Conical Tomography: A Method for the Study of Macromolecular Assemblies

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Keywords: conical tomography, 3D reconstruction, freeze-fracture, thin section, transmission electron microscopy.

ABSTRACT

We have developed conical tomography for the transmission electron microscope to study a wide range of structures at molecular resolution. Unlike the other related types of tomography (single and double-axis tilt), conical tomography has a higher throughput and more importantly, produces reconstructions that are isotropic in the x-y plane with a resolution of 2-3nm [1]. Using a custom made tilting and rotating stage, we have been able to collect complete conical series from a variety of sources, including thin sections and freeze-fractured replicas of chemical synapses from the rat somato-sensory cortex , isolated synaptic vesicle anti-SV2 complexes.

INTRODUCTION

We have developed conical tomography for the transmission electron microscope to study the structure of macromolecular assemblies and organelles in their native cellular environment. Unlike the other related types of tomography (single and double-axis tilt), conical tomography has a higher throughput and more importantly, produces reconstructions that are isotropic in the x-y plane with a resolution of 2-3nm [1].

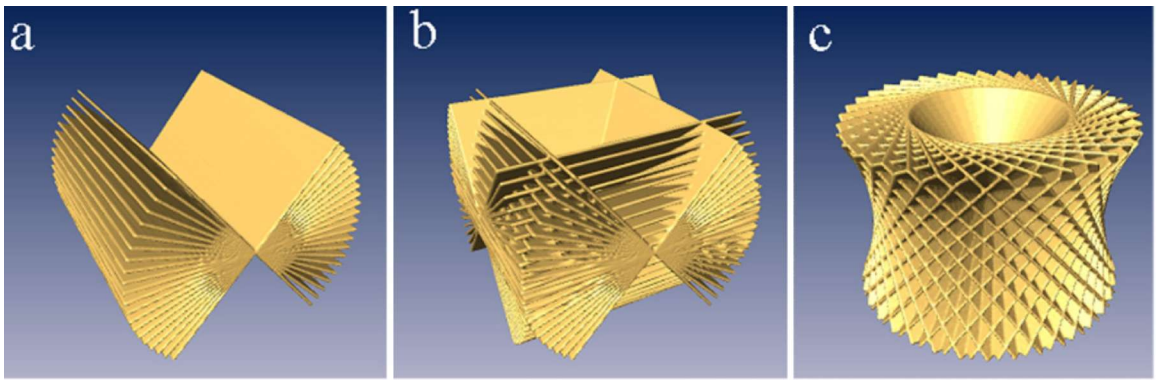

Figure 1. Geometrical representation of the imaged area in A. single-axis tilt, B. double-axis tilt, and C. conical tilt. Since the projection planes are evenly spaced, the resolution is isotropic in the x-y plane.

Using a custom made tilting and rotating stage, we have been able to collect complete conical series from a variety of sources, including thin sections and freeze-fractured replicas of chemical synapses from the rat somato-sensory cortex (Figure 4), isolated synaptic vesicle anti-SV2 complexes (Figures 7, 8, 9 10).

METHODS

The specimens were tilted at 38°, 50° or 55° and turned by 2.5°, 4°, or 5° increments until completing 360° rotations.

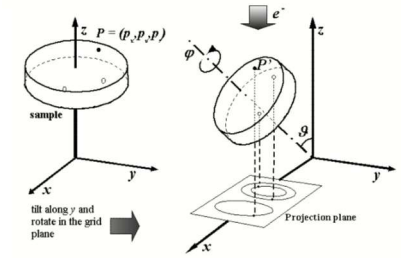

Figure 2: Shows the conical geometry in detail. The specimens are tilted at 55 degrees and projected on to a plane.

With software developed by our laboratory, we used the elliptical paths of 3-12 fiduciary markers to align the images to a common coordinate system.

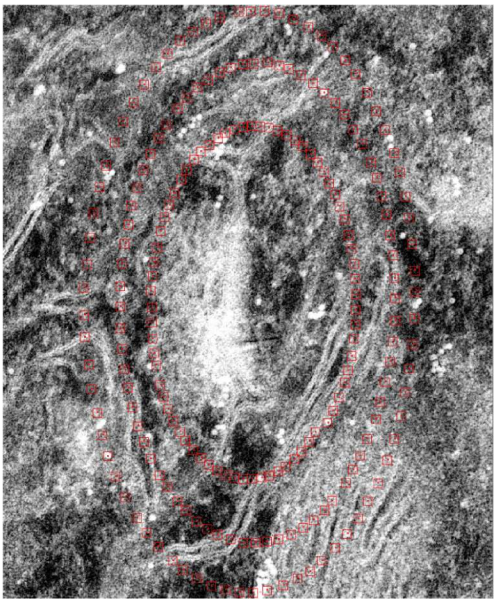

Figure 3. Projection with the ellipses of three fiduciary markers.

The field was reconstructed, using the weighted back projection algorithm in order compute a 3D reconstruction from a series of projections. The 3D maps were then refined through the process of projection matching, a technique for improving the alignment by comparing collected projections with possible solutions. It was also necessary to develop a further index based on variance with which we could monitor the refinement process. With current processor speeds, the final reconstructions were often obtained in less than 24 hours, the main limitation being the time needed to collect all the images in the series.

RESULTS

To illustrate the authority of conical tomography, we used volumetric representations ("voltex")

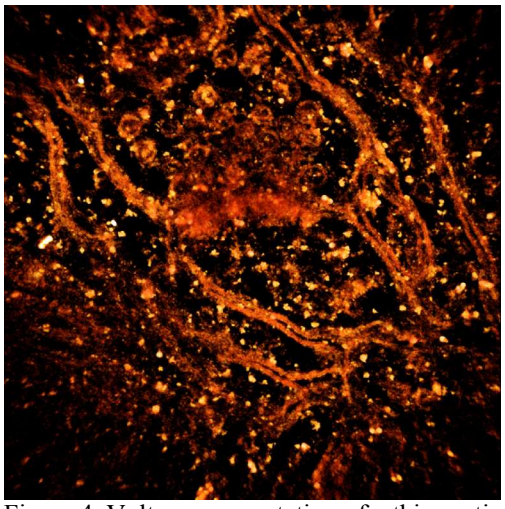

Figure 4. Voltex representation of a thin section synaptic junction, oriented such that the presynaptic vesicles can clearly be seen in the upper middle.

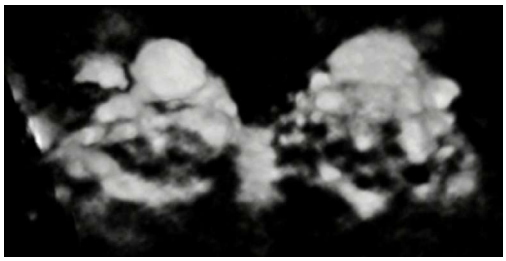

Figure 5. Voltex representation of two freeze-fracture synaptic vesicles.

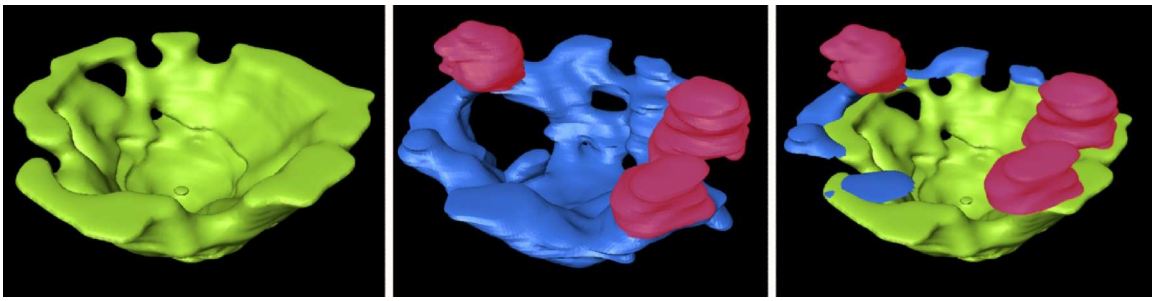

Figure 7. The segmentation of two "down-facing" vesicles, one clearly exhibiting Anti-SV2-gold particle complexes (red areas). Panel A. Vesicle without the Anti-SV2-gold particle complexes. Panel B. Vesicle with Anti-SV2-gold particle complexes. Panel C. The two vesicles superimposed on each other.

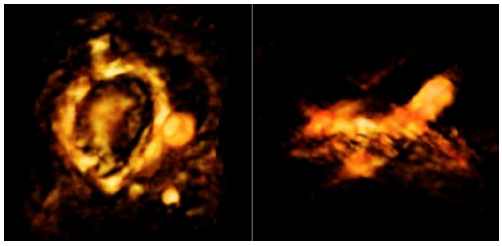

Figure 8. Top and side view of a vesicle labelled with Anti-SV2-gold particle complexes.

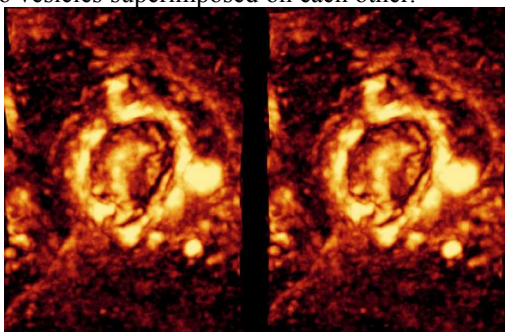

Figure 9. Stereoscopic view of the same vesicle as Figure 8.

and density segmentation (Figure 5, 8, 10) to analyze the sections, revealing a number of highly detailed structures: tubes and cisterns were found in the presynaptic terminal, as well as protein cages formed of SV-2 rings, circumscribing the synaptic vesicles, and a number of *docked vesicles* and *omega figures*.

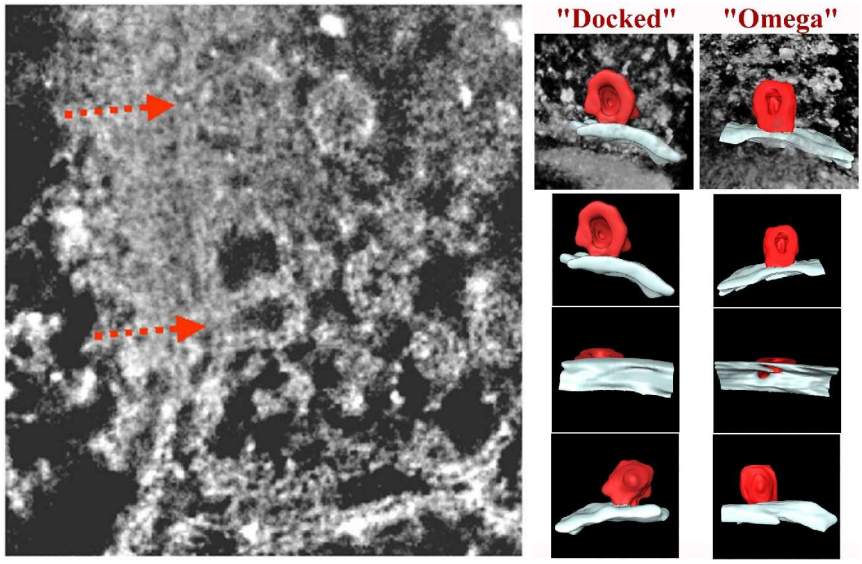

Figure 10. Orthoslice of docked and omega figures and their segmentation.

Furthermore, in contrast to single particle reconstruction, imaged using random conical tilt, reconstructions using conical tomography allow the size and shape of individual particles to be

identified (figures 6-10) without the need for averaging or imposing symmetry [2].

CONCLUSION

While Conical Tomography is a general technique, theoretically applicable to any types of structure on the molecular scale, our immediate goal is to reconstruct the synaptic terminal under a variety of different experimental conditions. Using Conical Tomography, we can now study the synaptic terminal and the distribution of the ~60 proteins associated with it in different neuronal environments at high resolution. Subsequent analysis promises to provide information about it's structure/function, including clues as to it's role in Alzheimer's, Parkinson's, drug addiction, and other diseases. Eventually, we hope to further the development of conical tomography in other areas and in conjunction with other laboratories, since the technique should prove quite useful for anyone involved in biotechnology field.

REFERENCES

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