10th Anniversary Light Sheet Fluorescence Microscopy Conference

Report of Contributions

Contribution ID : **5** Type : **Short Talk**

Imaging Intestinal Invasion by Vibrio cholerae, and other Stories from the Gut

Wednesday, 15 August 2018 12:15 (15)

Our digestive tracts are home to trillions of microbes that immigrate, emigrate, reproduce, and compete with one another. Little is known about the physical structure and temporal dynamics of gut microbial communities, which must necessarily influence the function not only of normal, commensal communities but also community invasion by pathogens. To address this, my lab applies light sheet fluorescence microscopy to a model system that combines a realistic *in vivo* environment with a high degree of experimental control: larval zebrafish with defined subsets of commensal bacterial species. I will focus here on experiments in which a native bacterial species is challenged by the invasion of a second species, specifically *Vibrio cholerae*, the pathogen that causes cholera. Using live imaging and genetic manipulation of *Vibrio*'s Type VI Secretion System (T6SS), with which the bacterium stabs adjacent cells, we have found that *Vibrio cholerae* can displace resident bacteria through a surprising ability to induce strong mechanical contractions of the host gut [S. L. Logan, J. Thomas, J. Yan, R. P. Baker, D. S. Shields, J. B. Xavier, B. K. Hammer, and R. Parthasarathy. *Proc. Natl. Acad. Sci.* **115:** E3779-E3787 (2018)]. This suggests not only previously unknown mechanisms for bacterial manipulation of animal physiology, but also potential paths for microbiome engineering. I will also describe other experiments in which the spatial and temporal dynamics of gut microbes are key determinants of responses to such challenges as antibiotic perturbation and inhibition of motility.

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Session Classification : Miscellaneous applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **6** Type : **Poster**

Shareable microscopy: a robust and compact platform adaptable to a multitude of applications

Despite the successes of entry-level open source projects, with new frontiers for light sheet imaging apparent, the demand for the technology outstrips supply. Furthermore, these projects are in need of rejuvenation, do not reflect the state of the art and remain dependent on an abundance of adventurous life-scientists capable of tackling the technical challenges. Similarly, commercial platforms are, by their nature, costly and generic

To overcome these challenges, we hope to foster a new model of advanced microscopy, based on shareable, modular instruments configurable to a broad range of applications. Employing modularity in the design facilitates reconfiguration and allows easy upgradability and an expandable functional palette. In turn, shareability provides financially prudent widespread access to cutting edge technologies.

The virtues of light sheet fluorescence microscopy justify its primacy as the foundation of this effort. Nevertheless, the core values of shareability and multi-applicability are inherently linked to the underlying microscopy platform, which must be robust, reconfigurable and compact, besides the more usual performance requirements. Light sheet microscopes capable of imaging samples spanning many orders of magnitude in size can be constructed from a remarkably small set of components. This ensures that any one microscope can be dismantled and reconfigured and that the obsolescence of one component does not result in expensive technologies languishing.

We report a compact, reconfigurable microscopy platform realized by a confluence of optical modelling, bespoke embedded control, and rapid prototyping that can be adapted to a plurality of applications. We illustrate how different microscope geometries, sample sizes and mounting methods can be accommodated within the framework of this platform, discuss specifics of the first implementations rolled out and provide a roadmap for future refinements and additions to the technology.

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Session Classification : Posters

Contribution ID : **7** Type : **Short Talk**

Smart adaptive multi-sample imaging for multi-view light sheet microscopy – when the microscope decides what to look at

Wednesday, 15 August 2018 10:00 (15)

Light sheet microscopy of early embryo developmental stages is challenging. A significant number of time-lapse acquisitions, started at the onset of development, need to be stopped due to suboptimal sample orientation, poor image quality, not-fertilised eggs or because the development process arrests due to the phototoxicity induced by the imaging process itself. To increase the likelihood of success, we devised a strategy based on simultaneous multi-sample imaging at a low frame rate coupled with smart, image-aware microscope control. The open source ClearControl framework allows flexible user-defined assembly of instructions, such as device-based instructions (e.g. image acquisition), GPU-accelerated image post-processing and analysis, adaptive instructions (e.g. auto focus) and smart instructions (e.g. sample selection).

The advantages of our strategy are twofold: first, it reduces phototoxic effects by minimizing sample exposure, and second, it allows the screening of several samples in parallel, until an operator-independent algorithm decides for a sample to image in more temporal detail and with higher signal-to-noise-ratio.

We implemented our multi-sample imaging strategy on a multi-view light sheet microscope – the XWingScope. FEP tubes with typically five embryos of *Drosophila melanogaster* in the early cleavage stage were mounted and serially imaged by using a motorised stage. The acquired volumetric images were analysed by searching for a rapid rise in an entropy based image quality metric, which is used to detect the formation of the syncytial blastoderm and to predict when the first cells will invaginate. The presented strategy allows to select a sample automatically, auto-focus it, and continue imaging to capture the beginning of gastrulation with increased temporal resolution. Further improvements, more sophisticated control and decision-making algorithms, and other applications are made possible by our highly modular and extensible framework.

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Session Classification : Developmental biology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **8** Type : **Poster**

4 D imaging of insulin secretory granule dynamics and secretion in primary beta cells with lattice light sheet microscopy

Total internal reflection microscopy (TIRFM) has been the method of choice for many years to image insulin secretory granule (SG) dynamics and secretion in primary beta cells and insulinoma cell lines. However, it only allows for imaging of SGs located <200 nm from the surface of the cell attached to the glass, thereby restricting the view only to events happening on one side of the cell. Since beta cells have a polyhedral shape with a diameter of several µm, by TIRFM imaging events happening in the major part of the cell remain invisible. Furthermore, prior to TIRFM imaging pancreatic islets are usually dissociated into single cells – a procedure that affects cell-to-cell interaction and signaling.
These limitations can be overcome with novel microscopy techniques that allow for imaging insulin SGs within primary beta cells of isolated islets at sub-cellular resolution and high speed. Specifically, we have used lattice light sheet microscopy (LLSM) to resolve insulin SGs, which have a mean diameter of 250 nm. LLSM allows for fast TIRFM-like sectioning of cells in 3 dimensions with low photo-toxicity. In this way we could image SNAP-labelled insulin SGs in isolated SOFIA (Study of Insulin Ageing) mouse islets within the whole cell volume. Use of a novel pH-sensitive SNAP-substrate further enabled us to image insulin SGs undergoing exocytosis. Hence, this is the first report for the use of LLSM in a primary mouse tissue at sub-cellular resolution in order to address insulin SG turnover within whole beta cells. Ultimately, this approach might be exploited to study peptide hormone turnover in other model systems, thus providing novel insights into the physiology of regulated secretion in health and disease.

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Session Classification : Posters

Contribution ID : **9** Type : **Poster**

A 4 dimensional analysis of actomyosin dynamics during extraembryonic tissue development in Tribolium castaneum

Extra embryonic tissues in insects show highly diverse morphogenetic strategies. Unlike *Drosophila melanogaster*, the short germ insect *Tribolium castaneum* undergoes dramatic epiboly like extraembryonic tissue expansion and ventral window closure. This offers exceptional material to study the interplay of conserved cellular and molecular mechanisms in creating novel tissue morphologies. We imaged live *Tribolium* embryogenesis using multi-view fluorescence light-sheet microscopy(SPIM), and characterized nuclear, membrane and actomyosin dynamics in wild type and genetically perturbed conditions. We unfold our 3D data into 2D cartographic maps to compare and quantify cellular events across tissues in the entire embryo. Using our 4D imaging and image analysis pipeline, we report a contractile actomyosin cable that forms during serosa window closure and shows unprecedented cellular dynamics. It appears as a 3D enrichment spanning the dorsoventral axis of the embryo, initially demarcating the boundary between the embryonic and extraembryonic tissues, showing diverse shape changes over time. Laser ablations of the serosa tissue indicate that it expands due to a pull generated by the embryonic region, leading to an increase in membrane tensions at the cable as the window closure proceeds. Interestingly, the actomyosin cable is formed as a shifting boundary of autonomously contractile cells, which intercalate into the serosa contributing to its area. Genetic perturbations of serosa affect the cable and embryo morphology, indicating towards a morphogenetic role of serosa in embryo development. Together, our results indicate towards a novel actomyosin cable type that could show conserved cellular dynamics across insects which undergo serosa window closure.

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Session Classification : Posters

Contribution ID : **10** Type : **Poster**

In toto light sheet imaging for the reconstruction of developing vertebrate embryos

Long-term in toto imaging of developing vertebrate embryos to achieve the full reconstruction of their lineage tree is still a major challenge. Based on Single Plane Illumination Microscopy (SPIM by PhaseView http://phaseview.com/alpha3/), we developed a methodology for long-term in toto imaging of zebrafish and rabbit embryos and demonstrate its performance in terms of cell detection and tracking. We achieved an optimal trade off between spatial and temporal resolution, signal to noise ratio and field of view to accurately follow cell displacements and cell divisions in whole embryos, while minimizing phototoxicity. We developed specific molds and protocols for live specimen mounting and the maintenance of homeostatic conditions for up to 4 days in the case of mammalian embryos. Following our main goal of reconstructing cell dynamics in embryonic morphogenesis to reveal pattern formation, 3D+time image data was processed for cell detection and tracking with the BioEmergences workflow (http://bioemergences.eu). Validation and annotation of the reconstructed data was done with Mov-IT, our custom-made visualization tool [1]. Comparing the cell lineage accuracy between our SPIM and 2-photon point laser scanning data demonstrates the advantage of our SPIM based strategy. In addition, we propose that our protocol for in toto imaging and reconstruction of zebrafish early embryogenesis performs far beyond the current state of the art. Our SPIM imaging strategy together with the BioEmergences image processing workflow is meant to build large cohorts of 3D+time datasets of the highest quality to open the way to large scale *in silico* embryology.

[1] E. Faure et al., "A workflow to process 3D+time microscopy images of developing organisms and reconstruct their cell lineage", *Nat. Commun.* 7, 8674, (2016).

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Session Classification : Posters

Contribution ID : **11** Type : **Short Talk**

High-Throughput LSFM Imaging Of 3D-3-Culture Models To Unveil Macrophage Plasticity In The Tumour Microenvironment

Tuesday, 14 August 2018 12:15 (15)

Drug screens on complex cell models and organisms are a key factor to understand and treat human diseases. However, fast and effective conclusions have been hindered by the lack of robust and predictable models amenable to high-throughput (HT) analysis. Recently, important advances have been made towards the development of 3D co-culture models using distinct cell types that better recapitulates its in vivo features. These models bridge the gap between adherent cell culture and animal models, providing a powerful in vitro model for preclinical research.

A major hurdle, hampering the widespread utilization of complex in vitro models, is the lack of robust imaging tools. Light sheet fluorescence microscopy (LSFM) has been proposed to overcome those limitations [1, 2]. Few years ago, we created the first flow cytometry system based on LSFM, SPIM-Fluid [3, 4], allowing the massive interrogation of a large set of biological parameters in hundreds of 3D cell cultures, thus providing statistical relevance. Now we have developed a new LSFM platform, Flexi-SPIM, which combines automatic fluidic loading of the samples and traditional scanning, overcoming the limitations of previous systems while keeping its HT capabilities.

Using Flexi-SPIM, we are able to image more than 150 sample in only two imaging sessions of complex 3D-3 culture models including a co-culture of tumour cell spheroids of a non-small cell lung carcinoma cell line (tdTomato); cancer-associated fibroblasts (GFP) and a monocytic cell line (THP-1) (Cell tracker) in alginate capsules [5]. We observed phenotypic changes over time as well as how myeloid cells infiltrate into the tumour spheroids and display an immunosuppressive phenotype typical of tumour-associated macrophages.

Ref
[1] Gualda EJ,et al. Nat Methods 10 (2013)
[2] Gualda EJ,et al. Front Cell Neurosci 8 (2014)
[3] Gualda EJ,et al. Biomed Opt Express 6 (2015)
[4] Estrada MF,et al. Biomaterials 78 (2016)
[5] Rebelo SP,et al. Biomaterials 163 (2018)

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Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **12** Type : **Short Talk**

Molecular mapping of developmental disease progression using HiTS-FAST

Wednesday, 15 August 2018 12:30 (15)

Three-dimensional molecular mapping of RNA expression within intact biological tissue is allowing for new insight into the relationship structure and function. Current RNA quantification approaches are depth-limited to less than 200 μm by the requirement for single-molecule read out of sequential barcoded RNA fluorescence in-situ hybridization (RNA-FISH) or hydrogel embedded in-situ RNA sequencing. An alternative approach to single-molecule readout is to sacrifice intracellular localization by using amplified RNA-FISH to detect RNA expression on a per cell basis. This strategy has the potential to extend three-dimensional molecular mapping of RNA expression to cubic millimeters or centimeters of tissue. Building on our previous work on autofocusing, inertia-free light sheet fluorescence microscopy for cleared tissue, we designed a platform for rapid multiplexed molecular interrogation of cleared tissue samples. The High Throughput Scalable Fluorescence Assay for Spatial Transcriptomics (HiTS-FAST) platform combines closed-loop feedback to maintain co-planar alignment of the exciting light sheet and optical detection plane in thick samples, programmable fluidics for sample clearing and sequential multiplex labeling, and third generation single-molecule hairpin chain reaction (smHCR v3.0) to reliably label RNA expression millimeters deep in cleared tissue samples. I will present our hardware and software implementation to maintain co-planarity, automated sample handling, and present initial results from multiplexed labeling of both RNA and protein in healthy and developmentally disrupted whole rat lungs.

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Session Classification : Miscellaneous applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **13** Type : **Short Talk**

The mesoSPIM initiative – open-source light-sheet microscopes for imaging in cleared tissue

Monday, 13 August 2018 10:00 (15)

Tissue clearing methods have recently seen a renaissance with a wide variety of clearing approaches now available. In neuroscience, the combination of tissue clearing with light-sheet microscopy is ideal to bridge scales from the μm to cm-level, thus providing a link on the mesoscale for detailed 3D anatomical investigations. To optimally image cleared samples, we set out to design a modular light-sheet microscope that combines extremely simple sample mounting and exchange with large field-of-views (FOV) of 2-22 mm to provide users with overview datasets within minutes. Especially for such large FOVs, common light-sheet microscopes suffer from non-uniform axial resolution due to the varying thickness of the light-sheet. To circumvent this problem, we are using tuneable lenses to shift the excitation beam waist through the sample in synchrony with the rolling shutter of the camera. For whole mouse brains, typical datasets are isotropic (5 μm sampling), small (12-16 GB), and generated quickly (7-8 minutes). Together with standardized quick-exchange sample holders, these features allow fast screening of samples for clearing, imaging, and labelling quality and thus speed up data acquisition considerably.
After creating overview datasets, users can zoom in and acquire high-resolution data.
The microscope has been tested and validated in combination with common clearing methods ranging from hydrogel-based techniques such as CLARITY to organic solvent approaches such as iDISCO – by using a modular design of the imaging chambers, switching between different imaging media can be done in less than a minute. Recently, we have realized four such microscopes at various institutions across Switzerland as part of the mesoSPIM initiative (mesospim.org) – a project aimed at creating a community to accelerate the exchange of tissue clearing and mesoscale imaging expertise. Microscope hard- and software are open-source and we welcome suggestions for improvements.

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Session Classification : Light sheet hardware 1

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **14** Type : **Poster**

In vivo localization of annexin 1 during post-embryonic Arabidopsis development

ANNEXIN 1 (ANN1) is the most abundant member of the evolutionary conserved multigene protein superfamily of annexins. Annexins participate in diverse cellular processes, such as cell growth, differentiation, vesicle trafficking and stress responses. Moreover, they can associate with cytoskeleton and membrane phospholipids in a calcium dependent manner. Expression of annexins is developmentally regulated and it is sensitive to the external environment. ANN1 is expressed in almost all Arabidopsis tissues, while it is the most abundant in the root, hypocotyl epidermal cells and in the root hairs. Study of developmental expression patterns and subcellular localization of ANN1 could help to clarify its role during early stages of Arabidopsis seedling development. Using modern microscopy techniques, including spinning disc microscopy and advanced light-sheet fluorescence microscopy (LSFM), we followed developmental expression and subcellular localization of ANN1-GFP under natural conditions. By contrast to conventional microscopy, LSFM allows long-term imaging of plants in near-environmental conditions without affecting plant viability. Overall, live imaging of tissue-specific and developmentally regulated localization of ANN1-GFP in young Arabidopsis root showed accumulation of ANN1 in the root cap and epidermal cells. However, ANN1-GFP was absent in the root meristematic zone. During root hair development, ANN1-GFP accumulated in emerging tips of root hairs, which was accompanied by decreased abundance in trichoblasts. In aerial plant parts ANN1-GFP was localized mainly in epidermal cells of hypocotyls, leaves and petioles.

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Session Classification : Posters

Contribution ID : **15** Type : **Short Talk**

Brainwide optical circuit interrogation guided by online analysis of neuronal function

Tuesday, 14 August 2018 12:00 (15)

Understanding the brain requires measuring and perturbing neuronal activity. Tools for this are typically applied locally, but behavior is generated by the coordinated activity of neurons widely distributed across the brain. Thus, ideally we want to measure activity patterns of all neurons in the brain during behavior, use this information to decide which neurons to perturb, and record the brainwide effects of the perturbation.

We introduce an experimental and computational system that enables such experiments at the brainwide scale. In behaving larval zebrafish, we measure neuronal activity in the entire brain during behavior using light-sheet imaging. Concurrently, through fast distributed computational analysis, we generate whole-brain functional maps relating neuronal activity to stimuli/behavior. Any subset of neurons can be selected from the maps and then optically ablated with a two-photon laser. The resulting changes in whole-brain activity and behavior are subsequently analyzed, all in the same animal.

We apply this method to brainwide neuronal responses during visually-evoked swimming and find that a widely distributed set of nuclei mediate the behavior. Deleting specific functional neuron types from any of these nuclei has profound effects on brainwide responses consistent with a distributed implementation of the sensorimotor transformation.

We extend the method to cellular-resolution targeted optogenetic activation during whole-brain imaging. These methods allow for concurrent whole-brain activity and causality mapping in the same animal, which will enable delineating the contributions of neurons to brainwide circuit dynamics and behavior.

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Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **16** Type : **Poster**

Characterization of the distribution and dynamics of the antigen-presenting cells using light sheet miscroscopy

The aim of our project is to depict the dynamics of mouse antigen presenting cells (APCs) in a mammary gland during the ontogenesis and breastfeeding period. Additionally, the theory postulating transport of bacteria from the small intestine to the mammary gland will be tested.
Recent studies show that a subset of bacteria in milk could be transported by dendritic cells (DCs) from the maternal small intestine. This fact is in agreement with our observations of massive imigration of immune cells to the mammary gland at the beginning of the breastfeeding period. To understand the mechanism, immune cell populations will be precisely phenotyped in the maternal mammary gland and small intestine, with focus on changes during the breastfeeding period. The phenotypic and functional connection between the mammary gland and the small intestine will be explored using flow cytometry and immunodetection in situ. Our goal is to characterize complex cellular interaction in histological context using fluorescent techniques compatible with 3D reconstruction and quantification tools, ideally in the whole organ context. Light sheet microscopy is in this view optimal tool for our research.
The role of the interaction with bacteria and adaptive immunity in APC tissue localization will be investigated using appropriate mouse models. We routinely use MHC II-EGFP knock-in mouse model, which allows us to direct visualization of professional APC.

Differences in a gut microbiota composition are linked to pathological conditions. Understanding the mechanism of the transmission of bacteria from the maternal intestine to the infant digestive system via mammary gland may change the perception of differences in the fitness of breastfeeded non-breastfeeded youth. Exploration of this phenomenon using combination of mouse models could be translated in the human therapeutics.

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Session Classification : Posters

Contribution ID : **17** Type : **Poster**

Combining Deep Learning and Active Contours Opens The Way to Robust, Automated Analysis of Brain Cytoarchitectonics.

Deep learning has thoroughly changed the field of image analysis yielding impressive results whenever enough annotated data can be gathered. While partial annotation can be very fast, manual segmentation of 3D biological structures is tedious and error-prone. Additionally, high-level shape concepts such as topology or boundary smoothness are hard if not impossible to encode in Feedforward Neural Networks. Here we present a modular strategy for the accurate segmentation of neural cell bodies from light-sheet microscopy combining mixed-scale convolutional neural networks and topology-preserving geometric deformable models. We show that the network can be trained efficiently from simple cell centroid annotations, and that the final segmentation provides accurate cell detection and smooth segmentations that do not introduce further cell splitting or merging.

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Session Classification : Posters

Contribution ID : **18** Type : **Poster**

MASH: a method for scalable cytoarchitectonic characterization of large optically cleared human neocortex samples in 3D

With the introduction of optical clearing in neuroscience, considerable advances in tissue clearing and large volume microscopy have been made1-4. However, volume imaging and cytoarchitectonic characterization of large human brain samples, scalable in terms of time and cost to cover a significant portion of a cortical area, has so far remained challenging. This is especially true for adult formalin-fixed tissue. We recently reported on MASH (Multiscale Architectonic Staining of Human cortex)5: a scalable nuclear and cytoplasmic labelling and optical clearing approach suitable for 5 mm thick archival, adult human cortex samples. Here we show results of MASH processed brain tissue from the level of visual areas down to the single cell. We also present an economic solution to further scale up this approach for robust and rapid histological processing of an entire human occipital lobe. To this end we build a custom-made cutting device to acquire consistent 5 mm thick coronal slices of an agarose-embedded occipital lobe. Clearing and labelling could be robustly performed in a glass jar with Teflon spacing elements under constant stirring. This is an important step for mapping and cytoarchitectural characterization of entire sub-systems of the human brain in 3D.

References:

1 Liebmann, T. et al. (2016) Three-Dimensional Study of Alzheimer's Disease Hallmarks Using the iDISCO Clearing Method.
2 Murakami, T. C. et al. (2018) A three-dimensional single-cell-resolution whole-brain atlas using CUBIC-X expansion microscopy and tissue clearing.
3 Renier, N. et al. (2016) Mapping of Brain Activity by Automated Volume Analysis of Immediate Early Genes.
4 Ye, L. et al. (2016) Wiring and Molecular Features of Prefrontal Ensembles Representing Distinct Experiences.
5 Hildebrand, S., Schueth, A., Herrler, A., Galuske, R. & Roebroeck, A. Scalable cytoarchitectonic characterization of large intact human neocortex samples. bioRxiv (2018).

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Session Classification : Posters

Contribution ID : **19** Type : **Poster**

Light-Sheet Microscopy: studying zebrafish microtubules during embryonic development

The microtubule network is an essential part of the cell, providing structure and shape. It is also important for intracellular transport of cargos, which is crucial for correct embryo morphogenesis. Microtubules are dynamic structures that undergo continual assembly and disassembly within the cell.
In particular, yolk microtubule organization undergoes several changes over the various developmental stages in zebrafish. Nowadays, its arrangement in the yolk is still poorly understood since the problem has been undertaken only partially, in time and space. The primary approach has been immunostaining on fixed samples, hiding the rich variety of phenotypes over time [1]. Recently, the use of transgenic lines and multiphoton and confocal microscopy allowed the dynamical study of those processes, although only in restricted areas and with several side effects [2].
Light-Sheet Fluorescence Microscopy (LSFM) offers uniques capabilities for the live imaging, such as low photodamage, fast acquisition rate, and the possibility to reconstruct high quality images of whole organisms. The aim of our project is to obtain a mesoscopic 3D view of the microtubule skeleton dynamics of the zebrafish yolk throughout all epiboly stages using a home-made implementation of LSFM, so called Flexi-SPIM. Our system allows, on a single microscope, to operate in different modalities. Samples can be either embedded in agarose blocks to perform multi-view time-lapse movies of the whole embryo, or be transported through FEP tubes [3], increasing the imaging throughput.
Different zebrafish transgenic lines are investigated in order to unveil the microtubule dynamics. We observed a high variability between embryos, showing various phenotypes during epiboly stages. In order to establish statistical value to our findings, we exploited the unique high-throughput capabilities of our system by imaging tens of samples, in a straightforward manner, through a semi-automated fluidic sample loading system.

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Session Classification : Posters

Contribution ID : **20** Type : **Short Talk**

Multi-sample SPIM image acquisition, processing and analysis of embryonic zebrafish vasculature

Tuesday, 14 August 2018 10:15 (15)

To quantitatively understand biological processes that occur over long time periods, it is desirable to image multiple samples simultaneously, and automatically process and analyze the resulting datasets. Here, we present a comprehensive and dedicated multi-sample image acquisition and processing workflow using selective plane illumination microscopy (SPIM) to image several embryos up to 4 days and demonstrate its value for understanding the formation of embryonic zebrafish vasculature.

To process and analyze the large amount of data generated, we designed customized, automated and parallelized image processing tools in Fiji and FunImageJ. With a novel approach of vascular segmentation, a precise quantification of the vascular network's growth over the first days of development was obtained. Further analysis of the imagery data revealed that parts of the vasculature showed different degrees of symmetry and variation. Moreover, analysis of calcium signaling suggested that variation on a macroscopic level was already established on a signaling level.

Our multi-sample imaging pipeline further paves the way for many other quantitative long-term imaging studies such as xenotransplantation experiments or small-scale screens. It advocates a holistic approach based on multi-sample imaging using SPIM with integrated data processing and analysis to reveal and understand biological processes that occur over long time periods.

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Session Classification : Sample preparation, clearing and expansion

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **21** Type : **Poster**

A Two-Color Scanned Light-Sheet Microscope for Expanded Mouse Brain Sections

In Expansion Microscopy (ExM) a sample with fluorophores linked to a swellable gel is expanded homogeneously by a factor of approx. 4 [1]. This leads to a virtual optical resolution of up to 60 nm laterally and 250 nm axially. Applied to mouse brain samples this allows for resolution of neuronal network details on length scales of 100 nm, which are normally below the diffraction limit of optical microscopes. Combining ExM and Light-Sheet Fluorescence Microscopy (LSFM) results in an imaging technique, which features low phototoxicity, high frame rates and super resolution. Compared to super resolution point-scanning confocal microscopes the data acquisition time can be reduced by a factor of 20 [2].
Here, we constructed a scanned LSFM specifically for Expansion LSFM. The instrument features a water-dipping, high numerical aperture, long working distance objective lens, simultaneous two-color detection and a confocal data acquisition mode [3]. The instrument was devised to investigate expanded gel samples with dimensions of up to 20x20x2.5 mm^3. The fragile gels require a gentle sample handling, which was considered for the design of the sample holder. For fast image acquisition in two colors a simultaneous detection with the GEMINI-2C from Hamamatsu was installed. Combined with two sCMOS cameras in rolling shutter mode, a confocal image acquisition is feasible. An automated image acquisition with frame rates up to 40 Hz is implemented, which will reduce the imaging duration for a 1 mm^3 sample (before expansion) from currently 110 to only 5 hours.
The instrument will be used to analyze the 3D structure of extended, dense and sparsely labeled neuronal networks in subregions of mouse brains in super-resolution.

[1] Chozinsky et al., "Expansion microscopy with conventional antibodies and fluorescent proteins", Nat. Methods 13(6), 2016
[2] Bürgers et al., submitted
[3] Baumgart et al., "Scanned light sheet microscope with confocal slit detection", Opt. Expr. 20(18), 2012

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Session Classification : Posters

Contribution ID : **22** Type : **Short Talk**

Fast nearly isotropic imaging of large samples with light sheets beyond the diffraction limit

Monday, 13 August 2018 12:00 (15)

We were able to generate extremely long thin sheets of light in the one micron range and a vastly increased Rayleigh length by breaking the diffraction limit of light sheets of low numerical aperture. We measured the thickness of the light sheets with different methods including standard point spread function measurement with fluorescent beads. By using these light sheets in our ultramicroscope fast 3D imaging of whole mouse brains with objectives with a large field of view was possible. Due to the extremely low divergence of the light sheets mouse brains could be reconstructed from a single stack of optical sections with nearly isotropic resolution. The light sheets used were essentially non-Gaussian generated by new optics we developed. Compared to a Gaussian light sheet of the same NA our new light sheet is much thinner. Thus the diffraction limit which holds also for low NA Gaussian light sheets was significantly surpassed. These optics will allow the application of ultramicroscopy to ever increasing samples beyond the whole mouse brain range including human cancers.
Besides mouse brains we imaged also cleared whole adult drosophilae. We were able to get good transparency for all developmental stages of the insect from larvae to adult animals with fully preserved GFP signal. We showed that also dualview imaging of cleared adult drosophilae is possible and and allows easy isotropic resolution also with standard light sheet microscopy for such kind of specimens.
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Session Classification : Light sheet hardware 2

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **23** Type : **Poster**

Light Sheet Fluorescence Expansion Microscopy: Fast Mapping of Neuronal Connectivity at Super Resolution

Understanding the architecture of neural circuits is an important but formidable task. Critical details of neuronal connectivity - the synapses - occur on length scales of about 100 nm. Thus, imaging techniques reaching optical super resolution are required. However, neurites extend over distances of millimeters and centimeters, thus optical sectioning, a large field of view and a high imaging speed is required to cope with these complex 3D structures in an acceptable amount of time. Here we combined tissue expansion and light sheet fluorescence microscopy to allow volumetric of large mouse brain samples. These two methods are an ideal match to obtain super-resolved images of extended neuronal circuits with three distinctive features, namely high imaging rates of up to 40 Hz, high contrast and low photobleaching. We demonstrate the capabilities of our method by comparing it with high resolution confocal laser scanning microscopy using an Airyscan detector to obtain detailed high-resolution images of extended neuronal networks from the hippocampal mouse dentate gyrus (DG). The Airyscan approach delivers high-resolution images featuring a lateral and axial resolution of 120 and 400 nm, respectively, for green fluorescence, but with limited contrast and a prohibitively low frame rate of 0.1 Hz, considering the necessity to image the complete DG region of ~1mm^3. Using light sheet fluorescence expansion microscopy we achieved a virtual lateral and axial optical resolution of 75 and 450 nm, respectively, thus performing fast volumetric super resolution imaging of mouse dentate gyrus. Our approach allows us to observe autofluorescent proteins, thus avoiding antibody staining. In combination with Rabies virus staining, specific cell types and selected connections between neurons may be studied. In this manner neural connections can be mapped throughout axially extended brain sections allowing a better segmentation of DG granule cell neurites for further morphology analysis.

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Session Classification : Posters

Contribution ID : **24** Type : **Poster**

Multi-purpose SLM-light-sheet microscope

A classical Selective Plane Illumination Microscope (single light-sheet, generated using a cylindrical lens) suffers from a number of issues, such as shadow artefacts, scattered out-of-focus background and limited FoV (Field of View). A variety of advanced techniques in light-sheet microscopy have been proposed to tackle these issues, and previous publications have shown how image quality can be improved by rejecting out of focus light (structured illumination and pencil beam scanning), reducing shadow effects (light-sheet pivoting), or increasing the effective FoV by moving the focus of the light-sheet across the imaging FoV (tiling).

We have developed a SLM-SPIM system which is able to perform all the above-mentioned techniques. To obtain such a versatile system, we integrated a phase-only Spatial Light Modulator (SLM) into the illumination arm of a classical SPIM. We will present our system and discuss how the SLM, placed in a Fourier plane, allows to modulate the microscope's light-sheet in an easy and programmable manner. We will illustrate the results obtained performing different imaging techniques on samples of fluorescent beads, Zebrafish (*Danio rerio*) embryos, and optically cleared whole mouse brain samples.

With its simple design and the use of a computer-reconfigurable SLM, we believe our system represents an ideal platform for manipulating the illuminating light-sheet to apply a range of advanced imaging techniques on a single microscope, and also to explore combinations of multiple techniques and potentially trial new ones. The modular nature of our system also offers the possibility to choose between three slightly different setups, which result in different light-sheets, in thickness and height, and in a different conjugation of the SLM with the sample plane. We will discuss the consequences of the different conjugations and how the setup can be selected according to the characteristic of the sample and the imaging technique to be performed.

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Session Classification : Posters

Contribution ID : **25** Type : **Poster**

How thick is your light sheet? A guide to quantitative measures

Several novel Light Sheet Fluorescence Microscopy techniques have emerged in recent years in pursue of good axial resolution over a long field of view. These include Bessel, Lattice and Airy beam light-sheet microscopy, among others. There has not been a direct comparison in the literature of their dimensions, and often different criteria are used among publications. Most of them present complex geometries that, in addition, are rapidly changing along the propagation direction, which makes the measurement troublesome. Here we propose a unified criterion to measure the length and thickness for all the currently available variants of light sheet. We have applied this methodology to our results of numerical simulations of the propagation of beams with the geometries stated above. Considering gaussian beams as the basis, we offer quantitative measurements and a direct comparison of the beams considered. With this we aim at providing a guideline for the scientific community on the choice of the most appropriate technique for their specific application.

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Session Classification : Posters

Contribution ID : **26** Type : **Poster**

Multi-view and multi-sample light-sheet imaging from a microscopy facility point of view

The Francis Crick Institute is a young, large and ambitious biomedical research centre that aims to understand the biology underpinning human health. The Crick Advanced Light Microscopy facility (CALM) supports basic and advanced light microscopy with the institute. We recently acquired a Luxendo-Bruker MuVi light-sheet microscope in order to support research in many different fields, including developmental biology, stem cells, infections, cancer, immunology, neuroscience. This microscope is characterized by a double illumination and detection system and is ideal for imaging small living samples. However, due to the large variety of sample (and users) that we have to deal with (early embryos, organoids, small fixed organs, etc.) optimization of imaging settings can be challenging and not always possible. Here we describe the strategy we adopted for helping scientists to best exploit the potentiality of the MuVi SPIM and we discuss the limits and benefits of this commercial microscope. First of all, introduction to light-sheet theory and microscope training strongly benefited from intense visual supports and 3D models of both Gaussian beam and biological samples. Then, specimen preparation has been optimized using different glass capillaries, loading systems and FEP tubes-based chambers for imaging fragile, cleared or Matrigel-embedded objects. Specimen imaging has been improved using an additional small digital microscope and comparing different beam positions or sample orientations. Data processing has been optimized using a dedicated computer connected to the institute server by InfiniBand and easily accessible by desktop remote control. We are currently optimizing the image fusion process and the photo-stimulation module. Many of these expedients can help to better perform light-sheet imaging with the MuVi SPIM in a microscopy facility that is daily challenged by a wide range of different samples and biological questions.

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Session Classification : Posters

Contribution ID : **27** Type : **Poster**

Post-embryonic imaging of zebrafish semicircular canal morphogenesis

The vertebrate inner ear contains three orthogonally arranged semicircular canals that function to detect angular accelerations (turning movements of the head). Each canal comprises a curved duct with a swelling (ampulla) at the base that houses sensory hair cells. We are using light-sheet fluorescence imaging of transgenic zebrafish to examine formation of the semicircular canals from embryonic to adult stages. This technique is particularly well suited for this purpose as it allows cellular details to be resolved in relatively deep-lying tissue (>100 μm) within a large whole-mount specimen (1-10 mm). We are using 3D renderings of our data to visualise the changes that occur during the post-embryonic period. Additionally, we are making morphometric measurements of the tissue, including canal duct length, lumen diameter and angle between the canal ducts. We are using these measurements to generate an atlas of wild-type semicircular canal development as a reference point for comparison with mutant phenotypes.

Our imaging is revealing detail that has not previously been observed using paint-fill or histological techniques, to our knowledge. All three canal ducts have a distinct morphology from the time of their appearance at 3 days post fertilisation. The anterior and posterior semicircular canals form as ducts first and enlargement to form their ampullae happens at later stages. Conversely, in the lateral (horizontal) canal, the presumptive ampulla is already evident as a swelling around the lateral crista at 3dpf, and the lateral canal duct elongates during post-embryonic stages. These differences between the lateral canal and the other two canals correlate with different genetic requirements: for example, the lateral canal is uniquely dependent on the conserved function of otx1b. We have also observed other anatomical features including a notable seam along the length of each canal and a thickening of the epithelium at the sides of the ampulla in adult inner ears.

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Session Classification : Posters

Contribution ID : **28** Type : **Poster**

Hyperspectral, multimodal light microscopy

Complex developmental processes cannot be captured in single-color recordings. More data can be extracted from a single sample using multiple labels and contrasts. Implementing several modalities in one instrument provides comprehensive information from a single specimen without the need for registration and synchronization.
Selective Plane Illumination Microscopy (SPIM [1]) and Optical Projection Tomography (OPT [2]) are highly suited for 3D imaging of embryonic development and provide complementary optical contrasts: one fluorescence, the other transmission. We demonstrate three examples how SPIM and OPT were combined and extended to collect more data from a single sample:
1) OPT requires large depths of field in contrast to high NAs desirable for fluorescence microscopy [3]. We implemented a spiral acquisition OPT to enhance depth of field on any standard SPIM setup. The 3D transmission data provides morphological context to the sparse fluorescence [4].
2) We added a descanned detection and an imaging spectrograph to a scanned light sheet microscope to acquire spectrally resolved fluorescence data. Up to five overlapping fluorophores and autofluorescence were distinguished in living zebrafish and fruit fly embryos [5].
3) We equipped an OPT setup with supercontinuum source and AOTF to sweep through the wavelengths used for transmission. We found strong evidence that OPT image formation in living zebrafish embryos is governed by refraction, whereas absorption and scattering play minor roles.
By harvesting spectral information with two modalities we have extended the capabilities of a single instrument. Ideally all modalities will benefit from each other when combined. If care is taken that imaging speed matches sample development, detailed insights into the dynamic processes are obtained.

[1] Huisken, J et al, Science 2004
[2] Sharpe, J et al, Science 2002
[3] Mayer, J et al, Opt. Lett. 2014
[4] Bassi, A et al, Development 2015
[5] Jahr, W et al, Nat. Commun. 2015

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Session Classification : Posters

Contribution ID : **29** Type : **Poster**

Enhancing in vivo-imaging in medaka by objectively improving pigmentation, anesthesia and fluorescent proteins

Medaka (Oryzias latipes) is amenable to *in vivo*-imaging by light-sheet microscopy due to its comparably slow development, its very large available toolbox and mostly transparent embryos. Its lifelong growth allows extensive studies of stem cells. However, following these stem cells and their descendants by *in vivo*-imaging is very challenging and imaging conditions needed optimization.
In order to improve these imaging conditions we addressed the largest challenges for *in vivo*-imaging in medaka: retinal and peritoneal pigmentation, anesthesia and the choice of fluorescent protein. We addressed the latter two by a medium-throughput screen using 96-well plates and time-lapse imaging in an Acquifer plate imaging machine.
The retinal and peritoneal pigmentation was largely reduced by introducing a double knockout of *Oca2* and *Pnp4a* using the CRISPR/Cas9-system. This double knockout abolishes retinal pigment epithelium and iridophore pigments completely.
Anesthesia was improved by substituting the commonly used MS222 with injections of α-Bungarotoxin mRNA, as suggested by similar experiments in zebrafish.
Fluorescent proteins were scored in green and red. The results indicate that eGFP and mCherry are the best conservative choices, if you are considering *in vivo*-fluorescence and the available toolsets such as antibodies or nanobodies. However, mVenusNB and mGFPmut2 showed a higher fluorescence intensity *in vivo* compared to eGFP and can be used depending on the used analysis methods.
During this study we improved imaging conditions in medaka dramatically and are thus able to perform light-sheet microscopy of the developing retina, brain, gills and inner organs, without the use of toxic chemicals. This is achieved by using a combination of pigment double knockout fish, α-Bungarotoxin mRNA anesthesia and suitable fluorescent proteins.

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Session Classification : Posters

Contribution ID : **30** Type : **Short Talk**

Hybrid Optically-Gated Light Sheet Microscopy Allows Long-term Timelapse Imaging in the Developing Zebrafish Heart

Tuesday, 14 August 2018 12:30 (15)

Although the low-impact nature of light sheet microscopy has opened up new avenues for developmental timelapse imaging, the heart remains a particularly challenging organ to image in 3D timelapse. To image processes on timescales of minutes to hours (such as heart development, cell migration, repair and regeneration) demands some form of synchronized image acquisition in order to separate the high-frequency heartbeat motion from the lower-speed morphological changes of interest. Although current postacquisition synchronization methods are attractive for imaging the beat process, or for acquiring small numbers of timepoints, the accumulated light dose precludes longer-term timelapse imaging. Indeed, we will show that this rapidly induces catastrophic photobleaching, phototoxicity and heart arrhythmia.

We have previously developed prospective optically-gated light sheet microscopy, to allow synchronised 3D imaging of the in vivo beating zebrafish heart with a laser dose no higher than required for imaging static tissue. However, sustained timelapse imaging over 24h or more presents significant additional challenges, since the dramatic morphological changes undergone by the heart frustrate existing synchronization approaches. We will describe how we have been able to overcome this barrier by using hybrid prospective-retrospective optical gating technologies, and present 24h 3D-timelapse video imaging of cardiac development and immune response to cardiac injury.

Just as light sheet microscopy minimizes the distribution of the light dose to the specimen in the spatial domain, our approach offers the same gain in the time domain. Our work opens up the unperturbed, beating heart to direct timelapse imaging studies that have until now been restricted to stationary organs. Our new approach also points the way towards integrated light-sheet microscopy studies of the developmental coupling between heart structure, fluid flow and electrical activity.

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Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **31** Type : **Poster**

Analysing 3D cell dynamics in the developing zebrafish inner ear

The labyrinthine structure of the vertebrate inner ear is vital for the perception of gravity, and linear and angular acceleration, to help control balance. The formation of this complex organ involves dynamic changes in cell shape and movement in the otic epithelia during embryo development. Optical sectioning microscopy, in particular light-sheet fluorescence microscopy coupled with the generation of transgenic lines, have made it possible to follow these developmental processes in the relatively transparent zebrafish (*Danio rerio*) embryo [1]. Analysing the large volumes of multi-dimensional data acquired through light-sheet microscopy has, however, been a significant challenge [2]. Additionally, while software solutions have been developed for semi-automated 3D segmentation and obtaining volumetric data from such images [3,4], tools for analysing cell shape have continued to be limited to 2D image information [5]. We present an image analysis pipeline for fully automated 3D cell segmentation using 3D-UNet (a Deep Learning Convolutional Neural Network) [6], and measurement of 3D cell shape and membrane dynamics, including apicobasal asymmetry (without labelling for cell polarity descriptors), in the zebrafish otic epithelium. Such 3D analysis of cell shape during epithelial folding and remodelling is a critical step forward in understanding organ formation events in embryogenesis across study organisms.

1. Alsina, B. & Whitfield, T. T. *Semin. Cell Dev. Biol.* **65**, 47–59 (2017).
2. Reynaud, E. G., *et al. Nat Meth* **12**, 30–34 (2015).
3. Mosaliganti, K. R., *et al. PLoS Comput. Biol.* **8**, e1002780 (2012).
4. Stegmaier, J., *et al. Dev. Cell* **36**, 225–40 (2016).
5. Farrell, D. L., *et al. Development* **144**, 1725–1734 (2017).
6. Ronneberger, O., *et al.* in *Lecture Notes in Computer Science (including subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)* **9351**, 234–241 (Springer, Cham, 2015).

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Session Classification : Posters

Contribution ID : **32** Type : **Poster**

4D in vivo blood flow mapping using SPIM-μPIV in the zebrafish heart

Light sheet microscopy is an ideal tool for precision mapping of flow fields on a microscopic scale, due to its optical similarities with particle image velocimetry, which on a macroscopic scale is a mature technique in fields such as aerospace engineering. Several challenges exist that mean it is difficult to obtain high-quality 3D velocity data in a living sample, but we will show how optical gating allows us to enhance the measurement quality dramatically, by applying statistically rigourous correlation-averaging techniques on the periodic flow within the beating heart.

We show how our approach enables high-precision mapping of blood flow throughout the heartbeat, and quantification of actual pumped volumes in the presence of flow regurgitation. We explore the repeatability of the flow across heartbeats, as well as the distinction between blood plasma flow and transport of the red blood cells themselves. Finally, we present preliminary results showing recovery of the out-of-plane velocity component, permitting full 3-dimension, 3-component velocity mapping throughout the heartbeat.

Our novel results illustrate the applicability of SPIM-μPIV as a reliable, truly non-invasive in vivo microfluidics imaging modality, with no need for micro-injection of fluorescent beads. We propose this will enable researchers to merge quantitative flow and structure information in 4D as an input and validation for computational models of heart mechanics.

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Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **33** Type : **Short Talk**

High content 3-D light sheet microscopy in 96 and 384-well plate formats applied to study cancer cell size, and invasiveness and morphology in 3D matrices

Tuesday, 14 August 2018 18:15 (15)

Conventional light sheet fluorescence microscopy (LSFM) requires two microscope objective lenses orientated at 90° to one another. However, their proximity to one another and the sample makes high content imaging of samples mounted on conventional 96 and 384-well plates difficult. Oblique plane microscopy (OPM)1 uses a single high numerical aperture microscope objective to provide both fluorescence excitation and detection whilst maintaining the advantages of LSFM.

We present the development and application of a stage scanning OPM (ssOPM)2 approach for high content light sheet fluorescence imaging in commercially available glass and plastic-bottomed 96 and 384-well plates. 3D images of cells were acquired by scanning the sample through the tilted light sheet at a constant velocity. Methods for implementing autofocus during acquisition together with the data acquisition pipeline will be discussed.

The ssOPM system was used to perform functional screens for regulators of cell size and 3D invasiveness. In the screen for regulators of size, melanoma cells were grown as 2D cultures in 384-well plates and genes were systematically knocked down with a library of 120 siRNAs. For 3D invasion assays, 9 siRNAs were used and plates were incubated for 24 hours allowing cells to invade vertically into the gel prior to fixation and staining. For both assays, 100s-1000s cells were quantified per condition to allow 3D cytometric data analysis.

We developed a MATLAB 3D image analysis pipeline for automated segmentation and morphological quantification of the image data. This allowed determination of cell size in 2D and 3D, measurement of cell invasiveness into the collagen matrix, and quantification of cell morphology of invading cells. The ssOPM approach will enable a better understanding of which genes are responsible for cancer cell size determination and invasion in 3D cultures.

1 Dunsby, C. Opt. Express 16.25 (2008): 20306-20316; 2 Maioli, V., et al. Sci Rep 6:37777 (2016).

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Session Classification : Cell biology applications of light sheet microcopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **34** Type : **Poster**

Using HPC as a Service for Remote Parallel Processing on the Fiji Platform

Tackling current biomedical challenges calls for in-depth understanding of biological systems, particularly their structures, functions, and interactions on both the molecular and the cellular level. Biological imaging constitutes an important field of scientific investigation and one of its most valuable techniques is fluorescence microscopy. State-of-the-art imaging devices, such as light sheet microscopes, produce datasets so large that they can only be effectively analyzed by employing methods of image processing on high-performance computing (HPC) clusters. To address this issue, an HPC plugin for Fiji, one of the most popular open-source software tools for image processing, has been developed. The plugin enables end users to make use of HPC clusters to analyze large scale image data remotely and via the standard Fiji user interface. Seamless interaction between the remote HPC infrastructure and the user is substantially facilitated by the HPC as a Service Middleware. To demonstrate the performance of the plugin, it has been benchmarked on a Snakemake pipeline, performing complex registration and fusion tasks on sizable Selective Plane Illumination Microscopy (SPIM) time-lapse in toto recordings of developing embryos. The presented plugin offers a graphical user interface which allows the user to smoothly define pipeline job parameters, start execution, monitor progress, download results, and debug errors of the SPIM image processing pipeline. The presented framework will form a foundation for parallel deployment of any Fiji/ImageJ2 command on a remote HPC resource, greatly facilitating big data analysis.

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Session Classification : Posters

Contribution ID : **35** Type : **Poster**

Implementation of a commercial Lattice Light Sheet Microscope (LLSM) in an Imaging Facility (PICT-IBiSA)

Lattice Light Sheet Microscope (LLSM) represents the novel generation of 3D fluorescence microscopes dedicated to live single-cell analysis. LLSM[1] uses ultrathin light sheets derived from 2D optical lattices. These are scanned plane-by-plane through the specimen to generate a 3D image. The thinness of the sheet leads to high axial resolution and negligible photobleaching and background outside of the focal plane. By dithering the lattice to create a uniform light sheet, single cells can be imaged in 3D, often at sub-second intervals, from hundreds to thousands of time points at the diffraction limit (300 x 300 x 700 nm, 40 to 100 planes per second per cell). Photo-bleaching and photo-toxicity are typically reduced by one order of magnitude relative to that seen with a 1D scanned Bessel beam or spinning disk confocal microscopy. This allows 3D images to be captured over longer periods of time, and enables the study of signaling, transport, and stochastic self-assembly in complex environments with single molecule sensitivity. However, facing the amount of information provided by LLSM, cutting-edge image processing algorithms need to be investigated, at a time regime compatible with live cell imaging.
LLSM is commercialized by Intelligent Imaging Innovations (3i). However, as it is, the commercial version of LLSM, shows a number of drawbacks deserving a strict metrology control and alignment protocols. As early adopters of the 3i LLSM project, this is one of our aim to determine how this sophisticated system could be useful at the level of a large Imaging facility. Another goal of our project consists to optimize the LLSM for different biological live samples in 3D. In a second part of the project, it is planned to develop different software to help the reconstruction, visualization and analysis of data produced in the microscope.
[1] B.-C. Chen et al., Science 346, 1257998 (2014).

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Session Classification : Posters

Contribution ID : **36** Type : **Short Talk**

Interplay between cell size and cell polarity

Tuesday, 14 August 2018 18:00 (15)

The PAR network polarizes a broad range of cell types by localizing proteins to opposing membrane domains. Despite its abundance, we know almost nothing about how the PAR proteins adapt to this vast diversity of cell sizes and shapes. In many systems, maintenance of polarity has been described as a reaction-diffusion network of the proteins involved.

Here, by first using theoretical modelling, we show that these reaction diffusion systems break below a certain cell size, resulting in a uniform, unpolarized membrane distribution. This predicts that cells below a size threshold should be unable to maintain polarity *in vivo*. The precise nature of this threshold depends on parameters such as membrane diffusion and turnover.

Next, by combining light sheet microscopy-based 3D reconstruction of the plasma membrane with single molecule measurements of key biophysical parameters, we have revealed this size limit *in vivo*, in a developmental lineage of the *C. elegans* embryo. These findings are in remarkable quantitative agreement with our theoretical predictions.

Thus, intrinsic properties of polarity proteins impose physical limits on the ability of cells to polarize, pointing to an unappreciated link between the size of a cell and its ability to polarize and establish cell fate.

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Session Classification : Cell biology applications of light sheet microcopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **37** Type : **Poster**

Three-dimensional imaging and uptake of anticancer drugs in multicellular spheroids by light sheet fluorescence microscopy

Multicellular spheroids (MCS) are increasingly being used as tissue models by converting two-dimensional cell monolayers into three-dimensional cultures to mimic the physiology and functions of living tissues. Such natural cellular networks created through cell-cell contacts together with cell signalling enriched environments could be used in tumour biology for morphology and drug screening applications. MCS models are widely used in cancer research where cell signalling pathways, as for example the mammalian Target of Rapamycin (mTOR) pathway, responsible for the regulation of cell growth and proliferation, can be elucidated. Currently, some tumours have developed resistance to first generation mTOR inhibitors. AZD2014, a second generation mTOR inhibitor, is undergoing active clinical trials, but its mechanism of action within live cell environment is unknown. We report the study of the uptake of AZD2014 in Human Embyronic Kidney 293 (HEK293) MCS utilising the natural fluorescence of the drug by Light Sheet Fluorescence Microscopy (LSFM). LSFM was chosen for its capability of monitoring large volumes at high speed, as well as for its superior detection of fluorescence from MCS and lower phototoxicity compared to conventional microscopy. HEK293 MCS were cultured and transferred to agar wells in petri dishes. Different doses of AZD2014 were administrated while the MCS was imaged in real-time with a 10X/0.3 NA objective under excitation with a 405 nm laser. Z-stacks of 250 μm thickness were recorded every 15 s for at least 90 min. The uptake rate was determined for different depths inside the MCS and compared to that from monolayers. Volumetric changes difficult to observe in 2D cell cultures were also characterized. We observed an increase of 25% in the MCS size upon drug administration. Comparison with other drugs, including Combretastatin, was also performed. Altogether, this study highlights the significance of the combined use of MCS and LSFM for drug discovery.

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Session Classification : Posters

Contribution ID : **38** Type : **Poster**

Automated Cell Counting and Region-specific Mapping in Whole Mouse Brain Light Microscopy Data

ariadne.ai offers tailored solutions for the analysis of increasingly demanding high-throughput light and electron microscopy datasets by combining the state-of-the-art convolutional neural networks with the experience of our professional image annotator team. Fed with high quality ground truth, our novel machine-learning tool for the automated segmentation of somata analyzes whole mouse-brain imaging data. In a second step, all detected somata were mapped onto distinct brain regions in correspondence to a neuroanatomical reference atlas.
In one of our test datasets, we automatically counted more than 1.8 million cfos-expressing neurons in a single brain, distributed across many brain regions. Quality scoring yielded an F1-score of greater than 0.97 and a false merger rate of less than 5% for both low- and high-density regions. In contrast to that, more than 500 hours of manual work would have been necessary to achieve comparable results, even if performed by highly motivated and skilled professionals at an annotation rate of one soma per second.
Here, we present our workflow, detailed accuracy measurements and 3D reconstructions of the analyzed data.

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Session Classification : Posters

Contribution ID : **39** Type : **Poster**

Automated Cell Counting and Region-specific Mapping in Whole Mouse Brain Light Microscopy Data

With increasingly demanding microscopy techniques, also the handling of data amounts and high-throughput analysis becomes more and more challenging. Utilizing latest convolutional neural networks and the experience of our professional annotator team, we join forces of artificial and human intelligence to turn these challenges into scientific results.
For the automatic segmentation of somata in whole mouse-brain imaging data, we developed a novel deep-learning workflow. With this method, we drastically reduce manual effort necessary for large-scale image analysis. Moreover, we propose a high-throughput method for the assignment of individual cell bodies to distinct brain regions referring to the Allen Reference Atlas. Based on this, we facilitate detailed and accurate measurements as well as 3D reconstructions of the whole organ.
Here, we present our image analysis pipeline as well as detailed results of the corresponding accuracy measurements. We further demonstrate the application of the soma-mapping plugin in the open-source software KNOSSOS.

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Session Classification : Posters

Contribution ID : **40** Type : **Poster**

LIGHT SHEET FLUORESCENCE MICROSCOPY FOR HIGH RESOLUTION WHOLE-BRAIN 3D MAPPING

Rabies virus-based retrograde tracing is a powerful approach for visualizing synaptically connected neurons. Combined with a refined tissue clearing technology [1], this approach enables e.g. the visualization of transplanted neurons and synaptically connected host cells in whole-mouse brain preparations [2]. In order to visualize and 3D reconstruct such a transplant connectome we optimized a light sheet fluorescence microscope (LSFM) for high resolution imaging of complete cleared mouse brains.

The instrument features two long working distance, refractive index corrected objective lenses suitable for organic clearing solutions such as BABB. The objectives have magnifications of 4.44 and 12, and numerical apertures of 0.3 and 0.53, respectively. Due to the two-sided Gaussian beam illumination of the sample the image quality in both brain hemispheres is identical. A pivoting of the scanned light sheets significantly reduces shadow artefacts. An autofocus routine based on Vollath's F4-function is used to optimize illumination and detection planes. We used submicrometer fluorescent beads embedded in epoxy resin to characterize the experimental point spread function and compared the achievable optical resolution to that realized in mouse brain samples. Finally, we compared the effect of Bessel and Gaussian beams for imaging in strongly scattering samples. Bessel beam illumination yielded a clearly superior contrast in such samples.

[1] M. K. Schwarz, A. Scherbarth, R. Sprengel, J. Engelhardt, P. Theer, G. Giese (2015) Fluorescent-Protein Stabilization and High-Resolution Imaging of Cleared, Intact Mouse Brains. PlosOne, DOI:10.1371

[2] J. Doerr, M. K. Schwarz, D. Wiedermann, A. Leinhaas, A. Jakobs, F. Schloen, I. Schwarz, M. Diedenhofen, N. C. Braun, P. Koch, D. A. Peterson, U. Kubitscheck, M. Hoehn, and O. Brüstle (2017). Whole-brain 3D mapping of human neural transplant innervation. Nat. Comm. 8, 19 January 2017

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Session Classification : Posters

Contribution ID : **41** Type : **Poster**

High resolution 3D imaging using tiling light sheet microscopy

To understand the structure and function of a living matter, scientists rely on microscopic imaging methods. The behavior and interactions between cells in the developing embryos happen in a three dimensional environment. Hence, we need a microscope to record data in three dimensions. Light sheet microscopy is a novel technique which has the capability to acquire a 3D images with high resolution and high imaging speed. The main purpose of the light sheet microscope is the investigation of large biological samples. For this purpose, the imaging field of view should be large enough to cover the whole sample. On the other hand, a large field of view requires an illumination beam with large beam waist, which causes a weak optical sectioning and low axial resolution. Hence, the trade-off between the field of view and axial resolution is remained a challenge. Here we implemented a new method which is called tiling method to acquire a 3D image of Drosophila embryo as a large sample with high spatial resolution in all directions. Instead of trying to generate a light sheet with thick beam waist, a thinner illumination beam which has smaller field of view was tiled in several positions to compensation the field of view. Hence, in each plane were imaged several times, related to the number of tiles. Then the final imaged can be easily achieved by stitching the tiles. With this method, we are able to record the whole functional 3D image from a multicellular organism with cellular resolution.

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Session Classification : Posters

Contribution ID : **42** Type : **Poster**

Long-term light sheet imaging reveals kinesin light chain function in zebrafish axonal development

Precise regulation of cargo trafficking and axonal transport is critical for neuronal development and function. Cargos are delivered to specific locations through the activity of diverse kinesins and their cargo-linking adaptor proteins. However, the roles of these kinesins and adaptor proteins as well as the mechanisms of cargo localization during neuronal morphogenesis remain poorly understood. To elucidate the specialized functions of kinesin light chain (KLC), a subunit of kinesin-1, we developed an assay to determine effects of KLC manipulation using transgenic zebrafish (Tg:ngn1-GFP-caax) with fluorescently labeled sensory neurons. Image stacks are acquired on a home-built mSPIM system. Preliminary image analysis reveal visible developmental defects in the axonal projections of Rohon-Beard sensory neurons in embryos treated with Kinesore, a KLC-binding drug. A significant difference in the axonal projection density as well as branching angles during development can be observed. Moreover, Kinesore treated embryos also exhibit posterior lateral line pathfinding and fasiculation defects. We are currently developing a custom image analysis workflow to perform phenotyping on the fly. We aim to incorporate the workflow into an upright V-SPIM system based on the Flamingo framework to enable mid-throughput screening for up to 25 samples at a time. The platform will be used to screen KLC null mutants for defects in sensory neuron development.

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Session Classification : Posters

Contribution ID : **43** Type : **Short Talk**

Imaging axial emergence though self-organization in embryonic organoids.

Wednesday, 15 August 2018 10:15 (15)

Emergence of multicellular forms (tissues, organs and organisms) from cells through changes in their shape, size, number and organization is central to understanding the process of morphogenesis. While molecular players are known, we do not know how the activity of genes and proteins is translated into 3D structures in space and time. Preexisting spatial cues, species-specific geometry and extraembryonic signaling centers, confound studying these processes *in vivo*. We have recently shown that 3D cell aggregates from different species (mouse embryonic stem cells and zebrafish blastula cells, which we term *gastruloids* and *pescoids* respectively) generate spatial asymmetries in gene expression and cell behavior within otherwise equivalent groups of cells, to develop a global coordinate system (body axes) *de novo*. Combining light-sheet imaging with germ-layer specific labelling of cells we are now gaining some insights into the spatio-temporal precision and species-independent manner, with which such 3D embryonic cell aggregates generate the major body axes even in the absence of any embryonic information. Using these embryonic organoids, as a minimal alternate system, sufficient to generate embryonic axes, we aim to understand early development in embryos as an emergent phenomenon of the self-organization of pluripotent cells.

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Session Classification : Developmental biology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **44** Type : **Poster**

Adaptive light-sheet microscopy for the systematic analysis of mitotic spindle scaling in vivo

Cells need to regulate the size and shape of their cellular structures for proper functioning. One classic example of such processes is the scaling of the mitotic spindle during early zebrafish embryogenesis. The mechanisms of how these spindles scale, however, are still unknown partly due to the lack of quantitative measurements of spindle dynamics. During early zebrafish development, the mitotic spindle only appears for three minutes out of the twenty minutes of cell cycle. Quantifying this short-lived event in an intact living embryo requires flexible and adaptive multi-resolution imaging, which is impossible with any state-of-the-art microscope. Here, we present a new type of light-sheet microscope, which autonomously adapts its detection scheme to the sample state. This microscope includes adaptable magnification to map the development of the millimeter-sized embryo and measure single-molecule dynamics of all individual spindles from the whole embryo in a single experiment. To automatically adapt the detection scheme, we have trained a Convolution Neural Network to detect the cell cycle state of individual cells from acquired fluorescence images. Using this new type of light-sheet microscope, we are studying the mitotic spindle scaling in freely developing zebrafish embryos. We will map the length, size, orientation, microtubule architecture and dynamics of all mitotic spindles in millimeter-sized zebrafish embryos with the necessary spatial and temporal resolution to solve the question of spindle scaling *in vivo*.

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Session Classification : Posters

Contribution ID : **45** Type : **Short Talk**

Dual illumination inverted light sheet microscope for long term live imaging

Monday, 13 August 2018 09:45 (15)

Organoid cultures have been recently established to study organ formation and tissue morphogenesis. However, imaging of these samples has been hampered by their long and often inefficient development and their light sensitivity. Light sheet microscopy would be the imaging technique of choice due to its low photo-toxicity and high acquisition speed. However, many current light sheet microscopes suffer from complicated sample mounting that also limits sample survival (e.g. mounting in FEP tube or agarose embedding) and lack of multi-position imaging. To overcome these limitations we have built an inverted light sheet microscope system with two illumination objectives, one high NA imaging objective and a beam scanning and alignment module. The sample is easily accessible and completely isolated from immersion medium and multiple samples can be imaged in parallel. Using this microscope we were able to acquire a continuous five-day long time-lapse capturing the formation of fully grown intestinal organoid starting from a single stem cell embedded in a matrigel. By imaging the stem cell marker (LGR5-GFP) we could follow for the first time the dynamics of stem cells during a complete intestinal organoid development at single cell resolution. Moreover, we demonstrated the versatility of this microscope by imaging different organoid models and the development of several living samples across different scales (e.g. zebrafish, mouse embryo and *C. elegans*).

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Session Classification : Light sheet hardware 1

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **46** Type : **Short Talk**

Towards next generation imaging pipelines using the Adaptive Particle Representation.

Monday, 13 August 2018 17:45 (15)

The Adaptive Particle Representation (APR) is an alternative image representation to pixel images for realizing the next generation of imaging pipelines using light-sheet fluorescence microscopy. The APR addresses computational, memory, and storage bottlenecks by adapting the image resolution to the local image content. Unlike standard image compression, the adaptive computational and memory benefits of the APR can be used for all processing and visualization tasks, without returning to the original full pixel image. Here we will present recent developments and extensions for the APR including GPU pipeline and processing acceleration, adaptation through time, and block-wise APR transforms for large images. Also, we will discuss current software support including, Python and Java wrappers for the C++ LibAPR library, and integration with Fiji and BigDataViewer.

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Session Classification : Image analysis of light sheet data

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **47** Type : **Short Talk**

Single- and multi-photon shaped illumination for light-sheet fluorescence microscopy

Monday, 13 August 2018 10:15 (15)

The use of exotic optical modes is becoming increasingly widespread in microscopy. Particularly, propagation-invariant beams, such as Airy and Bessel beams and optical lattices, have been particularly useful in light-sheet fluorescence microscopy (LSFM) as they enable high-resolution imaging over a large field-of-view (FOV), possess a resistance to the deleterious effects of specimen induced light scattering, and can potentially reduce photo-toxicity (e.g. [1]).

Although these propagation-invariant beams can resist the effects of light scattering to some degree, and there has been some interest in adaptive-optical methods to correct for beam aberrations when they cannot, scattering and absorption of the illuminating light-sheet limit the penetration of LSFM into tissues and results in non-uniform intensity across the FOV.

A new degree of control over the intensity evolution of propagation-invariant beams can overcome beam losses across the FOV, restoring uniform illumination intensity and therefore image quality. This concept is compatible with all types of propagation-invariant beams and is characterised in the context of light-sheet image quality [2].

Another property to control is the wavelength of light used. Optical transmission through tissue is greatly improved at longer wavelengths into the near-infrared due to reduced Rayleigh scattering and two-photon excitation has proved beneficial for imaging at greater depth in LSFM. Three-photon excitation has already been demonstrated as a powerful tool to increase tissue penetration in deep brain confocal microscopy, and when combined with beam shaping can also be a powerful illumination strategy for LSFM [3].

Recent progress in shaping optical fields for LSFM will be presented.

[1] T. Vettenburg et al, Nat. Methods 11, 541-544 (2014), doi:10.1038/nmeth.2922
[2] J. Nylk et al, Sci. Adv. 4, eaar4817 (2018), doi:10.1126/sciadv.aar4817
[3] A. Escobet-Montalbán et al, bioRxiv 323790 (2018), doi: 10.1101/323790

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Session Classification : Light sheet hardware 1

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **48** Type : **Poster**

Tissue clearing and imaging using CLARITY and Ce3D

Until recently, imaging deep into intact organs using fluorescence microscopy posed a significant challenge. Imaging depth for conventional one-photon excitation microscopy is limited to a few tens of microns due to appreciable light scattering and absorbance in dense, turbid tissue. This limited penetration depth is not significantly improved even with the use of genetically encoded fluorescent reporters or the incorporation of molecular labeling methodologies of targets expressed throughout the tissue. The recent advent of tissue clearing methods has enabled optical transparency of whole intact organs. Utilizing high-speed light-sheet microscopy on these cleared samples provides full, volumetric visualization with cellular resolution.

Here, we present a combination of two clearing techniques, namely CLARITY and Ce3D, for imaging a diverse set of intact tissues. We first exploit the active removal of lipids out of the sample using an externally applied electric field, and then clear the sample passively using Ce3D chemistry. This results in a more optically transparent tissue, allowing for deeper 3-D imaging using a commercially available light-sheet microscope. To this end, we have successfully applied this technique to image several intact adult mouse organs, such as the brain, heart, kidney and liver. We also present the image-processing pipeline which, includes the generation of large seamless image datasets, preliminary analyses, and image visualizations.

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Session Classification : Posters

Contribution ID : **49** Type : **Poster**

Simulator of Benchmarking Image Datasets for Time-Lapse Lightsheet Microscopy

In fluorescence microscopy image analysis, cell segmentation and tracking algorithms are indispensable tools to, e.g. reconstruct lineages or for time-resolved analysis of cell characteristics or events. Although there are such algorithms in everyday use, most of them are not properly validated and their accuracy limits are not well understood. Provided testing data together with expected results (so called ground-truth annotations, GT) and suitable metrics would exist, much of the questions above could be addressed.

Lightsheet microscopy images come obviously without GT. Acquired datasets are displaying often time-lapse embryonic development in high-resolution, and easily reach 1 TB per one experiment. The number of displayed cells can be in thousands per single frame. In this setting, it is extremely difficult to manually annotate real datasets to obtain GT.

Here, I present the current status of my work on a generic simulator of many-cells biological systems. In particular, the simulator produces GT annotated, time-lapse image sequences with artificial yet realistically looking and developing populations of nuclei-stained (simulation) cells. The development includes division and mutual interaction of cells, and known motion patterns for respective embryos. The GT will be useful for benchmarking segmentation, tracking and multi-view registration algorithms. Free benchmarking datasets will be published as well as the simulator itself.

The recently published results of the Cell Tracking Challenge indicated a gap in all embryonic development datasets used in the challenge. The gap is both in the number of submitted segmentation and tracking algorithms, and in the overall scores achieved. The field, for now more the algorithm developers than algorithm users, could benefit from freely available challenging data with GT. The developers could examine their solutions to discover and analyze much easier where and why their algorithms are not performing well.

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Presenter(s) : ULMAN, Vladimir

Session Classification : Posters

Contribution ID : **50** Type : **Short Talk**

Conditional control of fluorescent protein degradation by an auxin-dependent nanobody

Wednesday, 15 August 2018 12:00 (15)

Most biological processes involve spatial-temporal changes in the concentration of proteins that ensure that the right protein acts at the right place at the right time. Due to its high temporal resolution and minimal photo bleaching light sheet microscopy is ideally suited to visualize such protein dynamics given that the protein of interest is labelled with a fluorescent probe. Indeed, GFP-traps and increasingly CRISPR/Cas9-mediated fluorescent knock in's exist in several experimental systems ranging from tissue cell culture to model organisms and thus are great resources for light sheet microscopy experiments. To understand complex biological systems, however, we do not only need to visualize the emergent behaviour of protein, cells or organisms, but also to have to ability to interrogate the system. Here, we present an auxin-dependent GFP-nanobody to regulate the levels of overexpressed and endogenous GFP-tagged proteins in a conditional and reversible manner. We demonstrate efficient and reversible inactivation of the anaphase promoting complex/cyclosome (APC/C) in human tissue cell culture and thus provide new means to study the functions of this essential ubiquitin E3 ligase. Further, utilizing light sheet imaging, we show that the auxin-dependent GFP-nanobody can be applied to zebrafish. Hence, in principle the auxin-dependent GFP-nanobody has the potential to make any existing GFP-line in this and other model organisms compatible with auxin-mediated protein degradation thus enabling advanced functional studies.

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Session Classification : Miscellaneous applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **51** Type : **Poster**

Inverted Lattice-Light Sheet Microscope for long-term imaging on embryonic development

Lattice light-sheet microscopy [1] provides fast volumetric images with subcellular resolution from cells to embryos. To expand its capacity to a wider range of biological applications, we implemented an inverted version of a lattice light-sheet microscope [2]. The inverted lattice light-sheet microscope adopts a two chamber-design, which separates the biological samples from the immersion liquid of the two objectives. The open-top design further allows for atmospheric control (N2, O2, CO2 and humidity) besides the temperature to support the long-time embryonic development over days, and suppress the photobleaching effect. Two different sample mounting schemes are available for iLLS: a) an adaptor to hold the coverslip with samples attached upside down and dipped into the isolation chamber, and b) a sample carrier made from a 50⊠m/25⊠m thick FEP folio carrying larger samples such as embryos or cultured cells. We demonstrate live images of Drosaphila embryos, mouse embryos and cultured cells. iLLS together with DNA-PAINT (point accumulation for imaging in nanoscale topography) also to super-resolve tubulin structure inside cultured cells is also shown.

[1] Science, vol.346, 1257998-1, 2014
[2] Nat. Meth., vol. 13, 139, 2016

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Session Classification : Posters

Contribution ID : **52** Type : **Short Talk**

A non-cell autonomous actin redistribution enables isotropic retinal growth

Tuesday, 14 August 2018 11:45 (15)

For many developing tissues, their shape is established early in development. In order to maintain this shape during subsequent growth, these tissues need to scale isotropically. The way by which cells inside tissues enable coordinated, isotropic tissue scaling is not understood, however, as most studies focused on changing, rather than maintaining tissue shapes during development. In this study, using light sheet fluorescence microscopy of both fixed and live samples, we follow tissue shape with cellular resolution in the zebrafish retinal neuroepithelium. This vertebrate neural progenitor tissue forms a smooth cup early in development and keeps its architecture as it grows. By combining 3D analysis and theory, we identify global cell elongation as a cellular mechanism to maintain retinal shape during growth. Timely cell height increase occurs concurrently with a non-cell autonomous actin redistribution, during which actin gets depleted from the lateral cell-cell interfaces. Blocking actin redistribution and cell height increase perturbs isotropic tissue scaling and we observe, using long light sheet timelapses, the emergence of the resulting disturbed, folded tissue shape. Taken together, from our whole-tissue imaging and analysis, we propose a model in which timely tissue-wide actin redistribution permits global cell elongation, which enables isotropic growth of the developing retinal neuroepithelium, a concept that could be applied to other systems.

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Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **53** Type : **Poster**

Evaluation of dopaminergic neurons using lightsheet microscopy

Evaluation of dopaminergic neurons using lightsheet microscopy

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Dopaminergic neurons in the midbrain play important roles in the brain function including learning and memory, reward and movement. Specially, the dopaminergic neurons in substantia nigra compose nigrostriatal tract that is a pathogenic neural circuit in Parkinson's disease (PD). In preclinical in vivo research, the precise quantitation of dopaminergic neurons in substantia nigra (SN) is essential to evaluate the defect in PD in vivo models as well as the effect of new treatment on it. However, the calculated number of neuronal fragments in the brain sections using manual counting would be different from the original number of neurons in the tissue. Thus, to make the most reasonable estimation of the total dopaminergic neuron number in SN, unbiased stereology method has been employed. The optical fractionator, one of the most common method to count dopaminergic neurons in SN, uses thick brain sections to estimate the total number of neurons from the number of neurons sampled. Since the sampling process usually takes significant time under the microscope, quenching would be a problem for the specimen labeled with fluorescence. Lightsheet microscopy is 100-1000 times faster than point-scanning methods and could minimize the modification of the original fluorescence signal. Stereological method using lightsheet microscopy would be beneficial for the quantification of dopaminergic neurons labeled with fluorescence

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Session Classification : Posters

Contribution ID : **54** Type : **Poster**

Genetic control of the cephalic furrow morphogenesis in Drosophila

The evolution of distinct head and trunk domains revolutionized animal morphology. Sophisticated anterior structures evolved to sense the environment while powerful posterior appendages propelled bilaterians to diversify and occupy every ecosystem on the planet. Pioneering work on developmental genetics revealed that the head and the trunk identities are specified by distinct regulatory landscapes early in embryogenesis. However, the developmental mechanisms that establish the boundary between such territories remain poorly understood. Here I investigate this key bilaterian trait using as a model the cephalic furrow – an understudied structure that demarcates the embryonic head/trunk boundary in the fly *Drosophila melanogaster*. The cephalic furrow is one of the first morphogenetic events during gastrulation. It begins when a single row of lateral cells undergo cell shortening forming a deep invagination in the embryonic epithelium. However, the specification of these initiator cells and the signaling cascade controlling the cephalic furrow morphogenesis remain unclear. To uncover potential regulators, I compiled a genome-wide list of candidate genes expressed at the cephalic furrow, and I am screening for genes that are disrupted in the only mutant strains known to affect the furrow, *buttonhead* and *even skipped*. To understand how the molecular patterning relates to the morphogenetic movements, I am using lightsheet microscopy to visualize the tissue and protein expression dynamics *in toto* between wild type and mutant embryos. This approach will reveal new patterning genes and their role in the cephalic furrow formation in *Drosophila*, bridging the gap between molecular patterning and morphogenesis, and help to uncover the developmental mechanisms that establish the embryonic head/trunk boundary, a key trait for bilaterian evolution.

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Session Classification : Posters

Contribution ID : **55** Type : **Poster**

Light Sheet Illumination can enhance the resolution of Light Field microscopy

Light sheet fluorescence microscopes have high spatial resolution but sequential scanning of the planes limits the maximum imaging speed that can be achieved. On the other hand, light field microscopes and other extended depth of field imaging methods have high temporal resolution but suffer from low spatial resolution. Here we show a method to dynamically increase the spatial resolution of a light field microscope using multiple light sheet illumination. This method essentially allows trading the temporal resolution of the light field for increased spatial resolution, with the possibility of making this tradeoff on the fly with software. This approach extends the versatility and the range of applicability of light field microscopy, without additional hardware changes, enabling fast adaptation of the microscope to the requirements of different biological experiments.

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Session Classification : Posters

Contribution ID : **56** Type : **Poster**

Whole-tissue phenotyping of clinical samples using light sheet microscopy

Intratumoral heterogeneity is a critical factor when diagnosing and treating patients with cancer. Marked differences in the genetic and epigenetic backgrounds of cancer cells have been revealed by advances in genome sequencing, yet little is known about the phenotypic landscape and the spatial distribution of intratumoral heterogeneity within solid tumours. Here, we developed a pipeline for three-dimensional light-sheet microscopy of whole formalin-fixed paraffin-embedded (FFPE) biopsy samples, which allow us to clear solid tumours and identify unique patterns of phenotypic heterogeneity at single-cell resolution. We also show that cleared FFPE samples can be re-embedded in paraffin after examination for future use, and that our tumour-phenotyping pipeline can determine tumour stage and stratify patient prognosis from clinical samples with higher accuracy than current diagnostic methods, thus facilitating the design of more efficient cancer therapies.

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Session Classification : Posters

Contribution ID : **57** Type : **Short Talk**

Mastodon – a large-scale track-editing framework for light sheet data

Tuesday, 14 August 2018 09:00 (15)

Light sheet microscopy allows live 3D imaging of entire developing embryos with high spatial and temporal resolution. Computational analysis of these recordings promises new insights in developmental biology. However, a single dataset often comprises many terabytes, which makes storage, processing, and visualization of the data a challenging problem. The open-source Fiji platform provides tools to address this challenge. In this talk, I will present Mastodon, a track-editing framework for cell tracking and lineage tracing in Fiji.

Large-scale automated tracking in biological datasets is a very active field of research. To support machine learning methods, editing tools are needed to facilitate curation, proof-reading, and the manual generation of ground truth data. To make such tools accessible to biologist researchers, they should be easy to obtain, learn, and use. Additionally they must be intuitively usable and remain responsive in the face of millions of tracked objects and terabytes of image data. To make them useful for researchers in automated tracking, they need to be open source, adaptable, and extensible. Mastodon is our effort to provide such a tool in Fiji.

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Session Classification : Sample preparation, clearing and expansion

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **58** Type : **Poster**

Interactive Analysis of Cell Tracks in Light Sheet Microscopy Images using EmbryoMiner

Light sheet microscopy imaging allows recording entire embryos in 3D and over time (3D+t) for many hours. Fluorescently labeled structures can be tracked automatically in these 3D+t images [1-4]. Analyzing the resulting cell migration trajectories can provide detailed insights in large-scale tissue reorganization and morphological changes in early developmental stages at the cellular level. With the open-source framework EmbryoMiner in-depth analyses and comparisons of entire embryos in unprecedented detail are possible [5]. Iteratively focusing on a region of interest within the embryo allows investigating and testing specific trajectory-based hypotheses. After a selection process, quantitative features can be computed from isolated trajectories. All steps can be interactively refined to cope with different data sets. A study with unexperienced users indicated that even complex analysis tasks can be done in a time range less than 5 minutes. In future works, deep learning approaches for 3D+t microscopy data will be applied in order to improve the segmentation accuracy and to yield more valid cell trajectories for the subsequent analysis with EmbryoMiner.

References:

1. Ulman et al., 2017, Nat. Meth., 14, 1141-1152
2. Bartschat et al., 2016, Bioinformatics, 32, 315-317
3. Stegmaier et al., 2016, Dev. Cell., 36, 225-240
4. Amat et al., 2014, Nat. Meth., 11, 951-958
5. Schott et al., 2018, PLoS Comput Biol, 14(4)

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Session Classification : Posters

Contribution ID : **59** Type : **Short Talk**

Cellular basis of limb morphogenesis: lessons from the crustacean Parhyale hawaiensis

Tuesday, 14 August 2018 09:15 (15)

During development coordinated cell behaviors orchestrate tissue and organ morphogenesis to suit the lifestyle of the organism. We have used here the crustacean *Parhyale hawaiensis* to study the cellular basis of limb development. Transgenic *Parhyale* embryos with fluorescently labeled nuclei were imaged at high spatiotemporal resolution using multi-view light-sheet fluorescence microscopy over several days of embryogenesis spanning appendage morphogenesis from early specification up to late differentiation stages. To be able to analyze the terabyte-sized data sets we used a new tool called *Massive Multi-view Tracker* (*MaMuT*) for cell tracking that enabled us to reconstruct the complete cell lineage of an outgrowing thoracic limb with single-cell resolution. The quantitative analyses about cell behaviors show that the limb primordium in *Parhyale* becomes subdivided from an early stage into anterior-posterior and dorsal-ventral compartments whose boundaries intersect at the distal tip of the growing limb. Limb bud formation is associated with the spatial modulation of cell proliferation, while limb elongation is also driven by the preferential orientation of division of epidermal cells along the proximal-distal axis of growth. Our findings validate the boundary model originally proposed by Hans Meinhardt. This model postulates that a secondary developmental field, i.e. the proximodistal axis of a limb that is specified during embryogenesis de novo relative to the main anteroposterior and dorsoventral body axes, and is initiated around the intersection of the anteroposterior and dorsoventral compartment boundaries.

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Session Classification : Sample preparation, clearing and expansion

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **60** Type : **Short Talk**

The Physics of Blastoderm Flow during Early Gastrulation of Tribolium castaneum

Wednesday, 15 August 2018 09:45 (15)

The early embryo of the red flour beetle, Tribolium castaneum, initially consists of a single-layered blastoderm covering the yolk uniformly that differentiates into an embryonic rudiment as well as extraembryonic amnion and serosa. The germband anlage forms inside the egg during gastrulation when the embryonic rudiment condenses and folds along the ventral midline; this process is accompanied by large-scale flow and expansion of the extraembryonic serosa which ultimately covers the entire surface of the egg, thus engulfing the growing embryo. The mechanical properties of these tissues and the forces governing these processes in Tribolium, as well as in other species, are poorly understood. Here, we present our findings on the dynamics of myosin in the early blastoderm of Tribolium using multiview lightsheet live imaging of transiently labeled wild type embryos. We quantitatively measure the global distribution of myosin throughout the flow phase and present a physical description that couples the contractile forces generated by myosin to the mechanical properties of the blastoderm. In particular, we describe the overall tissue as a thin, actively contractile, viscous bulk medium that exhibits friction with the vitelline membrane. This description accurately captures the large-scale deformation the tissue undergoes during the initial stages of gastrulation. Our findings lay a foundation for the physical description of gastrulation in Tribolium and will allow, in combination with the well-studied Drosophila paradigm, for the first time the comparative analysis of blastoderm tissue morphogenesis

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Session Classification : Developmental biology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **61** Type : **Poster**

Advanced Light sheet Imaging Center (ALICE) Development of a full-service imaging platform – from sample clarification to 3D VR visualization

Lightsheet microscopy is a fluorescence imaging technique that allows visualization of whole organs or small organisms while preserving their physical integrity i.e. without the need to slice them prior imaging. Although the principle of operation of this technology was developed more than 100 years ago, it is only in the last fifteen years that biologists have commonly started to use such microscopes. Since that time, this type of microscopy has become a standalone field of research that has never been as active as it is now. At the Wyss Center for Bio and Neuroengineering in Geneva, Switzerland, we have created an imaging center, available to everyone, which integrates a series of cutting edge and custom-tailored tools into a single working pipeline aimed at imaging whole organs at high temporal or spatial resolution. We then analyze the data in a Virtual Reality environment. The center includes a customized version of the COLM/SPED (originally designed by Prof. Raju Tomer - Columbia University) microscope for near diffraction-limited resolution imaging of large clarified samples (cm range). Recently we expanded the capabilities of lightsheet microscopy, setting up a large-scale imaging system: mesoSPIM (see poster from Fabian F. Voigt). This customized system enables whole brain imaging at cellular resolution, in a few minutes with no need for further stitching processes. Finally, we are developing innovative 3D exploration and analysis tools that will enable researchers to navigate and segment their own lightsheet data in a virtual reality environment. This complete pipeline offers to the researcher the possibility for large scale screening, high resolution imaging and data visualization and analysis.

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Session Classification : Posters

Contribution ID : **62** Type : **Poster**

in toto live imaging and cell lineage analysis of the spiralian development

Metazoans specify germ layers during early development in a process called gastrulation. Gastrulation involves massive cell movements during which the specified germ layers are divided into molecularly distinct domains, which later gives rise to diverse differentiated cell types. Gastrulation movements have been described at both the cellular and molecular levels in vertebrates and insects in considerable detail. Similar in-depth analysis in other animals is hampered by technical difficulties, both concerning imaging techniques and available molecular tools. This generates a noticeable gap of biological knowledge for larger branches of the metazoan phylogenetic tree, e.g. the spiralians, which constitute 1/3rd of the metazoan phyla (Zhang, Z (2013), Laumer et al., 2015). To address this issue, we have first recorded the embryological development of the spiralian ragworm *Platynereis dumerilii* at cellular resolution for more than one day. We have used SiMView microscopy for high-speed *in vivo* and *in toto* imaging (Tomer et al., 2012). Second, through a combination of semi-automated cell segmentation and tracking (TGMM, Amat et al., 2014) and manual correction by using CATMAID (Saalfeld et al., 2009, Schneider-Mizell et al., 2016), we have generated the entire cell lineages of a one-day old *Platynereis* embryo. Third, we have produced gene expression data for key developmental genes in the one-day old *Platynereis* embryo. Overall, this allows us to present an analysis of the cellular movements and behavior during gastrulation and the mapping of genes important for gastrulation and axis formation onto the cell lineages. This work presents the first whole-embryo analysis at cellular resolution of the gastrulation process in a spiralian. It offers a resource for analyzing molecular underpinning of cell behavior in wild-type and functionally perturbed spiralian embryos.

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Session Classification : Posters

Contribution ID : **63** Type : **Poster**

Imaging of 3D neuronal cell cultures in hydrogels under iSPIM with an electrically tunable lens

Three-dimensional in vitro neuronal networks could give better insights to the functioning of the brain, mimicking extracellular conditions more accurately than two-dimensional ones. Moreover, the development of genetically encoded calcium indicators together with fluorescence microscopy has facilitated the recording of neuronal activity as sharp calcium changes upon neuronal firing. Nonetheless, imaging such fast events, in three-dimensional cultures and at high resolution, requires instrumentation capable of acquiring wide fields of view along large depths and high frame rates.
In our study we image 3D neurons from rat primary cell cultures placed in hydrogels. We use an inverted SPIM configuration, allowing for the imaging of samples on standard petri dishes. In our iSPIM, the sample remains static while we scan the light sheet along it with a galvanometric mirror. By including an electrically tunable lens in the detection path of the microscope, we rapidly refocus on the illuminated section of the sample, avoiding the need to displace the detection objective and reducing vibrations on the sample. We present our observations of these three-dimensional cultures under our optical setup.

This research is part of MESOBRAIN. MESOBRAIN has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 713140.
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Session Classification : Posters

Contribution ID : **64** Type : **Poster**

Exploring the _scenery_ of LSFM with virtual reality

With the advent of lightsheet microscopes, and their unprecedented ability to generate 3D and 4D data of biological specimen with high spatiotemporal resolution, the question of how to interact with both the data produced, and the measuring instrument itself becomes more and more important.

In this talk, we present scenery, an open-source realtime 3D visualisation framework that can be used to quickly prototype visualisations or even build your own applications. We are going to detail some case studies in which we have used the framework, such as visualisation of Drosophila development or whole-organism vasculature development in VR headsets or even room-scale CAVE systems. Further, we will show ideas for future interaction with microscopes, such as virtual reality-based laser ablation.

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Presenter(s) : GUNTHER, Ulrik

Session Classification : Posters

Contribution ID : **65** Type : **Short Talk**

12 years with Light Sheet Microscopy in core imaging facilities on Dresden campus

Monday, 13 August 2018 12:30 (15)

Light Sheet Microscopy made great progress during last 15 years. We can now see its transition from the hands of developers to the hands of ''ordinary" scientists who work in the field of biomedical research. Core imaging facilities can, in our opinion, play great role in supporting such transition. In our talk we will share the lessons which we learned during last 12 years when we have been bringing light sheet microscopy technology to users of core imaging facilities on Dresden campus. We will reflect the perspective of core imaging facilities who provide open access to broad range of imaging technologies to hundreds of users per year.

Our light sheet story started in 2006, when the first Zeiss light sheet ''concept" system got installed in the the multi user environment of the core Light Microscopy Facility (LMF) of the Max Planck Institute of Molecular Cell Biology and Genetics (MPI-CBG) in Dresden (Germany). And then our journey continued with the first Z.1 system (Zeiss) which was installed in January 2013. This system was used around 9.500 hours during first two years of its operation. Facing the capacity limits of this system, we installed second Z.1.system in March 2015 (modified also for imaging of cleared samples). Total usage of both Z.1 systems from January 2015 till June 2018 reached circa 15 300 hours reflecting popularity of this technology among our users.

Two Z.1. setups at the MPI-CBG got complemented by the Ultramicroscope setup (LaVision Biotech Germany) which was installed at the core imaging facility of our partner institute on Dresden campus – Center of Molecular and Cellular Bioengineering (CMCB) in 2014. In 2017 the MPI-CBG established new Advanced Imaging Facility which provides users with an access to home-built lattice light sheet setup. All four light sheet setups are now available (together with other imaging technologies) via Biopolis Dresden Imaging Platform (www.biodip.de) to more then 600 users on Dresden campus and beyond.

Affiliation

Biopolis Dresden Imaging Platform, BioDIP Dresden, www.biodip.de

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Presenter(s) : Dr PEYCHL, Jan (Head, Light Microscopy Facility, MPI-CBG, Dresden, Germany)

Session Classification : Light sheet hardware 2

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **67** Type : **Short Talk**

BigStitcher: Reconstructing high-resolution image datasets of cleared and expanded samples

Monday, 13 August 2018 18:15 (15)

Continuous advancements in microscopy and sample preparation methods such as clearing or expansion allow for the investigation of ever larger samples at high resolution. This entails increasingly large datasets that may consist of hundreds of images of one sample that are not aligned, suffer from optical disturbances and often cannot even be opened as a whole, which can pose a serious bottleneck to scientific inquiries. With terabyte-sized datasets becoming more and more common, development of software tools that make handling and analysis of large and complex image data available to the broader scientific community is an urgent issue.

To allow efficient handling and reconstruction of large multi-tile and multi-view image data, we developed the BigStitcher software. It enables import from most file formats, interactive handling, fast and precise alignment, as well as deconvolution and real-time fusion of large image datasets. We additionally support the alignment of multi-tile acquisitions taken from different orientations, effectively doubling the size of objects that can be imaged. We also enable the correction of a variety of optical distortions, e.g. via automatic illumination selection, flat-field correction and interest-point-based correction of chromatic aberrations.

We implemented BigStitcher using the state-of-the-art frameworks ImgLib2 and BigDataViewer. By combining multi-resolution data representation and sub-pixel accurate registration algorithms, even very large datasets can be reconstructed on conventional, off-the-shelf hardware. In an effort to make large sample reconstruction available as a routine task, we provide a user-friendly graphical user interface (GUI) to manually guide the alignment and interactively display the intermediate results using BigDataViewer. BigStitcher is open-source and provided as a Fiji-plugin, making it a powerful, scalable tool for automated processing of very large image datasets.

Affiliation

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Presenter(s) : HÖRL, David (Department of Biology II, Ludwig-Maximilians-Universität München)

Session Classification : Image analysis of light sheet data

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **68** Type : **Short Talk**

Light propagation in retinal tissue and in silico

Monday, 13 August 2018 18:00 (15)

Light penetration and formation of image inside biological tissues is of physiological relevance in the eye and practical importance in the context of tissue and whole organism microscopy. We present here how the vertebrate retina, having a counter intuitive inverted structure deals with incoming light. We show by direct transmission measurements how the unique chromatin arrangement within photoreceptor nuclei impacts image quality at the level of the photoreceptors. The experimental findings are complemented by wave optical simulations of forward scattering. The simulations from the anatomically faithful tissue models successfully predicts the loss of image contrast due to the large angle scattering occurring in the tissue and also provides a physical and mechanistic understanding of the image formation process.

We further show, how these simulations can also be used to mimic the imaging process in tissue microscopy. Our open source software, biobeam [1] has the flexibility to implement multiple modalities ranging from laser scanning to light sheet fluorescence imaging. It can reproduce aberrations, distortions, adaptive optical effects and most intricate wave optical phenomena relevant to microscopy. With the multiplexed, GPU accelerated implementation of the in silico light propagation our software pushes the frontiers of computer model guided microcopy enabling highest resolution deep tissue imaging.

[1] Weigert, Martin, Kaushikaram Subramanian, Sebastian T. Bundschuh, Eugene W. Myers, and Moritz Kreysing. "Biobeam—Multiplexed wave-optical simulations of light-sheet microscopy." PLoS computational biology 14, no. 4 (2018): e1006079.

Affiliation

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Presenter(s) : SUBRAMANIAN, Kaushikaram

Session Classification : Image analysis of light sheet data

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **69** Type : **Poster**

Symmetric light-sheet microscope for subcellular imaging

A symmetric light-sheet microscope is presented, featuring two high numerical aperture objectives arranged in 120°. Both objectives are capable of illuminating the sample with a tilted light-sheet and detecting the fluorescence signal. This configuration allows for multi-view, isotropic imaging of delicate samples where rotation is not possible, while collecting more than twice as much light as conventional, perpendicular setups. The optical properties of the microscope are characterized, and its imaging capabilities are demonstrated on *Drosophila melanogaster* embryos and mouse zygotes.

Affiliation

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Presenter(s) : BALAZS, Balint (European Molecular Biology Laboratory, Cell Biology and Biophysics Unit)

Session Classification : Posters

Contribution ID : **70** Type : **Short Talk**

Focused-light-induced cytoplasmic streaming (FLUCS). A new paradigm to probe the physiology on intracellular transport.

Monday, 13 August 2018 12:15 (15)

Throughout the last decades, access to genetic perturbations, fluorescent labels and modern microscopy advanced our molecular understanding of cell-biological processes tremendously.

The spatio-temporal organization of cells and developing embryos that we observe under these microscopes is widely believed to depend on physical processes such as diffusion and motor-driven intracellular flows. Thus far, however, it remains a challenge to unravel physiology of these physical transport processes, which is due to the lack of suitable perturbation methods.

Here, we exploit thermoviscous expansion phenomena to optically induce hydrodynamic flow in single cells and developing embryos. By controlling such flows inside the cytoplasm of the C. elegans zygote, we reveal the causal implications of intracellular flows during PAR polarization. Specifically, we show i) that hydrodynamic flows inside the cytoplasm localize PAR-2 proteins at the posterior membrane. ii) Induced cortical flows transported membrane-bound PAR molecules and rotated the membrane polarization, leading to iii) the down-stream phenotype of an inverted body axis.

Furthermore, we utilize flow perturbations for probe-free active micro-rheology of the cytoplasm and within subcellular compartments. We conclude by emphasizing the opportunities and challenges of combining FLUCS with light-sheet-microscopy.

Mittasch et al., Nat Cell Biol 20 (2018)
Kruse, Chiaruttini, and Roux, Nat Cell Biol 20 (2018)

Affiliation

MPI-CBG

Terms and Conditions

Yes

Primary author(s) : KREYSING, Moritz

Presenter(s) : KREYSING, Moritz

Session Classification : Light sheet hardware 2

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **71** Type : **Poster**

Mechanisms driving symmetry-breaking in intestinal organoids

Symmetry-breaking events are fundamental biological processes for the formation of specialized tissues. In particular for intestinal organoids, symmetry-breaking is a paradoxical event: only a fraction of cells, part of a genetically identical population forming a cyst and immersed in unchanged medium, undergo differentiation. Although striking, the underlying mechanisms that drive symmetry-breaking are so far not known. In order to understand these mechanisms within a population of cells we need to be able to record with high spatiotemporal resolution the early growth of intestinal organoids as they form from single cells until around day 3, the usual time when symmetry-breaking has already occurred. This way we can extract important dynamical parameters such as cell distribution dynamics, synchronicity of cell divisions and cytoskeletal dynamics in order to answer the main causal relations needed for the appearance of the first differentiated cell. Such recordings are made possible by using a custom-built light-sheet microscope tailored for organoid imaging and developed in the lab. It not only allows the full recording of organoid formation with subcellular resolution but also gives potential to observe the tissues reaction to different perturbations in 3D over long periods of time, with the combination of e.g. a photoablation module. Furthermore, when combined with high content multivariate timecourse imaging, light-sheet microscopy allows the in-depth study of previously selected main mechanisms that might be driving symmetry-breaking in intestinal organoids.

Affiliation

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Presenter(s) : QUINTAS GLASNER DE MEDEIROS, Gustavo

Session Classification : Posters

Contribution ID : **73** Type : **Poster**

Deciphering the Cellular and Physical Mechanisms in Spiral Cleavage

Spiral cleavage is the ancestral developmental mode within the Spiralia. It covers a period of the early development characterized by asymmetric cell divisions with alternating division angles giving the embryo a spiral looking appearance. However, the mechanisms controlling spiral cleavage are poorly understood. To elucidate the molecular, cellular and physical mechanisms of spiral cleavage, we use the marine annelid Platynereis dumerilii as a model. We unravel the role of the cytoskeleton during spiral cleavage through mRNA injections of fluorescently labeled tubulin and histone into the zygote to label cytoplasmic elements. We imaged the live embryos with selective plane illumination microscopy, processed the data with Fiji softwares, and reconstructed this way early cell cleavages in Platynereis. Next, we measured dynamic cellular events such as inclination of the mitotic spindles, transportation of the nuclei within the cells, and membrane deformation during cell division. Furthermore, we monitored the cortical actomyosin dynamics through syn21-lifeact-mKate2 mRNA or protein injection. We described that actomyosin polarizes in the first two asymmetric divisions as well as toward the micromeres prior to each spiral cleavage of the macromeres. A counter chiral flow of the cortical actomyosin is observed in the macromeres during the first spiral cleavage. Moreover, cell orientation remains normal when the embryo develops in an eggshell-free status or when cells are dissociated. Drug treatments showed that membrane deformation and actomyosin dynamins were not interfered with exogenous colchicine or nocodazole. However, actin polymerization inhibition with latrunculin A severely disrupted spindle position, suggesting that the actomyosin plays critical roles in both establishing cell polarity and controlling division pattern. This study provides mechanistic insights into the spiralian development and a base to compare the degree of conservation among spiralians.

Affiliation

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Terms and Conditions

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Presenter(s) : HSIEH, Yu-Wen

Session Classification : Posters

Contribution ID : **74** Type : **Poster**

Refractive Index Matching for in vivo Light-Sheet Microscopy

The emergence of Selective Plane Illumination Microscopy (SPIM) about a decade ago enabled scientists to study the development of whole embryos (e.g. fruit fly, zebrafish, mouse) with unmatched spatiotemporal resolution and low phototoxicity. Yet, true in toto recordings of only few model organisms have been realized thus far. As any light microscopy technique, SPIM suffers from optical aberrations, light absorption and scattering – especially when imaging large samples, e.g. embryos. Our goal is to push further the current depth limitations in light-sheet microscopy and enable the study of in toto organogenesis and development in frequently used model organisms. To achieve this, it is crucial to minimize aberrations induced by the optical setup, the interface between sample and mounting medium, and the sample itself.
In this work, we present our results of refractive index tuning in light-sheet microscopy to minimize aberrations arising from the refractive index mismatch between sample and mounting medium. We show that by adjusting the refractive index in light-sheet microscopy one can reduce aberrations and restore image quality in deep tissue layers of mouse and Medaka embryos, as well as in the root tip of Arabidopsis. Further, we discuss our results of matching the refractive index to specimens with different optical properties as the fruit fly embryo.

Affiliation

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Presenter(s) : KROMM, Dimitri (European Molecular Biology Laboratory, Heidelberg, Germany)

Session Classification : Posters

Contribution ID : **75** Type : **Poster**

Light sheet fluorescence microscopy in plant developmental imaging

Plant growth and development is a complex process evolving through continuous qualitative and quantitative changes in four (x-, y-, z- and t-) dimensions. Classical microscopy methods pose some serious limitations for long-term live cell and developmental plant imaging. Out-of-focus fluorescence, phototoxicity, photobleaching, restricted temporal resolution and limitations in imaging depth are compromises of widefield epifluorescence, confocal laser scanning and spinning disk modalities. The recent emergence of mesoscopic imaging methods and especially of light sheet fluorescence microscopy (LSFM) provided a new tool for fast and long-term imaging of animal and plant development. Importantly, plants growing in culture medium are positioned vertically in the microscope. All these parameters favour LSFM for long-term developmental plant imaging at the subcellular, cellular, tissue, organ, and whole-organism levels in a natural orientation maintaining undisturbed plant growth in near-environmental conditions. Using currently developed protocols for preparation of living plants for long-term LSFM, plant imaging is possible at diverse scales, ranging from subcellular compartments up to whole seedlings. We will present examples from LSFM imaging of individual plant organs such as roots, hypocotyls and cotyledons, different tissues and cell types as well as observation and tracking of different subcellular organelles

This work was supported by grant no. LO1204 (Sustainable development of research in the Centre of the Region Haná) from the National Program of Sustainability I, Ministry of Education, Youth and Sports, Czech Republic.

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Session Classification : Posters

Contribution ID : **76** Type : **Invited Speaker**

Applications and extensions of SCAPE microscopy

Monday, 13 August 2018 09:15 (15)

Affiliation

Terms and Conditions

Primary author(s) : HILLMAN, Elizabeth (Columbia University)

Presenter(s) : HILLMAN, Elizabeth (Columbia University)

Session Classification : Light sheet hardware 1

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **77** Type : **Invited Speaker**

Light-sheet microscopy with subcellular resolution: technology and applications

Tuesday, 14 August 2018 17:30 (15)

Affiliation

Terms and Conditions

Primary author(s) : FIOLKA, Reto (UT Southwestern Medical Center)

Presenter(s) : FIOLKA, Reto (UT Southwestern Medical Center)

Session Classification : Cell biology applications of light sheet microcopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **78** Type : **Invited Speaker**

Imaging organoids in diSPIM

Tuesday, 14 August 2018 17:15 (15)

Affiliation

Terms and Conditions

Primary author(s) : CONRAD, Christian (DKFZ)

Presenter(s) : CONRAD, Christian (DKFZ)

Session Classification : Cell biology applications of light sheet microcopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **79** Type : **Invited Speaker**

Content-Aware Image Restoration and Quantitative Downstream Analysis

Monday, 13 August 2018 17:30 (15)

Affiliation

Terms and Conditions

Primary author(s) : JUG, Florian

Presenter(s) : JUG, Florian

Session Classification : Image analysis of light sheet data

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **80** Type : **Invited Speaker**

Photoreceptors catching the light(sheet): Fate, movements and lamination

Tuesday, 14 August 2018 11:30 (15)

Affiliation

Terms and Conditions

Primary author(s) : Dr NORDEN, Caren (Max Planck Institute of Molecular Cell Biology and Genetics)

Presenter(s) : Dr NORDEN, Caren (Max Planck Institute of Molecular Cell Biology and Genetics)

Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **81** Type : **Invited Speaker**

Light sheet microscopy in Nature Methods

Monday, 13 August 2018 11:45 (15)

Affiliation

Terms and Conditions

Primary author(s) : STRACK, Rita (Nature Methods)

Presenter(s) : STRACK, Rita (Nature Methods)

Session Classification : Light sheet hardware 2

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **82** Type : **Invited Speaker**

Attenuation Artifacts in LSFM Corrected by OPTiSPIM

Tuesday, 14 August 2018 10:00 (15)

Affiliation

Terms and Conditions

Primary author(s) : SWOGER, Jim (EMBL Barcelona)

Presenter(s) : SWOGER, Jim (EMBL Barcelona)

Session Classification : Sample preparation, clearing and expansion

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **83** Type : **Invited Speaker**

Whole-body/organ Cell Profiling by a Hydrophilic Tissue-clearing method, CUBIC

Tuesday, 14 August 2018 09:30 (15)

Affiliation

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Primary author(s) : UEDA, Hiroki (The University of Tokyo/RIKEN (BDR))

Presenter(s) : UEDA, Hiroki (The University of Tokyo/RIKEN (BDR))

Session Classification : Sample preparation, clearing and expansion

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **84** Type : **Invited Speaker**

In Vivo Cellular Dynamics imaged using Adaptive Optics Lattice Light Sheet Microscopy

Tuesday, 14 August 2018 17:00 (15)

Affiliation

Terms and Conditions

Primary author(s) : UPADHYAYULA, Srigokul (Harvard Medical School)

Presenter(s) : UPADHYAYULA, Srigokul (Harvard Medical School)

Session Classification : Cell biology applications of light sheet microcopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **85** Type : **Invited Speaker**

Root organogenesis is biased towards the availability of water

Wednesday, 15 August 2018 11:30 (15)

Affiliation

Terms and Conditions

Primary author(s) : VON WANGENHEIM, Daniel (University of Nottingham)

Presenter(s) : VON WANGENHEIM, Daniel (University of Nottingham)

Session Classification : Miscellaneous applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **86** Type : **Invited Speaker**

Resolution and applications of LSFM in the life sciences

Monday, 13 August 2018 09:00 (15)

Affiliation

Terms and Conditions

Primary author(s) : Prof. STELZER, Ernst

Presenter(s) : Prof. STELZER, Ernst

Session Classification : Light sheet hardware 1

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **87** Type : **Invited Speaker**

Adding Dimensions to Light Sheet Imaging

Monday, 13 August 2018 09:30 (15)

Affiliation

Terms and Conditions

Primary author(s) : Prof. FRASER, Scott

Presenter(s) : Prof. FRASER, Scott

Session Classification : Light sheet hardware 1

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **88** Type : **Invited Speaker**

Flamingos for you and me

Monday, 13 August 2018 11:15 (15)

Affiliation

Terms and Conditions

Primary author(s) : Dr HUISKEN, Jan (Morgridge Institute for Research)

Presenter(s) : Dr HUISKEN, Jan (Morgridge Institute for Research)

Session Classification : Light sheet hardware 2

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **89** Type : **Invited Speaker**

Lightwedge and Lightsheet-Raman Microscopy

Monday, 13 August 2018 11:30 (15)

Affiliation

Terms and Conditions

Primary author(s) : Prof. HEINTZMANN, Rainer

Presenter(s) : Prof. HEINTZMANN, Rainer

Session Classification : Light sheet hardware 2

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **90** Type : **Invited Speaker**

Different Rigs for Different Gigs

Monday, 13 August 2018 17:00 (15)

Affiliation

Terms and Conditions

Presenter(s) : Prof. MYERS, Eugene W.

Session Classification : Image analysis of light sheet data

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **91** Type : **Invited Speaker**

Bioimaging information: combining light sheet imaging with single cell transcriptomics

Monday, 13 August 2018 17:15 (15)

Affiliation

Terms and Conditions

Primary author(s) : Dr HUFNAGEL, Lars

Presenter(s) : Dr HUFNAGEL, Lars

Session Classification : Image analysis of light sheet data

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **92** Type : **Invited Speaker**

High resolution and quantitative super-resolution microscopy: from cells to tissue imaging

Tuesday, 14 August 2018 09:45 (15)

Affiliation

Terms and Conditions

Primary author(s) : ZANACCHI, Francesca Cella

Presenter(s) : ZANACCHI, Francesca Cella

Session Classification : Sample preparation, clearing and expansion

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **94** Type : **Invited Speaker**

Light sheet theta microscopy for quantitative imaging of large cleared samples.

Tuesday, 14 August 2018 11:15 (15)

Affiliation

Terms and Conditions

Primary author(s) : Dr TOMER, Raju (Department of Biological Sciences, Columbia University)

Presenter(s) : Dr TOMER, Raju (Department of Biological Sciences, Columbia University)

Session Classification : Neurobiology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **95** Type : **Invited Speaker**

3D+time imaging of developing embryos for the reconstruction of their cell lineage

Wednesday, 15 August 2018 09:00 (15)

Affiliation

Terms and Conditions

Primary author(s) : Dr PEYRIERAS, Nadine (BioEmergences, CNRS USR3695 Gif-sur-Yvette, France)

Presenter(s) : Dr PEYRIERAS, Nadine (BioEmergences, CNRS USR3695 Gif-sur-Yvette, France)

Session Classification : Developmental biology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **96** Type : **Invited Speaker**

Exploring a new contrast mechanism for light-sheet microscopy: fast harmonic generation imaging

Wednesday, 15 August 2018 09:15 (15)

Affiliation

Terms and Conditions

Primary author(s) : SUPATTO, Willy

Presenter(s) : SUPATTO, Willy

Session Classification : Developmental biology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **97** Type : **Invited Speaker**

Imaging and dynamic analysis of the post-implantation mouse embryo at the cellular level using light-sheet microscopy

Wednesday, 15 August 2018 09:30 (15)

Affiliation

Terms and Conditions

Primary author(s) : Dr MCDOLE, Katie

Presenter(s) : Dr MCDOLE, Katie

Session Classification : Developmental biology applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **98** Type : **Invited Speaker**

Imaging photosensitive reef-building corals

Wednesday, 15 August 2018 11:15 (15)

Affiliation

Terms and Conditions

Primary author(s) : Dr LAISSUE, Philippe

Presenter(s) : Dr LAISSUE, Philippe

Session Classification : Miscellaneous applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **99** Type : **Invited Speaker**

Macro LSFM with sTSLIM (scanning Thin-Sheet Laser Microscopy)

Wednesday, 15 August 2018 11:45 (15)

Affiliation

Terms and Conditions

Primary author(s) : SANTI, Peter (University of Minnesota)

Presenter(s) : SANTI, Peter (University of Minnesota)

Session Classification : Miscellaneous applications of light sheet microscopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **100** Type : **Invited Speaker**

Integrated development of a methodology for studying the spatio-temporal dynamic of the DNA damage response within Multi Cellular Tumor Spheroids

Tuesday, 14 August 2018 17:45 (15)

Affiliation

Terms and Conditions

Primary author(s) : LORENZO, Corinne

Presenter(s) : LORENZO, Corinne

Session Classification : Cell biology applications of light sheet microcopy

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **101** Type : **Short Talk**

Zeiss

Monday, 13 August 2018 14:00 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **102** Type : **Short Talk**

Intelligent Imaging Innovations GmbH: Imaging Life with 3i LightSheet Systems

Monday, 13 August 2018 14:50 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **103** Type : **Short Talk**

Acquifer: Large Image Data Solutions by Acquifer

Monday, 13 August 2018 14:05 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **104** Type : **Short Talk**

Applied Scientific Instrumentation: ASI makes DIY light sheet easy

Monday, 13 August 2018 14:10 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **105** Type : **Short Talk**

ANDOR/Bitplane: Quantitative 3D and 4D image analysis for Light Sheet Microscopy in Imaris

Monday, 13 August 2018 14:15 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **106** Type : **Short Talk**

Hamamatsu: Extended Focus Device

Monday, 13 August 2018 14:20 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **107** Type : **Short Talk**

LaVision BioTec: Cleared tissue imaging

Monday, 13 August 2018 14:25 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **108** Type : **Short Talk**

Leica: SP8 DLS – Leica‘s Lightsheet on a Confocal at a glance

Monday, 13 August 2018 14:30 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **109** Type : **Short Talk**

Luxendo: Luxendo Light-Sheet Microscopes: Seeing Life from a Different Angle

Monday, 13 August 2018 14:35 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **110** Type : **Short Talk**

PCO: To cool or not to cool? How cooling influences the performance of sCMOS-cameras

Monday, 13 August 2018 14:40 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy

Contribution ID : **111** Type : **Short Talk**

PhaseView: Alpha3, a new generation of light sheet microscope

Monday, 13 August 2018 14:45 (5)

Affiliation

Terms and Conditions

Session Classification : Techno bites

Track Classification : Light sheet fluorescence microscopy