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3rd International Workshop on

Image-based Systems Biology

September 2016, 29-30

IbSB 2016 Program

Thursday 29 September

11.30 Registration opens
12.30 Lunch
13.00 Opening

Session I: High-Resolution Microscopy

Session chair: Stefanie Dietrich

13.10–13.50 **Ernst Stelzer,** Goethe University Frankfurt, Germany
Light sheet-based illumination provides the basis for highly corrected, sensor-based and fully automated microscopy

13.50–14.10 **Zoltán Cseresnyés,** German Rheumatism Research Center Berlin, Germany
Striped illumination microscopy with an enhanced iterative processing algorithm provides a novel high-resolution deep-tissue imaging technique

14.10–14.30 **Ronny Förster,** Friedrich Schiller University Jena, Germany
Motion artefact detection in structured illumination microscopy

14.30–15.10 ***Coffee break***

Session II: Quantitative Bioimage Analysis

Session chair: Carl-Magnus Svensson

15.10–15.50 **Jean-Christophe Olivo-Marin,** Institut Pasteur, France
Quantitative bioimage analysis: from cell to numbers

15.50–16.30 **Katrin Heinze,** University of Würzburg, Germany
Robust spatiotemporal analysis of cell-vessel interplay in large tissue specimen

16.30–16.50 **Leena Latonen,** University of Tampere, Finland
Feature based comparison of normal tissue and early pathological lesions in histological images of mouse prostate cancer models

16.50–17.10 **Sabine Fischer,** Goethe University Frankfurt, Germany
Three-dimensional cell-graph analysis reveals distinct spatial patterns of the fate markers NANOG and GATA6 in mouse pre-implantation embryos

17.30 ***Poster session and barbecue***

Friday 30 September

Session III: Cell Dynamics: Motility and Morphology I

Session chair: Naim Al-Zaben

09.00–09.40 **Erik Meijering,** Erasmus University Medical Center, Rotterdam, The Netherlands
Model-based bioimage analysis of cell shape and motion

09.40–10.00 **Stefanie Dietrich,** Friedrich Schiller University Jena, Germany
Migration and interaction tracking for quantitative analysis of phagocyte-pathogen confrontation assays

10.00–10.20 **Feng Wei Yang,** University of Sussex, United Kingdom
A computational analysis framework for cellular and nuclear dynamics driven by experiments

10.20–11.00 ***Coffee break***

Session IV: Cell Dynamics: Motility and Morphology II

Session chair: Anna Medyukhina

11.00–11.40 **Johannes Textor,** Radboud University Medical Center, Nijmegen, The Netherlands
MotilityLab: storing, sharing, and analyzing immune cell migration data

11.40–12.00 **Michael Haberl,** University Medical Center Göttingen, Germany
T-cell and myeloid cell motility at the BBB: a mathematical approach to understand inflammatory processes

12.00-13.00 ***Lunch break***

Session V: Image-derived Modeling

Session chair: Sandra Timme

13.00–13.40 **Niels Grabe,** National Center for Tumor Diseases, Heidelberg, Germany
Wound healing revised: a novel reepithelialization mechanism revealed by in vitro and in silico models

13.40–14.00 **Alvaro Gomariz,** ETH Zürich, Switzerland
Applying 3D quantitative microscopy to study global topography and cellular interactions in the bone marrow

14.00–14.20 **Philip Kollmannsberger,** University of Würzburg, Germany
Spatiotemporal mapping of proliferation and extracellular matrix stretch during microtissue growth under geometric confinement

14.20-14.40 **Christine Lang,** ETH Zürich, Switzerland
Image-based modeling of organogenesis

14.40 Closing

Abstracts

Talks

Light sheet-based illumination provides the basis for highly corrected, sensor-based and fully automated microscopy

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In light sheet–based fluorescence microscopy (LSFM), optical sectioning in the excitation process minimizes fluorophore bleaching and phototoxic effects [1]. Since biological specimens survive long-term three-dimensional imaging at high spatiotemporal resolution, light sheet-based microscopes (LSM) have become an indispensable tool in developmental [2], three-dimensional cell [3] and plant biology [4, 5]. LSFM is based on two main optical paths [6]. The detection path consists of a microscope objective, a spectral filter, a tube lens and a camera. The excitation path is perpendicular to the detection path and directs a light sheet into the side of the specimen [7, 8]. The thin light sheet and the focal plane of the detection objective overlap. LSFM provides at least three important degrees of freedom, which are usually not available in an epifluorescence microscope: 1) the axial and 2) lateral locations of the light sheet and 3) the axial location of the focal plane. Further, probably less important, degrees of freedom are the tilt and the incline of the light sheet. LSFM takes full advantage of modern cameras, massively parallelizing the data acquisition process [9] and recording ten to one hundred images per second with a high dynamic range. LSFM does not rely on traditional features that are required for ergonomic reasons. A powerful multiple-sensors-based image processing pipeline is, therefore, an inherent feature.

Traditional fluorescence microscopy enforces specimen preparation schemes that rely on hard and flat surfaces. LSFM places the specimen in the center and arranges the optics around it. Specimens can be prepared in new ways, their three-dimensional integrity is maintained and they can be used in experiments hitherto regarded as impossible. Fluorescence microscopy has several basic limitations. First, the excitation light is absorbed not only by fluorophores but also by many endogenous organic compounds, which are degraded much like fluorophores and thus unavailable for vital metabolic processes. Second, the number of fluorophores in any volume element at any given time is finite, and fluorophores can degrade upon excitation. As a consequence, the number of photons that are retrieved from a fluorophore-labeled specimen is limited. Finally, life on Earth is adapted to the solar flux, which is less than $1.4kW/m^2$. This might not be a hard limit, but it indicates that irradiance should not exceed $1nW/\mu m^2 = 100mW/cm^2$ when dynamic biological processes are observed. When imaging living biological samples, these challenges must be addressed. LSFM is perhaps the best technology we have so far, which makes a sincere and honest effort to address these challenges: 1) it provides optical sectioning, 2) a true axial resolution, 3) reduces fluorophore bleaching and 4) photo toxicity at almost any scale, 5) allows one to record millions of pixels in parallel and 6) dramatically improves the viability of the specimen.

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Striped Illumination Microscopy with an enhanced iterative processing algorithm provides a novel high-resolution deep-tissue imaging technique

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In this work,we introduce a cost-effective and simple method to generate 2-photon excitation fluorescence images with lateral and axial resolutions well beyond the actual diffraction limit. The method, called Striped-Illumination Microscopy (SI) [1, 2], is based on splitting an infrared pulsed laser beam 2^n ($n = 1..5$) beamlets, each beamlet pair being separated by $2.8\mu m$, and then scanning the sample with this linear grid. The beamlet array is then shifted by 250-300 nm perpendicularly to the direction of the scan, and the sample is scanned again. The process is repeated until the entire sample area is scanned without gaps. The detected raw images, which are the result of the true fluorescence structure multiplied by the grid-shaped illumination pattern, and convolved by the system's Point Spread Function, need to be processed in order to receive the superresolution image. As a first approach, the Max-Min algorithm can be applied [1, 2], which results in a 3-fold improvement in the axial resolution, with a 20% enhancement in the lateral resolution [1, 2]. In order to further improve the lateral resolution, we developed a new processing algorithm, called SIBILLA (**S**triped **I**llumination-**B**lind **ILL**umination **A**lgorithm).

The algorithm starts with the Blind SIM principle [3] and adds our *a piori* knowledge of the original (undistorted) illumination pattern, the most likely light scattering mechanism (Rayleigh scattering) and the typical optical aberrations as described by the first few Zernike polynomials (astigmatism at 0° and 45°, coma at 0° and 90°, and third order spherical aberration). Using genetic algorithm and gradient descent, the iterative SIBILLA approach approximates the "ideal" image by minimizing a quadratic functional, built from the differences between the measured and the expected images. The starting values for the iterations are provided by the intact illumination pattern (measured in a homogenous fluorescent medium) and the MAX-MIN approach of the improved resolution images. The method converges robustly and provides images with 1.7 to 1.9-times better lateral and 3-times better axial resolution when compared with CCD camera-based 2-photon microscopy data.

In summary, SI and SBILLA provide vastly improved lateral and axial resolution at up to $250\mu m$ depth in live tissue. The technique can be applied with an Optical Parametric Oscillator, thus increasing the penetration depth even further. Furthermore, SIBILLA provides an estimation of the light aberration and -scattering parameters of the examined tissue, together with the high-resolution images.

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Motion Artefact Detection in Structured Illumination Microscopy

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Using structured illumination microscopy (SIM) an image resolution as high as 100nm is possible [1]. SIM was shown to be the superior high resolution method for live cell imaging, due to its high acquisition speed, a large possible region of interest and lower necessary exposure intensities. In addition, there are no restrictions for the particular labelling fluorophores of the specimen. However, SIM requires an extensive image processing to calculate the high-resolution images from the raw data. The reconstruction is prone to artefacts in case of experimental imperfections, aberrations or a movement of the specimen. The latter is unavoidable in live cell imaging and leads to unpredictable artefacts. Those cannot be distinguished from high resolution features. Thus, many scientists doubt that SIM is able to image their specimen with the necessary credibility [2].

We present an algorithm that detects motion in the SIM raw data. This is done by a modified frame-difference-method and a probability theory based analysis of the noise. Since there is no need for acquiring additional images, the cell is protected against unnecessary phototoxicity. Moving fluorophores are tagged in the SIM image, so that the end user can distinguished between hidden artefacts and true high-resolution. Exemplary, figure 1a shows a living cell with a fixed stoma (bottom) and a moving chloroplast (top). The movement of the latter is detected by our algorithm and illustrated using a heat-map (figure 1b).

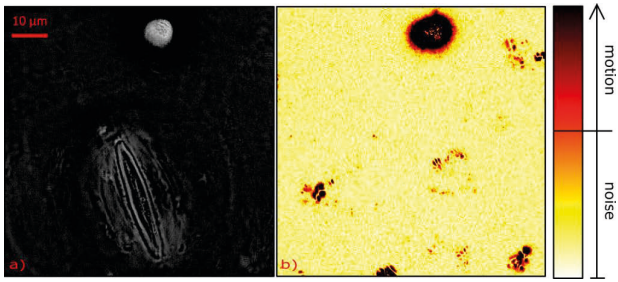

Fig. 1: SIM image of a a) chloroplast(top) and a stoma(bottom) ; b)corresponding motion map with scale.

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Quantitative bioimage analysis: from cell to numbers

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An increasing number of biological projects aim at elucidating the links between biological function and phenotype through imaging and modelling the spatiotemporal characteristics of cellular or organism dynamics. This requires the automatic quantification of dynamics parameters and the characterization of phenotypic and morphological changes occurring during of such diverse events such as cell motility, host/pathogen interaction, organism development or social interactions between animals. We will present and discuss some recent developments of algorithms and software for robust quantitative assessment of dynamic bioimaging data. We will also demonstrate on a number of examples how the use of these tools to extract quantitative data from bioimages has enabled the understanding of biological information contained therein.

Robust spatiotemporal analysis of cell-vessel interplay in large tissue specimen

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Three-dimensional fluorescence imaging is not only a powerful, but also a challenging tool when it comes to quantifying cellular interactions. Here, we show analysis pipelines for a large set of image stacks that correct for imaging artefacts, high background and sample inhomogeneity. We demonstrate robust object segmentation even in 'difficult' samples with large size and shape variations within one individual class of objects. As proper segmentation is a prerequisite for further statistical data analysis and physiologically meaningful computational simulations, we exemplify our concepts on cell-vessel interactions in murine bone marrow (BM). In complete and intact bone we investigate giant BM precursor cells, the megakaryocytes (MKs), interacting with blood vessels. MKs are known to vary in size and shape in more than three orders of magnitude and produce anucleate blood platelets. MKs originate from hematopoietic stem cells and are thought to migrate from an endosteal niche towards the vascular sinusoids during their maturation. By combining *in situ* light-sheet fluorescence, *in vivo* multi-photon microscopy and computational simulations we found that MKs are homogeneously distributed within the dense BM blood vessel network, leaving no space for such vessel-distant niches. Our data reveal surprisingly slow MK migration, limited intervascular space and a vessel-biased MK pool. This effectively eliminates the necessity for MKs to migrate to reach the vessel and contradicts the current concept of directed MK migration during thrombopoiesis.

Feature based comparison of normal tissue and early pathological lesions in histological images of mouse prostate cancer models

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Recent development of digital pathology has enabled development of image analysis tools for quantitating differences between tissue phenotypes. Recognition of cancer from normal tissue at an early stage is a desirable goal for such method development. As different genetic backgrounds are often connected to differences in cancer histological phenotypes and to patient prognosis, also descriptors for subtyping cancer histologically based on image-derived features would be useful. In the attempts to understand oncogenesis in the human prostate, neoplastic development is often modelled in mouse with genetic manipulation of certain tumor suppressor genes or oncogenes.

In here, we used images of normal and pathologically affected prostates of mice heterozygous of tumor suppressor *PTEN*, forming prostate intraepithelial neoplasia (PIN), and mice expressing transgenic oncogene *Myc* in their prostates, inducing prostate cancer. Pathologies are of early stage, when the lesions are confined in the lumen of prostatic acini (10-11 months for *PTEN+/-* and 1 month for *Myc+*). As with many pathological changes, early lesions in the mouse prostate are macroscopically small, but microscopically spanning areas larger than single high magnification focus fields in microscopy.

To visualize and analyse the microscopic information spanning whole lesions, we utilize automated whole slide scanning and develop quantitative image analysis tools to describe morphological changes in histological images. We implemented an image analysis pipeline for describing early morphological changes in hematoxylin and eosin (H&E) stained histological images. The model is based on large, generic feature set and supervised learning with random forest model. For training, we used a set of lesions of abnormal epithelial cell growth and glands of normal tissue segmented by an expert. The extracted features include 102 local descriptors related to tissue texture and spatial arrangement and distribution of nuclei. These features form a numerical representation of a tissue sample and were used to learn a discriminative model using machine learning. The implemented random forest model is an ensemble of 50 classification trees and it uses bootstrap aggregation to improve stability and accuracy. Leave-one-out cross-validation (LOOCV) was used to evaluate the performance of the random forest model. The classification model was able to discriminate normal tissue segments from the early neoplastic lesions and also to describe the spatial heterogeneity of the tissue samples. The model can be easily interpreted and used to assess the contribution of individual features. This feature significance provides information about differences in the histology between normal glands of the prostate and early histological changes in neoplastic prostate pathologies.

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Three-dimensional cell-graph analysis reveals distinct spatial patterns of the fate markers NANOG and GATA6 in mouse pre-implantation embryos

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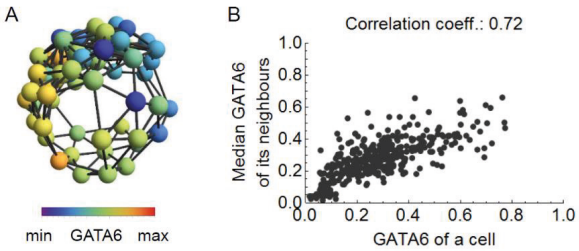
The specification of cells into embryonic and extraembryonic endoderm tissue in mouse embryos occurs before the embryo is implanted into the uterus. Starting from a co-expression of the two transcription factors NANOG and GATA6, the cells downregulate one or the other, resulting in a salt-and-pepper distribution of mutually exclusive expression. At this stage, a pattern in the three-dimensional spatial arrangement of NANOG and GATA6 levels within the embryo is not obvious and a quantification of the spatial distribution has not been conducted so far.

We applied a combination of three-dimensional fluorescence microscopy, image segmentation and cell graph analysis[1] to reveal distinct spatial patterns for NANOG and GATA6 in the pre-implantation mouse embryo. We find that high levels of cellular NANOG require an optimal number of neighbouring cells, while GATA6 does not depend on the number of neighbours. On the other hand, the expression levels of NANOG do not correlate between neighbouring cells, while for GATA6 we obtain strong correlations between neighbouring cells. These results suggest that NANOG levels are affected by local cell density, while GATA6 levels are affected by intercellular signalling.

Our results provide the quantitative information for refining and testing mathematical models taking into account three-dimensional cell arrangements and cell fate marker distributions. Moreover, they establish the significance of studying neighbour interactions during mammalian development.



Quantification of the spatial distribution of cell fate markers in the pre-implantation mouse embryo. (A) Example cell graph for one mouse embryo at mid blastocyst stage. The colours represent the relative expression of GATA6. (B) Correlation analysis of the GATA6 level of a cell versus the average GATA6 level of its neighbours.

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Model-based bioimage analysis of cell shape and motion

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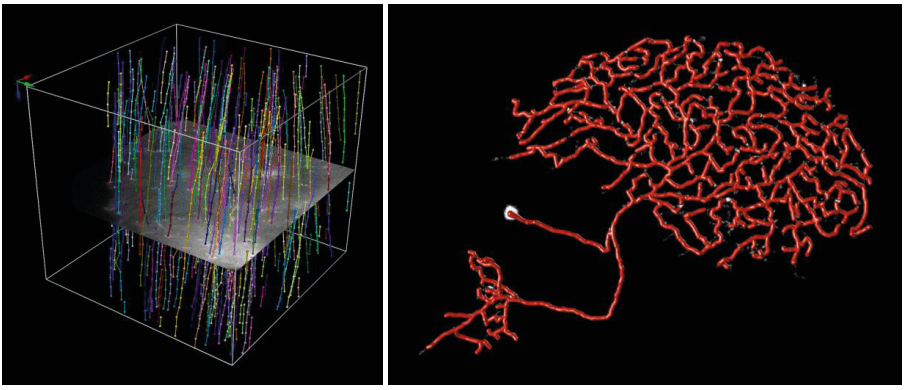
Comprehensive knowledge of the cellular mechanisms involved in healthy and diseased conditions is of fundamental importance in developing effective medicines and therapies. Thanks to groundbreaking advances in microscopic imaging and digital computing technology in the past decades we are now able to study these mechanisms at an unprecedented scale. In this process, mathematical or computational models are increasingly used to make sense of the data, but also to design simulation experiments for testing biological hypotheses or rigorously evaluating methods under controlled conditions, which would otherwise be infeasible.

Here we discuss several examples of bioimage analysis problems and how models were used in designing and testing advanced solutions. Specifically, we consider the problems of particle and cell motion tracking, and the model-based methods that performed best in large-scale international studies aimed at rigorously evaluating and comparing these methods. In addition, we discuss examples where similar computational methods were used for the very different problem of cell shape segmentation and analysis, in particular neuronal morphology. Finally, we explore how the output of many different methods can be combined to build consensus findings that are potentially more accurate and complete than those produced by individual methods alone. Ultimately this will contribute not only to better bioimage analysis methods but also to a more accurate model of the cell.



Example results of intracellular tracking (left) and neuron reconstruction (right) generated by methods using a model-based Bayesian estimation framework. The methods were/are contenders in, respectively, the particle tracking challenge[1] and the BigNeuron project[2].

[1] http://bioimageanalysis.org/track/
[2] http://bigneuron.org/

Migration and interaction tracking for quantitative analysis of phagocyte-pathogen confrontation assays

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Invasive fungal infections are emerging as a significant health risk for humans. The innate immune system is the first line of defense against invading micro-organisms and involves the recruitment of phagocytes, which engulf and kill pathogens, to the site of infection. To gain a quantitative understanding of the interplay between phagocytes and fungal pathogens, live-cell imaging is a modern approach to monitor the dynamic process of phagocytosis in time and space. However, this requires the processing of large amounts of video data that is tedious to be performed manually.

Here, we present a novel framework, called AMIT (algorithm for migration and interaction tracking), that enables automated high-throughput analysis of multi-channel time-lapse microscopy videos of phagocyte–pathogen confrontation assays. The framework is based on our previously developed segmentation and tracking framework for non-rigid cells in brightfield microscopy [1]. We here present an advancement of this framework to segment and track different cell types in different video channels as well as to track the interactions between different cell types. For the confrontation assays of polymorphonuclear neutrophils (PMNs) and *Candida glabrata* considered in this work, the main focus lies on the correct detection of phagocytic events. To achieve this, we introduced different PMN states and a state-transition model that represents the basic principles of phagocyte–pathogen interactions. The framework is validated by a direct comparison of the automatically detected phagocytic activity of PMNs to a manual analysis and by a qualitative comparison with previously published analyses [2, 3]. We demonstrate the potential of our algorithm by comprehensive quantitative and multivariate analyses of confrontation assays involving human PMNs and the fungus *Candida glabrata*.

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A computational analysis framework for cellular and nuclear dynamics driven by experiments

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Computational frames with automatic algorithms are desirable for and have become a requisite component of the study of many complex processes in biology. These complexities mainly reside because of the vast amount of experimental data and noisy laboratory environments. Combining experiments with mathematical and computerised algorithms allows a rigorous validation of biological hypotheses, thereby providing a powerful and inexpensive investigation tool in biology. Here we would like to present our research where we collaborate with biologists in order to understand cellular and nuclear dynamics from the experimental data. Our work may be separated into two parts. Firstly, we focus on cells from phase contrast microscopy, where we employ active contour and background reconstruction methods for detection. We then perform cell centroid tracking and extract information from trajectories in order to understand cell migration. In our recent work [1], we provided a solution to analyse data from directed cell migration experiments. We also include mathematical modelling [2] to reconstruct cell morphology to investigate forcing associated with cell migration. Secondly, within one of our ongoing projects, we look at cell nuclei from fluorescent microscopy, where we include image analysis to explain nuclear morphological changes due to their softness and pressure.

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MotilityLab: storing, sharing, and analyzing immune cell migration data

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Leukocyte trafficking is regulated by complex tissue-dependent factors, and leukocyte behaviors are often heterogeneous and asynchronous. Intravital imaging is a powerful approach for studying cell migration in its physiological in vivo environment. However, imaging delivers rich 3D single-cell tracking data sets that are difficult to quantify. To address this need, we developed MotilityLab, a resource for the easy, robust and flexible analysis of cell movement that consists of three components: (1) an easy-to-use web interface for calculating common motility parameters & statistics and generating publication-quality plots from uploaded tracking data sets; (2) a flexible open-source software package for the R platform, which allows advanced users to perform more complicated and customized analyses; and (3) an online database, coupled to the web interface, which allows comparisons between a given data set and previously uploaded sets. With this three-pronged strategy, MotilityLab will directly advance cell migration analysis in the fields of cell biology, microbiology and immunology, and over time, may become the first comprehensive cell motility database. Such a database would provide an invaluable resource for systems-level or computational biology studies of cell migration and chemotaxis. For instance, 'systems'-studies could aim to parse out cell-type intrinsic motility behaviors from tissue-specific or experimental model-specific behaviors.

MotilityLab is an international project led by Johannes Textor (Tumor Immunology, Radboud University Medical Center, Nijmegen, The Netherlands), Mark J. Miller (Department of Medicine, Washington University, St Louis, USA), and Rob J. de Boer (Theoretical Biology, Utrecht University, The Netherlands).

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- Francesco Marangoni, Thorsten R. Mempelm Harvard Medical School, USa
- Michael J. Hickey, Monash U, Australia
- Matthew Fricke, Judy Cannon, U of New Mexico, USA

T-cell and myeloid cell motility at the BBB: a mathematical approach to understand inflammatory processes

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Experimental autoimmune encephalomyelitis (EAE), the classical animal model of multiple sclerosis, is caused by T cells directed against myelin antigens. Upon intravenous transfer these cells appear in the vessels of the blood-brain-barrier (BBB) where they crawl extensively before entering the CNS (Bartholomäus *et al.*, Nature 2009). Once in the target tissue these cells are locally reactivated and start an inflammatory process leading to further recruitment of other inflammatory cells such as myeloid cells (Lodygin *et al.*, Nat. Med 2013). Myeloid cells are known to play an important role in the effector phase of the disease, however, how they move in the vessels of the BBB and which are the molecular cues that determine their motility is still unknown. Here, in the EAE in Lewis rat, we performed intravital two-photon microscopy to investigate the motility pattern of antigen specific T cells and myeloid cells in the CNS vessels during the different phases of EAE by simultaneously recording their movement. We then applied a mathematical/statistical method to characterize the migration behavior of the recorded cell populations in an unbiased fashion. In this way we could detect that: 1) T cells and myeloid cells exhibit a different motility pattern at the BBB; 2) This observed motility pattern changes during the different phases of EAE; 3) These changes are not correlated with intrinsic properties of the cells but with different inflammatory states of the BBB; 4) Different molecular cues determine T cells and myeloid cells motility on the BBB over time. Our data suggests that in vivo processes such as inflammation being determined by the interaction of many different parameters necessarily require a mathematical/statistical approach to be interpreted. With this approach we were able to track several of these parameters and their interplay over the course of a CNS inflammatory response. The same approach could be of help for detailed investigation of inflammatory processes in the context of other disease models.

Wound healing revised: a novel reepithelialization mechanism revealed by *in vitro* and *in silico* models

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Wound healing is a complex process in which a tissue's individual cells have to be orchestrated in an efficient and robust way. Multi-cellular systems biology allows a systems perspective on this process integrating 3D tissue culture and 3D multi-cellular computational simulation. Although 2D monolayer experiments are ideal for analyzing individual cellular functions such as migration mechanistically on the single cell level, wound healing cannot be reduced merely to cell migration. This can only be undertaken in 3D wound-healing models, which have to be systematically and quantitatively characterized both, in vitro and in silico.

To experimentally derive a consistent mechanistic model of wound closure, we set up a dedicated technical analysis pipeline. Central steps comprise 3D organotypic wound models, standardized immunohistology, fluorescent whole-slide imaging, image analysis, multiplex protein analytics, and 3D multi-cellular computational systems biological modeling. We applied our pipeline on large numbers (92) of 3D organotypic full-thickness skin wound models comprising keratinocytes and fibroblasts, which we tracked in time by a novel two-step time-lag fluorescence staining. We quantitatively captured proliferation, differentiation, and migration in our in vitro wound models. Using fluorescent time-lag staining, we were able to infer source and final destination of keratinocytes in the healing epidermis. With our EPISIM modelling and simulation platform we created a multi-cellular in silico reepithelialization model allowing to develop alternative mechanistic theories which we then experimentally validated.

We discovered the novel extending shield reepithelialization mechanism (ESM), which we confirmed by computational multicellular modeling and perturbation of tongue extension. The ESM is a mechanistic theory of how a three dimensional tissue is incrementally reconstructed from streams of in-migrating cells from the tissue surrounding the wound. This work provides a consistent experimental and theoretical model for epidermal wound closure in 3D, negating the previously proposed concepts of epidermal tongue extension and highlighting the so far underestimated role of the surrounding tissue. Based on our findings, epidermal wound closure is a process in which cell behavior is orchestrated by a higher level of tissue control that 2D monolayer assays are not able to capture.

Applying 3D quantitative microscopy to study global topography and cellular interactions in the bone marrow

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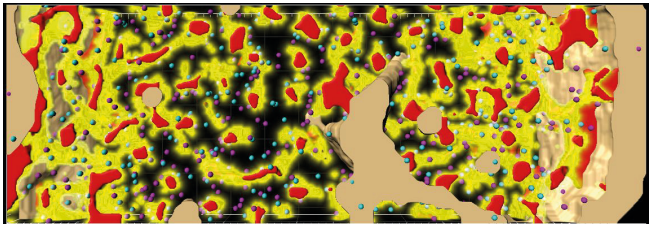
The bone marrow (BM) is the primary site of adult hematopoiesis. This process consists in the generation of huge amounts of mature blood cellular components from a population of rare hematopoietic stem cells. In the BM a nonhematopoietic heterogenous compartment, collectively known as stroma, adds a level of complexity by providing regulation and support for hematopoiesis. So far, inherent difficulties in image acquisition of the BM have limited quantification to flow cytometry studies, in which different cell populations are counted, but spatial information is not available. Now that novel techniques exist to image the BM, extensive 3D datasets are available which allow to examine how different hematopoietic and stromal components are spatially regulated. However, up until now, conclusions have been drawn based solely on visual inspection of these images. Now, the challenge resides in the development of computational tools which extract quantitative information from these images and provide information about the spatial context in an automatic and unbiased fashion. In this work we address these shortcomings by implementing image analysis and spatial statistics tools to study the global topography and spatial relationships at an organwide scale in the context of the BM. For this purpose, we produce and use a segmented representation of 3D BM samples, including isolated objects (cells) and spatially extended and connected structures (sinusoidal blood vessels). This representation is used in 3D Monte Carlo simulations to compare the observed spatial patterns to biologically relevant phenotypes. To use the framework of classical point processes, that are usually 2D, we adapted them to 3D and also included special boundary conditions imposed by our biomedical datasets. We have employed these newly developed tools to describe for the first time the spatial distribution and perivascular nature of the BM stroma. Our framework, based on 3D imagery data of BM, allowed us to quantify spatial differences arising in the BM architecture as well as intercellular interactions under different treatments. One, surprising, result is that flow cytometry, hitherto considered the standard method for analysis of this kind of samples, fails to detect the vast majority of the cells when compared to the present imagebased approach.



Depiction of modeling and simulation of stroma cells in a 3D image of BM. Structures are first segmented (sinusoids are shown in red and bone in brown). The local probability of finding a cell is modeled as a function of the distance to sinusoids and represented in yellow. Cells are simulated with different models in cyan and magenta.

Spatiotemporal mapping of proliferation and extracellular matrix stretch during microtissue growth under geometric confinement

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Tissue growth and remodeling are important in development, regeneration and disease, but it is not well understood how these processes are orchestrated. While single cells use forces to sense local geometric cues, it is less clear how multicellular assemblies respond to macroscopic geometric confinement during growth. We developed a fully automated image analysis pipeline to study how local cell behavior and global geometry are related in an invitro scaffoldbased microtissue growth assay. First, confocal stacks of microtissues are automatically aligned and segmented to identify the tissue surfaces. Since new matrix is deposited on top of the existing tissue [1], the distance to the outer surface can be used as a proxy for tissue age, resulting in a 3D agedistance map. We used this approach to determine how cell proliferation and extracellular matrix (ECM) stretch change from the growth surface to the more mature bulk. Cell proliferation was measured by quantifying the percentage of EdUpositive cells, while ECM stretch was determined by incorporating FRETlabelled Fibronectin (Fn) into the growing tissue [2]. We found that both proliferation and Fn fiber stretch are highest on the growth surface and decay with increasing tissue depth and age. Stimulating or inhibiting cellular contractility resulted in an corresponding increase or decrease of both proliferation and fiber stretch as well as changes in overall tissue volume. Agentbased modeling of the growth process using the Cellular Potts Model shows that cellgenerated forces and tissue curvature give rise to an effective surface tension resulting in increased cell proliferation. Our results suggest that collective tension patterns and cellmatrix interaction link local cell behavior to macroscopic geometry during geometrically confined tissue growth. The fully automated image analysis pipeline can be used to systematically map cellular properties and protein expression patterns in 3D under different conditions to study the underlying signalling mechanisms during growth.

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[2] Smith ML, Gourdon D, Little WC, Kubow KE, Eguiluz RA, LunaMorris S, Vogel V, PLOS Biology 2;5(10):e268 (2007)

Image-based modeling of organogenesis

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To achieve a high surface-to-volume ratio, branched organs such as the lung and the kidney undergo a complex, yet highly stereotyped morphogenetic process. It is an open question how the branching program can be both stereotypic, yet capable to adjust to the available space. The molecular players underlying bud specification and outgrowth have been extensively studied. Thus, FGF10 and GDNF signaling are both necessary and sufficient to guide the outgrowth of new branches in the lung and kidney respectively. Several mechanisms have been proposed to explain how signaling can self-organize into spots to define new branch points. We have combined 3D microscopy and image-based spatiotemporal modeling to test the validity of the proposed models. We find that of all proposed mechanisms only a receptorligand based Turing-type model can reproduce the measured embryonic growth fields and guide the outgrowth of new branches to recapitulate the embryonic 3D branching process (Menshykau et al. 2014, Georgieva et al. in preparation). The ligand-receptor based Turing mechanism recapitulates all reported qualitative lung and kidney branching mutant phenotypes (Celliere et al. 2012). Additionally, we have now used 2D lung and kidney cultures to quantitatively test the ability of the Turing mechanism to recapitulate the branching behavior in mutants and in the presence of inhibitors (Menshykau et al. in preparation; Kull et al. in preparation). The ligand-receptor based Turing mechanism not only recapitulates all data, but also offers an explanation of how stereotyped patterning can be achieved in spite of molecular noise and how a space-filling tree can be generated (Menshykau et al. 2014, Georgieva et al. in preparation). In conclusion, image-based spatiotemporal modeling allows us to identify, test, and refine mechanisms to explain the control of the complex branching program during mouse lung development.

[ ] Menshykau, D., P. Blanc, E. Unal, V. Sapin, and D. Iber. "An Interplay of Geometry and Signaling Enables Robust Lung Branching Morphogenesis." Development 141, no. 23 (December 1, 2014): 4526–36. doi:10.1242/dev.116202.

[ ] Celliere, G., D. Menshykau, and D. Iber. "Simulations Demonstrate a Simple Network to Be Sufficient to Control Branch Point Selection, Smooth Muscle and Vasculature Formation during Lung Branching Morphogenesis." Biology Open 1, no. 8 (August 15, 2012): 775–88. doi:10.1242/bio.20121339.

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Posters

Enhanced detection of unstained cells in an existing segmentation and tracking framework

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During the last decade, various areas in the biological sciences experienced a tremendous boost owing to the application of live cell imaging techniques that allow visualizing biological processes in real-time. Today, time-lapse microscopy experiments are routinely performed in biological research, however, in many cases the acquired image data are eventually used for illustrative purposes only. This disregard of quantitative information on dynamical, functional and morphological aspects of the biological system under consideration is due largely to the lack of systematic strategies for the appropriate processing and for the statistical analysis of image data.

We present enhancements to an existing automated segmentation and tracking framework for videos from live cell imaging [1]. The framework handles highly variable cell shapes and does not rely on any cell staining. The segmentation approach is based on a combination of spatial and temporal image variations in pixel intensities to detect moving cells in the microscopy videos. Although the existing framework performs well in detecting and tracking cells, it fails to do so in cases where some of the cells become undetectable due to a low spatial variance. This mostly happens for flattening cells causing transient failures in the track detection associated with a fragmentation of cell tracks.

The enhancements we have introduced affect both segmentation and tracking of the cells. The existing framework uses a Gaussian Mixture Model (GMM) to identify cells, where the input is a combination of the temporal and spatial intensity variances. The output is split into three classes: (i) mobile elements, i.e. moving cells, (ii) static elements that are possibly present in the petri dish and (iii) background. This approach, however, failed to detect the cells that transiently undergo flattening by detecting them as static elements that are neglected in the subsequent tracking. We enhanced the detection of cells by revising the GMM classification in such a way that flat cells, which were previously falsely detected as static elements, are captured and integrated into the subsequent tracking procedure. To avoid over-detection, we consider only tracks of flat cells that can be combined with previously existing tracks. This enhancement results into improved continuous tracking of cells with considerably less track fragmentation.

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[1] Brandes S, Mokhtari Z, Essig F, Hünniger K, Kurzai O, Figge MT (2015) Automated segmentation and tracking of non-rigid objects in time-lapse microscopy videos of polymorphonuclear neutrophils. Medical Image Analysis 20(1), 34-51.

Whole stack visualization of megakaryopoiesis in intact murine bone

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When blood platelets are generated the cell-vessel interplay inside the bone marrow is the key to understand these complex processes. Fortunately, recent progress in large volume microscopy and computational image analysis allows to sufficiently upscale complex biological and biomedical modeling, and thus provides correlations within a broader tissue context. Particularly high-resolution fluorescence approaches, often based on Light Sheet Fluorescence Microscopy (LSFM), open the door for precise thick tissue and whole-organ imaging. While imaging of mechanically sectioned samples may be prone to cutting artifacts, and only provides a limited three-dimensional view, LSFM even allows 3D-imaging of intact murine bone. LSFM covers the complete bone marrow volume with its dense vasculature and its complex platelet generation machinery we would like to tackle.

Blood platelets are generated from small anucleated cell fragments in the bone marrow, the megakaryocytes (MKs). The location and nature of MK maturation and migration (megakaryopoiesis; [1, 2]), particularly the details about the formation and release of blood platelets remain elusive thus far. Here we demonstrate how to visualize spatio-temporal responses of the different MK differentiation stages upon platelet depletion by LSFM in the intact murine bone marrow of the whole bone. To prepare the sample, we triggered megakaryopoiesis in mice by depleting circulating platelets. Samples were antibody-stained in vivo for MKs and endothelial cells. While imaging was straight forward, image processing and analysis was challenging: Besides common background related problems, MKs identification was initially difficult as cell size varies over 2 orders of magnitude with unpredictable shape and irregular membrane staining. In a tailored semi-automated routine using Imaris and FIJI, we succeeded to segment blood vessels and MKs and finally determined MK number, volume and localization in respect to the blood vessels with typical samples sizes of $>10^3$ MKs per database. This individual object recognition combined with mapping of cell-to-vessel and vessel-to-vessel distances lead to advanced statistical analysis of megakaryopoiesis with minimized bias. Furthermore, we show that segmented objects can serve as close-to-reality templates for computational simulations. We found that MKs are homogenously distributed within the dense BM blood vessel network, leaving no space for vessel-distant niches. The limited intervascular space and the vessel-biased MK pool eliminate the necessity for MKs to migrate to reach the vessel, which could be confirmed by in vivo imaging. These results challenge the concept of directed MK migration during thrombopoiesis.

[1] Bluteau et al., JTH 2009.
[2] Patel et al., J Clin Invest. 2005.

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Influence of cell density on migratory behavior of breast cancer cells

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The ability of cancer cells to invade neighboring tissue from primary tumors is an important determinant of metastatic behavior. Quantification of migration characteristics such as migration speed helps to predict such invasiveness. Preliminary analysis of in-house data on MDA-MB-231, Hs578t, and HCC38, three aggressive Triple Negative Breast Cancer (TNBC) cell lines, suggests that migration speed depends on cell density. In this study we investigated the size and the significance of this density-dependent speed effect and we developed realistic spatial simulations using the Cellular Potts Model (CPM) to find a mechanistic explanation for this effect. We analyzed the data with several tracking algorithms and compared them to manual tracking. The density dependence of the measured speed depended quite strongly on the tracking algorithm because of differences in the number of segmentation errors. Thus, the employed tracking algorithm is crucial to understand the true effect size and significance. Our CPM simulations in which we included a general persistence of cells are able to reproduce the observed migration speed increase. However, future work is needed to include realistic behavior of pseudopodia, because we expect these to determine cellular persistence.

Supervoxel-based image segmentation

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During the last decade, advances in imaging technology led to an enormous increase in the amount of produced image data. A multitude of microscopy techniques generates a variety of image types such as two-dimensional whole slide scans, three-dimensional image stacks or time resolved image sequences. In order to obtain segmentations image processing pipelines often are specifically tailored towards a certain image setup, for example in terms of dimensionality and staining. Hence, such pipelines are typically limited in their applicability to differing image setups. Engineering of accordingly adapted pipelines can be a time-intensive process. Additionally, image processing pipelines often require the user to be trained and to have an understanding of the inner workings of the algorithms, which greatly hampers their usability in the wet lab. Therefore, it is indispensable to develop image processing methods, capable of processing a variety of image types and segmenting diverse tissue structures requiring only few user interventions in a unified, easy to use and intuitive image analysis tool that can be used by virtually everyone.

We present a novel image segmentation methodology implemented in our image processing and analysis tool TiQuant that allows untrained users without image processing expertise to segment two- and three-dimensional images in an interactive, nearly parameter-free and standardized way. The tool is used to produce an initial oversegmentation of an image into so called supervoxels, which are represented in a dual data structure: as a collection of connected image regions and as a graph, representing those image regions and their neighborhood relationships. Users are enabled to provide labeled training data through a convenient drawing interface. The labeled data is used to train SVM or random forest classifiers to generate a probability graph holding predicted class membership probabilities for supervoxels based on their respective features, which comprise local, and neighborhood color histograms as well as texture and gradient features. Segmentations are produced by application of an energy minimizing graph cuts algorithm, making simultaneous use of the supervoxels' class-membership probabilities as well as similarities between neighboring supervoxels' features. The segmentation results can be post-processed with smoothing or watershed filters to clean up boundaries and separate clustered objects. TiQuant provides a graphical user interface to guide this workflow as well as a batch mode to process a large amount of images using previously trained and saved classifiers. The tool will be freely available upon publication.

In the contribution to the IbSB 2016 we will introduce our supervoxel-based image processing module with an emphasis on the underlying methods as well as strengths, weaknesses and potentials of the approach.

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Combined ^{18}F-fluoride PET/CT imaging in experimental arthritis*

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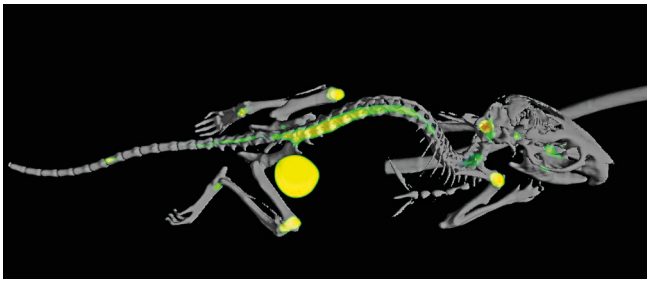
Although innumerous scientists have investigated Rheumatoid Arthritis (RA) in the past decades, the causes of this disease are still not resolved and there exists no cure. RA is an autoimmune disease leading to painful inflammation of the joints, bone erosion and eventually loss of joint function and life quality. In the meanwhile, many experimental models of the human disease could be established which are extensively used to study arthritic processes and potential treatments *in vivo*. We could already show that combined positron emission tomography/computed tomography (PET/CT) is able to capture tissue inflammation and, to a certain degree, bone degradation in a murine model of glucose-6-phosphate isomerase(G6PI)-induced arthritis [1]. In this study, we aim to quantify pathological bone metabolism and bone erosion based on ^{18}F-fluoride PET and high-resolution CT imaging. The uptake of the PET tracer is quantified longitudinally throughout the course of experimental arthritis. Additionally, the surface roughness of volumes of interest (VOIs) is calculated based on the CT images, where the VOIs comprise the bony and cartilage structures of the animals hind paws. These metabolic and anatomic markers of disease severity can help to understand the arthritic processes and assist in the evaluation of new drugs.



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Raman-based identification of circulating tumor cells*

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Raman micro-spectroscopy is a powerful technique for biological research, because it allows to detect variations in the biomolecular composition of cells. Methods of machine learning allow to train classification models that can automatically identify specific cell types based on their Raman spectra provided a sufficiently large training dataset is available.

In this contribution, a set of ≈ 8000 Raman spectra has been collected from a large population of single cells, namely 15 cancer cell lines plus leukocytes from healthy volunteers. All cells were grown in the same cell medium to eliminate its influence on the spectra. The spectra have been acquired using a Raman system developed in-house [1] that has a 785 nm excitation laser, a custom-built microscope with a 60× NA 1.0 water-immersion objective lens and a lens-based spectrograph with an open electrode CCD camera. We developed a software package that controls the instrument, interacts with the user and automates instrument calibration, data acquisition and organization processes. The software stores the spectra in a central network database. Each spectrum gets metadata associated with it, such as information about experimental parameters, sample preparation, date of the experiment or instrument calibration, as well as a bright-field image from the microscope. These metadata could be used to figure out the sources of variance and their relative contributions, such as variation between batches or instrument stability. This knowledge is essential for design of future experiments, since the effort should be focused on the important confounders that contribute high variation to classifiers.

Collected spectra have been imported into `R` and handled using the package `hyperSpec` [2]. The data underwent automated preprocessing, i.e. removal of cosmic spikes, background subtraction, baseline correction and normalization. Then, partial least squares (PLS) regression has been applied to the preprocessed data to maximize the distance between the cell lines. Noticeably, already two PLS components provide reasonably good separation between leukocytes and tumor cells. We are going to train a classification model for an automated identification of circulating tumor cells and test whether it can be applied to spectra of samples coming from real patients.

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On-chip-imaging flow cytometry and cell sorting*

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Imaging Flow Cytometry extends conventional approaches in flow cytometry to detailed and dense information on texture and intracellular distribution of markers and features. It perfectly fits the requirements of the in-depth characterization of arbitrary collections of bio particles. Combined with microfluidic sorting and in-line decision making it allows the viability preserving separation of hit candidates from the sample collections. However, microscopy in flow chambers is challenged by the low focal depth of large magnification microscopy objectives in the order between 1 and 6 μm. Small positional deviation of the imaged objects from the focal position causes optical distortion and even identical objects give different images. Alignment can be forced utilizing capillary slit channels with a small height in the size of the target objects. Although this approach is feasible the channels become very sensitive to clogging. Channels with increased hight can solve this problem, but require a preceding hydrodynamic focusing step, which automatically aligns all the particles within the focal plane of the imaging system. In our work we report on the implementation of a microfluidic operation unit for 3D flow focusing which automatically aligns all particles as a 2D-sheet within the focal plane of the microscope objective.

For imaging flow cytometry particles are readout as a 2D arrangement within a measured ROI of $400x300$ μm. This allows the parallel capturing of 300 blood cells with sub cellular resolution within a single image. Hence, about 18000 cell images can be captured per second with an optical resolution of 1.2 μm at camera frame rate of 60 FPS. Sorting is implemented independently by a piezo-actuated system where the actor becomes a permanent part of the system platform. For sorting the particles pass the imaging region one by one. Therefore, the sorting rate up to now is limited to about 30 Hz with more than 99.5% sorting accuracy.

The system can be applied to a wide range of applications. Examples are given for Pollen grain Imaging Flow Cytometry, assessment of micro algae populations from natural sources and eukaryote cell analysis. It can be operated on nearly all inverted microscopy platforms with some minor extensions for fluid management and sorter control. Summarizing, a reliably working imaging flow cytometry chip device and an image activated sorting platform have been developed, making these application available to standard laboratories as tool for the daily work. This is confirmed and reported by the results from the application tests.

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Benchmarking and selection of algorithms and software in bioimage analysis

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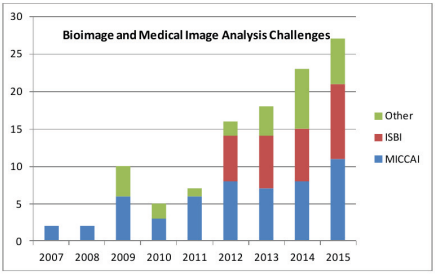
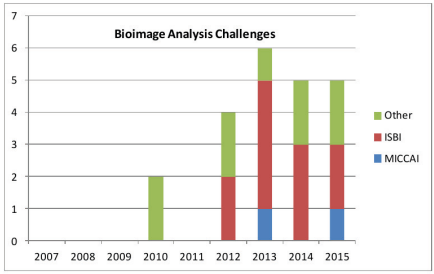
Computer analysis of microscopy images has been an indispensable part of biological research for decades. While in the last century semi-automatic methods were sufficient for many applications, it is no longer true for the 21st century due to the tremendous increase of the amount of acquired data that can be processed only using fully automatic methods tuned for a particular application. Biologists have thus to rely on the correctness of results obtained by computer analysis. This in turn requires paying proper attention to the quality control of the available open source as well as commercial software for bioimage analysis.

The common way of checking the performance of image analysis software is to use benchmark image datasets of known properties and evaluate various analysis methods on the same data. Unfortunately, in biological imaging community there has been a lack of publicly available reference images for a long time, both simulated with precisely known ground truth as well as real ones annotated by experts. Nevertheless, inspired by medical imaging community, also bioimaging community has recently realized the strong need for benchmarking various image analysis methods and software packages in order to compare their performance and assess their suitability for specific applications.

This presentation summarizes the recent progress in this respect, reviews available benchmarks and describes the general process of designing a bioimage analysis benchmark or challenge (benchmarking effort associated to a particular conference), including proper selection of reference image datasets and evaluation metrics. Finally, examples of biological applications will be shown for which benchmarking of relevant image analysis methods have already been performed including the latest comparison of 34 software packages for localization microscopy.





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An image-based systems biology approach for the quantitative analysis of phagocytosis assays

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Fungus-host interactions have gained a lot of interest in the last decade. This is due to the increasing number of life-threatening infections caused by different human-pathogenic fungi in immunocompromised patients, and also due to the limitation of the available therapies. Therefore, inspecting the pathobiology of these fungi is of key importance for ultimately possible treatments. As the last advances of microscopy techniques allow for viewing the pathogen-host interactions (micrometer resolution) and consequently for producing a lot of images, automated image analysis raises as one of the best approaches for quantifying these interactions. We here show how we exploit automated image analysis to quantify this kind of interactions through two in vitro phagocytosis assays: (1) *Lichtheimia corymbifera* confronted with murine alveolar macrophages and (2) *Aspergillus fumigatus* confronted with murine alveolar macrophages. A variety of thresholding, segmentation and classification methods are employed in one final algorithm that automatically analyses the relevant images. Quantifying the interactions is done using different measures for phagocytosis, adhesion and aggregation. This quantification allows for conducting comparative statistical studies between different strains and under different conditions: resting, swollen and opsonized. The comparison shows significant differences in both examples. As the results are objective, it is expected that this kind of studies will play a big role in exploring the different strategies used by fungi during an infection process.

Identification of single neural stem cells in the zebrafish brain

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Neural stem cells (NSCs) divide to maintain the stem cell population in the brain and generate neural progenitors that eventually replenish mature neurons and glial cells. The modalities of this division process are not fully understood. We thus analyze the number and position of dividing NSCs and progenitor cells in a neurogenic area of an adult zebrafish brain. To that end, we develop a single cell identification pipeline (SCIP) using prior biological knowledge, which identifies GFP expressing NSCs in threedimensional (3D) image stacks of zebrafish brains from wholemount preparations. We exploit the fact that NSCs in the zebrafish brain are located on a twodimensional (2D) surface by fitting a polynomial regression model. We are thus able to exclude outliers and image artifacts, which would otherwise impair the NSC identification. We compare our results with other identification approaches that do not use the prior biological knowledge of the hemisphere surface and find an improved fraction of manually identified cells with SCIP. We apply SCIP to three zebrafish hemispheres and identify around 2000 NSCs in the dorsal part of every hemisphere. Using a staining that marks dividing cells, we estimate the length of the Sphase of NSCs and determine the position of dividing NSCs and progenitors relative to the hemisphere surface. Our approach enhances the identification of single cells positioned on 2D surfaces and can improve the understanding of spatial properties of NSC divisions in the zebrafish telencephalon. This aspect is important for deeper insights into the maintenance of the NSC population and its implications in neurodegenerative diseases.

Morphological restoration: a fast alternative to deconvolution of 3D images of cells

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Two-photon microscopy (TPM) is a powerful tool for intra-vital imaging and is widely employed in a lot of biomedical applications [1]. Any microscopy image represents a convolution of the actual sample with the point spread function (PSF) of the microscope, which depends on both the microscope characteristics and the sample properties. The shape of a PSF from TPM is close to a Gaussian, which is elongated along the optical axis relative to the lateral axes, resulting in an elongated shape of the visualized cells. For the analysis of cell shape or detection of interactions, it is crucial to first restore the original cell shape. For this, a number of image deconvolution techniques exist [2], but most of them require enormous computational resources in terms of computation time and memory. Often, the quantitative analysis of cells employs only binary cell masks obtained in the process of image segmentation. For such cases, we propose a novel fast morphological restoration approach, where first cell segmentation is performed, and then the cell boundaries are shifted towards the cell center by the distance, by which the cell image has enlarged due to the PSF. We propose two approaches for estimating this enlargement: an approach based on PSF parameters and a "blind" approach based on computing the eccentricity of the "average cell" in an ensemble of cells. We validated our restoration technique in comparison with the "Iterative Deconvolve 3D" plugin of the Fiji software [3] using (i) synthetic cell images and (ii) experimental TPM images of mast cells in murine ear skin. Both PSF-based and blind versions of our approach yielded similar restoration results with respect to the Fiji implementation, however, at the benefit of reducing the computation time by two orders of magnitude.

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Track descriptors – automated characterization of cell tracks

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Cell migration is a fundamental process in cell biology and is tightly linked to many important physiological and pathological events such as the immune response, wound healing, tissue differentiation, metastasis, embryo genesis, inflammation and tumor invasion. Experimental advances provide techniques to observe migrating cells both in vitro and vivo. These imaging techniques provide two-dimensional or even three-dimensional movie data (image stacks) to be analyzed. These image stacks are the primary data source used to identify certain features (shapes within the image) as cells. By tracking the displacement of the so defined cells over the time axis, which defines their relative movement from time frame to time frame, the movement path of the cell is derived (a trajectory). These coordinate vector lists describing the track positions can be mathematically described / translated as feature vectors. Using the feature vector representations we demonstrate the feasibility to derive population classifications for analyzing cell track data and the concurrent related statistical analysis to characterize differential conditions.

Reconstruction of muscle fascicle architecture from an image stack: a combined texture mapping and streamline approach

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Skeletal muscle models are used for a better understanding of movement and force generation in both biological and bioengineering research. Yet, they often lack a realistic representation of the muscle's internal architecture which is primarily composed of muscle fibre bundles, known as fascicles. Here, we present a reconstruction method of the fascicular spatial arrangement and geometry of an exemplary facial muscle, based on a combination of pattern recognition and streamline computation. From our preprocessed μCT-data a vector field was created based on a pattern recognition algorithm, which represents the fascicle directions. The pattern recognition algorithm uses the different statistical gray value distributions along or across the fascicles to find their main direction. After this, the resulting vector field was transformed into a realistic muscle fascicle representation based on a streamline approach. We conclude that the presented approach allows for implementing realistic fascicle information into finite element models of skeletal muscles for a better understanding of the function of the musculoskeletal system. Additionally, it can be used to analyse intraspecific or interspecific muscle architectures.

Image and data analysis in a core facility

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What is it like to work as an image and data analyst in a facility setting? What set of skills should you have? How does it compare to working as a research scientist?

To answer these questions, we give an overview of our work at ETH in the Scientific Center for Electron and Light Microscopy. There, we teach image analysis, offer consulting, provide access to commercial and open-source software, and are involved in the analysis of data from research projects ranging from the quantification of individual microscopy images to performing end-to-end analysis of image-based high-content screening campaigns.

Rather than listing the pros and cons we will highlight the differences between a position in a facility as opposed to working as a research scientist in an academic group: What one person perceives as a drawback another will see as an advantage - from writing papers and applying for grants, to pursuing your own research interests versus being involved in many and diverse projects.

Automated enumeration of circulating tumor cells: machine learning techniques and interobserver variability

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The enumeration of circulating tumor cells (CTCs) is an important diagnostic tool in the treatment of various types of cancer. The determination of the extremely low number number of CTCs by liquid biopsy gives information on the progression of the disease and the effectiveness of their treatments [1]. The use of computer vision and machine learning techniques is an attractive approach to identify CTCs in a reliable and automated fashion after they are collected with a functionalized medical wire and are stained for fluorescence microscopy. However, for training of supervised machine learning algorithms, such as support vector machines (SVMs) or random forests (RFs), we need manually annotated training data. Also for unsupervised algorithms, in this case a naive Bayesian classifier (NBC) based on a probabilistic generative mixture model [2], we need annotated data to evaluate the performance of the classifier. The manual annotation of our data set revealed that two human observers agreed on average on 85% of the data set, giving an uncertainty in annotation will that will naturally affect the performance of all classifiers during evaluation of performance. It will also affect the training of SVMs and RFs as label noise is introduced by possibly contradictory annotation.

Here we demonstrate the usefulness of an unsupervised NBC for CTC classification and compare its performance with a SVM and RF. We then look into the interobserver variability and determine a consensus limit for which we can certainly say whether we have a CTC or not, thereby being able to control the label noise. Finally we investigate the effect of uncertain annotation during training on the supervised algorithms and find the SVM to be considerably more sensitive to label noise than the RF[3].

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MUCIC: Masaryk University Cell Image Collection for evaluation of bioimage analysis methods*

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In recent years, the first large bioimage benchmark datasets [Gelasca-2009, Ljosa-2013] have started to emerge. Their existence enabled the researchers and developers to validate the newly designed image analysis methods (segmentation, tracking or classification ones) in an easy and simple way. Commonly, such databases contain real image data with the ground truth obtained by manual or semi-automatic annotation. In this project, we introduce a new collection of benchmark data that were generated synthetically by a computer. In this sence, the collection serves as a complement to the existing benchmark datasets as it is commonly understood that the validation requires both testing sets: the real and the synthetic one. The real datasets with annotated ground truth offer a realistic variability that is difficult to reach in the synthetic data. Vice versa, the synthetic data can be generated in huge amount and the parameters of the acquisition process can be repeatedly changed with no additional costs. The advantage of our collection is its flexibility. All the generated data are supported by the journal or conference papers, however, based on the individual requirements, we are capable of producing modified datasets simulating the acquisition under different conditions (amount of cells in the volume of interest, clustering effect, amount of noise, uneven illumination, magnification, etc.). Most of the data included in this collection are three-dimensional. In the very beginning, we focused our interests on the generation of fixed cells [Svoboda-2009]. In the course of time, we started to simulate the dynamic processes as well, hence time-lapse image sequences are also included. At this moment the collection of synthetic images contains: DAPI stained nuclei of HL60 cell line (3D), DAPI stained nuclei of granulocytes (3D), Cy3 stained healthy colon tissue (3D), Hoechst labeled living population of HL60 cells (2D+t, 3D+t), the evolution of tubular network formed of endothelial cells (2D+t). The methods and algorithms responsible for the generation of synthetic data mostly use parametric image based modeling. However, the newly introduced image dataset depicting the development of tubular network during vasculogenesis is based on predictive modeling.

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Modelling bacterial single-cell growth and division

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The mechanisms that regulate cell size control, the balance between growth and division, is a long-standing question in biology. In the case of microorganisms, in addition to "sizer" and "timer" models suggested previously, recent studies have proposed alternate models such as elongation control [1] (a.k.a. "adder" model [2]), rekindling the debate on cell size control [2]. In this study, we evaluated these three different models of cell size control and assessed their potential relevance for Mycobacterium smegmatis, which we use as a surrogate model for the human pathogen Mycobacterium tuberculosis. We performed simulations to analyse the mathematical stability and predictive power of the three models with linear or exponential growth modes. We found that "timer" control over cell division is possible only if single-cell growth is linear but not exponential. Otherwise, we found that cell size homeostasis and realistic distributions of interdivision times could be obtained with all of the other models and growth modes. By culturing M. smegmatis in microfluidic devices and following their growth and division processes at the single-cell level using time-lapse microscopy, we generated quantitative data for growth and division of thousands of individual bacteria. We then found that the "adder" model provides a better fit to the experimental data than either the "timer" or "sizer" model of cell size control. Moreover, our preliminary analysis of bacterial growth at the single-cell level suggests that the growth mode may vary as a function of the culture medium.

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Random forests for detection of cancer tissue from whole slide images

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Digital pathology has led to a demand for automated detection of regions of interest, such as cancerous tissue, from scanned whole slide images. With reliable and computationally efficient methods, speed-up and improved accuracy in screening of samples could be achieved. We present an approach to detection of cancerous tissue from scanned whole slide images using supervised machine learning. Our method is based on feature engineering and random forest based classification. The feature set is generic, allowing the use of different stainings, such as H&E or immunostaining, making the method applicable in various applications. So far, we have evaluated our method in breast cancer metastasis detection from scanned whole slide images of lymph node samples. The results show that the method detects cancer tissue with high accuracy (AUC = 0.98 for tumor detection within whole image area, AUC = 0.88 for tumor vs. normal tissue detection). Further tests on generalizability are underway. Our anticipation is that the method will be widely applicable in automated analysis of scanned whole slide images in different applications, whenever enough training data is available.

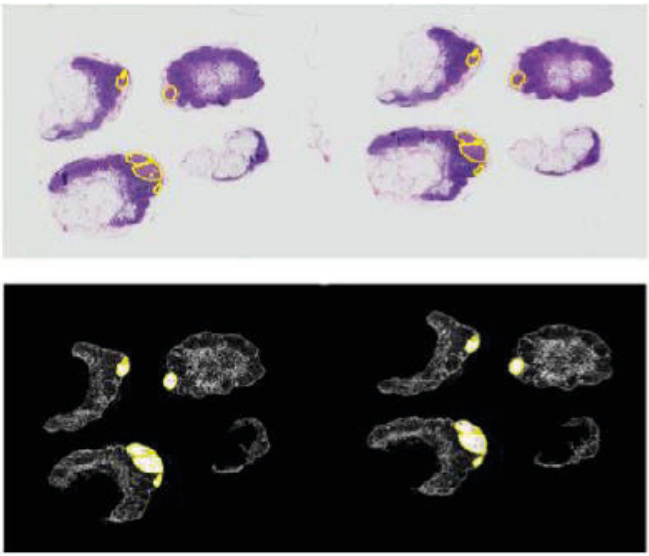

Figure 1. An example image with cancer tissue areas outlined in yellow, and the output from classifier model as confidence values for the image below.

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