3D reconstruction of cellular microtubule networks using time-resolved live cell imaging and serial electron tomography

J.-M. Verbavatz[1,2*], S. Redeman[3], T. Müller-Reichert[3], S. Prohaska[4], A.A. Hyman[1]

[1] *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*
[1] *Institut Jacques Monod, CNRS, Université Paris Diderot Sorbonne Paris Cité, Paris, France*
[3] *Medizinisch Theoretisches Zentrum, TU-Dresden, Dresden, Germany*
[4] *Zuse Institute Berlin, Berlin, Germany*

During cell division of most eukaryotes, the microtubule spindle, which is essential for the segregation of chromosomes between two daughter cells, is organized at the two centrosomes. We have developed an approach to reconstruct the 3D network of microtubule at the centrosome in *C. elegans* embryo during cell division. This method combines live cell imaging, serial-section electron tomography of high-pressure frozen samples, automated segmentation of microtubules in single tomograms and stitching of microtubule segments in consecutive tomograms to reconstruct complete centrosomes and their 3D microtubule network. Using this approach, we have engaged into the quantitative analysis and comparison of microtubules at the centrosomes of *C.elegans* embryo at different stages of embryogenesis and in response to the inhibition of the expression of important proteins for the regulation of microtubule dynamics by a RNAi approach. Our result show a rather robust organization of microtubules and microtubule nucleation at the centrosome, with an extremely reproducible ring of microtubules around a rather microtubule-free centrosomal core. We also find significant effects of the inactivation of some relevant proteins.