Data Collection with EPU Independence Test Evaluator Checklist - CryoEM Merit Badges

Trainee: _____

Microscope and Software checks

- ❑ ❑ Check if any errors on TEMUI
- ❑ ❑ Check microscope states, such as vacuum, temperature or CFEG
- ❑ ❑ Cassette with grids have been loaded
- ❑ ❑ Autoloader Inventory is finished

Starting Atlas in EPU

- ❑ ❑ Open column valves and check beam before starting Atlas
- ❑ ❑ Create an Atlas session
- ❑ ❑ Start Atlas

Screening grids in EPU

- ❑ ❑ Load a grid to screen
- ❑ ❑ Move stage to a square and use GridSquare Preset to acquire an image
- ❑ ❑ Eucentric height is found using AutoFunctions-AutoEucentric by Beam Tilt
- ❑ ❑ Acquire an image of foil holes
- ❑ ❑ Perform AutoFocus on carbon film
- ❑ ❑ Move stage and image samples in a hole
- ❑ ❑ Evaluate the quality of ice and samples

Image Shift Calibration

- ❑ ❑ Find a suitable feature on the grid atlas
- ❑ ❑ Move stage to the feature and use GridSquare Preset to acquire an image
- ❑ ❑ Find Eucentric height using AutoFunctions-AutoEucentric by Beam Tilt
- ❑ ❑ Acquire an image of the feature under Hole/EucentricHeight
- ❑ ❑ Center the feature under Hole/EucentricHeight
- ❑ ❑ Acquire an image of the feature under DataAcquisition. Move stage to find the feature if it is not visible
- ❑ ❑ Start Image shift calibration

Energy Filter Tuning (BioQuantum)

- ❑ ❑ Find an empty or broken square and move stage
- ❑ ❑ Acquire a grid square image to make sure the stage is over vacuum
- ❑ ❑ Set the microscope to the DataAquisition optics
- ❑ ❑ Insert Screen and examine the beam
- ❑ ❑ Change Spot Size to 1 and C2 aperture to 150
- ❑ ❑ Select Tune GIF and Full Tune on Digital Micrograph

Energy Filter Tuning (Selectris)

- ❑ ❑ Find an empty or broken square and move stage
- ❑ ❑ Acquire a grid square image to make sure the stage is over vacuum
- ❑ ❑ Set the microscope to the DataAquisition optics
- ❑ ❑ Insert Screen and examine the beam
- ❑ ❑ Open Sherpa and select Energy Filter
- ❑ ❑ Tune the Energy Filter by clicking the buttons one by one

CryoEM Merit Badges were developed with support from NIH Common Fund Merit Badge supplement to 3U24 GM129539-02

Cryo-EM Merit Badge Independence Checklist

A Collaboration Among the NIH-Funded Cryo-EM National Centers

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Gain Reference Collection (K3)

- ❑ ❑ Find an empty or broken square and move stage
- ❑ ❑ Acquire a grid square image to make sure the stage is over vacuum
- ❑ ❑ Set the microscope to the DataAquisition optics
- ❑ ❑ Insert Screen and examine the beam
- ❑ ❑ Change Spot Size to 1 and C2 aperture to 150
- ❑ ❑ Select Prepare Gain Reference under the Camera menu on Digital Micrograph
- ❑ ❑ Collect linear and counting gain references

Gain Reference Collection (Falcon 4)

- ❑ ❑ Find an empty or broken square and move stage
- ❑ ❑ Acquire a grid square image to make sure the stage is over vacuum
- ❑ ❑ Set the microscope to the DataAquisition optics
- ❑ ❑ Insert Screen and examine the beam
- ❑ ❑ Open Falcon 4(i) Reference Image Manager
- ❑ ❑ Measure dose before collecting postcounting gain reference
- ❑ ❑ Click Acquire

Dose Fractionation (K3)

- ❑ ❑ Find an empty or broken square and move stage
- ❑ ❑ Acquire a grid square image to make sure the stage is over vacuum
- ❑ ❑ Set the microscope to the DataAquisition optics
- ❑ ❑ Measure the dose rate by capture an image under counting mode with 1 second exposure, binning=1x
- ❑ ❑ Make sure the dose rate is between 15-25 e-/pixel/s
- ❑ ❑ Determine the total dose, exposure time and number of frames

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Dose Fractionation (Falcon 4)

- ❑ ❑ Find an empty or broken square and move stage
- ❑ ❑ Acquire a grid square image to make sure the stage is over vacuum
- ❑ ❑ Set the microscope to the DataAquisition optics
- ❑ ❑ Measure the dose rate by click MEASURE in EPU
- ❑ ❑ Make sure the dose rate is within the green range
- ❑ ❑ Determine the file format (EER, TIFF, or MRC)
- ❑ ❑ Determine the total dose, exposure time and number of frames

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Microscope Alignment

- ❑ ❑ Move stage to a square with carbon film
- ❑ ❑ Find Eucentric height using AutoFunctions-AutoEucentric by Beam Tilt
- ❑ ❑ Preview at Hole/EucentricHeight
- ❑ ❑ Move stage to carbon film
- ❑ ❑ Run Autofocus
- ❑ ❑ Under Direct Alignment, center the beam and adjust PP
- ❑ ❑ Autostigmate
- ❑ ❑ Autocoma
- ❑ ❑ Insert objective aperture and center the aperture at diffraction mode
- ❑ ❑ Autostigmate

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Set up an EPU Session

- ❑ ❑ Create an EPU Session
- ❑ ❑ Pick squares for data collection
- ❑ ❑ Select holes on the first square, adjust ice filter and prepare the rest of the squares
- ❑ ❑ Define template (Autofocus area, Acquisition area and defocus)
- ❑ ❑ Check the number of exposures and the estimated data collection time
- ❑ ❑ Select more squares if needed
- ❑ ❑ Start Run

Supervising Staff 1: _____ **Date:** _____

Supervising Staff 2: _____ **Date:** _____

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