

## Zero-loss P-contrast in EFTEM

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Biological samples consist mainly of C, N, O, P, and S atoms. Varying ratios of these atoms produce different contrast in EM images of native material. The increased content of phosphorus should allow to distinguish DNA/RNA from protein. To test whether this prediction holds for the experimental situation of cryo-EM we simulated images of samples with different P atom content. These images were then processed like experimental data.

Images were simulated using a multislice program [2,3] with the following parameters: electron energy  $U_0 = 120$  kV, spherical aberration  $C_s = 2.7$  mm, chromatic aberration  $C_c = 2.7$  mm, energy spread  $\Delta E = 0.75$  eV, resolution aperture 12.0 mrad, focus spread 0.0  $\mu\text{m}$ , and ideal zero-loss filtering. The used phantom (figure 2 a,b) consists of five spheres (radius  $R = 30\text{\AA}$ ) which were filled with cubes cut out of the molecular model of g-actin. Three spheres of the phantom contain 0.7 % P atoms like the original actin. In the top sphere 5 % and in the bottom sphere 10 % of all C, N, and O atoms were evenly replaced by phosphorus. For the simulations this phantom was embedded in a 300  $\text{\AA}$  ice layer. All the simulations were computed for 50 different ice embeddings and averaged. A tilt series of 11 tilt angles ( $0^\circ \pm 24.7^\circ \pm 33.6^\circ \pm 44.4^\circ \pm 54.0^\circ \pm 60.0^\circ$ ) and 8 azimuthal angles ( $0^\circ \pm 45^\circ \pm 90^\circ \pm 135^\circ \pm 180^\circ$ ) was simulated. The defocus values of the simulated series are - 0.97  $\mu\text{m}$  and - 1.97  $\mu\text{m}$ . Each averaged projection was CTF corrected according to [3].

The higher electron optical density of phosphorus compared to the other atoms in the phantom is not obvious in the images (figure 1a,b). However, regions masked in the images where the corresponding object area contains P atoms have a lower mean in the gray value histograms than non P areas (data not shown). The biggest difference between mean (P) to mean (non P) is found in the CTF corrected images. Because those histograms have a wide overlap no segmentation is possible by simple comparison of histograms. This is also the case for reconstructed volumes [4], which is shown in figure 2 c-f for resolutions of 3  $\text{\AA}$  and 13  $\text{\AA}$ . The reconstructed density increases with P content. With decreasing resolution the differences in density are more obvious but the reconstructed volumes become smaller (figure 2 c,e).

A more promising approach is segmentation. The CTF corrected images were processed without using any previous knowledge about the phantom: First a threshold was set to remove the contrast formed by the ice embedding (figure 3a). Then closing (erode, dilate) was applied (figure 3b). The rest of the embedding could be removed by labeling. In this way a mask was obtained and applied to the original CTF corrected image (figure 3c). Each masked area is represented by its average gray value per pixel (figure 3d,e). The areas found by segmentation are more than 80% of the expected areas. These preliminary results show that after segmentation an appropriate thresholding allows to distinguish the P and non P regions.

### References

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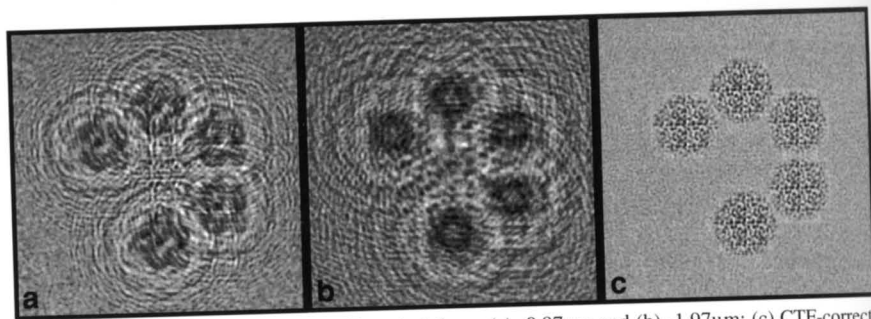


FIG.1 - Simulated images [1,2], zero-loss energy filtered, defocus (a)  $-0.97\mu\text{m}$  and (b)  $-1.97\mu\text{m}$ ; (c) CTF-corrected image [3], resolution better than  $2\text{\AA}$

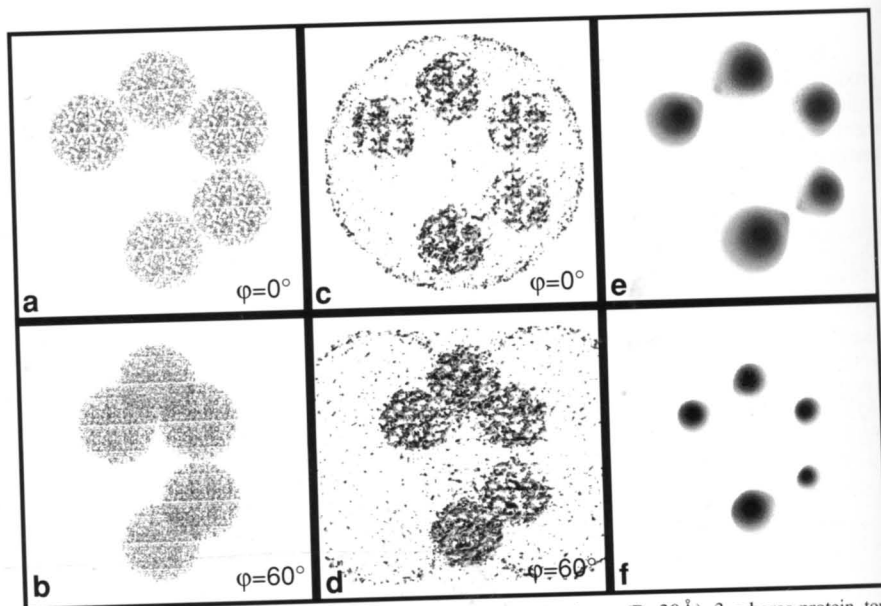


FIG.2 - Rotated 3D views of (a,b) pdb-phantom used for simulations: 5 spheres ( $R=30\text{\AA}$ ), 3 spheres protein, top sphere containing 5% P, bottom sphere containing 10% P; (c,d) 3D-reconstruction [4] of zero-loss energy filtered, CTF-correct-