NANOSCALE IMAGING BY SUPER-RESOLUTION EXPANSION MICROSCOPY

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By linking a protein of interest into a dense, cross-linked network of a swellable polyelectrolyte hydrogel, biological specimen can be physically expanded allowing for magnified imaging with subdiffraction-resolution on conventional microscopes. Since its first introduction in 2015[1], expansion microscopy (ExM) has shown impressive results including the magnified visualization of pre- or post-expansion labeled proteins and RNAs with fluorescent proteins, antibodies, and oligonucleotides, respectively, in isolated organelles, cells, pathogens, tissues, and human clinical specimen[2,3]. In addition, various protocols have been developed to anchor proteins or RNA into charged polyacrylamide hydrogels enabling expansion factors of up to 20-fold[4,5]. Thus, ExM enables confocal diffraction-limited fluorescence imaging with spatial resolutions comparable to that of super-resolution microscopy methods. By careful optimization of the expansion protocol U-ExM demonstrated that even ultrastructural details of multiprotein complexes such as centrioles can be truthfully preserved[6]. Combined with super-resolution microscopy methods such as STED and SIM, ExM provides a simple and efficient way for three-dimensional (3D) multicolor nanoscale imaging with 10-20 nm spatial resolution. In combination with single-molecule localization microscopy method such as *d*STORM, ExM has the potential to approach the resolution of electron microscopy. However, current attempts to demonstrate ExM-*d*STORM remained challenging because of protein and fluorophore loss during digestion or denaturation, gelation, and the incompatibility of expanded polyelectrolyte hydrogels with photoswitching buffers. I will summarize our recent efforts to track down the molecular architecture of the synaptonemal complex by ExM-SIM. Furthermore, I will show nanoscale imaging of cellular and bacterial membranes by ExM-SIM and finally show that ExM-*d*STORM with post-expansion immunolabeling enables super-resolution imaging of endogenous proteins with minimal linkage error.

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