Combined STED-microscopy, x-ray holography and x-ray scanning diffraction studies of biological cells

Marten Bernhardt[1*], Jan-David Nicolas[1], Markus Osterhoff[1], Haugen Mittelstädt[2], Matthias Reuss[2], Benjamin Harke[2], Andrew Wittmeier[1], Michael Sprung[3], Sarah Köster[1], and Tim Salditt[1]

[1]*Institute for x-ray physics, university of Göttingen, Germany*

[2]*Abberior Instruments, Göttingen, Germany*

[3]*Deutsches Elektronen-Synchrotron (DESY), Hamburg, Germany*

[*]*mbernha2@gwdg.de*

We present a correlative imaging approach based on stimulated emission depletion(STED)-microscopy, holographic x-ray imaging and x-ray scanning diffraction to resolve nanoscopic structures in biological cells. The approach is based on intrinsically different and complementary contrast schemes: The fluorescence signal in STED-micrographs depends on the photon emission of a fluorophore of an explicitly labeled target structure. In contrast, the x-ray singals originate from the electron density of the entire ensemble of constituents in the illuminated area regardless of any labeling.

In particular, we correlate superresolution fluorescence data with the x-ray holographic phase maps, as well as the local diffraction patterns recorded in the regime of small angle x-ray scattering (SAXS). This is made feasible by using a dedicated STED microscope built by Abberior Instruments (Göttingen), which with its special design can be aligned (anti-)parallel to the x-ray beam axis. Results are obtained from different biological systems, with the main focus on studying the actin cytoskeleton of eukaryotic cell types. In this study, we aim at the well-ordered systems of sarcomers in cardiac muscle cells, which enable to generate forces responsible for heart muscle contraction. The size of the length scales of the underlying actomyosin-composition is in the nanometer range thus requiring high and superresolution imaging techniques. Superposition of data recorded on the same sample area shows that actin stands out in the phase maps and that the structure orientation of local SAXS patterns, as found by principal component analysis (PCA), can indeed be correlated to the orientation of actin filaments or filament bundles [1]. Data indicate, that the x-ray signals of sarcomers are strongly dependent on the maturation state of the cardiac muscle cells [2]. We also underline the advantages of combined STED- and x-ray microscopy by two further examples, namely the structure of the actin cortex of the amobae *D. discoideum* as a model for cellular motililty, as well as the compactification of DNA in procaryotic *D. radiodurans*. In the future, and based on the localization constrains, the *in situ* STED-recoding scheme introduced here will help to frame suitable models for the diffraction data. Conversely, the STED micrographs can be complemented by the high resolution phase contrast maps rendered from x-ray holography, thus clarifying the structural context in which a labeled target structure is embedded. Experiments were performed at the synchrotron nanofocus GINIX-instrument of the P10 endstation at the DESY campus in Hamburg.

References

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