THE VERTICO-SMI – ANALYSING NUCLEAR NANOSTRUCTURES IN-VIVO

U. J. Birk[*1], J. Reymann[1], D. Baddeley[1], M. Kemner-v. d. Corput[2], F. Grosveld[2], T. Jégou[1], K. Rippe[1], G. Gerisch[3], and C. Cremer[1]*
** presenting author*

[1] Kirchhoff Institut für Physik, Universität Heidelberg, Germany
[2] MGC-Department of Cell Biology and Genetics, EMC, Rotterdam, The Netherlands
[3] Max Planck Institut of Biochemistry, Martinsried, Germany
uspoeri@kip.uni-heidelberg.de

For the Spatially Modulated Illumination (SMI) Microscope two counter propagating laser beams are brought to interference, establishing a standing wavefield. In combination with high precision axial displacement this technique of analysing biological objects allows size measurements in molecular dimensions of some ten nanometres.

By the extension of this microscope for the realisation of in-vivo measurements, i.e. analysis of the living cell with a vertical setup of the SMI (the so called Vertico-SMI), the advantages of the SMI Microscope can be extended to vital biological systems. The construction of the Vertico-SMI and its characterisation, including the recording of beads and a first look at fixed and live cells, has been carried out. A present application is aiming at the detection of gene activation induced conformational changes in the β-globin locus (Collaboration F. Grosveld / M. Kemner-v. d. Corput, Rotterdam). As a starting point, SMI-nanosizing of active and inactive mouse β-globin gene regions has been performed.

Presently we are further optimising the Vertico-SMI Microscope with respect to its in-vivo operation. For that an Incubator Chamber has been developed which surrounds the complete setup to provide a stable temperature. For CO_2 and nutrition control, a microperfusion chamber has been developed. One of the most important requirements in order to realise in-vivo measurements is the consideration of the mobility of intracellular components. As studies show, the relative movement of specific chromatin domains lies in the order of up to 500nm per minute and it is therefore of utmost interest to avoid the influence of this dynamics during data acquisition. Presently, the Vertico-SMI allows the registration of a 3D data stack of a single object within 2s, corresponding to about 15fps.

Additionally to the above mentioned collaborative project, we present first measurements of nuclear nanostructures using in-vivo labelling with GFP variants. As a first "semi-" quantitative example for in-vivo nuclear nanostructure analysis with the SMI-technology, we are presently using the tet-operator system in U2OS cells. The next planned in-vivo experiments with these specimens will address the energy dependant analysis of nanosized structures under the presence and absence of ATP respectively (Collaboration with K. Rippe, Heidelberg). Future plans regard the "in vivo" analysis of distances between two fluorescently labelled sub-complexes during and before the conversion of clathrin-coated pits into vesicles in the SMI (and/or CLSM) microscope. First single colour measurements have been performed. Dual colour specimens will make use of the double labelled clathrin-coated vesicles with GFP as well as mRFP. These double labelled clathrin light chains will serve as calibration objects for the two colour distance measurements (Collaboration with G. Gerisch, Martinsried).