Imaging & Microscopy

RESEARCH • DEVELOPMENT • PRODUCTION

4
VOLUME 26
NOVEMBER
2024

Official Partner of the EMS

Read our eMagazine

WILEY

WILEY

Microscopy on Wiley Analytical Science

Read all contents of our journals Imaging & Microscopy and Microscopy & Analysis in the microscopy channel on Wiley Analytical Science. All issues for reading in magazine format can also be found here.

https://bit.ly/WAS-microscopy

WILEY Analytical Science

Dear Readers,

The landscape of the world is evolving at a rapid pace, and the publishing industry is no exception. For many years, Wiley has been serving the microscopy community with two esteemed publications: *Imaging & Microscopy* and *Microscopy and Analysis*. With a vision to deliver the utmost value to our readers, we are excited to announce the amalgamation of these publications into a single, robust periodical starting in 2025.

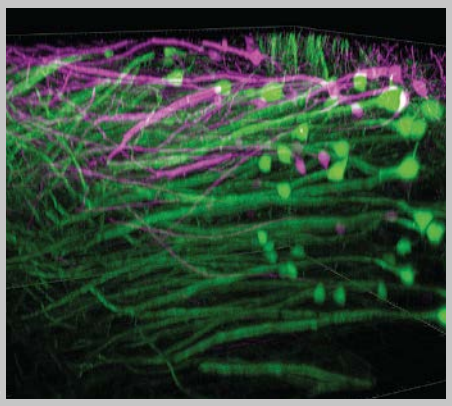
After careful consideration, we have chosen to retain the name *Microscopy and Analysis*, honoring its legacy of over 38 years in the market. This consolidation will not only preserve but also enhance the publication by integrating the most acclaimed features of *Imaging & Microscopy*. Furthermore, *Microscopy and Analysis* will proudly serve as the partner publication of the European Microscopy Society (EMS). Starting next March, all EMS members will receive the new edition of *Microscopy and Analysis*. Our collaboration with the Royal Microscopy Society will continue, and we have also forged new partnerships. You can look forward to new insights from Global Bioimaging and the Global BioImage Analysts' Society, who will be collaborating more closely with us from 2025.

The inaugural issue of the revamped *Microscopy and Analysis* is set to debut in March 2025, and we invite you to anticipate a fresh design and enriched reading experience. The magazine will continue to be an essential resource, providing a comprehensive mix of scientific and industry news, event updates, society information, profiles, interviews, application stories, and expert articles from international contributors from science and industry, ensuring you remain thoroughly informed about all facets of microscopy.

Our esteemed external editors, Chris Parmenter, Rebecca Pool, and Martin Friedrich, will maintain their commitment to delivering compelling content, leveraging their specialized knowledge to captivate our audience.
We are enthusiastic about the opportunity to keep you abreast of the cutting-edge advancements in microscopy in the forthcoming years. Additionally, we aim to provide our customers with a valuable platform to effectively reach their target audience and engage with 50,000 readers worldwide, both in print and digital formats.

See you in 2025!
Birgit Foltas

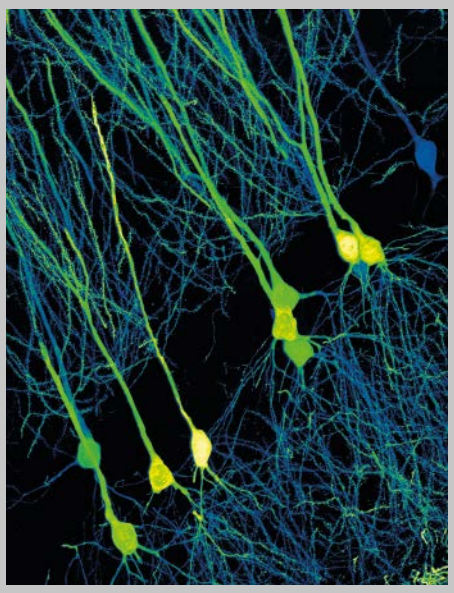
© FOM


© Polisseni


© This image was generated by DALL-E


© Giada Franceschi


© CryoCapCell



© C. Polisseni, MPI for biol. Intelligence

COVER

Recent Developments in Multi-Photon Microscopy

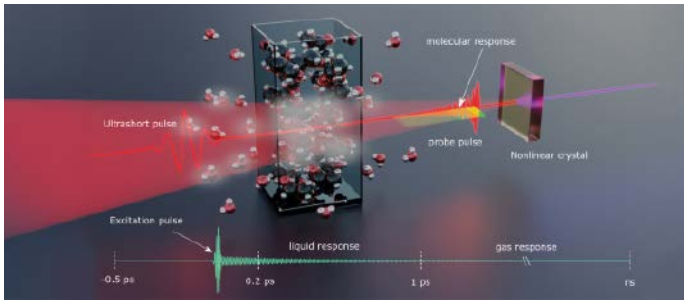
Pushing the Limits of Speed, Field-of-View, and Miniaturization

Decoding the neuronal network requires the latest tools capable of acquiring images at a high resolution, deep within the brain. Among these, multi-photon fluorescence microscopy is a popular method which, thanks to the non-linearity of the excitation, can capture background-free structural, functional and dynamic processes inside living biological tissue. Recent studies have been advancing this technique pushing the limits of speed, field-of-view, depth and miniaturization.

Newsticker

Femtosecond Fieldoscopy

Enabling High-Resolution Detection of Biomolecules in Liquid Samples



© Florian Sterl

Researchers at the Max Planck Institute for the Science of Light have developed a novel method that facilitates the precise detection of extremely small liquid quantities, down to micromolar levels, with exceptional sensitivity in the near-infrared spectrum. By achieving this, the scientists have opened doors to highly advanced, label-free bio-imaging techniques for detecting target molecules in aqueous environments, for example, pushing the boundaries of biomedical applications. The new femtosecond fieldoscopy relies on ultrashort laser pulses that induce rapid molecular vibrations, much like a quick tap that causes a bell to ring. When the molecules are excited by these brief pulses, they emit a unique signal known as 'free-induction decay' (FID). This signal, lasting for just a trillionth of a second, carries a precise "fingerprint" of the molecule's vibration. By using an ultrashort laser pulse, the researcher team has managed to isolate the molecule's signal from the laser's own pulse, significantly enhancing the detection of vibrational responses without interference. This groundbreaking achievement allows scientists to identify specific molecules with remarkable accuracy, setting a new standard for biological marker detection.

Original publication:
doi: 10.1038/s41566-024-01548-2
More information:
https://bit.ly/IM-042024-a

Time-Lapse Microscopy

A "Google Earth" for Mapping Zebrafish Embryonic Development

© Dale Ramo

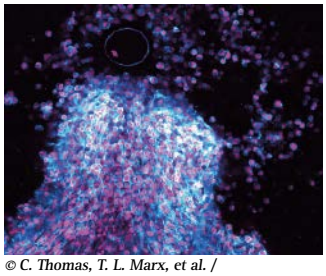
Researchers at the Chan Zuckerberg Biohub San Francisco (CZ Biohub SF) have created "Zebrahub," a revolutionary tool for mapping the development of zebrafish embryos. Dubbed the "Google Earth of developmental biology," Zebrahub presents a navigable, detailed map that tracks each cell journey as it contributes to the body structure of an adult fish. Using zebrafish, a small freshwater species native to South Asia known for its genetic similarities to humans, Zebrahub leverages time-lapse microscopy and sophisticated cell-tracking software to visualize the transformation from a single cell into a complete organism. With its transparent embryos, the zebrafish is an ideal model for studying the intricate choreography of cellular division and differentiation, offering insights into developmental processes and disease mechanisms relevant to human health.

Original publication:
doi: 10.1016/j.cell.2024.09.047
More information:
https://bit.ly/IM-042024-c

Real-Time Visualization

Observing the Entire Ovulation Process in Mouse Follicles with Unprecedented Clarity

In a breakthrough for fertility science, researchers from the Max Planck Institute for Multidisciplinary Sciences have visualized the entire ovulation process in mouse follicles in real-time. The research team developed a novel live imaging technique that allows ovulation to be observed with unparalleled spatial and temporal precision. This advancement provides valuable insights into the mechanisms behind ovulation, a complex process crucial for reproduction. Ovulation is a finely tuned, hormone-driven process during which a mature egg is released from a follicle in the ovary and enters the fallopian tube. During a lifetime, women will ovulate around 400 times, releasing one egg per menstrual cycle for potential fertilization. Each cycle sees the maturation of 15 to 30 eggs within fluid-filled follicles, though only the largest and best-developed follicle ultimately releases its egg.

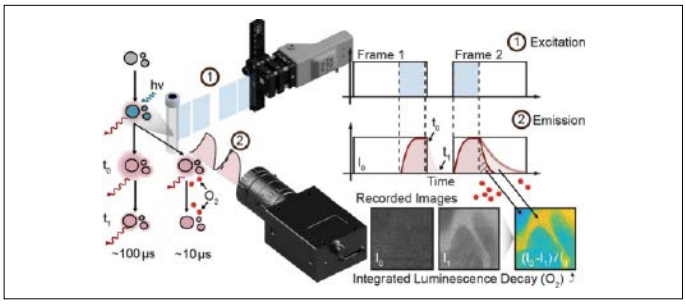


© C. Thomas, T. L. Marx, et al. / MPI for Multidisciplinary Sciences

Original publication:
doi: 10.1038/s41556-024-01524-6
More information:
https://bit.ly/IM-042024-b

Luminescence Imaging

Tracking Oxygen Dynamics from Microscopic Particles to Entire Ecosystems



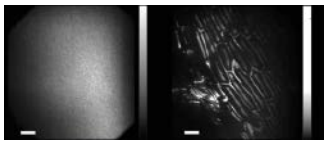
© S. Ahmerkamp/MPI for Marine Microbiology

A team of researchers from the Max Planck Institute for Marine Microbiology, the Leibniz Institute for Baltic Sea Research, and the University of Copenhagen has introduced a pioneering method that uses readily available and cost-effective equipment for imaging luminescence lifetimes. This innovative technique allows for the recording of oxygen dynamics with unprecedented temporal and spatial precision, crucial for understanding ecosystem functions. Oxygen is essential for life, and tracking its behavior is key for advancing ecological research. Optical sensors that use luminescent dyes have traditionally mapped oxygen levels in marine environments, relying on the principle that oxygen reduces the phosphorescence lifetimes of these dyes. However, conventional imaging of luminescence lifetimes requires expensive, specialized equipment, hindering its real-world application. The innovative imaging method employs readily available camera systems and facilitates high-speed luminescence lifetime measurements.

Original publication:
doi: 10.1021/acssensors.4c01828
More information:
https://bit.ly/IM-042024-d

Computational Holography

Fast, Non-Invasive Method for Optical Imaging through Scattering Media



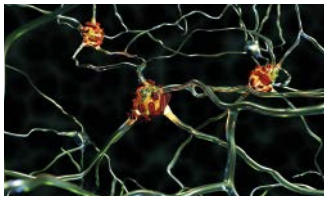
© O. Haim and J. Boger-Lombard

Scientists at the Hebrew University of Jerusalem have introduced a new method for non-invasive imaging through highly scattering media. The research led by Prof. Ori Katz describes a novel computational holography-based technique that bypasses the need for traditional tools like spatial light modulators (SLMs) or guide stars, marking a major leap forward in optical imaging technology. This new method stands out for its versatility and flexibility, allowing for high-resolution imaging across various modalities, such as epi-illumination and lensless endoscopy. The key breakthrough is the ability to correct over 190,000 scattered modes using only 25 holographic frames. Unlike traditional techniques, which rely heavily on physical hardware and prior knowledge of the scattering environment, this approach relies purely on computational optimization, making it faster and more adaptable. The efficiency of this new technique is another crucial aspect. By reducing the need for extensive measurements and the computational load usually associated with wavefront shaping or reflection matrices, it drastically accelerates the imaging process. This shift from physical hardware to software-based solutions not only speeds up the process but also makes it scalable for a wide range of applications, from biological tissue imaging and multi-core fiber endoscopy to acousto-optic tomography and even geophysics and radar systems.

Original publication:
doi: 10.1038/s41566-024-01544-6
More information:
https://bit.ly/IM-042024-f

Atomic Force Microscopy

Revealing Toxic 'Superspreader' Potentially Linked to Alzheimer's Progression



© EMPA

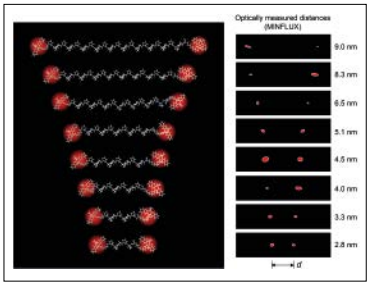
In an exciting development for dementia research, researchers have visualized the formation of toxic protein structures linked to dementia with unprecedented precision. Using advanced imaging, they monitored protein fibril formation in real-time, uncovering distinct types of fibrils, including a particularly active form termed "superspreaders." These fibrils, capable of catalyzing additional protein accumulation, play a suspected role in the rapid spread of Alzheimer's pathology through brain tissue. The team led by Peter Nirmalraj from Empa›s Transport at Nanoscale Interfaces laboratory and scientists from the Irish University of Limerick analyzed the fibrils formed by amyloid β under conditions that mimic the human brain. Traditional staining methods can distort protein structures, but by employing atomic force microscopy in a salt solution, the researchers preserved the fibrils in their natural state. This breakthrough allowed them to track fibril formation from initiation through 250 hours of progression. Their observations revealed that fibril "superspreaders" feature highly active edges and surfaces where new protein molecules accumulate, eventually forming longer fibrils that may spread across brain tissue. Unlike standard fibrils, these superspreaders catalyze further aggregation, effectively facilitating Alzheimer's progression.

Original publication:
doi: 10.1126/sciadv.adp5059
More information:
https://bit.ly/IM-042024-g

MINFLUX Microscopy

Unlocking Ångström Precision in Direct Biomolecule Measurement

A research team led by physicists Steffen Sahl and Nobel laureate Stefan Hell from the Max Planck Institute for Multidisciplinary Sciences in Göttingen and the Max Planck Institute for Medical Research in Heidelberg has successfully measured distances within biomolecules down to one nanometer, achieving Ångström precision using light microscopy. The team's breakthrough demonstrates that MINFLUX can not only optically record the spatial distances between subunits in biomolecules but also detect different conformations of individual proteins with unprecedented precision. This advancement moves MINFLUX microscopy into the resolution range of Förster Resonance Energy Transfer (FRET), a method widely used in molecular biology. Unlike FRET, which estimates distances indirectly via energy transfer between dye molecules, MINFLUX directly measures the positions of individual fluorescent markers, resulting in far greater accuracy. This new capability could revolutionize our understanding of biomolecular structures and interactions, aiding in fields ranging from drug development to protein engineering.



© I. Böttcher-Gajewski / MPI for Multidisciplinary Sciences

Original publication:
doi: 10.1126/science.adj7368
More information:
https://bit.ly/IM-042024-e

Focus On Microscopy

Taipei, Taiwan, April 13 - 16, 2025

It is a great pleasure to announce FOM2025, the next in a series of unique interdisciplinary meetings on advanced and multi-dimensional light microscopy and image processing. The conference will take place from Sunday, April 13, to Wednesday, April 16, 2025, during the week before Easter.

FOM2025 continues a long-standing (since 1988) yearly conference series on the latest innovations and developments in (optical) microscopy and its applications in biology, medicine, and the material sciences.

FOM2025 will be hosted at the National Taiwan University Hospital Conference Center (NTUHCC), centrally located in Taipei and near major landmarks. Taipei is recognized as an exceptionally safe city and celebrated for its rich cultural heritage, vibrant night markets, and breathtaking natural scenery.

FOM Taipei will start on Sunday morning, April 13, with tutorials, followed by parallel sessions, Flash poster presentations, and a plenary opening session with invited speakers at the end of the afternoon. The first day will be closed with a welcome reception. A technical exhibition will also be part of the conference.

All details concerning registration, abstract submission, and deadlines are or will be available on the conference website.

A wide range of microscopy and related subjects will be addressed, ranging from the physics of microscopy to advanced applications.

Topics of the Upcoming FOM Conference Include:

- Theory and practice of confocal and multi-photon-excitation microscopy
- Instrumentation and microscope design
- Super-resolution microscopy
- 3D and 4D live cell and tissue imaging
- Adaptive optics for microscopy
- Light sheet microscopy
- Phase/interference-based microscopies
- OCT, holography, endoscopy
- Advanced fluorescence imaging/spectroscopy: FRET, FRAP, FLIM, FCS, SOFI
- New fluorescent probes, proteins, quantum dots, single-molecule imaging
- Clearing and expansion techniques
- Coherent non-linear microscopies: SHG, THG, SFG, CARS
- Multi-dimensional fluorescence and Raman spectroscopy imaging
- Correlated microscopies, e.g., light/electron
- Laser manipulation and tracking, photo-activation
- Fast acquisition, automated and high-content microscopy
- 3D image processing and visualization for multidimensional data

For information on the program schedule, conference venue, and background on the present and previous FOM conferences, please visit the conference website.

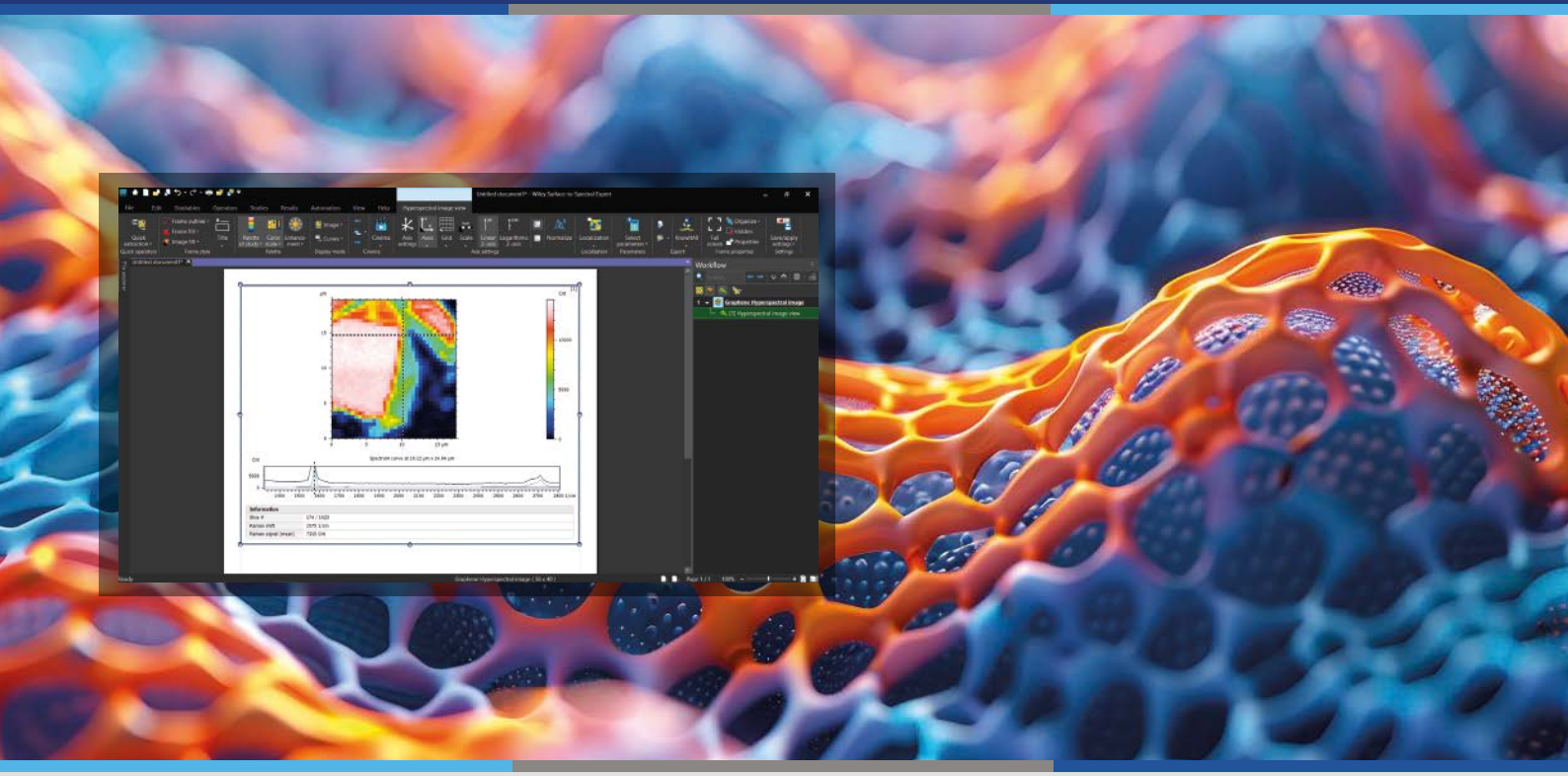
Welcoming you to Taipei for the FOM2025 Conference and Exhibition, the Organizers:

- Fu-Jen Kao, National Yang Ming Chiao Tung University, Taiwan
- Chi-Kuang Sun, Graduate Inst. of. Photonics & Opto ELectronics, National Taiwan University, Taiwan
- Hans Gerritsen, University of Utrecht, The Netherlands
- Fred Brakenhoff, Honorary member

More information and registration:
www.FocusOnMicroscopy.org

Contact the organizers:
info2025@FocusOnMicroscopy.org

Go from microscopy to spectral analysis in a single workflow



The complete correlation & spectroscopy analysis software solution for microscopy

The new **Wiley Surface-to-Spectral Analysis** is a comprehensive, powerful software solution to **process**, **combine**, and **analyze** images and data from various spectroscopic techniques.

Designed to make your lab more efficient with a direct workflow from surface to spectral analysis, ***all in a single interface***.

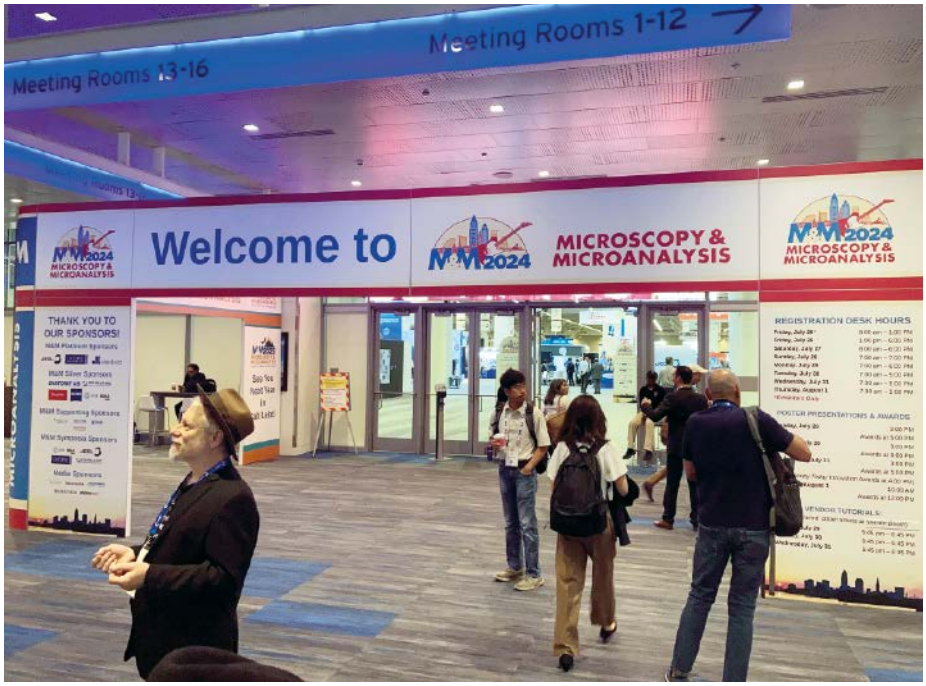
WILEY

Learn more
sciencesolutions.wiley.com

RMS Reaches Out on Both Sides of the Atlantic

© RMS

RMS staff member Noelle Knight on the stand at the Oxford Biomedical Imaging Festival.



© RMS

The RMS joined exhibitors this summer at M&M 2024 in Cleveland, Ohio.

In recent months the Royal Microscopical Society (RMS) has been busy reaching out to new audiences at high-profile events on the international stage.

The Society was proud to be among the exhibitors at Microscopy and Microanalysis 2024 – the USA's premier microscopy event - which took place in Cleveland, Ohio, from 28 July to 1 August. And just a few weeks later, the events team was packing its bags for Copenhagen, Denmark, to take part in the European Microscopy Congress 2024.

Both events featured an extensive and dynamic conference program, plus many of the world's leading companies exhibiting their latest, cutting-edge instrumentation and accessories. As such, they presented the perfect platform for the Society to make new connections and grow its international membership.

RMS Chief Executive Sali Davis, who attended both events, said: "As a truly international society, it's so important to reach out to our overseas audience, and nothing beats the personal interaction of an exhibition stand."

She added: "It was particularly pleasing to speak to so many students and Early Career attendees about submitting their papers to the Journal of Microscopy – as well as all the benefits of RMS membership."

Meanwhile, within the UK, the RMS made its first appearance as an exhibitor at this year's Advanced Materials Show at the NEC Birmingham. The event brought together leaders in engineering, science and innovation - along with the technologies used to develop new applications.

The Society also joined exhibitors at the Oxford Biomedical Imaging Festival at Oxford University in September. This proved to be another great opportunity to engage with those undertaking biomedical imaging research at the university and its partner institutions.

© RMS

The RMS stand at emc 2024 in Copenhagen.

Wheel of Fortune

A popular feature of the Society's recent exhibition appearances has been the 'Wheel of Fortune' (pictured above left) – which visitors are encouraged to spin for the chance of winning a modest prize.

Sali said: "Everyone seems to love spinning the wheel. It's a nice little ice-breaker, and really helps us to engage with people on our exhibition stand. No doubt we'll be taking it on tour with us in the future – so why not come and pay us a visit!"

Visit the RMS website to find out more about RMS membership and all our other activities in support of microscopy, imaging, and flow cytometry.

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

More information:
www.rms.org.uk

Vladislav Krzyžánek,
EMS President

Catherine Vénien-Bryan,
EMS General Secretary

EMS Newsletter #87

November 2024

Dear EMS Members,

Many of us had the privilege of attending the 17th European Microscopy Congress, EMC2024, held in Copenhagen in August this year. EMC2024 attracted about 2,500 participants from 51 countries worldwide and had Europe's largest exhibition dedicated to microscopy. It also offered numerous training sessions and scientific activities, highlighting the vibrancy of European research. We would like to thank the exhibitors for their dedication and support, and the attendees for their participation and valuable scientific contributions.

On August 26, the EMS Executive Board convened for the final time in its 2020-2024 term. During this meeting, many key activities and issues of the society were discussed, evaluated, and concluded.

On August 27, the EM award ceremony took place, the winners Quentin Ramasse (France, UK) and Tomáš Čižmár (Czech Republic, Germany) were honored and presented their work.

At the EMS General Council on August 28, the proposal for the next venue of the European Microscopy Congress (EMC) in 2028 was presented, with Barcelona and Brno as the final bidding destinations. The General Council selected Barcelona as the host city for EMC2028. We eagerly look forward to this event and hope to see you all there!

The EMS General Assembly was held on August 29, with the participation of over 180 EMS members. The presidential and financial reports as well as the changes in the constitution were approved. Klaus Qvortrup gave a brief update on EMC2024. The members also approved the new EMS Executive Board, which took office following EMC2024.

- President: V. Krzyzanek, Czech Republic
- General Secretary: C. Venien-Bryan, France
- Treasurer: P. Leclère, Belgium
- Members: A. Kittel, Hungary; L. Jones, Ireland; T. Müller-Reichert, Germany; S. Sturm, Slovenia; S. Turan, Turkey; P. Verkade, UK; I. Weber, Croatia
- Past-president: J.M. Valpuesta, Spain
- Chair EMC2024: K. Qvortrup, Denmark
- Chair EMC2028: F. Peiró Martínez, Spain
- ECMA representative: M. Peeters, Thermo Fisher Scientific

Visit the EMS website to find the New Board.

The EMS Outstanding Paper Award ceremony took place following the General Assembly. The winners of the papers in the three categories: Instrumentation and Technique Development, Life Science, Materials Science, presented their work and received the prize.

Reports of the meeting, EM awards, OPAs, and EMS scholarships will be published in the next EMS Yearbook in 2025. We encourage all EMS members to send us reports to be included in this exciting new booklet!

Finally, we would like to extend our gratitude to José María Valpuesta for his invaluable contributions as President, to Virginie Serin for her dedicated support as General Secretary, and to Christian Schoefer for his outstanding work as Treasurer over the past years. We also wish to recognize all other leaving members for their valuable contributions.

We invite you to regularly visit the EMS website and we welcome any suggestions or ideas for future newsletters!

Contact
Dr. Vladislav Krzyžánek
President
Institute of Scientific Instruments of the Czech Academy of Sciences
Brno, Czech Republic
krzyzanek@isibrno.cz

Prof. Dr. Catherine Vénien-Bryan
EMS General Secretary
Sorbonne Université
Institut de Minéralogie, Physique des Matériaux et Cosmochimie
IMPMC
Paris, France
secr@eurmicsoc.org

Suggestions for EMS newsletters:
sec@eurmicsoc.org

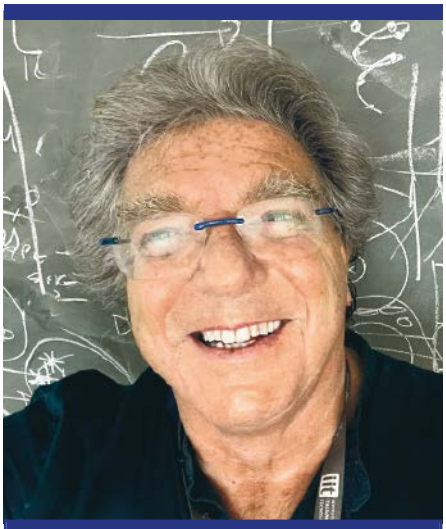
EMS webpage:
https://www.eurmicsoc.org/en/

New board of the EMS:
https://bit.ly/EMS-New-Board

A Ligurian Passion to Microscopy

From Electronic Engineering to Applied Biophysics

Alberto Diaspro's specific research experience in biophysics is related to the design, realization, and use of various optical and biophysical instruments to address key questions in cellular and molecular biology related to oncology and neuroscience. The focus has been and continues to be on three-dimensional imaging, two-photon excitation microscopy, super-resolved fluorescence, and Mueller matrix microscopy. In 2018, he and his team introduced liquid tunable microscopy (LIQUITOPY, liquid tunable optical microscopy) as a new paradigm in optical microscopy to address the central question of the structure-function relationship of chromatin-DNA in living cells using label-free and molecular imaging approaches [1]. A first step towards the current development of the so-called "artificial microscope" [2]. Based on image scanning microscopy and SPAD arrays, he combines the approaches into a multimodal microscope, including Mueller matrix microscopy, and is boosted by artificial intelligence (MOMIX, multimodal optical microscopy image correlation sensing). Most recently, he received the Enrico Fermi Prize of the Italian Physical Society (SIF), together with Francesco Saverio Pavone of the University of Florence, for experimental research in the field of physics applied to biological systems. The citation for his award reads: "For his remarkable original contributions to the development and application of optical microscopy and its decisive impact on cellular and molecular biophysics". Looking back on his long and unique career, Imaging & Microscopy took the opportunity to interview the Genoa Laureate and discuss an outstanding scientific life dedicated to microscopy.



Alberto Diaspro

is Full Professor of Applied Physics at the Department of Physics of the University of Genoa, Research Director of Nanoscopy at the IIT, and Full Academic of the Ligurian and Venetian Academy of Sciences and Humanities. Active in European and national projects, he has published more than 400 scientific articles with 25,000 citations (H = 67). He has been a SPIE Fellow since 2014 and an EOS Fellow since 2022. Alberto Diaspro co-founded the start-up Genoa Instruments in 2019. 2014, he received the Emily M. Gray Award from the Biophysical Society. In 2022, he received the Gregorio Weber Award for excellence in fluorescence studies and, this year, the SIF Enrico Fermi Prize for his experimental research in the field of physics applied to biological systems.

Professor Diaspro, you received the Enrico Fermi Prize from the Italian Physical Society (SIF) this year. What does this award mean to you, and how important is it compared to your other honors?

A. Diaspro: It is an important recognition not only for me but also for biophysics and optical microscopy as a discipline in physics. It is a recognition for those researchers who populated in the last 40 years my lab and for my "teachers", namely Bruno Bianco, Mario Bertero, Antonio Borsellino, and Enrico Gratton.

Why have you devoted your professional life to biophysics and microscopy?

A. Diaspro: Biophysics is in tune with the idea of trying to decipher the delicate and complex mechanisms of life. I was attracted by the fact that biophysics takes the general principles of physics and all the consequences deriving from them deductively as known starting data and aims to explain, based on them, the complex phenomenology of biological systems.

In which project did you first experience the feeling of having personally made a scientific breakthrough?

A. Diaspro: Starting from theory and from scratch, I designed and realized an optical architecture to address changes in chromatin condensation using CIDS (circular intensity differential scattering), and when I used my two-photon set-up to study aloe-modine [3] and RAB5A [4]. Moreover, I have two more achievements in terms of original optical architectures, namely: single molecule detection in thick samples using light sheet microscopy [5] and correlative nanoscopy [6]. I started with three-dimensional optical microscopy after reading a keystone paper by Agard and Sedat [7], and three fundamental books for my training, namely "Introduction to Fourier Optics" by Joseph W. Goodman [8], "Digital Image Processing by Ken Castleman [9] and the Handbook of Biological Confocal Microscopy by Jim Pawley [10].

What scientific projects took you much time and effort but brought the most value to you and your team?

A. Diaspro: Starting from two-photon excitation microscopy and adding single molecule imaging, even if the first approaches that I did alone with the support of Bruno Bianco (my supervisor at the University of Genoa) were on digital phase-contrast and holography and three-dimensional optical sectioning using the wide field microscope.

What scientific team achievement are you most grateful for?

A. Diaspro: The recent coupling of label-free with fluorescence boosted by an artificial intelligence approach both in terms of hardware and software, with the realization of the Genoa Instruments start-up and the relevant role in the brand-new Nikon microscope, NSPARC.

As a contemporary witness of advanced optical microscopy and super-resolution nanoscopy, what developments continue to impress you the most?

A. Diaspro: Quantitative label-free and fluorescence imaging down to the atomic level precision of localization, as implemented in the MINFLUX microscope, which also enables spectroscopy. I have to say that the theoretical developments of Colin Sheppard and the research activities of Stefan W. Hell, Jörg Enderlein, and Carlos Bustamante are the most impressive to me among the LFD people of Gratton. I miss Ignacio Tinoco Jr., Mats Gustafsson, Gabriel Popescu, and Pio Benedetti very much.

You have published over 400 scientific articles with 25,000 citations and written 5 books. Is there a particular paper/book you are most proud of?

A. Diaspro: The Nature methods papers on single molecule light sheet ("Live-cell 3D super-resolution imaging in thick biological samples") [5] and super-resolved lifetime ("A robust and versatile platform for image scanning microscopy enabling super-resolution FLIM") [11]. Moreover, I am very proud of a PNAS paper on super-resolved multiphoton coupled with STED ("Single-wavelength two-photon excitation stimulated emission depletion (SW2PE-STED) superresolution imaging") [12].

Your curriculum vitae shows you have remained loyal to your hometown of Genoa throughout your scientific career. What does your hometown mean to you, and what has prevented you from moving to other prestigious institutes during your career?

A. Diaspro: Well, I had some chances to move. However, for some reasons related to family members and to the decision – taken with my wife Teresa – to have school in Italy for our daughter Claudia, we decided to stay in Genoa, Italy. I had a good job opportunity, and my wife did too.

We know you have a warm relationship with your students and staff. It seems that you are not just an ordinary Ph.D. supervisor. What does the training of your staff and their careers mean to you?

A. Diaspro: The alumni list is long, and it is too long to mention them by name. They were and still are family members, and I still continue the tradition of inviting them with their family to Cavo's, a typical historic cafe and pastry shop, for the Christmas party. I am proud because most of them are successful researchers in microscopy and biophysics. I was lucky to have met them during my career. I have to admit that Enrico Gratton and Martin Chalfie are the persons of inspiration for the way they conduct research and interact with their own research teams.

How do you balance your personal and professional life? How has your family supported you over the years, and what are you most grateful for?

A. Diaspro: Teresa, since 1975, and my daughter Claudia (born in 1984) since she first came to the laboratory in 1988, have fully supported my research activities, even when it was very difficult (economically speaking) to insist on optical microscopy as a research and professional topic. I am also grateful for the fact that they accepted specimens in the home fridge and the second family formed by most of the researchers and graduate students working in my laboratory since 1984.

The name Alberto "Alby" Diaspro is widely known in the professional world for developing (optical) microscopy and image processing technologies and their applications in the life sciences. We know from a mutual acquaintance that you are also recognized and addressed as "Professore" by your fellow citizens on the street in your region. To what extent are you involved in your region, and what role does science play in this?

A. Diaspro: I think it is the duty of each researcher to find some time to communicate in a (popular) comprehensive way the research aims, results, and potential social impact of our activities. I was President of the Scientific Council of the Genoa Festival of Science; now, I am involved in the Festa di Scienza e Filosofia of Foligno and the Camogli Communication Festival. I am an elected member of the Ligurian and Venetian Academies of Science and Humanities, and I have organized events for many years, "Scienza Condivisa" (Shared Science) with the Society of Scientific Readings and Conversations (https://www.societaletturescientifiche.it). In 2009, with the support of the Genoa Chamber of Commerce, I launched the "Scientific Café" series. For all these activities, what matters is bringing science to the people. I have to say that I prefer to be addressed as "Alby", the nickname used by my daughter during the Erice School of the

© Alberto Diaspro

DiasproLab Early days at Department of Physics, University of Genoa, 2003 circa: (from the bottom, left to right) Ilaria Testa, Raffaella Magrassi, Francesca Cella Zanacchi – Giuseppe Vicidomini, Alby Diaspro, Silke Krol – Mattia Pesce, Davide Mazza, Paolo Bianchini.

Scientific Culture Foundation Ettore Majorana in 1986, the one used by Roger Johnston (Los Alamos) and Ignacio Tinoco (Berkely) to pose their questions following her example.

What are your scientific plans for the future? And at which scientific conferences can we meet Alberto Diaspro in person in 2025?

A. Diaspro: Like most of the researchers, my plans for the future lie in the next unknown results. The battlefield is multidisciplinary, namely: on one side, the goal of deciphering the way condensation and decondensation of chromatin in the nucleus affect cellular functions and, on the other side, how to manipulate, control, and realize in a new way the effects of light-matter interaction in terms of temporal and spatial resolution. Image scanning microscopy, single-photon detection, and Mueller matrix microscopy are great, but at the very same time, they are more than 10 years old; something new has to be found. My next 2025 conferences could be SPIE Photonics West, Biophysical Society Annual Meeting, and Focus on Microscopy for the very first part of the year. For sure, I will be at the 12th International "Weber Symposium" on Innovative Fluorescence Methodologies in Biochemistry and Medicine that will be held in Genoa, Italy, on June 15-20, 2025. The symposium honors the fundamental and far-reaching contributions of Professor Gregorio Weber. It provides an overview of modern fluorescence methodologies and applications in the biological and medical sciences. Weber symposia were held in Búzios, Brazil, in 2017, in Hawaii in 2014, 2011, 2008, 2005, 2002, 1999, and 1995, in Italy in 1986 and 1991, and in Uruguay in 2023. Please do not miss it!

The interview was conducted by Dr. Martin Friedich.

Contact
Alberto Diaspro
Department of Physics, University of Genoa, Italy

Nanoscopy, Center for Human Technologies, IIT, Genoa, Italy

ORCID: 0000-0002-4916-5928
Italyalberto.diaspro@iit.it

Online version:
https://bit.ly/IM-Diaspro

Recent Developments in Multi-Photon Microscopy

Pushing the Limits of Speed, Field-of-View, and Miniaturization

Claudio Polisseni

Recent biological studies have been moving towards 3D structures, with great advances in the field of organoids, *in vitro* 3D cell cultures, and *in vivo* awake animal research. Parallel advances in microscopy are necessary to provide reliable methods to visualize structural, functional, and dynamic processes deep inside living biological tissue. Multi-photon fluorescence microscopy is one of the promising techniques that meet this demand. Here I review the basic principles of multi-photon microscopy and its recent advances to push the limits of speed, field-of-view, and miniaturization.

A Basic Recipe for a Multi-Photon Microscope

Dating back to the 1990s [1], multi-photon microscopy has been one of the few methods capable of imaging deep inside living organisms. Thanks to the non-linearity of multi-photon excitation, this technique can produce background-free images deep inside the living tissue (Fig. 1).

In multi-photon microscopy, fluorescence is generated when two or more photons are absorbed (virtually) simultaneously by a fluorophore at the focal plane but to achieve this, one needs a very high excitation power density. Thus, at the heart of each multi-photon microscope is a laser that meets special requirements. For 2-photon microscopy, these are short pulses, high power (but well below the threshold for DNA damage), high repetition rates, and wavelengths that match the peak absorption cross-section of the fluorophore [2,3,4]. Typical lasers have a pulse duration in the range of 75-150 fs and operate in the mode-locked regime with fixed repetition rates between 50 and 100 MHz, high enough to have multiple excitation cycles within a pixel but low enough to allow the fluorophores to relax to the ground state (typical excited state lifetimes are between 3 and 5 ns). The 2-photon excitation wavelengths for the fluorophores, ranging from 750 nm to 1200 nm, match the range of tuneable Ti:Sapphire lasers, as well as recently developed more compact fiber lasers or optical parametric oscillators (OPO). For a comparison among these please see [5,6].

3-photon microscopy can push the imaging depth to more than a millimeter. To achieve 3-photon absorption, one requires even more power density, which is accomplished by setting a lower repetition rate, down to 1-4 MHz, and by squeezing the pulse width to less than 50 fs. The absorption spectrum of water plays an important role in limiting the possible excitation wavelengths to either 1300 nm or 1700 nm [7,8].

In a standard multi-photon microscope, the beam path is relatively simple (Fig. 2). A x-y galvanometric scanner (either a galvo-galvo, or, for faster image acquisition, a resonant-galvo) moves the beam through a scan lens and a tube lens to the back aperture of the objective. Here, a trade-off between penetration depth and resolution can be achieved by slightly underfilling (higher penetration depth) and overfilling (higher resolution) the back aperture, addressing different kinds of samples and experimental requirements. The intrinsic optical sectioning ability of the non-linear excitation means that a non-descanned detection path is sufficient to achieve background-free images without out-of-focus light.

The simple optical setup highlighted before is rare these days, with scientists developing novel techniques to improve the acquisition speed, the imaging volume, and

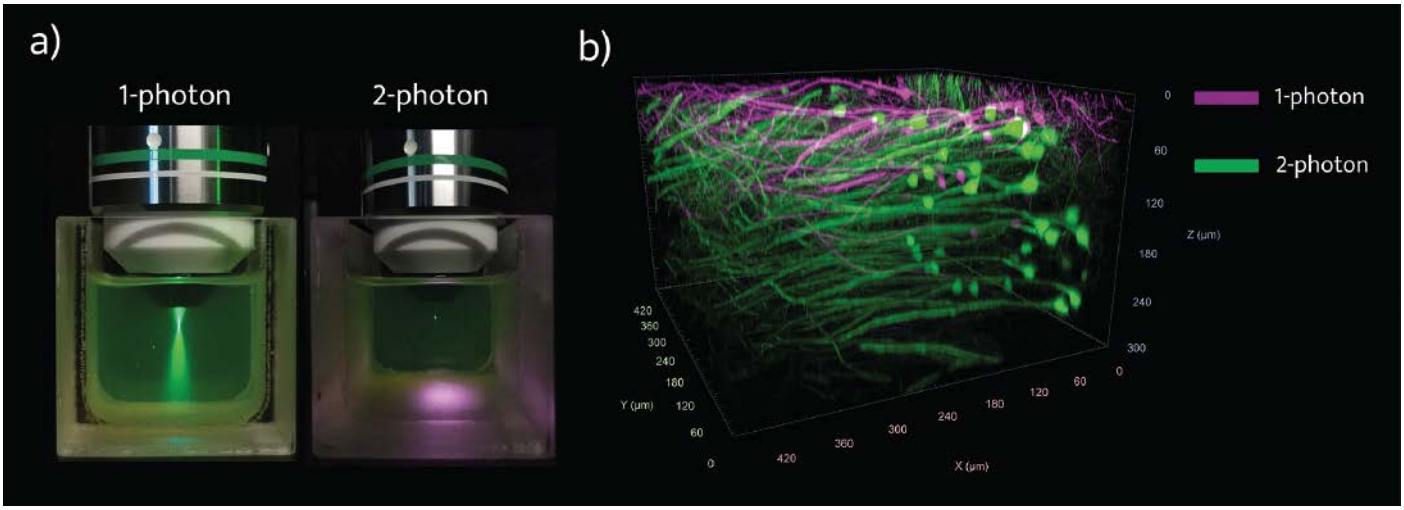


Fig. 1: a) Contrary to single-photon excitation, the absorption of two or more photons by a fluorophore requires such high power density that the fluorescence is only generated in the focal plane of the objective. b) This enables imaging at greater depths when using multi-photon imaging (green) compared to single-photon confocal microscopy (magenta).

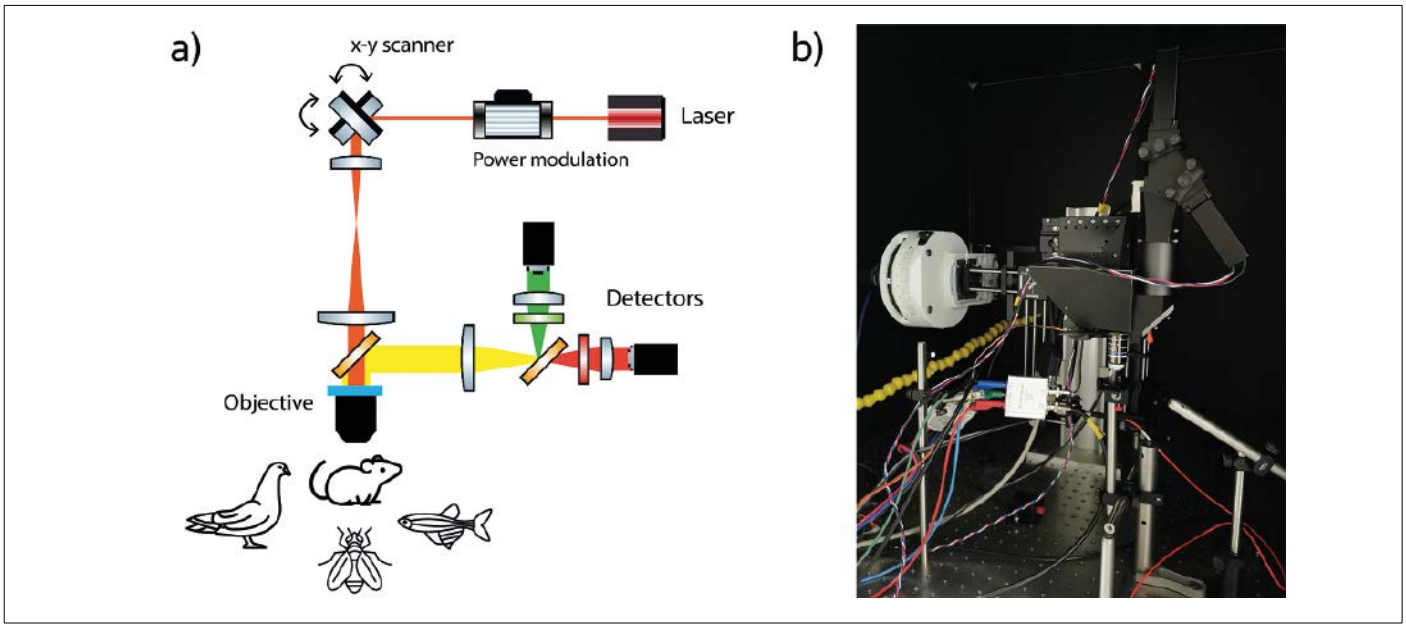


Fig. 2: a) A typical multi-photon microscope has a simple beam path. Light coming from femtosecond laser is scanned across the field of view of the objective lens. A non-descanned detection path collects as many non-ballistic emitted photons from the sample as possible. b) The simplicity of the beam path allows a wide range of in vivo studies such as at the Max-Planck Institute for Biological Intelligence.

even miniaturization to image freely moving organisms. Complex systems have low transmission efficiency (<10%) and the optical components introduce strong pulse dispersion which need to be accounted for to make sure a high-power and short pulse is delivered at the sample position.

Keeping Up with the Biology

A popular application of multi-photon microscopy is imaging neuronal activity through calcium indicators deep inside the brains of living organisms [9]. The most frequently used indicator is the genetically encoded GCaMP (gfp-tagged calmodulin), which is constantly under development with new versions being brighter and with faster response and decay times than previous ones [10,11]. To this end, the data acquisition needs to keep up with the biology. Being a raster-scanning technique, multi-photon microscopy is intrinsically slow. Since the early developments of multi-photon microscopy, many studies have addressed this.

Multiple beams have been created using a microlens array and scanned at the same time across the sample plane [12]. The microlens array was also rotated to achieve imaging at 225 Hz [13] or in combination with a diffractive optical element that limited transmission losses in the beam path [14].

More recently, scientists have employed temporally focused widefield illumination to remove the need for point scanning. This concept relies on shaping the laser beam in time and space by separating the laser pulse spectrally using a dispersive optical element, such as a grating. The components of the beam are then recombined at the focal plane of the objective lens, where the pulse reaches the shortest duration with power density high enough for multi-pho-

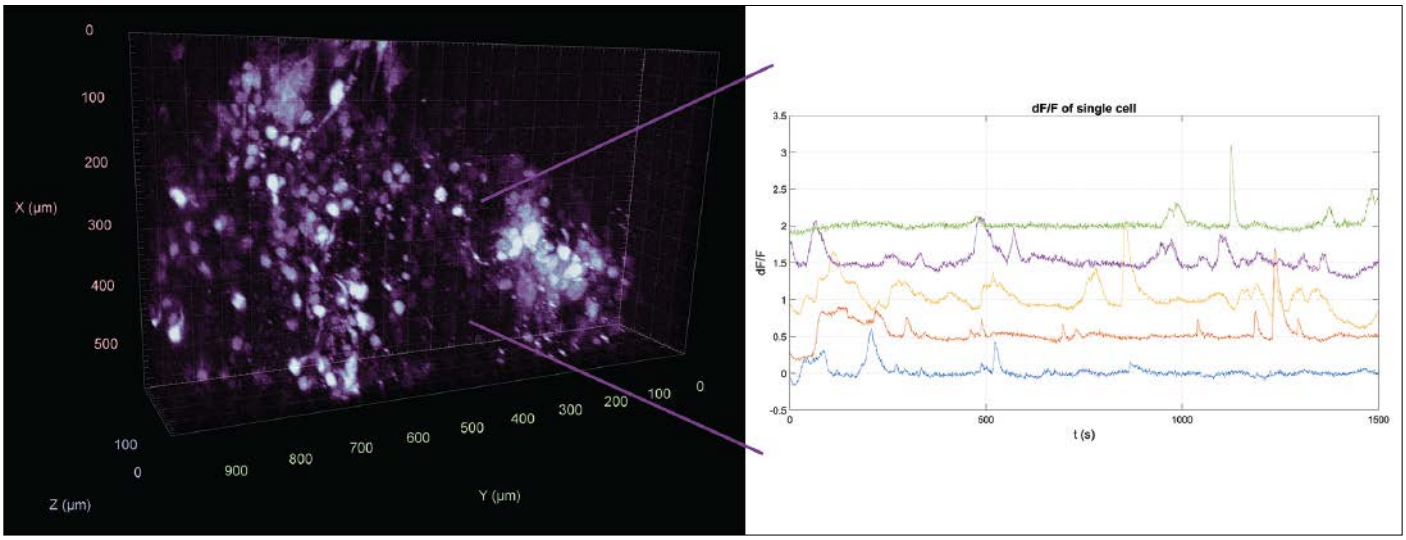


Fig. 3: GCaMP calcium imaging over a depth of 200 µm deep inside the brain of an axolotl. Imaged at 0.75 Hz.

ton absorption [15,16,17]. This technique has been used for imaging not only calcium but also voltage indicators, with speeds up to 1 kHz [18].

Fast Volumetric Imaging

Temporal focusing can be used to sculpt light to the shape of a single cell, and raster scan it across the focal plane [19]. To move the beam in the axial direction, researchers have different strategies.

The simplest way is to use a piezoelectric device, or piezo, either to move the sample stage or the objective lens. Figure 3 shows volumetric neuronal activity recorded 200 µm deep in the brain of an axolotl at 0.75 Hz thanks to such a device. Combining piezo scanning with sculpted light, researchers were able to achieve volumetric imaging over 0.5 mm × 0.5 mm × 0.5 mm at a single-cell resolution and a rate of 3–6 Hz [20].

Another approach is remote focusing. One can place a lightweight mirror in a conjugated sample plane and scan it much faster than a heavy objective lens [21].

Avoiding mechanical perturbations of the sample is particularly important for *in vivo* studies as moving the organisms can cause stress and bias the results. Similar to remote focusing, random access multi-photon microscopy steers the beam in all three dimensions without moving the sample or the objective. Thanks to acousto-optic deflectors or spatial light modulators one can also only probe the cells of interest without imaging "empty space" [22].

Miniature Multi-Photon Microscopy

To study biological dynamics in freely-moving organisms, researchers have developed miniature multi-photon microscopes that can be fitted on top of the region of interest of the living, moving animal. In neuroscience, this is typically the head. Femtosecond pulses are delivered through a hollow-core photonic crystal fiber. Scanning is implemented via a microelectromechanical systems (MEMS) scanner. The emitted light is collected via a multi-mode fiber and detected by external devices. Although in its infancy, this concept has been demonstrated for 2-photon imaging in freely behaving mice [23]. Using MEMS piezo devices for volumetric imaging is possible [24] and researchers have even pushed the technology to a 3-photon head-mounted microscope [25].

Conclusion

Visualizing dynamic biological processes in a 3D context is crucial for a variety of studies, from probing cellular interactions across a large scale to drug discovery and disease research. Multi-photon microscopy provides intrinsic optical sectioning and can deliver images of large volumes deep inside the living tissue.

Since its early conception in the 1990s, the microscopy community has been developing new tricks to improve the speed, field-of-view, and resolution with recent work demonstrating the possibility of miniature multi-photon microscopes. Exciting new developments are continuously being made and new biological discoveries await.

Acknowledgments

I thank Laura Loschek at the MPI for Biological Intelligence for providing and preparing Thy1-GFP mouse brain slices and Simone Horenkamp and Elly Tanaka at the Institute of Molecular Pathology (IMP) for providing resources for *in vivo* imaging of axolotls.

I also thank Stefanie Kiderlen and Danielle Paynter at the MPI for Biological Intelligence for proofreading this article. This work was supported by the Max Planck Society.

Contact
Claudio Polisseni
Max-Planck Institute for Biological Intelligence
Martinsried, Germany
claudio.polisseni@bi.mpg.de

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

Label-Free Microscopy for Studying Lipid Metabolism

A Glimpse into Living Cells to Uncover Hidden Dynamics of Biomolecules

Jong Min Lim[1] and Minhaeng Cho[2,3]

In recent years, significant advancements have been made in the field of microscopy, including the development of infrared photothermal microscopy (IPM). This technique leverages infrared wavelengths to examine biological specimens non-invasively and label-free, offering high spatial resolution beyond the diffraction limit of infrared wavelengths. IPM addresses the limitations of traditional IR microscopy by incorporating additional visible probes to enhance spatial resolution. The method has shown great promise in monitoring live brain cells and tracking protein-rich particles without external labels. Moreover, the two-color IPM (2C-IPM) technique has further advanced the field by enabling the simultaneous detection of photothermal responses from specimens using two discrete IR excitation beams, thus overcoming the limitations of conventional IPM. These innovations have opened new avenues for investigating living cells and biological processes, establishing IPM as a valuable tool for cytological and metabolic studies.

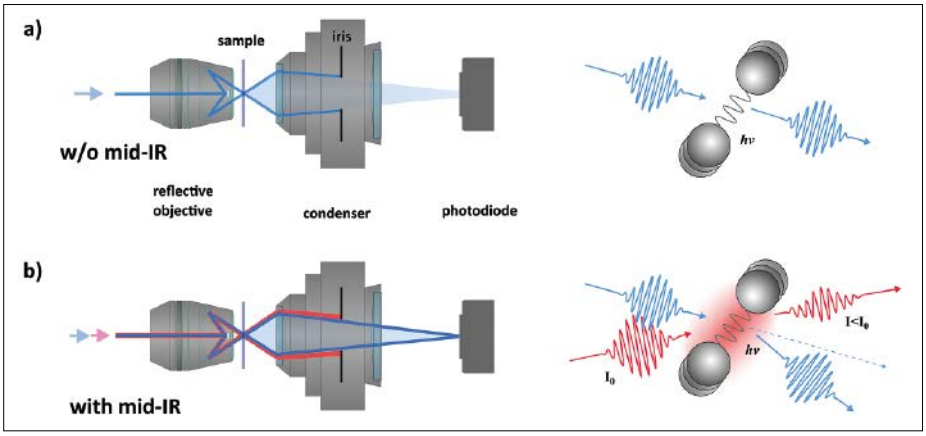


Fig. 1: Simplified optics scheme for infrared photothermal microscopy (IPM). a) In the absence of mid-infrared light, the visible probe beam passes through the sample slide but is largely blocked by the iris. b) When mid-infrared light is present, the sample absorbs the IR light, resulting in heat dissipation and the formation of a thermal gradient at the focal point. This thermal gradient, called the photothermal lens, causes the visible probe to deflect and reach the detector. The panels on the right depict the deflection of the visible probe beam due to the photothermal lens effect induced by infrared light absorption.

Infrared (IR) microscopy, utilizing wavelengths of a few micrometers, has long been recognized as a valuable tool for studying biological systems due to its chemical selectivity, non-destructive nature, and label-free approach [1]. However, it was previously believed that the technique could have been more effective for investigating small cellular spaces due to limitations in spatial and depth resolutions. Infrared photothermal microscopy (IPM) has since emerged as a revolutionary method that overcomes these limitations and introduces new possibilities for studying living cells [2-5].

IPM uses a pump-probe scheme that assigns distinct roles to incident lights for chemical selectivity and spatial information. This method achieves chemical contrast through vibrational excitation using frequency-tunable IR laser pulses. At the same time, the resulting photothermal effect—a refractive index change caused by local heating—is analyzed by detecting a deflected visible light beam. This versatile IR imaging method allows for noninvasive, nondestructive, and label-free examination of functional materials and biological specimens with a spatial resolution of less than a micrometer, providing a safe and reliable tool for live-cell imaging.

Recent work has demonstrated the biocompatibility of IPM by monitoring live brain cells over extended periods, successfully tracking protein-rich particles smaller than 1 µm in diameter without external labels [6]. Additionally, two-color IPM (2C-IPM) has been developed, integrating modulation-frequency multiplexing into the IPM signal, allowing for the simultaneous detection of photothermal responses to two distinct IR excitation beams. This article provides a comprehensive introduction to IPM, focusing on its methodology [7,8].

Mirage in the Microcosm

By studying cellular dynamics, such as changes in shape, microstructure, and the redistribution of biomolecules inside and outside cells over time, our understanding of cellular processes and their underlying mechanisms has deepened [9-11]. While various microscopic techniques using fluorescent labels have been employed to study these processes, there is a growing need for improved label-free observation of cellular processes throughout the cell's lifecycle. Label-free live-cell imaging, which relies on internal biomolecules for imaging contrast, is highly sought after due to the drawbacks of fluorescent labeling, such as phototoxicity and photobleaching [12-14]. Moreover, the complexity and dynamic nature of cytoplasmic activities make it challenging to study genuine cellular behaviors using external labels.

A schematic presentation of the IPM technique is shown in Figure 1. After incorporating collimation optics, IR and visible beams are collinearly combined and directed to a reflective objective lens to ensure the overlap of the two laser beams and avoid chromatic aberration. The mid-IR laser generates photothermal contrast at the focal spot when it interacts with target vibrational molecules, detected by a continuous wave visible probe laser. An iris is used to block non-deflected beams. In contrast, the deflected visible probe beam, affected by refractive index changes, is collected by a variable aperture condenser and directed to the photodiode detector. The photothermal contrast is modulated at the mid-IR pulse repetition rate and amplified by a lock-in amplifier [6].

The fundamental principle of IPM is closely related to the mirage effect caused by atmospheric thermal gradients, which dis-

tort vision. We have essentially harnessed this effect on lab benches by generating thermal gradients within microorganisms, where a similar mirage effect within living cells is induced by IR excitation.

One Sweep, Multiple Signals: Biomolecule Detection in Unison

Recent studies on IPM have employed a quantum cascade laser (QCL) as the IR source for vibrational excitation. While using QCL allows for the selective detection of chemical species, the narrow spectral width (<1 cm^{-1}) of the QCL pulse limits the molecular information that can be obtained from a specimen within a given observation time. Consequently, sampling scanning must be repeated on the same region to acquire comprehensive molecular information from multiple species, including external additives like drugs. However, this approach is time-consuming and unsuitable for live cells, as cell movement can create artificial correlations in sequential IP images [7].

A new IPM technique is needed to capture multiple chemical signatures from specific regions simultaneously, making it highly valuable for cytological and metabolic studies. The 2C-IPM meets this need by enabling the simultaneous detection of photothermal responses to two discrete IR excitation beams (Fig. 2), thus overcoming the limitations of conventional IPM. This technique utilizes classical frequency-division multiplexing, dividing a broader bandwidth channel's frequency spectrum into narrower bandwidth communication channels [7,8]. Here, photothermal responses from the photodiode detector are transferred simultaneously but at different carrier frequencies (IR modulations). Two QCLs provide the IR excitation pulses, while a continuous-wave probe beam generates and measures two independent pump-probe imaging contrasts. These three beams are combined in the same direction using a CaF_2 beamsplitter and a dichroic mirror. At the focal point, each IR excitation pulse train excites a specific molecular vibration, creating a time-dependent refractive index gradient that alters the visible beam's physical properties, such as scattering and divergence, matching the repetition rate of each IR excitation pulse.

2C-IPM has been used to investigate the biosynthesis of neutral lipids inside lipid droplets [8]. It was found that lipid droplets (LDs) are essential organelles in eukaryotic cells, featuring a neutral lipid core surrounded by a phospholipid monolayer. Once thought to be mere storage sites, LDs are now recognized for their crucial roles in processes like vitamin metabolism, lipotoxicity regulation, and cell signaling. However, studying LDs is challenging due to their dynamic nature, highlighting the need for innovative techniques. 2C-IPM enables the simultaneous detection of lipid contents in LDs with excellent spatial and spectral resolutions. For the first time, it was applied to study lipid dynamics within LDs of human cancer cells (U2OS and Huh-7 cells), specifically focusing on neutral lipids. This technique distinguishes intracellular LDs from other organelles and provides detailed information about their lipid contents. More specifically, using deuterated fatty acids. 2C-IPM could differentiate between newly biosynthesized and native fatty acids in real-time. Quantitative evaluation of lipid composition shifts within individual LDs over exposure time to excess fatty acids was achieved by analyzing IP responses corresponding to CH_2 stretch vibrations of native fatty acids and CD_2 stretching vibrations of deuterated palmitic acids, which provide invaluable information on the rate and mechanism of neutral lipid biosynthesis in living cells. This breakthrough in lipid research, enabled by IPM, opens up new avenues for understanding and potentially diagnosing and treating cancer.

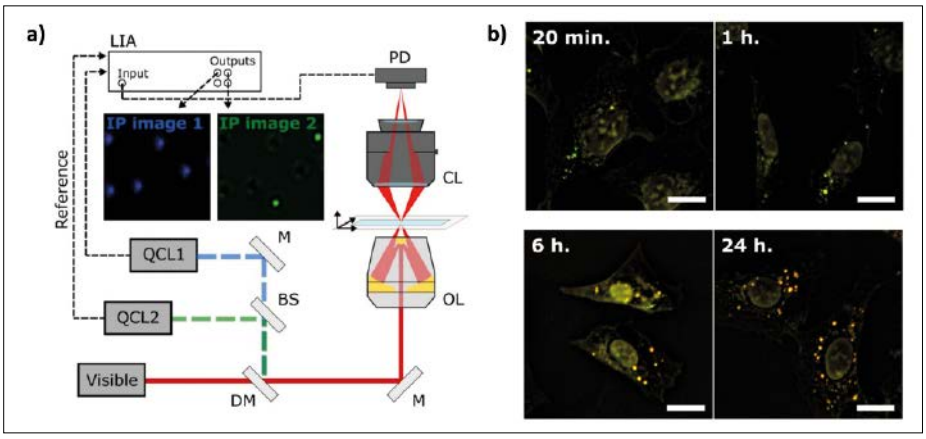


Fig. 2: Two-color IPM microscope. a) Schematic of the two-color IR photothermal microscope setup. b) Two-color (excitation: 2193 cm^{-1} and 2855 cm^{-1}) IPM images of fixed U2OS cells at various time points following PA-d_{31} administration. The PA-d_{31} treatment times are indicated in the upper left corner of each image. IR excitations at 2855 cm^{-1} (45 kHz) and 2193 cm^{-1} (50 kHz) are represented by false green and red colors, respectively. Adapted with permission from ref. 7 and 8. Copyright 2023, 2024 RSC publishing.

Summary

Infrared (IR) microscopy is a powerful tool for studying biological systems due to its chemical selectivity and non-destructive and label-free nature. However, traditional IR microscopy is limited by low spatial and depth resolution, reducing its effectiveness in examining small cellular structures. This article introduces the fundamental concept of IPM and highlights recent advancements in multiplexing modalities for investigating various biomolecules in live cells. IPM overcomes those limitations using a pump-probe scheme that combines frequency-tunable IR lasers with visible light detection, achieving sub-micrometer spatial resolution. This technique enables non-invasive, label-free imaging of live cells, avoiding issues like phototoxicity and photobleaching associated with fluorescent labels. Recent innovations, such as two-color IPM, allow for the simultaneous detection of multiple chemical signals, significantly enhancing the study of dynamic cellular processes like lipid metabolism in lipid droplets. This breakthrough offers detailed insights into cellular functions and disease mechanisms, establishing IPM as a crucial tool in biological and medical research.

Affiliation
[1] Department of Chemistry, Kyungpook National University, Daegu, Korea
[2] Center for Molecular Spectroscopy and Dynamics, Institute for Basic Science, Seoul, Korea
[3] Department of Chemistry, Korea University, Seoul, Korea

Contact
Minhaeng Cho, Professor
Center for Molecular Spectroscopy and Dynamics
Institute for Basic Science
Seoul, Korea

Department of Chemistry
Korea University
Seoul, Korea

mcho@korea.ac.kr
ORCID: 0000-0003-1618-1056

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

Enhancing Efficiency in AI-based Bioimage Analysis

Faster and Greener

Yu Zhou[1,2] and Jianxu Chen[1]

Artificial intelligence (AI) is widely used in analyzing biological images today. However, the efficiency of AI models, especially concerning energy consumption and speed, is a major concern due to increasing model size and complexity, as well as the growing demand for analyses in modern biomedical studies. Just as large images can be compressed for easier storage and sharing, AI models can be compressed for more efficient use and deployment. In this work, we introduce EfficientBioAI, a user-friendly toolbox (open source) designed to compress AI models for bioimage analysis. This allows them to run with much lower energy and faster processing times on CPUs and GPUs without losing accuracy. Our toolbox will make resource-limited bioimage AI applications more feasible, speed up large-scale biological studies in an eco-friendly way, and encourage further research into making bioimage AI more efficient.

This image was generated by DALL-E in December 2023. Prompt: make a simple illustration of the concept "energy efficient deep learning".

Introduction

Today, biologists can use various AI-based tools like DeepImageJ [1], ZeroCostDL4Mic [2], Cellpose [3], and mmv_im2im [4] for analyzing microscopy images. These tools have achieved top-notch performance in tasks like image segmentation, noise reduction, and resolution enhancement. However, there is a lack of awareness regarding how efficient these AI tools are. The "efficiency" here refers to two main aspects: latency and energy consumption.

Latency is the time it takes for AI models to process input data (like microscope images) and produce the final output (like segmentation masks). As AI models have become larger and more complex, especially with the emergence of Large Language Models (LLMs), the time it takes to get results has increased, particularly for huge images or when using devices like CPUs or small embedded systems (e.g., Smart Microscopes on an embedded system [5]). This increased complexity also means higher energy consumption, with the energy needed for a single analysis growing exponentially over the last decade [6].

One way to make AI models more efficient is through model compression. There are several techniques to achieve this, such as quantization and pruning. Quantization involves reducing the precision of the model's weights from higher precision (like floating point 32) to lower precision (like integer 8). The main benefit of using lower precision is that it speeds up computations and reduces memory usage. Pruning, on the other hand, involves removing "unimportant" parts of the model based on specific criteria. This makes the model smaller, requiring less computation, which speeds up processing and improves energy efficiency. While these techniques have been successfully used in fields like computer vision, they are still not widely adopted in bioimage AI.

Methodology

In this study, we introduce EfficientBioAI as a toolbox, which integrates advanced compression techniques to enhance the efficiency of AI-based bioimage analysis workflows. It is ready to use and available on GitHub and is designed to easily integrate with existing PyTorch codebases with minimal code changes, as long as the code does not include dynamic control flow. Additionally, this toolbox is highly customizable, allowing users to adjust various compression configurations such as CPU/GPU acceleration and compression levels.

The workflow of EfficientBioAI is illustrated in Figure 1 and involves two main

stages: compression and inference. In the compression stage, the tool takes a neural network the user has already trained. First, it uses pruning to reduce the model's size, which may require fine-tuning with a small set of labeled data to keep the model accurate. This pruning step is optional and can be skipped by setting the compression ratio to 1. After pruning, the model goes through quantization, where the precision of the model weights is reduced from fp32 to int8. During this step, the tool automatically calibrates to prevent loss of accuracy, using only sample input images (which do not need to be labeled) to capture the necessary quantization information. In the inference stage, users can select different backends (CPU/GPU) to perform inference. Notably, the open-source toolbox fully supports mmv_im2im and Cellpose, and it also supports custom PyTorch models.

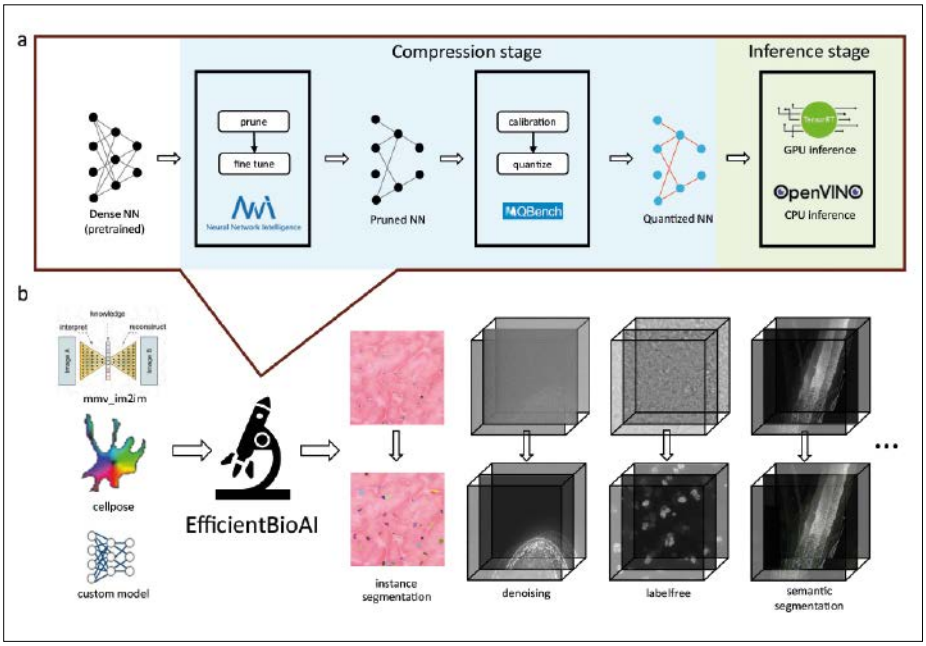


Fig. 1:EfficientBioAI aims to compress the model and accelerate the bioimage analysis tasks. a. Starting from a pre-trained neural network (NN) in PyTorch, the whole pipeline consists of two stages: compression (pruning, quantization) and inference. In the compression stage, various off-the-shelf compression strategies can be chained up to build the compression pipeline. Sample data can be utilized for calibration and fine-tuning to avoid performance drop. In the inference stage, the compressed models can run on different hardware with specific inference engines. b. The tool is compatible with various models, and the compression performance is justified on multiple bioimage analysis tasks.

Experiments

The effectiveness of our toolbox is validated by various experiments, characterized by speed improvements and reduced energy consumption across different hardware platforms, with minimal or no accuracy drop in various bioimaging analysis tasks. The results show that, on average, compression techniques can reduce latency by approximately 13.4% to 80.9% and save 12.5% to 80.6% in energy consumption. We will elaborate on two specific experiments and the resulting data in the following sections.

2D Resolution Enhancement of F-actin Fluorescence Microscopy Images

Resolution enhancement aims to convert low-resolution images to their high-resolution counterparts. This task is particularly challenging for compression since high-frequency information may be lost during the quantization process. In this study, we selected the WGAN model [7], which improves learning stability by introducing the Wasserstein distance loss function compared to the vanilla GAN model. We used F-actin fluorescence microscopy images from ZeroCostDL4Mic (originally from [8]) for training, comprising 250 pairs of 2D low-resolution (502 x 502 pixels) and high-resolution (1004 x 1004 pixels) images. We applied the quantization technique, and the results are shown in Figure 2. The predictions under int8 precision experienced nearly no accuracy drop compared to those predicted by the standard model (fp32 precision). Additionally, energy usage decreased by 67.7% on the GPU.

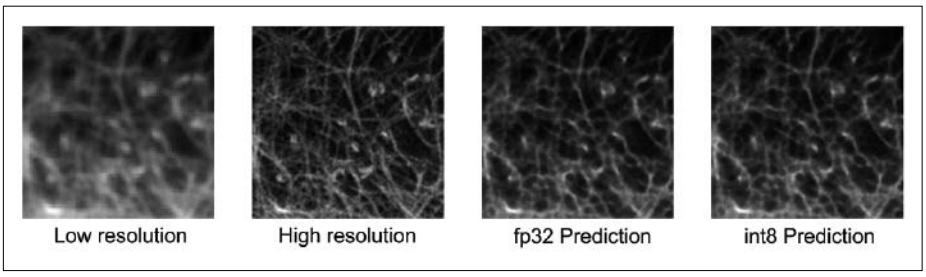


Fig. 2: Resolution Enhancement Experiment Results. Zoomed-in images (from left to right): original image (low quality), ground truth (high quality), inference output with float32 precision, inference output with integer 8 precision.

2D Nuclei Instance Segmentation with Cellpose

Cardiomyocyte size determination is essential for evaluating hypertrophic growth in cardiovascular research. Segmenting the nuclei within cardiomyocytes ensures accurate analysis. Therefore, we performed 2D nuclei

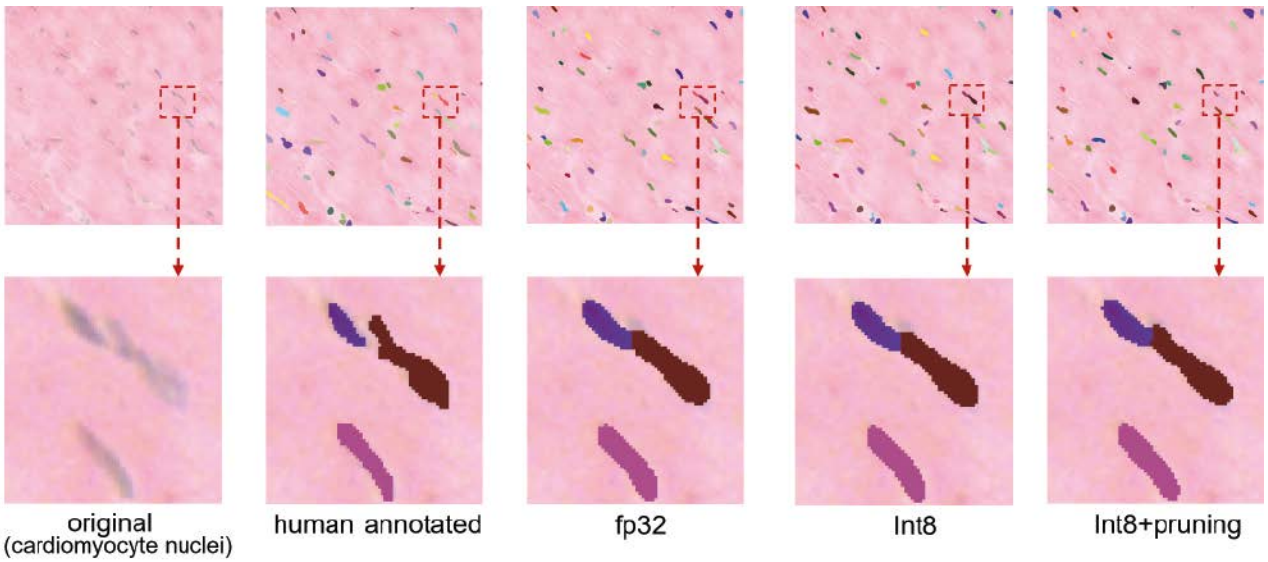


Fig. 3: Instance Segmentation Experiment Results. There is not much difference between the inference result after compression and before compression, implying the effectiveness of our methods. ***Top row:*** *original image, human-annotated labels (cardiomyocyte nuclei masks), predicted labels with float32 precision, predicted labels with integer8 precision, predicted labels with integer8 precision, and pruning.* ***Bottom row:*** *The corresponding zoomed-in images.*

instance segmentation on H&E-stained mice cardiomyocyte images. For this task, we used an in-house trained Cellpose model. Cellpose is one of the most widely used bioimage segmentation tools, making it an ideal candidate to demonstrate the compatibility and efficiency improvements of our EfficientBioAI toolbox with standard third-party bioimaging AI tools. Both pruning and quantization techniques were utilized in this task. The results are shown in Figure 3.

We tested the performance on four large images (approximately 11,578 × 11,660 pixels). On a CPU, 48.7% of energy savings were achieved with quantization alone, while 62.1% of energy savings were realized with both pruning and quantization. Meanwhile, the accuracy, measured as AP50, was maintained.

Discussion

In addition to effectiveness, we assessed our toolbox regarding robustness and extensibility. The robustness of our method was evaluated by executing a 3D semantic segmentation task using different models. Compression consistently produced stable results across all models, with significant reductions in latency and minimal decreases in accuracy. Furthermore, we enhanced our toolbox's extensibility by integrating advanced zero-shot quantization methods, such as ZeroQ [9]. These methods achieved results comparable to traditional quantization techniques without the need for sample data.

However, the toolbox has some limitations. For example, it lacks support for some common processors, such as Apple M-series chips, which may restrict its application scenarios. Additionally, it can only be utilized during inference, and the training step cannot be accelerated.

Summary

The rapid development of neural networks facilitates the study of bioimage analysis. However, the growing network complexity poses challenges for speed and energy consumption. In this study, we proposed a plug-and-play compression toolbox (open source) called EfficientBioAI to address these issues. The toolbox integrates various compression techniques, such as quantization and pruning, which can significantly accelerate bioimage analysis tasks and save energy while preserving accuracy. Despite some drawbacks, the tool holds great potential in the bioimaging AI field, making it accessible to non-expert users who can benefit from model compression advantages. Furthermore, this is the first attempt to focus on the efficiency of bioimaging neural networks, achieving tangible improvements in latency and energy footprint on real hardware beyond simulations. We hope the toolbox can foster advancements in the field by promoting efficient and sustainable bioimage analysis.

Affiliation
[1] Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Germany
[2] Ruhr University Bochum, Germany

Contact
Dr. Jianxu Chen
Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V.
Dortmund, Germany
jianxu.chen@isas.de
ORCID: 0000-0002-8500-1357

[1] References: https://bit.ly/IM-Chen2

Surface Details of a Common Ice-Nucleating Mineral

Using Non-Contact Atomic Force Microscopy in Ultra-High Vacuum

Giada Franceschi[1] and Ulrike Diebold[1]

Microcline feldspar ($KAlSi_3O_8$) is a common mineral that plays an important role in the Earth's ecological balance. It participates in the carbon, potassium, and water cycles, contributing to CO_2 sequestration, soil formation, and atmospheric ice nucleation. To understand the fundamentals of these processes, it is essential to determine its atomic surface structure and its interaction with the ubiquitous water molecules.

© Giada Franceschi

The Surface Physics Group at the Vienna University of Technology in Vienna, Austria, has succeeded in determining the intrinsic atomic-scale details of the lowest-energy surface of microcline and its interaction with water by combining ultrahigh vacuum studies using non-contact atomic force microscopy and X-ray photoelectron spectroscopy with density functional theory calculations. At room temperature, an ordered array of hydroxyls bonded to silicon or aluminum readily forms on the cleaved surface. The distinct proton affinities of these hydroxyls influence the arrangement and orientation of the first water molecules to attach to the surface, with potential implications for subsequent water condensation.

Introduction

The fundamental study of mineral surfaces is as exciting as it is challenging. Minerals make up most of the rocks on which we stand. They are active CO_2 sponges; through weathering processes, they transform into clays and form soils, providing essential nutrients for plants. They also exist as airborne dust particles in the atmosphere, influencing ice nucleation (IN) and cloud formation, profoundly affecting global weather patterns. By determining how mineral surfaces are structured at the atomic level, we can take a step toward truly understanding these important natural processes. However, there are challenges. Minerals often contain impurities and do not have the "perfect" shape of commercial crystals, creating obstacles to atomically resolved studies. In addition, they are often highly insulating and can expose surface charges that prevent atomic resolution by standard atomic force microscopy.

One system that exemplifies these challenges is microcline feldspar ($KAlSi_3O_8$), a common mineral with exceptionally high activity for atmospheric IN [1]. Theoretical studies have attempted to correlate surface chemistry and IN activity by investigating the atomic-scale interaction of "perfect" microcline surfaces with water. Density functional theory (DFT) calculations have shown that ice-like structures can grow on top of a non-ice, mediating water layer directly adsorbed on the lowest energy (001) surface of the microcline [2]. However, molecular dynamics studies have failed to reproduce spontaneous IN on the low-index facets of microcline. On the experimental front, studies have predominantly relied on observations of macroscopic ice crystals, focusing on the potential role of macroscopic defects on microcline rather than its surface chemistry. Direct atomic-scale studies of pristine microcline and its interaction with water have been lacking. Such studies may shed light on the ability of microcline to support hydrogen-bonded networks, an important factor for IN on silicate minerals.

Main Findings

In our work [3,4], we have successfully advanced the characterization of a heterogeneous, insulating, charged mineral such as microcline feldspar by using non-contact atomic force microscopy (nc-AFM) in ultrahigh vacuum (UHV) together with X-ray photoelectron spectroscopy (XPS) and DFT calculations. Cleaving a sample at room temperature (Fig. 1a, b) exposes low-energy surfaces that are atomically clean and flat (Fig. 1c, d). Single atoms can be imaged (Fig. 1d, Fig. 2) after irradiating the surface during XPS, which effectively removes the surface charges created during cleavage. This allows the AFM sensor to get close

enough to the surface to sense the short-range forces responsible for atomic contrast. Surprisingly, the cleaved surface appears hydroxylated even when cleaved at very low pressures of 10^{-11} mbar. It turns out that fluid inclusions within the mineral that rupture during cleavage release enough water for full hydroxylation. The hydroxyls bond to the Si and Al atoms of the cleaved surface, forming long-range ordered aluminol and silanol groups (Fig. 2a, b). Such a hydroxylated surface is expected to be present in the environment [3].

Determining the details of the cleaved surface is the first step in understanding how water binds at low temperatures. To this end, we introduced controlled amounts of water vapor into our UHV system while maintaining the sample at 100 K. Water molecules bind in a regular pattern dictated by the details of the hydroxylated surface (Fig. 2c, d): each molecule accepts a hydrogen bond from a silanol group and donates one to the adjacent aluminol group [3]. We hypothesize that the ordered anchoring of water molecules observed in UHV may provide an opportunity to create H-bonded water layers that may determine the unique IN activities of microcline in the atmosphere.

Methodology

The experiments were carried out in a UHV setup with base pressure $<1 \times 10^{-10}$ mbar. A natural specimen of microcline feldspar from Russia was characterized *ex situ* before being mounted (glued to Omicron-style stainless steel sample plates with UHV-compatible epoxy glue) and inserted into UHV. It was cleaved in UHV at room temperature by applying a tangential force with a wobble stick to a metal stud glued to the top of the sample. This removed the portion of the sample initially covered by the stud. Notably, the water partial pressure in the UHV chamber increased to $\approx 2 \times 10^{-7}$ mbar for a few seconds after each cleavage, resulting in complete hydroxylation of the cleaved surface.

Like other insulators [5], the as-cleaved samples exhibit surface charges that make AFM measurements difficult. The charge could be effectively removed by exposing the cleaved sample to X-rays from our XPS setup for one minute. This treatment did not introduce any contamination. Residual fields were compensated by applying a bias voltage between the tip and the sample, which minimized the electric field between the tip and the sample, as judged by local contact potential difference (LCPD) measurements using the Kelvin parabola method. In other words, the bias voltage was set to the maximum of the LCPD parabola. If the surface was not irradiated with X-rays prior to the AFM measurements, it was not possible to compensate the potential with the maximum ±10 V voltage range provided by the microscope controller, resulting in large absolute values of frequency shifts in the constant-height AFM images that dominated the atomic contrast.

The AFM measurements were performed at 4.7 K using a commercial Omicron qPlus head and a differential cryogenic amplifier. The frequency-modulated non-contact AFM mode was used. The tuning fork based AFM sensors (k = 2000–3500 N/m, $f_0 \approx 45$ kHz, Q ≈ 50000) had a separate contact for tunneling current attached to electrochemically etched W tips. Before each measurement, the tips were prepared on a clean Cu(110) single crystal by repeated indentation and voltage pulses. The coarse approach was performed with a set point of -0.8 Hz. The controller was switched off and the tip was gradually approached in constant height mode until a contrast was visible while scanning in x and y.

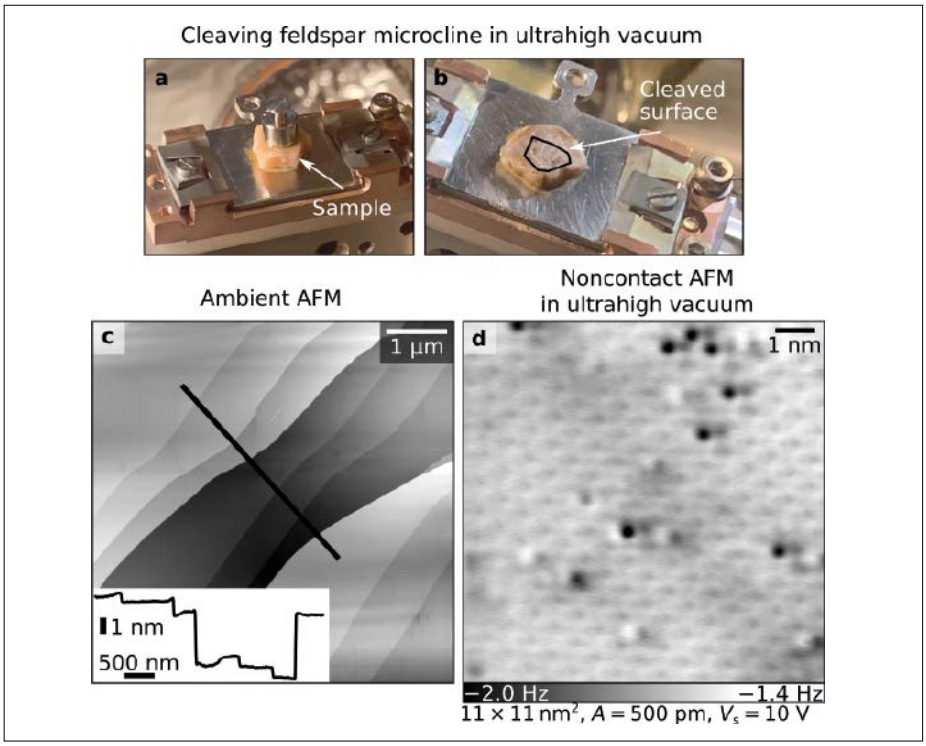


Fig. 1: (a, b) Cleavage of microcline feldspar in ultrahigh vacuum (UHV). A metal stud glued to the sample (a) is removed by applying a tangential force with a wobble stick (b). The resulting surface is atomically flat and clean and can be imaged with atomic resolution. (c, d) Surface morphology of cleaved microcline feldspar in ambient and non-contact UHV AFM. Point defects are visible on an otherwise atomically clean and periodic lattice. Adapted from ref. [3].

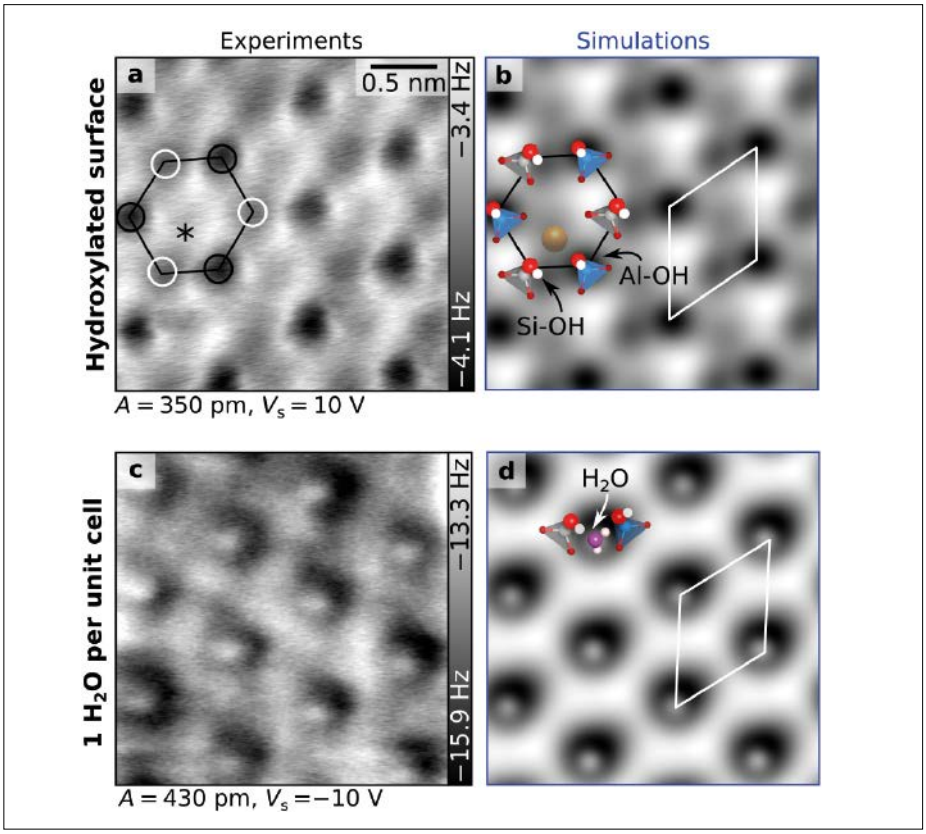


Fig. 2: Experimental non-contact AFM images and corresponding theoretical simulations of hydroxylated (a, b) and water-covered (c, d) microcline feldspar. Hydroxyls sit on Al and Si atoms that alternate on the surface, forming a regular honeycomb pattern. Water molecules sit between the Al- and Si-bonded hydroxyl groups. Adapted from ref. [3].

Images were acquired in constant height mode.

To study water adsorption at low temperatures, water vapor was dosed from a leak valve while keeping the sample holder on the manipulator of the preparation chamber at 100 K with liquid nitrogen.

DFT calculations were performed with the Vienna Ab-initio Simulation Package (VASP) [6] using the r^{2}SCAN-D3 metaGGA exchange-correlation functional. AFM images were simulated with the Probe Particle Model [7].

Conclusion

This study delves into the surface atomic details of microcline feldspar, $KAlSi_3O_8$, a mineral that plays a crucial role in atmospheric ice nucleation. By cleaving (001)-oriented microcline single crystals in UHV and using state-of-the-art ncAFM in combination with XPS and DFT, the structure of its hydroxylated surface was determined: the Si and Al atoms of the cleaved surface provide binding sites for hydroxyls, resulting in the formation of long-range ordered silanol and aluminol groups. The different acidity of these hydroxyl groups dictates a specific adsorption configuration for water molecules, potentially influencing the subsequent condensation of water. The results add another piece to the complex puzzle of atmospheric ice nucleation on feldspars.

Affiliation
[1] Institute of Applied Physics, TU Wien, Vienna, Austria

Contact
Dr. Giada Franceschi
Institute of Applied Physics
TU Wien
Vienna, Austria
ORCID: 0000-0003-3525-5399
franceschi@iap.tuwien.ac.at

[1] References: https://bit.ly/IM-Franceschi2

A New Era in Molecular Imaging

Liquid-Electron Microscopy

Deborah Kelly[1]

Imagine watching human pathogens live in solution as they would operate in the body. While still years away from being realized, this scientific goal is the biological capstone of the new liquid-Electron Microscopy (EM) technique. Researchers have recently advanced the technology to produce novel observations of hard materials in action, and applications for biological polymers are gaining momentum. Recent highlights for liquid-EM applications are demonstrated here that are accelerating real-time observations of soft polymers such as virus systems. New devices are also highlighted as scientists and engineers join forces to invent next-generation solutions to assess macromolecules under biomimetic conditions.

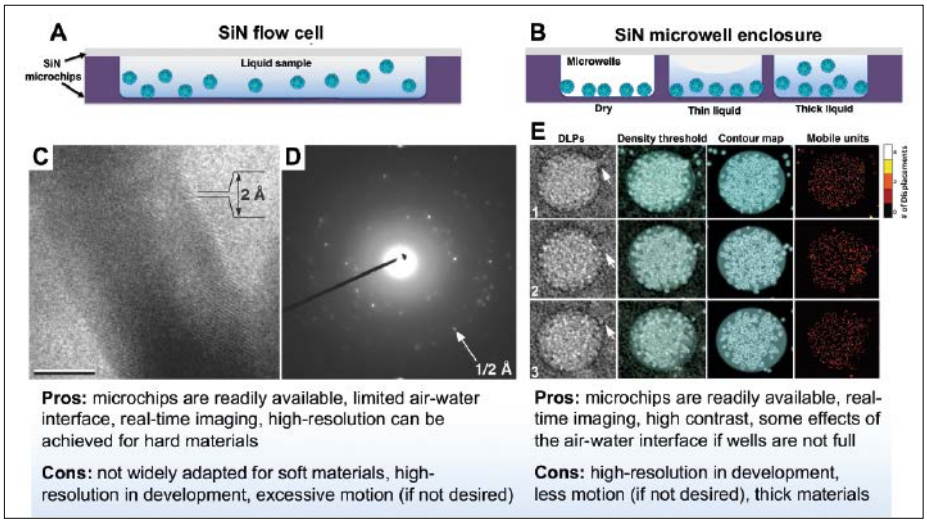


Fig. 1: Two varieties of microchip-based liquid enclosures and representative examples of liquid-EM applications. (A, B) Schematic drawing of SiN-based flow cell and microwell assemblies used to examine hard and soft polymer materials. (C, D) An up-close view of a pegylated gold nanorod with a defined lattice spacing of 2 Å is also displayed in an electron diffraction pattern. Images used with permission from the Royal Society of Chemistry [23]. (E) Representative rotavirus double-layered particles (DLPs, 1 – 3) displaying emerging strings of RNA (white arrows). Contrast was inverted from the original image data for ease of viewing the particle details. Virus particles were analyzed using a density threshold filter and contour mapping routine. Changes in pixel displacement values were assessed over a 10 second interval and further quantified by heat maps using a scale of 0 – 4. Images were used with permission from the Royal Society of Chemistry [21]. The pros and cons of each technique are also listed.

Introduction

We are entering a new era in analyzing macromolecules at the atomic scale. Just as the James Webb Space Telescope delivers captivating discoveries of far-off galaxies, liquid-electron microscopy (EM) reveals dynamic details of the never-before-seen nanoworld. Traditional knowledge of minute entities relies heavily on stitching together image sequences, generally devoid of real-time direct observations. While segmented interpolations may provide important high-resolution insights into macromolecular architectures, mechanistic data of their dynamic properties are often incomplete.

In situ electron microscopy is a fast-growing area of science, taking the imaging field by storm. Recent advances in high-frame-rate cameras, along with microfluidic specimen holders, permit researchers to study the intricate aspects of materials in motion. The technique has been a game-changer for studying molecular nuances in the energy section, battery research, mineralization processes, and other chemical reactions [1–6]. The number of applications has soared in the last decade since the inception of the technique (circa 1970s), with many recent results reported for soft polymers [7-21]. Although the new launch of Liquid-EM has been spearheaded by materials researchers, great opportunities exist to expand knowledge of dynamic biological entities.

Here, we recap a few success stories using liquid-EM to study bio-related assemblies at the nanoscale. The method's current limitation is achieving high-resolution structures, as in the case of cryo-EM. However, a key benefit of the liquid-EM technique is the direct visualization of macromolecules in a fluid, free-form environment. Equally important, exciting new devices are in the works to visualize macromolecules in a temperature-controlled manner (i.e., viewing biological entities at body temperature within the TEM). Notably, as innovations that progress the field forward are often faced with reluctance and challenges along the way, research in this scientific discipline is moving full steam ahead.

Experimental Methods

A range of samples were examined under different electron flux conditions ranging from ≤ 10 electrons/A^2) and up to 50 electrons/A^2/sec using variable time frames and instruments. Standard TEMs used were equipped with a FEI CCD camera (2k x 2k array) operating at 120 KV. Additional experiments were performed using higher-powered TEMs equipped with field-emission guns operating at 200 kV or 300 kV and with integrated direct electron detectors or CCD arrays. Pixel sizes varied at the specimen level depending upon the magnification range used for the image recordings and specimen thickness estimates were adjusted for the Z-height. Materials tested include silicon nitride (SiN) microchips, graphitized carbon films placed over gold grids, and graphene layers placed over top of amorphous holey carbon films fixed onto copper or gold grids. Samples were incubated using the different enclosure systems for a specified incubation time before conducting imaging experiments as indicated in prior work [e.g., 21-23].

Results and Discussion

Silicon Nitride Microchip Systems

Several configurations and materials have been explored to produce specimen enclosures

suitable for biological applications. Among these are SiN flow cells that require specialized holders to encase the liquid samples between two thinned-down microchips having an imaging window of approximately 50 nm thickness or less. The hermetically sealed system protects the liquid sample from the high vacuum inside the EM column (Fig. 1A). In this configuration, liquid flows through the microfluidic chamber using an external pumping system. Large-range movements can be detected in the sample during real-time recordings. An alternative approach involves the use of two SiN microchips with one chip being engineered with 10-micron-sized microwells rather than one large flow chamber (Fig. 1B). The advantage of the smaller microwells is that there is a more limited range of motion among the sample. However, there may be more variability in the liquid thicknesses contained in each microwell. [21,22].

One example of the flow cell configuration (Fig. 1C, D) includes dynamic recordings of pegylated gold nanorods in solution [23]. These therapeutic nanorods were allowed to free flow through the microfluidic solution and have been previously described and characterized [23]. Electron diffraction patterns show high-resolution features of gold nanorods in solution (Fig. 1D). Benefits of this system include the fact that the chips and flow cell device are commercially available, and there is little to no air-water interface during real-time recordings. This interface is known to be detrimental to fragile biological specimens. Minimizing its effects in specimen preparation is highly desired and an active area of research in the EM community.

A corresponding prominent example of the microwell configuration includes previous studies of simian rotavirus (SA-11 strain) double-layered particles (DLPs) (Fig. 1B, E). Real-time image series were recorded in 10-second bursts, and representative DLPs were quantified in terms of mobile units and individual pixel value variances during the imaging time course. In Figure 1E, the contrast values of the DLPs were inverted to better visualize strings of RNA emanating from the assemblies. Density threshold values at the 3-σ-cutoff were evaluated, and contour maps were determined as described in the original study [21]. Mobile units were quantified using heat maps based on the number of pixel displacements. This collective information revealed motion in the particles over the 10-second recording that was attributed to several experimental and fluid-based factors.

The benefits of the system are that the materials and specimen holders are readily available, and for thick liquid samples, there is little to no air-water interface. The original particle images also have strong contrast features that may be similar to phase-plate

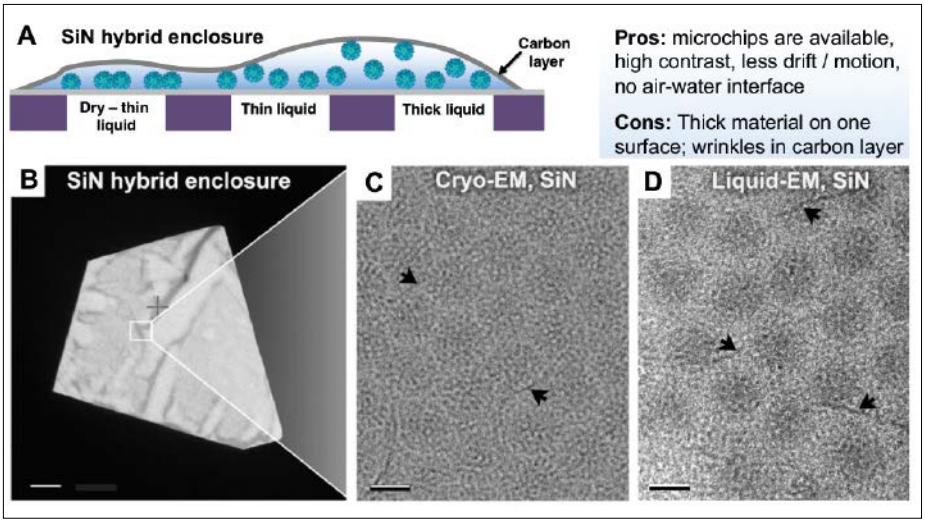


Fig. 2: Representative data showing virus assemblies contained in SiN hybrid enclosures. (A) Drawing of a SiN-carbon-based hybrid enclosure used to contain biological specimens. The pros and cons of the technique are listed. (B, C) Images of frozen-hydrated rotavirus DLPs at low magnification and at a higher magnification. Scale bar is approximately 5 microns in (B). (D) Parallel experiments were performed using liquid-EM for comparison to the cryo-EM images. Black arrows point to wrinkles in the carbon film. The scale bar is 50 nm in C, D. Images were used with permission and adapted from prior work [24].

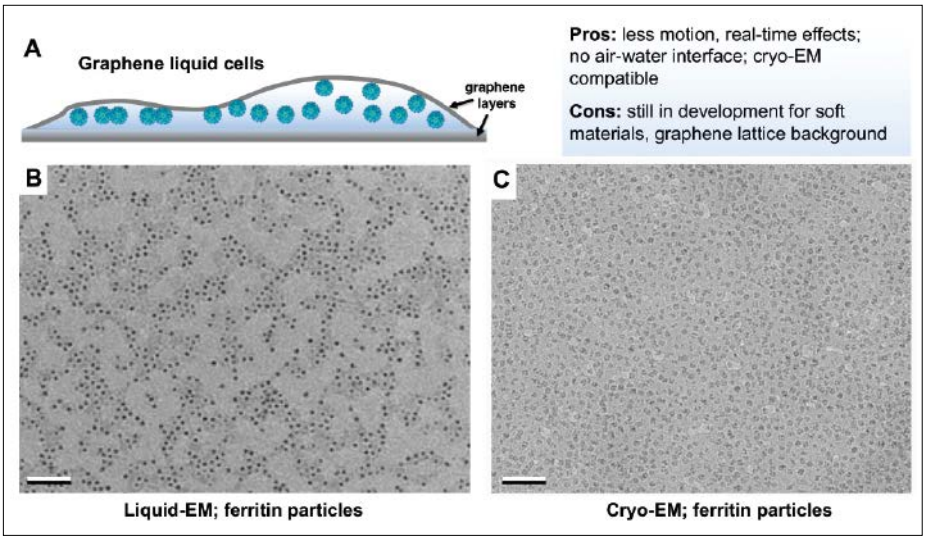


Fig. 3: Representative data for ferritin particles contained in GLC enclosures. (A) Schematic representation of a GLC enclosure containing biological particles. Pros and cons of the GLC method are indicated. (B) Ferritin particles shown in a graphene enclosure prepared using the Naiad-1 system. Scale bar is 50 nm. Image used with permission from VitroTEM (2024). (C) Cryo-EM image of ferritin particles flash-frozen in a GLC prepared using the Naiad-1 system. Scale bar is 50 nm. Image used with permission (www.nanoscience.com/products/naiad-1-automated-graphene-liquid-cells).

effects. The limitations are that the two-microchip enclosures are thicker than desired for atomic-level imaging of soft materials. The samples may have more motion than thinner substrate enclosures, such as carbon film or graphene layers.

Silicon Nitride Hybrid Enclosures

A more recent enclosure system encompasses a hybrid configuration of one SiN microchip and a thin (approximately 2 nm) carbon layer applied over the top of the liquid sample (Fig. 2A). Optimal sample volumes are about 500 nL, and the carbon film forms a snug-fitting blanket over the top of the sample while enclosing the specimen within the EM vacuum system. The "sandwich" enclosure may be assembled and sealed with a standard single-tilt holder at room temperature or clipped using standard grid clipping tools employed for cryo-EM grid preparation. The sealed/clipped assemblies

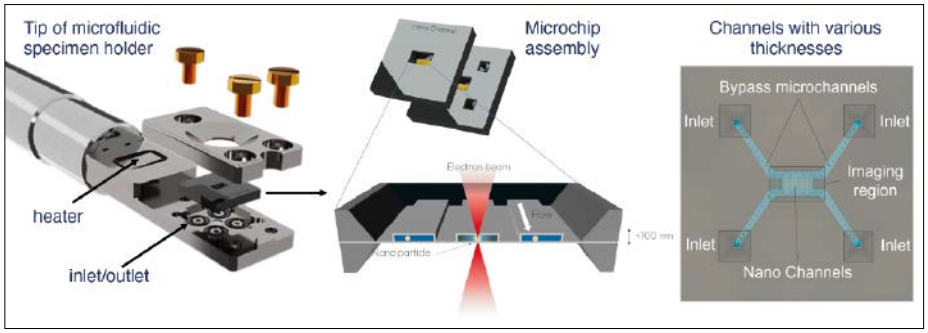


Fig. 4: The new microfluidic heating system in development at Insight Chips. The microfluidic system uses pre-sealed assemblies, and samples are injected through inlet lines for imaging experiments in the EM. The microchip assembly is clamped into the specimen holder using a metal lid and 3 brass screws. Each engineered microchannel allows for the sample solution to flow into subsequent nanochannels for mixing experiments. Multiple nanochannels are present within one assembly and they can accommodate similar volumes of sample as other commercial specimen holders. Full 3D data sets of thousands of images and movies can be acquired from one nanochannel with simultaneous liquid thickness and temperature control. Schematics used with permission.

were found to still retain suitable volumes of liquid for up to 6 months of storage. An additional benefit of using the clipped hybrid enclosure is that it can be flash-frozen in liquid nitrogen or ethane and examined using cryo-EM as previously demonstrated (Fig. 2B, C) [24].

Non-frozen samples can be examined in parallel using liquid-EM (Fig. 2D). The clipped SiN hybrid assemblies fit within the tip of most side entry cryo holders, such as a Gatan 626 holder, and an external temperature controller can be used to vary temperature settings according to application needs. For example, this configuration can be used to record movies of human viruses at 37° C in a manner they would exist in the human body. The benefits of the hybrid configuration are that the individual parts are readily available, and liquid-EM samples appear to have greater visual contrast than paired cryo-EM counterparts. Due to the blanketed carbon film, there is less sample drift and motion, and little to no air-water interface was observed. Again, as the microchip forms one of the enclosure substrates, sample thickness is limited, although high-resolution applications have shown promising preliminary results (Fig. 2D).

Graphene Liquid Cells

Another very popular liquid-EM enclosure is the graphene liquid cell (GLC). GLCs are comprised of thin graphene layers that are placed over top of reticulated carbon films that are fixed to conventional EM grids. Two such grids are used to sandwich small volumes of liquid, which are naturally sealed together through the adhesive forces of the graphene layers (Fig. 3A, B). The technique was first made popular by the Alivisatos team at Lawrence Berkeley National Laboratory, and numerous other researchers have reconfigured the method for a vast number of material applications [1,5,25–30]. GLCs are also used to produce cryo-EM specimens, and recent studies suggest their benefits in minimizing the damaging effects of the air-water interface.

Variations on GLC enclosures include using multi-layered graphene and graphene oxide (GO) layers to prepare specimens of interest. Early attempts to enclose virus samples in GLCs have proven successful, although initial specimens revealed background features and wrinkles in the graphene layers (data not shown). Nevertheless, as these enclosures have also given promising new results in cryo-EM experiments, they are worth pursuing as a means to expand observations of spatial properties in real-time imaging studies. Automated instruments, such as the Naiad-1 benchtop device developed by VitroTEM, are also available to prepare GLC specimens (e.g., Fig. 3B, C). Similar to hybrid enclosures, GLCs can be placed in specimen holders with external temperature control mechanisms to observe macromolecules in a fluidic environment under biomimetic conditions.

Temperature-Controlled Specimen Holders

One of the most intriguing devices made commercially available in the last few years is the temperature-controlled microfluidic specimen holder. These tools will undoubtedly push the boundaries of live imaging for hard and soft materials. Multiple iterations of these devices have been recently released by companies such as Protochips (Triton AX), Hummingbird Scientific, DENS Solutions (Stream), and the newcomer to the field, Insight Chips (Fig. 4). Each of these units uses its own proprietary means to encase liquid samples for data collection and promising new results are starting to surface for each system. For example, one major advantage of the new Insight Chips holder is the thinly engineered microchannels used to control the liquid thickness of the observed specimen in a highly reproducible manner. While biological polymers are still in the testing phase, the new technology has the potential to deliver dynamic imaging results due to a heating unit integrated into the specimen tip. This feature provides continuous and controlled temperature levels throughout the entire imaging process. Collectively, these new devices could provide next-level opportunities to discern dynamic observations of biological materials in action.

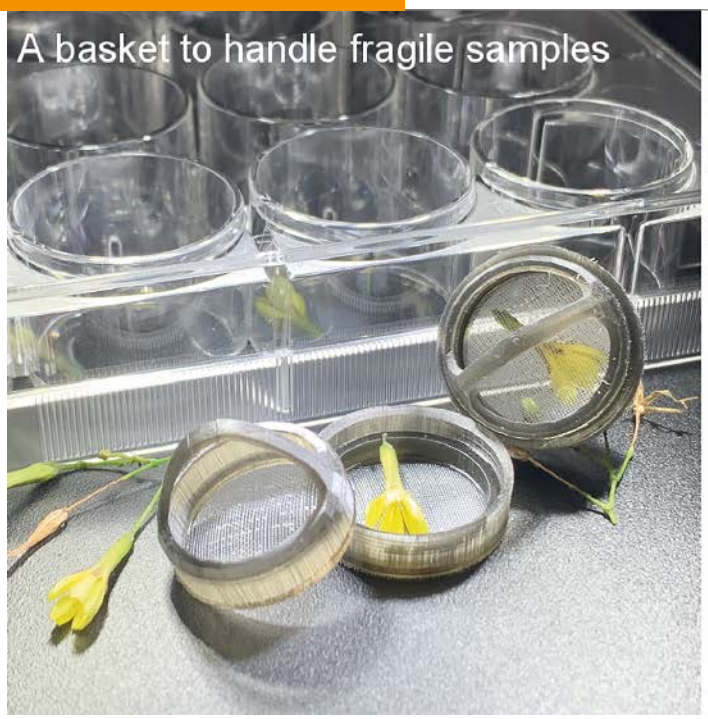
Summary

While still in its infancy compared to other modalities, much progress has been made in the liquid-EM field to break down barriers and inspire the "real-time revolution" that is upon us. Here, recent studies of macromolecules in solution have been discussed with particular focus on the potential for real-time experiments involving biological polymers. The tools that are currently in use to prepare microfluidic samples include a combination of advanced materials that have unlimited potential for unique dynamic observations. Coupled with new temperature-controlled modules, there are an endless number of possibilities to transform current knowledge of biological dynamics into novel observations relevant to the inner workings of the human body.

Affiliation
[1]Structural Oncology LLC, State College, Pennsylvania, USA

Contact
Prof. Dr. Deborah F. Kelly, PhD
Structural Oncology LLC
State College
Pennsylvania, USA
ORCID: 0000-0002-7341-7435
Debkelly@OncologyLLC.com
www.oncologyllc.com

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



FS-Basket for Freeze-Substitution and Embedding

Novel Multi-Purpose Tool for Electron Microscopy

Chie Kodera[1], Xavier Heiligenstein[1], Martin Belle[1]

Room-temperature electron microscopy implies dehydrating and embedding the sample into a resin after immobilization (vitrification or chemical fixation), transferring the sample into successive baths. Fragile samples may suffer from the transfer, impeding the experiment's success rate. We developed a novel tool series, FS-basket, that protects the sample during these transfer steps and hence increases the sample preparation reliability.

Introduction

Sample preparation for electron microscopy at room temperature implies rendering the sample resistant to the high vacuum of the electron microscope. The sample is first dehydrated in increasing concentrations of solvent (up to 100%) that is then progressively replaced by a resin and finally polymerized. At room temperature or in cryo-conditions (so-called freeze-substitution techniques) [1–3], the solution around the sample is replaced in successive rounds, or the sample is transferred in successive baths. These replacement or transfer steps are often prejudicial to the sample that may get dry (uncontrolled dehydration) or torn apart (mechanical damage. This promoted the development of various flow-through chambers or handmade solutions. To increase the reliability of those steps with diverse carriers including 3 mm and 6 mm carriers, as well as Cryocapsules, we invented a novel device, designated FS-basket [4]. This device is designed to securely trap the carrier which contains the sample, between two fine nylon meshes. This prevents unwanted sample movements during fluid exchange steps and facilitates performing the procedures with high reliability. This device has been introduced in detail [4] and applications are largely presented [5]. Introduction to the community suggested broadening the format and application range as presented in this manuscript with a wider and deeper basket, suitable to transfer large tissues across 12 well plates.

Results and Discussion

Whether conducting room temperature dehydration and embedding or freeze-substitution, electron microscopy is an essential research technique to investigate biological ultrastructure. Samples such as biopsies, small model organisms, or cell pellets are often fragilized by dehydration, especially mildly fixed material, which often leads to fragmentation and loss during washing and embedding steps. To compensate, researchers tend to increase the number of replicates when available (patient biopsies are unique), which increases cost and time.

Several flow-through chambers to automate this process have been developed (e.g., Leica AFS-2 + FSP or microscopy innovations mPrep ASP). However, those tools were primarily designed for 3 mm carriers coming from high-pressure frozen techniques and propose large pore sizes, where fine samples can flow through easily. Therefore, handling the most fragile samples typically remains a manual task entrusted to experts, and this is not easily achievable to newcomers. This limitation is particularly problematic when training a large group of next-generation electron microscopy users unfamiliar with cryo-methods.

In response to these challenges, we developed FS-basket with the following objectives:

- Maintain sample stability throughout fixation, washing, dehydration, and embedding steps
- Facilitate unhindered fluid exchange
- Be compatible with commonly available cryo-tubes or well plates
- Have minimal chemical reactivity with the chemicals involved in sample preparation

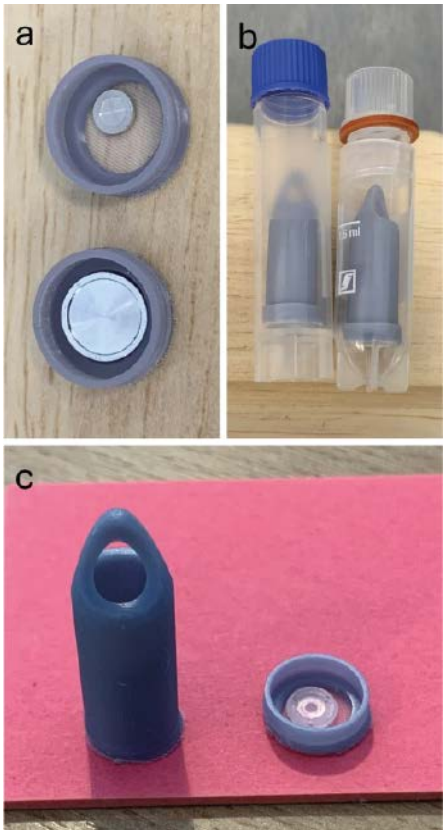


Fig. 1: The original version of the FS-basket. a) The lower part of the FS-basket with 3 mm (upper) and 6 mm (lower) carriers. b) Closed FS-baskets in cryo-tubes. c) The upper (left) and lower (right) part of the FS-basket with the Cryocapsule.

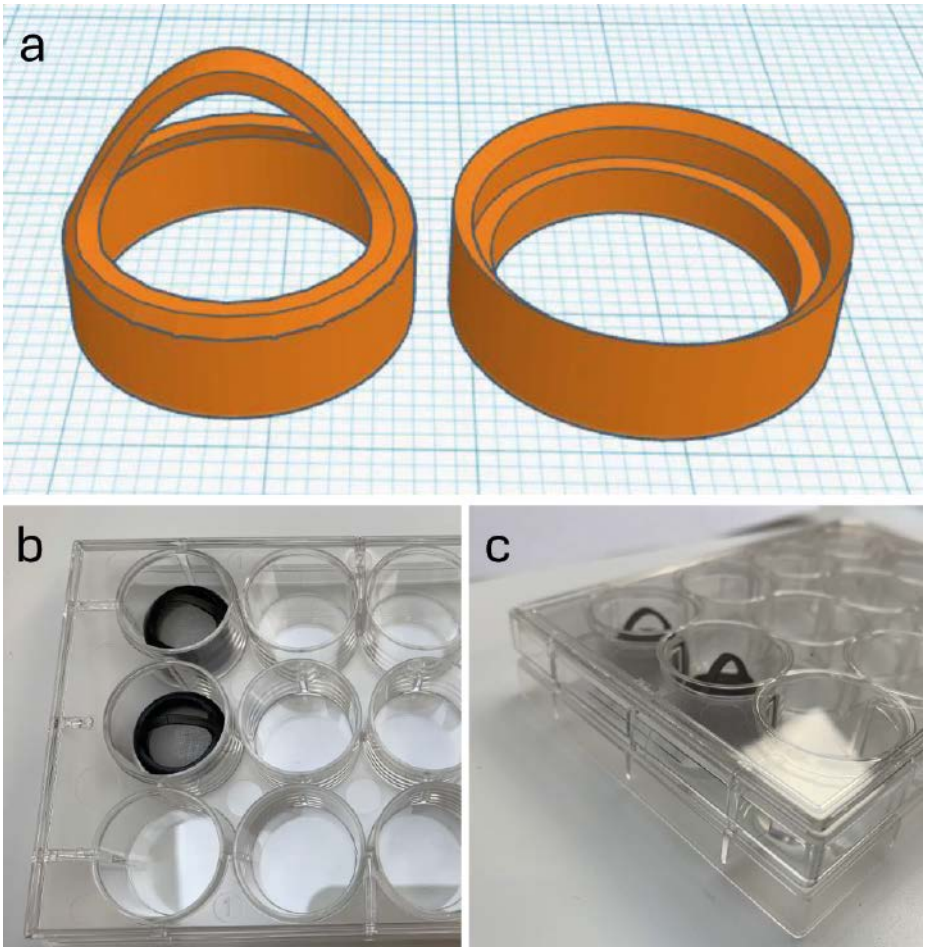


Fig. 2: The expanded version of the FS-basket. a) The schematic diagram of the new version of the FS-basket. b) The overview of the FS-baskets in a 12 well plate. c) The sideview of the FS-baskets in a 12 well plate.

- Enable manual handling, even in narrow and deep FS chambers
- Withstand the extreme cold temperatures of liquid nitrogen

We opted for a passive mesh that does not react with the chemicals commonly used in FS. The support structure to which the mesh is affixed is resistant to solvents. The primary device, designed for freeze-substitution protocols, proposes a cavity of 6.5 mm in diameter and 0.5 mm in depth to deposit the sample. These tight dimensions around a typical HPF carrier prevent sample resuspension and ensure optimal sample retention (Fig. 1).

To expand the basket's application range and adapt to larger specimens coming from room-temperature protocols, we adapted the basket's dimensions to accept entire organs from small model organisms. This novel device measures 16 mm in diameter and is 3 mm deep. It fits into a 12-well plate, allowing on-ice processing of samples in successive baths (Fig. 2). Its use and application follow the same procedure described above, only differing in the dimensions of the primary sample.

We have successfully tested embedding with various resins, including EPON Embed812 (EMS #14120 kit), LRWhite (EMS #14380 kit), and R221 (CryoCapCell # R221mono-step) [6,7], using flat embedding or en-bloc polymerization strategies directly within the cryotube [4]. Our observations report no alteration of the contrast nor the embedding quality using the FS basket. Beta tester customer also reported testing successfully EPON Embed812 (EMS #14120 kit) and LRWhite (EMS #14380 kit), but experienced issues using Agar-Low-viscosity-resin-kit (Agarscientific #AGR1078 - Spurr like). They reported that this resin caused denaturation of the FS basket, resulting in softening deformation after overnight exposure. We have not identified the chemical compound that specifically caused this denaturation. Therefore, we do not recommend using the FS-basket in its current form with Spurr-like resin.

Conclusion

We propose a tool series that increases the ease and reliability of sample dehydration and embedding for electron microscopy preparation. The concept entails maintaining the sample between two meshes with pore sizes smaller than the sample and maintaining a tight seal while conducting fluid exchanges. These tools prevent unnecessary movements of the carriers/samples throughout the process. The absence of flipping movement is particularly beneficial for large samples, facilitating recurrent orientation in multimodal imaging strategies such as CLEM or biopsy orientation.

Affiliation
[1] CryoCapCell, Le Kremlin-Bicêtre, France

Contact
Chie Kodera, PhD
Staff Scientist / Lab manager
CryoCapCell
Le Kremlin-Bicêtre, France
chie@cryocapcell.com
www.cryocapcell.com

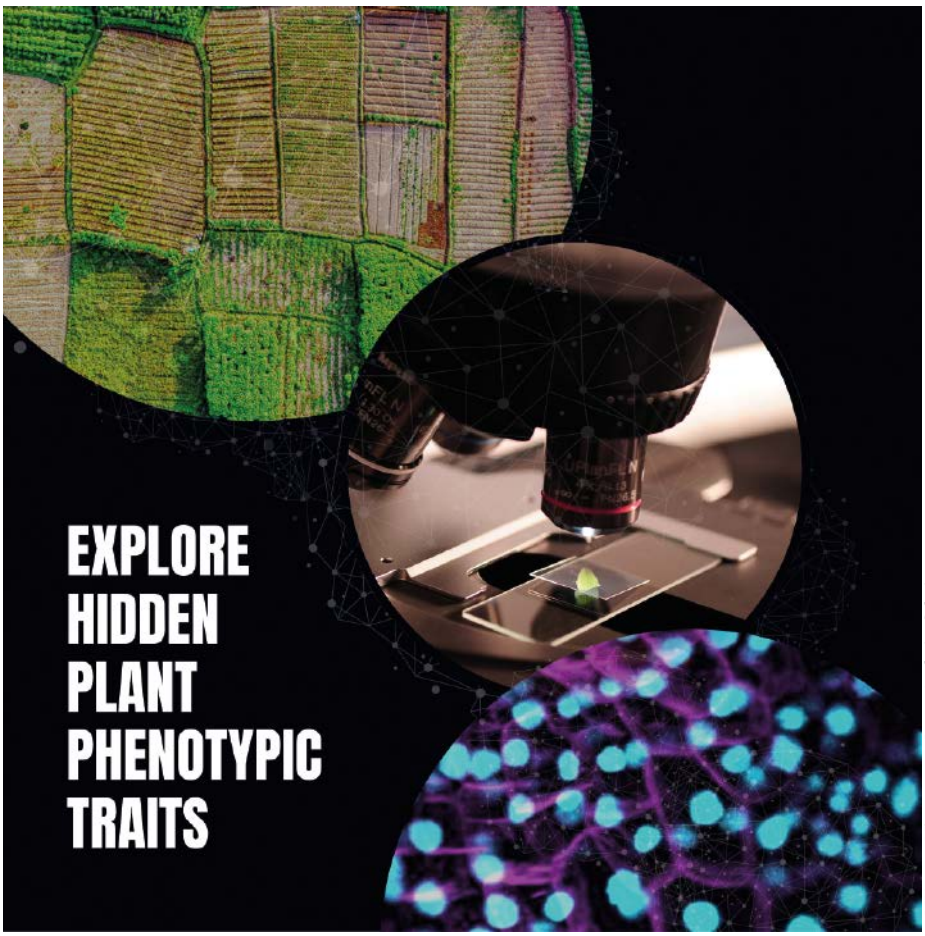
[1] References: https://bit.ly/IM-Kodera

MIAtecs: Plant Phenotyping at the Microscopic Scale

Plant phenotyping plays a crucial role in addressing the challenges of global food security and agricultural sustainability, particularly in the context of climate change. High-Throughput Plant Phenotyping (HTPP) companies and platforms typically focus on assessing observable traits and physical characteristics using advanced technologies such as satellites, drones, and remote sensing, primarily for aerial parts of plants. In contrast, root phenotyping requires distinct technologies and often operates at lower throughput levels.

At the microscopic scale, cellular phenotyping is already common in fields such as immunology and cancer research. However, microscopic-scale phenotyping in plant science remains underrepresented. To date, no commercial solutions or services have effectively addressed this gap. The reason is that HTPP and cellular biology are typically considered separate disciplines, especially in terms of the techniques used.

To bridge this gap, plant scientist Fabien Miart, with over a decade of experience in plant phenotyping and microscopy, founded MIAtecs in early 2024. MIAtecs offers High-Throughput Microscopic Plant Phenotyping (HTMPP) solutions that integrate plant physiology, HTPP methodologies, and advanced microscopy, from early-germinated seedlings to the subcellular level.



© MIAtecs - Microscopy & Image Analytics Solutions

MIAtecs Approach Consists of Four Key Steps

- We offer customized imaging systems and provide guidance on protocols and biological materials.
- We source the most suitable imaging technologies and acquire high-quality images to ensure optimal data acquisition.
- Through advanced 2D to 5D image analysis, we provide cutting-edge segmentation, object detection, and detailed morphological evaluations.
- We develop user-friendly graphical interfaces, along with statistical analysis, graphs, and data visualization.

Key Advantages of MIAtecs' HTMPP Services

- Elimination of gravitropic, mechanical, and osmotic stress caused by conventional plant microscopy techniques, using our vertical imaging solutions.
- Simultaneous, high-throughput analysis of both aerial and root parts of the same plants.
- Access to novel microscopic phenotypic traits in controlled environments, including analysis of stomata, trichomes, leaf veins, roots, root hairs dynamics, and more.
- Eco-friendly chemical treatments (e.g., pesticides, biocontrol products) with in vivo imaging of plant responses.
- High-throughput analysis of gene expression patterns over time.

The mission of MIAtecs is to unlock new phenotypic traits, providing deeper insights into the interactions between genetics and environmental factors. Our innovative solutions offer a more comprehensive understanding of plant development, adaptability, and performance. This will ultimately help breeders and researchers identify desirable traits at a microscopic level, leading to the development of more resilient and productive plant varieties.

Contact
Dr. Fabien Miart
MIAtecs - Microscopy & Image Analytics Solutions
Marseille, France
fabien.miart@live.fr
www.miatecs.com

MIAtecs

Supercontinuum Lasers

It was a milestone in laser technology when the first supercontinuum lasers were introduced over 15 years ago. Now you could get diffraction-limited light from a laser in the 400–2400 nm range. This breakthrough facilitated the characterization of exotic nanomaterials and the excitation of fluorophores. The supercontinuum technology proved versatile. However, the heart of the laser, the non-linear fiber, degraded over time and compromised the laser's reliability. In 2016, we began our quest to improve its longevity. Fast-forward to 2024: A client sent us a SuperK supercontinuum laser for maintenance. The log showed that it had been running non-stop for 50k hours! We replaced the booster and returned the laser, with the expectation of it running for another 35k hours. In our test lab, close to 100 SuperK supercontinuum lasers are running non-stop for 10k hours. In real life, our lasers typically run much longer – as this client can testify.

NKT Photonics
www.nktphotonics.com

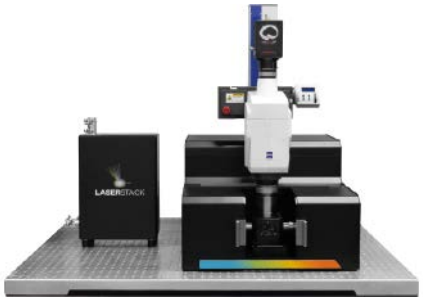
3D X-Ray Microscope

The VersaXRM 730 3D X-ray microscope by Zeiss offers resolution performance as well as faster throughput and time-to-results to accelerate productivity. The platform's guidance and control system, the ZEN navx, accommodates users of all skill levels. It is a systematic approach of built-in guidance, automated workflows, and intelligent system insights. It enables even the newest user in a busy environment to be immediately productive, achieve experimental results more easily and efficiently, and obtain the right data the first time without extensive training. Additionally, File Transfer Utility (FTU) automatically transfers data from the microscope to other locations so that users have their information where they need it when they need it. Fast Mode delivers one-minute tomographies, accelerating 3D imaging by using continuous sample motion data collection. The microscope is also equipped with AI.

Zeiss
www.zeiss.com

Axially Swept Light Sheet Microscopy System

AxL Cleared Tissue LightSheet (AxL CTLS) is a fully automated macro zoom microscope with high NA apochromatic objectives and dual-sided light sheet illumination for imaging whole organs to small animals. Custom-designed excitation objectives and patented axially swept light sheet microscopy (ASLM) produce an exceptionally thin, long and uniform light-sheet for large-scale high-resolution imaging. Using a back-thinned sCMOS camera, AxL CTLS images one cubic centimeter in less than a minute. ASLM scans the light sheet in its propagation direction using high-speed remote focusing synchronized to the rolling shutter readout of an sCMOS camera at the size of the beam waist. This approach creates an exceptionally thin light sheet across a large field of view, resulting in improved optical sectioning and signal-to-noise images. This scanned sheet features a constant laser intensity across the field of view for an evenly illuminated image. The 0.14NA excitation objective creates a 2µm thin waist for superior axial resolution. AxL CTLS is designed to operate optimally across a range of 1.33 to 1.56 refractive indices ensuring compatibility across all available clearing methods.



3i – Intelligent Imaging Innovations
www.intelligent-imaging.com

Bioimaging Platform

Tomocube introduces the HT-X1 Plus, a bioimaging platform, that provides researchers with insights into biological processes. The platform offers a 4x larger field of view and advanced illumination optics for phenotypic screening, tissue section imaging, and the study of fast-moving microorganisms. Equipped with a high-performance CXP camera and AI-powered image reconstruction algorithms, the platform reduces scan times, enabling comprehensive 3D imaging of a full 96-well plate in under 30 minutes, without sacrificing detail or quality. Key features include a larger field of view. Capture expansive areas without stitching, ideal for large-scale, high-content experiments. It also has faster image acquisition, is optimized for high-throughput screening, and is capable of scanning 96-well plates in just 30 minutes. And flexible light source options for configuring imaging with three wavelengths (R/G/B) for optimal contrast and penetration.

Tomocube
www.tomocube.com

Back-Illuminated sCMOS Camera

Oxford Instruments Andor has launched the Marana 4.2B-6 back-illuminated 4.2 Megapixel sCMOS camera. The performance of the model has been enhanced to widen its application appeal within Physical Sciences and Astronomy. The low noise mode reduces the read noise to 1.0e-. When combined with market-leading -45°C vacuum cooling and 95% QE, this pushes the limits of detection further, even under the most challenging, light-starved imaging applications, enabling tracking of smaller Space Debris or NEOs, shorter exposures, lower illumination powers to protect photosensitive samples or the detection of trace concentrations of species. The high-speed mode helps to meet the needs of fast imaging applications such as trapped ion/atom quantum computing, solar astronomy, fast spectroscopy, or hyperspectral imaging. Furthermore, a long exposure mode, markedly enhances the exposure flexibility of Marana 4.2B-6. A 'Global Clear' mode has now been implemented for the Rolling Shutter sensor type. This mode purges charge from all rows of the sensor simultaneously at the exposure start.

Oxford Instruments Andor
https://andor.oxinst.com

Spinning-Disk Confocal Solution

CrestOptics announced the collaboration with Leica Microsystems. The partnership enables Leica Microsystems to offer CrestOptics' Cicero spinning disk unit within its new Thunder Imager Cell Spinning Disk system. Using Cicero, users can transition between widefield and confocal imaging as required, allowing for deeper insights into subjects ranging from large cell monolayers to complex 3D structures, as well as the capture of fast cellular events such as chromosome segregation and organelle trafficking. The partnership provides a wider range of options and technologies for customers working with challenging large and 3D samples. The integration brings together Thunder's proprietary computational clearing technology, which enhances image quality, with the new intelligent automation of the DMi8 microscope. This includes features such as the automated sample finder for overviews, and adaptive immersion technology that maintains focus, switching between low magnification dry overview and water high-resolution views.

CrestOptics
https://crestoptics.com

Focus Scanner and Stage System for Inverted Microscopes

With the P-725.xCDE1S focus scanner and the U-781 PILine XY stage system, PI completes its range of positioning systems for fluorescence microscopy applications. The focus scanner is offered as a complete package including controller and allows travel ranges up to 400 µm. The U-781 XY stage system with controller and joystick is mechanically compatible with inverted microscopes of leading manufacturers and offers interfaces to common microscopy software tools. The contactless measuring capacitive sensors achieve a resolution in the sub-nanometer range. This results in good motion linearity, high long-term stability, and a bandwidth in the kHz range. The zero-play flexure guides provide high guidance accuracy and are maintenance-free, friction-free, wear-free, and lubricant-free. Their stiffness makes them highly resilient and insensitive to shock loads and vibration. In addition to high-resolution microscopy, the PIFOC focus scanner is also used in semiconductor inspection, interferometry, and screening, as well as in many other areas.

Physik Instrumente (PI)
www.physikinstrumente.com

Confocal Raman Microscope

Craic Technologies announced the Apollo M Confocal Raman Microscope. Key features include high-resolution confocal imaging. The microscope's confocal imaging system enables researchers to acquire high-resolution, three-dimensional images of materials with exceptional spatial resolution and depth profiling capabilities. Furthermore, ultra-sensitive Raman spectroscopy. With its advanced Raman spectroscopy capabilities, it offers unparalleled sensitivity and signal-to-noise ratio, allowing researchers to detect and analyze even the smallest traces of molecular and structural information. It supports also multimodal imaging techniques, including confocal Raman spectroscopy, fluorescence imaging, and color microscopy, providing researchers with comprehensive insights into material composition, morphology, and behavior. Intuitive software interface and automated analysis workflows streamline data acquisition, processing, and interpretation, enabling researchers to maximize productivity and accelerate research outcomes.

Craic Technologies
www.microspectra.com

AI-Powered Digital Fluorescence Microscope

Leica Microsystems released Mateo FL, an AI-powered digital fluorescence microscope for cell culture checks in advanced research applications. Mateo FL combines transmitted light for label-free imaging with multi-channel fluorescence and automated analysis tools, optimizing the way labs approach downstream experiments using cell culture. It offers features for advanced cell checks, including multi-modal capabilities, AI-powered confluency checks, swift and accurate cell counting, and smart measurements for transfection efficiency. The range of automated workflows offers reproducible results, even in challenging cell culture samples. Users can obtain an accurate, automated cell count in 5 seconds, saving an average of 15 minutes of manual cell counting. The automated phase contrast function also saves time and effort in the process of image acquisition. The instrument's compact and integrated design reduces contamination risks by at least 50%. Workflow efficiency can be doubled by capturing both monochrome and color images without physically swapping devices mid-experiment.

Leica
www.leica-microsystems.com

Plasma FIB-SEM

Tescan, in collaboration with Orsay Physics, announced the launch of AMBER X 2, a plasma FIB-SEM system. AMBER X 2 leverages Orsay Physics' extensive expertise in plasma FIB-SEM technology, offering significant benefits for materials science applications, namely increased precision enabling fully automated TEM/STEM specimen preparation, and enhanced milling efficiency. These are complemented by improved FIB optical parameters, a field-free SEM column, and multimodal and multiscale materials characterization capabilities,

providing an all-encompassing solution for researchers. Key features include optimizing beam parameters for superior beam profiles, simplifying workflows and improving precision, beam placement accuracy at low ion beam energies, and milling performance. Fully automated workflow for precise and efficient TEM sample preparation. Furthermore, it allows high-resolution imaging of diverse materials, eliminating the limitations of immersion optics while offering a wide field of view and various scanning modes.

Tescan
www.tescan.com

Imprint

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

Managing Directors
Dr. Guido F. Herrmann

Group Vice President
Harriet Jeckells

Director, Business Strategy – Corporate Products and Services
Dr. Katja Habermüller

Product Manager Analytical Science
Ralf Kempf

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

Editorial Team
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

Tina Renner
Ph.: +49 6201 606-021
E-mail: tina.renner@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
E-Mail: WileyGIT@vuservice.de
Our service is available for you from Monday to Friday 8 am – 5 pm CET

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
Overall audience: 27,000
14,000 print copies, 4 times per annum
13,000 newsletter recipients, 4 issue alerts per annum
Advertising prize 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.

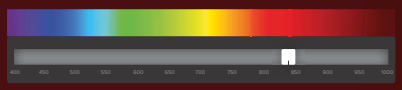
Bank account
J.P. Morgan AG, Frankfurt
Konto-Nr. 6161517443
BLZ: 501 108 00
BIC: CHAS DE FX
IBAN: DE55501108006161517443

Printed in Germany

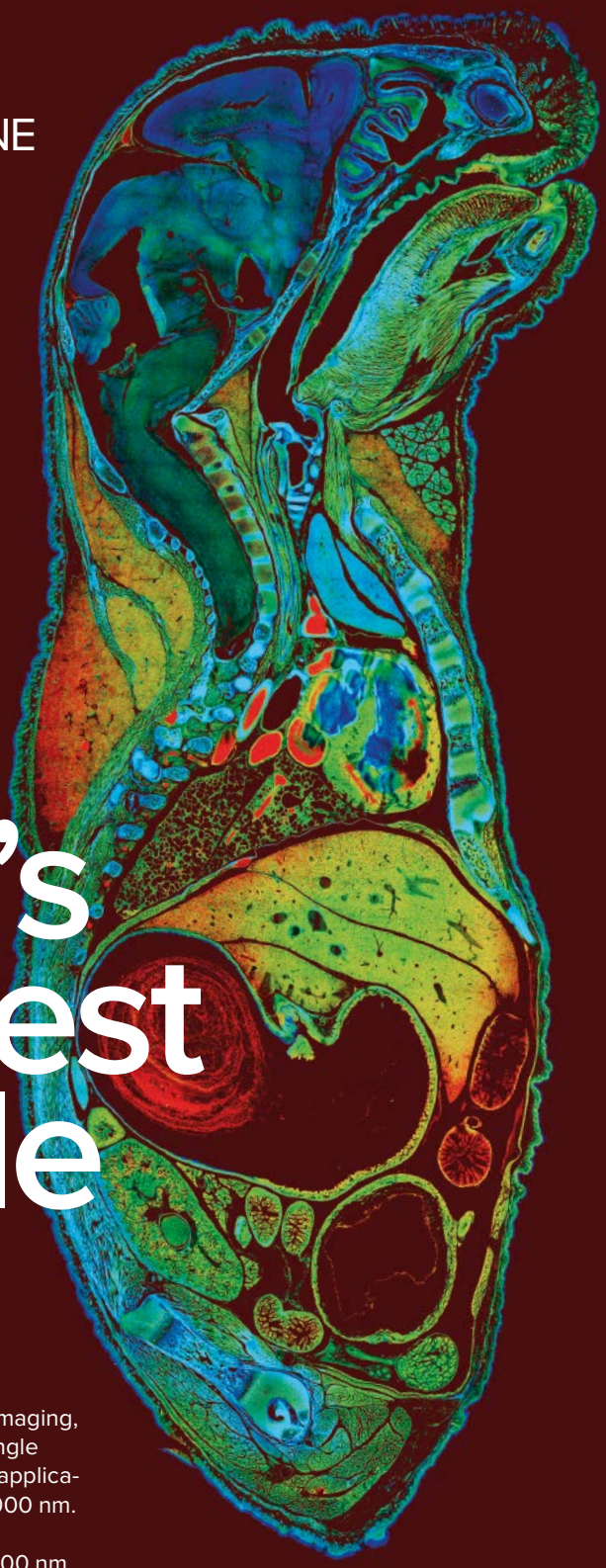
westermann DRUCK pva

ISSN 1439-4243
www.imaging-git.com

WILEY



SuperK CHROMATUNE
400-1000 nm



Confocal microscope image of a mouse. Credits: Leica Microsystems

New!

World's broadest tunable laser

Use your SuperK CHROMATUNE for bioimaging, fluorescence imaging & spectroscopy, single molecule imaging and other microscopy applications, at any wavelength between 400-1000 nm.

Excite at any wavelength in the 400 to 1000 nm range. Fiber delivered and diffraction limited. Zero maintenance with a lifetime of thousands of hours and two years warranty.

nktphotonics.com

NKT Photonics