

Image - based Systems Biology

2nd International Workshop

September 25-26, 2014

HKI - Center for Systems Biology of Infection Jena Germany

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IbSB 2014 Program

Thursday 25 September

- 12.00 Registration opens
- 13.00 Opening

Session: Image-derived Models of Spatial Organization

13.10-13.55 **Robert F. Murphy**, Carnegie Mellon University and Albert Ludwig University of Freiburg

Image-derived spatiotemporal models of subcellular organization differentiation and perturbation

13.55-14.40 Jean-Christophe Olivo-Marin, Institut Pasteur

(presented by Thibault Lagache)

Colocalisation analysis of biomolecules

14.40-15.00 Thomas Zerjatke, Technical University Dresden

Comparison of image-based measures on spatial heterogeneity in ESC colonies

15.00-15.30 Coffee break

Session: Automated Image Segmentation and Classification

15.30-16.15 Stephen J. McKenna, University of Dundee

Automating the analysis of histopathology images

16.15-16.35 Haiyue Yu, University of Oxford

Automatic assessment of immuno-fluorescent tissue image quality using a machine learning approach

16.35-16.55 Adrian Friebel, University of Leipzig

Quantification of liver tissue by image processing

16.55-17.15 Stefan Helfrich, Forschungszentrum Jülich GmbH

Investigating single-cell growth of microbial cell factories: is counting cells enough?

17.30 Poster session and barbecue

Friday 26 September

Session: Cell Tracking Approaches

09.00-09.45 Karl Rohr, University of Heidelberg, BIOQUANT, IPMB, and DKFZ

Tracking and registration for automatic analysis of live cell image data

- 09.45-10.05 **Damian Stichel**, University of Heidelberg
 - Analysis of migrating NSCLC cells using particle image velocimetry
- 10.05-10.25 Konstantin Thierbach, Technical University Dresden

Cell tracking accuracy - quantification and consequences

10.25-10.55 Coffee break

Session: Analysis of Image-derived Cell Shape and Migration

10.55-11.40 Till Bretschneider, University of Warwick

Mapping amoeboid cell migration

11.40-12.00 **Zoltan Cseresnyes**, German Rheumatism Research Center (DRFZ)

Fourier transform-based fast characterization of cell shapes as a tool for intelligent classification of cells in intravital microscopy

12.00-12.20 Marco Körner, Friedrich Schiller University of Jena

Behavioral classification of cells by observing their spatial dynamics: a self-similarity approach

12.20-13.45 Lunch break

Session: Image-derived Models of Cellular Dynamics and Interactions

13.45-14.30 Joost Beltman, Netherlands Cancer Institute

Analyzing and modelling immune cell migration

14.30-14.50 Szymon Stoma, INRIA and ETH Zürich

Image-based modeling of drug delivery in HeLa multicellular spheroids

14.50-15.10 Jan Hasenauer, Helmholtz Zentrum München

Model-based outliers treatment in image-based systems biology: filtering vs. noise model

15.10 Closing

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Image-derived spatiotemporal models of subcellular organization, differentiation and perturbation

Robert F. Murphy^{1,2}

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Given the complexity of biological systems, machine-learning methods are critically needed for building systems models of cell and tissue behavior and for studying their perturbations. Such models require accurate information about the subcellular distributions of proteins, RNAs and other macromolecules in order to be able to capture and simulate their spatiotemporal dynamics. Microscope images provide the best source of this information, and we have developed tools to build *generative* models of cell organization directly from such images. Generative models are capable of producing new instances of a pattern that are expected to be drawn from the same underlying distribution as those it was trained with. Our open source system, CellOrganizer (http://CellOrganizer.org), currently contains components that can build probabilistic generative models of cell, nuclear and organelle shape, organelle position, and microtubule distribution. These models capture heterogeneity within cell populations, and can be dependent upon each other and can be combined to create new higher level models. The parameters of these models can be used as a highly interpretable basis for analyzing perturbations (e.g., induced by drug addition), and generative models of cell organization can be used as a framework for cell simulations to identify mechanisms underlying cell behavior. Results for analysis of systems ranging from neuronal differentiation to perturbation of plant protoplast organization will be presented.

Colocalisation analysis of biomolecules

T. Lagache¹ and J.-C. Olivo-Marin¹ ¹BioImage Analysis Unit, Institut Pasteur

We will present a review of methods for studying the spatial colocalisation distribution of two different biomolecule populations. We will consider first standard methods based on intensity and/or objects, and then proceed to statistical methods and modeling, while a final section will consider a prospective topic related to the characterization of interactions. We will illustrate the methods on some case studies related to endocytosis.

Comparison of image-based measures on spatial heterogeneity in ESC colonies

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Pluripotent embryonic stem cells (ESCs) have the potential to differentiate into all cell lineages of the body. This unique property is mainly studied on the intracellular, transcriptional level. However, cultured ESCs form cell clusters of different albeit distinct shape and establish spatial structures that are essential for the maintenance of pluripotency. Even though it is recognized that the cells' arrangement and local interactions play a role in fate decision processes, correlations between transcriptional and spatial patterns have not yet been studied.

We present a systems biology approach which combines a quantitative analysis of live-cell imaging data with a multi-scale, mathematical model of ESC growth. In particular, we develop quantitative measures on the morphology and on the spatial clustering of ESCs with different expression levels and apply them to images of both in vitro and in silico cultures. Using the same measures, we are able to directly compare model scenarios with different assumptions on cell-cell adhesions and on (de-)stabilizing feedback mechanisms between single ESCs with experimental data.

Applying our methodology to experimental image data of cultured ESCs, we demonstrate that the emerging colonies are highly variable regarding both morphological and spatial fluorescence patterns. Moreover, we can show that ESCs with a high self-renewing capacity are more likely located in the interior of a colony structure than close to its border. In combination with our modeling results, we argue that transcription factor-correlated cell adhesion facilitates heterogeneous ESC morphologies. Our analysis demonstrates that a comparison between real and simulated colony structures based on a set of quantitative measures is a suitable strategy to evaluate the consistency of functional models.

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Automating the analysis of histopathology images

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Advances in quantitative analysis of histopathology image data are needed; while staining, capture, and organisation are largely automated, analysis of image content still constitutes a bottleneck and exhibits significant intra- and inter-observer variability.

One approach to analysis involves first detecting and segmenting specific cells, nuclei or other subcellular components and then building a representation based on these detected 'objects'. Apart from the difficulties associated with segmenting such objects, focusing attention on epithelial nuclei, for example, risks ignoring contextual information of predictive value.

This talk describes algorithms for identifying regions of interest (cancer, dysplasia) that (i) are based on descriptions of image patches or superpixels without explicit segmentation of cellular components, and (ii) incorporate contextual information from surrounding tissue when performing classification.

Results will be presented for two applications on data captured with imaging modalities of different scale and dimensionality: immunohistochemical scoring of breast cancer tissue from 2D tissue microarray images, and analysis of colorectal polyps from optical projection tomography images.

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Automatic Assessment of Immuno-Fluorescent Tissue Image Quality Using a Machine Learning Approach

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Automatic assessment of tissue microarray quality through microscopic images can greatly reduce human bias in sample selection and increase the value of image analysis algorithms and automatic microscopy. Previous research has determined that several factors may have considerable impact on the quality of the immunohistochemistry [1]. The existence of different non-tumor types of cells in tumor biopsies can also result in failure of the image analysis software and introduce significant noise in quantitative analysis. In practice, the evaluation of TMA quality has to be performed by pathologists who observe either cell quality or tissue 'appearance' under microscopy and select image regions which mainly contain tumor cells for further study. In order to select image regions automatically, we developed a machine learning based method using image features.

In this experiment, we studied the sample quality criteria used in Ewing sarcoma tissue microarray and developed image feature based tissue quality metrics. As shown in (Fig. 1), the TMA images in this dataset contain five types of image regions related to sample quality. We used an ensemble learning method to classify image features, which are extracted at two different scales from the original im-At each scale, an independent ages. multi-class random forest model was trained (the dataset contains a total of 900 patches). The predictions from the two random forest models were assem-



Figure 1: The upper row displays the 5 different types of substances in tissue samples. The lower row shows the prediction outcome of the ensemble classifier when it was applied to each data point in the validation dataset. Datapoints 1-20 are images of clumpy nuclei, 20-40 stromal tissue, 40-60, degraded nuclei, 60-80 normal nuclei, and 80-100, background.

bled using weight factors learned from the training data. Image regions which contains clumpy nuclei, stromal tissue, degraded nuclei and background are considered as poor and uninformative quality, and image regions that contain tumor cells considered as good and informative quality for subsequent biomarker analysis.

The method was tested on 100 independent samples from training dataset within which image patches were manually selected by experts . The overall accuracy of the ensemble classifier was 93%, while a single random forest model achieved 82% accuracy and a multiclass support vector machine model achieved 70% accuracy, respectively. This analysis demonstrates an ensemble learning method with image based features that appeared able to assess the tissue image quality, and indentify regions of tumor nuclei within complex tissue images.

[1] Economou M, ni LS, Hammer C, n JAG, Mueller D-E, Zlobec I. Proper paraffin slide storage is crucial for translational research projects involving immunohistochemistry stains. 2014;3:1–3.

Quantification of liver tissue by image processing

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During the last years imaging technology advanced significantly, especially with regard to the amount of data which can be obtained. Different microscopy techniques, such as brightfield, fluorescent confocal or two-photon microscopy and a variety of imaging methods, such as two-dimensional whole slide scans, three-dimensional image stacks or time series made it possible to quickly acquire large amounts of image data in the order of several hundreds of gigabytes. In order to obtain detailed quantifications of the imaged tissue structures, it is indispensable to have efficient, flexible and easy to use image analysis tools.

We present the novel image processing and analysis tool TiQuant that allows the reconstruction and quantification of structures within liver lobules, such as hepatic nuclei, the sinusoidal and bile canaliculi networks and larger hepatic vessels from three-dimensional fluorescent confocal microscopy image stacks of different magnifications. The tool can be used to predict borders of liver lobules and the shape of hepatocytes. TiQuant can be applied to tissue blocks comprising full lobules and the margins of neighboring lobules. Thereby, we can obtain comprehensive statistical data on the physiology of liver lobules from a sub-cellular to a lobular level. The obtained reconstructions and quantifications can either be used to translate an in-vivo specimen into an in-silico specimen or to generate statistically representative liver lobules. Thereby calibrated three-dimensional spatio- temporal models enable us to study the mechanisms that control e.g. liver regeneration after intoxication or flow and perfusion within the organ.

TiQuant provides a graphical user interface facilitating its employment in wet labs and it is already successfully used by experimentalists. The tool is freely available at www.msysbio.com/tiquant.

In the contribution to the IbSB 2014 we will introduce our image processing tool TiQuant with an emphasis on a novel method for cell shape reconstruction. We will show how this method utilizes already acquired information, like nuclei, sinusoidal and bile networks, and thereby supersedes a decided cell membrane staining. Additionally, we will show how adaptation of the method allows for lobule shape reconstruction.

Investigating single-cell growth of microbial cell factories: is counting cells enough?

Stefan Helfrich,^{1, *} Alexander Grünberger,¹ Dietrich Kohlheyer,¹ Wolfgang Wiechert,¹ and Katharina Nöh^{1, †} ¹IBG-1: Biotechnology, JARA-HPC, Forschungszentrum Jülich GmbH

Bacterial growth has attracted scientists since the first studies by Monod more than 60 years ago (Monod, 1949). Since then, a plethora of empirical laws and theoretical growth models have been postulated (Peleg and Corradini, 2011). These models mainly focus on the growth of populations in connection with data aggregates. Emerging image-based single-cell technologies with microfluidic lab-on-a-chip devices allow for monitoring the development of individual cells in high-throughput (Wang and Bodovitz, 2010). With such single-cell data at hand, challenging population growth models has become feasible on a statistically sound basis.

Based on a specifically developed experimental setup (Grünberger et al., 2012) we have implemented an image analysis pipeline for the evaluation of time- lapse videos of the industrially relevant aminoacid producer C. glutamicum. The pipeline has been optimized for the identification of cells in crowded environments, tracking of cells with large spatial displacements over time, and the extraction of a multitude of cellular characteristics, i.e. cell length and cell area, for further use.

The extracted data are used to test the applicability of single-cell read-outs from low-density lab-ona-chip cultivations to published growth models based on experimental data. Quantitative differences between the use of cellular characteristics and cell number per distinct cultivation (equivalent of optical density) for computing growth measures are revealed. The implications are not specific to the bacterium C. glutamicum but generally apply to single cell investigations of other relevant organisms, for example E. coli. In combination with a suitable experimental setup the whole image analysis and modeling pipeline can be used to further investigate microbial growth and its inherent heterogeneity.

References

Grünberger, A., Paczia, N., Probst, C., Schendzielorz, G., Eggeling, L., Noack, S., Wiechert, W., Kohlheyer, D., 2012. Lab Chip 12, 2060–8.

Monod, J., 1949. Annu. Rev. Microbiol.

Peleg, M., Corradini, M.G., 2011. Crit. Rev. Food Sci. Nutr. 51, 917-45.

Wang, D., Bodovitz, S., 2010. Trends Biotechnol. 28, 281-90.

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Tracking and Registration for Automatic Analysis of Live Cell Image Data

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Automatic analysis of high-content and high-throughput image-based screens of living cells requires suitable image analysis methods. This talk presents computational approaches for cell tracking, particle tracking, and registration of dynamic live cell image data. To analyze the movement of cells and to determine cell lineages from 2D and 3D time-lapse microscopy images we have developed a cell tracking approach which integrates a mitosis detection measure. We also combined this approach with feature-based classification to quantify cell cycle delays. To correct classification errors we introduced a state transition model which exploits the temporal context. Our approach can cope with normal as well as abnormal phenotypes and was applied to RNAi knockdown image data of HeLa cells, and more recently was extended to high-throughput screens of Neuroblastoma cells.

To analyze the spatial-temporal behavior of biological particles, we have developed a probabilistic tracking approach based on particle filters or Kalman filters. The approach was applied to study virus particles in multi-channel fluorescence microscopy images and to investigate pathways of virus entry and release. An extension of this approach allows automatic identification of rare events like virus-cell fusion based on a layered probabilistic approach. We have also developed an image registration approach for spatial normalization of live cell microscopy images and accurate classification of particle motion. The approach was applied for rigid and non-rigid registration of 2D and 3D dynamic microscopy images to study the motion of nuclear particles and viral particles.

References

Harder N, Mora-Bermúdez F, Godinez WJ, Wünsche A, Eils R, Ellenberg J, and Rohr K: Automatic Analysis of Dividing Cells in Live Cell Movies to Detect Mitotic Delays and Correlate Phenotypes in Time, Genome Research 19:11 (2009) 2113-2124

Batra, R., Harder, N., Gogolin, S., Diessl, N., Soons, Z., Jäger-Schmidt, C., Lawerenz, C., Eils, R., Rohr, K., Westermann, F., and König, R. Time-Lapse Imaging of Neuroblastoma Cells to Determine Cell Fate upon Gene Knockdown. PLoS ONE 7:12 (2012) e50988

Godinez WJ, Lampe M, Wörz S, Müller B, Eils R, and Rohr K: Deterministic and Probabilistic Approaches for Tracking Virus Particles in Timelapse Fluorescence Microscopy Image Sequences, Medical Image Analysis 13 (2009) 325-342

Godinez WJ, Lampe M, Koch R, Eils R, Müller B, and Rohr K. Identifying Virus-Cell Fusion in Two-Channel Fluorescence Microscopy Image Sequences Based on a Layered Probabilistic Approach, IEEE Transactions on Medical Imaging 31:9 (2012) 1786-1808

Chang, L., Godinez, W.J., Kim, I.H., Tektonidis, M., de Lanerolle, P., Eils, R., Rohr, K., Knipe, D.M. Herpesviral replication compartments move and coalesce at nuclear speckles to enhance export of viral late mRNA. Proceedings of the National Academy of Sciences of the United States of America (PNAS) 108:21 (2011) E136-E144

Kim I-H, Chen YC. Spector DL, Eils R, and Rohr, K: Non-Rigid Registration of 2D and 3D Dynamic Cell Nuclei Images for Improved Classification of Subcellular Particle Motion. IEEE Transactions on Image Processing 20:4 (2011) 1011-1022

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Analysis of migrating NSCLC cells using Particle Image Velocimetry

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We are interested in the interplay between intracellular signaling and migratory behaviour in nonsmall cell lung cancer. For this we analyse data from migration assays involving lung cancer cells under different growth factor stimulations and inhibitor treatments. We use particle image velocimetry (PIV) to infer migratory characteristics. The PIV is a method that originates from Fluid Dynamics, where in an experimental setup tracer particles are added to a fluid and 2 photographs are taken in a short period of time. The first image is divided into small boxes. Each box is then pixelwise shifted in all directions (on the second image) and a two-dimensional cross-correlation function is computed, from which the average displacement of the objects in the box is obtained. Thus, the method yields velocity fields, similar to single cell tracking approaches, however, not for single but for groups of cells. PIV has several advantages in comparison to single-cell-tracking, for instance when the cell density is high or cells cluster to the extend that segmentation is no longer possible. Furthermore, the method also works for low-contrast bright field images and does not require nucleus staining. From the velocity fields we obtain spatiotemporal speed distributions and correlation lengths, and investigate how these change under different treatments. The obtained data is then used to fine-tune a mathematical model of collective cell migration which incorporates mechanical properties and is able to explain most of the observed migratory behavior.

Cell tracking accuracy - quantification and consequences

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Due to the progression of computer and microscopy technology, biological imaging became a true Big Data application, during the last two decades increasing quantities, resolution, and duration of biological image data enhanced the necessity of automated data processing to master the analysis of single cell fates or embryonic development in specimen such as in vitro cultured cells or in vivo imaging of organisms such as the zebrafish (danio rerio) or the fruit fly (drosophila m.).

The investigation of such biological systems is usually tackled by the analysis on a single cell basis. Usually, many specimen have to be analysed to reliably analyse the dynamics and variances. Depending on the particular goal, the difficulty to process the data sets automatically spans from lower complexity, such as cell counting, to very high complexity, such as extraction of clonal genealogies. For questions, where temporal dynamics are in the focus of interest, cells have to be tracked. Although many automatic tracking algorithms are available, most of them will supposedly perform differently on different data sets. In order to select the best suited tracking algorithm for a specific problem, tracking accuracy has to be measured by appropriate metrics, which reliably estimate the error of analyses based on the tracking data.

We present a survey on cell tracking accuracy, using the example of the endodermal development of the early zebrafish embryo. First, we introduce a set of metrics to measure cell detection as well as cell tracking accuracy. These measures are then set in relation to general questions, which may be investigated in any experiment on single cell systems.

We show that different measures are necessary to describe the accuracy of a given cell tracking algorithm in order to estimate the error of the analyses, which are based on the outcome of the algorithm. A given measure for cell tracking accuracy may be either to weak or to strict to give a useful error estimate for the subsequent analysis, if it is not specifically tailored for the given subject of research. For example, an error measure which expresses cell tracking accuracy by means of correct cell to cell associations from one image to the subsequent image is not informative, if the subject of research is cell cycle time. On the other hand, a measure expressing the cell tracking accuracy by means of correctly extracted clonal genealogies, is too strict for the same subject of research.

For selected questions related to the endodermal development of the early zebrafish embryo, we show that for different scenarios, different measures give a more informative overview on the reliability of the analysis done. Consequently, we conclude from our analysis that for each subject of research, which can be answered by tracking single cells, the correct measure for cell tracking accuracy has to be chosen in order to give information about the expected error in the resulting analysis.

Mapping amoeboid cell migration

Till Bretschneider^{1,*}

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Extracellular gradients of chemoattractants or mechanical signals can guide cell movement by directing polymerization of actin, the cellular motor, to the leading edge. Various mathematical models have been proposed which describe gradient sensing and cell polarization, mostly in terms of local excitation and global inhibition mechanisms related to original work on biological pattern formation by Turing. I will present approaches how imaging data can be used to put these models to the test. We parameterize competing reaction-diffusion models employing experimental data of actin redistributions in Dictyostelium cells, which reorient to alternating gradients of shear flow. Incorporating different types of experimental data such as random motility can successively improve parameter identifiability, while numerical simulations and theoretical analysis help constraining model parameters further.

In another mode of cell motility, namely blebbing, hydrostatic pressure forces the cell membrane to detach from the underlying actin cortex, creating protrusions in form of hemispherical blebs. Dictyostelium cells sandwiched between a coverslip and a sheet of agar chemotax by employing a mixed mode of actin protrusions and blebs. A detailed analysis of cell shape dynamics allowed us to study how blebs and actin driven protrusions interact. Results suggest that changes in geometry incurred by actin driven protrusions could direct blebs to the cell front thus underpinning a feedback mechanism that acts in concert with signaling. The examples I give highlight the demand for novel computational and statistical methods for synchronizing single cell dynamics in silico, in time and in space.

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Fourier transform-based fast characterisation of cell shapes as a tool for intelligent classification of cells in intravital microscopy

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In numerous tissue types, the kinetic behavior of cells as well as the complexity and the aspect ratio of their shapes adjust to short- and long-term changes in the actual physiological and pathological conditions of the tissue. Generally, the kinetic behavior of cells is characterized by the so called "cell tracks", i.e. the time course of the 3-D location of the centroids of the cells, as well as by various parameters characterizing the volume of the tissue occupied by the cell and those describing the surface corresponding to the outer membrane of the cell. Cells are then often classified based on a number of these parameters, often guided by a priori knowledge provided by expert biologists or medical professionals. Whilst such approach will provide an excellent starting point for analyzing complex intravital image data, ultimately we will need faster methods for automated characterization and more intelligent tools for unbiased analysis that may also reveal hidden correlations amongst the track- and shape-describing parameters.

Here we propose the use of Discrete Fourier Transform (DFT) applied on multiple-angle cross sections of cells prepared automatically by a set of ImageJ/Fiji macros. We find that the first 10 DFT parameters (F_0 – F_9) are sufficient to describe even the complex shape of microglia in the healthy or cancerous tissue of the mouse brain, or that of the macrophages in the mouse gut under physiological or pathological conditions, imaged via two-photon intravital microscopy. After normalizing the DFT components (using the amplitude $F_i = SQRT\{Re(F_i)^2 + Im(F_i)^2\}$) so that they are independent of the cell size (by dividing F_i (i = 2..9) by F_1), we were able to find clusters in the Fourier parameter space by using methods of Artificial Intelligence (AI), e.g. Self-Organizing Maps (SOM). These AI methods have also been successfully applied here to identify correlated cell tracks by training an SVM on a selected group of cell tracking parameters. In more complex cases, a 3-D SOM was necessary in order to provide adequate separation of track groups; however, for visualization purposes the 2-D version may be preferred in order to make comprehension easier for the observer.

We further propose that such automated and unbiased methods may be used as part of the design of intelligent microscopes, where the actual flow of experiments can be continually adjusted based on the instantaneous analysis of the recorded 3-D and 4-D image stacks.

Behavioral Classification of Cells by Observing their Spatial Dynamics: A Self-Similarity Approach

Marco Körner^{1, *} and Joachim Denzler¹ ¹Computer Vision Group, Friedrich Schiller University of Jena, Germany

We give a formal motivation for employing frame-wise similarity features for describing motion patterns of cells. Furthermore, we present a framework for weakly-supervised classification of individual cells by means of their kinematic behavior. Experimental results argue for the validity and applicability of our approach.

Introduction & Motivation. Cells are the smallest discrete and self-contained units of living organisms and show stand-alone metabolism. In higher organisms, cells are diversified enabling them to perform various structural or functional tasks. For instance, *neutrophil granulocytes* – i.e., representatives of white blood cells – play an important role for the *innate immune system*. As different functional behaviors are expressed by characteristic migration types, it turns out to be promising to classify arbitrary cells by means of their kinematics observed during video microscopy. While the assessment of migration types was performed manually in the past due to the lack of automatic methods, recent research focused on investigating methods for providing quantitative evaluation in a fully- or semi-automatic fashion. For instance, Mokhtari et al. [2] propose to evaluate frame-wise migration similarity based on novel distance measures.

Following their idea, we present a theoretical justification of the correctness of this approach by taking reference from the theory of *dynamical systems* and suggest a framework for extracting valuable information from these similarities. Experiments performed of synthetic data proof the validity of our methods, while their applicability is shown by real-world experiments on *in-vitro* neutrophil cell tracks.

Methods. Cell migration trajectories can be described as sequences of model configurations or *states* $\mathbf{x}_t \in \mathbb{R}^n$ evaluated at time discrete indices $t \in \mathbb{R}$. Newton's second law [3] allows to describe the driving forces of these *dynamical systems* by *first-order ordinary differential equation systems*

$$\dot{\mathbf{x}}_t = \partial \mathbf{x}_t / \partial t = (\partial \mathbf{x}_{t,1} / \partial t, \dots, \partial \mathbf{x}_{t,n} / \partial t)^\top, \mathbf{x}_t, \dot{\mathbf{x}}_t \in \mathbb{R}^n.$$

Hence, a temporal self-similarity map (SSM) [1]

$$\mathbf{S} = (s_{i,j})_{i,j} \in \mathbb{R}^{N \times N}, s_{i,j} = d(f(\mathbf{x}_i), f(\mathbf{x}_j)),$$

representing the frame-wise system state similarities obtained by appropriate distance functions $d(\cdot, \cdot)$ of arbitrary features $f(\cdot)$ at time indices $1 \le i, j \le N$ contains all dynamic information of the inducing physical system. For this reason, we propose to describe the appearance of these SSMs by means of various quantitative measures, for instance, their Fourier spectra or velocity distributions. Classification for cell types based on these features vectors is further conducted by pre-trained Support Vector Machines (SVM).

Experimental Evaluation. In order to demonstrate the validity as well as the applicability of our method, we performed two sets of experiments. For simplicity, we used the actual 3d positions of the cells them- selves as features f and Euclidean distances $d(\mathbf{x}, \mathbf{y}) = \sqrt{\mathbf{x}^{\top} \mathbf{y}}$ as similarity measure in all experiments. Average accuracy numbers where obtained after *10-fold cross validation*.

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First, we reproduced the *proof-of-concept* experiment of Mokhtari et al. [2] on their synthetic data. In conformity to them, we obtained an accuracy of 99.7% when using the labels of 1% of the dataset as ground-truth for training.

Furthermore, we ran *real-world* experiments on trajec- tories of *in-vitro* neutrophils, again the same as used in [2]. Employing 10% of their classification results as training evi- dence yielded an overall consensus in 85.4% of the cases.

Summary. Next to a formal motivation for employing frame-wise similar- ities for the use of cell behaviors classification, we presented a framework for extracting meaningful features from *temporal self-similarity maps*.

Acknowledgments. We thank the authors of [2] for providing the data used in our experiments and all the invaluable discussions.

References

[1] M. Körner. Methods for Model-based and Model-free Recognition of Articulated Actions in Multi-Camera Environments. PhD thesis, Friedrich Schiller University of Jena, Germany, 2014. (to appear).

[2] Z. Mokhtari, F. Mech, C. Zitzmann, M. Hasenberg, M. Gunzer, and M. T. Figge. Automated characterization and parameter-free classification of cell tracks based on local migration behavior. PLoS One, 8(12):e80808, 2013.

[3] G. Teschl. Ordinary Differential Equations and Dynamical Systems. Graduate studies in mathematics. 2012.

Analyzing and modelling immune cell migration

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Since the beginning of this century immunologists have applied time-lapse fluorescence microscopy imaging to living tissues in mice. This has resulted in a highly dynamic picture of how immune cells migrate and interact in vivo. For example, T cells of the immune system need to interact with dendritic cells (DCs) to become activated and it was shown that the search for DCs within lymphoid organs occurs in a roughly random manner. The vivid movies and rich data sets resulting from imaging can be incorporated into modelling approaches that try to explain how immune responses develop in an efficient and timely manner. We have developed realistic, 3D simulation models of immune cell migration and interactions within lymph nodes using the Cellular Potts Model. These simulations showed that random migration within organs is indeed a very efficient strategy for T cells. Further modelling showed that a random search for the correct lymphoid organ (i.e., preceding the migration within the organ) also results in a timely immune response and that our predictions for the kinetics of T cell recruitment closely matched experimental data. Finally, we imaged the migration of killer cells in the skin amidst growing foci of Herpes Simplex Virus infection. Detailed analysis of short-term videos demonstrated that the killer cells exhibit a subtle CXCR3-dependent chemotaxis towards the foci. Modelling predicted that this subtle effect was critical for the T cells to accumulate close to infected cells. Long-term long-interval imaging subsequently confirmed that the subtle chemotaxis could explain the accumulation patterns observed in vivo. In summary, immune cells frequently adopt an efficient random search strategy, whereas sometimes subtle chemotaxis makes this even more effective.

Image -based modeling of drug delivery in HeLa multicellular spheroids

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Though the majority of tumor cells grow in three-dimensional (3D) structures in vivo, their development and response to drug treatments is mainly studied in monolayer cultures (2D). However, the cells' physiology and drug response is markedly different in 2D or 3D. Resistance is often associated with the microenvironment present in 3D structures, but its origin is currently poorly understood. The potentially different outcomes from anti-neoplastic drug application in 2D and 3D systems might be to some extent the result of a complex and non-uniform spatio-temporal drug distribution emerging due to tumor geometry. In our work we focus on understanding quantitatively the influence of spatial organization of cells in 3D structures and its impact on drug delivery. Our study utilizes HeLa multicellular tumor spheroids (MTS) and uses the following tools: i) high throughput, semi-automatic spheroid handling, imaging and data-extraction ii) biophysical, agent-based model of intercellular and environment interactions and iii) intracellular model of cell signaling. In this work we present how to calibrate the biophysical growth model to experimentally measured data. Additionally, we discuss how different mechanisms of drug delivery inside tumor affect the spatio-temporal drug distribution. We strongly believe that the development of this quantitative and image-based in silico model will greatly aid future exploration of drug delivery into solid tumors.

References

Hoehme and Drasdo, A cell-based simulation software for multi-cellular systems, Bioinformatics, 2010.

Thoma et al., 3D cell culture systems modeling tumor growth determinants in cancer target discovery, Adv. Drug Delivery Rev., 2014.

Bertaux et al., Dynamics of cell-to-cell variability in TRAIL-induced apoptosis explains fractional killing and predicts reversible resistance, in revision, 2014. Available as INRIA research report.

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Model-based outliers treatment in image-based systems biology: filtering vs. noise model

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Background: Biological tissues are dynamical systems with a complex spatial organization. To study these systems, imaging methods are used and constantly improved to allow for a quantitative assessment. Image-based modeling aims at systematically using this imaging data to create spatio-temporal models of the underlying biological processes and to understand their working modes. It is however not clear how imaging artifacts, e.g. outliers, can be treated in the context of image-based modeling.

Methods: We illustrate the process of data-driven tissue-scale modeling imaging data containing outliers using the example of gradient formation for dendritic cell guidance towards the lymphoid vessels. The elementary steps are model development, parameter estimation and uncertainty analysis. To account for outliers, we developed a model- based approach relying on a reformulated likelihood function for the parameter estimation. This approach has been compared to common image filtering methods using synthetic data. We found that the model-based approach outperforms available methods and significantly reduces bias and variance in the estimated model parameters.

Results and Conclusion: We applied the developed method to staining images of the chemokine CCL21, which is responsible for dendritic cell guidance towards the lymphoid vessel. The images contain outliers created by migrating fibroblasts and our novel method could substantially reduce the bias in the estimated parameters. The developed method is widely applicable to parameter estimation problems from images and does, in contrast to filtering methods, not require the fine tuning of parameters.

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Patchy biofilm formation: resource-directed motility of the microalga Seminavis robusta

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Biofilms are temporal and spatial heterogenous communities that can be formed by phototrophic organisms. Ephemeral, local hotspots of limiting nutrients are among the factors affecting the patchy distribution of such communities on a micro-scale. In this study, we explore the behavior and motility patterns of a marine benthic diatom Seminavis robusta towards a Si-loaded bead as a hotspot nutrient source. Semi-automatic tracking of cells and their accumulation was monitored within 10 min intervals. Tracks and motility parameters were analyzed to determine factors affecting the chemo-attraction. Starved cells moved faster, turned more frequently and accumulated towards a Si-loaded bead in comparison with control beads. Changing the nutrient source to GeO_2 elicited negative response, indicating that the attraction was guided by specific nutrient requirement of the cells. Longer starvation slowed the response of the cells toward the Si-source. Our results suggest that chemotactic behavior of this diatom increases its chance to detect and benefit from high nutrient micro-environments. We therefore show for the first time a chemoattraction of a photosynthetically active organism towards dissolved inorganic salts and provide a new explanation for the often observed patchiness of biofilms.

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Cell Tracking of Neutrophils in Phagocytosis Assays

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Within the field of Image-based Systems Biology, microscopy imaging is a substantial approach to study dynamic biological systems. Visualization of cell motility, tracking, and statistical analysis is necessary to increase the understanding of the functionality of complex biological systems. However, accurate segmentation and tracking of cells in image data is still a challenging problem. Here, we propose a fully automated cell segmentation and tracking algorithm for analysis of microscopy time-lapse videos. The algorithm is applied to microscopy data of motility and phagocytosis studies of polymorphnuclear neutrophils (PMNs) in assays with Candida albicans and Candida glabrata. The automated tracking is validated using reference tracks obtained from manual tracking.

Complement Factor H-Related Protein 3 (CFHR3) inhibits co-receptor complex mediated B cell activation by preventing crosslinking of Complement Receptor Type 2 (CD21/CR2) and the B cell receptor

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The complement system is part of the innate immune system and plays an important role in the protection from invading microorganisms, clearance of immune complexes and modified cells, as well as the modulation of the adaptive immunity. Complement activation leads to opsonization of foreign cell surfaces with C3b and its cleavage products iC3b and C3d. These cleavage products are recognized by the Complement Receptor Type 2 (CR2/CD21), which is part of the co-receptor complex of the B cell receptor (BCR) on mature B cells and links innate and adaptive immunity. Ligand binding to CR2 leads to a cross-linking of the co-receptor complex with the BCR, which substantially lowers the antigen threshold for B cell activation and enhances inflammatory reactions. However, no such B-cell response is induced by opsonized self cells like apoptotic particles. In this case complement activation is tightly controlled by a variety of membrane bound and soluble complement regulators like factor H (FH). So far little is known about the factor H-related (CFHR) proteins CFHR1, CFHR2 and CFHR3 on the adaptive immune response. All three proteins bind to self-structures via heparin interaction and to complement C3 cleavage products C3b, iC3b and C3d, the ligands of CR2. Thus we asked, whether CFHR1, CFHR2 or CFHR3 modulate B cell activation via the co-receptor complex on B cells and thereby prevent autoimmunity.

Here we show that CFHR3, but not FH, CFHR1 or CFHR2, inhibits the intracellular signaling cascade via CR2 as shown by blockade of phosphorylation events as well as Ca2+ release. Using an image-based approach, cross-linking of CR2 with the BCR upon B cell activation in the presence of CFHR3 was analyzed. Addition of a construct, including C3d associated with anti-IgM, to the B cells leads to visible clustering of the BCR and CR2. An image based analysis procedure was developed, that automatically detects clusters of BCR and CR2, and the regions of their overlap. With this tool co-localization of CR2 and the BCR was followed in presence and absence of CFHR3 and the area of overlapping clusters relative to the total area of BCR and CR2 clusters was statistically analyzed.

CFHR3, but not FH or CFHR1, reduced co-localization of BCR and CR2 significantly. These results demonstrate that CFHR3 blocks complement induced sensitization of B cells by preventing the cluster formation of the BCR with its co-receptor complex. This new function of CFHR3 may explain why microorganisms like the human pathogenic yeast Candida albicans bind CFHR3 to their outer surfaces.

Automated Image Analysis of Microfluidic Droplets

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Secondary metabolites of Actinobacteria and other microbia are valuable natural substances for the production of new antibiotics. As most of those species are still unknown and conventional cultivation methods have their limits, the development of new and innovative methods is essential. We are developing a high-throughput screening method based on a microfluidic system on a chip to discover a large amount of new species and their metabolites in a short time. Picolitre droplets with bacteria spores are automatically generated, incubated and can be dosed with different substances. Coloured beads inside the droplets are used as a barcoding system. Automated image analysis at different stages of the process is used to analyse and assess the droplets, their shape and content. The determination of the volume of the added substance and thereby the comparison of the droplet volume before and after the dosing is subject of this work.

In vivo quantification of arthritic severity in a murine model by micro-CT imaging

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Rheumatoid arthritis (RA) is an autoimmune disease that affects approximately 1% of the adult population leading to pain, disability and, if not treated, significantly decreased life span. Although the exact aetiology of RA is not known, evidence suggests that autoreactive T cells together with other immune cells are creating an inflammatory cytokine milieu around the joints of the patient. The chronic inflammation causes destruction of tissue, especially cartilage and bone, which at early stages are painful and at later stages severely debilitating. Although RA is an exclusively human disease, animal models of arthritis are an invaluable tool to hypothesise about the aetiology and to test therapeutic strategies. The degree of arthritis, RA or animal models, in a limb is today scored semi-quantitatively by the physician or experimentalist, based on bone degradation from X-ray images. Sometimes metabolic data recorded by PET or fluorescence imaging is available for the scoring and in the case of RA the patient answers questions about pain levels. Although the scoring methods are clinically well motivated and tested, the score will still be dependent on the individual's judgement and any evaluation of the disease progress will be subjective. We will present quantitative measures based on the cortical bone thickness and surface roughness in a longitudinal study of arthritis in a murine model. The mice are immunised with a glucose-6-phosphate isomerase peptide (G6PI) inducing arthritis and their paws are imaged using a micro-CT scanner throughout the experiment. This gives us three dimensional image data of the arthritic paw during the different stages of the disease which is the basis of our analysis. Both cortical bone thickness and roughness have their own merits and by combining them we have a sensitive, yet robust, method to determine the structural damage caused by arthritis. We also discuss how the quantification of arthritic bone degradation can help with the evaluation of therapy strategies in animal models and RA diagnostics. We predict that non-subjective quantification of bone degradation, which our analysis is an important step in, will become an important tool for both experimentalists and clinicians.

Blind reconstruction of Structured Illumination Microscopy data (blind-SIM)

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Structured Illumination Microscopy (SIM) is a super-resolution optical microscopy method which enables imaging of biological samples down to a resolution of \sim 100 nm. The fluorescent sample is excited by a light pattern, typically a fine sinusoidal grating. Computational treatment must be applied to process the raw data. The classical reconstruction algorithm requires knowledge of the illumination pattern and is prone to artefacts in the cases where the period and phase parameters of the excitation fringes cannot be precisely defined.

Blind-SIM is a novel reconstruction method which enables the reconstruction of SIM data without prior knowledge of the illumination pattern and only minimal assumptions [1]. This approach promises to widen the range of application of the SIM method to more complicated and biologically relevant samples. For instance, we successfully reconstructed some SIM data where the excitation fringes were distorted [2].

A least square deconvolution approach is used to reconstruct the fluorophore density ρ along with the N illumination patterns I_{illu} ,n (n = 1 to N), if we describe the imaging process by:

$$I_{det,n} = (I_{illu,n} \cdot \rho) \otimes h,$$

where $I_{det,n}$ is the detected intensity for each illumination pattern, \otimes the convolution operator and h the point spread function (PSF).

Recently, we developed *thick slice* blind-SIM, a method that rejects the out-of-focus light out of a single plane of data [3]. It uses a 3D PSF but places the 2D data at the middle of an extended stack. This method is fitted for the treatment of 2D SIM data acquired in thick samples, providing super-resolution as well as optical sectioning.

This poster will introduce the working principle of the blind-SIM algorithm, explain how it can be used to process challenging SIM data, and present its latest achievements.

References

[1] E. Mudry; et al., Nature Photonics, 6, 312-315 (2012).

[2] R. Ayuk; et al., Optics Letters, 38, 4723-4726 (2013).

[3] A. Jost; et al., Optics Letters, (submitted).

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Automated image analysis of the host-pathogen interaction between immune cells and human-pathogenic fungi

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Since fungal infections have drastically increased during the last decades, it is a major goal to quantify the interactions between immune cells and human-pathogen fungi. In this work we shed the light on the fungus Lichtheimia corymbifera as an example of the most dangerous fungi to humans. L. corymbifera is a ubiquitous soilborne zygomycete fungus, which is an opportunistic human pathogen in immunocompromised patients. The fungus can cause life-threatening diseases by attacking the lung during early stages of invasion and by disseminating during later phases causing systemic infection. One of the first barriers, which the fungus needs to cope with in the lung tissue, is phagocytosis by alveolar macrophages. Here, we report on phagocytosis assays for murine alveolar macro-phages co-incubated with resting, swollen and opsonised spores of a virulent and an attenuated L. corymbifera strain. A major finding of this study is the significantly increased phagocytosis ratio of the virulent strain if compared to the attenuated strain. We quantify the phagocytosis by performing automated analysis of fluorescence microscopy images and by computing ratios for (i) fungal phagocytosis, (ii) fungal adhesion to phagocytes and (iii) fungal aggregation and spore cluster distribution in space. Automation of the image analysis yields objective results that overcome the disadvantages of manual analyses being time consuming, error-prone and subjective. Therefore, it can be expected that automated image analysis of confrontation assays will play a crucial role in future investigations of host-pathogen interactions.

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NDICE: Noisy Discriminative Immune-Cell Extractor

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Live-cell imaging has become a powerful method to determine the number, motility and behaviour of cells. Analysis of the pathogens' behaviour is important for drug and disease research. In order to automatically determine a cell's motility in live-cell images, several image-segmentation and tracking algorithms have been developed. While images of stained cells can be analyzed with high accuracy, there is still a lack of automated tracking algorithms for grayscale timelapse images. The proposed approach aims to provide a full environment for the tracking of ellipse-like shaped cells in any grayscale timelapse images created with a light microscope. The algorithm is embedded into a graphical user interface for easy use. It performs several image-segmentation steps and a final tracking step to estimate the motility of single cells, as well as clustered cells. To obtain segmentation, the images are preprocessed at first to segregate the objects from the background, using intensity and variance information. Secondly, a rough estimation of the number of objects and their positions is performed by convolving the image with several circles. Using this estimations, single cells and clusters of cells are detected. Inside the clusters, single cells are validated using an active contour algorithm which also provides a shape of the object. The final segmentation is achieved by eliminating false positive objects with a watershed-like process and a more detailed look at the shape of the objects in a postprocessing step. In the Tracking step, consecutive images are analyzed for nearest-neighbor associations to build up trajectories for the objects. After this short trajectories are build, lost and found objects are compared and trajectories are combined if possible to track the objects over the whole video. The parameters for the algorithm can be detected and optimized semiautomatically by labeling one example image.

Epithelial invasion outcompetes hypha development during *Candida albicans* infection as revealed by an Image-based Systems Biology approach

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Candida albicans is the most common fungal pathogen of the human mucosal flora and can become invasive in immunosuppressed patients and thus frequently causes systemic infections such as sepsis. The transition from yeast to hypha and the subsequent invasion of host-tissue represent major determinants in the switch from benign colonizer to invasive pathogen. We applied an Image-based Systems Biology approach to quantify the kinetics of hyphal length growth, hyphae development and epithelial invasion. First, fluorescence microscopy images of C. albicans undergoing epithelial invasion during a time course of 6 hours were generated and then automatically analysed. This automated image analysis provided quantities that allow for the time-resolved characterization of the morphological and invasive state of fungal cells. Based on these data, we generated two mathematical models, the kinetic growth model and the kinetic transition model, that were formulated as differential equation systems. The kinetic growth model describes the dynamics of hyphal length and revealed that hyphae undergo massive invasion of epithelial cells. Applying the kinetic transition model, we could quantify the route of invasion in the state-space of non-invasive and invasive fungal cells depending on their number of hyphae. Our analysis revealed that after initiation of hyphae formation the directly followed invasion outcompetes formation of further hyphae. This indicates that in vivo, the yeast to hypha transition must be under exquisitely tight negative regulation to avoid the transition from commensal to pathogen invading the epithelium.

Image analysis of immune cell interactions from two-photon microscopy data

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Immune cells are intrinsic constituents of the immune system, which keeps us healthy. Dysfunctions of the immune system – due to infection, immune deficiencies or allergy – can lead to severe disease. Therefore, the study of the immune cell interactions is essential. The behavior of immune cells can be extremely complex and not always accessible for study in an experiment. This problem can be overcome by image-based systems biology approach: computer simulations of image-derived models can help to predict the behavior of immune cells and suggest new settings for the further experimental study. Automated image analysis is an essential step of the image-based systems biology approach. Combined with live-cell imaging, it allows to quantitatively characterize the immune cells interactions in their natural environment, which can be further employed to construct computer models. This work presents an image analysis workflow for two-photon microscopy data of different immune cells. It is shown what problems can arise and how they can be solved in order to quantitatively describe the immune cells and their interactions.

Automated characterization of cell tracks based on local migration

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Cell migration is a critical parameter for a wide variety of physiological and pathophysiological processes. Even though microscopy experiments are routinely performed today by which populations of cells are visualized in space and time, valuable information contained in image data is often disregarded because statistical analyses are performed at the level of cell populations rather than at the single-cell level. Image-based systems biology is a modern approach that aims at quantitatively analyzing and modeling biological processes by developing novel strategies and tools for the interpretation of image data. In this study, we take first steps towards a fully automated characterization and parameter-free classification of cell track data that can be generally applied to tracked objects as obtained from image data. The requirements to achieve this aim include: (i) combination of different measures for single cell tracks, such as the confinement ratio and the asphericity of the track volume, and (ii) computation of these measures in a staggered fashion to retrieve local information from all possible combinations of track segments. We demonstrate for a population of synthetic cell tracks as well as for in vitro neutrophil tracks obtained from microscopy experiment that the information contained in the track data is fully exploited in this way and does not require any prior knowledge, which keeps the analysis unbiased and general. The identification of cells that show the same type of migration behavior within the population of all cells is achieved via agglomerative hierarchical clustering of cell tracks 1 in the parameter space of the staggered measures. The recognition of characteristic patterns is highly desired to advance our knowledge about the dynamics of biological processes.

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Quantitative image analysis of cell colocalization in murine bone marrow

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Long-term antibody production is a key property of humoral immunity and is accomplished by longlived plasma cells. They mainly reside in the bone marrow, whose importance as an organ hosting immunological memory is becoming increasingly evident. Signals provided by stromal cells and eosinophils may play an important role for plasma cell maintenance, constituting a survival microenvironment. In this joint study of experiment and theory, we investigated the spatial colocalization of plasma cells, eosinophils and B cells by applying an image-based systems biology approach. To this end, we generated confocal fluorescence microscopy images of histological sections from murine bone marrow that were subsequently analyzed in an automated fashion. This quantitative analysis was combined with computer simulations of the experimental system for hypothesis testing. In particular, we tested the observed spatial colocalization of cells in the bone marrow against the hypothesis that cells are randomly distributed. We find that B cells and plasma cells highly colocalize with stromal cells, to an extent larger than in the simulated random situation. While B cells are preferentially in contact with each other, i.e. form clusters among themselves, plasma cells seem to be solitary or organized in aggregates, i.e. loosely defined groups of cells that are not necessarily in direct contact. Our data suggest that the plasma cell bone marrow survival niche facilitates colocalization of plasma cells with stromal cells and eosinophils, respectively, promoting plasma cell longevity.

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CCEAN: A Tool for Cell Count Estimation aided by Area Calculation

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Image analysis plays a large role in biological research. Although error-prone and tedious, it is still best practice to do this analysis manually.

One of the most common tasks in biological image analysis is cell detection and counting. Existing cell counting tools often have issues when facing clustered cells.

The Cell Count Estimation aided by Area Calculation (CCEAN) tool addresses these problems by using a hybrid approach of cell detection and area calculation.

We are studying the effect of different BMP-2 mutants to muscle growth using immunofluorescence microscopy. Images show C2C12 cells stimulated for myogenic differentiation and stained using DAPI (nuclei, blue) and myosin heavy chain an-



Figure 1: Use case of CCEAN. A used microscopy image is depicted in (a). Cells are colored white (b), muscle tissue is detected and cells within the tissue displayed (c). A resulting overlay image is shown in (d).

tibody (green, see Fig. 1 a). The percentage of cells within muscular tissue (fusion index) is a measure of myogenic differentiation potential.

We use the image processing library OpenCV [1] to estimate the fusion index. We differentiate nuclei from background by using a modified version of the original Otsu algorithm [2], which works on overlapping sub-images to mitigate the adverse effects of varying background intensities. Singular nuclei are detected using contour detection and ellipse fitting. The average area of a single nucleus is calculated and a total count is extrapolated based on the area of all nuclei. In a second pass, the muscular tissue is detected semi-automatically, taking advantage of the human ability to intuitively capture large, connected structure. Finally, the fusion index is calculated using the nucleus area within myotubes and the overall nucleus area (Fig. 1 b-d).

In our future work we plan to further improve the tool and to find other use cases, since CCEAN generally performs well with images containing large numbers of (partially) clustered cells.

References

[1] G. Bradski, "The OpenCV Library", Dr. Dobb's Journal of Software Tools (2000)

[2] N. Otsu, "A threshold selection method from gray-level histograms", IEEE Transactions on Systems, Man and Cybernetics, Vol. 9, Issue 1 (1979)

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Segmentation of individual cells at phase contrast microscopy images

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Time-lapse microscopy imaging is a commonly used modern method for observing the dynamics of cells and tissues. A large number of images that time-lapse microscopy generates is difficult to evaluate manually, and computer methods of image processing would be highly advantageous. Quantifying of mammalian cancer cell images captured by phase contrast microscopy is especially challenging. Cancer cells have irregular shapes that change over time and the mottled background pattern is partially visible through the cells. In addition, the images contain artifacts such as white areas around the cell edges - so called halos.

Phase contrast microscopy is often used in the study of mammalian cancer cells to assess biotoxicity of materials in vitro. A common approach in biotoxicity assessment is measuring cell growth rate or counting the number of living cells. Phase contrast microscopy provides a nearly natural environment for cells due to small invasiveness and a low-level of exposure.

We developed a novel algorithm for segmentation of individual cells. First part separate the cells from the background and it is based on the differences in time between consecutive images and a combination of sophisticated thresholding, blurring, and morphological operations. It is fast and precise. The second part of our algorithm separates individual cells in the clusters. It uses the halos between cells (thresholding and modified skeletonization) and fills the missing parts by connecting the hanging branches of the skeleton via Dijkstra algorithm.

We created the software which implements our segmentation method. We added the possibility to modify the resulting segmentation. User can modify the result by merging or splitting the cell regions that was found by out algorithm.



Yeast Image Toolkit: A benchmark and evaluation strategy for yeast segmentation and tracking algorithms

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Many biological research questions are answered through the use of time-lapse microscopy and automated tracking of cells. Brightfield imaging is often employed to minimise the undesirable effects of phototoxicity during extended image-acquisition. Brightfield images are more difficult to segment than, e.g., fluorescence images. Consequently, a number of software solutions have been developed independently of each other, attempting to solve this problem. However, due to the lack of broadly available benchmarks and comparison methodologies, the objective software's quality assessment is barely possible leaving the end user with little information for choosing an optimal software solution adapted to his problem. To overcome this issues we provide a benchmark dedicated to yeast monolayer cultures together with a platform for evaluating the performance of segmentation and tracking algorithms. The benchmark data-sets were obtained by manual annotation of brightfield images of Saccharomyces cerevisiae (budding yeast), imaged with a 100x objective every 2-6 min for 20-360 min (frequency varies in datasets). Experimental conditions cover typical scenarios encountered in monolayer cultures i) individual cells and small colonies, ii) colony translations and merging, and iii) large colonies with significant clustering of cells. For software evaluation, we provide a python-based software facilitating the numerical and visual evaluation of benchmarks. Aditionally, we provide a web-based resources, available at http://yeast-image-toolkit.biosim.eu/, where the benchmark data-set and the software tool can be downloaded. Using these resources we evaluated five widespread software tools dedicated to yeast segmentation and tracking in brightfield images: CellProfiler, CellTracer, CellID, Tracker, and CellSerpent. We kindly invite the community to contribute to the number of benchmark data-sets available at the above mentioned site, thus increasing the number of use-cases that existing and new software can be tested against.

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Automated detection of circulating tumor cells with naive Bayesian classifiers

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Personalized medicine is a modern healthcare approach where information on each person's unique clinical constitution is exploited to realize early disease intervention based on more informed medical decisions. The application of diagnostic tools in combination with measurement evaluation that can be performed in a reliable and automated fashion plays a key role in this context. As the progression of various cancer diseases and the effectiveness of their treatments are related to a varying number of tumor cells that circulate in blood, the determination of their extremely low numbers by liquid biopsy is a decisive prognostic marker. To detect and enumerate circulating tumor cells (CTCs) in a reliable and automated fashion plays are collected with a functionalized medical wire and are stained for fluorescence microscopy so that their color signature can be used for classification through the construction of Red-Green-Blue (RGB) color histograms. Exploiting the information on the fluorescence signature of CTCs by the NBC does not only allow going beyond previous approaches but also provides a method of unsupervised learning that is required for unlabeled training data. A quantitative comparison with a state-of-the-art support vector machine, which requires labeled data, demonstrates the competitiveness of the NBC method.

Vinculin Binding Angle in Podosomes Revealed by High Resolution Microscopy

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Podosomes are highly dynamic actin-rich adhesive structures formed predominantly by cells of the monocytic lineage, which degrade the extracellular matrix. They consist of a core of F-actin and actin-regulating proteins, surrounded by a ring of adhesion-associated proteins such as vinculin, talin and paxillin. We have characterised the structure of podosomes in macrophages, particularly the structure of the ring, using three super-resolution fluorescence microscopy techniques: stimulated emission depletion microscopy, structured illumination microscopy and localisation microscopy. Rather than being round, as previously assumed, we found the vinculin ring to be created from relatively straight strands of vinculin, resulting in a distinctly polygonal shape. The strands bind preferentially at angles between 116° and 135°. Furthermore, adjacent vinculin strands are observed nucleating at the corners of the podosomes, suggesting a mechanism for podosome growth.

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