging future events organisers to explore this issue more fully. Options do exist and we need to embrace them, for those organisers that do there is much to gain for so many people.

vEM Technology Forum: gathering the community to tackle tech issues

Volume electron microscopy (vEM) is a remarkable group of techniques used to image the structure of cells and tissues through continuous depths of at least 1 micrometer. But while vEM is fast-becoming THE tool to understand biological complexity across scales, myriad technical issues still exist. With this in mind, the vEM Technology Forum, taking place from 21 to 24 October at the UK-based Rosalind Franklin Institute (Franklin), will explore the issues and also build the community. Technical presentations, posters, panel discussions, as well as hardware and software demo lounges will all feature at the conference. And topics to be covered include multimodal and/or correlative imaging, AI and volume EM, microscopy software and hardware, new workflows for microscopy or data analysis and translational vEM – and more. Michele Darrow, Franklin's Head of Data Strategy for Cryo Electron Imaging, tells Rebecca Pool what attendees can expect.



HUMAN PLACENTA *imaged using X-ray microCT with segmentation of intervillous space overlayed in light green. [Rosalind Franklin Institute]*

Why launch the vEM Technology Forum now?

The techniques for volume electron microscopy (vEM) are decades old but in the last five years we've seen our vEM community really come together. Really exciting things have come out of this, including the Volume Electron Microscopy Gordon Research Conference (GRC), which [launched] last Summer and will now take place every two years. As well as this, we wanted to have an event in the alternate years that focused on the technology and brought in industry partners and early-career researchers – we really want to make sure we're bringing the whole community together. Luckily, the Chan Zuckerberg Institute (CZI) has had a funding call for community building. Myself, Kirk Czymmek [Donald Danforth Plant Science Center], Paul Verkade [University of Bristol] and co-investigators put together a grant proposal [Enabling Volume Electron Microscopy: Building a Global Community and Resources] and thanks to the resources we've received associated with the grant, we've been able to go to industry partners and say, 'look, we have this idea - will you support us?' Our sponsors are now ThermoFisher Scientific, JEOL, Zeiss, Leica, Oxford Instruments, Delmic, Tescan, Sirius XT, ConnectomX, Webknossos, Microscopy Innovations and Quantifoil.

MEMBERS *of the team that has organised vEM Technology Forum. [Rosalind Franklin Institute]*

What are the key vEM issues that you hope to address with the conference?

I surveyed our organising committees... and three things came up over and over and over again. First was facility management - interacting with facilities, either as a user or as a manager of the facility. Second was data struggles - handling and processing your data, and deriving meaning from your data. And then there was sample preparation challenges - bringing consistency to sample preparation and understanding what each step is actually doing. We're focusing on these in our 'Challenge Panel Discussions' and with these, hope to create a space where people can really shout about the things they struggle with. At the vEM Tech Forum, we're going to have rooms full of software developers, hardware developers, academics and industry [partners] – I think it will be really helpful for everyone to hear what people still struggle with, because that can help to focus the community on those areas.

Why bring in early-career researchers?

We want to encourage lots of early career researchers to present at the conference. From a personal perspective, I've found it frustrating hearing a keynote talk about the research that's taken place in their lab – three years of a postdoc's life is essentially on one slide in a

pretty picture, and you don't always understand the details. So what I really want is to be able to dig into pictures and understand what went into them - and I think for that you have to hear from the researcher who's actually doing the work. So, it's a little risky but we've chosen not to have keynote speakers – everyone will talk for around fifteen minutes so there's enough time for everyone to speak.

Who will attend the conference?
We have space for 150 people. The vEM community is very broad and diverse – we have five different techniques and a collection of associated techniques - but we have enough commonalities to all come together. We also want to include the industry partners, the use cases and the end users who maybe aren't experts in the techniques but want to use the science to benefit their research. We're trying to ensure that everyone has a place in the room as that's the best way to have these broad conversations and come up with solutions.

Where in the world will people come from?
Many conferences tend to bounce back and forth between Europe and America, which is kind of rough for people in the Asia Pacific region. So we're committed to having our conference bouncing between a triangle of the Americas, Europe and Asia Pacific. We have Gregory Kitten from Universidade Federal de Minas Gerais in Brazil on our scientific organizing committee and we've also been reaching out to people in South Africa who have a volume-scope. There's also a pretty big vEM community in Australia and South Asia. We're hoping that people from these groups will join us - we really want to bring people in from 'the rest of the world', so we're providing travel bursaries for those travelling a farther distance – and we're trying to advertise in the parts of the world that don't normally see advertisements for such conferences.

What are you looking forward to most at the conference?
I'm a very hands-on learner, so I'm looking forward to our demo lounges, which will give people the opportunity to demonstrate their software and their hardware. Many industry partners are going to have a demo lounge but we're also looking forward to welcoming open source developers – they will get to sit alongside industry partners and have just as much exposure of their software and tools. I'm also excited about the social aspects of our conference - I think it's going to be a lot of fun.

VEM TECHNOLOGY FORUM *basic info, including logo (A), Rosalind Franklin Institute (B), sponsors (C), and conference QR code (D). [Rosalind Franklin Institute]*

What are your hopes for vEM Technology Forum?
I will consider our inaugural conference a success, if out of it comes a discussion that leads to new software or a tool or product that addresses a challenge. I think bringing everyone into one room to have these conversations is going to spark opportunities - for whoever happens to be present. I also hope that the conference continues to exist in the opposite years to the Volume Electron Microscopy Gordon Research Conference – and for this, we need community support. We're entirely funded by our industry partners, and will need their willingness to continue that funding. Our goal is to have vEM Technology Forum as a long-term resource for our community.

Early Bird Registration closes on Wednesday 31st July. Find out more: https://www.rfi.ac.uk/volume-em-technology-forum/

HUNTINGTON
Convention Center of Cleveland. [Tim Evanson]

Getting ready for M&M in Cleveland

The Microscopy and Microanalysis - M&M - conference from the Microscopy Society of America and the Microanalysis Society, is running from Jul 28 - Aug 1, 2024, and as always, is set to be the largest scientific meeting and gathering of microscopy and microanalysis professionals, academics, technicians, students and exhibitors in the world. Located in the Huntington Convention Center of Cleveland, Ohio, this year, M&M provides a forum for the presentation and discussion of a wide range of microscopy and microanalysis techniques and their application to the biological and physical sciences.

Plenary lectures by Professor Edward Boyden from the Massachusetts Institute of Technology and Dr Wren Carr from the National Ignition Facility at Lawrence Livermore National Laboratory will start off the Microscopy & Microanalysis 2024 event. The program will highlight the latest techniques, methodologies and findings spanning nano-to-macroscopic scales and advances in fields such as nanotechnology, biological and clinical sciences, materials science, 3D manufacturing, and metallurgy. Organizers have assembled 11 symposia in the physical sciences, 11 in analytical sciences, 9 on biological sciences, as well as 7 cross-cutting symposia. Sessions will look at atom probe tomography, cultural heritage studies, correlative analysis, quantitative label-free imaging, volume electron microscopy, magnetic imaging, emerging electron microscopy methods to understand quantum materials, frontiers in electron tomography, diversity, facilities management and very much more.

A Memorial Symposium for Lena Fitting Kourkoutis - who made foundational contributions to advanced and high-resolution electron microscopy - will also highlight topics impacted by her work, and pay tribute to the far-reaching impacts of her research and community leadership. Invited speakers include David Muller and Judy Cha, Cornell University, Ondrej Krivanek, Nion, Peter Ercius, Lawrence Berkeley National Laboratory, Max Haider, CEOS, Wolfgang Baumeister, Max Planck Institute of Biochemistry, Peter Crozier, Arizona State University and many more world-leading scientists.

Short courses include 'Guidelines for Performing 4D-STEM Characterization from the Atomic to Micrometer Scales: Experimental Considerations, Data Analysis', 'Cryo-EM for Materials Sciences: Hardware, Applications, and Data Acquisition', and 'Transmission Electron Microscopy and Spectroscopy from First Principles'. Other courses also include 'Automated Experiments in Electron Microscopy', 'From Obscure to Clear: A Dive into Tissue Clearing and Expansion Microscopy' and 'Automation for the Microscopy Workflow - Serial Sectioning of Materials at the Meso-scale'.

Meeting highlights

A round-up of the conferences, meetings and courses taking place later this year.

JULY 28 TO AUGUST 1:
MICROSCOPY & MICROANALYSIS 2024

Celebrating and showcasing the latest advancements in microscopy and microanalysis, (see 'Getting ready for M&M in Cleveland').

25 TO 30 AUGUST:
17TH EUROPEAN MICROSCOPY CONGRESS EMC2024

Following the postponement of an in-person EMC2020 due to the pandemic, EMC2024 will be held at the Bella Center in Copenhagen, Denmark. The conference program includes microscopy in both physical and life sciences, and microscopists, manufacturers, and suppliers will come together to share new techniques, applications, and technology.

1 TO 13 SEPTEMBER:
SCHOOL ON NANO-OPTICS WITH FREE ELECTRONS EBEAM 2024

Taking place in Aussois, France, the meeting aims to spread knowledge about electron spectroscopies for nano-optics. Courses will cover the basics of electron instrumentation and spectroscopies, electron-matter-light interaction, electron spectroscopies of optical material as well as time, space, and quantum coherence in electron spectroscopy, advanced EELS, CL and PINEM and photoemission. EBeam 2024 is aimed at PhDs, Post Docs and any researchers willing to dive in this new field.

2 TO 6 SEPTEMBER:
RMS FLOW CYTOMETRY COURSE

Held at the University of York, UK, the course is aimed at both clinical applications and applications in cell biology, with the common fundamentals covered on Day 1 and 2. The course then splits into clinical applications and applications in cell biology streams, from practical demonstrations to lectures highlighting not just the applications, but best practise as well.

9 TO 13 SEPTEMBER:
CONFERENCE ON ELECTRON MICROSCOPY OF NANOSTRUCTURES ELMINA2024

ELMINA2024 will be focused on electron microscopy methods applied to nanoscience and nanotechnology (physics, chemistry, physical metallurgy, materials science, earth and life sciences). Taking place in Belgrade, Serbia, the conference will highlight recent progress in instrumentation, imaging and data analysis, large data set handling, as well as time and environment dependent processes.

24 TO 25 OCTOBER:
RMS FACILITY MANAGEMENT TRAINING COURSE 2024

This is an intensive in-person course equipped to give attendees the basic knowledge to run a core facility effectively. There will be tips and tricks throughout from experienced experts in the area. It is geared towards all levels, from those experienced in running a core lab to those looking to move in to this field as a career. This course will take place at the University of York, UK.

11 TO 12 NOVEMBER:
RMS FRONTIERS IN BIOIMAGING 2024

Set in Oxford, Frontiers in Bioimaging 2024 will focus on the latest developments in optical and electron microscopy as well as image analysis. Sessions will cover novel technical developments and applications of these microscopy-based approaches to key cell and molecular biology questions with an overarching aim to bring insights on how they participate in our understanding of human health and disease.

Electron dose plummets with turboTEM's Tempo

An event-responsive imaging approach, 'Tempo', from Trinity College Dublin spin-out, turboTEM, is available from JEOL subsidiary, Integrated Dynamic Electron Solution (IDES), developer of high-speed electrostatic beam blanking and deflection, and other technologies, for ultrafast TEM.

Tempo is designed to increase the amount of information obtained for a given electron dose during STEM experiments. While STEM imaging accumulates electrons scattered across different detectors by waiting, Tempo, instead, measures the time needed for a set number of electron events to output the rate of electron hitting the detector.

When Tempo is combined with IDES's beam blanking system - the Electrostatic Dose Modulator (EDM) - on a STEM, users can dynamically modulate the number of electrons delivered per pixel as data is collected from a sample. Once enough electrons have been counted to accurately characterise a pixel, the blanking system can then prevent excess damage from further beam exposure. So, in this dose-optimised set-up, pixel intensity is defined by the time taken to detect a fixed number of electrons, rather than the number of electrons in a fixed time.

"The irradiation savings with Tempo are massive," highlights Dr Lewys Jones, CEO and co-founder of turboTEM, and principal investigator of the Ultramicroscopy group at TCD. "We can save more than 95% of the beam-dose [on biological tissues] whilst retaining a brightfield-like contrast."

THE TURBOTEM *team from left: Dr Jonathan Peters, CTO and Co-founder, Dr Lewys Jones, CEO and Co-founder, Fletcher Thompson, COO and Co-founder, and Germano Motta from TCD, who works on early stage R&D. [Lewys Jones]*

Jones has patented the technology with Brian Reed, CTO of IDES, and Tempo is available on JEOL systems. "We believe this is a very important development for the imaging community," says Jones. "It's retrofittable to existing microscopes that have the [IDES] shutter... it's also very simple and elegant in its implementation, and will change how imaging takes place."

turboTEM also manufactures the 'Pulse' signal digitiser, a modular retrofittable hardware upgrade to increase the functionality and performance of old STEM detectors, and the User Adjustable Pole-piece that offers a user-selectable pole-gap from 1.5mm to 6.5mm.

Zeiss to open new optics unit

Zeiss is to establish a strategic business unit, effective 1 October 2024. So-called Zeiss Photonics & Optics will include existing businesses outside of the Zeiss segments, which have growth potential.

The units of the Consumer Products strategic business unit from the Consumer Markets segment will move to Zeiss Photonics & Optics, including Cinematography, Mobile Imaging, Photo, and the optics business for hunting and nature observation. Additionally, Microoptics, Spectroscopy, Planetariums, and Simulation Projection Solutions from the Shared Production Unit of the Zeiss Group will go into the new strategic business unit.

With a combined annual revenue of approximately Euro 200 million and nearly 900 employees, Zeiss Photonics & Optics will have locations in Germany, Hungary, the UK, the US, India, and China.

"The goal of this new strategic business unit is to make the individual businesses thrive economically and to support the further development of the Zeiss Brand," says Stefan Müller, CFO of the Zeiss Group.

Refeyn opens US headquarters in Massachusetts

Mass photometry pioneer, Refeyn, has opened a 10,000 sq ft US Headquarters and Customer Interaction Center in Waltham's Biotech Hub in Greater Boston, Massachusetts.

Refeyn CEO Gerry Mackay – along with company co-founder, Philipp Kukura, and colleagues – helped to cut the ribbon at the opening event. According to the company, the grand opening is a significant milestone in the rapid growth of Refeyn since it was spun out of Oxford University six years ago.

Kukura led the research team that invented mass photometry, a light-based bioanalytical tool for rapid, single-particle analysis. As he commented at the opening event: "In the early days, mass photometry's potential was obvious and the key question wasn't 'What can mass photometry do?' but 'What can mass photometry not do?'"

"It is amazing to witness the opening of Refeyn's state-of-the-art US HQ, having started in an early 19th century building above a burger joint in central Oxford, which feels like only yesterday," he added.

Serving as Refeyn's US Customer Interaction Center, alongside offices, meeting and training rooms, the laboratory space in the new headquarters enables customers across North America to visit to run samples and test applications. The laboratory space includes a BSL-2 lab, service center, R&D center and applications lab. The new site also facilitates instrument maintenance, customer training sessions, and workshops.

REFEYN *US Headquarters and Customer Interaction Center. [Refeyn]*

Refeyn now has more than 180 employees globally and is currently recruiting more to support our expanding US customer-base from our new facility. Hundreds of Refeyn instruments are installed in laboratories globally, whose use has been cited in over 300 scientific papers to date. The company claims this literature highlights the ever-growing capabilities of mass photometry in rapid single-particle analysis, providing vital insights for scientific discovery, R&D, and therapeutic production.

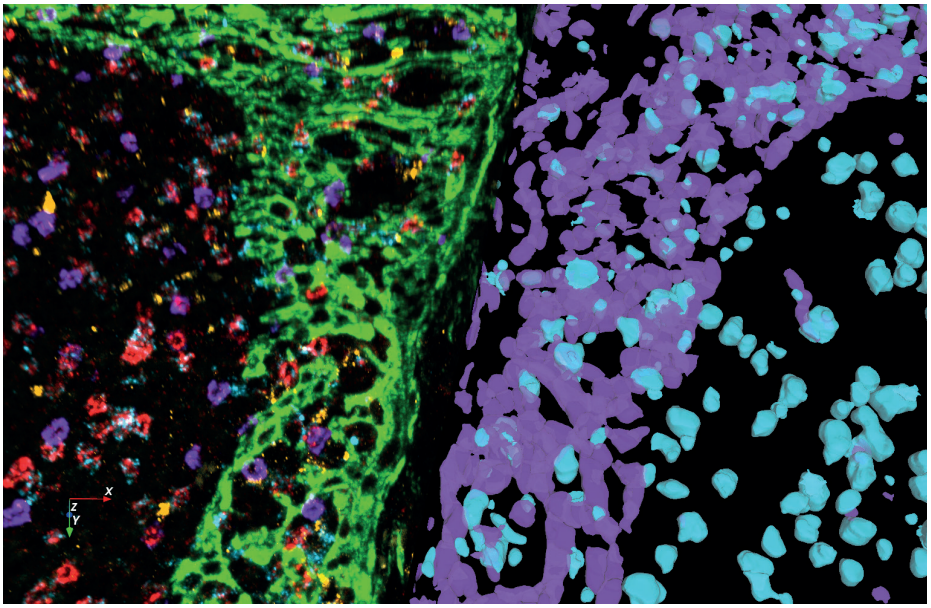
FROM LEFT TO RIGHT, *Refeyn's: CEO, Gerry Mackay; Co-founder, Philipp Kukura; Key Account Manager, Gael Nicolas; and US Sales Director, Candi Mach, cut the ribbon to open Refeyn's new US headquarters in Waltham's Biotech Hub in Massachusetts. [Alto Marketing for Refeyn]*

NKT Photonics joins Hamamatsu

NKT Photonics is now part of Hamamatsu Photonics, home to more than 5,800 employees worldwide and an annual revenue of some Euro 1.3 billion. The company will continue as a subsidiary with responsibility for fiber and laser development, manufacturing, and commercialization within the Hamamatsu Photonics group.

"We are excited and honored to become part of Hamamatsu Photonics," says Basil Garabet, CEO of NKT Photonics. "We share common goals and a vision of providing the best solutions to our customers and innovators. Together, and with our complementary technology platforms, we will be stronger than ever."

"[NKT Photonics]... will form the backbone of our laser division. By combining Hamamatsu's expertise within photodetectors with NKT Photonics' lasers and fibers, we will be able to provide unique system solutions to our core markets," adds Tadashi Maruno, CEO Hamamatsu Photonics.



MULTIPLEXED *human tonsil tissue labeled with a panel of 8 OPAL dyes and DAPI acquired on a Stellaris system, cell segmentation performed with Aivia 14. [Leica]*

Leica launches latest version of Aivia

Leica Microsystems has released version 14 of Aivia, its image analysis solution. This update introduces new features and enhancements for accurate deep-learning based cell segmentation, automated phenotyping and spatial data analysis in 3D multiplexed images. Researchers can visualize up to 15 channels in 3D multiplexed images simultaneously, providing a comprehensive view of complex biological processes.

"This new version of Aivia is particularly well suited to contribute to drug development and will catalyze advances in cancer research, immunology and personalized medicine," highlights Luciano Lucas, Director Data & Analysis at Leica Microsystems. "Aivia 14 enables users to systematically segment, phenotype and explore heterogeneities in healthy and pathological tissue microenvironments, and this will play a crucial role in determining treatment outcomes."

Aivia's improved deep learning model accelerates cell detection by up to 78%, resulting in faster and more accurate detection and partition of cells. This enhancement enables characterization of tissue microenvironments and different phenotypes based on the expression of multiple biomarkers such as disease state or cell type. With the software's updated dendrogram and dimensionality reduction tools, users can interactively explore phenotypes and gain a deeper understanding of 3D multiplexed image data.

"Dealing with massive numbers of data points in complex biological images can be daunting for researchers," says Won Yung Choi, Product Manager, Data & Analysis at Leica Microsystems. "Aivia 14 automates this process by leveraging advanced AI algorithms, allowing scientists to seamlessly identify and analyze phenotypes without the need to train deep learning models or code. This not only accelerates their research but also uncovers insights that might have otherwise been missed."

Zeiss reveals arivis Pro 4.2

Zeiss has unveiled the latest version of its image analysis software, arivis Pro 4.2, which includes AI-powered segmentation tools, enhanced 3D analysis capabilities, and the ability to handle large datasets. Described as 'a universal solution optimized for any imaging workflow', Zeiss says the software provides unprecedented flexibility to tailor microscopy image analysis workflows to answer research questions.

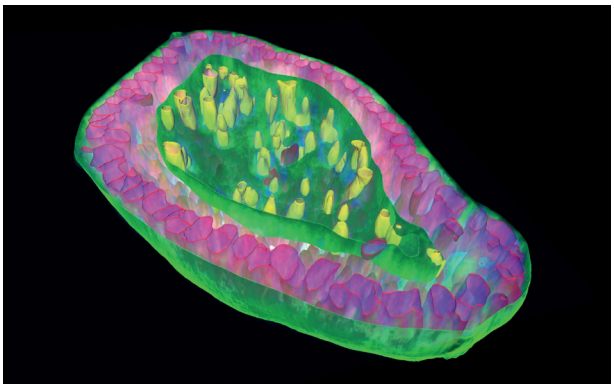
"Our goal with arivis Pro 4.2 is to put the power of customization directly into the user's hands," says Dr Sreenivas Bhattiprolu, Head of Digital Solutions at Zeiss. "Whether working with small datasets or incredibly large 3D volumes, researchers can now leverage the most appropriate tools for their specific analysis requirements."

According to the company, a cornerstone of the new version is the integration of cutting-edge instance segmentation models powered by deep learning. Trained on the Zeiss arivis Cloud, these models enable users to precisely segment individual objects within images using AI - without any coding expertise required. Additionally, pre-trained models from the open-source Cellpose library enable users to segment cells and nuclei in most images.

"In microscopy, no two datasets are alike," explains Bhattiprolu. "Some demand traditional segmentation methods, while others benefit from AI-driven approaches. With arivis Pro 4.2, we empower researchers to choose the ideal techniques for each unique scenario."

arivis Pro 4.2 also allows users to seamlessly load, visualize, and extract rich insights from massive 3D datasets containing a vast number of objects, promising unprecedented exploration of complex 3D volumes at scale.

A CROSS-SECTIONAL *view of an intestinal gut organoid captured at 20X magnification on the Zeiss Celldiscoverer 7 and segmented using arivis Pro image analysis software. The image highlights the outer cell layer nuclei in pink and the inner luminal nuclei in yellow. [Zeiss]*



Horiba delivers InverTau for fluorescence lifetime imaging

Horiba has launched InverTau, a new platform for confocal fluorescence lifetime imaging. InverTau extends the capability of conventional inverted microscopes by adding confocal high-speed laser scanning while seamlessly integrating all elements required for high performance FLIM.

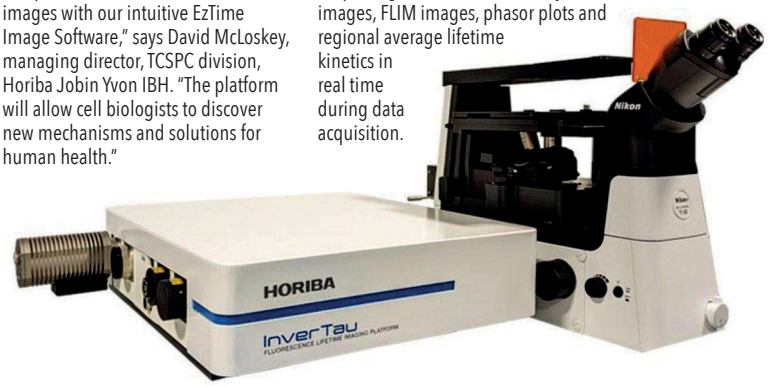
As Horiba points out, InverTau is a fully software-controlled platform that mounts to the side port of an inverted fluorescence microscope to add fluorescence lifetime imaging (FLIM) capability. The system can be customized around any microscope or purchased as a complete system.

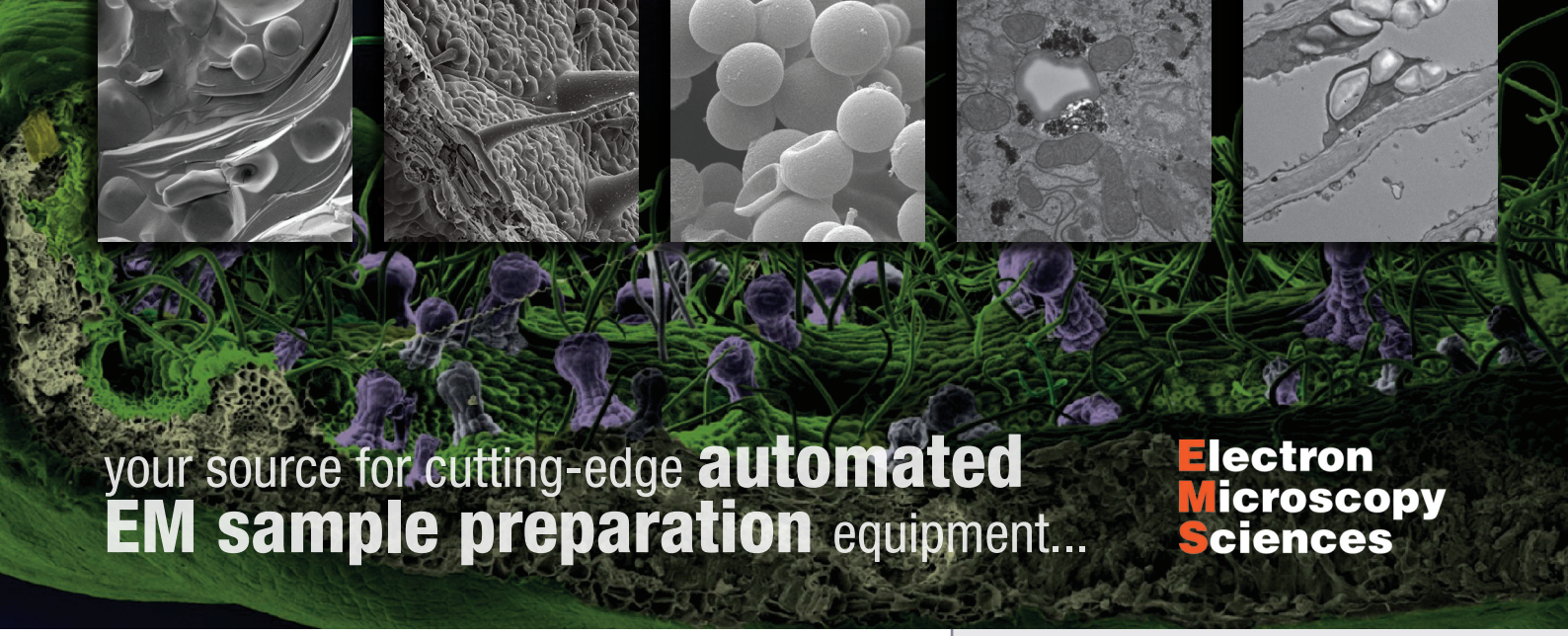
A typical complete system includes the InverTau confocal fluorescence lifetime imaging platform, a Nikon Ti2-U manual inverted fluorescence microscope, Computer, FiPho TCSPC electronics and EzTime Image software. It also includes the HPPD-720 TCSPC detector and DeltaDiode lasers from 375 to 595 nm. Optionally, the FLIMera widefield video rate FLIM camera for real time visualization and lifetime kinetics can also be added to combine laser scanning and widefield modalities on the same instrument. Additionally, InverTau offers the ability to acquire real time FLIM dynamics at up to 30fps with the addition of HORIBA's award winning FLIMera camera.

"InverTau has been developed utilizing our core TCSPC technology and delivers high-quality, fully computer-controlled confocal FLIM images with our intuitive EzTime Image Software," says David McLoskey, managing director, TCSPC division, Horiba Jobin Yvon IBH. "The platform will allow cell biologists to discover new mechanisms and solutions for human health."

Fluorescence lifetime imaging provides more information about molecular interactions than is available with traditional fluorescence intensity images. EzTime Image is designed for use with a touchscreen monitor and enables complete control, data acquisition and analysis, while outputting fluorescence intensity images, FLIM images, phasor plots and regional average lifetime kinetics in real time during data acquisition.

THE INVERTAU *platform is a laser scanning confocal unit for Fluorescence Lifetime Imaging (FLIM). [Horiba]*





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Electron Microscopy Sciences

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- **Status display to indicate time remaining.**
- **Pre-set profiles cover different sample types allowing easy start for new samples.**
- **Screw top chamber for easy sample loading.**
- **Large viewing window.**
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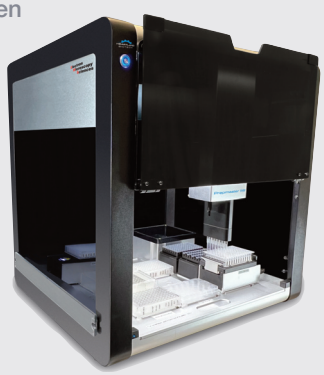
EMS Quorum

Prepmaster™ 5100 Specimen Preparation Robot

Walk-away specimen prep automation



The Prepmaster™ 5100 is a fully automated system that uses advanced robotics and liquid handling to prepare biological specimens for TEM or SEM. It reliably accomplishes your repetitive tasks, increasing consistency in specimen preparation and giving you confidence in your results compared to manual processing.

- **Easy to set up and clean up**
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- **Prepare up to 8 kidney specimens in less than 1 hour.**
- **Excellent choice to run Ellisman rOTO protocol for vEM specimen prep.**
- **Up to 24 unique reagents or rinses.**
- **Reliable unattended overnight operation.**
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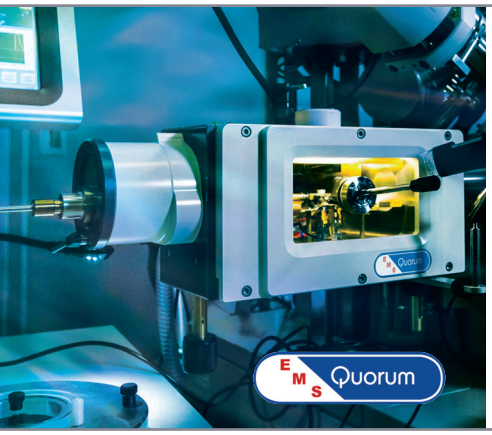

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A highly automated, easy-to-use, column-mounted, gas-cooled cryo preparation system suitable for most makes and models of SEM, FE-SEM and FIB/SEM.

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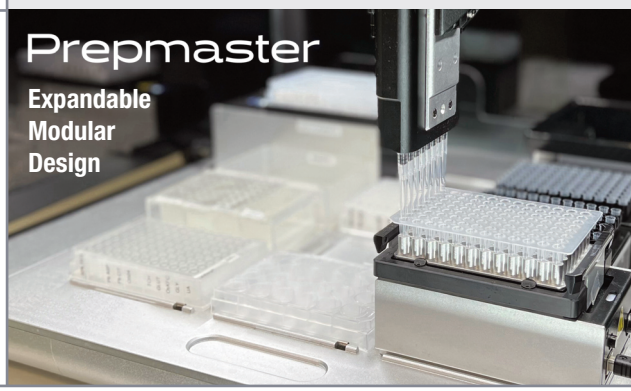
The unique Cryo Rotate Stage facilitates cryo-FIB lamella preparation, cryo-FIB lift out and cryo-tomography.



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DON'T MISS THE BIG ANNOUNCEMENT...

SCAN AGAIN ON JULY 22 FOR EXCITING NEWS!

Electron Microscopy Sciences and Azer Scientific will be making a special announcement in conjunction with M&M 2024.