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Efficient Single Particle and Tilt Series Workflow for a Cryo-EM Core

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Setting up an efficient workflow is crucial for any cryo-EM core facility. Core facilities expect to have many users and projects, need to keep track of information over time, and need to be able to process data as it comes in so decisions about collection can be made. Enough processing power and storage space needs to be available on-hand to accomplish this, as well as a software suite which can be automated as much as possible. At the NYU Cryo-EM core, we have two high powered microscopes: a Talos Arctica for screening and data collection, and a Titan Krios for high resolution data collection. Each is equipped with a Gatan K3 detector, and collection rates are generally 210 super-resolution images per hour. A 9 PB storage server, allocated for all of NYU Langone, is available for long-term frame storage of raw data.

We have found that the Leginon software suite [1], when combined with Appion [2] for pre-processing, works very well for project tracking, user tracking, and permanent storage of experimental metadata, particularly for single particle projects. The core has 200 TB of high performance GPFS disk space for short term image storage and processing. Each microscope has a dedicated 4-GPU workstation for on-the-fly frame alignment and CTF estimation, which is sufficient to keep fully caught up at the collection pace. Aligned, dose-weighted images are transferred to a Windows workstation for particle picking, per-particle CTF, and stack creation using Warp [3] software. These stacks are uploaded to a CryoSPARC [4] server for initial 2D analysis and further processing if required. They can easily be shared with users of the facility, since the output is a standard star file and an associated Particles directory containing the stacks. The Appion server is set up to make jpg snapshots of all images taken. Once the experiment is completed and frames are backed up, all raw frames and images can be deleted. The Core policy is to delete frames immediately after transfer to long-term storage. Non-processed images (lower magnification grid, square, and subsquare images as well as non-dose weighted high magnification images) can be deleted once the session is over. Dose-weighted images are generally kept for 3 months in order to allow for additional processing time. The snapshots are kept indefinitely so that experiments can be reviewed for long-term analysis.

For tilt series collection, we have found that a modified workflow has worked best for us. We collect grid atlases, square, and subsquare images using Leginon. This allows for easy long-term analysis of the grids used for collection. When good ares are found, we switch out of Leginon and into SerialEM [5] for tilt series collection. SerialEM is widely used and the stacks created can be used in several software suites, including Warp, EMAN2 [6], and eTomo [7]. Medium magnification maps are made using SerialEM's Navigator, and areas for collection are marked there. The raw frames and mdoc files are transferred from the K3 PC to the Warp PC for frame alignment in Warp. As with single particle data, the Warp PC can keep up with data collection, and frame alignment is automatic. Warp is then used to create tilt series stacks from the aligned frames and mdoc files, which are then exported to eTomo. eTomo is used to align the tilt series and generate initial tomograms. Optionally, the alignment parameters can be imported into Warp for tomogram generation. The raw frames, mdoc files, stacks, and initial tomograms are shared with the core users.

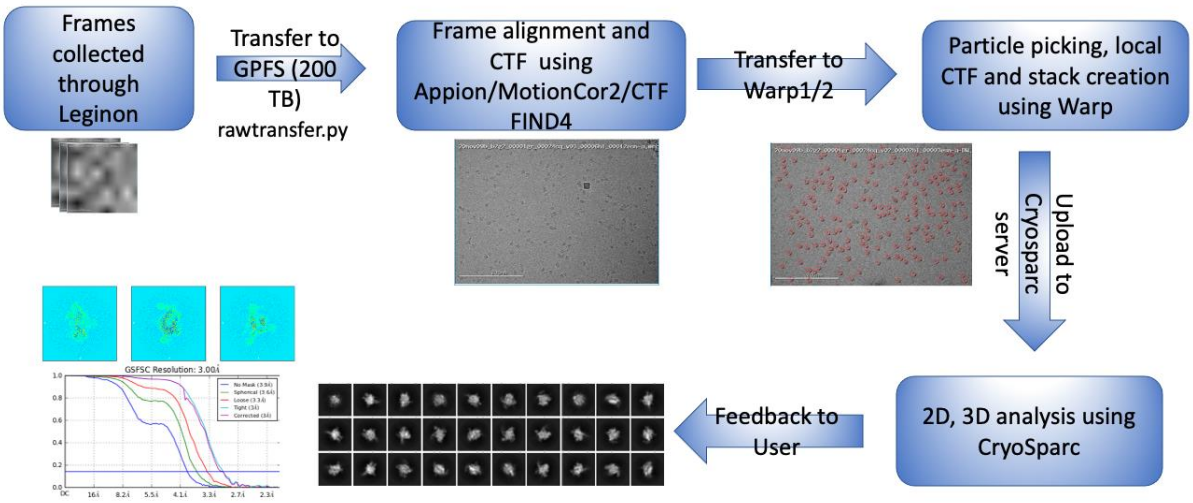

Figure 1. Standard workflow for single particle data collection. Images are collected using Leginon and frame alignment is done using Appion's MotionCorr2 interface. Aligned and dose-weighted imaegs are automatically transferred to a Warp server, where particles are picked. The particle stacks are uploaded in chunks to CryoSPARC for initial processing.

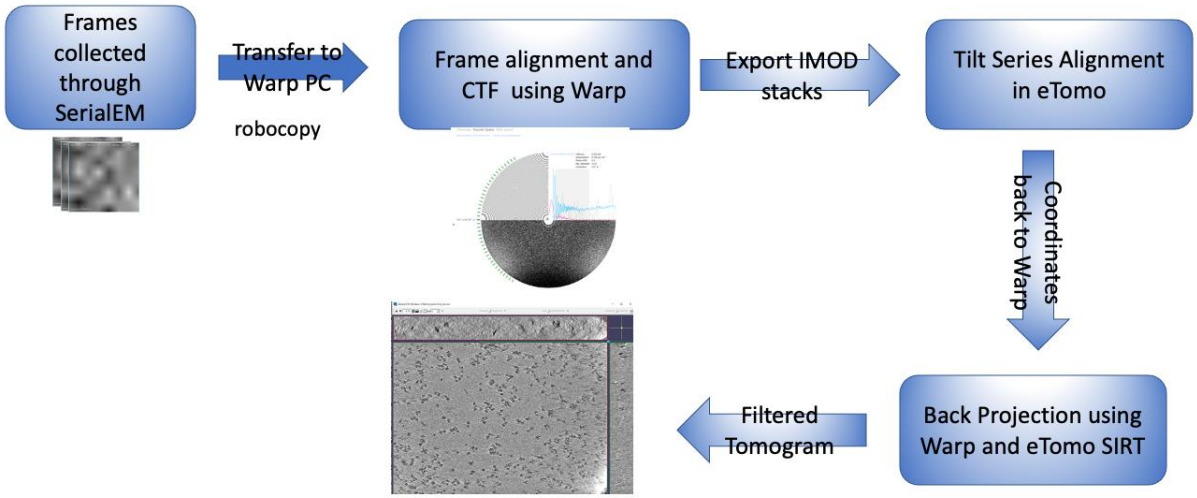

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Figure 2. Standard workflow for tilt series collection. Tilt series are collected in SerialEM following initial targeting in Leginon. Frames are transferred directly to the Warp PC for alignment and assembly into stacks. Etomo is used for tilt series alignment and initial tomogram reconstruction.

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