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doi:10.1017/S1431927622005268
Microsc. Microanal. 28 (Suppl 1), 2022

Using Inelastically Scattered Electrons to Enhance Imaging of Biological Macromolecules from Any Layer of a Thick Specimen

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As the rate at which structural biology accumulates structures continues to accelerate [1], an increasingly important frontier of electron cryomicroscopy (cryoEM) is the imaging of biological molecules within their native environments. In the context of structural abundance, the goal of cryomicroscopy of cellular specimens will increasingly shift from structure determination to the identification of the position and orientation of particular molecules relative to other structures present in a cell. Electron cryotomography (cryoET), in which a series of tilted cryomicrographs is collected and reconstructed into a 3D tomogram [2], has been used for many years for this purpose, but the direct identification of specific molecules is limited to large macromolecular complexes that are of order 10^6 Daltons in mass. More recently, 2D template matching has also emerged as another approach to this problem which omits the tilting and 3D reconstruction and identifies the molecules directly from their projected images in a single micrograph [3].

Imaging macromolecules *in situ* requires specimens that are thicker than those used to obtain high resolution structures by single particle cryoEM, which causes a major limitation on the attainable resolution since it results in a larger proportion of electrons suffering inelastic and multiple elastic scattering. Electrons that have lost some energy to the specimen are incorrectly focused in the image plane due to the chromatic aberration (C_c) of the objective lens. As a result, electrons that carry elastically scattered information but also inelastically scatter may not provide high resolution information in typical phase contrast images; the current practice in both single particle cryoEM and cryoET is to remove these electrons with an electron energy filter to reduce the background. Figure 1, which was taken using exclusively inelastically scattered electrons, demonstrates that high resolution signal is typically lost by energy filtering. Given the advent of practical chromatic aberration correction [4], there is now a potential to incorporate these inelastically scattered electrons in phase contrast imaging.

To quantify the amount of potential improvement in signal from C_c correction, we experimentally determined the loss of signal from inelastically scattered electrons through a thick carbon specimen by measuring the intensity in the reflection from the 111 lattice planes of gold particles as a function of defocus. Simulations guided by the experimental data were then used to predict the rate of signal loss from inelastic scattering in biological specimens as a function of defocus (Figure 2). We have thus characterized the amount of loss in spatial coherence caused by interaction with the specimen itself, which we call “specimen induced decoherence”. We conclude that inelastically scattered electrons can be used to enhance signal provided the sample is imaged as close to zero defocus as possible. This indicates that a method of generating phase contrast, such as a phase plate, may be particularly useful with an achromatic objective lens. The potential improvement in signal is greatest for specimens

between 100-500 nm thick. Together, a C_c corrector with a phase plate could decrease the size of molecules that can be directly identified *in situ* by cryoEM.

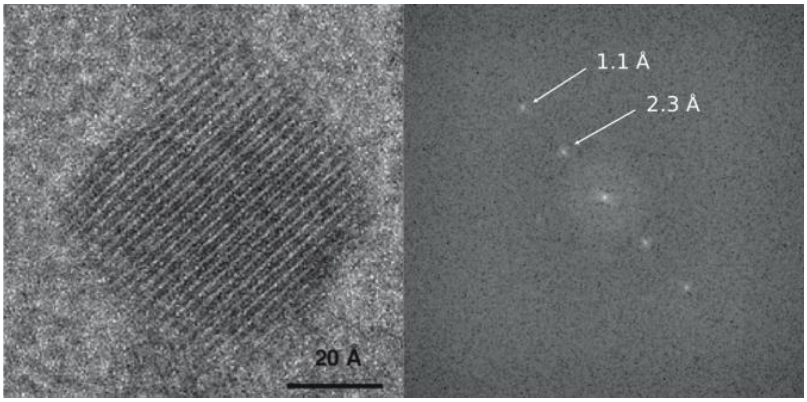

Figure 1. Phase contrast from a platinum particle on a thick aluminium/carbon foil, with a mass density equivalent to 4000 Å thick water ice. The micrograph was taken at 15 eV energy loss and 300 keV primary energy. The 2.3 Å and 1.1 Å reflections are clearly visible in the power spectrum.

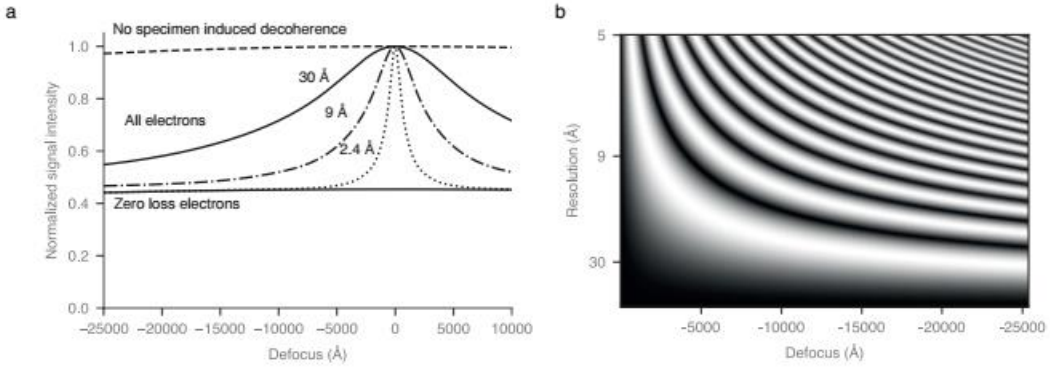

Figure 2. (a) Simulations of the fading of intensity for different resolutions from a protein specimen embedded in amorphous water ice. The simulated electron energy was 300 keV, the specimen was 2000 Å thick, and the protein to water ratio was taken as 30:70. The series of curves show the additional power at 2.4, 9 and 30 Å resolution from both elastic and inelastic phase contrast (~perfect C_c correction and phase contrast). The top dashed curve represents the total potential improvement in power if there were no specimen induced decoherence. (b) The absolute amplitude of the contrast transfer function for a given defocus and resolution (black = 0 and white =1). The minimum amount of defocus required for visualising particles (~-5000 Å) will cause a significant loss of signal at both 2.4 and 9 Å resolution.

References:

[1] W. Chiu, M. F. Schmid, G. D. Pintilie, C. L. Lawson, PDB, and EMDB, Journal of Biological Chemistry **296** (2021) 100560.
[2] L. Gan, G. J. Jensen, Quarterly Reviews of Biophysics **45** (2012) 2756.
[3] B. A. Lucas, B. A. Himes, L. Xue, T. Grant, J. Mahamid, N. Grigorieff, eLife **10** (2021) e68946.

https://doi.org/10.1017/S1431927622005268 Published online by Cambridge University Press

[4] B. Kabius, P. Hartel, M. Haider, H. Müller, S. Uhlemann, U. Loebau, J. Zach, H. Rose, Journal of Electron Microscopy **58** (2009) 147–155.

https://doi.org/10.1017/S1431927622005268 Published online by Cambridge University Press