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

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1. INTRODUCTION

Microtubules are part of the cell cytoskeleton and play crucial roles in cellular processes like intracellular transport and cell division. During cell division, the centrosome, found in most eukaryotes and also know as the microtubule organizing center, duplicates and the mitotic spindle is organized by microtubules which are nucleated and polymerized from tubulin at the two centrosomes. This process can be followed by light microscopy in live cells using fluorescently labelled tubulin. Due their small diameter (~25 nm) and high density at the centrosome, microtubules cannot be resolved by light microscopy and no precise quantitative analysis of tubulin polymerized in microtubules, on the ultrastructure of the network of tubes and their dependance on cell division stage, cell size, cell type can be obtained. To model the 3D organization of centrosomal microtubules in *C. elegans* embryo mitosis, we have chosen to use electron tomography on serial- 300nm-thick, sections, followed by segmentation of microtubules in the serial tomography stacks. In the perspective of doing a large-scale statistical analysis of multiple samples, we have developed software in the ZIBAmira (ZIB, Germany)/Amira (FEI, The Netherlands) framework for automated tracing of microtubules centerlines in electron tomograms based on template matching [1]. Then, we have developed a method for automated stitching of microtubules across serial sections, using the previously segmented network of lines to register consecutive sections [2]. Finally we have applied this image processing workflow – from serial electron tomography to complete microtubule network reconstructions - to cells from *C. elegans* embryo after live cell imaging and cell division staging.

2. RESULTS

Materials and Methods

Early *C. elegans* embryo from wild type (Bristol N2) or ß-tubulin-GFP expressing worm lines were imaged using light microscopy until metaphase of the first (P0 cells) or second (P1 cells) was reached. The embryos were placed in 100µm-deep flat membrane carriers (Leica) and rapidly high-pressure frozen (EMPACT2, Leica). Samples were freeze substituted and flat-embedded between 2 microscope slides in Epon resin. 300nm-thick sections were cut, stained with 2% uranyl acetate in 70% methanol, followed by lead citrate. The surface of sections was covered with 15 nm colloidal gold particles and electron tomograms (+- 60°, dual axis) were acquired on a 300kV Tecnai F30 TEM (FEI), using SerialEM [3]. Each tomogram was reconstructed using the IMOD software [4], followed by tracing and joining as previously described [1,2].

Results

Consistent with data from fluorescence microscopy, results show a remarkably high density of microtubule at the immediate periphery of centrosomes, up to the equivalent of 1µM polymerized tubulin. This peri-centrosomal region also exhibited a large number of small microtubules (<500 nm in length), consistent with nucleation of spindle microtubules at the periphery of centrosomes. However, the density of microtubules in the center of the centrosome, where the total concentration of tubulin is also high, was extremely low (Figure 1, right). Preliminary data suggest that the number of microtubules at the centrosome decreases with blastomere size during *C. elegans* embryogenesis. However, in contrast to previous reports using fluorescence microscopy [5], there was no apparent change in the size of centrosomes in the first 2 cycles of cell division during embryogenesis.

Following the analysis of WT *C. elegans* cells, the consequences of decreased expression of candidate proteins for the regulation of microtubule dynamics during cell division using a RNAi approach has been studied and shows that the 3D organization of microtubules is dependent on multiple proteins and enzymes.

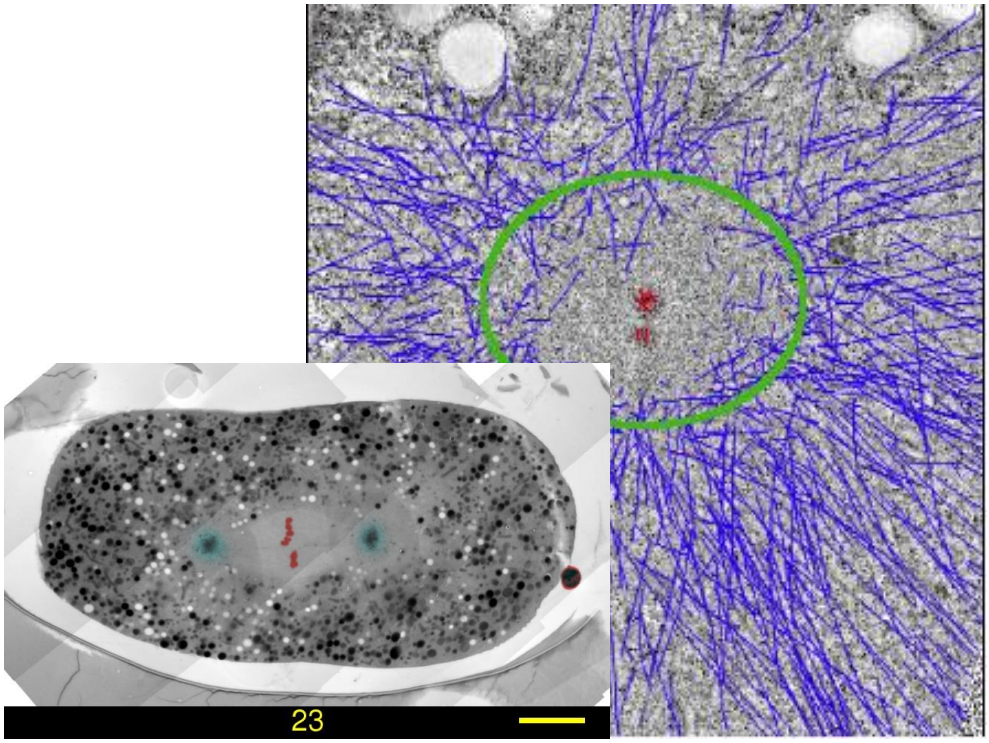

Figure. A single cell embryo of C. elegans was imaged by live fluorescence microscopy (top left), high-pressure frozen and processed for electron tomography (bottom left). In order to reconstruct the microtubule networks from serial semi-thin sections, microtubules were first traced using automated tracing (right) and the microtubule centerlines were used to register consecutive tomograms (not shown).

3. CONCLUSION

In conclusion, this work illustrates the combination of live cell imaging, electron tomography and image processing for a large-scale analysis of the extremely complex 3D organization of microtubules during cell division. In combination with other cell biology approaches, including control of gene expression, it will allow to model the important parameters that govern the ultrastructure and dynamics of the mitotic spindle.

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