2566
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Montage cryo-electron tomography: imaging a large field-of-view without sacrificing resolution

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Cryo-electron tomography (cryo-ET) is a powerful tool for studying macromolecular structures in their near-native context of frozen-hydrated cells, unperturbed by stains or fixatives. Recently, subtomogram averaging algorithms have enabled structure determination to near-atomic resolution *in situ* [1]. However, retaining high-resolution signal requires collecting data at high magnification. The trade-off is a smaller field-of-view, which limits the region that can be imaged to a fraction of a cell. Montage tomography overcomes this challenge and permits imaging a large field-of-view without sacrificing high-resolution details. During montage data collection, the beam is tiled across a specimen and the recorded images are computationally stitched together during reconstruction. To date, montage tomography has only been performed on resin-embedded samples, which suffer from artifacts induced by chemical fixation but resist radiation damage [2-3]. By contrast, cryo-preserved specimens can tolerate only a limited dose before being destroyed [4]. Historically this dose sensitivity prohibited montage tomography of vitrified samples, as portions of the sample must be exposed multiple times for reconstruction. However, the stable optics of modern microscopes, increased sensitivity of current detectors, and ability to collect data using fringe-free illumination motivate revisiting the potential of this technique [5-6].

Here we describe our development of montage cryo-tomography to image large regions of cells at high magnification. We used simulations to determine the most efficient strategy to distribute dose throughout the sample when collecting data using a circular beam equipped with fringe-free illumination (Fig. 1). We then collected and reconstructed montage tomograms from the thin edges of eukaryotic cells. These tomograms featured multilamellar vesicles, mitochondrial calcium-phosphate granules, and microtubules (Fig. 2). In addition, reconstruction quality was assessed by subtomogram averaging of ribosomes from different regions of the montage tilt-series. We anticipate that montage data collection will further the impact of cryo-ET by providing significantly more cellular context for macromolecules of interest, maximizing the amount of data that can be collected from focus ion beam-milled specimens, and increasing the likelihood of imaging transient biological events.

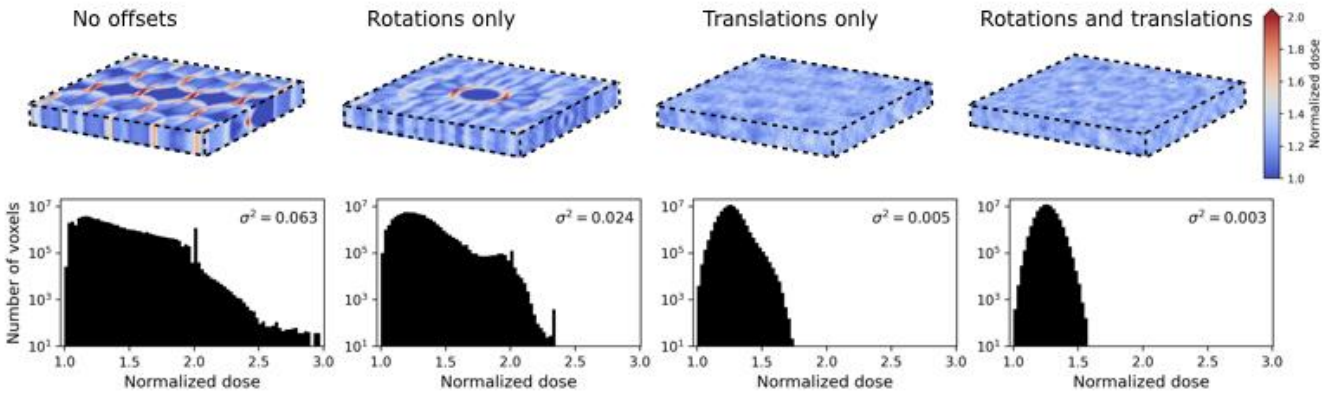

Figure 1. Figure 1. Optimization of a montage tiling strategy. At each tilt angle, a hexagonally-packed set of circular tiles is imaged. By rotating and translating the centers of these tiles between images, the dose

can be efficiently distributed throughout the sample to minimize the regions of the sample that receive excess dose. The upper panel visualizes the spatial distribution of normalized dose throughout the sample after all tilt images are collected, comparing the least ("no offsets") and most efficient ("translations and rotations") data collection schemes. Hundreds of tiling strategies were simulated and scored by the variance of the dose distribution, which is noted in the upper right of each histogram in the lower panel.

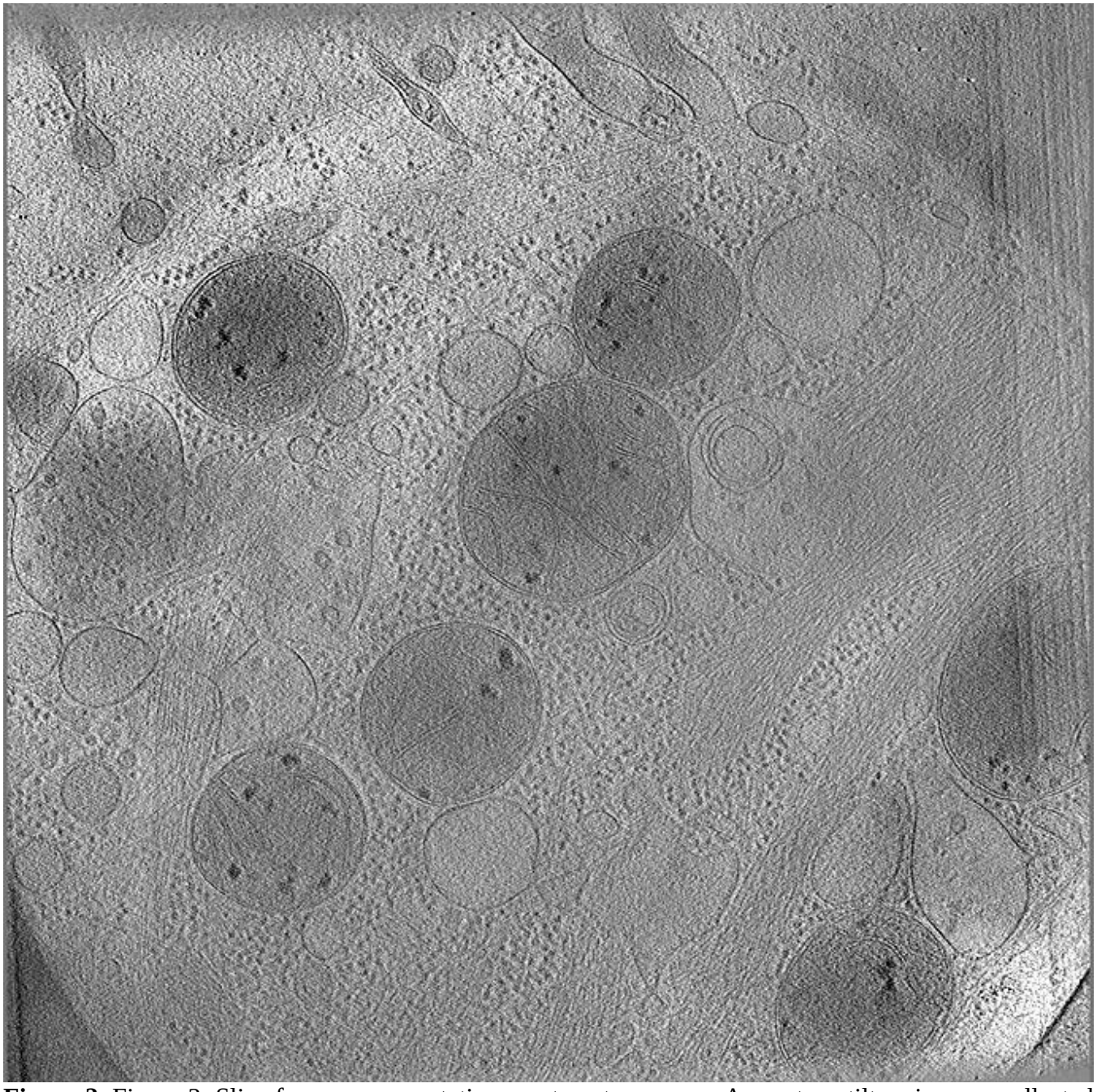

Figure 2. Figure 2. Slice from a representative montage tomogram. A montage tilt-series was collected from the thin edge of a eukaryotic cell and reconstructed into a tomogram.

References

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1. D. Tegunov, L. Xue, C. Dienemann, P. Cramer, and J. Mahamid, Multi-particle cryo-em refinement with M visualizes ribosome-antibiotic complex at 3.5 Å inside cells, Nature Methods 18 (2021).
2. A. B. Noske, A. J. Costin, G. P. Morgan, and B. J. Marsh, Expedited approaches to whole cell electron to- mography and organelle mark-up in situ in high-pressure frozen pancreatic islets, J. Struct. Biol. 161, 298 (2008).
3. R. I. Koning, S. Zovko, M. Ba´rcena, G. T. Ooster- getel, H. K. Koerten, N. Galjart, A. J. Koster, and A. Mieke Mommaas, Cryo electron tomography of vitri- fied fibroblasts: microtubule plus ends in situ, J. Struct. Biol. 161, 459 (2008).
4. L. A. Baker and J. L. Rubinstein, Radiation damage in electron cryomicroscopy, Meth. Enzymol. 481, 371 (2010).
5. A. C. Milazzo, A. Cheng, A. Moeller, D. Lyumkis, E. Ja- covetty, J. Polukas, M. H. Ellisman, N. H. Xuong, B. Carragher, and C. S. Potter, Initial evaluation of a direct detection device detector for single particle cryo-electron microscopy, J. Struct. Biol. 176, 404 (2011).
6. S. Konings, M. Kuijper, J. Keizer, F. Grollios, T. Spanjer, and P. Tiemeijer, Advances in Single Particle Analy- sis Data Acquisition, Microscopy and Microanalysis 25, 1012 (2019).

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