1534 CD
DOI: 10.1017/S1431927606069388
Microsc Microanal 12(Supp 2), 2006

Development of Electron Tomography Methods to Link Cellular Architecture to Function for Cell Biological Applications

*A.J. Verkleij, *W.J.C. Geerts, **M. Barcena-Martin, **K.M. Valentijn, **K.A. Jansen, *T.P. van der Krift, *J.A. Post, *A. Yukashevska, *M.N. Lebbink, *B.H. Humbel, **A.J. Koster

*Institute of Biomembranes, Molecular Cell Biology, Utrecht University, The Netherlands.
**Division Molecular Cell Biology, Leiden University Medical Center.

Electron tomography is a technique with TEM to image specimens in 3D with nm-scale resolution. The technique involves the acquisition of a set of tilt images, and can be applied to a wide variety of samples. A restriction is that the recorded image contrast has to be a projection of some physical characteristic of the sample, e.g. its mass/electron density [1]. Besides conventional bright-field TEM, other modes of image acquisition, like energy filtering (zero-loss or element specific) or high angular annular dark-field scanning transmission electron microscopy (HAADF-STEM), can be utilized in electron tomography [2,3,4].

Method development in our laboratory is directed toward optimization of electron tomography for applications using immunogold-labeled, high-pressure frozen, freeze-substituted, stained and resin-embedded specimens. This type of specimen preparation is highly suitable for questions for which 3D imaging of the general cellular architecture is required, or for questions for which invasive preparation steps needed for labeling with small gold are not a disadvantage. In our vision, this is a powerful approach for general questions within cell biology as well as for interactive genomics [5,6]. Optimization of methods for specimen preparation, data collection, data processing and analysis, will be necessary to localize proteins and lipids with high sensitivity and high reliability in cells within tissue material as well as in suspension.

We focus on three technological developments:

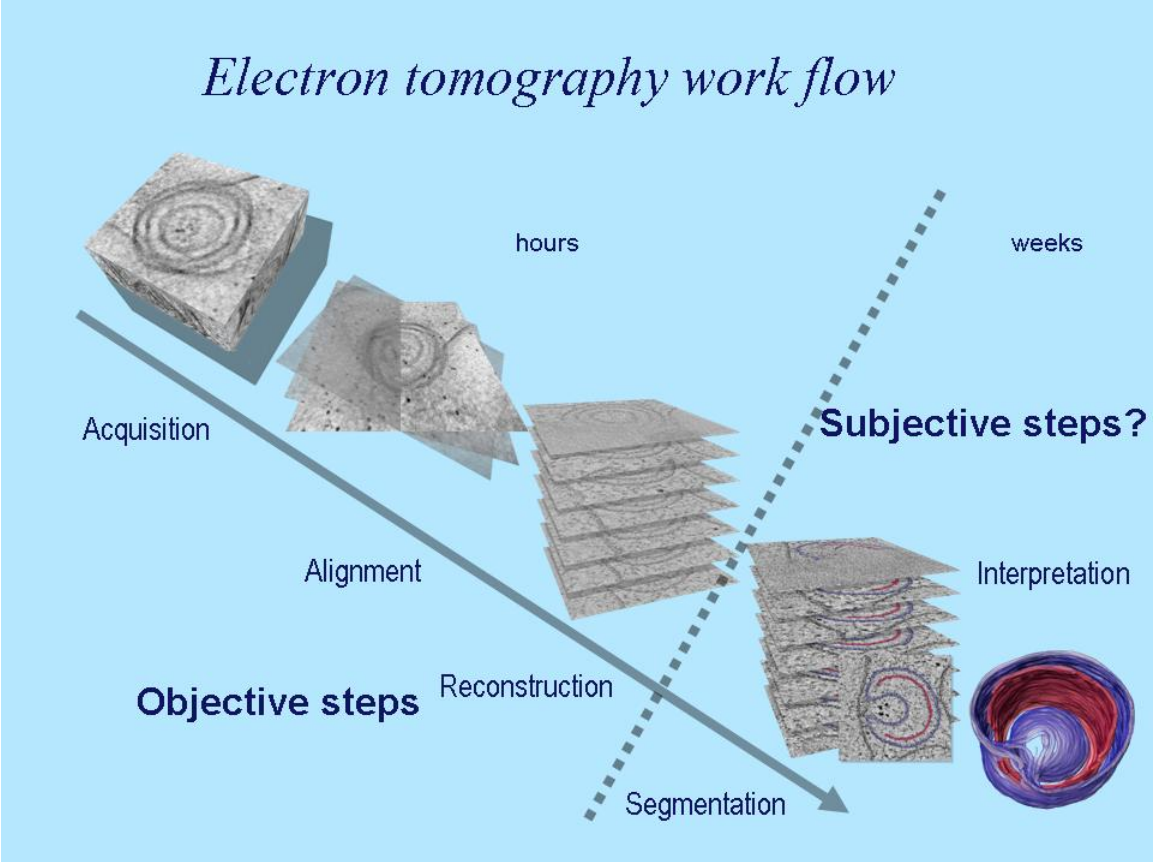
(1) Development of robust and reproducible preparation methods that allow 3D immunogold labeling, e.g. by using small antibody fragments with high affinity, and by tagging specific proteins and lipids.

(2) Optimization of automated high-resolution tomography data collection methods combining TEM with (dark-field) STEM imaging for detection of ultra-small gold labels plastic-embedded sections of tissue.

(3) Development of pattern recognition methods suitable for electron tomography that can annotate cellular structures within tomograms based on their shape or based upon specific (immuno-gold) labeling.

The power of tomography will be exemplified with several studies related to membrane-trafficking, e.g. ER-Golgi and ER-peroxisome.

[1] Lucic et al. *Annu. Rev. Biochem.* 74 (2005) 833.
[2] McIntosh et al. *Trends Cell Biol.* 15 (2005) 43.
[3] Ziese et al. *J. Struct Biol.* 138 (2002) 58.
[4] Midgley PA and Weyland M. *Ultramicroscopy* 96 (2003) 413.
[5] Koster AJ, Klumperman *J. Nat Rev Mol Cell Biol.* Suppl. (2003) SS6.
[6] Zeuschner et al., *Nature Cell Biology*, in press (2006).



https://doi.org/10.1017/S1431927606069388 Published online by Cambridge University Press