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Computational Tools for Interpreting Electron Tomograms

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To understand the function of biological macromolecules in their cellular context, it is essential to link high-resolution information with cell biology. We will discuss a number of computational tools that assist in bridging the gap between the information coming from atomic structures of individual macromolecules and higher order structural entities obtained by electron tomography.

Due to dramatic improvements in experimental methods and computational techniques in the past few years electron tomography has become a powerful tool for elucidating the three-dimensional architecture of cellular and sub-cellular systems at resolution of about 5-8 nm [1-3]. Electron tomography can depict unique structures and scenes, but an implication of this is that it cannot take advantage of image-averaging techniques for noise reduction. Thus, in addition to the relatively low resolution, electron tomograms inevitably suffer from a low signal-to-noise ratio. Consequently, the resulting three-dimensional maps are difficult to interpret. The main difficulty is the assignment of density within the tomographic reconstruction to a particular molecular component. In addition to innovative experimental labeling techniques (see for example Tom Deerinck's tutorial in this session), a number of complementary algorithms have been developed over the years to help directly identifying these molecular components. Examples include detection of macromolecular footprints by template matching, auto-segmentation approaches, docking of crystallographic structures, and noise-reduction approaches. In this tutorial we will discuss a number of specific algorithms that have proven to be helpful in this regard:

Noise reduction based on iterative median filtering:
Noise reduction is often used as a pre-processing step for template matching or segmentation approaches. Here, we will describe a noise-reduction approach based on iterative application of median filtering. The application of this algorithm produces encouraging results for a wide variety of experimental and synthetic electron tomographic reconstructions.

Segmentation of fine features:
Segmentation is an essential step for interpreting tomographic reconstructions. We will describe the use of a three-dimensional watershed algorithm [4], which is specifically designed to separate touching entities such as monomers inside an actin helix, monomers within membrane receptors or other interacting molecules.

Correlation-based template matching and docking of crystal structures:
Template matching can be used to compare the content of the tomographic reconstruction (or segments thereof) with known structures obtained by other methods. We will describe the use of the correlation-based template matching algorithm as implemented in the package CoAn [5] and show how the algorithm can be used for docking atomic models into low-resolution experimental densities [6,7].

Template matching using reduced representations:
Reduced representations consist of small sets of three-dimensional points that capture the characteristics of the underlying structure. The use of these representations results in a reduction of computational complexity that allows scanning relatively large volumes in real space in a relatively short time [8]. This approach is specifically useful for structures with higher order such as filaments and bundles. It also contains a built-in way to code for variations in stain distribution, a factor that may hamper detection using correlation based template matching. The approach was also used for detection of macro-molecular projections in electron micrographs, where the ability to avoid negative hits (false positives) can match that of a human operator [8].

References:

[1] Baumeister, W., Grimm, R., and Walz, J. (1999). Electron tomography of molecules and cells. Trends Cell Biol *9*, 81-85.

[2] McEwen, B. F., and Frank, J. (2001). Electron tomographic and other approaches for imaging molecular machines. Curr Opin Neurobiol *11*, 594-600.

[3] McIntosh, J. R. (2001). Electron microscopy of cells: a new beginning for a new century. J Cell Biol *153*, F25-32.

[4] Volkmann, N. (2002). A novel three-dimensional variant of the watershed transform for segmentation of electron density maps. J Struct Biol. *138*, 123-128.

[5] Volkmann, N. (2002). Atomic model of the cell: docking in a tomographic environment. Biophysical Discussion, http://www.biophysics.org/discussions/volkmann-speaker.pdf.

[6] Volkmann, N. (2004). An approach to automated particle picking from electron micrographs based on reduced representation templates. J Struct Biol. *145*, 152-156.

[7] Volkmann, N., and Hanein, D. (1999). Quantitative fitting of atomic models into observed densities derived by electron microscopy. J Struct Biol *125*, 176-184.

[8] Volkmann, N., and Hanein, D. (2003). Docking of atomic models into reconstructions from electron microscopy. Methods Enzymol *374*, 204-225.

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