BP 17: Bioimaging

Time: Wednesday 9:30–12:45 Location: H 2032

BP 17.1 Wed 9:30 H 2032

X-ray tomography techniques for predicting medical implants performance — •Tatiana Akhmetshina, Robin E. Schäublin, Andrea M. Rich, and Jörg F. Löffler — Laboratory of Metal Physics and Technology, Department of Materials, ETH Zurich, Switzerland

Mg-based temporary implants that can be resorbed after fracture healing are beneficial for patients and necessary in certain clinical applications. However, since Mg reacts with body fluids and dissolves, we need reliable data to predict the implants performance in vivo. X-ray tomography techniques can provide valuable insights into the degradation behavior, but they also show limits in materials characterization. While absorption contrast works very well for some materials, in Mg-based alloys we often have features (second-phase particles) that cannot be resolved due to their low contrast. Additionally, the resolution required to characterize the material and understand the microstructure-property relationships is below 100 nm. In this study, we compare two distinct X-ray tomography techniques (Zernike and ptychography) to examine their advantages and disadvantages and present a case study focused on Mg-based alloys (WE43 and Mg-Ca: X0). Our results show that ptychographic tomography resolves features of less than 10% difference in their densities, such as a Mg2Ca phase in a Mg-Ca alloy, which is not possible with the Zernike. The 3D resolution reached is 23 nm, which allowed us to distinguish fine microstructural details. This illustrates the suitability of ptychographic X-ray computed tomography for the characterization of Mg alloys.

BP 17.2 Wed 9:45 H 2032

Helium Ion Microscopy for Morphological Analysis of Thrombi Extracted via Thrombectomy for Acute Stroke — •Michael Westphal[1], Natalie Frese[1], Clemens Sommer[2], Alkisti Kitsiou[3], Wolf-Rüdiger Schäbitz[3], André Beyer[1], and Armin Gölzhäuser[1] — [1]University Bielefeld — [2]Institut für Neuropathologie, Universitätsklinik Mainz — [3]Universitätsklinik für Neurologie, Evangelisches Klinikum Bethel gGmbH, Universitätsklinikum OWL

Strokes are one of the leading causes of death in the aging Western society. Especially in elderly patients, strokes are frequently recurring events. An essential component of stroke management after acute therapy is to diagnose the cause of the stroke forsecondary prevention. More than 50 thrombi extracted via thrombectomy were examined by chargecompensated helium ion microscopy to investigate possible correlations between their morphology and the origin.

BP 17.3 Wed 10:00 H 2032

The behaviour of vital mitochondria in response to nanoprobing using Scanning Ion Conductance Microscopy (SICM) — •Eric Lieberwirth[1], Christian Völkner[1], Regina Lange[1], Anja Schaeper[2], Magdalena Otte[2], Rica Waterstradt[2], Annett Kott[2], Ingo Barke[1], Simone Baltrusch[2], and Sylvia Speller[1] — [1]University of Rostock, Institute of Physics — [2]Rostock University Medical Center, Institute of Medical Biochemistry and Molecular Biology

The mitochondrial network maintains contacts with cell organelles such as the peroxisome, endoplasmic reticulum (ER) or cytoskeleton, but also undergoes constant remodelling of its structure and shape. In order to understand the processes further, we examine vital, metabolizing mitochondria extracted from HeLa cells using the non-contact Scanning Ion Conductance Microscope (SICM). The lateral resolution is approx. 50 nm - 100 nm and the resolution in the z-direction is approx. 1 nm. Besides the known diversity of shapes of the mitochondrial network, simple image processing techniques are sufficient to visualize structures, compatible with cristae folds. We observed a peculiar effect at the edges of mitochondria which decreases with time and which we interpret as a signature of vitality. An attempt of interpretation is based on the mistaking of the glass nanopipette probe as an organelle, e. g. as ER or microtubule.

BP 17.4 Wed 10:15 H 2032

High-resolution chemical imaging in mid-infrared photo-induced force microscopy (PiF-IR) — •Maryam Ali[1], Selema Buzhala[1,2], Sebastian Unger[1,2], Christoph Krafft[1,2], Rainer Heintzmann[1,2], and Daniela Täuber[1,2] — [1]Friedrich Schiller University, Jena — [2]Leibniz Institute of Photonic Technology, Jena, Germany

Non-contact force microscopy is able to report changes in the attractive Van-der-Waals (VdW) force between a metallic tip and a sample. In mid-IR photo-induced force microcopy (PiF-IR), thermal expansion due to absorption leads to a change in the VdW force, which is reported via a heterodyne detection scheme. This results in an unprecedented resolution < 5 nm for chemical imaging of surfaces including bacteria walls and cellular tissue[1]. In biomaterials, important chemical variations have to be identified above a rather heterogeneous background. Topological Data Analysis (TDA) is a promising approach for extracting signals of interests in hyperspectral PiF-IR images. We compare the analysis using TDA with other approaches including Principal Component Analysis (PCA), Hierarchical Clustering Analysis (HCA) and to a guided approach using known spectral components. [1] J. Joseph, L. Spantzel, M. Ali, D. Moonnukandathil Joseph, S. Unger, K. Reglinski, C. Krafft, A.-D. Müller, C. Eggeling, R. Heintzmann, et al., Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2024, 306, 123612.

Invited Talk BP 17.5 Wed 10:30 H 2032

Red photocontrollable fluorescent proteins in nanoscopy — •Francesca Pennacchietti — KTH Royal Institute of Technology, Stockholm, Sweden

The observation of organelles dynamics and macromolecular complex interactions inside living cells and tissues, requires minimally invasive imaging strategies. In this context, photocontrollable fluorescent proteins (FPs) play a crucial role as tags in optical super-resolution microscopy and functional live cell imaging. To this end we have previously shown that reversibly switchable FPs enable fast (1 Hz for a 50 x 50 um2) and gentler (< 1 kW/cm2) nanoscopy (Masullo et al, Nat Comm, 2018). Additionally, irreversibly photoconvertible FPs can achieve photolabeling with high spatiotemporal precision. Nevertheless, their photophysical complexity poses some challenges in expanding such techniques toward multiplexing and in vivo imaging. Here, we explore novel photoswitching mechanism for fluorescent proteins in the red and near-infrared region of the spectra and assess their compatibility with live cell imaging at the nanoscale (Pennacchietti et al, Nat. Meth, 2018). Finally, we present strategies to combine the spectral and photophysical fingerprint of distinct photocontrollable FPs to achieve multiplexing in live cell imaging at the nanoscale and photolabeling studies (Pennacchietti et al, Nat Comm, 2023).

15 min. break

BP 17.6 Wed 11:15 H 2032

eSRRF: Super-resolution radial fluctuation is going 3D — R. F. Laine[1], •H. S. Heil[2], S. Coelho[2], J. Nixon-Abell[3], A. Jimenez[4], T. Wiesner[4], D. Martinez[2], T. Galgani[5], L. Régnier[5], A. Stubb[6], G. Follain[6], S. Webster[7], J. Goyette[7], A. Dauphin[5], A. Salles[8], S. Culley[1], G. Jacquemet[6], B. Hajj[5], C. Leterier[4], and R. Henriques[2] — [1]UCL, London, UK — [2]IGC, Oeiras, PT — [3]Cambridge University, Cambridge, UK — [4]CNRS-AMU, Marseille, FR — [5]Institut Curie, Sorbonne Université, Paris, FR — [6]University of Turku and Åbo Akademi University, Turku, FI — [7]University of New South Wales, Sydney, AU — [8]Institut Pasteur, Université de Paris, Paris, FR

Fluctuation-based image reconstruction extracts super-resolution details from brief wide-field image sequences, even under low light conditions (Gustafsson, Nat. Com. 2016). In the latest eSRRF version (Laine & Heil, Nat. Methods 2023, 10.1038/s41592-023-02057-w), reconstruction fidelity is significantly improved, with automated parameter optimization for image fidelity and resolution, crucial for avoiding artifacts (Culley, Nat. Methods 2018) and reducing user bias. While eSRRF excels in 2D super-resolution across microscopy techniques and biological systems, we've expanded its capability to 3D imaging.

For high-fidelity 3D live-cell nanoscopy with eSRRF, simultaneous detection of fluorescence fluctuations across multiple focal planes is crucial, achieved through a multifocus microscope (MFM, Hajj, PNAS 2014). This method allows volumetric super-resolution imaging of live cells at ~ 1 vol./s.

BP 17.7 Wed 11:30 H 2032

Quantitative visualisation immune cell interactions in complex three-dimensional environments — •Anna Schepers[1,2], Narain Karedla[1,2], Joannah Fergusson[2], Helena Coker[2], Kaitlyn Purdie[3], Robert Koechl[2], and Marco Fritzsche[1,2] — [1]The Rosalind Franklin Institue, Harwell, UK — [2]Kennedy Istitute of Rheumatology, Oxford, UK — [3]King's College London, UK

The intricate dynamics of the immune system, regulated by diverse cell interactions across tissues, present challenges for multiscale and real-time observation of the immune response, from tissues down to single cells and subcellular structures. A technological leap has been achieved with the introduction of lattice light sheet (LLSM) technology, allowing fast and gentle imaging of live samples while achieving subcellular resolution. By complementing LLSM-based volumetric imaging with advanced sample handling of biomimetic systems, *ex vivo* tissue samples, and custom-built fluidics, we provide a system that preserves critical physiological complexity. The perfusion system provides the necessary control over O_2 and nutrient supply while, at the same time, enabling imaging of the perfused samples. We show that in our setup, we can follow single cells and their interactions in volumes several cell layers deep in living samples within their environment, providing nuanced insights into the immune response.

BP 17.8 Wed 11:45 H 2032

Video-rate volumetric fluorescence lifetime imaging of living multicellular systems using single-objective lightsheet microscopy — •Valentin Dunsing-Eichenauer[1], Johan Hummert[2], Claire Chardès[1], Felix Koberling[2], Ivan Michel Antolovic[3], Léo Guignard[1], and Pierre-François Lenne[1] — [1]IBDM & CENTURI, Aix-Marseille University/ CNRS, Marseille, France — [2]PicoQuant GmbH, Berlin, Germany — [3]Pi Imaging Technology SA, EPFL Innovation Park, Lausanne, Switzerland

Fluorescence lifetime imaging microscopy (FLIM) is a widely used technique for functional and multiplexed bioimaging. It is commonly performed on confocal laser scanning microscopes equipped with time correlated single photon counting hardware. However, high excitation powers or long acquisition times are needed to obtain sufficient photon statistics, preventing applications on sensitive living specimen such as embryos or organoids. To overcome these limitations, we have combined single objective lightsheet microscopy with pulsed excitation and time-resolved detection on a 512x512 pixel gated SPAD array detector. We report excellent quantitative agreement with confocal FLIM at 100-1000-fold shorter acquisition times, down to 150 ms per image. We further demonstrate 3D FLIM on live embryonic organoids, lifetime unmixing of two spectrally overlapping fluorophore species, and time-lapse 3D FLIM of mechanosensitive tension probes. Our approach facilitates volumetric FLIM at unprecedented speed and throughput, providing a powerful tool for functional imaging of dynamic multicellular systems.

BP 17.9 Wed 12:00 H 2032

CRISPR screen to improve the optical properties of living tissues — •Susan Wagner, Venkat R. Krishnaswamy, Kaushikaram Subramanian, Heike Petzold, Benjamin Seelbinder, and Moritz Kreysing — Institute of Biological and Chemical Systems - Biological Information Processing, KIT, Karlsruhe

Optical microscopy has been massively advanced to deliver unprecedented resolution allowing discoveries down to the molecular level. Nevertheless, optical access of living biological samples by microscopes is usually restricted to the outer most surface owing to tissue-induced light scattering.

We successfully improved the optical properties of mammalian cells and found that evolved transparency frequently goes along with the reduction of nuclear granularity, while the gene expression profile reflects scattering properties of cells. To genetically clear living mammalian tissues, we are conducting a genome-wide CRISPR activation screen to find those genes which confer transparency.

As a next step, we are investigating how improved optical properties of individual cells influence the optical properties of 3D cell clusters, such as spheroids, using interspersed fluorescent microspheres to quantify imaging quality.

Understanding the full range of a tissue's optical plasticity will provide us with a broad toolkit, so that different genetic strategies can be applied depending on the specific nature of the various biological samples.

BP 17.10 Wed 12:15 H 2032

Illuminating Real-Time Plant Health: Optical Insights into Detecting Plant Stress and Metabolism Transitions — •Katarina Miletic[1], Marija Petković-Benazzouz[1], Sara Ristic[1], Dejan Jeremic[2], and Becko Kasalica[1] — [1]University of Belgrade, Faculty of Physics, Department of Metrology and Applied Physics, Studentski trg 12, 11000 Belgrade, Serbia — [2]University of Belgrade, Innovation Center of the Faculty of Chemistry, Studentski trg 12, 11000 Belgrade, Serbia

Global food security faces threats from plant stress caused by environmental factors. Traditional assessment methods are often invasive, time-consuming, and lack temporal resolution.

A novel developed nondestructive optical sensing method offers real-time insights into plant stress induced by light intensity, water scarcity, nutritional deficiency, and pathogen infection. This approach captures the values of the optical transmission coefficients, representing optical responses through the leaves of the plants, and presents them in graphs depicting the time dependence of circadian rhythms. The method, tested on various plants under diverse stressors, reveals distinct circadian rhythm changes, successfully detecting nutrient deficiencies, early pathogen presence, and metabolic shifts.

This innovative approach, providing continuous monitoring without causing harm to plants, holds significant potential for advancing plant research and improving agricultural practices.

BP 17.11 Wed 12:30 H 2032

Characterizing of complex random media and biologigal tissue with self-consistent quantum field theory — Andreas Lubatsch[1] and •Regine Frank[2,3] — [1]Physikalisches Institut, Rheinische Friedrich Wilhelms Universitaet Bonn — [2]College of Biomedical Sciences, Larkin University, Miami, Florida, USA — [3]Donostia International Physics Center, 20018 Donostia-San Sebastian, Spain

We present a quantum field theoretical method for characterizing disordered complex media with short laser pulses and (OCT). We introduce weighted essentially non-oszillatory solvers (WENO) for the analysis of highly nonlinear and discontinuous processes including interference effects and Anderson localization of light in time-of-flight (ToF) and pump-probe experiments. The results are a measure of the coherence of multiple scattering photons in passive matter as well as in soft matter and biological tissue.

[1] A. Lubatsch, R. Frank, Phys. Rev. Research 2, 013324 (2020) [2] D. Huang, et. al., Science 254, 1178 (1991) [3] K. C. Zhou, et. al., Nat. Photon. 13, 794 (2019)