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VOLUME 26
MARCH
2024

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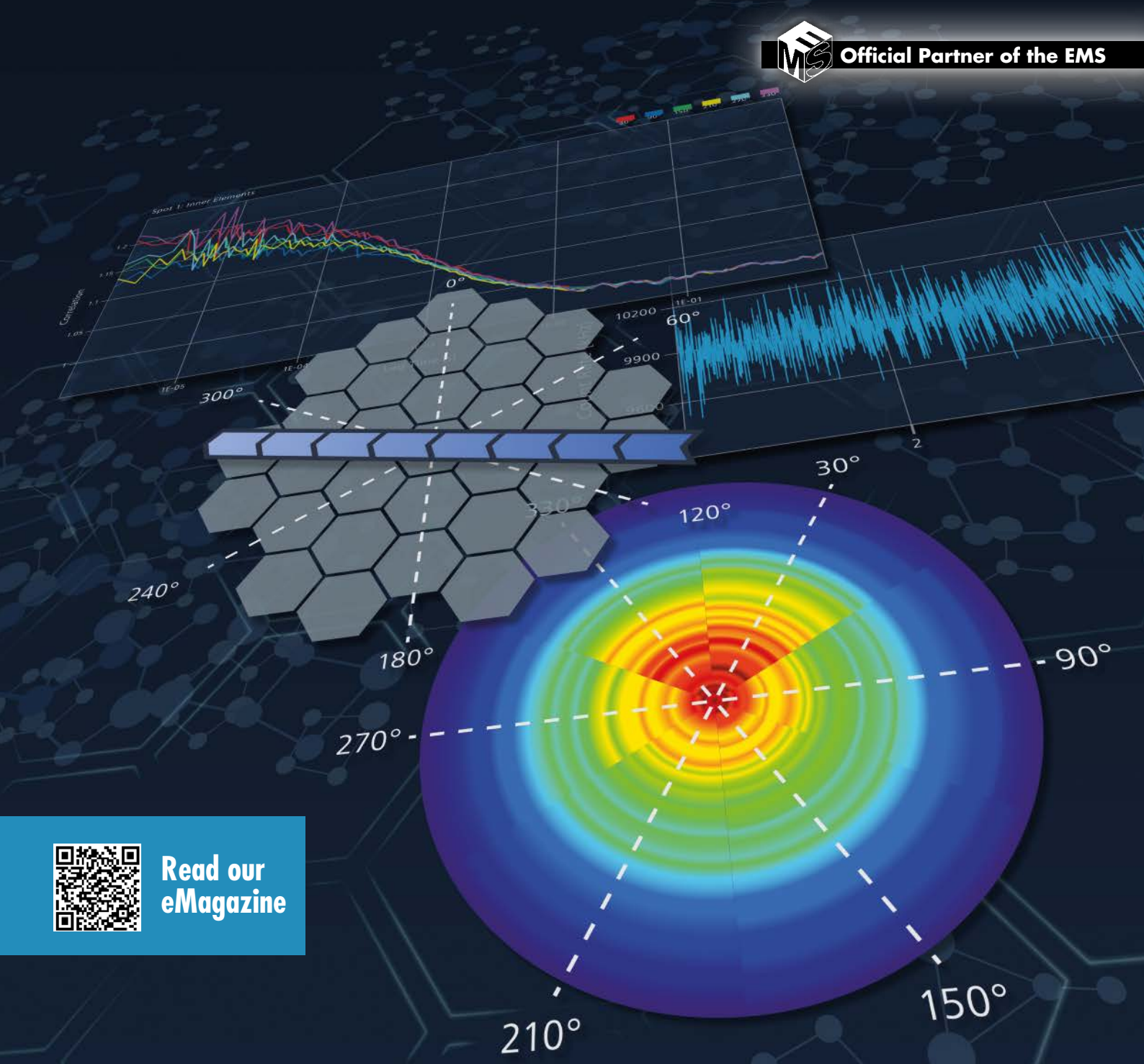

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Thank you and kind regards,

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Dear Readers,

It is with great pleasure that we welcome you to EMC24, Sunday, August 25 - Friday, August 30, 2024.

EMC2024 is the 17th of its kind and will bring the world of scientific imaging together in one of Europe's most beautiful cities - Copenhagen, Denmark.

Incorporating a balanced conference program of light and electron microscopy in both physical and life sciences - microscopists, manufacturers, and suppliers will come together to share new and exciting techniques, applications, and technology.

The EMC24 will take place at Bella Center, Center Boulevard 5, 2300 Copenhagen, Denmark, and will on Sunday, August 25 feature pre-congress workshops and an opening reception.

Monday the 26th, Eva Olsson IFSM President, will give the opening address followed by Prof. Carolyn Larabell as the first plenary speaker. The following days will feature Prof. Claus Ropers, Prof. Emma Lundberg, Prof. Vincenzo Grillo, and Prof. Moritz Helmstaedter as morning plenary speakers. The conference days will continue with six parallel sessions and each day will close with drinks in the Poster and Exhibition area. The last day of the Congress will conclude with a farewell reception.

The main symposia feature Life Science (Chair, Eija Jokitalo), Physical Science (Chair, Jakob Wagner), and Instrumentation and Methodology (Chairs, Julia Fernandez-Rodriguez and Randi Holmestad) – all session topics are described on the conference homepage. Abstract submission will start in early January 2024, but registration for the conference is already open – early bird registration is open until July 6, 2024.

Ongoing fundraising will attempt to fund as many travel bursaries (€ 400) as possible for young students and technicians.

The exhibition is one the largest of its kind featuring more than 100 exhibitors and a Nordic Corner for smaller enterprises, start-ups, and organizations, and 50% of the available exhibition space has already been reserved. Day passes will be available for a visit to the exhibition only. On January 17 the EMC2024 Corporate Advisory Board (Chair, Kornelia Weidemann, Thermo Scientific) will meet for an introduction of EMC2024 scientific content and not least an inspection of the venue.

The congress will among other address a range of microscopy-related subjects, ranging from dynamic interactions in cells, organoids, tissue, and entire organisms, pathology, immunocytochemistry, biomolecular labeling, volume Electron Microscopy in Life Sciences, semiconductors, heterostructures, and devices, geological materials, and bio-mineral systems, quantum materials, advances in 3-dimensional image reconstruction, new Instrumentation, and dynamic studies using micro-nano labs.

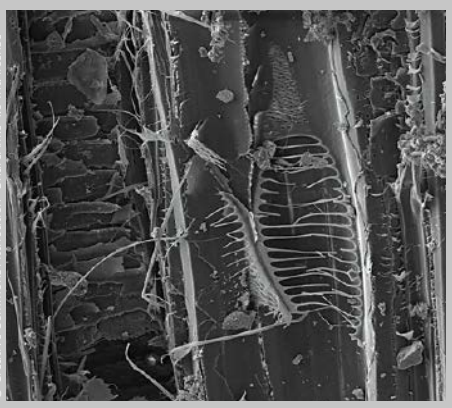
For information on the program, exhibition, sponsorship opportunities, and conference venue, please visit the conference website.

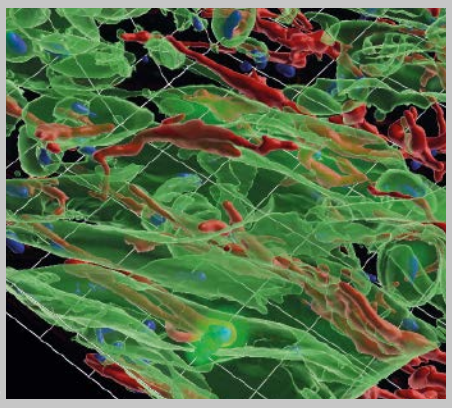
We welcome you to Copenhagen for the EMC2024 Congress and Exhibition.

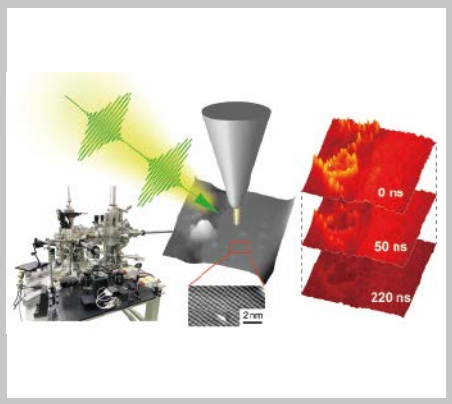
On behalf of the EMC2024 Local Organizers:

Prof. Klaus Qvortrup
Conference Chair

More information:
www.emc2024.eu







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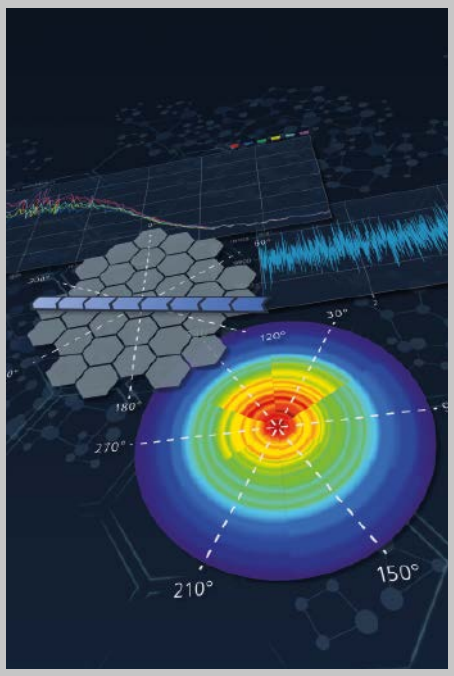


COVER STORY

Easy Access to Molecular Dynamics in Living Samples

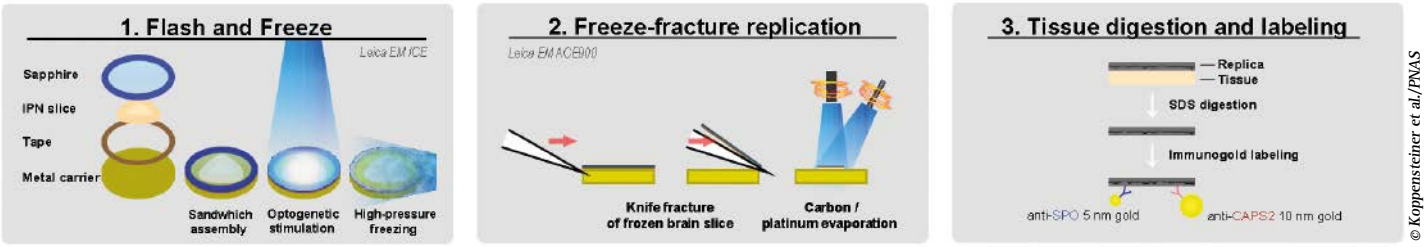
Investigation of Molecular Behavior in Combination with LSM Imaging Experiments Going Confocal

Understanding the dynamics of life is one of the driving forces in microscopy technology development. Resolving ever-so-small structures while reaching the needed temporal resolution allows to investigate subcellular and sub-organelle behavior in living samples in more detail and complexity than ever before [1]. However, molecular behavior in living samples is often overlooked, since image-based microscopy cannot always provide sufficient answers. But microscopy-based technology and techniques such as Fluorescence Correlation Spectroscopy (FCS) do allow deeper insights into molecular characteristics in vivo [2].

Newsticker

Electron Microscopy in Neuroscience Research

Analyzing Brain Region Using Light Flashes, High-Pressure Freezing and Fracturing



© Koppensteiner et al./PNAS

Fear and addiction exert significant influence within society. Managing them is often challenging, as they are driven by intricate neuronal circuits in our brains. Understanding the underlying molecular mechanisms is crucial to intervene when these processes malfunction. Pioneered by scientists at the Institute of Science and Technology Austria (ISTA), the novel "Flash and Freeze-fracture" technique provides a unique glimpse into the respective brain region. While looking for food, a bird encounters a fox. It gets away just in time, but the sight and the sound of the predator lingers. The negative experience will form a memory in its brain and will be associated with fear and stress from now on. Whenever it meets a fox again, the fear memory is revived. The bird's attention spikes, its heart rate goes up, and it changes its behavior to reduce the risk of predation. Such memory is mediated by a specific brain region called the medial habenula, one of the epicenters for emotional processing. The scientists investigated this particular part of the brain to understand how its neurons communicate with each other.

Original publication:
doi: 10.1073/pnas.2301449121
More information:
https://bit.ly/IM-012024-d

Beamline Analysis

A Dual-Functional Additive for Fast Charging and Long Cycle Life of Lithium Metal Batteries

© Jessica Rotkiewicz/BNL

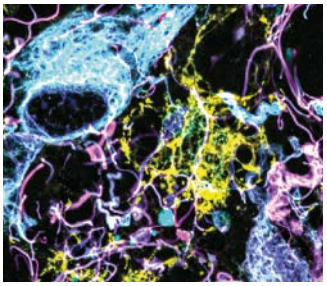
Researchers employed an electrolyte additive to improve the functionality of energy-dense lithium metal batteries. By adding cesium nitrate to the electrolyte that separates the battery's anode and cathode, the charging rate of lithium metal batteries significantly improved while maintaining a long cycle life. To better understand how the cesium nitrate additive influenced the electrolyte composition and battery performance, the scientists used four different beamlines at National Synchrotron Light Source II (NSLS-II), a DOE Office of Science user facility at Brookhaven Lab. The results using an X-ray Powder Diffraction (XPD) beamline showed that the cesium nitrate additive increased the presence of components known to make the interphase more protective. Remarkably, in addition to the typical crystalline components, a compound called cesium bis(fluorosulfonyl)imide was also identified.

Original publication:
doi: 10.1038/s41467-023-44282-z
More information:
https://bit.ly/IM-012024-b

Expansion Microscopy

Human Brain Tissue in Greater Detail than Ever Before

Using a novel microscopy technique, researchers have imaged human brain tissue in greater detail than ever before, revealing cells and structures that were not previously visible. The researchers hope this technique could eventually be deployed to diagnose tumors, generate more accurate prognoses, and help doctors choose treatments. The new imaging method is based on expansion microscopy, developed in Boyden's lab in 2015. By labeling the proteins with fluorescent antibodies before expansion, their location and identity could be visualized after the expansion. However, the antibodies commonly employed for this type of labeling face difficulty penetrating densely packed tissue before expansion. In the study the team created a different tissue-softening protocol that breaks up the tissue but preserves proteins in a sample. Once the tissue is expanded, commercially available fluorescent antibodies are used to label the proteins.

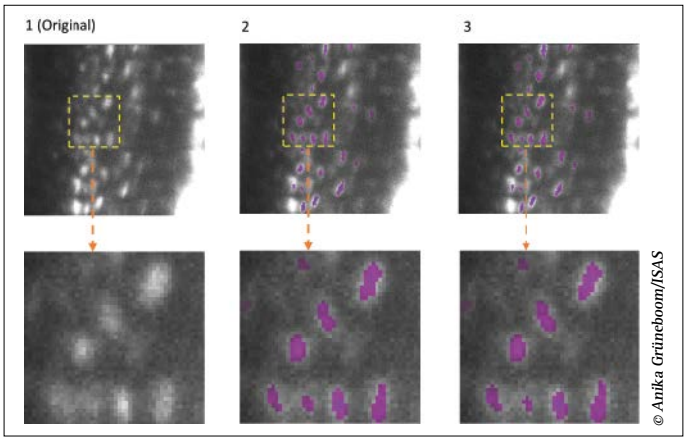


© Valdes, P.A., et al./MIT

Original publication:
doi: 10.1126/scitranslmed.abo0049
More information:
https://bit.ly/IM-012024-c

Open-Source Compression Software

EfficientBioAI Allows to Run Existing Bioimaging AI Models Faster with Significantly Lower Energy Consumption

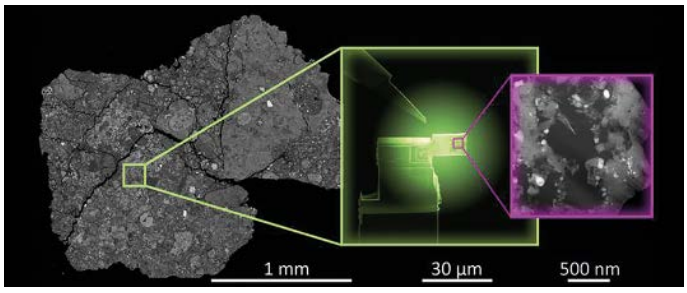


The open-source, user-friendly EfficientBioAI can dramatically increase the efficiency of neural networks in bioimaging without limiting the accuracy of the models. Artificial intelligence (AI) has become an indispensable component in the analysis of microscopic data. However, as AI models become more complex, the required latency (processing time) for images significantly increases, as does the associated energy consumption. To overcome the energy issue, sophisticated algorithms are used to compress the AI models and avoid high latency in image analysis, especially on devices with restricted computing power; i.e., the computational extent is reduced in these models while retaining comparable prediction accuracy. Several strategies are combined to reduce memory consumption, speed up model inference, and the 'thought process' of the model. Pruning, for example, can be used to remove excess nodes from the neural network.

Original publication:
doi: 10.1038/s41592-024-02167-z
More information:
https://bit.ly/IM-012024-e

SuperSTEM: *In Situ* Electron Microscopy

Insights into the Origin of Life Inside a Rare Carbonaceous Meteorite



© D.M. Kepaptsoglou, SuperSTEM

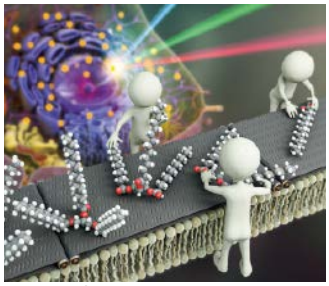
An international research team reported the first in-depth analysis of organic matter within the Winchcombe meteorite at the nanoscale. The study shows that the meteorite still contains pristine extraterrestrial organic molecules that might have been crucial to the advent of life on early Earth. They were able to uniquely correlate synchrotron-radiation data with complementary ultra-high resolution spectroscopic information about the nature of the functional chemical groups present in the organic matter, using one of the most powerful electron microscopes in the world at the SuperSTEM Laboratory—this allowed *in situ* detection of nitrogen-bearing biorelevant molecules, including amino acids and nucleobases.

Original publication:
doi: 10.1038/s41467-024-45064-x
More information:
https://bit.ly/IM-012024-a

Infrared Photothermal Microscopy

Monitoring Lipid Metabolism in Living Cells

Researchers have successfully developed a novel technology to investigate neutral lipids within lipid droplets of living cells. Two-Color Infrared Photothermal Microscopy (2C-IPM) can be used with isotope labeling, which allows for the detailed monitoring of neutral lipid synthesis within individual lipid droplets. Unlike previous fluorescence microscopy-based methods, the 2C-IPM technology relies on infrared spectroscopic (IR) and does not require the use of fluorescent dyes. The new approach directly monitors neutral lipids within LDs by detecting changes in IR absorbance. Remarkably, it allows researchers to monitor the synthesis of neutral lipids within individual LDs in living cells over a long period. The team investigated the synthesis of neutral lipids in cells exposed to excess fatty acids. They could distinguish freshly synthesized neutral lipids from pre-existing neutral lipids within cells by subjecting deuterium-labeled fatty acids, which have distinct spectroscopic properties from non-deuterated forms. The results demonstrated that excess fatty acids led to lipid toxicity, and cells responded by increasing the synthesis of neutral lipids.



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Original publication:
doi: 10.1039/D3SC04705A
More information:
https://bit.ly/IM-012024-f

RMS Events: Action-Packed Spring and Summer Ahead

A wide range of fantastic RMS meetings, conferences, and courses are lined up for the microscopy, imaging, and flow cytometry community in the coming months.

Our Spring offerings get underway with AFM & SPM Meeting 2024 (25 – 28 March, Durham, UK). This international conference on Scanning Probe Microscopies is perfect for graduate students and early career scientists, as well as renowned experts in the field of SPM.

Another essential Spring event, EBSD 2024, takes place on 29 – 30 April, in Glasgow, UK. This annual gathering draws together the whole Electron Backscatter Diffraction (EBSD) community, including leading international research scientists and engineers, early career researchers, and students.

Fast forward to June, and the RMS will be proudly hosting elmi2024 in Liverpool, UK. This is an essential event for the Light Microscopy

community – bringing together both research scientists and the manufacturers of the equipment. Find out more and book your ticket online at the elmi2024 website:

Throughout the summer, the RMS is also hosting a series of courses and summer schools covering a host of different branches of microscopy, techniques, and applications.

Find out more about the following RMS courses, and book your place:

- Light Microscopy Summer School 2024
- Getting the most from your Confocal Course 2024
- Electron Microscopy Summer School 2024
- Strathclyde Optical Microscopy Course 2024 (RMS-sponsored event)
- Flow Cytometry Course 2024

All upcoming RMS events: www.rms.org.uk/rms-event-calendar.html

Super Resolution Optical Imaging and Microscopy: Methods, Algorithms, and Applications

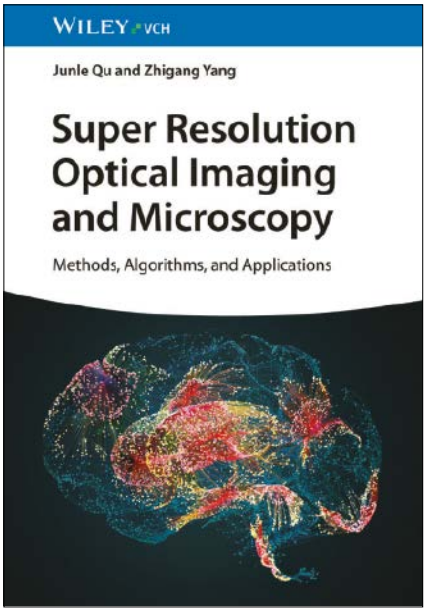
This book covers both the basic principles and specific technical details of super-resolution microscopy techniques. It covers the criteria to choose different fluorophores for various SRM methods and critically assesses the nitty-gritty of associated problems that are often encountered in practical applications. A progressive guide to designing the next generation of advanced fluorophores to meet the goal of advanced SR imaging studies is also put forward.

Written by two well-qualified authors, the book contains exclusive content to enhance readers' understanding on innovation of newer SRM technologies. Sample topics covered in the book include:

- Optical techniques, fluorescent probe design, and algorithm development
- Recent highlight and breakthroughs in biology using SRM methods
- The overall success of SRM in biological inventions
- The future direction and scope of the field

This book is an invaluable resource for chemists and researchers/scientists involved in designing newer fluorescent materials for SRM studies. It can also assist biologists engaged in advanced biological studies using SRM by guiding them through sample preparation, image processing, and precautions to be taken in practical imaging studies.

Editor(s): Junle Qu, Zhigang Yang
First published:10 January 2024
Print ISBN:9783527349869
Online ISBN:9783527835539
DOI:10.1002/9783527835539



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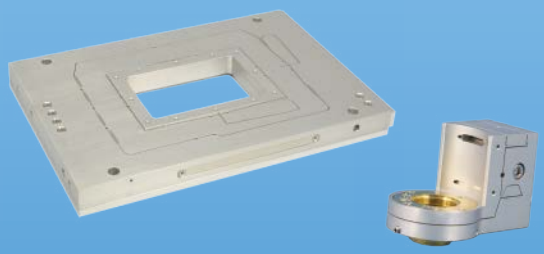
Nanopositioning Systems



Features
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- Sub-nanometer precision
- High stability, low noise
- Smooth, continuous motion
- Analog & Digital controllers

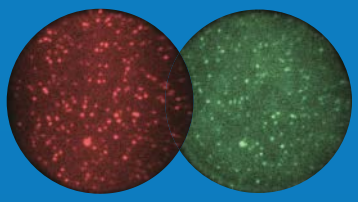
Applications
- Single Molecule Microscopy
- Particle Tracking
- Optical Microscopy
- Force Measurements & AFM

Micropositioning Systems

Products
- Mad-Deck™ XYZ platform
- MMP Series - compact & modular
- Microscope stages

Features
- Intelligent control with 95nm steps
- Nanopositioner compatible
- High stability design
- High native precision & accuracy

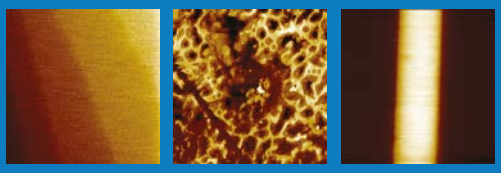
RM21® Single Molecule Microscopes



Features
- Open optical pathway access
- Nanopositioner compatible
- Unique MicroMirror TIRF method with outstanding signal-noise ratios
- Ideal for microscopy innovation

Applications
- MicroMirror TIRF Microscopy
- Single Molecule Localization Microscopy
- TIRF/HiLo Microscopy
- Interferometric Scattering Microscopy
- Single Particle Tracking

Atomic Force Microscopes (AFM)



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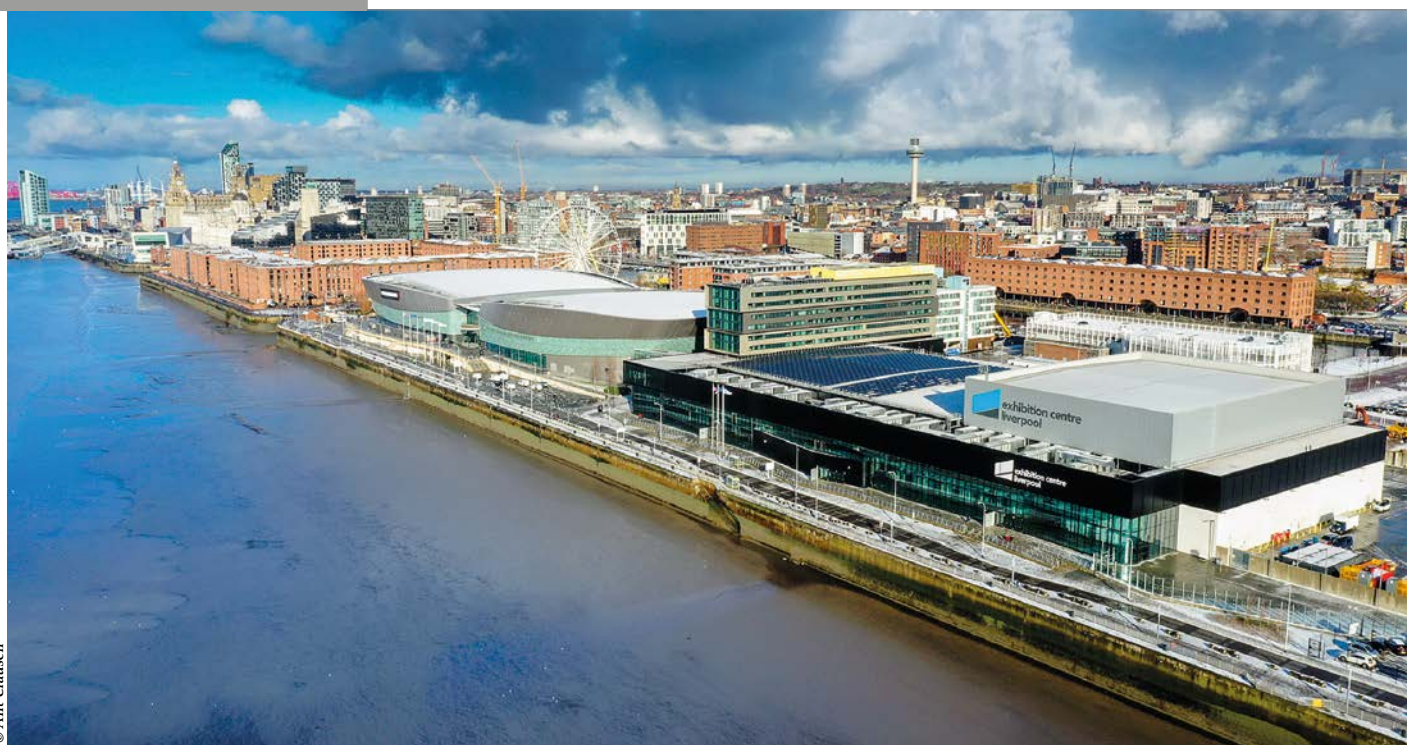

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ACC Convention Centre and Exhibition Centre.

Register Now for elmi2024!

The RMS Is Very Proud to Be Hosting elmi2024, Taking Place in Liverpool, UK, from 4 - 7 June

With a blockbuster scientific programme, world-class exhibition, 'hands-on' workshops and more, the annual meeting has become regarded as an essential event for the Light Microscopy community.

'Early bird' booking rates are available until 9 April – providing a 23% discount on the cost of a standard ticket, and 30% off for students. Find out more about registration rates and book now on the event page (link in crossmedia bar).

Unique Communication Network

The European Light Microscopy Initiative (elmi) was created in 2001 to establish a unique communication network between European scientists working in the field of light microscopy and the manufacturers of their equipment. Its aim is to promote the quickly developing field of light microscopy as a fundamental research tool for the life sciences and to strengthen the channels of communication between researchers, core facilities and industry.

The event has been running for two decades at various venues across Europe, and has an excellent reputation within the microscopy community, making it a key fixture in the calendar of hundreds of scientists and developers.

The strength of this meeting lies in the mixture of scientific lectures on state-of-the-art, high-end microscopy, combined with 'hands-on' workshops and an exhibition of the latest technology, organised by the leading companies in the field.

Great Venue in the Heart of Liverpool

elmi2024 is being held at ACC Liverpool, a purpose-built arena and convention centre in the heart of the iconic city, on the King's Dock. The venue has great transport links as well as being within walking distance from a large number of hotels, restaurants, shops and bars. Liverpool is one of the UK's most iconic cities with a rich, cultural heritage, a wide range of attractions and buzzing nightlife. With the conference venue just a short walk from the bustling waterfront and commercial centre, there is no shortage of things to see and do during your stay.

What you Can Expect at elmi2024:

- A blockbuster meeting programme covering all the latest techniques, applications and technology. Topics include: New Technologies, Imaging Across Scales, Super-resolution and Nanoscale Imaging, The AI Revolution, The Science of Tomorrow Today, and Multimodal Imaging.
- A wide range of companies showcasing their latest technology and running workshops timetabled outside of the main meeting programme.
- An accompanying exhibition in the purpose-built hall alongside posters, food and drink;
- A community workshop space at the heart of the exhibition, with many groups hosting meetings and running workshops.
- An event dinner with networking at the Rum Warehouse.

european light microscopy initiative (elmi)

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

More information and registration:
www.elmi2024.org

José Maria Valpuesta,
EMS President

Virginie Serin,
EMS Secretary

EMS Newsletter #84

March 2024

Dear EMS members,

Let me first wish you a happy 2024. Last year our society celebrated its 25th anniversary. To commemorate this milestone, a booklet containing articles written by leading scientists of our community will be distributed at the upcoming European Microscopy Congress (EMC2024), to be held in Copenhagen from August 25th to August 30th, 2024.

The new EMS year has started with the call for nominations for the EMS Outstanding Paper Awards 2023 and for the EMS awards, which will be presented at EMC2024.

As usual, the EMS is committed to supporting the activities of our members, so please visit our webpage for the various meetings and workshops that will take place over the next year, some of which are going to be financially supported by EMS:

In addition, EMS will offer scholarships (link in cross-media bar) to young researchers participating at EMC2024. The application deadline is April 17, 2024. Visit our webpage for more information. We look forward to receiving your applications.

Hoping we can meet next August at EMC2024!

Contact

Prof. Dr. Virginie Serin
EMS Secretary
Toulouse, France
sec@eurmicsoc.org

Prof. Dr. José Maria Valpuesta
EMS President
Madrid, Spain
jmv@cnb.csic.es

- Molecular Diagnostics Training School (MDTS) & Digital Pathology and Image Analysis Training School (DP & IATS)
  25 to 28 February 2024, Vienna – Austria

- BIST Symposium on Microscopy, Nanoscopy, and Imaging Science 2024,
  10 March 2024, ICFO - Auditorium – Barcelona

- Winter School 2024 – Practical course in advanced microscopy
  22 to 26 January 2024, University of Zurich Center for Microscopy and Image Analysis – Zurich – Switzerland

- Electron Microscopy Spring School 2024
  03 to 06 March 2024, Berlin – Germany

EMS scholarships:
www.eurmicsoc.org/en/funding/scholarships/

All EMS events:
https://bit.ly/EMS-spons-Events

EMS web page:
https://bit.ly/EuroMicr

Easy Access to Molecular Dynamics in Living Samples

Investigation of Molecular Behavior in Combination with LSM Imaging Experiments

Annette Bergter[1] and Hanna Gut[1]

Understanding the dynamics of life is one of the driving forces in microscopy technology development. Resolving small structures while reaching the needed temporal resolution allows investigation of subcellular and sub-organelle behavior in living samples in more detail and complexity than ever before [1]. However, molecular behavior in living samples is often overlooked, since image-based microscopy cannot always provide sufficient answers. But microscopy-based technology and techniques such as Fluorescence Correlation Spectroscopy (FCS) do allow deeper insights into molecular characteristics *in vivo* [2].

Laser Scanning Microscopes (LSM) are high-end microscopy systems that provide great flexibility in imaging experiments for a wide variety of samples [3,4]. The single excitation volume of a laser beam, which is conventionally used in the process to generate instant high-resolution optical sections of one or several fluorescent labels, is the perfect tool for FCS measurements. The excitation volume is kept stationary and positioned at a point of interest within the sample (Fig. 1). Fluorescently labeled molecules move through the excitation volume and the constantly changing emission intensity is recorded. With a matching analysis, the pattern of this intensity trace can reveal molecular characteristics, such as concentration or velocity.

Until recently, FCS was often disconnected from imaging experiments routinely conducted with an LSM, since FCS only worked with extremely low fluorescent expression levels in the sample, needed specialized hardware, and required specific training.

Zeiss Dynamics Profiler uniquely overcomes this limitation and gives easy access to molecular dynamics with a wizard-based workflow. It utilizes the Zeiss Airyscan detector to collect 32 individual FCS intensity traces per measurement for enhanced molecular information, even in bright samples.

Adding spatial information to FCS measurements with the Airyscan area detector [5] expands the opportunities from straightforward mobility and concentration measurements. Using multiple Airyscan detector elements allows investigation of asymmetric diffusion as in biological condensates and determines flow speed and direction in blood vessels or microfluidic systems (Fig. 2) [6].

Remarkably Easy Access to Molecular Dynamics

To ensure reliable and reproducible data, Dynamics Profiler guides the user by a 3-step wizard. It ensures correct hardware selection and detector alignment and supports steps to improve data quality. A reference image is taken in which the positions to be analyzed can be chosen. Each of the measurement spots is color-coded and its displayed dimensions precisely depict the investigated area in the sample. After data acquisition, the results are provided as a combination of images, graphs, and tables. All data can be exported to be used for further investigation, documentation, or publication.

Thus, information about the concentration and the dynamic behavior of a fluorescently labeled protein can be easily added to a live cell or live organism experiment, as in the example depicted in Figure 3. mCherry is expressed under the control of the enhancer for the *hand* gene in the heart cells of the *Drosophila melanogaster* embryo. The AI Sample Finder was used to create an overview image and identify an embryo of the correct age and orientation (Fig. 3, top left). A z-stack was taken with the Airyscan detector, providing super-resolution structural information while keeping light exposure to a minimum (Fig. 3, bottom left). Several cells of different intensities were chosen for Dynamics Profiler measurements, recording their precise concentration levels of the expressed protein (Fig. 3, right).

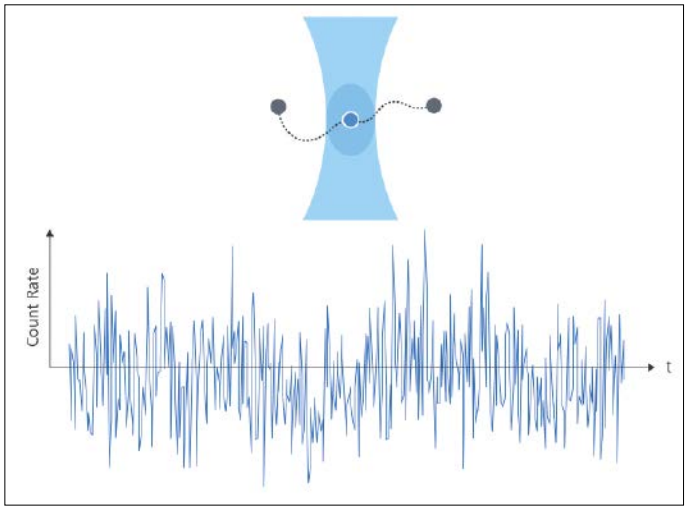


Fig. 1: A stationary excitation volume, created by an objective lens focused laser beam (top) is positioned in a sample. Fluorescence intensity fluctuations are recorded for this spot over several seconds, resulting in an intensity trace measurement (bottom).

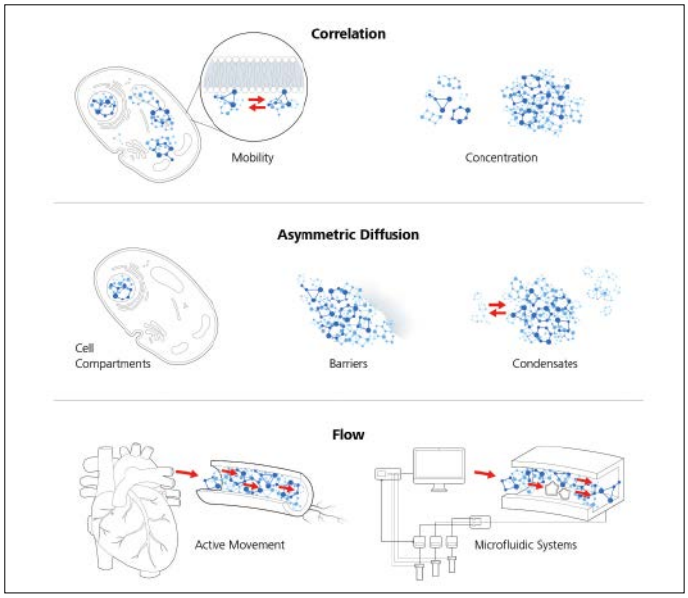


Fig. 2: Applications enabled by Dynamics Profiler.

Access to New Molecular Discoveries

Utilizing the Airyscan area detector, the Dynamics Profiler adds spatial information to every FCS measurement, providing the unique opportunity to measure liquid flow at a precise position within a volume. The flow direction and speed (µm/s) of fluorescent proteins or dyes that are actively transported in a liquid can be determined in single measurement spots.

The behavior of blood flow in vessels can be investigated, resulting in exact speed values for individual positions within the bloodstream. The conditions for cells, organoids, or tissue cultures in microfluidic systems can be documented and included in the experiment (Fig. 4).

New Insights from Living Samples

Due to the dramatic increase in brightness during the process of protein accumulation into condensates, it is impossible to study these high concentrations of molecules using conventional FCS methods. Dynamics Profiler can measure even these bright condensates. Furthermore, by utilizing element pairs of the Airyscan detector, differences caused by cellular compartments, barriers, or molecular binding processes can now be assessed (Fig. 5). The changing behavior of proteins inside and outside cellular condensates formed by liquid-liquid phase separation can be studied to analyze their changing dynamic behavior.

Summary

Zeiss Dynamics Profiler provides a new method to investigate molecular behavior in combination with LSM imaging experiments. In addition to the integrated analysis tools, raw data of 32 individual detector elements is always saved, allowing for seemingly endless possibilities to investigate molecular movement.

Affiliation
[1]Zeiss Research Microscopy Solutions, Jena, Germany

Contact
Dr. Annette Bergter
ZEISS Research Microscopy Solutions
Jena, Germany
annette.bergter@zeiss.com

Online version and references:
https://bit.ly/IM-Zeiss-0124

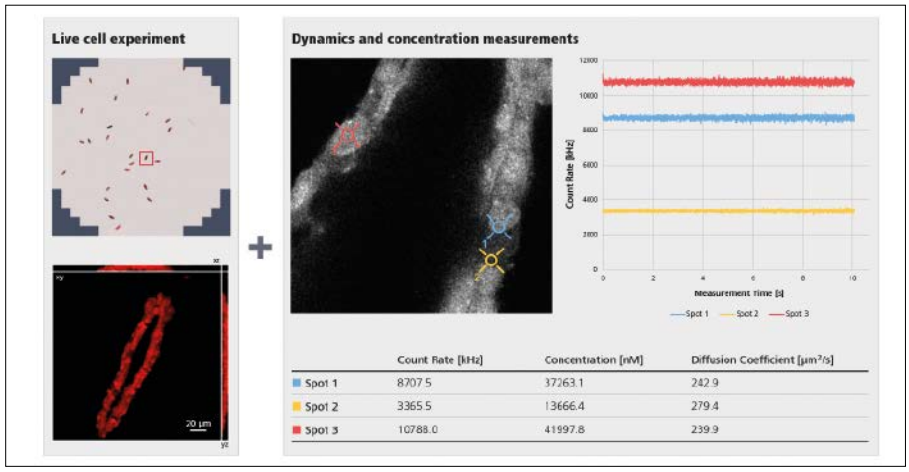


Fig. 3: Live imaging experiment of Drosophila melanogaster embryos. Top left: Overview scan. Bottom left: Z-Stack acquisition with Airyscan, Ortho Maximum Intensity Projection. Right: Dynamics Profiler experiment with 3 spots, showing the individual and color-coded intensity traces (graph) and analysis results (table). Sample courtesy of Prof. Dr. Achim Paululat and Dr. Christian Meyer, Osnabrück University, Department of Zoology and Developmental Biology, Germany.

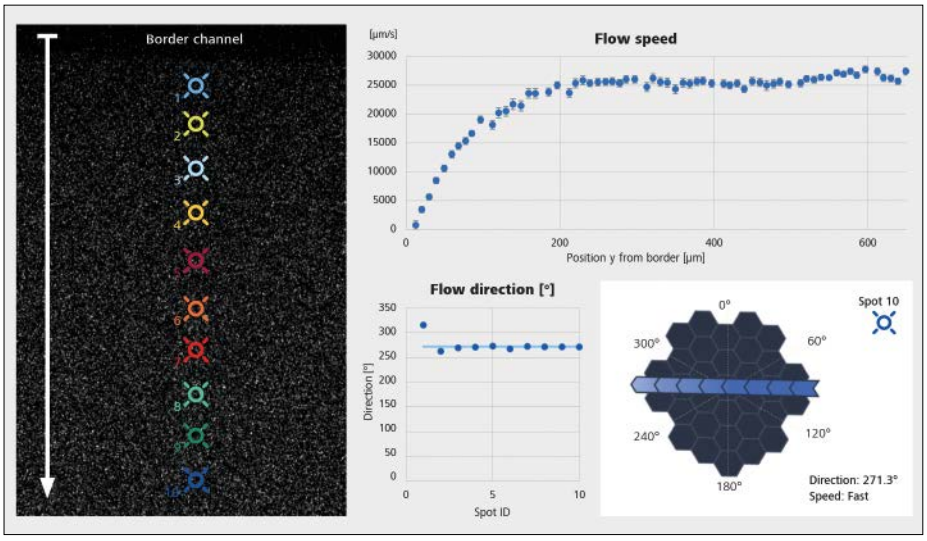


Fig. 4: Analysis of flow characteristics in a microfluidics channel. The Automated Sequential Injection System Aria by Fluigent was used to pump a rhodamine 110 solution through a microfluidic flow cell (1000 µm channel width).

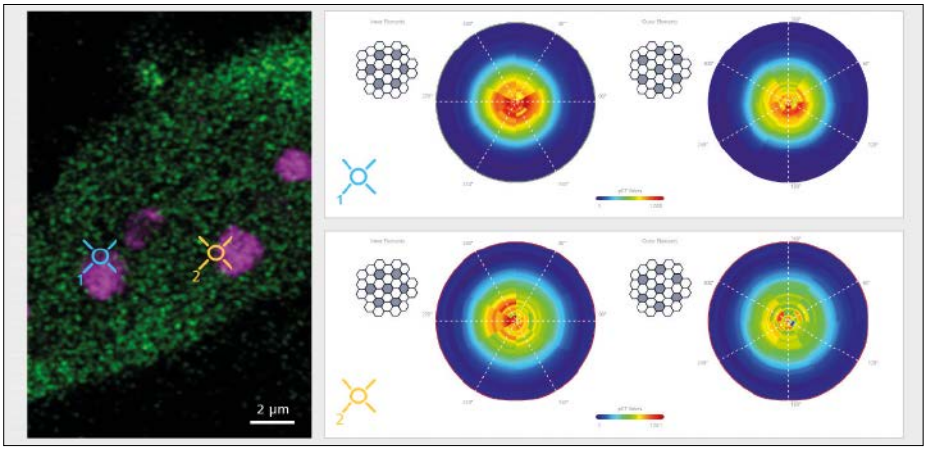
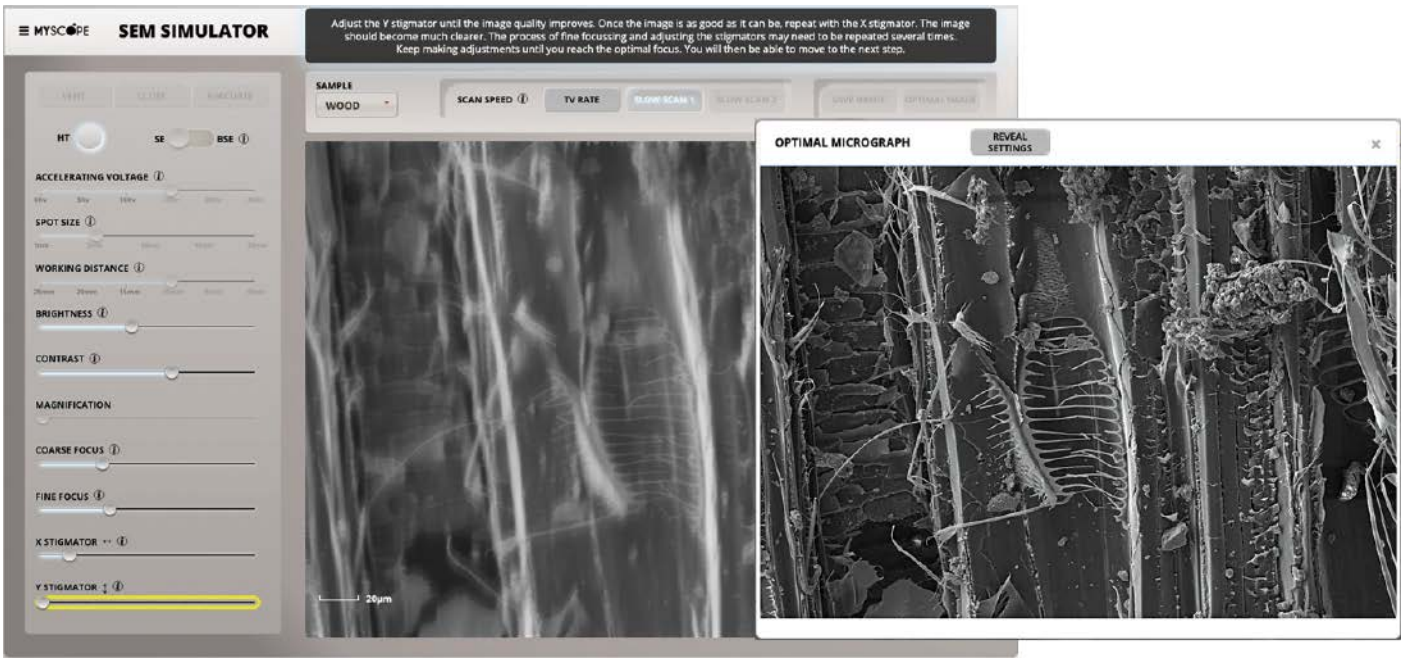


Fig. 5: Live U-2 OS cells transiently co-expressing tetrameric EGFP and the nucleolar protein Fibrillarin tagged with monomeric Red Fluorescent Protein. Measurements at the border of nucleoli (left) reveal an asymmetric diffusion behavior (heatmaps, right). Sample kindly provided by P. Hemmerich and T. Ulbricht, Core Facility Imaging, Leibniz Institute on Aging, Jena, Germany.



MyScope: Free Online Microscopy Training

A Comprehensive, Open-Access, Training Resource

Jenny Whiting[1], Peta Clode[2], Lisa Yen[1], Nikki Stanford[3]

Microscopy training is a time-consuming and labor-intensive process and tools that support this training can aid efficiencies for staff in microscopy core facilities. MyScope is one such resource that has been developed by Microscopy Australia, a provider of open-access research infrastructure for microscopy and microanalysis in Australia. The content is completely free, openly accessible, and doesn't require any registration. This comprehensive online tool covers ten different broad microscopy techniques such as SEM, TEM, Light, and Fluorescence Microscopy as well as basic concepts, research data management, and work health and safety. MyScope has been developed by experienced trainers and is structured into separate modules that can be tailored further if required. The modules are structured to contain well-illustrated theory, instrument simulators, and assessments that can generate a certificate if a high-level pass is achieved. The use of, and rationale for, MyScope development are discussed.

Introduction

Microscopy Australia developed the free, open-access online microscopy training website MyScope in 2011. It was developed to be part of a blended learning approach to microscopy training, providing a preliminary online component for new microscopists, thereby saving time during subsequent hands-on training. Although developed to support Microscopy Australia users, the platform is freely available to everyone and has emerged as a world-leading resource for the international microscopy community.

Rationale for MyScope

Microscopy Australia is Australia's national research infrastructure for microscopy and microanalysis and is composed of an Australia-wide network of university-based microscopy facilities. This organization provides open access to microscopy instruments and expertise to researchers across Australia. A major part of the facility staff's time is involved with training new users and supporting more experienced users to get the most out of the instruments. To increase the efficiency of this new user training, MyScope was developed and is used across Microscopy Australia. It has been shown to reduce hands-on training time by 11–25% (depending on training regimes at different facilities) [1] by bringing inexperienced new users up to speed on the fundamentals of specific microscopy techniques and, through simulators, giving them a feel for using a real instrument.

MyScope contains ten technique-specific modules, plus others on Microscopy Concepts, Research Data Management, and Work Health and Safety. Initially, it focused on six of the major microscopy techniques offered to our users. As the microscopy landscape has diversified, the platform has been developed to where it is now (Fig. 1).

The approach to developing content is to provide a range of complementary pedagogical approaches: clear theory and diagrams, real images and data, animations, flowcharts, interactive instrument simulators, assessments, glossary, and links to more resources, including videos. This approach remains front of mind when extending MyScope.

Instrument simulators are at the core of many of the modules and are designed to

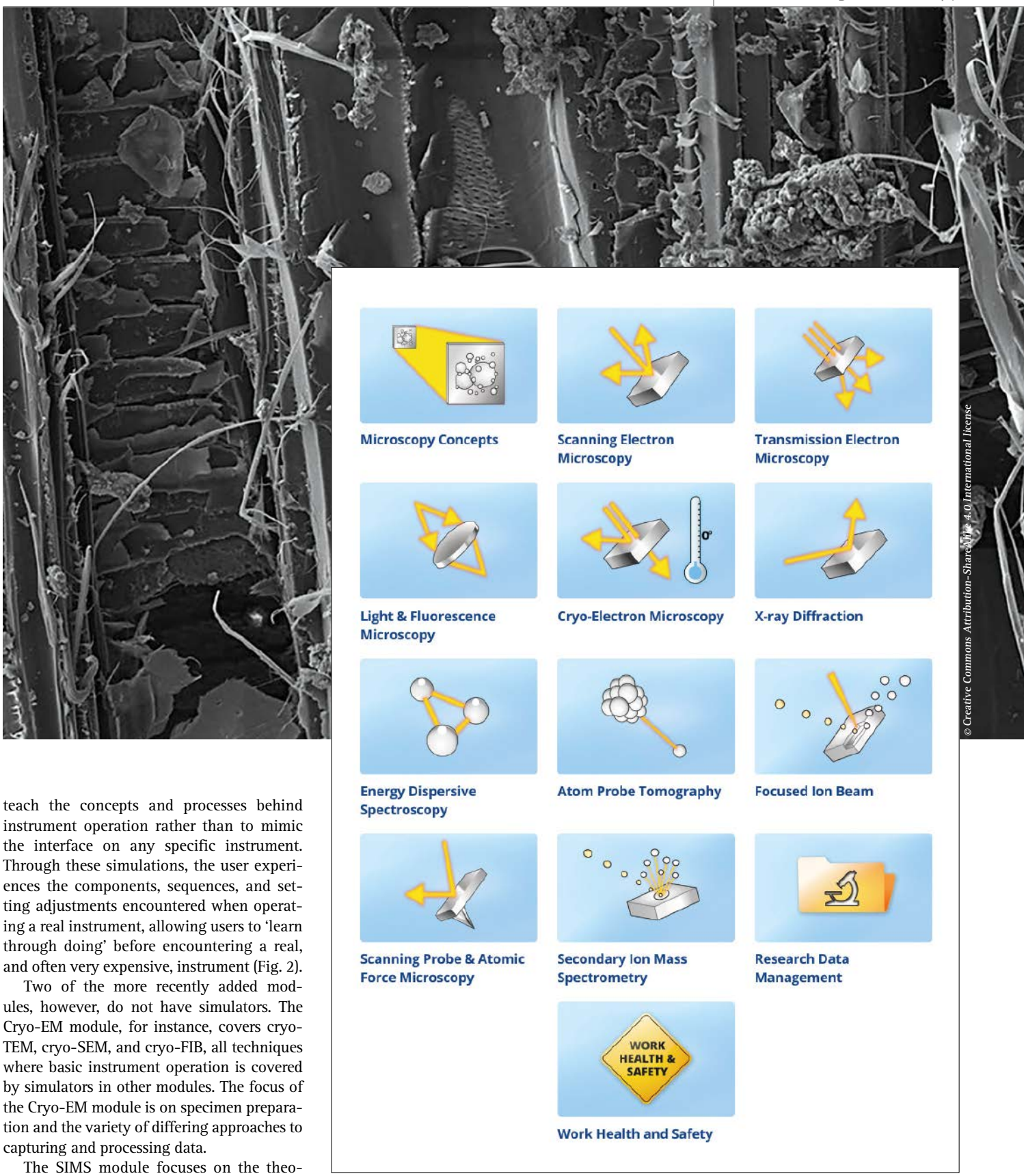


Fig. 1: The 13 MyScope training modules.

teach the concepts and processes behind instrument operation rather than to mimic the interface on any specific instrument. Through these simulations, the user experiences the components, sequences, and setting adjustments encountered when operating a real instrument, allowing users to 'learn through doing' before encountering a real, and often very expensive, instrument (Fig. 2).

Two of the more recently added modules, however, do not have simulators. The Cryo-EM module, for instance, covers cryo-TEM, cryo-SEM, and cryo-FIB, all techniques where basic instrument operation is covered by simulators in other modules. The focus of the Cryo-EM module is on specimen preparation and the variety of differing approaches to capturing and processing data.

The SIMS module focuses on the theoretical basis of the different types of SIMS instruments, how they work, what is achievable, and why. Although our network has several SIMS instruments, the majority are too advanced and complex for users to learn in a time- and cost-effective way. The staff runs these instruments for the researchers so that optimal outcomes are obtained. This module gives users an understanding and appreciation of the technique, as well as provides an opportunity for the global SIMS community of facility staff and researchers to learn more about the fundamentals of SIMS.

In both these cases, the developers have chosen to include several interactive components to enhance learner engagement and reinforce critical concepts addressed in the text. Figure 3 shows examples of these. In cryo-EM, single particle analysis depends heavily on 2D projections of 3D molecules, and this concept is illustrated in the Cryo-EM module by an illuminated 3D shape that can be rotated so that the chang-

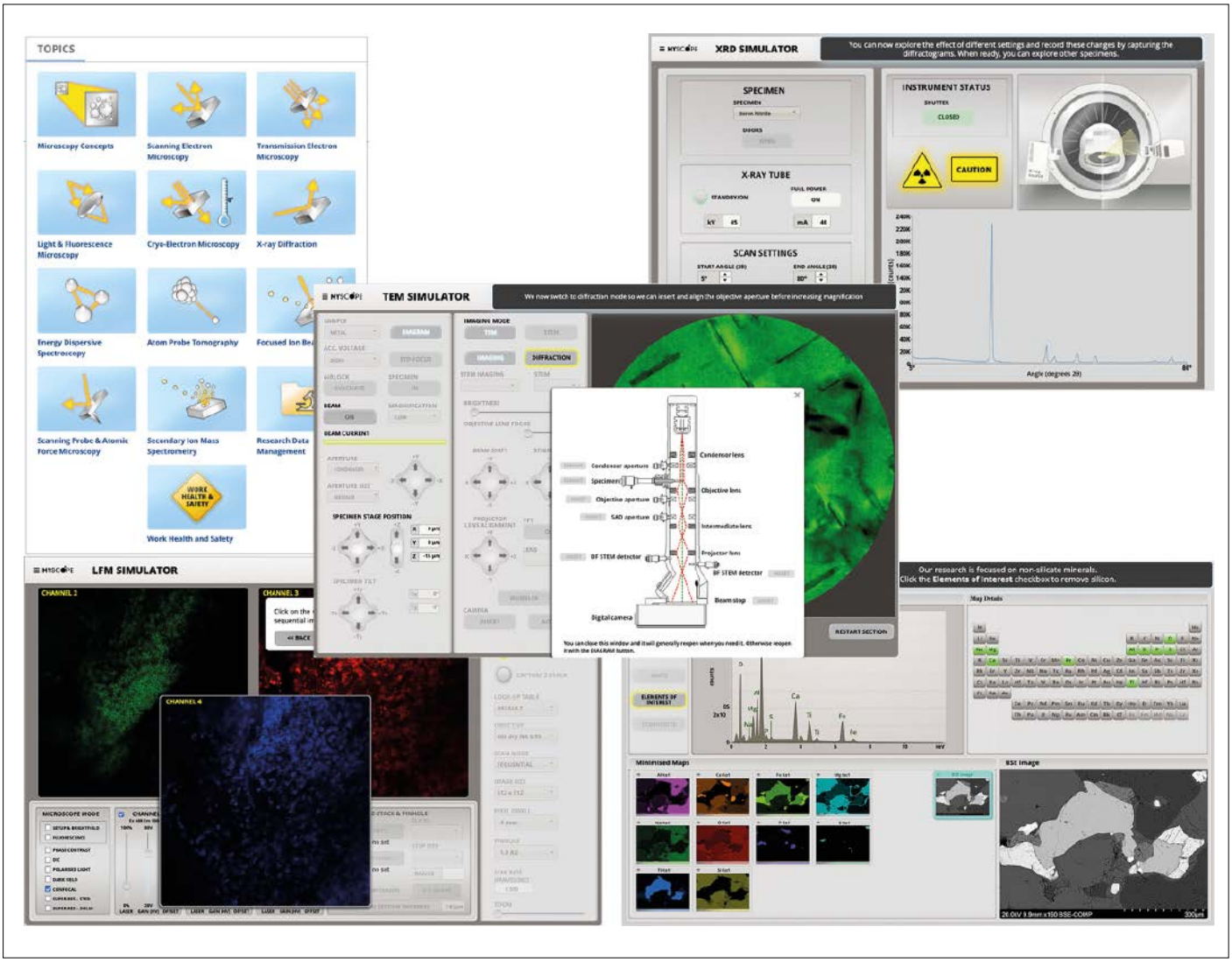


Fig. 2: Selected screen grabs from MyScope and some of its simulators.

ing projection can be observed. Another requires the user to sort 2D projections into classes corresponding to different protein orientations (Fig. 3).

Assessment is an essential component of MyScope. It allows users to monitor their learning and demonstrates to facilities that relevant knowledge has been acquired. When a user achieves an 80% pass on the assessment quizzes, a certificate is earned. Achievement of this high level was determined to be necessary if significant time savings in hands-on training were to result. This certificate can be presented to facility staff as evidence of completion of the online training component and hands-on training can then be booked. Each assessment session draws ten questions randomly from a bank of multiple-choice questions. All answers, whether right or wrong, have instructive feedback to help the learner understand the correct answer as part of the learning process.

Underpinned by Expertise

The content for MyScope has been developed by Microscopy Australia expert staff largely through in-kind contributions from our network of facilities. Highly skilled in microscopy and user training, the staff maintain their expertise through a range of national and international collaborations and exchanges through which they share best practices and innovative approaches. Central coordination of the platform content ensures a level of consistency across the resource.

Uptake and Usage of MyScope

MyScope has developed a global reputation for excellence in training and has thousands of users each year from around 100 countries with the majority from Australia, the US, China, the UK, and Mexico.

One of the powerful aspects of the platform is that it accommodates the diverse backgrounds and levels of experience of the learners who come to use it. It is self-paced and freely available, making it a valuable complementary resource for use before and during the early stages of hands-on training.

Another helpful feature is that modules can be tailored to just those components that are relevant to the students in question. Teachers and trainers who include microscopy in their courses provide us with very positive feedback.

Challenges and Outlook

Tracking the use of MyScope consistently over time is currently challenging due to the change of Google Analytics to GA4. However, many thousands of users from nearly 100 countries are engaging with all the platform modules with extended periods of use per session. A

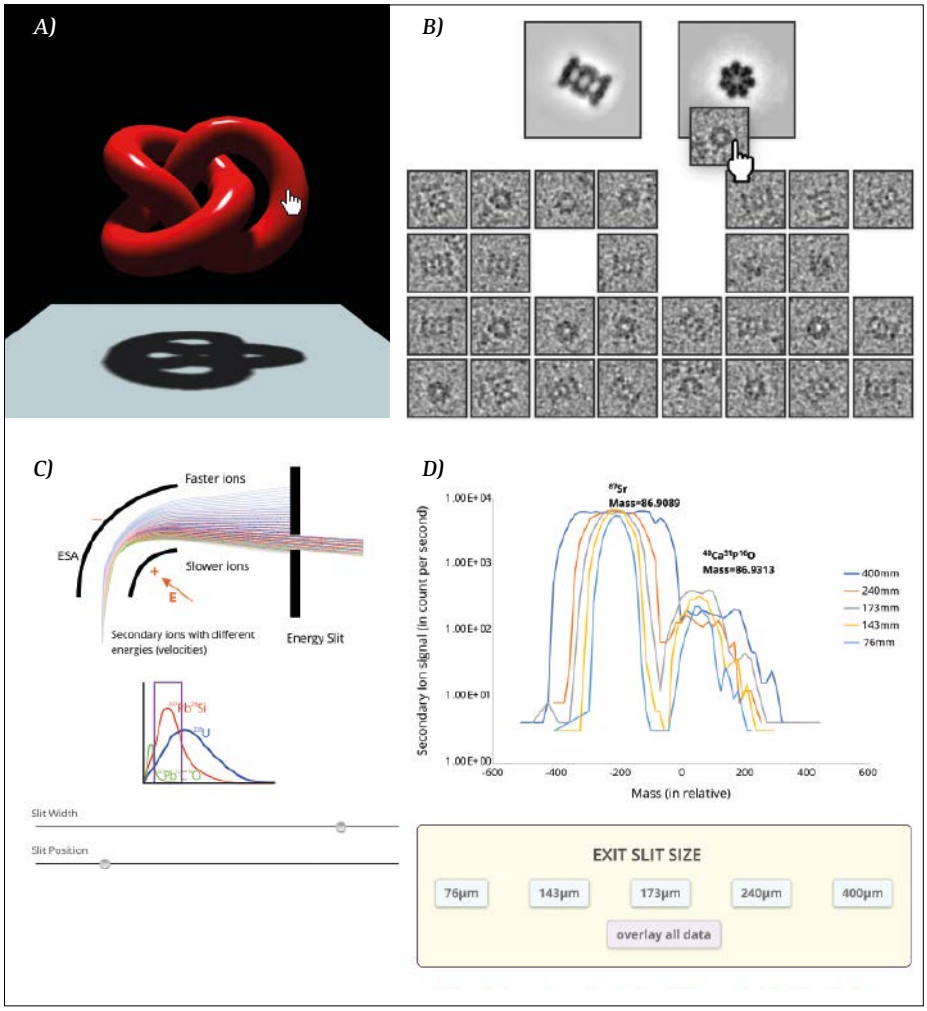


Fig. 3: Examples of interactive components in the Cryo-EM (A & B) and SIMS (C & D) modules. A. Projection of a rotatable 3D object (image courtesy: Martyn Cook). B. Class sorting activity. C. Activity where the user adjusts and selects the optimal slit size for an electrostatic analyzer. D. Activity where the user selects the optimal slit size for a magnetic sector analyzer.

clearer pattern of use will emerge as GA4 analytics are collected going forward. Also, with constraints on staff availability and funding, it is increasingly challenging for us to continue developing MyScope, with impacts felt in content preparation and online development. However, we are committed to improving and maintaining the platform, including more integration of Microscopy Australia videos and links to external resources available through reputable international sources.

To finish, this comment from an independent external review of Microscopy Australia summarises the impact of MyScope: "The MyScope microscope simulators and online training modules are one of Microscopy Australia's most internationally visible and cost-effective investments ... There is no comparable resource to MyScope anywhere else in the world."

Affiliation
[1]Microscopy Australia, University of Sydney, Sydney, Australia
[2]Centre for Microscopy, Characterisation and Analysis, University of Western Australia, Perth, Australia
[3]Future Industries Institute, University of South Australia, Adelaide, Australia

Contact
Jenny Whiting, Ph.D.
Microscopy Australia
University of Sydney
jenny.whiting@sydney.edu.au
ORCID: 0009-0006-4377-0859

MyScope Training: https://myscope.training

More information: https://micro.org.au

[1] References: https://bit.ly/IM-Whiting

A Global Reproducibility Initiative in Light Microscopy

Community Standards for Instruments, Metadata, and Publications

Roland Nitschke[1] and Hella Hartmann[2]

Reproducing published research results can be challenging. In the field of light microscopy, there is a global open community bridging the gap between academia and industry to improve the reproducibility of light microscopy experiments in the life and materials sciences. The community is working towards establishing standards for quality assessment and quality control (QA & QC) for imaging instrumentation, improving reporting of imaging experiments, and data quality regarding metadata contained within. The development of the initiative QUality Assessment and REProducibility for instruments and images in Light Microscopy (QUAREP-LiMi) is presented, and the current topics and achievements of the 15 working groups are summarized. Several accessible online resources utilized by QUAREP-LiMi are introduced here, as well as the future goals, followed by an invitation to join and contribute to the success story of QUAREP-LiMi.

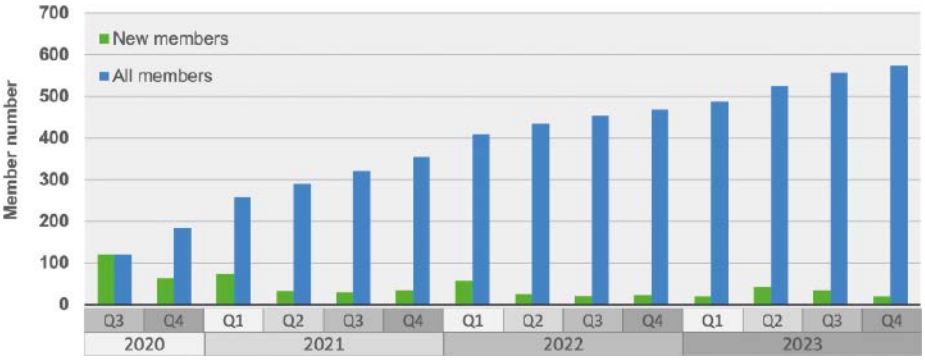


Fig. 1: Dynamic development of the QUAREP-LiMi memberships from the foundation in April 2020 until the end of 2023.

Introduction

The reproducibility crisis of research results has emerged as a major challenge in science [1], resulting in increased resource consumption due to additional experiments required. A lack of awareness, knowledge, and agreement on common standards and guidelines for quality assessment and reproducibility in the field of light microscopy and image data analysis is the main reason for the under-reporting of imaging methods. The community-driven initiative "Quality Assessment and Reproducibility for Instruments & Images in Light Microscopy (QUAREP-LiMi)" addresses this crisis by (1) defining robust and easy-to-implement Standard Operating Procedures (SOPs) characterizing relevant instrument parameters for data acquisition, (2) establishing a common metadata model for image data, including quality control data and associated analysis, (3) developing community agreed guidelines for publication of image data and its analysis, ensuring reproducibility and AI-compatible data mining. Ultimately, these efforts will lead to more robust results

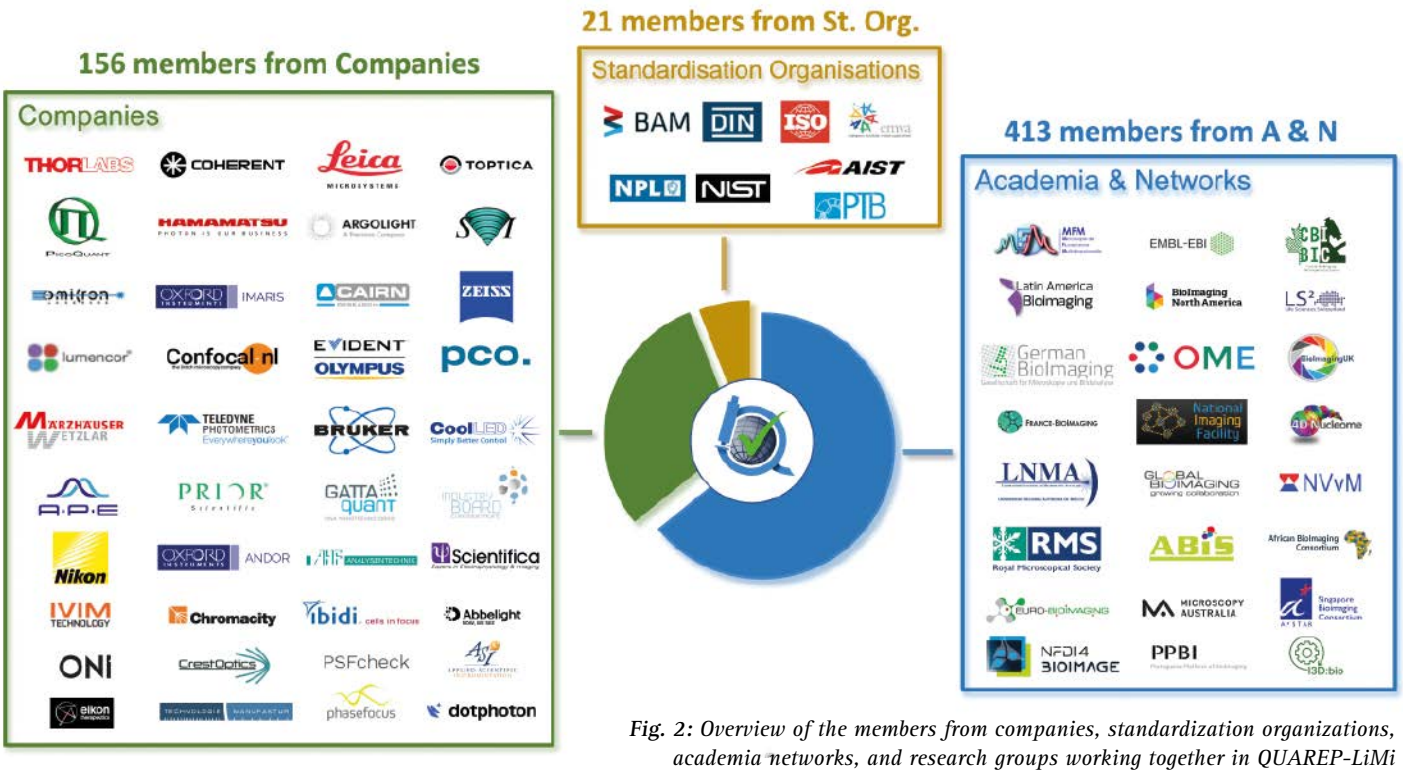


Fig. 2: Overview of the members from companies, standardization organizations, academia networks, and research groups working together in QUAREP-LiMi (due to space restrictions, not all member logos were included).

and a significant reduction in the use of resources. This will be achieved by providing better conditions for new research projects based on previous, more profound findings.

Groundwork and Organization

Initially, QUAREP-LiMi started as a grassroots collective within the German BioImaging Society and informal meetings of small groups of scientists at ELMI and other conferences. After an initial international online meeting with about 50 participants during the early Covid pandemic, it quickly gained traction and, since then, has continuously grown. Figure 1 summarizes the tremendous development of the QUAREP-LiMi membership numbers since 2020. Today, 590 people from 41 countries have joined QUAREP-LiMi, and 30% of the members are females. The majority of the 413 academic members work in light microscopy core facilities or research labs and have a strong focus on light microscopy techniques. The 156 members from equipment and software manufacturers are designated specialists in their field of expertise like optics engineers, specific product experts, and hardware or software developers. From those, 21 QUAREP-LiMi members are involved in national and international standardization organizations such as DIN or ISO or work as scientific editors for journals. Figure 2 provides an overview of the various organizations, networks, and industry partners working together in QUAREP-LiMi. All members participate voluntarily and without any financial contribution or benefit, ensuring complete independence for the best possible progress.

QUAREP-LiMi is organized in working groups (WGs) focusing on specific topics and tasks. Interested individuals can subscribe to specific QUAREP-LiMi WGs via online forms. About 250 members are actively contributing to 15 WGs, as listed in Table 1 with the main topics they are focusing on. WGs 1 to 6 are dedicated to technical parameters described in the ISO norm 21073:2019 - Optical data of fluorescence confocal microscopes for biological imaging. WG 7 - Microscopy data provenance and QC metadata and WG 10 - Image quality interact with most of the other WGs, as their topics are overarching. WG 8 - Education, training, and outreach, WG 14 -Environment, and WG 15 - FLIM have recently been established. WG 11 - Microscopy publication standards and WG 12 - Image visualization and analysis concentrate on establishing agreed-upon guidelines for extensive description and visualization of light microscopy data. WG 13 - Phototoxicity is raising awareness of the phototoxicity induced by microscopy and evaluating the best methods for assessing, minimizing, and reporting phototoxicity.

The work within each WG is coordinated and guided by at least two elected co-chairs in charge of all WG-related organizational tasks. Most WGs come together online monthly, and dates are published in an online calendar. All meetings are recorded, and meeting agendas and minutes, shared working documents, literature, recorded image data sets, and measurements are collected on a Nextcloud server that is openly accessible to all QUAREP-LiMi members. QUAREP-LiMi is active on social media channels like LinkedIn and X, with about 800 followers. Further details and information are summarized on the initiative's extensive webpage.

Achievements and Work in Progress

The QUAREP-LiMi WGs 1-6 are working on or have already published detailed protocols for the assessment, control, and analysis of important imaging system parameters and properties:

- Illumination power WG 1
- Detection system performance WG2
- Uniformity of illumination field-flatness WG 3
- System chromatic aberration and co-registration WG 4
- Lateral and axial resolution WG 5
- Stage and focus precision WG 6

QUAREP-LiMi uses the platform protocols.io for the joint creation, management, and publication of the protocols. PDF versions of the protocols are also available for download from the QUAREP-LiMi protocol website. Moreover, WGs have developed macros for many protocols to automate image acquisition and instrument control such as microscopes and their devices or power meters. In addition, WGs created various data and image analysis program routines together with further tools for visualization of the measured data over time and their storage. All information is publicly available on the QUAREP-LiMi Github account. Working groups 1, 4,

and 5 also make use of Airtable, an online platform for gathering and presenting data. WG 1 collected hundreds of laser power measurements on confocal microscope systems, which are publicly available, and new measurements can be conveniently uploaded via a form or as bulk data. QUAREP-LiMi operates a YouTube channel that currently holds 20 videos. Each WG has published short videos summarizing their work and achievements, and additional videos explain protocols and measurement routines developed in different WGs. Steered by the newly founded WG 8 - Education, training, and outreach, more teaching and training videos will be put together in the future. In general, QUAREP-LiMi is putting a lot of effort into spreading our message within the light microscopy community by organizing sessions during the last three ELMI Core Facility days. Moreover, an ELMI community room has been established that serves as an informal meeting and discussion point and playground to see demos of protocols in use, and newly developed tools and devices.

As a work in progress, a QUAREP-LiMi OMERO image database server has been set up. All image data currently stored on the Nextcloud will be transferred to OMERO and will be annotated according to FAIR data standards. In the future, the QUAREP-LiMi OMERO database will be open to the general imaging community and can serve as a repository for standard image data sets of QA & QC measurements.

The QUAREP-LiMi community initiative has accomplished an impressive number of 12 high-impact publications since 2020. The overall aims and the organization of QUAREP-LiMi were described in two whitepapers in the Journal of Microscopy [2] and Nature Methods [3] and are setting the frame for future QUAREP-LiMi developments. Most remarkably, Nature Methods highlighted in the December 2021 issue the importance of improving rigor and reproducibility in microscopy as well as proper reporting of metadata in a collection of nine articles with significant contributions from QUAREP-LiMi members. Four publications described the efforts of finding and setting standards for open image data formats [4], recommended metadata standards for light microscopy [5,6], and the development of a next-generation file format to expand bioimage data-access strategies [7]. Another set of publications focused on tools for collecting, annotating, and publication of image data including rich metadata as specified by the community and that comply with FAIR image data principles [8–10]. That impressively demonstrates that the initiative evolved into a productive consortium uniting researchers, facility managers, as well as companies, standardization organization representatives, and publishers. In July 2022, two editorials in Nature Methods [11,12] highlighted the joint work of QUAREP-LiMi on developing the revision of the 4DN-BINA-OME-QUAREP (NBO-Q) Microscopy Metadata specifications data model related to cameras. This major task was achieved in more than 10 online meetings of camera specialists from academia and industry and shall be finalized by the end of 2024. Recently, the QUAREP-LiMi WG 12 - Image Visualization and Analysis published "Community-developed checklists for publishing images and image analyses" in Nature Methods [13]. It was further highlighted in the Journal of Cell Sciences [14], resulting in several publishers expressing their strong interest in implementing the suggested guidelines. The Journal of Histochemistry and Cell Biology already implemented the guidelines [15].

WG	Working Group Topic	Members	Meetings until 01/2024
1	Illumination Power	133	30
2	Detection System Performance	126	38
3	Uniformity of Illumination Field – Flatness	98	28
4	System Chromatic Aberration and Co-Registration	90	33
5	Lateral and Axial Resolution	149	35
6	Stage and Focus – Precision and Other	75	27
7	Microscopy data provenance and QC metadata	139	25 + 12 sub
8	Training, Education and Outreach (before white paper WG)	54	1 10
9	Overall Planning and Funding	77	31
10	Image Quality	160	29
11	Microscopy Publication Standards	137	27
12	Image Visualization and Analysis	122	32
13	Phototoxicity	53	10
14	Environment (start announced for spring 2024)	21	Founding phase
15	FLIM (start announced for spring 2024)	28	Founding phase

Table 1: QUAREP-LiMi members work together in fifteen working groups (WGs), each dedicated to specific topics and tasks. Meetings of the WGs are usually every month with about 10-15% of the total WG members attending each time. Members are often part of more than one WG.

Conclusions

The QUAREP-LiMi initiative has gained tremendous momentum in the light microscopy community in a relatively short period to advance the QA & QC issues that have been difficult to address for the last 20 years. The breakthrough seems to have been achieved by the very open and productive collaborative approach taken by all people involved, including academia, industry, standards organizations, and scientific publishers. The goal of improving the reproducibility and quality of research results based on imaging data by monitoring and documenting instrument quality parameters over time with scientifically accepted, harmonized SOPs, and thoroughly tested tools is coming closer.

QUAREP-LiMi aims to become a visible and essential actor in the scientific landscape. It stands for sustainable use of imaging infrastructure, robust and reproducible results, and transparent reporting. High-quality FAIR image data, rich in metadata, will facilitate data reuse. It will also be a prerequisite for innovative, next-generation AI-supported data analysis methods.

Would you like to be part of this amazing new community? Participating in QUAREP-LiMi is easy and free – please complete the membership form. Contributing actively can be quite some work, but you will meet an open community and amazing colleagues along the way. Every expertise counts, and you could be part of the next success story

Acknowledgment

We want to thank all the QUAREP-LiMi members who dedicate a part of their free time to this initiative. R.N. would like to thank the team at the Life Imaging Center for their support of QUAREP-LiMi (in particular, Arne Fallisch) and their comments on this manuscript. R.N. is supported by grant NI 451/10-1 from the German Research Foundation and grant 03TN0047B 'FluMiKal' from the German Federal Ministry for Economic Affairs and Climate Action.

Affiliation

[1] Life Imaging Center and Signalling Research Centres CIBSS and BIOSS, University of Freiburg, Germany

[2] Light Microscopy Facility, Technology Platform, Center for Molecular and Cellular Bioengineering (CMCB), University of Technology Dresden, Germany

Contact

Dr. Roland Nitschke

Life Imaging Center and Signalling Research Centres CIBSS and BIOSS

University of Freiburg, Germany

Roland.Nitschke@biologie.uni-freiburg.de

ORCID: 0000-0002-9397-8475

More information: https://quarep.org

[1] References: https://bit.ly/IM-Nitschke

OXFORD INSTRUMENTS

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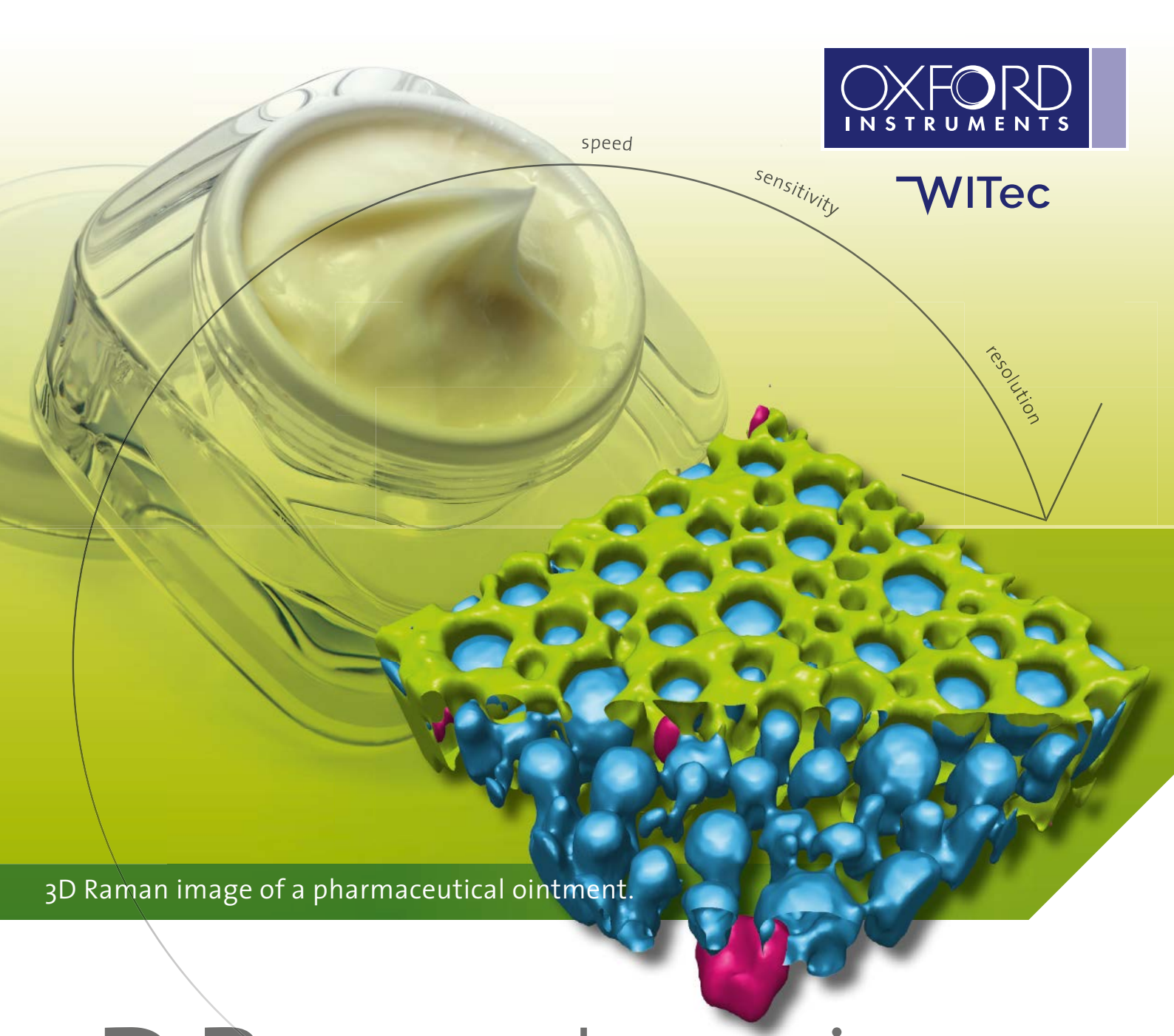

3D Raman image of a pharmaceutical ointment.

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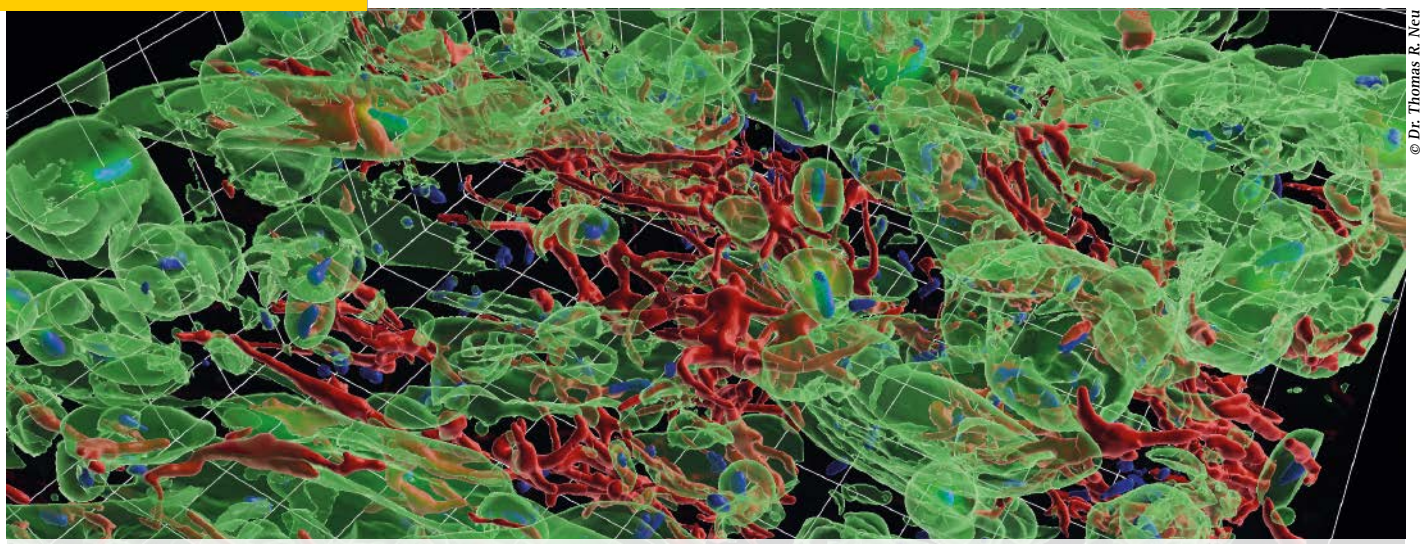

Three-dimensional semi-transparent and isosurface view after dual lectin staining in combination with nucleic acid staining. Take notice of the two lectin signals showing volumetric versus filamentous features and the large nucleic acid stained bacterial cells partly inside the green lectin signal. Color allocation: lectin AAL-Alexa488 (green, isosurface semi-transparent), lectin PNA-TRITC (red, isosurface), Syto60 (blue, isosurface). Grid size: 20 µm.

Out of this World – Hidden Biofilms Under the Microscope

The Matrix of Cave Snottites Studied by Lectins and Confocal Microscopy

Thomas R. Neu

Microbial biofilms are present in numerous locations including medical, technical, and natural habitats. A specific form of biofilms found in a semi-artificial cave is the subject of the present study. The biofilm formed pendulous snottites at the ceiling of the cave. Confocal laser scanning microscopy represented the ideal technique for investigating the snottite architecture and assessing their hydrated structure. Fluorescently labeled lectins proved to be a very useful probe for examining the extracellular matrix of microbial snottites. The lectin approach, comprising fluorescence lectin bar-coding (FLBC) and subsequent fluorescence lectin binding analysis (FLBA), identified the glycoconjugates that represented the main volume of the snotittes. In combination with other fluorochromes, the composition and structure of these snotittes were characterized for the first time.

Introduction

Biofilms are defined as microbial communities associated with an interface (solid, liquid, gas) together with their self-produced extracellular polymeric substances (EPS). Microbial biofilms come in many different shapes, textures, colors, and dimensions. They range from bacterial cell monolayers via micro-colonies to biofilms (immobile) and bio-aggregates (mobile). They may reach macroscopic dimensions in the form of massive biofouling layers (industrial) or microbial mats (natural). Biofilms are highly relevant in the environment, technical systems (positive and negative), and the medical field (mostly negative). Initially, the microscopic analysis of biofilms was done on dehydrated samples using scanning electron microscopy. However, the invention of confocal laser scanning microscopy (CLSM) revolutionized the view of this universally present microbial growth mode. Using CLSM, the fully hydrated biofilm system could be examined by employing several different fluorescent probes. Thereby, not only the bacterial cell distribution could be assessed but also the so-called "dark matter" of biofilms, the extracellular polymeric matrix [1,2].

Microbial biofilms in the environment are present in all kinds of habitats, including aquatic, sediment, soil, and rocks, and they may be associated with plants, fungi, and animals. A specific type of biofilm can be found in caves where they grow on humid rock surfaces, in flowing water, and in the form of snottites. The latter develop at the ceiling of caves and are characterized by soft, gel-like, pendulous structures up to a size of 10 cm and longer. The snottites investigated in the present study were collected in a semi-artificial, methane-driven cave, which was used as a former medicinal spring rich in iodate [3].

Methods

In the case of snottites, the finger-size gelatinous material was sectioned with a scalpel and mounted in a cover well chamber with the appropriate spacer. Samples were examined using 25x NA 0.95 or 63x NA 1.2 water immersion objective lenses.

The methodology of lectin staining has been published in detail [4,5]. It's a straightforward and easy approach, including staining and washing the sample. In short, the labeled lectin is applied to the sample at a concentration of 0.1 mg/mL. Usually, the biofilm sample is covered with a few droplets of the working solution. A time series revealed that an incubation in the dark for 20 min is appropriate. Then, the samples are carefully washed 3-4 times to remove the unbound lectin. Usually, a nucleic acid-specific fluorochrome is applied as a counterstain.

The examination of biofilms by CLSM is ideally performed with a common instrument having an upright microscope. This setup is most flexible for different types of biofilms. It will allow the use of 1) slides with coverslip and spacer, 2) cover well chambers with spacers of different dimensions, and 3) imaging the sample in a small Petri dish using water-immersible lenses.

Lectins			
Latin name	Common name	3-letter code	Inhibiting carbohydrate
Aleuria aurantia	fungus	AAL	α-Fuc
Musa paradisiaca	banana	Ban	Glc, Man
Lycopersicon esculentum	tomato	LEA	β-GlcNAc
Arachis hypogaea	peanut	PNA	β-Gal

Table 1: *Characteristics of the four lectins identified as useful for fluorescent lectin binding analysis (FLBA) after fluorescence lectin bar-coding (FLBC) screening.*

Main Developments

Microbial biofilms and bio-aggregates were examined by CLSM in numerous studies with a focus on bacterial cell distribution. For this purpose, nucleic acid-specific fluorochromes were usually employed. More recently, molecular techniques have taken advantage of fluorescent proteins such as GFP, YFP, RFP, or others for labeling bacteria in biofilms. Nevertheless, the extracellular matrix proved to be an intractable feature of biofilms [6]. The main reason is the complexity of the matrix consisting of polysaccharides, extracellular nucleic acids, various types of proteins (e.g., enzymes, structural proteins, amyloids), lipophilic constituents, as well as bacterial-derived refractory substances. For all these matrix compounds, there is no single fluorescent stain available. Even more, there is no universal fluorochrome on hand for one of the major compounds, polysaccharides. One fluorochrome, somehow specific for polysaccharides, is calcofluor white, but it will bind to β 1-3 or β 1-4 polysaccharides only. This linkage type is typically found in chitin and cellulose.

As a consequence, there is an urgent need to have a fluorescent probe for polysaccharides or, more specifically, for glycoconjugates. Such a probe is available in the form of fluorescently labeled lectins. Lectins have been used as a probe for several years, often as a single probe only. During our confocal work and the investigation of biofilm systems, we used more and more different types of lectins. Finally, we collected all commercially available lectins (about 80) to assess the biofilm glycoconjugate matrix. Thus, when we got a new type of biofilm sample, we tested each of the lectins individually on 80 pieces of biofilm. This screening, we named fluorescence lectin bar-coding (FLBC). Based on FLBC, we identified some useful lectins binding to the structure of interest. In a second step, we employed this panel of lectins for the actual study. This subsequent assessment of the samples from a defined experiment we named fluorescence lectin binding analysis (FLBA) [7,8].

Due to their specificity, lectins will bind differentially at various locations within microbial biofilms. They may stain the bacterial cell surface, bacterial footprints, extended capsules of bacteria, microenvironment of micro-colonies, or matrix of biofilms and bio-aggregates. As lectins are specific for carbohydrate residues, they do not always show the overall biofilm matrix. Despite this issue, they often result in a reasonably good signal showing glycoconjugate features of interest in microbial communities. Hence, lectins are not a general probe for all glycoconjugates present in a biofilm sample; however, they are currently the best compromise for glycoconjugate imaging via fluorescence in combination with CLSM.

To examine the snottite samples, we initially tried a range of different fluorochromes to get a first idea of their basic structure, e.g., with nucleic acid and protein-specific stains. As an example, SyproOrange showed different types of bacterial shapes as well as filamentous features (Fig. 1). Then we performed the screening via FLBC to identify the most valuable lectins. As a result, we found four lectins that were employed on various snottites as well as in different areas of the snottite. The lectins AAL, PNA, LEA, and Ban (Table 1) showed amazing voluminous structural features. Figures 2, 3, and 4 show lectin signals in the form of capsules and globules, tubular structures, and filamentous features together with bacterial cell distribution. The fluorochrome "stains all" allowed the separation of bacterial cells from globular signals in two emission channels (Fig. 5). The lead image shows a triple combination of fluorescent stains showing tube-like features, filaments, and large bacterial cells. The specificity of the four lectins suggested α-Fuc, β-Gal, β-GlcNAc, and α-Man as major glycoconjugates. Throughout the dominating lectin signal, bacteria of different sizes, shapes, and lengths were found. In fact, some of the filamentous bacteria formed a dense network and might be responsible for the additional strength of the snottites. Experiments with fluorescent beads showed an outer layer that was not penetrated by the beads (data not shown). This indicated a surface layer around the snottites, which could not be characterized in further detail. For more details on snottite architecture, the reader is referred to the original article [9].

Additional approaches might be suggested to reveal more detailed information. For example, non-commercial lectins from research labs could be tested. Another option is carbohydrate-binding modules (CBMs) as a probe. The next logical strategy would

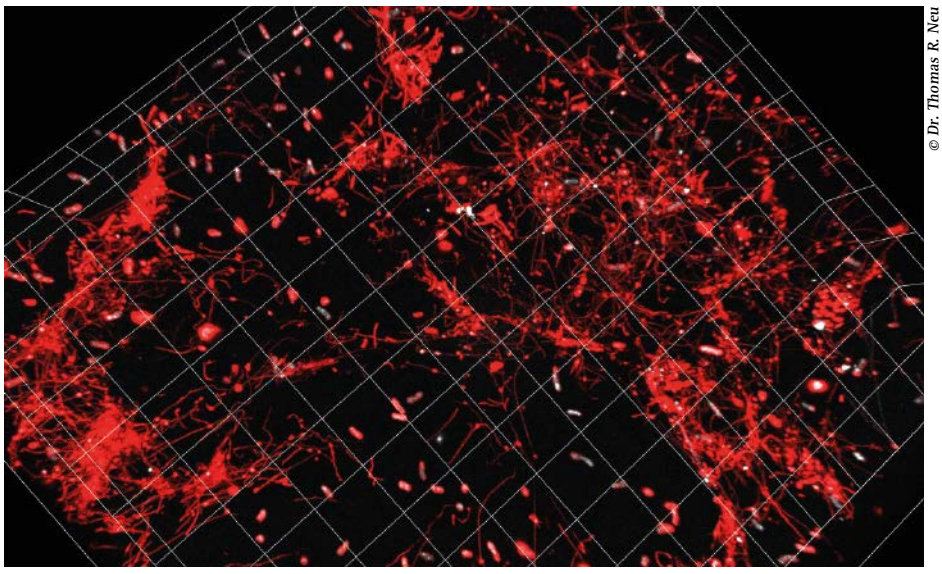

Fig. 1: *Transparent 3D view of a snottite stained with SyproOrange showing bacteria of different sizes and shapes together with filamentous structures. The reflection signal inside bacteria indicates solid inclusions. Color allocation: SyproOrange (red), reflection (grey). Grid size: 20 µm.*

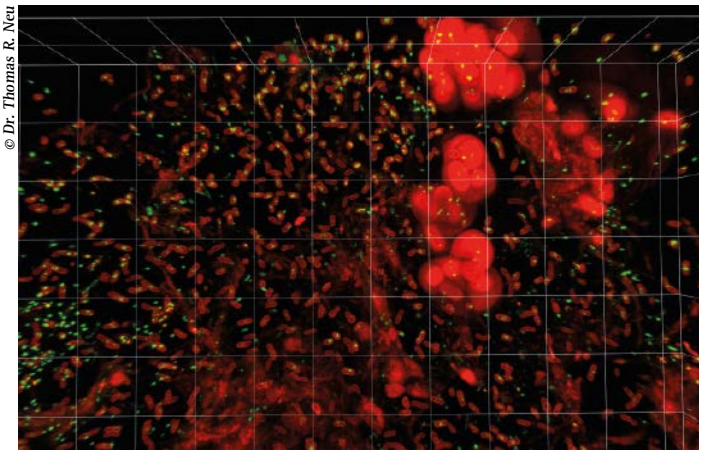

Fig. 2: Transparent 3D view of a snottite stained with a lectin and a nucleic acid specific counterstain showing bacteria with and without capsules as well as large globular lectin signals. Color allocation: AAL-Alexa568 (red), SybrGreen (green). Grid size: 20 µm.

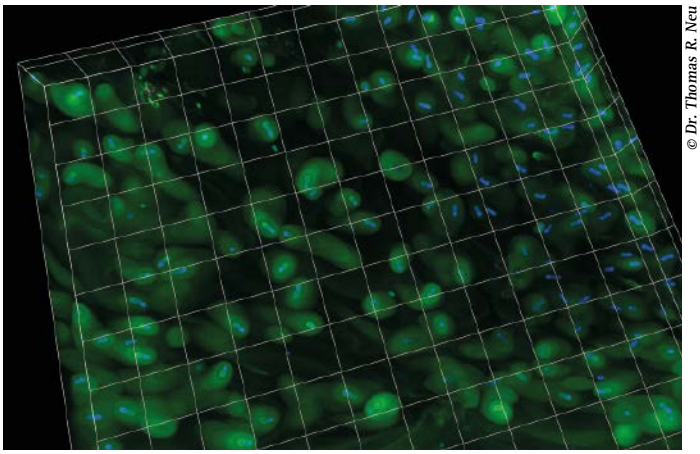

Fig. 3: Transparent 3D view of a snottite stained with the same lectin but with another label and a different nucleic acid-specific stain. The bacteria are embedded in extended glycoconjugates in the form of globules and tube-like shapes. Color allocation: AAL-Alexa488 (green), Syto60 (blue). Grid size: 20 µm.

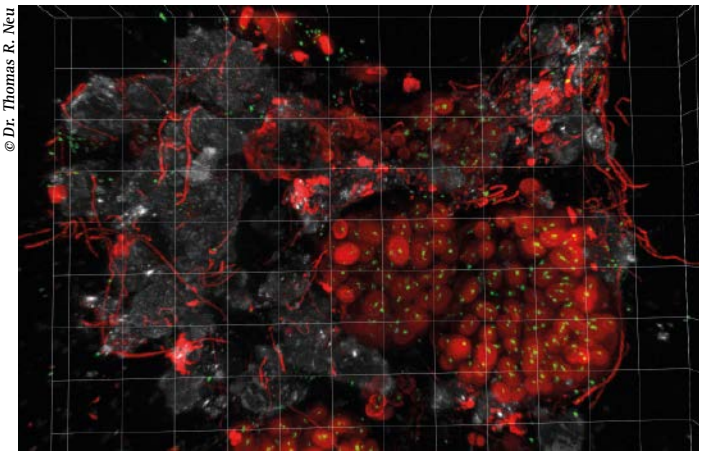

Fig.4: Transparent 3D view of a snottite showing a group of bacteria embedded in globules with filamentous structures and mineral particles. Color allocation: reflection (grey), AAL-Alexa568 (red), SybrGreen (green). Grid size: 20 µm.

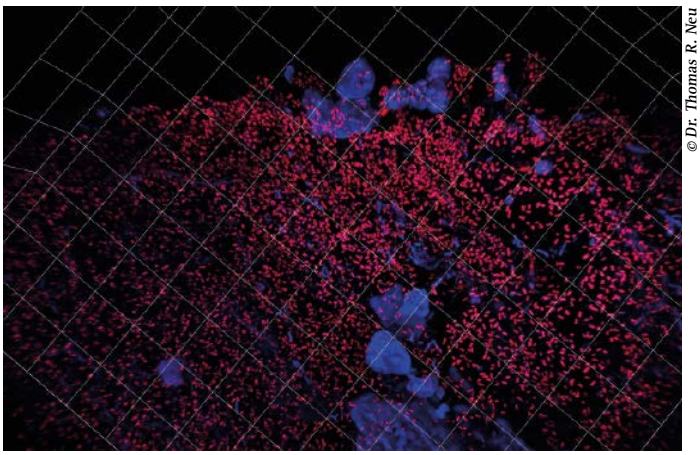

Fig. 5: Transparent 3D view showing the edge of a snottite treated with "stains all" and recorded in two emission channels. Take notice of the differentiation between bacterial cells and globular signals. Color allocation: emission 570-620 nm (red), emission 650-720 nm (blue). Grid size: 50 µm.

include gene probes for the different bacteria already identified by molecular analysis. This fluorescence *in situ* hybridization (FISH) or CARD-FISH approach can be combined with the lectin approach [10]. Thereby, the identity of the bacteria can be linked to the identity of the glycoconjugates. Lately, new probes for matrix constituents using oligothiophenes were suggested for so-called optotracing. Finally, other fluorescent techniques such as FRAP and FCS, may help measure cave snottites' diffusion characteristics.

Conclusions

Confocal laser scanning microscopy is an ideal technique for studying hydrated, gelatinous snottites. Fluorescence lectin bar-coding (FLBC) with subsequent fluorescence lectin binding analysis (FLBA) revealed the architecture of snottites. The lectins showed voluminous, globular, and tube-like structures interwoven with bacteria of different sizes and lengths. Of the 78 lectins tested, the lectins AAL, PNA, LEA, and Ban indicated α-Fuc, β-Gal, β-GlcNAc, and α-Man as major glycoconjugates. Methane and iodate, together with the microbial metabolism of methano- and methylotrophic bacteria, may be the primary reason for the snottite architecture.

Acknowledgments

The cooperation with Tillmann Lüders and Clemens Karwautz concerning the snottite project is highly appreciated. I am very thankful to Ute Kuhlicke, who skillfully worked with me at the laser microscope for over 25 years.

Contact
Dr. Thomas R. Neu
Department of River Research
Helmholtz Centre for Environmental Research – UFZ
Magdeburg, Germany
ORCID: 0000-0001-6990-8787
thomas.neu@ufz.de

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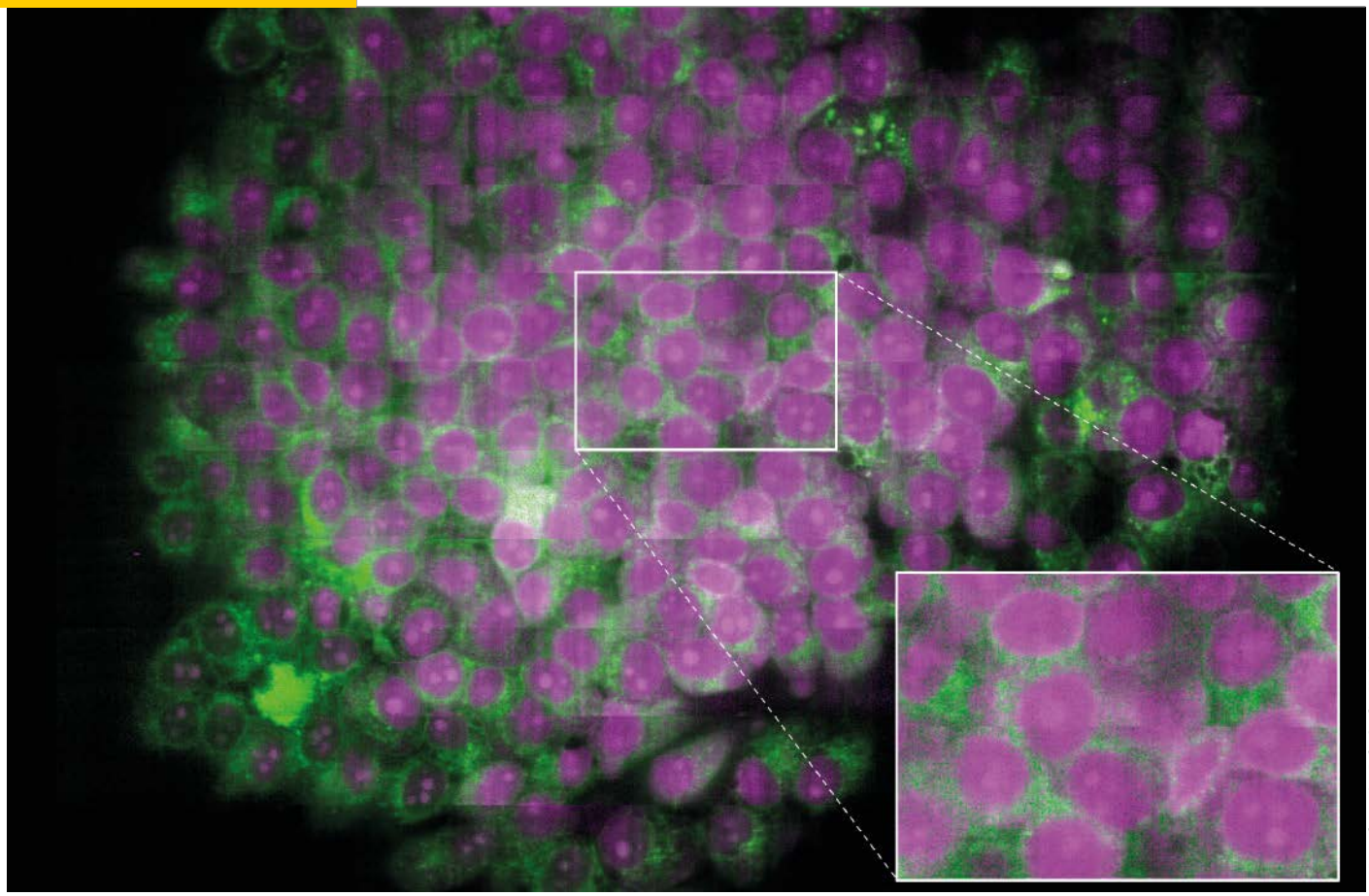

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High-Speed Molecular Imaging

Line-Illumination Raman Microscopy with Slit Array Detection

Tomoaki Okumura[1,2], Yasuaki Kumamoto[1,3], Katsumasa Fujita[1,2,3]

Raman microscopy is an emerging molecular imaging technique that does not require the labeling of targeted molecules in specimens. A key to the emergence of Raman microscopy is the development of high-speed imaging techniques that enable the analysis of biological specimens susceptible to temporal changes or alterations. Here, we introduce a high-speed Raman hyperspectral imaging technique developed based on line-illumination Raman microscopy. The technique, namely multiline-illumination Raman microscopy, equips periodic arrays of line-shaped laser focus and confocal slits, enabling simultaneous spatial sampling of 10,000–20,000 Raman spectra and spectral sampling of 100 wavenumbers per exposure, which performs high-throughput Raman hyperspectral image acquisition of (10^5 spectra × 10^2 wavenumbers)/min. We anticipate that our technique will enhance the utility of Raman microscopy in practical applications in biology and medicine.

Introduction

Raman microscopy has emerged in the last decade as a molecular imaging technique enabling label-free molecular analysis [1]. The capability of Raman microscopy has been studied for a variety of biomedical applications, *i.e.*, observing drug response in living cells at the molecular level [2], cell sorting [3], intraoperative rapid diagnosis [4], and identification of cell differentiation [5]. However, the slow imaging speed of Raman microscopy hampers the examination of practical applications of Raman microscopy in the life sciences, mainly due to the small Raman scattering cross-section and point-by-point scanning scheme for image construction. Although multiplexing spatial sampling, e.g., by line-illumination [6] and multifocal illumination [7], has been applied to increase the speed by 2-3 orders of magnitude, Raman hyperspectral imaging often takes a few tens of minutes or even longer than an hour [6, 8].

Strategy for Accelerating Raman Imaging

In conventional confocal Raman microscopy, a laser beam in the form of a spot illuminates the sample, and a single Raman spectrum is recorded using sensors only in several lines of a two-dimensional sensor array (Fig. 1A). In multiplex spatial sampling technique using line-shaped illumination, the spatial distribution of the spectra in the sample is recorded on the sensor array by expanding the spatial information along y-axis, which is orthogonal to the wavelength dispersion axis (x-axis), as shown in Figure 1B. The number of spectra simultaneously acquired here is determined by the number of sensors in the y-axis used for recording, *i.e.*, typically 100-1,000, resulting in the imaging speed faster by two or three orders of magnitude compared to confocal Raman microscopy [9]. To further improve the imaging speed by multiplex spatial sampling, we increased the number of illumination lines in the x-axis to acquire

more spatial information simultaneously in a trade-off with reducing the wavenumber information (Fig. 1C).

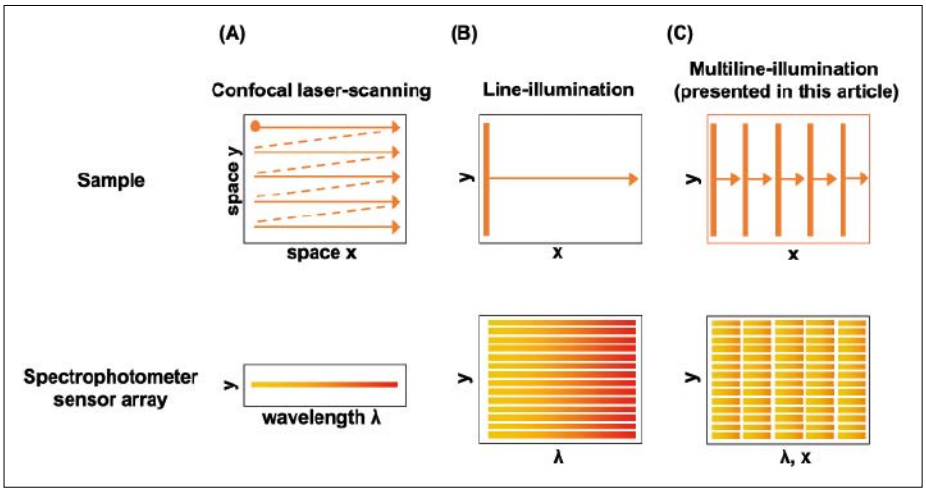

Fig. 1: Schematic illustration of laser-scanning and spectral detection in three different types of laser-scanning Raman microscopy, i.e., (A) confocal, (B) line-illumination, and (C) multiline-illumination Raman microscopy. In line-illumination Raman microscopy, spatial information is recorded along y direction on the spectrophotometer sensor array. In multiline-illumination Raman microscopy, spatial information is expanded in the x direction as well for further multiplexing spatial sampling.

Multiline-illumination Raman Microscopy with Slit Array Detection

We have developed a multiline-illumination Raman microscope equipped with a confocal slit array (Fig. 2) [10]. A cylindrical lens array formed a high-power laser beam with a wavelength of 532 nm into multiple line-shaped laser beams. The multiple line-shaped beams were relayed to the sample plane and excited Raman scattering in a sample. The Raman scattering was collected with an objective lens, separated from the excitation beam path by using an edge filter, and refocused at the entrance of the spectrophotometer, where a periodic slit array was located instead of an ordinary entrance single slit. Each slit was conjugated to one of the irradiated regions at the sample and worked as a confocal slit. To avoid unwanted spectral mixtures among different sample regions in the sensors of the spectrophotometer, the wavelength range of Raman scattering entering the spectrophotometer was limited by a bandpass filter. A comb-like Raman hyperspectral image excited by multiple illumination lines is acquired by a single sensor exposure, where each Raman spectrum includes specific Raman peaks from target molecules in the sample.

Raman hyperspectral image from polystyrene (PS) and polymethylmethacrylate (PMMA) beads dispersed on a quartz coverslip is shown in Figure 2. In the spectra corresponding to the CH vibration region (2,850–3,100 cm^{-1}), the characteristic Raman peaks of PMMA (green) and PS (magenta) are found at 2,953 and 3,047 cm^{-1}, respectively. To acquire a two-dimensional Raman hyperspectral image (Raman spectra distributed in two-dimensional space), the sample was scanned with the slit array and multiple illumination lines by steering a galvanometer mirror. A shutter in the beam path opened

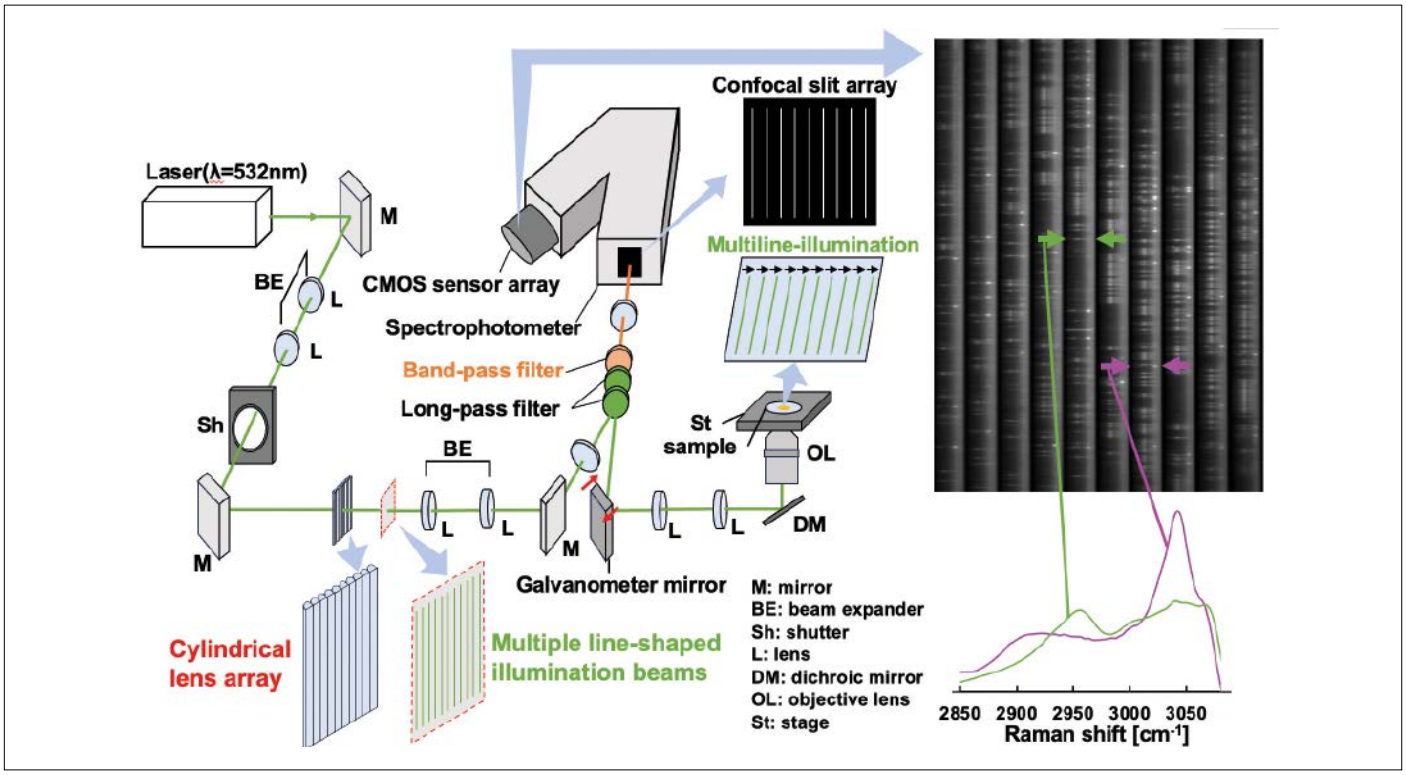

Fig. 2: A schematic diagram of the multiline-illumination Raman microscope equipped with a confocal slit array. Each Raman band in the Raman hyperspectral image corresponds to the CH region, where characteristic Raman peaks from polystyrene (magenta) and polymethylmethacrylate (green) beads are found at 2,953 and 3,047 cm^{-1}, respectively.

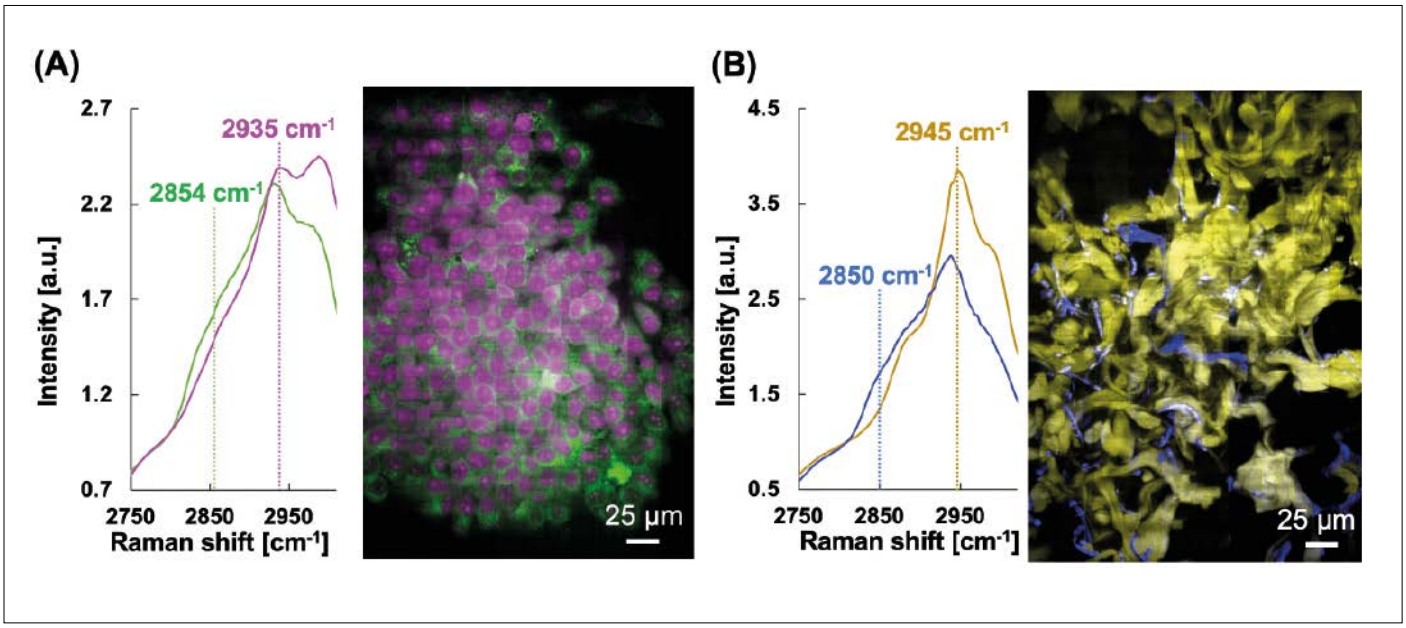

Fig. 3: (A) Raman spectra and image of living cultured HeLa cells. The image shows the distributions of the Raman signal at 2,854 cm^{-1} (magenta) and 2,935 cm^{-1} (green). (B) Raman spectra and images of human dermal tissue. The Raman image was reconstructed using the Raman intensities of 2,850 cm^{-1} (blue) and 2,945 cm^{-1} (yellow). All the protocols for treating the clinical specimen were performed by the declaration of Helsinki and were approved by the Institutional Review Board in Osaka University Hospital (20108). The written consent form was obtained from the participant who underwent skin transplant surgery, and a surgical discard was used.

and closed synchronously with the start and end of a sensor frame exposure, respectively, to prevent the excitation laser beams from excessively irradiating the sample.

High-throughput Raman Hyperspectral Imaging of Cell and Tissue

Here, we present Raman images of biological specimens to show the utility of the multiline-illumination Raman microscope for molecular imaging of biological samples. Figure 3A displays the Raman spectra and image for living cultured HeLa cells. In the spectra, the band at 2,854 cm^{-1} can be assigned to the CH_2 asymmetric stretching vibrational mode, while the band at 2,935 cm^{-1} can be assigned to the CH_3 symmetric stretching vibrational mode. The Raman images reconstructed using the intensities at 2,854 and 2,935 cm^{-1} can illustrate the distributions of lipid and protein, respectively, in the cells [11]. Small dots of lipid, *i.e.*, lipid droplets, are imaged without significant distortion, indicating that the multiline-illumination Raman microscope has sufficiently high spatial resolution to observe organelles in living cells.

A measurement result for clinical specimens of frozen dermal tissue is shown in Fig. 3B. In the spectra, two characteristic Raman bands were identified at 2,850 and 2,945 cm^{-1}. The Raman image reconstructed with the intensity at 2,945 cm^{-1} shows collagenous fibrous structures that occupy most of the part in the dermal tissue [12], while the Raman image reconstructed at 2,850 cm^{-1}, exhibiting a warped shape, presumably shows elastin fibers that connect collagen structures which distribute among them [13]. These results indicate that the multiline-illumination Raman microscope visualizes tissue structures that can be useful for studies of tissue chemistry and morphology in a label-free manner [14,15].

Each Raman hyperspectral image in Figure 3 is composed of 920 × 1,300 spectra. The data acquisition time was 8.0 min (5.0-s exposure and 0.2-s readout per single frame acquisition of the sensors) for each dataset, corresponding to a throughput of 2×10^5 spectra/min. This throughput is an order of magnitude higher than that in line-illumination Raman microscopy with single-slit detection, which can realize the ultrafast Raman imaging of biological specimens in practical conditions.

Conclusion

We have developed a high-speed line-illumination Raman microscope equipped with a confocal slit array, where the image acquisition speed was improved by increasing the number of spectra that can be acquired with a single exposure. With the developed technique, we achieved a throughput with the order of 10^5 spectra/min in cell and tissue imaging, marking a 10-fold improvement in speed compared to the single-line-illumination technique. Currently, to accelerate the image acquisition further, we are developing a technique capable of achieving speeds on the order of 10^6 spectra/min by efficiently detecting Raman scattering in a shorter exposure time. We believe that the presented high-speed molecular imaging technique will further expand the utility of Raman microscopy in the fields of biology and medicine, not only for pursuing basic science but also for developing new applications in clinics and related industries.

Affiliation
[1]Department of Applied Physics, Graduate School of Engineering, Osaka University, Osaka, Japan
[2]AIST-Osaka University Advanced Photonics and Biosensing Open Innovation laboratory, National Institute of Advanced Industrial Science and Technology (AIST), Osaka, Japan
[3]Institute for Open and Transdisciplinary Research Initiatives (OTRI), Osaka University, Osaka, Japan

Contact
Prof. Dr. Katsumasa Fujita
Department of Applied Physics
Graduate School of Engineering
Osaka University, Japan
ORCID: 0000-0002-2284-375X
fujita@ap.eng.osaka-u.ac.jp

[1] References: https://bit.ly/IM-Fujita

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Attosecond Electron Microscopy

Movies of Light in Space and Time

David Nabben[1], Joel Kuttruff[1], Peter Baum[1]

The fundamental process behind almost any light-matter interaction is the motion of electrons in the extremely fast oscillations of the electromagnetic wave. By advancing electron microscopy to attosecond time resolution [1], we can now measure light waves and their interactions with complex materials as a movie in space and time. We simultaneously achieve a spatial resolution of nanometers and a time resolution in the attosecond range, smaller and faster than the oscillations of light. Selected results in nanophotonics and metamaterials show the potential implications of our technique for future research.

Introduction

Almost all matter around us consists of atomic nuclei surrounded by a shell of electrons. The arrangement of these constituents into molecules, solids, or nanostructures determines almost all of the macroscopic functionality. When a reaction occurs, or a material interacts with light, the atoms and electrons must shift from initial to final configurations. For example, the outcome and properties of phase transitions, chemical reactions, or optical phenomena are all determined by microscopic movements and rearrangements of the atoms and electrons within.

Knowing as much as possible about such processes on an atomic scale is therefore crucial for modern research, not only to provide a fundamental perspective on complex material dynamics but also for developing innovative electronic and photonic devices for novel application regimes. However, the direct spatial and temporal observation of electronic motion in atoms, molecules, solids, or nanostructures is still an unreached milestone in modern physics.

The difficulty lies primarily in the extremely small spatial and temporal scales on which all these elementary processes are taking place. An atom is approximately 10^{-10} m in size, ten thousand times smaller than the wavelength of light. The time scales on which electronic processes take place are attoseconds, that is 10^{-18} s, which is ten to hundred times faster than one oscillation period of light. Experimental access to these unimaginably fast domains of time poses an enormous challenge, in particular when the spatial resolution of the atoms has to be maintained. Figure 1 shows some fundamental physical processes in their relevant dimensions with the limits of current experimental measurement techniques.

Attosecond Electron Microscopy

Recently we have succeeded in finding a novel approach to tackle this challenge [1]. We combine a transmission electron microscope which is capable of atomic spatial resolution with a continuous-wave laser that advances the time resolution into the attosecond domain. With this new technology, we can now create movies of light-matter interactions in nanophotonic objects and metamaterials with attosecond and nanometer resolutions in space and time [1]. This new methodology can help researchers observe and understand fundamental atomic and electronic processes in complex materials and then utilize these results for advanced material design.

Figure 2 shows the principles of our attosecond electron microscopy. It is based on a stroboscopic measurement principle, similar to a classical pump-probe experiment, only that the optical oscillation periods of continuous laser light rather than ultrashort laser pulses are used for the excitation of the specimen. We drive the process under investigation repetitively with the optical cycles of laser light and then interrogate the system response with high-energy electron pulses that have short enough de Broglie wavelength to resolve the atomic details of the specimen. To achieve the necessary time resolution as well, the probe pulses in the electron microscope must be shorter than half an optical cycle of the excitation light, that is, attoseconds.

To generate our attosecond electron pulses, we cross the continuous electron

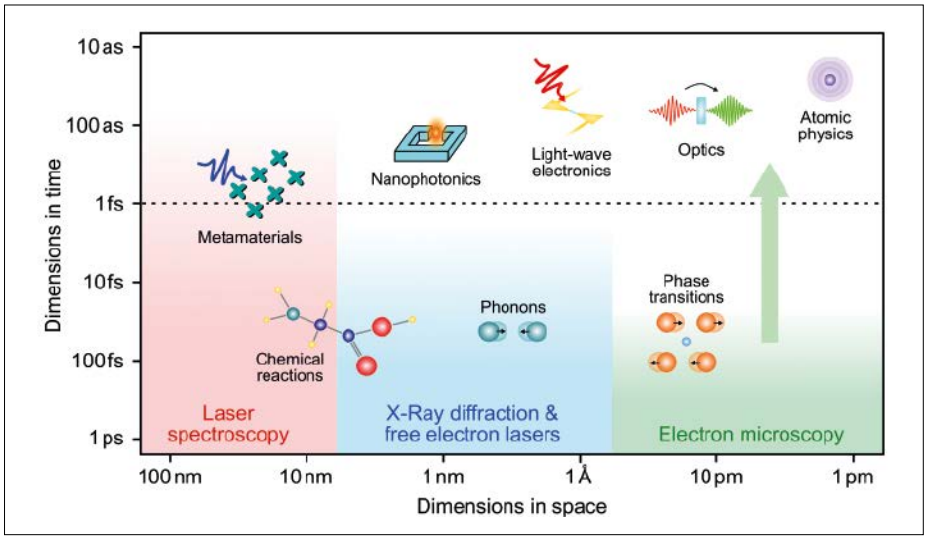


Fig. 1: Fundamental physical processes and their dimensions in space and time. Color shades denote the available measurement methods, that is, laser spectroscopy (red), X-ray diffraction (blue), and electron microscopy (green). Most processes above the dashed line at one femtosecond cannot be addressed as a movie in space and time with existing technologies because either the temporal or the spatial resolution is too low. However, our recently developed attosecond electron microscopy (green arrow) can now provide access.

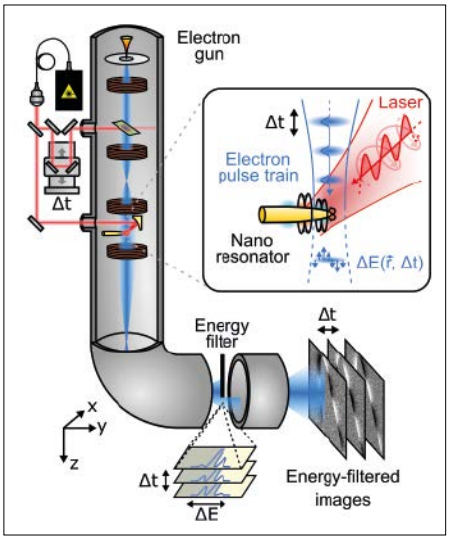


Fig. 2: Concept of our attosecond electron microscope. A continuous wave laser (red) modulates the continuous electron beam (blue) of a transmission electron microscope (gray) at an ultra-thin dielectric membrane (green) into a sequence of attosecond pulses. A second part of the laser beam excites the object to be examined (yellow) and generates location-dependent and time-dependent energy changes ΔE(r, Δt). An energy-filtered image and variation of the time delay Δt between the two laser paths can then be used to measure the electromagnetic response of the sample as a movie in space and time.

beam of the transmission electron microscope with the electromagnetic fields of a continuous-wave laser. The extremely fast oscillations of the laser wave then periodically modulate the energy and thus the speed of the electrons. Later/faster electrons eventually catch up with earlier/slower electrons [2], and the electron beam transforms into a rapid sequence of attosecond electron pulses which we then use to interrogate the ultrafast processes and material reactions at the sample site [1].

In more detail (Fig. 2), a continuous-wave laser (red) is split into two paths that are directed into the transmission electron microscope (gray) at two different points. The first part of the laser modulates the electron beam (blue) into a sequence of attosecond electron pulses with the help of an ultra-thin dielectric membrane (green) that is needed to provide energy and momentum conservation [3-4]. The second part of the laser, phase-locked in time, triggers the dynamics to be investigated in the specimen (yellow). The enlarged section in Figure 2 shows the ultrafast excitation of electromagnetic near-fields (orange and turquoise) in the optical cycles of the laser light (red) in the example of a nanometer-sized needle resonator (black). Depending on their arrival time, the attosecond electrons (blue) are either accelerated or decelerated in the temporally frozen near-fields, and the energy ΔE(r, Δt) of the electrons changes as a function of space and time (r and Δt).

A magnetic spectrometer in the electron microscope (the 90° curve) then spectrally separates the electrons in the image and selects only those electrons for imaging that have gained energy at the specimen. The magnified image then shows the spatial distribution of the time-frozen electric near-fields in and around the sample at the selected time Δt. By varying Δt with a micromechanical mirror (gray arrows), we obtain a movie of the dynamics at the nanostructure with attosecond time resolution. The head figure on the first page shows a photograph of the experiment.

Extremely sharp metal needles with a tip radius of only a few nanometers have a wide range of applications in classical and ultrafast electron microscopy, but also in nanoplasmonics, where they are used in near-field scanning electron microscopes for optical surface analysis. The curved surface of our tungsten needle and its small radius of 150 nm enables the formation of surface waves in the form of plasmon-polaritons, whose propagation along the needle tip we can film in space and time (Fig. 3). Complete slow-motion movies of the measured dynamics can be found in the supplementary material of our original report [1].

Diagnostics of Metamaterials

By cleverly arranging many such resonators on nanometer dimensions in the form of so-called metamaterials, researchers can create macroscopic optical responses that cannot be achieved with any natural materials. Examples include flat lenses, cloaking layers, sensors for chiral molecules, condensers for solar cells, or quantum optical circuitry. In realistic metamaterials, however, only the overall optical response can be measured, while the functionality of the individual resonators remains unclear. For the effective development of such structures, however, the field and emission dynamics of the individual elementary building blocks, the so-called meta-atoms, must be understood and the time delays and interferences between several meta-atoms must be determined and taken into account.

Figure 4 displays a series of time-frozen snapshots from our attosecond electron microscope. The specimen is an arrangement of four dielectric slot resonators in a tilted, zig-zag geometry which has potential applications in the field of meta-lenses. In the attosecond movies, we see that localized near-field resonances appear within all of the slits, like in radio antennas, but smaller than the wavelength of light. We also observe how a complex, dynamic interference pattern of the radiated fields arises around the nano-antennas which determines and defines the macroscopic optical response. In particular, we see a temporal symmetry break between the upper and lower halves that is probably generated by non-planar components in the material. We conclude that our attosecond electron microscopy can

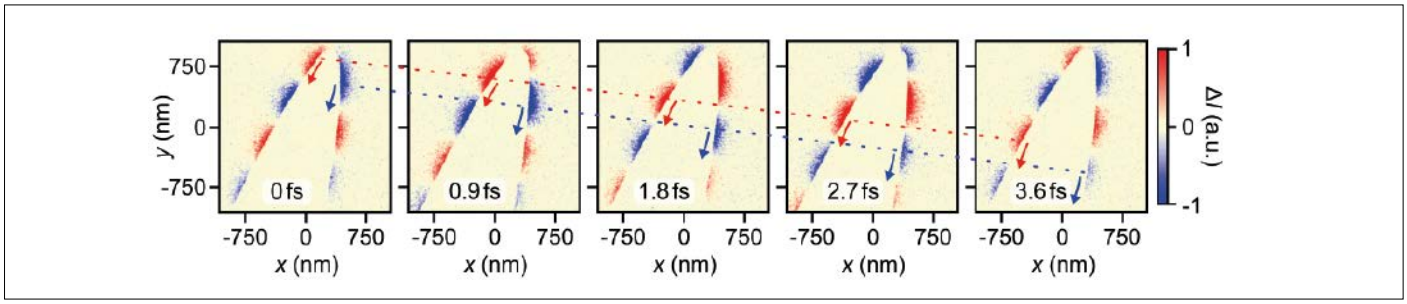


Fig. 3: Movies of light-matter interactions with the attosecond electron microscope. Plasmon-polaritons are excited with a laser on a tungsten needle and propagate downwards along the surface. Attosecond electron microscopy provides a movie of the electric fields (blue, red) in space and time.

not only measure the functionality of individual antennas but also trace the collective optical responses of nanophotonic superstructures back to their elementary components. Our measurement principle can therefore contribute to the development of novel and better nanophotonic materials by understanding their functionality on fundamental dimensions in space and time. More advanced results, in particular on the chirality of nanometer light, can be looked up in our original report [1].

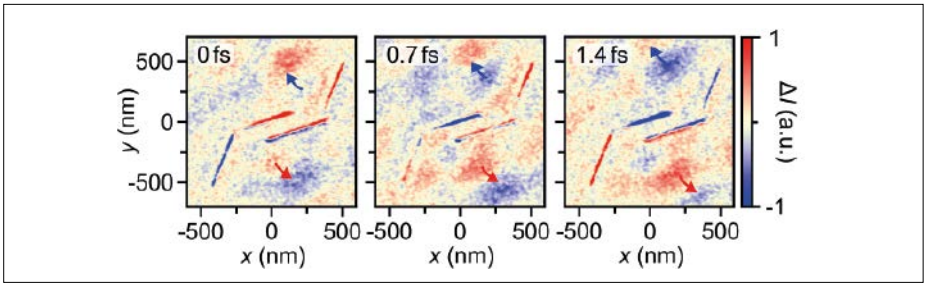


Fig. 4: Optical field-cycle dynamics in a dielectric metamaterial. The functionality of the individual nanoresonators and their collective response in the far-field is measured with our attosecond electron microscope as a movie in space and time.

Conclusions and Outlook

So far, the reported results [1] are only a first demonstration of the new capabilities of our attosecond electron microscope on selected materials from optics and nanophotonics. However, we believe that attosecond electron microscopy and the insight it delivers into light-matter interaction can open up many more application fields.

For example, a long-standing goal in chemistry is to increase the speed of chemical reactions through the use of catalysts. However, most industrial catalysis requires high temperatures and/or pressures that often limit the reactivity, selectivity, stability, or throughput rate. An emerging area of research in catalysis is therefore the utilization of plasma resonances in metallic nanoresonators in combination with light and aims to use hot charge carriers or tailored electromagnetic near-fields in nanomaterials to specifically control chemical reactions. The distribution and dynamics of the laser-induced optical near-fields in and around the resonators are crucial here. As nanotechnology approaches the atomic frontier and plasmonic decay processes become as fast as one oscillation period of the exciting light, the problem becomes highly involved. Here, our attosecond electron microscopy could make a decisive contribution, because nanometer structures and their attosecond dynamics can be measured explicitly in space and time, to understand the influence of local optical fields on chemical processes and in turn design more effective catalysts.

Another area of research where we foresee significant practical benefits is computer science. Our desire for ever smaller and ever faster electronic circuitry, operating ultimately at the frequency of light, creates a fundamental problem: How can one measure a world-fastest prototype when electronic methods themselves are obviously too slow? We believe that ultrafast electron microscopy can become a crucial tool here, because electron-optical measurement methods are already standard in chip manufacturing and quality control, and nothing fundamental stands in the way of adding time resolution here.

In addition to the use of continuous laser light for attosecond pulse formation in our electron microscope, pulsed lasers can also be applied and will provide stronger specimen excitations at the expense of the average signal strength. Forthcoming publications from other research groups [5,6] already show some of these emerging possibilities, for example, in ultrafast scanning electron microscopy [5] or in attosecond electron interferometry [6]. In our laboratories, the next steps for further optimization of the measurement procedures will include the improvement of the signal-to-noise behavior using improved modulation membranes, the use of femtosecond sample excitation for investigating nonlinear optical responses as well as measurements of fully three-dimensional electric and magnetic fields by attosecond electron tomography.

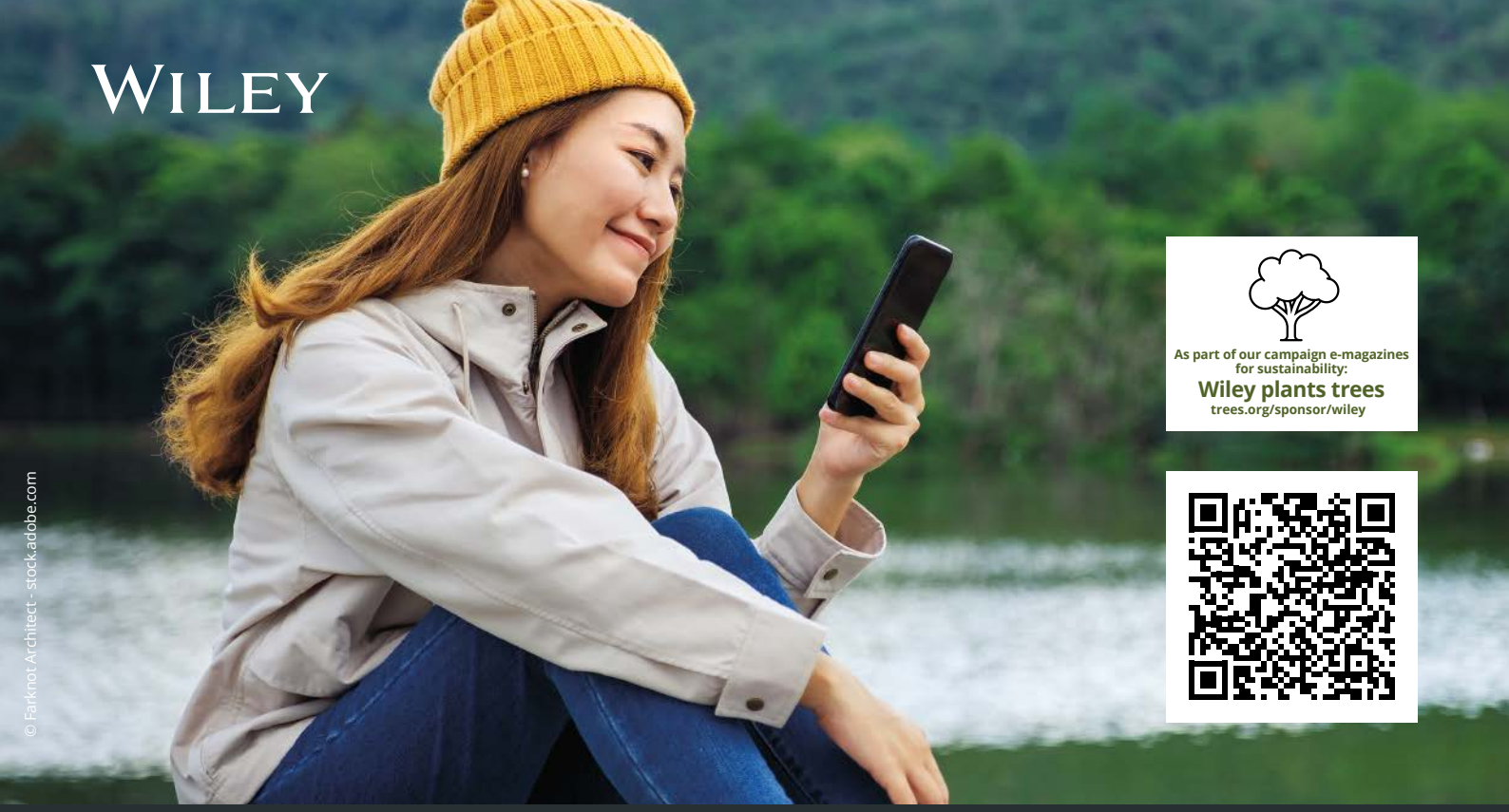
In all these activities, it seems important to us to recall that the electron microscope is one of the most versatile measuring instruments of our time, as is the laser. We therefore expect a very broad application range of our new attosecond imaging approach to many open questions of modern research, by providing space-time access to optical components, lasers, metamaterials, chemical reactions, solar cells, or photocatalysis on an attosecond and atomic scale.

Parts of this text and of the figures are adapted from the PhD thesis of David Nabben, University of Konstanz, 2023.

Affiliation
[1]Universität Konstanz, Germany

Contact
Prof. Dr. Peter Baum
Universität Konstanz
Konstanz, Germany
peter.baum@uni-konstanz.de

[1] References: https://bit.ly/IM-Baum



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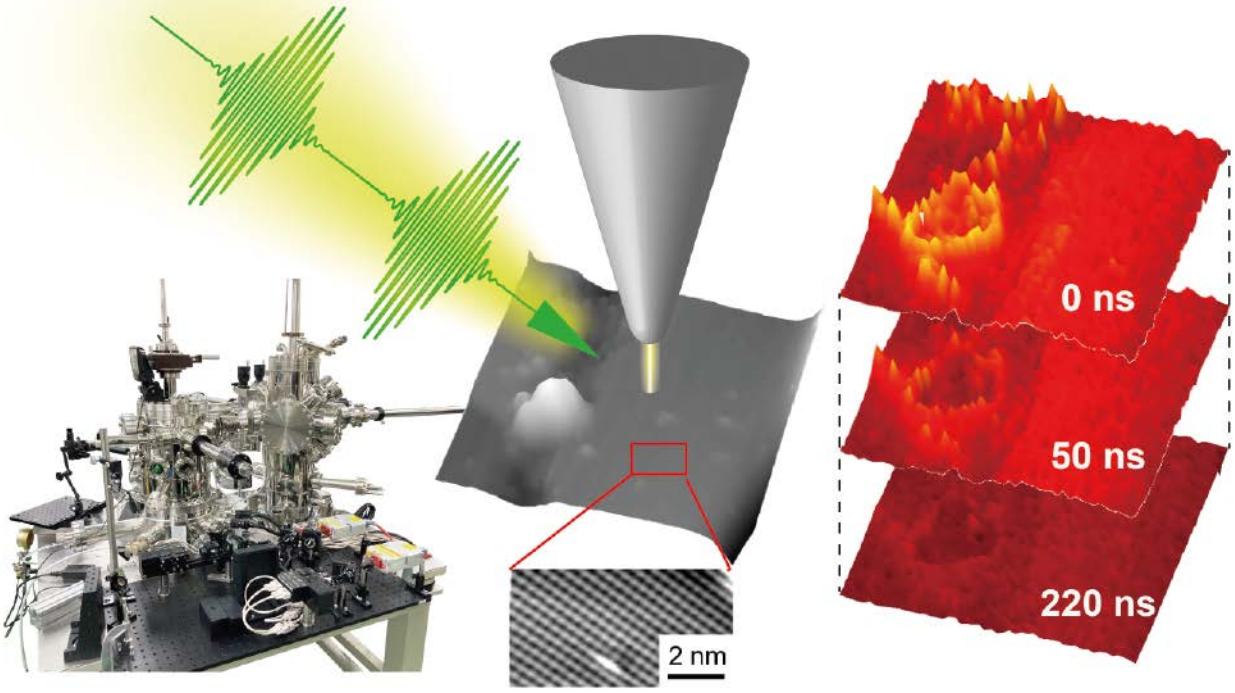

Next-Generation Time-Resolved Scanning Probe Microscopy

Ushering in a New Era of Nanoscale Exploration with Enhanced Usability

Katsuya Iwaya[1], Hiroyuki Mogi[2], Shoji Yoshida[2], Yusuke Arashida[2], Osamu Takeuchi[2], Hidemi Shigekawa[2]

Understanding the nanoscale carrier dynamics induced by light excitation is the key to unlocking futuristic devices and innovative functionalities in advanced materials. Optical pump-probe scanning tunneling microscopy (OPP-STM) has opened a window to these phenomena. However, mastering the combination of ultrafast pulsed lasers with STM requires high expertise and effort. We have shattered this barrier and developed a compact OPP-STM system accessible to all. This system precisely controls laser pulse timing electrically and enables stable laser irradiation on sample surfaces. Furthermore, by applying this technique to atomic force microscopy (AFM), we have captured time-resolved force signals with an exceptionally high signal-to-noise ratio. Originating from the dipole-dipole interactions, these signals provide insights into the carrier dynamics on sample surfaces, which are activated by photo-illumination. These technologies are promising as powerful tools for exploring a wide range of photoinduced phenomena in conductive and insulating materials.

Introduction

The need for exceptional spatial and temporal resolutions is at the heart of deciphering the intricate carrier dynamics in nanoscale materials. Conventional scanning tunneling microscopy (STM), although providing unparalleled spatial and energy resolutions, hits a temporal resolution ceiling at sub-millisecond levels owing to preamplifier bandwidth constraints. With the integration of transformative OPP techniques into STM, such barriers have been overcome, realizing higher temporal resolutions. This fusion is pivotal in probing materials' complex non-equilibrium carrier and spin dynamics [1,2]. Another technique, the use of a subcycle electric field as bias voltage, has been incorporated as an electric-field-driven STM. This technique provides temporal resolutions of less than 1 ps and 30 fs, maintaining the spatial resolution of STM using terahertz (THz) and mid-infrared pulses [3-6]. This leap in technology widens the horizon of time-resolved STM; however, it still requires high expertise and simplification of the system to make it accessible for a broad spectrum of research endeavors. In addition, since the application of STM is limited to conducting materials, extending this OPP technique to atomic force microscopy (AFM) would further expand the capabilities of this time-resolved measurement technique.

Optical Pump-Probe Scanning Tunneling Microscopy (OPP-STM)

In the conventional OPP method, pump light and probe light, which are delayed in time, are irradiated onto a sample, as shown in Figure 1a. When carriers such as electrons and holes excited by the pump light remain in an excited state, the excitation by the probe light is suppressed (absorption bleaching). Therefore, by measuring the reflectivity of the probe light as a function of the delay time, one can investigate the dynamics of the states excited by the pump light with a time resolution corresponding to the pulse width of the excitation light. However, the spatial resolution is limited to the diffraction and light-spot size (around micrometer order).

In OPP-STM, the sample surface under the STM tip is first excited by a pump pulse and subsequently by a probe pulse with a delay time t_d, and the tunneling current is detected using a conventional preamplifier (Fig. 1b) [1]. When t_d is sufficiently long ((1) in Fig. 1c), most of the photocarriers excited by the pump pulse relax to the ground state before the subsequent probe pulse illumination so that a similar number of carriers would be excited by the probe pulse as with the pump pulse, resulting in a large transient current I^*_{probe}. In contrast, when t_d is short, the excited states remain occupied with the photocarriers excited by the pump pulse when the probe pulse illuminates the sample so that the optical absorption saturates, resulting in a small I^*_{probe} ((3) in Fig. 1c). By illuminating a pair of pump and probe pulses sequentially and by varying t_d, one can detect a time-averaged tunneling current $\langle I \rangle$ as a function of t_d (Fig. 1d). By fitting the time-resolved tunneling current with an exponential function, we can obtain a decay time at the tip location, as examples are shown in Figure 1e [1].

In the macroscopic OPP technique, the modulation of optical intensity is conventionally utilized to detect a weak OPP-induced signal. However, the optical intensity modulation causes severe problems, such as the thermal expansion of the STM tip. Since changes in tip-sample distance are exponentially multiplied in the tunneling current, the optical intensity modulation cannot be directly applied to STM. To suppress the thermal expansion effect, we have developed an excellent delay-time modulation technique [1]. In this technique, we use two delay times (t_d and t_{max} in Fig. 1b). The longer delay time t_{max} is generally set to one-half of the laser pulse interval (for example, 0.5 µs for 1 MHz repetition rate), corresponding to the longest delay time available for the selected repetition rate. We modulate the delay time between t_d and t_{max} at, for example, 1 kHz and detect the resultant tunneling current $\Delta I(t_d) = \langle I(t_d) \rangle - \langle I(t_{max}) \rangle$ using the lock-in amplifier (Fig. 1b). This modulation technique enables us to keep the thermal load at the tunnel junction constant, substantially suppressing the thermal expansion effect.

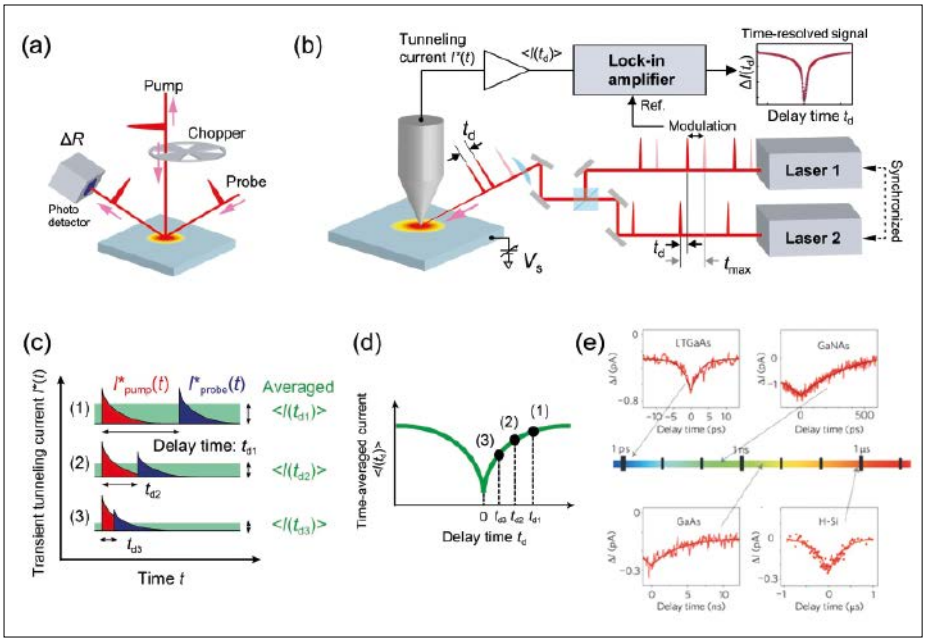

Fig. 1: (a) Conventional OPP configuration. (b) Schematic of OPP-STM with the delay-time modulation method. (c) Transient tunneling current I induced by the pump light and probe light at the representative delay time t_d. Time-averaged tunneling current $\langle I(t_d) \rangle$ is shown for each case (green rectangular). (d) $\langle I \rangle$ as a function of t_d. The time-averaged $\langle I \rangle$ corresponding to each case in (c) is plotted. (e) Time-resolved signals obtained for several samples. [1]*

Compact and Stable OPP-STM

The delay time modulation technique has enabled reliable OPP-STM measurements. Since then, many studies such as the atomic scale carrier dynamics around a single impurity on a GaAs surface [7,8] and the visualization of the ultrafast carrier dynamics in a GaAs-PIN junction [9] have been reported. However, the optical system has been generally complex and large in scale, hindering the widespread use of this technique. To overcome this difficulty, the OPP-STM system, whose laser-pulse timing is electrically controlled by external triggers, has significantly improved the ease of use, but its temporal resolution has been limited to the nanosecond range [10]. In addition, fluctuations in optical intensity cause unexpected issues, such as the thermal expansion effect, making the accurate observation of physical phenomena challenging.

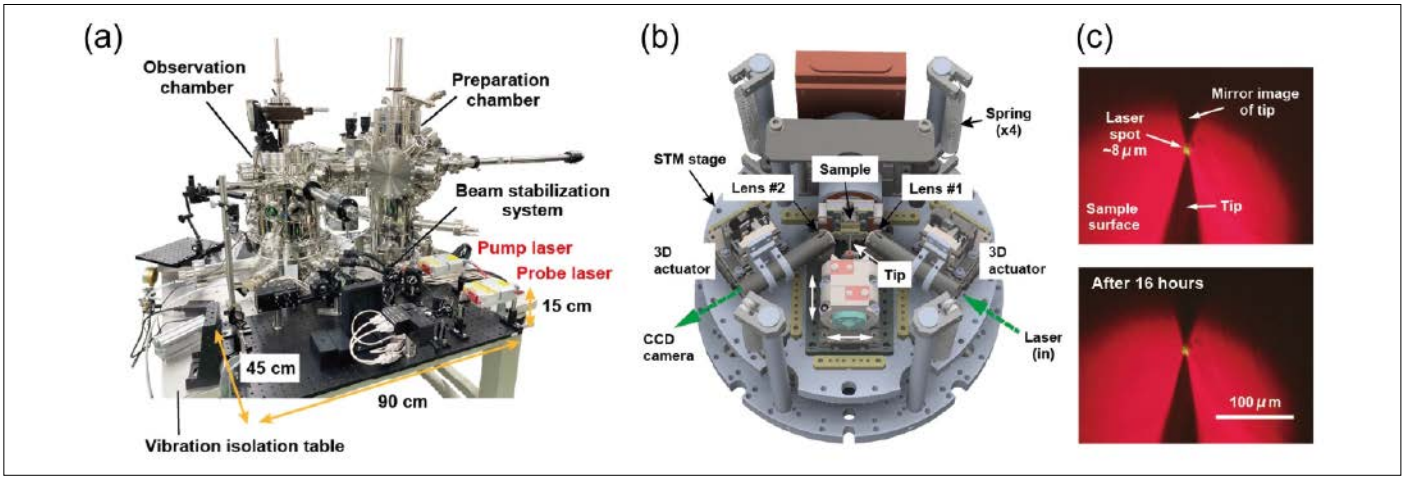

Fig. 2: (a) Photograph of the compact OPP-STM system. (b) Three-dimensional illustration of the OPP-STM unit. (c) Optical image of the tip and its mirror image on GaAs(110) surface with a laser spot illuminated at the tunneling junction before (top) and after 16 h (bottom), showing the stability of the laser spot. [11]

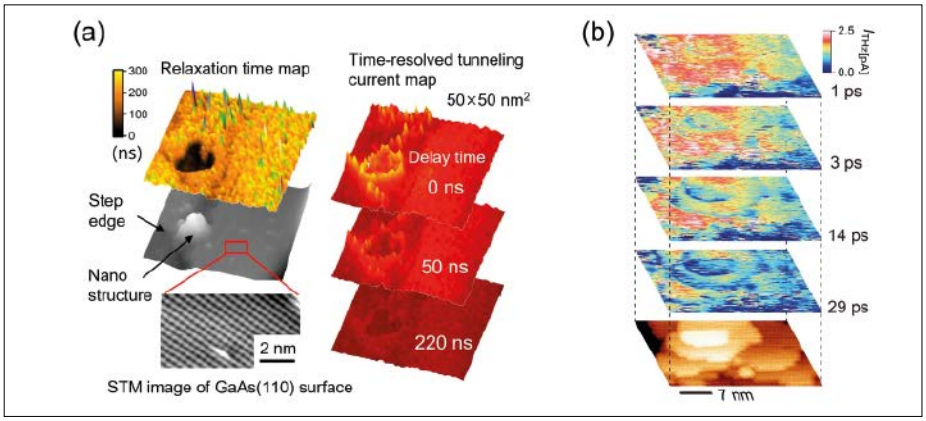

Fig. 3: (a) Grid-point time-resolved tunneling current measurement on GaAs(110) cleaved surface [11]. Characteristic features along the step edge and the perimeter of the nanoscale bump structure are identified in the time-resolved tunneling current map (right). By fitting a time-resolved tunneling current curve at each grid point, we can obtain the nanoscale relaxation time map (top left). (b) Snapshots of ultrafast motion of photoinjected electrons in a C_{60} multilayer/Au sample obtained by THz-STM. [5]

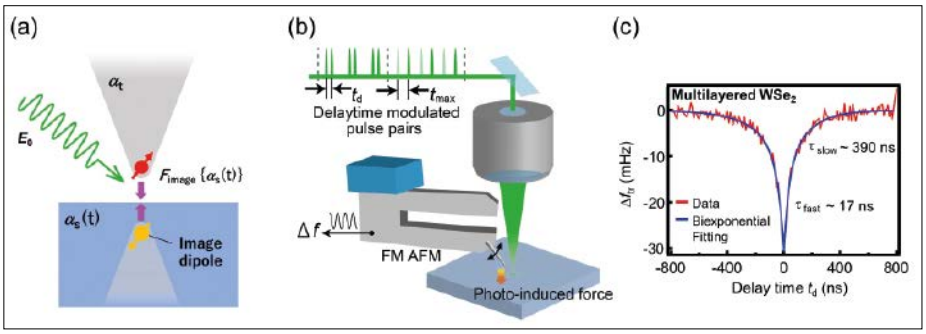

Fig. 4: (a) Dipole-dipole interaction mechanism. α_t, α_s, and E_0 are the complex effective polarizabilities of the tip and sample, the incident light electric field vector, respectively. (b) Schematic of tuning-fork-type time-resolved FM AFM setup. (c) Time-resolved signal obtained for a multilayer WSe_2 sample and fitting results. [14]

To improve the temporal resolution and the stability of laser illumination based on a compact electrically controlled laser system (Fig. 2a), the OPP-STM system with a temporal resolution of tens of picoseconds has recently been developed [11]. The long-term stability of laser illumination was realized also by placing the focus lens on the STM stage (Fig. 2b) and confirmed by monitoring the tip and its mirror image on the sample surface together with a laser spot focused on the tip-sample junction (Fig. 2c).

To demonstrate the performance of the system, we conducted a grid-point time-resolved tunneling current measurement on GaAs(110) surfaces at T = 6 K (Fig. 3a). By measuring the time-resolved tunneling current at each grid point, we can compile the time-resolved tunneling current maps at each delay time and determine characteristic features along the nanostructure (Fig. 3a, right). Furthermore, it is possible to map a relaxation time by fitting each curve with an exponential function. The relaxation time map (Fig. 3a, top left) demonstrates that the relaxation time inside the nanoscale bump structure is substantially shorter than that of its surroundings. This mapping technique is powerful in visualizing carrier dynamics associated with nanoscale structures. Figure 3b shows an example of a time-resolved signal map obtained by THz-STM. The improvement in the usability of the optical system can be extended to electric-field-driven STM, which is currently our ongoing investigation.

OPP Atomic Force Microscopy

The optical system we have developed is also applicable to AFM, expanding its potential beyond conductive materials such as semiconductors and metals, traditionally targeted in STM. Integrating AFM into our repertoire significantly broadens the horizons of nanoscale time-resolved microscopic techniques. Previous studies have clarified the photoexcited dynamics of forces originating from surface photovoltage and dipole-dipole interactions (Fig. 4a) based on optical intensity modulation (Fig. 1a) [12,13]. We have advanced these achievements by combining electrically controlled delay-time modulation with tuning-fork-type frequency modulation (FM) AFM (Fig. 4b) [14]. By focusing optical pulse pairs onto the apex of the AFM probe, we were able to detect the amplitude of the frequency shift Δf as a time-resolved signal. The markedly stable time-resolved measurements on bulk WSe_2, a layered semiconductor, have unveiled dynamics with two decay components through an ultrafast photoinduced force (Fig. 4c). These signals, decoded as the surface recombination and diffusion of photocarriers through tunneling current and force spectroscopy, mark a significant stride in our understanding of material properties. This innovative approach not only resolves the limitations encountered in time-resolved STM due to tunneling current but also paves the way for its application across a diverse array of materials, setting a new standard in nanoscale imaging.

Conclusion

Our streamlined optical system markedly eases researchers' path to harness the transformative power of the OPP-STM technique. With the multiprobe (MP) system, features such as small islands on an insulating substrate can be observed by using one tip as an electrode while conducting STM measurements with the other tip. The OPP-MP measurements have already been demonstrated on monolayer transition metal dichalcogenides to investigate nanoscale exciton dynamics [15,16]. Regarding the limitations of wavelength and temporal resolutions, cutting-edge laser technology, which has been rapidly developing, may overcome these limitations, thereby realizing a higher-performance optical system enabling a wavelength-variable, externally controllable laser system with a smaller pulse width in the future. The development of easy-to-use optical systems and their applications to various scanning probe techniques will expand the capability of OPP-SPM techniques and contribute to a deeper understanding of various photo-induced phenomena.

Affiliation
[1]UNISOKU Co., Ltd., Hirakata, Osaka, Japan
[2]University of Tsukuba, Tennnodai, Tsukuba, Japan

Contact
Prof. Dr. Hidemi Shigekawa
University of Tsukuba
Tsukuba, Ibaraki, Japan
hidemi@ims.tsukuba.ac.jp

[1] References: https://bit.ly/IM-Shigekawa

Data-Driven Microscopy Improves Image Acquisition

Finding the Right Cells to Image

Oscar André[1] and Pontus Nordenfelt[1]

Deciding where to take images in light microscopy is commonly realized by manual decision-making or complete scanning. By using information about the objects in the sample, a data-driven approach, it is possible to steer a motorized microscope to the best spots at the right time. This provides better data at a much faster rate and can be applied to most microscopy scenarios.

Introduction

Today, researchers can quickly amass vast amounts of cellular process data through high-speed image collection. However, current methods for microscope image acquisition rely on manual decisions or brute-force techniques like slide scanning. High content screening (HCS), when combined with automated image analysis, helps extract valuable information from overwhelming datasets [1–9]. HCS is the primary approach for analyzing large single-cell datasets in a population-wide context [10–14]. Yet, in high-resolution microscopy, images are often captured from specific points, lacking population context and risking bias due to human selection. This challenge intensifies during live imaging, like tracking cell migration or host-pathogen interactions.

Integrating image analysis, including machine learning, into data selection has enhanced efficiency and reduced bias, termed event-driven feedback microscopy or intelligent microscopy [15, 16]. Various proprietary (Nikon, Zeiss, Leica, Olympus) and open-source solutions control microscope hardware [17–19] and enable feedback microscopy [16, 20–22], allowing real-time response to image content. However, these decisions usually rely on predetermined image characteristics and do not adapt to sample population data distribution. Notably, no solutions currently focus on targeted image acquisition based on analyzing population characteristics to identify relevant and representative objects or events.

Methodology

We have recently introduced a general microscopy framework, data-driven microscopy (DDM) (Fig.1), which uses population-wide data to improve and control microscopy and enables cross-experiment image data validation [23]. A data-independent acquisition phase performs high-throughput imaging and generates a population-wide phenotype assessment. This data includes the relative coordinates of each data point for the system, which feeds into the automated data-dependent acquisition of selected phenotypes. Combining the two data sets yields population-wide data with high-fidelity object characterization data. DDM also inherently includes what constitutes an objective representation of the sample population. The general DDM framework applies to any motorized microscope, and all steps can be fully automated, including phenotype targeting. Placing the combined data in a population context yields a more robust, reproducible, and efficient method for selecting and acquiring data in microscopy.

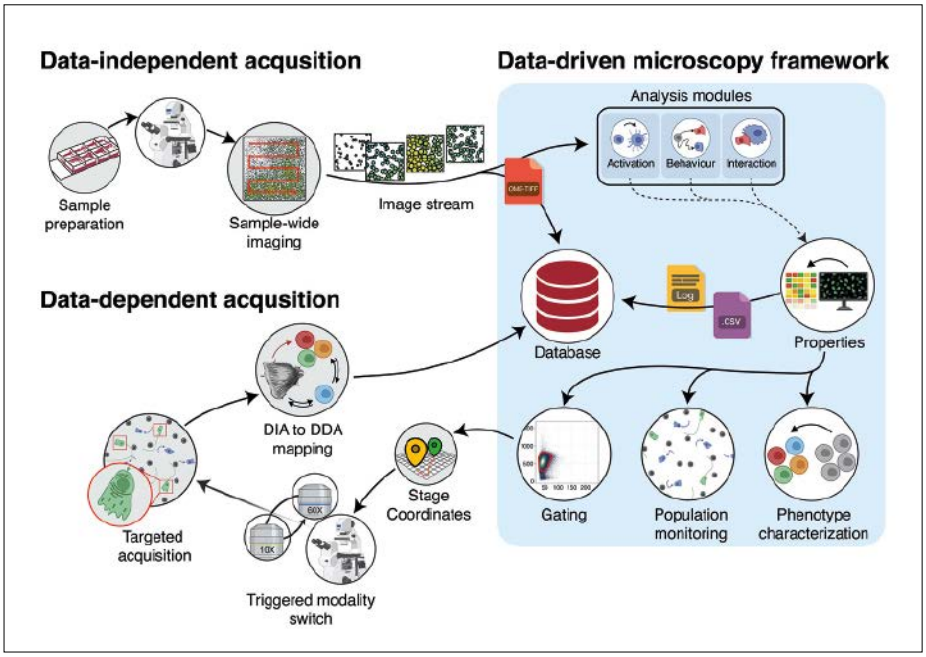

Fig. 1: Principle of data-driven microscopy. Data-independent acquisition (DIA) aims to characterize the full sample population in real-time. Generated single-cell data is continuously stored in a database, allowing for data filtering and single-cell targeting. Predefined or generated targeting criteria are fed back to the microscopy for data-dependent acquisition (DDA). The high-fidelity data is stored in a database interconnected to the DIA database. The interconnection of DIA and DDA databases allows for high-fidelity data to be placed in the sample-wide context, allowing for potential new insights that motivate future experiments.

Targeting and Capturing Infection Events

In live imaging of host-pathogen interactions, predicting rare events is a challenge. To validate DDM's capabilities, we used HeLa cells expressing mScarlet-Lifeact and *Yersinia pseudotuberculosis* expressing GFP. Interactions were categorized based on distance. DIA monitored HeLa cells (N = 16,988) and *Y. pseudotuberculosis* (N = 990), achieving a 100% hit rate in identifying interactions [23]. Traditional manual monitoring would only yield a 1.4% hit rate, demonstrating DDM's adaptability in acquiring population-wide data and targeting rare events for live-cell imaging.

Targeting and Capturing Migratory Phenotypes

To assess DDM's capacity in live feedback microscopy, we studied cancer cell migration at high spatiotemporal resolution using an adaptive feedback microscopy module with DDM. We imaged H1299 mKate-paxillin cells (N = 24,940) through DIA every 10 minutes for 6 hours, characterizing single-cell migration and collapsing the parameter space using UMAP analysis to categorize migration modes. Cells exhibited diverse migratory behaviors, categorized based on mean migration speed and meandering index (Fig. 2A and 2B). In low-resolution DIA data, a wide heterogeneity in cell migratory behavior was observed.

To gain deeper insights, we conducted high-magnification DIA on select cells, tracking them every 10 minutes for 100 minutes (Fig. 2C). Based on mean speed, we targeted the fastest cells for DDA (N = 100) and tracked their migration at higher temporal resolution (Fig. 2D). Continuous tracking between DIA and DDA was seamless (Fig. 2E), and we expanded the dataset by tracking neighboring cells, resulting in a total of 1,336 observations. (Fig. 2F). DDA provided increased resolution and variance in cell tracking (Fig. 2G), showing that fast-migrating cells maintained their speed profile over time, while DIA data indicated an overall regression to the population mean without altering speed group allocation. In summary, DDM enhances cell monitoring on a population scale with targeted high-spatiotemporal acquisition, ensuring data fidelity and integrity.

Conclusion

Light microscopy, a potent single-cell method, provides subcellular resolution for quantitative spatial data. However, unlike flow cytometry and single-cell sequencing techniques, it faces challenges in achieving high-quality population-wide sample characterization while preserving resolution. We introduce a versatile approach called da-

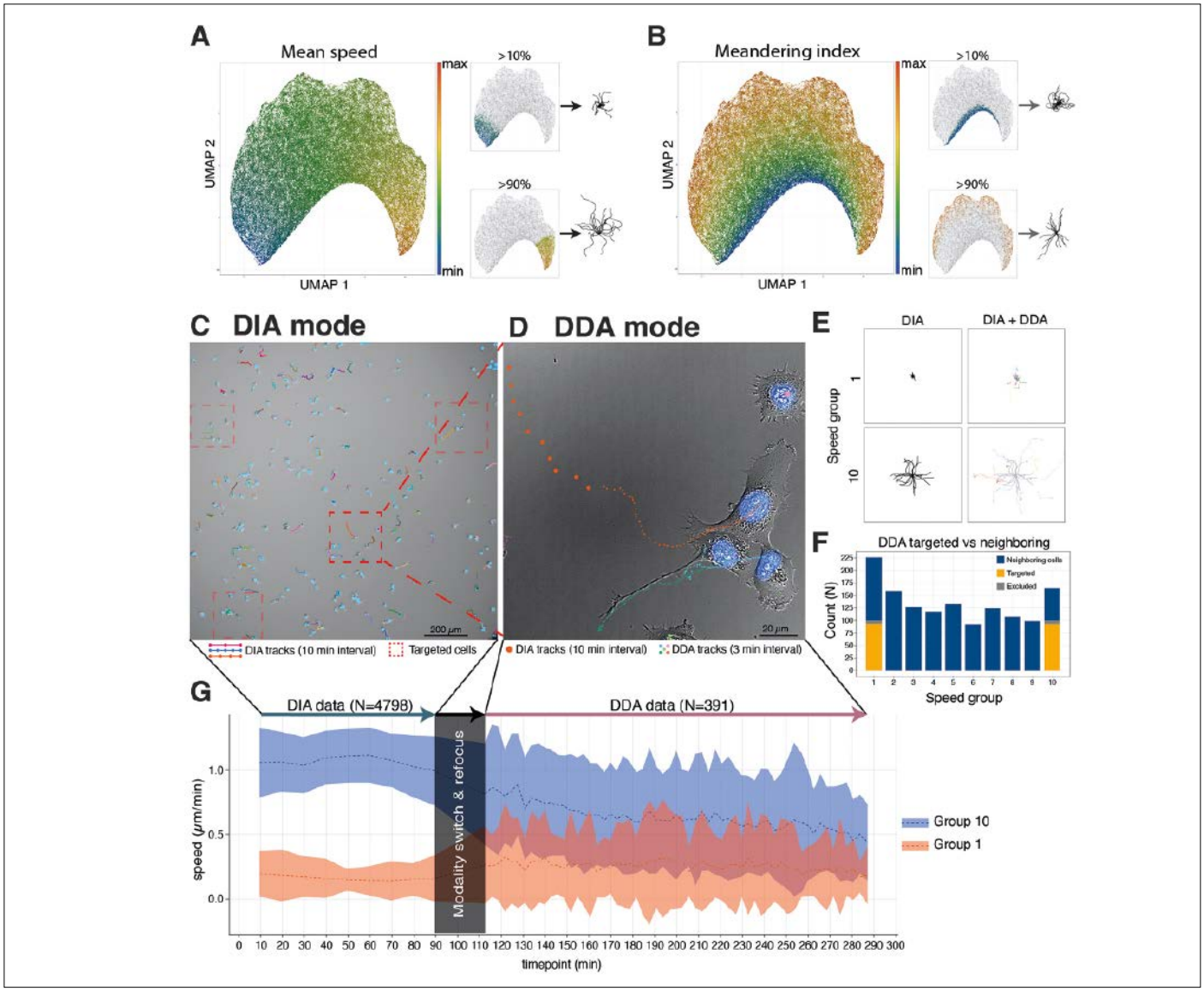

Fig. 2: Targeting and capturing migratory phenotypes. Cells were imaged every 10 min and analyzed (total N = 24 940) in multiple migration properties and categorized into slow and fast (<10th and >90th quantile, respectively) in terms of mean speed. a, b UMAP space colored using mean-speed (a) and meandering index (b) revealed distinct migratory phenotypes. c,d Example image of cells migrating at different speeds in DIA (c) and a targeted cell in high-magnification DDA (d) migrating in the top percentile with overlayed coordinates from the DIA and DDA (large and small markers, respectively). Highlighted are fast (group 10; top left, top right, and middle rectangle) and slow (group 1; bottom left rectangle) cells in terms of mean speed. e. Cell migration was continuously tracked between DIA and DDA. f. From the DDA datasets, neighboring cells (blue) to the targeted cells (yellow) significantly increased the overall cell count. A small portion of the cells (gray) were excluded during analysis due to autofocus or tracking error. g. Speed variation over time during DIA vs. DDA for the two speed groups.

ta-driven microscopy (DDM). DDM leverages real-time population-wide object analysis to facilitate data-driven, high-fidelity imaging of relevant phenotypes based on population context. This method synergistically combines data-independent and data-dependent steps, enhancing data collected through various imaging modalities.

As a proof of concept, we have developed and applied DDM along with plugins to enhance high-content screening and enable live adaptive microscopy for cell migration and infection studies. This approach allows for the precise capture of both rare and common events of interest with high precision and resolution. DDM has the potential to mitigate human bias, boost reproducibility, and provide single-cell characteristics within the context of the sample population during microscopy data interpretation. Consequently, it can significantly enhance overall microscopy data fidelity.

Acknowledgment

This project has received funding from the Swedish Research Council (Vetenskapsrådet), Cancer Foundation (Cancerfonden), and Strategic Research Foundation (Stiftelsen för Strategisk Forskning).

Affiliation
[1] Department of Clinical Sciences, Faculty of Medicine, Lund University, Lund, Sweden.

Contact
Dr. Pontus Nordenfelt
Biomedical Center
Medical Faculty
Lund University
Lund, Sweden
pontus.nordenfelt@med.lu.de
www.nordenfeltlab.com

[1] References: https://bit.ly/IM-Nordenfelt

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In 2022, Olympus made the decision to spin off its Scientific Solutions division into a new, independent corporation called Evident. By moving forward as a distinct Scientific Solutions entity, we now have the autonomy and focus to respond more effectively to the needs of our life science and industrial customers.

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EVIDENT Europe GmbH
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Ultrasonic Optical Flaw Detector

Shimadzu has released the MIV-X Ultrasonic Optical Flaw Detector in Europe. Unlike conventional ultrasonic flaw detection systems, the MIV-X detects and visualizes cracks, voids, delamination, and other hidden defects in areas where ultrasonic testing is difficult and which are normally impossible to check visually. The MIV-X also provides a clear visualization of any flaws detected. With the MIV-X, anyone can quickly and easily perform a visual surface and near-surface inspection. These and its other advantages make the MIV-X the new benchmark device for fast, accurate and easy flaw detection on the surface and below it.

Shimadzu
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CellVoyager High-Content Analysis System

Yokogawa Electric Corporation's CQ3000 has been designed to capture 3D microscopic images of live cell cultures in high definition at high speed. When used together with Yokogawa's CellPathfinder image analysis software, it can quantify and analyze intracellular organelles to assess cellular reactions and the effects of drug compounds. It enables highly efficient evaluation of cells in various applications, from basic research to drug discovery screening. The instrument enables the stable and precise observation of live cell cultures over long periods. Furthermore, it can capture images of microplates at high speed thanks to enhancements to its stage control and auto-focus functions. When used in combination with an optional dual camera and wide-field image capture feature, which uniformly captures the entire field of view, image capture time is reduced. This product enables the high-speed selection of promising chemical compounds from vast numbers of new drug candidates.

Yogogawa
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Camera-Based System for Fluorescence Lifetime Imaging Microscopy

Lambert Instrument's LIFA vTAU is a camera-based system for transforming Fluorescence Lifetime Imaging Microscopy (FLIM), particularly for live cell applications. The system, featuring the versatile SPAD detector, helps with near instantaneous acquisition of lifetime images at unprecedented frame rates and high accuracy. Whether for studying dynamic cellular processes, molecular interactions, or biological phenomena, it is engineered to meet FLIM application needs. The camera integrates with any brand of fluorescence microscope and the dedicated LIFA software records the images and analyses the data. From sample preparation to data analysis, this system streamlines the entire imaging process. Equipped with an ultra-high sensitive SPAD detector featuring micro exposures for dynamic range optimization, The system can capture up to an astonishing 100-lifetime images per second, even in challenging lighting conditions. It enables a range of applications, from molecular interactions and protein conformation to biosensors, oxygen imaging, and more.

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Long Exposure Cameras

Teledyne Photometrics extends its Retiga series with the Retiga E9 camera that can capture exposures from tens of microseconds to tens of minutes, delivering detailed, high-resolution images without extraneous noise. With its stacked CMOS sensor technology developed by Sony, the E9's square pixel array with 3.76 µm pixels and high dynamic range (>80 dB) combine to create a sensitive, versatile, and cost-effective low-noise camera ready for integration or benchtop use. With a combination of low readout noise, high quantum efficiency, small pixel size, and large full well capacity the Retiga E9 is an ideal solution for imaging low-light signals for the extended periods required in gel documentation, DNA and RNA sequencing, qPCR, fluorescence / phosphorescence imaging, and general microscopy applications. The 9-megapixel, 3k x 3k array matches the field of view of many scientific imaging instruments, microscopes, and other C-mount-based optical systems.

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www.photometrics.com

Raman Fiber Amplifier

Toptica's new highly reliable Raman fiber amplifiers (RFA) are based on patented technology. With their high power of up to 30 W the amplifiers cover the wavelength range from 1120 to 1370 nm that is not accessible by Yb or Er fiber amplifiers. The all-fiber design requires no re-alignment and provides a high degree of stability. The RFA offers a wide tuning range of up to 10nm, a relative intensity noise <1% RMS (10 Hz- 10 MHz) and excellent long-term RMS power stability of less than 0.5 % over 100 hours (with a TA pro seed laser). For wavelengths outside this range please enquire for a custom system. The RFA utilizes the proven and stable DL pro or TA pro as a seed laser for example wavelength stabilization options. Utilizing a third-party seed laser of your choice is possible. To reach visible and UV wavelengths the RFA can be integrated with a portfolio of frequency doubling and quadrupling systems.

Toptica
www.toptica.com

Compact Scanning Electron Microscopy

ModuleSci's new compact SEM, the PicoEye-100 is a compact SEM with full-size performance. Supplied with a robust tungsten filament source, the PE-100 has an effective magnification of over 100,000x with a resolution of 3.0 nm. With its generously sized sample chamber, the PE-100 is purpose-built for imaging larger samples, while the 5-axis motorized stage ensures swift and precise sample positioning. And the PE-100 comes with an impressive set of features typically reserved for more expensive microscopes, including both a Navigation Camera and a Chamber Camera for increased versatility. Available with SE, BSE, and EDS, the PE-100 is the latest in a line of imaging tools from a company committed to innovation and excellence.

ModuleSci
www.Modulesci.com

Portable Research-Grade Confocal Raman System

Oxford Instruments WITec has introduced alphaCART, a mobile confocal Raman system. The system lets researchers bring the laboratory to the sample for on-site chemical characterization with the speed, sensitivity, and resolution of WITec's stationary instruments. This novel instrument bundles a freely positionable, fiber-coupled Raman probe with a laser, spectrometer, and laptop computer into a robust rolling flight case. It provides high signal sensitivity as well as diffraction-limited resolution and confocality. These features enable measurements through and within transparent materials and render weak Raman scatterers visible even amidst a high background. alphaCART also offers integrated white-light illumination and a color video camera for a sample survey. Excitation lasers of various outputs, ultrahigh-throughput wavelength-optimized spectrometers, microscope objectives and positioning hardware are available.

WITec
https://Raman.oxinst.com

Imaging Photometer for Advanced Material Analysis

The GeoImage microscope imaging photometer by Craic Technologies is a high-performance instrument with advanced optical capabilities for analyzing materials at a microscale level. The device is designed to provide advanced material analysis for geology, mining, and environmental research. The Imaging Photometer is a imaging system that combines the capabilities of a microscope, software, and a high-resolution camera. The images are then analyzed using a range of advanced software tools, which provide detailed information about the samples' composition, structure, and properties. The device also includes a range of advanced features such as auto-exposure, auto-focus, and auto-color balance, which make it easy to use and ensure accurate results.

Craic Technologies
www.microspectra.com

Scientific Image Analysis Platform

Image-Pro, by Media Cybernetics, now supports all Teledyne Lumenera infinity microscopy cameras. From supercharged multi-channel capture to protocol-driven analysis, the system is designed to optimize every workstation in the imaging lab. It connects every aspect of the imaging workflow and easily capture, process, measure, analyze, report, automate, and share images and insights with the scientific image analysis platform. Media Cybernetics is committed to partnering with camera and microscope manufacturers to provide support for scientific cameras and ensure a superior capture experience for the users. The base features include image management and display; processing and adjustments; usability; data analytics and reporting; auditing and authentication, and scripting. Furthermore they offer new support for the Teledyne Lumenera Infinity8 cameras, completing support for all Lumenera Infinity microscopy cameras as the INFINITY8-3 and INFINITY8-9.

Media Cybernetics
www.mediacy.com

AI Microscopy Image Analysis Software

Oxford Instruments - Andor released Imaris 10.1, the latest version of its AI microscopy image analysis software. It integrates AI segmentation tools into its main image analysis workflows, improving ease of use and providing better and more versatile segmentation for all researchers across life science applications. A AI pixel classifier is an integral part of its big-data-capable surface segmentation model. Using the classifier simplifies and improves the segmentation and object detection in challenging fluorescent datasets. In addition, it opens doors for 3D Scanning Electron Microscopy (SEM) segmentation and shape recognition. The AI pixel classifier can be used by all researchers, regardless of their experience. Imaris is inclusive for all available microscopy file formats and provides seamless conversion to native IMS, using Bio-Formats.

Oxford Instruments – Andor
www.oxinst.com

Imprint

Published by
Wiley-VCH GmbH
A Company of John Wiley & Sons, Inc

Managing Directors
Sabine Haag, Dr. Guido F. Herrmann

Group Vice President
Harriet Jeckells

Publishing Director
Steffen Ebert

Regional Commercial Director
Dr. Katja Habermüller

Editor-in-chief
Dr. Birgit Foltas
Ph.: +49 6201 606-760
E-mail: bfoltas@wiley.com

Editorial Team
Dr. Cecilia Kruszynski de Assis
Ph.: +49 (0) 3047031 105
E-mail: ckruszynski@wiley.com

Róisín Murtagh
Ph.: +49 (0) 6201 606 042
E-mail: rmurtagh@wiley.com

Dr. Christina Poggel
Ph.: +49 6201 606-625
E-mail: christina.poggel@wiley.com

Isabel Brenneisen
Ph.: +49 6201 606-716
E-mail: isabel.brenneisen@wiley.com

Corinna Herbst
Ph.: +49 6201 606-752
E-mail: corinna.herbst@wiley.com

Freelancer
Dr. Martin Friedrich
Ph.: +49 178 4596064
E-mail: editor@redaktionsbuero-friedrich.de

Wiley GIT Reader Service
65341 Eltville / Germany
Phone: +49 6123 9238 246
Fax: +49 6123 9238 244
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Our service is available for you from
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Director – Global Sales, Sciences, Corporate Sales
Dan Nicholas
Tel.: +1 716 587 2181
dnicholas@wiley.com

Segment Sales Manager Laboratoty & Biotechnology
Vanessa Winde
Ph.: +49 6201 606-721
E-mail: vwinde@wiley.com

Media Consultant
Dr. Stefanie Krauth
Ph.: +49 6201 606-728
E-mail: stefanie.krauth@wiley.com

Layout & Lithography
Ramona Scheirich

Production Manager
Jörg Stenger
Ph.: +49 6201 606-742
E-mail: joerg.stenger@wiley.com

Sales Administrator
Kerstin Kunkel
Ph.: +49 6201 606-731 · Fax: -790
E-mail: kerstin.kunkel@wiley.com

Reprints
Dr. Stefanie Krauth
Ph.: +49 6201 606-728
E-mail: stefanie.krauth@wiley.com

Circulation
18,000 copies, 4 times per annum
Advertising price list from October 1st 2023

Subscription 2024
Four issues 40.00 €, single copy 15.30 € ca. plus postage. Pupils and students receive a discount of 50 % at sight of a valid certificate. Subscription orders can be revoked within 1 week in writing. Dispatch complaints are possible only within 4 weeks after publishing date. Subscription cancellations are accepted 6 weeks before end of year.

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Konto-Nr. 6161517443
BLZ: 501 108 00
BIC: CHAS DE FX
IBAN: DE55501108006161517443

Printed in Germany

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ISSN 1439-4243
www.imaging-git.com

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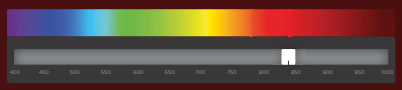

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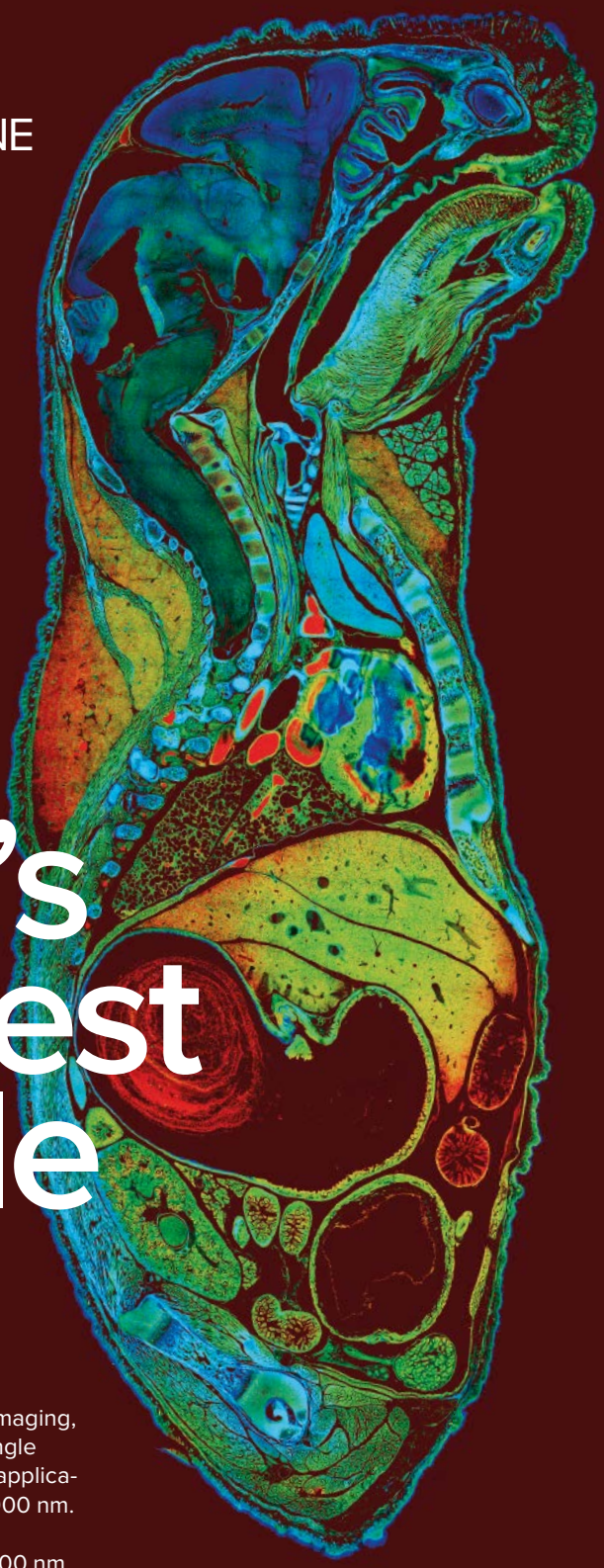

Confocal microscope image of a mouse. Credits: Leica Microsystems

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