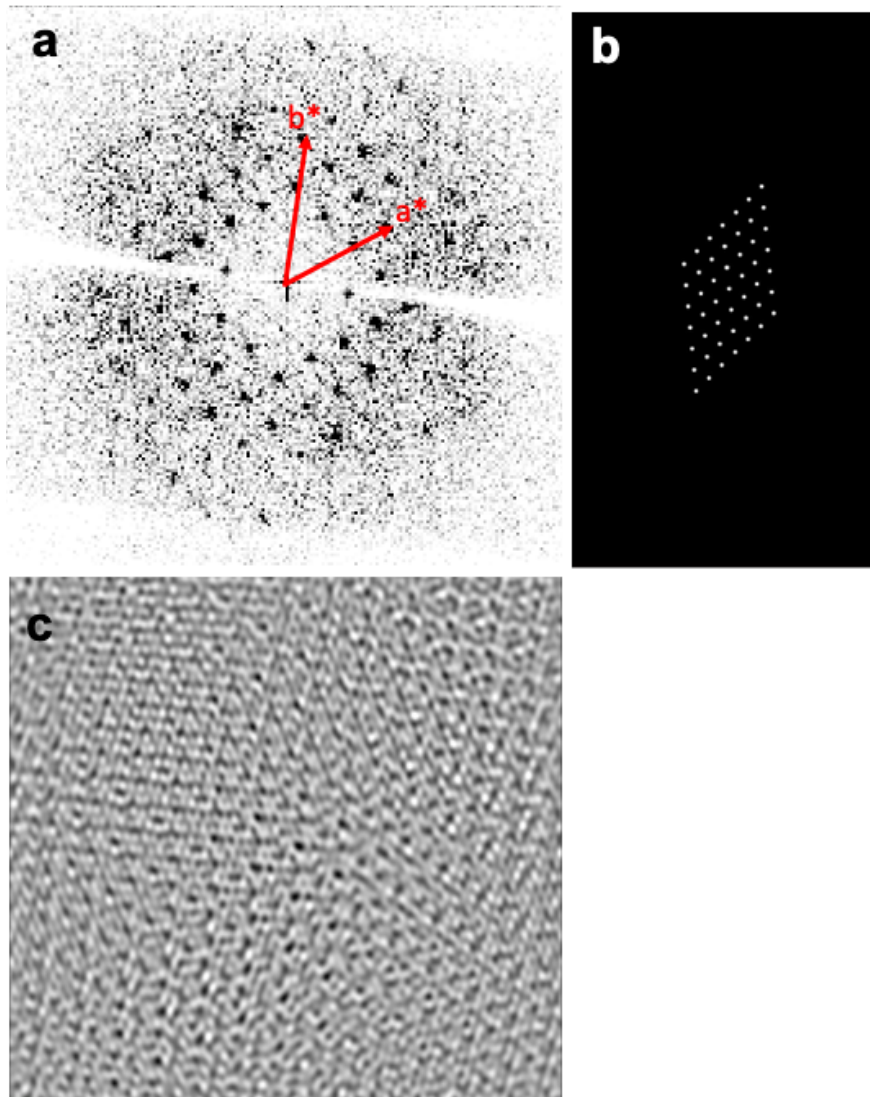


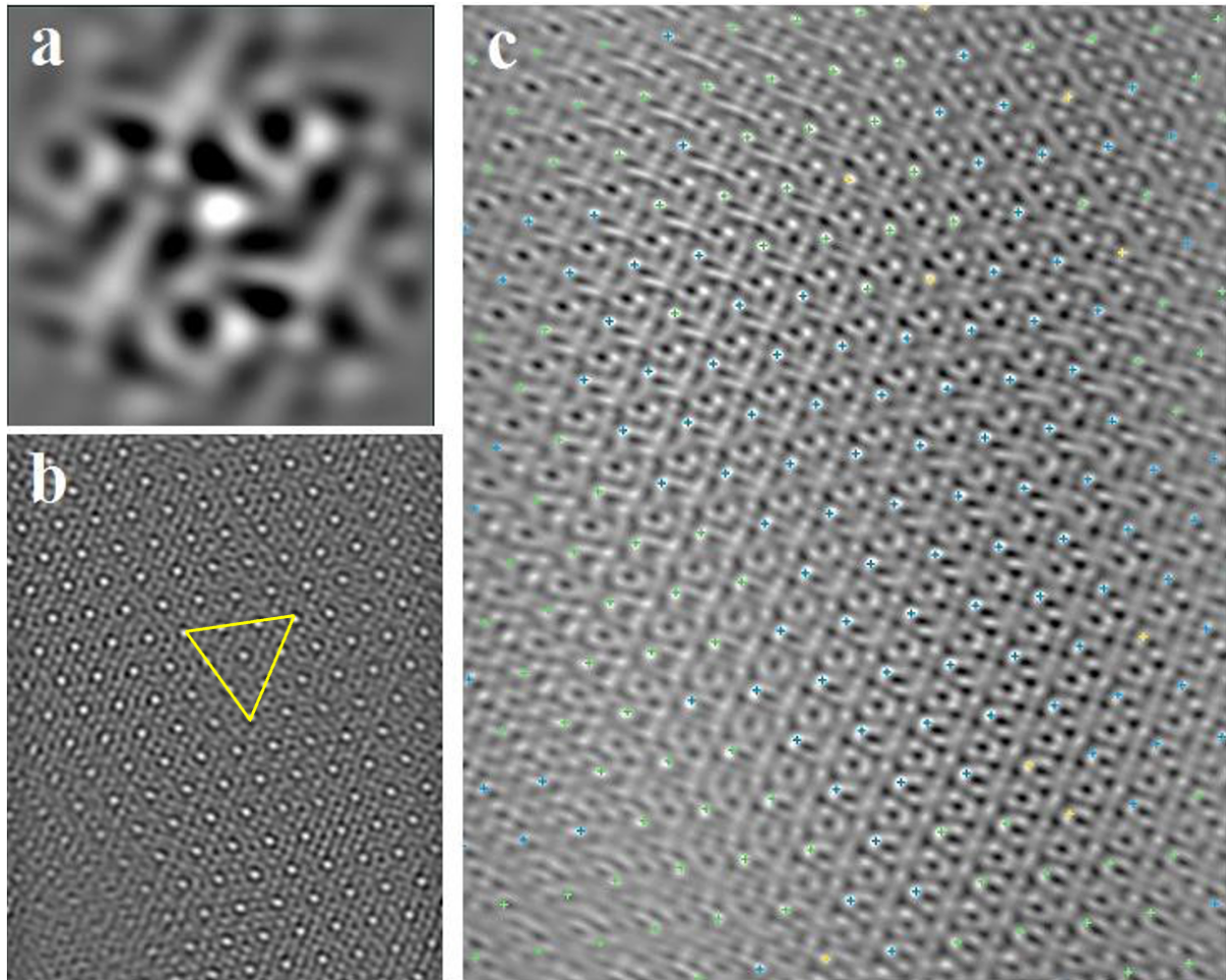
Supplemental Images

THE CRYO-EM 3D IMAGE RECONSTRUCTION OF ISOLATED *LETHOCERUS* *INDICUS* Z-DISCS

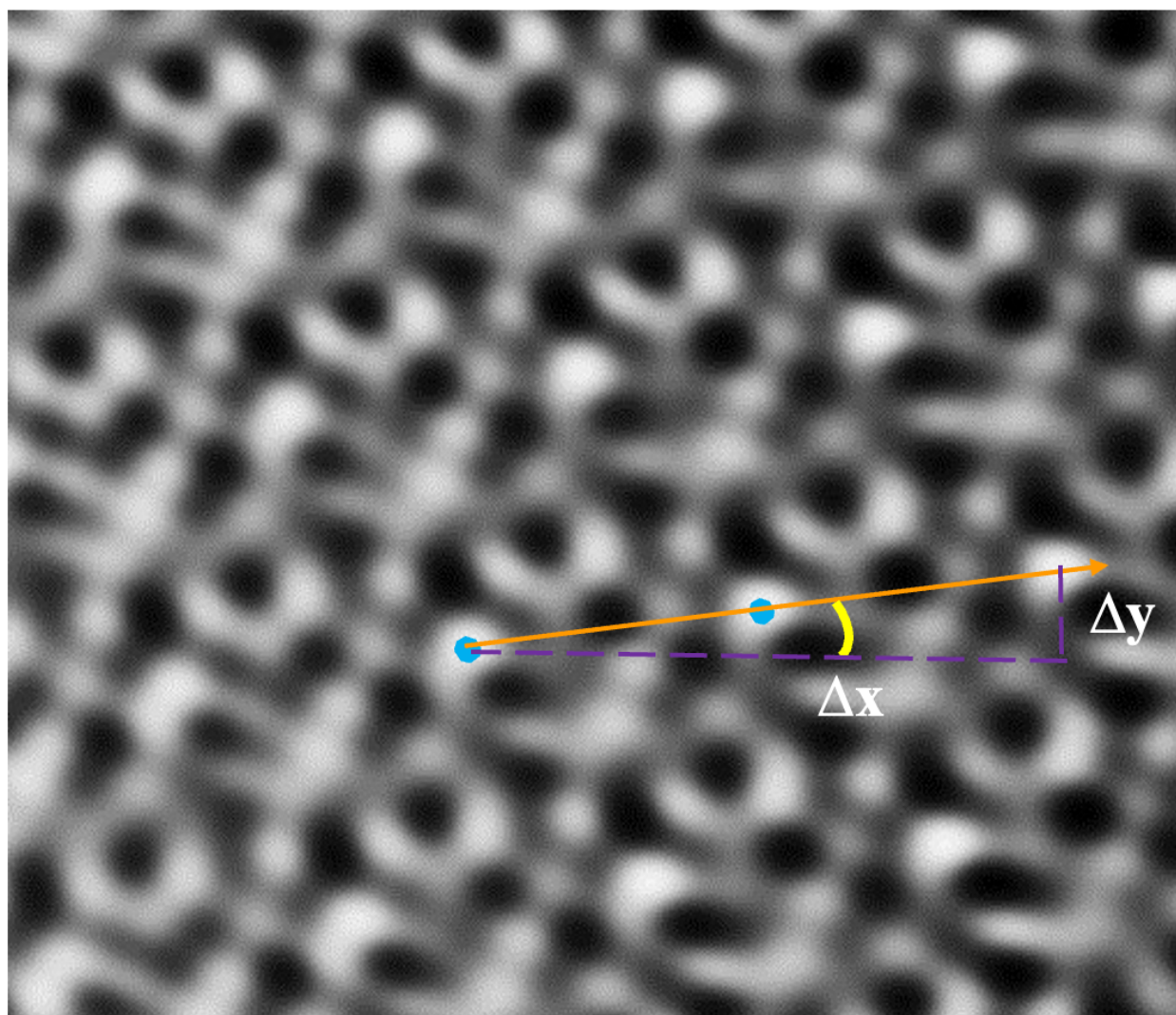
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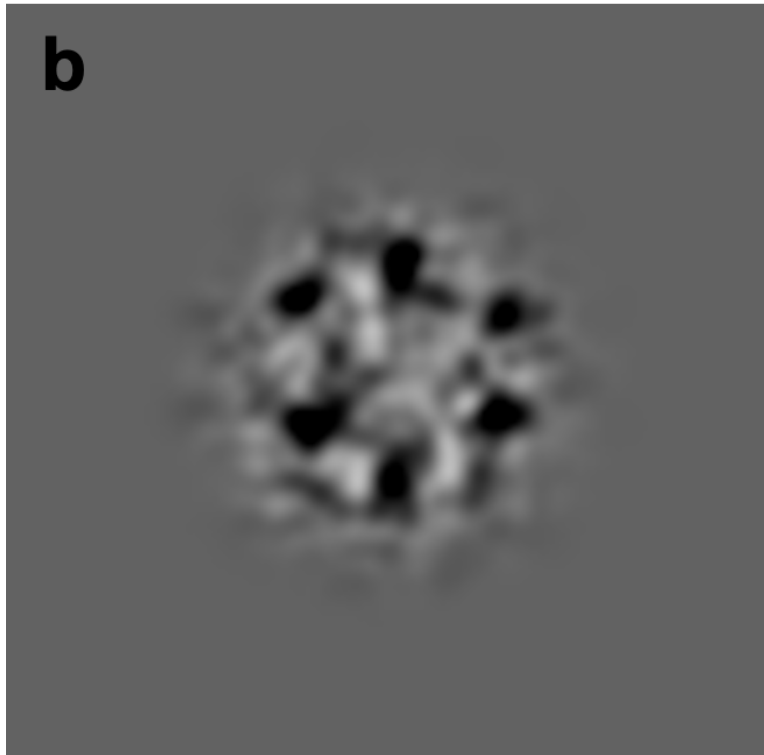
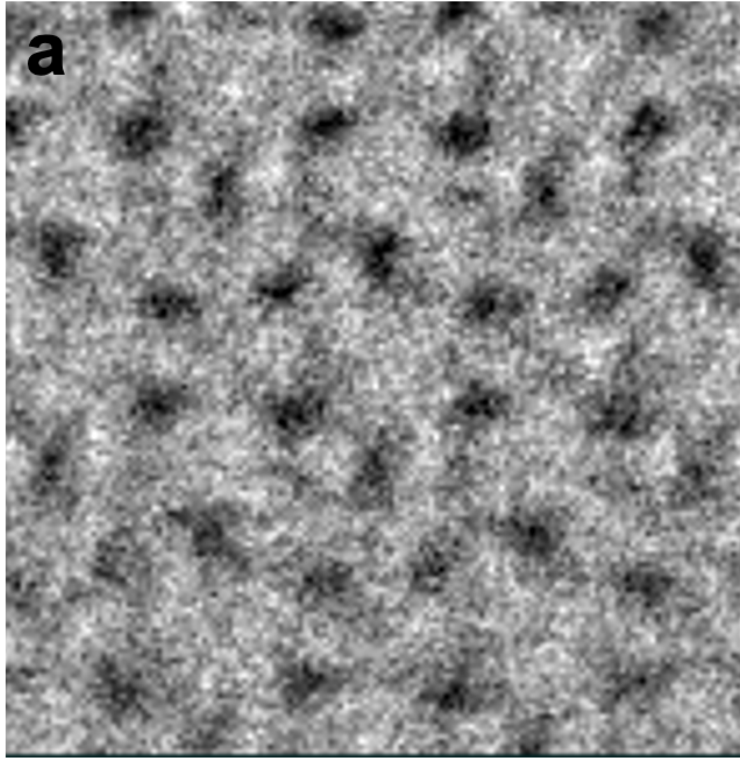
Supplemental Figure 1. Generation of a filtered tomogram. a) Fourier transform of tomogram showing the a^* and b^* vectors. The second order diffraction points were selected as the first order points were not visible. b) The vectors of (a) are used to generate a mask that is applied in the Fourier space of the tomogram. c) The resulting filtered, average image of the tomogram after the mask was applied.



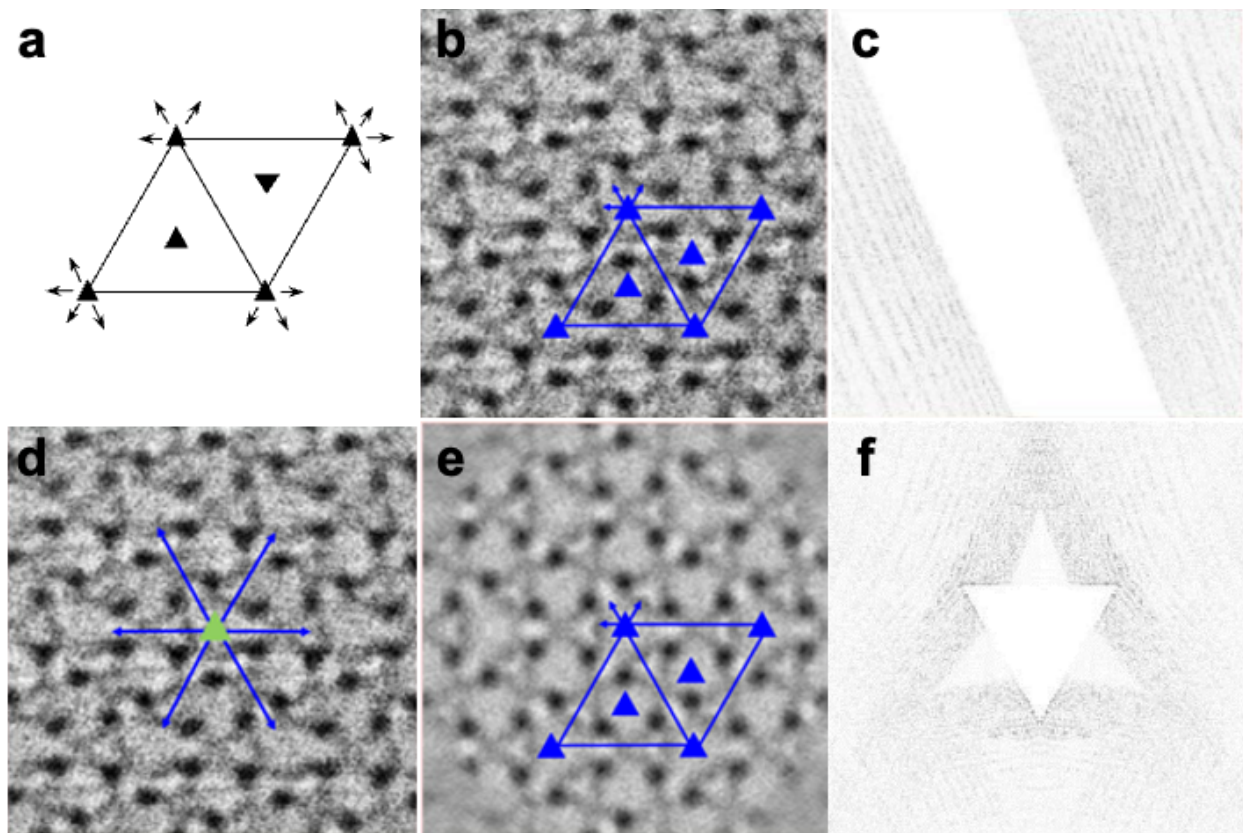
Supplemental Figure 2. Selection of subtomograms. a) The motif reference from the spatially averaged tomogram. b) The correlation map of the tomogram obtained by cross correlating the spatially average tomogram with the motif reference. The yellow triangle represents the real space vectors used for the peak search step. c) Peak coordinates on the averaged tomogram. The colors of the coordinates are keyed to the Z-coordinate (section number) of the plane in which the center is located. The orange ones in this image are in the same plane as the image, while the green and blue ones are out of plane.



Supplemental Figure 3. Reorientation of subtomograms prior to alignment. Magnified image of the filtered average tomogram. Two similar points from adjacent unit cells were selected in the tomogram (represented by the blue dots). The x and y coordinates of those points were used to find the slope (orange line) by calculating the rise (Δy) and run (Δx), which is represented with the purple dashed lines. Using the equation $\arctan(\Delta y/\Delta x)$, the Euler angle was determined (represented in yellow).



Supplemental Figure 4. Generation of initial reference. a) Projection of the global average of the raw subtomograms. b) The resulting single reference when the mask is applied to the global average. The reference in this example is enclosing the large solvent channel in the IFM Z-Disc.



Supplemental Figure 5. Symmetrization of the global average. (a) Symmetry elements of space group P321. 3-fold rotation axes occur at the corners of the unit cell, and at trigonal positions in the center of each triangle that are generated from the 3-fold axes at the corners. One of these has been flipped over to indicate that these 3-fold axes are not related by translation in the Z-disk. Running parallel with the unit cell axes and the body diagonal are 2-fold rotation axes within the plane of the Z-disk. (b) The unsymmetrized global average. (c) An upper level plane of the Fourier transform of the Z-disk showing the position of the missing wedge. (d) Symmetry positions applied to the reference. 2-fold rotations within the plane of the Z-disk will generate six equivalent, aligned copies of each structural element in the Z-disk. (e) Symmetrized global average after averaging the six equivalent copies. (f) Fourier transform of the global average showing the missing star-shaped/cone.