Single Particle CryoEM Screening and Data Collection using SerialEM

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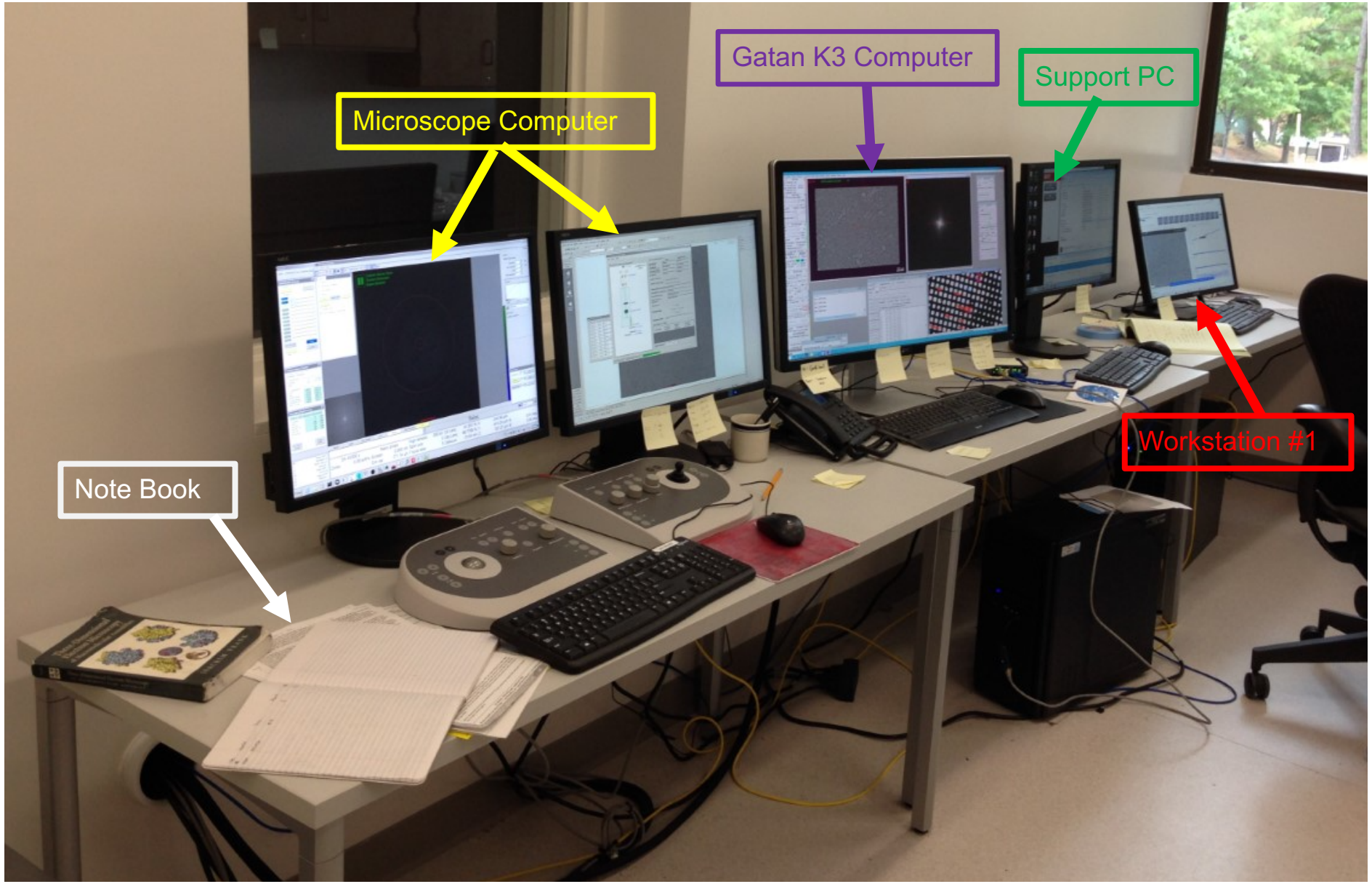
919-962-5979

Overview

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


Gatan K3 Computer
Support PC
Microscope Computer
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

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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/yjsbi

Automated electron microscope tomography using robust prediction of specimen movements

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

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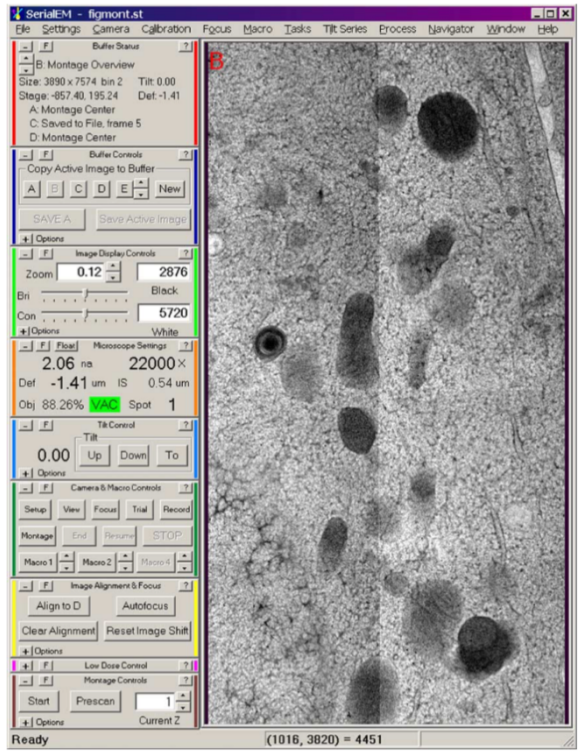
Available online 24 August 2005



Mastronarde 2005

Tomography Data Collection: Cellular Imaging

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Rapid Tilt-Series Acquisition for Electron Cryotomography

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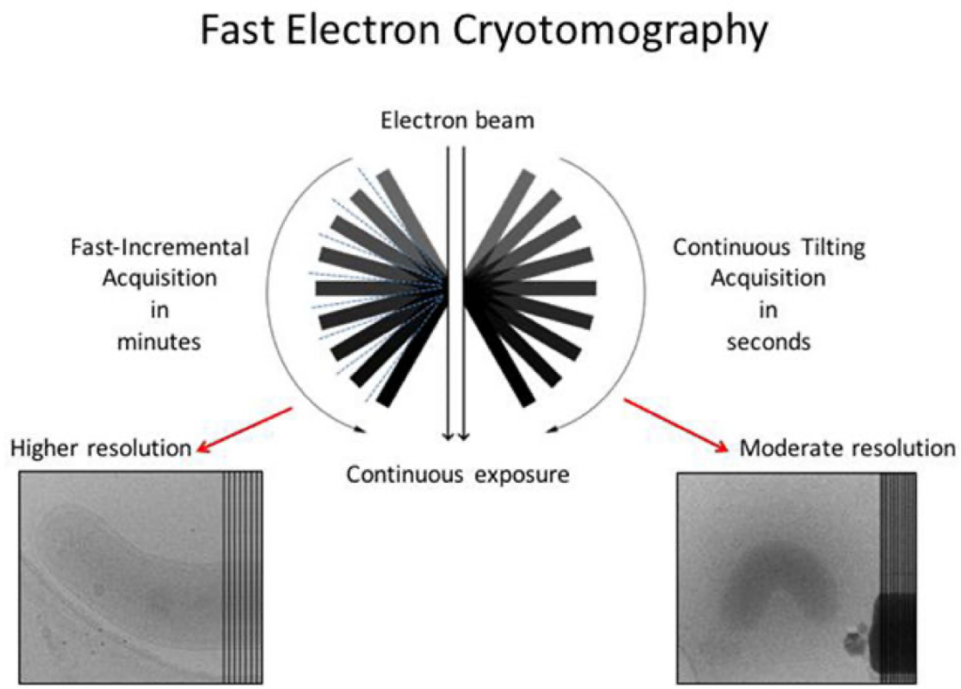
[#] These authors contributed equally to this work.

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.



Chrelf eta ., 2019

Correlative light electron microscopy

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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 Hammonds**[1], **Eric Alonas**[3], **Tanay M Desai**[1], **Mariana Marin**[1], **Rachel E Storms**[1], **Fredrick Leon**[1], **Gregory B Melikyan**[1], **Philip J Santangelo**[3], **Paul W Spearman**[1], and **Elizabeth R Wright**[1,4]

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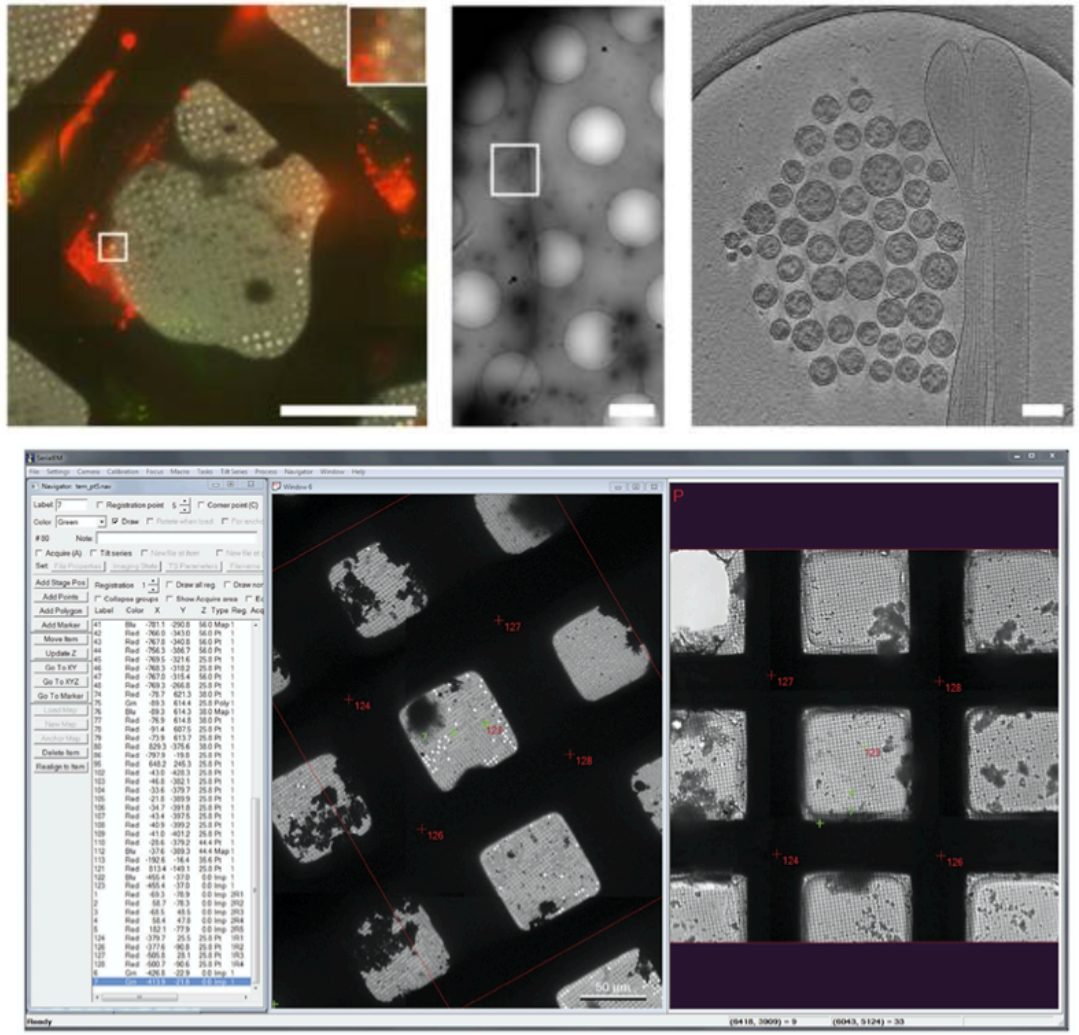
[4]Robert P. Apkarian Integrated Electron Microscopy Core, Emory University, Atlanta, Georgia, USA

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.



Campton et al., 2017

Tomography Data Collection: Subvolume Averaging

Journal of Structural Biology 197 (2017) 191–198

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

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

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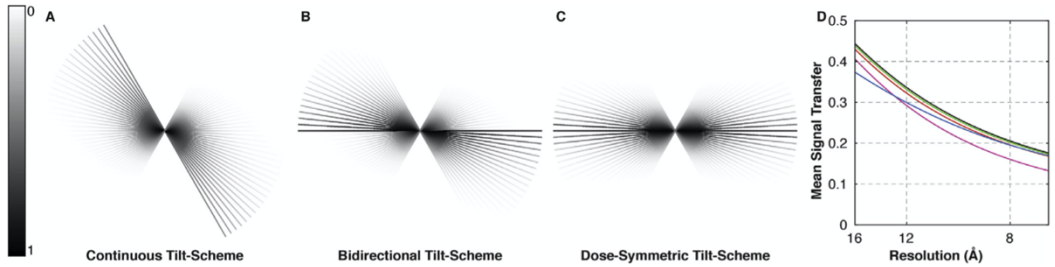
Tilt-scheme
Subtomogram averaging
Cryo-electron microscopy

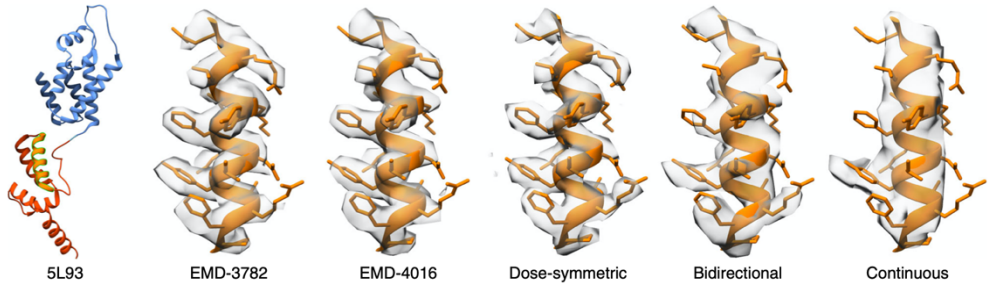
ABSTRACT

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.

Hagen et al., 2017



Hagen et al., 2017



Turonova et a., 2020

MicroED Data Collection

Ultramicroscopy 201 (2019) 77–80

Contents lists available at ScienceDirect

Ultramicroscopy

journal homepage: www.elsevier.com/locate/ultramic

MicroED data collection with SerialEM

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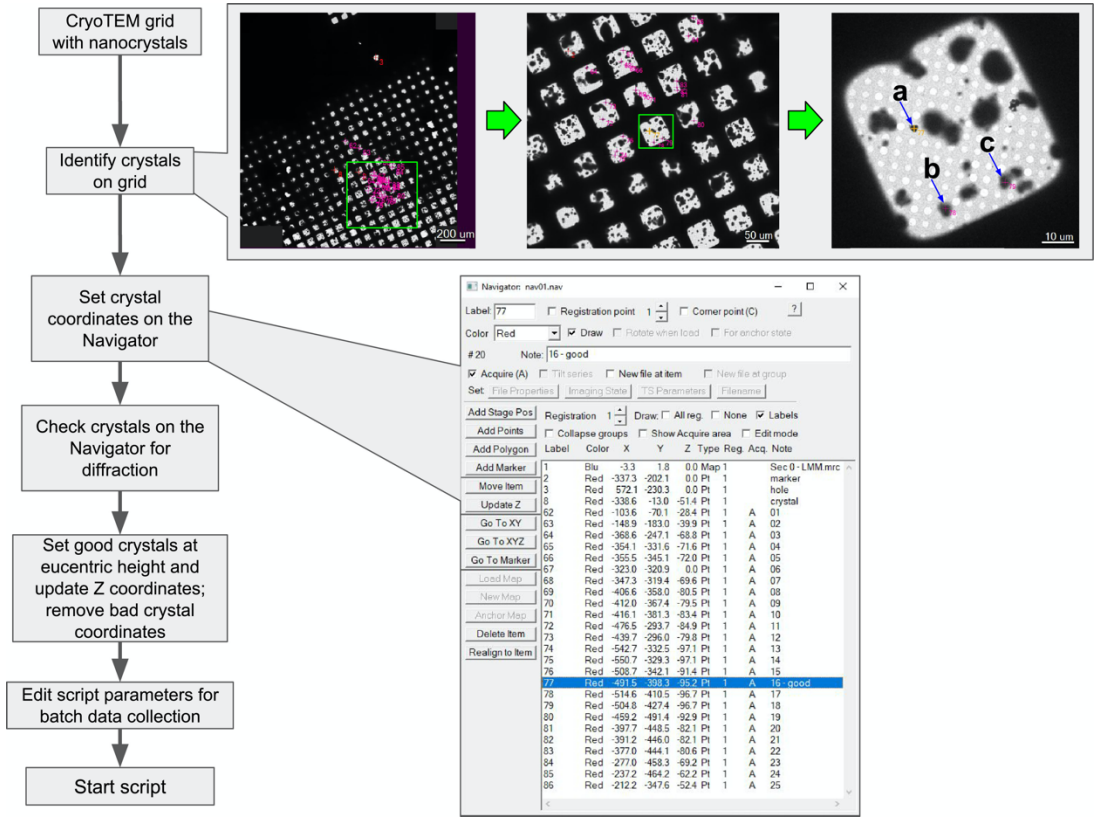
ARTICLE INFO

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

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

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 and 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 cryogrids 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.

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
*2019111_JS_TK_TK3_G1_Pt2_Search.mrc*Pt2 "navigator point 2"
2019111_JS_TK_TK3_G1_Pt2_View.mrc
2019111_JS_TK_TK3_G1_Pt2_Record.mrc

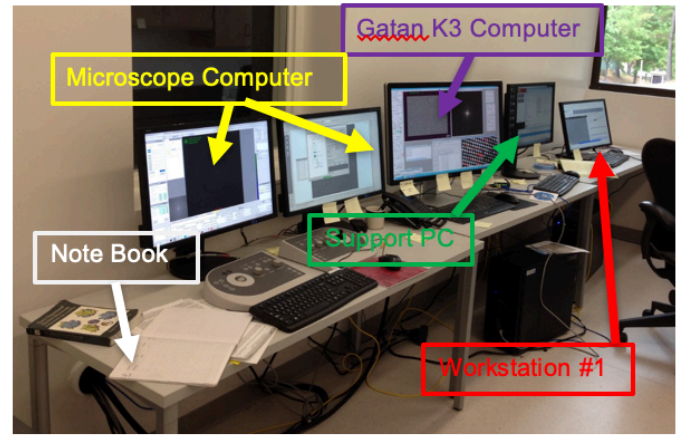
Make sure to save the montages and Navigator files, mdoc files and serialEM log file.

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

Check Microscope Status Before Starting

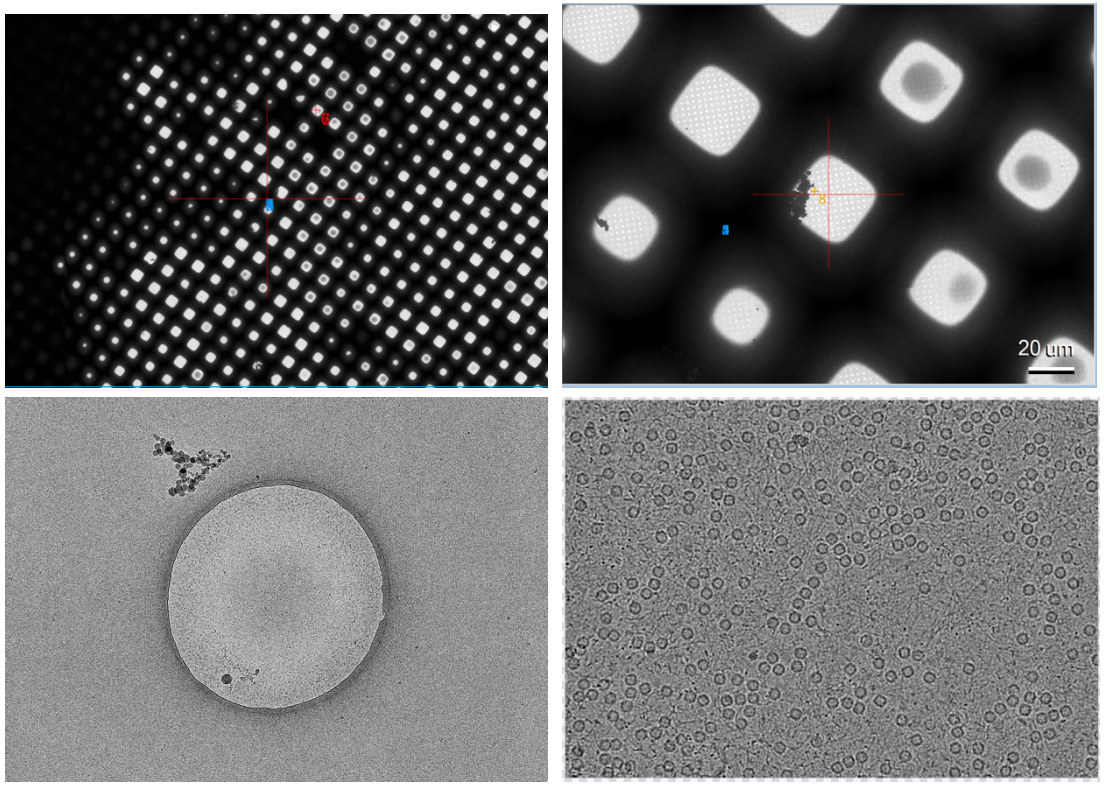
1. Microscope Computer
    a. TEM user interface is open
    b. Microscope status (ie. FEG, Vacuum, Autoloader, Temperature...ect)
    c. ***TEMSEM server*** is running (required for SerialEM)
    d. ***Gatan TEM server*** is running (required for Gatan K3 camera)
    e. ***Standalone camera*** is selected 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.



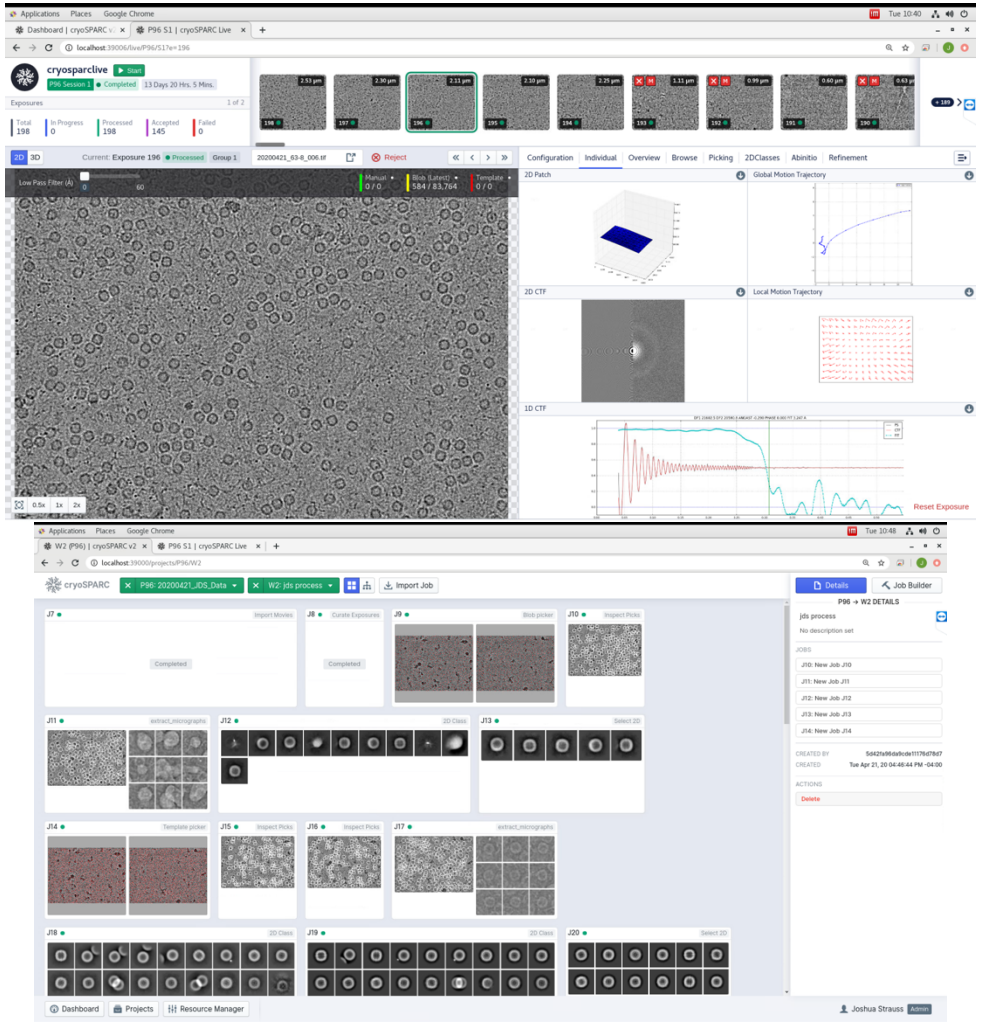
Multiple Levels of Screening and Data Collection

- 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 ?

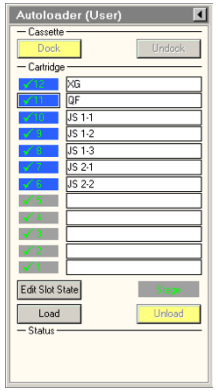


Multiple Levels of Screening and Data Collection: Processing

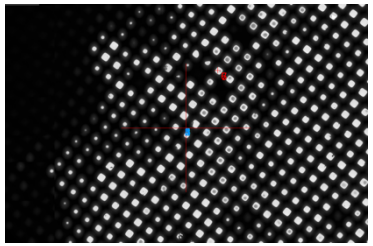
- Do the grids have vitreous ice ?
- Overall (low-mag) grid quality, how many good grid squares ?
- Are there particles in the ice ?
- What is the particle morphology, size, and distribution?
- How thin is the ice?
- Micrograph quality ?
- Do the 2D class averages have 2nd structure ?
- What is the 3D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

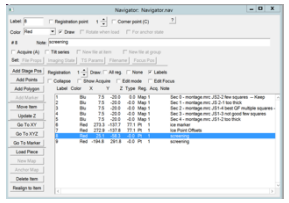


Typical Session

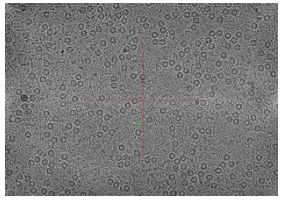


Autoloader Inventory





Map to Grids



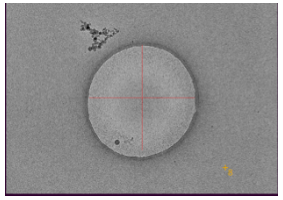
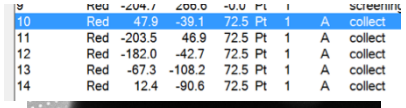
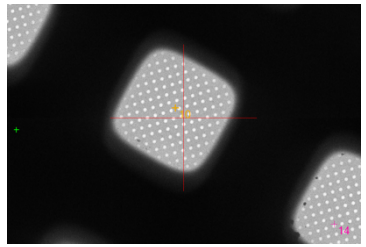


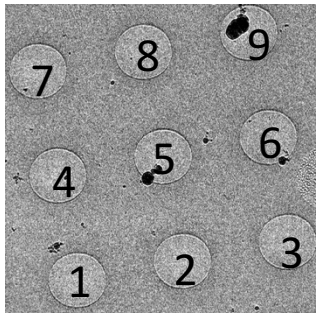
Image at High Mag



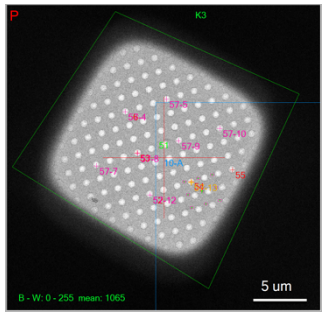


Montage Grid Squares

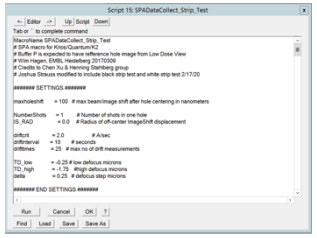
Setup Multishot Record



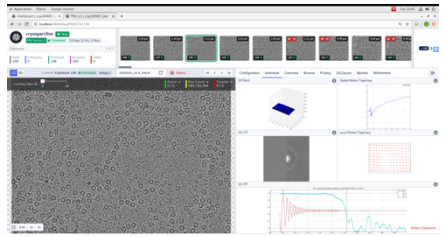
Add Grid of Points



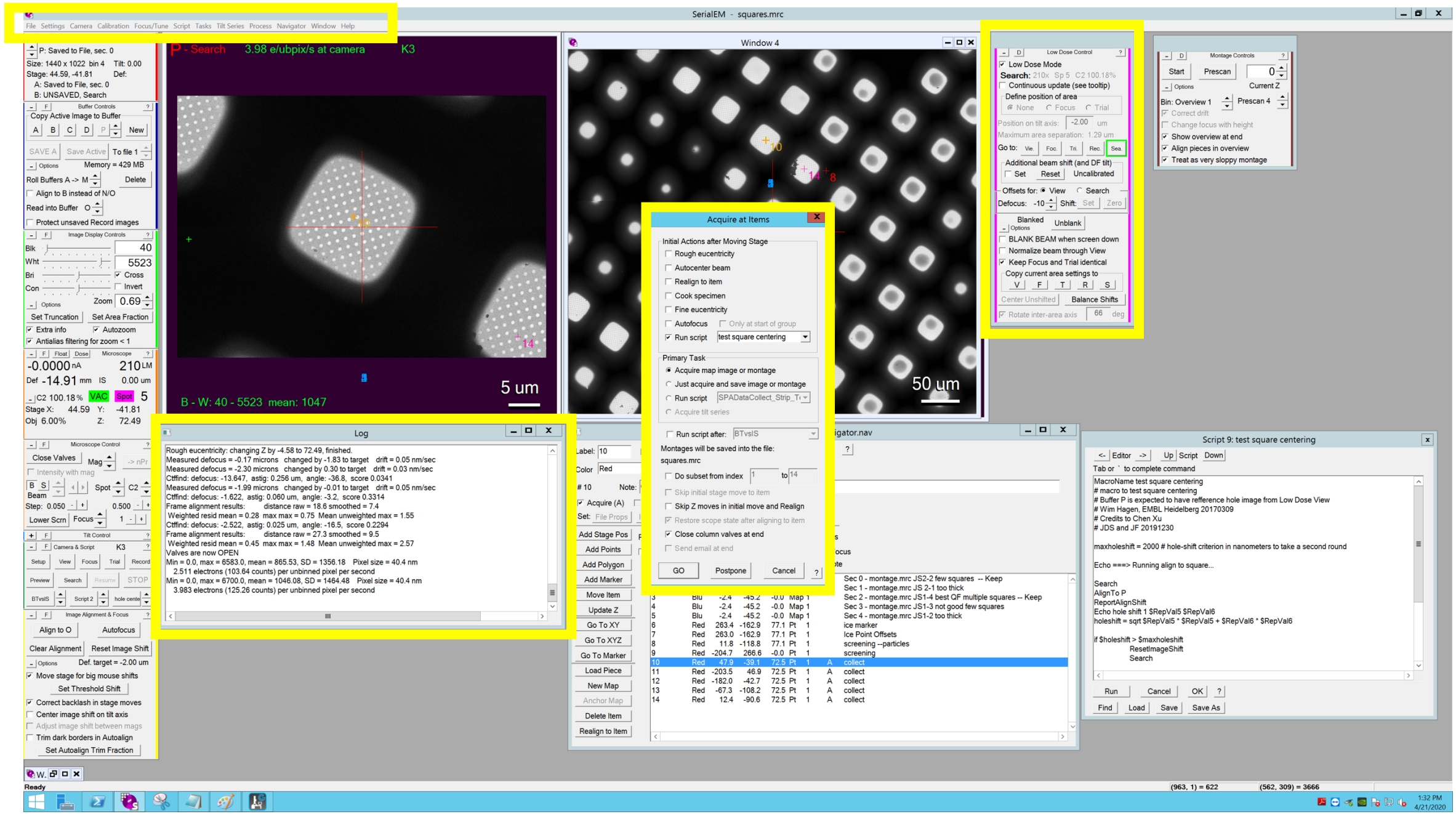
Start Data Collection



Pre-process Data

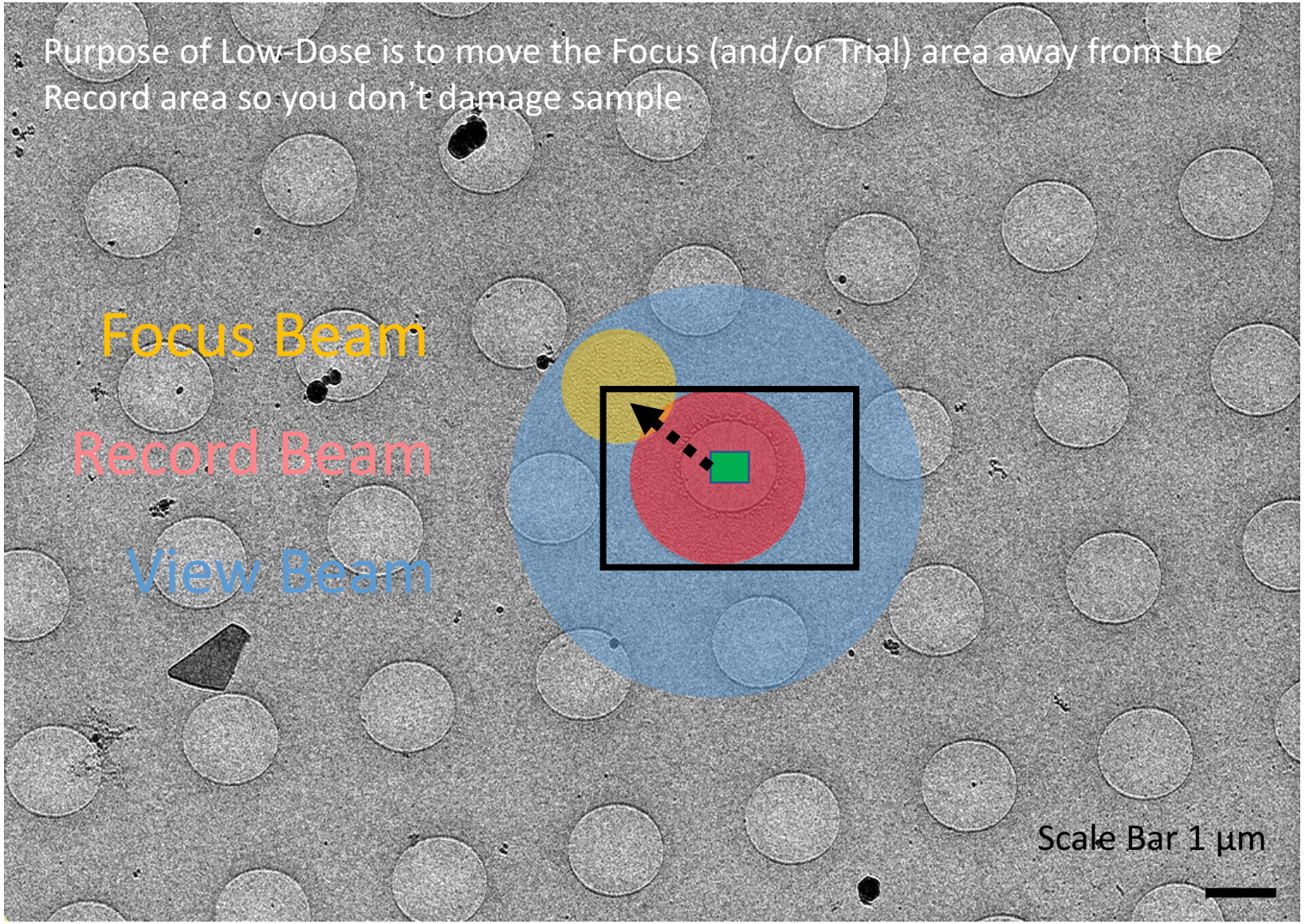


Align TEM and Collect Gain / Dark Reference Before Data Collection


SerialEM - squares.mrc
File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help
P: Saved to File, sec. 0
Size: 1440 x 1022 bin 4 Tilt: 0.00
Stage: 44.59, -41.81 Def:
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 Cross
Con Invert
Zoom 0.69
Set Truncation Set Area Fraction
Extra info Autozoom
Antialias filtering for zoom < 1
Float Dose 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
Microscope Control
Close Valves Mag -> nPr
Intensity with mag
B S Spot C2
Beam
Step: 0.050 0.500
Lower Scrn Focus 1
Tilt Control
Camera & Script K3
Setup View Focus Trial Record
Preview Search Resume STOP
BTvsIS Script 2 hole cente
Image Alignment & Focus
Align to O Autofocus
Clear Alignment Reset Image Shift
Options Def. target = -2.00 um
Move stage for big mouse shifts
Set Threshold Shift
Correct backlash in stage moves
Center image shift on tilt axis
Adjust image shift between mags
Trim dark borders in Autoalign
Set Autoalign Trim Fraction
P - Search 3.98 e/ubpix/s at camera K3
B - W: 40 - 5523 mean: 1047
5 um
Window 4
50 um
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
Options
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
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 SPADataCollect_Strip_T
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 ?
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
Ctffind: defocus: -13.647, 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
Ctffind: 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
Ctffind: 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
Label: 10
Color Red
10 Note:
Acquire (A)
Set: File Props
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
Realign to Item
igator.nav
Sec 0 - montage.mrc JS2-2 few squares -- Keep
Sec 1 - montage.mrc JS 2-1 too thick
Sec 2 - montage.mrc JS1-4 best QF multiple squares -- Keep
Sec 3 - montage.mrc JS1-3 not good few squares
Sec 4 - montage.mrc JS1-2 too thick
6 Red 263.4 -162.9 77.1 Pt 1 ice marker
7 Red 263.0 -162.9 77.1 Pt 1 Ice Point Offsets
8 Red 11.8 -118.8 77.1 Pt 1 screening --particles
9 Red -204.7 266.6 -0.0 Pt 1 screening
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 refference 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
Ready
(963, 1) = 622
(562, 309) = 3666
1:32 PM
4/21/2020

https://www.youtube.com/watch?v=_yxhBT-nR9c

Low-Dose Imaging



Low-Dose Control

On/Off

To Change

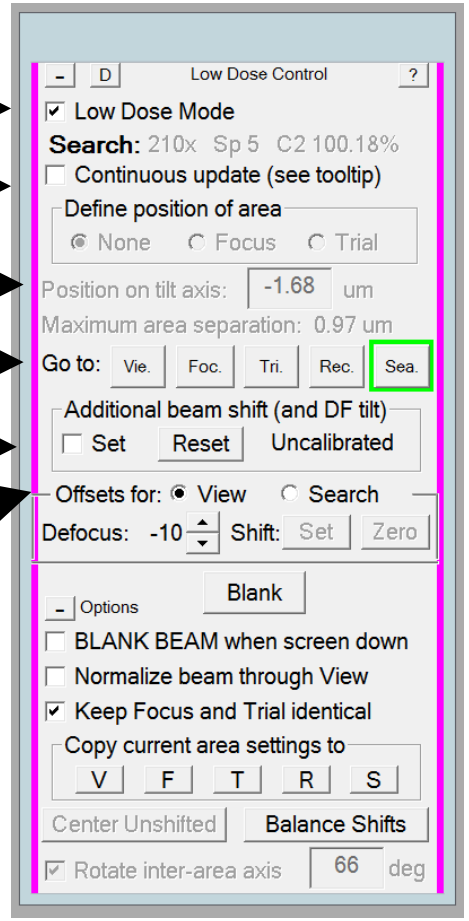
Position of Beam

Select

Moving beam

Record/View/Search

For Setting up Low-Dose



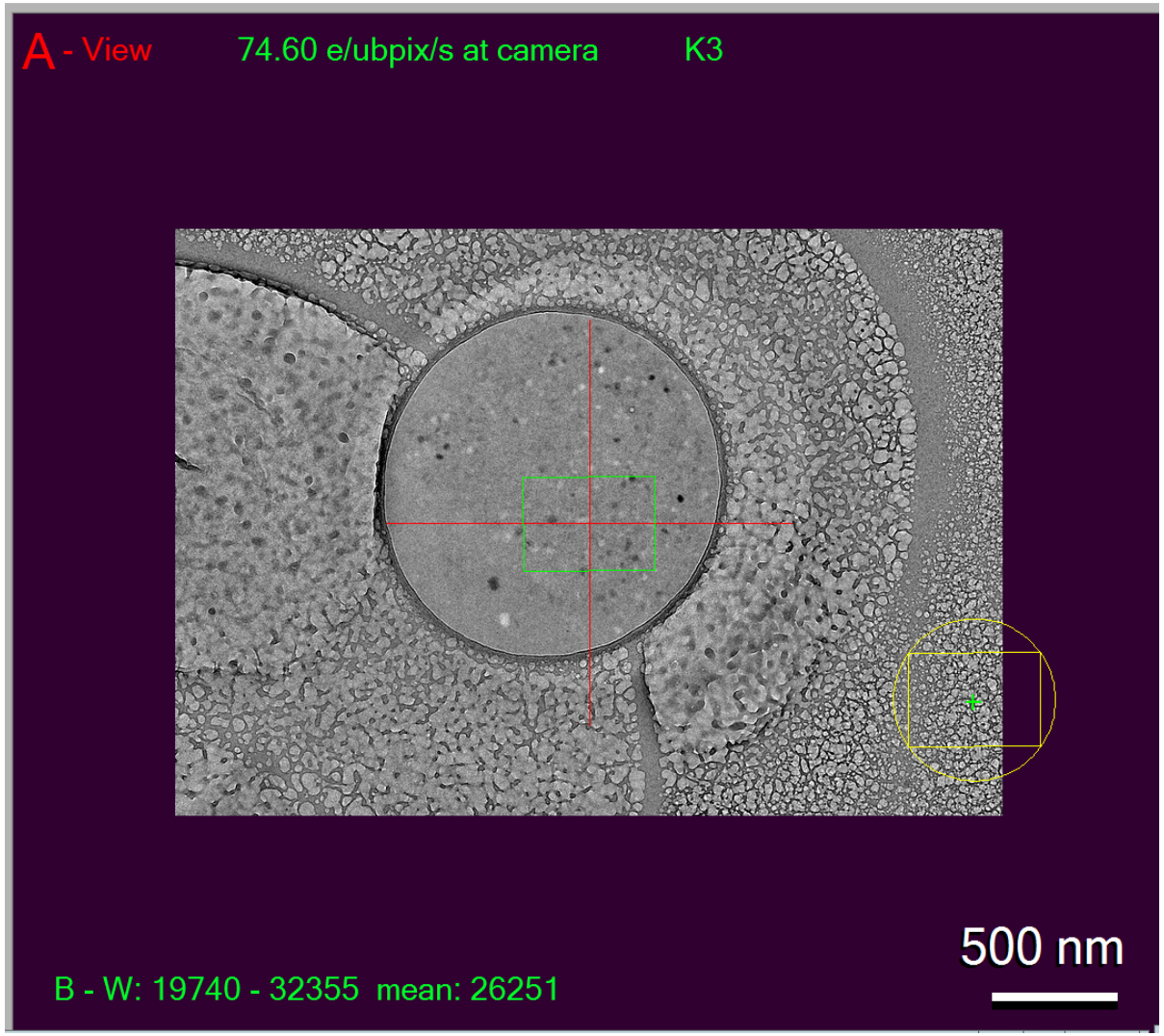
Focus (and/or Trial) away from the Record area so you don't damage sample!!!

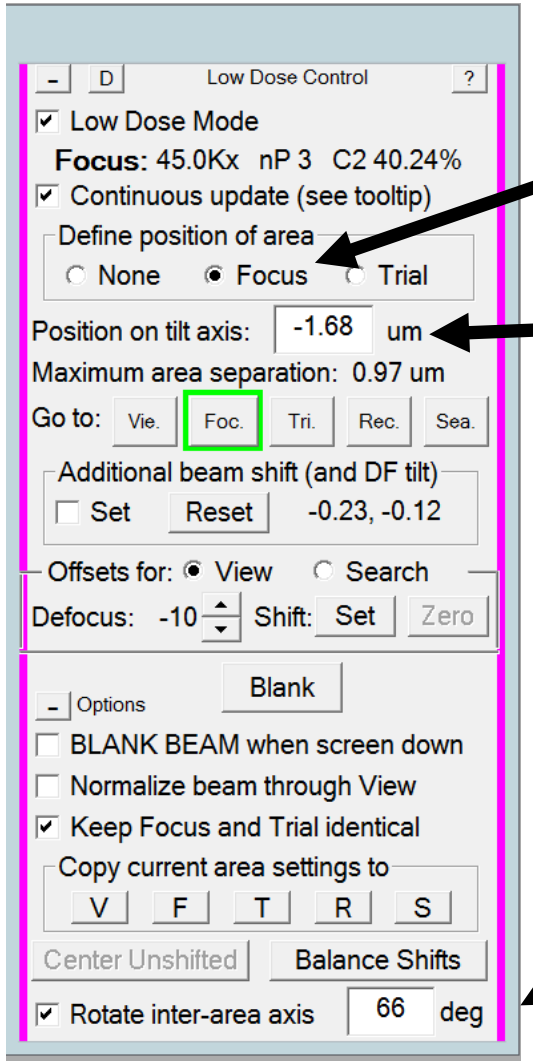
Setup 5 different microscope conditions (Mag, Spt size, probe, lenses strength…)

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





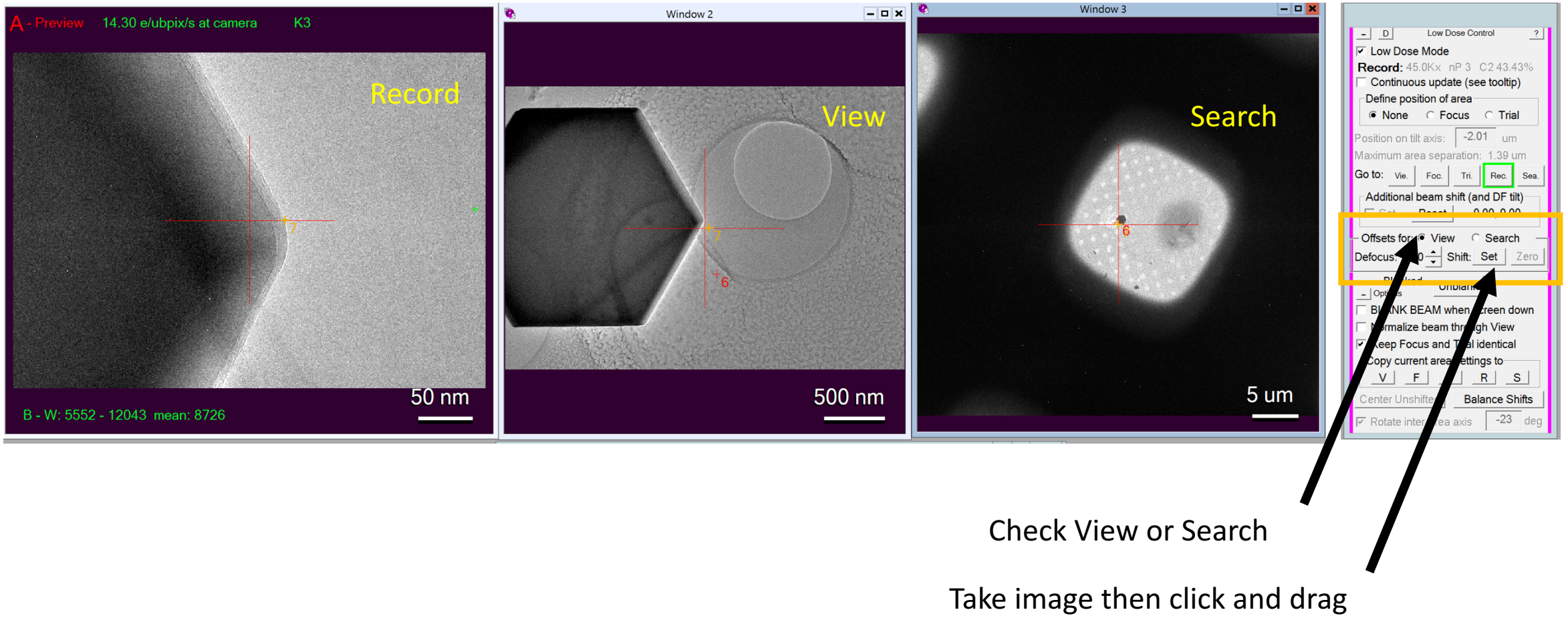
Click this

Adjust

Adjust

Additional Image Shift

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

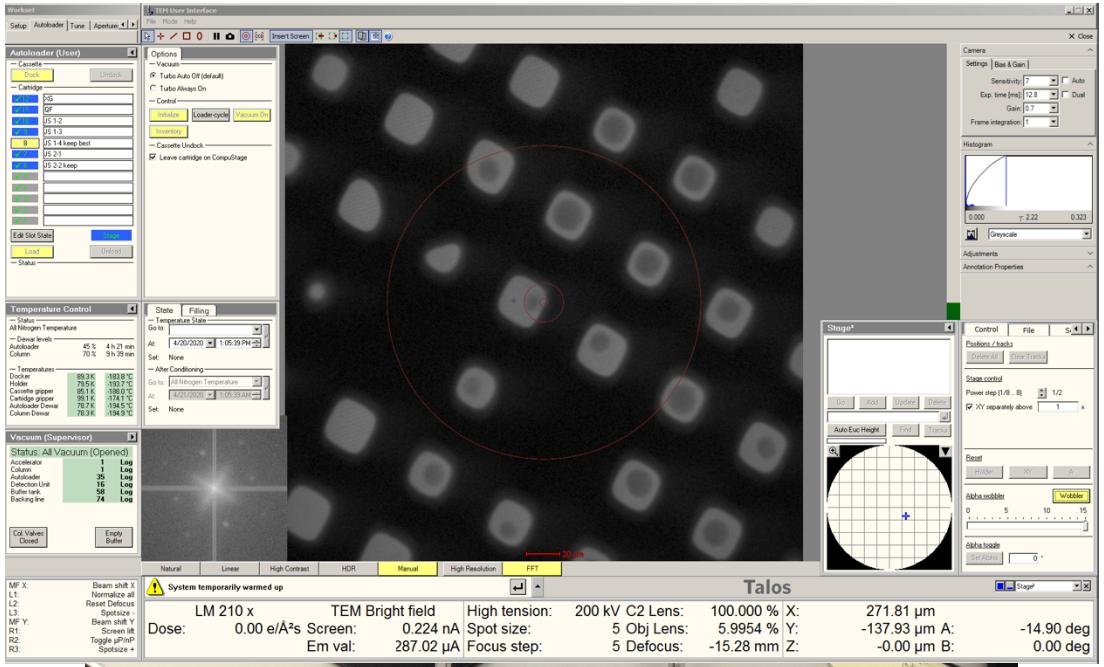


Check View or Search

Take image then click and drag

Eucentric Height

Set using TEM FluCam TEM User Interface



SerialEM

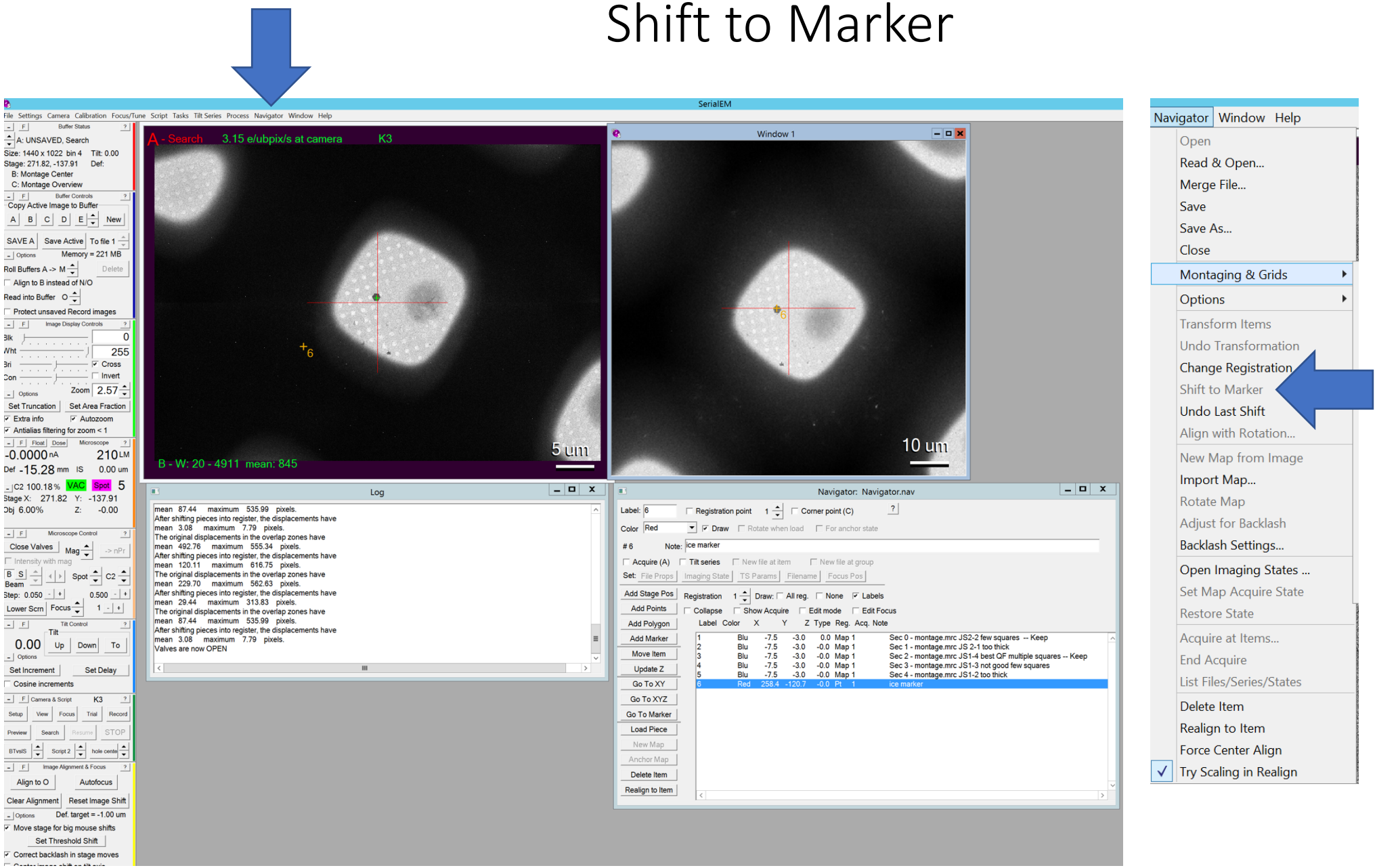
Eucentric Rought

Find Z height by stage tilting

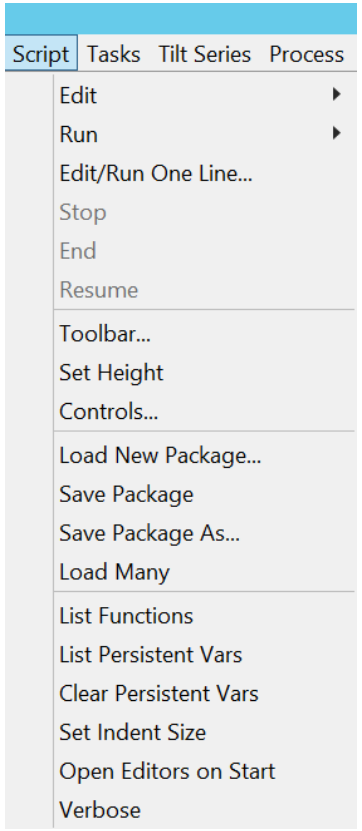
Accurate 5-10 µm

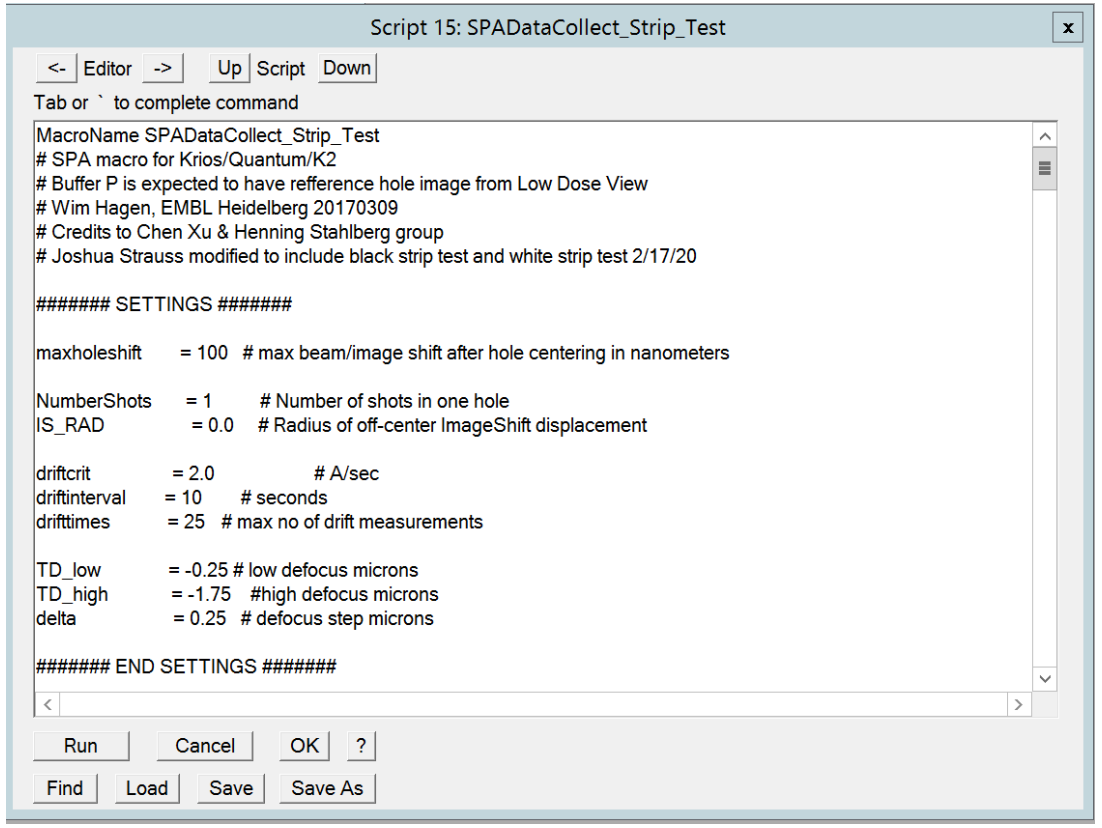
Best if “near” eucentric < 50 µm.

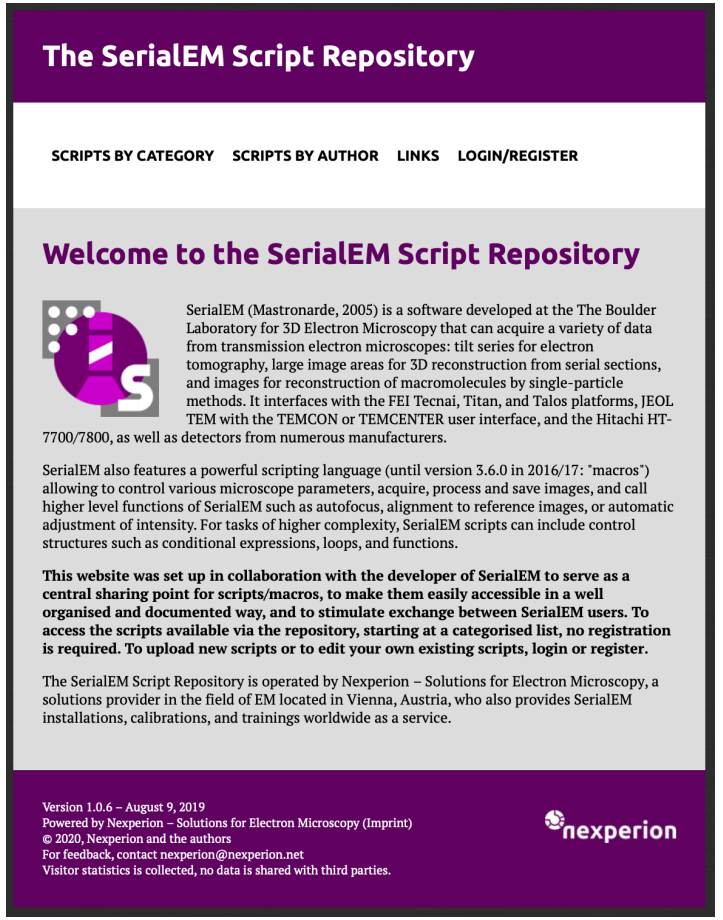
Shift to Marker



SerialEM Scripts

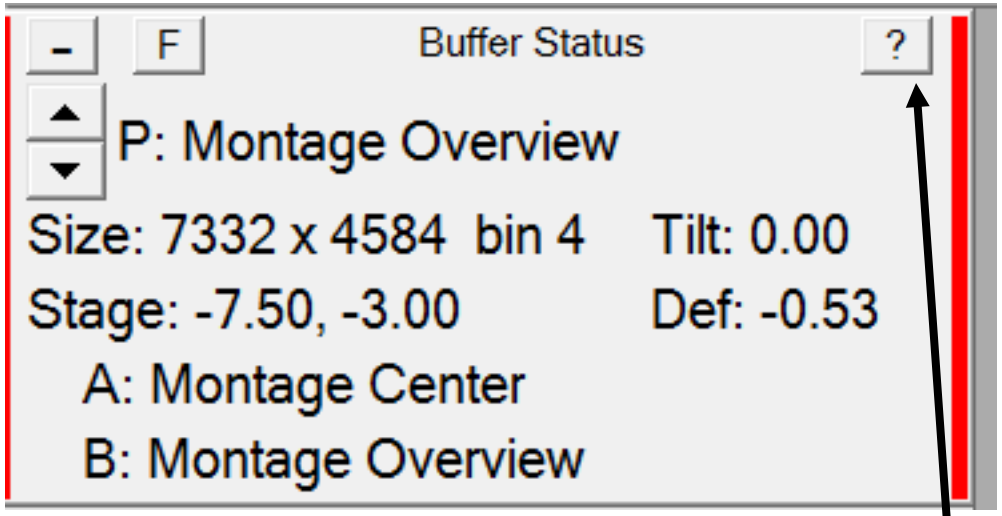






https://serialemscripts.nexperion.net

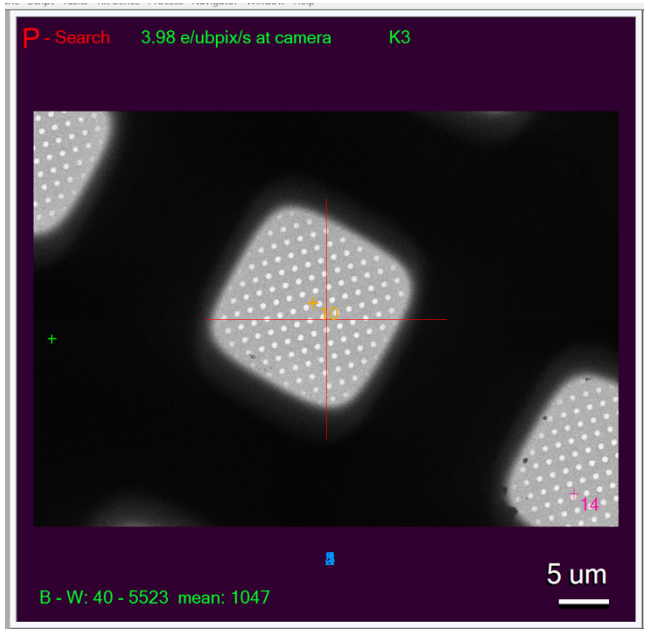
Buffer Status



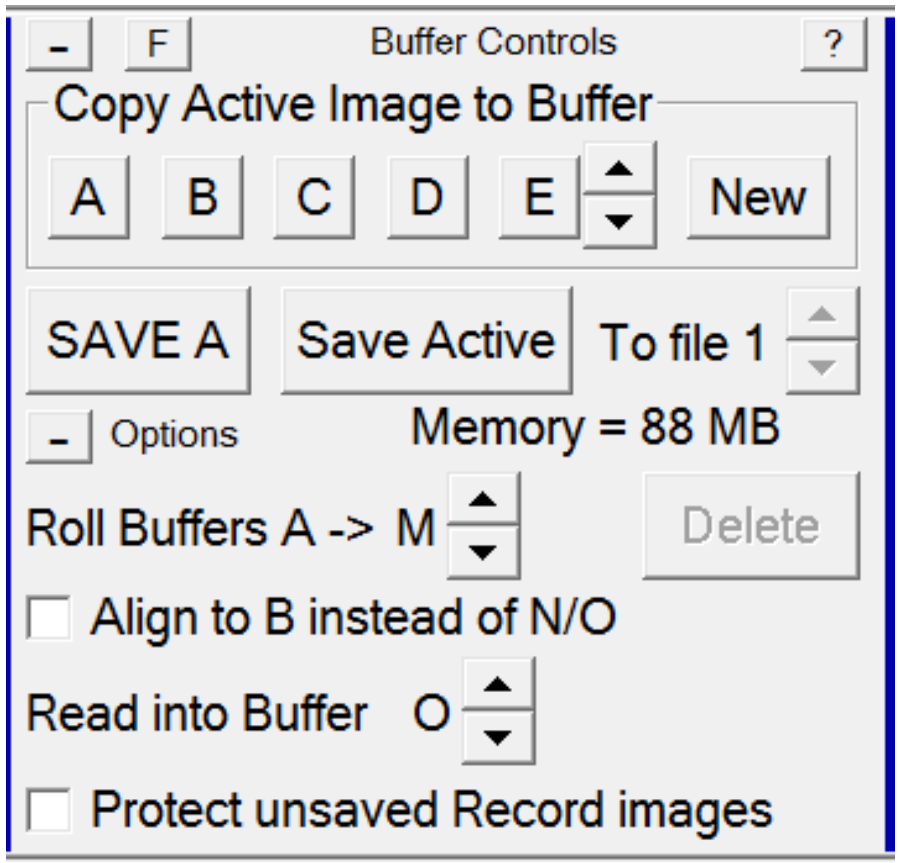
Buffer Status Panel
This panel shows detailed information about the image buffer currently shown in the main display window, and summary information about several other buffers. For an ordinary image acquired by the user, the summary line for a buffer will show what parameter set was used to acquire 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

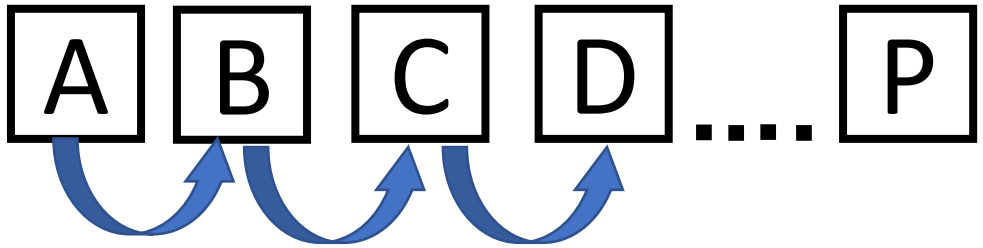
Current buffer spin button:
Display different buffers



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

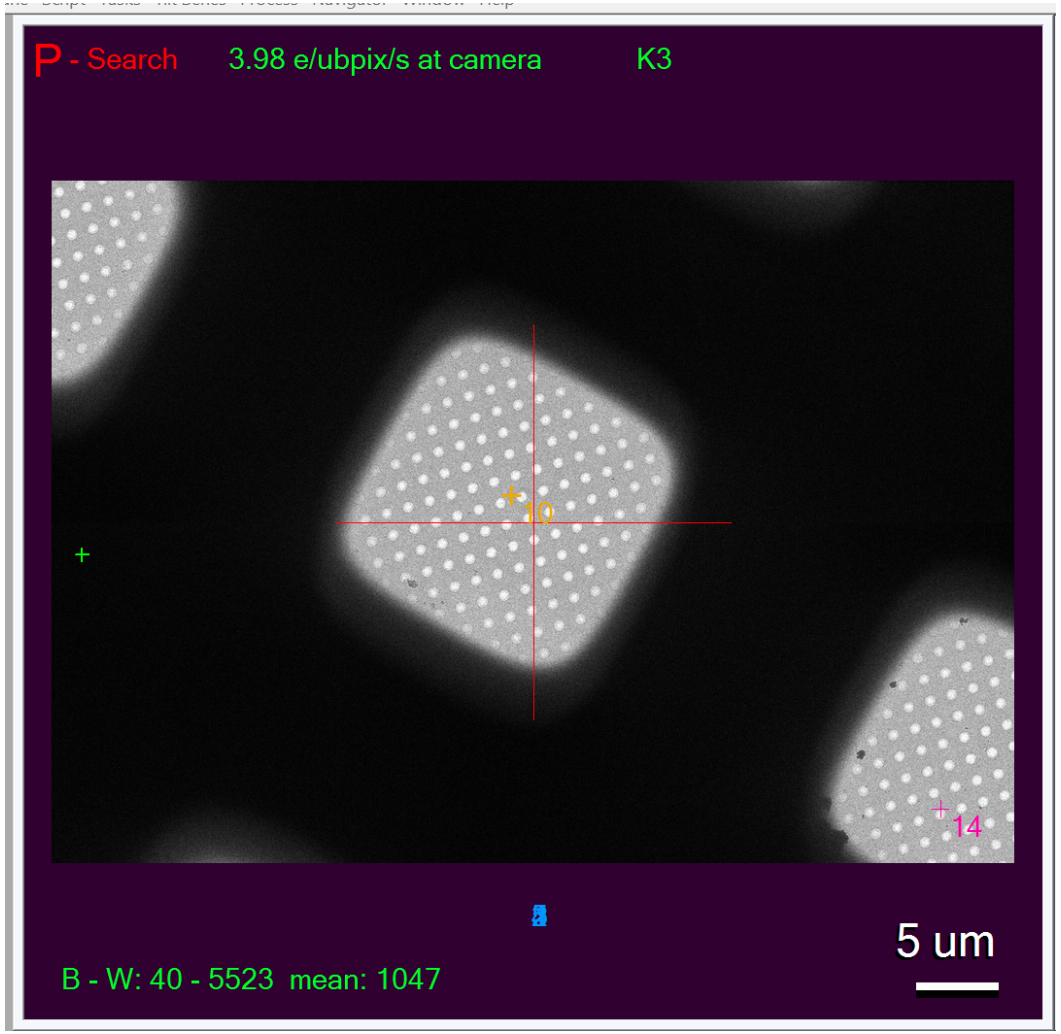
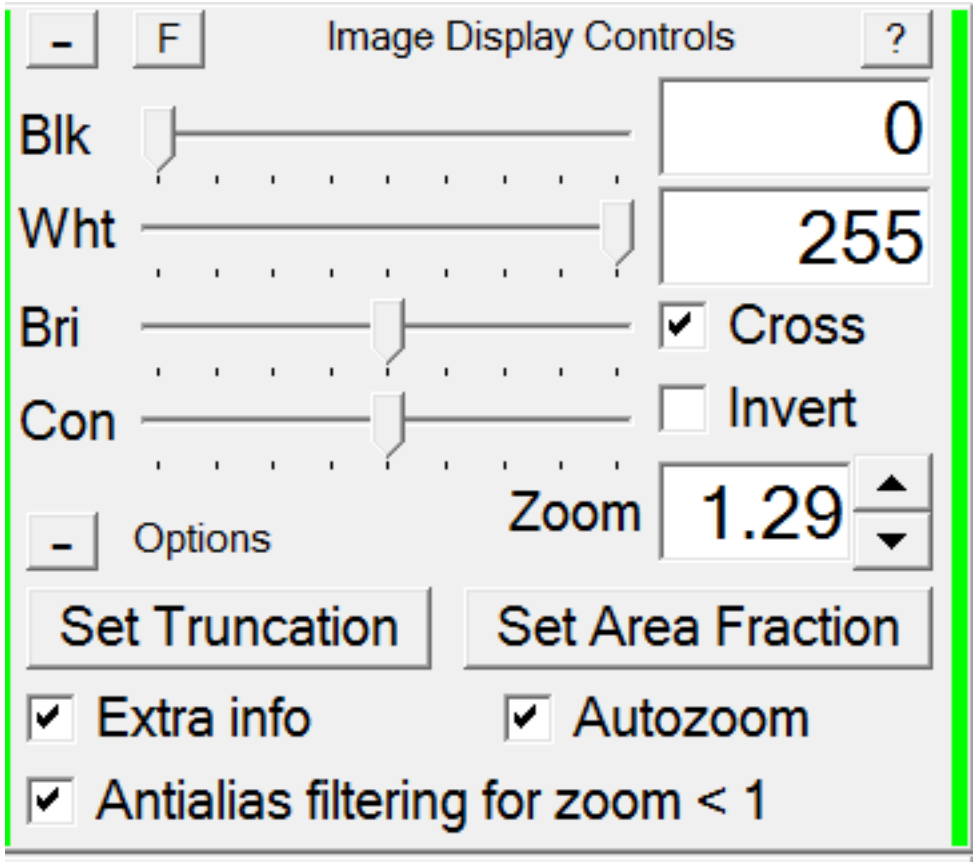

P - Search
3.98 e/ubpix/s at camera
K3
10
14
5 um
B - W: 40 - 5523 mean: 1047

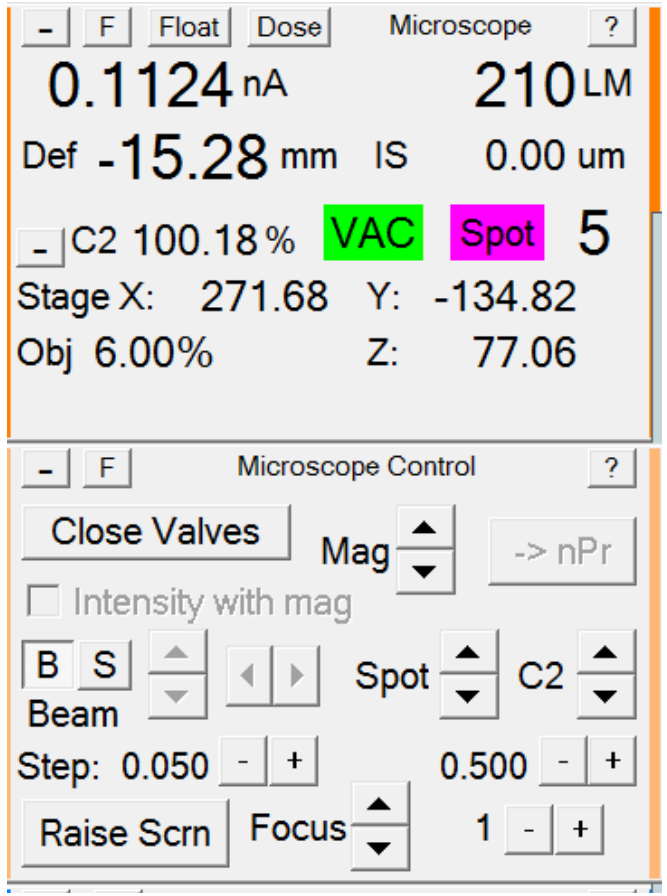
Image Display Controls



Controls how images are displayed

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

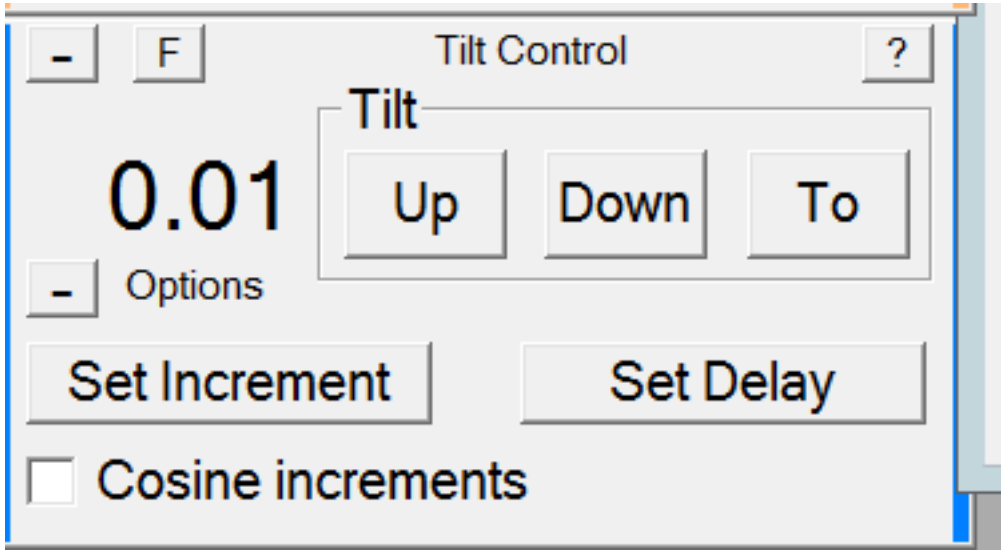
Microscope and Microscope Control



Microscope:
Display microscope status and imaging conditions

Microscope Control:
Allows you to control microscope and imaging conditions

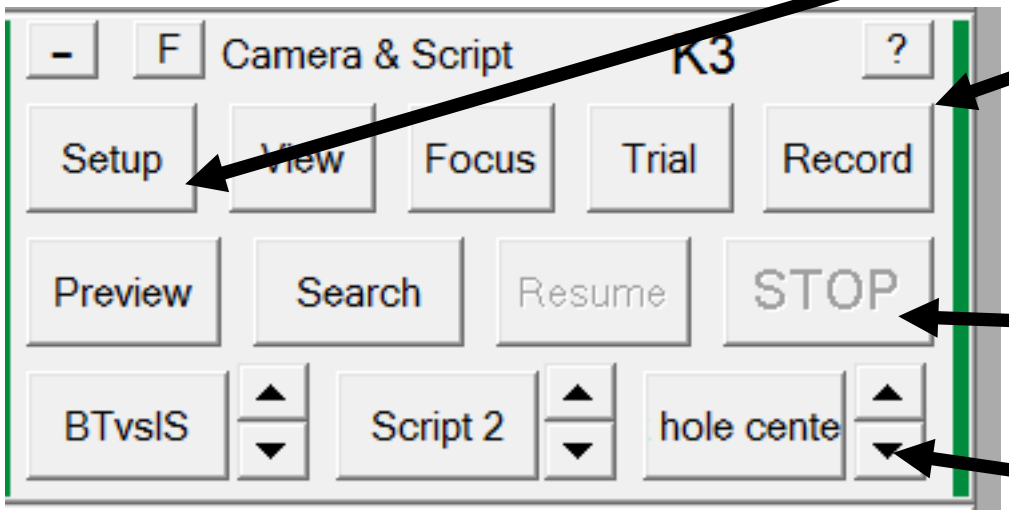
Tilt Control



Displays tilt angle of stage

Tilt the stage +/- 70

Camera & Script



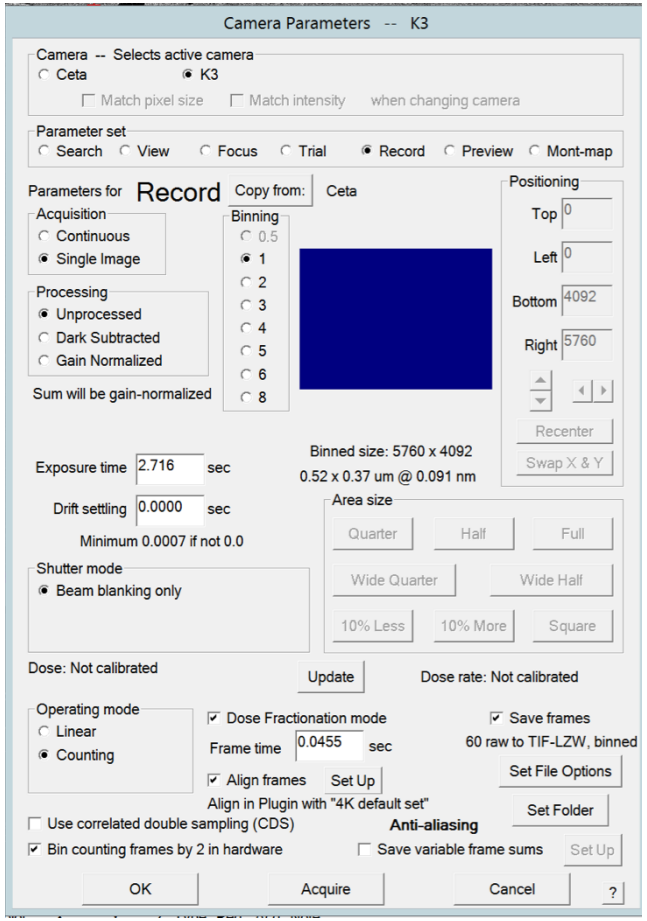
Setup camera parameters

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

Resume/Stop scripts

Select and Run different scripts

Camera Setup



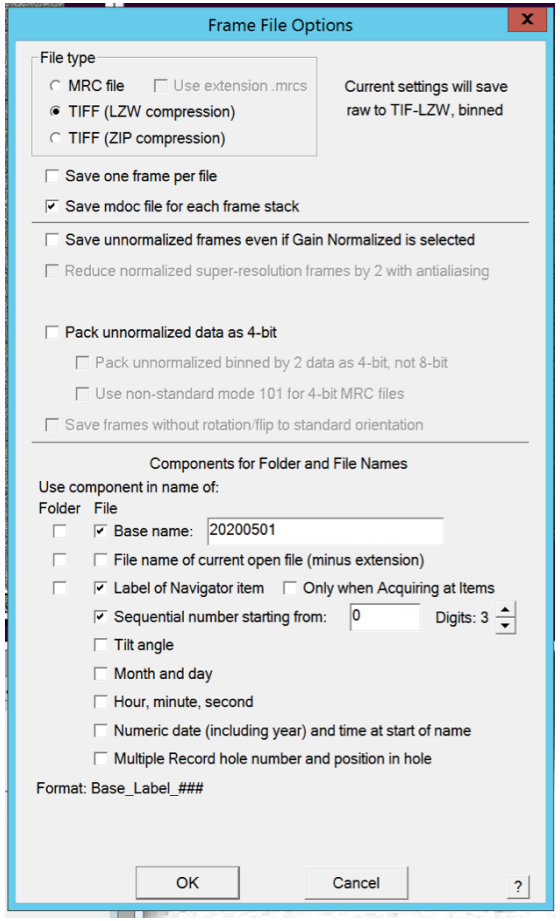
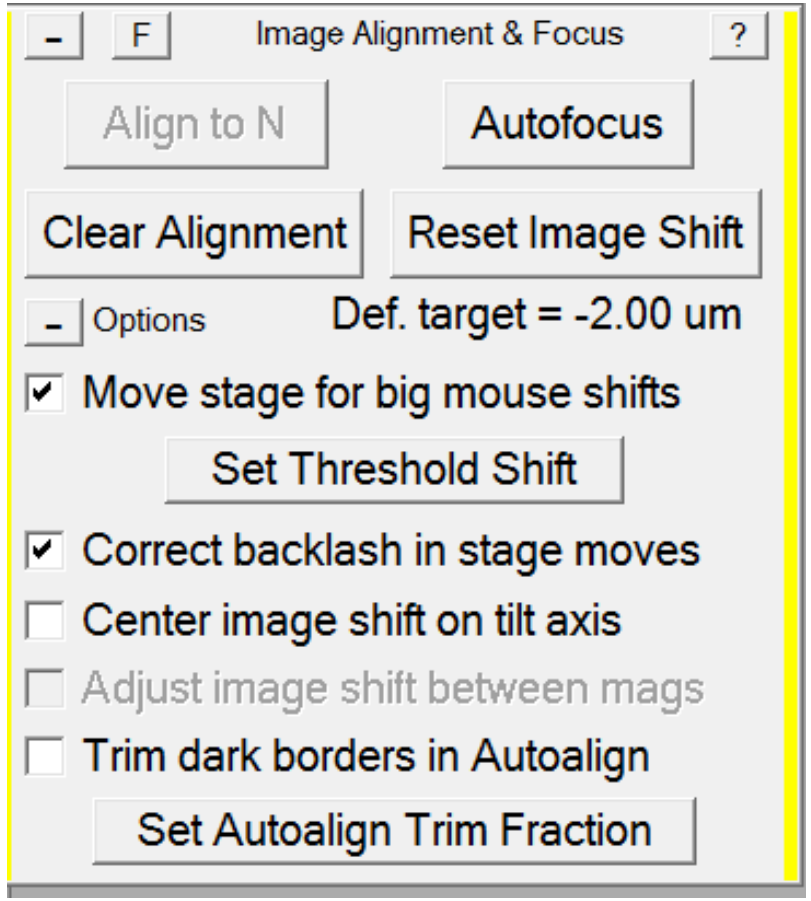


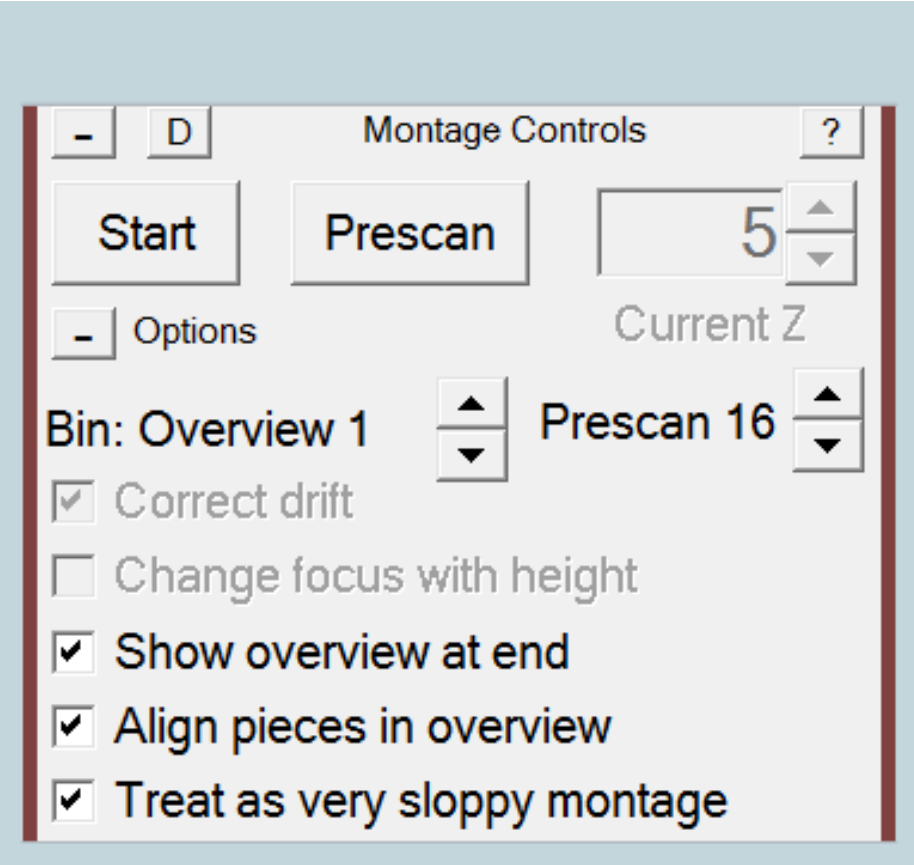
Image Alignment & Focus



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

Defocus Target
Movie stage for big mouse shift / Set Threshold Shift
Reset Image Shift

Montage Controls



Start: Start montaging

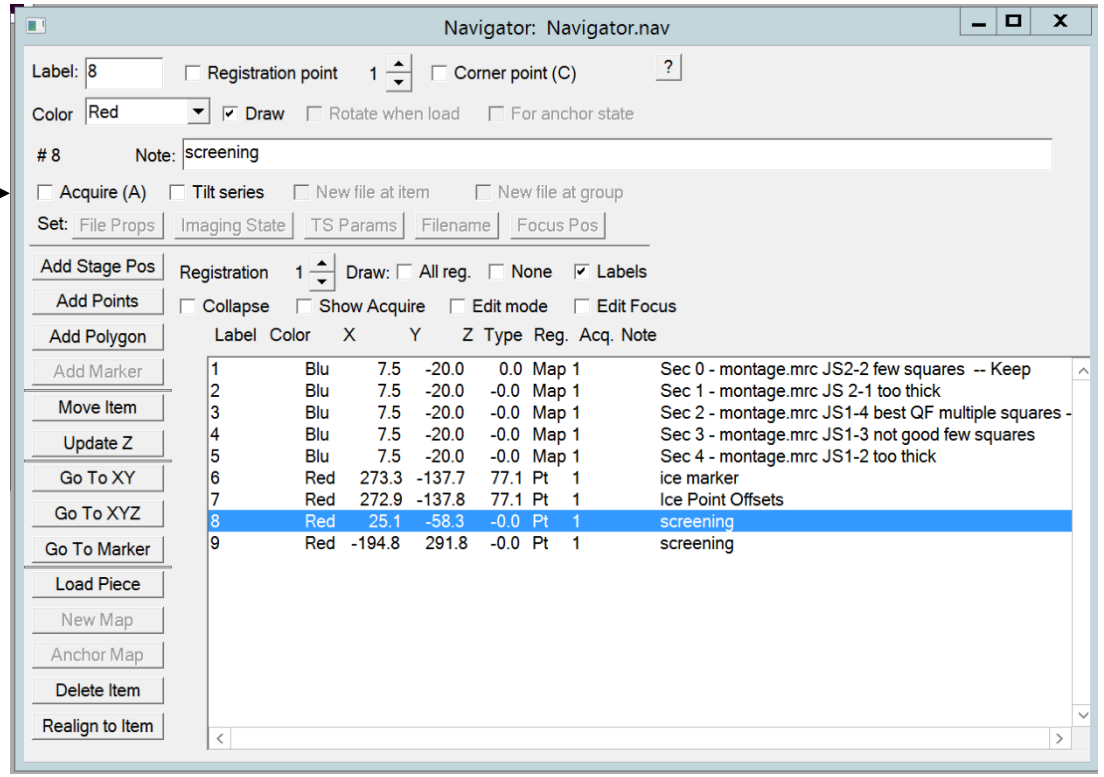
Treat as very sloppy montage: align edges (IMOD blendmont)

-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)



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

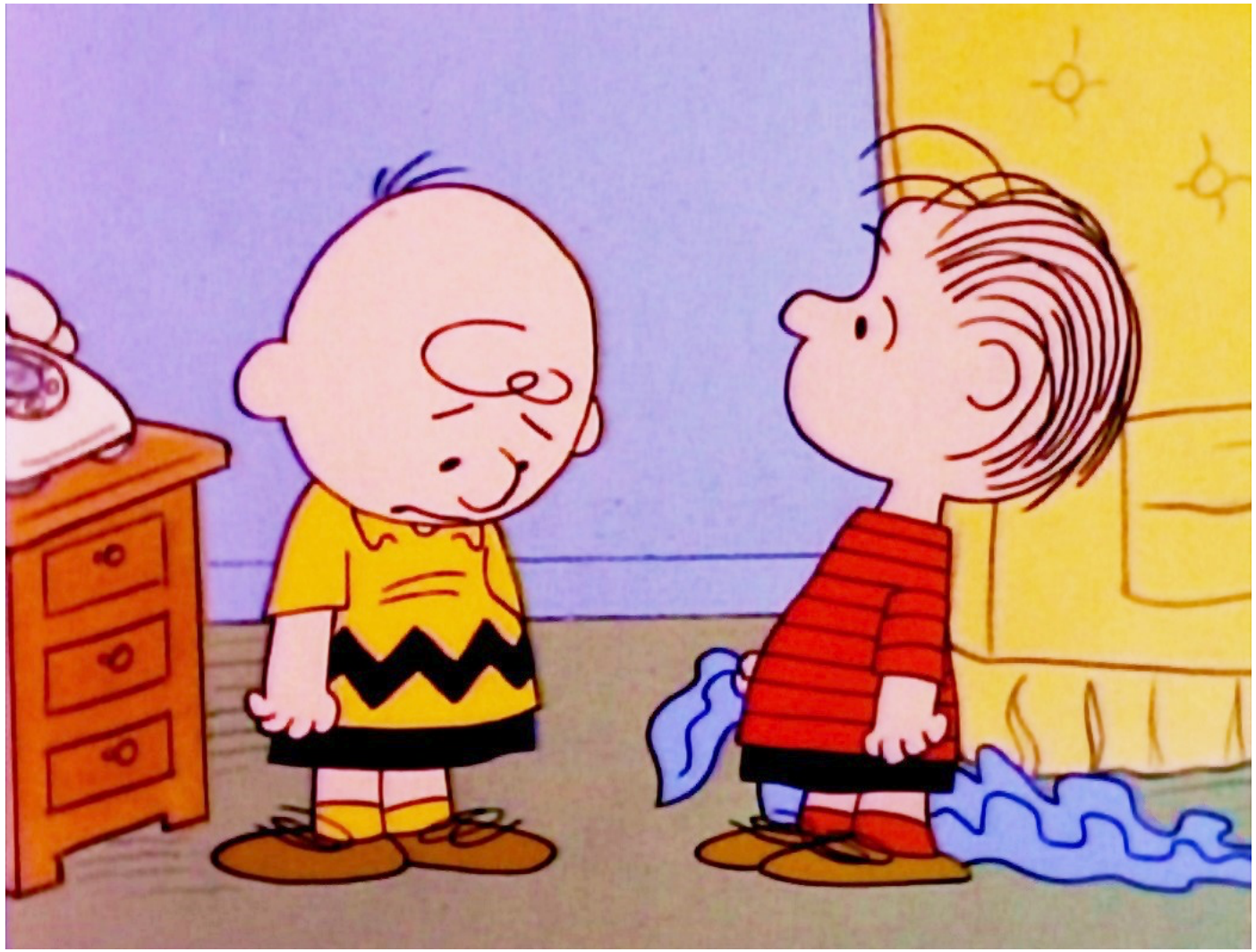
Save the stage position for each item.

Got To XY / Got To XYZ / Go To Maker

SerialEM Resources

- Webpage https://bio3d.colorado.edu/SerialEM/
- Youtube https://www.youtube.com/playlist?list=PLGggUwWmzvs-DV4jCapSl5XQ-hpAXtzMb
- Scripts https://bio3d.colorado.edu/SerialEM/Script depository
- Lectures Wim Hagen EMBL https://www.youtube.com/watch?v=yqsUeOIg-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/serialem
- Microscopy and Microanalysis Short Course https://www.microscopy.org/MandM/2020/program/short_courses.cfm

The End



https://weheartit.com/entry/329040315