Imaging cellular ultrastructures using expansion microscopy (U-ExM)

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For decades, electron microscopy (EM) was the only method able to reveal the ultrastructure of cellular organelles and molecular complexes because of the diffraction limit of optical microscopy. In recent past, the emergence of super-resolution fluorescence microscopy enabled the visualization of cellular structures with so far unmatched spatial resolution approaching virtually molecular dimensions. Despite these technological advances, currently super-resolution microscopy does not permit the same resolution level as provided by electron microscopy, impeding the attribution of a protein to an ultrastructural element. To circumvent this gap, we developed a new approach based on the innovative method of Expansion microscopy (ExM). In ExM, a specimen is embedded and crosslinked into a swellable polymer network that can physically expand. Importantly, upon polymer expansion, the specimen expands as well, up to a 4.5-fold in an isotropic manner. This allows super-resolution microscopy with conventional microscopes.

Our novel method of near-native expansion microscopy (U-ExM) enables the visualization of preserved ultrastructures of macromolecular assemblies with subdiffraction-resolution by standard optical microscopy. U-ExM revealed for the first time the ultrastructural localization of tubulin glutamylation in centrioles. Moreover, combined with stimulated emission depletion (STED) microscopy, U-ExM unveiled the centriolar chirality, an ultrastructural signature, which was only visualizable by electron microscopy. We also demonstrated the general applicability of U-ExM by imaging different cellular structures including microtubules and mitochondria *in cellulo*.