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# An Optimized Solution for Cryo-Electron Tomography

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Three dimensional electron tomography (3DET) has become an essential tool for determining the three-dimensional (3D) structures of cellular components at the nanometer resolution, i.e. between that of atomic structures, as determined by X-ray crystallography, and more general cell architecture observed by light and confocal microscopy. FEI has created Xplore3D™, a software suite that seamlessly integrates all the steps in electron tomography from automated data acquisition until visualization of the reconstructed volume.

Cryo-fixation is an accepted technique to study the natural state of biological specimens. The integrity of specimens obtained in this way should be maintained during transfer and data collection sessions in the microscope. Recent developments have made it possible to streamline the complete process of cryo-electron tomography so that high quality, high-resolution 3D data can be obtained in an efficient and automated manner.

Vitrification under controlled humidity and temperature using the Vitrobot™ provides reproducible thin frozen films with proper ice thickness. The optimized optical system, in combination with the data acquisition software, allow us to collect high quality low-dose cryo data sets with only minor corrections for focus and shift in a very efficient way. Solutions for dual-axis data sets are available to minimize the effect of the missing wedge from single axis tomography. Further processing consists of accurate alignment of the tilt series, either by cross correlation or by marker tracking and subsequent reconstruction. The often-used technique of weighted back-projection (WBP) reconstruction is limited by both noise and suppression of low and high frequency information by the weighting filter. To overcome these difficulties, iterative techniques such as ART (algebraic reconstruction technique) and SIRT (simultaneous iterative reconstruction technique) have been implemented. These techniques do not rely on filtering, but iteratively optimize the tomograms so that projections of the reconstructions best resemble the acquired data. SIRT is especially usefull for noisy data sets like those obtained in low-dose cryo-electron tomography. It clearly reveals structures that are lost in the noise in a WBP reconstruction, as shown in FIG. 1. Unfortunately, due to these iterations the reconstruction process is very slow compared to WBP. While data acquisition generally takes between 30 min and 1.5 hours and subsequent alignment less than an hour, SIRT reconstructions can take up to 24 hours. By using the computational power of commercial off-the-shelf GPU's (Graphics Processing Units, or Graphic cards) on a standard desktop PC, we are able to reduce the time needed for WBP and SIRT reconstruction by a factor of at least 100.

In conclusion, new developments and automation make cryo-electron tomography a practical technique for routine 3D imaging at the nanoscale level and thereby providing new insights in the 3D organization of cellular structures.

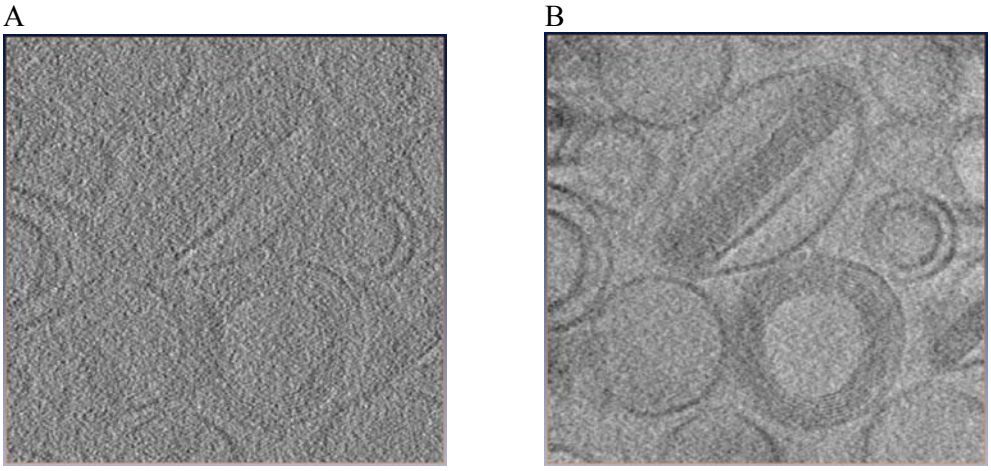


FIG. 1. 3D Reconstruction of Doxil loaded vesicles. Low-dose (total dose 80 e/Å$^2$), zero-loss (20eV slit) tilt series was recorded at liquid nitrogen temperature from –65 to +65 degrees with a pixel size of 0.75 nm. A) One slice through the reconstructed volume after weighted back-projection reconstruction. B) Slice at the same position after SIRT.

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