Project No.	**ILRS_04/2017**
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From single-cell to whole-organ monitoring: Morphokinetic characterisation of infection via quantitative image analysis

Project Abstract:

Infectious diseases manifest themselves across multiple scales, as reflected by the behavior of single cells, as well as by the functioning of whole organs. Using various imaging techniques, infection processes can be monitored at the single-cell level by fluorescence confocal or wide-field microscopy, as well as at the whole-organ level by lightsheet microscopy. The research group Applied Systems Biology (ASB) collaborates with multiple experimental groups – both within and outside of the ILRS – to study infectious diseases based on imaging experiments at various scales. In the context of kidney diseases, we study the development of the pneumococcal hemolytic uremic syndrome (HUS) at the cellular level by fluorescence microscopy [1]. We also focus on morphological aspects of kidneys of mice suffering from HUS that is induced by Shiga toxin derived from enterohemorrhagic Escherichia coli. These experiments are done at the whole-organ level using lightsheet microscopy [2]. All these studies require the development of mathematical algorithms for the automated analysis and objective quantification of the infection processes. The most challenging aspects of this type of image analysis include the handling of large amounts of data and the acceleration of the computations. As an example, whole-organ imaging by lightsheet microscopy produces huge amounts of data that can easily reach tens to hundreds of gigabytes per experiment. Currently, our analysis of a single lightsheet experiment requires between several hours and one day, which could be drastically reduced by using various methods of high-performance computing. For this purpose, ASB recently acquired a new high-performance server based on Graphics Processing Units (GPUs). Apart from the issues of data handling and computation speed, segmentation algorithms need to be developed that can analyze cellular and tissue-level behavior in near-intact biological conditions, e.g., by avoiding fluorescence labeling that may otherwise interfere with the infection scenarios. Taken together, this project will allow the reliable and fast measurements of cell- and organ-specific structures that can be correlated with the early recognition and progression of diseases such as HUS.

Work plan:

WP 1: Advanced analysis of fluorescence microscopy studies (1st year)

In previous work, we have developed a basic approach to characterize the tissue damage in pneumococcal HUS by identifying and measuring the areas of the endothelial cell layer from which the cells have retracted, leaving behind an exposed region of subendothelial matrix [1]. In addition to the areas and numbers of such discontinuities of the cell layer, a complete set of morphometric measures will be added to the analysis toolkit in this project. We will characterize the shapes and orientations of these exposed areas, which may reveal the potentially different action mechanisms of various pathogens that cause the cell retraction. We will also study the time-dependent development of the tissue damage to deduce morphokinetic measures that will further our understanding of the action mechanisms of the various pathogens. When cells and tissue samples are labeled for fluorescence microscopy, the labeling itself may interfere with the biological system. This issue will be resolved by developing novel methods to identify individual cells and tissue areas without fluorescence labeling, in order to enable a study of pneumococcal HUS in unlabeled intact tissue samples. Such label-free methods will be complemented by mathematical filters that are sensitive to edges, intensity gradients, curvature and other patterns. It will be equally important to develop a multi-step background correction workflow that will be applied to the images before processing. We will also use machine learning techniques to further increase the specificity and efficiency of the analysis procedure, especially when the exposed subendothelial matrix consists of areas of varied matrix content. A set of such sub-areas can be identified and used to train a network of artificial neurons in a deep-learning system to ultimately identify the complex structures of damage in pneumococcal HUS.

WP 2: Fast and efficient processing of lightsheet microscopy data (2nd year)

When analyzing lightsheet microscopy data recorded from whole organs in the three spatial dimensions, we face the severe problems of very high memory requirement and of low computational speed. In this project, the performance of the algorithm will be enhanced by (i) the implementation of the code in a machine-oriented programming language, e.g. C++, and (ii) the optimization of data handling, involving the task-dependent down-sampling of the data. An easy-to-use Graphical User Interface (GUI) will be implemented to allow the direct control of specific parameters of the image segmentation workflow.

WP 3: Segmentation and measurement of organ-specific structures (3rd year)

New and improved algorithms for the segmentation of organ-specific structures will be implemented. Regarding our previous work on lightsheet microscopy data of whole kidneys, we will go beyond determining the number and size of glomeruli, and we will measure their spatial distribution and relate this to the organ function under HUS induced by bacterial infection. This will require the application of various methods from graph analysis and/or Monte Carlo simulations of random cell position models. The analysis will be extended to identify and characterize tubular structures in the kidney, including morphological (e.g., thickness and length) and integrity measures that are expected to be affected by the disease. The automated segmentation of irregular and elongated tubules will rely on neuronal segmentation methods, superpixels, and skeletonization-based approaches. Several techniques need to be implemented and compared quantitatively in order to evaluate their overall performance. This will allow to measure organ-specific structures reliably, and to correlate the observed changes with the early recognition and progression of diseases such as HUS.

[1] Meinel C, *et al.* (2017) S. pneumoniae from patients with hemolytic uremic syndrome binds human plasminogen via the surface protein PspC and uses plasmin to damage human endothelial cells. The Journal of Infectious Diseases [DOI: 10.1093/infdis/jix305]

[2] Klingberg A, *et al.* (2017) Fully automated evaluation of total glomerular number and capillary tuft size in murine nephritic kidneys using lightsheet microscopy. *Journal of the American Society of Nephrology* 28(2), 452-459.