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IMAGE-BASED SYSTEMS BIOLOGY

4th International Symposium

September 6-7, 2018

Welcome by the speaker of the research group applied systems biology at the HKI-Center for Systems Biology of Infection

Marc Thilo Figge

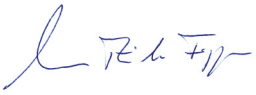
Dear participants of IbSB 2018,
dear scientists, dear colleagues,

It is my great pleasure to welcome you to the 4th International Symposium on Image-based Systems Biology in Jena, which is taking place biennially since the year 2012.

This year, our symposium is directly following the LIFE MEETS LIGHT conference, organized by the Leibniz ScienceCampus Infecto-Optics, with a focus on the development of novel optical technologies for life science applications. This is as well driving the field of Image-based Systems Biology, where we seek to take full advantage of the information in images in order to establish an essential connection between experimental and theoretical investigations of biological processes in space and time at a quantitative level.

The programme of IbSB 2018 covers five plenary lectures with excellent international speakers from different fields together with many talks from young engaged scientists as well as poster contributions. We hope to provide you with the great opportunity to have scientific discussions and to make steps into future collaborations that will push this field of research. In particular, IbSB 2018 will focus on quantitative bioimage analysis, *e.g.* supported by methods from machine learning, for dynamical systems at the tissue level, as well as at the level of cell populations and interactions between individual cells. To reflect the interdisciplinary nature of systems biology research, several presentations will have the format of *tandem talks*. These are given by two speakers, who are collaboration partners of their research topic, and presented in one talk from their respective perspectives.

The City of Light Jena provides the perfect interdisciplinary environment for an exchange of novel scientific methods and sharing recent achievements from image-driven research in biology. I wish all of us successful days in Jena.



Marc Thilo Figge

IBSB 2018 PROGRAM

Thursday, September 6th, 2018

12:00	**Registration opens**	
12:30	*Lunch*	
13:00	**Opening**	
Session I: Quantitative bioimage analysis		Session Chair: Anna Medyukhina
13:10	**Michael Unser,** EPFL Lausanne, Switzerland *Steerable detectors for bioimage analysis*	
13.55	**Pekka Ruusuvuori & Leena Latonen,** University of Tampere & Tampere University of Technology, Finland *3D reconstruction and quantitative analysis of histology for prostate cancer*	
14.25	**Ruman Gerst,** Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany *Towards an open high-performance platform for fully-automated analysis of whole organ light-sheet fluorescence microscopy data*	
14:45	*Coffee break*	
Session II: Image-based models of cell connectivity and dynamics		Session Chair: Marco Blickensdorf
15:10	**Pavel Tomancak,** Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany *Tissue morphogenesis in insects: a comparative and multidisciplinary approach*	
15:55	**Torsten Paul,** University of Würzburg, Germany *A framework for spatially embedded network growth*	
16:15	**Michael Kücken,** Technical University Dresden, Germany *Multi-scale modeling of planar cell polarity dynamics in planarians*	
16:35	*Coffee break*	
Session III: Machine learning assisted bioimage analysis		Session Chair: Stefanie Dietrich
17:00	**Carl-Magnus Svensson & Oksana Svydkiv,** Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany *Coding of experimental conditions in microfluidic droplet assays using colored beads and machine learning supported image analysis*	
17:30	**Benjamin Schmid,** Optical Imaging Centre Erlangen, Germany *Learning based interactive cell-counting of fluorescent labeled mouse tooth pulp nociceptors*	
17:50	**Maria Theiss,** University of Würzburg, Germany *Automated classification of synaptic vesicles in electron tomograms of C. elegans using machine learning*	
18:10	*Poster session and dinner*	

Friday, September 7th, 2018

Session IV: Growth and interactions of cell populations

Session Chair: Sandra Timme

9:00	**Dagmar Iber,** ETH Zurich, Switzerland *From Networks to Function - Image-Based Models of Organogenesis*	
9:45	**Valerio Lupperger,** Helmholtz Zentrum München, Germany *Increased re-division rate induces weak spatial correlations of NSC divisions in the zebrafish brain*	T
10:05	**Raimo Hartmann,** Max Planck Institute for Terrestrial Microbiology, Marburg, Germany *Physical determinants of Vibrio cholerae biofilm architectures at the single cell level*	T
10:25	*Coffee break*	

Session V: Medical image-based systems biology

Session Chair: Alexander Tille

10.50	**Jari Hyttinen,** Tampere University of Technology, Tampere, Finland *Image based assessment of human stem cell derived cardiomyocytes – from in vitro to in silico*	
11:35	**Pranita Pradhan & Melina Yarbakht,** Friedrich Schiller University & University Hospital, Jena, Germany *Non-linear multimodal imaging of early septic liver injury*	T T
12:05	*Lunch Break*	

Session VI: Image-based spatial models of cell-cell interactions

Session Chair: Teresa Lehnert

13:00	**Matthias Weiss,** University of Bayreuth, Germany *Illuminating physical cues for the early embryogenesis of a simple model organism*	
13:45	**Anastasios Siokis,** Helmholtz Centre for Infection Research, Braunschweig, Germany *An agent-based model for the F-actin driven spatial organization of the immunological synapse*	T
14:05	**Hannah Jeckel,** Max Planck Institute for Terrestrial Microbiology, Marburg, Germany *Learning the space-time phase diagram of bacterial swarm expansion*	T
14:25	**Claudia Sichting,** Friedrich Schiller University Jena, Germany *Simulation of virtual phagocytosis assays with alveolar macrophages and Aspergillus fumigatus conidia reveals immune reaction rates*	T
14:45	**Closing**	

TALKS

POSTERS

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KEYNOTE SPEAKERS

KEYNOTE SPEAKERS

Michael Unser is professor and director of EPFL's Biomedical Imaging Group, Lausanne, Switzerland. His primary area of investigation is biomedical image processing. He is internationally recognized for his research contributions to sampling theory, wavelets, the use of splines for image processing, and stochastic processes. He has published over 200 journal papers on those topics. From 1985 to 1997, he was with the Biomedical Engineering and Instrumentation Program, National Institutes of Health, Bethesda USA, conducting research on bioimaging. Prof. Unser is a fellow of the IEEE (1999), an EURASIP fellow (2009), and a member of the Swiss Academy of Engineering Sciences. He is the recipient of several international prizes including three IEEE-SPS Best Paper Awards and two Technical Achievement Awards from the IEEE (2008 SPS and EMBS 2010).

Pavel Tomancak started his career at the Masaryk University in Brno, Czech Republic, followed by a PhD in developmental biology at the EMBL in Heidelberg. After 5 years of postdoctoral research at the University of California, Berkeley, he started his own research group at the Max Planck Institute of Cell Biology and Genetics in Dresden. He is well-known for his studies on the development and evolution of Drosophila and his passion for open access. As one of the leads of the Fiji project, he helps in providing excellent image analysis software for biologists all around the world.

Jari Hyttinen studied biomedical engineering and completed his PhD in 1994 at Tampere University of Technology, Finland. Since 2001 he is professor at Tampere University of Technology, acting as head of the Department of Biomedical Engineering from 2007-2012 and as head of the Computational Biophysics and Imaging Group since 2013. Additionally, he was member of the board at BioMediTech, Tampere, as well as the president of the European Alliance for Medical and Biological Engineering & Science (EAMBES). His main research interests are computational biology and image-based modeling. Applying 3D imaging methods and computational modeling, he focuses on cellular biophysics, especially on bioelectric phenomena from cells and tissues and develops new tools for future personalized medicine.

Dagmar Iber studied mathematics and biochemistry in Regensburg, Cambridge, and Oxford and obtained PhDs in both disciplines. After three years as a Junior Research Fellow in St John's College in Oxford, Prof. Iber became a lecturer in applied mathematics at Imperial College London. Since 2008 she is a professor for computational biology at the ETH Zurich, where she develops data-based predictive models to understand the spatio-temporal dynamics of signaling networks. Her recent work focuses on mouse organogenesis (limb and brain development, lung and kidney branching morphogenesis) and simpler patterning systems to address more fundamental questions regarding the control of organ growth and the robustness of signaling mechanisms to evolutionary change.

Matthias Weiss received his PhD in physics at the University of Göttingen. After a two-year EMBO Long-Term Fellowship at the EMBL Heidelberg, he became Research Assistant Professor at MEMPHYS – Center for Biomembrane Physics at the University of Southern Denmark. In 2004, he went to Heidelberg and became leader of the Junior Department of Cellular Biophysics at the DKFZ and the Institute for Modeling and Simulation in the Biosciences (BIOMS). Since 2010, he is leader of the chair for Experimental Physics I at the University of Bayreuth and investigates biochemical reactions in crowded fluids as well as dynamics and development of cell membranes and organelles by advanced light microscopy techniques and mesoscopic modeling.

TALKS

Steerable detectors for bioimage analysis

Michael Unser[1], Virginie Uhlmann[1], Julien Fageot[1], Zsuzsanna Püspöki[1], Daniel Sage[1], Adrien Depeursinge[1]

[1] Biomedical Imaging Group, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Steerable filters are a powerful framework for detecting patterns that can appear at arbitrary orientations in images. The underlying idea, which goes back to Freeman and Adelson in 1991, is the ability to express any rotated version of a given template as a suitable linear combination of a fixed number of basis filters (or filterbank). One of the better known incarnation of the concept is the steerable pyramid, which provides a reversible decomposition of images in terms of steerable wavelets. We shall recall how the latter can be used effectively for image denoising, feature extraction, and morphological component analysis. We then introduce a mathematical framework for the optimal design of steerable detectors that explicitly takes into account the spectral properties of the underlying background signal. We also provide estimates on the angular accuracy of such detectors. We then describe a dedicated ImageJ plugin that facilitates the design and deployment of application-specific steerable detectors. We illustrate the usefulness of this tool for bio-image analysis; specific examples include the detection of DNA filaments in cryoelectron micrographs, the angular localization of double-helix PSF in super-resolution microscopy, and the detection/localization of bacteria in high-throughput microscopy.

3D reconstruction and quantitative analysis of histology for prostate cancer

Pekka Ruusuvuori[1,2], Leena Latonen[1], Kimmo Kartasalo[2], Mira Valkonen[1,2], Masi Valkonen[2], Tapio Visakorpi[1], Matti Nykter[1]

[1] Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland

[2] Tampere University of Technology, Tampere, Finland

The integration of serial sectioning of tissue, digital whole slide imaging (WSI) and computational reconstruction algorithms enable the examination of histological samples in 3D at subcellular resolution. This allows visualizing and analyzing the imaged sample in its true three-dimensional context, offering a more comprehensive view on its spatial and morphological characteristics than that obtained via typical 2D examination. The advantages of reconstructing 3D models computationally using WSI over direct 3D imaging include the combination of high resolution, large sample sizes and compatibility with existing biochemical techniques such as *in situ* hybridization, immunohistochemistry and established histological staining and interpretation protocols.

For this purpose, we developed a software pipeline to perform routine 3D reconstruction tasks for large image sizes and datasets in a fully automatic manner. The steps in our 3D reconstruction process are image acquisition, alignment of images to a shared coordinate space, and visualization of the reconstructed 3D data. We optimized algorithm selection and computationally intensive hyperparameter tuning using a quantitative benchmarking framework and Bayesian optimization. Further, we applied our existing pipeline for feature based analysis of tissue and extended it into 3D, allowing quantification and visalization of hundreds of features characterizing the tissue.

We currently apply this system to characterize prostate cancer tumors. Prostate cancer is a heterogenous, often multifocal disease. In order to understand why and how certain tumor foci develop to a life-threatening disease over others, the tissue growth patterns and evolution of tumors with different genetic backgrounds need to be studied. We use mouse models of prostate cancer to study early tumor development connected to common genetic cancer alterations. By computing hundreds of features from the histology, and studying these in the spatial context, we gain important information of tumor characteristics as well as intra- and intertumor heterogeneity. In the future, we will further scale up the protocol to perform 3D reconstruction for serially sectioned human prostates in the near future.

Towards an open high-performance platform for fully-automated analysis of whole organ lightsheet fluorescence microscopy data

Ruman Gerst[1,2], Anna Medyukhina[1], Marc Thilo Figge[1,2]

[1] Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Jena, Germany

[2] Faculty of Biological Sciences, Friedrich Schiller University, Jena, Germany

Lightsheet fluorescence microscopy (LSFM) of whole organs allows exact three-dimensional analysis of targeted cells. This includes the evaluation of structural changes such as a reduced number of glomeruli in kidneys or the formation of bronchus-associated lymphoid tissue (BALT) caused by lung inflammation. LSFM produces a large amount of high-resolution data, making manual analysis difficult. Therefore tools are required that offer reliable, fast and fully-automated detection and quantification of interesting structures. While such tools can be developed for each individual project, there are a large number of steps such as tissue segmentation that are common between all tools.

Here, we present our current effort in developing a high-performance and modular platform written in C++ to easily combine different analysis modules, as well as offering automated parallelization and an advanced input parameter system.

Using our platform, we were able to detect glomeruli in data by Klingberg *et al.* [1] up to 148 times faster compared to the published implementation. Based on our advanced implementation, we developed a new segmentation algorithm that reduces the relative difference in the number of glomeruli between manual counting and automated detection from on average 36% to on average 11%. We are currently expanding our platform to the analysis of BALT data provided by Mzinza *et al.* [2], which includes improved algorithms for the tissue segmentation module by using fuzziness-minimizing thresholding, graph-based object labeling and a new module to segment bronchioles.

[1] A Klingberg *et al.*, Fully Automated Evaluation of Total Glomerular Number and Capillary Tuft Size in Nephritic Kidneys Using Lightsheet Microscopy, *J Am Soc Nephrol 28*(2):452-459, 2017.

[2] DT Mzinza *et al.*, Application of light sheet microscopy for qualitative and quantitative analysis of bronchus-associated lymphoid tissue in mice, *Cell Mol Immunol*, 2018. [Epub ahead of print]

Tissue morphogenesis in insects: a comparative and multidisciplinary approach

Pavel Tomancak[1], Stefan Munster[1], Akanksha Jain[1], Alexander Mietke[1], Anastasios Pavlopoulos[1], Stephan Grill[1]

[1] Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

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.

A framework for spatially embedded network growth

Torsten Paul[1], Felix Repp[1], Philip Kollmannsberger[1]

[1] Center for Computational and Theoretical Biology, Faculty of Biology, University of Würzburg, Würzburg, Germany

Spatial biological networks are important for signaling, transportation and stability, and are found on many scales, from osteocytes and neuronal connections up to vascular or root like patterns.

By combining image analysis of such multicellular networks with graph theory, they can be compared quantitatively to different random or regular network models. To link image-derived connectivity patterns to the underlying biological growth process, these models need to explicitly account for the spatial embedding and the interaction of cells with their environment.

In a previous study, we used image analysis and network quantification to characterize the connectivity patterns of the osteocyte lacuno-canalicular network in different types of bone [1]. The interpretation of the results so far was limited, as we lack an appropriate model linking local cell behavior and tissue organization during growth to the resulting global network architecture.

Here, we introduce a new parallel simulation framework that aims to fill this gap. We model the process as a biased correlated random walk, where growth direction and branching probability depend on the local environment, *e.g.* soluble gradients, tissue anisotropy, or other cells. This is implemented by representing the environment as a multi-layer image from which gradients, structure tensors and other influencing parameters are derived by convolution operations. As a case study, we simulate the growth of the osteocyte network during lamellar bone formation.

Our generally applicable framework could help to better understand how observed network patterns depend on the growth process under different environmental conditions, and to identify the biological cause of deviations from healthy network function, *e.g.* in bone, neurons or the vascular system.

[1] Kollmannsberger P, Kerschnitzki M, Repp F, Wagermaier W, Weinkamer R, Fratzl P. *New J Phys* 19:073019, 2017.

Multi-scale modeling of planar cell polarity dynamics in planarians

Michael Kücken[1], Hanh Vu1, Karl B. Hofmann[1], Anja Voß-Böhme[1], Jochen Rink[1], Lutz Brusch[1]

[1] Center for Information Services and High-Performance Computing, TU Dresden, Dresden, Germany

Polarity is a universal design principle of biological systems that manifests at all organizational scales. Mechanistic understanding of polarity pattern formation can be gained from dynamic pattern reorganization in response to perturbations. Here, we make use of the extreme body plan plasticity of planarian flatworms and quantify their polarity patterns from ciliary rootlet orientation in the epidermis [1]. Based on these whole-animal image data with subcellular resolution, we first define a dynamically diluted alignment model linking three processes: entrainment of cell polarity by tissue-scale cues, local cell-cell coupling aligning polarity among neighbors, and cell turnover replacing polarized cells by initially unpolarized cells [2]. Combining analytical and computational approaches using the software Morpheus [3], we find that neighbor coupling retards polarity pattern reorganization, whereas cell turnover accelerates it and derive an effective neighbour coupling strength [2]. Second, we consider a continuum model as large scale approximation of the cell-based model and apply it to combinatorial perturbation experiments of planaria. We find that the superposition of separate anteroposterior and mediolateral polarity fields can explain the observed reorientations of the global polarity field. Overall, our study establishes a mechanistic framework for the multi-scale coordination of planar polarity in planarians and establishes the core PCP and Ft/Ds pathways as evolutionarily conserved 2D-polarization module [1].

[1] H T-K Vu *et al.*, Multi-scale coordination of planar cell polarity in planarians, *bioRxiv* 324822, 2018. doi:10.1101/324822

[2] KB Hoffmann *et al.*, A dynamically diluted alignment model reveals the impact of cell turnover on the plasticity of tissue polarity patterns, *J R Soc Interface* 14(135), 2017. doi:10.1098/rsif.2017.0466

[3] J Starruß *et al.*, Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology, *Bioinformatics 30*(9):1331-1332, 2014. doi:10.1093/bioinformatics/btt772

Coding of experimental conditions in microfluidic droplet assays using colored beads and machine learning supported image analysis

Carl-Magnus Svensson[1], Oksana Shvydkiv[2], Stefanie Dietrich[1,3] , Lisa Mahler[2,3] , Thomas Weber[2], Mahipal Choudhary[2], Miguel Tovar[2,3] , Marc Thilo Figge[1,3], Martin Roth[2]

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[2] Bio Pilot Plant, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany

[3] Faculty of Biological Sciences, Friedrich Schiller University, Jena, Germany

To exploit the potential of several millions of droplets that are the individual bioreactors in microfluidic experiments, methods to encode different experimental conditions in droplets are needed. We introduce an encoding approach based on co-encapsulation of colored polystyrene beads with biological samples. The decoding of the droplets, as well as content quantification, was performed by automated analysis of triggered images of individual droplets in-flow using bright-field microscopy. The encoding and decoding is a unique combination of engineering of a microfluidic device and development of an analysis pipeline. Microfluidic chips were designed to ensure stable generation of droplets and encapsulation of beads as well as optimal geometries for droplet imaging. Standard operating procedures were developed to make sure that the number of encapsulated beads, droplet integrity during incubation and distribution of microbes is stable across the entire experiment. The decoding strategy combines bead classification using a random forest classifier and Bayesian inference to identify different codes and thus experimental conditions. As a proof-of-principle, we demonstrated the coding method for the determination of the minimal inhibitory concentration of an antibiotic in a single experiment with one joint incubation vessel containing droplets with different antibiotic concentrations. We demonstrate that this method allows processing of 20 different experimental conditions within a large droplet population of more than 100000 droplets per condition. Our decoding strategy correctly assigned 99.6% of droplets to the correct condition and we established a method for the determination of minimal inhibitory concentration. Furthermore, we performed antibiotic susceptibility testing for the same species against nine different antibiotics. Our current encoding and decoding pipeline can readily be extended to more codes by adding more bead colors or color combinations.

Learning based interactive cell-counting of fluorescent labeled mouse tooth pulp nociceptors

Benjamin Schmid[1], Philipp Tripal[1], Tina Fraaß[1], Pamela Sotelo-Hitschfeld[2], Katharina Zimmermann[2], Ralf Palmisano[1]

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[2] Department of Anesthesiology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Recently, we established reproducible DiI labeling of dental primary afferent neurons (DPANs) in the mouse, which is important for investigating the molecular and functional specialization of DPANs within the trigeminal nociceptive system. To quantify the number of labeled neurons we developed a new ImageJ/Fiji plugin for interactively and semi-automatically detect and count cells in 3D fluorescent images. The datasets to analyze were imaged on a 2-photon microscope as multiple tiles which were subsequently stitched in Fiji. Possible cell candidates were identified as local maxima after an appropriate Difference-of-Gaussian filter. To distinguish true cells from false detections, we implemented an interactive learning environment. The user repetitively marks cell candidates either as correct or false. Each correction triggers the fitting of a Random-Forest-Classifier, based on local features of the user-provided ground truth. The classifier calculates predictions of so far unmarked cell candidates, and the result is displayed to the user. The process of interactive correction, learning and classification is iterated until the predicted outcome matches the user's expectation. Our plugin significantly accelerates cell counting in difficult settings where simple automatic methods such as thresholding fail. It is dedicated, but not limited to counting DiI labeled mouse DPANs in 2-photon images, although preprocessing will in general need to be adapted to the images at hand.

Automated classification of synaptic vesicles in electron tomograms of *C. elegans* using machine learning

Maria Theiss[1], Kristin V. Kaltdorf[1,2,3], Sebastian Matthias Markert[2], Mei Zhen[4] , Thomas Dandekar[3,*], Christian Stigloher[2,*], Philip Kollmannsberger[1,*]

[1] Center for Computational and Theoretical Biology, University of Würzburg, Würzburg, Germany

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[*] joint senior authors

Synaptic vesicles (SVs) are a key component of neuronal signaling and fulfil different roles depending on their composition. In electron micrograms of neurites, two types of vesicles can be distinguished by morphological criteria, the classical "clear core" vesicles (CCV) and the typically larger "dense core" vesicles (DCV), with differences in electron density due to their diverse cargos. Compared to CCVs, the precise function of DCVs is less defined. DCVs are known to store neuropeptides, which function as neuronal messengers and modulators. In *C. elegans*, they play a role in locomotion, dauer formation, egg-laying, and mechano- and chemosensation.

Another type of DCVs, also referred to as granulated vesicles, are known to transport Bassoon, Piccolo and further constituents of the presynaptic density in the center of the active zone (AZ), and therefore are important for synaptogenesis in mammals. To better understand the role of different types of SVs, we present here a new automated approach to classify vesicles. We combine machine learning with an extension of our previously developed vesicle segmentation workflow, the ImageJ macro 3D ART VeSElecT. With that, we reliably distinguish CCVs and DCVs in electron tomograms of *C. elegans* neuromusclular junctions using image-based features. Analysis of the underlying ground truth data shows an increased fraction of DCVs as well as a higher mean distance between DCVs and AZs in dauer larvae when compared to young adult hermaphrodites. Our machine learning based tools are adaptable and can be applied to study properties of different synaptic vesicle pools in electron tomograms of diverse model organisms.

From Networks to Function - Image-Based Models of Organogenesis

Dagmar Iber[1]

[1] Department of Biosystems Science and Engineering, ETH Zurich, Zürich, Switzerland

One of the major challenges in biology concerns the integration of data across length and time scales into a consistent framework: how do macroscopic properties and functionalities arise from the molecular regulatory networks – and how do they evolve? Morphogenesis provides an excellent model system to study how simple molecular networks robustly control complex pattern forming processes on the macroscopic scale in spite of molecular noise, and how important functional variants can evolve from small genetic changes. Recent advances in 3D imaging technologies, computer algorithms, and computer power now allow us to develop and analyse increasingly realistic models of biological control.

In the first part of my talk, I will focus on the image-based simulation of lung and kidney development. The branched trees of lungs, kidneys and many glands provide a fascinating example of complex shape formation. I will introduce the concept of the Turing mechanism and show how Turing patterns can arise from molecular interactions to control branching morphogenesis. I will then discuss the detailed properties of Turing mechanisms, and how to simulate those Turing models on growing domains that have been extracted from 3D microscopy images.

In the second part of my talk, I will focus on epithelial organization and growth control in epithelia. Several theories have been formulated to explain epithelial organization and growth control, but many key observations remain unexplained. Our recent work identifies the physical forces that drive apical epithelial organisation and provides a link between epithelial organisation, cell division, and growth.

Increased re-division rate induces weak spatial correlations of NSC divisions in the zebrafish brain

Valerio Lupperger[1], Prisca Chapouton[1], Carsten Marr[1]

[1] Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Computational Biology, Neuherberg, Germany

Understanding cell proliferation is one of the key issues when investigating the maintenance of cell populations. We have previously shown that cell divisions form spatially attractive patterns in the zebrafish brain by analysing neural stem cell (NSC) division patterns. To get further insight into spatial dependencies on a temporal scale we investigated the influence of dividing NSCs on later divisions. To evaluate the influence on later division we performed experiments with different time intervals, ranging from 9 hours to 72 hours using two different S-Phase markers (BrdU and EdU). Spatial statistics show that the two dividing cell populations are closer to each other compared to random division events. To quantify this observation, we used the data to infer parameters of a probabilistic model. We find an attractive influence for all time intervals, *i.e.* later divisions are spatially closer to early division locations than expected for independent divisions. Evaluating the model parameters over time, we find that the influence radius increases with temporal distance while its strength decreases. We furthermore find redivisions in one fourth of all daughter cells. Using an agent-based simulation for population growth we can show that redivisions are able to explain the observed patterns.

Physical determinants of *Vibrio cholerae* biofilm architectures at the single cell level

Raimo Hartmann[1], Praveen K. Singh[1], Phil Pearce[2], Rachel Mok[2], Boya Song[2], Jörn Dunkel[2], Knut Drescher[1,3]

[1] Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

[2] Department of Mathematics, Massachusetts Institute of Technology, Cambridge, USA

[3] Department of Physics, Philipps-University Marburg, Marburg, Germany

Bacterial biofilms undergo several developmental and architectural transitions from the initial attachment of individual cells to maturity and eventual dispersal. [1,2] We developed new methods to look inside living intact biofilms at the single cell level, which opens up new questions regarding physical determinants of growth, internal structure, and cell-cell interactions. In order to understand how the biofilm architecture and internal structure arises from mechanical interactions of individual cells in the human pathogen *Vibrio cholera*, we varied the cell-cell interactions experimentally by changing the composition of the extracellular matrix and monitored changes in biofilm architecture. We compared our findings with the results from individual-cell-based simulations and propose a cellular interaction potential, which captures the development of internal structure during early-stage biofilm formation. In addition, we perturbed the biofilm growth environment by changing the external fluid shear regimes, which also led to dramatic differences in internal biofilm structure that resulted in modified external biofilm morphologies.

[1] K Drescher *et al.*, Architectural transitions in *Vibrio cholerae* biofilms at single-cell resolution. *Proc Natl Acad Sci 113*(14):E2066-E2072, 2016.

[2] H-C Flemming *et al.*, Biofilms: an emergent form of bacterial life. *Nat Rev Microbiol* 14(9):563-575, 2016.

Image based assessment of human stem cell derived cardiomyocytes – from *in vitro* to *in silico*

Jari Hyttinen[1], Antti Ahola[1], Michelangelo Paci[1]

[1] BioMediTech and Faculty of Biosciences and Biomedical Engineering, Tampere University of Technology, Tampere, Finland

The future of personalized medicine is based on application of number of data sources depicting the patient geno- and phenotype. Human induced pluripotent stem cells (hIPSC) provide means to produce most human cell types and thus provide means to get patient own cells for *in vitro* disease models, models for drug development and personalized medicine. New technologies are needed to culture cells as well as to control and assess the cell functions including novel imaging methods. In addition to *in vitro* technologies, we can use computational models to mimic the cellular functions to gain insight into the cellular processes and use the *in silico* prior to wet lab works *e.g.* for drug testing.

We have developed a set of novel methods to produce hiPSC cell models and engineering environment including biomaterials, microfluidic culturing systems, sensors and imaging systems for 2D and 3D environment. For example we have developed imaging based methods to guide and assess functions of stem cell derived cardiomyocyte (CM) electrophysiology and mechanobiology *in vitro*. Further, we have developed *in silico* models of the hIPSC CM electrophysiology. We have shown that these *in vitro* and *in silico* models can also depict patient genotype with specific ion channel mutations, *e.g.*, long QT syndrome, showing that the pathological condition observed in cardiac scale is present also *in vitro* and can be effectively modelled *in silico*. Further, developing thousands of *in silico* cardiomyocytes - population of models – provide us means to look into variability of cellular functions on single patient or population level within a specific genotype and phenotype or over multiple genotypes.

The future use of stem cell technology for personalized medicine will need a number of various technologies. The multimodal sensing, imaging and computational modelling have their own role on understanding the causes of behavior initiated by the diseases, trauma and drugs. Our results highlight the potential of combination of *in silico* pre-screening and validation *in vitro* for patient group specific drug discovery and patient specific drug optimization.

Non-linear multimodal imaging of early septic liver injury

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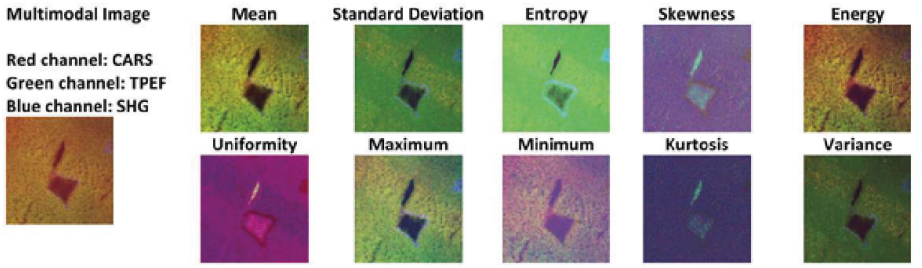
Sepsis is currently defined as a life-threatening organ dysfunction caused by a dysfunctional host response to infection. Biomolecular information may improve diagnosis and decision-making for patients with immanent organ failure and shed light on underlying pathological mechanisms involved. Non-linear multimodal imaging, combining coherent anti-Stokes Raman scattering (CARS), two-photon excited auto fluorescence (TPEF) and second-harmonic generation (SHG), can differentiate various biomolecules and might serve as a competent technique for real-time diagnosis of organ failure.

In order to investigate the molecular and morphological consequences of septic liver injury, mouse liver sections were studied 24 hours after the induction of abdominal sepsis (n=7) or peritoneal injection of NaCl as a control (n=7) using non-linear multimodal microscopy. Organ injury and inflammation were confirmed by qRT-PCR showing upregulation of cytokines and acute phase proteins and by immune fluorescence confirming liver-infiltrating myeloid cells. Tissue sections were displayed as RGB images with CARS, TPEF and SHG signal corresponding to red, green and blue channel, respectively. The analysis was restricted to 20 µm regions around the periportal fields and the central vein by using a binary mask generated by applying Otsu's thresholding on TPEF signal followed by morphological operations. Nine texture features were calculated for the three signals and Kruskal-Wallis test was applied.

We could determine significant differences between septic and control livers based on their textural features. Molecular changes and the chemical constituents could be visualized by calculating the texture features locally for each signal (Fig. 1) without differences in periportal or pericentral distribution. Based on these results, non-linear multimodal imaging along with image analysis techniques is a promising complementary real-time method for the label-free investigation of septic organ damage.

Fig. 1: Pseudo-colour images of ten texture features calculated locally for a septic tissue section.



Illuminating physical cues for the early embryogenesis of a simple model organism

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Embryogenesis is a remarkably robust, but still poorly understood self- organization phenomenon that is governed by a variety of biochemical and physical cues. Due to its simplicity, the small roundworm *Caenorhabditis elegans* is a superb model organism to study the role of physics during early developmental stages. Using single plane illumination microscopy (SPIM), we have explored how physical cues determine the cell arrangement during the early embryogenesis of *C. elegans*. In particular, we have studied the coupling of cellular volumes and cell cycle times, the nature of asymmetric cell divisions, and the mechanically driven cell arrangement process [1]. Going beyond mere imaging, we also have used pixel-wise fluorescence correlation spectroscopy (SPIM-FCS) to spatiotemporally quantify the diffusion of proteins in individual cells of the embryo in cytoplasm and on membranes [2]. As a result, we were able to monitor the emergence of intracellular diffusion and concentration gradients prior to the first cell division, which define the anterior-posterior body axis already in the single-cell state.

[1] Biophys. J. 105, 1805 (2013); Phys. Rev. Lett. 117, 188101 (2016); Sci. Rep. 7, 9369 (2017).

[2] J. Phys. D 49, 044002 (2016).

An agent-based model for the F-actin driven spatial organization of the immunological synapse

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Adaptive immune responses are driven by antigen recognition from T cells. A spatial characteristic pattern, known as immunological synapse (IS), forms in the cell-cell junction between T and Antigen Presenting cells (APCs). In the forming interface, signaling complexes, integrins, and costimulatory molecules like CD28 exhibit a specific pattern, essential for T cell activation and fate decision. Despite the extensive knowledge on T cell activation, the mechanisms that regulate the spatial organization of the surface molecules during IS formation are poorly understood.

We developed an agent based model for the investigation of the mechanisms leading to the IS formation. The *in silico* experiments simulate the dynamics of the critical surface molecules of the two interacting cells (T and APC). The model is calibrated based on experimental high resolution (including confocal and total internal reflection fluorescence – TIRF) microscopy imaging results. Our results show that F-actin driven centripetal flow is crucial for the formation of the characteristic IS pattern. The emergence of an LFA-1 gradient in the periphery of the contact region towards the center, impacts on the IS formation and affects molecular localization, also observed experimentally. CD28-CD80 complexes behave as passive followers of the TCR-pMHC movement, but nonetheless their characteristic annular structure around the cSMAC only emerges under an optimal actin coupling strength that induces centripetal motion.

The presented model shows that imaging data can be used to extract functional properties of the IS, and that actin forces are a major player in the formation of a proper synapse. The model is a cutting edge basis to predict the effect of potential therapeutics targeting actin-related pathways, and to decipher the strength of new mechanisms for molecular transport in the IS, where complex experimental set-ups are difficult to interpret.

Learning the space-time phase diagram of bacterial swarm expansion

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Coordinated dynamics of individual components in active matter are an essential aspect of life on all scales. Establishing a comprehensive, causal connection between intracellular, intercellular, and macroscopic behaviors has remained a major challenge due to limitations in data acquisition and analysis techniques suitable for multi-scale dynamics. Here, we combine a high-throughput adaptive microscopy approach with machine learning, to identify key biological and physical mechanisms that determine distinct microscopic and macroscopic dynamical phases which develop as *Bacillus subtilis* swarms expand over five orders of magnitude in space. Our experiments, continuum modeling, and particle-based simulations reveal that macroscopic swarm expansion is primarily driven by cellular growth kinetics, whereas the microscopic swarming motility phases are dominated by physical cell-cell interactions. These results provide a unified understanding of bacterial multi-scale behavioral complexity.

Simulation of virtual phagocytosis assays with alveolar macrophages and *Aspergillus fumigatus* conidia reveals immune reaction rates

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Image-based systems biology deploys the cycle of experiment, analysis and modeling onto microscopy images and can be applied to investigate host-pathogen interactions of the human immune system and pathogens. Several hundred airborne conidia of the fungus *A. fumigatus* are inhaled by every human every day. If not cleared by the resident immune cells, alveolar macrophages (AM), they can cause severe pulmonary infections in immunocompromised patients with high mortality rates. To investigate the interaction of the two wild-type strains ATCC 46645 and CEA10 of *A. fumigatus* with AM, fluorescent microscopy images of phagocytosis assays have been conducted, where differential staining allowed to distinguish phagocytosed, adherent and non-associated conidia.

To this end, we constructed a simulation framework CellRain for image-derived modeling to investigate immune reaction rates and to simulate virtual phagocytosis assays. Optimal parameters were determined by comparison of the symmetric phagocytic index between *in silico* and *in vitro* phagocytosis assays. First, virtual conidia were distributed randomly on images with segmented AM [1]. Subsequently, co-incubation and washing were simulated. If the conidia are located in the proximity of an AM, they can become adherent or phagocytosed with certain rates during co-incubation, which entails a position change of the conidia. Finally, washing is simulated in which non-associated cells are removed with a certain rate.

For parameter estimation we performed a grid-based screening of the immune reaction rates, as well as the range of AM, in which they can reach conidia. We performed 50 repetitions of the simulation for each parameter set in order to generate statistically sound results. Preliminary results for individual images suggest that strain-specific optimal parameter ranges can be found.

[1] Z Cseresnyes *et al.*, Hessian-based quantitative image analysis of host-pathogen confrontation assays. *Cytometry*, 93: 346-356, 2018.

POSTERS

Image-based Identification of Antibiotic Resistances in *E. coli* strains

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E. coli is a large and diverse bacterial species that can be found almost everywhere. These bacterial species vary excessively and many of these species are essential for the digestive tract, while others are pathogens and can cause complications in urinary and intestinal tracts. In order to cure such infections, antibiotics are utilized, which become complicated in the last years due to an increased incidence of antibiotic resistances.

In this contribution, we report on the acquisition and automated analysis of image data for the automated detection of antibiotic resistances in *E. coli*. Exemplarily, the image analysis algorithm is developed for the detection of susceptibility of *E. coli* strains towards two classes of antibiotics: the 3rd generation cephalosporin cefotaxime and the fluoroquinolone ciprofloxacin. Therefore, 7 *E. coli* strains were analyzed on dielectrophoretic (DEP) chips. Then the sensitivity of each strain was tested by inoculating with both antibiotics separately. Thereafter the DEP images of inoculated and control images were collected and preprocessed. After preprocessing, the first order statistical features of the histogram were calculated locally, and at the end a Mann Whitney test was applied to prove their significance.

However, the results of the susceptibility prediction based on the considered image analysis are summarized in table 1. In most cases the predicted susceptibility of a Mann Whitney test matched the reference value. For each antibiotic the prediction is set together with the reference value. In this table R indicates resistance, S represents sensitive and NA reflects missing reference values.

Based on the presented results, the image analysis techniques can be applied to predict the susceptibility of *E. coli* strains to antibiotics. This can be utilized to fast determine the antibiotic resistances before using a specific antibiotic.

Table 1. A comparison between the antibiotic susceptibility of *E. coli* strains

	Strain	416	416	539	544	545	579	AG 100
Cefotaxime	Reference value	NA	NA	R	S	S	R	S
	Susceptibility prediction	R	R	R	S	S	R	S
Ciprofloxacin	Reference value	NA	NA	S	R	S	R	S
	Susceptibility prediction	S	S	S	R	R	R	S

Holographic Region-of-Interest Illumination with Oblique Angles

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Candida albicans and *Aspergillus fumigatus* often cause infections in humans, especially in immunocompromised patients. Both fungi have developed a pathogen mechanism to alter the membrane of the phagosome/phagolyosome after being phagocytosed.

However, until now, even with Single-Molecule-Localization-Microscopy (SMLM), the super-resolution technique in wide-field fluorescence microscopy which provides the most precise position of fluorophores, it is not possible to measure the membrane before and after the infection process, preferably *in vivo*.

To improve the localization accuracy and to reduce the overall photo damage in SMLM we introduce a holographic based region-of-interest (ROI) illumination. This approach allows to adaptively change the size and the (excitation) angle of the ROI illumination resulting in reduced out-of-focus signal and less overall phototoxicity. The ROI illumination is realized by generating a spot in the back focal plane (BFP) of the objective using a spatial light modulator. This spot can be changed in size and can be moved laterally in the BFP. This results in different sizes of the ROI respectively with different, oblique illumination angles.

In combination with SMLM, this approach allows potentially higher localization accuracies and less photo damage in the sample. In addition, first experiments have shown promising results in the creation of multiple ROI's achieved by the creation of multiple spots in the BFP. This may allow multiple ROI imaging in SMLM or multiple particle tracking in live cell experiments.

Advanced imaging flow cytometry

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Imaging flow cytometry (IFC) is a hybrid technology which extends conventional flow cytometry with additional high resolution morphological information. The objective of our work is to develop a microfluidic system for conventional and tomographic IFC.

Both, conventional and tomographic IFC is realized by advanced 3D hydrodynamic focusing which automatically aligns all particles as a sheet at a controllable z-position. Tomographic imaging Flow Cytometry extends conventional imaging flow cytometry for the image based measurement of 3D-geometrical features of cells. The required multidirectional views are generated by rotating all cells while passing the imaging window of the developed microfluidic chip. Rotation is implemented by guiding them at a shear flow position of the parabolic velocity profile. All cells pass the detection chamber as a two dimensional sheet under controlled rotation where each cell is imaged multiple times.

Experimental results show a strong focusing quality even under flow velocities below 1 mm/s. For the tomographic IFC, white blood cells with fluorescent stained nuclei are been recorded in parallel for the bright field and the fluorescence channel. Different subtypes of white blood cells can be distinguished by the shape of its nucleus. The experiments show that the multidirectional imaging enhances the identification of these subtypes compare to a single 2D view. Ongoing experiments are focusing on a label free classification of a mixed population of eight allergic pollen types using a convolutional neuronal network (CNN). The whole process requires a high effort in data-processing containing algorithms for object detection, particle tracking and mapping (multi-channel applications) and a CNN-model for the particle classification.

In our work we report on a microfluidic system and method for tomographic imaging flow cytometry, where the angular velocity of a rotating cell is controlled by its z-position in the parabolic velocity profile of a carrier fluid. We also show the need of advanced data-processing tools for image analysis.

We acknowledge the microsystem group and the cleanroom staff at the IPHT for the development and realization of the microfluidic units. The funding from WaterChip (EU Era-NET-DLR 01DQ16009A) is gratefully acknowledged.

A dynamically diluted alignment model reveals the impact of cell turnover on the plasticity of tissue polarity patterns

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The polarization of cells and tissues is fundamental for tissue morphogenesis during biological development and regeneration. A deeper understanding of biological polarity pattern formation can be gained from the consideration of pattern reorganization in response to an opposing instructive cue, which we here consider using the example of experimentally inducible body axis inversions in planarian flatworms. We define a dynamically diluted alignment model linking three processes: entrainment of cell polarity by a global signal, local cell–cell coupling aligning polarity among neighbours, and cell turnover replacing polarized cells by initially unpolarized cells. We show that a persistent global orienting signal determines the final mean polarity orientation in this stochastic model. Combining numerical and analytical approaches, we find that neighbour coupling retards polarity pattern reorganization, whereas cell turnover accelerates it. We derive a formula for an effective neighbour coupling strength integrating both effects and find that the time of polarity reorganization depends linearly on this effective parameter and no abrupt transitions are observed. This allows us to determine neighbour coupling strengths from experimental observations. Our model is related to a dynamic 8-Potts model with annealed site-dilution and makes testable predictions regarding the polarization of dynamic systems, such as the planarian epithelium.

Morpheus: a user-friendly modeling environment for multiscale and multicellular systems biology

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Morpheus is a modeling environment for the simulation and integration of cell-based models with ordinary differential equations and reaction-diffusion systems. It allows rapid development of multiscale models in biological terms and mathematical expressions rather than programming code. Its graphical user interface supports the entire workflow from model construction and simulation to visualization, archiving and batch processing.

GriBMSO - Analysing Images using Grid-Based Mapping and Set Operations

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Different imaging techniques produce different aspects of the content to be measured and depending on what the object of the specific investigation is, different parts of the image are relevant for the given analysis task.

Yet the one common thing is, that the information to be retrieved from the object which is under investigation has a spacial and sometimes even temporal context to it. The relative grid position within the image defines thus the context. Estimating colocalization can therefore be derived as a measure defining the occupation of the same space and time. In order to fully utilize this knowledge one has to annotate the found feature to be located within a specific volume. Thus for rapid correlation between different feature lists (each annotating specific features as part of the image), one ideally needs a deterministic indexing scheme. This replaces time consuming distance calculation from the underlying reference coordinates, like postal codes do.

Here we present the R-package GriBMSO which is designed to facilitate the exploration of spacial annotation data. We do this by mapping the annotation to a grid-based indexing system and thus allowing fast list based set operation to replace the otherwise required distance calculations.

If users can pinpoint their region of interest using filters using their favorite tool (*e.g.* using a specific channel and a cutoff), this definition of occupancy in the resulting pixel map afterwards allows for mapping to the grid space. Our package contains multiple wrapper functions aimed to facilitate the focusing on the comparison step and even reconstruction tasks (*e.g.* layer interpolation).

We tested the system on a wide range of input tasks: from the simple analysis of patch distributions and sizes to the 3D-reconstruction of blood vessels and subsequent annotation of the vessel diameter and segmentation.

https://gitlab.gwdg.de/mnieter1/GriBMSO.git

Lung-on-a-chip microtechnologies for studies of host-pathogen interactions in Tuberculosis

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A majority of host-*M.tuberculosis* encounters lead to latent infections in which the bacteria exist in a poorly characterized host-pathogen equilibrium, usually within granulomatous lesions in the lung. Although the structure and components of these lesions are well characterised, the dynamics of this equilibrium, and the contribution of phenotypic heterogeneity within the bacterial population towards this process is less well understood. While much is known about the initial interactions of macrophages with *M. tuberculosis* through *in vitro* batch culture experiments, these experiments, by their very design, preclude the effects of the rest of the host environment, and many of the host-pathogen interactions that occur *in vivo*, especially as the infection progresses.

Here we report on experiments that successfully reconstitute, entirely *in vitro* and using primary cells, the murine alveolar interface using a lung-on-a-chip microfluidic device. This builds upon the previously reported human lung-on-a-chip system[1] by completely incorporating the innate immune system including alveolar macrophages and bone-marrow derived macrophages and monocytes. This system is then exposed to at low multiplicity of infection (MOI) to *M. tuberculosis*, and the progression of the infection is monitored in real-time through a combination of long-term live-cell time-lapse microscopy (7-10 days) followed by confocal microscopy.

This "bottom-up" approach allows for single-cell imaging and temporal tracking of host-pathogen interactions and enables us to closely follow the chain of events from initial infection. We are currently investigating unresolved aspects about the innate immune response to the initial infection. As the system develops further, we will extend it to study how granulomatous lesions begin to develop, and the roles played by bacterial phenotypic variants in granuloma formation and in entering and exiting the latent state. This approach will address challenging questions of immense significance for our understanding of latent TB and persistence during antibiotic therapy.

[1] D Huh, BD Matthews, A Mammoto, M Montoya-Zavala, HY Hsin, DE Ingber, Reconstituting organ-level lung functions on a chip, *Science* 328:1662-1668, 2010.

Prodrug nanoparticles as treatment for *Aspergillus fumigatus* conidia residing in macrophages

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Aspergillus fumigatus is the most important airborne fungal pathogen that infects immunocompromised patients. Several virulence factors are present in this fungus, which, in particular allow its conidia to survive inside macrophages. A combination of caspofungin and humidimycin has been proposed as a new potential drug combination against aspergillosis. Humidimycin prevents the caspofungin paradoxical effect by inhibiting the salvage pathway enabling *A. fumigatus* to resist a high dose of caspofungin.

We generated polymer based nanoparticles with a size range from 200 to 800 nm. Our main target are conidia residing in macrophages, therefore we decorated the nanoparticles with mannose moieties. The mannose facilitates the nanoparticles entering cells by mannose receptor-mediated endocytosis. The macrophage cell line RAW 264.7 was transfected to express GFP fused to the Rab7a, protein of the endosomal compartment. These cells were treated with a nanoparticle suspension containing Nile Red as cargo, and confocal laser scanning microscopy was performed to monitor endocytosis. Also tridimensional images were developed to determine the presence of nanoparticles at the conidium-phagolysosome compartment.

Preliminary results showed an uptake of mannose-functionalized nanoparticles recorded during 15 minutes of treatment. Accumulation of nanoparticles inside late endosomes was observed after few minutes. Also, it was observed how nanoparticles containing Nile Red co-localized at the conidium-phagolysosome compartment, by tridimensional reconstructions. By investigating the efficacy of nanoparticles containing antifungal drugs we aim to develop improved treatment options to fight *A. fumigatus* conidia inside macrophages.

DeconvTest: an *in silico* microscopy framework to evaluate the accuracy of deconvolution

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Microscopy is an indispensable tool to study biological processes. However, any microscopy image is a blurred version of the "ideal" image of the specimen, which results from convolution with the point spread function (PSF) of the microscope. To reconstruct the "ideal" image, the acquired microscopy image has to be de-blurred by the process known as deconvolution. Many deconvolution methods and software are available, which makes it challenging to select the most appropriate and accurate deconvolution method. New deconvolution techniques are also constantly being developed, but comparison between different approaches is often done qualitatively by visual inspection of the deconvolution results. We believe that to objectively compare different deconvolution approaches, it is crucial to characterize their performance quantitatively.

Here we present a python-based simulation framework DeconvTest that allows to quantify and compare the performance of different deconvolution algorithms. The framework consists of three main modules: (1) *in silico* microscopy, (2) deconvolution, and (3) quantification. The *in silico* microscopy module includes routines for generating synthetic PSFs, cells with ellipsoidal or complex shapes, convolution, downsizing, and adding synthetic noise. The deconvolution module contains several deconvolution plugins implemented in Fiji and allows to test different sets of input parameters for these plugins. The quantification module allows to compare the deconvolved images to the ground truth (initially generated synthetic cells) by computing various accuracy measures, such as, Jaccard index or overlap error. This allows to test the performance of various existing and new deconvolution methods and select the optimal set of deconvolution parameters. The modular structure of the framework allows to easily extend it by integrating other types of synthetic cells, deconvolution approaches or accuracy measures.

ACAQ: a Fiji and R toolkit targeted for automated confrontation assay quantification

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Host-pathogen interactions are often studied through imaging confrontation assays, which are then quantified via automated image segmentation and classification of the participating cells in order to unravel biological functions. While the segmentation of fluorescently labeled cells was already established in previous works, here we introduce a novel segmentation method of confrontation assays for unlabeled cells. Label-free imaging saves the time and expense spent on fluorescence labeling, and, most importantly, minimizes any side effects that fluorescence labeling may have on biological functions. Here we introduce ACAQ, a new and complete toolkit based on Fiji and R to analyze the images of both labeled and label-free confrontation assays and to visualize the extracted data.

We developed the fourth version of our algorithm for confrontation assay quantification (ACAQ-v4) together with the ACAQ Visualizer. ACAQ-v4 was designed to identify cells in two-dimensional microscopy images, using either fluorescence labels or bright field label-free microscopy images. In the latter case, a segmentation method based on a Hessian-filter is used, which requires that the types of cells differ in size and are fairly circular in order to be identifiable – a prerequisite that is typically met in host-pathogen confrontation assays. The ACAQ toolkit computes a collection of phagocytosis measures, as well as common morphological descriptors. The results are automatically visualized by the ACAQ Visualizer and can be exported for further data processing and presentation.

ACAQ-v4 was written in the Fiji macro language, and it runs as a Fiji plugin. ACAQ Visualizer was written in R on top of Shiny as a server-based online tool; it runs as an independent executable program. ACAQ-v4 and ACAQ Visualizer are both designed to be cross platform toolkits, working on both Windows and Linux operating systems.

Morphokinetic analysis of live-cell imaging data

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Cell morphology is a macroscopic (relative to cell-scale) manifestation of intracellular processes orchestrated by plenty of factors. It is clear, that time-correlated cell shapes repertoire (morphokinetic) can be used as a marker for cell population behavior analysis. In this work we will demonstrate, how to use these characteristics for identification of human polymorphonuclear neutrophil (PMN) populations affected by *Candida albicans*.

To identify changes in PMN behavior induced by *C. albicans*, fungal cells were added to human whole blood *in vitro* and compared to mock-infected control samples. Following a one-hour confrontation in human whole blood, PMN were purified and monitored by time-lapse microscopy.

Visual analysis of live cell imaging data reveals two types of morphological appearances: flattened and non-flattened cells. In the mock-infected scenario the last type dominates. This fact allows us to create a one-class classifier for non-flattened cells using the tracking data and simple shape descriptors (area, perimeter, Feret diameter) extracted from mock-infected experiments data. Soft Independent Modeling by Class Analogy (SIMCA) approach was chosen for model creation and classification.

The typical results of classification for each condition demonstrate that the fraction of flattened cells is different between the infection scenarios. Whereas mock-treated PMN showed predominantly a non-flattened shape, cells isolated from whole blood infected with *Candida* were found to be dominated by the flat phenotype. Currently, we are investigating the possibility to use the morphokinetics of immune cells as a biomarker to distinguish between infections by various *Candida* spp. and between fungal and bacterial pathogens.

Measured and simulated 3D spatial distribution of plasma cells in the mouse bone marrow

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Plasma cells are mature immune cells that are distributed into the bone marrow parenchyma via a vast vasculature, which also supports the bone marrow's need for nutrients and oxygen that helps to maintain the strongly proliferating and differentiating blood cell progenitors. The vasculature of the parenchyme can be divided into arterioral and sinusoidal systems. Vessels with permeable endothelial layers are responsible for trafficking cells between the circulation and the bone marrow parenchyme, *e.g.* plasma cells are being transported back into the bone marrow via this pathway. Consequently, the anatomical structure of the bone plays an important role in supporting this wide range of functions.

The 3-dimensional structure of the bone marrow was investigated via 2-photon microscopy in excised mouse long bones. The stroma was detected via its second harmonic generation signal, whereas the arterioles, the sinusoids and the plasma cells were tagged with fluorescence labels. All bone components, as well as the plasma cells were reconstructed as 3D surfaces using Imaris (Bitplane, Zürich, Switzerland). The resconstructed surfaces were used to measure the shortest distances between each plasma cell and the three bone marrow components (stroma, arterioles, sinusoids), as well as between the plasma cells. We then compared the distribution of the measured distances with those of a randomly positioned model system. For the latter, we exported all 3D surfaces from Imaris into Blender (www.blender.org), where we used a Python script to rebuild the bone marrow structure and to position the plasma cells as 3D surfaces from the measured dataset. Comparing the measured and simulated (random) distances between the plasma cells and the three bone marrow compartments provides information about whether the plasma cell distribution is guided, *e.g.* by their need to remain near the vasculature for oxygen and nutrients, or if they are distributed randomly.

Automated image analysis methods for the quantification of cell damage and adherent fungal cells

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Epithelial tissues build the first line of defence against many microbial invaders like human pathogenic fungi. An impaired functionality of these barriers can lead to invasion and infection, but the exact mechanisms on host and pathogen side are still unknown. Therefore, studying the interplay of fungal and epithelial cells under different conditions is an important step to understand the mechanisms of microbial invasion. The conducted experiments study the interplay of *Aspergillus fumigatus* aspf2 mutants and wildtype with human lung epithelial cells and the effect of mesenteric ischemia on the barrier function of enterocytes and adhesion of *Candida albicans*.

During these experiments, microscopic images were generated to analyse the biological processes. Because manual analysis of these images is tedious, automated image analysis was used to provide objective and reproducible results for the quantification of epithelial cell damage and adhesion of fungal cells. We developed a library of computational methods for the analysis of fluorescent and electron microscopy images using simple thresholding combined with texture-based machine learning and advanced segmentation methods like convexity-based clump splitting to quantify damaged cell area and count fungal cells.

In vivo quantification of arthritis-induced alterations of murine bones based on PET/CT image data

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In vivo imaging has become an essential methodology to examine pathological processes in clinical studies as well as in animal models. Major advantages of *in vivo* imaging modalities, such as positron emission tomography (PET) or computed tomography (CT), are the ability to perform longitudinal studies and to acquire time-resolved, three-dimensional and high-resolution data. To tackle challenges that arise from the increased complexity, automated image analysis is required to process data within reasonable time and in an objective way.

In this work, we present the automated analysis of PET/CT image data to assess alterations of the bones longitudinally in an experimental model of rheumatoid arthritis (RA) [1, 2]. Analysis of µCT images was performed to quantify the degree of bone damage throughout the course of disease. This analysis comprised the quantification of cortical bone thickness, detection of volumes of interest in 3d CT images and quantification of bone surface roughness. Especially the latter turned out to be highly sensitive for early bone destruction. Furthermore, with this approach we detected clear differences between periosteal and endosteal bone surface alteration. These differences indicate that the roughness analysis is able to recognize bone erosion as well as new bone formation, depending on the respective parameters used for image analysis.

The results of our work demonstrate the usefulness of PET/CT imaging combined with automated image analysis to quantify arthritic processes in experimental RA. Additionally, the presented image analysis is not restricted to RA but can be applied to a wide field of bone-related diseases.

[1] B Hoffmann *et al.*, Automated quantification of early bone alterations and pathological bone turnover in experimental arthritis by *in vivo* PET/CT imaging. *Sci Rep* 7:2217, 2017.

[2] CM Svensson *et al.*, Quantification of arthritic bone degradation by analysis of 3D micro-computed tomography data. *Sci Rep* 7:44434, 2017.

3D ultrastructure analysis of the central nervous system via focused ion beam – scanning electron microscopy

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In order to understand the nervous system at a systemic level high-throughput analysis are necessary to investigate the cellular properties and network of its major constituents - neurons and glia cells. Although classical transmission electron microscopy reveals ultrastructural information, serial image acquisition necessary to gather 3D information for connectivity analysis has been challenging.

To overcome this limitation we established a workflow to acquire volumetric ultrastructural data of the mouse (*Mus musculus*) and fly (*Drosophila melanogaster*) central nervous system by applying Focused Ion Beam Scanning Electron Microscopy (FIB-SEM). Biological sample preparation and FIB-SEM parameters were optimized to obtain continuous structural data series allowing for reliable semi-automated cell structure identification, segmentation and tracing via open-source software TrakEM (https://fiji.sc/) and CatMaid (http://www.catmaid.org).

In *Drosophila melanogaster*, we compare the synaptic connectivity of the major neuronal cell types of glomeruli that constitute morpho-functional units within the olfactory lobes of insects. To facilitate investigation of the microarchitecture of the glomeruli of interest, we developed a correlative approach, by combining genetically encoded fluorescence labels, 2-photon laser branding to mark the glomerulus of interest and volume targeting electron microscopy by FIB-SEM.

In *Mus musculus*, we study the interaction of astroglia cells with neuronal synapses, which is important for signal transmission control. In detail, we address whether the astrocyte synapse contact depends on the Actin binding protein Ezrin, which specifically localizes to peripheral astrocyte processes. We seek to compare astrocyte contacts quantified from 3D synapse models acquired from conditional Ezrin knockout and wild type mice.

To facilitate the evaluation process of large data sets we are further aiming to apply automated segmentation algorithms in the future.

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