

## Preliminary Results

Our preliminary results are entirely from the work on invertebrate thick filaments from the large waterbug *Lethocerus indicus*, which has been our main focus for the last 5 years.

The 5.5 Å thick filament reconstruction published in 2016 [2] was actually pushed to 4.3 Å by Dr. Hu before he graduated from 24,000 filament segments (particles) with enforced helical symmetry; it lacked the signal to noise improvement obtained with the recent 4.2 Å reconstruction. Arguably a subnanometer or even atomic resolution reconstruction will require fewer movies if they are of higher quality than the DE-20 movies which he used.

We did a feasibility test using data which produced the 4.2 Å structure recently published [1]. That data set consisted of 3,562 movies 8192 x 8192 pixels in size collected over a 3.5-day period using a DE-64 direct electron detector operated in integrating-mode. A total of 7650 filaments were picked for the structure with helical symmetry imposed. From these, 418 images, e.g. Figure 1, showed 542 crown-0 motifs, the first level of myosin heads in the A-band. Using crown-0 as the center, we obtained a 16 Å resolution reconstruction (Fig. 2, left). The result compares favorably against our first subnanometer reconstruction of the *Lethocerus* thick filament from 24,000 segments [2] when filtered to 16 Å resolution (Fig. 2, right). The preliminary results suggest that the major barrier to obtaining subnanometer and even atomic resolution without imposing helical symmetry is simply the number of movies of equal or higher quality.

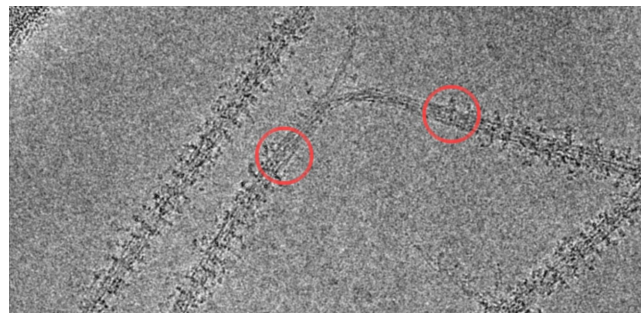


Figure 1. Electron micrograph of a frozen-hydrated *Lethocerus* thick filament recorded in integration mode on the DE-64. The red circles outline the first crown (crown-0). At the end of the bare zone.

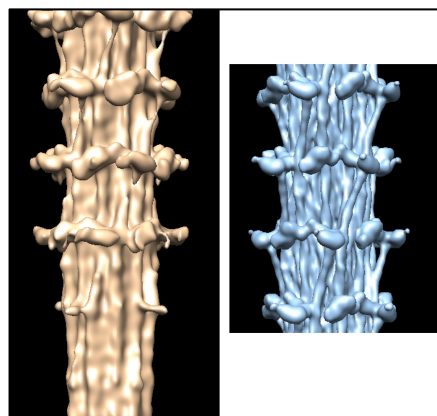


Figure 2. Comparison of *Lethocerus* thick filaments reconstructions. (left) The crown-0 reconstruction which imposed only rotational symmetry on 542 motifs, equal to 2,168 asymmetric units, reached a resolution of 16 Å by FSC criteria. Images were recorded in integration mode on a DE-64 detector. (right) Previously published sub-nanometer reconstruction from ~24,000 segments, 96,000 asymmetric units, low pass filtered to 16 Å resolution from images recorded on a DE-20 direct electron detector operated in integration mode with imposed helical symmetry. The DE-20 detector is inferior to both the DE-64 and K3 with regards to quality of image delivered as measured by detector quantum efficiency, DQE. Both reconstructions show the poorly ordered myosin heads projecting from the backbone about equally, but also resolve the S2 linkage from the heads to the backbone, though not as well as in the crown-0 reconstruction with symmetry imposed.