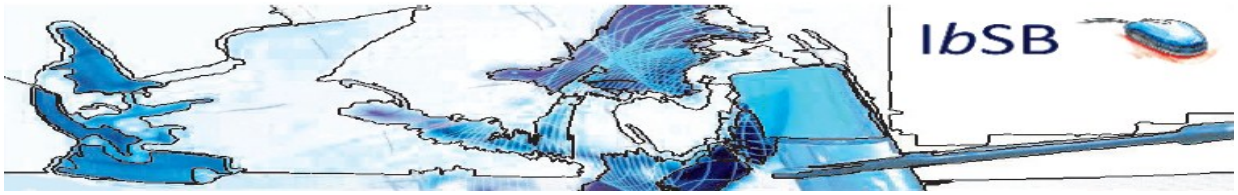

Image - based Systems Biology

Workshop

September 19, 2012
Jena
Germany

Satellite Workshop of the German Conference on Bioinformatics 2012

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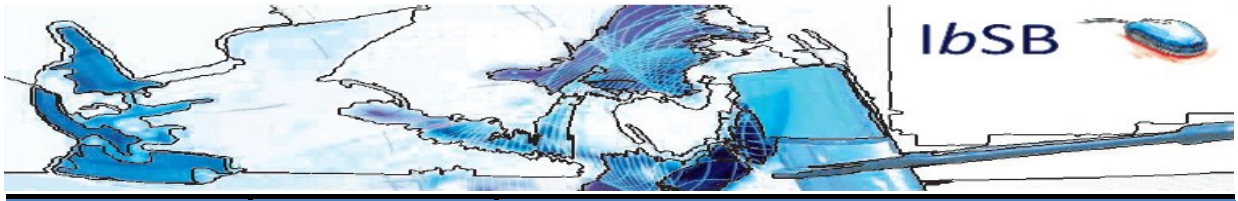
Program & Abstracts



Time	Speaker	Title
09:00		**reception opening**
09:20		**workshop opening**
09:30 – 10:15	**Prof. Dr. Joachim Denzler (invited speaker)**	Novelty Detection in Biological Data using Gaussian Processes
10:15 – 10:45	**Dr. Zoltan Cseresnyes**	Kinetic analysis of cell migration and cell membrane geometry in a migrating epithelial sheet
10:45 – 11:15		**coffee break**
11:15 – 11:45	**Jindřich Soukup**	Segmentation of time-lapse microscopy images of somatic cells for automatic cell cycle analysis
11:45 – 12:30	**Prof. Dr. Ingo Röder (invited speaker)**	Automatic tracking and quantification of dynamic cellular characteristics
12:30 – 14:00		**lunch break**
14:00 – 14:45	**Dr. Daniel Rapoport (invited speaker)**	Image based methods for turning cell culture into numbers
14:45 – 15:30	**Dr. Jörg Lücke (invited speaker)**	Representational Approaches for the Analysis of Microscopy Images
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16:30 – 17:00	**Hendrik Schäfer**	Automated Image Analysis of Hodgkin lymphoma
17:00 – 17:30	**Dr. Ivo Sbalzarini**	Image-based Systems Biology: Modeling and Simulation in Image-derived Geometries
17:30		**workshop closing**

Novelty Detection in Biological Data using Gaussian Processes

Joachim Denzler
Computer Vision,
Friedrich Schiller University Jena, Germany

Detecting a pattern of a previously unseen and thus unknown class is a topic that has been investigated in different disciplines in the past. It is called one class classification, out of vocabulary problem, or novelty detection. The goal is not just to reject a sample during classification but to identify a sample as a novel object or event. For incremental, life-long learning, novelty detection is one of the preliminaries and it is an important research area in computer vision and machine learning nowadays.

In this talk I will first introduce Gaussian Processes for classification and then explain, how to exploit this powerful framework for novelty detection. I will demonstrate its application for some computer vision tasks but also in the context of biological data. Experiments will demonstrate the suitability as well as the advantage compared to state of the art methods in this area.

Kinetic analysis of cell migration and cell membrane geometry in a migrating epithelial sheet

Zoltan Cseresnyes[1] and Marcus Bischoff[2]
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Here we describe custom written C# and XNA (Microsoft Visual Studio 2005 and 2008) software packages that calculate kinetics and population data of migrating cells, identify and characterize fluorescently labeled cell membranes, as well as visualize and simulate heart cell migration in developing zebrafish embryos.
The first software (see Ref.) uses 4D tracking data obtained from movies where the cell nuclei were followed manually using SIMI Biocell. The coordinates of the nuclei are saved at regular time intervals, both before and after mitosis. The following parameters are calculated using the program:
Cell division orientation: the angle of cell division between the sister cells relative to a given axis (e.g. the direction of movement) during anaphase (one frame after the metaphase plate is visible); ***sister cell rearrangement:*** the position of sister cells relative to each other, using their coordinates from either before the next mitosis or from the end of the movie; ***cell density:*** measures the distance of each cell to its N nearest neighbors, and the average distance is plotted at the position of the cell; ***neighborhood maps:*** the changes of distances to N closest neighbors at two time points are calculated and plotted at the position of the cell at the first time point; ***velocities:*** The velocities of each cell are plotted at their positions at the beginning of the time interval; ***trajectories of cell movements:*** this option shows the direction in which the cells moved in a given time interval. The color of the plotted lines represents the velocity of the cell.
Another part of the software uses fluorescence images of cell membranes to characterize cell geometry in migrating cells. Pixels belonging to cell membranes are identified with a "shooting rays" algorithm and assigned to one (individual cells) or two (continuous cell layer) cells. The resulting pixel populations are further defined by e.g. iterative smoothing that pulls in outliers. Various membrane parameters can then be calculated, including cell shape classification and membrane network characterization.
In zebrafish heart development, the gradient distribution of cell motility, combined with a random-walk model is shown here to be sufficient to describe the observed left-right directional movement of cardiac cells. We demonstrate the effect of the motility gradient on the cell migration pattern, and estimate the value of left-right motility difference that resulted in the observed data.

Ref: Bischoff and Cseresnyes, "Cell rearrangements, cell divisions and cell death in a migrating epithelial sheet in the abdomen of Drosophila." Development, 2009 Jul; 136 (14): 2403-11

Segmentation of time-lapse microscopy images of somatic cells for automatic cell cycle analysis

Jindřich Soukup, Petr Císař
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The automation of the analysis of image data series produced by microscopy experiments of biological processes is becoming more and more important in systems biology. The main reason for the need of the automatic data analysis is the enormous amount of information contained in the data. We are presenting here the first step (cell segmentation) of the integrated project for somatic cell behavior analysis under different conditions from raw (fluorescently unlabeled) images.

The segmentation of the phase contrast microscopy images of somatic cells is one of the most difficult tasks. The contrast between objects and background is poor and the image contains artifacts – halo (due to the microscopy technique), the shape of the cells vary dramatically during the cell cycle, see Fig.1. These are the reasons why the traditional approaches for image segmentation cannot be used.

We developed a new technique for somatic cell segmentation based on the variation of the image patterns during the cellular evolution. The approach is based on the simple assumption that the intensity of pixels which belong to the cell varies more than that of the pixels of the background. The level of signal variation in time is calculated for a given number of consecutive frames for each pattern in the image. The classification of pixels by variation into cellular or background pixels is obtained by a threshold that is generated by two different methods. The first method is the Gaussian mixture clustering method. This method requires no user parameters and is fully automatic. The second method is cluster based by minimizing the Mumford-Shah functional depending on one user parameter for determination of the compactness of the separated objects. Each method works differently, such that there is potential to improve the segmentation by their combination.

The method was tested on four different manually segmented experiments with MG63 cells. The segmentation accuracy of the method is between 88 and 98 percent of the pixels without any additional processing, see Fig.1.

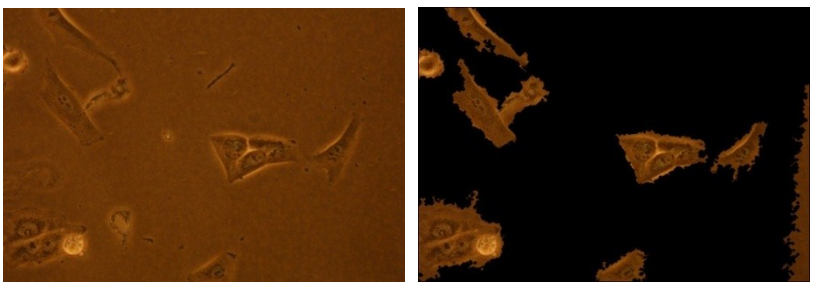

Fig 1. Example of MG63 segmentation. Left – original phase contrast image, Right – image with segmented cells

Automatic tracking and quantification of dynamic cellular characteristics

Ingo Röder
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Technical University Dresden, Germany

The continuous analysis of individual cell fates within a population of cells is still a major experimental challenge. However, new monitoring techniques, such as high-resolution time-lapse video microscopy, facilitate the tracking and the quantitative analysis of single cells and their progeny. The obtained information, e.g. on cellular development, divisional history, and differentiation of individual cells, can be comprised into a pedigree-like structure, denoted as cellular genealogy. To go beyond a pure descriptive analysis of this type of data and to statistically extract reliable information about effecting variables and control mechanisms underlying cell fate decisions, it is necessary to analyse large numbers of cellular genealogies. This requires the development and application of automatic cell tracking algorithms.

In the talk I will present computational methods that allow for the automatic segmentation (recognition) and tracking of cell ensembles and individual cells and, based here on, the reconstruction and analysis of behavioural characteristics and cellular genealogies from time-lapse video data. The talk will report about a study that applied the tracking algorithms to human haematopoietic stem and progenitor cells (HSPCs) in bioengineered culture conditions over several days and about the tracking of cells in developing zebrafish embryos.

Image based methods for turning cell culture into numbers

Daniel H. Rapoport
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Automated time-lapse microscopy can be used to non-invasively and label-free follow complex multi-cellular processes, such as cell migration, cell cycle, and cell differentiation. Not only can these processes be observed but also quantified in real time using image analysis tools. This way, many standard assays in cell culture can be analyzed with unprecedented accuracy, e.g. wound healing assays (scratch assays, migration assays), angiogenesis assays, apoptosis assays etc. Also, it can be used to develop standardized screening methods for substance testing in cell based test systems. The influence of these substances on e.g. cell migration speed, mitosis rates, cell growth etc. can readily be determined.

In this talk I will focus on two major aspects of these technical advancements: First, I will elaborate on independent validation algorithms for automated cell recognition / cell tracking. Validation is of utmost importance in this field, because the error propagation is particularly bad in tracking problems. However, a quantifiable and reliable tracking data set needs to be of high "trustworthiness", i.e. the error rate of the data must be small to extract true and biologically meaningful results. This can be achieved through a systematic search for tracking errors and subsequent rejection of untrustworthy results. In the second part of my talk, I will give a number of examples for the application of image based cell metrology to different well established tasks in cell biology, which nowadays are still being solved by hand. The use of image based analysis methods not only helps to speed up these assays but also considerably improves their reliability and accuracy.

Representational Approaches for the Analysis of Microscopy Images

Jörg Lücke
Computational Neuroscience and Machine Learning,
Frankfurt Institute for Advanced Studies (FIAS), Goethe University Frankfurt, Germany

The advancement of experimental methods in many areas of science is generating an increasing amount of data. Automated approaches to data analysis, therefore, become increasingly important. In recent years, Bayesian approaches to data analysis have been very successful as tools to reveal the underlying processes that have generated the data.

Instead of processing data directly, Bayesian approaches usually first try to build appropriate representations of the data before a task is accomplished. For a set of images as data, such a representation would consist of the typical objects appearing in them. The challenge is to automatically find appropriate object representations for a given set of images.

I first introduce the general Bayesian approach to data analysis and then discuss why it is promising to solve crucial problems occurring in the analysis of microscopy images. Then I present some example approaches developed in joint work by groups at Frankfurt and Jena. Using different types of data, we show results and discuss their implications for microscopy image analysis in general. Finally, open problems and typical limitations are discussed in relation to methods as they are currently used.

Feature Based Classification of Circulating Tumour Cells

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[2]Research Group Applied Systems Biology, Hans Knöll Institute, Beutenbergstr. 11a, 07745 Jena, Friedrich Schiller University Jena, Germany

The identification and counting of Circulating Tumour Cells (CTCs) is an important diagnostic tool for planning and evaluation of treatment for patients with various types of metastatic cancer. The CTC count is for example strongly correlated with the expected progression-free survival of the patients. The company Gilupi Nanomedizin Gmbh has developed a nanodetector that collects CTCs that are than imaged using fluorescence microscopy.

The annotation and counting of CTCs in the images are normally performed by an expert that makes the judgment based on factors such as cell fluorescence, shape and size. By working closely together with experimentalists we aim to develop and implement a model that will help clinicians in this classification task. We are combining segmentation methods that traditionally are used in biomedical imaging, such as thresholding and watershed segmentation, with a generative model of the cells. The generative model allows for a representation of features of cells which gives a deeper understanding of the images compared to classical segmentation methods. The features we are interested in, colour and shape, are learned using expectation maximization (EM). The parameter learning makes the model robust for changes in the experimental procedures, e.g. a change in the fluorescence can be learned without any adjustments to the basis of the model. This also makes the model easy to adjust to other data sets which require interpretation of fluorescent microscopy data.

Automated Image Analysis of Hodgkin lymphoma

Alexander Schmitz[1], Hendrik Schäfer[1], Tim Schäfer[1], Claudia Döring[2], Jörg Ackermann[1], Norbert Dichter[1], Sylvia Hartmann[2], Martin-Leo Hansmann[2], and Ina Koch[1]

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[2]Senckenberg Institute of Pathology, Johann Wolfgang Goethe-University Frankfurt, 60590 Frankfurt am Main, Germany

Hodgkin lymphoma (HL) is a common but unusual type of lymphoma, arising from malignant B-cells. Morphological and immunohistochemical features of malignant cells and their distribution differ from other cancer types. HL does not form solid tumors and only about 1 per cent of the cells in affected tissues are Hodgkin and Reed-Sternberg (HRS) cells, which are a typical feature of classical HL. They are embedded in inflammatory tissue which also contains a mixture of other cell types, e.g. small lymphocytes, macrophages, and mast cells. Currently, not much is known about the interactions of these cell types during the disease. Based on systematic tissue image analysis, computer-aided exploration can provide new insights into Hodgkin lymphoma pathology.

We report results from an image analysis of CD30 immunostained Hodgkin lymphoma tissue section images. CD30 is a cell surface protein which belongs to the tumor necrosis receptor family and is usually expressed by HRS cells. Even though non-lymphoma cells express it under rare conditions, it serves as a marker for HRS cells. To the best of our knowledge, this is the first systematic application of image analysis to a set of tissue sections of Hodgkin lymphoma. We have implemented an automatic procedure to handle and explore image data in Aperio's SVS format, a single-file pyramidal TIFF with non-standard metadata and compression.

We use pre-processing approaches on a down-scaled layer to separate the image objects from the background. An intensity threshold is used to eliminate background pixels, followed by region growing and filtering to remove small objects. This consistently identifies the tissue sections in the images. Then, we apply a supervised classification method to assign pixels to predefined tissue classes, such as Nuclei, CD30-positive and Unstained. We analyzed three immunohistologically defined groups: nonlymphoma and the two most common forms of Hodgkin lymphoma, nodular sclerosis and mixed type. We found that nodular sclerosis and non-lymphoma images exhibit different amounts of CD30 stain, whereas mixed type exhibits a large variance and overlaps with the other groups.

The results can be seen as a first step to computationally identify tumor regions in the images. This allows us to focus on these regions when performing computationally expensive tasks like object detection in the high-resolution layer. In the future, we will investigate the distribution of different cell types for several subtypes of HL.

Image-based Systems Biology: Modeling and Simulation in Image-derived Geometries

Ivo F. Sbalzarini
MOSAIC Group, Center for Systems Biology,
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Biological systems differ from engineering systems in a number of ways. Arguably the most evident is the geometric complexity and diversity of biological shapes. These shapes define the environment in which biochemical and biophysical processes take place. Moreover, the complex and dynamic shapes of biological entities often have functional relevance themselves. This is for example the case for the complex-shaped Endoplasmic Reticulum, but also for the arrangement of cells in developing tissues.

We model biological systems from images where their shapes are visible. Extracting the geometries and their dynamics from images allows us to build realistic models that take shape context into account. In addition, photometry can be used to directly quantify the spatiotemporal localization of fluorescent markers in the observed sample, allowing parameter identification of chemical network models in the reconstructed geometries [1].

We combine image processing with particle-based simulations in order to simulate dynamic processes such as diffusion, flows, and biochemical reactions directly in the image-derived geometries [2, 3]. Particle methods offer a unique and versatile numerical simulation framework with accuracy and computational cost that are not hampered by shape complexity. Moreover, particle methods can simulate both discrete and continuous models either stochastically or deterministically. When simulating discrete models, particles correspond to real-world objects that interact according to the model. When simulating continuous models, particles represent the Lagrangian tracer points of the continuous fields, or the collocation points of a mesh-less discretization scheme [4]. Particle methods can be efficiently implemented an large-scale parallel computer systems based on a common set of abstract data types and operators [5]. These are transparently implemented in the open-source Parallel Particle Mesh (PPM) library [6], reducing code development times for simulations in complex geometries.

We demonstrate examples ranging from diffusion in the Endoplasmic Reticulum to human brain electromagnetism to Drosophila wing disc development.

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[3] I. F. Sbalzarini et al., Biophys. J. 90 (2006) 878.
[4] S. Reboux, B. Schrader, and I. F. Sbalzarini., J. Comp. Phys. 231 (2012) 3623.
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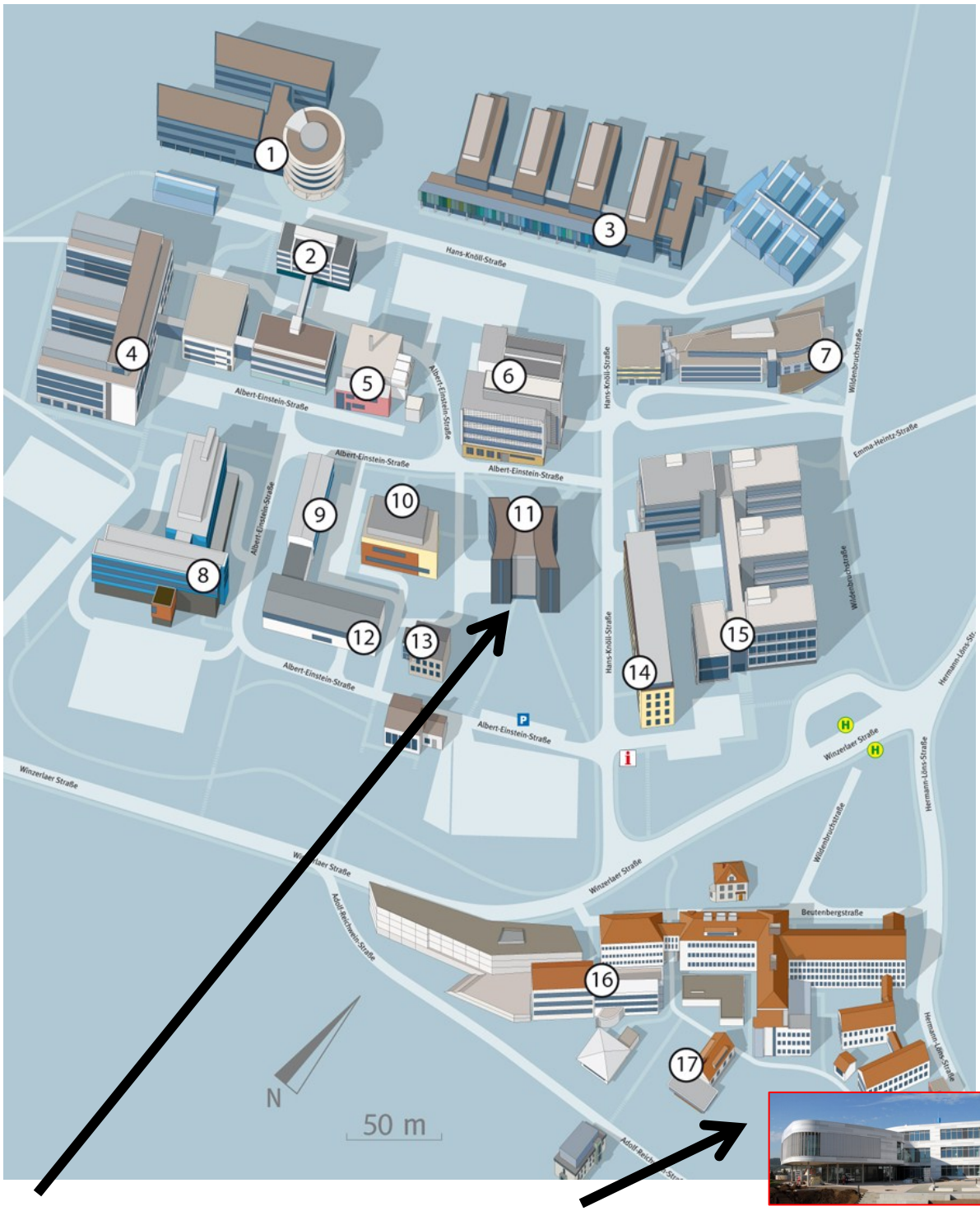
Local Information



Lunch:

Casino,
Hans-Knöll Strasse 1, 07745 Jena

Workshop:

HKI-Center for Systems Biology of Infection,
Beutenbergstr 11a, 07745 Jena
Please follow the signs inside the building!

Organization & Sponsoring

This workshop is organized by the Research Group *Applied Systems Biology* at the *HKI-Center for Systems Biology of Infection*, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Friedrich Schiller University Jena, Germany.

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