5th International Symposium on Image-based Systems Biology

October 1-2, 2020

HKI Center for Systems Biology of Infection, Jena, Germany

IbSB 2020 Program

Thursday 1 October

09.00 Opening

Session I: Deep learning for image enhancement and data augmentation

09.10-09.50 **Florian Jug**, Max-Planck Institute for Cell Biology and Genetics, Dresden, Germany

Image Restoration and Downstream Processing - old problems and new solutions

09.50-10.30 **Christophe Zimmer**, Institut Pasteur, Paris, France

Computational augmentations of single molecule super-resolution microscopy

10.30-10.50 **Takuya Ohmura**, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

3D segmentation of biofilms with convolutional neural networks

10.50-11.00 Break

11.00-11.30 Poster session & Networking I

Session II: Software and tools for quantitative image analysis, Part I

11.30-12.10 **Jason R. Swedlow**, University of Dundee, UK

OME's Bio-Formats, OME-TIFF, OMERO, & IDR: Making BioImage Data FAIR

12.10-12.30 **Hannah Jeckel**, Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

BiofilmQ, a quantiative image analysis tool

12.30-13.30 Break

Session II: Software and tools for quantitative image analysis, Part II

13.30-14.00 **Johannes Müller & Theresa Suckert**, Technical University Dresden, Germany

Slice2Volume - The next dimension of multi-modal image data fusion

14.00-14.20 **Ruman Gerst,** Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

JIPipe: a graphical batch-Processing language for ImageJ

14.20-14.40 **Lutz Brusch,** Technical University Dresden, Germany

Morpheus: A user-friendly simulation framework for multi-cellular systems biology

14.40-15.00 **Emad Alamoudi,** University of Bonn, Germany

FitMultiCell: Simulating and parameterizating computational models of multi-cellular processes

15.00-15.10 Break

15.10-15.40 Poster session & Networking II

Session III: Image analysis for advanced imaging systems and environments

15.40-16.20 **Susan Cox**, King's College London, UK

Faster and better: taking localisation microscopy into live cells

16.20-16.50 **Mai T.N. Hoang & Susann Hartung**, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

Quantitative characterisation of Aspergillus fumigatus hyphal growth and branching behaviour in an "Invasive aspergillosis-on-a-chip" disease model of the Human lung

16.50-17.20 **Zoltán Cseresnyés & Fatina Siwczak**, Leibniz Institute for Natural Product Research and Infection Biology & University Hospital Jena, Germany

Role of hepatic macrophage polarization in the acute phase of bacteraemia with Staphylococcus Aureus

17.20-18.00 Networking opportunities

Friday 2 October

Session IV: Deep learning for object detection and classification

09.00-09.40 **Carolina Wählby**, Uppsala University, Sweden

Deep Convolutional Neural Networks as a tool in image based Systems Biology

09.40-10.10 **Jan-Philipp Praetorius & Franziska Hoffmann**, Leibniz Institute for Natural Product Research and Infection Biology & University Hospital Jena, Germany

Automatic Segmentation and Classification of Fungal-Infected Tissue using Deep Learning

10.10-10.40 **Diego Ulisse Pizzagalli & Alain Pulfer**, University of Lugano, Switzerland

Action recognition in intravital microscopy: detecting cell death in vivo

10.40-10.50 ***Break***

10.50-11.20 ***Poster session & Networking III***

Session V: Image analysis and modelling of embryogenesis

11.20-12.00 **Sabine Fischer**, Julius-Maximilians University Würzburg, Germany

Spatio-temporal patterns of cell differentation in mouse blastocysts

12.00-12.20 **Justina Stark**, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Simulating morphogen gradients in zebrafish epiboly

12.20-13.20 ***Break***

Session VI: Image-based modelling

13.20-14.00 **Tiina Roose**, University of Southampton, UK

Image based modelling of plant-soil interaction

14.00-14.30 **Agustín Andrés Corbat & Mauro Silberberg**, University of Buenos Aires, Argentina

Unravelling apoptotic signalling cascade by co-imaging extrinsic, intrinsic and effector caspase activity by fluorescence anisotropy microscopy

14.30-14.50 **Daniel Wüstner**, University of Southern Denmark, Odense, Denmark

Quantitative imaging of membrane contact sites for sterol transfer between endo-lysosomes and mitochondria

14.50-15.10 **Muriel Heldring**, Leiden University, Netherlands

Data-driven kinetic modeling of p53 signaling linked to cell cycle progression

15.10-15.20 **Closing words**

15.20-17.20 ***Poster session & Networking IV***

Keynote Speakers

Faster and better: taking localisation microscopy into live cells

Susan Cox

King's College London, UK

Super-resolution microscopy is a powerful tool for imaging structures at a lengthscale of tens of nm, but its utility for live cell imaging is limited by the time it takes to acquire the data needed for an image. For localization microscopy the speed at which an image of a given structure can be acquired is directly linked to the structure being imaged, leading to a factor of more than 1000 difference in how fast a particular resolution can be achieved in different types of structure, even given identical performance of dyes and optics. Another result of this is that in almost all localization microscopy datasets there are images where fluorophores overlap. As an initial approach to this problem, we combined principle component analysis and a random forest classifier to allow data from overlapping fluorophores to be removed.

However, this approach can only deal with a small degree of overlap. For localisation microscopy the acquisition time can be cut by more than two orders of magnitude by using advanced algorithms which can analyse dense data, trading off acquisition and processing time. Information can be traded for resolution: for example, the whole dataset can by modelled as arising from blinking and bleaching fluorophores (Bayesian analysis of Blinking and Bleaching), although at a high computational cost. However, all these approaches will come with a risk of artefacts, which can mean that the image does not resemble the underlying sample. We have recently developed Harr Wavelet Kernel (HAWK) analysis, a multi-timescale prefiltering technique which enables high density imaging without artefacts. The results of benchmarking with other techniques reveal that at high activation densities many analysis approaches may achieve high apparent precision, but poor accuracy. However, HAWK analysis produces images free from sharpening artefacts allowing accurate images to be rapidly taken. Furthermore, this property of HAWK can be used to identify artificial sharpening artefacts and assess the quality of localisation microscopy images.

Spatio-temporal patterns of cell differentation in mouse blastocysts

Sabine Fischer

Julius-Maximilians University Würzburg, Germany

Tissue development and maintenance rely on coordinated interactions of individual cells. The correct composition of the three-dimensional cell neighbourhood is essential. However, often the details of the spatial arrangement of the cells are unknown and the processes underlying its establishment and maintenance are understudied. We focus on the development of the mouse blastocyst. Just before implantation, the cells of the inner cell mass differentiate into embryonic or extraembryonic precursor cells. Hallmarks of the cell fate decision are the expression levels of the two transcription factors NANOG and GATA6 in the cells. We quantitatively analysed the three-dimensional spatio-temporal arrangement of cells with different expression levels in ICM organoids and mouse embryos. We describe a so far unknown local clustering of cells with comparable expression levels that is already present at the early blastocyst stage. The cell fate decision occurs through a transition from such a local to a global pattern. Using mathematical modelling, we identify different mechanisms that could generate the observed patterns. Our results highlight the importance of analysing the three-dimensional cell neighbourhood while investigating cell fate decisions during early mouse embryonic development.

References:

Mathew *et al.* Biophys., 2019

Fischer *et al.* PLOS ONE, 2020

Image Restoration and Downstream Processing - old problems and new solutions

Florian Jug

Max-Planck Institute for Cell Biology and Genetics, Dresden, Germany

The necessity to analyze scientific images is as old as the ability to acquire such data. While this analysis did initially happen by observation only, modern microscopy techniques now enable us to image at unprecedented spatial and temporal resolutions, through the 'eyes' of many and very diverse imaging modalities.

The unfathomable amounts of data acquired in the context of biomedical research endeavors cannot any longer be analyzed by observation alone. Instead, algorithmic solutions help researchers to study and quantify large image data.

In the past 5 years, our abilities to use artificial neural networks (ANNs) for the automated analysis of scientific image data gained significant traction, and many important analysis problems have now much improved solutions based on ANNs. At the same time, we also become aware of the limitations that come with this new set of machine learning approaches.

In my talk I would like to update you on some of the latest algorithmic developments from our lab. More specifically, I will talk about improved and easy to use denoising and segmentation methods. Furthermore I will show how downstream processing tasks can benefit from the properties of our new methods. Finally, I will introduce you to the BioImage.IO Model Zoo -- a much needed and in our view very useful infrastructure we are currently building together with several other labs. The Model Zoo will help to improve the useability an cross-plattform sharability of ANN-based analysis solutions.

Image based modelling of plant-soil interaction

Tiina Roose

University of Southampton, UK

We rely on soil to support the crops on which we depend. Less obviously we also rely on soil for a host of 'free services' from which we benefit. For example, soil buffers the hydrological system greatly reducing the risk of flooding after heavy rain; soil contains very large quantities of carbon, which would otherwise be released into the atmosphere where it would contribute to climate change. Given its importance it is not surprising that soil, especially its interaction with plant roots, has been a focus of many researchers. However the complex and opaque nature of soil has always made it a difficult medium to study.

In this talk I will show how we can build a state of the art image based model of the physical and chemical properties of soil and soil-root interactions, i.e., a quantitative, model of the rhizosphere based on fundamental scientific laws. This will be realised by a combination of innovative, data rich fusion of structural and chemical imaging methods, integration of experimental efforts to both support and challenge modelling capabilities at the scale of underpinning bio-physical processes, and application of mathematically sound homogenisation/scale-up techniques to translate knowledge from rhizosphere to field scale. The specific science questions I will address with these techniques are: (1) how does the soil around the root, the rhizosphere, function and influence the soil ecosystems at multiple scales, (2) what is the role of root- soil interface micro morphology on plant nutrient uptake, (3) what is the effect of plant exuded mucilage on the soil morphology, mechanics and resulting field and ecosystem scale soil function and (4) how to translate this knowledge from the single root scale to root system, field and ecosystem scale in order to predict how the climate change, different soil management strategies and plant breeding will influence the soil fertility.

OME's Bio-Formats, OME-TIFF, OMERO, & IDR: Making BioImage Data FAIR

Jason R. Swedlow

University of Dundee, UK

Despite significant advances in biological imaging and analysis, major informatics challenges remain unsolved: file formats are proprietary, storage and analysis facilities are lacking, as are standards for sharing image data and results. The Open Microscopy Environment (OME; http://openmicroscopy.org) is an open-source software framework developed to address these challenges. OME releases specifications and software for managing image datasets and integrating them with other scientific data. OME's Bio-Formats and OMERO are used in 1000's of labs worldwide to enable discovery with imaging. OME-TIFF is an open, metadata-rich, multi-dimensional, multi-resolution data format for modern bioimaging that has been widely adopted across the bioimaging community.

We have used Bio-Formats and OMERO to build solutions for sharing and publishing imaging data. The Image Data Resource (IDR; https://idr.openmicroscopy.org) includes image data linked to >60 independent studies from genetic, RNAi, chemical, localisation and geographic high content screens, super-resolution microscopy, single cell profiling, light sheet microscopy of developing organisms and tissues, and digital pathology. Datasets range from several GBs to tens of TBs. Wherever possible, we have integrated image data with all relevant experimental, imaging and analytic metadata. These annotations make it possible to re-use IDR data, and to connect independent imaging datasets by molecular perturbations and phenotypes.

We have built cloud-based analysis tool portals to catalyse the re-use of published imaging data. These include notebooks and Docker containers that package well-known tools like ImageJ/Fiji, CellProfiler and Ilastik, making it easy to view and interact with IDR data. We are also trialling new data formats based on Zarr, N5 and other cloud-competent binary vessels to improve adoption of modern cloud technologies in bioimaging.

Deep Convolutional Neural Networks as a tool in image based Systems Biology

Carolina Wählby

Uppsala University, Sweden

Biological processes can be observed both in space and over time using imaging. Visual assessment becomes limiting as datasets grow, and complexity of data as well as subtleness of processes makes it difficult to draw confident conclusions without automated and quantitative measurement strategies. Traditionally, digital image processing has relied on engineering mathematical models of e.g. the size and shape of cell nuclei, surrounding cytoplasm and fluorescent signal distributions, to extract measurements and apply classification strategies. These methods are powerful, but they are also limited by how well we manage to find a good set of feature descriptors for what we observe. In the past ten years, learning-based approaches relying on deep convolutional neural networks (DCNNs) have gained enormous popularity in all fields of image-based science. The methods have great potential, but they also require care in their usage, where again, the traditional image processing methods play crucial role. We develop and apply combinations of traditional and learning-based methods in a range of areas of systems biology: We apply DCNNs in understanding of cell and bacterial dynamics, drug screening in model organisms, and in decoding and exploration of spatially resolved gene expression in tissue and show results on segmentation of time-lapse imaging of unstained cells, unsupervised feature extraction, and learning of localized gene expression patterns. Compared to traditional methods, where we have full control of the mathematics behind each step pin an analysis pipeline, DCNNs are more difficult to interpret and explain. We also see that learning-based approaches have their limitations, both when it comes to training approaches, data handling and quality control – these factors all become is crucial for successful methods implementation.

Computational augmentations of single molecule super-resolution microscopy

Christophe Zimmer

Institut Pasteur, Paris, France

Single molecule localization microscopy (SMLM) has matured into one of the most powerful and widely used super-resolution imaging methods. In this talk, we'll highlight recent developments of our lab to push the limits of SMLM using computational approaches.

One long-standing challenge is to visualize cells at high resolution and with high throughput. SMLM delivers exquisite spatial resolution, but at the price of very low throughput. Previous approaches to accelerate SMLM typically trade off spatial resolution. We present ANNA-PALM, a computational technique based on deep learning that can reconstruct high resolution views from strongly under-sampled SMLM data and widefield images, enabling considerable speed-ups without any compromise on spatial resolution. We illustrate ANNA-PALM's robustness and potential for high throughput super-resolution imaging and highlight a dedicated web platform (annapalm.pasteur.fr). We will also discuss limitations and perspectives of ANNA-PALM.

Another challenge is to extend SMLM to 3D imaging of entire cells. While many approaches for 3D SMLM have been proposed, the need remains for a more accessible and flexible technique. We present ZOLA-3D a combined optical and computational method that enables versatile 3D super-resolution imaging over up to ~5 um depth. Software and sample data are freely available from github.com/imodpasteur/ZOLA-3D.

Finally, the microscopy field could greatly benefit from easier access to SMLM data generated by the community, especially to train machine learning models. We will briefly highlight shareloc.xyz, an online platform to facilitate the sharing and reanalysis of SMLM data.

Talks

FitMultiCell: Simulating and parameterizating computational models of multi-cellular processes

Emad Alamoudi

University of Bonn, Germany

Biological tissues are often dynamic and highly organized. Its Spatio-temporal patterns are relevant in many biological processes such as tissue homeostasis, viral infection, or tumor development. These processes can be studied by imaging techniques including light and fluorescence microscopy. Quantitative information about biological systems can be inferred from the biological imaging data, however, the mechanisms that cause the spatial patterning often remain elusive. Computational modeling tools are getting more attention as a way to understand the multicellular Spatio-temporal processes. In recent years, there was a focus on imaging, data analysis, and simulation techniques, however, the rigorous parameterization of multicellular models is becoming accessible through the advance in the methods and the computational resources. The parametrization of the multicellular models form high-throughput and high-content (imaging) data is essential to compare competing hypotheses, to understand the multicellular processes, and to predict the perturbation experiments. A method that has been proven to be applicable to multi-cellular models is Approximate Bayesian Computation (ABC). Unfortunately, ABC is a computationally expensive approach, as it requires a large number of simulations. Thus, there is an increased need for a fast and general-purpose pipeline for modeling and simulating multi-cellular systems that can exploit HPC systems for faster computations. To this end, we started the development of a user-friendly, open-source, and scalable platform, called FitMultiCell, which aims to build and validate an open platform for modeling, simulation, and parameter estimation of multicellular systems, which will be utilized to mechanistically answer biomedical questions based on imaging data. To achieve the goal of FitMultiCell, we combine the modeling and simulation tool Morpheus with the advanced statistical inference tool pyABC. In this contribution, we present an overview of the FitMultiCell pipeline anddemonstrate an application example. An HCV model that was described by Kumberger et al. (Viruses, 10(4), 2018) was used to illustrate the flow of the platform.

Unravelling apoptotic signalling cascade by co-imaging extrinsic, intrinsic and effector caspase activity by fluorescence anisotropy microscopy

Agustín Andrés Corbat & Mauro Silberberg

University of Buenos Aires, Argentina

Intercellular variability can be both a source of information and a confounding factor in understanding signal propagation in biological networks. It is crucial to properly sample the population, which demands high-throughput methods. In turn, these require not only automated microscopy, but also automated analysis. Therefore, we developed a set of algorithms to address that challenge. Using a statistical approach, they leverage both spatial and temporal information for background estimation, segmentation and tracking of cells. To handle intercellular variability, it is also imperative to quantify multiple biological observables simultaneously. To that end, we designed three spectrally-separated anisotropy-based FRET biosensors to simultaneously observe the enzymatic activity of three key caspases in the apoptotic signalling network. While a biosensor provides a photophysical observable, it is not the readout of interest but a proxy of the biological observable. Properly transforming it, requires both accurate determination of the photophysical observable, and that the biosensor perturbs as little as possible the biological signal of interest. Regarding the former, in anisotropy quantification, it is important to properly subtract background noise, which our algorithm allows us to recover unbiasedly. For the latter, modelling showed that different concentrations of our biosensors affected the response of the system. Hence, we designed a single-plasmid triple-modality reporter to control stoichiometry of biosensors. Additional insight from modelling showed that estimating the maximum activity from the anisotropy readout is a robust proxy, which reduces the variation due to both absolute concentration and caspase functional profile. Finally, interplay between modelling biological observables and correlating with experimentally found ones allows better understanding of signal propagation as well as its refinement.

Morpheus: A user-friendly simulation framework for multi-cellular systems biology

Lutz Brusch

Technical University Dresden, Germany

Computational modeling and simulation become increasingly important to analyze tissue morphogenesis. A number of corresponding software tools have been developed but require scientists to encode their models in an imperative programming language. Morpheus [1,2], on the other hand, is an extensible open-source software framework that is entirely based on declarative modeling. It uses the domain-specific language MorpheusML to define multicellular models through a user-friendly GUI and has since proven applicable by a much broader community, including experimentalists and trainees.

We here present how MorpheusML and the open-source framework [3] allow for rapid model prototyping and advanced scientific work-flows. MorpheusML provides a bio-mathematical language in which symbolic identifiers in mathematical expressions describe the dynamics of and coupling between the various model components. It represents the spatial and mechanical aspects of interacting cells in terms of the cellular Potts model formalism and follows the software design rule of separation of model from implementation, enabling model sharing, versioning and archiving. A numerical simulation is then composed by parsing the MorpheusML model definition and automatic scheduling of predefined components in the simulator. Moreover, Morpheus supports simulations based on experimental data, e.g. segmented cell configurations, and offers a broad set of analysis tools to extract features right during simulation. A rich C++ API allows to extend MorpheusML and the simulator with user-tailored plugins.

Finally, we apply Morpheus and image-based modeling to study the regulatory mechanisms underlying liver tissue architecture [4] and flatworm regeneration [5].

[1] Starruß *et al.* Bioinformatics, 2014
[2] Morpheus homepage: https://morpheus.gitlab.io
[3] Source code: https://gitlab.com/morpheus.lab/morpheus
[4] Kolbe *et al.* Cell Reports, 2019
[5] Vu *et al.* Developmental Cell, 2019

Role of hepatic macrophage polarization in the acute phase of bacteraemia with Staphylococcus Aureus

Zoltán Cseresnyés & Fatina Siwczak

Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

University Hospital Jena, Germany

Invasive pulmonary aspergillosis is a great threat to immunocompromised patients as treatment options are limited and only successful upon early diagnosis. Infectious agents are conidia of the mold *Aspergillus fumigatus* that enter the lung alveoli but, in immunocompetent humans, are cleared by innate immune cells such as macrophages. In immunocompromised patients, however, conidia can grow into filamentous hyphae leading to tissue destruction and invasion of blood vessels. To date, complex human cell-based models to investigate invasive aspergillosis are rare and often lack the quantification of growth parameters of individual hyphae.

Our novel “invasive aspergillosis-on-a-chip” model is composed of human lung epithelial cells at an air-liquid-interface and human endothelial cells separated by a porous membrane. Models were infected by *A. fumigatus* conidia on the epithelial side and fungal growth was detected by confocal microscopy. 3D bioimage data were analysed using (semi)automated systems biology methods. The structure of the models was reconstructed by the fluorescence of Calcofluor White-labelled hyphae, FITC-labelled conidia and the reflected light image identifying the membrane pores. However, automated reconstruction of hyphae from fungal clusters could result in wrongly connected filaments and had to be manually corrected. Reconstructed hyphae allowed us to compute the morphometric measures of the individual hyphae (length, number of branches and branching levels) in 3D. Additionally, computational analysis of the 3D reconstructed membrane pores and hyphae revealed an interesting turning behavior of invading hyphal branches under physiologic and diseased conditions as well as under influence of antifungal drugs.

The development of this versatile “invasive aspergillosis on a chip” system is very promising regarding its potential applications in understanding pathophysiology in invasive aspergillosis and providing a tool for animal-free drug screening.

JIPipe: a graphical batch-Processing language for ImageJ

Ruman Gerst

Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

Bioimage analysis involves the development of novel algorithm pipelines that are easy to adapt to new data sets. A common way to create such tools is to develop scripts in languages like ImageJ macros [1], which requires programming experience and detailed knowledge of the available script functions. Alternatively, there are graphical programming languages like KNIME [2] and Icy protocols [3] that allow users to create complex pipelines by creating diagrams. Each tool comes with its own solutions for managing data sets that differ greatly in simplicity for beginners, verbosity in handling complex data sets, and how ImageJ is integrated into their own ecosystem.

Here we present Java Image Processing Pipeline (JIPipe), a plugin for ImageJ that provides a graphical image processing language that not only includes functions from ImageJ, but also lets developers and non-programmers create new functions that can be used from within ImageJ. JIPipe introduces a new data model as hybrid between the easy-to-learn design of Icy and script-like style used by KNIME. Data is organized in tables where one column contains binary data such as images and additional text columns are used to annotate data. Such annotations can be used by algorithms to process data without the need for complicated control structures. JIPipe comes with over 400 image, table, plot and ROI processing algorithms, automated parallelization and result-exporting, and well-illustrated training material.

[1] C. T. Rueden *et al.*, BMC Bioinformatics, 2017.
[2] M. R. Berthold *et al.*, “KNIME: The Konstanz Information Miner,” 2007.
[3] F. De Chaumont *et al.*, Nat. Methods, 2012.

Data-driven kinetic modeling of p53 signaling linked to cell cycle progression

Muriel Heldring

Leiden University, Netherlands

Cellular homeostasis is essential for maintaining a healthy and normal-functioning liver. Homeostatic disruption caused by chemical compounds can cause temporary or chronic liver injury and even complete liver failure. There is a vast array of proteins involved in cellular homeostasis that controls the response to diverse types of stress. Among the most important is the transcription factor p53, primarily known for its function to maintain genomic stability, regulate transient and permanent cell cycle arrest and apoptosis. Activated p53 transcriptionally regulates the expression of many proteins, among which are Mdm2, p21 and Btg2. Mdm2 functions as a direct inhibitor of p53 by targeting it for ubiquitination, which results in a p53-Mdm2 negative feedback loop. The protein p21 is known for its regulatory function in both G1 and G2 cell cycle arrest and Btg2 has been found to play a role in G1 arrest. Based on time-resolved protein expression and cell cycle data in HepG2 cells exposed to cisplatin, we developed an ODE model, aiming to describe the protein expression dynamics of p53, Mdm2, p21 and Btg2 and link the expression patterns to cell cycle progression. With the developed model, we are able to mechanistically describe the interactions between proteins involved in the DNA damage response with p53 as central regulator. We linked the modelled protein expression dynamics to cell cycle progression to determine the importance of p21 and Btg2 in their regulation of G1 and G2 cell cycle arrest. We found that the expression of p21 and Btg2 can predict G2 cell cycle arrest, but is not sufficient to explain subsequent cell cycle continuation. In the future, we will perform *in silico* protein knockdowns to study the effect of pathway perturbations on cell cycle progression.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002 (EU-ToxRisk).

BiofilmQ, a quantiative image analysis tool

Hannah Jeckel

Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Biofilms are a highly abundant form of microbial life on Earth, playing critical roles in biogeochemical cycles, agriculture, and health care. Phenotypic and genotypic variations in biofilms generally occur in three-dimensional (3D) space and time, and biofilms are therefore often investigated using microscopy. However, the quantitative analysis of 3D fluorescence microscopy images presents a key obstacle for phenotyping biofilm communities as a whole, and for phenotyping the cellular heterogeneity inside biofilms. Here we present BiofilmQ, a comprehensive image cytometry software tool for the automated, high-throughput quantification and visualization of community properties. The tool enables the quantification of numerous parameters for biofilm-internal properties and whole-biofilm properties in space and time. The quantified community properties can be visualized in spatiotemporal kymographs, demographs and a multitude of other editable figures within BiofilmQ by users without programming expertise. BiofilmQ is designed for analysing fluorescence images of a wide variety of spatially structured microbial communities and growth geometries, including microscopic, mesoscopic, macroscopic colonies and aggregates, as well as bacterial biofilms in the context of eukaryotic hosts.

Quantitative characterisation of *Aspergillus fumigatus* hyphal growth and branching behaviour in an "Invasive aspergillosis-on-a-chip" disease model of the Human lung

Mai T.N. Hoang & Susann Hartung

Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

Invasive pulmonary aspergillosis is a great threat to immunocompromised patients as treatment options are limited and only successful upon early diagnosis. Infectious agents are conidia of the mold *Aspergillus fumigatus* that enter the lung alveoli but, in immunocompetent humans, are cleared by innate immune cells such as macrophages. In immunocompromised patients, however, conidia can grow into filamentous hyphae leading to tissue destruction and invasion of blood vessels. To date, complex human cell-based models to investigate invasive aspergillosis are rare and often lack the quantification of growth parameters of individual hyphae.

Our novel "invasive aspergillosis-on-a-chip" model is composed of human lung epithelial cells at an air-liquid-interface and human endothelial cells separated by a porous membrane. Models were infected *by A. fumigatus* conidia on the epithelial side and fungal growth was detected by confocal microscopy. 3D bioimage data were analysed using (semi)automated systems biology methods. The structure of the models was reconstructed by the fluorescence of Calcofluor White-labelled hyphae, FITC-labelled conidia and the reflected light image identifying the membrane pores. However, automated reconstruction of hyphae from fungal clusters could result in wrongly connected filaments and had to be manually corrected. Reconstructed hyphae allowed us to compute the morphometric measures of the individual hyphae (length, number of branches and branching levels) in 3D. Additionally, computational analysis of the 3D reconstructed membrane pores and hyphae revealed an interesting turning behavior of invading hyphal branches under physiologic and diseased conditions as well as under influence of antifungal drugs.

The development of this versatile "invasive aspergillosis on a chip" system is very promising regarding its potential applications in understanding pathophysiology in invasive aspergillosis and providing a tool for animal-free drug screening.

Slice2Volume - The next dimension of multi-modal image data fusion

Johannes Müller & Theresa Suckert

Technical University Dresden, Germany

In recent years, bio-medical research has made tremendous progress regarding imaging modalities and techniques for accurate patient diagnostics. On the other hand, these staggering amounts of imaging data from various modalities pose new challenges. Additionally, the underlying biological mechanisms that bring forth macroscopic image changes remain uncertain. Patient biopsies or autopsies can offer valuable insights, but remain rare and precious. A way to bridge this knowledge gap is to establish a preclinical model for direct correlation of biological tissue properties and image features. To exploit the full potential of such experiments, methods are required for the fusion of multi-modal bio-medical imaging data, i.e. computed tomography (CT), magnetic resonance imaging (MRI), simulated dose distributions, and anatomical atlas data with histology. We irradiated mouse brain subvolumes with different doses and investigated the radiobiological response. All animals received a CT prior to radiation, which was used for a Monte-Carlo beam transport simulation to obtain the spatial dose-distribution. A brain atlas was co-aligned with the CT to resolve regional anatomical variances. Brains were excised after follow-up for histological evaluation; depending on the specific research question different stainings were applied such as H&E (general morphology), DNA damage (γH2AX), and cell-type specific markers (e.g. NeuN for neurons). To enable analysis with high spatial resolution, 30 – 40 planes were acquired per brain using a Zeiss AxioScan slide scanner for high throughput imaging. We established and implemented a robust and easy-to-use registration work-flow to fuse the generated data within reasonable time. Our tandem talk will focus on the recent progress of this project. First, we had to accomplish high-precision irradiation of mouse brain subvolumes. This was achieved with the in-house developed software RadiAIDD (github.com/jo-mueller/RadiAIDD), which combines different imaging data for optimal positioning [1], [2]. To analyze the biological radiation effect, we developed a fast and reliable algorithm for cell counting based on a spot detection (github.com/Theresa-S/Cell-ratio-detection) [3] and created a pipeline to align stacked 2D histological images, 3D imaging data (CT, MRI; github.com/jo-mueller/Slice2Volume), dose simulations, and volumetric atlases. With this platform, we can unravel the missing dimension from clinical data and apply this knowledge to improve patient care.

[1] J. Müller *et al.* Acta Oncol. (Madr.), 2017

[2] J. Müller *et al.* Biomed. Phys. Eng. Express,. 2020

[3] T. Suckert *et al.* Radiother. Oncol., 2020

3D segmentation of biofilms with convolutional neural networks

Takuya Ohmur

Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

To survive and grow efficiently in harsh environments, bacterial cells make 3D colonies on surfaces, called biofilms. Biofilms are considered as the most abundant form of microbial life on Earth. To obtain an understanding of the development and heterogeneity inside biofilms, it is important to extract quantitative parameters for biofilms in space and time during growth. Single-cell quantitative analysis of images has been required to obtain the spatio-temporal analysis. During the last decade, microscopic technique significantly has improved, which enabled us to acquire 3D images of biofilm with single-cell resolution. To extract spatio-temporal information from the 3D images, individual cell inside biofilm have to be segmented. Several cell-segmentation softwares already have been used for biofilm segmentation, but those softwares still could not detect all cells in biofilms without errors. To achieve a higher accuracy of segmentation, convolutional neural network (CNN) for cell segmentation has been developed rapidly in the last few years. The CNN requires experimental raw image data and manually annotated labels as training data. The amount of accurate training data is critical for the accuracy of segmentation, but manual annotation of accurate cell labels in 3D images consumes a lot of time and manpower. Therefore, we developed the protocol to multiply accurate training dataset, to obtain 18,000 annotated cells in 3D. As a result, our trained CNN model achieved higher accuracy of segmentation than the previous software solutions. We believe that such highly accurate segmented data provides the key for single-cell tracking during biofilm growth.

Action recognition in intravital microscopy: detecting cell death *in vivo*

Diego Ulisse Pizzagalli & Alain Pulfer

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Action recognition (AR) is a technique, widely used to analyze the behavior of humans from digital videos. AR detects specific motility patterns a displayed when performing certain actions (i.e. crossing a road, forming aggregates, or leaving a bag), with important applications in video-surveillance and autonomous driving.

Similar to humans, the cells of the immune system display a broad range of motility patterns (i.e. while patrolling, forming swarms, or during killing). These patterns reflect biological mechanisms that are crucial to understand the dynamics of the immune response.

Thanks to the development of intravital microscopy protocols, the behavior of cells can be captured inside organs of living organisms, and recorded in digital videos. However, the classical pipeline to analyze this type of data involves cell segmentation and tracking, which are particularly challenging. Interestingly, AR methods do not require these steps and represent a promising option to quantify cell behavior. However, the application of AR in microscopy remains poorly explored.

Amongst all the actions, we focus on apoptosis - a type of regulated cell death involved in several biological processes, including development, homeostasis, infection and cancer. Apoptotic cells display specific morphodynamic hallmarks, characterized by shrinkage, folding, and disruption of the cell membrane into blebs. We created the first dataset of intravital microscopy videos of cells undergoing and responding to apoptosis. Then, we developed a method to detect apoptotic cells based on morphodynamic hallmarks. This method employs state-of-the-art computer vision techniques for AR such as optical flow and deep neuronal networks, obtaining an accuracy similar to human experts. Finally, we evaluated the response of neighboring cells to apoptotic events, confirming the recently discovered “non-silent” properties of this type of cell death.

Automatic Segmentation and Classification of Fungal-Infected Tissue using Deep Learning

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If the human immune system is weakened, fungal infections can lead to a life-threatening condition in humans. In order to determine the type of fungus, the pathologist has to cut out a piece of tissue from the organ to be examined. A common method of highlighting the fungi is to stain the entire sample slices. An alternative and relatively new method is MALDI-imaging (matrix-assisted laser desorption ionization), in which the mass spectrum of the tissue is measured without destroying the sample. Regardless of which images are used for the pathologist's examination, the pathologist needs a lot of time to examine the entire tissue due to the far above-average size of the image.

In more recent research questions, deep learning has become increasingly important in the field of image-based systems biology. Here we learn a convolutional neuronal network that is able to learn features of images to distinguish between normal tissue and fungal-tissue on one or more tissue slices. Preliminary results on the such a neuronal network is capable to classify certain fungal species such as *Aspergillus fumigatus*, *Mucorales* and *Candida albicans*. We provide a framework that is able to examine these huge images and automatically segment and classify various types of fungi on various types of organs.

Simulating morphogen gradients in zebrafish epiboly

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Studying embryo and tissue morphogenesis is challenging because of the large number of factors influencing each other and the complex and dynamic geometries in which this happens. Computational methods provide a way to disentangle the different processes by gaining control over specific parameters. In this work, we construct a three dimensional (3D) model of a developing zebrafish embryo during epiboly from images. This model is then used to simulate morphogen gradient formation and growth.

Both geometry reconstruction and simulation are done using a level set method (LSM) and particle methods (PMs). The LSM facilitates tracking of topological changes, such as cell divisions - an important feature when simulating growth during embryonic development. One advantage of PMs over mesh based numerical schemes is that PMs are more straightforward in handling complex and deforming geometries, as they do not require the generation and maintenance of an (unstructured) mesh.

In particular, we apply this method to reconstruct the geometry of the complex-shaped 3D extracellular space of a zebrafish embryo undergoing epiboly. Reconstruction is based on confocal fluorescence microscopy images, which were acquired by Rohit Krishnan Harish from the lab of Prof. Michael Brand (CRTD, TU Dresden). In the subsequent simulation, we aim to numerically reconstitute the coupled processes of cell migration, space deformation, and gradient formation of Fibroblast growth factor 8 (Fgf8) during epiboly, deducing the spatio temporal information from light-sheet microscopy movies and live FCS measurements.

We believe that our approach provides a flexible and efficient way for gaining insight into biophysical processes of tissue formation in realistic, complex biological systems by mathematically formulating the information that we can derive from microscopy image data.

Quantitative imaging of membrane contact sites for sterol transfer between endo-lysosomes and mitochondria

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Mitochondria form contacts to endosomes and endoplasmic reticulum, but little is known about the dynamics of such membrane contact sites (MCSs). Mitochondria receive cholesterol from late endosomes and lysosomes (LE/LYSs) and the plasma membrane (PM) for production of oxysterols and steroid hormones in a process depending on the endo-lysosomal sterol transfer protein Niemann Pick C2 (NPC2). In this study, we present novel image analysis methods to automatically quantify the distance between LE/LYSs containing NPC2 and mitochondria from live-cell microscopy data. By localizing endo-lysosomes relative to mitochondria based on the Euclidian distance transform, we show that about 70% of all NPC2 containing LE/LYSs are in close proximity (< 150 nm) to mitochondria in human fibroblasts. Using Markov Chain Monte Carlo (MCMC) image simulations within the cell geometry, we show that such a spatial pattern can be caused by an attractive interaction between both organelle types, which is characteristic for MCSs. Using soft X-ray microscopy, we determine the ultrastructure of MCSs between both organelle types. Single particle tracking of MCSs over time shows that they either form transiently in a kiss-and-run fashion, or they remain stable for tens of seconds. Lasting MCSs containing NPC2 move very slowly along mitochondria by anomalous sub-diffusion. Using the intrinsically fluorescent cholesterol analog, cholestatrienol (CTL), we directly observe sterol transport to mitochondria in fibroblasts upon treating NPC2 deficient human fibroblasts with NPC2 protein. Our quantitative imaging strategy will be of high value for characterizing the dynamics and function of MCSs between various organelles in living cells

Posters

Image-based analysis of *Candida albicans* invasion in a gut-on-chip model

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Candida albicans is a commensal fungus of the human microbiota that, under certain circumstances, can become pathogenic and cause life-threatening systemic infections. Several factors influence *C. albicans* virulence, but the specific triggers to this commensal-to-pathogenic shift are poorly understood. To study this in a more physiological approach, we are using a gut-on-chip model. This consists of two compartments with flow which are separated by a porous membrane: the vascular with endothelial and immune cells; and the intestinal, with functional villi and crypts.

Our goal is to analyze the interactions of *C. albicans* with the host and the factors that influence them. The first experiments aim at studying the hyphal trajectories across the barrier to dissect influential chemotropic or thigmotropic factors.

Preliminary data showed that differential staining can be performed on the epithelial cells (E-Cadherin), endothelial cells (V-Cadherin), hyphae (calcofluoro white), and yeast cells (FITC). This enables a segmentation of all major components of the system, based on transmitted light and fluorescence imaging.

To extract a maximum of information from the segmented images at low computational effort, we transform the segmentation masks of *C. albicans* hyphae into directed graph structures. The graph roots are defined by the corresponding yeast cells. This transformation allows a flexible and fast analysis of hyphal growth characteristics and, in combination with a polygon representation of the segmented epithelial surface, a versatile analysis of the positional relations of all major components of the system.

The realistic rebuild of the intestinal epithelium in combination with the segmentation-based analysis should enable the investigation of *C. albicans* infections under *in vivo*-like circumstances, as well as to manipulate the environment, leading to changes in hyphal length, morphology or branching.

Enhanced segmentation of label-free cells for automated migration and interaction tracking

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In biomedical research, the migration behaviour of cells and interactions between various cell types are frequently studied subjects. An automated and quantitative analysis of time-lapse microscopy data is an essential component of these studies, especially when characteristic migration patterns need to be identified. Plenty of software tools have been developed to serve this need. However, the majority of algorithms are based on data with fluorescently labelled cells, even though it is well-known that fluorescent labels can substantially interfere with the physiological behaviour of interacting cells. We here present a fully revised version of our algorithm for migration and interaction tracking (AMIT), which includes a novel segmentation approach. This approach allows segmenting label-free cells with high accuracy and also enables detecting almost all cells within the field of view. With regard to cell tracking, we designed and implemented a new method for cluster detection and splitting. This method does not rely on any geometrical characteristics of individual objects inside a cluster, but relies on monitoring the events of cell-cell fusion from and cluster fission into single cells forward and backward in time. We demonstrate that focusing on these events provides accurate splitting of transient clusters. Furthermore, the substantially improved quantitative analysis of cell migration by the revised version of AMIT is more than two orders of magnitude faster than the previous implementation, which makes it feasible to process video data at higher spatial and temporal resolutions.

A Multimodality and Multiscale Imaging Pipeline for Image-Based Vascular Systems Biology

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Image-based systems biology is emerging as a powerful new paradigm in cancer research. However, there is an exigent need for a new image-based systems biology framework to better understand the role of the vasculature in cancer progression, metastasis and therapeutic response. In spite of recent advances in vascular imaging, there is a dearth of methods that make blood vessels simultaneously visible in the primary imaging modalities, i.e. magnetic resonance imaging (MRI), computed tomography (CT) and optical microscopy. This poses a hurdle to the integration of vascular data across multiple spatial scales or with tumor microenvironment (TME) data acquired using complementary image contrast mechanisms, such as white matter fiber from MRI (~40µm), bone contrast from CT (9µm), protein expression from multiphoton (MPM) (<1µm), all of which can function as invaluable inputs to image-based systems biology models. Moreover, since their spatial resolution span several orders of magnitude, integrating these data via conventional image co-registration approaches also remains a challenge. Therefore, we developed a “multicontrast” vascular contrast agent combination that makes vasculature visible in MRI, CT and optical imaging. This vascular contrast agent combination does not hinder conventional imaging contrast mechanisms and also provides ‘internal’ fiducials that enable co-registration of these multiscale, multimodality imaging data. Moreover, the high-fidelity 3D microvascular data generated from this imaging pipeline can be combined with computational hemodynamic modeling to simulate functional TME properties (e.g. tumor blood flow). We demonstrate the utility of our pipeline by conducting multiscale, multimodality imaging of the TME in an orthotopic breast cancer model. Finally, our imaging pipeline has direct applications in image-based systems biology of healthy tissues, and in other disease models involving the vasculature (e.g. stroke and traumatic brain injury).

Semi-supervised machine learning facilitates segmentation and tracking in fluorescence microscopy

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Fluorescence microscopy is one of the most useful techniques to investigate the location and the interactions between cells *in vivo* and *in vitro*. With that technique it's possible to study cellular mechanisms in a non-invasive method by using the light emitted by matter when it is excited with electromagnetic waves. The main subsequent pros are the possibility to use *in vivo* approaches and the extreme probes sensibility. On the other hand, several disadvantages come from the photobleaching effect and the environmental susceptibility. Infact, since a lot of natural disturbing elements can interfere with the video quality/stability, it's not rare to see target cells emit in more than one channel or with different intensity in time. If there is not a way to specify these information, errors in data interpretation and analysis could occur. Altogether, these challenges hamper automatic cell segmentation and tracking, making this process poorly reproducible and requiring manual correction. Here we propose a new method to easily identify target cells independently from the channel and morphology. It combines unsupervised machine learning and supervised machine learning methods. Using the former it is possible to group all the pixels into Super pixels and then simplify the image complexity. The latter allows to classify them accordingly with the user's accommodation as cells or background. This method overcomes heavy computational calculations relying on superpixel decomposition and uses the indication of the user as input of a superpixel classifier. This method facilitated cell segmentation and tracking in challenging cases like multichannel videos with autofluorescent bodies or when cells form masses hard to separe. Even when there are overlapping regions or poor visibility, super pixel classification is preferred to deep learning algorithms to identify the foreground and the background.

Automated detection of nascent myofibrils during myofibrillogenesis

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All our voluntary movements are actuated by striated muscle, where each muscle cell contains highly regular myofibrils comprising a periodic arrangement of sarcomere units built of actin filaments, myosin molecular motors and cross-linking proteins. How myofibrils self-assemble during myofibrillogenesis into such 'cytoskeletal crystals' remains poorly understood. To better understand this process, we have developed a Matlab-based feature detection algorithm which analyses multi-channel fluorescence images taken at distinct myofibrillogenesis time points. The automated analysis allows us to compute physical order parameters and cross-correlation functions of concentration profiles for a large statistics of nascent sarcomeres as a function of time. We apply our pipeline to fluorescence images of fixed flight muscle samples of Drosophila, provided by collaboration partners from the Schnorrer lab (IBDM, Marseilles). The aim of the algorithm is to track elongated myofibrils and to identify protein patterns along these myofibrils. Specifically, the giant protein titin appears at a characteristic position in each sarcomere to identify individual sarcomeres. Our algorithm first detects titin dots in the respective fluorescence channel. Myofibril by comparing the integrated signal of an actin channel of 'sarcomere candidates' between nearby titin dots. By combining the information from the two channels, our algorithm identifies individual sarcomeres in images with minimal user input. Our algorithm allows for fast identification of myofibrils in both two-dimensional slices, and three-dimensional data Z-stacks. Our analysis constrains the space of self-assembly mechanisms, e.g., by identifying which constituents drive the initial pattern formation and which follow subsequently. These results directly inform a parallel modelling approach in our group, as well as the design of experiments in our collaboration with the Schnorrer lab.

MISA++: a modular and high-performance framework for analysis of light sheet microscopy images

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Light sheet fluorescence microscopy (LSFM) produces very large data sets that are difficult to analyze manually. An alternative to such a time-consuming and biased process is a fully automated analysis. Due to the large data volume, it is essential to improve the efficiency via parallelization and optimized memory-management. The programming language C++ is ideal for such high-performance tasks, yet misses standardization of algorithm parameters, modularization, parallelization, and integration into established open-source frameworks such as ImageJ [1].

MISA++ is an open-source framework that allows creation of modularized, task-oriented and efficient C++ programs. It utilizes widely used libraries like OpenCV (https://opencv.org/) and OME [2] to allow developers to rapidly create multi-threaded and memory-efficient image analysis applications. It includes features such as a standardized input and output data structure, standardized sample metadata and algorithm parameters, automated memory management, performance analysis, and a standardized metadata and result storage.

We developed an ImageJ plugin that offers user interfaces that allow setting algorithm parameters, creating pipelines and managing input data such as images with the option to directly import data from ImageJ. The plugin takes advantage of the high standardization and ability to automatically generate a human- and machine-readable documentation to automatically extract information about the input and output data and the available algorithm and sample parameters.

[1] C. T. Rueden *et al.* BMC Bioinformatics, 2017.
[2] M. Linkert *et al.* J. Cell Biol., 2010.

Objective and quantitative assessment of liver function by pixel-wise kinetic clustering of MSOT images

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Multispectral optoacoustic tomography (MSOT) is an emerging, non-invasive imaging modality that has gained wide application due to its ability to gather functional tissue information at high spatio-temporal resolution and imaging depth. However, quantitative analysis of MSOT image data is to date almost exclusively limited to the extraction of basic features, e.g. mean and maximum intensity, from manually defined regions of interest (ROIs). As these ROIs usually comprise a very specific region of tissue, we refer to this approach as being tissue-oriented. Depending on the study setup, it can have multiple drawbacks, e.g. impeded transferability between datasets, limited reproducibility due to user bias and increased workload if ROIs are complex in shape.

Here we present a new, signal-oriented approach, enabling an objective analysis of MSOT images. Instead of confined ROIs, the entire sample is analyzed with regard to the observed signal kinetics in the images. Pixel-wise clustering is used to extract main kinetic curves and to assign each pixel position to a characteristic kinetic behavior. The kinetic curves and corresponding pixel frequencies are used to calculate area under the curve (AUC) statistics, serving as quantitative measures. Additionally, the spatial distribution of the kinetics can be visualized and used to examine signal distribution within the sample. This MSOT clustering analysis toolkit – Mcat – is implemented in Java and will be freely available as a plugin for ImageJ.

We exemplify our new approach by quantifying liver function in a preclinical model of sepsis. Liver function is evaluated by means of indocyanine green uptake and clearance, which is also used in clinical diagnostics. AUC values are calculated and compared between healthy and diseased animals. Finally, we can show that group differences found with our signal-oriented approach would have been overlooked with a tissue-oriented approach, emphasizing the advantage of our method.

Automated and high-throughput quantification of microglia activation to map radiation-induced inflammation

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Microglia are the resident immune cells of the brain. Cranial radiation therapy (CRT) can cause persistent microglia activation, reflected by microglia transcriptional and morphological changes, which may lead to neurotoxicity and neurocognitive disorders. To prevent and treat these late side effects, it is important to understand microglia activation and its impact on neurogenesis after CRT. We have recently established a preclinical model to reproduce clinical side effects after proton irradiation [1]. Mouse brains were treated with different radiation doses and excised after short- and long-term observation periods. Brain sections were stained with Iba1, a common marker of microglia activation, and DAPI, a DNA-specific dye used for nuclei detection. These sections were then imaged with an AxioScan slide scanner for high-throughput acquisition. To evaluate this large dataset of immunofluorescence images, we developed an image analysis method using Fiji.

The main steps of this algorithm are i) microglia and nuclei segmentation, ii) microglia count post co-localization, and iii) quantification of microglia activation based on two parameters: cell and cell soma circularity. Automated microglia counts were validated by comparison to manual counts from two observers and the analysis could show a strong correlation between the methods. The preliminary analysis of microglia morphology revealed a dose-dependent increase of microglia activation, i.e. more circular cells and less circular cell somas. In conclusion, this workflow enables rapid and unbiased analysis of microglia count and activation status.

Future studies will focus on spatial analysis and co-registration with the corresponding dose distribution and biomedical imaging modalities (github.com/jo-mueller/ Slice2Volume).

[1] T. Suckert *et al.* Radiother. Oncol., 2020

A role for Taok2 in Listeria monocytogenes vacuolar escape

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The bacterial pathogen Listeria monocytogenes (Lm) invades host cells, ruptures its internalization vacuole and reaches the cytosol for replication. A high-content siRNA microscopy screen allowed us to identify epithelial cell factors involved in Lm vacuolar rupture, among them the serine/threonine kinase Taok2. Kinase activity inhibition using a specific drug validated a role for Taok2 in favoring Lm cytoplasmic access. Furthermore, we showed that Taok2 recruitment to Lm vacuoles requires the presence of pore-forming toxin listeriolysin O. Overall, our study identifies the first set of host factors modulating Lm vacuolar rupture and cytoplasmic access in epithelial cells.

Reproducible 3D animations using natural language with 3Dscript

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Here we demonstrate the use of 3Dscript to create high-quality 3D animations of multi-dimensional imaging data sets intuitively and reproducibly. In 3Dscript, animations are represented as text written in natural English language. Arbitrarily complex motion paths can be described by concatenating multiple elementary instructions in the form of “From frame 0 to frame 100 rotate by 180 degrees horizontally”, which are processed by a parsing and a rendering engine to compose the final video. Unlike the keyframe-based approach, where the user interactively defines a set of consecutive key frames that store the spatial transformation and visual state at given time points, and the rendering engine interpolates transitions between them to obtain the final video, 3Dscript can handle more complex motions. Unlike video recordings of interactive freehand transformations, 3Dscript produces smooth, reproducible high-quality transitions. Unlike creating a movie by a scripting language to obtain the individual frames, 3Dscript’s animation text is intuitively comprehensible and applicable by end-users without programming experience. Furthermore, we particularly want to emphasize the batch processing mode which allows to integrate 3Dscript in automatic image processing pipelines, using, e.g., the ImageJ macro language.

Automatic recognition and counting of fossil pollen - how far are we?

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Palynology is one of the most powerful tools for vegetation reconstruction and provides valuable data for other disciplines. However, since the inception of the field more than 100 years ago, pollen samples are still routinely analysed manually by specialized personnel. Pollen analysis is thus time consuming and expensive. First attempts to automatize pollen counting using image analysis have started already 30 years ago. The field of image analysis is developing rapidly since the invention of deep learning convolutional networks only in 2012 by Krizhevsky, et al.

Today, first applications of automatic recognition are in use for analysis of honey and for pollen monitoring. However, despite the recent progress in the methodology, so far there is no working application for automatic analysis of fossil pollen. The major limitations probably are the larger number of pollen types that are of interest and degradation of fossil pollen grains.

Within the dig-it! Project, we aim to apply dcnns for true analysis of pollen samples. The first step is to prepare applications that focus on a few, characteristic pollen grains (e.g. Fagus and Picea) and allow for larger counting errors. Such approach is suitable to e.g. to detect mast in years in annually laminated lake sediments.

Machine learning part is done at fraunhofer igd using deep convolutional neural networks software. For the training data we used pure samples from flowers and fossil samples. Images were obtained using an automated slide scanner. From set of 10 000 images 80% is going to be used for training of program and 20% will be used to detect performance. Later using already existing algorithms we will be able to provide a well-founded basis for the development of an automated pollen concentration estimation system.

The poster will present results for automated recognition of selected pollen types in fossil pollen samples.

Dynamic Bayesian networks for the analysis of causal interactions in cell track data

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Dynamical Bayesian networks (DBNs) are used to find causal relationships in time resolved data. They have, for example, been used to investigate spike dynamics in neuroscience [1, 2] and we will here demonstrate how DBNs can be used to analyse cell track data to find interactions between different events that we can observe. In this way we can investigate if the heterogeneity that is often observed in cell tracks [3] is caused by previous interactions or natural variability. One common issue with track data is the limited amount of tracks, that the cells disappear from the field of view and that interactions occur because of the spatial distribution of the system. Therefore, we here use simulated data of a confrontation assay containing neutrophils and fungal cells based on an agent based model [4]. In this model we can introduce causal relationships and remove them to investigate how these actual causal are represented in the DBN. We give an example how DBNs can identify a causal relationship between neutrophil-fungal touching and the probability of individual neutrophils to phagocytose fungal cells. We also propose a strategy how a combination of data analysis using DBNs and agent based modelling can be used to analyse real cell track data.

[1] Eldawlatly *et al.* Neural Comput., 2010
[2] Guo *et al.* IEEE Transactions on Cybernetics, 2019
[3] Mokhtari *et al.* PLOS ONE, 2013
[4] Lehnert *et al.* Frontiers in Microbiology, 2015

Bacterial shape changes for fast detection of antibiotic susceptibility

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Antibiotic resistance in bacteria is currently developing into one of the most challenging global health threads. Novel fast and reliable antibiotic resistance tests are the key to avoid unnecessary use of broad-spectrum antibiotics in calculated therapy and to administer tailored narrow-spectrum antibiotic therapy earlier.

Detecting the effect of an antibiotic with phenotypic susceptibility tests relies on co-incubating the isolated pathogens with selected drugs. To speed up the time from sample drawing to therapy suggestion for the treating physician, new readouts for detecting antibiotic susceptibility need to be established. The recognition that many antibiotics cause major changes in the shape of pathogens within a few minutes to hours of incubation has prompted the exploration of the bacterial shape as a possible readout for their susceptibility.

Here we present a proof-of concept analysis for detecting the microbial susceptibility to the antibiotic cefotaxime in sensitive and resistant clinical isolates of E.coli, which when susceptible become elongated upon treatment. The pathogens were incubated with different antibiotic concentrations and bright field images were acquired every five minutes. Image analysis involved illumination correction and image segmentation into background and bacteria, which enabled feature extraction for each identified bacterium. The features feret diameter and roundness were used to evaluate the effects of the antibiotic over time. Using these parameters, we identified the minimal inhibitory concentration (MIC) of cefotaxime in several E. coli isolates after an incubation period of only 90 minutes. The obtained values were in quantitative agreement with the MIC values determined by classical gold-standard methods, like broth microdiluton or E-test, which take about 16-20 hours for detecting antibiotic susceptibility profiles. We conclude that analysing changes in bacterial shape, possibly along with other fast readout parameters like spectroscopic profiles, may well be implemented into new rapid antibiotic susceptibility testing procedures.

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Virtual phagocytosis assays reveal strain-specific differences in the microscopic parameters of the interaction between alveolar macrophages and two *Aspergillus fumigatus* strains

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Phagocytosis is a major immune response mechanism of professional phagocytes to combat invading pathogens. Therefore, the quantification and characterization of phagocytosis is essential for a better understanding of host-pathogen interactions. Typically, various phagocytosis measures, such as the phagocytosis ratio, the uptake ratio or the symmetrized phagocytic index are used for this characterization. However, these measures are limited in several ways: (i) they can give the same result despite different experimental results, (ii) different measures can give contradictory results, and (iii) they do not allow to obtain microscopic cell-based interaction rates. To overcome these limitations we developed a generalized C++ framework - CellRain - for modeling based on endpoint images.

The saprophyte *Aspergillus fumigatus* is a ubiquitous human pathogen. Its spores that are distributed via air are inhaled hundreds of times every day by most humans. If not efficiently cleared by the resident immune cells in the lung - the alveolar macrophages (AM) - they can cause severe pulmonary infections in immunocompromised patients, resulting in high mortality rates. Previously, phagocytosis assays with AM and two *A. fumigatus* wild-type strains, ATCC 46645 and CEA10, have been performed using differential staining. After one hour of co-incubation fluorescence microscopy images were taken and subsequent image analysis allowed for the quantification of phagocytosed, adherent and non-associated conidia [1,2].

Based on these data we simulated virtual phagocytosis assays using CellRain. First, virtual conidia were distributed randomly on images with segmented AM. Subsequently, experimental steps, such as co-incubation and washing, were simulated. During the co-incubation, the conidia that are located on or within the operating distance of an AM can become adherent or phagocytosed with certain probabilities. During the washing step non-associated conidia are removed with the respective probability. To estimate these microscopic parameters, we performed a grid-based screening and compared *in vitro* and *in silico* data. For statistically sound results we performed several repetitions for each parameter set. This image-based systems biology approach enables prediction of microscopic parameters and reveals strain-specific differences in the phagocytosis and adherence by AM.

[1] Kraibooj *et al.* Frontiers in Microbiology 2015

[2] Cseresnyés *et al.* Cytometry A 2018

Quantification of the dynamics of fungal confrontation assays

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Fungal infections are emerging as a significant health risk for humans. The innate immune system is the first line of defense against pathogens and broadly protects against invading microorganisms. In this study we investigate the dynamics of the interaction of the fungus Candida glabrata and polymorphonuclear neutrophils (PMNs). We use confrontation assays with live cell imaging and automated image analysis to gain a quantitative understanding of the interplay between phagocytes and fungal pathogens in time and space. Due to the experimental conditions, the proliferation of Candida glabrata cells leads to the formation of cell clusters. Although the phagocytosis of Candida glabrata clusters reduces the absolute cluster number, we observe a stationary fungi population that cannot be eliminated by the PMNs. To quantify the underlying processes we apply an image-based systems biology approach. We use a state-based model and random-selection dynamics to model the dynamics of the confrontation assay and calibrate the model predictions with experimental data obtained from the confrontation assays. We apply a strategy for reliable parameter estimation where different models with increasing complexity are used. In addition, we are testing possible immune evasion processes to explain why PMNs cannot eliminate the fungi population.

Intracellular Raman micro-spectroscopic detection of *Coxiella burnetii* and discrimination of their morphoforms in infected Buffalo Green Monkey cells

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The zoonotic obligate intracellular pathogen *Coxiella burnetii* causes infections in ruminants and humans. When untreated, the acute disease (Q fever) may lead to a chronic manifestation. During its biphasic life cycle, *C. burnetii* alternates between two morphoforms: the large cell variant (LCV) occurs exclusively inside the host cell; the spore-like small cell variant (SCV) is the major infectious form and is able to live outside the host cell [1]. Routinely, sample-manipulative, labor-intense and expensive electron and fluorescence microscopy are used to visualize intracellular infections. As an alternative, Raman spectroscopy, in combination with multivariate data analysis, offers a new possibility to study the process of infection in a label-free and non-invasive manner [2, 3]. In this study, image scans of infected Buffalo Green Monkey (BGM) cells are recorded at different times post infection (pi) by Raman micro-spectroscopy. In high-quality, false color Raman maps, both bacterial morphoforms can be distinguished during infection. Also valuable information about the metabolic state of *C. burnetii* can be extracted, possibly revealing the transition from LCV to SCV. The data obtained during this study will help to guide research in intracellular infections on the basis of the extracted spectral signatures of the infecting bacteria. This work was supported by the Leibniz Science Campus InfectoOptics Jena, which is financed by the funding line Strategic Networking of the Leibniz Association.

[1] Sandoz *et al.* PLOS ONE, 2016
[2] Stöckel *et al.* J. Raman Spectrosc, 2016
[3] Große *et al.* Anal. Chem., 2015

Organization

This symposium is organized by the research group Applied Systems Biology (ASB) at the Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany.

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