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Masters Theses in Biomedical Optics and Computational Image Analysis

Conventional epifluorescence microscopy is limited (by the nature of the light, i.e. by diffraction) in resolution and is therefore insufficient to study the nanostructure of subcellular components smaller than about 200 nm. The group of Prof. Cremer and Dr. Birk (IMB Mainz) has developed a number of super-resolution laser microscopy ("nanoscopic") methods that can overcome this limitation. With these microscopes we can obtain highly accurate information about cell components on the nanometer scale. Thus we can determine - among others - the position of fluorescently labeled proteins with an accuracy that was previously accessible only by electron or X-ray microscopy. Devices currently available for this 'Nano Imaging' of biomolecular structures include laser interference illumination (Spatially Modulated Illumination, SMI, or Patterned Excitation Microscopy, PEM) for high precision scanning of the sample preparations and Spectrally Assigned Localization Microscopy (SALM) for single fluorophore detection. These microscope systems can be applied to study the composition, function and metabolism of many biomolecular structures. The study of the architecture of various cellular components and their functional layout has profited from these revolutionary developments in light microscopy like few other areas of biology. In the following Masters theses, an existing SALM system will be used.

Project 1) Super-resolution laser microscopy of the architecture of drug receptors

In this master thesis, it is planned to transfer the approaches and results obtained so far in single cells to tissue sections. Until now, only commercial fluorescent dyes (and some fluorescent proteins) were used, in which the mechanisms of reversible photobleaching (which are essential for localization microscopy) are not well understood. The fluorescence behavior of different dyes to tunable laser sources (in the UV to 220nm, in the IR to 1000nm) shall be investigated to select suitable dyes. The immunostained sample preparations are prepared and provided by collaboration partners.

Highly innovative laser-based methods will be used to characterize the architecture of the components of an endogenous signaling system in various mammalian cell types. Through labeling with two or more different fluorophores we can characterize the individual receptor proteins and the metabolizing enzymes for the endogenous ligands in their spatial arrangement accurately. Thus the physiological processes of the underlying system can be investigated at the subcellular level.

Project 2) Molecular resolution by image analysis

Setting up novel microscope devices is only half of the solution to obtain super-resolution information. The raw data stacks need then to be analyzed with respect to the arrangement of the

fluorophores inside the cell nucleus. In this Masters thesis, cluster analysis of fluorescently labeled proteins will provide the basis to discriminate e.g. between cancer cells and healthy tissue at the single cell level, well before presently used cancer markers can be detected. Here, computational methods (in the Matlab programming environment) will be employed to evaluated experimental (microscopy) data from localization experiments. Several Matlab-based toolboxes are available in our group to automatize many of the tasks. However, training is required to use these algorithms to produce meaningful data. In addition, new functions need to be developed in order to study ensembles of cells.

Project 3) Localization microscopy of epigenetic regulators in active and inactive chromatin domains

Recent years have witnessed several molecular pathways that modify histones and DNA in a dynamic manner. Such modification patters are heritable, but do not involve changes in the DNA sequence and therefore are referred to as 'epigenetic' modifications. Thus, while genetic information provides the basic information to encode cellular contents, the epigenetic information defines how, when and where this information has to be used. We would like to study the localization of specific epigenetic regulators with respect to active and inactive chromatin domains in the genome using high resolution microscopy techniques and further reveal aberrations in the nuclear organization in their absence.