Epigenetics meets superresolution light microscopy

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Scientific background

Applied Optics for Studies of Biological Samples

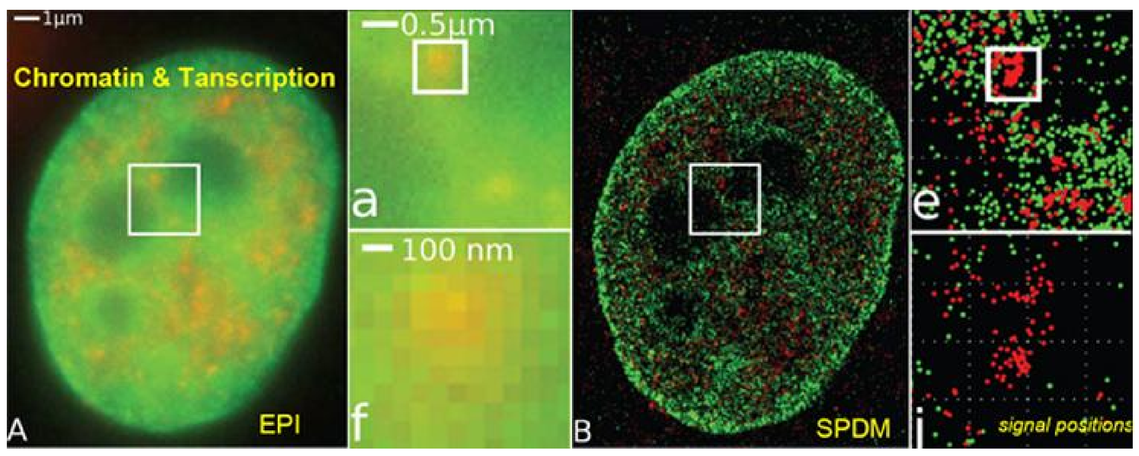
Few other fields of biology benefit as much from the revolutionary developments in light microscopy as the study of the architecture of the cell nucleus and of its functional layout. Chromatin folding, accessibility, e.g. to proteins of the replication and transcription machinery, and changes in chromatin condensation for a controlled “on-and-off switching” of genes are only a few important topics of epigenetics, which are presently investigated at IMB-Mainz. In this multi-disciplinary environment, we offer positions for physics students at the utmost forefront of what is currently possible in far field light microscopical analyses of the cell. Depending on the background and interest of the candidate, the focus of the PhD will be shifted either towards biophysics/optics or towards biology.

Physics

Conventional far field light microscopy provides an optical resolution of about 200 nm laterally (in the image plane) and 600 nm axially (along the optical axis i.e. along the direction of viewing). As in this case the specimens are investigated with a high-end objective lens, only a fraction of the light enters the detection light path through the front lens (basically acting as a single-”slit”), giving rise to a diffraction pattern. In spite of these physical limitations, at IMB we have established a variety of superresolution light microscopy (“nanoscopy”) methods for the “nanoimaging” of functional cellular structures. Such methods include 4Pi-microscopy, Spatially Modulated Illumination (SMI) microscopy, and Spectral Position Determination Microscopy (SPDM)/Localization Microscopy. 4Pi-microscopy provides an axial optical resolution of ~120 nm, and we have applied it to study e.g. nuclear pore complexes, replication complexes and other cell-nuclear nanostructures. Using SMI microscopy we measured the size of chromosome end-point regions (telomeric complexes) with a spatial resolution down to a few tens of nanometers and performed precise size measurements to estimate the compaction level of small regions of the DNA, as well as replication and transcription complexes (protein complexes that e.g. read the gene code off the DNA). Furthermore, SPDM has allowed imaging of nuclear nanostructures by means of imaging (detecting) individual fluorescent molecules. Thus, a lateral optical resolution of a few nanometers could be obtained, using standard fluorescence proteins/fluorochromes in cell nuclei of three dimensionally (3D) intact cells. Recently, we established an SPDM method making possible single molecule optical resolution in 3D.

Biology

Chromatin structure and especially its highly complex dynamics play a key role in the “on-and-off switching” of genes, i.e. in activation and silencing of gene expression. Present Spectral Position Determination Microscopy (SPDM) applications studied in the Cremer-Lab include the analysis of the spatial correlation between single histones and chromatin remodeling proteins; of the nuclear spatial relation between Polymerase II (Pol II) molecules and histones; between Pol II molecules and splicing factors; the spatial analysis of heterochromatin and Polycomb-protein clusters, as well as radiation induced repair protein complexes and radiation induced changes in chromatin nanostructure.



Distribution of H2B (labelled green) and Pol II proteins (labelled red) within a HeLa nucleus. A: Conventional Epifluorescence Image; B: SPDM image (the corresponding images have the same scale; “signal positions” denote the localization of single fluorophore molecules). From Markaki et al. , Cold Spring Harb. Symp. 75 (2010).

PhD and Masters Thesis in Physics available

Superresolution light microscopy of fluorescent nanostructures in the cell nucleus

In the study of the highly complex structure and dynamics of the cell e.g. DNA folding and repair, the successful application and further development of new microscopy methods holds a key position (as do technologies for epigenetics analysis which are also available at IMB). With these optical setups, the organization of the cell nucleus will be resolved at the nanostructure level in order to be able to understand the mechanisms and regulatory processes involved in the “on-and-off switching” of genes. Supported by data from other (e.g. non-imaging) analyses, this information presently enters into models used to describe and eventually determine how changes in chromatin organization alter the activation level of genes. The vast field of applications includes cancer research, cell differentiation (e.g. in the development of organs), drug development, radiation damage, ageing and many more.

Our group aims to further improve available methods of quantitative optical analysis of chromatin nanostructures. Furthermore and in collaboration with other groups inside and outside IMB and the university, we will use techniques such as localization microscopy, structured illumination and focused nanoscopy, to study chromatin structure and dynamics during processes such as DNA repair and gene regulation.

The subject of this project is to enhance localization microscopy (for reviews see Cremer et al. 2011, 2012) and to apply it in a biological context. The SPDM technique enables nanoscopy of biological samples with the same fluorophores used in conventional microscopy. This greatly facilitates specimen preparation as well as multi-modal imaging and allows the comparison and combination of results obtained using different microscopic techniques. In conjunction with spatially modulated illumination (SMI) microscopy (a technique of structured illumination), three dimensional nanoscopy of nuclear complexes will be performed. Together, these techniques allow to measure small features in 3D about 1,000 times better than what can be achieved with conventional confocal microscopy (regarding the observation volume). So far, this combinatorial microscopy (SPDM-SMI) has been established in the Cremer Lab for 3D nanoimaging of small membrane receptor clusters. The goal of the thesis will be to adapt and apply this novel 3D superresolution technique to epigenetically relevant nuclear nanostructure analysis and to extend its application to nanoimage three different types of fluorescent molecules simultaneously.

The successful candidate for the Masters/PhD thesis is envisaged to have an interest in optical microscopy, in image analysis, and in cellular biophysics.

Application for the PhD position:

www.imb-mainz.de/PhD

Contact and Application for a Masters thesis:

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Please feel free to contact us for more information and/or for a short lab tour.

Publications relevant to the project

Cremer C (2012). Optics far Beyond the Diffraction Limit: From Focused Nanoscopy to Spectrally Assigned Localization Microscopy. *Springer Handbook of Lasers and Optics*, 2nd edition (F. Träger, Edit.), 1351 – 1389.
Cremer C, Kaufmann R, Gunkel M, Pres S, Weiland Y, Müller P, Ruckelshausen T, Lemmer P, Geiger F, Degenhard S, Wege C, Lemmermann NAW, Holtappels R, Strickfaden H and Hausmann M (2011). Superresolution Imaging of Biological Nanostructures by Spectral Precision Distance Microscopy (SPDM). *Biotechnology J*, 6, 1037 – 1051.
Weiland Y, Lemmer P and Cremer C (2011). Combining FISH with Localisation Microscopy, Superresolution Imaging of Nuclear Genome Nanostructures. *Chromosome Res*, 19, 5 – 23.
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Baddeley D, Chagin VO, Schermelleh L, Martin S, Pombo A, Carlton PM, Gahl A, Domaing P, Birk U, Leonhardt H and Cremer C and Cardoso MC (2009). Measurement of replication structures at the nanometer scale using super-resolution light microscopy. *Nucleic Acids Res*, 38, e8 1-11.