Microscopy and Analysis

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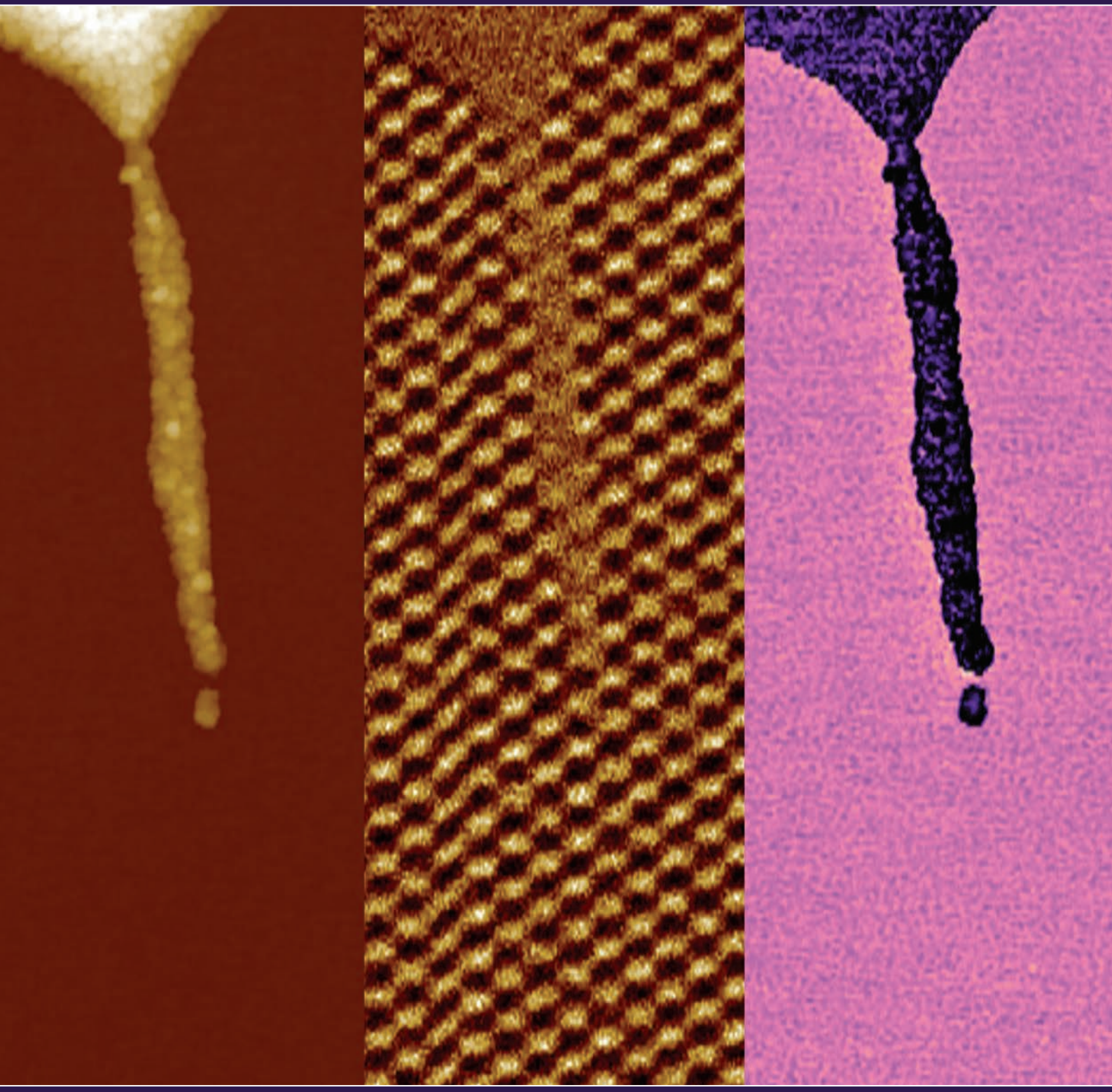
VOLUME 39 ISSUE 1 ▮ MARCH 2025 ▮ analyticalscience.wiley.com



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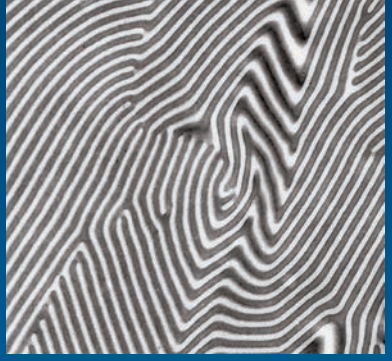

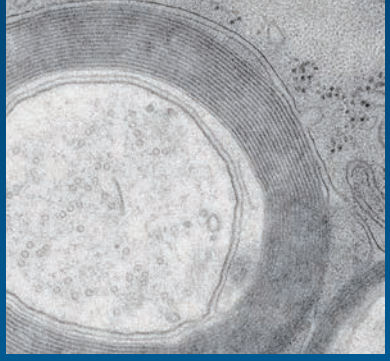

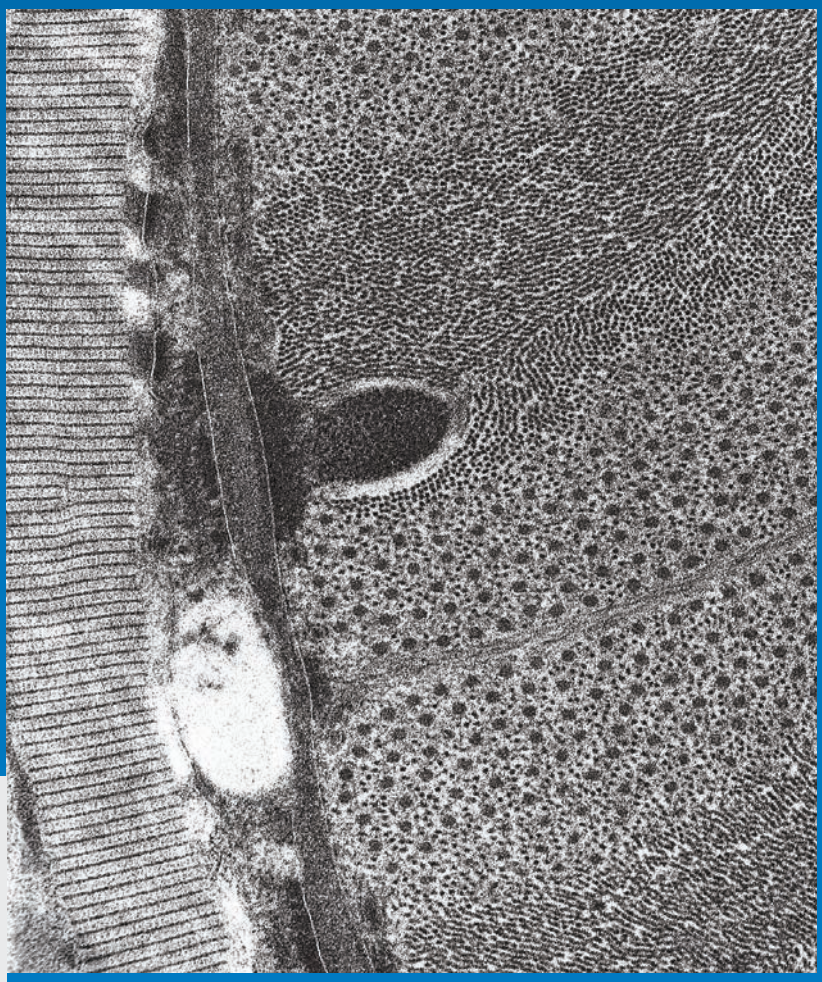

Ultramicrotomy in Biology

At Room Temperature:

- Dry sectioning for Nano SIMS and other imaging methods.
- Serial sectioning for Array Tomography, for Volume EM in general.
- Sectioning of difficult as well as hard materials: teeth, bone, implants,etc.
- Sectioning with the ultra sonic knife

At Cryo Temperatures:

- The Tokuyasu Technique
- CEMOVIS
- Cryo planing for cryo FIB

At Cryo Temperatures:

- The Tokuyasu Technique
- CEMOVIS
- Cryo planing for cryo FIB

Ultramicrotomy in Materials Research:

- Polymers at cryo temperature
- Polymers with the ultrasonic knife
- Battery materials
- Crystals
- Semiconductors
- Meteorite particles of many kinds
- Clam shells
- Concrete
- Soft wood, etc.

If you have any questions or are interested in having a workshop at your facility, please call or email us and we can discuss all of the particulars.

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Dear Readers,

Welcome to the new edition of Microscopy and Analysis, a result of the successful merger between Wiley's B2B magazines, Imaging & Microscopy and Microscopy and Analysis. Our dedicated editorial team is thrilled to bring you the latest trends and developments in the ever-evolving field of microscopy. The magazine's fresh and engaging layout symbolizes this exciting new beginning.

Microscopy techniques are advancing at a remarkable pace, offering diverse methods to address a wide range of scientific questions in both life and material sciences. As innovations in this field continue to unfold rapidly, our commitment is to keep the microscopy community well-informed about all significant breakthroughs. Our magazine features popular sections such as news, event updates, and important announcements from microscopy societies and initiatives.

We are proud to maintain our enduring partnerships with the European Microscopy Society and the Royal Microscopical Society, and we are pleased to welcome Global BioImaging as a new collaborator.

In addition, we will provide readers with insights into the latest techniques and applications in microscopy through articles from both industry and academia. Our profiles and interviews will introduce you to influential figures, companies, and research areas, offering a personal glimpse into the lives of researchers who are shaping the future of microscopy and its applications.

Our editorial team, comprising Chris Parmenter, Rebecca Pool, Martin Friedrich, and myself, is eager to share our combined expertise and years of experience with you. Together, we aim to keep you informed about all the important developments in microscopy. We are honored to be a vital part of the microscopy community and look forward to engaging with you on this exciting journey.

Enjoy the read of this issue!

Yours Birgit Foltas

Birgit Foltas,
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Chris Parmenter,
Freelance Editor

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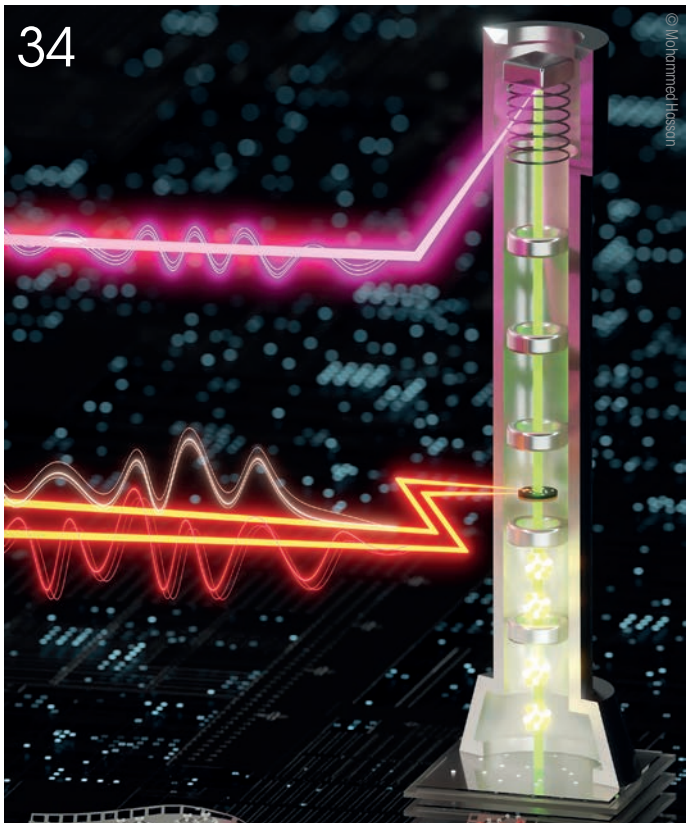
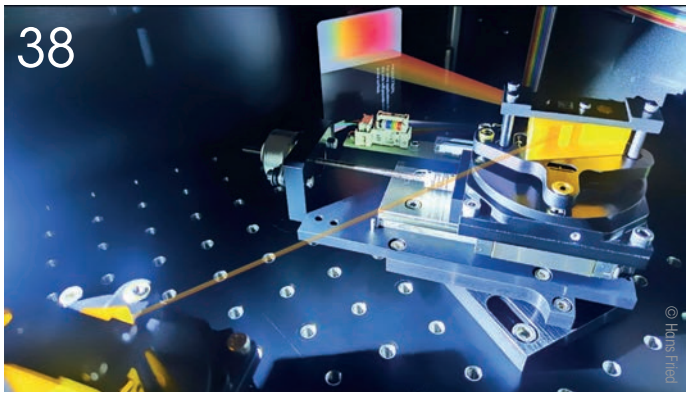
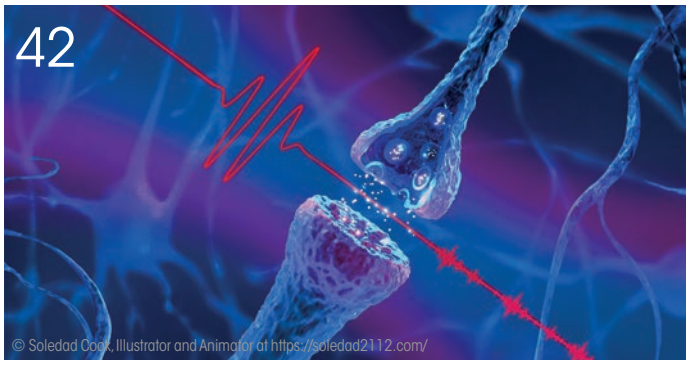
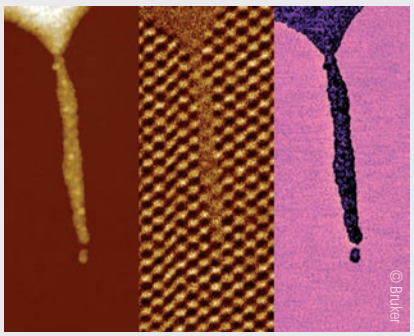






COVER STORY



Expanding Magnetic Force Microscopy

New Technology Advances High-Resolution Magnetic Imaging

This article presents PeakForce magnetic force microscopy (PF-MFM), a novel MFM variant where the initial topographic scan is conducted in PeakForce Tapping mode. This seemingly simple swap conveys numerous benefits: superior spatial resolution, enhanced sensitivity, added data channels, and the ability to image delicate samples with greater precision. MFM capabilities can be further enhanced by combining PF-MFM with torsional resonance MFM (TR-MFM) for 3D information about the magnetic field.

Solving the AFM-IR Resolution Mystery

The central challenge in microscopy is determining the smallest structures that can be identified without blurring. Traditional light microscopes have straightforward answers, but advanced techniques like atomic force microscopy-infrared spectroscopy (AFM-IR) have struggled with resolution issues. Researchers at TU Vienna have now resolved these challenges, providing a reliable method to calculate and predict AFM-IR's resolution. AFM-IR uses infrared radiation to identify molecules such as proteins by measuring their unique infrared spectra. Previously, the resolution varied unpredictably between 10 to 100 nanometers. The breakthrough involved integrating an atomic force microscope, which uses a fine tip to scan the sample's surface. This detects localized heating from infrared absorption, allowing researchers to determine both the identity and precise location of molecules. This advancement in AFM-IR enhances the ability to study molecular structures with greater accuracy.

DOI: 10.1073/pnas.2403079122

Combining Volumetric Expansion with Image-Based Transcriptomics

Researchers have achieved groundbreaking insights into bacterial gene expression using genomic-scale microscopy and an innovative technical approach. Published in *Science*, the study by Jeffrey Moffitt and colleagues at Boston Children's Hospital's PCMM maps bacterial gene activation across different spatial environments, marking a significant advancement in microbiology. The research utilized MERFISH, a molecular imaging technique developed by Moffitt, to profile thousands of bacterial mRNAs simultaneously. This approach enabled visualization of spatial gene expression patterns with unprecedented precision, overcoming challenges posed by the tiny size and densely packed RNAs in bacterial cells. The team employed expansion microscopy, a method pioneered by Ed Boyden at MIT, to expand bacterial RNA samples 50- to 1000-fold in volume, making individual RNAs distinguishable.

Traditionally, bacterial behavior was analyzed in bulk, averaging responses across populations. MERFISH now allows for the examination of individual bacterial gene activity, offering insights into bacterial interactions, antibiotic resistance, virulence, and biofilm formation. These discoveries could revolutionize understanding infections and developing new treatments. Moffitt emphasized the potential to explore host-microbe and microbe-microbe interactions, bacterial communication, competition for spatial niches, and microbial community structures. The technology also facilitates studying bacteria that are difficult to culture, allowing researchers to image them in their native environments without the need for cultivation.

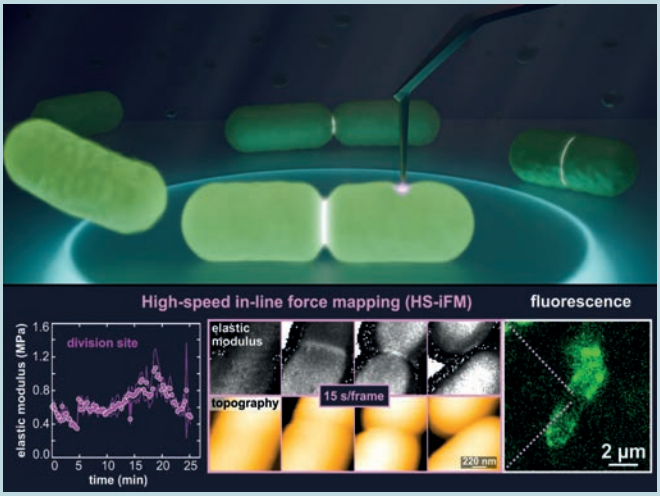
DOI: 10.1126/science.adr0932

Mapping ApoB100's Structure with Cryo-Electron Microscopy

A University of Missouri study has uncovered the detailed structure of ApoB100, a protein crucial for transporting low-density lipoproteins (LDL) through the bloodstream, offering new avenues for targeted cholesterol and heart disease therapies. Current treatments like statins are effective but often cause side effects. By understanding ApoB100's structure, scientists hope to develop precision treatments that maintain efficacy while reducing side effects, potentially revolutionizing cholesterol management and lowering cardiovascular risks. Researchers Zachary Berndsen and Keith Cassidy used cryo-electron microscopy at Mizzou's Electron Microscopy Core to achieve high-resolution images of ApoB100, allowing them to see molecular structures at an unprecedented scale. This advanced technology, complemented by artificial intelligence using the AI neural network AlphaFold, refined the structural model further, providing a more detailed understanding of ApoB100. The goal is to create treatments that target harmful LDL while preserving cholesterol's essential roles, such as hormone production and cell membrane maintenance. The research, driven by personal connections to heart disease, aims to connect fundamental science with practical health benefits, offering more accurate heart disease risk indicators and potential new treatments.

DOI: 10.1038/s41586-024-08467-w

Using High-Speed In-Line Force Mapping to Capture Cellular Processes

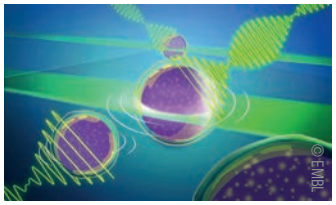


Researchers from the National Institutes of Natural Sciences (NINS) and Nagoya University have developed a new approach that enables real-time, high-resolution imaging of living biological samples. Their new method, using high-speed in-line force mapping (HS-iFM), overcomes the limitations of traditional light and electron microscopy by capturing both topography and mechanical properties at unprecedented speed and resolution.

Light microscopy falls short in resolving smaller cellular structures, while electron microscopy demands complex sample preparation that inevitably kills live specimens. Although atomic force microscopy (AFM) achieves exceptional resolution, its slow imaging speed limits its practicality. High-speed AFM (HS-AFM) enhances imaging speed but lacks the capability to measure mechanical properties. HS-iFM overcomes these limitations by capturing dynamic mechanical force measurements, providing researchers with an unprecedented ability to study living microorganisms in real-time.

In the study published in *Science Advances*, the researchers employed HS-iFM to observe significant mechanical stiffening at the *E. coli* cell division site, likely due to localized membrane tension and cell wall thickening. „The division site becomes much stiffer than the surrounding cell, hinting at large internal stresses that are needed to deform the membrane and separate the cells," explained Christian Ganser, assistant professor at the Exploratory Research Center on Life and Living Systems (ExCELLS) at NINS in Okazaki, Japan.

DOI: 10.1126/sciadv.ads3010



Faster Imaging with Minimal Light Exposure

Researchers at the European Molecular Biology Laboratory (EMBL) have achieved a significant breakthrough in microscopy, delivering a 1,000-fold improvement in speed and throughput in Brillouin microscopy. This advancement enhances the ability of life scientists to study light-sensitive organisms more efficiently, representing a major step forward in biological imaging technology.

Carlo Bevilacqua, lead author of the study published in *Nature Photonics* and an optical engineer in EMBL's Prevedel Team, explained the significance of the achievement: "We were on a quest to speed up image acquisition. Over the years, we have progressed from being able to see just a pixel at a time to a line of 100 pixels, to now a full plane that offers a view of approximately 10,000 pixels."

The technology is rooted in a principle first predicted in 1922 by French physicist Léon Brillouin, who demonstrated that when light interacts with a material, it exchanges energy with naturally occurring thermal vibrations, slightly shifting the frequency (or color) of the light. Analyzing the scattered light spectrum provides crucial information about a material's physical properties.

Brillouin scattering was first applied to microscopy in the early 2000s, made possible by advancements that enabled scientists to measure minuscule frequency shifts with high precision. This capability allowed researchers to determine the mechanical properties of biological samples. However, early versions of this technique could only capture one pixel at a time, making the process slow and limiting its practical application in biology.

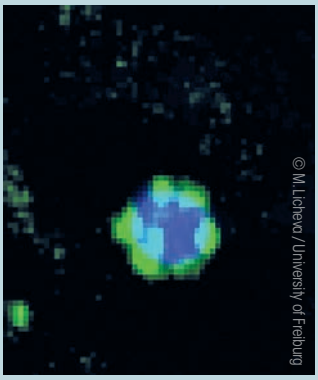
By 2022, Bevilacqua and the Prevedel group had expanded the field of view to a line. With their latest development, they have now achieved a full 2D field of view, significantly accelerating 3D imaging capabilities as well.

Robert Prevedel, Group Leader and senior author of the paper emphasized the impact of this advancement: "Just as the development of light-sheet microscopy here at EMBL marked a revolution in light microscopy because it allowed for faster, high-resolution, and minimally phototoxic imaging of biological samples, so too does this advance in the area of mechanical or Brillouin imaging. We hope this new technology – with minimal light intensity – opens one more 'window' for life scientists' exploration."

DOI: 10.1038/s41566-025-01619-y

Decoding the Mechanism Behind Autophagy with Advanced Imaging Techniques

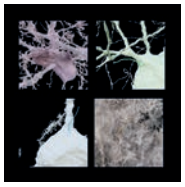
The research led by Prof. Claudine Kraft and Dr. Florian Wilfling used advanced imaging techniques to gain insights into autophagy, the cell's recycling system. The study employed fluorescence microscopy to track the dynamic behavior of receptors in living cells, allowing visualization of how receptors bind to cargo and cluster. Live cell imaging and time-lapse microscopy revealed the mobility and spontaneous clustering of receptor molecules, crucial for the early stages of autophagy. Additionally, cryo-scanning electron microscopy provided



high-resolution images of cellular structures, showing physical changes during autophagy. These techniques uncovered that weak binding allows receptors to remain mobile and form random clusters, eventually reaching a critical concentration that initiates degradation. The researchers demonstrated the ability to artificially trigger autophagy in yeast cells by modifying virus particles to bind weakly with autophagy receptors, inducing degradation. This breakthrough opens possibilities for therapeutic strategies targeting autophagy to treat diseases like Alzheimer's and improve cancer treatments.

DOI: 10.1038/s41556-024-01572-y

Large-Volume 3D Electron Microscopy to Study Cilia

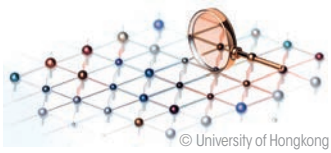


Researchers have utilized advanced 3D electron microscopy (EM) to study the elusive primary cilium in the mouse brain. By repurposing high-resolution 3D images originally created for mapping neural connections, they gained unprecedented insights into cilia structure and function in their natural environment. Volume EM, which stitches together nanometer-scale images to create ultra-high-resolution 3D visuals, enabled the detailed observation of cilia amidst dense brain tissue. This technique revealed not only neurons and their connections but also surrounding cellular structures, including cilia. The researchers analyzed the mouse primary visual cortex, identifying differences in cilia across various cell types. They also explored cilia in cerebellar granule cells, observing the development and disappearance of cilia during cell maturation. Volume EM provided detailed insights into how intermediary cells enclose cilia and how mature cells disassemble them, which could have implications for understanding brain tumors. These findings may explain the diverse symptoms of diseases involving cilia and open new avenues for treatment.

DOIs:
10.1073/pnas.2408083121
10.1016/j.cub.2024.04.043
10.1083/jcb.202404038

Novel Entanglement Microscopy

A research team from The University of Hong Kong has developed an algorithm called 'entanglement microscopy' to visualize and map quantum entanglement at a microscopic scale, offering new insights into quantum matter. Quantum entanglement, described by Einstein as "spooky action at a distance ", is crucial for quantum computing and cryptography, yet challenging to understand in complex systems. Led by Prof. Zi Yang Meng and colleagues, the study, published in *Nature Communications*, used quantum Monte Carlo simulations to explore entanglement in many-body systems, focusing on the transverse field Ising model and the fermionic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t-V model. Findings revealed that at the Ising quantum critical point, entanglement is short-range and can abruptly vanish ('sudden death'), while the fermionic transition shows a gradual decline in entanglement. The research also found that three-party entanglement is absent in two-dimensional Ising transitions but present in one-dimensional systems. This work promises advancements in quantum computing, materials, and simulations, enhancing technologies in energy, electronics, and fundamental physics.

DOI: 10.1038/s41467-024-55354-z

Using CryoNanoSIMS in Plant Science

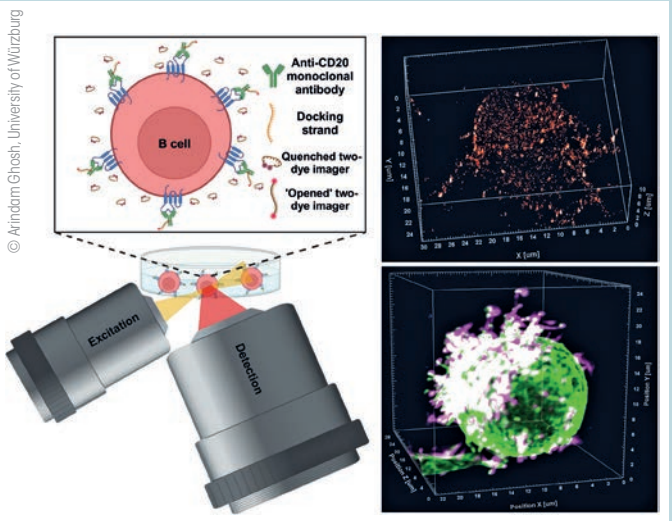
A research team from École Polytechnique Fédérale de Lausanne and the University of Lausanne has made significant advancements in understanding plant protection against salt stress using CryoNanoSIMS (Cryo Nanoscale Secondary Ion Mass Spectrometry). This innovative technique provided unprecedented cellular-level images, mapping sodium transport and storage within plant root cells. CryoNanoSIMS, a unique instrument globally, enabled the tracking of key elements like potassium, magnesium, calcium, and sodium in plant root tips. The study revealed that under moderate salt stress, the SOS1 transporter prevents sodium from entering cells. However, under high salt stress, SOS1 sequesters excess sodium into vacuoles, a temporary survival mechanism that slows growth and can lead to plant death if stress persists. The research, validated on mutant plants and rice, offers the first visual proof of plant sodium management at the cellular scale. CryoNanoSIMS could revolutionize understanding of salt tolerance and other plant protection mechanisms, such as resistance to heavy metals and microbes. This interdisciplinary collaboration, blending biology and engineering, allows for matching location with function and understanding previously unobserved mechanisms and processes, positioning the institutions as leaders in biological tissue imaging.

DOI: 10.1038/s41586-024-08403-y

New Super-Resolution Method for Cancer Research

Researchers at the University of Würzburg, Germany have visualized, for the first time in 3D and at a molecular resolution, how therapeutic antibodies attack and destroy cancer cells. This innovative discovery, published in *Science*, could revolutionize immunotherapy treatments for blood cancers such as chronic lymphocytic leukemia. Blood cancers like chronic lymphocytic leukemia develop when B cells of the immune system multiply uncontrollably. A common treatment involves using therapeutic antibodies that target the CD20 protein on the surface of B cells. This binding triggers a chain of immune reactions that ultimately leads to the destruction of the cancer cells. Despite the widespread use of such therapies for over three decades, the molecular mechanisms underlying their effectiveness have remained elusive.

A team led by Professor Markus Sauer at Julius-Maximilians-Universität (JMU) Würzburg has developed an super-resolution microscopy technique to address



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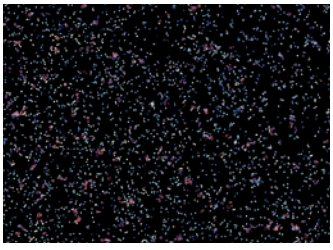
this gap in cancer research. The new method, LLS-TDI-DNA-PAINT, is based on fast volumetric fluorescence imaging with high spatiotemporal resolution, allowing researchers to visualize how therapeutic antibodies interact with cancer cells at a molecular level in 3D. The experiments were conducted using Raji B cells, a model system derived from Burkitt's lymphoma. The study revealed that antibodies such as RTX, OFA, OBZ, and 2H7 crosslink CD20 proteins in the cancer cell membrane, causing strong localized accumulations. This process activates the complement system, a critical component of the immune response, which leads to cell death. Contrary to previous classifications, the researchers discovered that this clustering occurs regardless of whether the antibody belongs to Type I or Type II, overturning long-held assumptions about their mechanisms of action.

DOI: 10.1126/science.adq4510

Pharmacoscopy for Rheumatoid Arthritis

A team from CeMM and the Medical University of Vienna, Austria has introduced an innovative microscopy-based technique that could revolutionize how rheumatoid arthritis (RA) is treated. By analyzing patients' blood samples, this method predicts the most effective drug for each individual, offering a personalized approach to treatment. Published in *EBioMedicine* the study highlights a major stride toward precision medicine for RA and potentially other autoimmune diseases. Current tools for predicting treatment outcomes are limited, leaving many patients in prolonged discomfort. The team developed a new technique that eliminates much of this guesswork. Using advanced microscopy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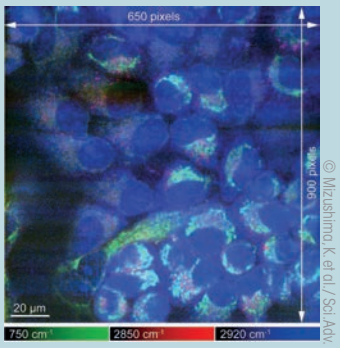
technology called „Pharmacoscopy," the researchers tested drugs directly on immune cells taken from patients' blood. This innovative method analyzes how individual immune cells respond to treatments, paving the way for faster and more precise therapy selection.

DOI: 10.1016/j.ebiom.2024.105522

Advancing Raman Imaging through Cryogenic Freezing

Researchers from Osaka University have developed a method to enhance Raman microscopy, significantly improving image quality for studying biological samples. Raman microscopy provides detailed chemical information about molecules like proteins, but its effectiveness is often hampered by weak signals and background noise. The Osaka team introduced a novel microscope that maintains frozen samples at consistent temperatures, allowing for longer exposure times without sample damage. This advancement results in images up to eight times brighter than those from



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traditional Raman microscopy. By imaging immobile frozen samples, researchers achieved high-resolution images with larger fields of view and high signal-to-noise ratios. This approach doesn't require staining or chemical fixation, offering a more accurate view of biological processes in their natural state. Moreover, freezing preserves the physicochemical properties of proteins, unlike chemical fixation methods that can alter these properties. This cryofixing technique ensures a faithful representation of proteins and their environments. The new method is versatile and can be combined with other microscopy techniques, enhancing the analysis of biological samples with potential applications in medicine and pharmaceuticals.

DOI: 10.1126/sciadv.adn011

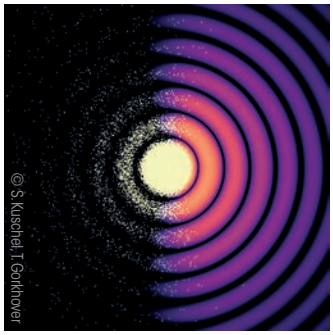
Advancing Solar Cell Design with Microgroove Technology

A study from the University of Sheffield and Power Roll Ltd. has introduced a new type of back-contact solar cell using perovskite material and embossed microgrooves in plastic film, offering a scalable, low-cost solar energy solution. This technology eliminates the need for rare materials like indium, making it sustainable and affordable. The lightweight, flexible solar films can be applied to surfaces unsuitable for traditional panels, such as warehouse rooftops. The design features all electrical contacts on the back, simplifying manufacturing and improving efficiency. Advanced imaging techniques were used to analyze the cells, marking a first in this field. The development results from a decade-long collaboration between the University and Power Roll, aiming to contribute to global net-zero targets. Further research is planned to enhance the stability and performance of perovskite-based solar cells.

DOI: 10.1021/acsaem.4c02734

High-Resolution Imaging of Ultrafast Phenomena

Researchers from the University of Hamburg, Germany, and collaborators have achieved a major milestone by capturing images of individual nanoparticles with single X-ray attosecond pulses. Published in *Nature Communications*, this new approach enables high-resolution imaging of ultrafast phenomena, such as chemical reactions and phase transitions, with unprecedented temporal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precision. Attosecond science, honored with the 2023 Nobel Prize in Physics, has reshaped our understanding of electron dynamics in atoms and molecules. An attosecond—a billionth of a billionth of a second—makes it possible to visualize processes occurring at incredible speeds. Until now, attosecond experiments have been limited mainly to spectroscopic studies. Using the X-ray Free Electron Laser (FEL) at SLAC National Laboratory, the team uncovered transient ion resonances that amplify X-ray diffraction images. These fleeting resonances, triggered by ultrashort FEL pulses, significantly enhance X-ray scattering efficiency.

DOI: 10.1038/s41467-025-56046-y

More Microscopy News on Wiley Analytical Science:

Back to the Roots: ELMI 2025 in Heidelberg

Heidelberg, Germany, June 3 - 6, 2025

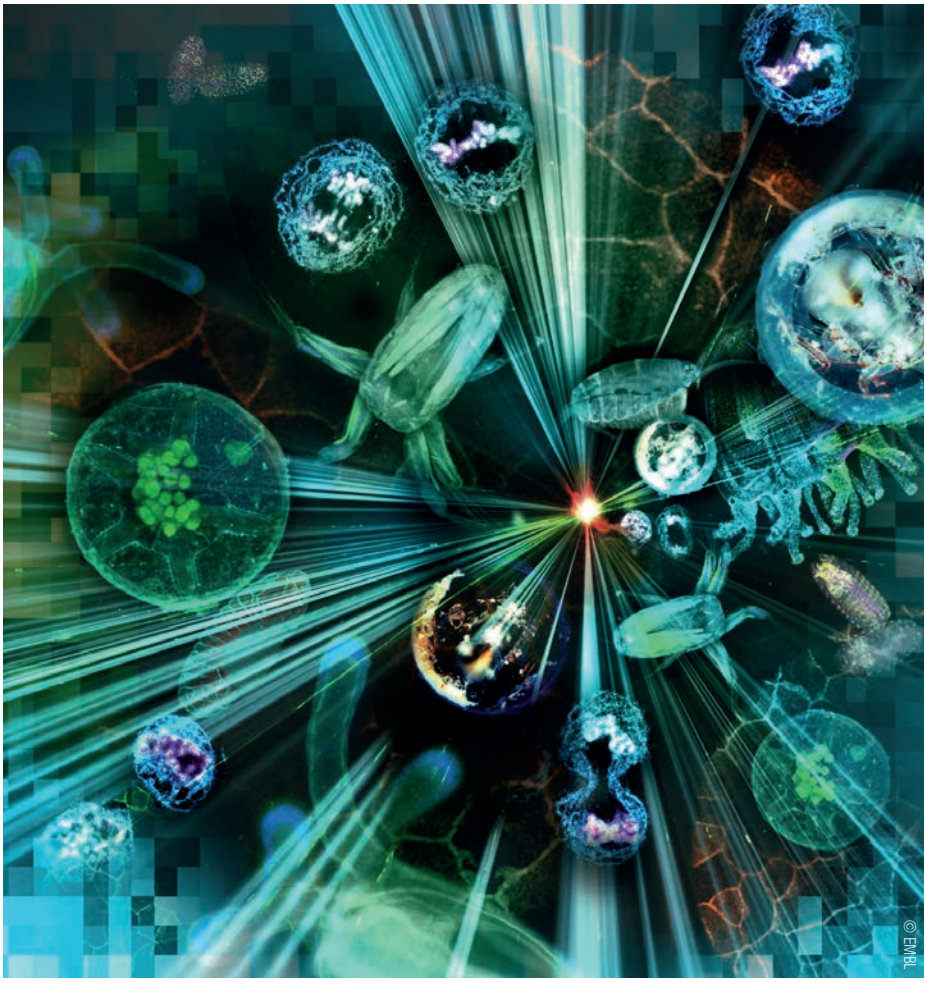
After the highly successful ELMI meeting in Liverpool last year, the 25th international meeting of the European Light Microscopy Initiative (ELMI) will take place from 3 – 6 June 2025 at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany and it will be a return to where it all started.

25 years ago, a group of imaging specialists from all over Europe met in a seminar room at EMBL in Heidelberg to get acquainted with each other's work, needs, and challenges and to discuss how one could efficiently network on such issues across Europe. The meeting was hosted by the newly created Advanced Light Microscopy Facility at EMBL and many of the participants actively provided imaging support for projects of other scientists in a form that subsequently crystallised into the current model of the core facility operation. The European Light Microscopy Initiative was born and one of its core activities since is the annual ELMI meeting that brings together imaging technology developers, service providers, users, and companies in the microscopy field.

The annual meeting provides information on the latest developments in light microscopy through plenary sessions on specific topics in the mornings, followed by workshops on new instrumentation and methods in the afternoon. With its unique format, the ELMI meeting is a perfect place for knowledge exchange and networking, both with academic partners and with companies.

The first day starts with the core facility day, which is an essential platform for service providers in the different institutes in Europe and worldwide. The core facility day is organised by and for core facility staff, but all registered participants interested in this topic are welcome to join. The main ELMI meeting starts in the evening of the first day with an opening lecture and a get-together.

As a major European science hub, Heidelberg's research landscape hosts many research centers with strong imaging groups and units and the ELMI meeting organisation brings together representatives of several such centres: Elisa d' Este (Max Planck Institute for Medical Research), Ulrike Engel (Heidelberg University), Elisa May (German Cancer Research Center) and Rainer Pepperkok, Stefan Terjung and Timo Zimmermann (EMBL).



This year's topics will range from nanoscopy over probes and biosensors, imaging in health applications, and tissue imaging to data analysis. The overarching impact of artificial intelligence technology will be reflected in all sessions. The scientific program will be complemented by ELMI's signature afternoon workshops and by the traditional "friendly" ELMI football match between two teams fielded by the academic and the company sides.

The 2025 meeting will take place at the Advanced Training Centre on the EMBL Heidelberg campus in the forest hills overlooking Heidelberg and the Rhine Valley.

We look forward to welcoming you to the place where the question "So, are we going to do it then?" started it all.

Dates

- Abstract submission deadline: 4 March 2025
- Registration deadline: 22 April 2025

More information:
https://s.embl.org/elmi2025

BNMI 2025 Symposium – 4th Annual Meeting of the Bridging Nordic Microscopy Infrastructure

Gothenburg, Sweden, August 19-22, 2025

The BNMI aims to strengthen the international competitiveness of Nordic advanced microscopy environments by fostering collaboration, knowledge exchange, and training. Through initiatives such as scientific symposia, workshops, shadowing programs for facility staff, and short-term scientific mobility, BNMI aspires to establish a sustainable Nordic platform that promotes efficient collaboration and long-term partnerships across the region. The BNMI is also an integral part of the Euro-Bioimaging-ERIC, which unites bioimaging infrastructures across Europe to enhance research, diagnostics, and patient care. One of BNMI's key activities is the organization of scientific symposia. This year, the Centre for Cellular Imaging at the University of Gothenburg, Sweden,

will proudly host the BNMI 2025 Symposium, a premier event that will convene imaging facility staff, leading scientists, early-career scientists, and industry representatives for 3 days of impactful discussions and collaboration. The symposium will offer an engaging and dynamic program tailored to imaging researchers across various disciplines. Attendees will have the opportunity to explore the latest advancements in imaging technologies and forge valuable connections within the global imaging community. Topics will span key areas of imaging research, including: Correlative Multimodal Imaging; Nanoscale Imaging; Mesoscopy; Smart Microscopy and New Frontiers in Artificial Intelligence for Microscopy

With ample opportunities for both formal discussions and informal networking, the symposium is designed to enrich scientific dialogue and foster meaningful collaborations. On behalf of the organizing committee, we look forward to welcoming you to this exciting meeting and wish you a memorable stay in Gothenburg!

BNMI

More information:
https://bnmi2025.gu.se

Microscopy Conference

Karlsruhe, Germany, August 31 - September 4, 2025

Discover the fascinating world of microscopy by attending the Microscopy Conference (MC 2025), from August 31st to September 4th, 2025, in the charming city of Karlsruhe, Germany. Whether you are a student, an experienced researcher or just curious – this is where experts from around the world meet to share the latest findings and technologies.

Following the tradition of the 'Dreiländertagung', the German, Austrian, and Swiss Society for Electron Microscopy (DGE, ASEM, SSOM) are excited to set the stage for vibrant scientific exchange and innovation sparked by the passion for science. There are expected over 1,000 participants from around the globe, making this a truly international celebration of electron microscopy.

The scientific program will feature plenary talks covering everything from the basics to current research topics. The attendees will hear about the latest advances in instrumentation and methods, materials science, and life sciences through invited talks, as well as oral and poster presentations. The event will be completed by the opening and closing ceremonies, award presentations, and prize lectures.

Be there and help shape the future of microscopy!

Further information and registration:
https://microscopy-conference.de

Göttingen Gears up for GEF25: Celebrating a Decade of Expansion Microscopy Innovations

Göttingen, Germany, September 27-30, 2025

© S. Rizzoli, University Göttingen

The historic university town of Göttingen is abuzz with anticipation as it prepares to host the prestigious Göttingen Expansion Microscopy Forum (GEF25), on September 27-30, 2025. This eagerly awaited conference will bring together the brightest minds in microscopy – scientists, journal editors, and biotech industry experts – to discuss the latest breakthroughs in super-resolution, expansion microscopy, and related innovations.

Super-resolution microscopy, invented in 1994 by Göttingen scientist Stefan Hell, has been a transformative force in biological imaging, enabling scientists to observe structures previously hidden by the diffraction limit. While initial super-resolution approaches were mostly based on optics inventions, expansion microscopy (ExM), pioneered in 2015 by Edward Boyden (at MIT), followed a radically different direction, by physically expanding the specimens. GEF25 celebrates a decade of innovations in this field, marking significant advancements that have shaped modern biological imaging.

Göttingen, with its impressive scientific landscape, provides the ideal setting for such an innovative event. The city is home to the prestigious Georg-August-Universität Göttingen, University Medical Center Göttingen, and the Max Planck Institute for Multidisciplinary Sciences, which are renowned for their contributions to ground-breaking discoveries, including Nobel Prize-winning research in electrophysiology and super-resolution microscopy. Göttingen's rich scientific heritage and vibrant academic community make it the ideal backdrop for GEF25, which is poised to be one of the biggest meetings on microscopy in the city's history, with contributions from speakers from all continents and over 25 countries.

GEF25 features an impressive line-up of experts who will share their insights and research. Attendees can look forward to presentations by leading figures in ExM and super-resolution, including Nobel laureate Stefan Hell, Edward Boyden, the father of expansion microscopy, Markus Sauer, the inventor of *d*STORM, and Ali Shaib and Silvio Rizzoli, the inventors of ONE microscopy. Joining them are several other leading experts in single-molecule fluorescence and molecular imaging, ExM leaders, editors of top-tier science journals, and prominent biotechnology representatives, all contributing to a comprehensive exploration of the latest advancements in microscopy.

The coordinators behind GEF25, Silvio Rizzoli and Ali Shaib, from the University of Göttingen Medical Center, have made substantial contributions to ExM. Their research focuses at the forefront of the ExM revolution, where they have successfully combined super-resolution techniques and artificial intelligence with expansion microscopy, achieving resolutions down to an astonishing one nanometer or better (see Shaib *et al.*, 2024 and Aguiar, 2024). This technology has been described as a means to democratize super-resolution (Ewen Callaway, 2023), a tool to "keep an eye on" (Eisenstein, 2024), and a "singular focus technology" (Dolgin, 2025).

The organizers of GEF25 extend a heartfelt invitation to scientists, researchers, and industry professionals to join them in Göttingen for what promises to be a milestone event in the field of fluorescence microscopy. GEF25 will be broadcast online.

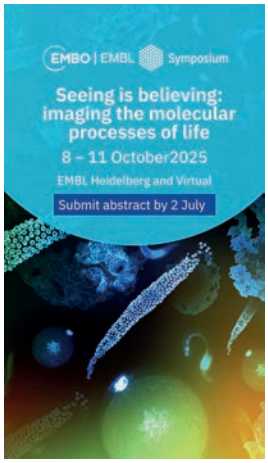
More information:
www.rizzoli-lab.de/gef25/

Article on ONE Microscopy:
https://www.nature.com/articles/s41587-024-02431-9

Seeing Is Believing: Imaging the Molecular Processes of Life

EMBL, Heidelberg, Germany, October 8-11, 2025

The molecular processes of life are naturally dynamic in space and time from the atomic to the organismal level. The rapid development of imaging methods across the full scale of biological organization is revolutionizing our ability to visualize the inner workings of macromolecular complexes, organelles, cells, tissues, organs, and whole organisms. Being able to see biological processes unfold in real time allows us to understand the mechanisms of life as well as disease.



The symposium will bring together the leading developers of imaging methods with cutting-edge applications that illustrate how imaging can answer biological questions. We will emphasize methods that can capture the dynamics of life, spanning the whole range from molecular resolution to imaging of whole organisms.

From its beginning in 2011, 'Seeing is believing' has embraced novel imaging technologies that open new windows for biological discovery, including single-molecule and super-resolution, light sheet, and correlative light electron microscopy.

The symposium provides many opportunities for presentations, discussions, and interactions between students, postdocs, junior and senior investigators.

Session Topics

- Imaging structure and function of molecules *in situ*
- Dynamic super-resolution microscopy
- Tissue and intravital imaging
- Probes and optical pertubation tools
- AI, image data and modelling
- New applications of imaging technologies

More information:
https://bit.ly/Seeing-2025

SPMConnect – Bringing Scanning Probe Microscopy to a More Applied and Industrial Audience

Austin, TX, USA, June 9-11, 2025

Dalia Yablon[1], Greg Haugstad[2]

A new conference dedicated to scanning probe microscopy will be in its third installment this summer. Named SPMConnect, its name is a play on the umbrella conference that hosts it, TechConnect World. Started in 2023, SPMConnect filled a void of a regular SPM conference in North America. For the previous ~15 years, SPMers had a home at the annual Fall MRS meeting in Boston. Additionally, there were other conferences that were occurring regularly with different focuses like SPM on SPM (SPM on soft and polymeric materials), ISPM (International SPM), and nc-AFM (non-contact AFM). For a variety of reasons, those conferences became more infrequent and irregular and SPMers lost their home-base.

TechConnect World was not a natural home as a landing place for an SPM-focused conference. It is a smallish ~2,000 member annual meeting in its 28th year dedicated to innovation, applied research, and entrepreneurship. It is not a typical research or technical society conference as it includes two meetings in one: a technical research conference that boasts over 30 technical symposia in varied technical sectors from advanced materials and AI to biotech and advanced manufacturing, as well as a business program featuring pitches from academics and startups about their latest innovation to an audience of corporate experts and funding agencies from government and industry.

TechConnect World features a fairly equal mix of academic researchers and applied/industrial scientists, an unusual audience for a technical conference of its size and quality. One of TechConnect's technical symposia is focused on "Materials Characterization and Imaging" and is led by Alex Norman of Princeton along with the authors of this article, veteran SPMers Greg Haugstad and Dalia Yablon. All three chairs, typical to TechConnect's theme, had significant industrial experience. While the symposium was broad and featured all forms of characterization (since its inception in 2006 including the efforts and insight of Pierre Panine as a founding organizer), it typically featured an SPM-focused session as well due to the chairs' area of expertise.

How did SPMConnect then arrive at TechConnect World? During Covid, leading SPMers Jason Killgore of NIST and Liam Collins of Oak Ridge National Labs ran a successful virtual conference I(SPM)^3 that combined ISPM and SPM on SPM. While including an outstanding array of research talks, Killgore and Collins put extra effort into organizing panel discussions about taking SPM into more applied directions, in line with the mission of their organizations. As Covid receded, Yablon and Haugstad approached Killgore and Collins about partnering to create a new arena for SPMers at TechConnect World. Killgore and Collins agreed and SPMConnect was born!

The rationale for housing SPMConnect at TechConnect was that SPM was finally ready to be pushed into a more commercial and industry-friendly space as the technique was maturing. Previously, it had mainly resided in the purview of academic and national lab research. (Some industrial labs did significantly publish open SPM research and participate in conferences, but these were exceptions.) At TechConnect World, scientists from a wide variety of applied and industrial backgrounds mingle and are exposed to the capabilities and advances in scanning probe microscopy. The scientific sectors represented at SPMConnect also provide an unusually broad spectrum of areas (e.g., materials, technologies) beyond what is represented at more focused technical society meetings, again providing exposure to a field that had been more confined to academic scientists.

Pushing the SPM community into a more applied and industrial venue did not come without resistance. Some scientists expressed concern that TechConnect World was too applied for SPM researchers, as there are many fundamental areas of basic science that they continue to pursue. However the organizers held firm feeling that SPMConnect would be a good home for SPMers of all backgrounds, and SPM was born at the 2023 annual meeting.

Now in its third year, SPMConnect is establishing itself as a successful home base for the SPM community. It typically lasts for the full 3- days of the conference and is a self-contained "conference within a conference." So far there has been one track for SPMConnect, which has provided an intimate and welcoming setting for SPMers to interact and share research over the week. True to its original design, attendees from other parts of the conference will pop in and out of sessions of interest. For example, at SPMConnect 2024, the graphene session drew a standing room only audience as it drew across the materials-heavy audience attending the entire TechConnect World spectrum of symposia, well beyond just SPMConnect attendees. Additionally, the SPMConnect poster session is integrated with the poster session of the overall conference to foster cross-disciplinary interactions.

One highlight of SPMConnect has been panel discussions, modeled after the original success of such discussions at ISPM^3. The original SPMConnect featured a panel discussion on shared user facilities from both academic and national labs, resulting in a lively discussion about training protocols, collaboration with professional scientists who manage facilities, IP barriers, ways to reduce obstacles to access, and how to acquire equipment improvements. The 2024 SPMConnect featured a panel discussion on applications to characterize semiconductor materials as well as a topic at the heart of SPM technology: cantilevers and trends in their fabrication/calibration. Panel discussions for 2025 SPMConnect will be announced later this spring.

An emphasis on industrial presenters and technological problems differentiates SPMConnect from other SPM conferences, but is well aligned with the mission of the overall TechConnect World conference. This conference has also enabled SPMers to explore fields within SPM that are not typically included in traditional SPM conferences - areas like FluidFM, a technology that integrates microfluidics into the atomic force microscopy instrumentation to enable ultrasensitive force measurements as well as the ability to aspirate femtoliters of volumes, with particular application to biological systems. An overarching goal of SPMConnect is to broaden the perspective of SPM users, not only in terms of science and technology but also as a connected user community. We strongly urge attendees to make new contacts for ongoing discussion and collaboration.

SPMConnect 2025 is being chaired by SPMers Filippo Mangolini of UT Austin, Simona Patange of NIST, and Jinhui Tao of PNNL and features an impressive array of speakers from national labs, industry and academia. Bringing together SPMers of various backgrounds fulfills the initial intention of the meeting as this community continues to push into new application areas and expand its audience to continue the growth of this remarkable instrumentation.

Figure 1: SPMConnect 2024 panel discussion on the use of SPM for semiconductor characterization.

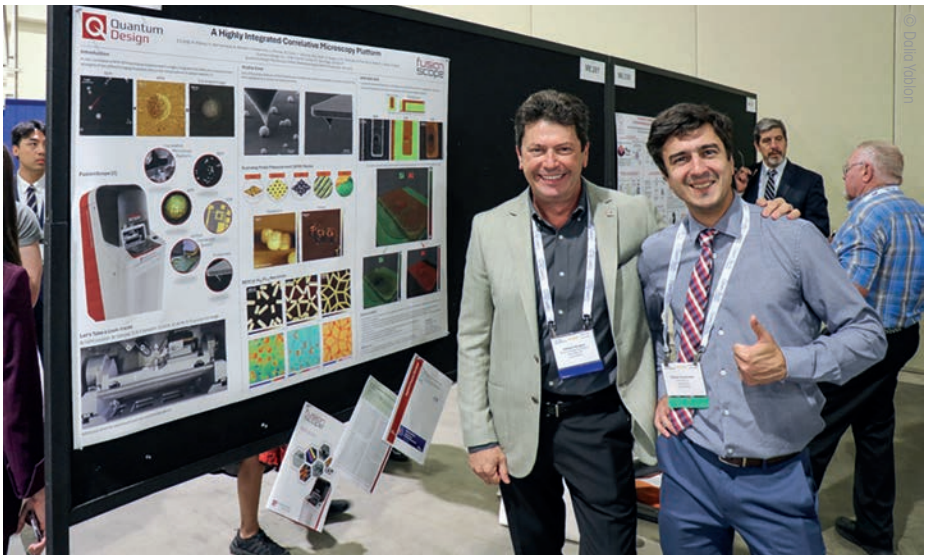


Figure 2: SPMConnect 2024 poster session.

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More information:
https://bit.ly/SPMConnect

mmc2025 Incorporating EMAG 2025

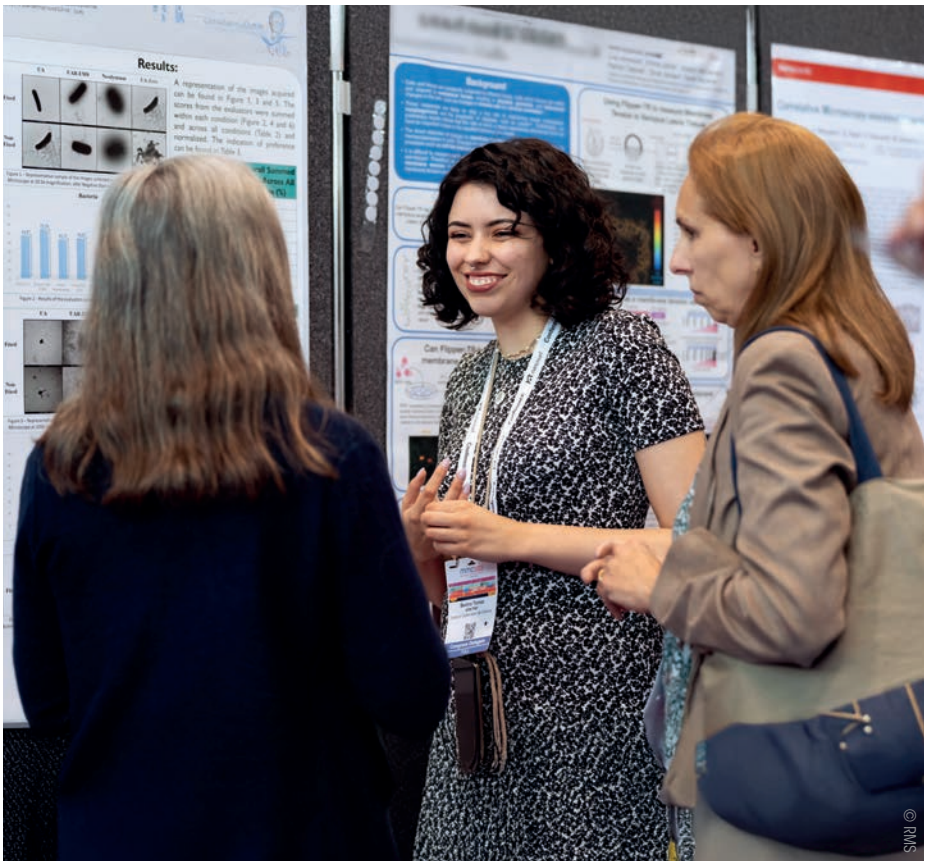
Registration Is Officially Open!

The Microscience Microscopy Congress is back for 2025!

One of the biggest events of its kind in Europe, mmc2025 incorporating EMAG 2025 will bring you the very best in microscopy, imaging, and cytometry from across the globe.

With six parallel conference sessions, a world-class exhibition, workshops, satellite meetings, an international Imaging Competition, and more, it is simply the place to be for anyone who uses a microscope for work, study, or pleasure.

Find out about our range of ticket options - including discount rates for RMS Members and students. As always attendance to the exhibition will be completely FREE throughout mmc2025.



World-Class Conference and Exhibition

mmc2025 incorporating EMAG 2025 will feature no fewer than 36 conference sessions across six parallel streams – with a fantastic range of speakers and poster sessions.

The program will cover the full range of the latest techniques, applications, and emerging topics in microscopy, imaging, and cytometry.

Congress registration also includes access to the huge mmc2025 incorporating EMAG 2025 exhibition which will house more than 80 exhibitors, including some of the biggest names in microscopy, cytometry, and imaging. Our sponsors will be eager to discuss their latest products and state-of-the-art technology, with representatives on hand to deliver practical demonstrations.

mmc2025 (incorporating EMAG 2025) will also bring together a number of smaller meetings, allowing you to meet with colleagues working in your field as well as with cross-disciplinary peers, all at the same event. They include:

- Pre-Conference Workshops (including ImageJ, EMAG, and EBSD) - Monday 30 June
- BioImagingUK Meeting - Monday 30 June
- Early Career Symposium - Monday 30 June
- Super-Resolution Workshop - Friday 4 July

Other Features

Other popular features include the RMS International Scientific Imaging Competition, with shortlisted entries – covering all microscopical techniques - displayed in an eye-catching gallery throughout the event.

The RMS will also be bringing its ever-popular Learning Zone to mmc, with experts on hand to share their knowledge with visitors and provide demonstrations.

Last – but by no means least – delegates will have the opportunity to register for one of our vibrant networking dinners during the week, to connect and socialize with others working in similar fields.

Register Now!

Registration is now open, and all the information on rates, accommodation, and transport can be found at www.mmc-series.org.uk.

We look forward to seeing you there!

Contact

Royal Microscopy Society
Oxford, UK
info@rms.org.uk

Registration for mmc2025:
www.mmc-series.org.uk

EMS Newsletter #88

March 2025

Vladislav Krzyžánek,
EMS President

Catherine Vénien-Bryan,
EMS General Secretary

Dear EMS members,

We would like to wish all of you a Happy New Year filled with opportunities and successes!

The new EMS year has started with the call for nominations for the EMS Outstanding Paper Awards 2024, which be presented at the 17th Multinational Congress on Microscopy (17MCM) at Portorož, Slovenia 7-12 September 2025.

As usual, the EMS is committed to supporting the activities of our members, so please visit our webpage for the various meetings and workshops taking place over the year, some of which are sponsored by the EMS, some others are patronaged:

- Winter School 2025 - Practical course in advanced microscopy, 20-24 January, Zurich, Switzerland
- Quantitative Electron microscopy 2025 (QEM2025), 11-23 May 2025, Port Barcares, France
- Latest Trends in In-situ and Correlative Electron Microscopy – Conference & Workshop ICEM 2025, 2 - 4 June 2025, Brno, Czech Republic
- BIST Symposium on Microscopy, Nanoscopy and Imaging Science 2025, 7 March 2025, Mediterranean Technology Park Castelldefels, Spain
- Microscopy Conference 2025, 31 Aug - 4 Sept 2025, Karlsruhe, Germany
- Women in Electron Microscopy – Breaking Barriers and Building Networks, 8 - 10 Oct 2025, Julich, Germany

And our extension meeting:

- 17th Multinational Congress on Microscopy, 7-12 September 2025, Portorož, Slovenia

We will launch the call for financial contributions very soon.

In addition, we invite all members of the EMS to update the information on your member's account in the "edit my preference" menu. This year, in particular, you will have the option of receiving a printed version of the yearbook or downloading it form the web page. The electronic version is the default option, so if you prefer the printed version, you must indicate your choice.

Finally, we still have many booklets celebrating the 25th anniversary of the EMS and containing articles written by leading scientists in our community. They are free to be offered during any EMS events, just let us know if you would like some!

Hoping to meet you next September in Slovenia at 17MCM.

Contact

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EMS event calendar:
https://bit.ly/EMS-calendar

Global BioImaging: An International Network of Imaging Scientists

Our 13 partner networks drive collaboration and innovation in bioimaging worldwide. Learn more about each on globalbioimaging.org.

Imaging science is at the heart of life sciences, from microscopy to biomedical imaging, computational imaging, and image data analysis. But behind every breakthrough image is a dedicated community of imaging professionals—scientists, facility managers, technical experts, and research coordinators—who ensure access to cutting-edge technology and drive innovation. Global BioImaging (GBI) is an international network uniting these professionals to advance imaging science through global collaboration, training, and shared resources.

Launched in 2016 through Horizon 2020 funding from the European Commission, GBI has since grown into a global initiative. Today, it is supported by the Chan Zuckerberg Initiative, enabling the network to expand its impact and provide programs for the international imaging community. Additionally, GBI coordinates the Imaging4All initiative, funded by Wellcome, which aims to promote equitable access to imaging technologies worldwide.

A Network Built for Imaging Scientists

Over the last two decades, imaging core facilities have become essential to modern research. Scientists specializing in imaging have found new career paths in facility management and technical support, yet recognition, funding, and structured training opportunities remain limited. To bridge these gaps,

GLOBAL BIOIMAGING
growing collaboration

GBI connects imaging scientists worldwide, providing a platform for knowledge exchange, training, and advocacy.

Founded by Euro-BioImaging, India BioImaging, and Microscopy Australia, the network has expanded to include members across Europe, North America, Latin America, Africa, Australia, and Asia, representing over 60 countries (see GBI partner network map). By bringing together imaging infrastructures and professionals, GBI strengthens the global imaging community, ensuring equitable access to imaging technologies and fostering international cooperation.

What We Do

Training & Capacity Building

- International Training Courses: Our courses cover topics of facility management, image data, and train-the-trainer formats. They are organized in collaboration with GBI partner organisations around the world.
- International Job Shadowing Program: Imaging core facility professionals can learn new aspects related to facility management, bioimage analysis, image data storage, quality management, software tools, user access, and more.
- Virtual Training Platform: A community-driven, curated repository of education resources focused on microscopy.
- Microtutor: An interactive light microscopy resource intended for independent learning tailored for early-career imaging scientists. Students can also engage with developers and peers on Microforum, a collaborative space for discussion and learning

Advancing Imaging Science Through Collaboration

- Working Groups: Sharing expertise and experience among experts in imaging-related fields and developing global image data standards and strategic policies.
- Exchange of Experience Conferences: Annual events where imaging professionals share expertise, discuss challenges, and shape the future of imaging science. This year's event will be held in Montreal, Canada, from 6-8 October! Sponsorship opportunities and travel grants are available.

Promoting Equitable Access to Imaging

- Imaging4All: Funded by Wellcome, this program supports efforts to ensure that imaging technologies are accessible to researchers worldwide, particularly in under-resourced regions.
- Advocacy for Imaging Infrastructures: GBI actively promotes the critical role of imaging facilities, engaging with policymakers and funding agencies to secure support for open-access imaging infrastructures worldwide.

Join the Global BioImaging Community

Whether you are a researcher, facility manager, technician, or industry professional, GBI offers opportunities to connect, learn, and collaborate. Visit our website to explore training courses, conferences, and funding opportunities.

GBI website:
https://globalbioimaging.org

Sodium-Ion Batteries: The Future of Energy Storage?

Interview with Magda Titirici

Sustainable alternatives to lithium-ion batteries are crucial to a carbon-neutral society, and in her Wiley Webinar, 'Beyond Li', at the upcoming Wiley Analytical Science Conference on Battery Technology, Professor Magda Titirici explores the options. Here, she tells ***Microscopy and Analysis*** about her passion for sodium-ion batteries and using renewable resources in energy storage.

Professor Magda Titirici develops sustainable materials and energy storage technologies. She is particularly known for her work in pioneering environmentally friendly alternatives to traditional energy storage systems, including sodium-ion batteries.

Born in Romania, you now hold the position of Chair in Sustainable Energy Materials at Imperial College London – tell us a little about your journey to the UK.

M. Titirici: I was born in Bucharest in Romania and went to university there to study Chemistry. I moved to Germany to pursue a PhD in molecularly imprinted polymers starting at Johannes Gutenberg Universität Mainz and graduating from Technical University of Dortmund having followed my PhD supervisor to Dortmund. I started my postdoctoral studies at the Max Planck Institute of Colloids and Interfaces, becoming an independent group leader, working on hydrothermal carbons. Then, in 2013 I moved to the UK - Queen Mary, University of London - where I was introduced to the British academic system, which I love. I love the collaborative nature and the friendliness of the academics here - so have stayed in the UK. In 2019 I moved to Imperial to the Department of Chemical Engineering as a Chair in Sustainable Energy Technologies and I also obtained a prestigious fellowship from the Royal Academy of Engineering as Chair in Emerging Technologies.

You discovered biomass could be used in batteries more than a decade ago – what set you on this road to sustainability?

M. Titirici: During my postdoctoral studies at the Max Planck Institute of Colloids and Interfaces, I discovered that if you treat biomass with a bit of pressure and temperature, you can form some interesting carbonaceous materials similar to coal – and an obvious application was battery electrodes. From the start of my research career, I had wanted to create more sustainable batteries, so it made sense to use such biomass-derived materials. I also knew that sodium ions love to insert into hard carbons - also known as disordered or non-graphitizable carbons - so investigating Na-ion batteries fitted like a glove. I published my first Na-ion battery paper, "Hollow carbon nanospheres with superior rate capability for sodium-based batteries", in *Advanced Energy Materials* in 2012, when the whole world was almost entirely focusing on Li-ion batteries, and it felt very exciting. There was not much known about hard carbon in Na-ion batteries at the time – we only knew it could work. At that time we proved if you nanostructure your carbon material into a hollow sphere, you get better rate capabilities. Looking back at my work critically - what I published wasn't going to revolutionize commercial Na-ion batteries, but it was a start.

Professor Magda Titirici and her team from the Sustainable Energy Materials Research Group, Imperial College London.

What progress on sodium-ion batteries has taken place since this time?

M. Titirici: We've gained an atomistic fundamental understanding of the Na storage mechanism into hard carbons and have now moved all the way to devices - we are constantly optimizing everything in terms of performance, cost, and sustainability. Energy storage is a very difficult market, and there is a lot of competition from China. But we are hoping that one way or another, we will get our hard carbons implemented in commercial Na-ion batteries, either via a spin-out company or by licensing out technology. This work is something that I'm very proud of, and I hope we can get it into the real world.

Why is hard carbon so important for sodium-ion batteries?

M. Titirici: Hard carbons are the only materials that currently work as anodes in Na-ion batteries. Na metal forms dendrites while metal alloys with tin or phosphorous expand their volume too much and crack. Of course, you need to design the hard carbon with the necessary properties - surface, structure, and pores - to maximize the Na ion storage and kinetics.

What advanced characterization methods does your research group use?

M. Titirici: Together with my collaborators, we are making great efforts to characterize the Na-ion battery interfaces. We use several techniques including electrochemical mass spectroscopy, Time of Flight Secondary Ion Mass Spectroscopy, X-Ray Photoelectron Spectroscopy with depth profiling, impedance spectroscopy and solid state NMR to only mention a few. Some are applied in *operando* and some *ex situ*. For cathode degradation we use X-ray Absorption Spectroscopy applied in *operando* going to high voltages to study degradation. We also look for cross-talk and cathode components on the anode. We study the intercalation of alkali metals into graphite, and use many techniques in concert, such as in *operando* optical microscopy with XRD.

Why should industry use sodium-ion batteries instead of lithium-ion batteries?

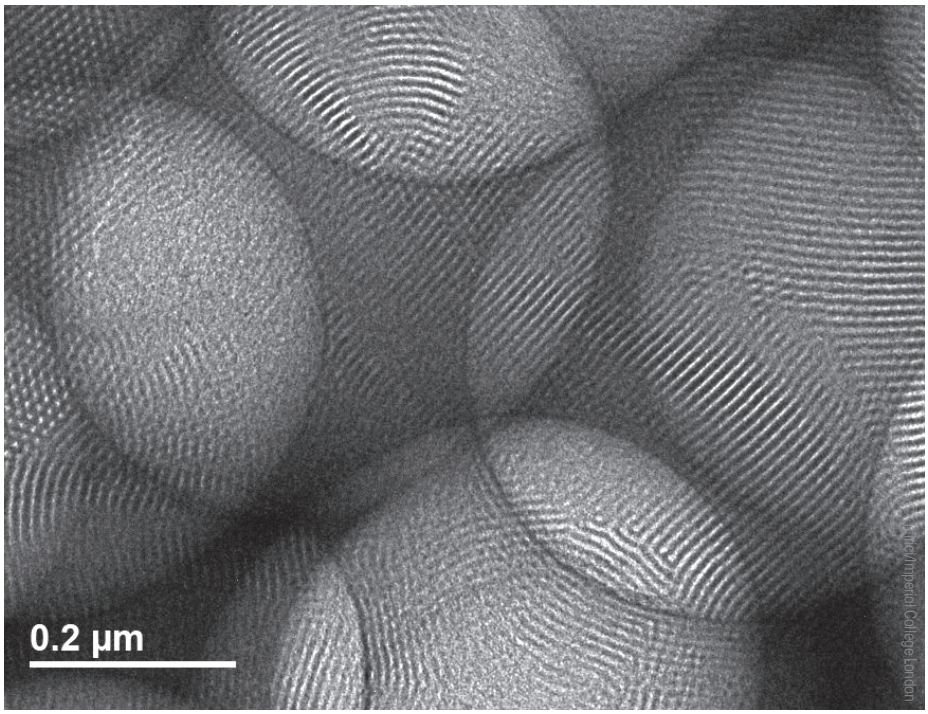
M. Titirici: Na-ion batteries are more sustainable and safer. Li-ion batteries need critical minerals that are mined elsewhere with severe social and ecological implications – these minerals affect everything from water usage to climate change to eutrophication of fresh water and biodiversity loss. Na-ion batteries can be transported and discharged at zero volts without short-circuiting.

Is industry eager to adopt sodium-ion batteries?

M. Titirici: That's a difficult question to answer - academics, NGOs, and various funders, keen on Na, are advising that everyone should be doing this. Recently I had a meeting with an industrial sponsor who basically said, 'we want performance - so why am I funding sodium if it doesn't work as well as lithium?' So I think there's still a perception from industry that performance is what counts - but we're seeing progress. In a recent visit to Na-ion battery manufacturer, HiNa Battery Technology Co in China, CEO, Professor Yong-Sheng Hu, showed me that their batteries can achieve the same performance as some of the lithium-ion batteries in commercial applications today - with the extra advantage of better low-temperature performance. If you look at some of the academic literature – Na-ion batteries have also either matched or beaten lithium ion (on some metrics). But the cost of Na-ion batteries at a commercial level remains much higher than lithium-ion batteries, and that's a big market barrier. I think we need bigger incentives for sustainability, and these have to come through legislation, otherwise, industry just won't change.

Where are sodium-ion batteries already being used?

M. Titirici: In China, you can already buy an electric vehicle - the JAC Yiwei - that is powered by Na-ion batteries and has a 250 km range. China also has a large-scale Na-ion battery storage plant in operation and, as far as I know, Germany is also currently working on demonstrating a Na stationary storage pilot plant. You can also buy a screwdriver powered by Tiamat's Na ion batteries. Of course, the commercial scale is very different from Li-ion batteries, but the more we develop commercial products that use Na-ion batteries, the more consumers will buy them and the more investment we will see in future years. I think in



TEM image of a hierarchically porous carbon material with macro and mesopores, as can be used in Na-ion battery electrodes.

three years we will see more Na-ion batteries in the market.

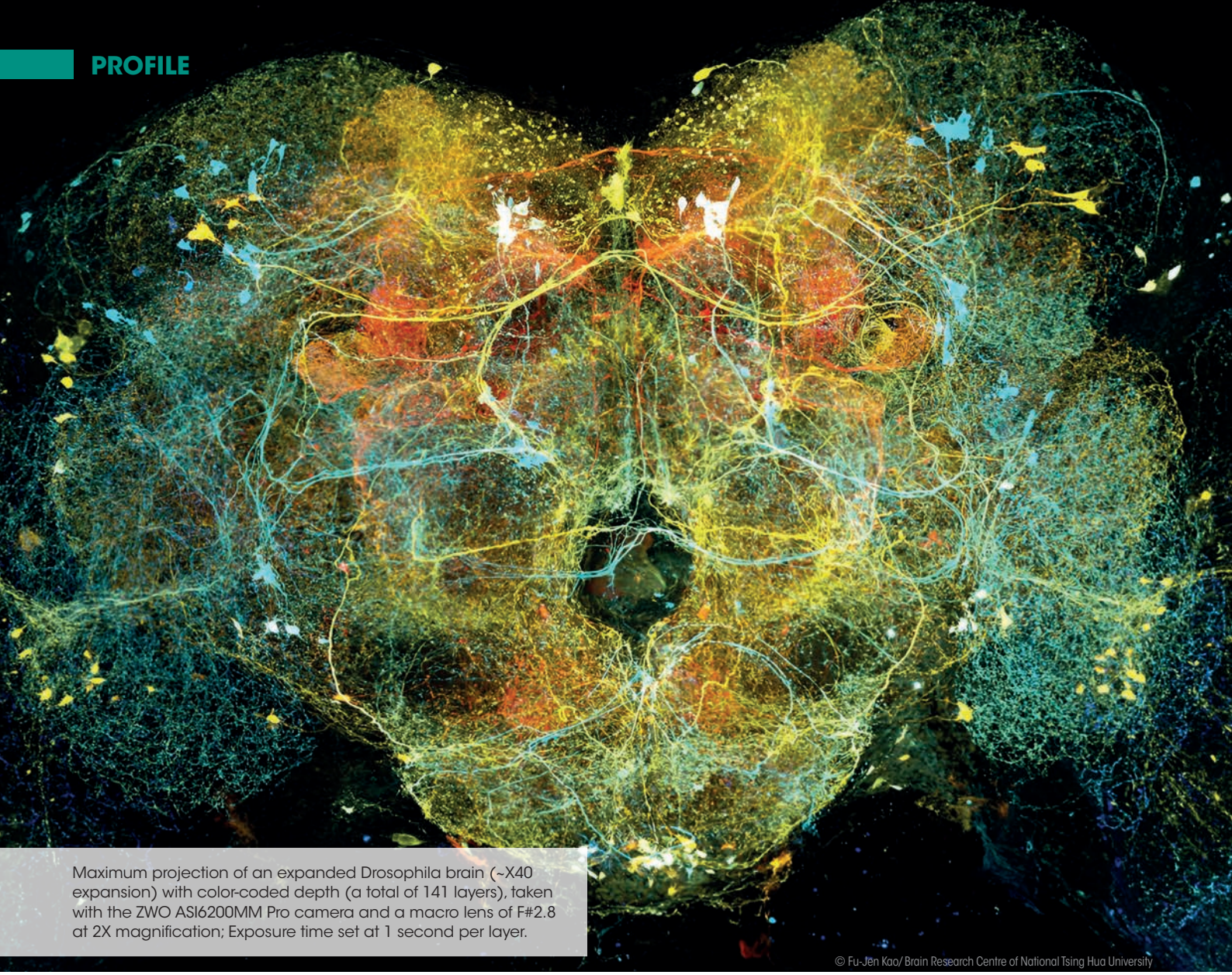
Besides sodium-ion batteries, what else does your research group work on at Imperial College London?

M. Titirici: We also study lithium-sulphur batteries, potassium-ion batteries, aluminium-graphite-dual ion batteries, hybrid supercapacitor-batteries-like devices, as well as supercapacitors. Besides batteries, the other side of my research includes 'Power to X' technologies where renewable electricity is used to convert small and abundant molecules into high value chemicals or fuels. I'm excited as I believe we have developed a new process with commercial potential. We started by putting a molecule derived from plastic waste, ethylene glycol, through our electrolyzer, to produce hydrogen and glycolic acid, used in cosmetics. Because of this, we've developed a new recycling process that derives ethylene glycol from low-grade polyethylene terephthalate (PET) plastic waste. (The process) doesn't use any chemicals, and at the same time isolates the main monomer, terephthalic acid, from the PET. So we can create three products for three markets simultaneously – terephthalic acid for plastics, hydrogen (for energy), and glycolic acid for cosmetics – but the challenge is that these are three markets that don't talk to each other.

You also lead the £1.6 million project, DIGBAT. What is this?

M. Titirici: Myself and colleagues at Imperial, alongside industrial partners and researchers from higher education institutions use robotic automation platforms to assemble and test battery electrodes and membrane electrode assembly electrocatalysts under real operating conditions in high throughput, to accelerate the development of sustainable battery technologies and Power to X technologies. A lot of research groups, worldwide, are moving in this direction, and it could be the start of a new era of research, where you maximize high throughput of experiments while eliminating errors from processes. We have a very short time to reach net zero, and my hope is that electrochemical labs around the world, working with these multi-user platforms, will share data workflows and collaborate closely. With the help of AI, we also hope to accelerate discoveries and bridge the gap between fundamental research and industrially-relevant processes. I'm very excited.

This interview was conducted by Dr. Rebecca Pool, freelance editor of Microscopy and Analysis.



Maximum projection of an expanded Drosophila brain (~X40 expansion) with color-coded depth (a total of 141 layers), taken with the ZWO ASI6200MM Pro camera and a macro lens of F#2.8 at 2X magnification; Exposure time set at 1 second per layer.

Fu-Jen Kao: Bringing Biophotonics to the World

Advanced optical microscopy pioneer, Professor Fu-Jen Kao, has revolutionized biophotonics in Taiwan and beyond. Here's how an early fascination with Maxwell's equations led to breakthroughs that have shaped biomedical imaging today.

When Professor Fu-Jen Kao was growing up, he loved Physics, especially electromagnetism. Fascinated by Richard Feynman's elegant explanation of Maxwell's equations - which form the foundation of electromagnetism and optics - the pioneer of myriad advanced optical microscopy methods wasn't immediately drawn to optics.

"At that time, I saw optics as mainly about optical path differences, which

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didn't excite me beyond imaging formation," he recalls. "Instead, astronomy, relying on advanced optical instruments, caught my attention as a fascinating frontier."

Without a doubt, very many researchers around the world will be thankful Kao later found his way to microscopy. In the last three decades, Kao has championed numerous laser-scanning and multi-photon microscopy techniques, including Asia's first two-photon second harmonic generation microscope, was instrumental in setting up Taiwan's first biophotonics institute, and thanks to his unwavering support for 'Focus on Microscopy' conference, has ensured his homeland retains a strong, international scientific voice. "It was only when I started working with ultrafast lasers that I realized optical science could itself be a research frontier – there have been so many opportunities," he says.

Early Days

Kao came from a family that valued education—his father was a surgeon

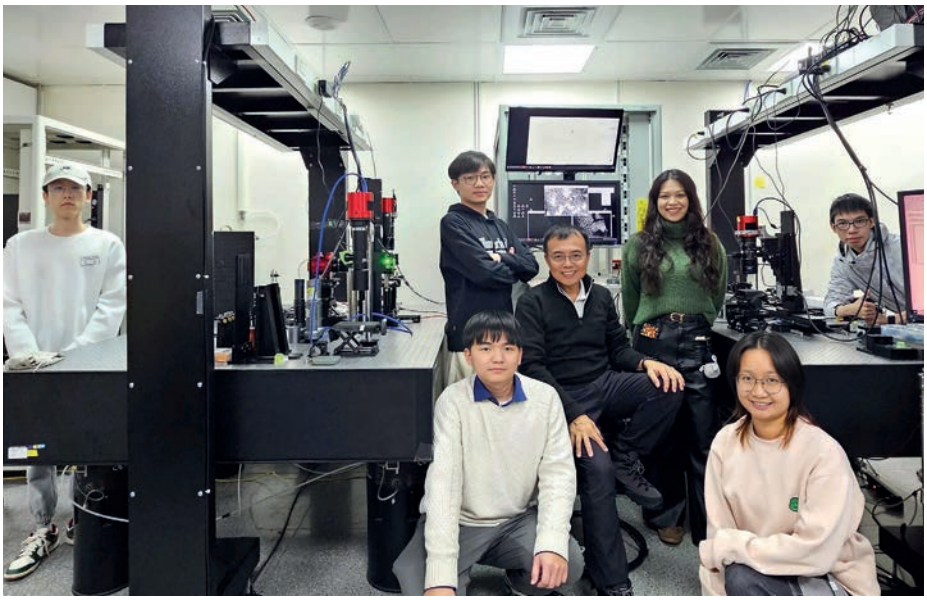
who considered knowledge and intellect to be of prime importance. As an undergraduate, he attended the National Taiwan University in Taipei, graduating with a Bachelor of Science in Physics in 1983.

"I was deeply influenced by Taiwan's transformative era at the time – we had not become a democracy, but our society was stable, and the economy was booming with double-digit growth rates," says Kao. "This was a time of intellectual enthusiasm – a feverish pursuit of new knowledge and technologies that created many opportunities."

On graduating, Kao completed two year's compulsory military service in the Republic of China Marine Corps and then embarked on a PhD at Cornell University. Here, Kao joined the research group of electron energy loss spectroscopy pioneer, Professor Wilson Ho, who was then a promising young faculty member with significant research funds – which Kao saw as a sign that he would be exploring cutting-edge topics. He was right.

In Ho's group, Kao used femtosecond lasers to investigate the ultrafast surface photochemistry of oxygen and carbon monoxide reactions on platinum (111) – critical for the petrochemical industry, which used platinum catalysts in refining reactions. Femtosecond lasers were a first for Ho's group. Fellow Cornell researcher, Andy Albrecht, had been using the devices to understand molecular vibrations, and according to Kao, Ho wanted to see how his group could use ultrafast lasers to study surface science.

"Building high-power femtosecond lasers was an exciting challenge since commercial options didn't yet exist," says Kao. "We had to construct our system from scratch using a liquid dye laser (Rhodamine 6G), as this was before the advent of Ti:sapphire lasers. I vividly remember the lab occasionally flooded with dye when the liquid circulation system malfunctioned."



© Fu-Jen Kao/ Brain Research Centre of National Tsing Hua University

Fu-Jen Kao team members in front of the Multiview Expansion Tomography setup.

Along the way, Kao also learned about nonlinear optics and nonlinear crystals from Chung Liang Tang's group, Frank Wise and Randy Ellington, and confocal microscopy from Watt W. Webb's group. "All of this hands-on and in-person experience taught me invaluable lessons about laser technology, nonlinear optics, experimental persistence, and critically, provided many scientific insights," he says.

Kao returned to Taiwan in 1993, securing a tenure at the National Sun Yat-sen University. It was a time of significant global shifts. The Soviet Union had fallen, China's economy was opening up, and democratization was taking place in Taiwan. Kao remembers a "strong sense of euphoria" as he, and many fellow researchers who had trained abroad, returned home. Still, as he also recalls: "There was no adequate research infrastructure – nothing."

Undeterred, the young researcher drew on the 'can-do American garage spirit' that had left an indelible mark on him whilst in the US. "Cornell had embodied this combination of risk-taking and doing things yourself, and it has guided me ever since," highlights Kao. "Sun Yat-sen didn't have the technical support of Cornell, so I went to factories and hardware stores, and built up my own infrastructure."

Remarkably, by 1998, Kao had developed the first two-photon and second harmonic generation (SHG) laser scanning microscope in Taiwan, and most likely Asia. The advent of mode-locked Ti:Sapphire lasers and frequency-doubled diode-pumped solid-state lasers had significantly eased ultrafast laser-based technology development while Olympus was promoting its laser scanning microscopy in Taiwan and loaned a system to Kao at a heavily discounted rate. For Kao, combining his lab-built ultrafast lasers with such an optical microscopy setup was a natural progression.

"I found the synergy between nonlinear optics and microscopy fascinating," he highlights. "Focused light under a microscope enhanced nonlinear optical effects, while nonlinear effects, in turn,

FOM 2025

Focus on Microscopy 2025 will take place in Taipei, Taiwan, from Sunday, April 13 to Wednesday, April 16. Fu-Jen Kao is one of the conference organizers, alongside Chi-Kuang Sun, NTU, Hans Gerritsen, University of Utrecht, and Fred Brakenhoff, Honorary member. This will be the 3rd FOM conference Kao has helped to organize in Taiwan. Very many topics are covered this year, ranging from instrumentation and microscope design, and clearing and expansion techniques to correlated microscopies and 3D image processing and visualization for multidimensional data. "I firmly believe that organizing conferences is one of the most effective ways to foster mutual learning and collaboration with the scientific community," highlights Kao. "Conferences are intellectual 'get-togethers' where new ideas, breakthroughs, and friendships are born."

www.focusonmicroscopy.org

improved spatial resolution and provided a broad range of non-labeling contrasts... opening up opportunities to study photonic devices – InGaN LEDs and laser diodes - as well as biological samples, including plant tissues."

Biophotonics Beginnings

Come 2004, Kao had moved to the National Yang-Ming University (NYMU), now National Yang Ming Chiao Tung University, in Taipei, attracted by its focus on biomedical research. He'd watched the rapid commercial development of semiconductor devices and realized more room for academic freedom existed in the biosciences. His feelings were confirmed on joining 'Focus on Microscopy', where he'd noted many fascinating bioscience applications emerging for researchers.

"I'd been promoted to full professorship at National Sun Yat-sen University and could have had a more laid-back life there – but then I thought, did I want to live my life that way?" says Kao. "No, I wanted to explore a different environment and different opportunities... I'd learned lasers from scratch – I could learn biomedical science from scratch too."

At NYMU Kao was instrumental in setting up Taiwan's first Institute of Biophotonics, reflecting the growing importance of light-based technologies in biomedical science – and here his instrumentation development and research flourished. He combined multiphoton microscopy with fluorescence lifetime imaging microscopy (FLIM), allowing himself and colleagues to monitor molecular metabolism and tissue regeneration in real-time – wound healing became a key application for his team.

As stimulated emission microscopy - pioneered by Nobel laureate Stefan Hell - gained traction, Kao and his team also began exploring stimulated emission-based FLIM and optical coherence tomography (OCT). They wanted to replace traditional incoherent fluorescence detection with coherent stimulated emission, enabling structural and lifetime imaging, and offering an alternative to TCSPC-based FLIM.

Along the way, Kao was always sure to transfer their technology developments to other research groups in Taiwan. "I thought the best way to increase our impact and accelerate biomedical studies in the region was to give our technology away... so we were always encouraging people to come to our labs and duplicate what we had built," he explains.

Fu-Jen Kao with Nobel Laureate, Eric Betzig, at Focus on Microscopy 2016, held in Taiwan.

Maturing Technologies

In the intervening years, confocal, multiphoton, and super-resolution microscopies matured commercially, and Kao's research group joined the Brain Research Centre of National Tsing Hua University. Now home to some 30 researchers – the team has pivoted towards addressing instrumentation limitations, most notably, field-of-view. The researchers now combine large-field macro lenses with expansion microscopy to image large samples, some several centimeters in size, at resolutions that rival electron microscopy.

"Using full-frame CMOS sensors, we further boost the efficiency of high-speed, high-resolution image acquisition – making our methodology applicable to diverse biomedical fields," points out Kao. "We've also explored endoscopy, which enables minimally invasive imaging and presents unique opportunities for optical modalities in clinical applications."

Looking to the future, Kao is excited about the opportunities that artificial intelligence will bring for advanced microscopies. "AI-driven image analysis, segmentation, restoration, and reconstruction can manage the massive datasets generated by new methods, improving accuracy, efficiency, and interpretation," he highlights. "In medical imaging applications, such as pathology, diagnostics, and surgical guidance, AI also enables real-time insights that were previously unattainable."

Kao also hopes that researchers can learn a lesson or two from how AI works. Highlighting how the technology coordinates very many computer processors to complete a task, he asks: "Why don't we actually coordinate ourselves more, and have more collaborations?"

"When I was young, the atmosphere amongst PIs was very competitive, but now I am seeing a shift to more cooperation with some competition," he says. "More coordination and collaboration will bring us forward in a very different way, and this will be good for us."

This profile has been written by Dr. Rebecca Pool, Freelance Editor.

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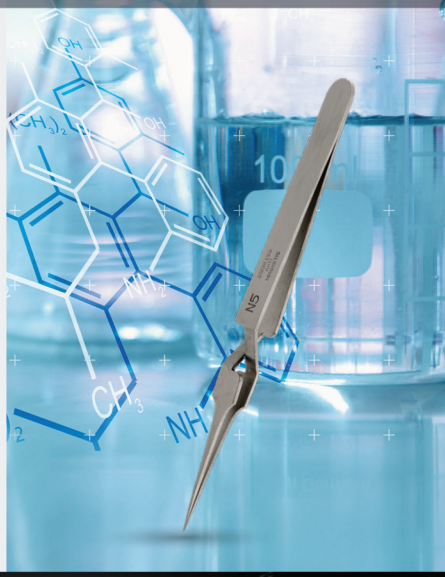

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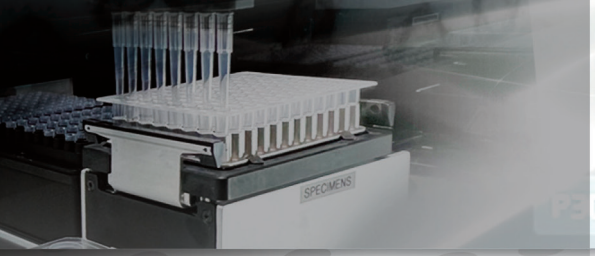

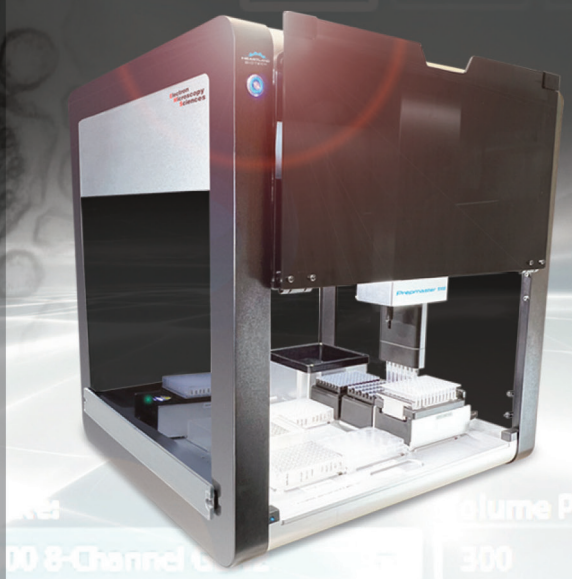

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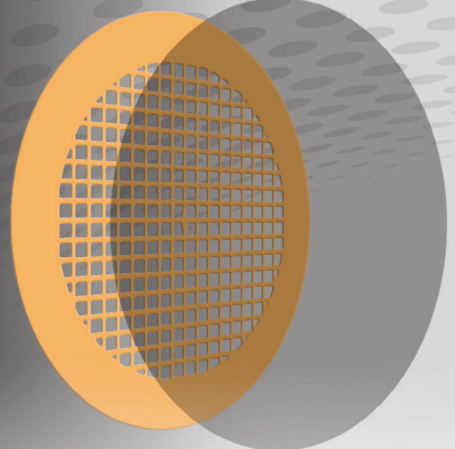

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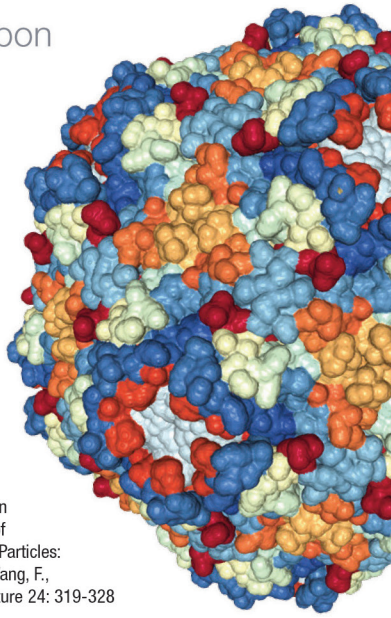

Right: 2.9 Angstrom Resolution Cryo-EM 3-D Reconstruction of Close-packed PCV2 Virus-like Particles: PDB ID: 3JCI Liu, Z., Guo, F., Wang, F., Li, T.C., Jiang, W. (2016) Structure 24: 319-328

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Expanding Magnetic Force Microscopy

New Technology Advances High-Resolution Magnetic Imaging

This article presents PeakForce magnetic force microscopy (PF-MFM), a novel MFM variant where the initial topographic scan is conducted in PeakForce Tapping mode. This seemingly simple swap conveys numerous benefits: superior spatial resolution, enhanced sensitivity, added data channels, and the ability to image delicate samples with greater precision. MFM capabilities can be further enhanced by combining PF-MFM with torsional resonance MFM (TR-MFM) for 3D information about the magnetic field.

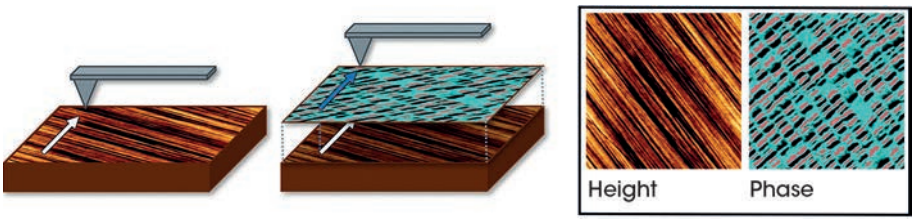

Figure 1: Schematic of MFM operation. A first pass establishes topography of the surface. The second pass is conducted at an established lift height and maps magnetic force gradient.

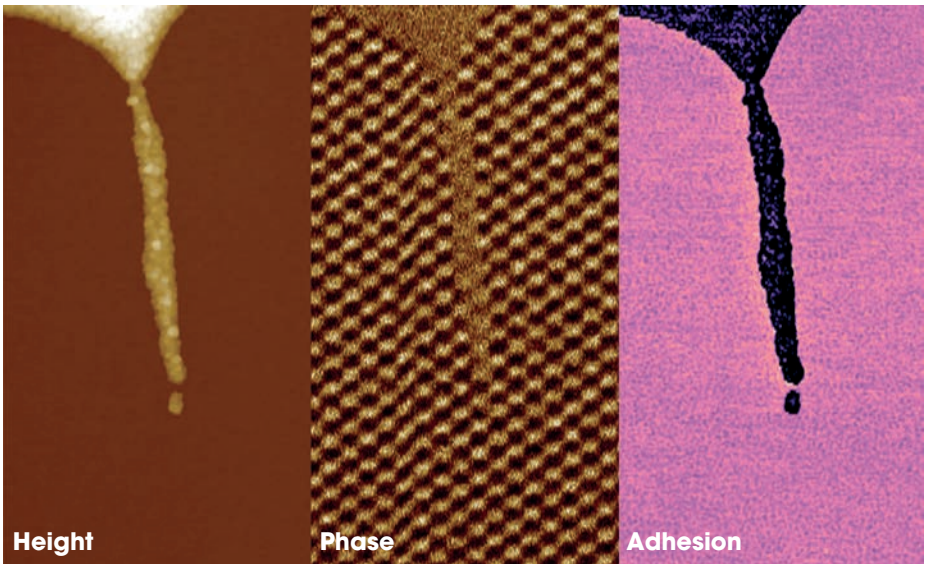

Figure 2: Cover image data; 20 TB HDD intentionally covered in the top right corner with ferrofluidic drops. Topography acquired during the first pass, and phase of the oscillating cantilever (representing the magnetic domains) acquired during the second pass (lift scan). Image size: 0.5x1 µm, lift height: 8 nm, oscillation amplitude: 10 nm. Sample courtesy of S. Montoya, University of California San Diego. Data collected on a Dimension Nexus AFM using ScanAsyst and a PFMFM-LM probe.

Mapping magnetic field distribution with high spatial resolution can be achieved using magnetic force microscopy (MFM). MFM is an atomic force microscopy method widely used in materials and life sciences, e.g., for thin films, patterned magnetic media, multiferroics, and magnetic nanomaterials.

MFM is typically performed in two passes, as shown in Figure 1: one for measuring topography and the other for measuring magnetic field gradients. Traditionally, the first pass uses tapping mode.

On the second pass (lift scan), the tip is maintained at a constant height above the sample. The magnetic force gradient is observed using the amplitude and phase of a cantilever driven at or near a resonant frequency. During this lift scan, the probe only experiences long-range magnetic (and electrostatic) interactions, not van der Waals forces. This enables the separation of topographic and magnetic signal contributions.

During the lift scan, the tip usually oscillates perpendicular to the sample plane, probing the vertical contributions to the magnetic field. An in-plane oscillation can be used to instead gather information about the lateral contributions to the magnetic field. This is called torsional resonance MFM (TR-MFM).

PeakForce MFM

In PeakForce magnetic force microscopy (PF-MFM), the first pass is conducted in PeakForce Tapping mode instead of tapping mode. PeakForce Tapping features direct force control and can operate at lower forces, resulting in higher spatial resolution, the ability to image fragile samples, and extremely long tip lifetimes.

PeakForce Tapping also uniquely enables the simultaneous acquisition of quantitative nanomechanical property data for adhesion, modulus, deformation, and dissipation. By conferring these property mapping capabilities onto MFM, PF-MFM enables the correlation of magnetic and mechanical property measurements at the nanoscale. The cover image (and Fig.2) showcase this data correlation as well as the high spatial resolution, resolving domains <30 nm on a 20 TB hard disk drive (HDD).

Probe Optimization

Resonance behavior of the cantilever is irrelevant for PeakForce Tapping, meaning that a broad range of cantilevers are compatible with the mode. This probe flexibility (granted also to PF-MFM) is a

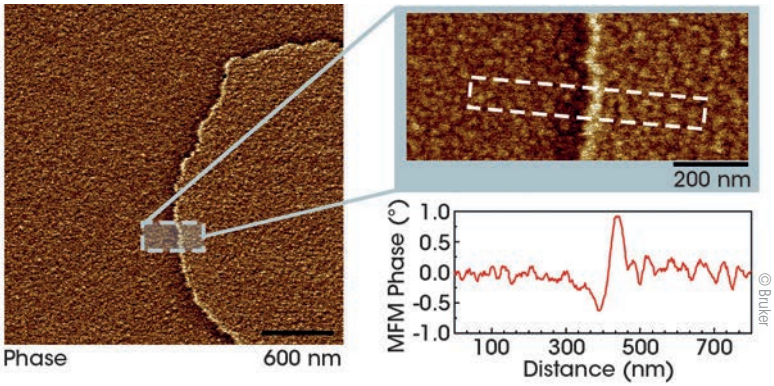

Figure 3: Perpendicular magnetization multilayer [Pt/Co/Pt]x5 domain walls. (left) MFM phase image (scan size 3x3 µm) and (right) phase data across the interface shows high sensitivity and spatial resolution. Sample is courtesy of K. Bouzehouane, Université Paris-Saclay, Thales.

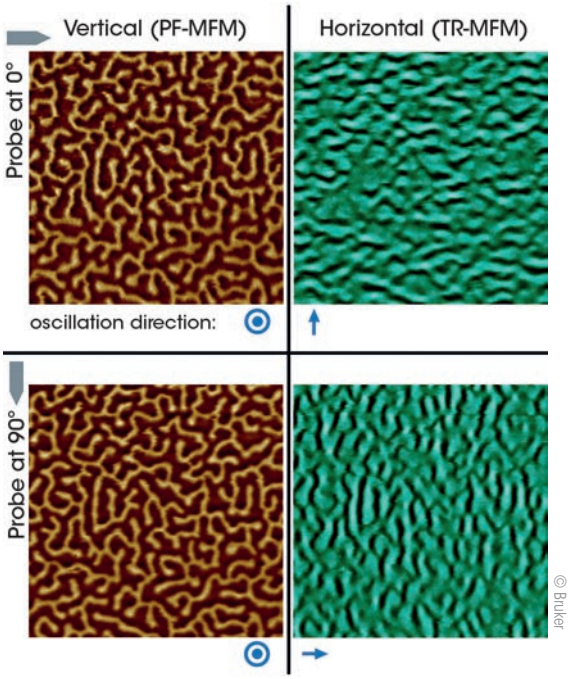

Figure 4: PeakForce MFM with vertical (left column) and lateral (right column) magnetic domain imaging. A torsional resonance was applied during the lift scan to acquire the lateral field images. In the top row, the probe was oriented at 0°. Upon rotating the sample 90°, the vertical field remains the same, but the lateral field rotates. Tip oscillation direction is indicated beneath each image. Scan size 3.8x3.8 µm. Sample is perpendicular magnetization multilayer [Pt/Co/Pt]x25. Sample is courtesy of K. Bouzehouane, Université Paris-Saclay, Thales.

critical advantage for nanoscale magnetic measurements since it increases design freedom for mode-optimized probes.

Cantilever sensitivity to phase or frequency variations during the lift scan of MFM scales directly with the cantilever's quality factor and detection frequency, and inversely with its spring constant. One way to optimize probe performance for PF-MFM would therefore be to reduce the cantilever spring constant, as was done with the PFMFM-LM probe. This probe has reduced cantilever thickness and width, with a paddle shape to accommodate conventional laser spot sizes.

With a PFMFM-LM probe, sensitivity in the lift pass was increased by three times for phase and by twelve times for frequency. Increased sensitivity allows operation at lower oscillation amplitudes and lift heights, leading to higher spatial resolution and higher throughput.

Increased sensitivity also enables the use of a magnetic coating with lower magnetic moment, since low magnetic moment reduces signal. The low-magnetic-moment coating can be thinner, leading to higher spatial resolution. Thinner coatings also prevent tip-induced magnetization changes on sensitive samples. Figure 3 shows a Pt/Co/Pt multilayer sample imaged using PF-MFM, illustrating the high sensitivity and spatial resolution of this mode.

Magnetic Field in Three Dimensions

Vertical cantilever oscillation is used for conventional and PeakForce MFM. This vertical oscillation is replaced by a horizontal oscillation in TR-MFM, resulting in selective detection of the components of the magnetic field gradient that are in the plane of the sample. In-plane measurements can be conducted with the same resolution and signal-to-noise ratio as flexural modes at the same location.

Obtaining both components of the force gradient leads to a more complete understanding of the force and its gradient source. Figure 4 is an example of co-localized vertical and lateral magnetic field measurements using a combination of PF-MFM and TR-MFM, providing insight into the field in all three dimensions. Lateral TR-MFM images at 0° and 90° probe orientation show a directional influence that is absent in the vertical magnetic field.

Optimizing Magnetic Force Microscopy

Replacing the traditional tapping mode with PeakForce Tapping on the first pass of MFM enables researchers to achieve superior spatial resolution, enhanced sensitivity, and the ability to image delicate samples. Probe freedom offered by PF-MFM permits the design of mode-optimized probes with reduced spring constants and better magnetic coatings. MFM capabilities can be further enhanced by combining PF-MFM with torsional resonance MFM (TR-MFM) for a three-dimensional understanding of the magnetic field. These and other technological advances are making magnetic force microscopy a powerful tool for nanoscale magnetic characterization.

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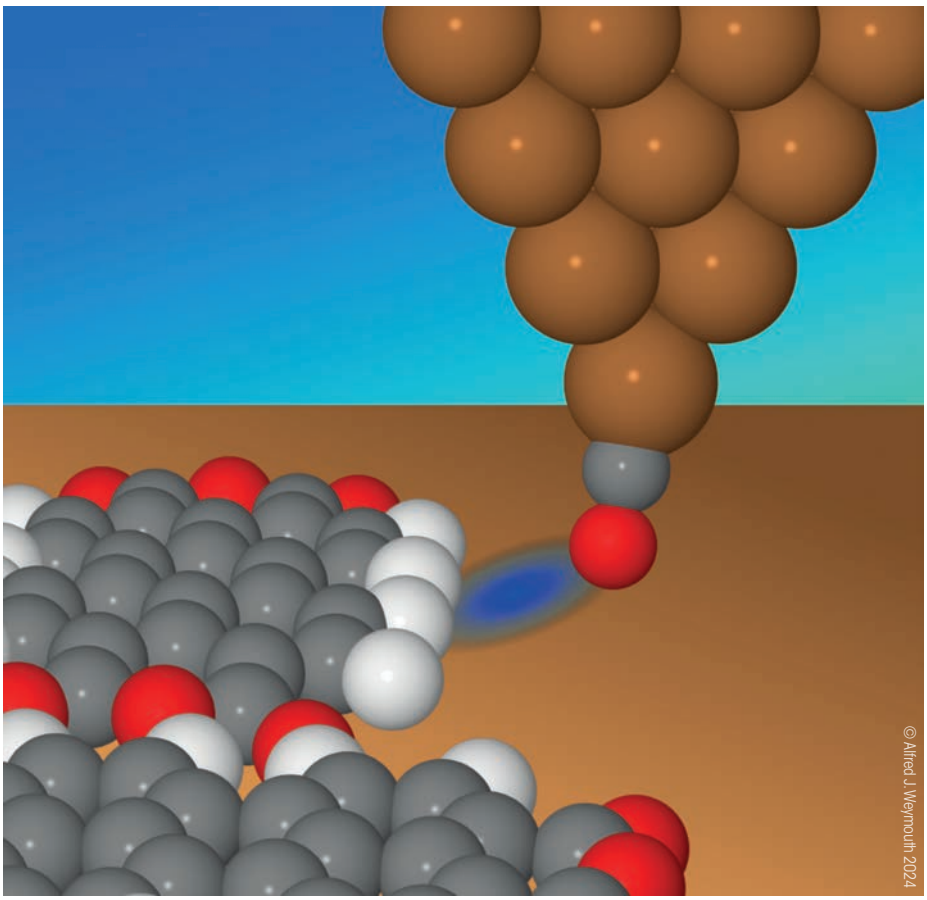
Lateral Force Microscopy to Study In-Plane Interactions Next to Adsorbed Molecules

Imaging of Hydrogen Atoms and Quantification of Lateral Interactions

Shinjae Nam[1], Alfred John Weymouth[1], Franz Josef Giessibl[1]

Lateral Force Microscopy (LFM), a variant of Atomic Force Microscopy (AFM), is well-suited for quantifying in-plane forces near an adsorbed molecule. Understanding these forces is crucial for understanding processes like adsorption, friction, and molecular self-assembly. Approaching closer to the sides of adsorbates requires careful consideration of the interaction with the tip. Notably, LFM enables the direct imaging of hydrogen atoms at the sides of planar molecules, despite their small size, by detecting lateral forces and short-range interaction with unprecedented resolution [1]. This capability is particularly significant for studying hydrogen bonds, where the presence and position of hydrogen atoms influence molecular interactions. The findings demonstrate LFM's potential to enhance the understanding of in-plane molecular interactions, paving the way for advancements in nanotechnology and materials science.



Introduction

In-plane molecular interactions, which can involve hydrogen bonding and van der Waals interactions, are crucial for adsorption, friction, and molecular self-assembly on surfaces. Frequency-modulation AFM (FM-AFM), which conventionally involves vertical oscillation of the tip, has been successfully applied to study in-plane interactions by deconvoluting the Δf signal of the 3D data set and integrating it to obtain potential energy, which can evaluate the lateral force between the tip and the sample. However, it is an indirect method due to the direction of the tip oscillation and is less sensitive to in-plane interactions. By modifying the AFM sensor to oscillate laterally, LFM becomes more sensitive to lateral force components, making it ideal for probing short-range in-plane interactions.

A notable advancement in AFM image resolution is the use of CO-functionalized tips, which allow for the direct imaging of internal molecular structures [2]. However, directly imaging hydrogen atoms within the locally polarized flat-lying molecule using this method has been challenging due to their small size. Recent LFM advancements have overcome these challenges, enabling the direct observation of H-atoms [1]. LFM was used to study a planar organic molecule adsorbed on Cu(111) and successfully observed each H-atom beside the molecule via their repulsive signature, using a CO-tip at imaging heights lower than those typically used in standard AFM.

To interpret any force microscopy data, a quantitative understanding of the tip is required. The CO-tip is often modeled by including CO bending with radially symmetric models of atomic interactions. At lower heights, interactions from the metal background have a much stronger effect and can be observed in the data.

This study explores the limitations of radially symmetric models (e.g., Morse potential) to establish a framework for understanding in-plane interactions. By characterizing the interaction between a

CO-tip and a single adsorbed CO molecule, we go beyond what can be measured with normal-force FM-AFM and develop a metric to assess the validity of these models as a function of tip height.

Materials and Methods

Several experimental methods have been introduced for performing frequency-modulation lateral force microscopy, which is referred to as LFM in this manuscript [3–5]. The first demonstration of LFM using a qPlus sensor with simultaneous force and current (STM) measurement dates back to 2002 [4], but significant progress has been made in noise reduction, tip quality, and modeling over the past years. The cantilever's high stiffness enables the control of oscillation amplitudes below one Angstrom, which enhances spatial sensitivity.

We collect LFM data on a low-temperature (He-bath) STM/AFM system manufactured by CreaTec Fischer & Co. GmbH. Figure 1 illustrates the building procedure of the LFM sensor. In Figure 1A, the quartz oscillator is shown. The middle contact corresponds to the STM channel, where a gold wire is attached using conductive epoxy glue (EPO-TEK H20E). As shown in Figure 1B, a heating plate is used under the microscope, and the gold wire is held in place with tweezers for precise micro-alignment, followed by immediate heating to harden the epoxy. This procedure is repeated for each gold wire, including those for the AFM and STM patches. To prevent electrical contact between the wires, non-conductive epoxy is applied over the conductive glue after it hardens.

Once the wires are attached, the entire sensor is mounted onto the home-built sensor holder, as shown in Figure 1C, using non-conductive epoxy glue (EPO-TEK H70E). The sensor holder is cleaned with acetone and isopropanol in an ultrasonic bath to remove contaminants and ensure proper adhesion of the glue. The tungsten wire, which will serve as the tip, is attached and electrochemically etched. We start with a longer wire and use the etching station provided by CreaTec Fischer, shown in Figure 1D. We used a 2M density of KOH as the etching solution. After etching, the tip is rinsed with diluted water and checked under the optical microscope. Tungsten is chosen as the tip material because of its high stiffness. This is important because extending the oscillating arm leads to a decreased effective spring constant, and a soft tip wire (because of material and/or geometry) can lead to an overall sensor stiffness that is too low.

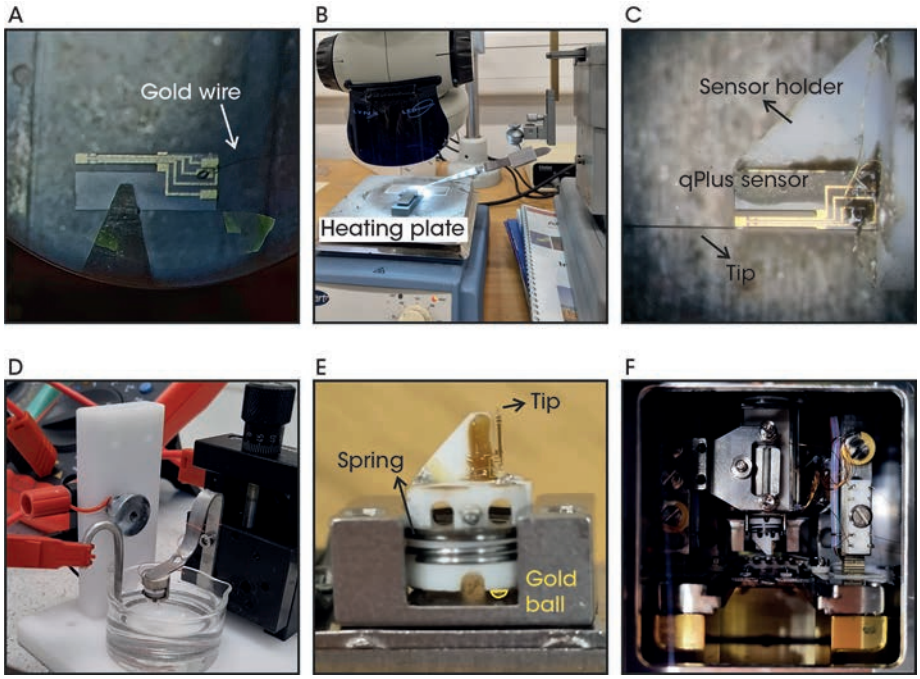


Figure 1: Assembly procedure of the LFM sensor. (A) The quartz oscillator with the STM channel contact, where a gold wire is attached using conductive epoxy glue. (B) Micro-alignment of the gold wire under a microscope with tweezers. The alignment was done over the heating plate, allowing direct heating without moving the component after alignment. (C) The sensor is mounted onto a custom-built holder using non-conductive epoxy. The white part is a custom-designed sensor holder made from macor. (D) Electrochemical etching of the tungsten wire, using 2M KOH solution, to sharpen the tip. The etching station includes a container for the etching solution, a ring-shaped cathode, and a sensor-holding part connected to the anode. During the electrochemical etching process, monitoring the decreasing current flowing through the solution and tip ensures the etching process. (E) The sensor holder is assembled with a spring for mounting. The three gold balls underneath the holder are electrically connected to the sensor's contacts through insulated copper wires. (F) The completed LFM sensor is positioned in the microscope head.

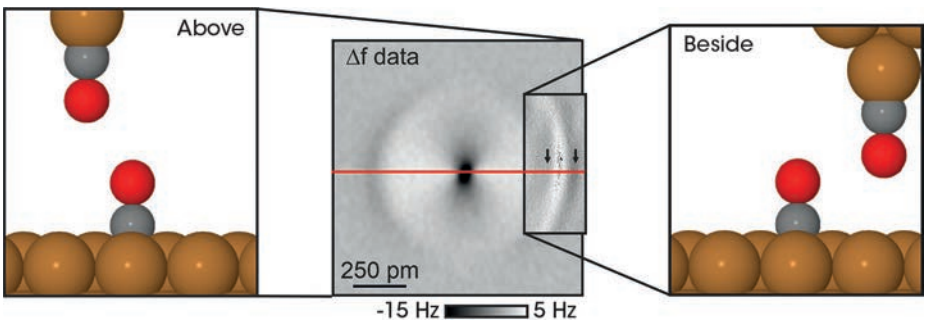


Figure 2: LFM data acquired both above and beside a single CO adsorbate on Cu(111) using a CO-tip. The tip oscillation direction is horizontal, along the red line. Black arrows indicate the additional dip beside the molecule. The oscillation amplitude was set to 50 pm, with a bias voltage of zero, operated in constant height mode.

The sensor holder assembled with a spring for mounting is shown in Figure 1E. It is essential that the spring is stiff for the quality factor of the sensor. Insulated copper wires are connected from the top to the bottom of the holder and are connected to the gold balls, which will be electrically connected to the sensor patches. After each assembly step, the conductivity is tested to confirm that the electrical pathways are intact.

Figure 1F shows the LFM sensor in the microscope head. To calibrate the amplitude accurately, we use the STM channel and collect data both without and with the tip oscillating above a local surface feature [5]. The amplitude is then determined by fitting the simu-

lation output to the data obtained with oscillation, as detailed in the referenced work. Currently, the sensor design fixes the oscillation direction and cannot be altered during operation. Changing from normal FM-AFM measurements to LFM is straightforward: The only modification required is the sensor. All other components including the actuator for oscillating the sensor and the detection electronics remain unchanged.

Data Collection and Analysis

Quantitative Understanding of the CO Tip Using Adsorbed CO on the Surface

Figure 2 shows data above and beside a single CO adsorbate on a copper surface with a CO tip, demonstrating the additional insight gained when approaching a surface. The tip oscillation direction is horizontal, along the red line. Both images are mirror-symmetric vertically and horizontally.

Besides the molecule, there is an additional dip indicated by black arrows. Calculations show that this additional dip originates from the electrostatic interactions between the metal tip and the adsorbate. It also indicates that surface dipoles of weakly polarized adsorbates can be investigated by acquiring LFM data closer to the surface.

Summary

Lateral Force Microscopy can directly investigate in-plane interactions with atomic precision, offering detailed insights into the forces beside adsorbed molecules on surfaces. The sensor-building procedure is straightforward and similar to a conventional qPlus sensor. There are established methods for calibrating the amplitude and stiffness of the sensor. Finally, we showed an example of a system that reveals new features when data are collected below the standard imaging heights.

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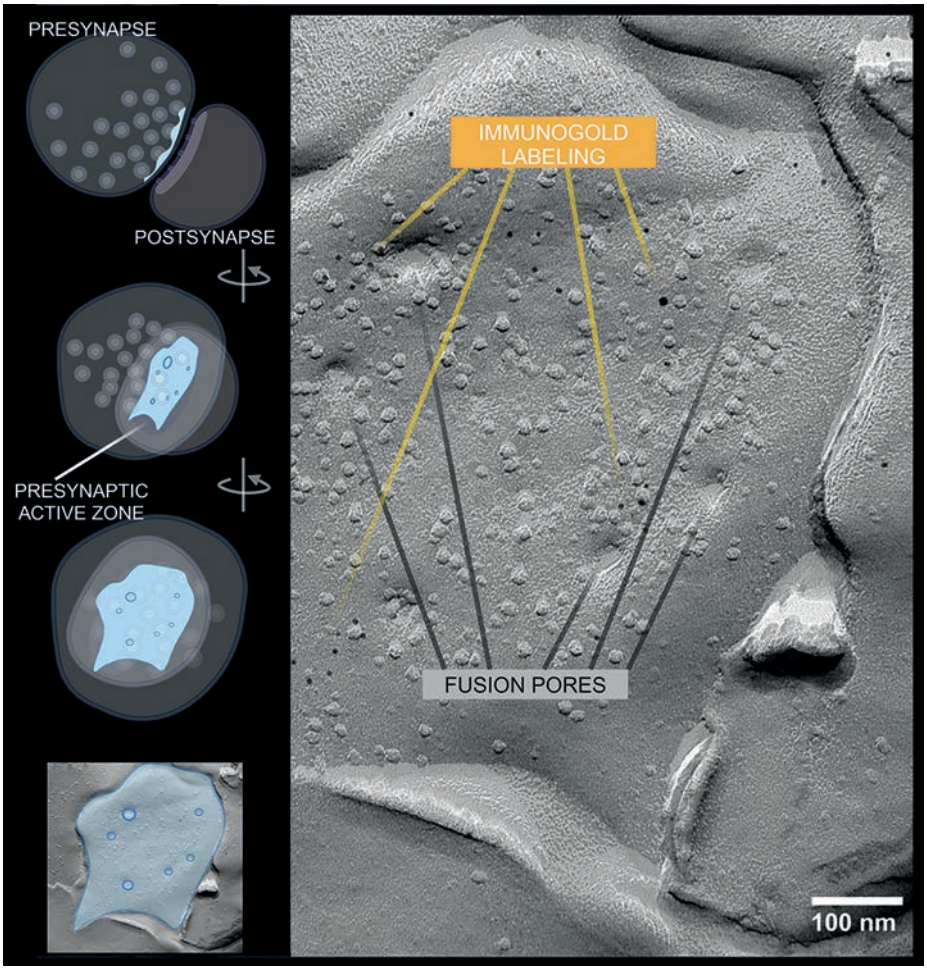
Flash and Freeze-Fracture Captures Fusion Moment

Nanometer-Scale Dynamics in Synaptic Transmission

Peter Koppensteiner[1] and Ryuichi Shigemoto[1]

The cell membrane is a fluid lipid bilayer that separates the intracellular and extracellular space and enables controlled interaction with the environment through dynamic processes, including endo- and exocytosis. This requires an intricate interplay of transmembrane, membrane-associated, and cytosolic proteins. When neurons communicate through synaptic connections in the brain, synaptic vesicle (SV) exocytosis occurs within milliseconds of a stimulus, presenting a major challenge for studying activity-dependent molecular and structural changes during neurotransmitter release. In our "Flash and Freeze-fracture" technique, we combine optogenetic stimulation of neurons with timed high-pressure freezing, followed by freeze-fracture replica immunolabeling and transmission electron microscopy. Our method captures molecular and structural dynamics snapshots during neurotransmission that occur on millisecond and nanometer scales.



Introduction to Freeze-Fracture Replication: From the 1960s to Today

Freeze-fracture replication is a method first described in 1966 and involves the breaking of a frozen biological sample under high vacuum, thereby separating the inner and outer leaflets of the lipid bilayer, followed by coating of the broken surface with carbon and platinum to create a "plaster print"-like replica [1]. Upon fracturing, proteins contained in the membrane (integral membrane proteins) are allocated to either the cytoplasm-facing leaflet (P-face) or the extracellular space-facing leaflet (E-face). In 1995, the method was first combined with the digestion of cells/tissues by a detergent, sodium-dodecyl-sulfate (SDS), and immunogold labeling to visualize integral membrane proteins [2], which ten years later was used in brain samples for quantitative analysis of synaptic proteins [3]. Recently, a combination of optogenetics and timed high-pressure freezing of living neurons, termed "Flash and Freeze", provided unprecedented insights into structural processes within milliseconds of neuronal activity [4]. Although "Flash and Freeze" is a powerful method, molecular investigations in the context of structural changes were not possible. Taking advantage of recent technical advances, we combined "Flash and Freeze" with freeze-fracture replica immunolabeling, allowing the visualization of structural and molecular dynamics at the highest temporal and spatial resolution [5]. Thus, "Flash and Freeze-fracture" is similar to the freeze-fracture method combined with quick freezing used by Heuser and Reese to study activity-dependent changes in the frog neuromuscular junction [6,7]. As a major advantage over the Heuser and Reese method, Flash and Freeze-fracture is more broadly applicable to brain slices combined with stimulation of specific cell types and immunolabeling.

The Flash and Freeze-Fracture Method

The concept of Flash and Freeze-fracture involves four principal steps (Fig.

1). First, 200-µm-thick living brain slices are prepared from mice expressing light-sensitive ion channels in specific neuronal cell types, and the brain region of interest is trimmed out. Following a recovery period in artificial cerebrospinal fluid (ACSF), each brain slice is briefly (3 – 5 min) incubated in a cryoprotective solution (15% polyvinylpyrrolidone in ACSF). Second, the brain slice is placed in the center of a ring of double-sided tape on a 6 mm metal carrier, and a 6 mm sapphire disk is tightly pressed on top of the tape, creating a closed compartment containing the brain slice. This metal-sapphire hybrid sandwich is then inserted into the high-pressure freezer (Leica EM ICE) and optogenetically stimulated, followed by rapid cryofixation at a user-defined time. Third, the frozen sandwich is then placed into the freeze-fracture device (Leica EM ACE900). After vacuum stabilization at 10-7 – 10-8 bar, a razor-type knife is moved through the double-sided tape between the sapphire and the metal carrier, fracturing the brain slice in the process. In the subsequent replication step, a 5 nm carbon layer at a 90° angle, a 2 nm platinum layer at a 60° angle, and a 20 nm carbon layer at a 90° angle are formed by Pt/C evaporation onto the fractured brain slice. Fourth, after taking the sample out of the machine, the tissue is digested in an SDS solution at 80°C, and immunogold labeling is performed using gold-conjugated antibodies of distinct gold particle sizes (typically 2 – 20 nm gold particle diameters). Immunogold-labeled replicas are then observed in a transmission electron microscope.

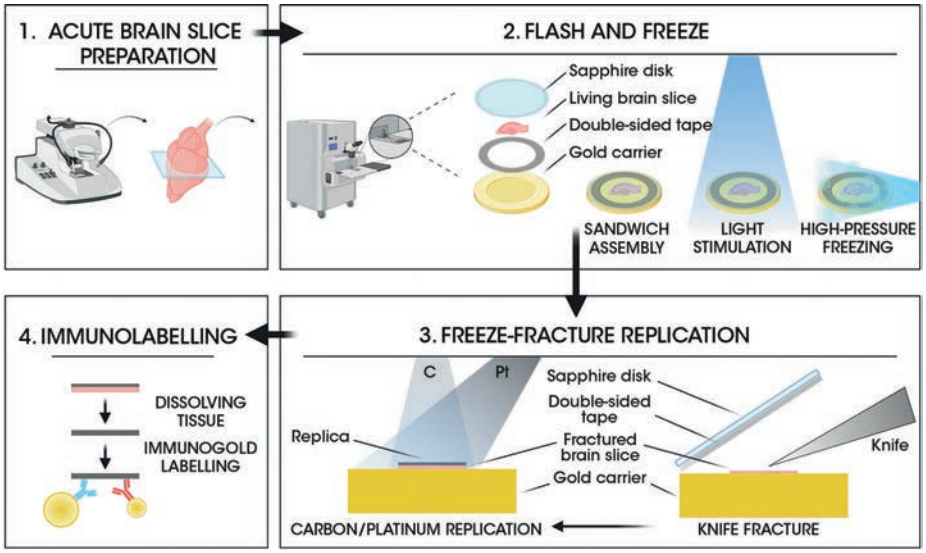


Figure 1: Step-by-step overview of Flash and Freeze-fracture method. Step 1: Acute brain slice preparation with a vibrating microtome. Step 2: Metal-sapphire sandwich preparation, optogenetic stimulation, and high-pressure freezing. Step 3: Knife fracture and freeze-fracture replication. Step 4: Dissolving tissue with SDS and immunogold labeling.

The Importance of Timing in Neurotransmission and Remaining Issues

Animals respond rapidly to stimuli because their brain cells are fine-tuned to transmit information within milliseconds of an electrical signal. To enable this, neurons form physical connections at specialized points, called synapses. At these synapses, the presynaptic side contains many round membrane compartments called synaptic vesicles (SVs, ~40 – 50 nm diameter) that contain neurotransmitters (Fig. 2A). These SVs fuse with the presynaptic membrane and release their contents into the synaptic cleft to activate or inhibit the postsynaptic target neuron. SV fusion is believed to occur less than 1 ms after calcium influx caused by membrane depolarization of the presynaptic axon terminal. Whether SV fusion results in a complete collapse of the vesicular membrane or a reversible kiss-and-run style event is still controversial. Following SV exocytosis and transmitter release, the SV membrane and its associated SV proteins remain in the presynaptic membrane until their reuptake via endocytosis. It is unclear if the collapsed SV membrane and the proteins contained therein retain a raft-like configuration or if the SV components dissociate upon collapse, followed by lateral diffusion before the endocytosis. In the case of ultrafast endocytosis [4], this process may take only 50-100 ms, whereas clathrin-mediated endocytosis requires several seconds [8]. Once endocytosis is complete, SV-proteins are located on clathrin-coated SVs and later in endosomal compartments. Whether ultrafast endocytosis contains SV-associated proteins remains elusive.

Compared to fast electrical stimulation, optogenetic stimulation delays SV exocytosis due to additional steps in the cascade of events triggering neurotransmitter release. Specifically, blue light first causes the opening of channelrhodopsin2 (ChR2), a non-selective cation channel, which depolarizes the membrane. This depolarization then leads to the opening of voltage-gated calcium channels, which triggers SV fusion. Importantly, the delay in light-triggered exocytosis may be advantageous when aiming to capture these transient, sub-millisecond structural representations of SV fusion with high-pressure freezing. However, the exact timings between light stimulation and SV exocytosis might be variable between synapses due to differences in ChR2 expression, differences in distances between ChR2 and calcium channels, as well as differences in excitability between terminals. Thus, the best timepoint for freezing after optogenetic stimulation must be determined individually for each brain region and preparation. In freeze-fracture replicas, the structural correlates of exo- and endocytosis might be readily distinguished by the presence of cross-fracture or intramembrane particles in the pore region (Fig. 2B – D) [6]. The molecular identity of these intramembrane particles in and around the pore regions should be examined to define these profiles.

Conclusion

We developed Flash and Freeze-fracture to visualize SV fusion and insertion of SV proteins into the active zone during distinct modes of neurotransmitter release [5]. We chose 8 ms after light onset as the freezing point to achieve full SV fusion and maximal SV protein insertion. An important goal for future developments of Flash and Freeze-fracture will be fine-tuning stimulation and freezing time points to capture precise moments of vesicle fusion and endocytotic events reliably. Establishing these

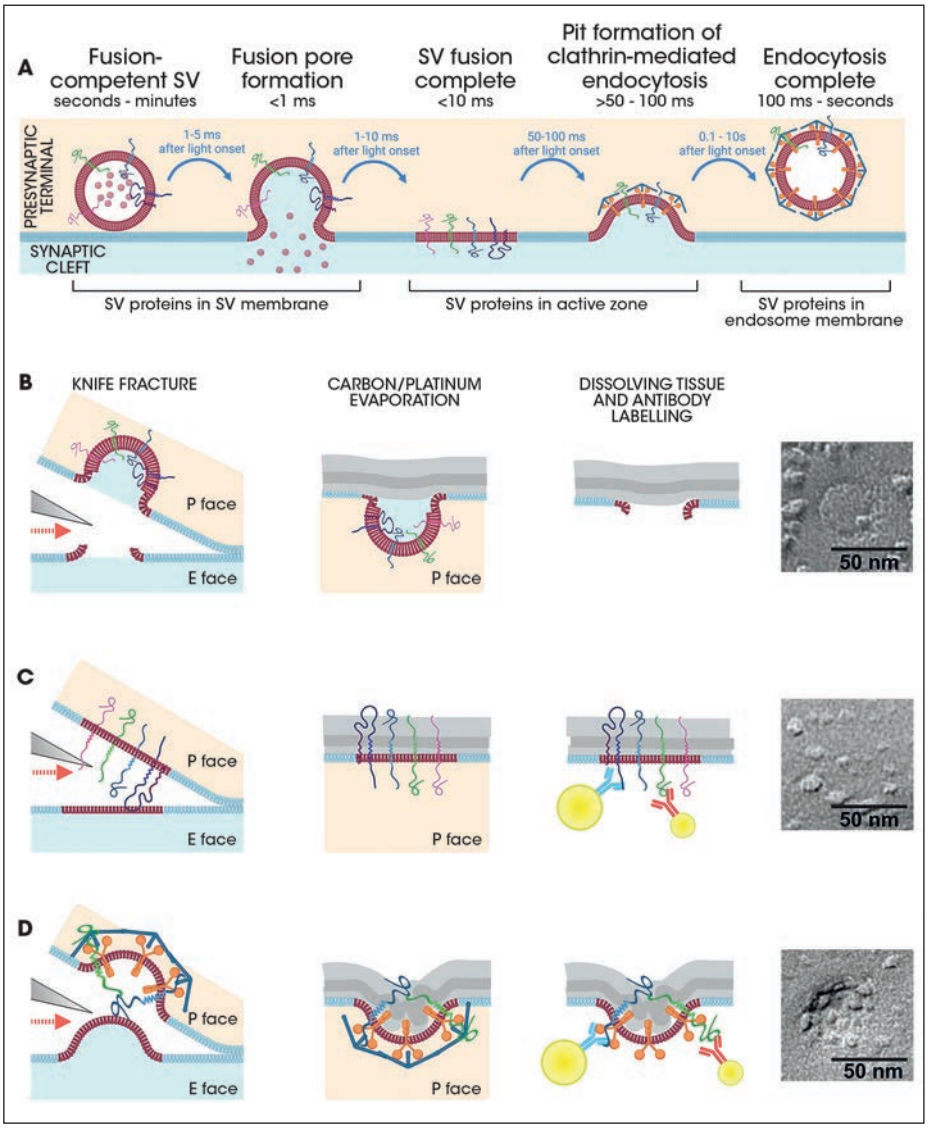


Figure 2: Timing of neurotransmitter release and freeze-fracture replica equivalents. A Timing of distinct steps in the neurotransmission process. SVs docked at the active zone are stable for seconds to minutes. Stimulation-induced SV fusion occurs within 1 ms. SV collapses, and its proteins appear in the active zone within 10 ms. The reuptake of SV and its proteins starts >50 ms and is complete 50 ms - 100 ms after SV fusion (for ultrafast endocytosis, not shown) or within several seconds (for clathrin-mediated endocytosis). Only clathrin-endocytosis is depicted for simplicity. Distinct vesicular membrane proteins are indicated with four colors. Clathrin and adaptor protein 2 complex with SV proteins are indicated with dark blue and orange, respectively. B – D Configuration of fracture planes of different SV states, including exocytosis (B), full collapse (C), and clathrin-mediated endocytosis (D). Example images of each type of fracture without antibody labeling are depicted on the right.

conditions will enable the investigation of activity-dependent nano-anatomical and molecular dynamics of neurotransmission in unprecedented detail. Among the many unknown aspects that could be investigated with Flash and Freeze-fracture are 1) locations of release machinery proteins and calcium channels relative to SV fusion sites, 2) changes in the activity-dependent molecular composition of synapses after physiological learning and under disease conditions, and 3) differences in SV fusion between spontaneous and evoked neurotransmitter release.

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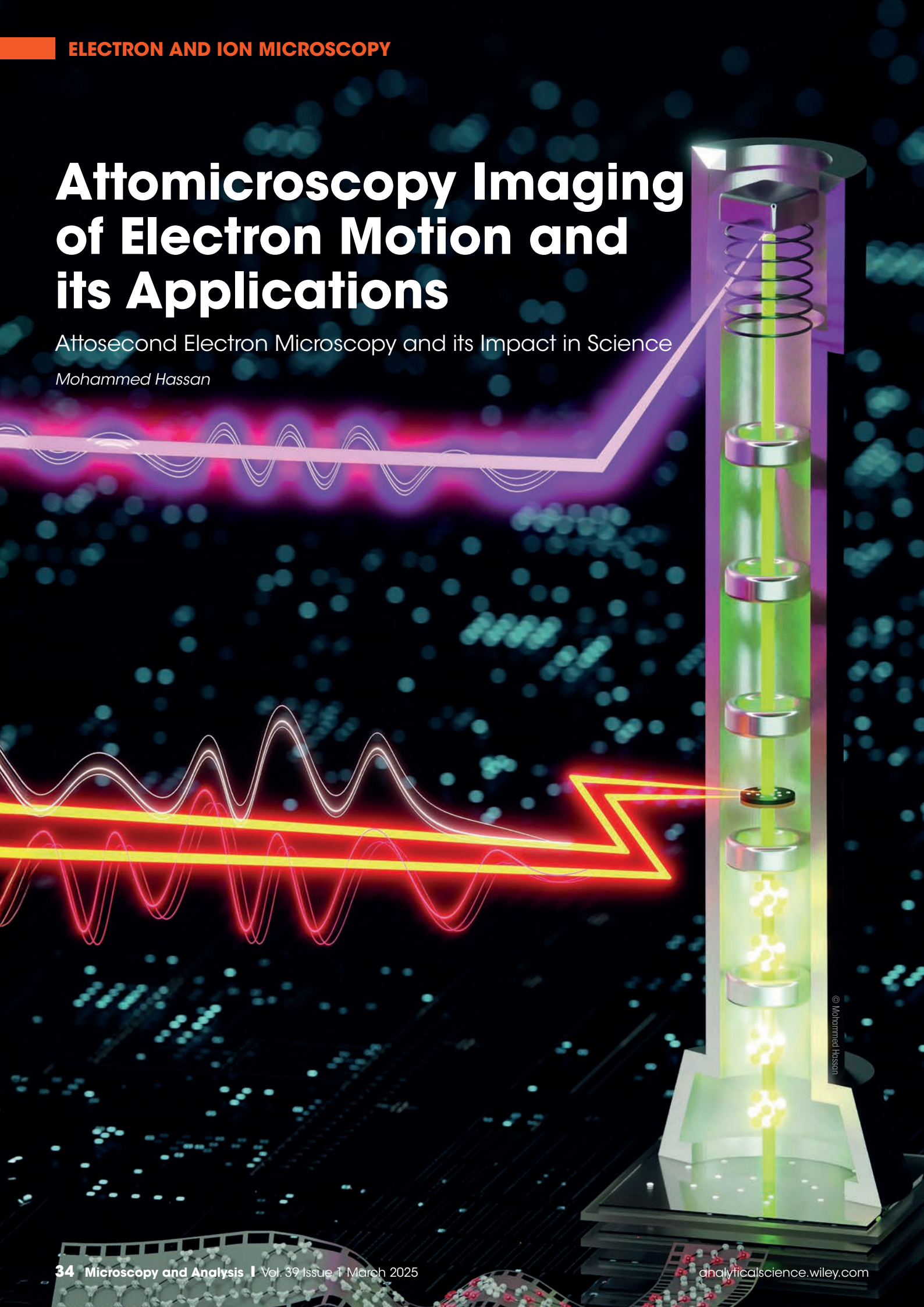
Attomicroscopy Imaging of Electron Motion and its Applications

Attosecond Electron Microscopy and its Impact in Science

Mohammed Hassan



The development of ultrafast electron microscopy (UEM) has enabled real-time imaging of atomic motion across a variety of scientific disciplines, including chemistry, physics, materials science, and biology. However, the temporal resolution of conventional UEM, typically in the femtosecond range, has limited its ability to capture the rapid dynamics of electron motion. In this work, we overcome these limitations by employing a novel optical gating technique to generate single attosecond (625 as) electron pulses. These pulses are produced by gating the electron wavepacket with a polarization-gated half-cycle laser pulse. The resulting attosecond pulses enable time-resolved diffraction measurements, allowing us to visualize electron dynamics in graphene for the first time. This achievement, realized in the "attomicroscope," sets the stage for new applications in electron imaging, ushering in a new era of attomicroscopy.

Introduction

The transmission electron microscope (TEM), since its invention in 1932 [1], has become one of the most powerful tools in scientific research. TEM has provided detailed structural imaging of materials and biological specimens and has been pivotal in revealing intricate atomic and molecular structures. Recent advancements in electron optics, such as electron energy loss spectroscopy and cryo-TEM, have expanded TEM's capabilities to observe biochemical and biological samples in native environments [2].

In the early 2000s, the introduction of time-domain measurements in TEM marked a significant leap. By using two ultrafast laser pulses—one to generate electron pulses from a photocathode and another to trigger the dynamics being studied—ultrafast electron microscopy (UEM) was born [3]. This development enhanced the temporal resolution of electron microscopy, allowing scientists to capture dynamic processes on the picosecond and nanosecond timescales. UEM has since been used to study various phenomena, such as phase transitions, laser-induced motions in nanostructures, and biological processes [4–10]. Despite these advances, the temporal resolution of UEM, limited by the electron pulse duration and space charge effects, has remained in the picosecond range, making it difficult to resolve ultrafast electron motions on timescales of femtoseconds and attoseconds [11–13]. Here, we introduce a breakthrough method that enhances the temporal resolution of UEM by generating single attosecond electron pulses through an optical gating technique [14,15]. This innovation, which we term the "attomicroscope," allows us to directly image electron motion in real-time at unprecedented temporal resolution [16,17].

Generation of Single Attosecond Electron Pulses

The key to achieving attosecond temporal resolution in UEM is the generation of single attosecond electron pulses. We employ a polarization-gated optical gating technique [18] (Fig. 1) to generate these pulses. A half-cycle laser pulse, created by passing a two-cycle pulse through a set of waveplates, is used to gate a few-hundred-femtosecond electron pulse. This gating pulse interacts with an aluminum grid to select a subset of electrons within a time window of 625 attoseconds [16], to obtain the attosecond imaging resolution in the electron microscope. The high-repetition rate [19] of the laser pulse (20 kHz) allows for the generation of a large number of attosecond electron pulses, which can be used to probe ultrafast dynamics in materials. In the attomicroscope, these attosecond pulses enable real-time observation of electron dynamics with unprecedented temporal precision. This advancement overcomes the space charge effects that have previously limited the temporal resolution of UEM [11,20].

First Imaging of Electron Motion with the Attomicroscope

To demonstrate the capabilities of the attomicroscope and the attomicros-

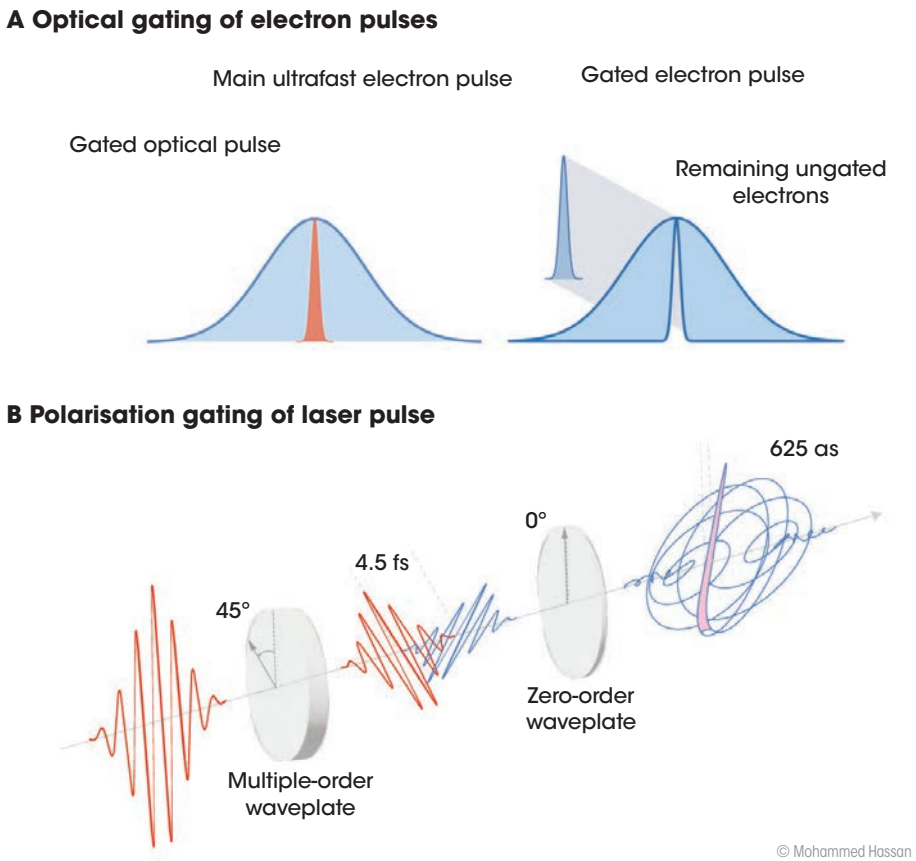


Figure 1: *(A) Optical Gating Approach:* Illustration of the optical gating method, where the electrons are gated by the optical gating pulse (OGP) generated via polarization gating. *(B) Polarization Gating Schematic:* Diagram of the polarization gating setup. The input laser pulses pass through multi-order and zero-order waveplates, set at 45° and 0°, respectively. The resulting optical gating pulse (OGP) consists of a half-cycle linearly polarized field in the center and a circularly polarized field at the edges. These OGPs are used to gate the electrons in the microscope, producing single attosecond electron pulses.

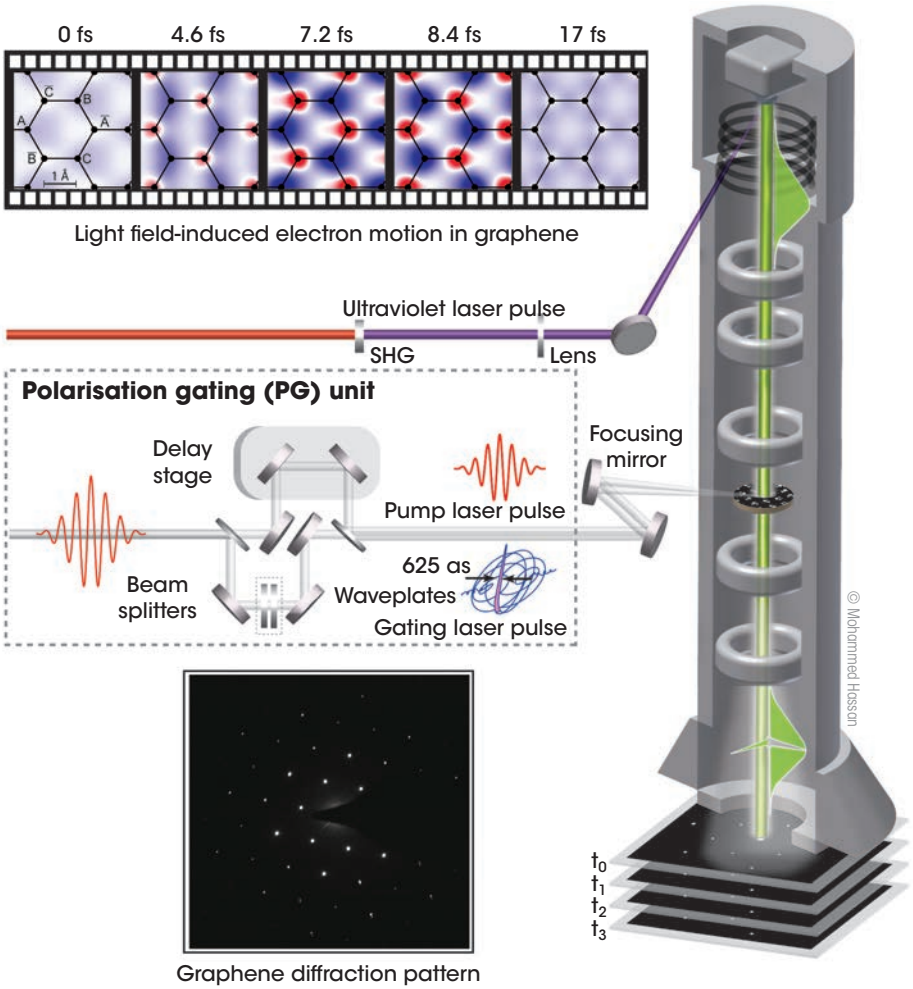


Figure 2: *Attomicroscopy Experiment Setup:* In the polarization gating (PG) unit, a 5-fs laser pulse is split by a beamsplitter into two beams. One beam is reflected off two mirrors mounted on a nanometer-precision delay stage and directed into the microscope, serving as the pump pulse to initiate electron motion in the system under investigation. The second beam undergoes polarization gating to generate the gating laser pulse, which is used to gate the electrons and produce attosecond electron pulses, enabling attosecond temporal resolution in the microscope.

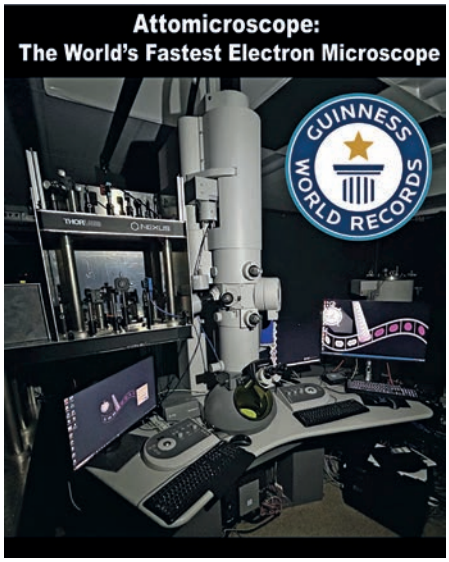


Figure 3: *Attomicroscope at Hassan's Lab:* Photograph of the attomicroscope in Hassan's Lab, recognized by the Guinness World Records as the world's fastest electron microscope.

copy imaging (Fig. 2), we performed the first-ever measurement of electron motion using attosecond pulses. We chose to study the electron dynamics in graphene, a material known for its unique electronic properties. In this experiment, the electron dynamics in graphene were excited by 5-fs laser pulses. The interaction between the pump laser and the electrons in graphene leads to both intraband and interband dynamics. Interband dynamics involve the excitation of charge carriers from the valence band to the conduction band, while intraband dynamics involve the movement of electrons within the conduction band [16].

To observe these dynamics, we measured the time-resolved diffraction patterns of graphene (Fig.2) using both gated and ungated electron pulses. The ungated electron pulse, spanning several hundred femtoseconds, was insufficient to resolve the sub-half-cycle electron dynamics. In contrast, the attosecond-gated electron pulse was able to track the evolution of electron motion in real-time (see the top panel in Fig.2). We observed changes in the scattered electron intensity corresponding to the waveform of the pump pulse, confirming that the diffraction measurements were sensitive to intraband dynamics. These results offer direct evidence of the attomicroscope's ability to image and control electron motion with attosecond resolution [21], and make it the world's fastest electron microcope (Fig. 3)

Impact of Attomicroscopy and Electron Imaging on Science

The attomicroscope represents a long-anticipated imaging tool that promises to significantly impact various fields of science and engineering. It serves as a foundational technology for developing other imaging tools with attosecond speed. For instance, the approach presented here can lead to the creation of a "cryo-attomicroscope," aimed at imaging electron motion in biological samples in their native environments. Additionally, we can adapt this concept to develop a quantum attomicroscope (Q-attomicroscope) for imaging the quantum behavior of electron motion in real-time. Together, these tools (attomicroscope, cryo-attomicroscope, and Q-attomicroscope) will form the core of a new field in science: "Ultrafast Quantum Imaging," unlocking unprecedented imaging applications (Fig. 4).

Attomicroscopy Imaging in Physics

Attomicroscopy imaging will enhance our understanding of electron behavior in real-time, enabling the control of quantum phenomena in materials, such as quantum phase transitions, superconductivity in twisted bilayer graphene, and electron spin dynamics for potential use as quantum bits (qubits). This advancement will contribute to the development of next-generation

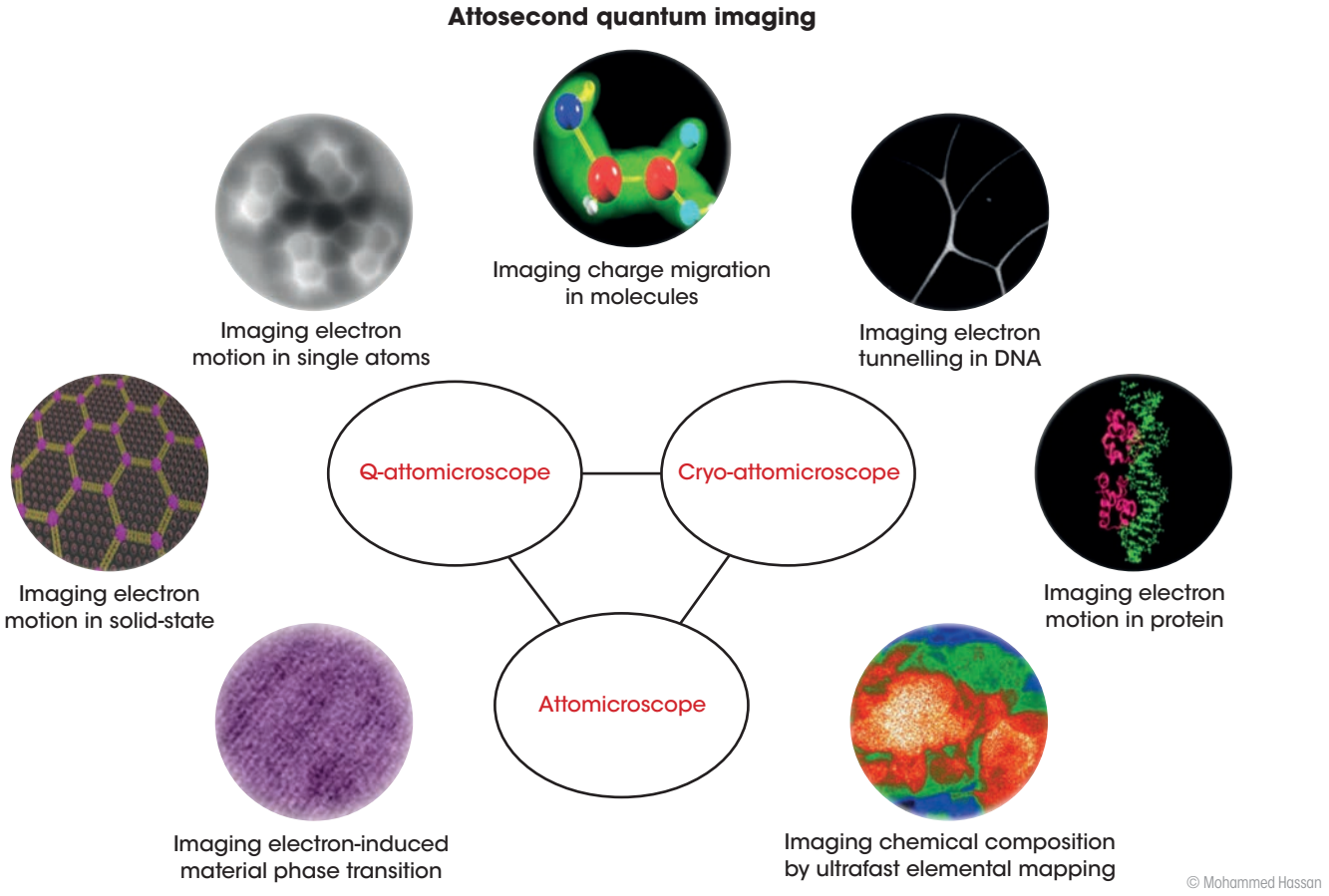


Figure 4: *Future Developments and Applications of Attomicroscopy:* Illustration of potential advances and applications of attomicroscopy in the emerging field of ultrafast quantum imaging.

quantum electronic devices and quantum computers. Moreover, the ability to visualize quantum electron dynamics in solid-state samples and correlate them with their morphology will facilitate the transition from scientific exploration to engineering applications, establishing the foundation for "ultrafast optoelectronics"—a million times faster than current electronics—crucial for the new era of information technology and AI.

Attomicroscopy Imaging in Chemistry and Biochemistry

Scientists have long sought to visualize and control chemical reactions, which occur through the breaking and forming of bonds between atoms mediated by electron motion. Achieving this requires an imaging tool with sub-atomic spatial resolution (sub-angstrom) and rapid imaging capabilities faster than the native timescale of electron motion (i.e., attoseconds). The Q-attomicroscope meets these criteria, fulfilling the dream of capturing chemical reactions in action. It could be used to visualize electron and charge migration in organic solar cells and the dynamics of charge transfer in revolutionary solid-state batteries, paving the way for a new generation of highly efficient solar cells and batteries that can help combat climate change. Furthermore, in photochemical reactions, imaging and controlling charge migration could influence reaction pathways and product formation, leading to more efficient synthesis methods or the development of new molecules for drug discovery.

Attomicroscopy Imaging in Quantum Biology

The cryo-attomicroscope is the long-awaited tool for imaging quantum processes in biological samples. For example, it could visualize the quantum electron tunneling process in DNA and related conformational changes, enhancing our understanding of DNA-DNA, DNA-protein, and DNA-drug interactions. This insight could significantly advance personalized medicine, elucidate mechanisms of carcinogenesis and mutagenesis, and shed light on DNA damage repair processes. Furthermore, quantum electron imaging of charge transfer dynamics in biological molecules may reveal the potential for using these molecules as building blocks in molecular electronics within the bioinformatics field. Additionally, in neuroscience, optogenetics allows researchers to control neuronal activity with light; the cryo-attomicroscope could be instrumental in imaging this process, revealing mechanisms that facilitate on-demand control of molecular interactions in living cells. This quantum imaging could deepen our understanding of brain function and contribute to developing treatments for disorders such as epilepsy and Parkinson's disease.

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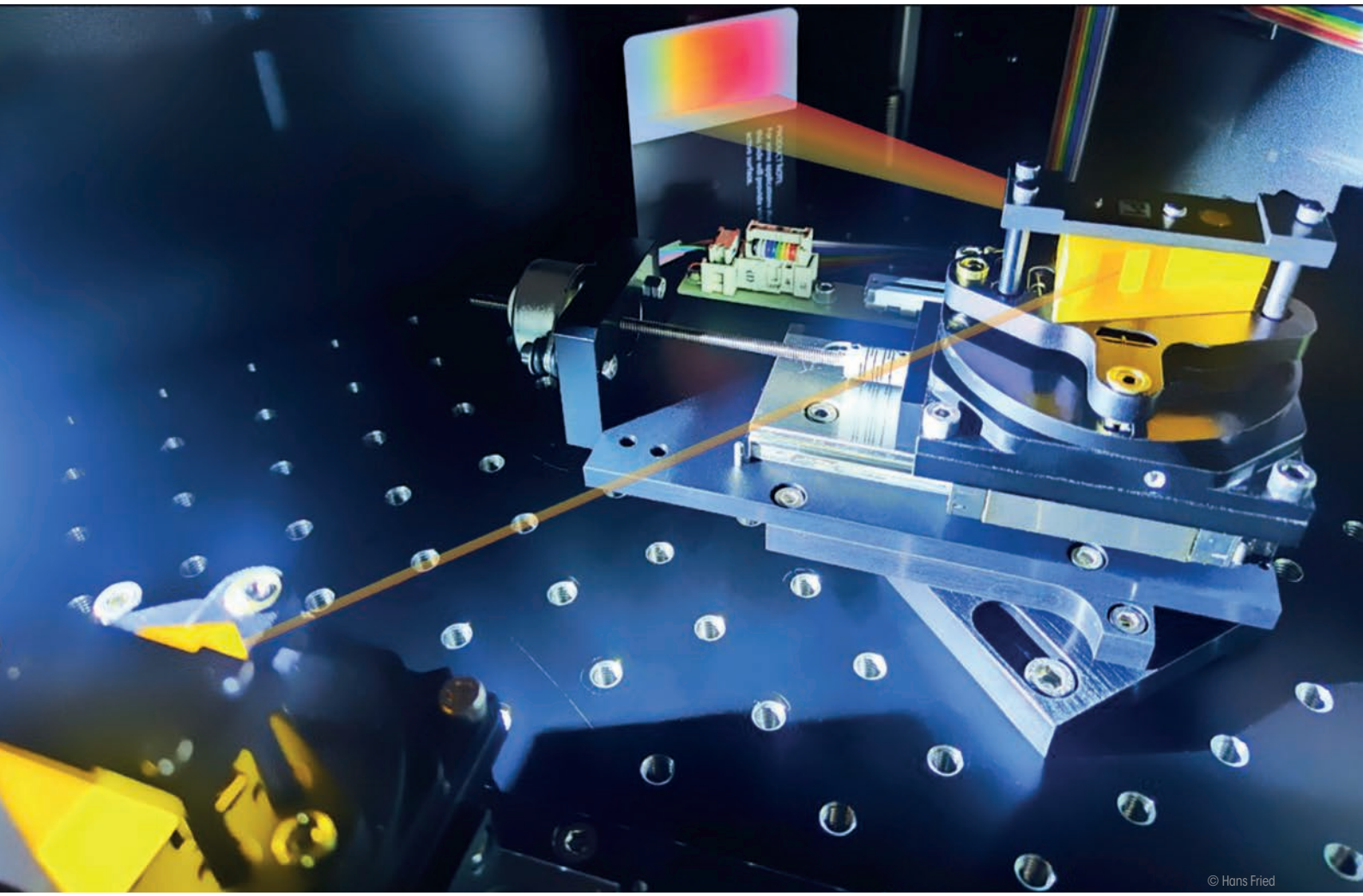

Deep into Tissue with Three Photons

Applying Three-Photon Microscopy to Access Deep Tissue Layers

Severin Filser[1], Yangting Fu[1], Eugenio Fava[1], Hans Fried[1]

Deep tissue imaging technologies are crucial for investigating biological processes in their natural environment in a non-invasive manner. Three-photon (3P) fluorescence excitation microscopy has been shown to provide deep optical access to various types of tissues, including the brain, bone, and kidney. After more than a decade of research and development, 3P microscopes have found ever-growing implementation in basic and clinical research. This article discusses the recent design concepts of current 3P microscopes and their applications in life sciences.

Introduction

Tracing back to Maria Göppert-Mayer's pioneering work in 1931, the potential of combining multiple photons to excite fluorescence has been well established. Fluorescence occurs when a fluorophore absorbs sufficient excitation energy. This specific amount of energy can be provided by a single photon or several photons that carry a fraction of this energy. For example: To excite the green fluorescent protein (GFP) an excitation energy of 2.6 eV is required. This amount of energy is carried by a single photon with a wavelength of 480 nm, based on the formula: $\lambda = 1239.8/\text{energy}$. Correspondingly, two photons with 1.25 eV (λ = 920 nm) or three photons with 0.867 eV (λ = 1300 nm) can excite GFP as well when they hit the fluorophore simultaneously, an effect known as multi-photon excitation. In microscopy, the penetration depth of photons entering the tissue is determined by absorption and scattering, with scattering being the dominant cause [1]. The distance before a photon gets scattered increases with wavelength. For example: In brain tissue, the average distance before photons at

480 nm get scattered is about 40 µm [2], about 150 µm at 920 nm, and about 350 µm at 1300 nm wavelength (1P, 2P, and 3P excitation of GFP, respectively) [3].

Necessary Optimizations and Components

While the concept appears straightforward, employing long-wavelength photons to achieve deeper tissue penetration presents significant challenges. The temporal window for multi-photon fluorescence excitation is exceptionally brief, necessitating the spatial and temporal condensation of photons. Femtosecond pulsed lasers with ultra-short pulse width and high peak power are needed to increase the probability that three photons arrive within the time window. About 100 fs to 200 fs pulse width for a 2P laser and about 50 fs for a 3P laser are common. However, when transmitting these pulses through thick glass elements like objectives, group delay dispersion causes a significant stretching of the pulse width, consequently reducing the 3P excitation efficiency. This phenomenon necessitates compensation for efficient deep tissue imaging (Fig. 1). We have measured that an originally 50-fs short pulse will be stretched to a width of 200 fs after the objective (Fig. 1), leading to a 10-fold reduction of fluorescence signal [4].

To further condense photons in time, high peak power is essential. If a standard 2P laser at 1300 nm is used for 3P excitation of GFP, the photodamage threshold will be exceeded before deep-tissue imaging is possible. Currently, turnkey solutions of specialized 3P lasers are available with reduced pulse repetition rates from 80 MHz (standard 2P laser) to 0.5 - 4 MHz. Each pulse contains significantly more peak power without exceeding the photodamage threshold in this configuration. However, slower repetition rates and, thus, longer acquisition times are disadvantageous for imaging fast dynamic processes, while higher repetition rates limit penetration depth due to less peak power. In our hands, 2 MHz represents a good compromise between speed and achievable imaging depth.

Concurrently, condensing photons in space necessitates using an objective, enabling the concentration of photons in a focused volume. Notably, the 3P volume is smaller than the 2P volume, resulting in improved resolution of densely labeled structures in 3P mode. Based on geometrical optics, the marginal rays of the focus

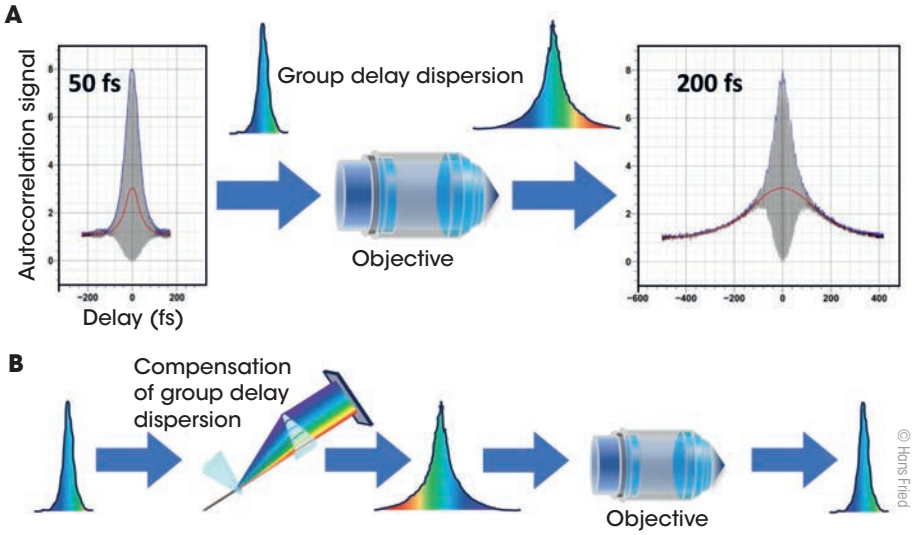

Figure 1: Group delay dispersion of an ultra-short laser pulse. A) Group delay dispersion measurement of an ultra-short laser pulse using an autocorrelator before and after an objective. An ultra-short laser pulse has a significant spectral bandwidth. Glass elements within a microscope (mostly those within the objective) delay the shorter wavelengths more than longer ones thereby stretching the laser pulse. B) Principle of compensation of the group delay dispersion: A prism splits the different wavelengths of an ultra-short laser pulse and the longer wavelengths have longer optical paths while traveling through the second prism, thereby being delayed. If the delay is exactly the same as produced within the microscope, the dispersion will be compensated after the objective.

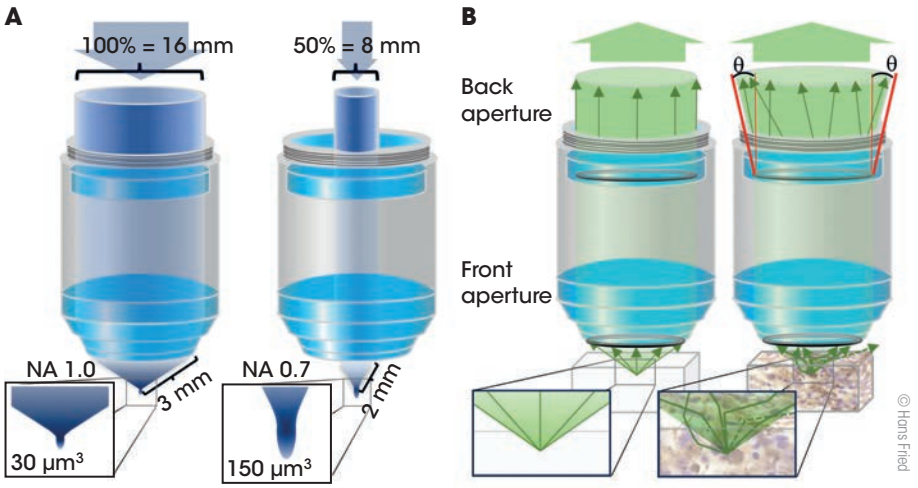

Figure 2: Schematic view of 3P laser focusing, fluorescence excitation, and collection through an objective. A) An objective with NA=1.05 focuses a laser beam with an effective 3P fluorescence excitation volume of approximately 30 µm³. With an objective working distance of 2 mm, the marginal rays of the focus cone are about 3 mm long. Underfilling the back aperture of the objective by 50% effectively reduces the NA to about 0.7, increases the 3P fluorescence excitation volume to approximately 150 µm³, and shortens the marginal rays of the focusing cone to about 2 mm. B) Scattering within the tissue will result in some photons reaching the objective front aperture along a non-ballistic trajectory. Those photons will leave the objective's back aperture deviating from a collimated beam at an angle (θ).

cone, especially from high NA objectives, have to travel much longer to the focus point than the paraxial rays (Fig. 2). Some of the marginal rays do not reach the focus spot due to scattering and cannot contribute to fluorescence excitation. Thus, for deep-tissue imaging, the laser beam is sent mainly through the central area of the objective by underfilling the back aperture to about 50%. However, simply choosing a lower NA objective would not be advisable since, in low NA objectives, too few of the emitted photons are collected to visualize weak fluorescence. In addition, in deep tissue imaging, the majority of emitted photons are scattered many times before reaching the objective. These so-called non-ballistic

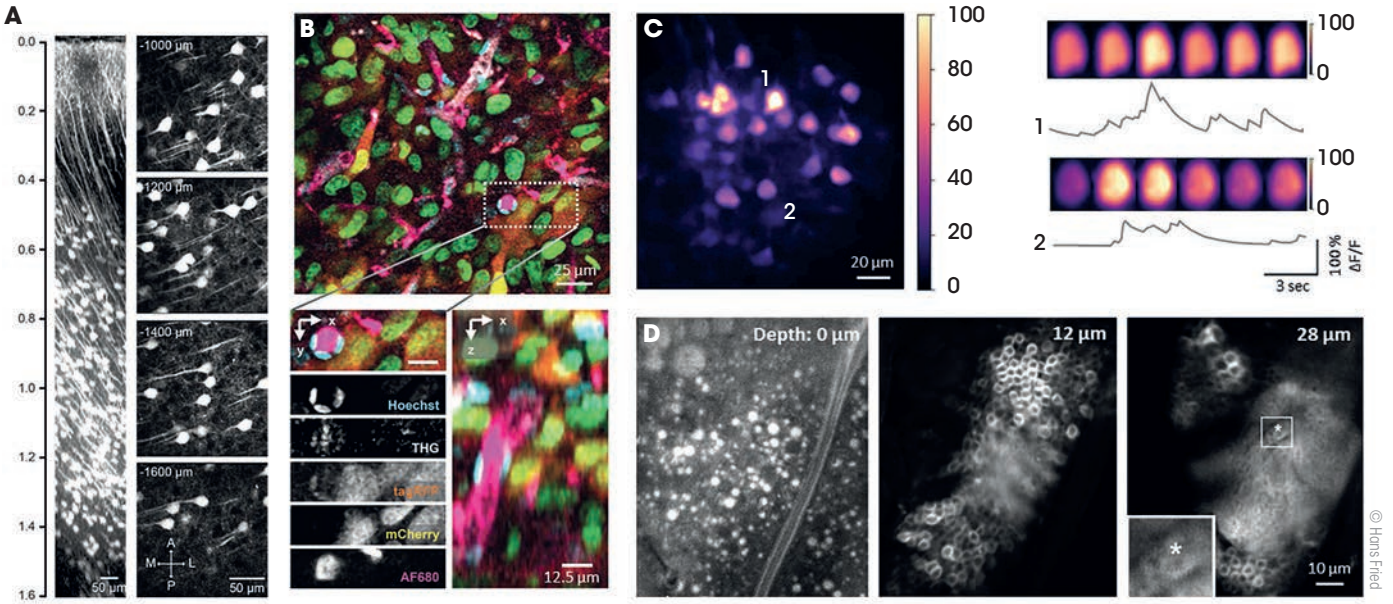

Figure 3: Examples of three-photon excitation fluorescence microscopy. A) 3D reconstruction of YFP labeled neurons imaged with 320 x-y frames from the surface of a mouse prefrontal cortex to 1600µm below. (Image taken from [11]). B) Multimodal 3P, 4P, and third harmonic generation (THG) microscopy of skin tumor xenografts at 1650 nm showing Hoechst (nuclei in cyan), tagRFP (cytosol in orange), mCherry (nuclei in yellow), Alexa Fluor 680 (vessels in purple). Note that cell nuclei containing mCherry and Hoechst appear as green (Image from [8]). C) 3P calcium imaging of neuronal activity about 1300 µm below the surface with GCaMP6s excited at 1300 nm. Fluorescence intensity changes are shown for two spontaneously active neurons. Data from [11], analysis kindly provided by Gian-Marco Calandra. D) 3P microscopy of Drosophila mushroom body. Neurons are labeled by mCD8-GFP expression and imaged at 1300 nm through the intact cuticle. The asterisk indicates an exemplary dendritic structure (microglomerulus) formed by multiple neurons. Images were kindly provided by Chi Wai Chan, AG Tavosanis.

photons leave the objective back aperture uncollimated (Fig. 2). Collecting those photons will increase signal intensity as well as achievable imaging depth. This can be realized by the appropriate design of the detection beam path and by a large back aperture of the objective. A large back aperture is found in low magnifying objectives. In deep tissue imaging, 10x to 25x objectives with a NA from 0.6 to 1.0 are commonly used.

Applications and Performance

Although several development efforts have been focused on strategies to further increase the achievable imaging depth limit using adaptive optics [5] or adaptive excitation sources [6], 3P microscopes with careful implementation of the above-described optimizations have proven to be very effective [7,8]. Moreover, due to their lower technical complexity, such setups are suited for a multi-user environment such as microscopy core facilities.

With the above-described setup, one can reach murine brain areas located well beyond 1 mm below the pial surface. Minute structures like dendritic spines (≤ 1µm) can be resolved at depths below 1 mm and chronically imaged in consecutive imaging sessions over days and weeks without obvious signs of phototoxicity. Furthermore, neuronal and astrocytic calcium dynamics in deep cortical areas could be studied via the expression of genetically encoded calcium sensors (Fig. 3). 3P microscopy enables non-invasive visualization of Drosophila mushroom bodies through the intact cuticle (Fig. 3) over extended periods of time [9]. In addition, 3P microscopy allows visualization of complete intact mouse lymph nodes [10], and deep layers of tumor tissue [8].

Due to the combined impact of tissue scattering and water absorption in tissue, only wavelengths around 1300 nm and 1700 nm are applicable for 3P microscopy. However, with these excitation wavelengths, many common green and red fluorophores like eGFP, YFP, GCaMP, mCherry, tdTomato, and tagRFP have been successfully imaged. Furthermore, together with fluorescence second and third harmonic generation can be acquired thus expanding the range of available 3P imaging modalities (Fig. 3).

Summary

With the herein described optimizations, 3P microscopy has enabled tissue imaging at depths well below 1 mm, visualization of subcellular structures, tracking of cell activity and metabolism. In essence, the multifaceted capabilities of 3P microscopy have significantly contributed to the exploration of intricate scientific queries within the depths of biological tissues. Such 3P microscopes are currently becoming commercially available and will find wider distribution in the scientific community due to the lower level of required technical expertise. 3P deep tissue microscopy opens new exciting avenues in basic and clinical research "to boldly go where no one has ever gone before".

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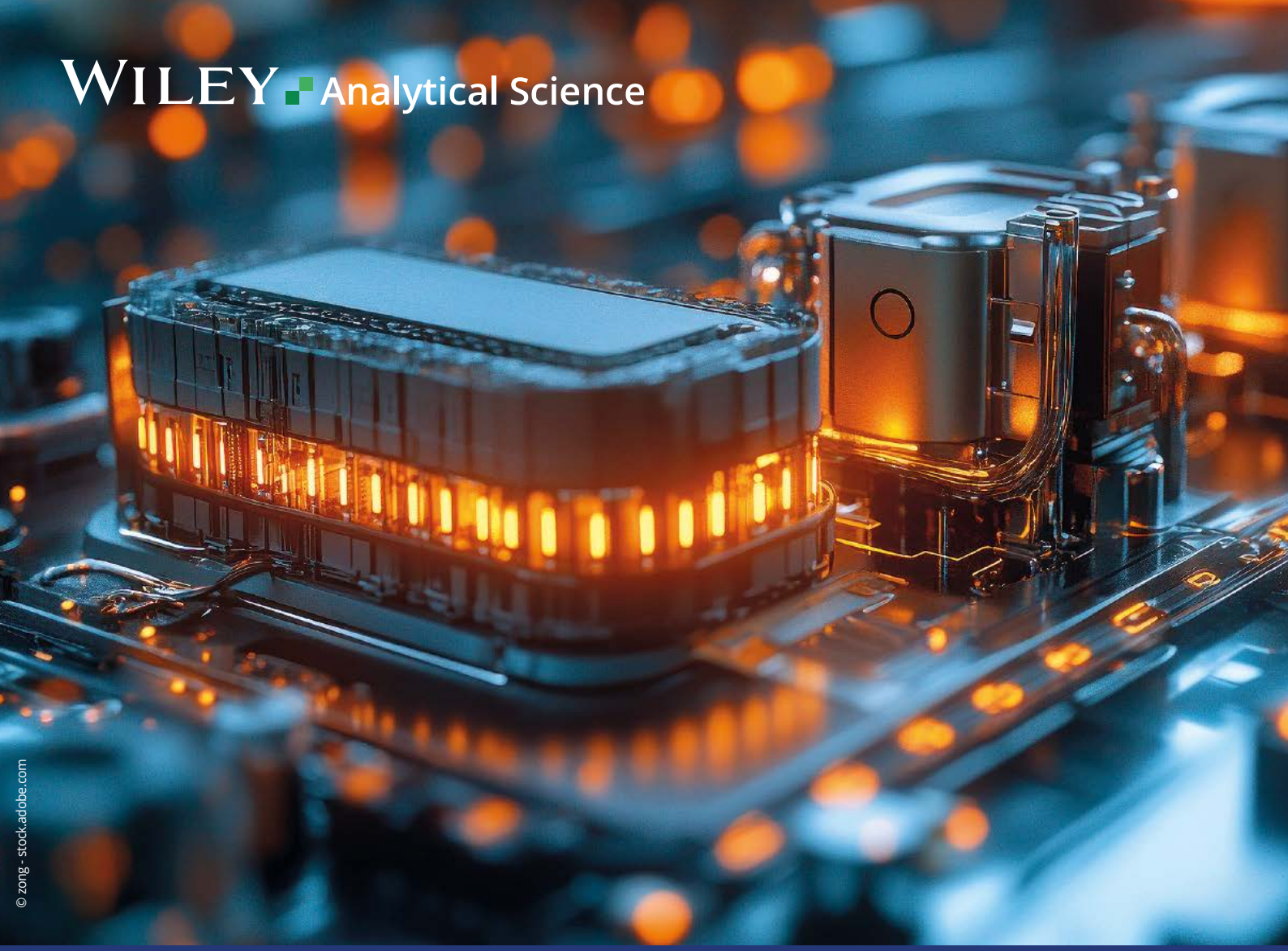
WILEY Analytical Science



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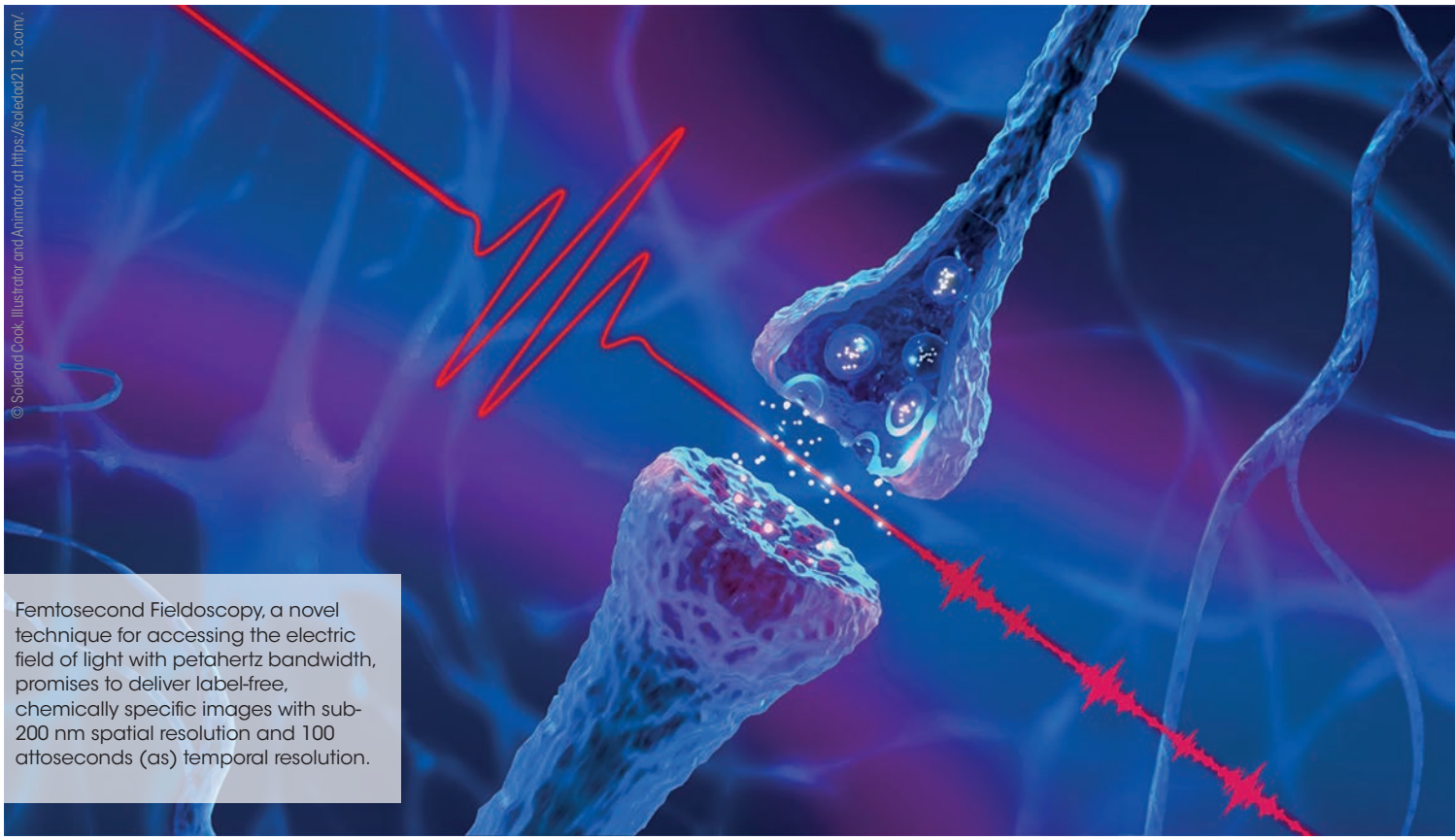

Femtosecond Fieldoscopy, a novel technique for accessing the electric field of light with petahertz bandwidth, promises to deliver label-free, chemically specific images with sub-200 nm spatial resolution and 100 attoseconds (as) temporal resolution.

Femtosecond Fieldoscopy

For Super-Resolution Label-Free Microscopy

Soyeon Jun[1,2,4], Andreas Herbst[1,2], Kilian Scheffter[1,2,4], Daniel Wehner[1,2,3], Anchit Srivastava[1,2], Hanieh Fattahi[1,2,4]

Accessing complete electric field information of a laser pulse interacting with a medium at visible to near-infrared (near-petahertz) frequencies has traditionally required complex laboratory systems operating in vacuum conditions. Recent advancements, however, have enabled the measurement of electric fields at near-petahertz frequencies in ambient air. This capability is critical for understanding ultrafast phenomena and for achieving quantitative detection of molecular species in various samples. This article introduces Femtosecond Fieldoscopy, a field-resolved detection technique for label-free spectroscopy and microscopy. This approach delivers exceptional detection sensitivity and dynamic range at petahertz bandwidths by combining attosecond temporal resolution with temporal isolation of target molecular responses from environmental and excitation pulse effects. Furthermore, Femtosecond Fieldoscopy holds promise for achieving sub-diffraction spatial resolution, opening new horizons for high-precision label-free spectro-microscopy.

Introduction

Vibrational microscopy is a powerful tool for non-perturbative, label-free identification of complex molecular compositions. It offers intrinsic chemical selectivity due to the specific vibrational frequencies of different molecules, known as molecular fingerprints. Among various label-free imaging techniques, stimulated Raman scattering (SRS) in the near-infrared spectral range stands out because of its low water absorption cross-section, high penetration depth, high spatial resolution, and linear relationship with molecular concentration. However, the spatial resolution of SRS label-free images is limited by the diffraction limit of the excitation pulse. In recent years, various strategies have been developed to achieve super-resolution SRS images by using Raman-active labels, structuring the excitation beams, exciting higher-order nonlinearity, or applying computational reconstruction methods, albeit with trade-offs. Figure 1 shows that the spatial resolution for current label-free SRS imaging exceeds 200 nm. The gray area in Figure 1 highlights an unexplored frontier in non-perturbative, label-free spectro-microscopy, where achieving resolutions beyond this threshold remains a significant scientific challenge.

Field-resolved detection has been a powerful method in the mid-infrared and terahertz spectral ranges, as it allows for the direct measurement of

light-matter interactions in sub-cycle regimes, capturing both amplitude and phase information. However, this method has not been employable in SRS detection due to the near-petahertz (PHz) frequency of excitation pulses in stimulated Raman interactions. For decades, attosecond streaking, which was awarded the Nobel Prize in 2023, has been the sole method to probe the electric field of light at frequencies approaching PHz. Yet, the requirement for vacuum conditions in attosecond streaking limits its applicability in biological contexts. Over the last few years, various techniques have been developed that enable near-PHz field-resolved light detection in ambient air. Among these techniques, electro-optic sampling (EOS) stands out for its unparalleled detection sensitivity and intrinsic sub-diffraction spatial resolution when used for imaging. In this article, we discuss how EOS can detect the molecular response in the short wavelength infrared and SRS, and how this concept advances the frontiers of non-perturbative, super-resolution, label-free microscopy.

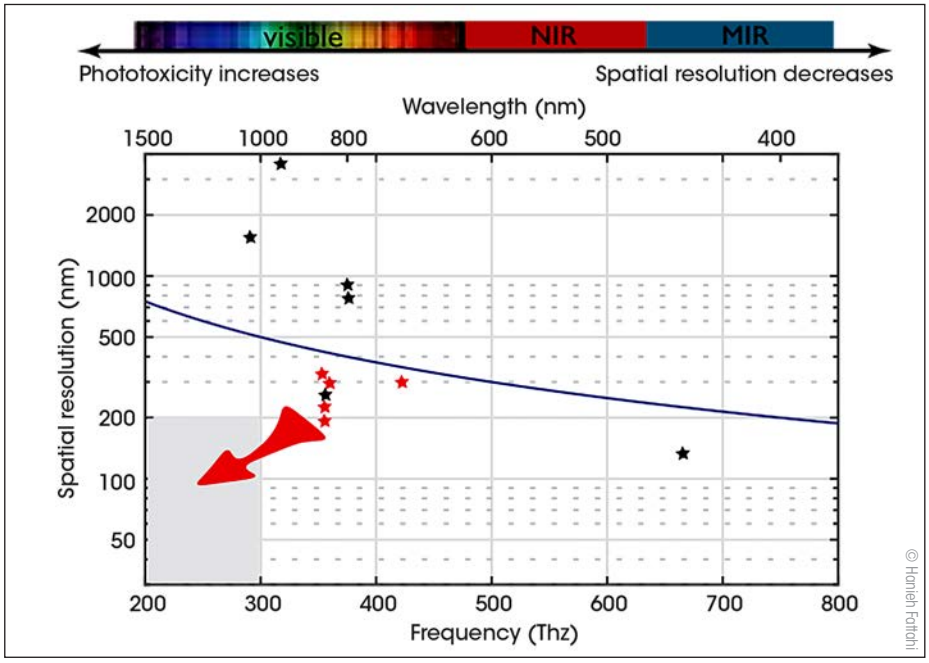

Figure 1: State-of-the-art label-free stimulated Raman microscopes. This figure compares the spatial resolution of these advanced microscopes. Black stars represent the SRS microscopes, while red stars indicate coherent anti-Stokes Raman scattering images. Increasing the excitation pulse frequency enhances spatial resolution, but also raises the risk of damage to soft tissue. The area marked by the red arrow highlights the optimum operation point.

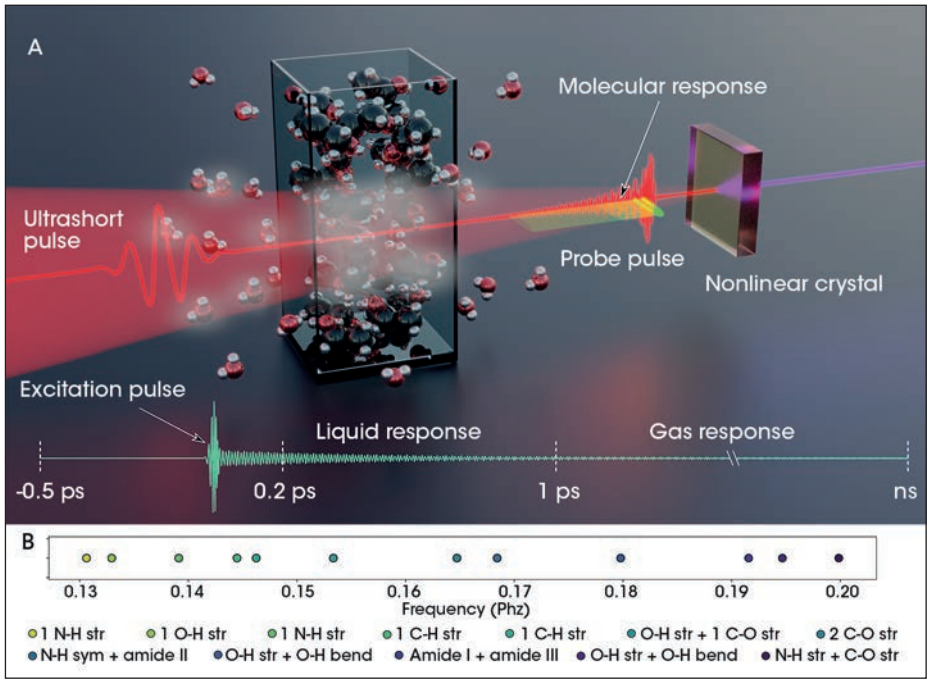

Figure 2: Near-Petahertz femtosecond fieldoscopy. (a) An ultrashort optical pulse impulsively excites all the in-resonance molecules along its beam path. The molecules in the cuvette represent the sample under test, while the surrounding molecules represent the environmental ones. As the transmitted field propagates, it accumulates the combined molecular responses of both the sample and the environment. A higher-frequency ultrashort probe pulse is employed for up-conversion, generating a delay-dependent signal in a quadratic medium. The measured electric field captures the excitation pulse, the liquid's picosecond-scale response, and the atmospheric gases' nanosecond-scale response. Time filtering analysis separates these into short-lived liquid and long-lived gas responses. (b) Biologically relevant vibrational modes in the near-infrared range correspond to compounds such as proteins, carbohydrates, lipids, polyphenols, and alcohols. Vibrational bands, including stretching ("str") and bending ("bend"), are detailed in the legend. First and second overtones [1,2] and combination bands (+) are noted. The image is taken from reference [2].

Electro-Optic Sampling at Near-Petahertz

In EOS, the electric field of a sample pulse is probed by a short pulse with a higher central frequency and a shorter temporal duration. When the two pulses interact in a phase-matched nonlinear medium, a sum frequency signal is generated, with its strength being proportional to the electric field strength of the sample pulse. Due to the broad spectral bandwidth of the interacting pulses, part of the probe pulses' spectrum overlaps with the generated sum frequency spectrum. This overlapped region is filtered spectrally and detected by a balanced detector. The electric field of the sample pulse is captured by temporally scanning the probe pulse over the sample pulse. The probe pulse plays a crucial role in EOS; it facilitates the upconversion of the signal and serves as a local oscillator, which enhances the detection signal-to-noise ratio and detection sensitivity through heterodyne detection, making the shot noise of the probe pulse the primary source of noise. By upconverting the signal's spectral bandwidth to higher frequencies, EOS enables the use of silicon detectors for broadband near-infrared detection.

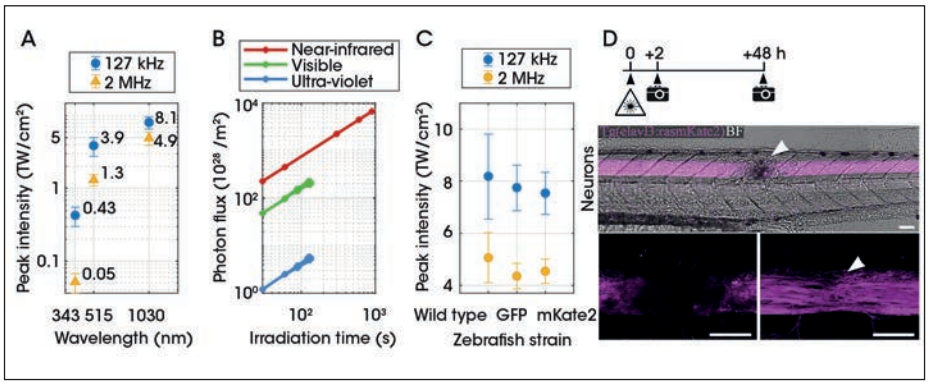

Figure 3: Femtosecond laser interaction with the central nervous system of zebrafish. (a) The damage threshold peak intensities are presented at various wavelengths and repetition rates. (b) The peak intensity for different irradiation wavelengths is maintained below the damage threshold, and the irradiation time is increased. It was noted that the damage site expanded gradually with increasing irradiation time at 343 nm and 515 nm. No damage was observed after 900 seconds of irradiation at 1030 nm. (c) The damage threshold peak intensity for wild type and differently labeled (elavl3:GFP-F and elavl3:RasmKate2) transgenic zebrafish larvae. (d) Top: Bright field image of the spinal cord of transgenically labeled zebrafish larvae after irradiation with 30 mW laser pulses at 1030 nm and at 2 MHz repetition rates. Bottom: Subsequent regrowth of white matter tracts post-irradiation. Scale bars: 50 µm. In panels (a, c), the error bars represent standard deviation. The figure is reproduced from reference [9].

Femtosecond Fieldsocopy

Field-resolved detection at near-petahertz frequencies provides exceptional detection sensitivity, bandwidth, and dynamic range while enabling attosecond temporal and sub-diffraction spatial resolution. In a novel scheme that we call "Femtosecond Fieldoscopy" (Fig. 2), ultrashort excitation laser pulses impulsively excite the in-resonance molecular composition of a sample. Subsequently, vibrational coherence is initiated at the trailing edge of the excitation pulses, decaying exponentially at a rate proportional to the vibrational dephasing time. The transmitted electric field contains the ultrashort excitation pulse, the sample's delayed response spanning several picoseconds, and a long-lasting response from atmospheric gases that lasts for hundreds of nanoseconds. One can access broadband information about molecular composition and concentration via field-resolved detection of the molecular response in the time domain and subsequent Fourier transformation. Specifically, by Fourier transforming the decaying trail of the electric field containing the pure molecular response, one gains spectroscopic information with unprecedented sensitivity and dynamic range, as this approach temporally gates the molecular response from the main excitation pulse. Femtosecond Fieldoscopy has recently been used to detect overtone, Raman, and combination bands in various liquid samples [2–4]. Furthermore, several techniques have been developed to accelerate the method for real-time detection [5–7] and extend its capabilities to label-free imaging [8].

Towards Super-Resolution Label-Free Imaging

The spatial resolution in label-free SRS microscopy is constrained by the diffraction limit of the Raman excitation pulses. Increasing the frequency of the excitation pulses enhances spatial resolution. However, the probability of damage also increases with the irradiation frequency, limiting in vivo microscopy. Our recent study revealed a different and nonlinear damage mechanism at 1030 nm, which has a higher damage threshold than UV pulses, making this wavelength range suitable for non-invasive in vivo interactions with biological samples (Fig. 3) [9]. However, imaging at longer wavelengths results in reduced spatial resolution. Femtosecond Fieldoscopy provides a solution to this challenge. In Femtosecond Fieldoscopy, the detected molecular response is confined to the overlapped area of the excitation and probe pulses. Here, the spatial resolution of the SRS image is determined by the diffraction limit of the short-wavelength probe pulses. Consequently, the Raman response of a sample can be coherently excited by femtosecond laser pulses in the near-infrared range, while the spatial gating of the EOS achieves spatial resolution at the sub-diffraction limit of the excitation Raman pulses [10].

Outlook

Label-free SRS microscopy enables imaging molecules in their natural state; however, its spatial resolution is limited by the diffraction limit of Raman excitation pulses, which restricts the unperturbed detection of small molecules. For instance, detecting neurotransmitters at synaptic clefts has been particularly challenging due to their small size, low detection sensitivity, and the limited spatial resolution of current SRS techniques, impeding accurate mapping of their distribution and concentration. Recent advancements in ytterbium laser technology [11] have facilitated Femtosecond Fieldoscopy, a groundbreaking approach that offers super-resolution imaging with unparalleled selectivity, sensitivity, enhanced spectral coverage, and high contrast [10]. By addressing the limitations of existing methods, it promises to unlock new possibilities for sub-cellular research, allowing precise, label-free studies of molecular dynamics at previously unattainable scales.

Author contribution

SJ, AH, KS, AS have contributed equally in advancing Femtosecond Fieldoscopy.

Acknowledgment

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Online version and references:
https://bit.ly/MA-Fattahi

Intuitive Workflows for Automated Image Analysis

JIPipe as an Open-Source Tool for FAIR Image Analysis

Ruman Gerst[1], Zoltán Cseresnyés[1], Marc Thilo Figge[1,2]

The increasing availability of complex imaging techniques requires scalable and reproducible software solutions that are capable of utilizing all information in high-resolution, multimodal image data. JIPipe is an open-source, metadata-driven image analysis platform for visually creating analysis pipelines without requiring programming expertise. The tool is designed for a wide range of applications and has already been successfully applied in studies concerning molecular and cellular level biology, drug delivery characterization, and materials sciences. This image analysis platform can also provide a versatile solution for imaging core facilities by promoting FAIR compliance when building workflows.

Introduction

In life sciences, image data analysis often presents a significant challenge due in part to the lack of analysis experience and to the huge variety of image types and associated metadata.

A popular tool that allows the interactive analysis of images is ImageJ [1, 2]. Here, users have access to a large pool of basic and advanced image analysis methods and many custom-designed tools called plugins. At the same time, with ImageJ, the relevant metadata can also be simply managed manually. However, this approach becomes increasingly impractical when hundreds or thousands of images need to be processed: the analysis is not only time-consuming but also prone to researcher bias, making it difficult to reproduce. A standard approach to handle this challenge is through batch processing, a feature available in ImageJ in the form of a macro language.

However, adapting to more complex data arrangements often requires considerable time and effort to implement data management features into a macro—a task that demands extensive programming knowledge. To address these challenges, we developed JIPipe [3], a tool designed to handle intricate data workflows without programming experience. Despite the relatively short time since its introduction, JIPipe has already been utilized numerous times in a wide range of applications, including biological tissue characterization [4, 5], bacterial

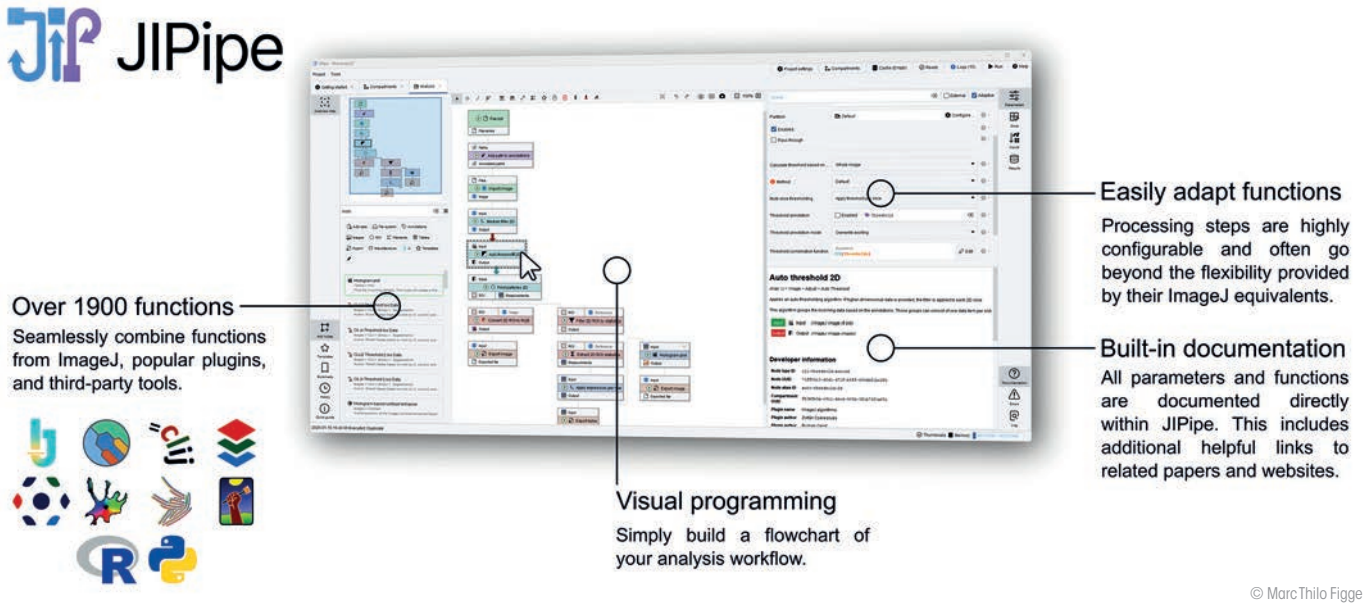

Figure 1: JIPipe allows researchers to visually build image analysis workflows that comprise functions from a huge variety of ImageJ-based and third-party tools. All included algorithms are highly configurable and fully documented via the built-in reference database.

and cell-level analysis [6–11], nanoparticle design [12–14], as well as materials science [15–17].

Seamlessly Connect Various Image Analysis Tools

JIPipe is designed to enable researchers of all skill levels to visually construct customizable image analysis pipelines. These pipelines consist of functions that address common tasks, including preprocessing, segmentation, object filtering, and the generation of statistics and visualizations. Users have access to functions from ImageJ and its plugins, such as CLIJ2 [18], 3D ImageJ Suite [19], TrackMate [20], and OMERO [21], as well as third-party tools like Cellpose [22], Python, and R. Additionally, JIPipe offers specialized features for filament processing and 3D scene generation, providing a comprehensive toolkit for diverse applications. It includes built-in documentation for all features, linking to related resources, publications, and authorship details. Validation checks and automatic configuration of third-party software are integrated into the platform, minimizing the technical challenges typically associated with setting up external tools. This integration was crucial for studying, for example, immune cell motility during infection responses in the human defense system [23]. Our image analysis platform was utilized to track neutrophils based on confocal microscopy time-series Z-stacks that were preprocessed to correct illumination artifacts, with segmentation performed using a pretrained Cellpose model (see Figure 2).

Effortlessly Manage Metadata to Guide Automation

Image metadata is essential to the contextualization of the information gained from the analysis, as well as to dynamically guide the pipeline behavior. JIPipe excels in extracting metadata from various sources and associating them with the corresponding data. It intuitively organizes data and metadata into an Excel-inspired table format. The user then modifies this table using the tools provided. For example, information can be added from folder names, image properties, preprocessing steps, and other sources. Similar to an Excel table, our image analysis platform provides tools to reuse, modify, and combine metadata, which can be used to guide batch processing. This way, the manual approach can be intuitively replaced by automated batch processing

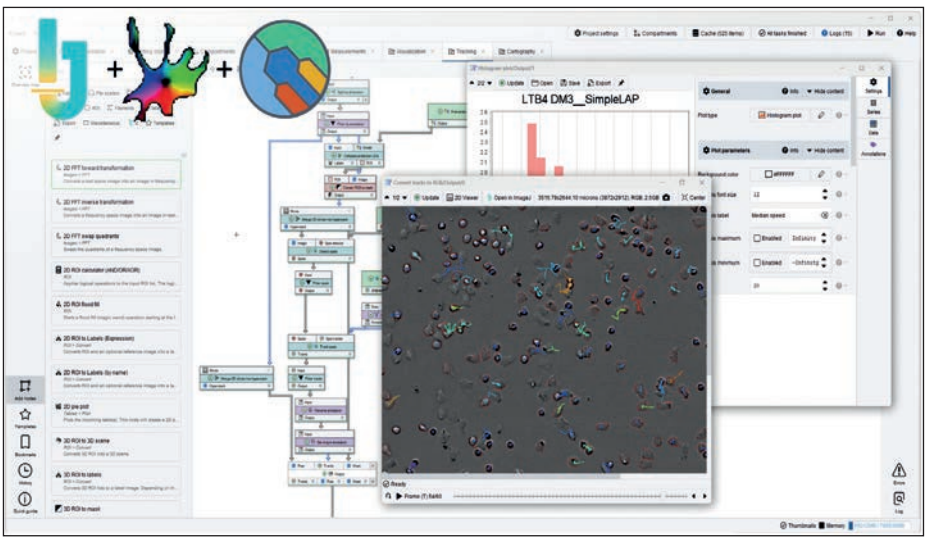

Figure 2: Human neutrophils were imaged over time using 3-dimensional transmitted-light microscopy to deduce their kinetic behavior under various immune-response conditions. JIPipe was used to segment and track the individual cells and to calculate their speed, velocity, cell density, and other kinetic parameters. The segmented cells are overlaid with the per-cell tracks (color lines in the inserted image).

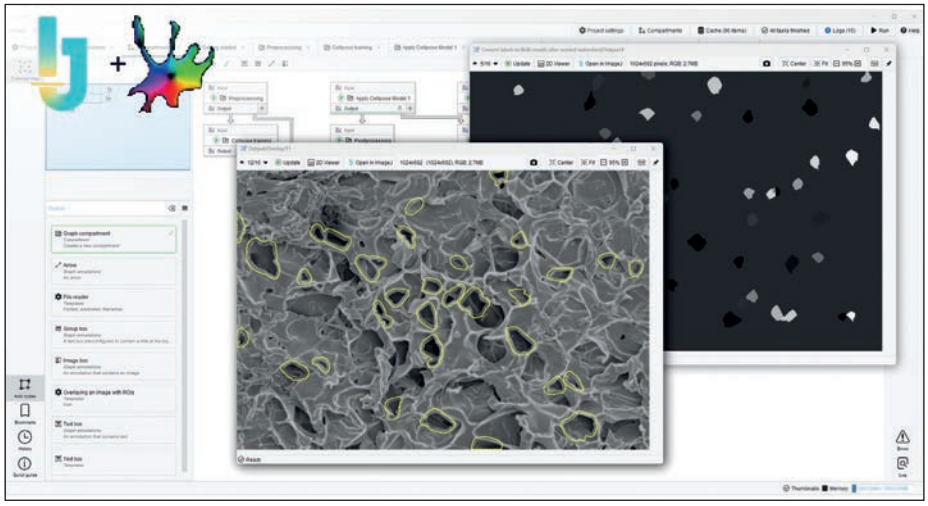

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Figure 3: The electron microscopy images of cross-cut surfaces of cryogels were used to characterize the pores formed in the gels during the fabrication procedure. The reproducibility of the size distribution of these pores (dark areas outlined with yellow edges in the center image) is of high importance when using cryogels as 3-dimensional scaffolding for cell growth and migration. The workflow (shown in the background) used Cellpose to identify the pores (yellow lines over the original image of the sample) and to create label images (gray-scale image in the top right) that were utilized to calculate various morphological measures of the pores.

without any programming by utilizing the already available metadata. The user simply selects the metadata relevant to the analysis, and the platform automatically carries out the batch processing around the available information. This data-driven approach was applied, for example, in a project where the reproducibility of cryogel preparation methods was tested for applications including tissue engineering and filtration (see Figure 3). Using JIPipe, scanning electron microscopy (SEM) images were analyzed to characterize cryogel pores, where the workflow incorporated a transfer-learning application of Cellpose models [24]. Two separate models were trained: one for large, shallow pores and another for smaller, darker pores, with the training conditions recorded in user-defined metadata. The experimental conditions determined the applicability of the two models, thus requiring the use of metadata extracted from the file system to decide on which model to prefer. Other metadata management applications, in combination with the platforms' building blocks, include combining various imaging modalities. For example, the augmentation of super-resolution images with a lower-resolution image to provide anatomical guidance is nowadays a common practice. Here, the system provides the essential tools for colocalization and registration, thus allowing the correct quantification of the super-resolution data.

Streamline Imaging Workflows for Core Facilities

With the rising complexity and cost of microscopy techniques, establishing core facilities has become the standard way to provide life science imaging support. Whilst core facility personnel are typically highly familiar with the hardware aspects, providing image analysis support beyond what the microscope software offers is often lacking. Proprietary software solutions are easy to use for many applications, but they are cost-restrictive and inflexible. Longevity is also a potential problem, with company support potentially phasing out over a long time. Our image analysis platform provides an open-source, highly flexible, community-supported, easy-to-install, and well-documented tool that can be mastered without actual programming knowledge, making it particularly suited for imaging core facilities. Its metadata-driven approach simplifies the creation and sharing of tailored workflows, enabling facility staff to efficiently support a wide range of imaging needs. By associating metadata with imaging data, this system provides a flexible framework to handle various modalities, from wide-field to super-resolution and electron microscopy, and seamlessly integrates data from different sources. In addition, the fully documented processing workflow provides a FAIR (Findable, Accessible, Interoperable, Reproducible) [25] way of analyzing images, which carries special importance for a multi-user facility. JIPipe is now also the main desktop workflow tool of the NFDI4BIOIMAGE [26], as well as part of the image.sc community.

Conclusion

JIPipe bridges the gap between the growing complexity of image analysis and the diverse needs of life scientists and imaging facilities. It offers an intuitive, metadata-driven platform that supports a wide range of imaging modalities and integrates seamlessly with tools like ImageJ, Cellpose, TrackMate, Python, and R. Thus, our image analysis platform helps researchers handle intricate workflows without requiring programming expertise.

For imaging core facilities, it provides a flexible, open-source solution that simplifies the creation and sharing of reproducible workflows. This enhances support for diverse imaging needs while maintaining compliance with FAIR principles. By enabling advanced analysis and automation, this software ensures that researchers can focus on scientific questions rather than technical barriers.

Whether supporting multiuser facilities or individual research projects, its versatile, community-supported platform is a valuable resource for advancing imaging-based research in the life sciences.

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Automated Inverted Microscope System

Evident has released the IXplore IX85, an automated inverted microscope system designed for speed, clarity, and reliability. It features a 26.5 mm field number (FN) and advanced imaging tools for clear and accurate results. The system offers high customizability, allowing users to create a tailored imaging platform. Built-in optics ensure consistent lighting across the field of view, while distortion correction and flatness provide accurate image representation. The IX85 includes silicone gel pad technology for improved objective usability, eliminating the need for oil replacement. Enhanced stitching capabilities enable capturing large samples with fewer images. The system includes automated acquisition features, customizable interfaces, and advanced image processing. An automatic correction collar fine-tunes objectives to reduce spherical aberrations. Dual built-in ports and an open frame design allow for easy module addition. Users can expand the platform with features like environmental control and imaging modalities. The IX85 adapts to various research needs, supporting labs in efficient data acquisition.

EVIDENT Europe
www.EvidentScientific.com

Field Emission Scanning Electron Microscopes

Hitachi High-Tech Europe has launched the SU3800SE and SU3900SE Schottky field emission scanning electron microscopes (SEMs), featuring fully automatic optical alignment for quick high-resolution imaging. These models handle a wide range of sample types with large sample handling and variable pressure operation. The SU3900SE supports specimens up to 300 mm in diameter and 130 mm in height, ideal for large materials like automotive components. The SU3800SE accommodates samples up to 200 mm in diameter and 80 mm in height. Both models include an advanced navigation system for precise specimen positioning and support the EM Flow Creator function for fully automated workflows. This feature allows easy creation of complex workflows with drag-and-drop flowchart tools. Users can achieve consistent, high-quality images with minimal training due to automatic alignment and sensitive detectors. Optional features include Hitachi Map 3D for surface reconstruction, a sample exchange chamber, and analysis accessories like EDX and EBSD.

HITACHI High-Tec
www.hitachi-hightech.com

Automated TEM Sample Preparation

Zeiss has launched the Crossbeam 550 Samplefab, a focused ion beam scanning electron microscope (FIB-SEM) designed for automated transmission electron microscopy (TEM) sample preparation. This system offers hands-free lamella preparation from bulk to thinned samples, producing up to 10 lamellae in under 8 hours with a >90% automation yield. It features new control software for improved stability and usability, capable of thinning samples down to 100 nm across various semiconductor types. The proprietary lift-out technology enhances automation yield, while the Gemini 2 electron column allows live SEM observation during FIB milling for high-quality lamellae. The system's user-friendly interface supports both novice and expert operation. The robust workflow and stable FIB column minimize operator intervention and setup time, increasing productive uptime and reducing consumable costs.

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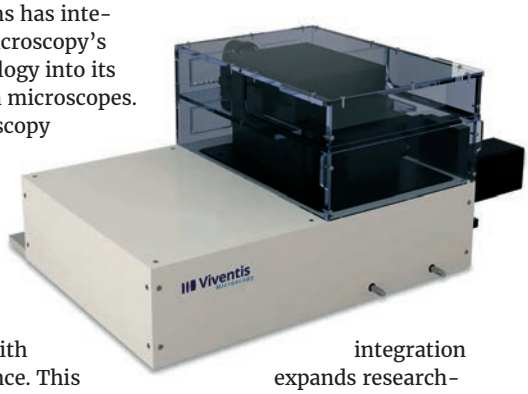
Light Sheet Technology Integrated Microscopes



Leica Microsystems has integrated Viventis Microscopy's light sheet technology into its advanced research microscopes. Light sheet microscopy enables detailed volumetric imaging, allowing researchers to study complex biological systems at the single-cell level with minimal disturbance. This technology offers an unbiased view of natural processes, aiding scientific exploration in biology, health, and disease. The Viventis LS2 Live light sheet fluorescence microscope provides multi-view and multi-position imaging, offering high spatio-temporal resolution and image quality, even for large, light-scattering samples. This integration expands researchers' capabilities in scientific analysis. Leica Microsystems is available for inquiries regarding the Viventis LS2 Live microscope and will provide worldwide support and service for all Viventis Microscopy products from now on.

Leica Microsystems
www.leica-microsystems.com

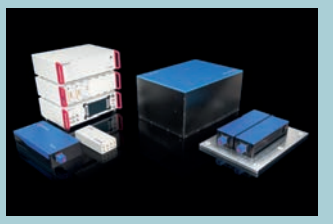
Cryo-Stage for Cryo-CLEM Microscopy

Linkam Scientific Instruments has introduced updates to its CMS196V4 cryo-stage, designed for cryo-correlative light and electron microscopy (cryo-CLEM). This stage allows sample investigation at cryogenic temperatures below -195 °C. Enhancements include a touch-panel and joystick interface, an encoded and motorized XY stage for precise automated mapping, and interchangeable optical bridge options. Improved cable management and simplified installation ease usage. Features include a cordless magnetic heated lid, auto-fill dewar with drip-feed, and an objective lens heater for better drift performance. The CMS196V4 supports full cryo-CLEM workflows, ensuring sample safety during handling and transfer. Additionally, Linkam's Modular Imaging Platform enhances sample access with motorized Z-axis control and a sliding mechanism, supporting various microscopy techniques. These updates aim to provide researchers with improved control and user experience in cryo-CLEM microscopy.

Linkam Scientific Instruments
www.linkam.co.uk

Clock Laser System for Quantum Applications

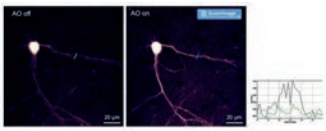
Toptica's Clock Laser System (CLS) is designed for quantum computing and optical clocks, offering exceptional frequency stability and ultra-narrow linewidths below 1 Hz. Stabilized with high-finesse optical ULE cavities, the CLS provides outstanding frequency stability beyond 1-second integration, making it suitable for driving narrow atomic clock transitions in neutral atoms like Yb and Sr, and ions like Yb^+, Sr^+, Ca^+, and Ba^+. The system is engineered with robust passive shielding, active vibration compensation, and advanced temperature stabilization, ensuring reliable performance even outside laboratory settings. The CLS is controlled via a single interface hosted by the DLC pro and accessible through the PC GUI Topas, supporting full remote operation and easy integration into user experiments with a programming SDK. Additionally, the CLS is available as a rack solution (MCLS) integrated into a Toptica T-RACK, ideal for space-constrained environments and easy transport.



TOPTICA Photonics
www.toptica.com

Adaptive Optics Boosts Imaging

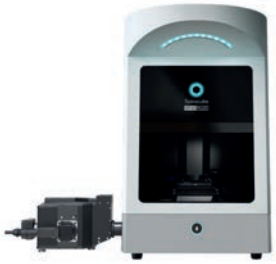
Phaseform has partnered with MBF Bioscience to integrate its Adaptive Optics (AO) solutions into ScanImage software for multiphoton and laser scanning microscopes. Using Phaseform's Deformable Phase Plate (DPP) technology, this integration offers a compact solution for correcting optical aberrations in deep tissue imaging. DPPs are placed directly in the illumination path, simplifying integration without major hardware changes. ScanImage now supports Phaseform's sensorless AO techniques for real-time aberration correction during image acquisition. Phaseform's AO control software interfaces with ScanImage to apply corrections seamlessly, allowing researchers to maintain standard microscopy workflows. This integration benefits research areas like neuroscience, developmental biology, and cancer research by enhancing imaging clarity and depth. The enhanced ScanImage platform will be available mid-2025, with previews at upcoming conferences.



Phaseform
www.phaseform.com

Bioimaging Platform

Tomocube and CrestOptics have created the HT-X1 Plus, a multimodal imaging platform that combines CrestOptics' spinning disk confocal technology with Tomocube's holotomography (HT). This integration enables high-resolution, 3D holotomographic imaging with fluorescence-based detection for advanced label-free biophysical imaging. HT imaging uses low-power visible light from various angles to measure phase delay and refractive index, visualizing 3D living cells and tissues without labeling. The HT-X1 Plus captures subcellular organelles with high spatial resolution while minimizing photodamage. By merging HT and confocal fluorescence imaging, the platform provides insights into molecular distribution within samples without invasive methods and aligns HT and fluorescence datasets, supporting researchers in studying molecular and phenotypic data for enhanced biophysical imaging.



Tomocube
www.tomocube.com

Operating System for Raman and Correlative Microscopy

Oxford Instruments has released Witec Suite Seven, an updated operating system for Raman and correlative microscopes. This update introduces several new features: DataTrace, Enhanced Project Manager, Spectral Certification, and Advanced Cosmic Ray Removal. DataTrace records all measurement settings and data processing steps, ensuring experiments are repeatable and results reproducible. The Enhanced Project Manager offers a streamlined overview of complex investigations with a color-coded hierarchical display and search capabilities. Spectral Certification verifies system calibration using reference sample spectra, useful in multi-user facilities and regulatory environments. Advanced Cosmic Ray Removal eliminates artifacts from high-energy particles in datasets, improving measurements with long acquisition times. These features enhance the software's transparency, intuitiveness, and precision, helping researchers maximize microscope performance and data clarity.

Oxford Instruments WITec
https://Raman.oxinst.com

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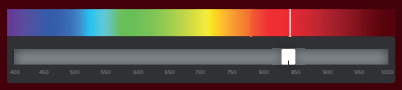

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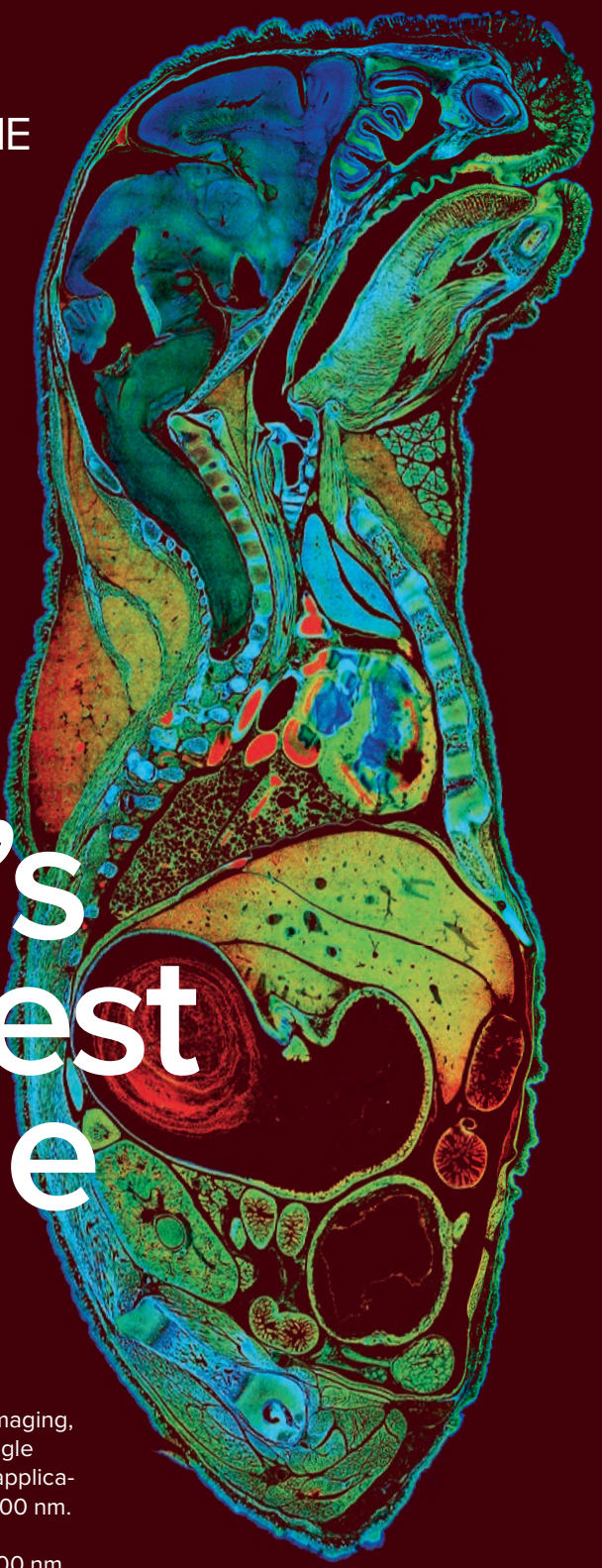

Confocal microscope image of a mouse. Credits: Leica Microsystems

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