Correlated Multimodal Imaging
BioImaging Austria - CMI

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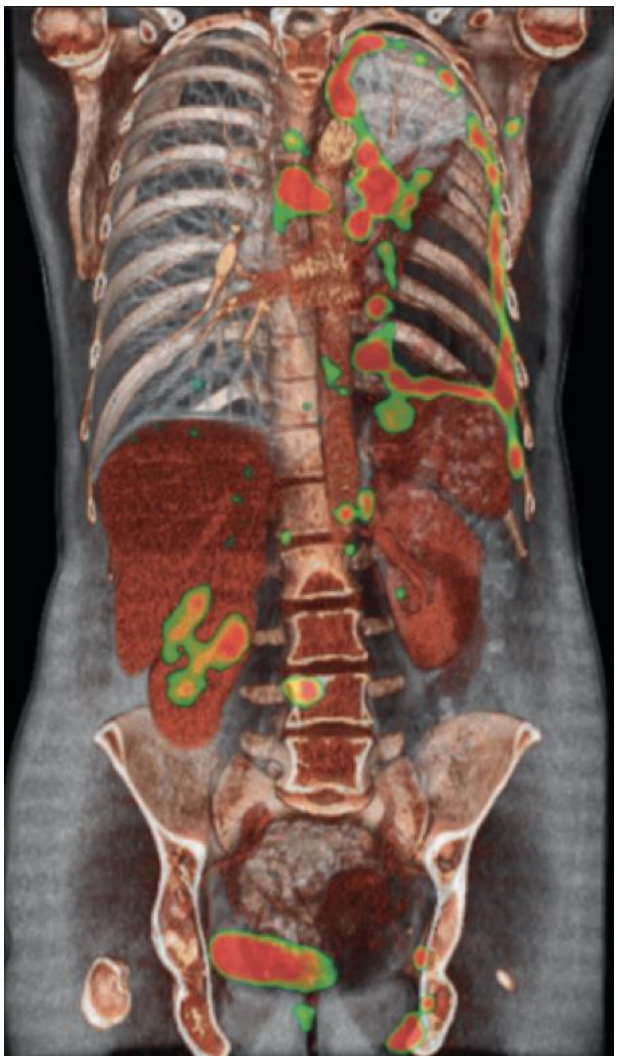
EUROPEAN COOPERATION IN SCIENCE & TECHNOLOGY

COMULIS
Correlated Multimodal Imaging in Life Sciences

Conference on Multimodality Imaging in Life Sciences

COMULIS & BioImaging Austria – CMI



21st – 22nd November 2019

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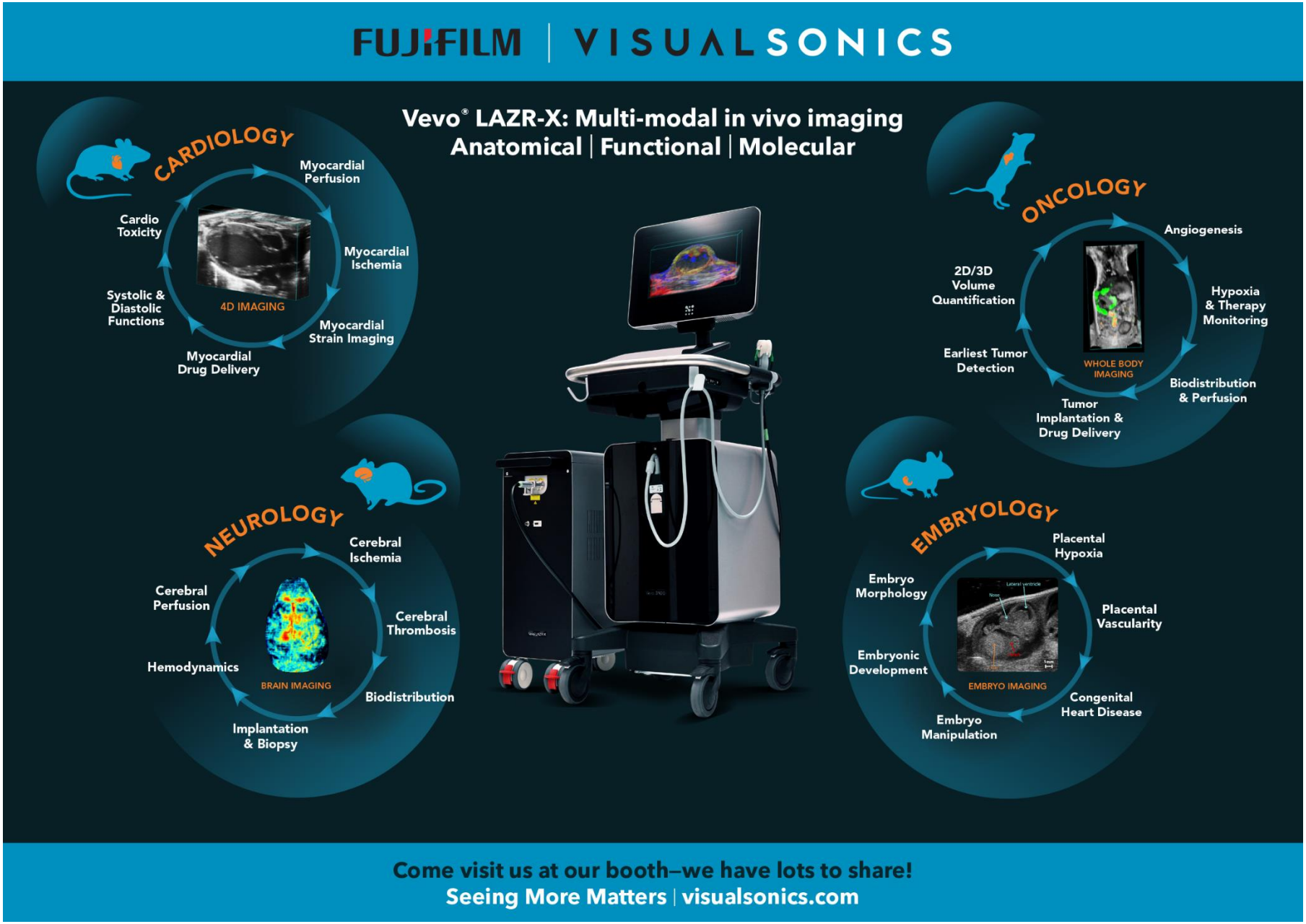
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Conference on Multimodality Imaging in Life Sciences

COMULIS & BioImaging Austria – CMI

Abstracts

PROGRAM

DAY 1 - NOVEMBER 21ST

09:00-09:45 **GENERAL ASSEMBLY BIOIMAGING AUSTRIA-CMI**
09:45-10:15 **GENERAL ASSEMBLY AUSTRIAN BIOIMAGING INDUSTRY BOARD (ABIB)**
10:15-11:45 **MC MEETING COMULIS & JOINT WG MEETING**

11:45-12:45 **COFFEE & LIGHT LUNCH RECEPTION**

12:45-13:00 **INTRODUCTION TO COMULIS & CMI** (A. Walter, BioImaging Austria/CMI & COMULIS)

13:00-14:50 **CORRELATIVE MICROSCOPY (WG1)** (Chair P. Verkade & A. Sartori)

13:00 **Paul Verkade** (University of Bristol, UK) – Introduction to Correlative Light and Electron Microscopy (CLEM)
13:20 **Anna Sartori-Rupp** (Institute Pasteur, France) – Correlative cryo-electron microscopy as a powerful tool to investigate cellular systems
13:45 **Lucy Collinson** (Francis Crick Institute, UK) – CLEM: Entering the third dimension
14:10 **Alberto Diaspro** (Istituto Italiano di Tecnologia, Italy) – The new horizon of multi-messenger optical microscopy for label-free correlative microscopy
14:35 **Manoel Veiga** (Olympus Soft Imaging Solutions GmbH, Germany) – scanR AI: Opening the gate to new applications

14:50-15:20 **COFFEE BREAK**

15:20-17:10 **PRECLINICAL HYBRID IMAGING (WG2)** (Chair L. Sefc & A. Pitiot)

15:20 **Alain Pitiot** (LBI for Traumatology & BioImaging Austria/CMI) – (Pre)clinical hybrid imaging: fusing modalities into meaning
15:40 **Sarah Bohndiek** (University of Cambridge, UK) – Shedding light on tumour oxygenation with multimodal imaging
16:05 **Manfred Ogris** (University of Vienna & BioImaging Austria/CMI) – Fusing optical tomography and morphological imaging in mice
16:30 **Uwe Himmelreich** (KU Leuven, Belgium) – Preclinical Hybrid PET/MRI – Do the benefits justify the investment?
16:55 **Dieter Fuchs** (Fujifilm VisualSonics, Inc, Germany) – The value of *in-vivo* imaging

17:10-17:30 **SHORT COFFEE BREAK**

17:30-18:50 **SELECTED POSTER & FLASH TALKS** (Chair M. Marchetti-Deschmann, P. Sampaio & J. Fernandez Rodriguez)

17:30 **Ana Laura Sousa** (COMULIS STSM, Instituto Gulbenkian de Ciencia, Portugal) – High-accuracy correlative light-electron microscopy for plants
17:40 **Rok Podlipec** (Poster talk, Helmholz Zentrum Dresden Rossendorf, Germany) – Correlative microscopy of lung epithelial in vitro model exposed to nanoparticles by using super-resolution optical and advanced ion/electron-based techniques
17:50 **Pieter De Beule** (Poster talk, INL, Portugal) – Simultaneous imaging for cellular biology using fluorescence microscopy and atomic force microscopy
18:00 **Martin Schorb** (Poster talk, EMBL, Germany) - Modular CLEM software: From microscopy acquisition to data publishing in one go
18:10 **Suman Khan** (COMULIS STSM, Weizmann Institute of Science, Israel) – Obtaining a mechanistic understanding of cell fusion using correlative microscopy
18:20 **Daniel Horák** (Poster talk, Institute of Macromolecular Chemistry, Czech Republic) – Surface-engineered upconversion and/or magnetic nanoprobes for multimodal bioimaging: Design, properties and applications
18:30 **Haider Sami** (Poster talk, University of Vienna, Austria) – Imaging techniques throw light on nucleic acid nanotherapeutics
18:40 **Irina Pradler** (Poster talk, Medical University of Vienna, Austria) – Non-invasive input function measurement for positron emission tomography using Doppler ultrasound imaging

19:00 **POSTER SESSION & FINGER FOOD**

DAY 2 - NOVEMBER 22ND

09:00-10:35	**CORRELATED MULTIMODAL IMAGING (WG3)** (Chair Y. Schwab & D. Fixler)
09:00	**Dror Fixler** (Bar-Ilan University, Israel) – Subcutaneous disorder detection with multi-modal imaging techniques (Showcase Project)
09:20	**Carsten Hopf** (Technical University Mannheim, Germany) - Infrared spectroscopy-guided mass spectrometry imaging in life science- and medical research
09:45	**Tim Salditt** (Georg August University of Goettingen, Germany) – Correlative hard X-ray and fluorescence microscopy of cells and tissues
10:10	**Anne Bonnin** (Paul Scherrer Institute, Switzerland) – Multi-scale and ultra-fast biomedical applications at the TOMCAT beam line
10:35	**Jan Giesebrecht** (Thermo Fisher Scientific, Germany) – Amira software for correlative imaging
10:50-11:20	**COFFEE BREAK**
11:20-12:30	**IMAGING ACROSS SCALES** (Chair A. Walter & W. Weninger)
11:20	**Jan Ellenberg** (EMBL, Germany) – Imaging across scales
11:50	**Jan Groen** (Poster talk, Alba Synchrotron (CELLS), Spain) – Correlative cryo-SIM and cryo soft X-ray tomography to localize specific structures or events within the 3D cellular ultrastructure
12:00	**Carmen Bedia** (Poster talk, Institute of Environmental Assessment and Water Research, Spain) – Multimodal chemical imaging using mass spectrometry, infrared and RGB information from tissues
12:10	**Florian Ganglberger** (Poster talk, VRVIS Research Center, Austria) – A web-based framework for the exploration of heterogeneous spatial big brain data
12:20	**Wolfgang Schwinger** (Carl Zeiss GmbH, Austria) – The ZEISS way of performing correlative microscopy
12:35-13:40	**LUNCH BREAK & COFFEE**
13:40-15:30	**CORRELATION SOFTWARE (WG4)** (Chair N. Sladoje & R. Maree)
13:40	**Perrine Paul-Gilloteaux** (University of Nantes, France) – Introduction to correlation software in CLEM
14:00	**Patrick Bouthemy** (INRIA Rennes, France) – Intensity-based methods for fully automated registration in 2D and 3D CLEM
14:25	**Katja Buehler** (VRVis & BioImaging Austria/CMI) – Visual computing for correlative imaging
14:50	**Gemma Piella Fenoy** (Universitat Pompeu Fabra, Spain) – Manifold learning for multimodal registration
15:15	**Roberto Spada** (FLUIDIGM, France) – Highly multiplexed immunohistochemistry - Visualize >40 functional and phenotypic markers simultaneously on the Hyperion™ Imaging System, powered by CyTOF® technology
15:30-16:00	**COFFEE BREAK**
16:00-17:15	**CONCLUSIONS, ROUND TABLE WITH COMPANIES & COMULIS WG MEETINGS**
17:15	**VISIT CHRISTMAS MARKET**

Poster abstracts

CORRELATIVE MICROSCOPY (WG1)

P1

Christian Tischer - Whole-organism cellular correlation of a gene-expression atlas with ultrastructural morphology in *Platynereis dumerili*

EMBL Heidelberg, Centre for Bioimage Analysis, Germany

Understanding how gene expression patterns correlate with cellular and tissue morphology is a fundamental aim of biological research. In previous work, we generated a fluorescence based cellular resolution 3D expression atlas for an entire *Platynereis dumerilii* larva [Vergara]. Here, we present the 3D mapping of this expression atlas into a whole-organism serial block-face electron microscopy (EM) stack (~2.5 TB at 10nm x 10nm x 25nm resolution) of *Platynereis*. To enable this mapping we first developed a Fiji plugin for trainable segmentation of big EM volume data, supporting HPC cluster computing. Using this plugin, we segmented nuclei, muscles, and neuropil in the EM stack at 100nm^3 voxel size. Next, we developed a Fiji user interface to the 3D registration suite elastix [Klein], which enabled us to simultaneously register the segmented nuclei, muscles and neuropil of the EM volume to fluorescence based nuclear staining and gene expression patterns in muscles and neuropil. Using the resulting 3D transform we mapped the expression of currently 190 genes to the EM volume, approaching cellular accuracy. To identify single cell instances in the EM volume, we employed a 3D U-Net [Ronneberger] to segment cell membranes followed by block-wise Multicut [Pape] for cell segmentation at full resolution (10nm x 10nm x 25nm). Finally, to interactively explore the expression of all genes within the full resolution EM volume on a cell instance level we developed a dedicated BigDataViewer [Pietzsch] based Fiji plugin. Taken together, we created an interactive resource for exploring correlations of gene expression patterns with (intra-)cellular morphologies in whole-organism *Platynereis* larva.

[Titze] Titze et al., “SBEMimage: Versatile Acquisition Control Software for Serial Block-Face Electron Microscopy”, Front. Neural Circuits, 31 July 2018

[Vergara] Vergara et al., ”Whole-organism cellular gene-expression atlas”, PNAS Jun 2017, 114 (23) 5878-5885

[Klein] S. Klein et al., "elastix: a toolbox for intensity based medical image registration", IEEE Transactions on Medical Imaging, vol. 29, no. 1, pp. 196 - 205, January 2010.

[Pietzsch] Pietzsch et al., "BigDataViewer: visualization and processing for large image data sets", Nature Methods 12(6): 481-483, 2015

[Ronneberger] Çiçek et al., “3D U-Net: Learning Dense Volumetric Segmentation from Sparse Annotation”. MICCAI, Springer, LNCS, Vol.9901, 424--432, Oct 2016

[Pape] Pape et al., “Solving Large Multicut Problems for Connectomics via Domain Decomposition”. ICCV Workshops, Oct 2017

P2 – Flash talk

Jan Groen - Correlative cryo-SIM and cryo soft X-ray tomography to localize specific structures or events within the 3D cellular ultrastructure

Alba Synchrotron (CELLS), Barcelona, Spain

In modern cell biology correlative microscopy is unavoidable when studying intracellular interactions. By using complementary techniques, the amount of information that can be obtained can be much greater than either technique alone. Using fluorescent markers and specialized equipment, features of interest such as molecules, structures or events can be localized in 3D, with high specificity and with a very high resolution. In order to place this information within the cellular context, other techniques are needed, like electron microscopy (EM) or cryo soft X-ray tomography (cryo-SXT). We used cryo structured illumination microscopy (cryoSIM, BL24 Diamond Synchrotron, UK), to obtain high resolution (100 nm lateral resolution) 3D fluorescent signal of modular protein repeats in NIH-3T3 fibroblast-like cells, in combination with cryo-SXT (MISTRAL, Alba Synchrotron, Spain) to obtain the 3D ultrastructural information of the cell (30 nm lateral resolution). Performing all parts of the experiments in cryo conditions has the advantages that there will be no movement of intracellular structures between the sessions, as well as that the cryo conditions can prevent, to some extent, possible artifact induction. In addition, both techniques are non-destructive, which means that other complementary techniques, such as EM for example, can be added to the workflow for additional information.

P3

Carles Bosch - Ultrastructure and function of a genetically-identified mouse glomerular column studied by correlative in vivo physiology, synchrotron X-ray tomography and volume electron microscopy

The Francis Crick Institute, London, UK

Brain function emerges from the activity of neuronal ensembles. It is encoded by physiological events mediated by nm-sized synapses that are distributed across mm-scale volumes of brain tissue. Therefore, an approach that efficiently bridges those temporal and spatial gaps is necessary in order to address current challenges in systems neuroscience.
Here we describe a correlative imaging experiment to study neural computation in the first processing stage of the mammalian olfactory sensory system: the glomerular columns in the olfactory bulb (OB). The projection neurons of the OB have a several µm-thick apical dendrite reaching into the glomerulus that defines the neuron's input signal. Their dendrites can be traced in near-micron-resolution volume datasets. This structural signature could be retrieved from neurons with known physiology by using a correlative in vivo – to – synchrotron-CT - to - EM approach: We presented odours in vivo while recording the activity of a neuronal population of interest using volume 2-photon microscopy. Then, that same circuit was dissected, landmarks recorded and the tissue was stained. At this point, synchrotron-CT datasets allowed tracing projection neuron apical dendrites across 3*3*0.6 mm-sized samples, providing key contextual information of the underlying neural circuit. Finally, these samples were trimmed down to ~1mm3 cubes so targeted regions could be imaged at nm resolution with EM. We show that consecutive imaging with these techniques is possible and can be conducted efficiently. Combining synchrotron-CT with volume EM holds significant promise for the wider systems neuroscience research. Here, we report biologically-relevant features in other brain areas that can be resolved by this technique: axon bundles in the striatum and CA1 pyramidal neuron apical dendrites in the hippocampus. With several synchrotron sources across the world being upgraded to provide higher flux and increased number of beamlines for biological imaging, synchrotron-CT can become a tool to bridge scales for neuroscience research in a routine manner.
Altogether, we present an efficient correlative workflow that allows interrogating a neural circuit of interest from both the functional and ultrastructural level, bridging volume in vivo physiology of hundreds of neurons, sub-µm histology across several mm3 and volume ultrastructure at the nm scale.

P4

Jan van der Beek - Immuno-localization updated: High-throughput correlative light-electron microscopy of endo-lysosomal markers

UMC Utrecht, Netherlands

Localization of molecules at the cellular and subcellular level is fundamental in understanding cell biology. Antibodies are a cornerstone of localization microscopy. They facilitate the endogenous study of proteins across different model systems without the need for genetic or molecular tools. Immunofluorescence (IF) and immuno-electron microscopy (EM) are two successful techniques that can localize proteins by relating their position to other proteins or structures. Conversely, once characterized, those proteins and antibodies can serve as established markers for specific structures. ImmunoEM gold labeling, which is typically done on ultrathin sections, depends on high specific signal and low background. Some antibodies work well for IF but perform poorly in ImmunoEM. We demonstrate that, in this case, the IF signal can be correlated to EM ultrastructure through correlative light-electron microscopy (CLEM). We performed IF on thawed cryosections and combined this with high-resolution stitched EM overviews for easy correlation of hundreds of organelles within a single section. We used this approach on a set of antibodies that is widely used in IF to mark early (EEA1, APPL1, Rab5) and late (Rab7) stages of the endo-lysosomal system, but perform poorly in immunoEM. For each marker, hundreds of organelles were categorized by EM morphology to distinct endo-lysosomal stages. This resulted in the striking finding that circa one-third of EEA1-labeled compartments are in fact late endosomes. By contrast, APPL1 predominantly marks small endocytic vesicles and tubules, whereas Rab5 labels both vesicles and early and late endosomes. Rab7 resides on late endosomes and lysosomes. These results provide the underlying morphology to findings in IF and shows that where APPL1 and EEA1 mark separate pools of endosomes, Rab5 labels both.

P5

Florian Kromp - Investigation of genetic tumor heterogeneity using correlated images of H&E and FISH stained consecutive tissue sections

Children's Cancer Research Institute, Austria

Interphase-fluorescence in situ hybridization (I-FISH) is a robust and powerful method to detect chromosomal aberrations and rearrangements at single-cell resolution. In neuroblastoma diagnostics, FISH is widely used to detect the stratifying MYCN amplification (MNA). The enormous power of I-FISH to resolve intra-tumor heterogeneity (ITH) can be fully exploited by simultaneous quantitative analysis and visualization of spot features in consecutive tissue sections. Nevertheless, registration between consecutive fluorescence images is challenging as cells and local structure change from section to section.
We developed an automated tool for I-FISH spot analysis in consecutive sections of neuroblastoma tumors consisting of image alignment, deep learning algorithms for nuclei- and spot-segmentation at the single cell level and Image Scatter plot (ISP) analysis, a method to visualize and further analyze image quantification results. Non-rigid image registration between consecutive tissue sections was performed by aligning multichannel FISH images with a brightfield image of an H&E stained section. As proof-of-principle we analyzed consecutive sections of a heterogeneously MYCN amplified tumor using MYCN-, telomere PNA- and MDM2- I-FISH and an H&E stain for alignment. The developed image analysis workflow allowed the simultaneous measurement of nuclear morphology features as well as spot count, spot size, spot intensity and mean spot distance of all three I-FISH images at single cell level. Comprehensive visualization in ISPs and hierarchical gating of genetically distinct regions demonstrated spatial heterogeneity of MYCN- and MDM2- copy-status.
A semi-automated user-driven image analysis workflow including non-rigid alignment of multichannel fluorescence images and H&E stained brightfield images was developed and validated on a use case, prospectively enabling improved diagnostics and a better understanding of spatial heterogeneity in neuroblastoma.

P6

Xavier Heiligenstein - R221 - A novel resin for Volume CLEM

CryoCapCell, Paris, France

Volume electron microscopy (vEM) and correlative light and electron microscopy are two major techniques to explore living material. Yet, no direct link between both technologies is available: a sample prepared for volume EM requires heavy metal staining that neutralizes the fluorescence required for high precision correlation post-embedding. We developed a novel resin that presents high EM contrast at very low concentrations of stain in biological material, allowing to preserve the fluorescence from the living sample while displaying fine contrast at high magnification in EM. Consisting of low temperature low oxygen embedding material, it is ideally used in combination with vitrification by high-pressure freezing to get close to native ultrastructure with in-resin fluorescence imaging techniques. We will illustrate the application range of the R221 resin through a couple of examples.

P7

Michal Dykas – Correlative imaging using standard microscopes.

Correscopy, Poland

Over €300B is spent by the EU on research every year. Around the globe, the number of science publications skyrocketed to 2 million. But quantity does not translate to quality, according to retraction watch as we face a dramatic rise in the number of scientific errors. Due to errors mainly in data correlation, around 70% of the results published in bioscientific studies contain the mistakes and cannot be repeated.1 The impact is massive, not only waste of money, but also a significant slowdown in development of drugs we desperately need. The catastrophe happens when the wrong results are used as a basis for other research. The data obtained and then combined with use of various microscopic techniques is important to understand the structure-function relations that drive most biological mechanisms. Such combinations of two or more different microscopy techniques are referred as correlative imaging. In this work we demonstrate universal solution which adopts any microscope and any imaging technique for correlative imaging. It will help to understand and solve the scientific and industrial problems where microscopy is required. It uses a specially designed sample holder, microscope adapters and software. The presented solution is device-independent, versatile (it works with all the imaging techniques), applicable to existing microscopy infrastructure of the imaging facility, easy to use, following the usual experimental/imaging workflows, and affordable. For the correlative imaging, we place the sample of interest on the sample holder and start the imaging process, moving from one microscope to the other, simply carrying the sample holder as we move between microscopes. Between the imaging sessions we can do additional sample preparation steps to optimize the sample for each imaging technique. Our solution fully integrates with the standard experimental and imaging workflows. It does not require any additional verification or validations prior to imaging. It enables to get scientific insights which could never be obtained before. In this work we present how we overcome the limitations for the correlative imaging. We show examples at various scales (from um to nm feature size) and applications (biological and material).

References:

1. Matthias Steinfath, Silvia Vogl, Norman Violet, Franziska Schwarz, Hans Mielke, Thomas Selhorst, Matthias Greiner, Gilbert Schönfelder. Simple changes of individual studies can improve the reproducibility of the biomedical scientific process as a whole. PLOS ONE, 2018; 13 (9): e0202762 DOI: 10.1371/journal.pone.0202762

P8

Paolo Bianchini - AFM-STED correlative nanoscopy

Istituto Italiano di Tecnologia, Genova, Italy

The presence of dyes can influence the dynamics of molecular processes. However, a method to verify the label distribution, and its influence in the studied processes, is missing. We coupled a stimulated emission depletion (STED) and atomic force microscopy in a single instrument to investigate the formation of amyloid aggregates. WE focused on the in vitro aggregation of insulin and two alloforms of b amyloid peptides. We followed standard methods to induce the aggregation and to label the molecules at different dye-to-protein ratios. Notably, STED imaging shows only a fraction of the fibrillar aggregates, indicating that the labeled molecules have a specific role in the aggregation process. This finding demonstrates that labeled molecules follow only selected pathways of aggregation, among the multiple that are present in the aggregation reaction.

P9

Andrzej Kubiak - Elasticity mapping with AFM allow to visualize mechanical properties of cancer cells cytoskeleton and nuclei

Andrzej Kubiak[1], Matteo Chighizola[2], Tomasz Zieliński[1], Kajangi Gnanachandran[1], Natalia Bryniarska[3], Justyna Bobrowska[1], Klaudia Suchy[1], Carsten Schulte[2], Agnieszka Basta-Kaim[3], Alessandro Podesta[2], Piotr Laidler[4], Malgorzata Lekka[1]

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[3] Institute of Pharmacology, Polish Academy of Sciences, Department of Experimental Neuroendocrinology, 31343 Krakow, Smetna Street 12, Kraków, Poland
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Atomic force microscopy (AFM) has been extensively used for studying mechanical properties of cancer cells. The general trend observed is that cancerous cells are more deformable than their healthy counterparts. It is generally explained by changes in actin cytoskeleton which makes cancer cells easier to give metastases. In most of studies, mechanical properties are obtained from AFM-based force spectroscopy in which data are acquired at small grids collected around the cell center. Such investigations averaged data thus they are not accurate enough to provide detailed description of mechanical properties of specific cell compartments.
In our studies, we performed elasticity mapping by means of the AFM. Matrix of force curves was acquired over the whole cell. It results in visualization of 3D distribution of elastic properties and allow us to correlate them with images obtained from confocal imaging. By elasticity mapping of human bladder cancer cells, we observed that results from large maps are in line with the data obtained for small maps. In particular, non-malignant cells are stiffer than those of transitional cell carcinoma. For metastatic prostate cancer cells treated with various microtubule interacting agents (vinflunine, colchicine and docetaxel) main differences in elasticity are localized in a nuclear region. They correlate with changes in microtubules organization. Our studies give evidence that correlation of elasticity mapping with confocal imaging allows for better understanding of the role of cytoskeleton mechanics.

P10

Tomasz Zieliński - Biomechanical properties of primary hippocampal neurons and SH-SY5Y cells in oxygen and glucose deprivation (OGD) model.

Tomasz Zieliński[1], Joanna Pabijan[1], Beata Strach[2], Bartosz Pomierny[2], Joanna Zemła[1], Joanna Pera[3], Małgorzata Lekka[1]
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[2] – Department of Biochemical Toxicology, Faculty of Pharmacy, Jagiellonian University Medical College, PL-31503 Krakow, Poland
[3] – Department of Neurology, Faculty of Medicine, Jagiellonian University Medical College, PL-31503 Krakow, Poland

Acute ischemic stroke caused by loss of blood supply to the brain is the second next to cardiovascular diseases cause of mortality worldwide. Despite intensive studies there is still no efficient neuroprotective pharmacotherapy. Novel approaches require both in vivo cerebral ischemia models and in vitro models of glucose and oxygen deprivation (OGD). Currently, a majority of studies is focused on molecular characterization of the pathophysiology of ischemic stroke. Little is known how biomechanical properties of brain tissue changes upon OGD, especially at the single cell level. In our study we have focused on elasticity alterations of primary hippocampal neurons isolated from E18 mouse embryos and of SH-SY5Y model cells. Changes were induced by oxygen and glucose deprivation. In our experiments, cells were cultured primary hippocampal neurons on plastic Petri dish (for neurons its surface was coated with poly-D-lysine for adhesion enhancement). Next, they were maintained in glucose free medium for 1 hour at the constant level of CO2 (5%) and two concentrations of O2 (i.e. 0.1% and 5%). SH-SY5Y cells were maintained in OGD conditions for 1 and 3 hours at the constant concentration of O2 (0.1%). Elastic properties of cells (quantified through Young's modulus) and organization of cytoskeleton were obtained using atomic force and fluorescence microscopes. Preliminary results have shown a correlation between oxygen level and stiffness of primary neurons and model cells. Cells become softer within a whole indentation range from 200 nm to 800 nm. These results suggest that in vitro ischemic stroke model conditions contribute to rearrangement of all cytoskeleton components including microfilament and microtubule networks. Thus, we conclude that remodeling process is one of the main phenomenon occurring during OGD.

P11 – Flash talk

Rok Podlipec - Correlative microscopy of lung epithelial in vitro model exposed to nanoparticles by using super-resolution optical and advanced ion/electron based techniques

Rok Podlipec[1,2], Nico Klingner[1], Rene Heller[1], Hana Majaron[2], Primoz Pelicon[2], Janez Strancar[2], Johannes von Borany[1]

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Clear understanding of molecular events followed by lung epithelial cells/tissue response to inhaled nanoparticles is still lacking. As these interaction events in lungs eventually lead to diseases and potentially persistent inflammation [1,2], one urgently needs new and relevant investigation methods which could provide new insights into the key mechanisms of interaction. In our latest research we have thus focused on this toxicology problem first by developing an appropriate in vitro lung epithelial model and second by developing and implementing relevant advanced correlative imaging techniques capable of gathering more insight of interaction properties on scales well below optical resolution limit. In order to understand the mechanisms of molecular initiative events we have first performed live cell imaging using STED super-resolution microscopy by which few tens to hundred nm resolution was achieved locally. As the technique is incapable of providing resolution further down to nm and lacks the visualization of non-labeled surrounding structures and morphology, we thus introduced suitable complementary correlative microscopy techniques with high surface contrast, SEM and Helium Ion Microscopy (HIM). Main focus, besides sample and sample holder preparation for these high vacuum techniques, was dedicated to HIM measurements which in general are capable of providing better resolution and sensitivity compared to SEM [3]. From this ongoing study we briefly present the first interesting results of correlative microscopy combining optical, electron and ion based techniques on the epithelial cells exposed to TiO2 nanoparticles from micro to nano scale.

References:

1. Li, X., Jin, L. & Kan, H. Air pollution: a global problem needs local fixes. Nature 570, 437–439 (2019).
2. Underwood, E. The polluted brain. Science 355, 342–345 (2017).
3. Hlawacek, G. et Al. Helium Ion Microscopy. J. Vac. Sci. Technol. 32, (2014)

P12

Daria Lazic - Deep Multi-Epitope Imaging of the Bone Marrow Disseminated Disease in Neuroblastoma

Children's Cancer Research Institute Vienna, Austria

Neuroblastoma (NB), an extracranial solid tumor, is the most common cancer diagnosed in children in their first year of life. Disseminated tumor cells (DTCs) in the bone marrow (BM) are detected in the majority of stage M (metastatic) NB patients who have a high risk of relapse and low survival rates. Currently, automatic immunofluorescence (IF) imaging is state-of-the-art to evaluate treatment response and identify DTCs in the BM, however this method allows visualization of only up to three biomarkers. Novel imaging techniques now enable simultaneous or sequential staining of multiple targets, which could improve our understanding of the cross-talk between DTCs and the BM microenvironment. Thus, the aim was to identify as well as validate additional relevant biomarkers and visualize these by multi-dimensional imaging. To explore single cells, we further sought to develop an image-processing and –analysis pipeline for generated multi-dimensional images.
Based on data mining of RNA-sequencing (seq) data-sets of stage M NB (tumors, DTCs, BM-derived non-tumor cells) and proteomics data (NB tumors, cell lines, fibroblasts), and guided by public databases, relevant biomarkers were selected. Sample preparation and IF-staining protocol were optimized to validate specificity of identified biomarkers and allow the assessment of protein expression profiles by multi-epitope ligand cartography (MELC). Generated MELC images were preprocessed using registration, illumination correction and background subtraction. Segmentation then allowed the generation of single-cell multi-channel images, based on which single-cell morphological and fluorescence features were computed to be further used for exploration of distinct cell populations in dimensionality reduction based methods.
Herein, three novel potential DTC biomarkers are proposed, namely DCLK1, FAIM2 and TAG1, which give new insight into NB heterogeneity. By addition and validation of further biomarkers to characterize the cell composition in the BM microenvironment and potential therapeutic targets, three individual 23 biomarker IF-panels are provided. Furthermore, an automated image processing and analysis pipeline allows single-cell exploration in the resulting 23-channel images. Obtained results demonstrate proof-of-principle for the assessment of cell composition in the metastatic BM microenvironment and will help to refine diagnostics for stage M NB patients.

P13

Mario Brameshuber - TCR-CD3 subunit ratio resolved by complementary fluorescence microscopy approaches

TU Wien Institute of Applied Physics, Austria

T-cell antigen recognition requires T-cell antigen receptors (TCRs) binding to MHC-embedded antigenic peptides (pMHCs) within the contact region of a T-cell with its conjugated antigen-presenting cell. Despite micromolar TCR:pMHC affinities, T-cells respond to even a single antigenic pMHC, and higher order TCR-structures have been postulated to maintain high antigen sensitivity and trigger TCR-proximal signaling. In our study we applied four complementary experimental approaches which unambiguously confirmed the accepted subunit ratio within TCR/CD3 complexes. We found exclusively monomeric TCR/CD3 complexes driving the recognition of antigenic pMHCs. Our findings underscore the exceptional capacity of single TCR/CD3 complexes to elicit robust intracellular signaling, which may prove critical for optimizing T-cell-based immunotherapies.

P14

Alexandre Lopes - OPenT + OpenSpi!n = Open Source Multimodal Mesoscopic Imaging

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Mesoscopic imaging refers to techniques which allow 3D fluorescence imaging of large samples at the mm-to-cm scale. Light-sheet microscopy (SPIM/LSM) is the reference optical imaging modality, allowing imaging inside large samples such as whole embryos much faster and with far less photo toxicity than other techniques such as confocal/two-photon microscopy. However, LSM datasets can easily ascend easily to hundreds of Gb or even TBs. OPT (Optical projection Tomography) also allows imaging of large samples, and though it does not easily allow high resolution, it has the advantage of producing isometric datasets and allowing 3D imaging of both fluorescent and non-fluorescent samples. We previously presented i) OpenSpin microscopy (Gualda et al 2013; http://sites.google.com/site/openspinmicroscopy), which allows LSM/OPT and ii) OPenT (Felix et al 2016), a platform simplified and optimized for OPT imaging. We have assembled a new setup with optimized versions of both systems sharing the same optical table and sample stage which renders it capable of multimodal imaging of large samples, in SPIM/dSLSM, and fluorescence or transmittance OPT. We discuss the advantages of OPT when compared to SPIM datasets with 3D tiles & stitching, present preliminary data on assembling and testing this new setup and on implementing a workflow to pre-process, fuse and analyse the multimodal datasets obtained using existing open-source software tools.

P15

Lukas Reissig - Using the Col4a2em1(IMPC)Wtsi mouse line as model for autosomal dominant porencephaly 2

Medical University of Vienna, Austria

Autosomal dominant porencephaly 2 (OMIM # 614483) is a rarely diagnosed disease, that is caused by mutations of the COL4A2 gene. We aim at providing phenotype descriptions of homozygous embryos of the Col4a2em1(IMPC)Wtsi line in order to define the role Col4a2 plays in organogenesis and to identify the spectrum of abnormalities associated with autosomal dominant porencephaly 2. Four homozygous Col4a2em1(IMPC)Wtsi mutants were harvested at embryonic day 14.5 and High Resolution Episcopic Microscopy (HREM) was employed to produce digital volume data with a resolution of 3x3x3µm3. Using the software package Amira® the morphological phenotype of the mutants was systematically screened following protocols developed in earlier studies.
All Col4a2em1(IMPC)Wtsi mutants had survived organogenesis, allowing the identification of the full spectrum of phenotype abnormalities associated with Col4a2 disruption. Characteristic defects of the brain, cranial nerves, visual system, lungs, endocrine glands, skeleton, subepithelial tissues and mild to severe cardiovascular malformations were diagnosed.
Embryos of the Col4a2em1(IMPC)Wtsi mouse line are therefore recommended as useful models for researching the mechanisms underlying autosomal dominant porencephaly 2.

P16

Brian Metscher - MicroCT in correlative imaging

University of Vienna, Austria

As a non-destructive whole-sample imaging method, X-ray microtomography (microCT, or XRM) is an effective complement to both traditional and specialized imaging modalities. MicroCT provides a 3D overview of the relevant morphology at microscopic resolution and can be used with practically any type of biological sample. This offers a supplement or even substitute for traditional dissection, as in the definitive description of the new millipede species *Ommatoiulus avatar*, whose defining characters are mainly in the internal male reproductive structures (Akkari et al. 2015, PLoS ONE 10(8):e0135243). MicroCT enables multiscale imaging, for example in a study of horse embryology using microCT imaging of resin blocks followed by sectioning for histology and TEM, with subsequent registration of histological and ultrastructural sections with the 3D images. Standard methods such as paraffin or ground-section histology can calibrate microCT image content: in a study of bat penis anatomy, we used labor-intensive ground sections of undecalcified samples to allow accurate interpretation of microCT images, which could be more easily obtained for a larger number of samples (Herdina et al. 2015, J Morph 276:695). With contrast enhancement, embryonic tissues can be imaged to corroborate cell-labeling and molecular assays, e.g. in characterizing the pre-oral gut contributions to anterior structures in basal fishes (Minarik et al. 2017, Nature 547:209). Current projects include refinement of tissue-specific contrasting for microCT, applications to histopathology, and investigation of heart development and metamorphosis.

P17

Norbert Cyran – Correlation of Light- and Transmission Electron Microscopy with NanoSIMS

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CIUS, University of Vienna

Both our Light-and Electron Microscopy Facility (CIUS) and the Large Instrument Facility for Advanced Isotope Research in the Life Sciences, with its 17enterpiece – the mass spectrometer NanoSIMS 50L (Cameca), are part of the „Vienna Life-Science Instruments“ (VLSI) initiative and an example of lively collaboration for the benefit of our users. Our work is focused on correlation of data obtained by laterally resolved isotope analysis with the underlying ultrastructure of cells and tissues revealed in the light- or electron microscope. By doing this we have to pay attention to sample preparation techniques that suit microscopy as well as NanoSIMS.
The requirements for NanoSIMS samples, in many aspects, are met by state-of-the-art TEM sample preparation. They both aim on rapid fixation of the living state and prevention of excessive wash-out of soluble components. This includes the isotopic labelling itself during sample dehydration by organic solvents, and embedding in epoxy resin. Studies of resin sections allow the semi-correlative exploration of the isotope distribution in the context of the cellular ultrastructure. For this, thin sections have to be mounted on TEM grids and on Sb-doped silicon wafers for NanoSIMS, respectively. The plane nature of the thin sections mounted on the wafers ensures that NanoSIMS analysis is undistorted by sample topography.
In practice, the diversity of research topics (reaching from intracellular localization of anti-cancer drugs to symbiosis research in animal and plant sciences) poses a challenge for TEM sample preparation; we meet it increasingly by inclusion of cryopreparation techniques and rapid low-temperature fixation and -dehydration. The latter we achieve by using patented agitation modules fitting in the cryochamber. Freeze substitution (FS) under agitation might be applied either to native or chemically fixed samples; both options take advantage of the fact that the wash-out of biological material is reduced at low temperatures if compared with processing at room temperature.

For more information: https://www.vlsi.at/ https://cius.univie.ac.at).

P18

Biliana Nikolova - In vitro and in vivo visualization of passive and electro-assisted delivery of quantum dot-labeled nanoparticles using fluorescent and magnetic resonance imaging

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The growing number of studies in the field of biomedical applications suggests that theranostic strategy becomes an important element of cancer treatment as it helps to develop the anticancer therapeutics based on combination of imaging and therapy. The selective disposition of nano-carriers into the target cells and tissues is an essential issue in drug delivery. In this context, hydrophilic and biocompatible nano-hydrogels are appropriate matrices for development of multimodal and multifunctional theranostic substances.
The present study describes a development of nanohydrogel, loaded with QD705 and manganese (QD705@Nanogel & QD705@Mn@Nanogel), and its passive and electro-assisted delivery in Colon 26 cancer cells and in solid tumours, visualized by fluorescence imaging and magnetic resonance imaging (MRI) on colon-cancer grafted mice as a model.
QD705@Nanogel was delivered passively predominantly into the tumor cells, which was visualized both in vitro, in vivo and ex vivo using fluorescent imaging. The applied electric field benefits the internalization of the nanosomes without significant cells viability reduction. The fluorescence intensity in the tumor area was about 2.5 times higher than the background fluorescence. A very weak fluorescent signal was detected in the liver area, but not in the areas of kidneys or bladder. We found that the embedding of a hard material (as QD) in nanohydrogel changes the physical properties of the soft material. QDs decreased the size and negative charge and changed the shape of nanohydrogel, which altered its pharmacodynamics.
Electroporation facilitated the delivery of the nanohydrogel in the tumor tissue, visualized by fluorescent imaging and MRI. Strong signal intensity was recorded in the tumor area shortly after the combined treatment (QD@Mn@Nanogel + electroporation) and it was observed even 48 hours after the electroporation. The data demonstrate more effective penetration of the nanoparticles in the tumor due to the increased permeability of blood vessels at the electroporated area. There was no rupture of blood vessels after electroporation and there were no artifacts in the images due to a bleeding.

Acknowledgment: COMULIS COST Action 17121
Key words: quantum dots; nanohydrogel; cancer; electroporation; fluorescence imaging; magnetic resonance imaging

P19

Mingdong Dong - Directly Mapping the Surface Charge Density

Aarhus University, Denmark

Scanning ion conductance microscopy (SICM) is an alternative scanning probe microscopy technique, which can reveal the 3D morphology of biological molecules under biological conditions. The recent development of SICM allows probing the surface charges quantitatively. Here we are able to map the local charge density of phase-separated lipid bilayers.

P20

Stefan Geyer - Morphology and topology of the veins of E14.5 mouse embryos

Medical University of Vienna, Austria

Characterising the effect that disrupted gene function has on the development of mouse embryos relies on precise detection of structural abnormalities. A prerequisite for this is a comprehensive understanding of the morphology and the range of morphological variation found in genetically normal control embryos at various developmental stages. This study provides reference data and metric characterisations of the venous system of C57BL/6 mouse embryos at embryonic day E14.5.
Using High Resolution Episcopic Microscopy (HREM), we created digital volume data of 200 genetically unaltered mouse embryos and 5 embryos with targeted gene deletions. Voxel size was 3 x 3 x 3 µm3. All embryos were part of the “Deciphering the Mechanisms of Developmental Disorders” (DMDD) programme (dmdd.org.uk) and were bred on the C57BL/6 background and harvested at embryonic day E14.5.
Our study provides highly detailed three-dimensional (3D) descriptions and surface models of the venous channels of embryos of all developmental stages between S21 and S23 according to Geyer et al. in respect to the embryonic bodies, tissues and organs. It also provides statistics of the occurrence of norm variations and developmental differences. Examples for diagnosing vascular malformations in single gene knock out embryos on the basis of the new reference data are shown.
We present comprehensive reference data based on over 200 individuals, which permit diagnosis of vascular abnormalities in mouse embryos with gene deletions, provide examples for their usefulness and demonstrate their value for identifying mouse models for inherited diseases.

P21

Mario Mairhofer – Biophysical characterization of extracellular bioparticles for therapeutical application – BioCETA

FH Oberösterreich, Austria

Bioparticles like extracellular vesicles (EVs) play a physiological or pathophysiological role - for example in tissue regeneration, cancer, infections and neurodegeneration - and they can be used as natural drug carriers for medicines. However, with current methods, it is not possible to analyze individual bioparticle populations specifically and with high resolution - although standardized quality control is essential for use in therapy, diagnostics and biotechnology. In the BioCETA project, we are establishing a novel multi-modal biophysical analysis of bioparticles. Properties such as particle size, morphology and protein composition as well as transport and uptake in cells are investigated. Existing techniques and expertise from high-speed atomic force microscopy (AFM), high-resolution and super-resolution fluorescence microscopy and bioinformatics (image analysis, data mining, machine learning) are combined and methodically expanded. As a model system, we are using fluorescently labeled EVs/exosomes, which are characterized in-depth by single molecule sensitive fluorescence microscopy to extract information like the number of fluorescent labels per particle and the photostability of the label. In the next step, from combined AFM/fluorescence microscopy analysis, informations about labeling efficiency, particle size and mechanical properties of the bioparticles are obtained and help to differentiate between membrane enclosed EVs and co-isolated impurities.
In addition to bioparticles isolated from tumor cell lines, therapeutically relevant EVs derived from mesenchymal stem cells are used in the project. The unique combination of the methods used in the project combined with state of the art bioinformatics analysis will enable us to advance our knowledge about medically highly relevant bioparticles.

PRECLINICAL HYBRID IMAGING (WG2)

P22

Jost Stergar - Challenges in hyperspectral imaging of thin tissue samples

Faculty of Mathematics and Physics, University of Ljubljana

When using hyperspectral imaging on thin and translucent samples of animal origin, a plethora of challenges can appear due to the weak interaction of light with the tissues. Additionally, time can be an important factor in a preclinical environment, where samples have to be processed fast for additional research. In this poster, specifics important for such studies are presented.
First, effects of the imaging substrate, which can play a key role in thin and translucent substrates were studied. Since most of the light passes through the sample, characteristics of the substrate, be it underlaying tissue or specially selected imaging substrate, have to be considered. Different geometries for imaging of such samples, including transmission and reflectance geometry are presented. Diffuse reflectance imaging is a tried and tested option, but processing of results can be challenging due to the strong substrate dependence on recorded spectra. Due to this, custom models have to be developed. Transmission imaging is shown as a promising option when dealing with thin samples of simple geometry; additional benefits of this approach are shorter integration times and readily available models of tissue-light interaction such as Beer-Lambert law and numerical Monte Carlo simulations. Performing measurements on readily available preserved samples from previous studies could increase the number of available samples. Imaging of preserved samples in current studies would also help by moving the imaging stage to a later point in the workflow, thus decreasing the deterioration of samples at critical points before molecular analyses. To achieve this, the effects of formaldehyde fixation on optical properties of tissues were studied.
Precise shape of the samples under consideration can play a key role in the data analysis, since a precise thickness of the sample must be known while analyzing the samples using optical models. Fine structural properties of the samples could also offer an additional insight into mesoscopic morphological changes, which in turn could relate to disease progress. To measure both sample thickness and its surface shape, a 3D laser profilometry setup has been used.

P23

Ludek Sefc - Compton camera based on single photon counting pixel detector in preclinical SPECT imaging

CAPI, First Faculty of Medicine, Charles University, Prague, Czech Republic

Gamma cameras are frequently used for SPECT (Single Photon Emission Computed Tomography) imaging both in preclinical research and in human medicine. SPECT visualizes biodistribution and accumulation of radiolabeled tracers in vivo. The main disadvantage of the standard SPECT examination is its low sensitivity (less than 1%) caused by losses of signal by use of collimators. Very high radiation activity of the tracer is needed for successful imaging which results in considerable irradiation of the imaged object. Compton camera can be used for increasing of SPECT sensitivity. Its principle is based on the use of Compton scattering, which makes it possible to create an image of a radiation source without using a heavy and signal weakening collimator. The Compton camera usually contains two layers of detectors. The first interacts with the primary gamma radiation and the second records secondary energy scattering. By capturing and evaluating a series of such scatterings, it is possible to reconstruct the shape and location of the original gamma radiation source. In our study, we used a commercially available photon counting pixel detector to construct the Compton camera. In the first part of the study, the camera consisted of two detectors - the first one had a CdTe sensor with a thickness of 1 mm and the second one had 1 mm thick Si sensor. The detectors were placed 8 mm apart and connected by a sync cable. The study determined that Compton camera-based cameras can display the position and shape of a gamma source with a resolution of about 6 times better than current available devices, in addition to a significantly wider display field. In the second part of the study, we managed to construct a single-layer Compton camera (with 2mm thick CdTe sensor) and we achieved similar results. The cost has been halved and the field of view was even wider. Based on experimental data, we designed (constructed) a prototype of SPECT camera using Compton scattering. The advantages mentioned above (higher resolution, wider display area) are complemented by a significant reduction in the overall size and weight of the device.

P24

Engin Ozcivici - Lensless Single Cell Densitometry

Izmir Institute of Technology, Izmir, Turkey

Magnetic levitation based single cell densitometry is a great biomedical tool for low-cost and high sensitivity detection of single cells in heterogeneous populations. However, single cell densitometry is a microfluidics-based technology, and biological imaging during the separation process requires limiting light microscopy components. Lensless digital inline holographic microscopy (LDIHM) systems are composed of a simple illumination system containing an LED, a pinhole, and an imaging sensor for high-resolution microscopic imaging lowering the costs and physical constraints of optical microscopy. Here, we integrated single cell densitometry system with LDIHM system for automated analysis of cell levitation heights and tested the system for several different cell-based biomedical applications.

P25

Petrus Dominicus van Oostrum - Holographic Phase Contrast Micro Cytometry

BOKU - DNBT – BiMat, Austria

Holographic Phase Contrast Micro Cytometry (HPCMC) is a label-free microscopy/cytometry technique that provides statistics of physical properties of individual microparticles and microorganisms in dispersion. HPCMC: a unique method for high-throughput measurements of size, shape, optical properties and motion of (sub-) micron objects.

We use collimated illumination and record so-called in-line holograms that we 'back propagate' to obtain the full 3D light-field. By combining the phase and intensity information we can generate phase contrast images of all the objects throughout the sample volume. Using our unique and proprietary algorithms, we can characterize all the objects in the sample in terms of the amount of light they scattered, their shape, orientation and so forth. In addition, we can determine dynamic properties from 3D trajectories.

HPCMC moreover combines this unique characterization power with a throughput that is similar to that of standard flow cytometry techniques (FC). Flow cytometry also measures signals from individual microparticles and microorganisms, but these have to be previously separated, typically by individual encapsulation in drops. Crucially, flow cytometry relies heavily on labeling the samples with fluorescent markers and combining this with analysis of the light scattered from the object. Thus, samples are subjected to invasive pre-labeling and no image is acquired. The latter means that decisive properties such as shape and size cannot be determined. There is also no dynamic information acquired, such as mobility (diffusion, rotation, propulsion, etc.), which are critical parameters in life science but unavailable using high-throughput methods today. HPCMC provides quantitative measurements of physical properties including mobility of individual objects at high acquisition rates and thus accurate statistics of entire sample populations with sub-population variations.

In contrast to whole population-based methods such as standard scattering techniques, HPCMC can refine datasets to identify sub-populations with distinguishing physical or motility properties within a population and thereby open new doors to investigators and in quality control.

P26 – Flash talk

Irina Pradler – Non-invasive input function measurement for positron emission tomography using Doppler ultrasound imaging

Medical University of Vienna, Austria

Positron emission tomography (PET) has become a premier tool in pre-clinical and clinical nuclearmedicine; major applications include small animal imaging for basic research and drug development as wellas oncologic diagnosis and brain research in humans. In short, PET is used to study metabolism using atracer – a substance doped with small amounts of a radioactive isotope - that is specific for a given physiological process under examination. The uptake and metabolization of this tracer is a dynamic process, and therefore an exact knowledge of the initial arterial tracer input is essential. Usually, this is achieved by taking blood samples invasively or with image derived input functions. In humans, blood sampling is hampered by patient discomfort and complication risk; in small animal imaging, taking blood samples leads to frequent loss of the specimen as a stroke is induced by the arterial puncture. Also, the blood volume is small. We plan to use a second imaging modality – Doppler ultrasound (US) imaging – and a matrix of scintillators each attached to highly integrated optical sensors for non-invasive arterial inputmeasurement. The US device is used to locate a large artery, for instance a carotid, and to aim the detectors onto that vessel by means of image-guidance techniques. By this approach, we propose that both- a measurement of the arterial input function and the background signal - is facilitated with high precision and high temporal resolution.

P27 – Flash talk

Daniel Horák - Surface-engineered upconversion and/or magnetic nanoprobes for multimodal bioimaging: Design, properties and applications

Institute of Macromolecular Chemistry AS CR, Czech Republic

Nanoprobes include superparamagnetic iron oxide and upconversion/magnetic NaY(Gd)F4:Yb3+/Er3+(Tm3+)@NaYF4:Nd3+ core-shell nanoparticles excitable at 808 or 980 nm. The latter, denoted as UCNPs, convert low-energy NIR irradiation, which is penetrable deep inside the living tissues, into high-energy visible light, with strongly reduced autofluorescence and light scattering and high photostability. The nanoparticles have controlled size and a very narrow size distribution to ensure homogeneous properties.

Surface particle engineering is needed to improve colloidal stability, biocompatibility (nontoxicity) and immobilization of target biomolecules, i.e., drugs, photosensitizers (e.g., phthalocyanine), streptavidin, permeation enhancers or targeting peptides, such as RGD or TAT, etc. We have developed poly(L-lysine), D-mannose or poly[N-(2-hydroxypropyl)methacrylamide] coatings and compared them with conventional silica-based modifications. In particular, newly designed poly(ethylene glycol)-neridronate coating provided excellent colloidal stability of the nanoparticles in biological media, preventing aggregation and ensuring a prolonged blood circulation time. Radiolabeling of the nanoparticles with 125I enabled to investigate their distribution in the blood stream.

Magnetic resonance imaging (MRI) enabled non-invasive in vivo monitoring of transplanted iron oxide-labeled cells in tissues, their biodistribution and both short- and long-term fate in the organism. MRI-monitored reverse transport of cholesterol could diagnose development of atherosclerosis.

Optical photoluminescent imaging examined internalization of the NaYF4:Yb3+/Er3+ nanoparticles in cancer cells and elucidated the mechanisms of particle internalization.
SPECT/CT using 125I-NaY(Gd)F4:Yb3+/Er3+ nanoparticles, which simultaneously integrate additional imaging modalities, photoluminescence and MRI, made investigation of their biokinetics possible.

Single molecule immunosorbent assay based on biotinylated anti-PSA antibody and streptavidin-conjugated NaYF4:Yb3+/Er3+@PEG nanoparticles served as a photoluminescent label for sensitive detection of cancer markers (prostate specific antigen PSA) under an upconversion wide-field microscope equipped with a 980-nm laser. The UCNPs thus offer a new platform for development of advanced diagnostic and therapeutic agents intended for highly efficient detection and treatment of various fatal diseases.

Single NaYF4:Yb3+/Er3+(Tm3+) particle tracking by the wide-field fluorescent microscopy is emerging as a new powerful tool for bioimaging and tracing of specific proteins in theranostic applications.

Nanothermometry using NaYF4:Yb3+/Er3+ nanoparticles is promising for temperature measurement of target cancer tissues during hyperthermia treatment.

■ **P28**

Rodica-Mariana Ion - Photonanomedicine – synergy between Photodynamic Therapy and Nanotechnology

ICECHIM, Bucharest, Romania

"Photodynamic therapy (PDT), has become nowadays a well-studied therapy for cancer which is based on the photoactivation of photosensitizers (PSs) in the presence of molecular oxygen [1]. PDT is a promising minimally invasive approach that is being used for treatment of different premalignant and malignant tumors, has a low toxicity in normal tissue, produce negligible systemic effects, and reduce acute and long-term morbidity. For such systems, extensive research on the design of PS molecules with optimized pharmaceutical properties, are imposed.

In this context, the photonanomedicine (PNM), appeared as a promised and personalized, image-guided therapeutic approach for cancer pathologies, is transforming the PNM formulations, into platforms that integrate molecular selectivity, the spatio-temporally controlled release of synergistic therapeutics, along with regulated, sustained drug dosing. High-resolution in vivo imaging technologies serve for quantitatively study the diseases in real time, at the molecular level, and they can be repeated and monitor disease progression or response to treatment.

In this paper have been considered some results of the clinical studies regarding PDT with AuNP-TS4PP on HUVEC cell lines, steady-state fluorescence and absorption measurements on the photosensitizers (gold functionalized with porphyrin as TS4PP formulations as a fluorescent PS that can serve as both a contrast agent in fluorescence imaging), singlet oxygen quantum yield measurements, in vitro photodynamic therapy on animal model, progresses made in the design of the photosensitizers, quantitative fluorescence spectroscopy in vivo, tumour distribution (optical imaging) and fluorescence imaging of tissue sections (confocal microscopy), photodynamic therapy results including so-called 'multicellular tumor spheroids'[2].

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P29

Joachim Friske – Synchronized PET/MRI at 9.4 Tesla – A pictorial essay of different organs and disease models

Preclinical Imaging Laboratory, Medical University of Vienna, Austria

Introduction: Synchronized PET/MRI has emerged as an exceptionally powerful and precise technique and visualizes complex interactions of different diseases in a clinical setting. The combination of several single parameters in a multiparametric (mp) imaging concept is necessary to maximize diagnostic accuracy and disease characterization [1-4]. With the development of a new PET insert which can be integrated in an MRI scanner synchronized mpPET/MRI can be introduced into field of preclinical imaging as well. This assay presents the potential of this new technique high lightening different disease models.
Methods: All mpPET/MRI measurements were performed using a horizontal bore 9.4T (BioSpec 94/30USR) system combined with a PET insert using a 1H volume coil (d=72 mm) (Bruker, Ettlingen, Germany). The PET insert consists of three rings of 8 octagonal orientated Silicon Photomultiplier (SiPMs) mounted inside the magnetic bore. Simultaneous PET and MRI scanning (with different sequences) is possible to acquire spatially and temporally registered images. Mice and rats were anesthetized using isoflurane (2-3%) in air (2 l/min). Respiratory triggering was used to limit movement artifacts. Different preclinical organ and disease models were imaged using the concept of mpPET/MRI spanning the spectrum from different tumor,heart vessel and brain diseases.
Results/Discussion: In this pictorial assay we will present a variety of organ and disease models imaged with mpPET/MRI using different tracers. A spectrum of different tumor, heart, vessel, and brain diseases will be shown. mpPET/MRI is feasible without quality impairment of PET and MR images. Tumours and organs of interest are clearly visible on both imaging techniques (Figure 1 and 2). This offers the ability to localize tissue of interest more precisely and differentiate it more exact from the surrounding area. In addition mpPET/MRI allows to synergistically visualizing complex interactions of different diseases.
Conclusions: This pictorial assay presents a variety of organ and disease models imaged with mpPET/MRI using different tracers. The first results are promising. The FOV is large enough to image both, rats and mice using appropriate coils and hardware. Future application can combine quantitative MRI methods like CEST, BOLD, spectroscopy or diffusion with new PET radiotracers.

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P30

Dante Maestre - LowPhi : A New Tool for Clinical Diagnostics

Faculty of Physics, University of Vienna, Germany

Phase contrast microscopy (PCM) has numerous applications in biological sciences and clinical diagnostics. However, traditional PCM sensitivity varies throughout a given sample and only provides qualitative information. In our approach, by using a Spatial Light Modulator (SLM) in a conjugate image plane, we modulate the wavefront to achieve maximal sensitivity across the entire field of view. After being initialized, local optimization of wavefronts for optimal phase imaging (LowPhi) allows for single frame quantitative imaging with minimal imaging artifacts. LowPhi can be set-up as an add-on to commercially available phase contrast microscopes. This scheme facilitates the study of dynamical systems without the need for staining as well as having the potential to provide quantitative data useful for the identification of novel biomarkers.

P31

Fatih Alioglu - Multimodal imaging to investigate the role of Thy1 in tumor growth in transgenic reporter mice

MMCT Laboratory of Macromolecular Cancer Therapeutics, University of Vienna, Austria

Bioimaging of reporter gene expression has demonstrated significant potential for studying in vivo events in the context of a variety of biological questions like promoter activity, longitudinal tracking of tumour growth and the activity developmental pathways. We have developed and characterised a transgenic mouse model (Thy1.2-Luc) by stable introduction of a firefly luciferase expression cassette under the control of a Thy1.2 promoter element. The Thy1.2 promoter element used have been shown to be active mostly in neuronal cells, but also in inflammation and wound healing. Thy1 (CD90) is a cell surface glycoprotein which is involved in cell adhesion, T-cell activation, and melanoma cell migration. Thy1 expression on endothelial cells plays a role in metastasis formation in melanoma. The present study employs two-dimensional BLI and optical tomography (diffuse light imaging tomography, DLIT) together with CT to investigate the transgenic mouse model for localization of luciferase expression and to visualize role of Thy1 in tumor growth via luciferase expression. B16f10 melanoma cells were injected into Thy1.2-Luc transgenic mice either subcutaneously or intravenously and bioluminescence signal imaged at different time points to visualize Thy1 activity. BLI imaging showed different patterns of Thy1 activity for subcutaneous vs. intravenous tumours. For subcutaneous injection, we could successfully and reproducibly show presence of Thy1 activity (bioluminescence signal) co-localized near the tumor mass. In 2D BLI, the signal appeared around day 8 after tumor cell implantation with increasing signal intensity. Activity appeared as a rim around the tumor mass at later time points. Interestingly, metastasis (in skin region distant from the injection site) could also be visualized at later time points, which was validated by DLIT/µCT and ex vivo organ/tumor imaging. DLIT signal was quantified using an algorithm considering the wavelength-dependent absorption of emitted light in conjunction with a surface analysis with CT data. Presence of Thy1 activity outside the nervous system and in association with melanoma tumor growth points at the role of Thy1 in tumorigenesis, which could be non-invasively imaged in a longitudinal fashion. Taken together, Thy1 dependent luciferase activity in reporter mouse strains can be precisely imaged and quantified using bioluminescence imaging and optical tomography.

P32 – Flash talk

Haider Sami - Imaging techniques throw light on nucleic acid nanotherapeutics

MMCT Laboratory of Macromolecular Cancer Therapeutics, University of Vienna, Austria

Nanomedicine offers innovative approaches for nucleic acid (NA) therapy. Real-time tracking of nanocarriers in vitro and in vivo is highly desirable and can further aid in identifying bottlenecks. Imaging techniques have constantly contributed to the development of NA therapeutics, both for investigating their interaction with biological systems and also for studying NA delivery outcomes. Nanoparticle (NP) based imaging contrast agents can further aid this concept if they are integrated within NA therapeutics. Nanoparticles used as imaging contrast agents include quantum dots (QDs) as excellent probes for near infrared (NIR) fluorescence imaging. Similarly, gold nanoparticles generate contrast in X-ray CT imaging based on their superior X-ray scattering properties. Towards this goal of integrating imaging within NA carriers, the present work reports on imaging enabled characterization of nanoparticle functionalized polyplexes (NA carrier based on cationic polymers). Imageable NA nanocarriers were prepared by synthesizing polyplexes functionalized with QDs (quantoplexes) or gold nanoparticles (auropolyplexes) for gene or siRNA delivery respectively.
Real-time 'visualization' of single nanoparticles in liquids via Brownian motion and light scattering (combined with high-sensitivity camera and microscope) can be performed by nanoparticle tracking analysis (NTA) to give a high-resolution measurement of biophysical properties and nanoparticle population characteristics. This is particularly relevant in case of multicomponent formulations like NP-functionalized polyplexes, where the polydispersity indices can be high. NTA showed a) optimized amounts of QDs in quantoplexes did not significantly change the biophysical properties and b) auropolyplexes offer tunable siRNA loading onto gold nanoparticles with good biophysical properties. In vivo studies included spatio-temporal tracking of NP-functionalized polyplexes by NIR based 2D epifluorescence imaging and fluorescence imaging tomography (FLIT/CT). Combination of tomographic fluorescence (for biodistribution) and bioluminescence (for gene delivery outcome) imaging together with CT based morphologic imaging indicated the potential of quantoplexes as theranostic gene carriers. Similarly, FLIT/CT imaging of AF750-labeled auropolyplexes in vivo showed presence of 20% of the siRNA dose in lungs (after 24h of administration) and also indicated crossing of air-blood-barrier. However, most of gold nanoparticles were retained in the lung. Taken together, imaging via multiple modalities can aid in deciphering important delivery/therapy concepts within the context of NA based nanotherapeutics.

CORRELATED MULTIMODAL IMAGING (WG3)

P33

José Javier Conesa Muñoz - 3D correlative X-ray microscopy to localize an iridium metallodrug in breast cancer cells

National Center of biotechnology CSIC

In recent years organometallic compounds with potent (nanomolar) cytotoxic activity have begun to emerge. Metallodrugs offer unprecedented versatility in medicinal chemistry because of the different building blocks from which they can be constructed, the variety of available interactions and their redox properties. The latter makes them extremely attractive as potential biocatalysts in cancer research. Understanding the intracellular fate of this class of drugs is crucial to further their development towards their clinical use. Previous work has confirmed intracellular accumulation and distribution inside the cells through cell fractionation and elemental quantification using Inductively Coupled Plasma Mass Spectrometry. However, cross-contamination during cell manipulation and fraction separation, metal efflux during sample handling, and sensitivity too close to the detection limit depending on the specific cell fraction, are major drawbacks of this type of experiments. Therefore, to elucidate the intracellular trafficking and the overall cellular accumulation of Ir compounds with potent cytotoxic activity we have chosen the following correlative approach. Cryo Soft X-ray tomography is used to obtain the 3D ultrastructural information of whole cells treated with an Ir metallodrug, at resolutions better than 50 nm. This 3D structural information is then correlated with elemental specific information obtained by cryo X-ray fluorescence. This novel strategy allow us to shed light on the cellular accumulation trafficking and localizing unambiguously which organelles are involved in the intracellular Ir metallodrug accumulation. Our data strikingly show a clear preference for the mitochondria, which can help design the next new generation of highly potent anticancer metallodrugs.

P34

Janis Spigulis - Multimodal in-vivo skin imaging

University of Latvia, Riga, Latvia

Previous and running projects of Riga group on macro-scale in-vivo skin imaging for clinical diagnostics and monitoring are briefly described. Designs of prototype imagers and results of their laboratory and clinical tests are discussed.

P35

Ruslan Hlushchuk - Correlative imaging of the microvasculature using microangioCT and light/fluorescence microscopy

Institute of Anatomy, University of Bern, Switzerland

Background/Rationale:

The cardiovascular diseases are the leading cause of death globally. Billions of cardiovascular patients would benefit from the effective angiomodulating treatments. The murine hind limb is a widely used model to study pro-angiogenic treatment strategies. Traditionally, the vasculature is evaluated based on histological sections, which lacks essential 3D information. A detailed vascular visualization and adequate quantification is essential for the proper assessment of novel angiomodulating strategies.

Aims

To develop an ex vivo microangioCT-based imaging approach for the 3D visualization of the entire vasculature down to the capillary level and rapid estimation of the vascular volume and vessel size distribution. Moreover, it should be suitable for the successive morphological analysis using light/fluorescence or transmission electron microscopy

Method/Results

A correlative imaging approach comprising two microCT scanning modalities followed by histology was introduced. After the perfusion with µAngiofil®, a novel polymerizing contrast agent, low- and high-resolution scans (Skyscan 1272; voxel side length: 2.58-0.66µm) of the entire vasculature were acquired. In a second step, samples were dehydrated and rescanned to delineate the muscle fiber architecture. Based on the microCT data, sites of interest were defined and samples further processed for correlative morphology. The solidified µAngiofil® remained in the vasculature and its autofluorescence allowed co-registering the histological sections with the corresponding microCT-stacks.

The perfusion efficiency of µAngiofil® was validated based on lectin-stained histological sections: 98±0.5% of the total blood vessels were µAngiofil®-positive, whereas 93±2.6% were lectin-positive.

By applying this approach we analyzed the angiogenesis induced by cell-based delivery of a controlled VEGF dose. VEGF increased vascular density by 426% predominantly by increasing the proportion of medium-sized vessels (20-40µm).

Conclusion

The introduced correlative and quantitative imaging approach is highly reproducible and allows a detailed 3D characterization of the vasculature and muscle tissue. Combined with histology, a broad range of complementary structural information can be generated."

P36

Philipp Schneider - Bridging biological and preclinical imaging through 3D X-ray histology

University of Southampton, United Kingdom

Living structures are an intricate three- dimensional (3D) arrangement of cells and tissue matrix across many length scales. However, structural analysis of tissues, whether for research or diagnostic purposes, remains overwhelmingly bounded and constrained by microscopic examination of relatively sparse 2D tissue sections, providing only a snapshot from which 3D spatial relationships can only be inferred. Therefore, whilst 3D medical imaging is commonplace, microscopic tissue structure analysis (i.e., histology) remains overwhelmingly wedded to 200-year-old practices of microscopic 2D examination of tissue sections.

We have demonstrated previously that X-ray imaging by micro-computed tomography (µCT) allows non-invasive 3D imaging of the microstructure of standard tissue biopsies (Scott et al. 2015, doi:10.1371/journal.pone.0126230). This yields details comparable to two-dimensional (2D) optical microscope sections but for the whole tissue volume, which can for example overturn misconceptions of disease development based on 2D assessment. One exemplar is the pathogenesis of idiopathic pulmonary fibrosis (Jones et al. 2016, doi:10.1172/jci.insight.86375), where 3D structural insight into co-localisation of tissue features suggested previously unrecognised fibroblast foci plasticity.

Based on this encouraging µCT results for soft tissues, in collaboration with an industrial partner, we developed a custom-design and soft-tissue optimised µCT scanner that can bridge the gap between biological and preclinical imaging (Katsamenis et al., doi:10.1016/j.ajpath.2019.05.004). Currently, we are establishing the foundations for routine 3D X-ray histology (http://www.xrayhistology.org), including new X-ray equipment and standardised & automated workflows and augmented sample throughput.

Applicable to vast existing sample archives and a wide range of soft tissue types, the technology will open new research areas, such as large-scale 3D histological phenotyping (i.e., histomics). Computing and data handling power is now more than capable of handling the image resolutions and processing required for 3D µCT data analysis and X-ray histology workflows. Furthermore, 3D X-ray histology can translate directly into next-generation clinical image-based diagnostics and patient stratification using artificial intelligence and deep learning, and time-critical intraoperative 3D examination of tissue biopsies will become a realistic future target in this research programme.

Here, we will present first results of our 3D X-ray histology approach and portray a vision, how high-throughput and non-destructive 3D histological assessment can offer new opportunities in basic biology, biomedical and translational research.

P37

György Vámosi - Simultaneous mapping of molecular proximity, co-mobility, dynamics and binding by SPIM-ALEX-FRET-FCCS in live cells

University of Debrecen, Hungary

Single Plane Illumination Microscopy (SPIM) revolutionized time lapse imaging of live cells and organisms due to its high speed and reduced photodamage. Quantitative mapping of molecular (co-)mobility by imaging fluorescence (cross-)correlation spectroscopy (F(C)CS) in a SPIM setup has been introduced to provide insight into the spatial distribution of diffusion properties and molecular binding. Another key aspect of molecular interactions is proximity, which can be studied by Förster resonance energy transfer (FRET). Here, we extend SPIM-FCCS by alternating laser excitation (ALEX), which reduces false positive cross-correlation by eliminating crosstalk, and allows for additional co-mapping of proximity by FRET. Thus, complementary aspects of interacting systems can be studied simultaneously, and several molecular subpopulations can be discriminated by multiparameter analysis. After demonstrating the benefits of the method on the AP-1 transcription factor, we show the way it improves the understanding of the dimerization and DNA binding behavior of retinoic acid receptor (RAR) and retinoid X receptor (RXR) upon ligand binding and we propose a refinement of the molecular switch model of nuclear receptor action. Our data imply that RAR agonist enhances heterodimerization between RAR and RXR, and that chromatin binding capacity and dimerization are positively correlated. We also propose a ligand induced conformational change bringing the N-termini of RAR and RXR closer together. RXR agonist increased not only heterodimerization with RAR, but also homodimerization of RXR suggesting a potential role for RXR as an autonomous transcription factor.

P38

Christina Streli - Analysis of zinc in osteosarcoma tissue by synchrotron radiation micro XRF

TU Wien Atominstitut, Vienna, Austria

Abnormal tissue levels of certain trace elements such as Zinc (Zn) were reported in various cancer types [1]. However, very little is known about the role of Zn in osteosarcoma. Using confocal synchrotron radiation micro X-ray fluorescence analysis (SR-µXRF) at the ANKA FLUO beamline (Karlsruhe, Germany), we characterized the spatial distribution of Zn in high-grade sclerosing osteosarcoma tissue of nine patients (4 women /5 men) following chemotherapy and wide surgical resection. Zn levels in mineralized osteosarcoma tissue were compared to levels in adjacent normal healthy tissue. Quantitative backscattered electron imaging (qBEI) as well as histological examinations were also performed.

We can report the following results: on average, the ratio of medians of Zn count rates (normalized to calcium) in mineralized tumor tissue was about 6 times higher than in normal tissue. There was no difference in Zn levels between tumor fraction areas with a low and a high fraction of mineralized tissue, which were clearly depicted using qBEI [2]. Moreover, we found no correlation between the Zn values and the type of tumor regression according to the Salzer-Kuntschik grading [3]. The underlying mechanism of Zn accumulation remains unclear. Given the emerging data on the role of trace elements in other types of cancer, our novel results warrant further studies on the role of trace elements in bone cancer.

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■ **P39**

Stephan Handschuh - In situ isotropic 3D imaging of vasculature using microscopic dual energy CT (microDECT)

University of Veterinary Medicine Vienna, Austria

Tomographic X-ray angiography is widely used in clinical settings to facilitate the diagnosis of vascular disorders and routinely involves the use of contrast agents supplied to the bloodstream. It is similarly exploited in small laboratory animal models. However, animal size and radiation dose ultimately limit the resolution of preclinical vascular imaging in vivo. In contrast, post-mortem angiography can provide higher resolution and thus information down to the level of capillaries. Sample preparation for ex vivo angiography starts with removal of blood from the vascular system followed by perfusion with a contrast medium mixed with a carrier such as gelatine or resin. Subsequently, the vascular micro-architecture of harvested organs is imaged either as a corrosion cast or in the intact fixed organ. In the present study we established novel X-ray imaging protocols based on dual energy CT, aiming at the visualization of microvasculature in situ. Mice were perfused with barium sulphate (Micropaque® CT). Tissues were dissected, postfixed, and counterstained with phosphotungstic acid or eosinY. Subsequently, samples were scanned using two X-ray energy spectra. Based on the different attenuation properties of the contrast agents, this allowed the separation of signal deriving from the vasculature and the surrounding tissue, providing isotropic 3D information on small blood vessels together with microscopic tissue architecture. The presented dual energy workflow is suited as a tool to screen and 3D quantify microvasculature and can be implemented in various correlative imaging pipelines to target regions of interest for downstream light microscopic investigation.

■ **P40**

Algimantas Kriščiukaitis - Multimodal in-vivo skin imaging

Neuroscience Institute, Lithuanian University of Health Sciences

Approximately two-thirds of population suffer from low back pain at certain period of their lives. Most commonly it happens at the age between 30 and 50 years. The reason for that in many cases are degenerative vertebral endplate and subchondral bone marrow changes, which were first noticed in MRI and described by de Roos et al. in 1987. The formal classification was subsequently provided by Modic et al. in 1988, based on a study of 474 patients, most of whom had chronic low back pain. The medical term “Modic changes” (MC) is now used to spinal degenerative diseases becoming one of parameters describing spinal degenerative diseases together with related morphological changes in spinal structures (bone marrow and endplate lesions). Different MC types reflect certain stages of degenerative processes starting with bone marrow oedema and inflammation (Type I), through conversion of normal red haemopoietic bone marrow into yellow fatty marrow as a result of marrow ischaemia (Type II), till subchondral bony sclerosis (Type III). All these stages are reflected as particular combinations of signal intensity in the lesion zones in different MRI sequences (T1, T2 and their variations). The aim of this study was to elaborate the method for multivariate analysis of images registered in several MRI modalities and test it in volumetric estimation of lesions of different type Modic changes in lumbar spine in patients with lower back pain.

We used 65 DICOM sets of standard MRI images obtained in T1, T2 and T2-TIRM sequences from the patients, who experienced lower back pain. Principal component analysis based method was elaborated to construct maximal contrast representation of particular type MC lesion in spinal structures in regard to healthy tissue or other types of degenerative changes. The method increased contrast of lesion zone at least twofold if compared to the highest contrast in single MRI modality images. Such representation allowed semi-automatic volumetry and 3D reconstruction of the lesion body.

P41

Maurizio Dabbicco - Scanless and Detectorless Chemical Imaging by Single-arm Interferometry: a proof of principle

Dipartimento di Fisica, Università di Bari, Italy

Optical Feedback Imaging (OFI) is a coherent scanning imaging technique with the unique feature of having the light source acting also as the signal detector. It is based on the perturbation of the laser emission produced by the radiation back-coupled inside the same laser cavity. Both the coherent and the incoherent part of the back-scattered radiation provide useful information about the optical properties of the sample, that are encoded into the voltage drop of the semiconductor laser. OFI is being increasingly applied to imaging of both inorganic and organic samples, especially in the mid-infrared and Terahertz spectral region, where compact and sensitive 2D detectors are not yet available. OFI as been recently adapted to collect Doppler images, super-resolution images, confocal depth resolved sections as well as photo-acoustic tomography acoustic waves images. OFI compact footprint makes it suitable for multi-wavelength and multi-modal simultaneous investigation of the sample.
We demonstrate the sensitivity of OFI to intensity, as well as to optical phase, contrast. Selective images of different organic pigments at three different visible wavelengths allowed for unambiguous identification of absorption features in back-scattering geometry. Translation of the technique to mid-infrared wavelengths, would candidate OFI for taking chemical pictures of epithelial tissues in reflection mode, at the same time with, for example, morphological and Doppler images, taken at visible and near-infrared wavelengths, respectively.
Lack of 2D detectors in mid-infrared and Terahertz region is also driving the development of new computational techniques aimed to the extrapolation of images from low-resolution detectors. Compressive Sensing (CS) algorithms allow to reconstruct an N-pixel image taking less than N measures. CS can be pushed up to the realization of Single Pixel Cameras, that produce spatially resolved image employing a bucket detector.
The laser cavity of a single mode semiconductor laser is an inherently single-pixel detector and the OFI spatial resolution is usually recovered scanning the sample pixel by pixel. Here we demonstrate, for the first time, the proof-of-principle of a scanless OFI, producing the image of a simple 2D object via CS techniques. The beam traveling in the common-path interferometer is expanded to the size of the sample surface, and its intensity profile is controlled by a Spatial Light Modulator (SLM). The CS algorithm evaluates the feedback for different intensity profiles and extrapolates an image of the object. The scanless imaging capability of CS along with the detectorless imaging capability and the versatile modality of OFI, are suggestive of a powerful unconventional multi-modal imaging approach with potential applications in materials science and biophotonics.

P42

Josef Prost - Establishment of a High Capacity X-ray Source in Austria – Bio-Imaging Capabilities

AC2T research GmbH, Austria

Constantly increasing demand on modern solutions for development of industrial technologies and high-performance analytical methods accelerates the competition between interdisciplinary research groups targeting new insights into the nature of materials.

An innovative development combines laser and electron-beam interaction (inverse Compton scattering) to design a specific laboratory-sized X-ray source (Compact Light Source, CLS). Despite its small size, the CLS offers the favourable features of synchrotron radiation, such as a continuously tuneable energy spectrum and high spatial resolution as well as angular divergence at high brilliance. Focusing the beam enables the operation with uniform area section within a distance of several meters from the input area. These properties make the CLS a promising solution for a wide range of X-ray applications related to tomography, diffraction, scattering and elemental analyses experiments.

An installation of such a CLS facility in Austria, embedded in further high-end analytical laboratory infrastructure, opens new perspectives for a broad suite of state-of-the-art scientific applications for multiple users including universities, scientific companies and research divisions of local high-tech industry.
In addition to a wide range of scientific applications, the assignment of the CLS for education purposes in combination with pre-characterization and evaluation of samples and experimental setups in preparation for a measurement at a large-scale European synchrotron facility cannot be underestimated. The installation of a CLS system will enable the flexible and tailored training for young scientists and professionals in research and industry.

The high beam intensity provided by the CLS allows a wide range of applications for high-quality imaging of biological materials, otherwise only possible on large-scale synchrotron facilities. Multimodal imaging allows the reconstruction of e.g. the three-dimensional absorption-, phase- and dark-field-contrast images from one single CT scan. This enhances the capabilities of biomedical imaging due to the dramatically improved contrast between tissues. Further analytical X-ray techniques such as 2D and 3D elemental imaging using micro X-ray fluorescence analysis complete the Bio-Imaging capabilities of the CLS system.

P43

Anna Turyanskaya - Correlation of µXRF and LA-ICP-MS in analysis of human bone-cartilage sample

TU Wien, Atominstitut, Austria

Within this project we aimed for the thorough elemental characterization of complex biological sample – biopsy of human femoral head, which includes both hard and soft tissue, i.e. bone and cartilage. Two elemental imaging methods employed - microbeam X-ray fluorescence (µXRF) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). µXRF and LA-ICP-MS are widely used in chemical analysis in various applications. Bone consists mostly of mineralized matter and its elemental composition is in focus of investigation (both in health and disease) of many research groups. Cartilage, however, is primarily composed of organic molecules – such as glycosaminoglycans, proteoglycans, collagen; and its elemental content is not well studied. In this proof-of-principle study µXRF was followed by LA-ICP-MS on the very same sample. The measurements resulted in the elemental maps of bone, as well as cartilaginous area of the biopsy (including the tidemark – interface between calcified and hyaline cartilage). In addition to the information on distribution of elements, we also obtained quantitative information by using matrix-matched standards.

P44

Christopher Kremslehner - Automated immuno-histo-enzymatic investigation of metabolic enzyme activity and spatial allocation of UVB-pretreated normal human keratinocytes in epidermal skin equivalents

Medical University Vienna / Dermatology, Austria

We here report the development of an automated microscopy method that allows relating the individual enzymatic activity of single cells to immuno-histochemical marker expression and its position within the epidermis of the human skin or a 3D skin equivalent (SE) model.
We adapted the StrataQuest software (TissueGnostics) to automatically predict the epidermis based on nuclear density-mapping and to further to allow distance-based distinction between the basal and low suprabasal epidermal strata, as well as the stratum corneum on automated microscopy scans of skin sections. To validate strata prediction the sections were immunofluorescence counter-stained for differentiation markers (KRT10, KRT14). A tetrazolium-based enzymatic activity assay for G6PD was established that allows relating the native enzymatic activity to the chromogenic signal in cryosections. To analyze the influence of UVB irradiation on the metabolic activity and spatial allocation of keratinocytes SE were generated including 20% of pre-irradiated, labelled cells.
The measured G6PD activity in the different strata revealed a significant increase from the basal to the suprabasal reflecting the histologic confirmation of strong tetrazolium salt signal in the granular layer of the epidermis. Further UVB-irradiated cells in the suprabasal strata showed a reduced enzymatic activity compared to surrounding untreated cells. Based on the distribution of labelled cells within the different strata a decreased presence of UVB-irradiated cells within the basal and low suprabasal strata was detected which may indicate an increased clearance of UVB-irradiated cells via differentiation. In conclusion, we are able to present an automated image analysis tool that reliably identifies the basal and suprabasal strata of the human epidermis, and can allocate both the spatial distribution as well as the enzyme-activity staining to pretreated or IF-detected cells with in the 3D microenvironment.

P45

Anna Nele Herdina - Pilot activities for mapping transcriptome information on cells representations of human organs

Medical University of Vienna, MIC, Division of Anatomy, Austria

Recently the Human Cell Atlas (HCA) project was established to create a reference map of molecular profiles of every single cell forming the human body. Its main challenge is to design methods which permit the creation of combined transcriptome and spatial single cell profiles that can be fed into a virtual framework representing the human body. In a first attempt to contribute to this project we designed a multimodal, multiscale imaging workflow, which permits harvesting liver cells for single cell RNA sequencing while maintaining the precise cell position information in respect to the liver of body donors. For this, we combined magnetic resonance tomography (MRT), contrast enhanced three-dimensional ultrasound (3D-US), high resolution episcopic microscopy (HREM) and histopathology. Blood channels are intended to be used as a highly individual spatial reference system, which provides precise cell position profiles on the macro-, meso- and microscopic level and as references for multiscale data fusion. As first concrete outcome dissection approaches were designed for quicky accessing and stabilising the liver in and pilot actions were initiated for evaluating the influence of contrast agents and post mortem intervals on the liver cell transcriptome. Our approach is tested for the liver, but the tools we are to develop can be modified for being used to harvest cells with spatial profiles of other organs of the human body. Beside other channels, our technology and results will be disseminated via EURO-Bioimaging and COMULIS.

P46 – Flash talk

Carmen Bedia - Multimodal chemical imaging using mass spectrometry, infrared and rgb information from tissues

Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Spain

In this work, we present a Multimodal Chemical Imaging (MCI) methodology to deepen into the chemical analysis of complex and heterogeneous tissues. This new approach uses the combination of two imaging technologies that provide highly valuable chemical information without any pre-labeling step: Mass Spectrometry Imaging (MSI) and Infrared Hyperspectral Imaging (IR-HSI). These data are also combined with the information coming from hematoxylin/eosin (HE) histological staining pictures. On the one hand, MSI has attracted growing interest in recent years to unravel the pathophysiology of organs and the pharmacodynamics of drugs. This technology allows to detect and identify different types of molecules in each pixel of the tissue analysed, providing structural and spatial information. On the other hand, vibrational technologies such as IR-HSI are currently emerging as an important tool for biomedical research with a spatial resolution at the cellular level. IR-HSI gives the information about the chemical functional groups present in each pixel, creating a distinct and unique fingerprint spectrum at each of the tissue positions analysed. When the study focuses on the abundance and distribution of specific molecules or chemical signatures, the analysis of the data provided by these technologies is rather simple. However, when the aim of the study is to discover the behaviour of a complex tissue without any previous hypothesis of the mechanisms underneath the processes, an untargeted analysis of the data generated is required. The vast amount of data obtained in MSI, IR-HSI and HE analyses needs for the application of chemometric tools to extract the hidden information. In this work, we present the data fusion of the three techniques and their simultaneous analysis by the means of multivariate resolution alternating least squares (MCR-ALS) methodology [1]. The application of MCR-ALS on imaging fused data enables the resolution of the chemical and staining signature and their distribution within the tissue of each of the components resolved. This analytical approach has also been used to analyse several tissues simultaneously, which enables a better understanding of the heterogeneity of tissues and their behaviour under different stimuli.

P47 – Flash talk

Pieter De Beule - Simultaneous imaging for cellular biology using fluorescence microscopy and atomic force microscopy

INL, Portugal

Since the introduction of the Atomic Force Microscope (AFM) in the field of cellular biology in 1990 there has been a continuous effort to improve AFM design to enable faster imaging, reduce undesirable sample interaction and provide mechanobiological information using chemically modified AFM cantilever probes. Also, many home-built laboratory set-ups have been developed integrating AFM with fluorescence imaging schemes such as CLSM, TIRFM, STED and PALM/STORM. One key challenge for such combined imaging platforms is simultaneous imaging obtaining fluorescence information near an imaging AFM cantilever. At INL we enabled this feature through the integration of AFM with fluorescence microscopy schemes that provide a constant time-integrated illumination of the AFM cantilever with the fluorescence excitation light: aperture correlation microscopy implemented in a Differential Spinning Disk (DSD) approach [1]https://aip.scitation.org/doi/10.1063/1.4931064 and Structured Illumination Microscopy (SIM) [2]http://biorxiv.org/cgi/content/short/638262v1.

SIM excels in the observation of dynamical biological processes as it provides a good compromise regarding resolution, phototoxicity, photon budget, and faster acquisition speeds for an extended duration, in contrast to other Super-Resolving (SR) fluorescence microscopy approaches such as STED and PALM/STORM microscopy. In addition, it is compatible with most conventional fluorophores. SIM illumination characteristics make this technique very suitable for integration with AFM as it does not interfere with cantilever operation therefore permitting simultaneous data acquisition. Most of the works using SR fluorescence microscopy use high intensity lasers and/or long imaging timeframes, making them quite incompatible with dynamical studies. Here we report SIM and AFM simultaneous imaging for the first time. We test the system with sub-resolution fluorescent beads and human bone osteosarcoma epithelial cells edited using the CRISPR/Cas9 system.

P48

Jaromir Gumulec - Mass redistribution – not accumulation characteristic for aggressive prostate cancer: simultaneous employment of quantitative phase microscopy techniques

Masaryk University, Czech Republic

Changes of biomechanical properties of the cells play a crucial role in the development of metastases in cancers. In most tumor types, decrease of cell stiffness is typically accompanied by epithelial-mesenchymal transition, a process crucial for tumor dissemination. We focused on an in-vitro multistep model of prostate cancer we previously developed (zinc-resistant PNT1A-22Rv1 and PC-3 cells). Using atomic force microscopy (AFM), we observed an inverse trend: more aggressive cells were characteristic by higher values of Young modulus.
With this regard, a combination of coherence-controlled holographic microscopy (quantitative phase imaging, QPI), confocal microscopy of actin and tubulin and refractive index tomography was performed to investigate this. Accordingly, this strategy was performed with aim to verify possibilities of correlative acquisition of those techniques.
It was found out that the cell dry mass (determined by QPI) followed the same trend as Young modulus determined by AFM. Nevertheless, data from confocal microscopy did not provided evidence that this increase of cell dry mass was caused by changes in actin or tubulin. Nevertheless, refractive index tomography provided data suggesting that this increase of stiffness is rather caused by mass redistribution; wild-type PC-3 cells were characteristic by no accumulation of perinuclear material (mitochondria/proteins) while (more aggressive) zinc-resistant counterparts shown massive perinuclear accumulation of mitochondria and endosplasmic reticulum and larger nucleoli in nucleus. Because QPI is label-free by nature and thus non-specific for structures, correlative QPI and fluorescence acquisition enabled us to further investigate the specificity of label-free QPI data by means of “synthetic staining” deep-learning reconstruction using U-net and in an unpaired concept with CycleGan. Using nuclear Hoechst 33342 staining we demonstrated that such approach is manageable with wither paired U-net strategy as well as with unpaired CycleGAN approach.
In sum we provided evidence that a combination of refractive index tomography, AFM and confocal microscopy provided more robust evidence that mass redistribution and increase of metabolic plasticity is characteristic for aggressive prostate cancer model rather than just increase of cell stiffness as seen by AFM.

This work was supported by Grant Agency of the Czech Republic (18–24089S).

P49

Veronika Huntosova - Time-resolved measurements of oxidative stress level in cancer cells

CIB TIP UPJS in Kosice, Slovakia

Fluorescence microscopy represents a visualization method that could rapidly achieve subcellular localization of fluorescent substances in real time. However, low concentration of substances could result in misleading information about its localization. On the other hand, fluorescence quenchers like molecular oxygen and ROS can significantly decrease the signal of detected fluorescence intensity. In our work, we suggest overcoming these obstacles with fluorescence and phosphorescence lifetime measurements of sensors. These parameters are concentration independent and sensitive to molecules and condition changes nearby. The basal level of oxidative stress is characteristic for each type of cells to maintain homeostasis. External or internal stimuli (like anticancer drugs) often break this balance. In the present work, we have demonstrated that MitorTracker Orange CMTM/Ros (MTO) besides relocalization into the nucleus dramatically changes its fluorescence lifetime in the ROS presence. Finally, we aimed to develop a new approach to simultaneously detect oxygenation and oxidative stress level in cancer cells during anticancer therapy.

P50

Luka Rogelj - Surface profile intensity correction for hyperspectral imaging of curved samples

Faculty of Mathematics and Physics, University of Ljubljana, Slovenia

Hyperspectral imaging (HSI) is a perspective imaging modality due to the possibility of acquiring spatial and spectral information simultaneously. It shows a lot of potential in various fields such as archaeology, art conservation, vegetation, food science and in recent years biomedicine. HSI can be used to extract optical parameters in every pixel of an image and therefore to analyze spatial distributions of geometrical, textural and chemical properties of a sample. Recorded spectral images are affected by a shape of a sample and by the distance between the sample surface and the camera lens, resulting in lower image intensity in the areas with the greater inclination and larger distance. In our study, the curvature and distance artifact is minimized by including the sample shape information from a 3D laser profilometry. Specifically, a Lambert cosine law (LCL), based on the angle between the surface normal and incident light direction, is used to correct for the inclination. Camera to surface distances and the surface normals are calculated from the results obtained by 3D laser profilometry. Height and angle correction maps for an image for each wavelength are determined and applied to the original image. To assess the performance of the correction algorithm, images of a homogeneous hemisphere and in-vivo human finger were recorded and corrected. A significant improvement of the images were obtained, the hemisphere cross-section closely resembling the ideal top-hat profile.

P51

Hector Dejea i Velardo - Multimodal imaging for cardiac anatomy and function studies in rodents

Paul Scherrer Institut and ETH Zürich, Aargau, Switzerland

The cardiac tissue is an example of dynamic hierarchical material, in which its components are arranged within several scales from whole organ to subcellular level, each participating towards the function of the system. This poses a difficulty when trying to fully investigate the cardiac structure and how it relates to the heart beat. In this context, multimodal imaging can be of great use to be able to assess features and validate the results obtained.

In this work, we present the multimodal investigation of cardiac anatomy and function in rodents thanks to the combined use of synchrotron propagation-based X-ray phase contrast imaging (PB X-PCI), ultrasound (US) and light microscopy.

A perfused isolated heart system was customized and implemented in the TOMCAT beamline of the Swiss Light Source. This allowed high resolution (2.75um or 5.8um pixel size) beating heart tomographic imaging using PB X-PCI, as well as 2D US imaging. Afterwards, hearts were fixed and imaged ex-vivo using a multiscale PB X-PCI setup that enables whole organ tomography at lower resolution (5.8um pixel size), and higher resolution (0.65um pixel size) imaging of selected regions of interested. Finally, histological sections were stained and scanned with light microscopy.

With this pipeline, 3D high resolution PB X-PCI data can be used to assess cardiac structure dynamics, which can be then compared to current established technique such as US. Then, cardiac tissue components at different length-scales can be investigated thanks to the multiscale PB X-PCI in ex-vivo static hearts and its comparison to histological images. Therefore, a complete study of cardiac structure and function in rodents can be achieved from millimetre (myocardium) down to the micrometer level (subcellular structures).

P52

Berta Cillero Pastor – Specific lipid and metabolic profiles of R-CHOP–resistant diffuse large B-cell lymphoma elucidated by matrix-assisted laser desorption ionization mass spectrometry imaging and in vivo imaging

M4I, Maastricht University, Netherlands

Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin lymphoma. To treat this aggressive disease, R-CHOP, a combination of immunotherapy (R; rituximab) and chemotherapy (CHOP; cyclophosphamide, doxorubicin, vincristine and prednisone), remains the most commonly used regimen for newly diagnosed DLBCLs. However, up to one-third of patients ultimately becomes refractory to initial therapy or relapses after treatment, and the high mortality rate highlights the urgent need for novel therapeutic approaches based upon selective molecular targets. In order to understand the molecular mechanisms underlying relapsed DLBCL and to find better therapeutic targets, we studied differences in the chemical composition after R-CHOP treatment and tumor relapse, using a combination of in vivo DLBCL xenograft models and mass spectrometry imaging. Together, these techniques provide information regarding analyte composition and molecular distributions of therapy-resistant and sensitive areas. We found specific lipid and metabolic profiles for R-CHOP–resistant tumors such as a higher presence of phosphatidylinositol and sphingomyelin fragments. In addition, we investigated intratumor heterogeneity and identified specific lipid markers of viable and necrotic areas. Furthermore, we could monitor metabolic changes, and found reduced adenosine triphosphate and increased adenosine monophosphate in the R-CHOP–resistant tumors. This work highlights the power of combining in vivo imaging and MSI to track molecular signatures in DLBCL, which has potential application for other diseases.

CORRELATION SOFTWARE (WG4)

P53

Barbara Geist - Quantification Software for Multimodalic Images

Meduni Vienna, Austria

"Image series from dynamic scans performed with PET/MR, PET/CT or SPECT/CT systems contain more than visual information, because the underlying biochemical processes of PET or SPECT tracers can be uncovered in any body region due to the high anatomical resolution of the CT or the MR. These processes depend on the used tracer, the body region and the health status of the patient. Their understanding therefore is indispensable for e.g. developing drugs, studying diseases or tracking the function of certain transporters. Consequently, a mathematical description of the biochemical processes is needed.

In nuclear medicine, this description is usually performed with so-called kinetic compartment models. Such a model is a set of differential equations, where the main biochemical processes are expressed as partially measurable compartments and their interactions as calculable constants, representing the quantification of any biochemical processes.

Depending on the problem, a compartment model can be sophisticated and tricky to solve. Therefore, a software (GhostKineticToolkit) was developed allowing to create and solve any compartment model of interest without needing knowledge about mathematical solutions or differential equations. The compartments are realized as clickable boxes, the interactions as arrows. By using sliders to adjust the intensity of the interactions, a real-time simulation is displayed showing the possible outcome of the entered model.

The main application of this software is on the one hand for not mathematically trained users to deepen their understanding of the quantification of biochemical processes with the simulation tool. On the other hand, data can be loaded from the most frequently used image viewers commercially available in nuclear medicine. Any model of interest can be created and fitted to the loaded data, allowing to quantify the imaging data according to their biochemical processes.

Further developments of the software will be an algorithm which allows to recognize the underlying main processes of loaded data as well as a tool which fits created models to each voxel in a 3D image."

P54

Guillaume Potier – Registration and error estimation in correlated multimodal imaging

INSERM, Nantes, France

Image registration methods are used in a wide range of applications, in particular in correlated multi-modal imaging in life science, yet we often lack an estimate of the associated registration error. In this work we aim to provide such estimates as a quality metric for image registration. Our method relies on multivariate multiple linear regression analysis which provides both image registration itself and registration error estimates. Since linear regression is flexible, models can be extended to integrate constraints such as rigid transformations. This is also known as the orthogonal Procrustes problem. We present the different methods for error estimation used in the correlated multi-modal imaging field, but also the ones used in the registration literature. Finally we provide an implementation of our registration framework as a plugin under Icy software.

P55

Raphaël Maree – Cytomine for collaborative analysis of large imaging: latest developments

University of Liège, Belgium

The Cytomine project (http://www.cytomine.org/; Maree et al., Bioinformatics 2016) started in 2010 at ULiège to build a rich web environment for multi-gigapixel imaging data. This tool has been designed with the following objectives in mind: provide remote and collaborative principles, rely on data models that allow to easily organize and semantically annotate imaging datasets in a standardized way, efficiently support high-resolution multi-gigapixel images (e.g. digital slide scanner image formats), and provide mechanisms to readily proofread and share image quantifications produced by machine/deep learning-based image recognition algorithms. By emphasizing collaborative principles, our aim with Cytomine is to accelerate scientific progress and to significantly promote image data and algorithm accessibility and reusability. We want to break common practices in this domain where imaging datasets, quantification results, and associated knowledge are still often stored and analyzed within the restricted circle of a specific laboratory.

In this talk we will present our latest developments (Rubens et al., Proteomics 2019; Rubens et al. biorxiv 2019). It includes 1) new developments in the Cytomine core and image management system for the support of video and multispectral data, 2) new front-end interfaces for efficient visualization of video and multimodal datasets, 3) new external software container architecture for seamless integration of algorithms from any existing image analysis platform.

P56

Nebojsa Nesic - 3D reconstruction of Cell Organelles

Univerzitet Singidunum, Serbia

With the emergence of volume electron microscopy (EM), and more widely of large volume microscopy and bioimaging (3D of thick tissues or entire organisms, time, channels), data analysis becomes the new bottleneck in biological and biomedical sciences. Automated data annotation and segmentation becomes necessary to extract meaningful information from these high-content multivariate data in reasonable time. We apply Yolo, which is a deep learning algorithm to EM cell datasets to identify rough regions of interest in each layer, such as the nuclei or mitochondria. We then apply image processing techniques to refine the regions of interest to the boundaries of the cell organelles, and recompose them as 3D structures. Segmentation is achieved in the order of days for a previously unseen EM dataset, as opposed to the tremendous human effort currently required to achieve a similar result for the segmentation of an entire cell and its organelles. Importantly, our approach will be applicable to other 3D imaging modalities in biology, such as Soft X-Ray Tomography. The software achieves 80% accuracy with respect to ground truth data when reconstructing 3D cell organelles, but allows the user to fix errors manually in order to be able to achieve the desired accuracy within a short time frame.

P57

Sevil Maghsadhagh - Characterization of Brain Cerebral Cortex Morphology Using Topology-Encoding Graphs

Independent researcher, Vienna, Austria

The graph spectrum is considered as a fingerprint that provides structural information. The human cerebral cortex exhibits a complex morphology that is unique to each individual. Characterization of the cortical structure is important in studies of structural brain changes also classification of healthy and unhealthy individuals. We introduce a method to represent an individual's cerebral cortex as a graph and apply this model for quantifying hemispheric asymmetry and gender variation based on cortical morphology.
For an undirected, unweighted graph, the normalized Laplacian matrix L is defined as L = I - D^-(1/2) 1/2 AD^-(1/2) where A and D denote the graph's adjacency and degree matrices, respectively. L is symmetric and positive semi-definite, its eigenvalues are limited to a range [0, λ"#], where Ng denotes size of the graph and λ"# ≤ 2 denotes its largest eigenvalue. These eigenvalues define the graph's Laplacian spectrum. Left and right hemispheres were analyzed separately. For each hemisphere, a Global Cerebral Hemisphere Cortex (GCHC) graph is constructed. Moreover, using spectral clustering, each hemisphere was parcellated into a set of equally sized parcells, using which a set of Localized Cerebral Hemisphere Cortex (LCHC) graphs were constructed. Varying parcellation resolutions, 5k to 10k voxels, were investigated. In both graph types, voxels within cerebral cortex were treated as graph nodes and edges were defined based on 26-neighborhood connectivity between adjacent voxels in 3D space. The construction was implemented at 1 mm cubic resolution, resulting in GCHC graphs of size approximately 300000 nodes. We quantified graph spectra by counting the number of eigenvalues falling within narrow sub-bands across the spectrum. The counts were treated as metrics and subsequently used for statistical hypothesis testing by Wilcoxon rank-sum test. We analyzed structural MRI scans of 75 female and 75 male healthy individuals, aged between 31 to 35, from the Human Connectome Project. For hemispheric asymmetry, we observed that at upper parts of the spectra, GCHC graphs provide higher discriminative power than LCHC graphs, whereas the opposite was observed at the lower end of the spectra. When testing variations across gender, spectral metrics of LCHC graphs conferred better discrimination than corresponding metrics from the GCHC graphs.

P58 – Flash talk

Martin Schorb - Modular CLEM software: From microscopy acquisition to data publishing in one go

EMBL Heidelberg, Germany

A typical CLEM experiment requires many dedicated software tools that serve different purposes. We present a software approach that integrates a number of existing common software tools to create a streamlined user experience in handling multimodal imaging data. Based on PyEM and BigDataViewer, we can now link and interface software tools reaching from the acquisition of correlated light microscopy and TEM data (using SerialEM) across registration of mutimodal data (Icy/ec-CLEM, elastix) all the way to visualizing and annotating the data in a consistent publication-ready collection (CoMuViewer). I will present the approach using a typical on-section CLEM experiment from our facility.

<<---- Back-to-Back submission with Christian Tischer who will show the concepts of CoMuViewer --->>

P59

Lindsey Marshall - syGlass: The Future of Data Exploration

syGlass, USA

syGlass is a carefully optimized visualization tool for direct volume rendering with no segmentation required.

At the forefront of exploiting advances in virtual reality technology, syGlass enables users to view, annotate and analyze volumetric data in its true three- or four-dimensional form. syGlass is primarily used by renowned researchers, medical professionals, and educators worldwide. Three-dimensional image visualization and analysis go from daunting, time-consuming tasks, to a precise and efficient workflow in an exciting virtual world.

Users can load data from many sources (confocal, light sheet, EM, MRI, CT, microCT…).

syGlass can render up to 20 TB of data at a time without crashing! Data may even include up to four channels or 4D data that moves through time.

Count, measure, trace tree-like structures, annotate and label your data.

Communicate your data by taking high quality photos, keyframe videos or record narrations while manipulating volumes to educate others or even for publications.

For collaborative analysis with anyone from around the world, view your data in MultiView mode.

Our software allows for true 3D visualization of 3D data, compared to such volumetric data being viewed on a 2D screen and having to understand the perspective and depths of what you are trying to analyze.

Website: www.syglass.io

Training academy: www.syglass.io/academy/home

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#ExploreYourData
Find us to load and explore your own data in syGlass during this meeting!

P60

Mitko Kostov - NM Images Denoising

St. Kliment Ohridski University, Faculty of Technical Sciences, Nord Macedonia

An approach for estimation of a non-uniform threshold used for denoising of nuclear medicine images in the wavelet transform domain will be presented. The wavelet coefficients are filtered by an estimated threshold that is adjusted to the noise level in the images wavelet coefficients. The estimated threshold is proportional to the noise level and hence it has an advantage over a uniform threshold which shrinks all the coefficients for an equal portion. Results of comparison of the presented approach with wavelet denoising methods that use a global threshold will be presented.

P61 – Flash talk

Florian Ganglberger - A Web-based Framework for the Exploration of Heterogeneous Spatial Big Brain Data

VRVIS Research Center, Austria

Recent advances in neuro-imaging allowed big brain-initiatives and consortia to create vast resources of brain data that can be mined by researchers for their own projects. Exploring the relationship between genes, brain circuitry, and behavior is one of key elements of neuroscience research. This requires joint analysis of a heterogeneous set of spatial brain data, including 3D imaging data, anatomical data, and brain networks at varying scales, resolutions, and modalities. With ever-increasing resolution, those exceed the past state-of-the art in several orders of magnitude in size and complexity. Current analytical workflows in neuroscience involve time-consuming manual aggregation of the data and only sparsely incorporate spatial context to operate continuously on multiple scales. Incorporating techniques for handling spatial brain data is therefore a necessity.

We present a novel web-based framework to explore heterogeneous neurobiological spatial data of different types, modalities and scale for interactive visual analytics workflows. It enables domain experts to combine data from large-scale brain initiative by utilizing the hierarchical and spatial organization of the data. Connectivity data at different resolutions, such as mesoscale structural connectivity and region-wise functional connectivity can be accessed on different levels on a common hierarchical reference space. On-demand queries on volumetric gene expression and connectivity data enable an interactive dissection of networks, with billions of edges, in real-time, and based on their spatial context. Additionally, 3D visualizations have been optimized to accommodate domain experts' needs for publishable network figures.

We demonstrate the relevance of our approach by reproducing findings of known microcircuits in fear and reward-system related functional neuroanatomy in mice. Further, we show its versatility by comparing multimodal brain networks linked to autism. Importantly, we achieve cross-species congruence in retrieving human psychiatric traits networks, which facilitates the selection of neural substrates to be further studied in mouse models.

For the future, we are aiming to extend this this framework in a holistic way. This should not only allow to access the data, but also include the import of user-generated data, preprocessing, as well as computing network statistics in the web.

Company talks

■ T1

Manoel Veiga – scanR AI: Opening the gate to new applications

Olympus Soft Imaging Solutions GmbH, Germany

Deep Learning

Within the field of artificial intelligence (AI) there has been a recent outstanding chain of continuous breakthroughs in vision applications driven by deep neural networks (DNNs) and deep learning. Still, the lack of easily usable training tools and the difficulty to make a reasonable judgement on the robustness and behaviour of the DNNs is a hurdle in the practical application. Additionally, the seemingly need to gather large human annoted datasets is discouraging.

Self Learning Microscopy

We show examples of various deep learning network models. They were trained using the new deep learning driven Olympus scanR AI high content screening system, with a self learning microscopy approach with minimal human supervision. The transparent cytometric visualization and data navigation allows straightforward judgement of the training step.
Among other examples, we show that it is possible to perform extremely robust segmentation on low contrast brightfield images with various sources of optical disturbances, shading and inhomogenities (Fig. 1). We also present accurate segmentation on flourescence signals well below a single count of the cameras pixel sensors (Fig. 2).
The examples show that it is practically feasible to get DNNs trained easily without technical expertise in the field, such that the DNNs can robustly perform segmentation tasks in the most challenging scenarios, with a performance far beyond traditional approaches. We expect this to open a door to new life science microscopy applications.

KEY WORDS: artificial intelligence, AI, deep learning, convolutional neural networks, high content analysis, life cell analysis, scanR

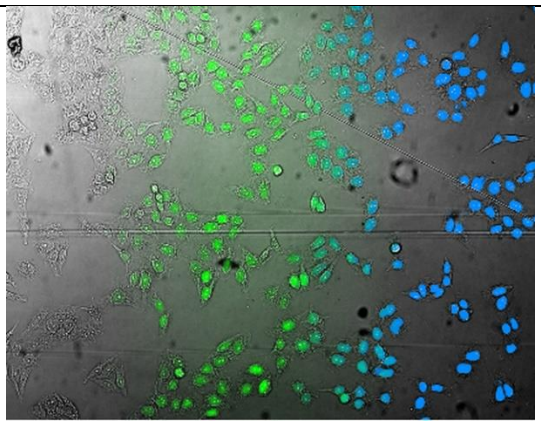

Fig.1: Transmission Image (left), fluorescence marker (green) and AI segmentation based only on the transmission (blue)

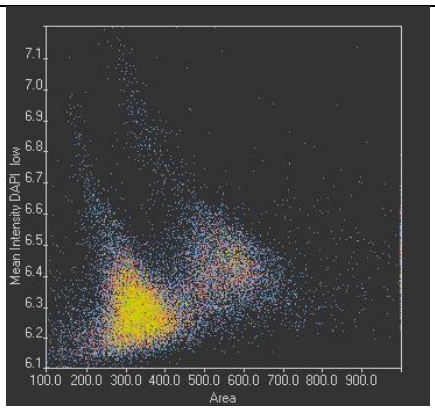

Fig.2: Cell cycle G1 and G2 phases based on segmentation with fluorescence signals below a single camera count

T2

Dieter Fuchs - The Value of In Vivo Imaging

FUJIFILM Visualsonics, Inc., Germany

Various animal models form an essential part in many research areas such as developmental biology, cancer research, cardiology, inflammation, or virology. These different animal models are needed to validate findings from in vitro experiments but also to provide novel clinically relevant information which in time has the potential to change patient care. This can be achieved with state of the art dedicated preclinical in vivo imaging, forming the basis of translational research. This seminar will focus on the latest multimodality imaging capabilities, covering fast, non-invasive and pain free applications for early detection, sizing, volumetric quantification, dynamic imaging, hypoxia assessment, etc. in various animal models with an outlook to clinical use. A strong focus on the principles of the 3Rs (reduction, refinement, replacement) and the acquisition of reliable and reproducible research data without compromising animal welfare will be maintained. The talk will outline various research areas showing possibilities and published results with the multimodality imaging equipment from Fujifilm VisualSonics.

■ T3

ThermoFisher SCIENTIFIC

Jan Giesebrecht - Amira Software for Correlative Imaging

Thermo Fisher Scientific, Germany

Correlative imaging is a field of high interest since fusing data obtained through different imaging techniques is an increasingly current practice in many life science applications. The Amira software has enhanced features for addressing this topic, such as simultaneous visualization of multiple data, multiplanar viewer to assist in data alignment and various alignment techniques even for different acquisition modalities (automatic, landmark-based, manual). Attend our presentation and learn how Amira can help you with your correlative imaging challenges.

■ T4

Seeing beyond

Wolfgang Schwinger - The ZEISS way of performing correlative microscopy

Robert Krimse[1], Eric Hummel[1], Alexandra F. Elli[1], Wolfgang Schwinger[2]

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The quest to understand the complex interactions of structure and function in biology continues to fuel efforts in advancing correlative methods combining light, electron and X-Ray microscopy imaging. The ability to combine information gathered using different imaging modalities through correlative microscopy has opened doors for new scientific discoveries and improved productivity of sample investigations. One of the largest challenges in these types of experiments is the time and effort involved in relocating the same area of the sample in successive instruments. Commercial solutions from ZEISS provide streamlined workflows and ensure quick and easy sample relocation to facilitate access to these multi-modal types of information. These solutions include Shuttle & Find for 2D and ZEN Correlative Array Tomography software modules as well as the use of ZEISS Atlas 5 for 3D correlation. We will present different workflows by using state of the art ZEISS imaging and correlations technology for real world life scientific applications.

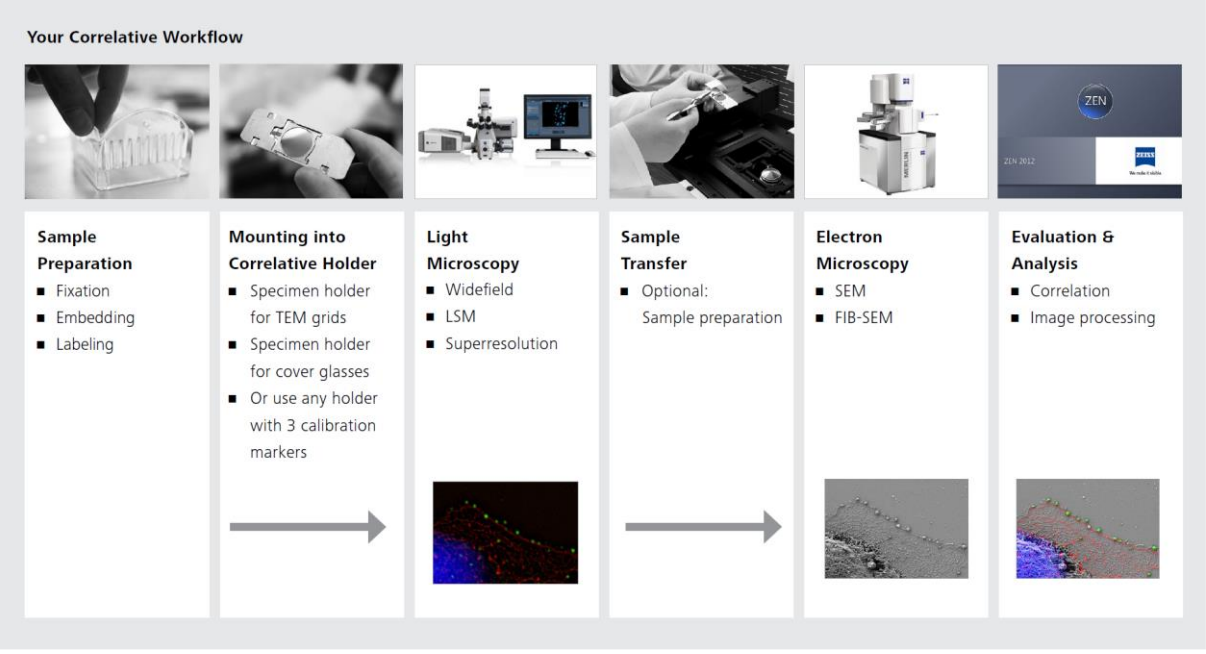

Figure 1: Correlative Workflow example using ZEISS hard and software called “Shuttle & Find”.

■ **T5**

Roberto Spada – Highly multiplexed immunohistochemistry - Visualize >40 functional and phenotypic markers simultaneously on the Hyperion™ Imaging System, powered by CyTOF® technology

FLUIDIGM, France

Changing the course of how disease is treated and ultimately cured requires a comprehensive understanding of complex cellular phenotypes and their interrelationships in the spatial context of the tissue or tumor microenvironment.

Imaging Mass Cytometry™ using the Hyperion Imaging System enables new biomarkers and cellular phenotypes to be uncovered using standard immunohistochemistry protocols, without signal overlap, autofluorescence or a cyclical workflow.

In this presentation, you will learn how to achieve unrivaled high multiplexing capability at subcellular resolution in a single scan with Imaging Mass Cytometry.

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Seeing beyond