

Single Particle CryoEM Screening and Data Collection using SerialEM

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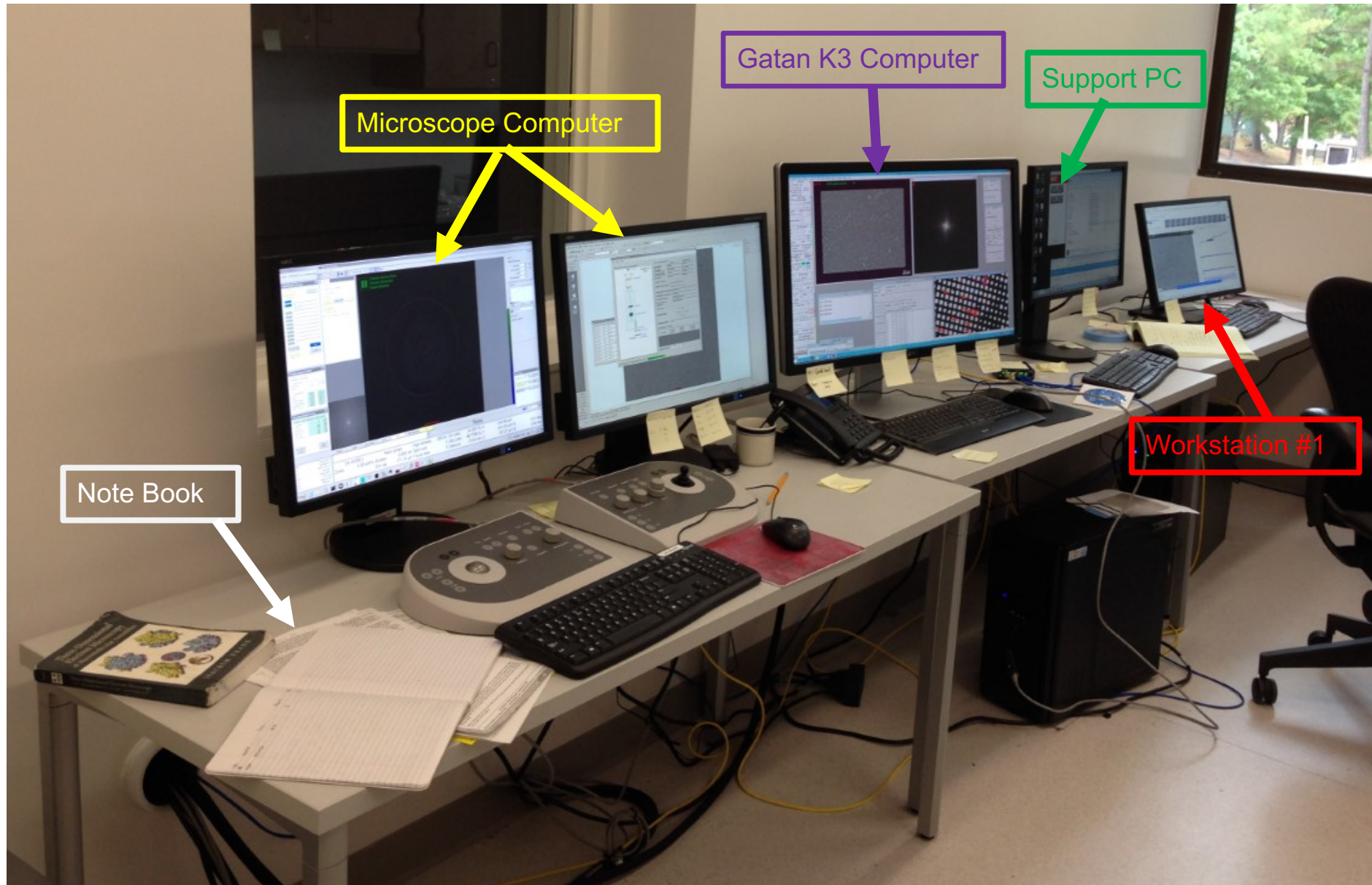
Glaxo Research Building, room 008

<https://www.med.unc.edu/cryo-em/>

919-962-5979

Overview

- Microscope Setup
- SerialEM Background Info
- Screening and Data Collection
- SerialEM Controls
- SerialEM Resources



Microscope Computer

Gatan K3 Computer

Support PC

Note Book

Workstation #1

History of SerialEM

- Developed by David Mastronarde University of Colorado in 2005.
 - <https://bio3d.colorado.edu/SerialEM/serialEMJSB.pdf>
- Proto version was developed for montaging and acquiring tilt series of plastic sections for HVTEM
- Windows
- Open Source
 - <https://bio3d.colorado.edu/SerialEM/OpenSerialEM/>
- Installed on > 500 microscopes



Available online at www.sciencedirect.com

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Journal of Structural Biology 152 (2005) 36–51

Journal of
Structural
Biology

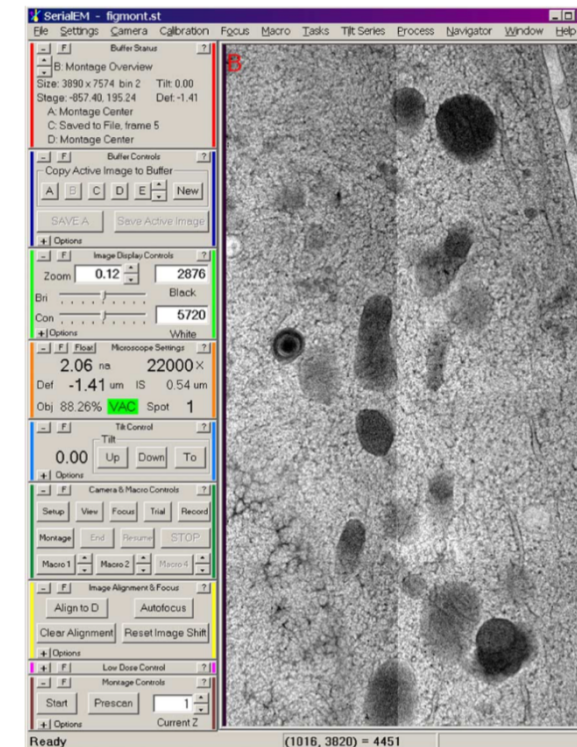
www.elsevier.com/locate/jysbi

Automated electron microscope tomography using robust prediction of specimen movements

David N. Mastronarde*

Boulder Laboratory for Three-Dimensional Electron Microscopy of Cells, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309, USA

Received 5 April 2005; received in revised form 14 July 2005; accepted 20 July 2005
Available online 24 August 2005



Mastronarde 2005

Tomography Data Collection: Cellular Imaging



HHS Public Access

Author manuscript

J Struct Biol. Author manuscript; available in PMC 2020 February 01.

Published in final edited form as:

J Struct Biol. 2019 February 01; 205(2): 163–169. doi:10.1016/j.jsb.2018.12.008.

Rapid Tilt-Series Acquisition for Electron Cryotomography

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^bBeckman Institute, California Institute of Technology, Pasadena, CA 91125

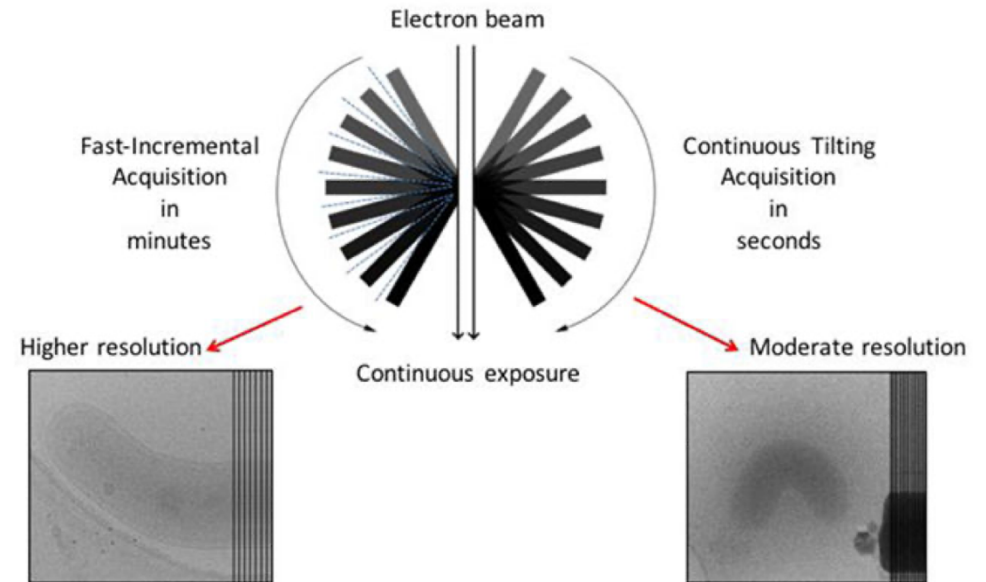
^cHoward Hughes Medical Institute³, California Institute of Technology, Pasadena, CA 91125

[#] These authors contributed equally to this work.

Abstract

Using a new Titan Krios stage equipped with a single-axis holder, we developed two methods to accelerate the collection of tilt-series. We demonstrate a continuous-tilting method that can record a tilt-series in seconds, but with loss of details finer than ~4 nm. We also demonstrate a fast-incremental method that can record a tilt-series several-fold faster than current methods and with similar resolution. We characterize the utility of both methods in real biological electron cryotomography workflows. We identify opportunities for further improvements in hardware and software and speculate on the impact such advances could have on structural biology.

Fast Electron Cryotomography



Chreifi et al., 2019

Correlative light electron microscopy



HHS Public Access

Author manuscript

Nat Protoc. Author manuscript; available in PMC 2018 January 01.

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Nat Protoc. 2017 January ; 12(1): 150–167. doi:10.1038/nprot.2016.168.

Correlated fluorescence microscopy and cryo-electron tomography of virus-infected or transfected mammalian cells

Cheri M Hampton^{1,5}, Joshua D Strauss^{1,5}, Zunlong Ke^{2,5}, Rebecca S Dillard^{1,5}, Jason E Hammonds¹, Eric Alonas³, Tanay M Desai¹, Mariana Marin¹, Rachel E Storms¹, Fredrick Leon¹, Gregory B Melikyan¹, Philip J Santangelo³, Paul W Spearman¹, and Elizabeth R Wright^{1,4}

¹Division of Infectious Diseases, Department of Pediatrics, Emory University School of Medicine, Children's Healthcare of Atlanta, Atlanta, Georgia, USA

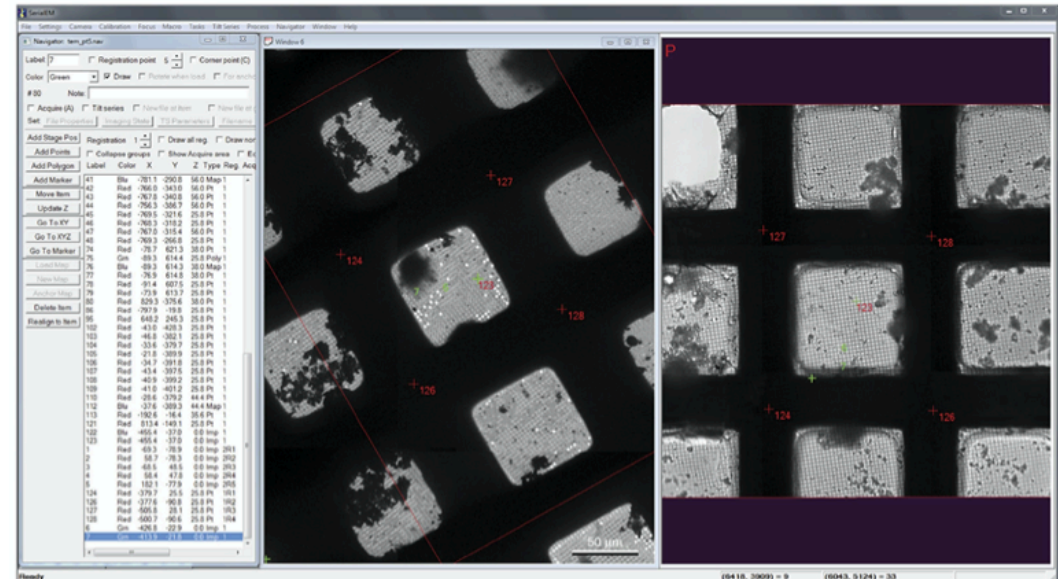
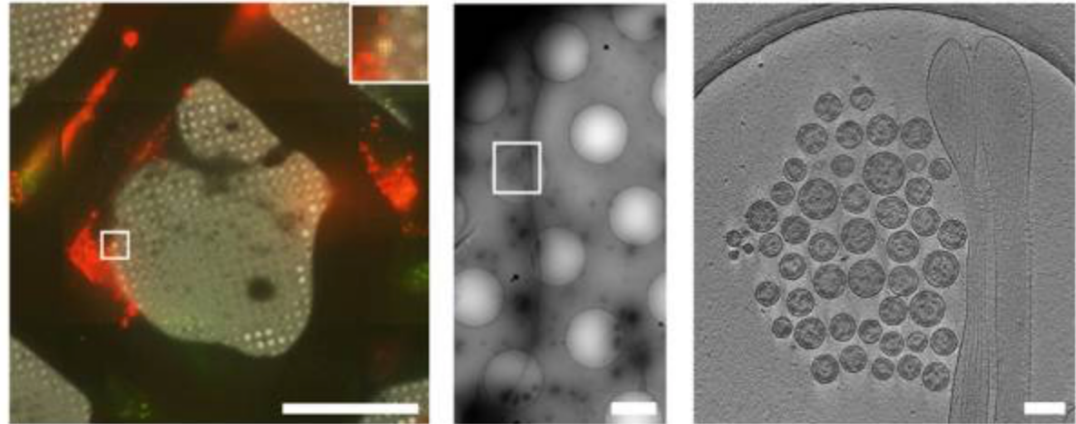
²School of Biology, Georgia Institute of Technology, Atlanta, Georgia, USA

³Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University, Atlanta, Georgia, USA

⁴Robert P. Apkarian Integrated Electron Microscopy Core, Emory University, Atlanta, Georgia, USA

Abstract

Correlative light and electron microscopy (CLEM) combines spatiotemporal information from fluorescence light microscopy (fLM) with high-resolution structural data from cryo-electron tomography (cryo-ET). These technologies provide opportunities to bridge knowledge gaps between cell and structural biology. Here we describe our protocol for correlated cryo-fLM, cryo-electron microscopy (cryo-EM), and cryo-ET (i.e., cryo-CLEM) of virus-infected or transfected mammalian cells. Mammalian-derived cells are cultured on EM substrates, using optimized conditions that ensure that the cells are spread thinly across the substrate and are not physically disrupted. The cells are then screened by fLM and vitrified before acquisition of cryo-fLM and cryo-ET images, which is followed by data processing. A complete session from grid preparation through data collection and processing takes 5–15 d for an individual experienced in cryo-EM.



Tomography Data Collection: Subvolume Averaging

Journal of Structural Biology 197 (2017) 191–198



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Implementation of a cryo-electron tomography tilt-scheme optimized for high resolution subtomogram averaging

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Structural and Computational Biology Unit, European Molecular Biology Laboratory, Meyerhofstrasse 1, Heidelberg, Germany



ARTICLE INFO

Article history:

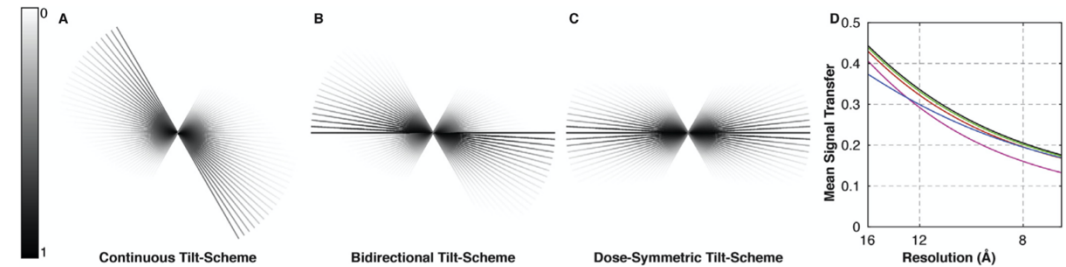
Received 1 April 2016
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Keywords:

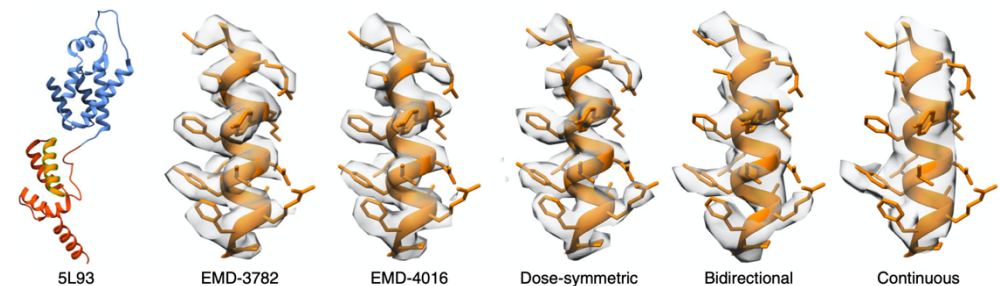
Electron tomography
Tilt-scheme
Subtomogram averaging
Cryo-electron microscopy

ABSTRACT

Cryo-electron tomography (cryoET) allows 3D structural information to be obtained from cells and other biological samples in their close-to-native state. In combination with subtomogram averaging, detailed structures of repeating features can be resolved. CryoET data is collected as a series of images of the sample from different tilt angles; this is performed by physically rotating the sample in the microscope between each image. The angles at which the images are collected, and the order in which they are collected, together are called the tilt-scheme. Here we describe a "dose-symmetric tilt-scheme" that begins at low tilt and then alternates between increasingly positive and negative tilts. This tilt-scheme maximizes the amount of high-resolution information maintained in the tomogram for subsequent subtomogram averaging, and may also be advantageous for other applications. We describe implementation of the tilt-scheme in combination with further data-collection refinements including setting thresholds on acceptable drift and improving focus accuracy. Requirements for microscope set-up are introduced, and a macro is provided which automates the application of the tilt-scheme within SerialEM. © 2016 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).



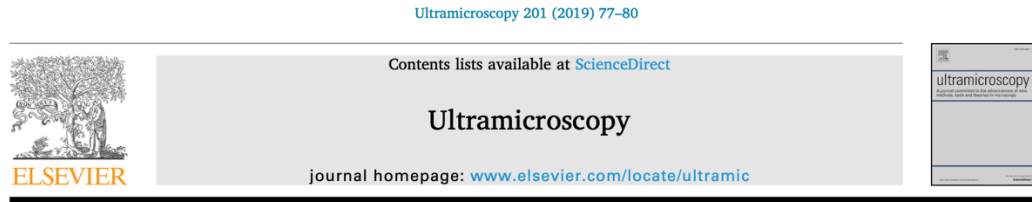
Hagen et al., 2017



Turonova et al., 2020

Hagen et al., 2017

MicroED Data Collection



MicroED data collection with SerialEM

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^c Howard Hughes Medical Institute and Departments of Biological Chemistry and Physiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA 90095, USA

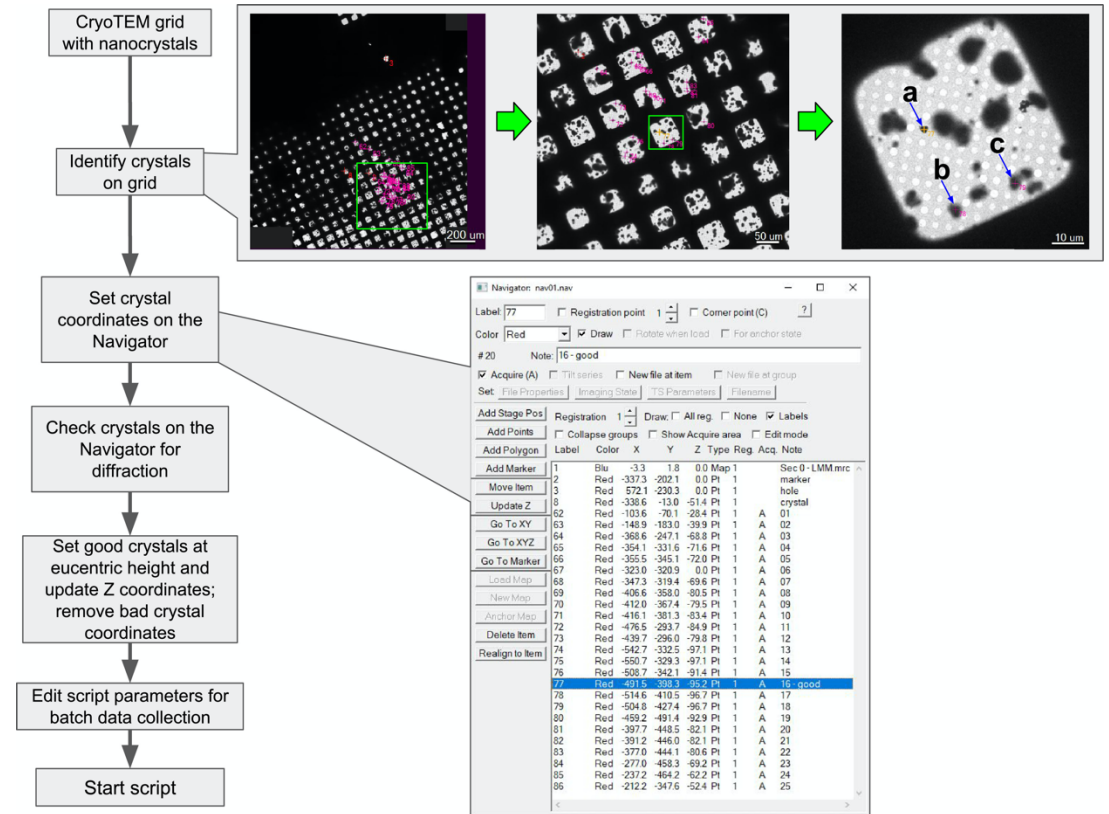
ARTICLE INFO

Keywords:
 MicroED
 Microcrystal
 Electron diffraction
 cryoEM
 Transmission electron microscopy
 SerialEM

ABSTRACT

The cryoEM method Microcrystal Electron Diffraction (MicroED) involves transmission electron microscope (TEM) and electron detector working in synchrony to collect electron diffraction data by continuous rotation. We previously reported several protein, peptide, and small molecule structures by MicroED using manual control of the microscope and detector to collect data. Here we present a procedure to automate this process using a script developed for the popular open-source software package SerialEM. With this approach, SerialEM coordinates stage rotation, microscope operation, and camera functions for automated continuous-rotation MicroED data collection. Depending on crystal and substrate geometry, more than 300 datasets can be collected overnight in this way, facilitating high-throughput MicroED data collection for large-scale data analyses.

Collect diffraction data of micron sized crystals during continuous tilting of the stage.



Cruz et al., 2019



Created by god Hephaestus and given to Minos as a gift. Talos the living robot and guardian of the island Crete, forged from bronze and fueled by ichor...defeated by the sorceress Medea. This is in reference to Greek mythology and is not the same Talos at the cryoEM core

Illustration take from <https://mikemyler.com/2018/10/07/dd-5e-in-ancient-greece-talos/>

Table of Context

- I. Basic information before operating the microscope
- II. Using the Autoloader
- III. Quickly Inspect a grid with the FluScreen
- IV. Using SerialEM, with the Ceta Camera to Screen Cryo-grids
- V. Using SerialEM, with the K3 camera
- VI. Map grids

I. Basic information before operating the microscope

This document is for trained users, contact the CryoEM Core Director Joshua Strauss to schedule a training session before using the microscope.

- Do not contact GTS or fix the problem without supervision.
- Before and after using the TEM fill out the log-book.
- Do not make your own settings files or change the setting files without supervision.
- Do not download or install software on any of the cryoEM core computers unsupervised.
- Do not align the microscope or FEG.
- If you noticed an error or if the alignments are not ideal or the microscope is not working contact Joshua Strauss ASPA. If you think that there is a problem with the microscope chances are you correct.
- Do not transfer data from the microscope computer to a flash-drive.
- If you have any questions please contact Joshua Strauss (Joshua_Strauss@med.unc.edu, 518-708-4234, Glaxo room 008).

Procedure for screening cryo-grids using SerialEM and the Ceta Camera.

In most cases the Ceta Camera is just great for screening, taking a full grid montages, finding areas of the TEM that have thin vitreous ice and collecting high mag images to visualize particle morphology.

This is general guide for screening cryo-grids with the microscope. Don't treat this document as a to do list or list of commandments. Before you do anything with the microscope really think about what you are doing before you do it.

Operation of the UNC Talos Arctica
Created by Dr. Joshua Strauss

Screening and Data Collection using the Gatan K3

Workflow is as follows: (1) Check Microscope Status, (2) Autoloader Inventory, (3) Map to grids, (4) screen/image cryo-grids, and (5) Setup data collection.

The Gatan K3 camera is used mainly for high-resolution data collection, and can also be used to screen cryo-grids. Basically this is the same as using the Ceta Camera, before you start using the K3 make sure you understand how to use SerialEM with the Ceta Camera.

Once you are ready to collect images at high magnification, ask the CryoEM core Staff to help setup the pre-processing pipe-line to assess micrograph quality.

Recommended file structure and saving data from a screening session

Be sure to save the data on the K3 Server X: drive and not the C: drive!!!

Make directory in /K3data/
20191111_JS_TK/"date" "PI initials" "Users Initials"

For each grid make a new directory to save images and navigator
20191111_JS_TK_TK3_G1/

File naming...save as tif or mrc or jpg
20191111_JS_TK_TK3_G1_Montage.mrc Full grid montage
20191111_JS_TK_TK3_G1_Pt2_Search.mrc Pt2 "navigator point 2"
20191111_JS_TK_TK3_G1_Pt2_View.mrc
20191111_JS_TK_TK3_G1_Pt2_Record.mrc

Make sure to save the montages and Navigator files, mdoc files and serialEM log file.

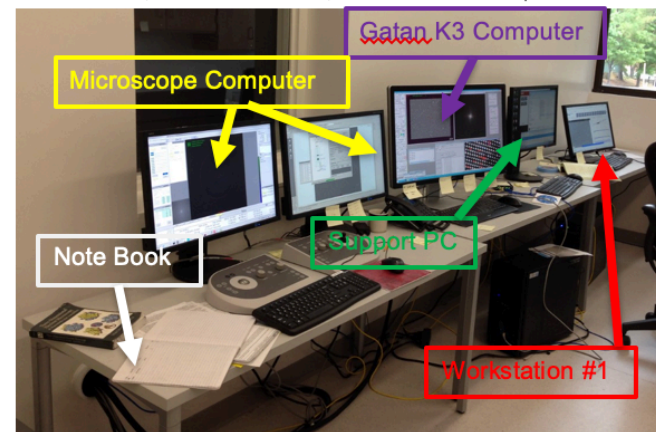
Operation of the UNC Talos Arctica
Created by Dr. Joshua Strauss

Check Microscope Status Before Starting

1. Microscope Computer
 - a. TEM user interface is open
 - b. Microscope status (ie. FEG, Vacuum, Autoloader, Temperature...ect)
 - c. **TEMSEM server** is running (required for SerialEM)
 - d. **Gatan TEM server** is running (required for Gatan K3 camera)
 - e. **Standalone camera** is selected when using the K3, select **pre-specimen shutter**
 - f. The Column Valves should be closed
2. Gatan K3 Computer
 - a. **Digital Micrograph** is open and the Gatan K3 Camera is active
 - b. **SerialEM** is on
 - i. load the settings file 20200421_LD_K3 (should be loaded)
 - ii. To do this select Settings → Open
 - iii. The settings are important and if you change them some of the scripts may not work. If you need to change or modify the SerialEM settings check with the CryoEM Core Director Dr. Joshua Strauss and write down the changes in the Microscope Notebook.
3. Support PC: Should be running, other than that don't worry about this computer
4. Workstation #1: Used to pre-process data, don't worry about this computer

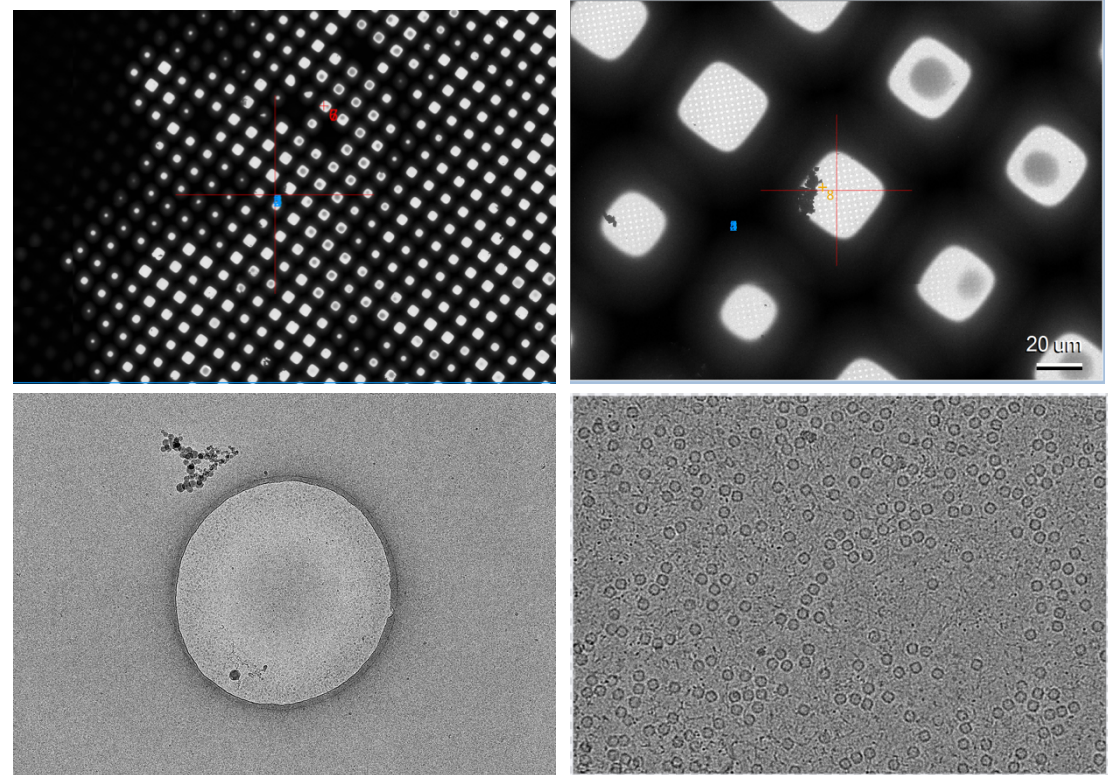
Enter your information into the microscope note book

Enter the date, your name, PI, number of grids loaded, microscope current, start and end time, if data was collected, or other information, if there was microscope error.



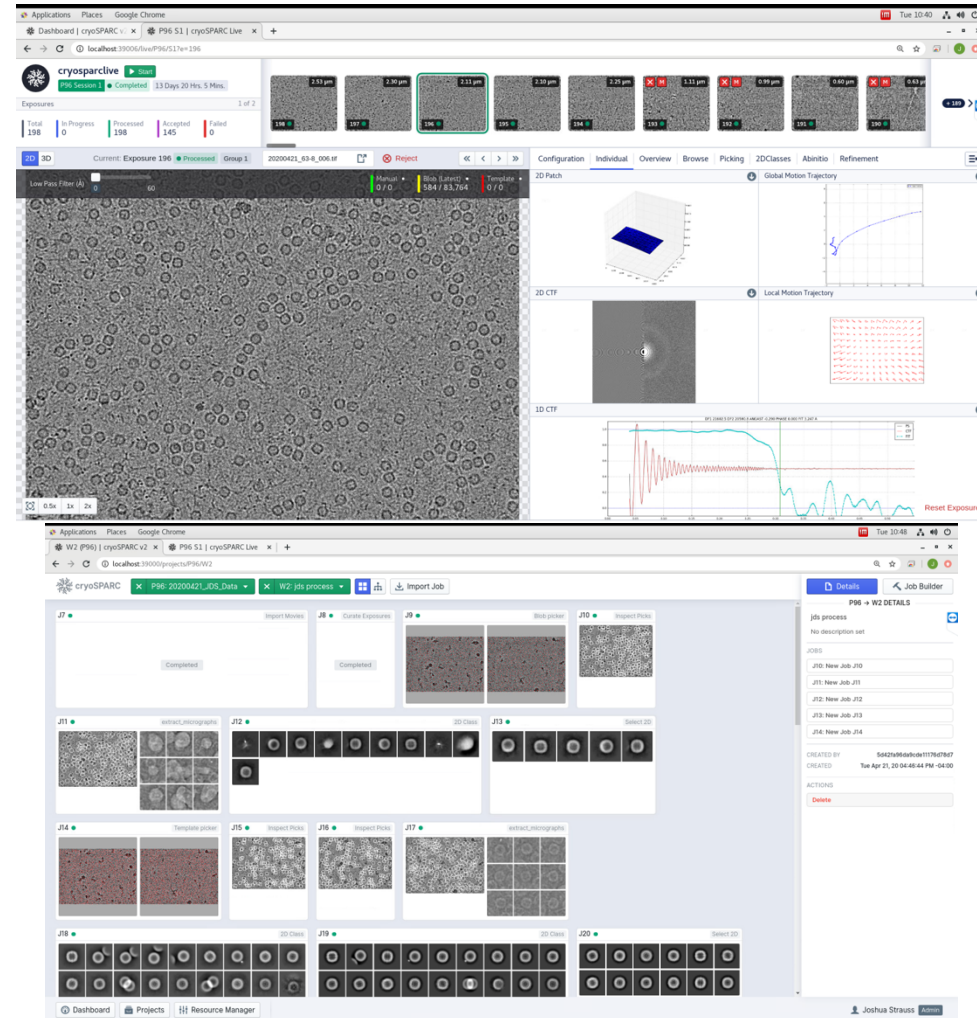
Multiple Levels of Screening and Data Collection

- Do the grids have vitreous ice?
- Overall grid quality, how many good grid squares ?
- Are there particles in the ice ?
- What is the particle morphology, size, and distribution?
- How thin is the ice?
- Micrograph quality ?
- Do the 2D class averages have 2nd structure ?
- What is the 3D structure ?



Multiple Levels of Screening and Data Collection: Processing

- Do the grids have vitreous ice ?
- Overall (low-mag) grid quality, how many good grid squares ?
- Are there particles in the ice ?
- What is the particle morphology, size, and distribution?
- How thin is the ice?
- Micrograph quality ?
- Do the 2D class averages have 2nd structure ?
- What is the 3D structure ?



P: Saved to File, sec. 0
 Size: 1440 x 1022 bin 4 Tilt: 0.00
 Stage: 44.59, -41.81 Def: 0
 A: Saved to File, sec. 0
 B: UNSAVED, Search

Buffer Controls
 Copy Active Image to Buffer
 A B C D P New

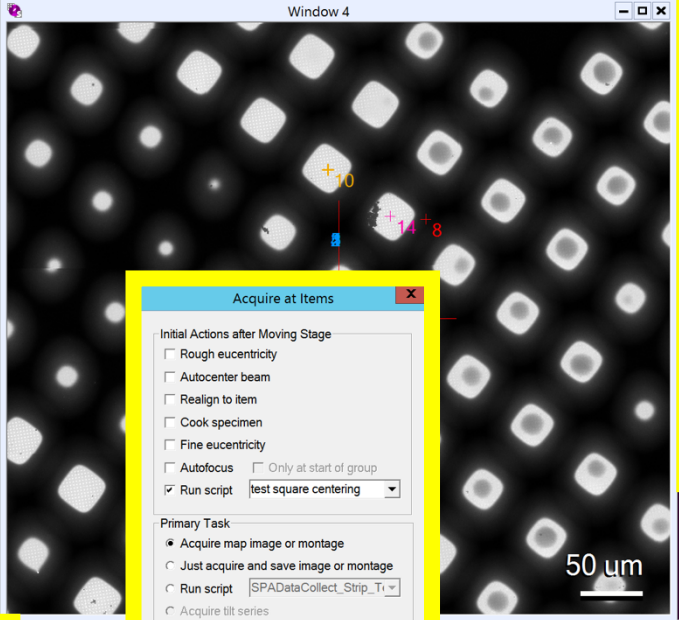
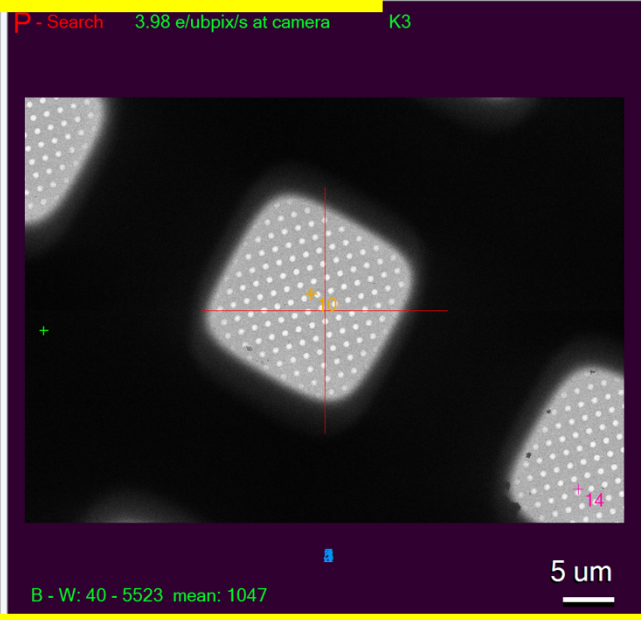
SAVE A Save Active To file 1
 Options Memory = 429 MB

Roll Buffers A -> M Delete
 Align to B instead of N/O

Read into Buffer O
 Protect unsaved Record images

Image Display Controls
 Blk 40
 Wht 5523
 Bri
 Con
 Options Zoom 0.69
 Set Truncation Set Area Fraction
 Extra info Autozoom
 Antialias filtering for zoom < 1

Microscope
 -0.0000 nA 210 LM
 Def -14.91 mm IS 0.00 um
 C2 100.18% VAC Spot 5
 Stage X: 44.59 Y: -41.81
 Obj 6.00% Z: 72.49



Acquire at Items

Initial Actions after Moving Stage
 Rough eucentricity
 Autocenter beam
 Realign to item
 Cook specimen
 Fine eucentricity
 Autofocus Only at start of group
 Run script test square centering

Primary Task
 Acquire map image or montage
 Just acquire and save image or montage
 Run script SPADDataCollect_Strip_Ti
 Acquire tilt series

Run script after: BTvsIS

Montages will be saved into the file:
 squares.mrc

Do subset from index 1 to 14
 Skip initial stage move to item
 Skip Z moves in initial move and Realign
 Restore scope state after aligning to item
 Close column valves at end
 Send email at end

GO Postpone Cancel ?

Low Dose Control

Low Dose Mode
 Search: 210x Sp 5 C2 100.18%
 Continuous update (see tooltip)
 Define position of area
 None Focus Trial
 Position on tilt axis: -2.00 um
 Maximum area separation: 1.29 um
 Go to: Vie Foc Tri Rec. **Sea**
 Additional beam shift (and DF tilt)
 Set Reset Uncalibrated

Offsets for: View Search
 Defocus: -10 Shift: Set Zero

Blanked Unblank
 BLANK BEAM when screen down
 Normalize beam through View
 Keep Focus and Trial identical
 Copy current area settings to
 V F T R S
 Center Unshifted Balance Shifts
 Rotate inter-area axis 66 deg

Montage Controls

Start Prescan 0
 Options Current Z
 Bin: Overview 1 Prescan 4
 Correct drift
 Change focus with height
 Show overview at end
 Align pieces in overview
 Treat as very sloppy montage

Log

Rough eucentricity: changing Z by -4.58 to 72.49, finished.
 Measured defocus = -0.17 microns changed by -1.83 to target drift = 0.05 nm/sec
 Measured defocus = -2.30 microns changed by 0.30 to target drift = 0.03 nm/sec
 Ctfind: defocus: -13.847, astig: 0.256 um, angle: -36.8, score 0.0341
 Measured defocus = -1.99 microns changed by -0.01 to target drift = 0.05 nm/sec
 Ctfind: defocus: -1.622, astig: 0.060 um, angle: -3.2, score 0.3314
 Frame alignment results: distance raw = 18.6 smoothed = 7.4
 Weighted resid mean = 0.28 max max = 0.75 Mean unweighted max = 1.55
 Ctfind: defocus: -2.522, astig: 0.025 um, angle: -16.5, score 0.2294
 Frame alignment results: distance raw = 27.3 smoothed = 9.5
 Weighted resid mean = 0.45 max max = 1.48 Mean unweighted max = 2.57
 Valves are now OPEN
 Min = 0.0, max = 6583.0, mean = 865.53, SD = 1356.18 Pixel size = 40.4 nm
 2.511 electrons (103.64 counts) per unbinned pixel per second
 Min = 0.0, max = 6700.0, mean = 1046.08, SD = 1464.48 Pixel size = 40.4 nm
 3.983 electrons (125.26 counts) per unbinned pixel per second

Navigator.nav

3	Blu	-2.4	-45.2	-0.0	Map	1
4	Blu	-2.4	-45.2	-0.0	Map	1
5	Blu	-2.4	-45.2	-0.0	Map	1
6	Red	263.4	-162.9	77.1	Pt	1
7	Red	263.0	-162.9	77.1	Pt	1
8	Red	11.8	-118.8	77.1	Pt	1
9	Red	-204.7	266.6	-0.0	Pt	1
10	Red	47.9	-39.1	72.5	Pt	1 A collect
11	Red	-203.5	46.9	72.5	Pt	1 A collect
12	Red	-182.0	42.7	72.5	Pt	1 A collect
13	Red	-67.3	-108.2	72.5	Pt	1 A collect
14	Red	12.4	-90.6	72.5	Pt	1 A collect

Script 9: test square centering

Editor Up Script Down

Tab or ` to complete command
 MacroName test square centering
 # macro to test square centering
 # Buffer P is expected to have reference hole image from Low Dose View
 # Wim Hagen, EMBL Heidelberg 20170309
 # Credits to Chen Xu
 # JDS and JF 20191230

maxholeshift = 2000 # hole-shift criterion in nanometers to take a second round

Echo ==> Running align to square...

Search
 AlignTo P
 ReportAlignShift
 Echo hole shift 1 \$RepVal5 \$RepVal6
 holeshift = sqrt \$RepVal5 * \$RepVal5 + \$RepVal6 * \$RepVal6

if \$holeshift > \$maxholeshift
 ResetImageShift
 Search

Run Cancel OK ?
 Find Load Save Save As

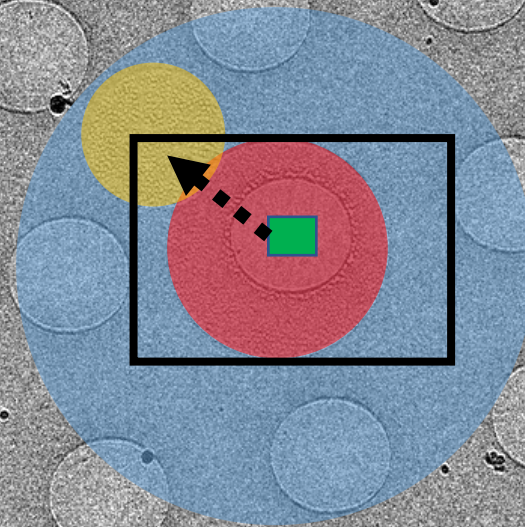
Low-Dose Imaging

Purpose of Low-Dose is to move the Focus (and/or Trial) area away from the Record area so you don't damage sample

Focus Beam

Record Beam

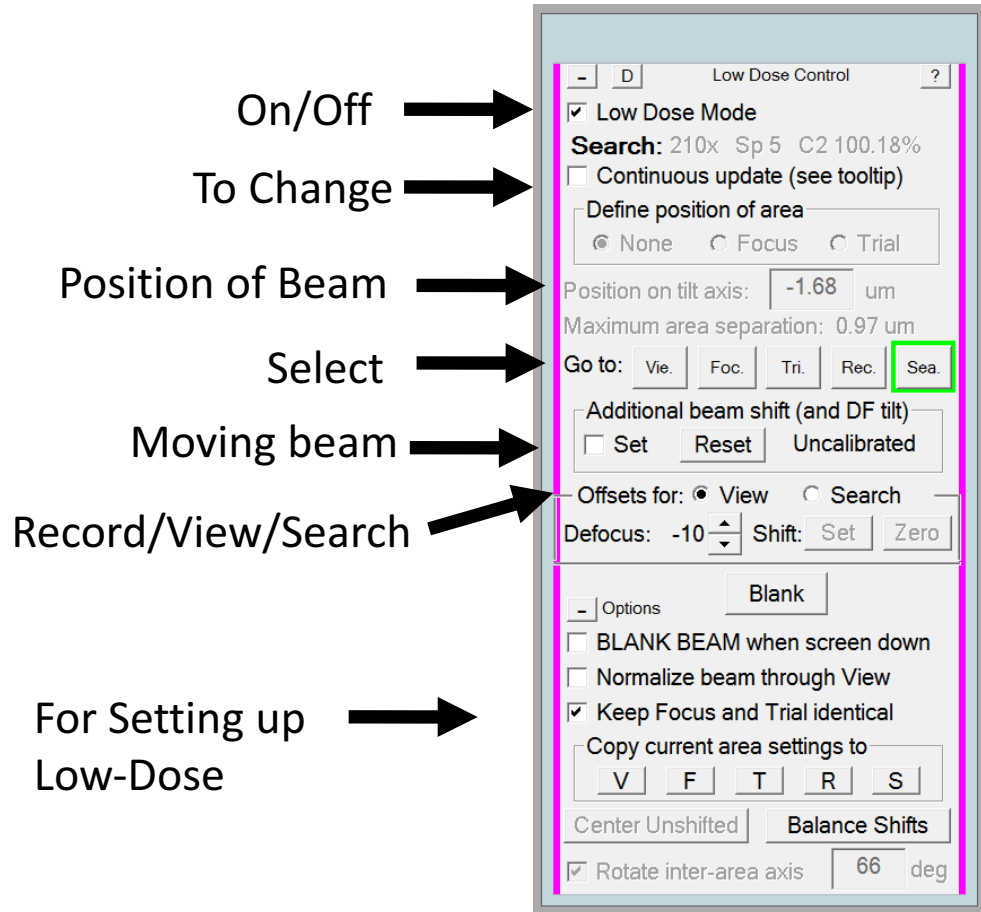
View Beam



Scale Bar 1 μm



Low-Dose Control



Focus (and/or Trial) away from the Record area so you don't damage sample!!!

Setup 5 different microscope conditions (Mag, Spt size, probe, lenses strength...)

View: 8,500 X Mag for eucentric height and centering stage/image shift

Focus: 45,000 X Mag for Autofocus

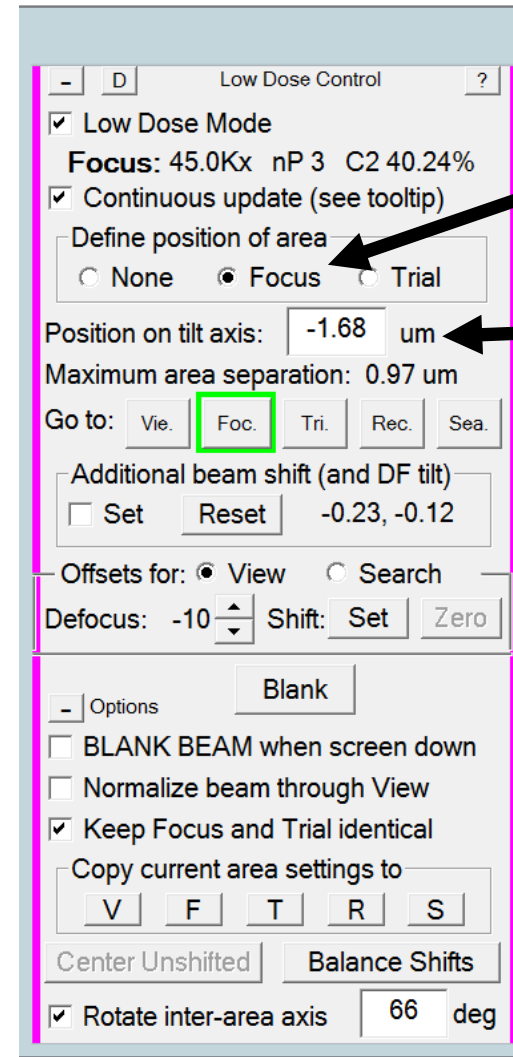
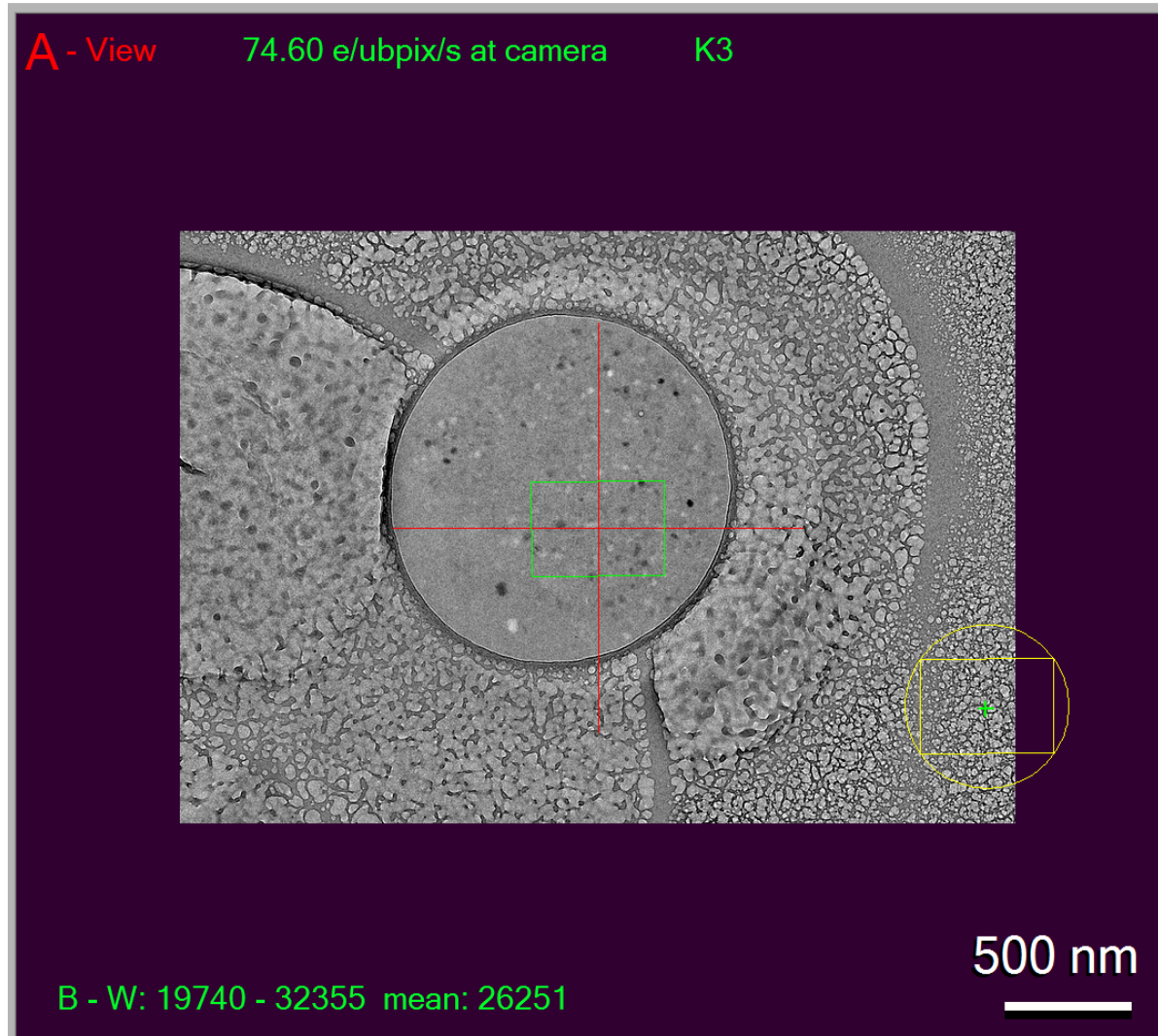
Trial: 45,000 X Drift measurement

Record: 45,000 X Collect data

Search: 62-210 X Mag for montaging

Offsets: defocus, beam shift and beam tilt

Low-Dose Control: Adjust position of Focus Area



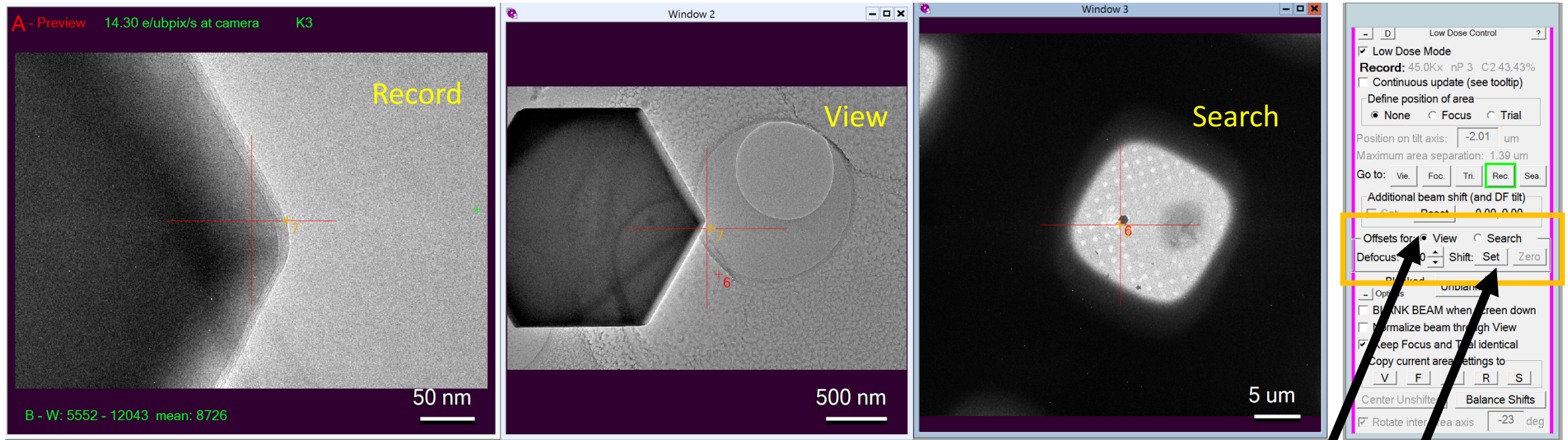
Click this

Adjust

Adjust

Additional Image Shift

Add image shift relative to Record for View and Search so all modes are centered

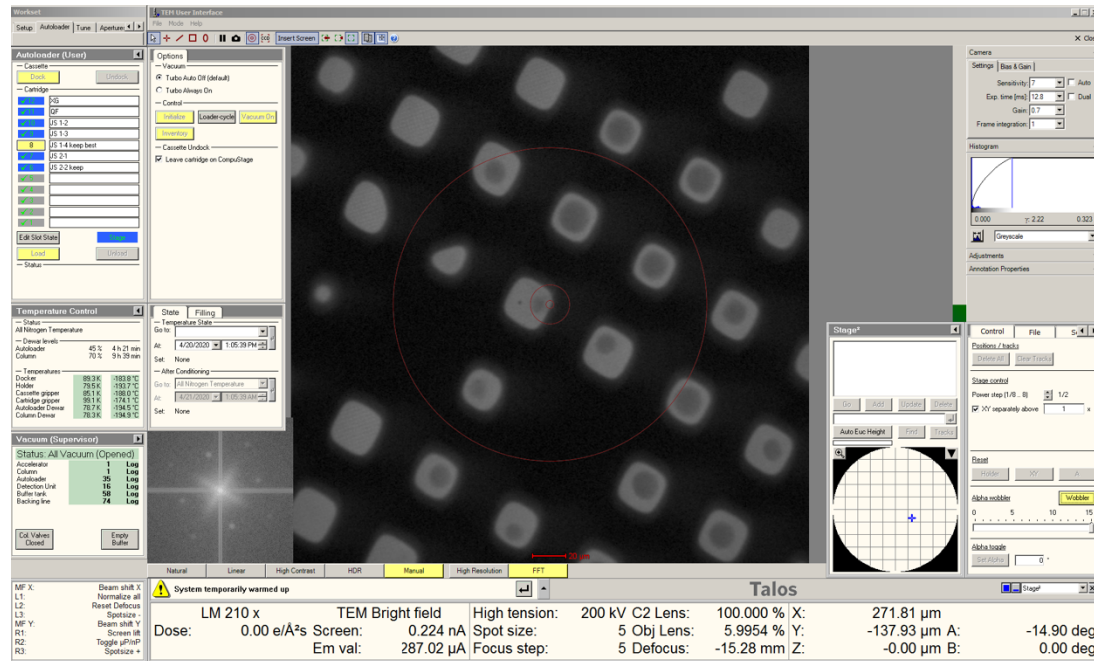


Check View or Search

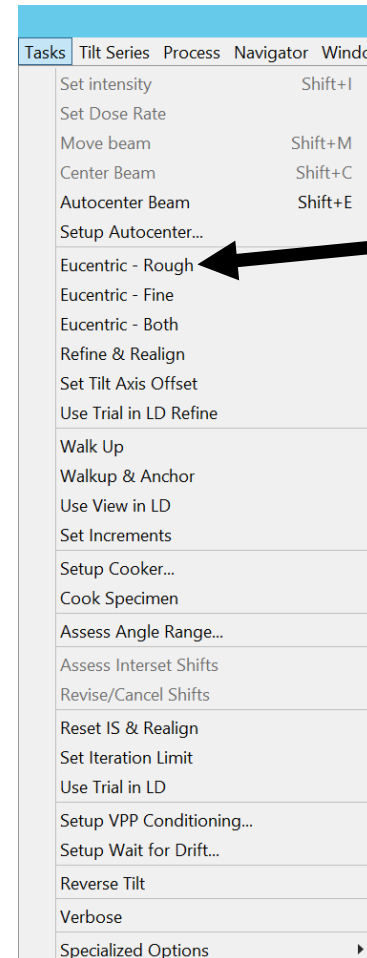
Take image then click and drag

Eucentric Height

Set using TEM FluCam TEM User Interface

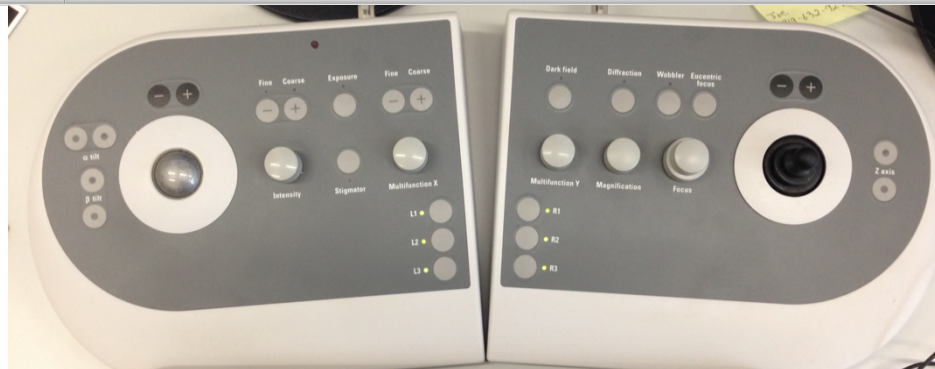


SerialEM



Eucentric Rough

Find Z height by stage tilting
Accurate 5-10 μ m
Best if "near" eucentric < 50 μ m.



Shift to Marker



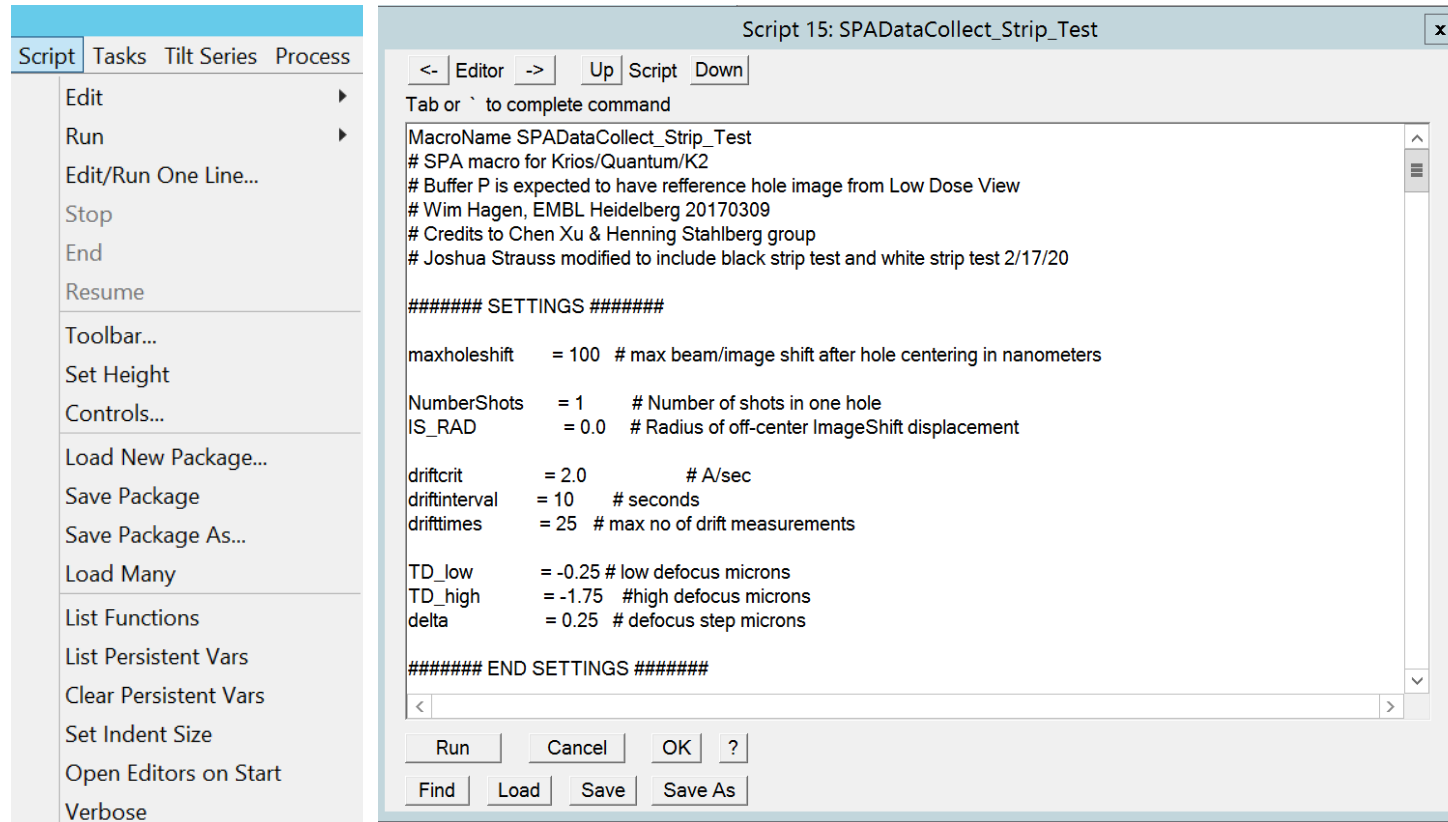
The screenshot shows the SerialEM software interface. The main window displays a grayscale image of a specimen with a red crosshair registration point and a yellow marker labeled '6'. The image has a 5 μm scale bar. The top status bar shows 'A - Search 3.15 e/ubpix/s at camera K3'. The left sidebar contains various control panels: Buffer Status, Buffer Controls, Image Display Controls, Microscope Control, Tilt Control, and Image Alignment & Focus. The bottom right shows the Navigator window with a table of items.

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Blu	-7.5	-3.0	0.0	Map 1			Sec 0 - montage.mrc JS2-2 few squares -- Keep
2	Blu	-7.5	-3.0	-0.0	Map 1			Sec 1 - montage.mrc JS 2-1 too thick
3	Blu	-7.5	-3.0	-0.0	Map 1			Sec 2 - montage.mrc JS1-4 best QF multiple squares -- Keep
4	Blu	-7.5	-3.0	-0.0	Map 1			Sec 3 - montage.mrc JS1-3 not good few squares
5	Blu	-7.5	-3.0	-0.0	Map 1			Sec 4 - montage.mrc JS1-2 too thick
6	Red	258.4	-120.7	-0.0	Pt 1			ice marker

The screenshot shows the SerialEM software menu. The 'Shift to Marker' option is highlighted with a blue arrow. The menu items are:

- Open
- Read & Open...
- Merge File...
- Save
- Save As...
- Close
- Montaging & Grids
- Options
- Transform Items
- Undo Transformation
- Change Registration
- Shift to Marker
- Undo Last Shift
- Align with Rotation...
- New Map from Image
- Import Map...
- Rotate Map
- Adjust for Backlash
- Backlash Settings...
- Open Imaging States ...
- Set Map Acquire State
- Restore State
- Acquire at Items...
- End Acquire
- List Files/Series/States
- Delete Item
- Realign to Item
- Force Center Align
- Try Scaling in Realign

SerialEM Scripts



The screenshot shows the SerialEM software interface. On the left is a sidebar menu with options: Script, Tasks, Tilt Series, Process, Edit, Run, Edit/Run One Line..., Stop, End, Resume, Toolbar..., Set Height, Controls..., Load New Package..., Save Package, Save Package As..., Load Many, List Functions, List Persistent Vars, Clear Persistent Vars, Set Indent Size, Open Editors on Start, and Verbose. The main window is titled "Script 15: SPADDataCollect_Strip_Test" and contains the following script content:

```
MacroName SPADDataCollect_Strip_Test
# SPA macro for Krios/Quantum/K2
# Buffer P is expected to have reference hole image from Low Dose View
# Wim Hagen, EMBL Heidelberg 20170309
# Credits to Chen Xu & Henning Stahlberg group
# Joshua Strauss modified to include black strip test and white strip test 2/17/20

##### SETTINGS #####

maxholeshift = 100 # max beam/image shift after hole centering in nanometers

NumberShots = 1 # Number of shots in one hole
IS_RAD = 0.0 # Radius of off-center ImageShift displacement

driftcrit = 2.0 # A/sec
driftinterval = 10 # seconds
drifttimes = 25 # max no of drift measurements

TD_low = -0.25 # low defocus microns
TD_high = -1.75 #high defocus microns
delta = 0.25 # defocus step microns

##### END SETTINGS #####
```

At the bottom of the script editor window are buttons for Run, Cancel, OK, ?, Find, Load, Save, and Save As.



The screenshot shows the SerialEM Script Repository website. The header is purple with the text "The SerialEM Script Repository". Below the header are navigation links: "SCRIPTS BY CATEGORY", "SCRIPTS BY AUTHOR", "LINKS", and "LOGIN/REGISTER". The main content area has a grey background and features a purple and white logo on the left. The text reads:

Welcome to the SerialEM Script Repository

SerialEM (Mastrorarde, 2005) is a software developed at the The Boulder Laboratory for 3D Electron Microscopy that can acquire a variety of data from transmission electron microscopes: tilt series for electron tomography, large image areas for 3D reconstruction from serial sections, and images for reconstruction of macromolecules by single-particle methods. It interfaces with the FEI Tecnai, Titan, and Talos platforms, JEOL TEM with the TEMCON or TEMCENTER user interface, and the Hitachi HT-7700/7800, as well as detectors from numerous manufacturers.

SerialEM also features a powerful scripting language (until version 3.6.0 in 2016/17: 'macros') allowing to control various microscope parameters, acquire, process and save images, and call higher level functions of SerialEM such as autofocus, alignment to reference images, or automatic adjustment of intensity. For tasks of higher complexity, SerialEM scripts can include control structures such as conditional expressions, loops, and functions.

This website was set up in collaboration with the developer of SerialEM to serve as a central sharing point for scripts/macros, to make them easily accessible in a well organised and documented way, and to stimulate exchange between SerialEM users. To access the scripts available via the repository, starting at a categorised list, no registration is required. To upload new scripts or to edit your own existing scripts, login or register.

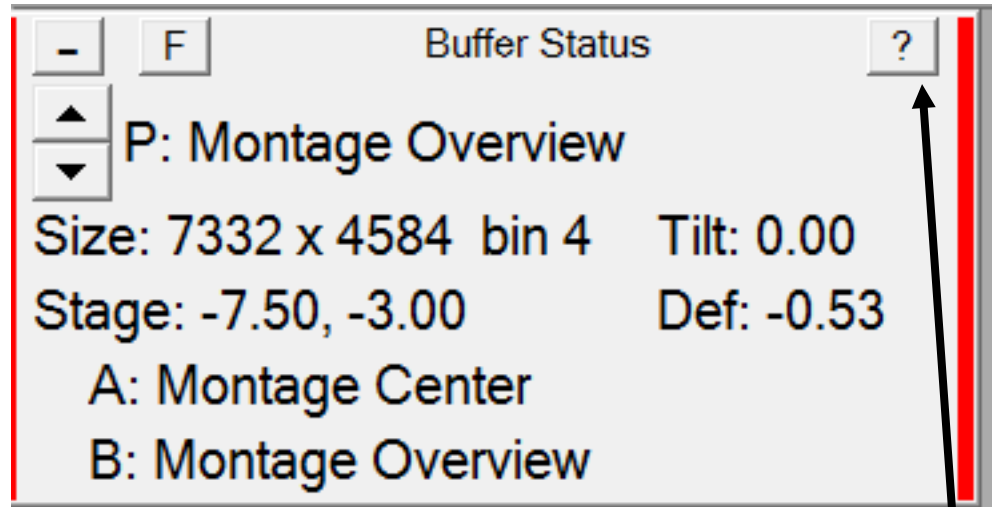
The SerialEM Script Repository is operated by Nexperion – Solutions for Electron Microscopy, a solutions provider in the field of EM located in Vienna, Austria, who also provides SerialEM installations, calibrations, and trainings worldwide as a service.

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Powered by Nexperion – Solutions for Electron Microscopy (Imprint)
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For feedback, contact nexperion@nexperion.net
Visitor statistics is collected, no data is shared with third parties.

 nexperion

<https://serialscripts.nexperion.net>

Buffer Status

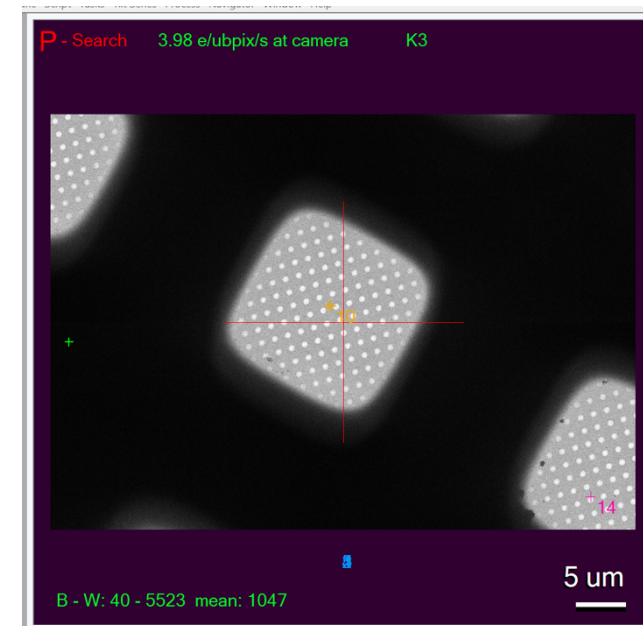


Buffer Status Panel

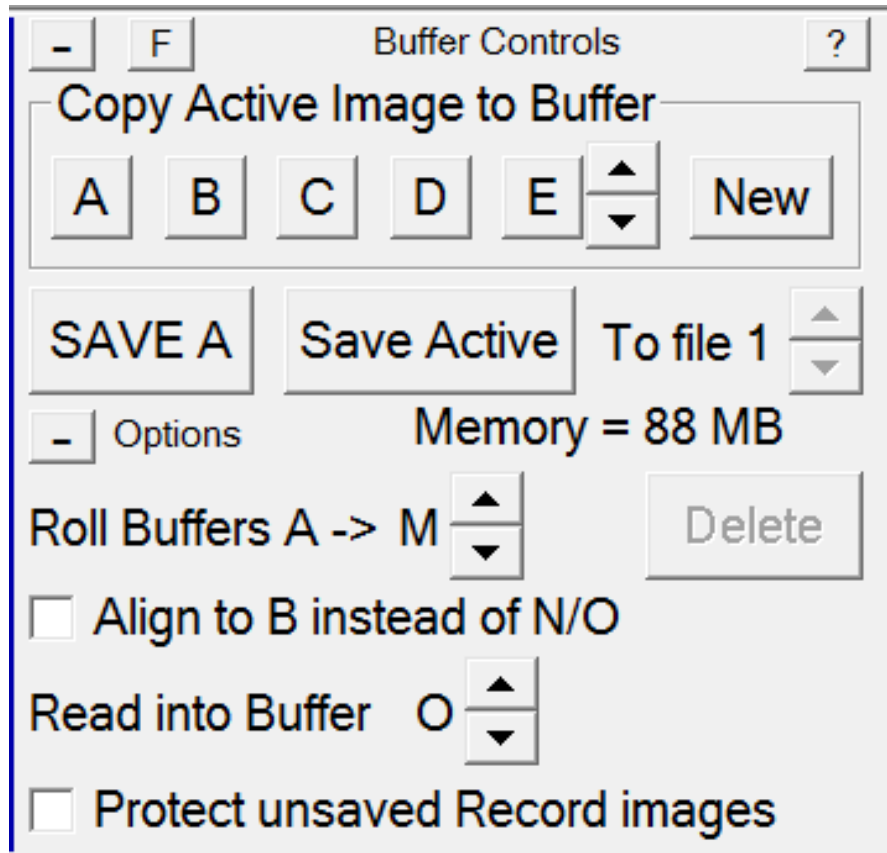
This panel shows detailed information about the image buffer currently shown in the main display window, and summary information about several other buffers. For an ordinary image acquired by the user, the summary line for a buffer will show what parameter set was used to acquire the image and whether it is saved to file or not. Other descriptive terms will appear when the program has acquired an image for calibration or tracking, when an image has been processed, or when the image was composed by the montage acquisition routine.

Shows image buffer information

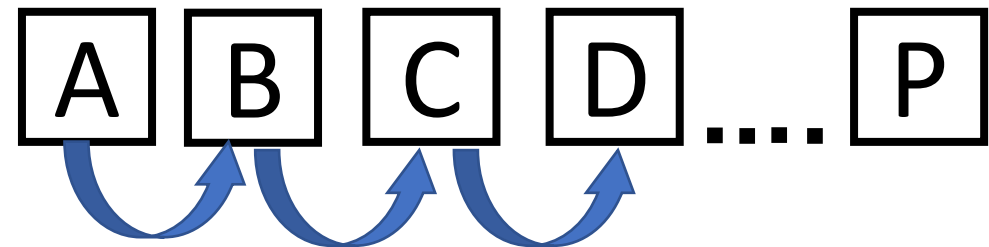
Current buffer spin button:
Display different buffers



Buffer Controls



- Copy images from one buffer to another
 - **Copy Active Image to Buffer**
- Saving images to file
 - **Save A**
 - **Save Active**
- Buffer settings
 - **Roll Buffer**
 - **Read into Buffer**



Window

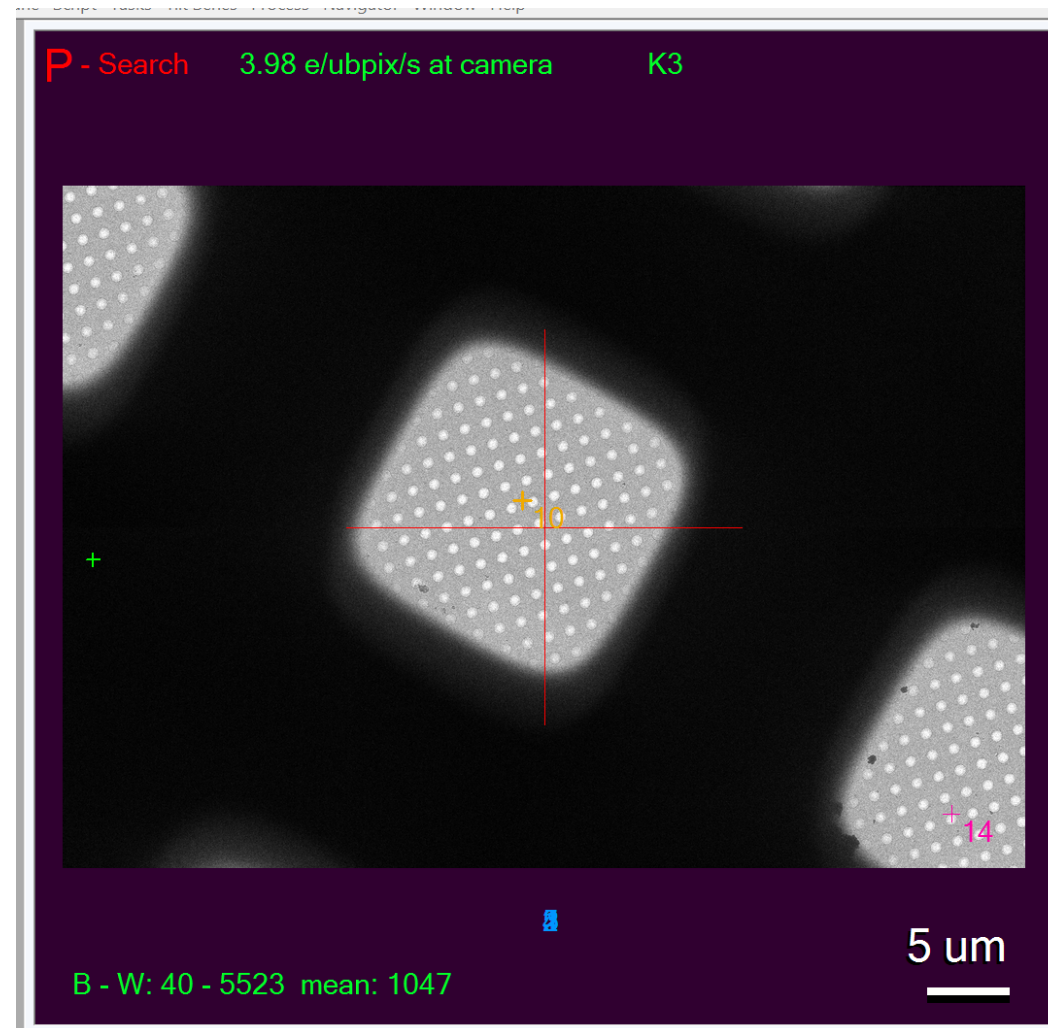
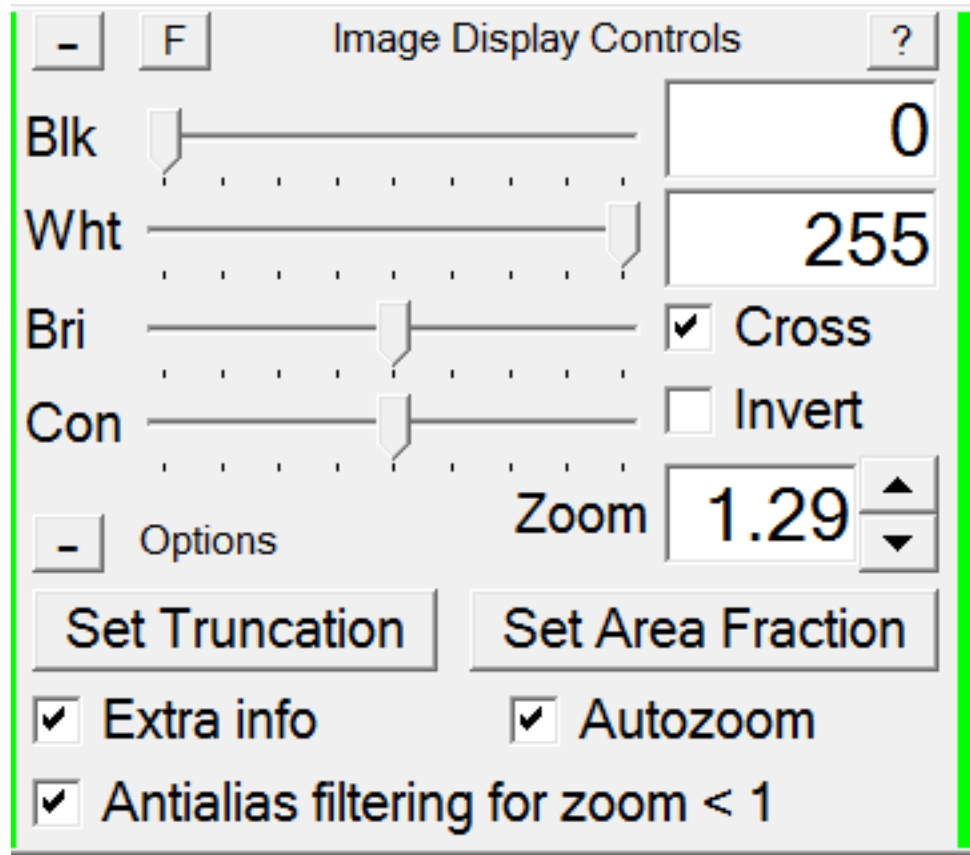


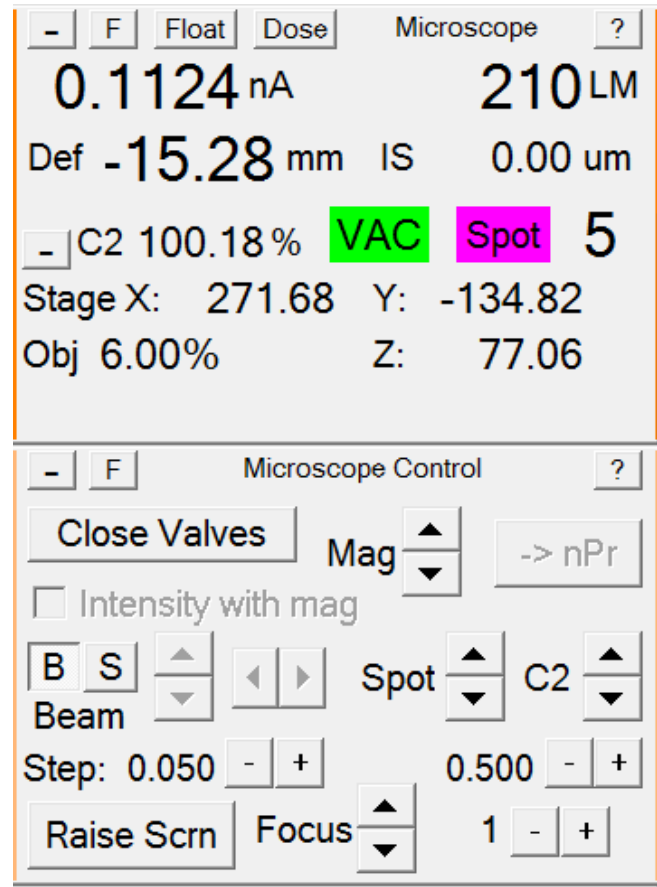
Image Display Controls



Controls how images are displayed

- Black and white levels
- Brightness and Contrast levels
- Cross
- Zoom
 - Middle mouse button

Microscope and Microscope Control



Microscope:

Display microscope status and imaging conditions

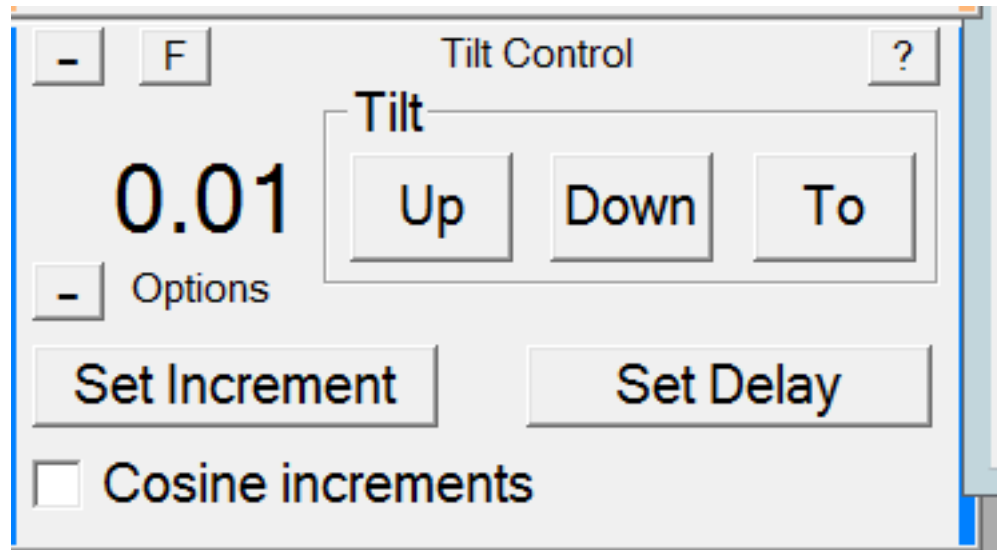
Microscope Control:

Allows you to control microscope and imaging conditions

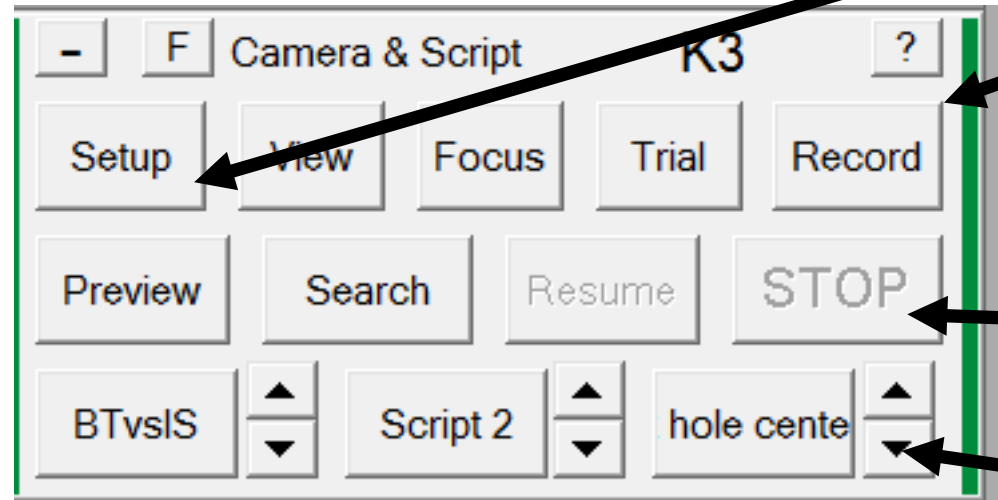
Tilt Control

Displays tilt angle of stage

Tilt the stage +/- 70



Camera & Script



Setup camera parameters

Acquire images for View, Focus, Trial, Record, Preview, and Search

Resume/Stop scripts

Select and Run different scripts

Camera Setup

Camera Parameters -- K3

Camera -- Selects active camera
 Ceta K3
 Match pixel size Match intensity when changing camera

Parameter set
 Search View Focus Trial Record Preview Mont-map

Parameters for **Record** Copy from: Ceta

Acquisition
 Continuous
 Single Image

Processing
 Unprocessed
 Dark Subtracted
 Gain Normalized

Sum will be gain-normalized

Binning
 0.5
 1
 2
 3
 4
 5
 6
 8

Positioning
Top
Left
Bottom
Right
Recenter
Swap X & Y

Exposure time sec
Drift settling sec
Minimum 0.0007 if not 0.0

Binned size: 5760 x 4092
0.52 x 0.37 um @ 0.091 nm

Area size
Quarter Half Full
Wide Quarter Wide Half
10% Less 10% More Square

Dose: Not calibrated Update Dose rate: Not calibrated

Operating mode
 Linear
 Counting

Dose Fractionation mode
Frame time sec
 Save frames
60 raw to TIF-LZW, binned

Align frames Set Up
Align in Plugin with "4K default set" Set File Options Set Folder

Use correlated double sampling (CDS) **Anti-aliasing**
 Bin counting frames by 2 in hardware Save variable frame sums Set Up

OK Acquire Cancel ?

Frame File Options

File type
 MRC file Use extension .mrcs
 TIFF (LZW compression)
 TIFF (ZIP compression)

Current settings will save raw to TIF-LZW, binned

Save one frame per file
 Save mdoc file for each frame stack

Save unnormalized frames even if Gain Normalized is selected
 Reduce normalized super-resolution frames by 2 with antialiasing

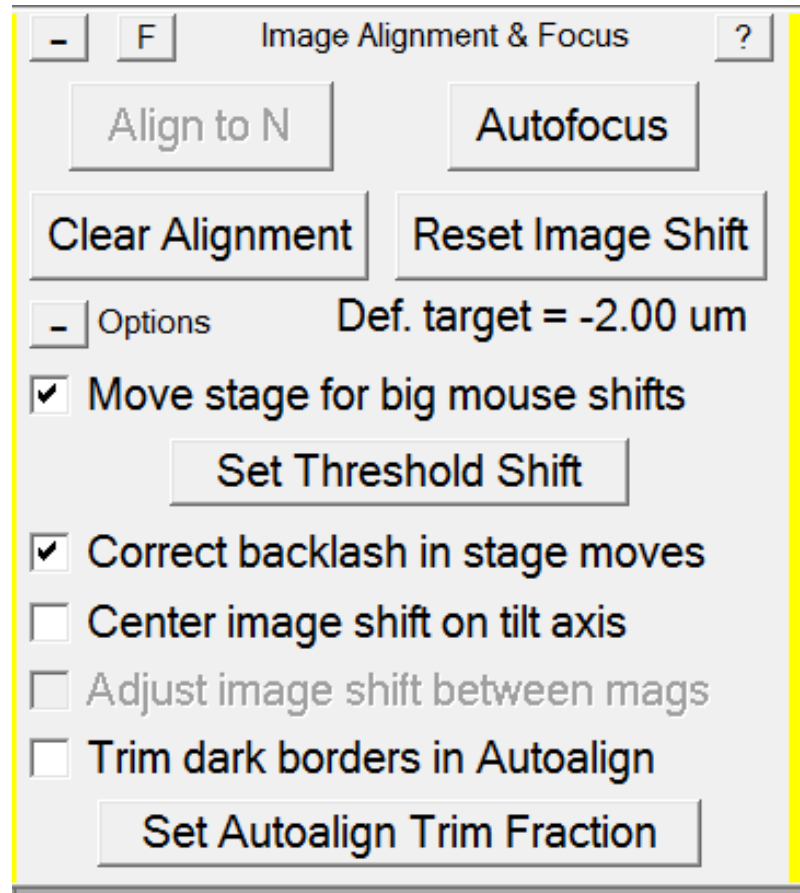
Pack unnormalized data as 4-bit
 Pack unnormalized binned by 2 data as 4-bit, not 8-bit
 Use non-standard mode 101 for 4-bit MRC files
 Save frames without rotation/flip to standard orientation

Components for Folder and File Names
Use component in name of:
Folder File
 Base name:
 File name of current open file (minus extension)
 Label of Navigator item Only when Acquiring at Items
 Sequential number starting from: Digits: 3
 Tilt angle
 Month and day
 Hour, minute, second
 Numeric date (including year) and time at start of name
 Multiple Record hole number and position in hole

Format: Base_Label_###

OK Cancel ?

Image Alignment & Focus



Autofocus: will take focus images (different beam tilts) to set the defocus to desired target. To Set target Menu: Focus → Set Target

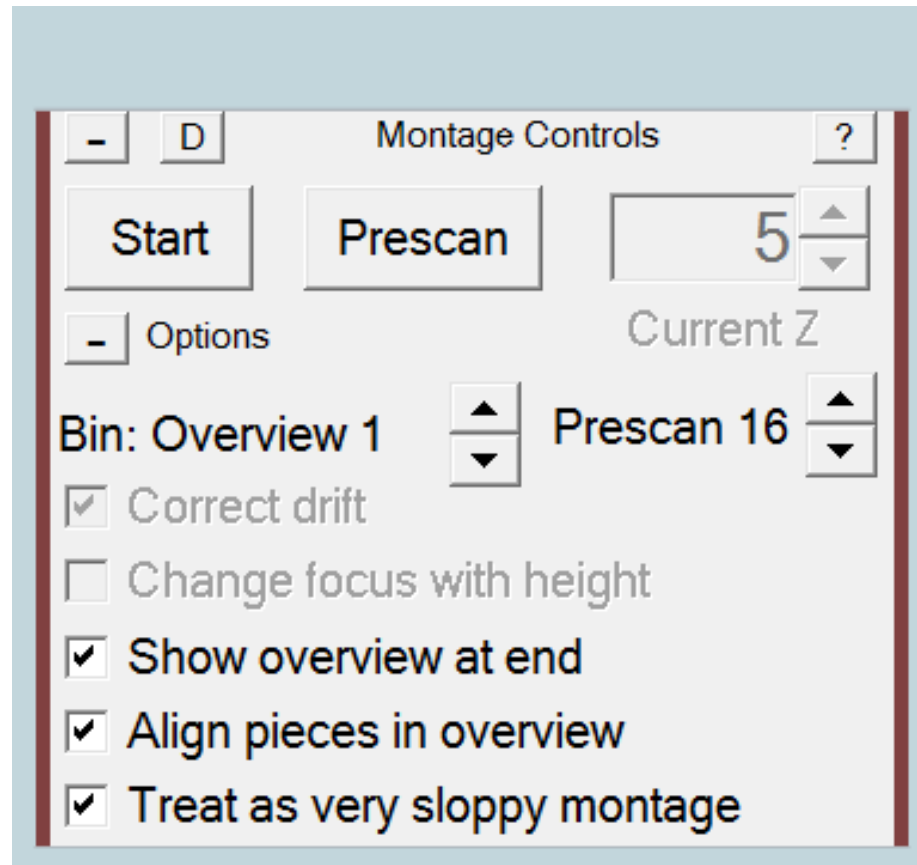
Defocus Target

Move stage for big mouse shift /

Set Threshold Shift

Reset Image Shift

Montage Controls



Start: Start montaging

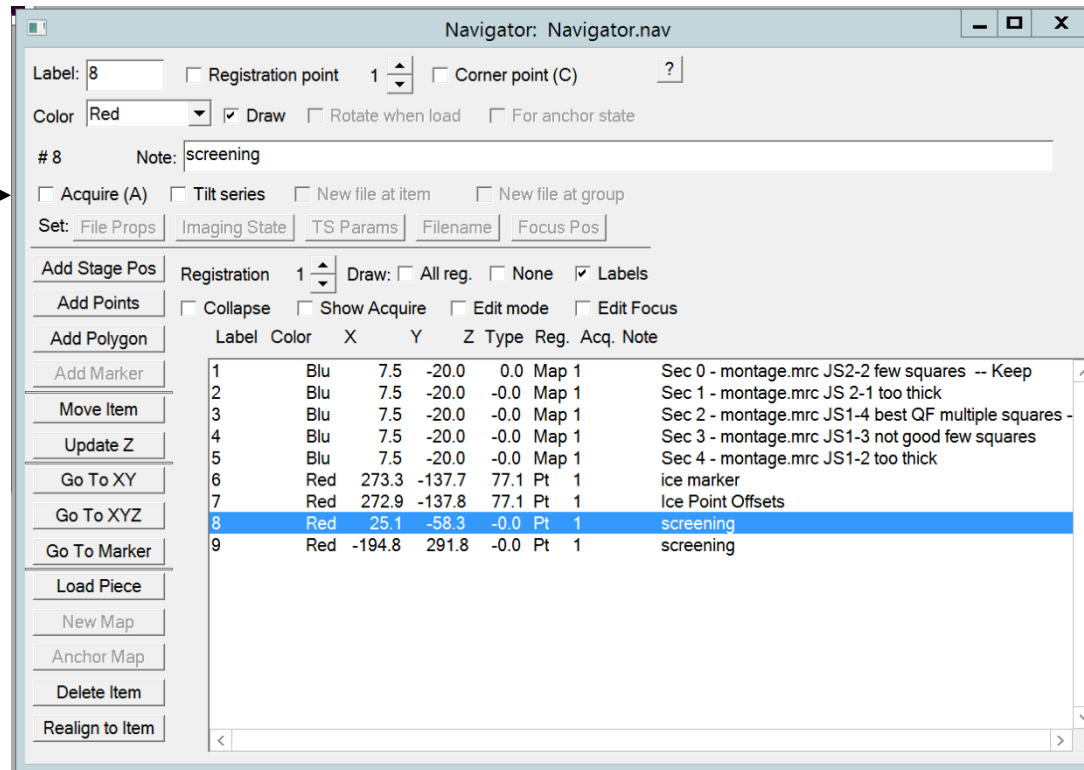
Treat as very sloppy montage:
align edges (IMOD blendmont)

-sloppy (-sl) OR -SloppyMontage Do initial cross-correlations for finding edge functions and shift pieces to minimize displacements in the overlap zones

<https://bio3d.colorado.edu/imod/doc/man/blendmont.html>

Navigator

Mark points for data collection (Acquire Points)



The screenshot shows the Navigator software interface. At the top, there are fields for 'Label: 8', 'Registration point 1', and 'Corner point (C)'. Below these are options for 'Color Red', 'Draw', 'Rotate when load', and 'For anchor state'. A 'Note: screening' field is present. A sidebar on the left contains buttons: 'Acquire (A)', 'Tilt series', 'New file at item', 'New file at group', 'Set: File Props', 'Imaging State', 'TS Params', 'Filename', 'Focus Pos', 'Add Stage Pos', 'Add Points', 'Add Polygon', 'Add Marker', 'Move Item', 'Update Z', 'Go To XY', 'Go To XYZ', 'Go To Marker', 'Load Piece', 'New Map', 'Anchor Map', 'Delete Item', and 'Realign to Item'. The main area displays a table of items with columns: Label, Color, X, Y, Z, Type, Reg., Acq., and Note. Item 8 is highlighted in blue.

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Blu	7.5	-20.0	0.0	Map	1		Sec 0 - montage.mrc JS2-2 few squares -- Keep
2	Blu	7.5	-20.0	-0.0	Map	1		Sec 1 - montage.mrc JS 2-1 too thick
3	Blu	7.5	-20.0	-0.0	Map	1		Sec 2 - montage.mrc JS1-4 best QF multiple squares -
4	Blu	7.5	-20.0	-0.0	Map	1		Sec 3 - montage.mrc JS1-3 not good few squares
5	Blu	7.5	-20.0	-0.0	Map	1		Sec 4 - montage.mrc JS1-2 too thick
6	Red	273.3	-137.7	77.1	Pt	1		ice marker
7	Red	272.9	-137.8	77.1	Pt	1		Ice Point Offsets
8	Red	25.1	-58.3	-0.0	Pt	1		screening
9	Red	-194.8	291.8	-0.0	Pt	1		screening

List of Items

Points: single location

- Add Stage Pos
- Add points

Polygons: set of connected points around a region

- Add Polygon

Maps: imaged saved to file (single image, montage, other microscope).

- Add Map

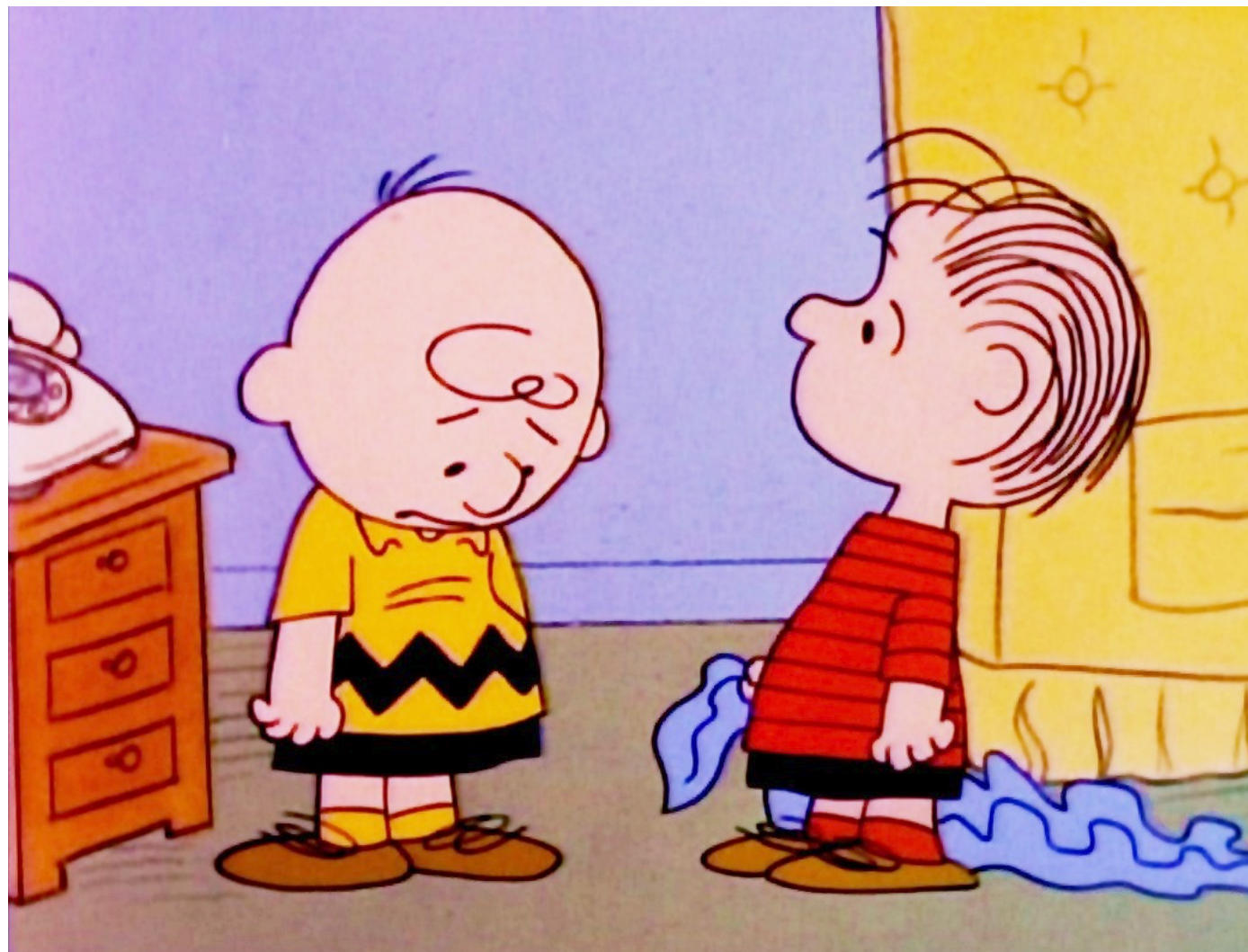
Save the stage position for each item.

Got To XY / Got To XYZ / Go To Marker

SerialEM Resources

- Webpage <https://bio3d.colorado.edu/SerialEM/>
- Youtube <https://www.youtube.com/playlist?list=PLGggUwWmzvs-DV4jCapSI5XQ-hpAXtzMb>
- Scripts [https://bio3d.colorado.edu/SerialEM/Script depository](https://bio3d.colorado.edu/SerialEM/Script%20depository)
- Lectures Wim Hagen EMBL <https://www.youtube.com/watch?v=yqsUeOlg-0Q>
- Umass CryoEM Core Website Chen Xu <https://sphinx-emdocs.readthedocs.io/en/latest/index.html>
- Google Group <https://groups.google.com/a/colorado.edu/forum/#!forum/serial-em>
- Microscopy and Microanalysis Short Course https://www.microscopy.org/MandM/2020/program/short_courses.cfm

The End



<https://weheartit.com/entry/329040315>