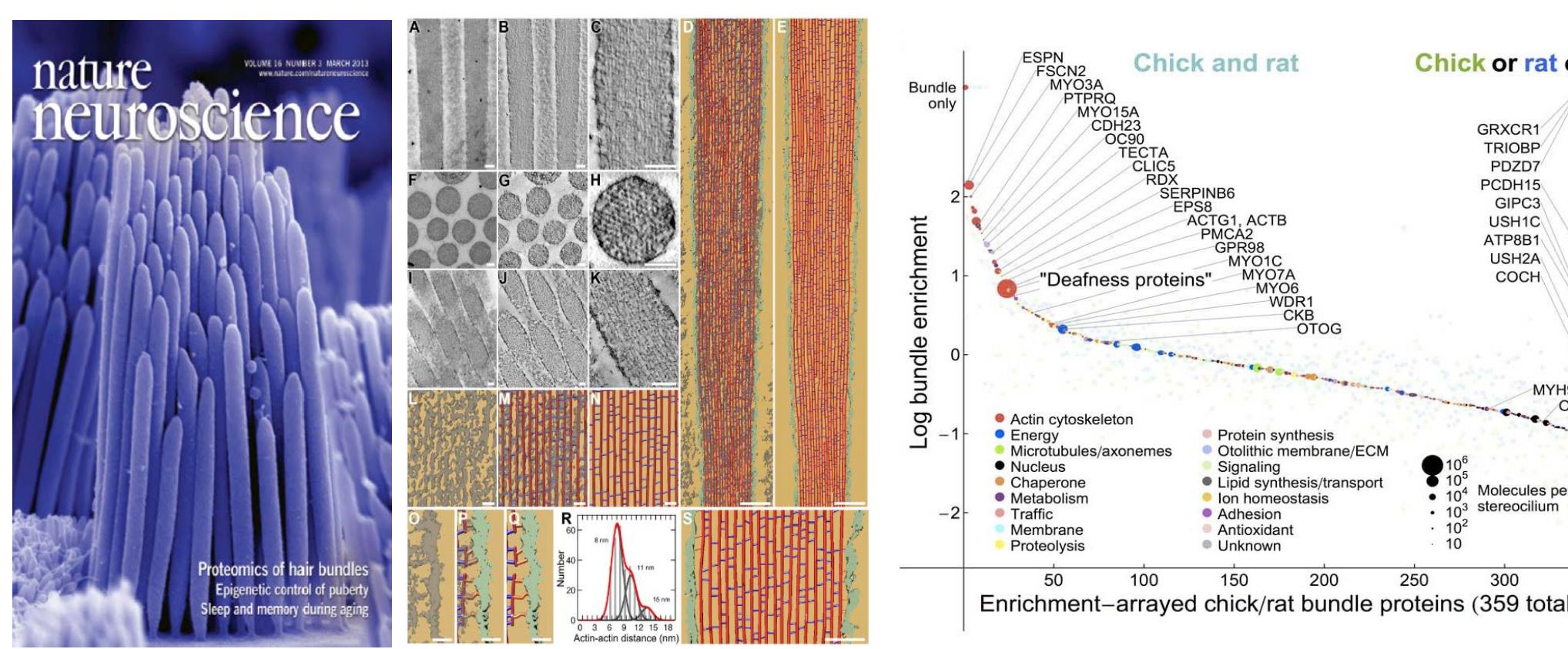


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## ABSTRACTS

Stereocilia ultrastructure to date is based on transmission electron microscopy (TEM) of resin-embedded, ultra-thin sections, which provide longitudinal or cross-sectional views. Using electron tomography of resin-sections we had shown 3D ultrastructural insight into the actin-rich stereocilium, including actin-actin crosslinkers and actin-membrane connectors (1). We found that while the stereocilia actin core was less ordered than expected, our tomography-based estimate of connector proteins was in excellent agreement with estimates from quantitative proteomics (1).



Being concerned that high pressure freezing/freeze-substitution and resin-embedding may have been prone to sample preparation artifacts, we developed a cryo-EM approach that allows imaging of intact vitrified stereocilia. Stereocilia were blotted off the sensory epithelial apical surface onto an electron microscopy grid and immediately plunge-frozen. Cryo-EM/tomography of such unstained frozen-hydrated intact stereocilia revealed that the membrane resealed at the point of insertion into the cell body. Judging by visual inspection as well as 2D Fourier analysis of tomographic slabs, wild type stereocilia contained both regions of high order, as well as regions of low order. We found evidence of gaps and forking in the actin core, suggesting that the actin filaments were structured more like a gel rather than a paracrystalline monolith. Given the higher order of the actin core in *Pls1*<sup>-/-</sup> mice (2), we imaged *Pls1*<sup>-/-</sup> stereocilia, which are currently used for development of automated filament tracking approaches. We have obtained simplified volumetric models of the actin core as well as an unexpected 3D volumetric arrangement of the actin in the taper and rootlet region. We are currently probing the space between the actin core and the membrane for unconventional myosin macromolecules. Furthermore, data collection is currently underway using beam-induced specimen motion-correcting direct electron detector- and phase plate imaging, which promise even higher resolution density maps. Such maps may be of high enough quality to detect known protein structures via template matching. While our focus lies on the 3D cryo-EM imaging of stereocilia, we have begun to use focused ion beam SEM imaging to reveal the 3D architecture of entire hair bundles and hair cells. We are currently developing multiscale, multimodal imaging work-flows that will allow correlative linking of fluorescence imaging studies of entire animals (e.g. zebrafish larvae) at the millimeter scale and submicron resolution to FIBSEM at the 10-50 micron scale and ~10 nanometer resolution imaging of individual cells and organelles via advanced X-ray tomography of resin-embedded tissues or entire small animals.

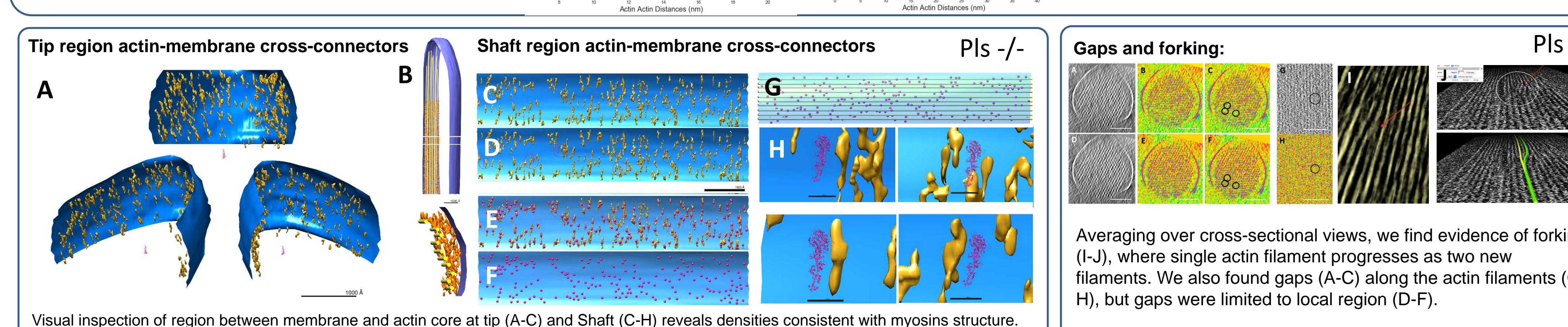
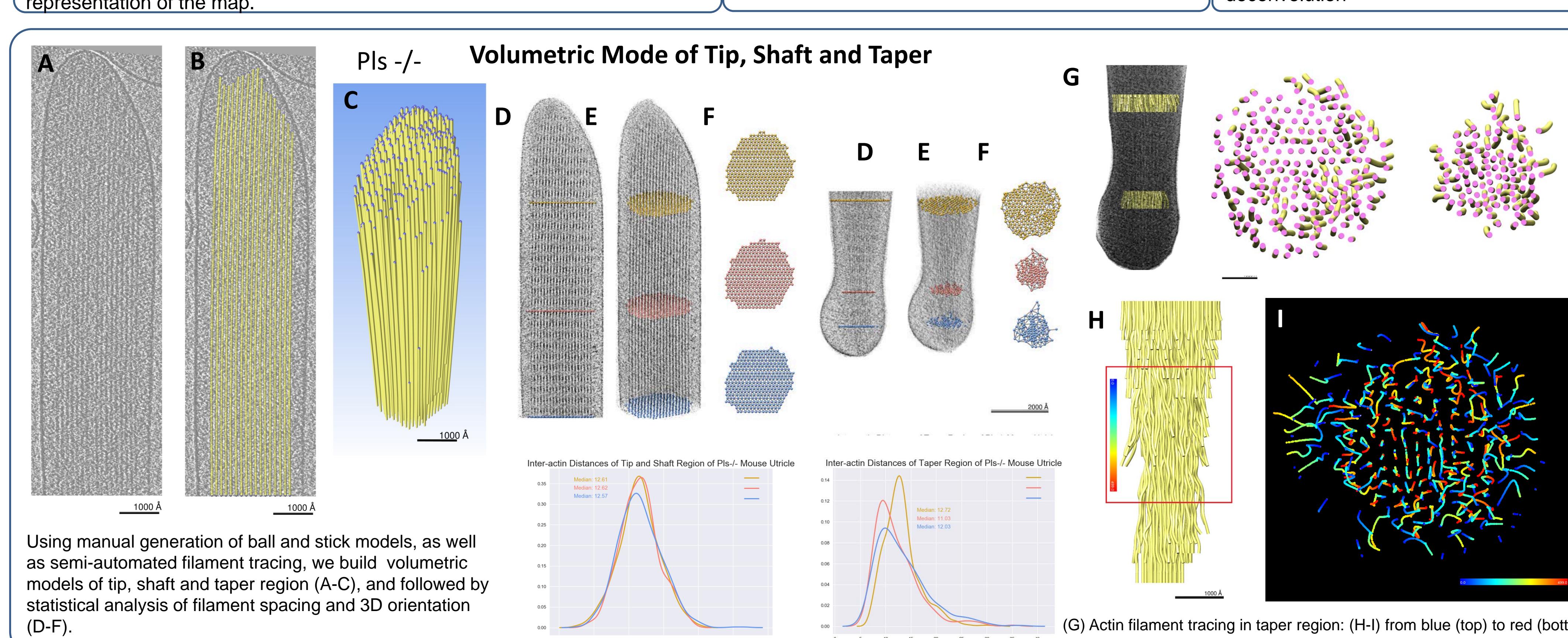
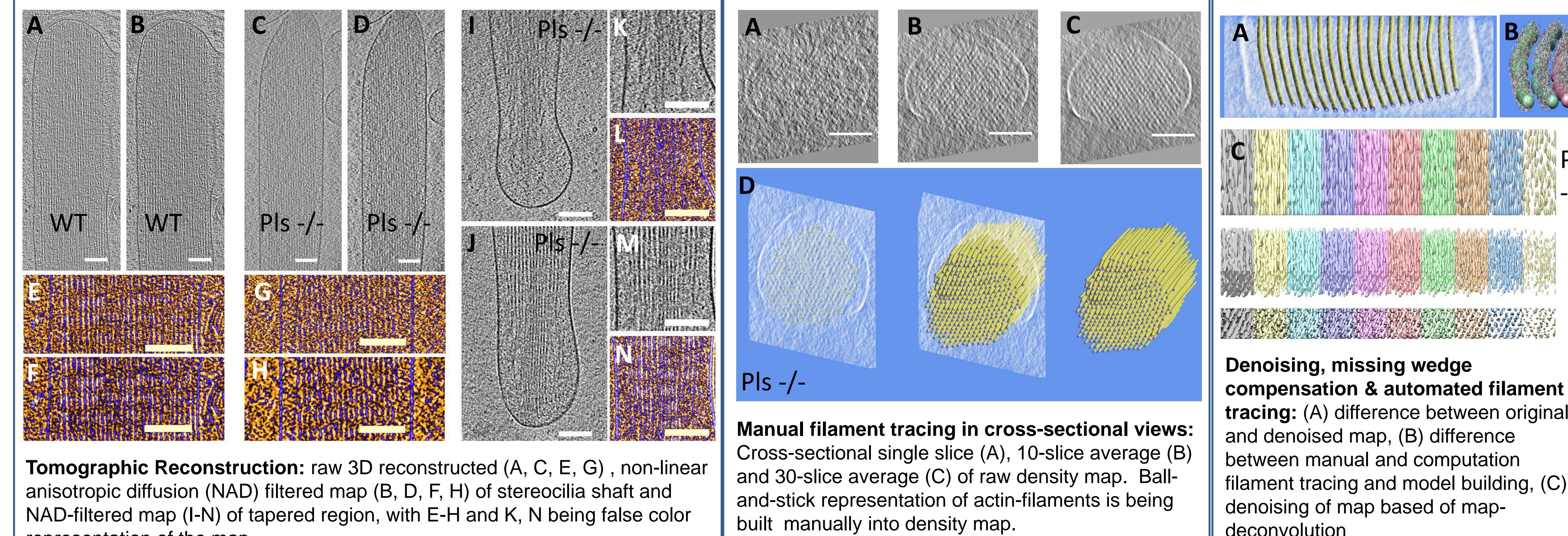
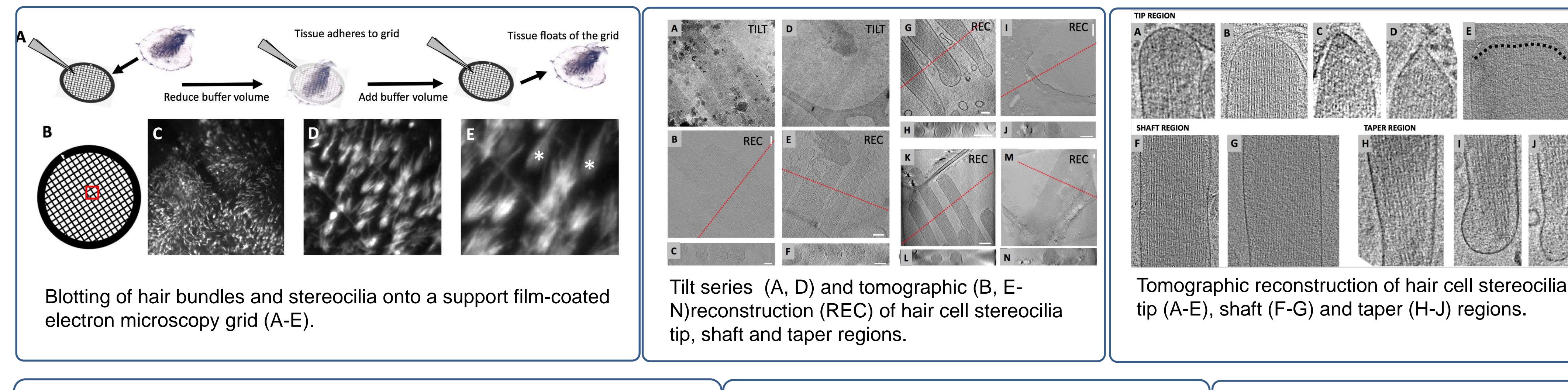
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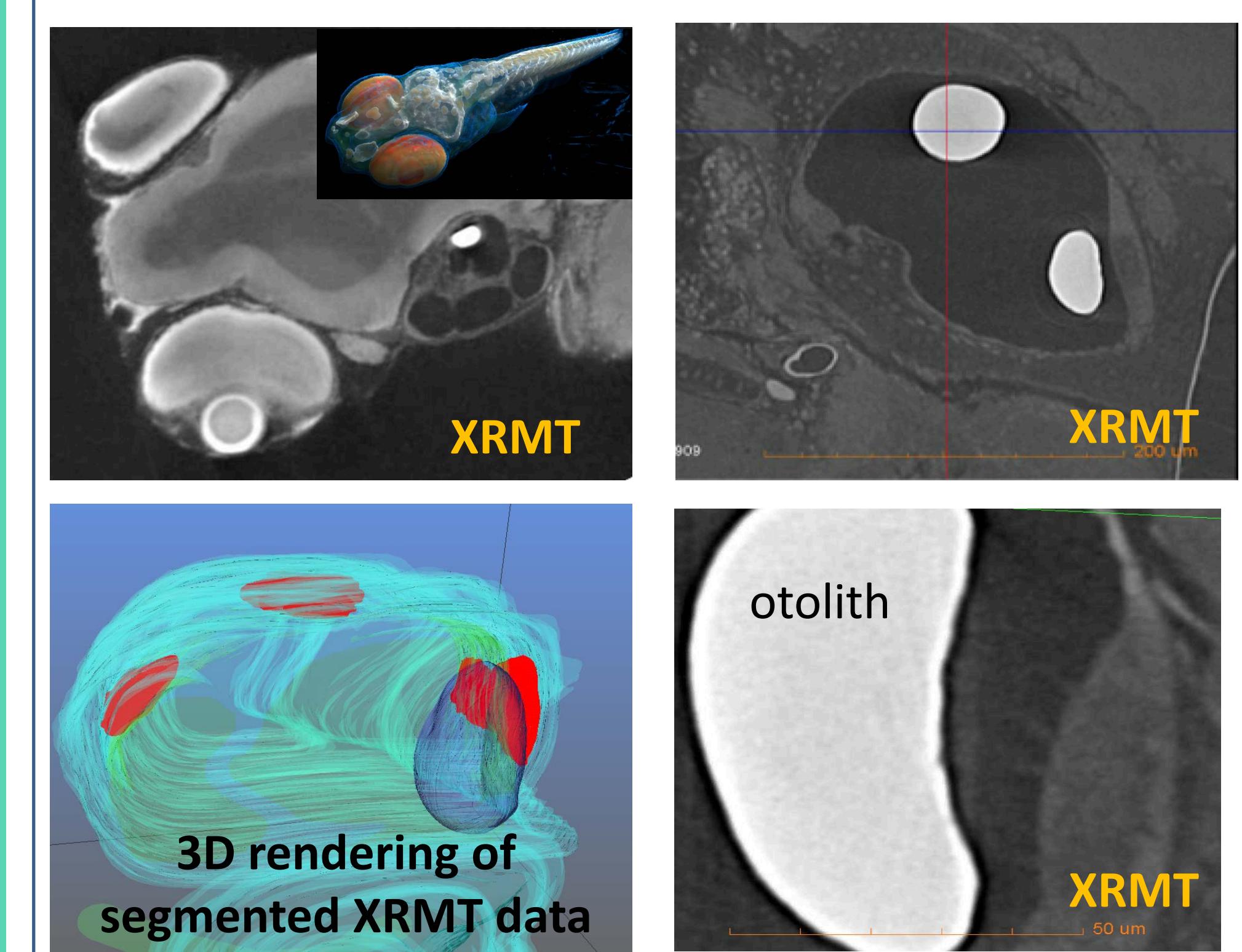
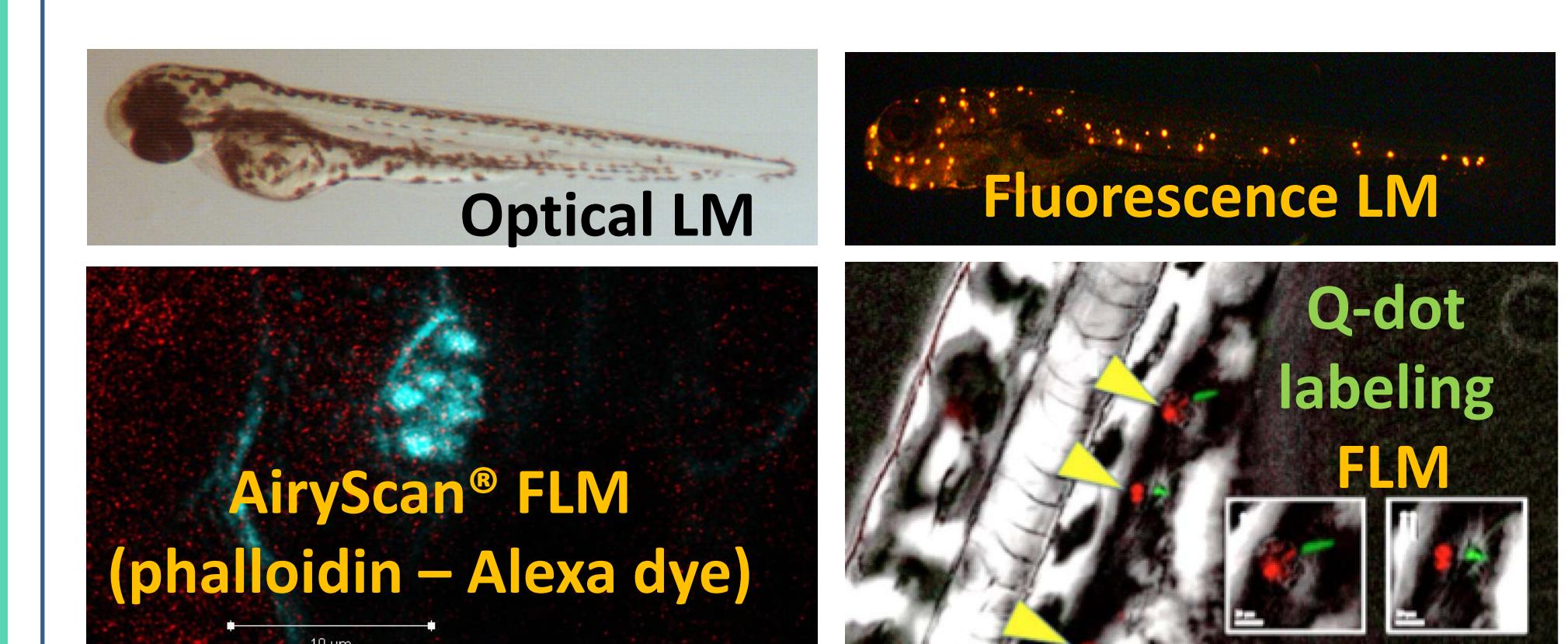
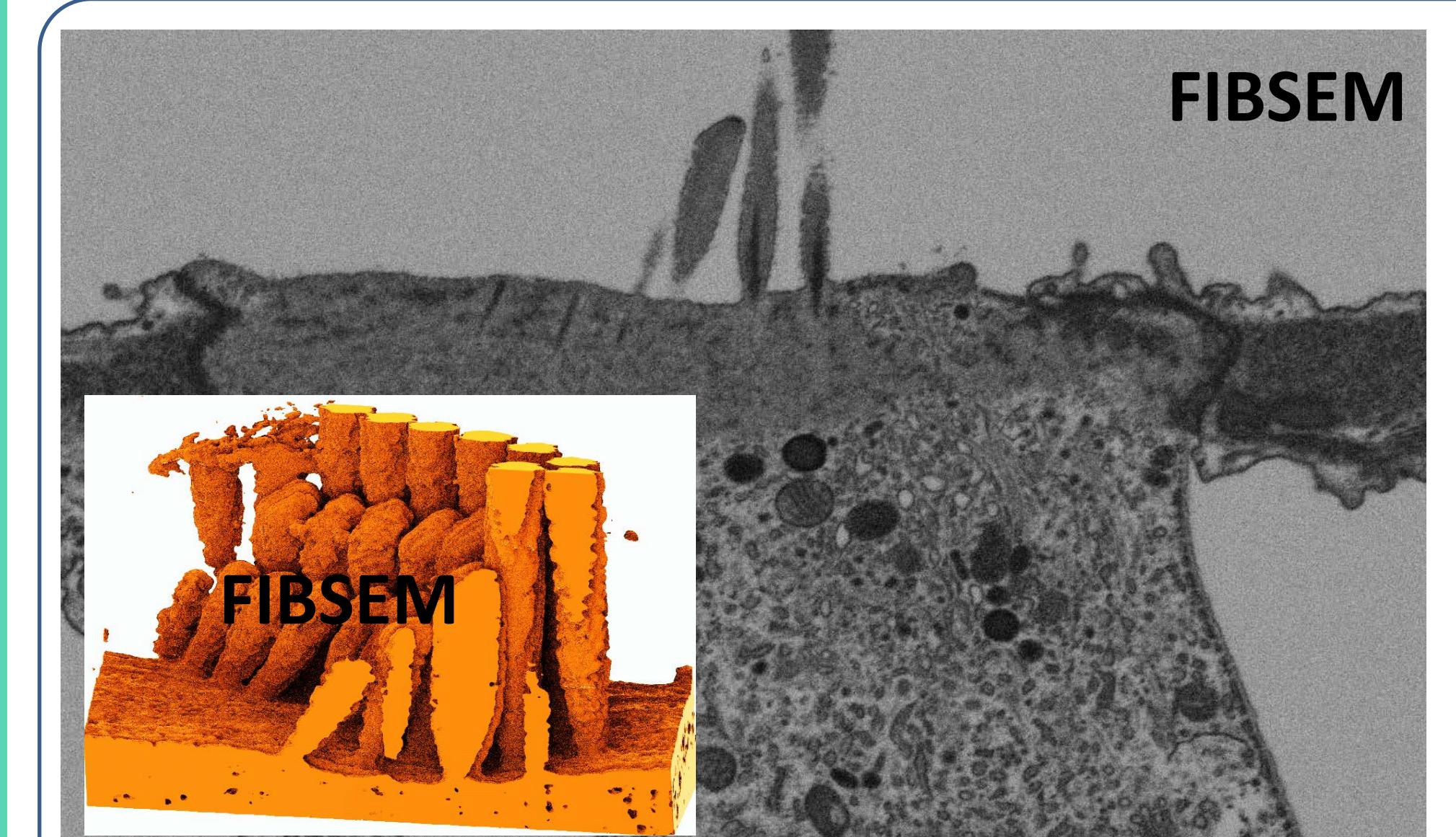
## ACKNOWLEDGEMENTS

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## RESULTS



## FUTURE DIRECTION: MULTISCALE IMAGING



X-ray Microscope Tomography (XRMT) is a powerful new technology that allows the visualization of entire (small) animals, such as zebrafish at the organ (inner ear), tissue (sensory epithelia), as well as cellular (hair cells) and subcellular (hair bundle). It also serves as an excellent bridging technology between (fluorescence) optical microscopy and high resolution FIBSEM imaging.