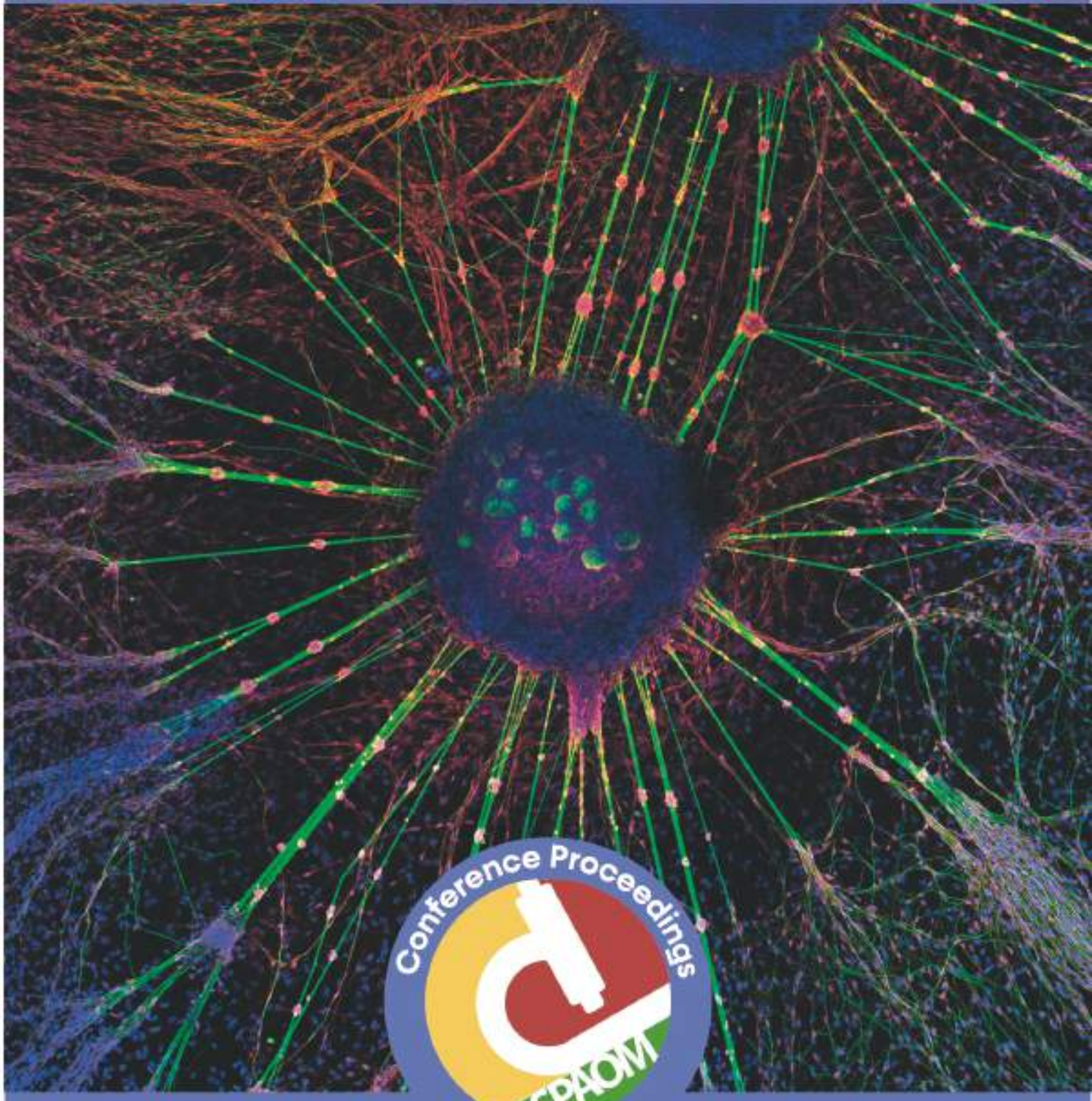


Conference Proceedings

SPAOM

8th EDITION

Spanish & Portuguese Advanced Optical Microscopy Meeting

Toledo (Spain),
20th-22nd November 2024

REMOA

PPBI

spaom2024.org

8th EDITION

Spanish & Portuguese Advanced Optical Microscopy Meeting

SPAOM24

G. Cristobal and T. Correia (Editors)

Toledo (Spain), 20th-22nd November 2024

FOREWORD

The 8th edition of the Spanish-Portuguese Advanced Optical Microscopy Meeting (SPAOM, by its English acronym) took place at the Toledo Conference Center, from November 20 to 22, 2024 in Toledo (Spain). The Conference Center was designed by the renowned architect Rafael Moneo, recipient of the Prince of Asturias Award for the Arts. A place rich in history, located in the heart of Toledo's historic center, just 30 minutes from Madrid by high-speed train (AVE). SPAOM is a joint initiative of the Spanish Network of Advanced Optical Microscopy (REMOA) and the Portuguese Platform of Biomedical Imaging (PPBI) and is organized by scientists from various Spanish and Portuguese institutions. The main objective of SPAOM is to organize an annual conference covering the latest applications in optical microscopy and image analysis, promoting collaboration and interaction in the field of advanced microscopy.

Optical microscopy is one of the most widely used technologies in scientific research due to its versatility, accessibility, and ability to provide highly relevant spatial and temporal data. Numerous scientific fields employ its many techniques and resources, thus establishing this technology as an essential tool for advancing scientific knowledge. Another noteworthy aspect of optical microscopy is its dynamism and constant evolution. New applications are developed each year and are quickly integrated into both the market and scientific research. SPAOM, therefore, serves as a forum for discussing this continuous innovation. At the meeting, industry experts present and analyze the latest trends, offering a space where industry and researchers can share results, needs, questions, and, in general, knowledge. SPAOM aims to create synergies that enhance the utility, accessibility, and functionality of this crucial tool for scientific progress.

Recent years have seen an explosion of innovation in microscopy techniques, with AI further transforming the field by enhancing image interpretation, enabling large-scale data analysis, and accelerating discovery. From visualizing the intricate details of cellular structures to revealing functional processes in real-time, the integration of AI with microscopy is revolutionizing our understanding and exploration of both the seen and unseen world.

The abstracts in this volume highlight the latest research and applications spanning several key areas of microscopy, including:

- **Advances in Mesoscopy Imaging**: Techniques that bridge microscopic and macroscopic imaging scales, providing powerful tools to visualize structures and systems within their larger biological or material context.

- **Functional Microscopy**: Innovations that enable real-time observation of dynamic biological and chemical processes, illuminating function alongside structure to deepen our understanding of living systems.

- **Nanoscopy**: Advances in super-resolution microscopy, pushing past traditional diffraction limits to reveal nanoscale structures with remarkable clarity.

- **Correlative Microscopy**: Methods for combining multiple imaging modalities, allowing researchers to integrate diverse data types for a comprehensive view of complex specimens.

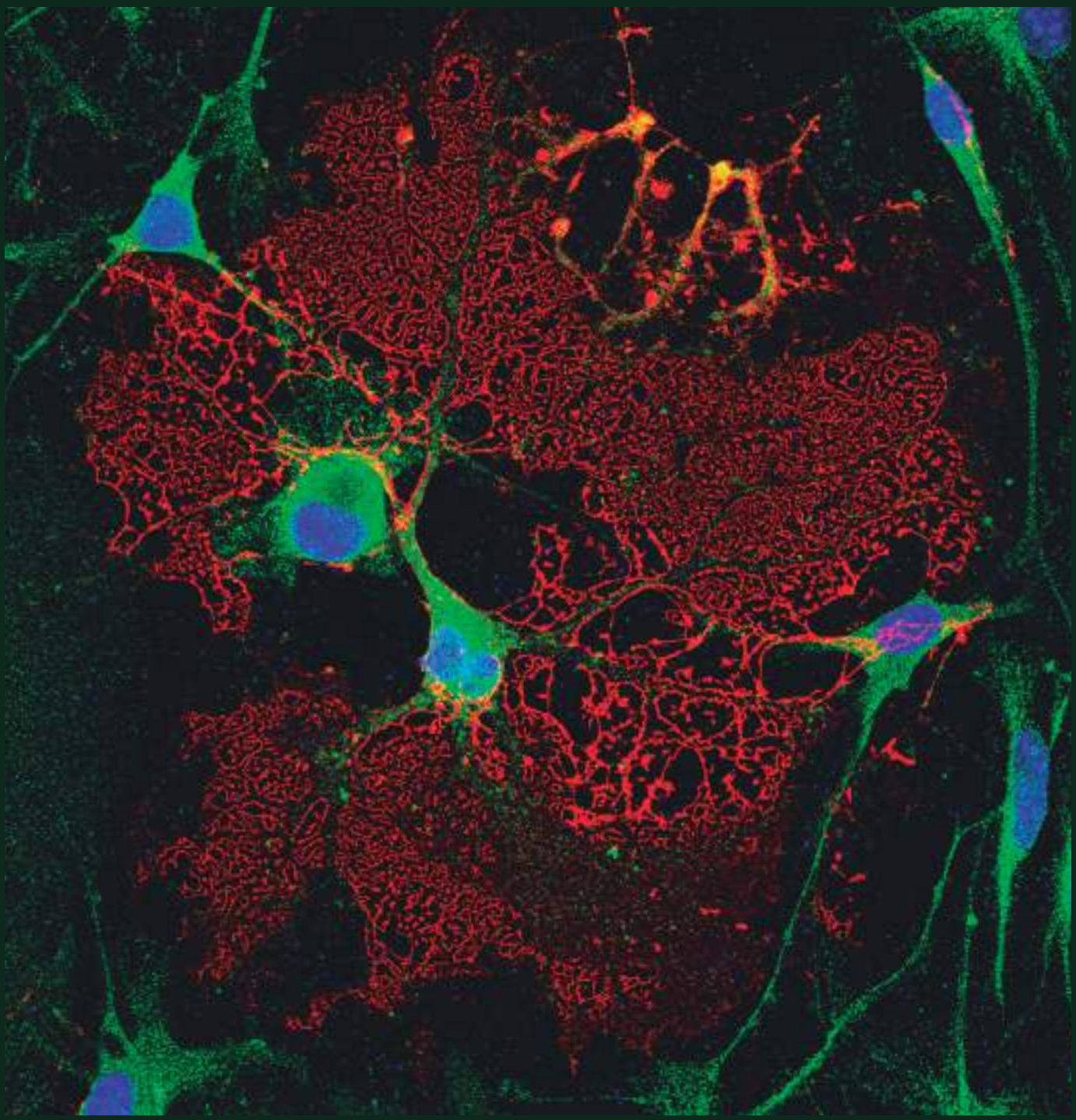
- **Image Analysis**: AI-driven techniques for processing and analyzing microscopy images, from automated segmentation and quantification to advanced pattern recognition and predictive modeling.

The conference had 228 attendees, 20 plenary presentations, 50 posters, 7 Community Workshops (CW), 10 Industry Workshops (IW), 2 sessions on running a core facility, 1 session on Microscopy Networks and for the first time 2 sessions on user-developer round tables. The CW are the signature session of SPAOM and they aim to feature balanced sessions made of a mix of talks and hands-on demos, given by scientists to scientists, and mostly focusing on imaging techniques, tools, sample prep, hints, shareable projects, protocols, open source etc.

We hope this book of abstracts serves as an inspiring and insightful resource, capturing the spirit of innovation and collaboration that defines this exciting field. Our gratitude extends to the sponsors, contributors, scientific committee, reviewers, and participants who have made this event possible.

A new Special Issue has been approved in the *Journal of Imaging* (MDPI), titled *New Trends in Image Analysis for Next-Generation Microscopy*. This issue will showcase a selection of contributions from the SPAOM 2024 Conference, along with insights from the broader microscopy community. For more details, visit the Announcements section in the app or check the SPAOM24 website. Finally, it has officially been announced that the 9th edition of SPAOM will take place in Braga, Portugal, in the fall of 2025. We look forward to seeing you in Braga next year!

G. Cristobal & T. Correia
On behalf of the organizing committee
November 2024



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Julien Colombelli
Institute for Reserach in Biomedicine (IRB)

INVITED SPEAKERS

Kate Grieve
Vision Institute, France

Javier Conesa
CNB, Spain

Steffen Dietzel
LMU, Germany

Ruslan Dimitirev
Ghent University, Belgium

Georgina Fletcher
Royal Microscopy Society (RMS), UK

Christel Geroud
IoL, Switzerland

Anja Hauser
DRFZ, Germany

Marine Laporte
Univ. Lyon, France

Giovanni Volpe
Gothenburg Univ. Sweden

James Swoger
EMBL, Spain

Ignacio Arganda
UPV/EHU, Spain

Scientific Program

8th Spanish & Portuguese Advanced Optical Microscopy Meeting
Toledo (Spain), November 20-22, 2024

Day 1: November 20th, 2024

Time	Event	Location
08:00 - 09:00	Registration & Poster Setup	Zocodover room
09:00 - 09:15	Welcome	Toledo room
09:15	SESSION 1: Advances in Mesoscopic Imaging (Chair: J. Colombelli)	Toledo room
09:16 - 10:00	**Keynote Address**: Imaging retinal organoids with dynamic full field optical coherence tomography (K. Grieve)	Toledo room
10:00 - 10:30	Cell 3D Positioning by Optical encoding (C3PO) and its application to spatial transcriptomics (J. Swoger)	Toledo room
10:30 - 10:45	Spatial protein localization in photoreceptor discs revealed by expansion microscopy and nanoscopy (S. Mortal)	Toledo room
10:45 - 11:00	Advanced imaging for visualizing the functional circuitry of retinas in visual disorders therapies (E. Perez)	Toledo room
11:00 - 11:30	COFFEE BREAK (Sponsored by Stratocore)	Zocodover exhibition room
11:30	SESSION 2: Multi-mode functional imaging (Chair: V. Caiolfa)	Toledo room
11:31- 12:00	Application of functional, multidimensional optical microscopy to analyze the function of myeloid cells during inflammation and bone regeneration (A. Hauser)	Toledo room
12:00- 12:30	Imaging oxygenation and metabolism in organoids using FLIM and PLIM (R. Dimitriev)	Toledo room
12:30- 12:45	Anthracyclines accumulation in cardiac tissue in situ detection by phasor-flim (L. Cadiz)	Toledo room

Time	Event	Location
12:45- 13:00	Advancing Diagnosis of COL6-CMD. AI and Transfer Learning in Muscle Tissue Analysis (M. Roldan)	Toledo room
13:00- 13:25	**Industry Talk**: Characterisation of biocondensate microfluidic flow using array-detector FCS (J. Hendrix, Zeiss)	Toledo room
13:25- 13:35	**Poster Flash Talks**	Toledo room
13:30- 14:30	**LUNCH**	Zocodover room
14:30- 15:30	**Poster Session I**	Zocodover room
15:30- 16:15	**Industry Workshop**: Reviewing Tissue Clearing: Hands-on with Clarification and Imaging (Miltenyi)	El Greco-A room
15:30- 16:15	**Industry Workshop** Microscopic precision, reproducibility and versatility. Automation as a harmonisation procedure (Eppendorf)	El Greco-B room
15:30- 16:15	**Industry Workshop**: The Power of Airyscan 2 for Live Dynamics (Zeiss)	El Greco-C2 room
15:30- 16:15	**Industry Workshop**: Microscope Performance Monitor with the new FLUOVIEW™ FV4000 (Evident)	El Greco-D1 room
15:30- 16:15	**Industry Workshop**: TauSTED Xtend ? New tools for gentle live imaging at remarkable nanoscale (Leica)	El Greco-D2 room
16:15 - 16:45	COFFEE BREAK	Zocodover room
16:45 - 17:45	**Community Workshop I**: Advancements and Applications of Open Hardware in Microscopy (M.Vasconcelos & H. Wang)– Chairs: G.Bueno & G.Cristobal	El Greco-B room
16:45 - 17:45	**Community Workshop II**: Live Cell Imaging (M.del Rosario & J. Nieder)– Chair: M. Vinas	El Greco-A room
16:45 - 17:45	**Community Workshop IV**: Monitoring and Analyzing Microscope Performance through PSF Measurements (N.Halidi & J. Diaz &G. Martins)	Toledo room
17:45 - 18:45	**Running a Microscopy Core Facility – General Session** Chairs: N. Moreno & G. Fletcher	Toledo room
18:30 - 19:15	**Social Activity:** Toledo tour by train – 1st group	Cuesta de Capuchinos Street

Time	Event	Location
19:00 - 19:45	**Social Activity:** Toledo tour by train – 2nd group	Cuesta de Capuchinos Street

Day 2: November 21st, 2024

Time	Event	Location
08:30 - 09:00	Registration & Poster Setup	Zocodover room
09:00	SESSION 3: Nanoscopy (Chair: J. Fernandez)	Toledo room
09:01 - 09:30	Five color super resolution microscopy with FLIM STED (S. Dietzel)	Toledo room
09:30 - 10:00	Visualizing the native cellular organization by coupling cryo-fixation with expansion microscopy (Cryo-ExM) (M. Laporte)	Toledo room
10:00 - 10:15	Super resolution microscopy reveals the interaction between nascent RNA, transcription factories and chromatin (A. Castells-Garcia)	Toledo room
10:15 - 10:30	Zero-Shot Object Detection with Foundational Models: A Similarity-Based Approach (A. Gonzalez-Marfil)	Toledo room
10:30 - 11:00	COFFEE BREAK	Zocodover room
11:00	SESSION 4: Correlative Microscopy (Chair: J. Fernandez)	Toledo room
11:01- 11:30	From plastic to cryo: workflows to explore tissue at ultrastructural level (C. Genoud)	Toledo room
11:30- 12:00	Cryo-correlative light and electron Microscopy (J. Conesa)	Toledo room
12:00- 12:15	Correlating Raman microspectroscopy with standard histology to demonstrate its potential in breast cancer diagnosis (E. Calatayud)	Toledo room
12:15- 12:30	Automated Alignment Strategies for Integrating Cryo 3D-SIM and SXT Data (J. Oton)	Toledo room
12:30- 12:45	**Industry Talk**: New technologies in imaging and image analysis for high-end microscopy, M. Abanto (online) (IZASA)	Toledo room
12:45- 12:55	**Poster Flash Talks II**	Toledo room
12:55- 13:00	**Industry Pitch** Elevate your imaging with new developments from Evident, B. Sieberer (Evident)	Toledo room

Time	Event	Location
13:00- 13:05	**Industry Pitch:** Smart Microscopy: High quality imaging for research and teaching, E. Martin (IESMAT)	Toledo room
13:05- 14:00	**LUNCH**	Zocodover room
14:00- 15:00	**Poster Session II**	Zocodover room
15:00- 15:45	**Industry Workshop**: Smart Microscopy with Celldiscoverer 7, S. Prag (Zeiss)	El Greco-C2 room
15:00- 15:45	**Industry Workshop**: Microscope Performance Monitor with the new FLUOVIEW™ FV4000 (Evident), B. Sieberer (Evident)	El Greco-D1 room
15:00- 15:45	**Industry Workshop**: TauSTED Xtend ? New tools for gentle live imaging at remarkable nanoscale, J.R. Pearson (Leica)	El Greco-D2 room
15:30- 15:45	**Industry Workshop**: To label, or not to label: Label-free three-dimensional imaging and quantitative analysis of live cells using holotomography, B. Combettes (Tomocube)	El Greco-B room
15:45 - 16:15	COFFEE BREAK	Zocodover room
16:15 - 17:15	**Community Workshop III**: Spatial Biology: a game changer (D. Megias & I. Peset & P. Rodrigues)	El Greco-B room
16:15 - 17:15	**Community Workshop V**: Optimising STORM technology: Hands-on workflow (A. Ona & G. Dagostino & J. Fernandez)	El Greco-D2 room
16:15 - 17:15	**Community Workshop VI**: Cryo-correlative workflows (J. Conesa)	El Greco-B room
16:15 - 17:15	**Community Workshop VII**: BiaPy: deep learning based Bioimage Analysis for all audiences (L. Backova & I. Arganda)	Toledo room
17:15 - 18:15	**Running a Microscopy Core Facility – Technical Session** Chairs: G. Martins & D. Megias	Toledo room
18:15 - 18:45	REMOA-PPBI Meeting	Toledo room
20:30 - 22:30	**Social Activity** Cocktail dinner	Hacienda del Cardenal Restaurant

Day 3: November 22nd, 2024

Time	Event	Location
08:30 - 09:00	Registration	Zocodover room
09:00 - 10:15	Microscopy Networks. National & International Level – RMS (G. Fletcher) & other networks – Chair: A. Sahun	Toledo room
10:15	SESSION 5: Image Analysis (Chair: P. Sampaio)	Toledo room
10:16- 10:45	How can deep learning enhance microscopy? (G. Volpe)	Toledo room
10:45- 11:15	BiaPy: Bioimage Analysis workflows for all audiences (I. Arganda)	Toledo room
11:15- 11:30	SAMJ: Fast annotation with foundation models on Java-based Bioimage Platforms (A. Munoz-Barrutia)	Toledo room
11:30- 11:45	PhotoFiTT: A Framework for Assessing Phototoxicity in Live-Cell Microscopy Experiments (M. del Rosario)	Toledo room
11:45 - 12:15	COFFEE BREAK	Zocodover room
12:15- 13:00	Introduction to AI4Life and BioImage Model Zoo (C. Fuster & A. Munoz-Barrutia)	Toledo room
12:15- 13:00	**User-Developer Session & Roundtable discussion I – Zeiss: C2 – Evident: D1 – Telstar: B**	El Greco rooms
13:00- 13:45	**Image Analysis Industry Workshop** "Accelerating Image Analysis with TruAI DL Technology", E. Escola (Evident)– "Scale up your image analysis with AI ZEISS Arivis solutions", D. Garcia (Zeiss)– "Label free imaging: image without disturbing your samples. Practical session", J. Recasens &Z. Novkova (Izasa)	Toledo room
13:00- 13:45	**User-Developer Session & Roundtable discussion II – Leica: D2– Izasa: A**	El Greco rooms
13:45- 14:00	**Awards & Closing remarks**	Toledo room
14:00- 15:00	**Leaving LUNCH**	Zocodover room

Posters I

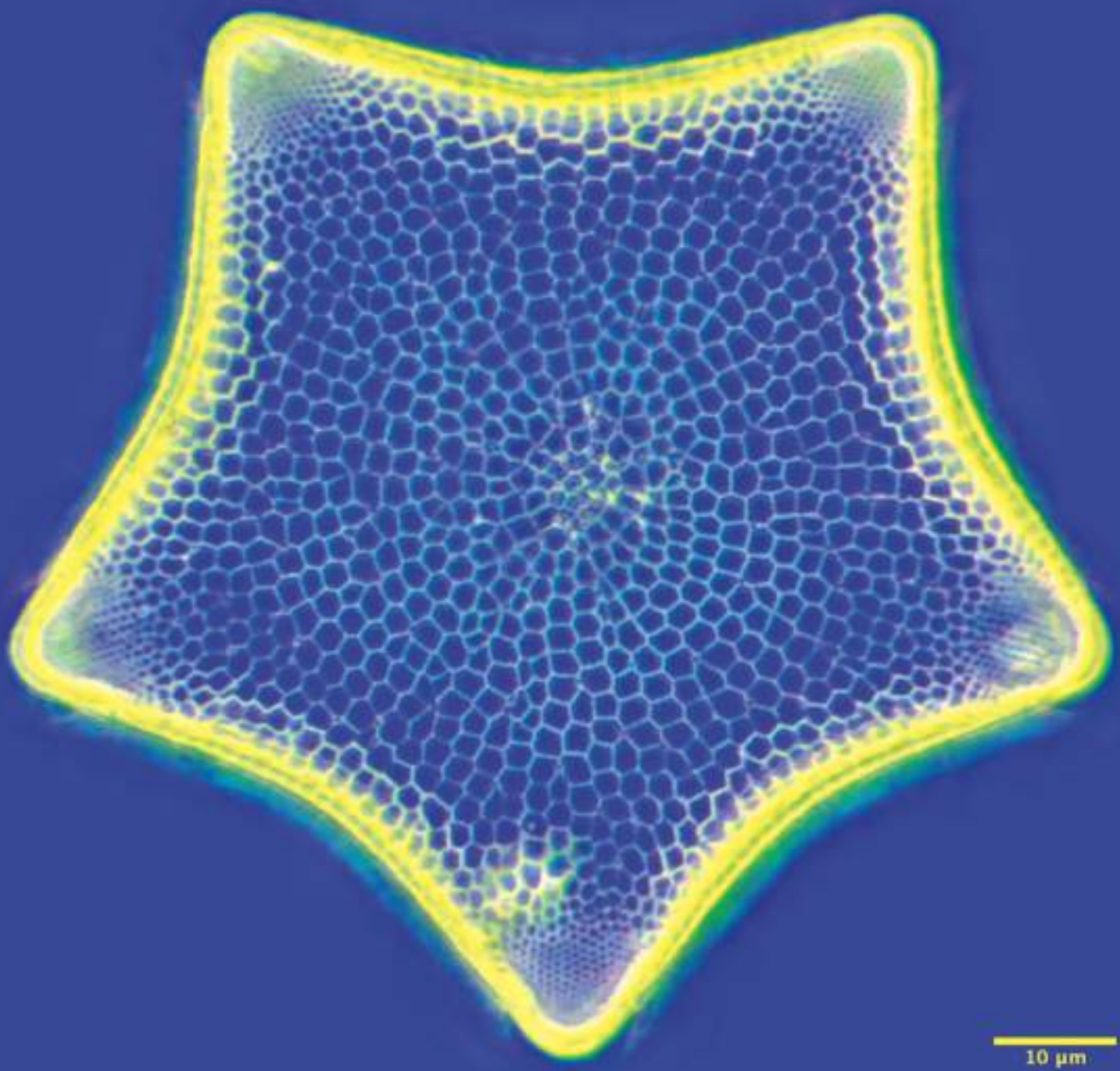
#	Author — Title
1	Guillermo Solís Fernández — Rapid stem cell spreading induced by high affinity a5b1 integrin-selective bicyclic RGD peptide in biomimetic hydrogels
3	Cristina Ederra — Integrating multiplex immunofluorescence staining and analysis for immune landscape of tumor samples
5	Pau Carrillo Barberá — Advancing myelin research through deep learning-enhanced microscopy
7	Ana Cayuela López — Leveraging nextflow for scalable processing in fluorescence microscopy: a novel approach in cancer research
9	Aleksa Dencevski — Development of a two-dimensional superresolution microscope using transmission diffraction grating obtained by analogue microfilming method
11	Caterina Fuster-Barceló — Empowering Researchers with the BioImage.IO Chatbot: an interactive tool for Bioimage Analysis
13	Jesús Gómez Alonso — QuPath implementation in a cancer research microscopy core unit
15	Yolanda Guerrero Sánchez — Detection of brain gene expression images based on image processing techniques
17	Lelde Hermane — A New Angle on Coral Imaging: Lightsheet Microscopy for Enhanced 3D Morphological Studies
19	Jovana Jelic — Development of a FLIM-FCS System: Single-Molecule Sensitivity and Enhanced Fluorescence Life-Time Measurements
21	Ainhoa Urbiola — Imaging core facility at CIMA beyond microscopy. Exploring new frontiers: the imaging facility as a center for innovation in biotechnology
23	Irantzu Llarena — Characterization of 3D materials for biotechnological applications
25	Biagio Mandracchia — High-speed sCMOS acquisition with optical pixel reassignment
27	Bruno Monteiro — RI_Hubs project: amplifying the cooperation among Bioimaging & Structural Biology Research Infrastructures from Europe and Latin America
29	Tien Nguyen — A deep learning framework for quality assessment of histopathological whole slide images
31	Gianluca D Agostino — Optimising STORM technology: hands-on workflow demo
33	Manuel Pérez — Implementation and validation of modified cell painting technique for customized screening of anticancer compounds
35	Arturas Polita — Revealing Lipid Order Differences Between Cancerous and Non-Cancerous Cells Using Fluorescent Viscosity Probes
37	Jose Rino — GIMM Bioimaging — A novel State-of-the-art, Multi-user, Multiscale Bioimaging Facility in the Lisbon area

#	Author — Title
39	María Teresa Vallejo-Cremades — Comparative study of different image analysis systems
41	Katrin Walter — The hidden architecture of the giant unicellular algae *Caulerpa*
43	Julien Colombelli — Dual-view oblique plane microscopy (dOPM) for high-content imaging of complex and heterogeneous 3D cancer organoid models
45	Gloria Bueno — AI-driven automated identification and genetic characterization of planktonic cyanobacteria
47	Ruta Bagdonaite — Effect of alkylphospholipids on the biophysical properties of model lipid bilayers and plasma membranes of live cells
51	Francisca Molina — Comparative study of collagen fibers in clinical samples of esophageal epithelium using epifluorescence combined with deconvolution and multiphoton microscopes

Posters II

#	Author — Title
2	Lenka Backova — Bioimage analysis and biophysical modeling of multicellular systems
4	Alvaro Carrasco-Carmona — Enhancing Tissue Analysis through Fluorescence Microscopy with Laser Microdissection and Spatial Omics
6	Mariana Carvalho — Combining Holotomography and Confocal Microscopy for Cellular Nanodiamond Localization Studies
8	Jakub Chojnacki — Super-resolution microscopy within high-level biosafety facility for live studies of pathogen-cell/tissue interactions
12	Raquel García Olivas — Optimizing Core Facility Workflows for User Training and Reproducibility in Quantitative Imaging
14	Esther González Almela — Viral infection reshapes host genome structure by sequestering RNAP II
16	Nadia Halidi — QUAREP-LiMi: A global community-driven initiative developing common quality assessment and quality control guidelines, protocols, and tools for light microscopy
18	Marta Huerta Ortuno — High-resolution cryo-correlative microscopy to study immunogenic cell death mechanisms
20	Hyun-Su Kim — Tomographic Imaging Using Reflective Fourier Ptychography
22	Sumin Lee — Segmentation and Analysis Method for 3D Cell Structures Enhanced with Open-Source AI Tools in Holotomography Images
24	Dolores López Maderuelo — 3D-analysis of single cell clonal expansion in whole adult mouse hearts

#	Author — Title
26	Monica Martín Belinchon — Optical and confocal microscopy facility at the IIBM
28	Nuno Moreno — Biomedical Data Hub ? A CaixaResearch initiative
30	Alicia Nieto Valle — Identifying specific macrophages as predictive biomarkers in human melanoma: older vs newer confocal acquisition and segmentation approaches
32	Julia Rubio Loscertales — Can we trust the diffusion coefficient in biological membranes?
34	Joan Repullés — Imaging infectious diseases: the IRTA-CReSA BSL3 Bioimaging Platform
36	Ana M. Santos — Microscopic features of fiber suture deposition patterns in common bean pods during domestication using fluorescent stains and TEM analysis
38	Aliana Vairinhos — Impact of polystyrene nanoplastics and bisphenol A on skin fibroblast morphology
40	Beatriz Villarejo Zori — A novel plugin for the extensive organelle features detection in the three-dimensional space
42	Gabriel Martins — Instrumentation Quality Control in the Portuguese Platform for Bioimaging - PPBI
44	Julien Colombelli — Scattered lightsheet microscopy for label free cleared tissue imaging
46	Haoran Wang — An open source super-resolution microscopy extension with real time reconstruction capability
48	Denitza Denkova — Developing a 2-photon light-sheet imaging methodology for functional imaging of life retinal explants
50	Ioannis Georvasilis — Advancing Diagnosis of COL6-CMD: AI and Transfer Learning in Muscle Tissue Analysis
52	Clara Pertusa — Increased resolution of organelles and cytoskeleton imaging by combining STED with fluorescence lifetime of fluorochromes



INVITED TALKS

KEYNOTE

IMAGING RETINAL ORGANOIDS WITH DYNAMIC FULL FIELD OPTICAL COHERENCE TOMOGRAPHY

Kate Grieve

Vision Insitute, France

Dynamic full field optical coherence tomography (DFFOCT) allows label free live imaging of 2D and 3D cell cultures in the lab. I highlight recent technical developments, including strategies for long term imaging of retinal organoids cultured for disease modeling, and present results on retinal degeneration models.

INVITED TALK

APPLICATION OF FUNCTIONAL, MULTIDIMENSIONAL OPTICAL MICROSCOPY TO ANALYZE THE FUNCTION OF MYELOID CELLS DURING INFLAMMATION AND BONE REGENERATION

Anja E. Hauser

DRFZ; Germany

My lab is interested in analyzing immune cells in the tissue context in health and disease. Focusing on bone regeneration after injury, we aim to understand how the tissue microenvironment affects the metabolism of myeloid cells in the bone marrow over time, and how that impacts on their function. We previously demonstrated that CX3CR1+ myeloid cells act as trailblazers for osteogenic type H vessels in the bone marrow. In order to analyze this process in 3D, we developed a tissue clearing, staining and light sheet fluorescence microscopy imaging pipeline called MarShie, optimized to image the entire intact femur at subcellular resolution down to the deepest bone marrow regions. To analyze the three-dimensional dataset, we applied a machine learning approach, enabling us to segment thousands of cells. We show that in aged mice the draining sinus massively decreases in volume while transcortical vessels also decline. We find that during homeostasis CX3CR1+ myeloid cells localize in perivascular niches, whereas CD169+ myeloid cells are dispersed in the parenchyma. After injury, CX3CR1+ myeloid cells relocate and sequester the injury site prior to vascularization. Analysis of the femur after full osteotomy reveals that vessel sprouting is initiated at periosteal regions.

Phenotypes and functions of immune cells are tightly linked to their metabolic profile, which in turn is affected by changes in the tissue microenvironment. We developed longitudinal intravital imaging of the mouse femur, to enable longitudinal micro-endoscopic fluorescence lifetime imaging (FLIM) for metabolic profiling ("MetaFLIMB"). Using our previously developed reference system of enzyme-dependent fluorescence lifetimes derived from the ubiquitous metabolic co-enzymes NADH and NADPH (NAD(P)H), we can determine preferential enzymatic activities in vivo. We stratify enzymatic activities to identify dominant metabolic pathways for energy production. Additionally, we distinguish pathways associated to cellular function and cellular state, i.e. oxidative burst (NADPH oxidase activity) and dormancy or death, indicated by low/no NAD(P)H-dependent enzymatic activity, reflected by an increase in unbound NAD(P)H.

Using MetaFLIMB in osteotomized femurs of mice with red fluorescent myeloid cells, we demonstrate that myeloid cells display highly heterogeneous metabolic profiles both spatially and temporally during bone regeneration. Our results go beyond the binary paradigm of myeloid cells using either glycolytic or oxidative signaling pathways (linked to pro- or anti-inflammatory functions, respectively) derived from in vitro experiments. Under in vivo conditions, myeloid cells with various metabolic profiles, i.e. using other pathways for energy production than the anaerobic pathway associated with pro-inflammatory cells, performed the oxidative burst necessary for the process of phagocytosis.

This demonstrates that a high metabolic flexibility of myeloid cells in vivo is related to their functional flexibility. It suggests that myeloid cells, which perform an oxidative burst and are responsible for clearing of debris at early stages after bone injury, and those responsible for vascular or bone remodeling during the later phases, use distinct pathways for energy production.

INVITED TALK

FROM PLASTIC TO CRYO: WORKFLOWS TO EXPLORE TISSUE AT ULTRASTRUCTURAL LEVEL

Christel Genoud

UoL, Switzerland

In this talk, a set of workflows designed to assist researchers in choosing and applying the most suitable electron microscopy (EM) methods for their specific biological questions will be discusses. The focus will be on demonstrating how these workflows, ranging from traditional plastic embedding to advanced cryo-preservation techniques, enable the detailed exploration of tissue at the ultrastructural level.

By showcasing various examples, I will illustrate how our platforms facilitates the optimal use of different EM techniques, ensuring that our users can obtain the most accurate and insightful results for their research. Whether addressing structural biology, cellular interactions, or molecular mechanisms, this presentation will highlight the versatility and precision of our EM workflows in advancing scientific understanding.

INVITED TALK

INTRODUCING THE RMS AND BIOIMAGINGUK

Georgina Fletcher
Royal Microscopy Society (RMS), UK

I will discuss the aims and activities of the RMS, an international microscopy society that began in 1839, as well as the more recent UK bioimaging community network, BioImagingUK.

INVITED TALK

HOW CAN DEEP LEARNING ENHANCE MICROSCOPY?

Giovanni Volpe
GU, Sweden

Video microscopy has a long history of providing insights and breakthroughs for a broad range of disciplines, from physics to biology. Image analysis to extract quantitative information from video microscopy data has traditionally relied on algorithmic approaches, which are often difficult to implement, time consuming, and computationally expensive. Recently, alternative data-driven approaches using deep learning have greatly improved quantitative digital microscopy, potentially offering automatized, accurate, and fast image analysis. However, the combination of deep learning and video microscopy remains underutilized primarily due to the steep learning curve involved in developing custom deep-learning solutions. To overcome this issue, we have introduced a software, currently at version DeepTrack 2.1, to design, train and validate deep-learning solutions for digital microscopy.

INVITED TALK

CELL 3D POSITIONING BY OPTICAL ENCODING (C3PO) AND ITS APPLICATION TO SPATIAL TRANSCRIPTOMICS

James Swoger

EMBL Barcelona, Spain

Most current state-of-the-art spatial -omics techniques require physically cutting the biological tissue of interest into thin slices, and are therefore intrinsically 2D methods. However, for many modern biological questions the 3D context is key. I will present our work on Cell 3D Positioning by Optical encoding (C3PO), in which we use a light-sheet microscope to optically write a pattern of 3D fluorescent gradients into an intact sample. Once this is done, the sample can be dissociated into individual cells on which transcriptomics, for example, can be performed. By dissociating the cells we lose the information about their original spatial positioning. However, because we have written the 3D fluorescence gradients into the cells before dissociation, after dissociation these fluorescent levels can be read by a FACS machine and used to map each individual cell back into the 3D space of the sample. Transcriptomics can then be done on cells whose original 3D positions in the sample are known.

INVITED TALK

CRYO-CORRELATIVE LIGHT AND ELECTRON MICROSCOPY

Javier Conesa

CNB, Spain

Correlative light and electron microscopy (CLEM) is a structural biology methodology to solve biological problems. This methodology, applied in cryogenic conditions, allows the extraction of high resolution structural 3D information in the native cellular context avoiding artefacts related to chemical fixation and dehydration. CLEM allows the study of cellular samples by means of cryo-fluorescence microscopy to locate interesting cellular events while cryo-focus ion beam-scanning electron microscopy (cryo-FIB-SEM) serial sectioning is used to analyse the cellular ultrastructure at the level of cellular organelles or, alternatively, cryo-electron tomography is used to analyse the 3D structure of protein complexes and their distribution at molecular resolution. At CNB-CSIC we are implementing a cryo-correlative platform aiming to provide users with dedicated tools to perform CLEM in cryogenic conditions in a variety of workflows, including the usage of cryo-epifluorescence, cryo-confocal fluorescence, cryo-FIB-SEM and cryo-ET, to investigate and answer questions to biological events occurring in the cell.

INVITED TALK

VISUALIZING THE NATIVE CELLULAR ORGANIZATION BY COUPLING CRYO-FIXATION WITH EXPANSION MICROSCOPY (CRYO-EXM)

Marine Laporte

Univ-Lyon, France

Super-resolution fluorescent microscopy (SRM), encompassing expansion microscopy (ExM) since few years now, allows to locate proteins with nanometer resolution in a cellular context. However, SRM often requires cell fixation with aldehyde-based chemical crosslinkers, such as paraformaldehyde, or protein precipitation with cold methanol which potentially alter the native cellular state and the following interpretations. Cryo-fixation has proven to be the gold standard for efficient preservation of the native cell ultrastructure compared to chemical fixation, however it is not widely used in fluorescence microscopy owing to implementation. We recently developed a method combining cryo-fixation to ExM (Cryo-ExM), which allow nanoscale observation of a wide cellular compartment in their native state. We could demonstrate that Cryo-ExM allows the native preservation of membrane-based organelles such as mitochondria, endoplasmic reticulum, golgi and lysosomes together with the cytoskeleton component actin and microtubules and preserve all the structure in the same way, contrary to chemical fixations. Moreover, direct comparison with the gold-standard chemical fixation PFA-GA for the preservation of cellular structure, demonstrate that cryo-fixation bypassed drawbacks associated with this chemical fixation such as antigen accessibility due to strong protein-protein crosslinking. Overall.

In summary, we introduce a new method to perform super-resolution expansion microscopy by coupling cryo-fixation of a biological specimen with ExM, providing a universal framework to visualize subcellular compartments without chemical fixation artefacts. Importantly, this method also demonstrates that the classical cryo-substitution protocols developed for electron microscopy are compatible with expansion microscopy by replacing the EM resin with hydrogel monomer solutions. Therefore, this approach may also be applicable on tissues cryo-fixed by high-pressure freezing as well as in hydrogel-based tissue clearing. Finally, as expansion microscopy is also compatible with SIM, STED or dSTORM 18–20, our method now allows all these microscopy modalities to image cells in their native state, paving the way for further studies of complex and rapid dynamic cellular processes.

INVITED TALK

IMAGING OXYGENATION AND METABOLISM IN ORGANOIDS USING FLIM AND PLIM

Ruslan Dmitriev

Tissue Engineering and Biomaterials Group, Ghent University, Ghent, Belgium

Ghent Light Microscopy Core (GLiM), Ghent University, Ghent, Belgium

Success in engineering of organoids, spheroids and related 3D microtissues is often hampered by their intrinsic heterogeneity and dynamic gradients of biomolecules. Live changes of cell metabolism, mitochondrial activity, physical characteristics and O2 are hard to predict and control without dynamic microscopy approaches. My group addresses the challenge of non-destructive quantitative multi-parametric imaging of 3D tissue models by using high-performance nanosensors, small molecule conjugates and live fluorescence (FLIM) and phosphorescence (PLIM) lifetime imaging microscopies. In my talk I will discuss how the imaging of autofluorescence, hypoxia, and other (bio)markers can be performed in stem cell-derived small intestinal organoids. I will discuss how “FLIM of organoids” methodology can address the emerging biomedical problem of the micro- and nanoplastic pollution.

INVITED TALK

FIVE COLOR SUPER RESOLUTION MICROSCOPY WITH FLIM STED

Steffen Dietzel

LMU, Germany

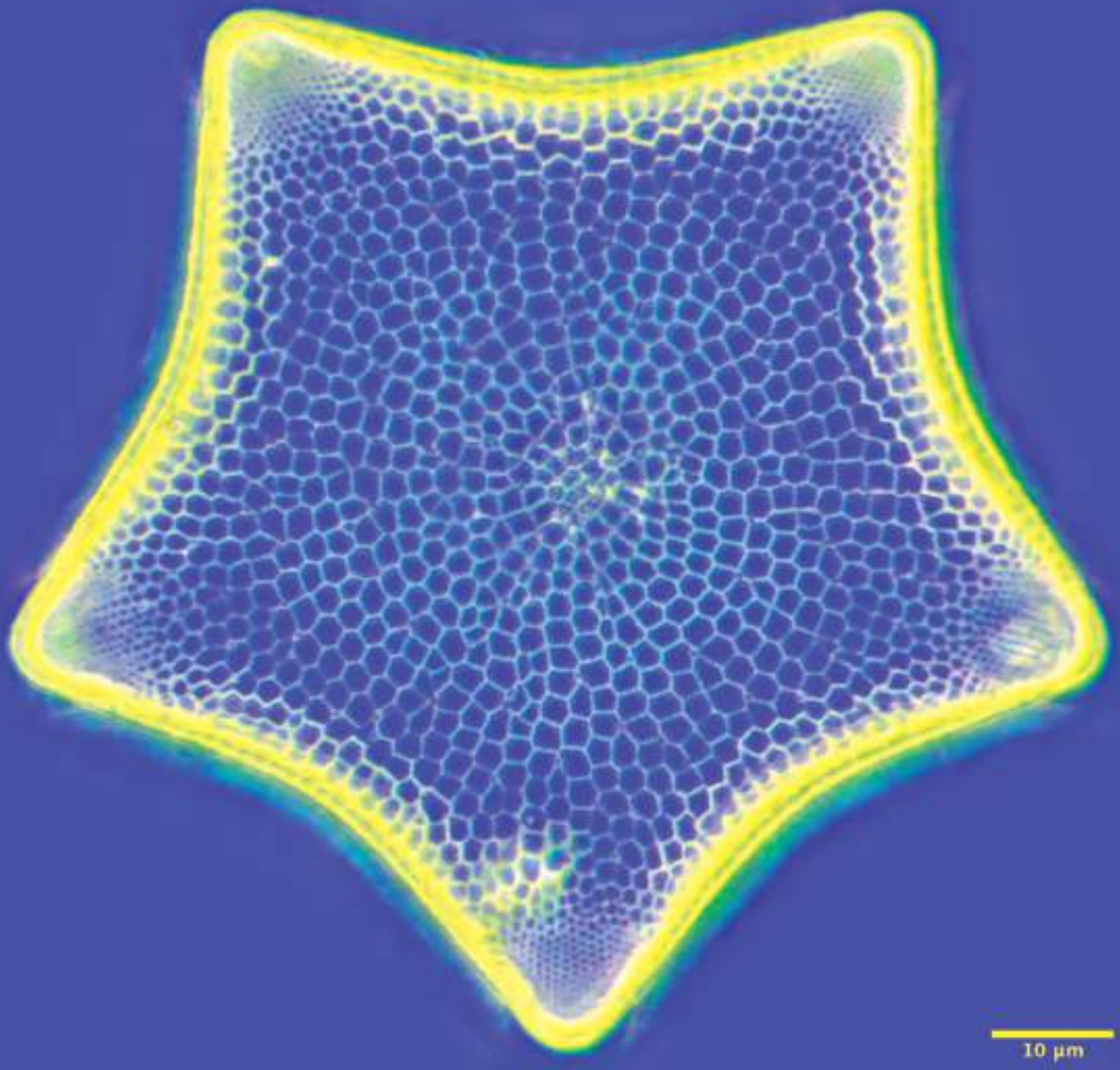
For multi-color STED (Stimulated emission depletion microscopy) with multiple depletion laser wavelengths, alignment of depletion lasers requires highest precision in the nanometer range. It is thus preferable to apply a single depletion wavelength for several fluorochromes. But this limits the number of color channels that can be used.

Using fluorescence lifetime imaging (FLIM) to separate labels was introduced to STED in 2011 by the group of Stefan Hell. However, FLIM equipment at that time was slow and difficult to operate compared to a confocal microscope and not many studies followed that path.

Technical progress and the development of phasor based analysis of FLIM data nowadays permit easy and fast fluorochrome separation in confocal and STED microscopy. Moreover phasor based separation requires much smaller photon numbers when compared to curve fitting when there is background present.

A labeling scheme will be presented that can be applied in life science environments, e.g. on cultured human cells, with common staining protocols, commercially available fluorochromes and a turn-key FLIM confocal or FLIM-STED microscope. The number of usable fluorochromes in STED or confocal microscopy can generally be doubled by phasor based fluorescence lifetime separation of two dyes with similar emission spectrum but different fluorescent lifetimes.

In a five color FLIM-STED approach we depleted with 775 nm two fluorochromes each in the near red (exc. 594 nm, em. 600-630) and far red channel (633/641-680), supplemented by a single fluorochrome even further redshifted (670/701-750). This allowed us to record five color STED samples with a single depletion laser. Current data suggest that eight color FLIM-STED with a single depletion laser would be possible if suitable fluorochromes were identified



CONTRIBUTED TALKS

CONTRIBUTED TALK

Advanced imaging for visualizing the functional circuitry of retinas in visual disorders therapies

Enric Pérez Parets[1], Denitza Denkova[1], Gustavo Castro Olvera[1], Marina Cunquero[1], Simone Mortal[1], Anna Duarri[2], Pablo Loza-Alvarez[1]

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KEY WORDS: light-sheet microscopy, two-photon microscopy, fast volumetric imaging, retina calcium dynamics, photoreceptor cell therapy

Visual disorders such as Age-related Macular Disease and Retinitis Pigmentosa stem from disruptions in retinal circuitry. Thus, to advance treatment strategies, it is of paramount importance to study such disruptions in the retinal circuitry – a challenging task. Our research investigates retinal calcium dynamics in response to luminous stimuli, employing advanced imaging techniques. Initial 2D imaging of the retina, focusing on the ganglion cell layer (GCL), is being conducted via calcium imaging in a two-photon scanning microscope while stimulating the retina with an LED in the visible range. This foundational data will form the basis for subsequent imaging using light sheet fast volumetric microscopy. First, we will assess the feasibility of reproducing similar data with two-photon light sheet microscopy in 2D, emphasizing its potential for studying dynamic cellular processes while minimizing phototoxicity and photobleaching. Second, taking advantage of the volumetric information given by light-sheet microscopes, the last step of the project will consist in performing the experiments in 3D. The obtained data will be studied through connectivity maps, unravelling the functional organization of the retinal circuitry in the three different study cases. Therefore, our project aims to deepen the understanding of retinal physiology and pathology, contributing to the development of effective tools to study visual disorders and asses their treatments.

CONTRIBUTED TALK

Advancing Diagnosis of COL6-CMD: AI and Transfer Learning in Muscle Tissue Analysis

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KEY WORDS: rare diseases, collagen VI, confocal microscopy, mapping, artificial intelligence, cellpose

Diagnosing rare neuromuscular disorders, such as Collagen VI-related Congenital Muscular Dystrophy (COL6-RD), presents significant challenges due to its low prevalence and the broad spectrum of clinical presentations, which often overlap with other forms of muscular dystrophy. Although genetic testing is the definitive diagnostic method, it is time-consuming and not always immediately available. A common diagnostic approach involves immunofluorescence analysis to evaluate collagen VI expression patterns. However, this method is laborious, subjective, and depends heavily on the clinician's expertise.

The incorporation of Artificial Intelligence (AI) offers promising potential to streamline and standardize the diagnostic process for rare diseases like COL6-RD [4-5]. Nevertheless, effective AI algorithms require large and representative datasets that capture the full heterogeneity of clinical presentations. This presents a major obstacle in the context of rare diseases, where limited data availability hinders the development of robust AI models. Specifically, utilizing a dataset comprising 154 confocal microscopy images of muscular tissue from a mouse animal model, categorized into three genotypic groups, the implementation of data augmentation techniques, including patching, appears insufficient to provide the necessary heterogeneity and statistical robustness essential for the reliable training of deep learning (DL) models.

To address the challenges in diagnosing COL6-RD, we leverage a deep learning-based approach using Cellpose, a pretrained model for cellular segmentation [1-3]. Cellpose's encoder, which is highly effective at extracting generalizable features from biological images, serves as the backbone of our model. By utilizing the pretrained weights of the Cellpose encoder, we exploit its extensive knowledge of cellular structures, thereby augmenting our model's capacity to identify patterns specific to COL6-related congenital muscular dystrophy (COL6-RD). To adapt the model for classification, we append fully connected layers to the encoder and fine-tune the entire network using our dataset. This transfer learning strategy enhances classification accuracy by

incorporating the robust feature extraction capabilities of Cellpose, which mitigates the challenges posed by limited data availability in rare disease diagnosis.

Our fine-tuned model demonstrates superior performance, surpassing models trained from scratch. By leveraging the pretrained weights of the Cellpose encoder, the model inherits robust feature extraction capabilities, which improves its ability to capture disease-specific patterns in the classification of COL6-RD images. This transfer learning approach has demonstrated superior classification accuracy compared to models built from random initialization, particularly in scenarios with scarce data. These findings suggest that incorporating pretrained models significantly enhances performance and mitigates the challenges posed by data scarcity in rare disease diagnosis.

Acknowledgments

We gratefully acknowledge the support of the European Commission, whose funding through the HORIZON-MSCA BE-LIGHT project (GA nº 101119924) has been crucial for advancing AI-driven diagnostics for rare neuromuscular disorders.

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CONTRIBUTED TALK

ANTHRACYCLINES ACCUMULATION IN CARDIAC TISSUE: IN SITU DETECTION BY PHASOR-FLIM

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KEY WORDS: multiphoton, fluorescence lifetime imaging, doxorubicin, anthracyclines, cancer

STYLE: Anthracyclines such as doxorubicin induce cardiotoxicity deteriorating quality of life of cancer survivors. Despite it is known for several decades that cumulative doses of doxorubicin lead to increased risk for cardiotoxicity, the intracellular distribution of doxorubicin and its metabolites in the heart has not been fully understood yet. In this study, we took advantage of the fluorescent properties of the drug and its predominant metabolites using for the first time a 2-photon phasor-FLIM approach to evaluate the distribution of doxorubicin-related molecules in-vivo. CD1 mice underwent once weekly intraperitoneal injections (5 mg/kg) of doxorubicin for 5 weeks. At selected time points, mice were sacrificed and hearts harvested for imaging on fresh slices (3x3x0.5mm). The combination of calibrated single photon counting detection and phasor analysis of the fluorescence lifetime distributions provided an estimate of the type and in-situ accumulation doxo-related molecules. These results complement standard Mass Spectrometry batch quantification from homogenates of fresh tissue, adding a real-time detection of the complex doxorubicin interactions occurring in the tissue at the intracellular level and as a function of the in-vivo treatment. Our advanced 2-photon Phasor-FLIM analysis of doxorubicin in-vivo offers a powerful tool to better understand the relationship between drug exposure (at clinically relevant concentrations) and its cellular detrimental effects on cardiac tissue.

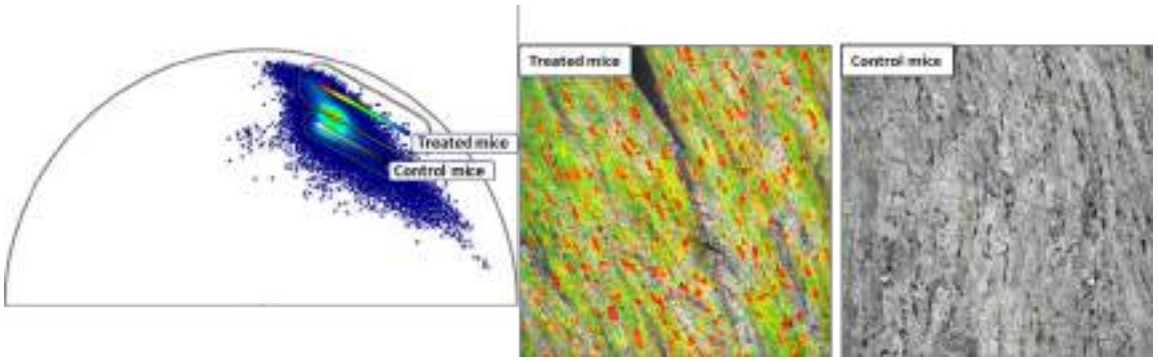

Figure 1. 2P-Phasor-FLIM analysis of heart sections after in-vivo doxorubicin administration in mice. (A) Phasor plot of doxorubicin-positive (color LUT) and doxorubicin-negative (grey LUT) pixels superimposed to the average intensity images from a treated and an untreated example. Colors depict the changes in doxorubicin fluorescence lifetime as a function of the local environment of the drug.

CONTRIBUTED TALK

Automated Alignment Strategies for Integrating Cryo 3D-SIM and SXT Data

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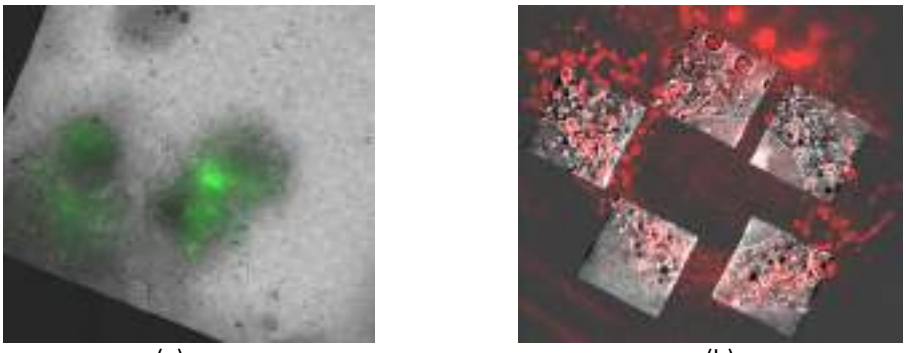
KEY WORDS: Super-resolution, correlative microscopy, cryo 3D-SIM, Soft X-ray Tomography, automated alignment, cellular structures

The correlation between Cryo 3D Structured Illumination Microscopy (3D-SIM) and Soft X-ray Tomography (SXT) offers a comprehensive tool for exploring complex biological systems, but aligning datasets from these distinct imaging modalities presents significant challenges. Our study aims to develop novel algorithms for automated alignment to enhance the integration of cryo 3D-SIM and SXT data, improving the accuracy and efficiency of correlative microscopy. We utilize feature-based matching to identify common structural landmarks within both datasets, followed by a two-step alignment process involving coarse 2D alignment and fine 3D refinement. The initial 2D alignment aligns a Soft X-ray mosaic view with a 2D projection of the 3D fluorescence data, while the subsequent fine alignment refines spatial correspondence between the two 3D datasets. Additionally, we address the sparsity of SXT data in the Fourier domain with a novel 3D correlation process based on 2D-correlation backprojection, focusing solely on effective data through tomographic reconstruction of 2D projection correlations.

Our methods proved effective in accurately aligning 2D and 3D data, using samples like Huh-7.5 carcinoma cells and NIH-3T3 cells. The automated alignments closely matched manual results, confirming the reliability of our approach and enhancing investigations into cellular structures.



(a) (b)

Figure 1. (a) Automated 2D correlation of Huh-7.5 cells using cryo-3D fluorescent microscopy projection showing the mitochondria in green (mitotracker) and cryo-SXT mosaic projection mosaic; (b) Automated 3D correlation of a TPR-Hsp90-AuNC treated NIH-3T3 cell using cryo-3D-SIM data showing the mitochondria in red (cmxros mitotracker) and cryo-SXT showing the reconstructed linear absorption coefficient. A single slice of the combined tomographic reconstruction in gray levels is shown overlaid with a single slice of the cryo-3D-SIM.

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CONTRIBUTED TALK

CORRELATING RAMAN MICROSPECTROSCOPY WITH STANDARD HISTOLOGY TO DEMONSTRATE ITS POTENTIAL IN BREAST CANCER DIAGNOSIS

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KEY WORDS: Raman microspectroscopy, breast cancer, multivariate analysis, histology

Early and accurate diagnosis of breast cancer is crucial for improving patient outcomes. Currently, breast cancer diagnosis relies on complex histological studies, which are time-consuming. This study explores the potential of Raman microspectroscopy (RMS) as a label-free alternative to speed up diagnosis and provide additional chemical information. As a first step, we aim to demonstrate the capability of RMS to distinguish between different tissue components of tumoral biopsies.

For this, frozen unstained sections of breast tumors from surgical resections were prepared on quartz slides. RMS images were then acquired, and the Multivariate Curve Resolution (MCR) algorithm[1] was applied to identify characteristic bands associated with different biological components. Posterior histological hematoxylin and eosin (H&E) staining, the gold standard method, was then compared with the RMS results, demonstrating a strong correlation between the cytochrome c distribution and the presence of tumoral nests (Fig. 1).

Our results highlight the potential of RMS as a diagnostic tool, capable of providing detailed molecular profiles that complement traditional histological methods.

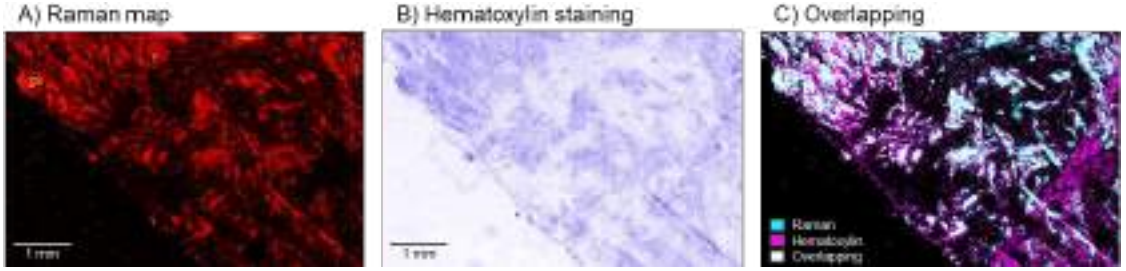

Figure 1. Comparison between Raman map and H&E staining.

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CONTRIBUTED TALK

SPATIAL PROTEIN LOCALIZATION IN PHOTORECEPTOR DISCS REVEALED BY EXPANSION MICROSCOPY AND NANOSCOPY

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KEY WORDS: Retina, super-resolution, expansion microscopy, photoreceptors, cytoskeleton,d

Vision originates within a stack of tightly packed disc-shaped membranes, or 'discs', located in the outer segment of photoreceptor cells, where the most abundant protein is rhodopsin, the key player in phototransduction. Traditionally, discs have been observed using electron microscopy, which can reveal their structure but cannot provide a precise protein localization, leaving their exact distribution uncertain. To overcome this limitation, we combined expansion microscopy with STED nanoscopy, achieving a resolution below 15 nm, thereby surpassing previous limitations in observing discs. This enabled us to precisely identify proteins and outline their distribution in photoreceptors. Analyzing the disc composition, we observed that around 90% of the disc area is decorated by rhodopsin, and the inter-disc spacing is more irregular compared to what is observed in electron microscopy observations. Furthermore, we localized proteins such as peripherin-2, responsible for maintaining the disc shape and its structural components. Lastly, we investigated how the connecting cilium penetrates within the rod outer segment and its relationship with disc proteins. Our results collectively suggest a larger spatial distribution of rhodopsin in the disc than previously described, leading to a lower protein concentration. Moreover, we found that the connecting cilium plays a significant role in determining the final shape and orientation of the disc. These findings provide new insights into the molecular architecture of photoreceptor discs, which could have significant implications for better understanding visual processes and the pathogenesis of retinal diseases like retinitis pigmentosa.

CONTRIBUTED TALK

"PhotoFiTT: A Framework for Assessing Phototoxicity in Live-Cell Microscopy Experiments"

***Mario Del Rosario*[1,*], *Estibaliz Gómez-de-Mariscal*[1,*], *Leonor Morgado*[1], *Guillaume Jacquemet*[2, 3, 4, 5], *Pedro M. Pereira*[6], *and Ricardo Henriques*[1,7]**

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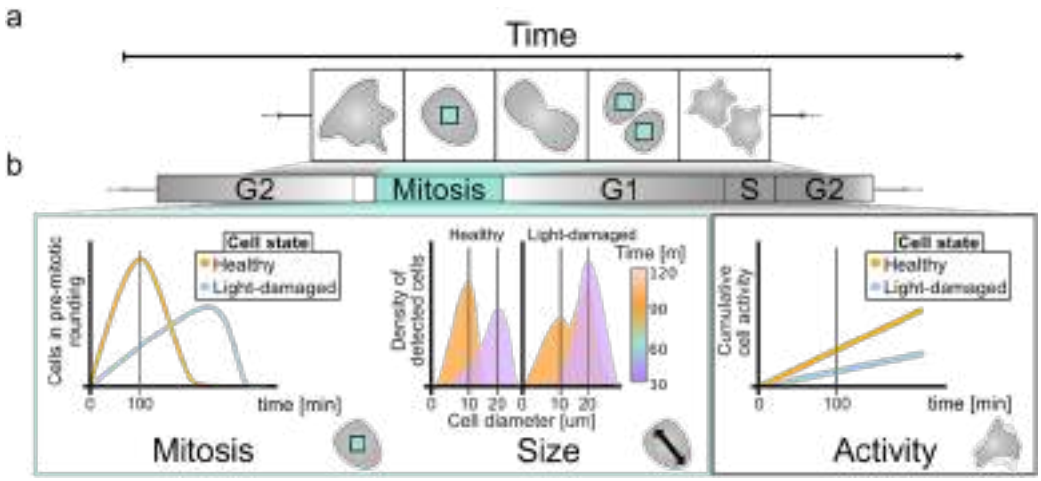
Keywords: phototoxicity, live-cell microscopy, AI, cell behaviour

Abstract

Fluorescence microscopy is indispensable for live-cell studies, yet the intense illumination required, particularly in advanced techniques, can induce phototoxicity, distorting cellular behaviour and compromising experimental outcomes. To address this critical challenge, we introduce the Phototoxicity Fitness Time Trial (PhotoFiTT) assay - a novel, label-free method that leverages machine learning and sophisticated image analysis to quantify phototoxic effects by measuring subtle changes in cell behaviour. Furthermore, we propose a paradigm shift in data acquisition and analysis. Rather than attempting to correct data compromised by excessive light exposure, we advocate for the integration of artificial intelligence to enhance data obtained through gentle imaging protocols. This approach uses readily available frameworks such as ZeroCostDL4Mic, enabling researchers to extract quantitative, robust, and highly reproducible data while minimising photodamage. The PhotoFiTT assay is a powerful tool for optimising imaging protocols, ensuring that biological insights derived from live-cell fluorescence microscopy are accurate and physiologically relevant.



Overview of the Phototoxicity Fitness Time Trial (PhotoFiTT) assay for quantifying phototoxicity effects in live-cell fluorescence microscopy. (a) The biological assay involves performing live-cell imaging after exposing cells to light irradiation. (b) The image analysis pipeline measures key cellular behaviors, including the mitotic rate, cell diameter (teal), and cellular activity outside of mitotic rounding events (grey). By detecting deviations from the expected sequence of these cellular events, PhotoFiTT measures the extent of cellular photodamage induced by the imaging conditions.

CONTRIBUTED TALK

SAMJ: Fast annotation with foundation models on Java-based Bioimage Platforms

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KEY WORDS: image annotation, segment anything model, java deep learning library, appose, fiji, icy

STYLE: The SAMJ Annotator [1] introduces a transformative plugin tailored for compatibility with ImageJ, Fiji, and Icy, providing advanced solutions in image-segmentation through state-of-the-art transformer-based models, such as the Segment Anything Model (SAM) variants [2]. These models are specifically effective in handling the complexities of diverse objects within optical microscopy images. The tool is crafted for user-friendly operation, allowing for manual annotations within Fiji to achieve precise contouring and efficient segmentation without the need for a GPU.

Significantly, the SAMJ Annotator leverages the power of SAM models, optimized for real-time performance. The installation and operational efficiencies are further enhanced by JDLL [3, 4], facilitating a streamlined interface between Python and Java via shared memory techniques, supported by Appose [5].

One of our focus is SAMJ Annotator's impact on the field of bioimage analysis, demonstrating its utility in significantly improving the annotation of extensive 2D images. This innovation is crucial for generating accurately labeled datasets essential for advanced research and application. Our discussion extends to the seamless integration and practical deployment of SAMJ, underscoring its potential to revolutionize image analysis workflows and enhance collaborative research.

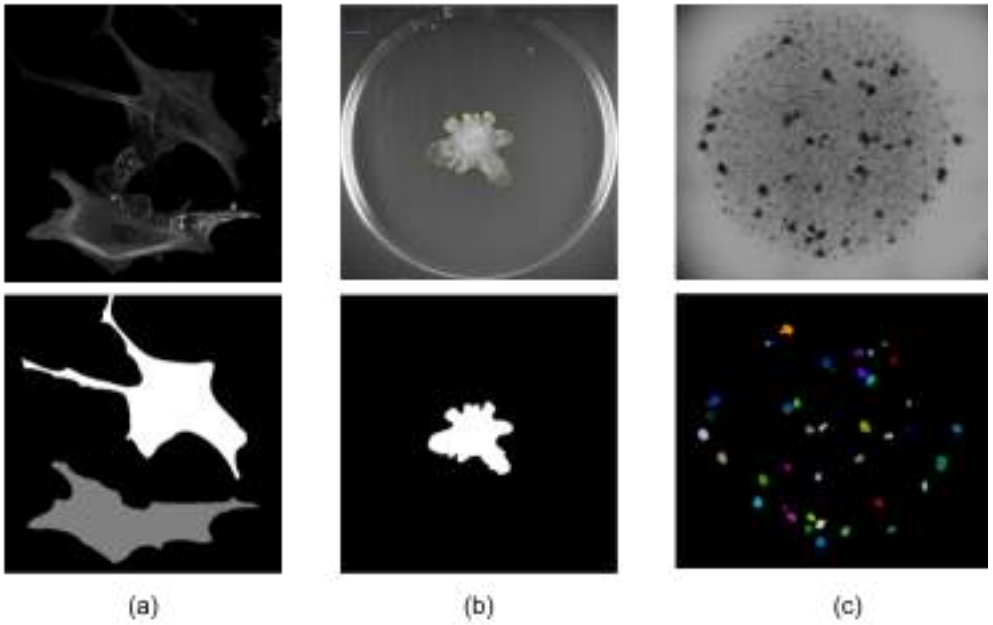

Figure 1. Illustrative Examples of Semi-Automatic Annotation Using SAMJ in Fiji. (a) Astrocytes stained for actin, post-deformation annotation, sourced from reference [6]. (b) Annotation of bacterial motility on agar plates, sourced from reference [7]. (c) Annotation of organoids to assess segmentation, counting, and measurement of eccentricity areas, sourced from reference [8].

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[8] Segmentation, counting, measurement of area and eccentricity (circularity) of organoids in image.sc forum

CONTRIBUTED TALK

Super resolution microscopy reveals the interaction between nascent RNA, transcription factories and chromatin.

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KEY WORDS: Super-resolution, quantitative microscopy,STORM, transcription, RNA.

Transcription and genome architecture are interdependent, but it is still unclear how nucleosomes in the chromatin fiber interact with nascent RNA, and which is the relative nuclear distribution of these RNAs and elongating RNA polymerase II (RNAP II). Using super-resolution (SR) microscopy, we visualized the nascent transcriptome in the nucleus with nanoscale resolution. We found that nascent RNAs organize in structures we termed RNA nanodomains, whose characteristics are independent of the number of transcripts produced over time. Dual-color SR imaging of nascent RNAs, together with elongating RNAP II and H2B, shows the physical relation between nucleosome clutches, RNAP II, and RNA nanodomains. The distance between nucleosome clutches and RNA nanodomains is larger than the distance measured between elongating RNAP II and RNA nanodomains. Elongating RNAP II stands between nascent RNAs and the small, transcriptionally active, nucleosome clutches. Finally, we describe a novel approach to quantify the transcriptional activity at an individual gene locus. By measuring local nascent RNA accumulation upon transcriptional activation at single alleles, we confirm the measurements made at the global nuclear level.

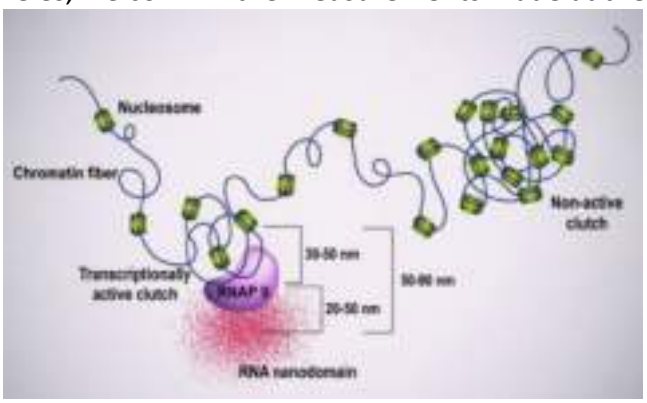

Figure 1. Schematic representation depicting the association between nucleosome clutches, RNAP II clusters and RNA nanodomains. Chromatin is organized in clutches. Active nucleosome clutches are associated with elongating RNAP II clusters (in purple) and small RNA nanodomains (in red).

CONTRIBUTED TALK

Zero-Shot Object Detection with Foundational Models: A Similarity-Based Approach

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KEY WORDS: Electron microscopy, object detection, semantic segmentation, foundational model

Foundational models, such as DINOv2 [1], have demonstrated significant potential to perform image processing tasks downstream across domains with minimal fine-tuning. Here we present a new method leveraging DINOv2 pertained encoder, for zero-shot object detection and segmentation in biomedical imaging, specifically targeting the lack of sufficiently annotated complex datasets. Our approach focuses on measuring the similarity between each patch and a chosen reference patch. Our findings demonstrate the method's ability to detect and segment unseen objects in electron microscopy images. We also examine the influence of prompts on model accuracy and generalization, offering insights into prompt selection strategies. Furthermore, we introduce an open-source Napari plugin to streamline the application of this method in scientific research. Our approach, accessible through a user-friendly interface, provides a flexible and generalizable out-of-the-box solution for object detection, providing an advanced solution for those domains with limited labelled data and contributing to diminishing the carbon footprint generated by current AI advances.

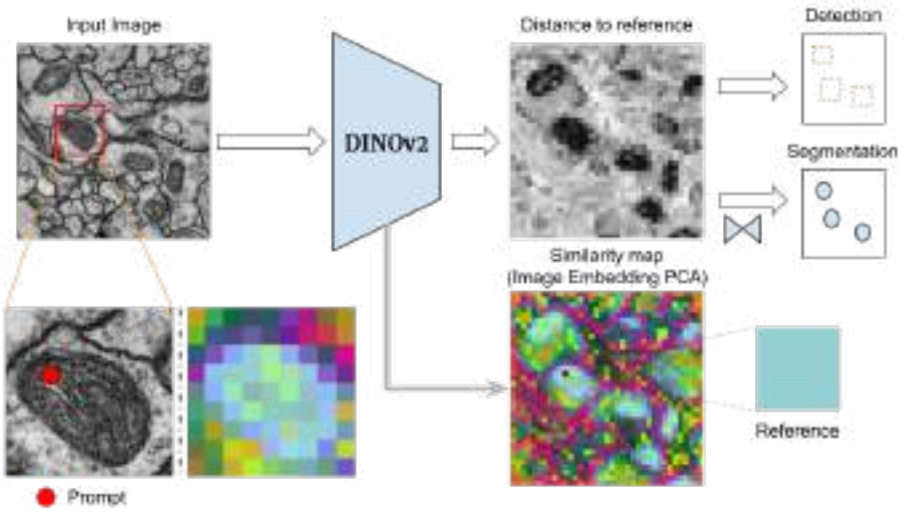

Figure 1. Zero-shot workflow representation: From left to right, we see the input image with the given prompt and the distance map to the selected reference patch. The image embedding is also visualized using its three principal components.

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CONTRIBUTED TALK

INTRODUCTION TO AI4LIFE AND BIOIMAGE MODEL ZOO

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KEY WORDS: DL models, AI, microscopy, image analysis, community partners, FAIR repository, open source, chatbot.

Together with participants, we will dive into the BioImage Model Zoo (bioimage.io) [1], an accessible and user-friendly repository of FAIR, pre-trained deep learning models designed for the life sciences. Participants will explore the current collection of models, interact with detailed model descriptions via cards, and test models directly on the website and through partner desktop tools like Ilastik [2] and deepImageJ [3]. We will demonstrate how to leverage pre-trained models in end-user software without needing code, enhancing usability and reproducibility. Additionally, attendees will engage with the AI chatbot [4] for expert tips on image analysis and learn about complementary projects from the AI4Life ecosystem, including FAIR annotation practices, reproducible model training environments, and the development of the BioImage.IO platform.

Figure 1. Building bridges: The AI4Life ecosystem connecting life science and computer science communities through open calls, AI models, standards, and collaborative challenges.

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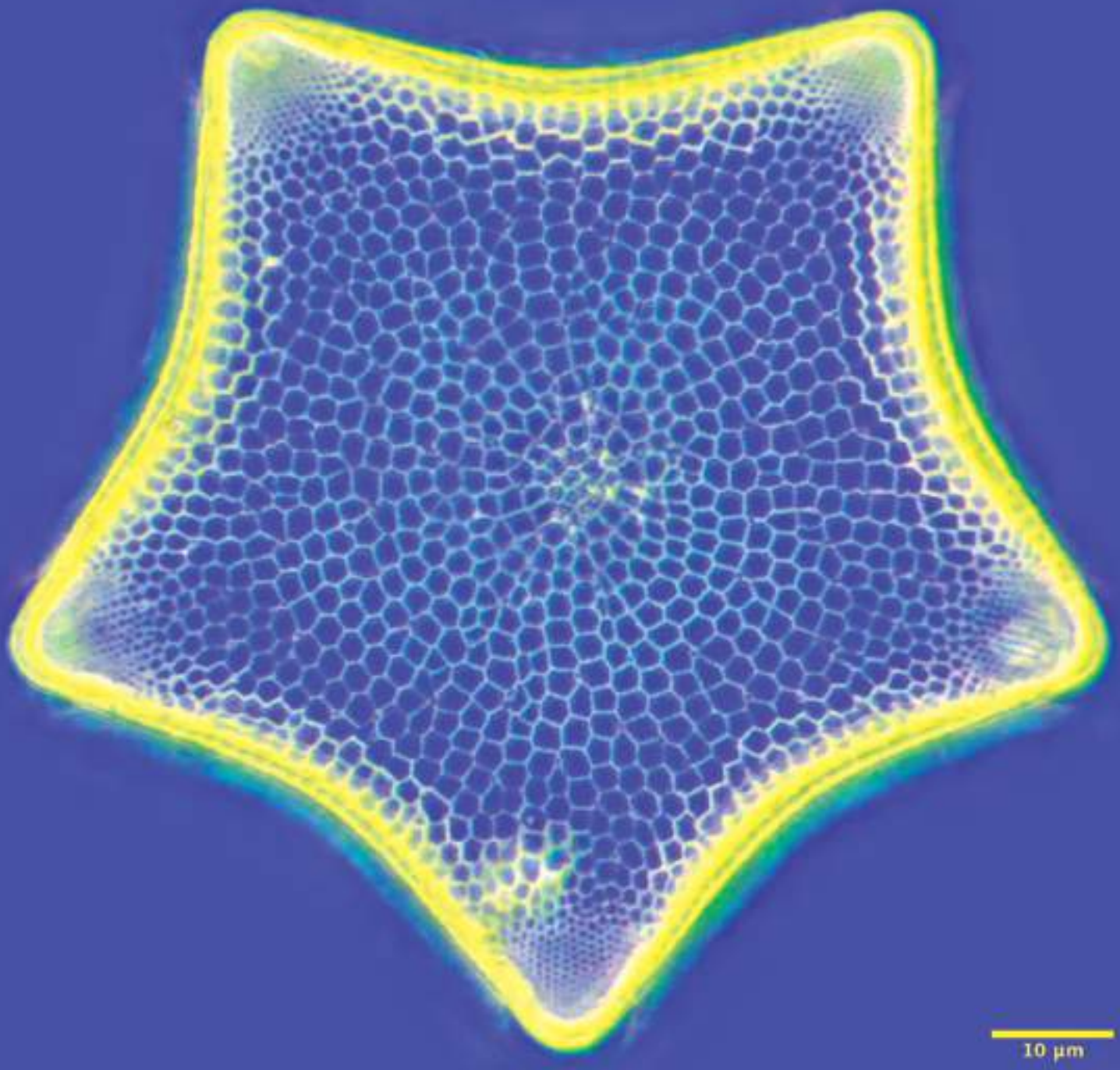

COMMUNITY WORKSHOPS

"How to track metabolic states, temperature and magnetic fields at subcellular resolution in live cells"

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Keywords: Intracellular temperature, metabolic imaging, nanotechnology, near field super resolution microscopy

Abstract

Fluorescence microscopy can be used to extract more than bare morphological information from live cells, but instead allows us to extract relevant biophysical parameters such as temperature or magnetic fields which have relevance for metabolism or neuronal signalling.

In this session we will explain how fluorescence-based microscopes can be turned into functional bioimaging technologies, either by modification of the setup - such as the addition of a femtosecond laser [1,2], or by unconventional read-out of fluorescence data taking advantage of two colour channel detection to measure intracellular temperature information for example [3].

Furthermore, we aim to showcase some nanotechnology approaches that render your fluorescence lifetime microscope (FLIM) into a nearfield super resolution microscope able to visualize for example details of the nuclear membrane organization one the 3-4 nm length scale [4] or allowing you to study droplet drying dynamics at the nanoscale [5], as well as nanoparticle based approaches for intracellular sensing using either fluorescence lifetime approaches [6] or quantum sensing approaches [7] via optically detected magnetic resonance experiments.

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COMMUNITY WORKSHOP

CRYO-CORRELATIVE WORKFLOWS

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KEY WORDS: Cryogenic conditions, correlative light-electron microscopy, TEM, SEM

Cryo-correlative light and electron microscopy is an integrative and powerful tool for studying biological processes in cells in a fully hydrated state. The combination of spatial and functional information provided by visible light microscopy, along with the high-resolution details obtained through electron microscopy—both performed on the same cell—yields insights that are not accessible through either technique alone.

In this workshop, we would like to engage with the scientific community and share our experience with the correlative workflow used to analyze cryopreserved cells. This will include discussions on sample preparation, data acquisition, and data processing.

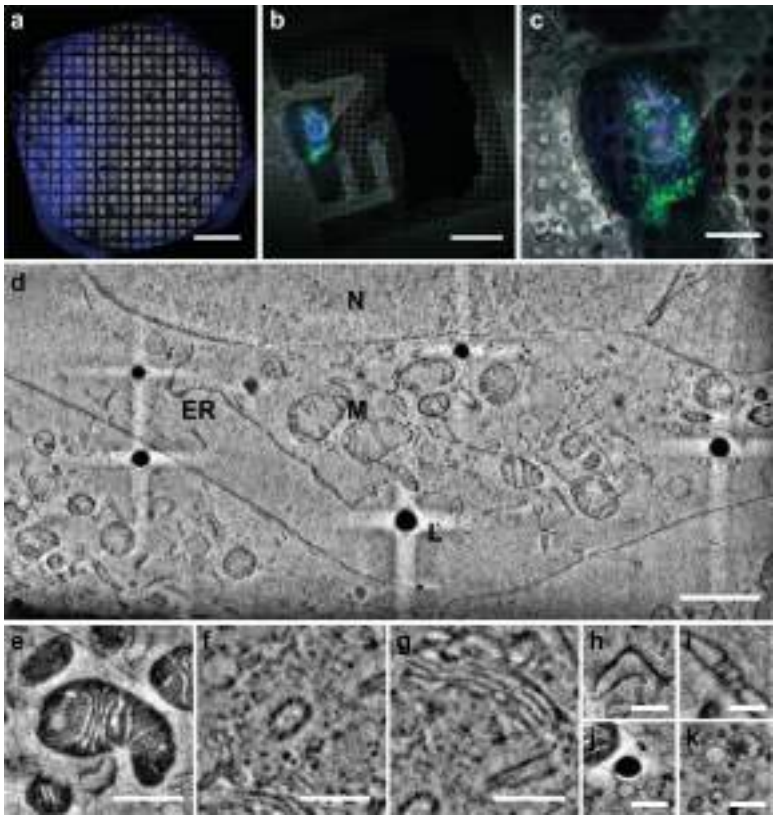

Figure 1. Cryocorrelative microscopy with FIB-SEM tomography. a. Overlay of an image of a TEM grid where GFP-EB3 protein (green) expressing cells are attached. Blue, nucleus**. b.** 5X magnification. **c.** 63X magnification of the cell in (b). **d.** 3D FIB-SEM reconstruction slice of the cell in (c). The internal structure of cellular organelles is observed: lipid droplets (L), mitochondria (M) and endoplasmic reticulum (ER) and nucleus (N). e. mitochondria. f, centriole. g, Golgi apparatus. H, endoplasmic reticulum. i, nuclear pores. j, lipid droplets. k, endocytic compartment vesicles. Bars: a, 500 µm; b, 25 µm; c, 10 µm; d, 2 µm; e-g, 500 nm; h-k, 250 nm.

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COMMUNITY WORKSHOP

BIAPY: DEEP LEARNING BASED BIOIMAGE ANALYSIS FOR ALL AUDIENCES

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KEY WORDS: Bioimage analysis, deep learning, image segmentation, object detection, image denoising, single image super-resolution, self-supervised learning, image classification, image-to-image translation.

BiaPy stands as a versatile and open-source bioimage analysis library designed to meet the diverse needs of users and developers in the field of computational biology. With an intuitive interface, zero-code notebooks, and Docker integration, BiaPy offers accessibility across a wide spectrum of technical expertise. This workshop will delve into the core functionalities of BiaPy, highlighting its capacity to address bioimage analysis challenges at different scales. From beginners to experts, BiaPy provides customizable solutions for a range of tasks, including semantic segmentation, instance segmentation, object detection, and more. Through its robust support for multi-GPU configurations and compatibility with large datasets, BiaPy aims to democratize the use of sophisticated bioimage analysis workflows. Join us to explore how BiaPy empowers researchers to harness the power of deep learning and advanced computational methods in their work, facilitating breakthroughs in computational biology.

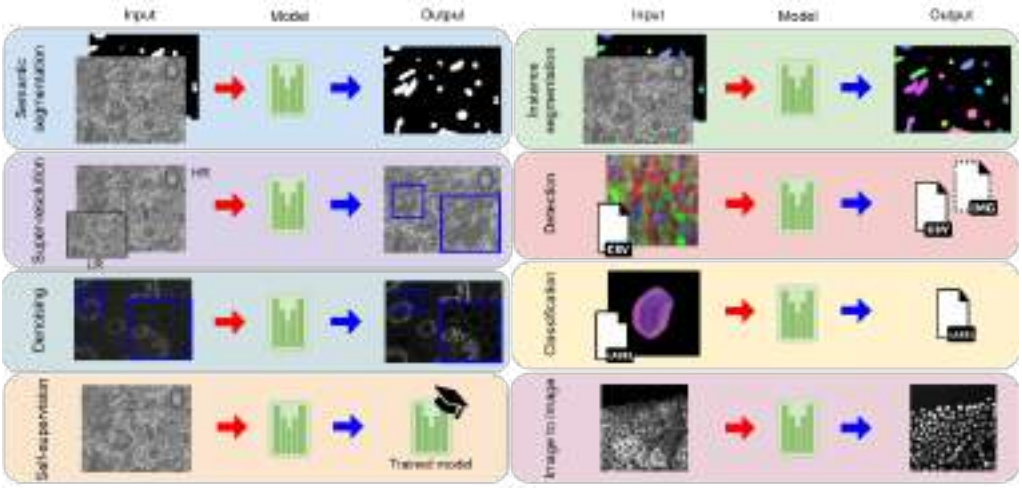

Figure 1. BiaPy available workflows. BiaPy provides deep-learning workflows for a large variety of bioimage analysis tasks, including 2D and 3D semantic segmentation, instance segmentation, object detection, image denoising, single image super-resolution, self-supervised learning (for model pretraining), image classification and image-to-image translation.

UC2 - AN OPEN-SOURCE MODULAR ECOSYSTEM FOR OPTICS

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KEY WORDS: quantitative microscopy, open-source, modular

The evolution of microscopy from rudimentary single-lens instruments to sophisticated, automated high-throughput systems has been transformative for scientific inquiry. Concurrent advances in computational analysis have enabled digital enhancement of image quality and the extraction of diagnostic information. Accessibility to these advances, however, remains hindered by the high cost and proprietary nature of most modern microscopes. Our initiatives, such as UC2 (You.See.Too.)[1] and the Anglerfish/ESPressoscope[2], aim to disrupt this status quo by equipping a diverse array of researchers, including amateur scientists, with the means to capture high-quality data in the field, thereby fostering inclusivity in scientific dialogue and innovation.

Our development ethos prioritizes the accessibility and modularity of components, ensuring that any user can replicate our devices using comprehensive open-access documentation, 3D printing technology, and readily available parts. This approach not only spurs creativity in overcoming design constraints but also employs unorthodox strategies to achieve high-resolution imaging capabilities traditionally reserved for costly equipment.

We demonstrate the ability of our modular microscopy system to application of different microscopic modalities from standard transmission microscope to fluorescence microscope. People can learn microscopy starting from basic ray optics and understand advanced imaging system. With the concept of open-source we build up a worldwide user community to contribute and maintain the system.

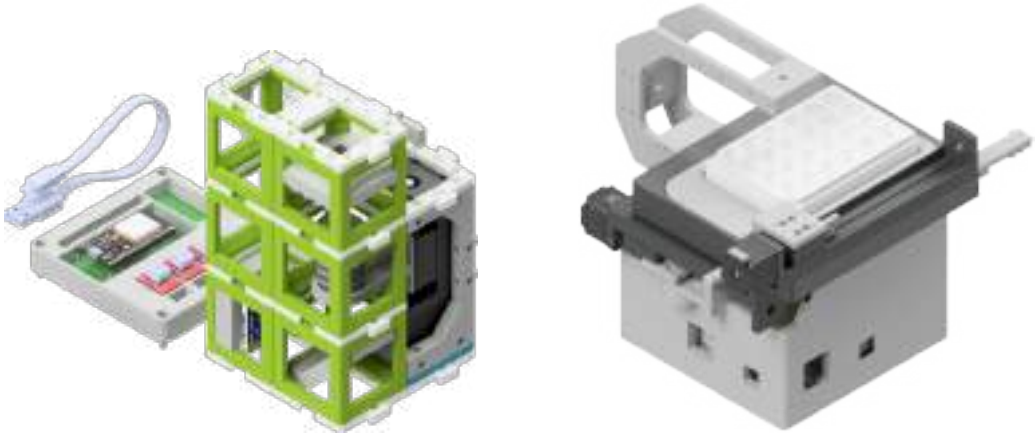

Figure 1. Different microscopy techniques can be built with the modular system. An example case of transmission brightfield (left) and motorized widefield fluorescence microscope (right) are shown here.

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COMMUNITY WORKSHOP

STORM IMAGING OPTIMIZATION

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KEY WORDS: STORM, Resolution, Subcellular.

Stochastic optical reconstruction microscopy (STORM) is a high resolution microscopy technique, developed by X. Zhuang and colleagues in 2006, which allowed to reveal unresolved details of many cellular structures at nanoscale resolution. It is based on the consecutive emission of single photons from the activated state of a photosensitive molecule to allow its precise localization before it enters a dark state or is deactivated by photobleaching. Each fluorophore is activated separately, and by adjusting the point spread function (PSF), the centre of mass can be calculated to determine the location of a molecule down to a resolution of 20 nm. The parallel registration of many individual emitting fluorophores, each with its different set of coordinates, allows the reconstruction of an image with a high degree of resolution [1, 2, 3, 4].

Sample preparation and acquisition conditions require fine-tuning to allow a correct image reconstruction. At the CNB-CSIC Advanced Optical Microscopy Facility, we have optimized a protocol for sample preparation and acquisition conditions that has allowed us to visualize different subcellular structures including the actin cytoskeleton, mitochondria, tubulin, and lysosomes with a resolution in the range of than 50 nm. In addition, we were able to set up an acquisition protocol to perform multicolor STORM for the observation of mitochondria in combination with microtubules, or actin. In the current practical workshop, we want to share with the scientific microscopy community our optimized STORM workflow including crucial steps for sample preparations, live image series acquisition, as well as data processing and image reconstructions using the open source plugin tool ThunderSTORM [5] available for ImageJ.

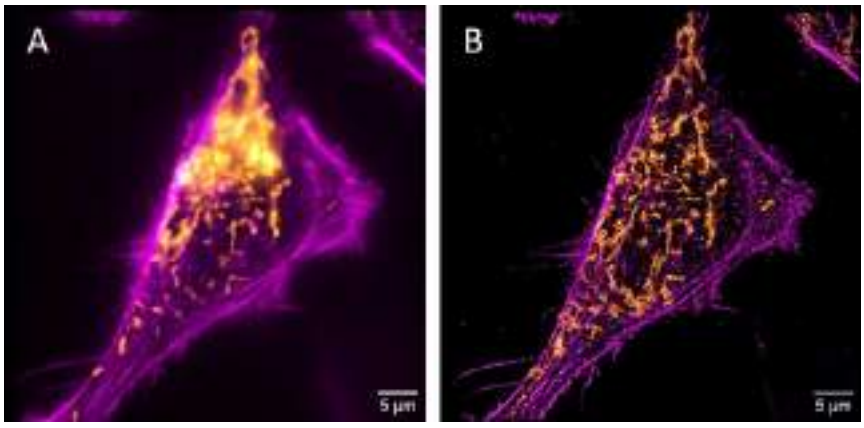

Figure 1. Comparison between TIRF (A) and STORM (B) images of filamentous actin (magenta) and mitochondria (yellow). Scale bar is 5 µm.

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COMPUTER-AIDED DIAGNOSTIC SYSTEMS IN MICROSCOPY:
µSMARTSCOPE AND ARTIFICIAL INTELLIGENCE

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KEYWORDS: Intelligent microscopy, mobile health, decision support systems

Fraunhofer Portugal AICOS has been performing research in the field of Mobile Microscopy since 2013, which started with the development a fully automated 3D-printed smartphone microscope, termed µSmartScope. Through the usage of a motorised automated stage fully powered and controlled by a smartphone, the µSmartScope allows autonomous acquisition of microscopic images, with the goal of decreasing the burden of manual microscopy examination [1].

µSmartScope has been evolving throughout the years, not only in terms of design and hardware [2, 3] but also in terms of parallel and equally important functionalities [4-8]. The µSmartScope has already been tested for NTDs like Malaria, Chagas, Cervical Cancer and Microfilaria [4, 5, 7].

The µSmartScope also aims to reduce dependence on experts in microscopy diagnosis available on-site, by allowing straightforward integration with Artificial Intelligence (AI). Particularly, computer vision modules can be easily embedded in the µSmartScope framework to support the diagnosis of target pathologies. Moreover, in the recent years Fraunhofer Portugal AICOS has been focusing on responsible AI, by designing and building data driven solutions based on the needs of healthcare environments [8].

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COMMUNITY WORKSHOP

Monitoring and Analyzing Microscope Performance through PSF Measurements

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KEY WORDS: Quality assessment, system performance, protocols and tools, reproducibility, axial and lateral resolution, point spread function, image quality, quantitative bioimaging.

To ensure quantifiable and reproducible microscopy data, it is essential to monitor the performance of the microscope over time. Monitoring performance stability begins with measuring the Point Spread Function (PSF) as an indicator of the optical lateral and axial resolution. The PSF's size, shape, and symmetry, when compared to the theoretical ideal resolution, provide a comprehensive assessment of the optical setup, including the objective. This, in turn, impacts image quality and the accuracy of subsequent experimental analyses, particularly in advanced microscopy techniques.
In this workshop, we discuss and demonstrate how to prepare, acquire tetraspeck beads images and how to analyze the data obtained using an open-source plugin (MetroloJ_QC). We will look at essential metrics such as: Nyquist-Shannon sampling, image quality, shape, size and symmetry of the PSF and the parameters that may influence the shape of PSF.

COMMUNITY WORKSHOP

Spatial Biology: a game changer

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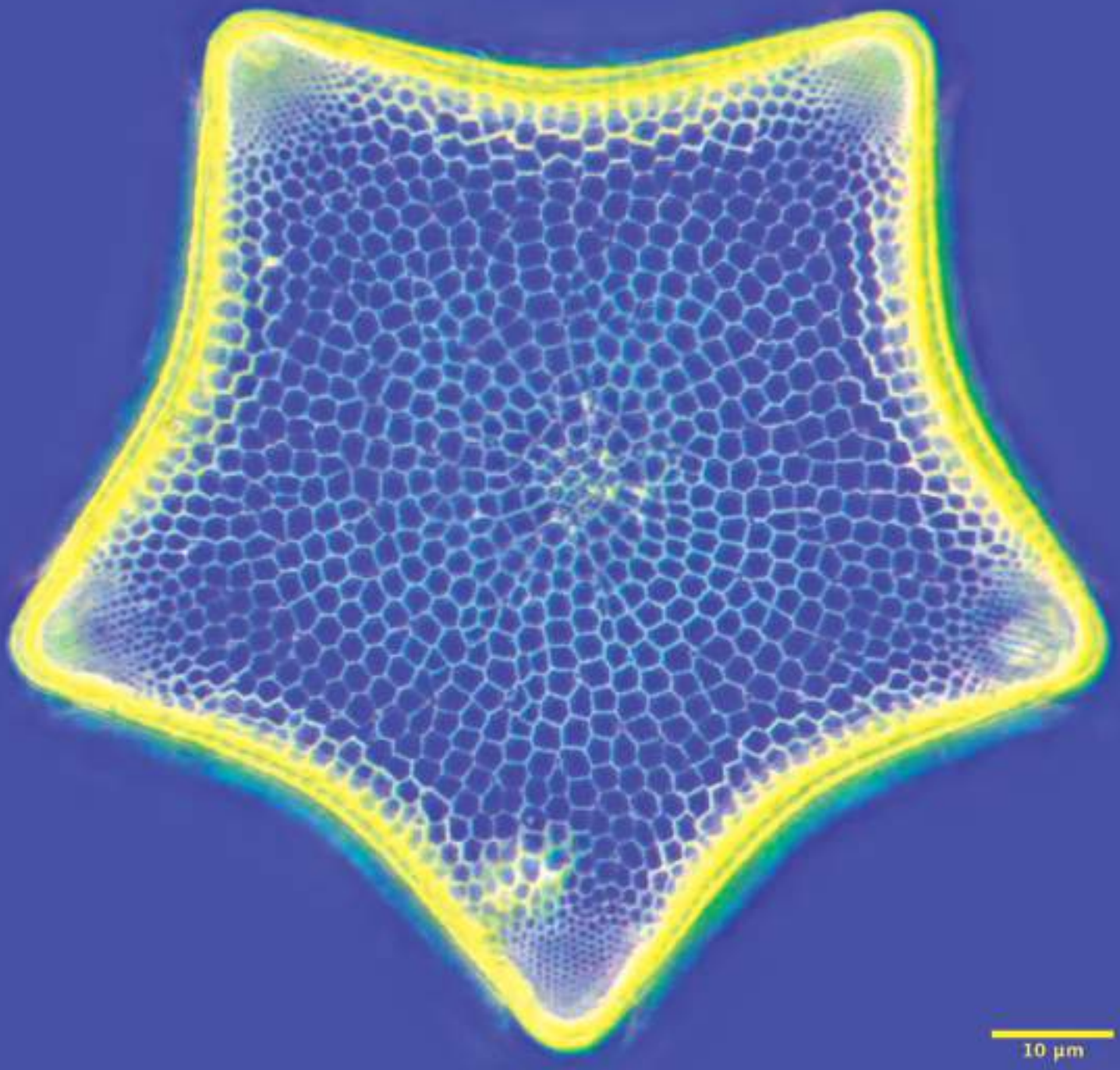
KEYWORDS: Fluorescence multiplex, Spatial analysis, Image analysis, Cell phenotyping

Spatial biology is transforming our ability to study complex biological systems by preserving the spatial relationships between cells and tissues. This workshop will focus on advanced microscopy multiplex techniques, including multi-colour immunohistochemistry and cyclic immunofluorescence staining, providing an overview of the different multiplex staining workflows, and will include experimental design, tissue preparation, imaging strategies and advanced image analysis open-source tools. Participants will learn image analysis methods for cell phenotyping, allowing the identification and characterization of cellular subsets within their native tissue environments with applications in cancer, immunology, and neuroscience and will demonstrate how these multiplex and spatial biology techniques are used to decode cellular interactions, tissue microenvironments, and disease mechanisms. Attendees will exchange their understanding of this transformative field and learn how multiplex spatial biology techniques and advanced image analysis can be applied in their research.



INDUSTRY TALKS

CHARACTERISATION OF BIOCONDENSATE MICROFLUIDIC FLOW USING ARRAY-DETECTOR FCS

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Biomedical Research Institute, Hasselt University, Belgium

Biomolecular condensation via liquid-liquid phase separation (LLPS) is crucial for orchestrating cellular activities temporospatially. Although the rheological heterogeneity of biocondensates and the structural dynamics of their constituents carry critical functional information, methods to quantitatively study biocondensates are lacking. Single-molecule fluorescence research can offer insights into biocondensation mechanisms. Unfortunately, as dense condensates tend to sink inside their dilute aqueous surroundings, studying their properties via methods relying on Brownian diffusion may fail.

We take a first step towards single-molecule research on condensates of Tau protein under flow in a microfluidic channel of an in-house developed microfluidic chip. Fluorescence correlation spectroscopy (FCS), a well-known technique to collect molecular characteristics within a sample, was employed with a newly commercialised technology, where FCS is performed on an array detector (AD-FCS), providing detailed diffusion and flow information. We show that AD-FCS is a valuable tool for advancing research on understanding and characterising LLPS properties of biocondensates.

MICROSCOPIC PRECISION, REPRODUCIBILITY AND VERSATILITY. AUTOMATION AS A HARMONISATION PROCEDURE

Adolfo Molejón, PhD, Application Specialist, Eppendorf

Automation is fundamental in multiple aspects of microscopy. Forgetting the fine control of lenses, aperture and other hardware features, automated XYZ movement of the stage, or High Content Screening analysis is becoming the norm. However, quality of samples is usually an important topic highlighted. How do you prepare your samples? In this workshop, we wish to discuss a setup for the routine procedures you may have and prove in a live software demo where we will use an example, or a real scenario you face daily and how you can load and run an experimental setup with confidence of harmonisation between users and different experiments.

ELEVATE YOUR IMAGING WITH NEW DEVELOPMENTS FROM EVIDENT

Dr. Bjoern Sieberer, Senior Application Specialist, Evident/Olympus

At Evident, we are guided by the scientific spirit, and we support our customers with solutions to solve their challenges and advance their work. We empower scientists and researchers through collaboration and cutting-edge life science solutions such as the new FLUOVIEW™ confocal laser scanning microscope with revolutionary SILVIR™ detectors technology, the SILA™ device integrated into our VS200 research slide-scanner system to obtain high-contrast images in widefield mode and too many other solutions. Are you interested in exploring the latest advancements? Don’t miss the chance to join us for our industrial pitch.

MICROSCOPE PERFORMANCE MONITOR WITH THE NEW FLUOVIEW™ FV4000

Dr. Bjoern Sieberer, Senior Application Specialist, Evident/Olympus
M. David Peña, Application Specialist, Evident/Olympus

Assessing the status of a microscope and controlling its according performance is critical, especially in a multi-user environment like an imaging core facility. Being able to quickly conclude where unexpected results originate from, and which countermeasures need to be taken, will help to maintain operational status and to act to even prevent breakdowns and malfunctions. In this EVIDENT workshop we will introduce the Fluoview Microscope Performance Monitor, a new software embedded performance assessment tool inclusive specific hardware module that will help to evaluate and monitor the microscope performance.

SMART MICROSCOPY : HIGH QUALITY IMAGING FOR RESEARCH AND TEACHING

Emilio Martín
IESMAT

In the course of the ever-increasing demand for high-quality images on optical microscopes, smart microscopy is the key technology for the refinement of easy use, ensuring their functionality, permitting remote control the device from anywhere, and sharing images to get second opinions. All the functionality of digital microscopes is enclosed as a perfect solution for research and teaching

NEW TECHNOLOGIES IN IMAGING AND IMAGE ANALYSIS FOR HIGH-END MICROSCOPY

Michael Abanto,
Department of Biomedicine University of Basel

A new approach using techniques like artificial intelligence and deep learning methods, facilitate imaging, automate tasks, decrease errors and increase cell viability in high-end microscopy experiments. All these techniques together make impossible tasks now possible. These new technologies also allow researchers to minimize human intervention and accelerate throughput, making them ideal for large-scale live-cell imaging applications.

In addition, these new techniques, when applied to image analysis allows automating challenging applications previously considered difficult or nearly impossible. The deployment of these new technologies represents a significant leap forward in automating and optimizing image capture processes, thereby fostering innovation and improving outcomes in high-end microscopy.

REVIEWING TISSUE CLEARING: HANDS-ON WITH CLARIFICATION AND IMAGING

Luis Muñiz, Imaging Application Specialist, Miltenyi Biotec
Edwin Hernandez, CSIC

Due to their inherent complexity and size, biological samples often present challenges for detailed imaging. Light-sheet microscopy has emerged as a crucial tool for capturing these intricate structures. However, to achieve optimal results, whole-sample tissue clearing is essential for rendering specimens transparent. This workshop provides a comprehensive overview of the latest tissue-clearing techniques, both aqueous and solvent-based, used to prepare samples for light-sheet microscopy. Participants will engage in a hands-on clearing session to learn essential tips and tricks to optimize the process for various sample types. Following the clearing demonstration, attendees will experience live imaging of stained samples using the cutting-edge UltraMicroscope Blaze, featuring its innovative lightspeed acquisition mode. This immersive session is designed to deepen understanding and practical skills in tissue clearing and imaging, equipping researchers with the knowledge to apply these techniques to their own complex biological samples.

TAUSTED XTEND – NEW TOOLS FOR GENTLE LIVE IMAGING AT REMARKABLE NANOSCALE

John R. Pearson
Advanced Workflow Specialist, Leica

The goal of scientific research is to understand the workings of nature. Given the complex interplay of biomolecules, molecular machines, and higher-order cellular structures, confocal imaging emerged as a fundamental tool owing to the optical sectioning, sensitivity, and the temporal and spatial resolution capabilities. Imaging intricate cellular structures at nanoscale resolution while characterizing the dynamics of multiple species in the context of live specimens are emerging avenues followed to shed light on biological processes. With the advent of STED (Stimulated Emission Depletion), researchers have realized the visualization of intracellular structures at the nanoscale, unveiling insights into cellular behavior, interactions, and function. In this workshop, we will demonstrate how our innovative TauSTED Xtend enables gentle imaging of live and fixed samples at the nanoscale. We will show how advances in our TauSTED1 approach to optical nanoscopy deliver cutting-edge resolution and image quality at low light dose, key to accessing fast nanoscale dynamics of cellular processes. We will also show how fluorescence lifetime information can be used for multiplex imaging of different markers, keeping the nanoscopic resolution.

Reference

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MEET THE ENHANCED DMI8 INVERTED MICROSCOPE: INTELLIGENT AUTOMATION, SPINNING DISK AND THUNDER

Francisco Porto
Advanced Workflow Specialist, Leica

As our understanding of cell biology deepens, the demands on microscopy to address challenging research questions are increasing. This is evident in the growing complexity of microscopy workflows and the need for quantitative imaging data that is derived from higher quality images.

The need for rigor and reproducibility when acquiring and analyzing microscopy images means that even slight deviations in workflows can introduce significant errors into your data. Constant manual adjustments, such as optimization of correction collars or maintaining immersion with water objectives can be frustrating, often necessitating repeated experiments. Even after the system is set up, the time-consuming task of finding the right region of your sample at the right time, and navigating between different magnifications, remains.

In this talk, we will introduce the new, enhanced DMi8 inverted microscope and its expanded functionality and intelligent automation, which streamlines complex workflows. We will showcase the patented Adaptive Immersion technology that ensures reliable use of optimal water immersion objectives in live cell experiments through an embedded sensor that maintains immersion, without worrying about evaporation. We will demonstrate how combining this with the new Integrated Sample Finder and fully integrated LAS X Navigator experience creates the ultimate synergistic workflow. You'll see how to identify key regions of interest (ROIs) from low magnification sample overviews and transition to high-resolution imaging with immersion objectives, all while keeping your sample on the stage and providing spatial context to your data throughout your workflow.

We will also discuss the DMi8's new advanced technology suite including SmartCORR, which automatically optimizes the objective correction collar settings to match your experiment, significantly improving image quality by reducing spherical aberrations. Additionally, high-speed confocal imaging with the new Spinning Disk Confocal Scanner, combined with technologies such as THUNDER and Aivia AI Image Analysis, offers deeper insights from 3D and live samples.

Don't miss this exciting session to see how these fundamental improvements allow you focus more on producing quality, publishable data and less on managing your microscope.

TO LABEL, OR NOT TO LABEL: LABEL-FREE THREE-DIMENSIONAL IMAGING AND QUANTITATIVE ANALYSIS OF LIVE CELLS USING HOLOTOMOGRAPHY

Bruno Combettes
Sales Director, Tomocube Inc

Holotomography (HT) has emerged as a useful tool for imaging live specimens without additional pre-treatment such as fixation, staining, and fluorescence excitation. HT can achieve long-term three-dimensional (3D) observation of live specimens for weeks without any cellular damage caused by photoactivation. The high resolution under 150 nm lateral achieved through synthetic numerical aperture provides sufficient spatial information to distinguish various subcellular compartments such as nucleus, nucleoli, mitochondria, lipid droplets, etc.

Furthermore, the measured individual cell data can be analyzed to elucidate the temporal 3D volumetric dynamics with the dry mass information. In this workshop, we will present the latest development of a low-coherence holotomography imaging system, HT-X1, and its numerous applications to different types of biological specimens, ranging from unicellular organisms to multicellular specimens. Several case studies will be introduced, such as 3D visualization of subcellular structures in living microorganisms, real-time quantification of cell volume and subcellular lipid contents, deep learning-based cell type classification, etc. We will also discuss how holotomography can be a versatile tool for various research fields combining downstream molecular analysis, such as live cell biology, immunology, microbiology, organoids, stem cells, and in vitro diagnosis.

THE POWER OF AIRYSCAN 2 FOR LIVE DYNAMICS

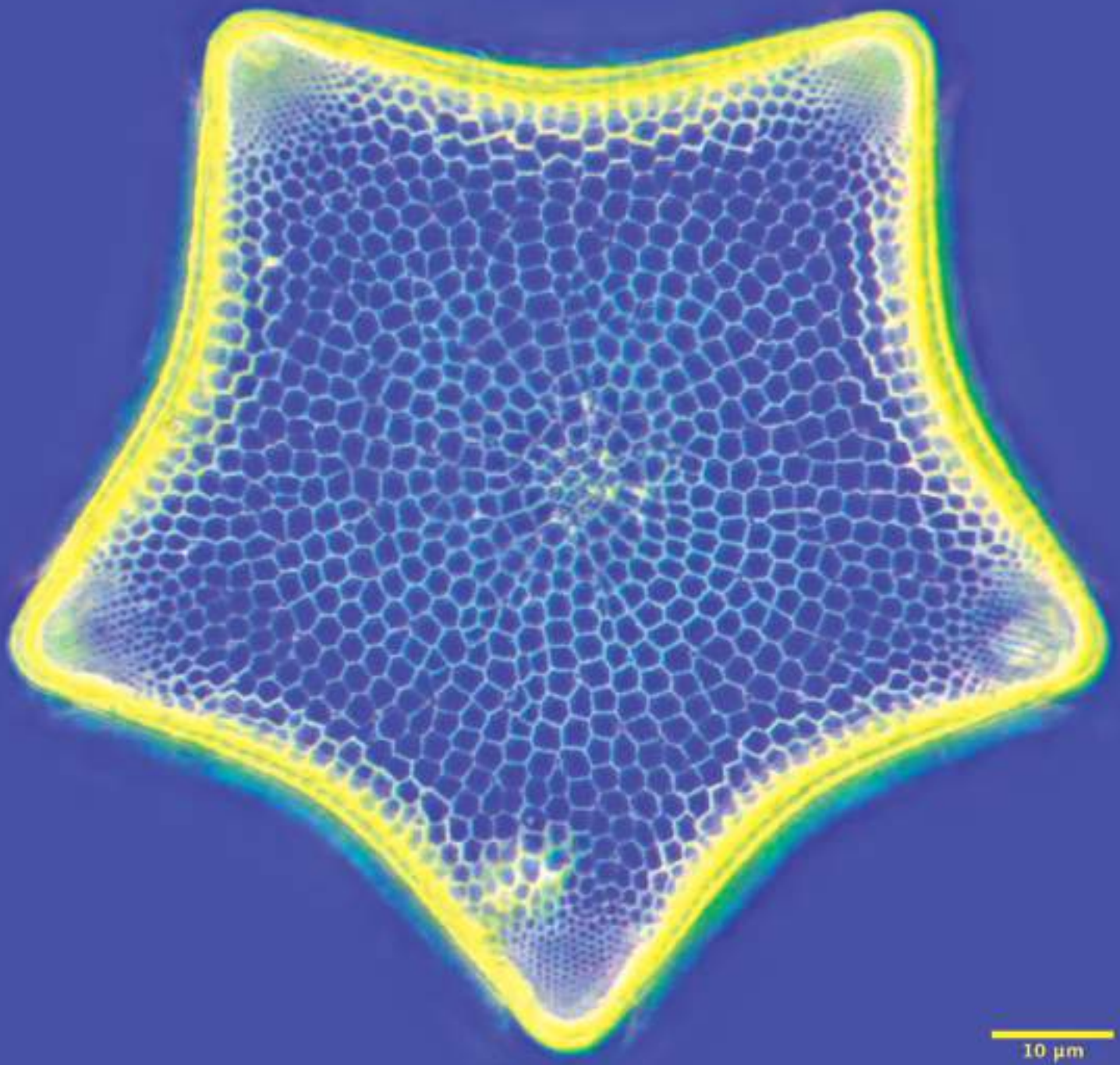
Dr. Verona Villar
Product Application Specialist at Carl Zeiss Iberia

In the field of molecular imaging, researchers have long been challenged by the inability to easily obtain valuable information about the behavior of molecules of interest. Traditional imaging techniques have been limited in their ability to provide insights into molecular concentration, flow dynamics, and directionality. ZEISS has developed two powerful modules for the confocal microscope LSM9 family, in addition to the long-standing FCS module: Dynamics Profiler, and SpectralRICS. These modules offer researchers unique insights into the behavior of their molecules of interest without the need of understanding complex algorithms or having in-depth knowledge of biophysics.During this Industry Workshop (November 20th at 3:30 pm in Room C2), we will show how ZEISS Dynamics Profiler can uncover molecular concentration, asymmetric diffusion, and flow dynamics of fluorescent proteins in your living samples in a single, easy measurement by employing advanced algorithms in combination with the unique Airyscan 2 detector. Develop a more in-depth profile of the molecules in your current experiments, from cell cultures to organoids to whole organisms.

SMART MICROSCOPY WITH CELLDISCOVERER 7

Soren Prag, Product and Sales Application Specialist, EMEA/LA
ZEISS RMS

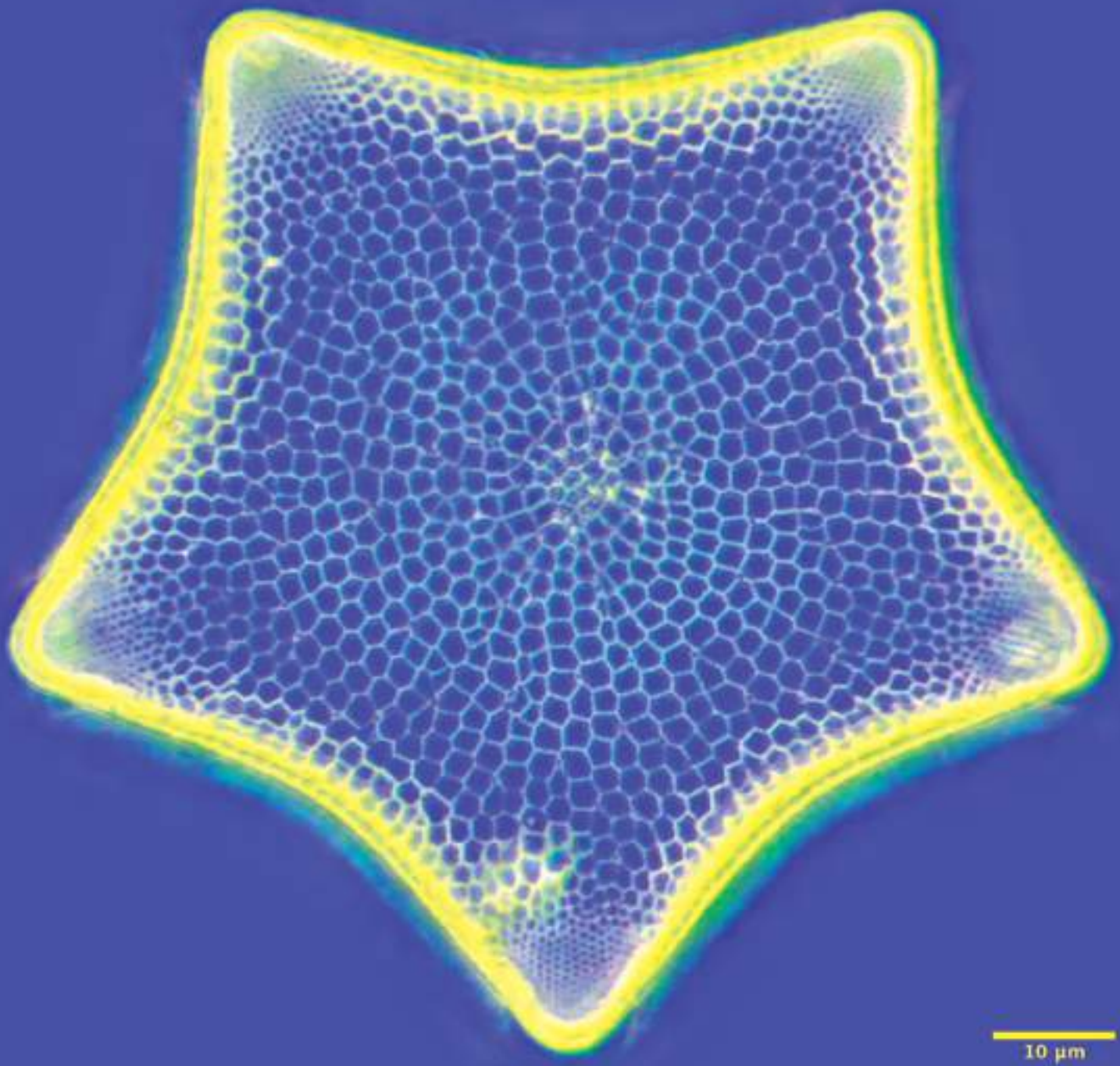
Light microscopes provide a research tool that is universally used across samples and scientific questions. In conventional microscopy, finding regions of interest, capturing them, and making changes to acquisition parameters are time-consuming and involve continuous user intervention. The evolution on microscope automation and image analysis tools has open a new era, the Smart Microscopy, providing new imaging systems that can automate complex workflows. Smart Microscopy combines automated workflows with real-time image analysis, allowing microscopes to monitor samples and adjust their imaging parameters without human intervention, enhancing efficiency and accuracy of data collection. During this workshop we will perform a live demonstration on Smart Microscopy with ZEISS Celldicoverer 7, our adaptable automation system for advance workflows.



POSTER • FLASH • TALKS

Posters Flash Talks

#	Author — Title
1	Lelde Hermane — A New Angle on Coral Imaging: Lightsheet Microscopy for Enhanced 3D Morphological Studies
2	Mariana Carvalho — Combining Holotomography and Confocal Microscopy for Cellular Nanodiamond Localization Studies
3	Pau Carrillo Barberá — Advancing myelin research through deep learning-enhanced microscopy
4	Biagio Mandracchia — High-speed sCMOS acquisition with optical pixel reassignment



POSTERS

3D-ANALYSIS OF SINGLE CELL CLONAL EXPANSION IN WHOLE ADULT MOUSE HEARTS

Luis Diago [1], Dolores López [2], Elvira Arza Cuesta [2], Verónica Labrador Cantarero [2], Olga Giménez [2], Rui Benedito [1], Valeria R Caiolfa [2,3]

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3. Centre for Experimental Imaging, IRCCS-Ospedale San Raffaele, Milan, Italy

KEY WORDS: Multiphoton, SPIM, angiogenesis

Myocardial infarction is a leading cause of death worldwide and current treatments or surgery often do not recover myocardial function. Therefore, there is the need to find new treatments and mechanisms to promote heart regeneration. It has been shown that promoting heart angiogenesis contributes to myocardial regeneration and function in some disease models and settings.

We aim to explore how heart vasculature changes post-infarction and how genetic pathways influence angiogenesis and vascular architecture. We are using advanced genetic approaches to study the clonal expansion of single ECs within the entire mouse heart vasculature, using 3D-2-photon laser scanning and single plane illumination (SPIM) microscopy. We aim at determining the real size of the endothelial clonal populations (number of expanded nuclei from an initially labelled single EC) and mapping the location of the clones relatively to arteries and other vascular structures in whole mouse hearts in 3D. Here we show how we troubleshooted several heart clearing protocols and how we determined the resolution, speed and penetration depth of imaging with a novel 2P-spectral NDD microscope and a SPIM. Both EZ Clear and iDisco effectively cleared adult mouse hearts and allowed to capture fluorescently labelled nuclei, arteries or capillaries in whole mounted organs. We could identify structures down to 3 mm in tissue, and the EZ clearing allowed for capillary visualization using endogenous fluorescence down to 800 μm into the tissue. The ongoing work is focussed on optimizing a multicolour labelling and detection protocol to distinguish up to 5 markers in whole mouse hearts, with single cell resolution and in 3D.

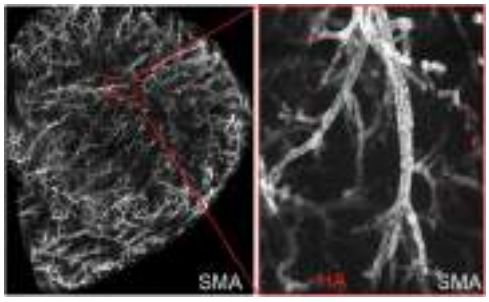

Figure
Whole adult mouse heart captured using 2-photon laser scanning technology with a Leica 4-Tune-Dive-Stellaris 8 device. White signal is smooth muscle actin, labelling coronary arteries, red signal is HA-tag, labelling single lineage-traced endothelial cell nuclei.

Microscopy was conducted at the Microscopy & Dynamic Imaging, CNIC, ICTS-ReDib,Grant EQC2021-007527-P funded by MCIN/AEI/10.13039/501100011033 and by "European Union NextGenerationEU/PRTR.

A DEEP LEARNING FRAMEWORK FOR QUALITY ASSESSMENT OF HISTOPATHOLOGICAL WHOLE SLIDE IMAGES

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KEYWORDS: Artificial intelligence, whole slide images, quality assessment, digital pathology

Clinical pathology is undergoing a significant transformation, shifting from traditional glass tissue slides viewed under optical microscopes to capturing digital whole slide images (WSIs). Digital pathology allows the acquisition of high-resolution images, enhancing disease diagnosis through easier and more accessible visualization. Like other medical images, whole slide images (WSIs) are susceptible to specific artifacts such as blurriness, folds, bubbles, and dirt, which can significantly impact histopathological diagnoses. Therefore, prior to pathologist examinations, it is essential to assess the quality of these WSIs to determine promptly whether image retakes are necessary which requires substantial human effort, precision, and time. The utilization of deep learning techniques effectively streamlines this practice, enabling the automatic detection of artifacts with high accuracy. We adopted two different learning-based approaches for WSI artifact detection including supervised and unsupervised models. The models can detect two common artifacts of out-of-focus blur and fold artifacts with high accuracy.

In the supervised approach, we deployed two models including Deep Focus (DF)[1] and Lightweight Densely Connected with Squeeze-and-Excitation Network (LDSE-Net)[2.] Due to limited sample availability, synthetic blur patches were generated at different levels and coverages. Our results show that the LDSE-Net outperformed DF, achieving 99% accuracy compared to DF's 87%. It is also observed that the LDSE-Net enhances generalizability, as tissue patterns vary among different organs. For instance, utilizing a model initially trained with lung and kidney images, LDSE-Net achieved 98% classification accuracy for distorted breast and skin tissues.

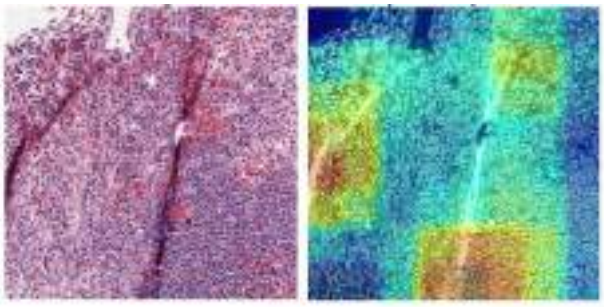

Figure 1. Patch with fold artefacts (*Left*) and artefact regions detected by the network (*Right*)

Detection of artifacts in unsupervised pathways is more challenging due to their nature. Here we deployed Contrastive Unpaired Translation (CUT)[3] model as a generative model, aiming to reconstruct pristine patches from those with artefacts. Finally, we used

the reconstructed and the original patches to segment the artifact region. To enhance the accuracy of the detected artifact regions, BASNet[4] model was used that refines the masks obtained from the initial thresholding. We achieved more than 95% accuracy in detection using our unsupervised model.

Ongoing work focuses on addressing various artifact types and implementing other techniques to provide quality scores to pathologists for evaluating specific images.

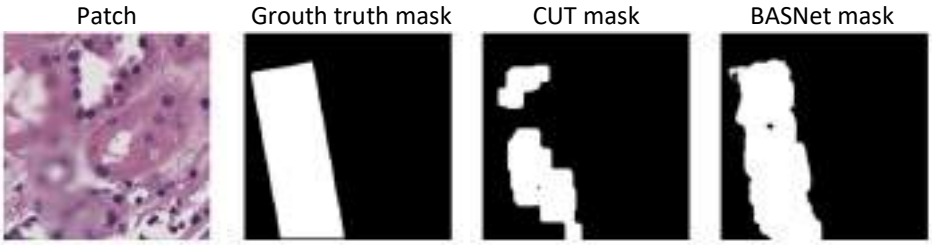

Figure 2. Masks generated by CUT, Basnet and the ground truth version.

The NextGen Digital Pathology project (VLAIO HBC.2022.0585) is funded by VLAIO, EU and is led by Barco NV, Kortrijk, Belgium

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A New Angle on Coral Imaging: Lightsheet Microscopy for Enhanced 3D Morphological Studies

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KEYWORDS: Corals, Lightsheet Fluorescence Microscopy, Segmentation, Image Analysis

In recent years, advancements in microscopy techniques have significantly enhanced our ability to visualize and analyse complex biological structures. This study presents a novel approach for imaging coral sclerites using Lightsheet Fluorescence Microscopy (LSFM), which could serve as a valuable tool for coral health monitoring and conservation efforts.

Sclerites are small calcium carbonate structures of soft corals that provide structural support [1], protection, and contribute to ecological interactions with various microorganisms. In this study, samples of the gorgonian *Paramuricea sp.* were labelled with a calcium binding fluorescent dye (ex: 577 *n*m; em: 590 *n*m). LSFM (ZEISS Lightsheet Z.1, Carl Zeiss, Microscopy GmbH, Jena, Germany) and ZEISS arivis Pro ver. 2.12.6 x64 (formerly known as Vision4D; Carl Zeiss Microscopy Software Center Rostock GmbH) was used for high-resolution 3D imaging [2] of intact corals and their sclerites to obtain new insights into their structure and distribution (Fig. 1). More specifically, image segmentation and analysis methods were used to extract quantitative metrics such as size, volume, shape, location and arrangement of the sclerites. Results were compared against measurements performed on isolated sclerites.

The integration of LSFM with image processing and analysis not only enhances our understanding of sclerite structure but also provides a robust framework for future taxonomical, developmental, evolutionary and paleontology studies.

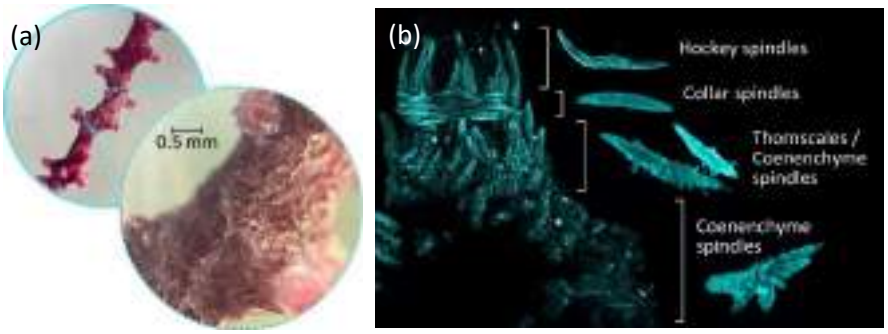

Figure 1. (a) Representative images of *Paramuricea cf. grayi* coral polyps acquired with a Stereo Microscope. (b) Coral polyp and visual representation of common sclerite types acquired with the ZEISS Lightsheet Z.1.

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A NOVEL PLUGIN FOR THE EXTENSIVE ORGANELLE FEATURES DETECTION IN THE THREE-DIMENSIONAL SPACE

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KEY WORDS: Colocalization, quantification, quantitative microscopy, signal overlap, data visualization, automated analysis, software development.

In this study, we present the development of a novel plugin designed to quantify the colocalization of various organelles and signaling markers in microscopy images. The plugin begins with an essential preprocessing step to enhance image quality, ensuring that subsequent analyses are accurate and reliable. Users can then select regions of interest (ROIs), focusing on specific cells or areas within the images for detailed examination.

Once the ROIs are defined, the plugin calculates their volume by summing the area of the ROI across each Z-plane. Additionally, it computes the volume of each object or channel by applying a threshold to filter the area of each object, followed by summing the area in each Z-plane. To determine the number of objects present, the plugin utilizes the 3D Objects Counter, which counts the number of objects in a Z-stack based on the specified threshold. In the case of reporters as *mito*-QC[1], we first filter out red pixels that do not correspond to green, applying the 3D Objects Counter to the resulting mask for accurate counting.

Furthermore, the plugin facilitates the calculation of colocalization metrics, such as Pearson's and Manders' coefficients, between each pair of channels using the JaCoP plugin. The results include a comprehensive output image that displays each channel used along with the thresholds applied for volume and object calculations. Finally, all measured parameters are systematically recorded in an Excel file for easy access and further analysis. This plugin represents a significant advancement in the automated quantification of organelle colocalization and signaling markers both in cells and tissues, providing researchers with a powerful tool for exploring complex biological interactions in microscopy data.

[1]McWilliams TG, Prescott AR, Allen GF, Tamjar J, Munson MJ, Thomson C*, et al.* mito-QC illuminates mitophagy and mitochondrial architecture in vivo. *J Cell Biol* 2016, 214(3)**:** 333-345.

Advancing Diagnosis of COL6-CMD: AI and Transfer Learning in Muscle Tissue Analysis

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KEY WORDS: rare diseases, collagen VI, confocal microscopy, mapping, artificial intelligence, cellpose

Diagnosing rare neuromuscular disorders, such as Collagen VI-related Congenital Muscular Dystrophy (COL6-RD), presents significant challenges due to its low prevalence and the broad spectrum of clinical presentations, which often overlap with other forms of muscular dystrophy. Although genetic testing is the definitive diagnostic method, it is time-consuming and not always immediately available. A common diagnostic approach involves immunofluorescence analysis to evaluate collagen VI expression patterns. However, this method is laborious, subjective, and depends heavily on the clinician's expertise.

The incorporation of Artificial Intelligence (AI) offers promising potential to streamline and standardize the diagnostic process for rare diseases like COL6-RD [4-5]. Nevertheless, effective AI algorithms require large and representative datasets that capture the full heterogeneity of clinical presentations. This presents a major obstacle in the context of rare diseases, where limited data availability hinders the development of robust AI models. Specifically, utilizing a dataset comprising 154 confocal microscopy images of muscular tissue from a mouse animal model, categorized into three genotypic groups, the implementation of data augmentation techniques, including patching, appears insufficient to provide the necessary heterogeneity and statistical robustness essential for the reliable training of deep learning (DL) models.

To address the challenges in diagnosing COL6-RD, we leverage a deep learning-based approach using Cellpose, a pretrained model for cellular segmentation [1-3]. Cellpose's encoder, which is highly effective at extracting generalizable features from biological images, serves as the backbone of our model. By utilizing the pretrained weights of the Cellpose encoder, we exploit its extensive knowledge of cellular structures, thereby augmenting our model's capacity to identify patterns specific to COL6-related congenital muscular dystrophy (COL6-RD). To adapt the model for classification, we append fully connected layers to the encoder and fine-tune the entire network using our dataset. This transfer learning strategy enhances classification accuracy by

incorporating the robust feature extraction capabilities of Cellpose, which mitigates the challenges posed by limited data availability in rare disease diagnosis.

Our fine-tuned model demonstrates superior performance, surpassing models trained from scratch. By leveraging the pretrained weights of the Cellpose encoder, the model inherits robust feature extraction capabilities, which improves its ability to capture disease-specific patterns in the classification of COL6-RD images. This transfer learning approach has demonstrated superior classification accuracy compared to models built from random initialization, particularly in scenarios with scarce data. These findings suggest that incorporating pretrained models significantly enhances performance and mitigates the challenges posed by data scarcity in rare disease diagnosis.

Acknowledgments

We gratefully acknowledge the support of the European Commission, whose funding through the HORIZON-MSCA BE-LIGHT project (GA nº 101119924) has been crucial for advancing AI-driven diagnostics for rare neuromuscular disorders.

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ADVANCING MYELIN RESEARCH THROUGH DEEP LEARNING-ENHANCED MICROSCOPY

Pau Carrillo-Barberà[1-3], Thibaut Goldsborough[3], Andrea Poveda Sabuco[4,5], Jose Antonio Gómez Sánchez[4,5], Ana Rondelli[6,7], Anna Williams[6,7], Peter Bankhead[3,8]

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KEY WORDS: bioimage analysis, deep learning, myelin sheath, electron microscopy, optical microscopy

The myelin sheath is created by specialized cells wrapping their processes around axons and is pivotal for nerve function, protection, and maintenance. Quantifying myelin thickness on axon transversal cross-sections stands as the gold standard for assessing myelin development, disease-related degeneration, and tissue regeneration through remyelination. Consequently, annotating image data to distinguish between compact myelin, uncompact myelin (inner tongue), and axon is a common bottleneck in myelin research. In an effort to provide a versatile solution for quickly and accurately determining key metrics in myelin research, we've created AimSeg, a bioimage analysis tool designed for the segmentation of myelinated axons and the extraction of various myelin morphometric features, including the traditional g-ratio, as well as innovative ones that account for the often-overlooked inner tongue. Originally based on random forest pixel classification, we are now expanding AimSeg to incorporate a Convolutional Neural Network model based on U-Net for a panoptic segmentation of myelinated axons: instance segmentation of the fiber, and semantic, multi-class segmentation of compact myelin, inner tongue, and axon. This, combined with a QuPath extension providing a user interface, offers a user-friendly tool for fiber segmentation and comprehensive analysis. We have trained and tested this model on a diverse dataset obtained through various microscopy techniques, species, and tissues from multiple research groups, ensuring robust and reliable performance across different experimental conditions.

AI-DRIVEN AUTOMATED IDENTIFICATION AND GENETIC CHARACTERIZATION OF PLANKTONIC CYANOBACTERIA

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KEY WORDS: Automatic identification, cyanobacteria identification, genetic characterization, environmental analysis, taxonomy, morphology, artificial intelligence

Characterizing cyanobacteria is gaining importance as the effects of climate change lead to more frequent cyanobacterial blooms, which in some cases, can be toxic and harmful to both the environment and human health. This study has two main objectives: firstly, to automatically identify different genera of cyanobacteria using artificial intelligence techniques; and secondly, to examine the effects on the morphology of some cyanobacterial strains exposed to three environmental variables (light intensity, nutrient concentration, and temperature)

Automatic identification was conducted across 15 different genera: *Aphanocapsa*, *Chroococcus*, *Microcystis*, *Snowella*, *Woronichinia*, *Anabaena*, *Aphanizomenon*, *Dichothrix*, *Dolichospermum*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Phormidium*, *Planktothrix*, and *Schizothrix*. Subsequently, three of these genera were used for genetic analysis and environmental study: the two worst-performing (*Aphanizomenon* and *Planktothrix*) and the best-performing one in automatic identification (*Dolichospermum*)

Limitations in the automatic identification of cyanobacteria, partly due to morphological variations, as well as the limited number of datasets with a reduced sample size [1], can be complemented by genetic studies. The findings underscore the need to combine molecular techniques with morphological analysis, due to phenotypic plasticity complicates morphology-based identification, while genetic similarity limits molecular techniques. Environmental analysis revealed that changes in biomass, cell width, and pigment production were the most affected morphological characters. Increases in nutrient concentration was the primary environmental factor modifying morphology, highlighting the role of cyanobacteria's environment in biodiversity studies.

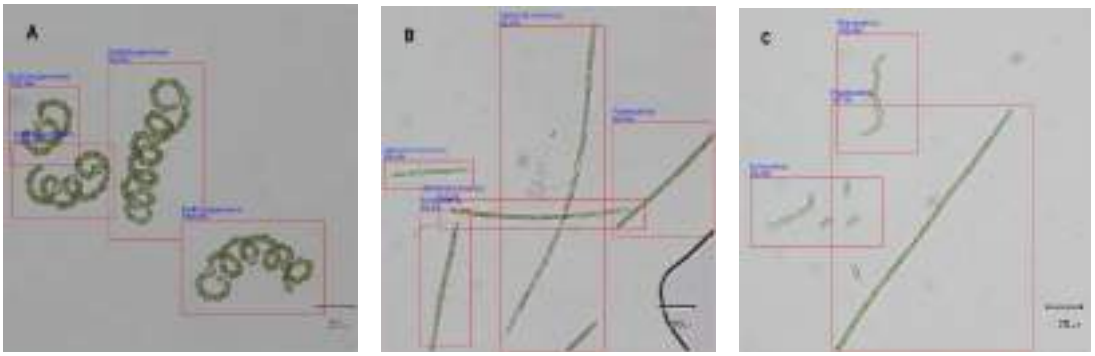

Figure 1. Cyanobacteria identification: A) *Dolichospermum,* B) *Aphanizomenon* and C) *Planktothrix* genus

[1] G. Bueno, L. Sanchez, E. Perona, et al., Microscopic image quality in few-shot GAN-generated cyano-bacteria images and its impact on classification networks. In *Optics, Photonics, and Digital Technologies for Imaging Applications VIII*, Proc. SPIE 12998, pp. 1299807 (2024).

An open source super-resolution microscopy extension with real time reconstruction capability

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Topic: Fluorescence microscopy, Super-resolution, Structured illumination microscopy.

Abstract

This work demonstrates a structured illumination extension for general epi-fluorescence microscope body gaining super-resolution ability. Its low photon-dose and speed makes structured illumination microscopy (SIM) an ideal super-resolution (up to 2x) fluorescence microscopy for live cell imaging. However, the high price tag of commercial and home build devices makes it a rare and exclusive tool not available to a large group of researchers.

In our setup, a digital mirror device (DMD) is used as a spatial light modulator operating at video frame rates with two excitation laser lines. The setup is fully controlled by the open source software "ImSwitch" [1], and thanks to the SIM reconstruction algorithm, the setup can reconstruct the raw images in real time. The self-contained module adapts to many commercial microscope bodies and can be replicated using off-the-shelf tools and hardware to provide high-resolution microscopy techniques on a small budget. This project is fully open sourced and a detail assembly tutorial is online available.

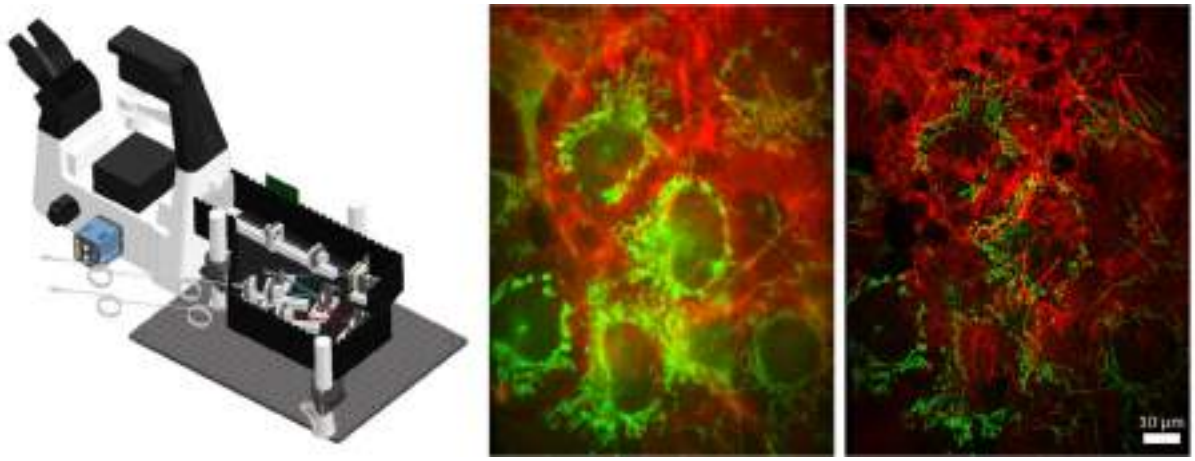

Figure 1: Left: 3D model of the structured illumination extension. Right: Cell sample with fluorescence labeled actin and mitochondrion. Left image: widefield microscopy, right image: structured illumination microscopy.

[1] Moreno et al., (2021). ImSwitch: Generalizing microscope control in Python. Journal of Open Source Software, 6(64), 3394.

BIOIMAGE ANALYSIS AND BIOPHYSICAL MODELING OF MULTICELLULAR SYSTEMS

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KEY WORDS: *Drosophila melanogaster*, spinning disk confocal microscopy, biophysical modeling, wound healing, tissue deformation.

Uncovering the mechanism of wound healing is crucial for unraveling fundamental processes in developmental biology and advancing biomedical applications [1]. In our research, we focus on the mechanics of cell and tissue dynamics during embryonic wound healing, employing *Drosophila melanogaster* as a model system. We acquire 3-dimensional time-lapse sequences of embryos regenerating their tissue after laser ablation using a spinning disk confocal microscope. We perform quantitative analysis of the process and the changes occurring after the sudden impact of the cut on the cells close to the wound and their intra-cellular organization. We observe changes at the intra-cellular, cellular and tissue-level, including cell shape changes and their reorientation, as well as anisotropic tissue deformation. We have implemented a biophysical model describing the spatio-temporal behavior of cells utilizing a modified Cellular Potts model [2]. By modulating the contractile forces at the wound edge or the mechanical properties of cells, we can control the kinetics of closure and cell shape changes. We have started to compare quantitatively our model to experiments performed in the laboratory on wounds of different sizes and shapes.

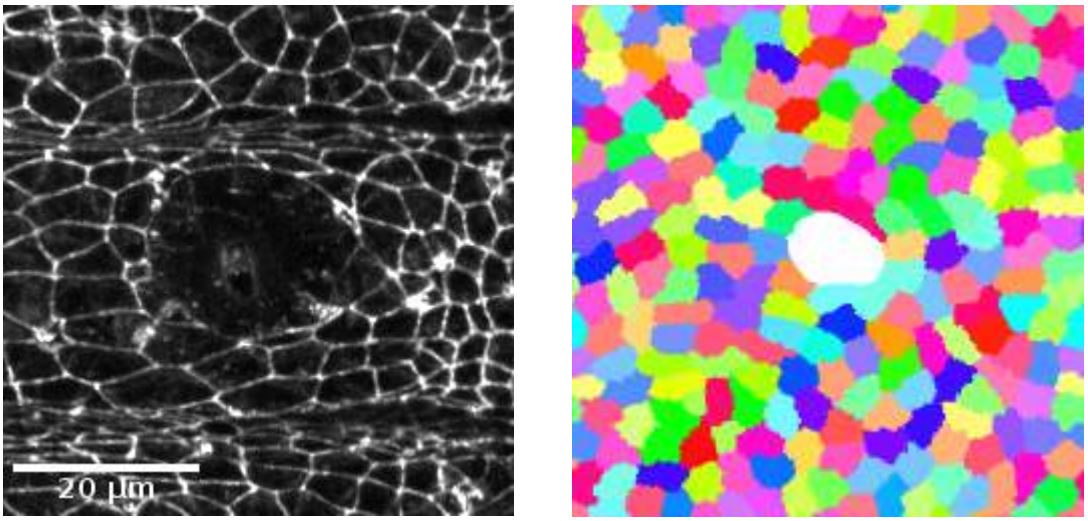

Figure 1. left) Image of epithelial tissue of *Drosophila melanogaster* embryo after wound generation (fluorescently labeled cell junctions), right) example frame from a simulation of the studied process by our Cellular Potts model.

[1] Y. Belacortu, and N. Paricio, Drosophila as a model of wound healing and tissue regeneration in vertebrates, *Developmental Dynamics*, (2011).

[2] F. Graner, and J. Glazier, Simulation of biological cell sorting using a two-dimensional extended Potts model, *Physical Review Letter*, (1992).

Biomedical Data Hub – A CaixaResearch initiative

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KEYWORDS: Research Data Management, FAIR[1] data, data sharing, data congruence

Effective data management represents a challenge within the scientific community, characterized by an excessively complex value chain that is untenable when managed on an isolated, project-specific basis. This process is further complicated by considerable bureaucratic demands related to data security and privacy concerns. Consequently, there is a critical need for academic and research institutions to establish robust initiatives aimed at the development and implementation of comprehensive, long-term data strategies. Such strategies should transcend the scope of singular research endeavors and include the provision of specialized training and the allocation of necessary resources to support these objectives.

The Biomedical Data Hub is designed to enhance data sharing across institutes associated with CaixaResearch, addressing the need for effective data management. A particular focus of one working group will be on imaging datasets, including microscopy. This group's mandate includes establishing best practices for the annotation and management of metadata, which are essential for improving data discoverability, usability, and integration with various technologies, under the FAIR data principles and close alignment with the forthcoming regulation of the European Health Data Space.

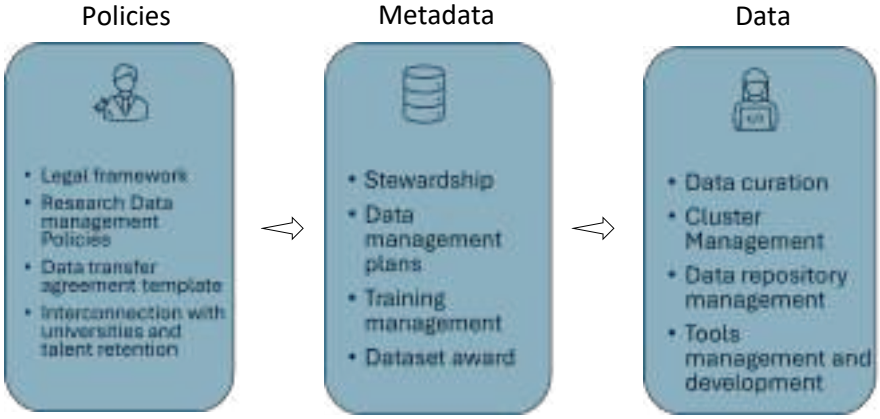

Figure 1. This initiative is structured around three foundational pillars: policies, metadata, and data. Specific activities aligned with these pillars will be implemented by dedicated working groups and supervised by a steering committee composed of representatives from each participating center.

[1] Wilkinson, M. D., et all The FAIR Guiding Principles for scientific data management and stewardship. Scientific Data, 3(1), 160018. https://doi.org/10.1038/sdata.2016.18 Ka

Can we trust the diffusion coefficient in biological membranes?

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The study of molecular events at the membrane is frequently undertaken using organic fluorophores tagged to a phospholipid. The choice of fluorophore is most commonly dictated by the fluorophore excitation and emission spectra, its brightness and its photostability. This is particularly true for Single-Molecule Tracking (SMT) studies where fluorophore prerequisites are long bleaching times and low-to-no blinking, whereas the potential effects of the fluorophore charge and hydrophobicity on the lipid behaviour are not often considered. In this work, we report on the diffusional behaviour of fluorescent phospholipids with different charge, hydrophobicity and fluorophore-lipid distance at the single-molecule level. Using SMT we have performed a systematic study of the diffusion of lipids tagged with different fluorophores on uniform supported lipid bilayers.

We describe three different diffusional regimes in uniform lipid bilayers. Fluorescent lipid probes participate of all three diffusion regimes, sometimes in the same trajectory. We present our results for commonly used fluorophores with negative charge (StarRed and Atto 488), positive charge (Rhodamine B and Atto647N), and a zwitterionic fluorophore with net zero charge (Atto 647). We note that the effect of the probe charge in diffusion has also been addressed in the past using ensemble methods such as confocal and STED FCS, but the observed trajectory richness can only be detected using single-molecule methods.

Since diffusion is the most significative read-out parameter in single-molecule studies, artefactual changes in diffusion may yield to the wrong conclusions on the phenomena under study. Our experiments provide valuable insight into fluorophore behaviour required for rigorous investigation of molecular events at the membrane.

CHARACTERIZATION OF 3D MATERIALS FOR BIOTECHNOLOGICAL APPLICATIONS

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KEY WORDS: Living cells, 3D cell culture, organoids, functional imaging

Materials for the development of 3D architectures have several biotechnological applications, from implants, regeneration of tissue, or models for drug discovery. These engineered materials are designed to replicate the complexity and functionality of native tissues for various research and therapeutic purposes.

In the construct of the 3D models several key aspects must be considered, biocompatibility, degradation rate, cell compatibility, mechanical properties, stimulus responsiveness and morphology among others, to suit appropriate scaffolds to the desire model.

The use of soft materials complies with most of the requirements, such hydrogels, biopolymers (1) or extracellular matrix. They´re shaped to form thin films, spheroids or larger structures such as organoids. All of them can be described as tissue structures engineered to mimic the three-dimensional architecture of the desired model

We would like to present some examples (2, 3) of these materials which proved successfully the use of biomaterials for the construct of 3D models. Their characterization by optical microscopy was key to assess functionality and viability under physiological conditions.

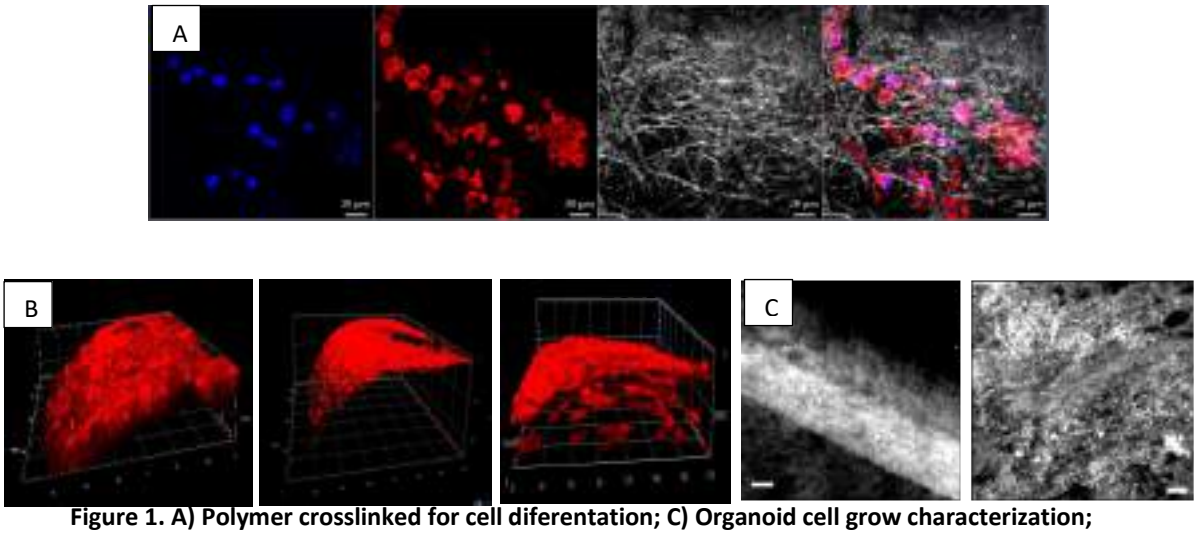

Figure 1. A) Polymer crosslinked for cell diferentation; C) Organoid cell grow characterization; D) SHG image collagen fiber organization for fibrosis model.

[1] **Khan, S.A., Shah, L.A., Shah, M. *et al.*** Engineering of 3D polymer network hydrogels for biomedical applications:a review. *Polym. Bull.* **79**, 2685–2705 (2022).

[2] **Aires, A. L. Cortajarena. *et al.*** Engineering multifunctional metal/protein hybrid nanomaterials as tools for therapeutic intervention and high-sensitivity detection. *Chem Science* **2021,** 12/7(2480-2487**)**

[3] **Arcos, J., Andres S, et al** A heterogeneous chemoenzymatic route toward the continuous transformation of γ-alkynoic acids into γ-hydroxy acids, *Cell Report Physical Science*, **2024** 5/6(102015)

Combining Holotomography and Confocal Microscopy for Cellular Nanodiamond Localization Studies

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KEYWORDS: Correlative Microscopy, 3D imaging, Phase Holotomography, Confocal Microscopy

Phase holotomography (HT) is an interesting technique for studying long-term motility, and morphologic analysis of cells in physiological conditions. This imaging technique allows label-free high-resolution 3D live cell imaging, however, the definite recognition of added particles in phase imaging is highly dependent on their optical characteristics. Fluorescent nanodiamonds (FND) with nitrogen-vacancy centres have strong fluorescence properties, as these point defects are known for their stability, also allowing the detection of magnetic fields [1]. Since action potential in neuronal signalling creates local magnetic fields, these particles are potentially interesting sensors for neurosciences. Thus, the interaction between FNDs and neurons, and their relative positions, are important factors to consider.

The FNDs' high refraction index lies outside the detection range of a traditional holo-tomograph, only their fluorescence signature can unmistakably locate them. HT microscopes can be upgraded with a widefield fluorescence detection channel, but this feature does not allow 3D localisation and is not sensitive enough to observe smaller particles. Nevertheless, these can be achieved with confocal fluorescence Imaging. We aim to use phase holotomography and confocal microscopy to study the impact of the FNDs addition in SH-SY5Y cultures - neurons. For that, after keeping the cell culture incubated with FNDs in the HT microscope, and imaging them over many days, the sample was fixed to stop its development and imaged with a confocal microscope. Combining the two sets of 3D images, an extra layer of characterisation of the FND localisation within the cell culture is achieved. We observe and locate the strong-fluorescent agglomerates, and also verify the presence of the smaller particles attached to the cells' membrane, that form a 3D cluster.

This work was supported by FCT (Diamond4Brain LCF/PR/HP20/52300001) and La Caixa Banking Foundation.

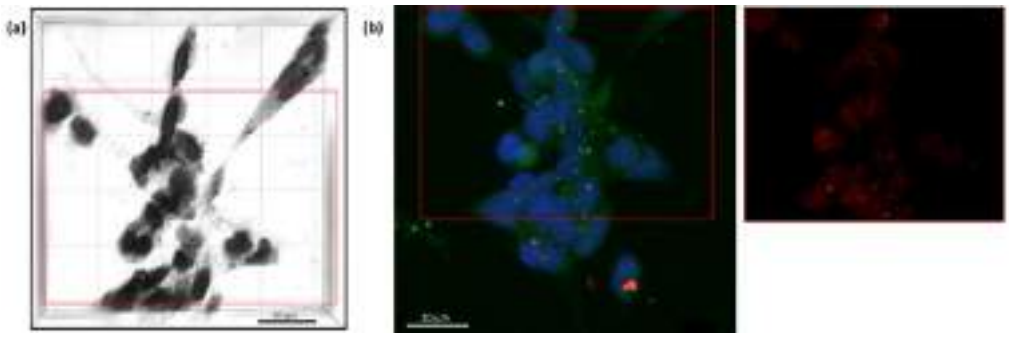

Figure. (a) Holotomography rendering, and (b) Confocal reconstruction and FND signal. The overlapping region are indicated. Comparing the images, details of the cell morphology can be seen with the HT, and the FND positioning can be defined with the confocal microscopes.

[1] **D. Glenn** *et al.* **Single-cell magnetic imaging using a quantum diamond microscope**. *Nat Methods* 12, 736–738 (2015). https://doi.org/10.1038/nmeth.3449

POSTERS

Title: COMPARATIVE STUDY OF COLLAGEN FIBERS IN CLINICAL SAMPLES OF ESOPHAGEAL EPITHELIUM USING EPIFLUORESCENCE COMBINED WITH DECONVOLUTION AND MULTIPHOTON MICROSCOPES

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7. Servicio de Anatomía Patológica. Hospital Unversitario de la Princesa, Madrid.
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KEY WORDS: Eosinophilic esophagitis; Fibrosis; Extracellular matrix; Second Harmonic Generation; Multiphoton.

Eosinophilic esophagitis (EoE) is a chronic disease of the oesophagus characterized by eosinophilic infiltration of the esophageal epithelium leading to remodeling and the development of fibrostenosis. The goals of treatment for EoE are to improve symptoms and reduce eosinophilic inflammation to prevent persistent histological activity from progressing to esophageal remodeling and fibrostenotic complications that may require esophageal dilation.

Disease diagnosis is typically performed by Anatomical Pathology services, who examine esophageal biopsies taken during upper endoscopy for the presence of ≥15 cells per high-power field, corresponding to an area of 0.24 mm², in one or more biopsy specimens after hematoxylin and eosin (H&E) staining. In this study, we analyzed paraffin sections of clinical biopsies provided by the Anatomical Pathology services of Hospital de La Princesa and the Niño Jesús Hospital, with the aim of investigating the possibility of objectively quantifying the extracellular matrix (ECM) in the epithelium, for possible future implementation into clinical practice. Samples were stained with H&E, Masson's trichrome stain or immunofluorescence staining against collagen type 6. Samples were then analyzed using a THUNDER Imager Tissue (Leica Microsystems) equipped with a color camera (H&E and Masson's stains) and a monochrome fluorescence camera (collagen) with a 40x objective and subsequently with a STELLARIS DIVE multiphoton microscope (Leica Microsystems) with a 25x water immersion objective to detect Second Harmonic Generation (SHG) associated with fibers in the lamina propia of the esophageal epithelium. **Fig.1** shows SHG as an effective method for investigating the deposition of collagen in the matrix of unstained specimens. As this is a secondary use of clinical biopsies from patients generated by Anatomical Pathology services, this workflow represents a valuable, flexible and objective detection method, although it requires experienced staff and a dedicated 2-photon microscope.

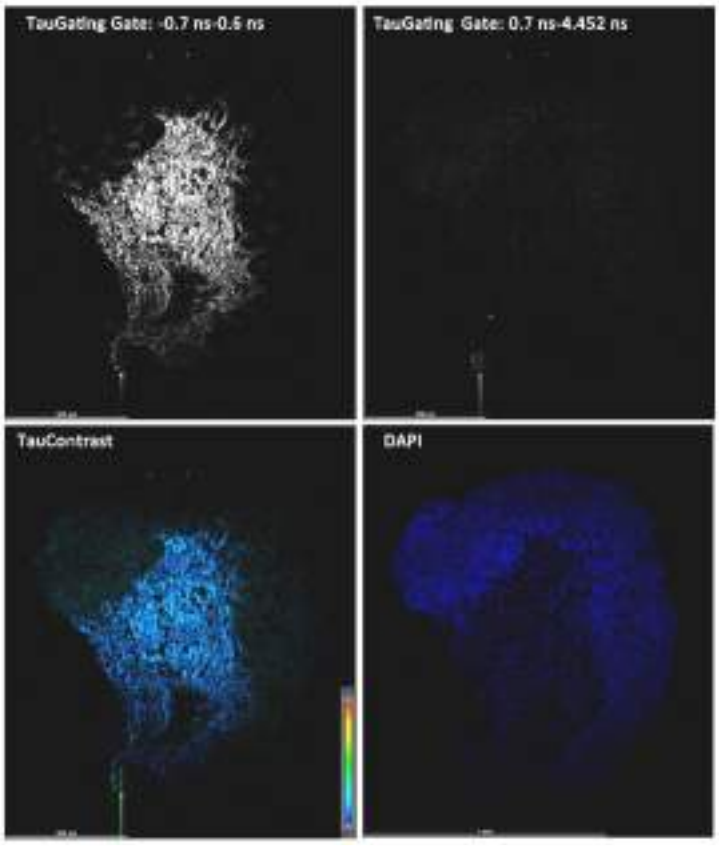

Figure 1. **SHG and fluorescence detection in a pediatric esophageal specimen using fluorescence lifetime.** Top images show fluorescence-lifetime (TauGating) separation; left – SHG collagen fibers gated at -0.7-0.6 ns of lifetime; right, DAPI fluorescence-stained nuclei gated at 0.7-4.45 ns of lifetime. Bottom left image shows the TauContrast detection of fluorescence lifetimes for SHG detection. Bottom right shows nucleus localization by DAPI staining.

COMPARATIVE STUDY OF DIFFERENT IMAGE ANALYSIS SYSTEMS.

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KEY WORDS: image analysis, Image j, Aivia

The comparison of image analysis tools in the fields of life sciences and biomedical research is crucial for selecting the most suitable methodology based on study objectives. Manual methods, while allowing detailed and precise control, are prone to limitations in result reproducibility. ImageJ, a widely used open-source tool, offers flexibility through its extensive range of plugins and segmentation tools but may not be ideal for automated analysis of large data volumes. On the other hand, Aivia, provides artificial intelligence algorithms that facilitate automated analysis, reducing human intervention and increasing speed and accuracy in the quantification of complex structures This study explores and compares three different approaches: manual image analysis methods, ImageJ software, and the advanced Aivia platform in a rat model of congenic diagphagmatic hernia where immune differential expression has been found. Embryonic paraffined brain was cut (5 μm) and stained following a standard immunohistochemistry protocol with a mouse antibody against CD68 (dil. 1/300). Images of the brain were taken with a confocal Stellaris 8 and a LASX software, analyzed with two blind observers, with Fiji Image J and Aivia softwares (3). Image J software analyzed sections with deconvolution and threshold plugin. Statistical analysis was made with t-student method and outcome correlation analysis. When the results were analyzed, no significant differences were found between these methods.This comparative analysis highlights the importance of selecting the appropriate analysis tool to optimize efficiency and accuracy in biomedical image research.

DETECTION OF BRAIN GENE EXPRESSION IMAGES BASED ON IMAGE PROCESSING TECHNIQUES

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KEY WORDS: brain gene, brain Allen, segmentation, clustering, registration.

The brain is organized into neuronal populations that display respective molecular identities, that is, they are characterized by the expression of specific gene combinations. Therefore, it is important to analyze gene expression in the different brain regions taking into account the full genome, as far as possible, as well the precise expression level of these genes in each brain location.

In our study, we obtained images corresponding to 334 experiments of situ hybridization from the Brain Allen Institute website (http://mouse.brain-map.org/) that belong to diverse gene families. From each experiment, we selected three representative coronal planes of the medulla oblongata, that were used for the subsequent analysis.

To this end a semantic segmentation of the cerebelum region was done previous the image registration of the entire dataset. The segmentation was done using a Deeplabv3-ResNet18, constructed by a Deeplabv3 model using a ResNet-18 backbone, defining the contour of the selected study area that corresponded to the aforementioned medulla oblongata. .

The registration was done using the Matlab Image Processing Toolbox with nonrigid technique for each image with respect to respective control images for each coronal plane. Subsequently, a clustering was done. The clustering consisted of a K-means analysis with R software, so that the pixels from each of the three representative planes were clustered into areas according to the quantitative expression level for each of the genes. This step was carried out trying different numbers of clusters, yielding subdivisions of the brain images into areas which were compared to the known classical anatomical subdivisions.

Therefore we have designed a procedure to retrieve brain images showing gene expression, and perform their segmentation, registration and clustering, allowing to define brain areas according to their molecular identity, based on the expression level of multiple genes in each precise location.

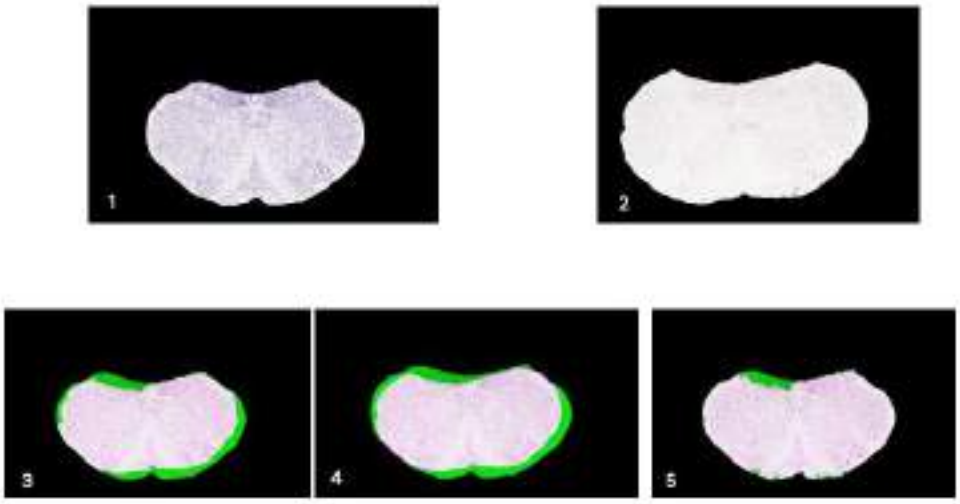

Figure 1. 1. Reference image; 2. Original image; 3. Rigid registration; 4. Deformable registration 1000 iterations; 5. Deformable registration 1500 iterations

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[2] **Jagalur M, Pal C, Learned-Miller E, Zoeller RT, Kulp D.** Analyzing in situ gene expression in the mouse brain with image registration, feature extraction and block clustering. BMC Bioinformatics. 2007;8 Suppl 10(Suppl 10):S5. doi: 10.1186/1471-2105-8-S10-S5. PMID: 18269699; PMCID: PMC2230506.

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[5] **Abbasi M, Sanderford CR, Raghu N, Pasha M, Bartelle BB.** Sparse representation learning derives biological features with explicit gene weights from the Allen Mouse Brain Atlas. PLoS One. 2023 Mar 6;18(3):e0282171. doi: 10.1371/journal.pone.0282171. PMID: 36877707; PMCID: PMC9987823.

Developing a 2-photon light-sheet imaging methodology for functional imaging of life retinal explants

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KEY WORDS: two-photon light-sheet microscopy, fast volumetric imaging, calcium dynamics, retinal explants

The retina is a complex three-dimensional neural tissue with light-sensing capabilities. Ideally, studying its functionality, understanding retinal disorders and developing potential therapies requires an imaging method capable of visualizing the neural signaling within the different retinal layers in its full three-dimensional functional complexity.

Typically, the sample of choice when studying retinal functionality are retinal explants – live retinas extracted from animal models, rats in our case. Studying the light response of such samples offers several advantages, but also presents specific challenges. To prevent the retinal explants from being "blinded", they should not be exposed to visible light once out of the eye. Once presented a carefully chosen visual stimuli, the neural activity in the retinal explants can be optically recorded through imaging a Ca2+ reporter, such as GCaMP. The latter allows us to observe flashes of fluorescence from the neurons whenever an action potential is fired. To resolve the temporal dynamics of the reporter, speeds of 5-10 fps are required. Typically, electrophysiology or 2P scanning point microscopy is used to record the neural activity. However, both are only limited to the top neural layer and do not have the capability to image in 3D at the required speeds.

Here, we aim to overcome this limitation and develop an imaging methodology capable to visualize in 3D neural signaling in live retinal explants. For this, we plan to use a 2P LS microscope. A digitally scanned LS will be formed by a Galvo mirror, while a second galvo mirror will scan the whole LS through the volume of the sample. An ETL will synchronously change the detection focal plane. We envision that this approach will allow us to image the live retinal explants at 5-10 volumes per second, enabling us to capture their functional response to light stimuli, introduced through a separate light path. Mounting of the sample will be particularly challenging and we are currently researching different possibilities.

Altogether, we aim to develop a methodology for fast 3D (5-10 volumes per second) Ca2+ dynamics imaging of retinal explants. We envision that this methodology will advance the understanding of the retinal neural circuit and open new avenues for developing novel therapies for vision restoration.

Development of a FLIM-FCS System: Single-Molecule Sensitivity and Enhanced Fluorescence Life-Time Measurements

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KEY WORDS: Fluorescence correlation spectroscopy, autocorrelation, diffusion, fluorescence life time

Fluorescence Correlation Spectroscopy (FCS) is an advanced and non-invasive technique for the quantitative analysis of fluorescent/fluorescently labeled molecules, providing insights into their concentration, mobility, and interactions both *in vitro* and *in vivo* [1]. Leveraging the high temporal resolution of time-correlated single photon counting (TCSPC) and the spatial precision of confocal microscopy, FCS achieves picosecond-scale temporal resolution and diffraction-limited spatial resolution (≈ 200 nm) with single-molecule sensitivity. Conventional Fluorescence Correlation Spectroscopy (FCS) utilizes temporal autocorrelation analysis of fluctuations in the recorded fluorescence signal caused by the motion of molecules (concentrations in nM range) through the small sample volume, often referred to as the focal volume (typically 0.2 – 1 fL) [2]. This approach is valuable for probing local microenvironmental factors, such as viscosity and pH, and for studying molecular processes affecting fluorescence signals.

Here, we present our custom-made Fluorescence Correlation Spectroscopy (FCS) system, which utilizes a laser diode operating at 488 nm to effectively excite fluorescent dyes such as Alexa 488, FITC, Atto 488, and Rhodamine 110. We conducted a comprehensive characterization of the system's performance by studying Rhodamine 110 in both aqueous solutions and sucrose solutions with varying mass percentages. In the case of a 2 nM aqueous solution of Rhodamine 110, we obtained a diffusion time of 30 µs. Further analysis of the obtained results clearly demonstrates that our home-built FCS system achieves single-molecule sensitivity and possesses a focal volume that is comparable to that of commercial instruments. Integration of the picosecond laser diode allows for simultaneous acquisition of fluorescence correlation and lifetime data, providing a more detailed understanding of molecular dynamics and environmental interactions.

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Development of a two-dimensional superresolution microscope using transmission diffraction grating obtained by analogue microfilming method

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KEY WORDS: super-resolution microscopy, structured illumination microscopy, diffraction grating

Structured illumination microscopy (SIM) is an advanced optical microscopy technique that can improve the resolution of images beyond the diffraction limit of conventional fluorescence microscopy. The technique is based on the illumination of the sample with a structured light pattern and the subsequent software reconstruction of the high-resolution image from several recorded images. We present the development of a custom-built setup for SIM equipped with a specially developed transmission diffraction grating. Using the analogue microfilming method, we have produced transmission diffraction gratings tailored to the specific requirements of our system. This robust and cost-effective method enables the production of diffraction gratings with customised constants that ensure excellent transmission in both the visible and near-infrared spectrum. To evaluate the performance of our system, we measured the resolution in both epifluorescent and super-resolution imaging modalities by applying the knife-edge technique to the MoS_2 monolayer flakes. We confirmed an improvement in the resolution of SIM over the epifluorescent imaging modality. Furthermore, we successfully demonstrated the capabilities of our microscope by imaging fluorescently labelled astrocytes *in vitro*, specifically targeting the vimentin filament protein in these cells. The super-resolved images reveal fine structures of the vimentin cytoskeleton that remain unresolved in the epifluorescent image.

Dual-view oblique plane microscopy (dOPM) for high-content imaging of complex and heterogeneous 3D cancer organoid models

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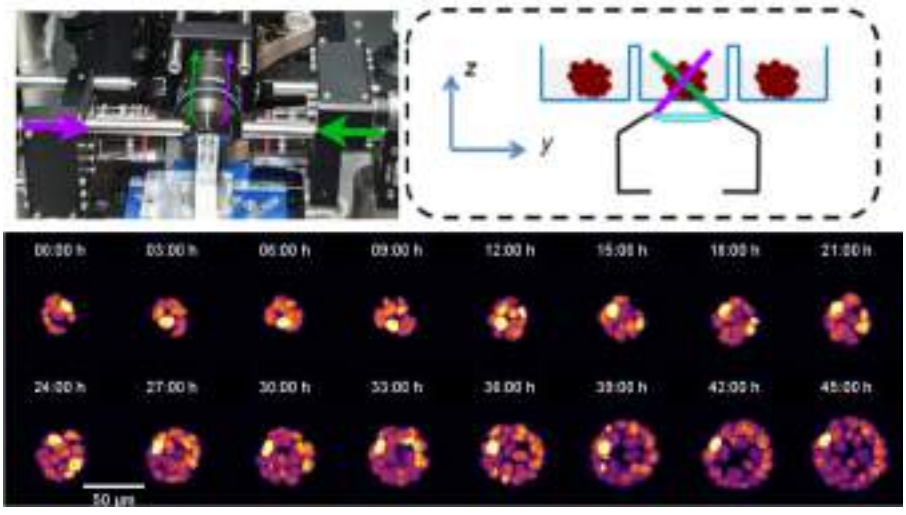
High-content analysis (HCA) of three-dimensional cancer models can provide insight into the complexity of cancer, and in vitro cultures such as mouse- or patient- derived organoids may improve our ability to probe cancer cells fate and behaviour and thereby characterize the efficacy of targeted treatment. Conventional high-throughput confocal microscopes, however, are less compatible with longterm 3D timelapse imaging of live samples due to phototoxicity and speed limitations that limits throughput. Light-sheet fluorescence microscopy (LSFM), on the other hand, enables fast and gentle optically sectioned imaging but common dual-objective LSFM systems typically require the positioning of two lenses in close proximity to the sample, which is not compatible with conventional sample carriers, e.g. multiwell plates used for HCA. However, Oblique Plane Microscopy (OPM) [1] uses a single objective for both light-sheet excitation and fluorescence detection and is compatible with conventional microscope frames and sample mounting. Moreover, Dual-view OPM (dOPM) [2] acquires two orthogonal views of the sample with the same objective lens, by translating a pair of tilted mirrors in refocussing space, and further image fusion of the views enables more uniform spatial resolution and reduced sample-induced image artefacts. Hence dOPM allows long-term single cell-resolved imaging of 3D cancer organoid models and can be applied to develop established workflows in drug discovery and cancer research, with the added value of single cell analysis and tracking, at high pace and over many days.

As part of the MACH3CANCER CRUK Accelerator Award [3], co-funded by the AECC in Spain, near-identical dOPM systems have been implemented at Imperial College London, Institute of Cancer Research and Francis Crick Institute in London, as well as the Institute of Genetics and Cancer in Edinburgh and the Institute for Research in Biomedicine, Barcelona. The custom-built systems consist of OPM-optics and hardware integrated with a commercial Nikon Ti2 frame, and are capable of multicolour, high-resolution, remotely-refocused 3D timelapse acquisitions of live 3D samples in multiwell plates.

We briefly introduce the recent developments in the design, construction, and image reconstruction with dual-view oblique plane microscopes at the partner institutes. We will share our experience in tackling the practical challenges associated with long-term timelapse image acquisition, as well as approaches to HCA pipelines developed as part of the MACH3CANCER project. To our knowledge, this version of dOPM is also the first OPM system that has been implemented, in an open-access platform, in Spain and we aim to raise awareness of its capabilities and discuss opportunities for scientists, in cancer research and beyond, to test and use it.



Top-left: The two light-sheets are coupled into the remote-refocusing relay (secondary objective lens) using two tilted mirrors, held by a (blue) piezo motor whose lateral translation enables switching between the two views and performing remote refocus of the detection plane. *Top-right:* scheme of dOPM illumination from the primary objective lens. *Bottom*: 2-days timelapse imaging of mouse organoid holding a nuclear label, at 15min. frame rate (Max.

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[3] www.mach3cancer.org

EFFECT OF ALKYLPHOSPHOLIPIDS ON THE BIOPHYSICAL PROPERTIES OF MODEL LIPID BILAYERS AND PLASMA MEMBRANES OF LIVE CELLS

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KEY WORDS: Fluorescence lifetime imaging microscopy, microviscosity, alkylphospholipids, supported lipid bilayers, living cells

Microviscosity measurements are a convenient way to monitor membrane-modulatory effects of small molecules. In this work, we investigated the structural changes of supported lipid bilayers (SLBs) and live cell plasma membranes induced by alkylphospholipids (APLs) – miltefosine and perifosine. APLs represent a promising class of antitumor agents, that unlike most of the chemotherapeutic drugs do not target DNA, but act on cell membranes and selectively induce apoptosis in tumour cells [1]. However, a detailed understanding of APLs' interaction with lipid membranes is still lacking.

Using a novel membrane-targeting viscosity probe BODIPY-PM, [2] we show that APLs induce an initial lipid ordering effect and increase membrane viscosity in both SLBs and live cells (Figure 1). We also observed the formation of dynamic fluorescent structures above the lipid bilayer in SLBs, likely indicating a weak detergent activity of APLs. Furthermore, incubation with miltefosine, but not perifosine, eventually lead to the decrease of membrane viscosities.

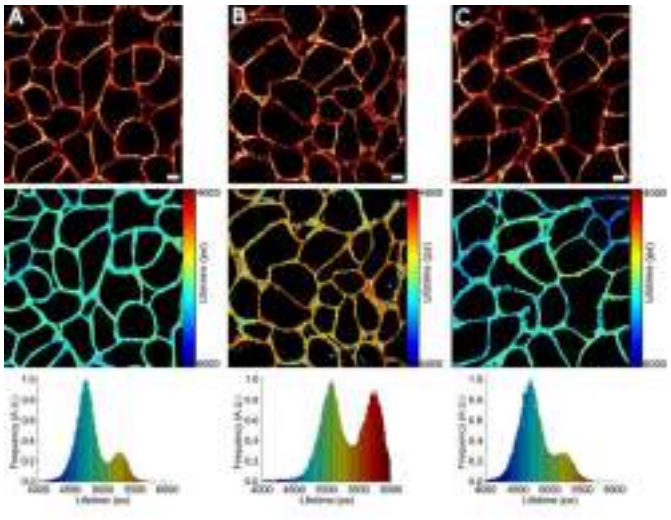

Figure 1. FLIM of BODIPY-PM in live MCF-7 cells (A) before, (B) 10 min and (C) 180 min after addition of 10 µM miltefosine. The top images show fluorescence intensity, the middle images show FLIM and corresponding lifetime histograms are at the bottom. Scale bars are 5 µm.

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Empowering Researchers with the BioImage.IO Chatbot: an interactive tool for Bioimage Analysis

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KEY WORDS: bioimage analysis, artificial intelligence, large language models, retrieval augmented generation, documentation, microscopy images

The dynamic field of bioimage analysis, particularly in microscopy imaging, continually seeks innovative tools to democratize access to sophisticated analysis capabilities and documentation. The BioImage.IO Chatbot [1], leveraging state-of-the-art AI technologies including Large Language Models (LLMs) and Retrieval Augmented Generation (RAG) [2], offers an interactive platform that significantly enhances the exploration and application of microscopy image analysis tools and models. This work highlights the BioImage.IO Chatbot's capabilities, emphasizing how it not only facilitates access to advanced analysis tools and documentation but also supports the direct analysis of microscopy images. Attendees will explore how to utilize the chatbot for interpreting complex image data and creating customized extensions tailored to specific microscopy challenges. Discover how the BioImage.IO Chatbot can transform your microscopy research by making sophisticated image analysis more intuitive and accessible.

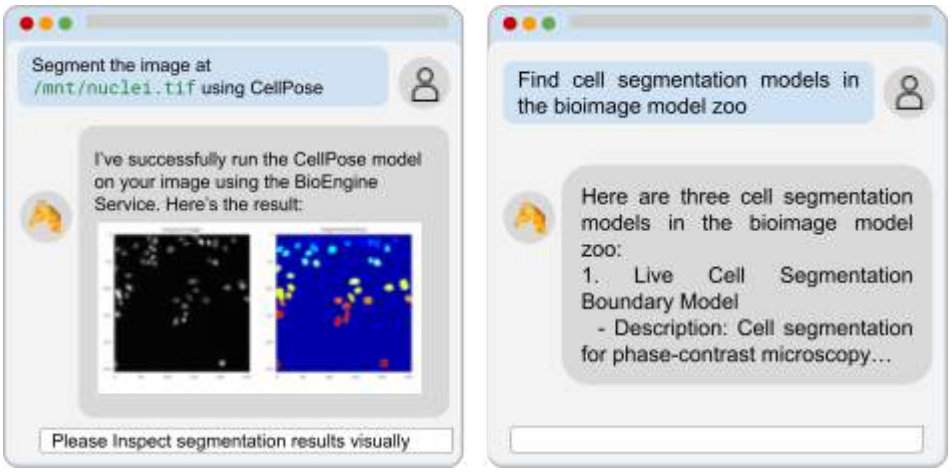

Figure 1. Illustrations of User Interactions with the BioImage.IO Chatbot. The left image depicts the BioImage.IO Chatbot executing successful segmentation on a microscopy image, requiring no coding from the user. The right image shows the BioImage.IO Chatbot querying various models from the BioImage Model Zoo to retrieve specific information.

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Enhancing Tissue Analysis through Fluorescence Microscopy with Laser Microdissection and Spatial Omics

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KEY WORDS: Acridine orange, fluorescence microscopy, spatial omics, Laser microdissection.

Acridine orange is a common fluorescent dye that has gained importance in recent years due to its versatility in clinical applications. Its ability to accumulate in the acidic environments of tumor tissues has enabled the classification of tumor types through fluorescence microscopy with rapid biopsy staining, offering an alternative to classic hematoxylin and eosin stains [1].

Spatial Omics combines a wide range of techniques that can revolutionize the simultaneous quantification of both physical and molecular tissue properties. Its emergence is linked to laser microdissection, enabling precise quantification and study of tissue sample distribution [2].

In this work, we present an alternative approach by combining mass spectrometry-based Spatial Omics with laser microdissection using fluorescence microscopy. This approach saves time and achieves better spatial information. The main advantage of this workflow is that all procedures can be performed on the same tissue section using acridine orange [3]. This allows for the identification of anatomical structures through optical fluorescence acquisition, followed by spatial profiling of lipids (MALDI MS-Imaging), the associated proteomics identification and quantification (LC-MS/MS) after selecting areas of interest via bisecting k-means segmentation of the tissue post-lipid location and subsequent laser microdissection. As a result, the presented workflow could be used as a new tool for high-precision data for basic and translational research projects.

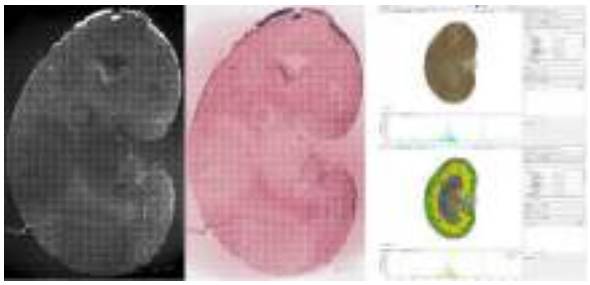

Figure. Tissue analysis workflow

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GIMM Bioimaging – A novel State-of-the-art, Multi-user, Multiscale Bioimaging Facility in the Lisbon area.

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KEY WORDS: Bioimaging services, imaging technology platform, super-resolution, microscopy, mesoscopy.

Abstract.

Biological imaging technology plays a pivotal role in advancing our understanding of living organisms at the molecular, cell, tissue, organ and organism levels. Bioimaging tools, from microscopy to medical and pre-clinical imaging modalities, give life-scientists and medical professionals the possibility to study cellular structures, biological processes, track organism and disease development and progression. The applications extend across numerous fields including biology, development, neuroscience, physiology, medicine and drug discovery, providing valuable insights for fundamental research, diagnostics and treatment development. Here we introduce the Bioimaging facility of the Gulbenkian Institute for Molecular Medicine, resulting from the merging of the former Advanced Imaging Facility of IGC and the Bioimaging U from iMM. This novel multi-user facility operates with open access and is equipped to address complex bioimaging projects spanning a wide range of scales, from nano to mesoscopy, offering fully dedicated and personalized support on experimental design, sample preparation, image processing and acquisition, instrumentation and bioimage data analysis, including a strong component also of advanced training in Bioimaging. Here we provide a quick overview of the vision, the team and the available technologies and how they are being used to address different biological questions across scales.

HIGH-RESOLUTION CRYO-CORRELATIVE MICROSCOPY TO STUDY IMMUNOGENIC CELL DEATH MECHANISMS

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KEY WORDS: Immunogenic cell death, oxaliplatin, cryo-correlative light-electron microscopy, volume imaging.

Immunogenic cell death (ICD) is a type of cell death characterized by the release of damage-associated molecular patterns that are sensed by dendritic cells and therefore able to induce an immunogenic response. In some cases, tumor cells undergoing ICD can release extracellular vesicles with bioactive elements able to reprogram dendritic cells. However, there is no clear hallmark to identify it, making it difficult to understand this process. Oxaliplatin induces ICD through a still unknown mechanism [1]. Interestingly, its counterpart cisplatin does not induce ICD.

We plan to address the mechanisms through which oxaliplatin induces ICD using cryo correlative light and electron microscopy (cryoCLEM). This approach will allow us to study cell ultrastructure at nearly native state. For that, MC38 cells non-treated and treated with either oxaliplatin or cisplatin will be vitrified, analyzed at the cryo confocal fluorescence microscope to select cells of interest and gain functional information, and transferred to a focused ion beam-scanning electron microscope. In this microscope, we will alternatively image the whole cell volume (Fig. 1), at about 10 nm resolution, or generate cryolamellae, which will be transferred to a cryo transmission electron microscope to perform cryo electron tomography, providing higher resolution and allowing the structural analysis at the molecular level.

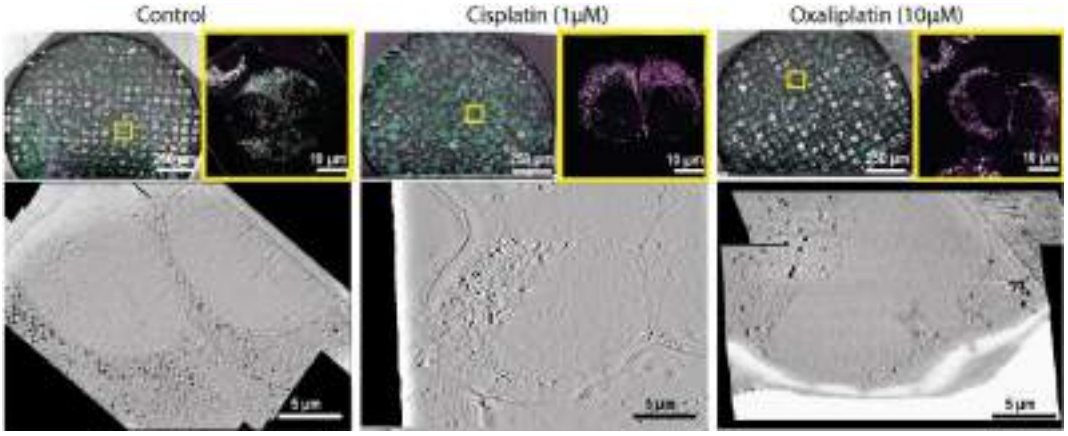

Fig 1. CryoCLEM workflow to study ICD induction. Non-treated, cisplatin and oxaliplatin-treated MC38 cells were submitted to a cryoCLEM workflow. Upper panels are the overlays of SEM and cryo confocal characterization with mitotracker Red (magenta signal) and NAO (green signal) for each treatment. Botton panels are the cryoFIB-SEM volume images of the selected areas (yellow-squared).

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High-speed sCMOS acquisition with optical pixel reassignment

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KEY WORDS: Living cells, fast acquisition, instrumentation and microscope design

Fluorescence microscopy has undergone rapid advancements yielding unprecedented visualization of biological events and shedding light on the intricate mechanisms governing living organisms. Scientific-grade CMOS (sCMOS) sensors offer high signal-to-noise ratios, quantum efficiency over 90%, and low readout noise under $2e^-$, making them suitable for low-light imaging. However, the trade-off between speed and field of view often limits their full potential, making it challenging to capture rapid biological events below the millisecond scale. Here, we introduce sHAPR, a high-speed acquisition technique that leverages the operating principles of sCMOS cameras to capture fast cellular and subcellular processes. The system utilizes a custom fiber bundle to compress a 2D image into a linear output, enabling ultra-high-speed recording (>25kHz). Attached to an epifluorescence microscope, the sHAPR system demonstrated its potential in applications such as high-throughput flow cytometry, cardiomyocyte contraction imaging, and neuronal calcium wave tracking.

Figure 1. Unprocessed video stack containing >10 excitations of the same Fluo-4 stained neuron. Right plot: Normalized signal from a single pixel indicated by white arrow fitted to a sigmoidal curve.

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Identifying specific macrophages as predictive biomarkers in human melanoma: older vs newer confocal acquisition and segmentation approaches.

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KEY WORDS: confocal microscopy, cell segmentation, prognostic biomarkers, artificial intelligence

Clinical diagnosis, identification of therapeutic targets and/or predictive marker estimation are tough tasks manually performed by experienced pathologists normally upon tissues stained chromogenically, which is an inevitably subjective and tiring process. During the last decade, we thoroughly screened a paraffin-embedded human primary melanoma collection and identified a specific macrophage phenotype with prognostic disease evolution value[1,2,3]. These studies were done by using multicolor fluorescence confocal microscopy and semi-automatic single-cell quantification, using the 'analyze particle' plugging of the ImageJ2 software. Simultaneously, an impressive technical (r)evolution has occurred concerning both the development of rapid-scanning fluorescence microscopes and the integration of artificial intelligence to cell segmentation applications. This coincidence in time has led us to challenge our 'classical' pinhole-based confocal + ImageJ procedure of identification of prognostic markers with a newer procedure that allows the screening of extended areas acquired by rapid fluorescence microscopes followed by AI-based segmentation. The current advantages and disadvantages of both pipelines will be discussed from the clinical view.

THIS WORK IS CURRENTLY IN PROGRESS!!! THIS IS A PRELIMINAR ABSTRACT THAT SHOULD BE MODIFIED IN OCTOBER.

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IMAGING CORE FACILITY AT CIMA: BEYOND MICROSCOPY.EXPLORING NEW FRONTIERS: THE IMAGING FACILITY AS A CENTER FOR INNOVATION IN BIOTECHNOLOGY

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KEY WORDS: Advanced Light microscopy, Animal imaging, 3D printing, microfluidics, biomedical image analysis

CIMA (Centro de Investigación Médica Aplicada) is a biomedical research institution of the Universidad de Navarra. Its main mission is to bring scientific discoveries from the laboratory to clinical practice, improving the diagnosis, treatment, and prevention of prevalent diseases. Located in a unique environment that encompasses both the Clínica Universidad de Navarra (CUN) and the Universidad de Navarra (UN), it creates has become an ideal ecosystem for the advancement of translational research [2,3].

CIMA's Imaging Facility is an advanced infrastructure that provides imaging services for biomedical research [4]. The facility is organized into five key areas: advanced light microscopy, animal imaging, analysis and development of advanced software, Prototyping with 3D printing, and design and fabrication of microfluidic devices. Its goal is not only to facilitate access to cutting-edge technologies for image acquisition and analysis but also to offer technical support and advice in the development of research projects, allowing researchers to optimize their studies and obtain more accurate results. This integration of services enables researchers to tackle their projects more effectively and efficiently, from image acquisition to data analysis and the development of experimental models.

Figure 1. CIMA's Imaging Facility key areas: microscopy, animal imaging, analysis and development of advanced software, 3D printing, and microfluidics

[1] https://cima.cun.es/

[2] https://www.cun.es/

[3] https://www.unav.edu/

[4] https://cima.cun.es/investigacion/plataformas-tecnologicas/imagen

IMAGING INFECTIOUS DISEASES: THE IRTA-CReSA BSL3 BIOIMAGING PLATFORM

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KEY WORDS: Biosafety level 3 (BSL3), Imaging facility, Confocal microscopy, Digital Lightsheet, Infectious agents, Zoonotic Diseases, High-biocontainment.

The IRTA-CReSA BSL3 Bioimaging Platform, operational since September 2023, is a pioneering facility in Spain, being the first within an ICTS (Infrastructura Científico-Técnica Singular) to offer live imaging of Biosafety Level 3 (BSL3) pathogens. The Platform is uniquely positioned within a BSL3 facility that includes experimental isolation rooms and laboratories, allowing for both *in vivo* and *in vitro* studies with high-risk pathogens. IRTA-CReSA, known for its focus on zoonotic diseases and animal health, also supports a wide range of research projects, from infectious diseases (such as SARS-CoV2) to drug development and other studies requiring high-biocontainment. Thus, the Bioimaging Platform stands out for its ability to handle and image live, high-risk agents under advanced biosafety measures. This makes it a distinctively safe resource for studying infectious agents and other biohazardous materials in controlled environments.

The facility is equipped with a Leica Stellaris 8 confocal system with Digital Lightsheet (DLS) module, allowing detailed 3D imaging of fixed and live samples. This technology has already been applied to organoid cultures, Arabidopsis plants, and mosquitoes, providing fast, high-resolution imaging of complex biological structures.

Additionally, the Sartorius IncuCyte SX5 offers continuous live-cell imaging and analysis, monitoring cellular behaviors in real time across up to six multi-well plates, offering valuable insights into pathogen dynamics. The BSL3 Bioimaging Platform also provides a dedicated image analysis workstation with support for software such as Imaris, MATLAB, Fiji, QuPath, and Python, ensuring comprehensive resources for data analysis and interpretation.

The IRTA-CReSA BSL3 Bioimaging Platform has supported research on pathogens such as the African Swine Fever virus, tuberculosis, prion diseases, SARS-CoV2, MERS-CoV, and West Nile Virus, highlighting its versatility. Accessible to both researchers and private companies, it promotes collaboration and innovation. Its advanced imaging technology and high-security setting provide a unique opportunity for safely studying high-risk pathogens, advancing Spain's infectious disease research infrastructure.

Impact of polystyrene nanoplastics and bisphenol A on skin fibroblast morphology

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KEY WORDS: Fluorescence microscopy, Immunofluorescence, Cell morphology

Nanoplastics (NPs) can interact with environmental pollutants, such as bisphenol A (BPA), posing an emerging global threat to ecosystems and human health. Previous studies have demonstrated that co-exposure to NPs and BPA enhance their toxic effects [1, 2]. In this study, we evaluated the cellular effects of polystyrene nanoplastics (PS-NPs) and BPA using cell-based assays, focusing on microscopy-driven analysis of cellular morphology. Human dermal fibroblasts (BJ5ta) were exposed to environmentally relevant concentrations of BPA and 1000 nm PS-NPs, both individually and in combination. Cell viability, proliferation and immunofluorescence assays targeting the cytoskeleton (alpha-tubulin, actin and nuclei) were used to assess the effects of single- (NPs or BPA) and co-exposure (NPs & BPA) on cell morphology. Our results revealed a dose- and time-dependent reduction in cell viability and proliferation for both treatments, with co-exposure leading to a significant intensification of these adverse effects. Morphologically, PS-NPs alone induced no notable changes in cells. In contrast, BPA exposure resulted in reduced cell density and cytoskeletal filament disruption, an effect further exacerbated by co-exposure to BPA and PS-NPs, leading to abnormal cytoskeleton morphology. These findings highlight the intensified cytotoxic and morphological effects on skin fibroblasts of co-exposure to PS-NPs and BPA. Further research using advanced microscopy techniques will contribute to bring insight into the cellular basis of the underlying mechanisms driving the observed adverse effects.

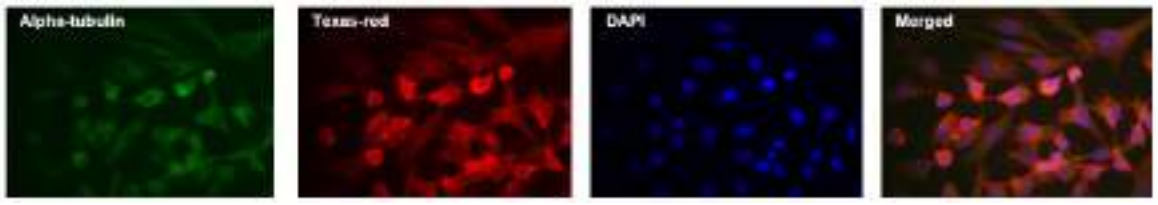

Figure 1. Representative images of BJ5ta cells under an inverted microscope.

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IMPLEMENTATION AND VALIDATION OF MODIFIED CELL PAINTING TECHNIQUE FOR CUSTOMIZED SCREENING OF ANTICANCER COMPOUNDS

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KEY WORDS: Cell Painting, High Content Screening, Fluorescence Microscopy, Cell profiling, Phenotypic Fingerprint, Image Analysis, Data Processing, Compound Screening, Cancer

ABSTRACT: The Cell Painting technique, an unbiassed high-content screening method broadly used in new compound screening campaigns, integrates multi-parametric profiling of cellular phenotypes to generate 'phenotypic fingerprints' of individual cells. This high-content screening method uses a tailored cocktail of fluorescent dyes to illuminate various cellular compartments and structures, thereby enabling a comprehensive analysis of cellular phenotypes in response to compound treatment. By combining image analysis and bioinformatic tools this approach allows conducting non-linear studies and detecting phenotypes that would otherwise go unnoticed using more targeted strategies.

Our group has innovatively adapted and validated the technique for a personalized screening project aimed at identifying compounds with anticancer action. The modification of the technique has significantly enhanced the sensitivity of detecting subtle phenotypic changes associated with anticancer activity. The successful implementation of this technique has been validated through rigorous testing of reference anticancer treatments, and comparison with the reference treatments of the original Cell Painting technique; and has shown promising results in identifying new potential anticancer compounds. This has profound implications for drug discovery and the development of personalized cancer therapies. Future work will focus on refining the technique to increase its specificity and applicability across a broader range of cell types and cancer conditions. This customized Cell Painting screening technique provides a robust platform for target deconvolution of anticancer compounds, paving the way for novel therapeutic strategies and personalized treatments.

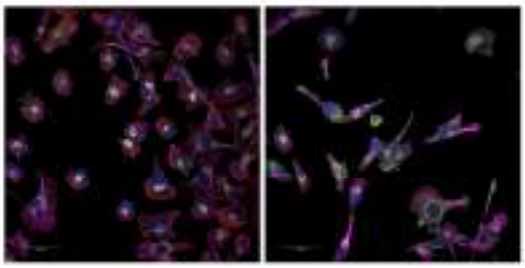

Figure 1.
Left panel: Control cells with customized Cell Painting staining. **Right Panel:** Treated cells with customized Cell Painting staining.

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POSTERS

Title: Increased resolution of organelles and cytoskeleton imaging by combining STED with fluorescence lifetime of fluorochromes

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KEY WORDS: cytoskeleton, mitochondria, actin, tomm20, STED

Understanding cellular behaviour such as signal transduction, transportation, and cell growth and migration is critically dependent on imaging and tracking subcellular structures at the nanometer scale. Super-resolution microscopy techniques such as STED (Stimulated Emission Depletion Microscopy) allow the diffraction limit imposed by the wavelength of light (approximately 250 nm for visible light) to be overcome by using a secondary laser to deplete fluorescence at the periphery of the excitation point of a scanning system. TauSTED, a method developed by Leica Microsystems that combines the optical information from STED with the fluorescence lifetime, allows increased resolution for a given STED light dose. TauSTED Xtend further enhances the resolutive potential of TauSTED images by obtaining their point spread function and performing deconvolution with an accelerated implementation of a Richardson-Lucy algorithm, providing higher quality images of biological samples at lower light doses.

Here, we present C57BL6 murine colon adenocarcinoma (MC38) cells stained with anti-TOMM20 (11802-1-AP, 1:200), followed by an Alexa Fluor 568-conjugated secondary antibody; and phalloidin conjugated to Alexa Fluor 488 (A-12379; 1:40). Fluorescence images were captured using a STELLARIS 8 STED confocal microscope (Leica Microsystems) with an HCX PL APO CS 100x/1.40 oil objective. Images were collected in confocal mode (10x zoom) with excitation at 488 and 568nm, and 2D STED depletion at 592 and 775 nm, respectively. Tomm20 is part of the outer mitochondrial membrane transporter complex that incorporates mitochondrial proteins synthesised in the cytosol (**Fig. 1A**). Our results show a 2-3 fold reduction in the size of the structures detected with TauSTED Xtend compared to confocal imaging, highlighting the improved clarity and resolution of TauSTED Xtend imaging for precise organelle analysis. This effect is also observed for actin filaments (**Fig. 1B**), allowing a more detailed study of subcellular structures with fluorescence microscopy.

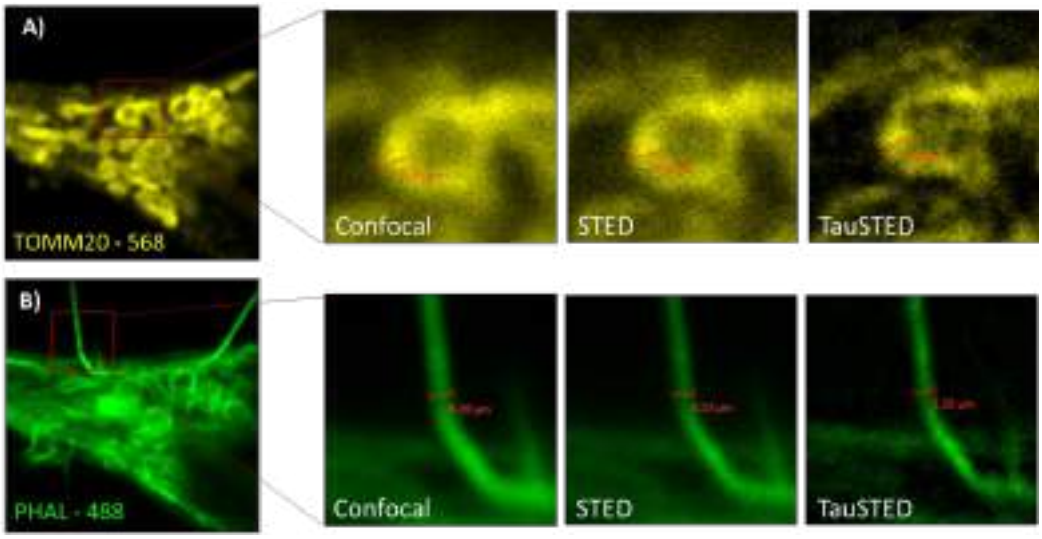

Figure 1. Comparative image analysis using confocal, STED, and TauSTED imaging of A) TOMM20 (yellow), translocase located in the outer mitochondrial membrane; and B) F-actin (green). The relative size of the structures is indicated.

Instrumentation Quality Control in the Portuguese Platform for Bioimaging - PPBI

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KEY WORDS: Bioimaging services, imaging technology platform, super-resolution, microscopy, mesoscopy.

Abstract.

Bioimaging data is crucial in fields such as cell and developmental biology, neuroscience, pathology, and drug discovery. Therefore, rigorous quality control (QC) of microscope equipment is essential to ensure accuracy, reliability, reproducibility, and the overall integrity of research. High-quality biological imaging relies on the precise calibration and upkeep of microscope systems, as even minor issues—such as degradation or misalignment of optics or illumination, or inconsistencies in light-detector response—can significantly affect or introduce errors in bioimaging data. Regular quality control procedures not only help detect and address potential issues before they impact research results but also ensure microscopes are operating at peak performance, thereby extending their lifespan and minimizing the risk of costly repairs or downtime.

At PPBI—the Portuguese Platform for Bioimaging—we have implemented minimum QC procedures across all national node imaging core facilities. The PPBI QC group is also involved and contributing to the international initiative QUAREP-LiMi. Additionally, we have organized our 1st workshop dedicated to training bioimaging facility staff, which covered not only the fundamentals of microscope operation and maintenance but also practical QC procedures. In this context, we present an overview of this novel workshop designed for facility staff, our proposed PPBI QC procedures, including standard operating procedures (SOPs), and some preliminary QC data from our node(s).

INTEGRATING MULTIPLEX IMMUNOFLUORESCENCE STAINING AND ANALYSIS FOR IMMUNE LANDSCAPE OF TUMOR SAMPLES.

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KEY WORDS*:* Multiplex, Tumor microenvironment, Machine learning, Deep learning, image analysis.

Recent advances in fluorescence microscopy, driven by multiplex immunostaining technologies and the integration of artificial intelligence, are changing the way we can understand the immunological landscape of tumors. In this presentation, we showcase our work in this field at CIMA's Imaging Facility. First, we show our protocol for multispectral scanning of tumor samples using the PhenoImager HT platform (Akoya)[1], enabling simultaneous detection of multiple biomarkers to obtain a comprehensive view of the tumor microenvironment. We describe the selection of antibodies, staining and image acquisition. Then we provide examples of data analysis using various software tools available for our users, including classical methods such as FIJI[2], machine learning approaches as Qupath[3], and AI-driven analysis using Naronet[4]. These diverse analytical strategies enhance our ability to identify unique immune cell populations and their interactions within the tumors, ultimately advancing in our understanding of tumor biology and informing personalized medicine strategies.

As we look to the future, the integration of these technologies will continue to enhance our capabilities in cancer research, paving the way for breakthroughs in diagnosis and treatment. By fostering collaboration between microscopy experts and data scientists, we can unlock the full potential of multiplex fluorescence microscopy in elucidating the complexities of tumor immunology.

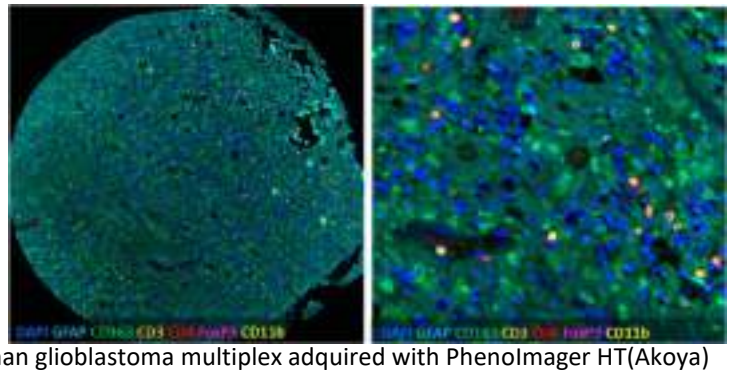

Figure1. Human glioblastoma multiplex adquired with PhenoImager HT(Akoya)

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[2] **CE. de Andrea; M.C. Ochoa; M. Villalba-Esparza et al. Heterogenous presence of neutrophil extracellular traps in human solid tumours is partially dependent on IL-8, J Pathol.,** 255(2):190-201 (2021)**.**

[3] **P.Bankhead, , M.B. Loughrey, J.A. Fernández et al**. **QuPath: Open source software for digital pathology image analysis**. **Sci Rep**, 7, 16878 (2017).

[4] **D. Jiménez-Sánchez et al., NaroNet: Discovery of tumor microenvironment elements from highly multiplexed images**, **Med Image Anal.**, 78:102384, (2022).

LEVERAGING NEXTFLOW FOR SCALABLE PROCESSING IN FLUORESCENCE MICROSCOPY: A Novel Approach in Cancer Research

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KEY WORDS: fluorescence, microscopy, image processing, Nextflow, spot, scalable, Cellpose, big data

In the rapidly evolving field of big data in fluorescence microscopy, the demand for efficient and scalable processing of large datasets is ever-increasing. Our methodology leverages the power of bioinformatics tools such as Nextflow[1] for handling data dependencies between tasks and parallelizing them to perform high-throughput data analysis.

Our approach focuses on the identification and in-depth characterization of spot signals that could explain relevant molecular mechanisms in cancer research such as DNA damage or Telomere responses. Using Cellpose[2], a generalist DL tool for segmentation, for spot and nuclei identification, we have trained a model that ensures precise spot localization in a wide range of 2D and 3D microscopy datasets despite the variability in signal and size. We have also developed a custom pipeline that establishes 3D/2D spot-nucleus pairs and extracts features for each pair, including fluorescence signal, size, morphology and spatial distribution. Our Nextflow-powered approach is efficient, reproducible and adaptable to various imaging conditions. It provides a framework for integrating additional modules, offering extensibility, and allowing to delve deeper into molecular mechanisms at play in cancer research.

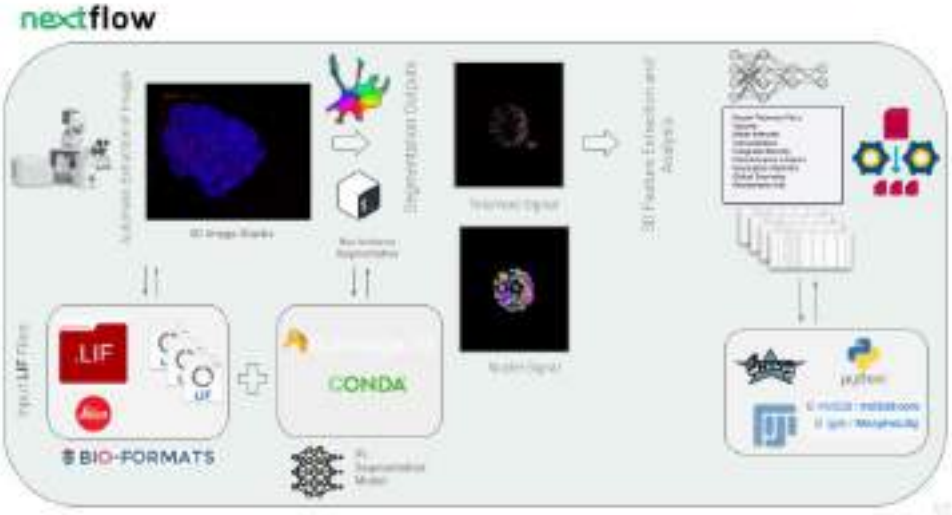

Figure 1. Schematic representation of our Nextflow-powered image processing pipeline.

[1] Stringer, C., Wang, T., Michaelos, M., & Pachitariu, M. (2021). Cellpose: a generalist algorithm for cellular segmentation. *Nature methods, 18*(1), 100-106.

[2] P. Di Tommaso, et al. Nextflow enables reproducible computational workflows. *Nature Biotechnology 35*, 316–319 (2017) doi:10.1038/nbt.3820

Microscopic features of fiber suture deposition patterns in common bean pods during domestication using fluorescent stains and TEM analysis.

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KEY WORDS: *Phaseolus vulgaris*, sutures, domestication, confocal microscopy, calcofluor white, auramine, cell wall, anatomy, lignin.

Common bean (*Phaseolus vulgaris*) has gained attention as a model species for legume biology, but little information is available about the histological anatomy of its pods and the relation of this morphology to seed dispersal or shattering. Pod shattering is a key agronomic trait of domestication since genotypes with reduced shattering lead to an increased crop productivity. Shattering depends on the differential patterns of lignified and non-lignified tissues in the ventral suture of the pod(1). Lignin and other secondary cell wall products exhibit an intense autofluorescence, and confocal microscopy approaches are being developed in plant anatomy studies (2). We used two different fluorescent stains, Calcofluor White and Auramine, to study the distribution patterns of primary and secondary cell walls in the pod ventral suture of two contrasting bean genotypes using confocal microscopy. Our results show that the secondary cell wall distribution is clearly different between the shattering-susceptible wild-type PHA1037 and the shattering-resistant domesticated PHA0595 genotype. This differences in the cell wall were also confirmed by TEM analysis of the suture cell walls. Both techniques could be of great value in the future identification of phenotypical shattering traits that took place along the evolution with an impact on crop improvement.

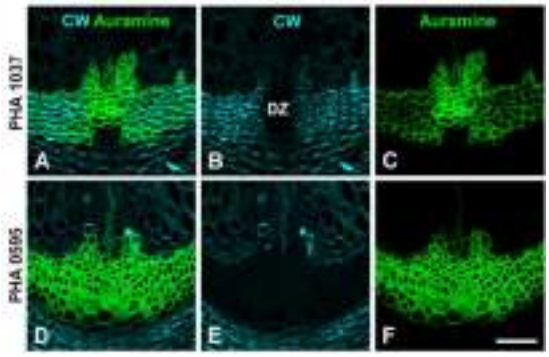

Figure 1. Anatomical comparison of wild-type shattering-susceptible PHA1037(A) and domesticated shattering-resistant PHA0595(B) genotypes. Representative confocal images at the ventral suture level show clear phenotypical differences in the distribution patterns of primary (CW) and secondary (Auramine) cell walls along the suture (scale 50um).

[1] **Parker TA *et al.* Pod shattering in grain legumes: emerging genetic and environment-related patterns.** *The Plant Cell* 33, 179–199 (2021).

[2] **Donaldson LA & Radotic K. Fluorescence lifetime imaging of lignin autofluorescence in normal and compression wood.** *Journal of Microscopy* 251:178–187 (2013

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OPTICAL AND CONFOCAL MICROSCOPY FACILITY AT THE IIBM

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KEY WORDS: wide field, confocal microscopy, super-resolution, STED.

The Sols-Morreale Biomedical Research Institute is a joint research center of the Spanish National Research Council (CSIC) and the Autonomous University of Madrid (UAM) [1].

The research lines of the IIBM groups are aimed at the study of the cellular and molecular mechanisms involved in different human pathologies, covering a wide range that includes cancer, metabolic and immune diseases, cardiovascular and neurodegenerative diseases, as well as congenital pathologies with a genetic basis. These projects range from basic studies of the molecular mechanisms underlying these diseases to translational studies that will advance their diagnosis and treatment, which is carried out through numerous collaborations with hospitals.

The Optical and Confocal Microscopy Facility (SEMOC) provides state-of-the-art technologies for wide field, confocal laser scanning and super-resolution microscopy through STED, covering most multi-colour fluorescence imaging studies with fixed and live samples [2]. This technology is not only applied to classical assays, but we go further, for example, applying confocal microscopy to electrophysiology assays, or using transmitted light microscopy in the acquisition of samples with polarized light.

The SEMOC also provides technical support to users, both in the acquisition and analysis of the images obtained, as well as training in the use of the equipment, thus contributing to the improvement of the quality of research. Our management commitment is focused on continuous improvement to help researchers obtain solid results.

All the equipment is available both to IIBM users and to researchers from other public or private institutions.

The facility has implemented a Quality Management system according to the UNE-EN ISO 9100:2015 regulation, certified by AENOR in 2009.

- www.iib.uam.es
- www.iib.uam.es/web/iibm/microscopia

OPTIMISING STORM TECHNOLOGY: HANDS-ON WORKFLOW DEMO

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KEY WORDS: STORM, Resolution, Subcellular.

Stochastic Optical Reconstruction Microscopy (STORM) is a high resolution microscopy technique, developed by X. Zhuang and colleagues in 2006, which allowed to reveal unresolved details of many cellular structures at nanoscale resolution. It is based on the consecutive emission of single photons from the activated state of a photosensitive molecule to allow its precise localization before it enters a dark state or is deactivated by photobleaching. Each fluorophore is activated separately, and by adjusting the point spread function (PSF), the centre of mass can be calculated to determine the location of a molecule down to a resolution of 20 nm. The parallel registration of many individual emitting fluorophores, each with its different set of coordinates, allows the reconstruction of an image with a high degree of resolution [1, 2, 3, 4].

Sample preparation and acquisition conditions require fine-tuning to allow a correct image reconstruction. At the CNB-CSIC Advanced Optical Microscopy Facility, we have optimized a protocol for sample preparation and acquisition conditions that has allowed us to visualize different subcellular structures including the actin cytoskeleton, mitochondria, tubulin, and lysosomes with a resolution in the range of than 50 nm. In addition, we were able to set up an acquisition protocol to perform multicolor STORM for the observation of mitochondria in combination with microtubules, or actin. In the current practical workshop, we want to share with the scientific microscopy community our optimized STORM **complete workflow including crucial steps** for **sample preparation**, **microscope configuration**, **live image acquisition**, as well as **data processing** and **image reconstruction** using the open source plugin tool ThunderSTORM [5] available for ImageJ.

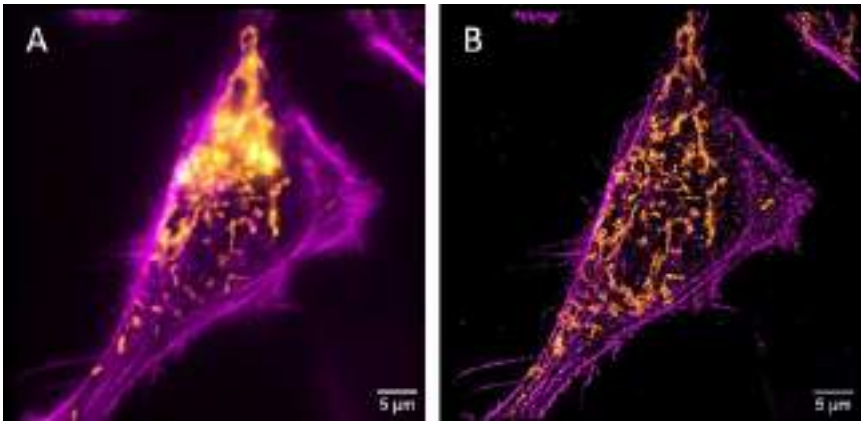

Figure 1. Comparison between TIRF (A) and STORM (B) images of filamentous actin (magenta) and mitochondria (yellow). Scale bar is 5 µm.

[1] **Rust, J.M.** *et al.*, **Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM).** ***Nature methods*** (2006).

[2] **Xu, K.** *et al.,* **Actin, Spectrin, and Associated Proteins Form a Periodic Cytoskeletal Structure in Axons.** ***Science*** (2012).

[3] **Wang, W.** *et al.,* **Chromosome Organization by a Nucleoid-Associated Protein in Live Bacteria.** ***Science*** (2011).

[4] **Bates, M.** *et al.*, **Multicolor super-resolution imaging with photo-switchable fluorescent probes. Science** (2007).

[5] **Ovesný, M.** *et al.***, ThunderSTORM: a comprehensive ImageJ plugin for PALM and STORM data analysis and super-resolution imaging.** Bioinformatics (2014).

Optimizing Core Facility Workflows for User Training and Reproducibility in Quantitative Imaging

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Established Quality Control (QC) protocols in a core facility ensure that microscopes perform reliably for users and that the obtained images are both quantitative and reproducible. QC can be assessed at various stages, from sample preparation and acquisition to image analysis, visualization, and validation. At ALMU, when users contact us for training, we ask them to fill out a request with detailed information about their sample (e.g. target proteins, fluorophores, mounting medium or imaging media in live specimens). This information helps us determine the most appropriate microscope for their training needs, considering specific lasers, filters, objectives, detectors and the structure of their sample. During training sessions, we assist users in defining settings for their samples and designing control experiments to ensure they can acquire high-quality images suitable for subsequent quantification.

To ensure reproducibility in imaging for reliable quantitative measurements, we conduct several regular tests to check quality control and system performance. We routinely conduct laser power measurements and monitor changes over time. We assess field illumination and check stage planarity. We measure point spread functions (PSFs) and assess their size, shape, and symmetry and measure resolution. We perform spectral registration for chromatic corrections to eliminate distortions and verify the alignment of invisible lasers with visible lasers in our confocal systems using sub-resolution TetraSpeck beads. These tests help us characterize our systems, monitor emerging issues, and correct them promptly. Here, we outline the workflow implemented in our core facility to ensure reproducibility in quantitative imaging.

QUAREP-LiMi: A global community-driven initiative developing common quality assessment and quality control guidelines, protocols, and tools for light microscopy

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KEY WORDS: Quality assessment, system performance, protocols and tools, reliability, reproducibility, standardization, quantitative bioimaging.

Comparing light microscopy measurements acquired with the same type of microscope across different laboratories or over a six-month period within the same lab remains challenging, making it difficult to determine the reliability of the results. What parameters and metadata must be measured and reported to allow reproducibility and comparability of experiments and data? These topics have been discussed for many years without a clearly coordinated community-wide effort.

In 2020, QUAREP-LiMi – Quality Assessment and Reproducibility for Instruments and Images in Light Microscopy was founded. This initiative has grown to more than 620 members globally and is hosted in Freiburg. QUAREP-LiMi members include academics, core facility staff, imaging scientists, national and international microscopy communities, microscopy and supply companies, and standards organizations on national and global levels. The following common goals unite these microscopy experts:

- Develop quality assessment tools and protocols that the entire global imaging community can adopt.
- Use standardized vocabulary and, when possible, standardized formats to report light microscopy instrumentation and configuration.
- Publish the QUAREP-LiMi guidelines and protocols.
- Work together with scientific publishers, national and international grant funding agencies, and scientific networks and associations to achieve a broad agreement, acceptance and usage within the scientific community.

QUPATH IMPLEMENTATION IN A CANCER RESEARCH MICROSCOPY CORE UNIT

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KEY WORDS: QuPath, microscopy, image analysis, tissue, cells, cancer, tumor, Cellpose, fluorescence microscopy, brightfield microscopy, AI, artificial intelligence, deep learning.

QuPath is a powerful open-source tool for image analysis that can provide an easy overview of big datasets such as entire tissue sections, with a very intuitive approach. In our facility, by generating whole tissue imaging using a Leica Thunder Imaging system, we can get very detailed and fast overview of a whole fluorescently stained tissue, crucial to extract all the biological information. We have developed a variety of pipelines using different strategies to understand molecular mechanisms involved in cancer progression such as vascularization around tumors, vessel structure, tumor microenvironment or transcription factors. We have also done RNAscope analysis to detect cells expressing a fluorescently tagged RNA. And interestingly, QuPath was also applied for brightfield segmentation of cell clones, to correlate area of a clone to its cell number. The integration of Cellpose-Qupath extension in our pipelines has made possible to segment cells with high precision.

QuPath has given us the possibility to handle and analyze big images of tissue sections and generate easily readable data outputs from those images, increasing the statistics and results robustness. We can teach researchers to run their own analysis after workflow development for a successful QuPath implementation in our microscopy Unit.

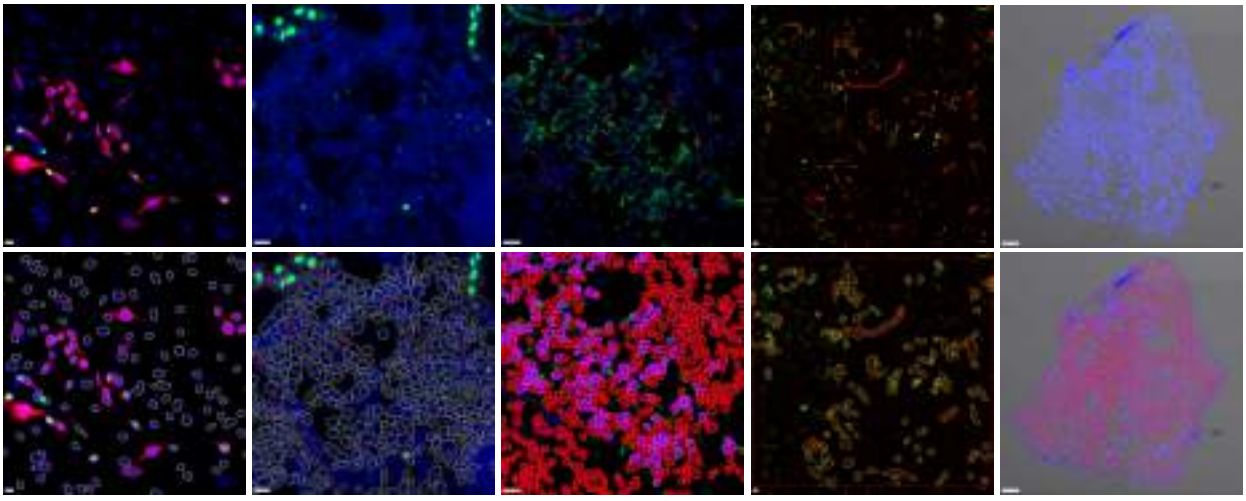

Figure 1. Representative images of QuPath segmentation and classification of different biological structures and cells. Images above are crops of the original image; images below are the same original images with the generated masks. Scale bars are 20 microns for 4 first panels, and 100 microns for last panel

[1] **Bankhead, P.** et al. **QuPath: Open source software for digital pathology image analysis**. **Scientific Reports**. https://doi.org/10.1038/s41598-017-17204-5 (2017).

[2] **Stringer, C., Wang, T., Michaelos, M.** et al. **Cellpose: a generalist algorithm for cellular segmentation**, **Nat Methods** 18, 100–106. https://doi.org/10.1038/s41592-020-01018-x (2021).

Rapid stem cell spreading induced by high affinity $\alpha_5\beta_1$ integrin-selective bicyclic RGD peptide in biomimetic hydrogels

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KEY WORDS: Living cells, cell-ECM interactions, Proteomics, functional microscopy

Cell-matrix interactions are important factors to consider when designing synthetic extracellular matrices. The most used approach to functionalize (synthetic) biomaterials is the conjugation of a linear or cyclic Arg-Gly-Asp (RGD) peptide, a common binder for integrins, the main receptor involved in ECM recognition. Polyisocianopeptide (PIC)-based hydrogels stand as a promising synthetic alternative due to their mechanical similarity to natural polymers.

Through a combination of proteomic and fluorescence microscopy our aim was to characterize the effect of different synthetic ECM replacements to develop better tools for 3D cell culture.

Proteomic analysis human adipose-derived stem cells (hASCs) revealed that the presence of RGD inside PIC polymers can overrule the effect of changes in matrix stiffness. Further evaluation of bicyclic peptides with a strong affinity towards specific integrin subtypes showed that hASCs spread within 24 hours in gels functionalized with one specific bicyclic α5β1 binder peptide. This was much faster than when using the same PIC gels decorated with other cyclic and linear RGD peptides or Matrigel. YAP/TAZ staining showed that the rapid morphological change in the 3D microenvironments was YAP-independent. Proteomic analysis of hASCs revealed that cells growing in the α5β1 binder peptide coated PIC overexpressed proteins involved in migration and actin remodeling, while those growing in Matrigel overexpressed proteins more related to matrix remodeling. Our results show that this synthetic PIC polymers result in an ECM with properties that are well beyond what is possible with biological materials. Our system has the potential to be a powerful tool to manipulate stem cell behavior and for tissue engineering.

Revealing Lipid Order Differences Between Cancerous and Non-Cancerous Cells Using Fluorescent Viscosity Probes

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KEY WORDS: Molecular rotors, microviscosity, FLIM, viscosity probes, fluorescence lifetime

Microviscosity is a key property of biological membranes, influencing passive solute diffusion, lipid raft formation, and overall membrane fluidity. Microviscosity measurements provide a convenient way for estimation of lipid order and observation of compositional changes in lipid systems. Viscosity-sensitive dyes, known as molecular rotors, can quantify microviscosity variations that result from cholesterol differences or phase separations in lipid bilayers or amorphous lipid systems [1]. In the case of cancer, multiple mutations produce cell signaling and metabolic aberrations, which change the overall composition of intracellular lipids and may allow for the identification of malignancies via microviscosity measurements [2]. In this study, we investigate the potential of BODIPY viscosity-sensitive dyes as diagnostic tools for cancer detection. Using fluorescence lifetime imaging microscopy (FLIM) combined with organelle-specific BODIPY dyes, we examine the lipid order in lysosomes and lipid droplets of live cancerous and non-cancerous cells. Our findings reveal significant variability in lipid order within lipid droplets across different cancerous cells in the same culture (Fig. 1A). In contrast, lipid droplets in non-malignant cells exhibit a uniform lipid order (Fig. 1B).

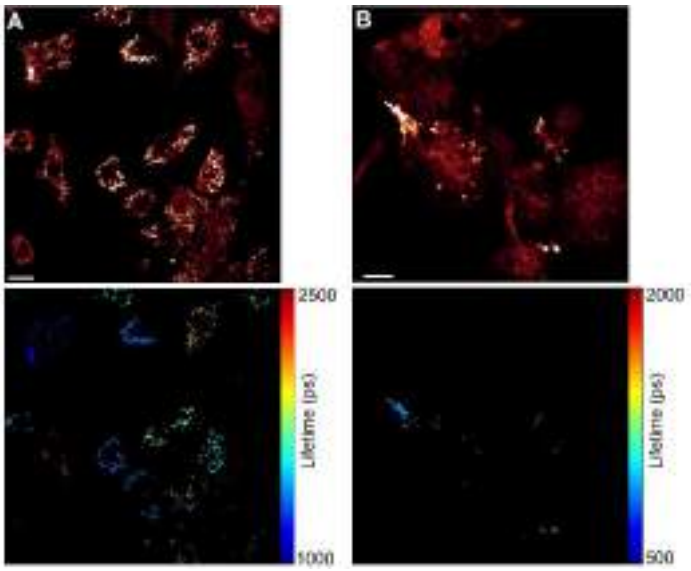

Figure 1. FLIM of BODIPY viscosity probe in lipid droplets of human lung cancer cells A549 (A) and human embryonic kidney cells HEK 293T (B). The top panel shows images of fluorescence intensity. FLIM images are shown in the bottom panel. Scale bars are 10 µm.

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[2] A. Polita; R. Žvirblis; J. Dodonova-Vaitkūnienė; K. Maleckaitė and G. Valinčius, Bimodal effects on lipid droplets induced in cancer and non-cancer cells by chemotherapy drugs as revealed with a green-emitting BODIPY fluorescent probe, *J. Mater. Chem. B*, 12, 3022-3030 (2024).

RI_Hubs project: amplifying the cooperation among Bioimaging & Structural Biology Research Infrastructures from Europe and Latin America

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KEY WORDS: Research infrastructures, microscopy facilities, super-resolution microscopy, Structural Biology

Research Infrastructures (RIs) are a key driver of scientific and technological advancement. However, the financial burden of developing infrastructure for exclusive use is often beyond the capacity of a single country. Additionally, the development of infrastructure in a single region is constrained not only by financial resources but also by human resources, including training, management, specialized researchers, and technicians. Shared use and cooperation of infrastructures can therefore generate a more significant impact in driving scientific and technological innovation.

The RI-HUBs project has the objective of establishing a bi-regional initiative involving Bioimaging & Structural Biology RIs in the European Union (Portugal, Spain, Italy) and in Latin America (Brazil, Peru and Uruguay) countries, together with two pan-European RI Consortia (Instruct and Euro-Bioimaging ERICs). Cooperation between these RIs will facilitate the following: (1) adoption of state-of-the-art imaging technologies, (2) recruitment and training of skilled professionals, (3) implementation of standard research infrastructure best practices, (4) collaboration with local industry partners, and (5) streamlined access to advanced RI. Additionally, the project will reinforce networking among RI teams through staff exchanges, visits, and workshops, thus strengthening international collaboration.

SCATTERED LIGHTSHEET MICROSCOPY FOR LABEL FREE CLEARED TISSUE IMAGING

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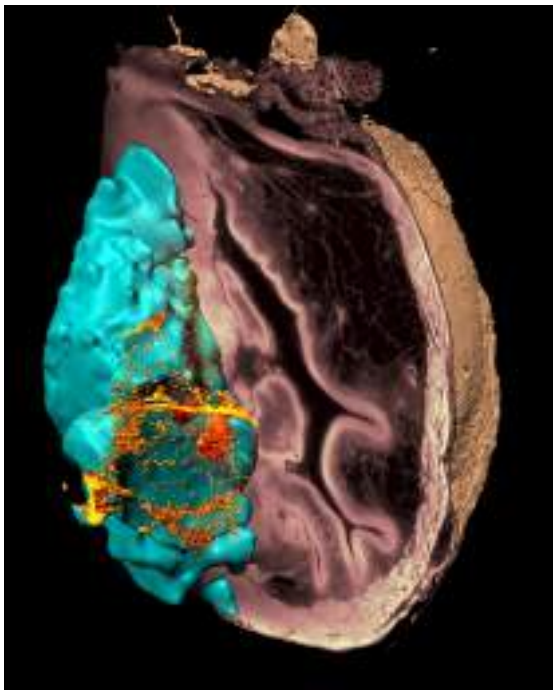
The combination of tissue optical clearing with lightsheet fluorescence microscopy at mesoscopic scale (mm to cm) greatly evolved in the past decade and became a routine assay to complement slice-based histological studies with slice-free 3D tissue imaging capability. However, the efficient delivery of fluorescent labels (e.g. antibody-based immunofluorescence) into large tissue samples is still a primary challenge and often, full penetration of fluorescent labels is greatly impaired. On the other hand, label free imaging of cleared tissues by autofluorescence may help providing contextual information in 3D (e.g. recognizing tissue morphology, type, layers, etc…), but the interpretation of autofluorescence signals in e.g. *ex vivo* adult mouse tissues is still challenging and lacks molecular or subcellular specificity. Thus 3D lightsheet imaging of cleared tissues would benefit from complementary readouts via label free imaging.

Scattered imaging is a label free method, applicable to many imaging modalities, that enables one to generate complementary information by imaging the response of a sample to the illumination light source that scatters through it, with the main difference to (auto)fluorescence imaging that this scattered "illumination" light is intentionally not rejected, but directly imaged. Reflection Microscopy, Confocal Reflection Microscopy or interference scattering microscopy are known contrasts that exploit backscattered laser light. In a similar fashion, lightsheet microscopy was recently shown [1,2] to reveal complementary 3D information by directly imaging the photons orthogonally scattered from the laser lightsheet field. Although still in its infancy, this contrast method is new and promising. The image quality directly depends on the light source noise levels (e.g. laser speckle), and it was shown to be also light-polarization dependent [1]. Here, we use scattered lightsheet imaging in cleared tissues and explore the nature of the scattered signal in several compartments of mouse tissues and organs. By exploiting double sided illumination and detection, and polarization control in excitation and detection, we reveal a polarization-dependent response that suggests that different parts of the tissues respond differently to one-another, suggesting a tissue-specific signal. We use this know-how [3] to optimize scattered imaging in mouse bladder tumours tissues, in the presence of unlabelled gold-coated silica nanoparticles (NPs), urease-powered nanorobots, used to target tumour tissues. In this context, we show that label-free scattered imaging reveals unlabelled nanoparticles throughout entire organs, but that polarization control is essential to differentiate NPs signal from that of the rest of the healthy and tumour tissues.



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[2] Zhou X, *et al*. Front Immunol. 2022 Jan 28;13:828634. doi: 10.3389/fimmu.2022.828634.
[3] Simó C, *et al.* Nature Nanotech. 2024 19, 554–564.

Segmentation and Analysis Method for 3D Cell Structures Enhanced with Open-Source AI Tools in Holotomography Images.

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Holotomography (HT) imaging enables label-free, high-resolution four-dimensional (XYZ and time) observation of cell morphology and subcellular components by measuring their refractive index (RI). However, extracting specific features from HT images can be challenging without manual annotation. To address this, we developed an image analysis pipeline combining RI-based thresholding algorithms, post-processing procedures, and AI models trained with RI information for precise segmentation and analysis. We employed Cellpose and StarDist for 2D and 3D cell and nucleus segmentation, generating robust predictions across various cell types. The ilastik model was trained for mitochondria segmentation, creating cell line-specific models to improve prediction accuracy. Lipid droplets were segmented using rule-based algorithms based on their distinct RI. For long-term imaging, TrackMate was used for object tracking, allowing the measurement of temporal dynamics of biological objects. This approach enables the extraction of morphological and biophysical properties from HT images, enhancing the analysis of cellular structures and dynamics. In conclusion, our method effectively isolates individual cells and organelles from HT images, maximizing HT's capabilities for real-time, high-resolution, and quantitative analysis.

SUPER-RESOLUTION MICROSCOPY WITHIN HIGH-LEVEL BIOSAFETY FACILITY FOR LIVE STUDIES OF PATHOGEN-CELL/TISSUE INTERACTIONS

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KEY WORDS: Infectious diseases, live cell imaging, tissue imaging, super-resolution, BSL3.

The Coronavirus pandemic highlights the need for advanced tools to study emerging high-level biosafety pathogens. Super-resolution fluorescence microscopy is one such tool with a proven record of driving novel discoveries in the field of infectious diseases [1]. Here, we present a super-resolution fluorescence microscopy platform built inside biosafety level 3 facility (BSL3) in the Comparative Medicine and Bioimage Centre (CMCiB) at Can Ruti Campus, Badalona, Barcelona. This platform is comprised of a highly upgradable open-bench 3D STED super-resolution microscope optimised for, but not limited to, live cell imaging studies covering high-level biosafety pathogens and animal model tissues. It will complement and enhance infectious diseases and pathogen research (SARS-CoV-2, HIV-1 and TB, among others) as well as *in vitro* laboratories and animal housing infrastructure, already existing within the CMCiB BSL3 facility. This platform, open to all internal and external academic and industry projects, represents a unique opportunity for studies of pathogen-cell/tissue interactions in the context of highly relevant fully infectious animal models, organoids or cell cultures. Moreover, it provides a microscopy facility R&D opportunity for adapting and deploying multiple microscopy techniques (such as multi-photon microscopy, adaptive optics, intravital imaging or remote microscopy) on a single machine in the constrained environment of a high biosecurity infrastructure.

[1] **Chojnacki, J.** and Eggeling, C. Super-resolution fluorescence microscopy studies of human immunodeficiency virus. *Retrovirology* 15(1):41 (2018)

POSTERS

The hidden architecture of the giant unicellular algae *Caulerpa*

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KEY WORDS: Caulerpa, LightSheet Microscopy, cellular organization, morphological diversity

Caulerpa is a fascinating genus of green macroalgae capable of reaching extraordinary sizes, consisting of a giant single cell. This unique organism plays a significant role in marine ecosystems and offers promising opportunities for biotechnology and industry [1]. While certain *Caulerpa* species can be invasive, disrupting native habitats, others have the potential to be valuable resources for food, pharmaceuticals, and industrial applications [2]. However, to fully understand *Caulerpa*'s ecological impact and biotechnological potential, we must delve into its internal organization.

Using a ZEISS Z.1 LightSheet Microscope (Carl Zeiss, Microscopy GmbH, Germany), we are creating a framework to visualize the three-dimensional structure of *Caulerpa* species. Our focus lies on understanding how the diverse morphologies of this genus, ranging from leaf-like to branched to grape-like, influence the distribution and organization of essential cellular components such as plastids, nucleus and filaments. By investigating the correlation between form and organization, we aim to shed light on the cellular structures that contribute to the remarkable morphological diversity within this genus and how these variations may impact cellular function.

Developing an approach to image *Caulerpa* using lightsheet microscopy presents unique challenges due to its large unicellular size and delicate structures. These include optimizing sample preparation, minimizing autofluorescence, and effectively visualizing the intricate details of these giant single cells. Our preliminary results suggest that *Caulerpa* exhibits complex internal organization, highlighting the importance of advanced imaging techniques for studying this remarkable organism.

Our initial imaging experiments have revealed structures and networks within *Caulerpa*, that challenge easy categorization. As we refine the imaging process, we anticipate uncovering additional organizational patterns and gaining a deeper understanding of this remarkable organism's internal complexity.

Fig. 1 One single cell of *Caulerpa lentillifera* (ZEISS Stemi 305 Microscope)

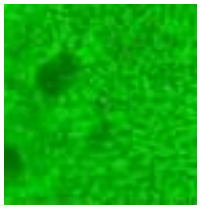

Fig. 2 Cytoplasma of *Caulerpa prolifera* (ZEISS Axiovert 5, 10x/0,2 HD)

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[2] Kohen R, Nsyka A, Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and method for their quantification, *Toxicol Pathol*, 30:620-650 (2002)

Tomographic Imaging Using Reflective Fourier Ptychography

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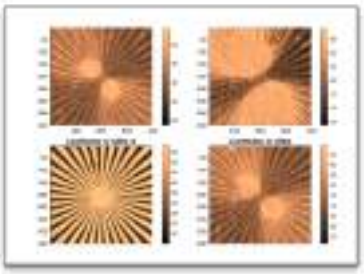
KEY WORDS: super-resolution, quantitative microscopy, Ptychography, coherent illumination, 2.5D imaging

This paper presents a reflective mode ptychographic microscope with substantial potential for life sciences research. Fourier ptychography, a recently introduced computational imaging technique, facilitates the reconstruction of the complex amplitude of a sample surface with high resolution and a large field of view. Initially developed for transmission mode setups, this technique has now shown promise for applications requiring reflective mode imaging.

We focus on biosamples that necessitate reflective mode imaging to acquire information that is inaccessible through transmission mode. Our primary objectives are to achieve tomographic and multilayer imaging. Utilizing coherent illumination from multiple LED sources, we reflect light at the sample's boundary from various angles to capture extended diffraction signals. This approach enhances image quality by optimizing the combination of bright-field and dark-field signals in Fourier space.

Currently, we are working with resolution target samples, but we anticipate applying this tool to biological samples in the near future.



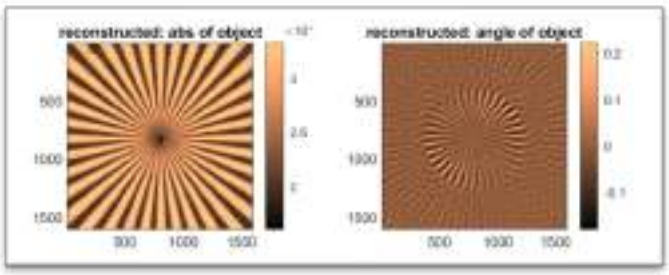


Figure 1. microscope images from different angle of illumination, **Figure 2.** High resolution reconstruction microscope images

[1] G. Zheng, R. Horstmeyer, C. Yang, "Wide-field, high-resolution Fourier ptychographic microscopy". *Nature Photon* 7, 739–745 (2013).
[2] H. Lee, B. Chon, H. Ahn, "Reflective Fourier ptychographic microscopy using a parabolic mirror," *Opt. Express* 27, 34382-34391 (2019).

SPAOM 2024 ABSTRACT
(Viral infection reshapes host genome structure by sequestering RNAP II)

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ABSTRACT: Herpes simplex virus type 1 (HSV-1) remodels the host chromatin structure and induces a host-to-virus transcriptional switch during lytic infection. The mechanism of remodelling and the specific kind of folding produced on the host chromatin are still unknown. Using Super Resolution microscopy to identify the HSV-1 induced mechanism of chromatin compaction can offer us insights into the underlying mechanisms regulating the high order chromatin folding.

We found that the host chromatin massive condensation is caused by the hijacking of RNA polymerase II (RNAP II) to the viral replication compartment (VRC) by the immediate early viral protein ICP4. Furthermore, sequestered RNAP II was found to be highly associated to individual viral DNA (vDNA) clusters. This viral mechanism of host chromatin rewiring sheds light on the role of transcription in chromatin architecture.

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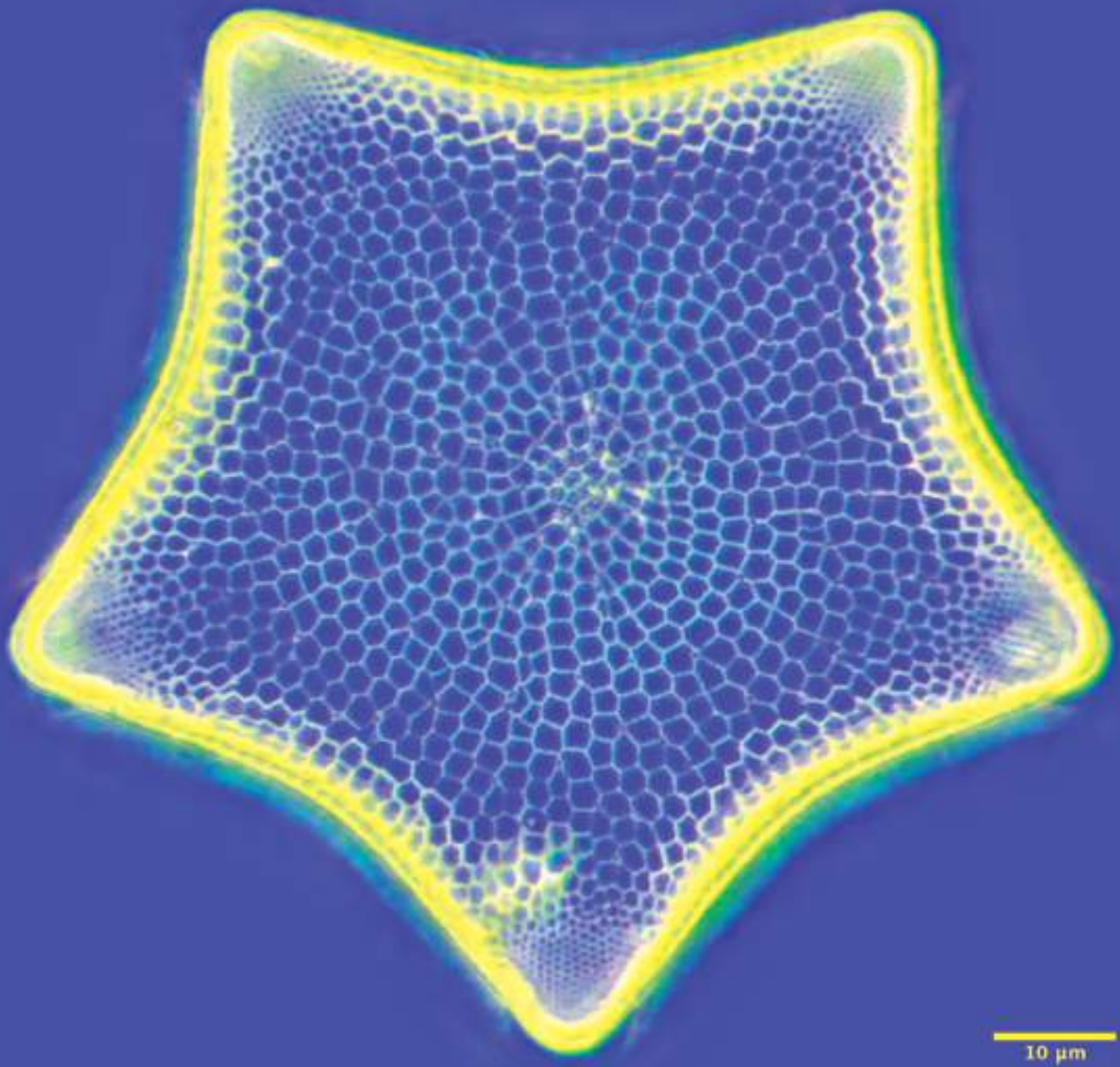

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Awards

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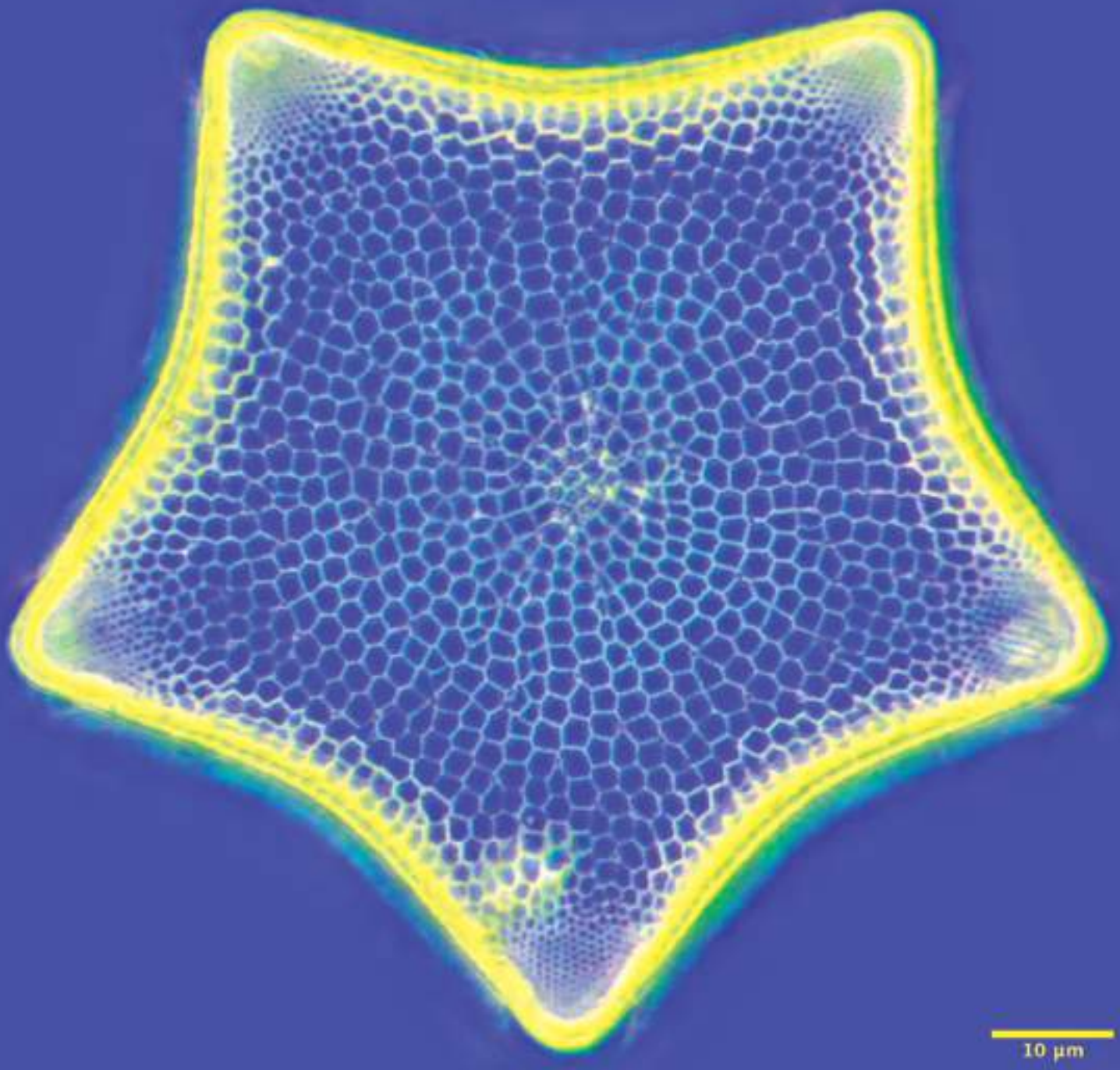

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