Molecular Structures and High Resolution TEM

LS.4.125
A control freak's approach to CTF corrected tomography

L. Voortman[1], E. Franken[2], A. Voigt[2], L. van Vliet[1], B. Rieger[1]

[1]Delft University of Technology, Delft, Netherlands
[2]FEI Company, Eindhoven, Netherlands

L.M.Voortman@TUDelft.NL
Keywords: TEM, cryo-electron tomography, CTF correction, defocus gradient

Cryo-electron tomography (CryoET) is an essential tool to study the three-dimensional (3D) structure of biological specimens at molecular resolution. The primary contrast mechanism for biological specimens is weak phase contrast. In this weak phase contrast regime, image formation is for an important part described by the contrast transfer function (CTF). The CTF is an oscillating function of the spatial frequency and depends, among other parameters of the imaging system, on the applied defocus.

In CryoET many two-dimensional (2D) projections of the 3D structure of a specimen are recorded at different tilt-angles. Tilting of the specimen induces a defocus gradient which depends on the specimen orientation and the orienation of the tilt-axis. We recently introduced a new global CTF correction method (to process the entire field-of-view at once) which can use different inverse filters, e.g. phase-flipping or Wiener filter, and do so within a reasonable time for large image sizes (4k x 4k) [1].

Contrast Transfer Function (CTF) correction increases the interpretability of the images up to higher resolutions. However, to use CTF correction on tomography data, one needs to know at each tilt-angle the exact defocus distribution over the specimen with an accuracy of ~ 50 nm [2]. A simple solution would be to trust the information from the microscope interface, since the selected defocus and tilt-angles are in principle sufficient. This approach, however, ignores that the actual defocus is not only (substantially) different from the requested defocus but it also varies during acquisition. These defocus variations can be due to the non-perfect mechanics of the stage, the accuracy with which the eucentric height can be adjusted, the auto-focus area being offset from the exposure area or unpredictable fluctuations. In order to account for these variations, the defocus needs to be measured throughout a tilt-series.

Furthermore, we do not know the orientation of the specimen when the stage is at zero tilt. It is not uncommon, however, to observe that the specimen is tilted/warped even up to 10 degrees in an arbitrary direction. This orientation influences the direction and magnitude of the defocus gradient throughout the tilt-series.

We decided to measure the defocus and specimen orientation throughout the tilt-series acquisition. This is achieved by taking four additional high-dose exposures at each tilt-angle on which we measure the defocus. These high-dose exposures are positioned on the carbon grid, away from the area-of-interest, two on the tilt axis and two off-axis (see Figure 1). The defocus at these high-dose areas is measured using the method described in [3].

The defocus measurements on the high-dose exposures are used to estimate the defocus on the exposure area. For this purpose we developed a robust algorithm that automatically detects faulty measurements. Assuming that the specimen is locally flat, we use the redundancy introduced by the four defocus estimation areas to detect outliers. Figure 2 shows the measured defocus, the estimated specimen orientation and the estimated defocus on the exposure area. With this procedure we can robustly estimate the defocus and specimen orientation (which defines the defocus gradient) at each tilt-angle. We are currently processing CTF corrected tomograms to show that this approach can increase the attainable resolution.

1. L.M. Voortman, E.M. Franken, L.J. van Vliet and B. Rieger, Ultramicroscopy 118 (2012), p. 26 – 34.
2. L.M. Voortman, S. Stallinga, R.H.M. Schoenmakers, L.J. van Vliet and B. Rieger, Ultramicroscopy 111 (2011), p. 1029 – 1036.
3. M. Vulović, E.M. Franken, R.B.G. Ravelli, L.J. van Vliet and B. Rieger, Ultramicroscopy 116 (2012), p. 115 – 134.
4. We acknowledge support from the FOM Industrial Partnership program No. 07.0559.

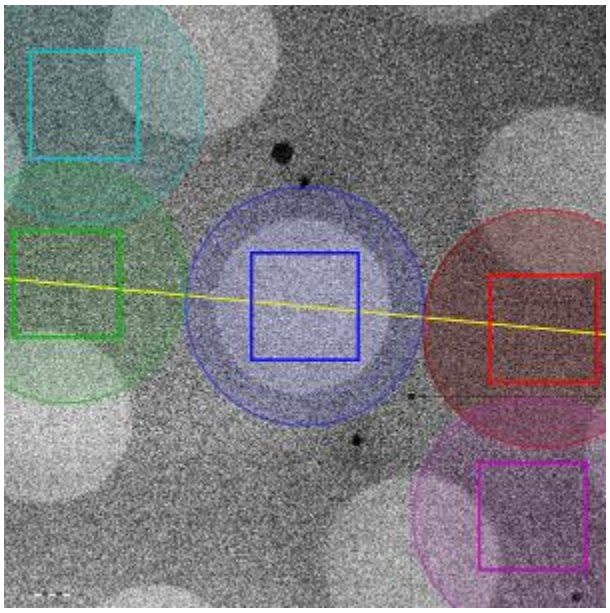

Figure 1. The area-of-interest (in blue) is located on the ice. Two high-dose areas are positioned on the tilt-axis (green and red) and two are positioned away from the tilt-axis (cyan and magenta). The locations are chosen such that also at high tilt-angles the high-dose beam does not overlap with the exposure area.

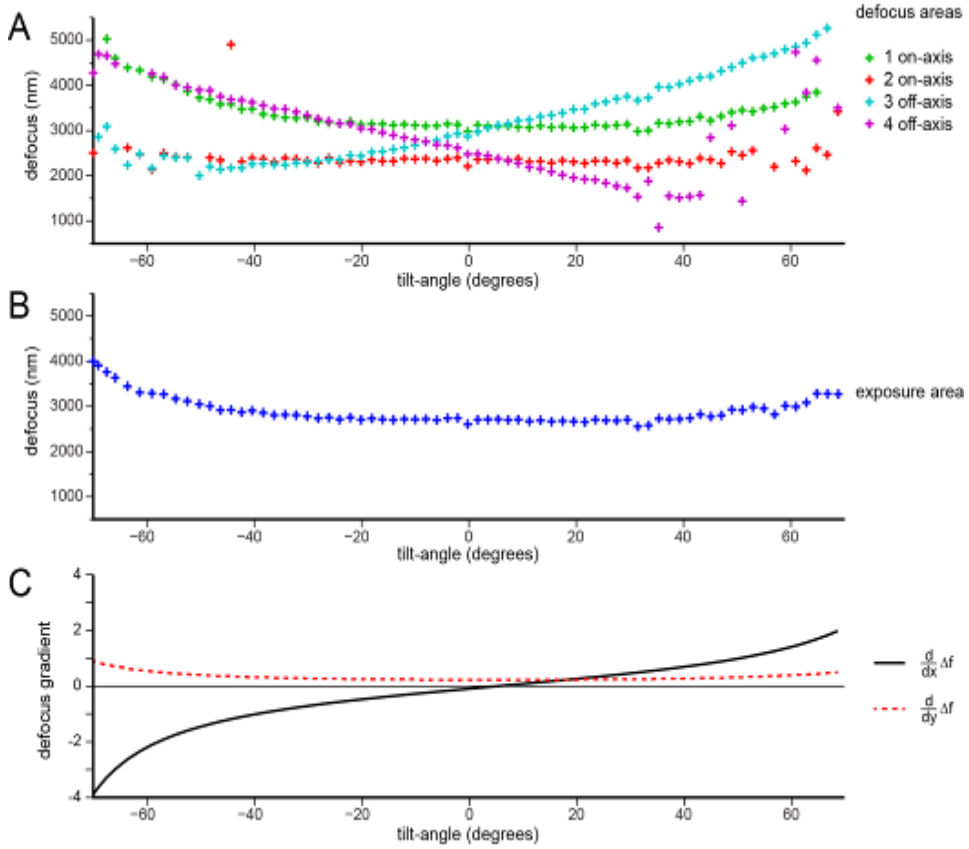

Figure 2. a) Measured defocus on the four high-dose exposure areas for each tilt-angle. b) The defocus at the tilt-axis in the area-of-interest, estimated using the location of the high-dose areas (Figure 1) and the measured defocus values (A). c) The estimated specimen orientation together with the tilt-angle defines the defocus gradient perpendicular to and along the tilt-axis. At high tilt-angles the defocus gradient is dominated by the applied tilt-angle, whereas at low tilt-angles the defocus gradient is a by-product of the non-zero specimen orientation.