doi:10.1017/S1431927610060368
Microsc. Microanal. 16 (Suppl 2), 2010
© Microscopy Society of America 2010

Electron Tomography of *C. elegans* - Cytoskeletal Dynamics Viewed in 3D

David H. Hall*, KD Derr**, William J. Rice**, Kristin A. Politi*, Ashleigh C. Bouchelion*, Chris Crocker*, Kevin Fisher*, Lei Sun*, Leslie Gunther***, and Ken C.Q. Nguyen*

*Center for *C. elegans* Anatomy, AECOM, Bronx, NY 10461
**New York Structural Biology Center, 89 Convent Ave, New York, NY 10027
***Analytical Imaging Facility, AECOM, Bronx, NY 10461

The nematode Caenorhabditis elegans is a model organism where the anatomy of all somatic cells is well established from serial thin sections [1,2]. However the advent of high pressure freeze fixation (HPF), freeze substitution, and electron tomography can now help in preserving and annotating individual organelles and cytoskeletal elements that escaped notice in earlier TEM images. Currently we are examining details of the microtubule bundle of the touch neuron, the terminal web beneath the microvilli of the intestine's apical membrane, and the canaliculi of the worm's excretory canal (kidney) cell (Figure 1). We have also identified a new organelle in the plasma membrane of the hypodermis. In each case electron tomography offers superior resolution of the normal cytoskeleton, its relationship to the plasma membrane, and the chance to look for specific anatomical changes in mutant animals or after introduced cell damage.

Multiple images are collected in a tilting series through semi-thick sections (80 -150 nm) using *SerialEM* [3]. *Protomo* [4] is used to compute a 3D tomographic reconstruction using weighted back-projection. Larger volumes can be stitched together from dual tilt tomograms of serial semi-thick sections. Thus it is reasonable to model a volume of one micron in depth, in one nm steps. Annotation and modeling is done by hand using *IMOD* [5] and *Amira* (Visage Imaging).

Figure 1 shows the 3D reconstruction of a portion of the excretory canal. Panel A shows one view within the tomogram of the excretory canal, where individual plasma membranes have been traced by hand in IMOD as colored lines. The white arrow in panels A,C indicates a wispy cytoskeletal structure lying within the canal lumen, which becomes visible only by HPF and electron tomography. Panel B shows a collage of overlapping membrane tracings of the branched canaliculi that surround the lumen, using separate colors (green, pink, white, purple) in IMOD for different groups of canaliculi; each group resembles beads on a string, connected by narrow necks. Panel C shows a 3D model of the tomogram for the canal, canaliculi, and neighboring cells. Ribosomes are shown as red spheres. The shapes of the lumen and canaliculi are known to undergo dramatic changes depending on the physiology of the whole animal, and the canaliculi can disappear or become malformed in mutations affecting cytoskeletal components [6,7]. Electron tomography will be used to compare details of these shape changes in mutants. Supported by NIH RR 12596 to DHH.

[1] J. G. White et al., *Phil. Trans. R. Soc. Lond* 314 (1986) 1-340.
[2] D. H. Hall and Z. F. Altun, *C. elegans Atlas*, 1st ed., CSHL Press, Cold Spring Harbor, 2008.
[3] D. N. Mastronarde, *J. Struct. Biol.* 152 (2005) 36.
[4] H. Winkler and K. A. Taylor, *Ultramicroscopy* 106 (2006) 240.
[5] J. R. Kremer et al., *J. Struct. Biol.* 116 (1996) 71.
[6] M. Buechner et al., *Dev. Biol.* 214 (1999) 227.
[6] V. Gobel et al., *Dev. Cell* 6 (2004) 865.

https://doi.org/10.1017/S1431927610060368 Published online by Cambridge University Press

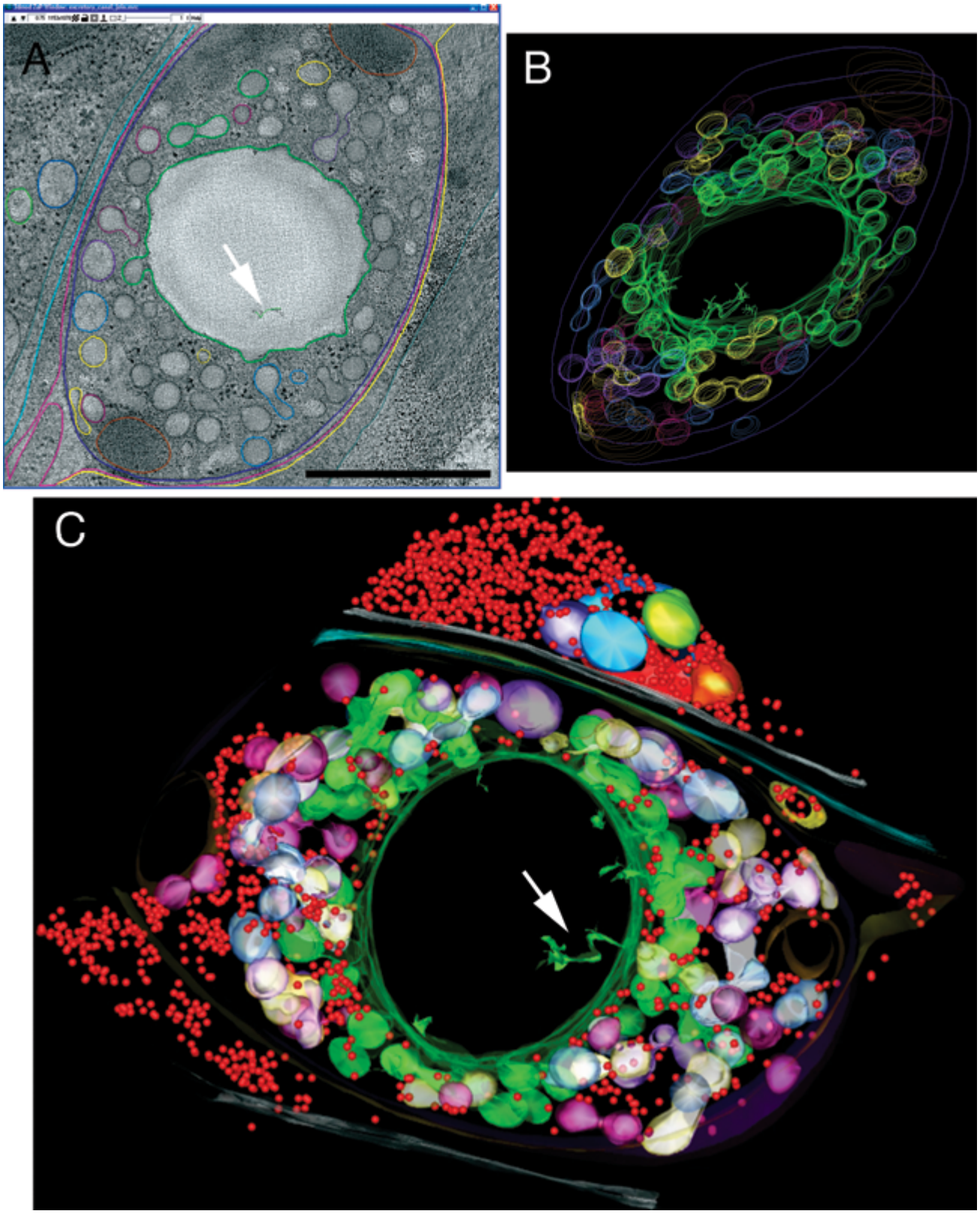

FIGURE 1. 3D model of the *C. elegans* adult excretory canal from a dual tilt tomogram, 150 nm semi-thick section. Scale bar in A, 0.5 microns.

https://doi.org/10.1017/S1431927610060368 Published online by Cambridge University Press