

MULTI-WAVELENGTH 3D IMAGING OF NUCLEAR STRUCTURES WITH SUBDIFFRACTION RESOLUTION USING STRUCTURED ILLUMINATION MICROSCOPY

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Studies of subcellular structures using state-of-the-art light microscopy have been restricted by the diffraction barrier of optical resolution that is 200-300 nm in the xy-plane and 500-800 nm along the z-axis. Recent advances have made possible to surpass the diffraction limit in axial direction using 4Pi microscopy [1] and additionally in lateral direction when combined with stimulated emission depletion (STED) microscopy [2]. An alternative approach developed at the UCSF uses the principle of structured illumination (SI) to improve lateral as well as axial resolution by a factor of two below the diffraction limit [3,4]. This technology has been implemented in a specially designed microscope platform, termed OMX, which provides unprecedented sensitivity and mechanical stability.

To explore the potential of the SI technology we have tested the OMX prototype on a wide variety of biological structures. We show here the conceptual basics of the OMX microscope and present high-resolution data on various structural features in mammalian cell nuclei. We show for the first time multi-fluorescence 3-dimensional (3D) data on the ultra-structural organization of the nuclear envelope and chromatin. In addition, we provide first light microscopical evidence for the organization of 300-800 nm sized DNA replication foci into smaller subunits of ~120 nm size. These results clearly demonstrate the potential of the OMX microscope for multi-wavelength 3D-imaging of biological samples with subdiffraction resolution that will allow new insights in biological structures and will help to narrow the gap between light and electron microscopy.

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