



UMass Cryo-EM Documentation

Release 1.2

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Mar 10, 2024

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Date-created 2017-2-13

Last-updated 2023-7-10

This is list of Frequently Asked Questions about Umass Cryo-EM. Some questions are technical, while others are more general.

1.1 How do I ...

1.1.1 ... get access to your facility?

We are a core facility and open to everyone including industry customers.

What is the logistics? Normally, you can start inquiry via email or phone call. If our facility is suitable for your projects, we will ask you to submit electronic forms for sample submission and request of instruments. The sample submission form is required by bio-safety office, for any sample coming to Umass Chan Med Sch campus. After we receive your submitted forms and things seem to be in order, our administration will send you a quote for you to obtain a PO for the work or/and instrument time. And we usually can schedule time for you shortly. Our waiting time is usually long, so it is good to plan early.

We have price rate policy for each year. It is not published on the web page, but is available upon request.

1.1.2 ... get to your facility location?

Please follow this site for main direction and the campus map - <https://www.umassmed.edu/about/directions/>

We are located at Medical School Building - A floor SA-106. You enter the “SCHOOLS” building from “medical school lobby” indicated on the campus map. After exiting elevator at A floor, turn left. Half way down the hallway, you will see a sign for “CryoEM Core Facility”. Call facility office # (774)455-4049 if you need help.

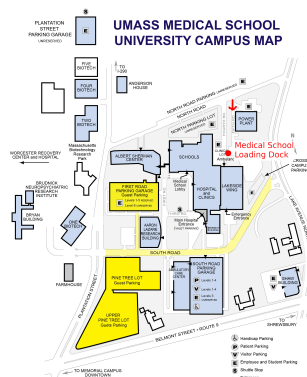
For parking, you should use “South Road Parking Garage” on South Road, the opposite side of two flags.

For packaging delivery by local carrier, the drop-off location should be

<https://goo.gl/maps/o4gKm9LQic3XybGG7>

It is the loading dock of Medical School Building Receiving. It is on Fifth Rd, right off North Rd. Please see the indication of dropping location (red) in the campus map below.

Fig.1 Campus Map with Dropping Location indicated in Red



For for nearby hotel to stay, there are a couple of possibilities within walking distance.

- 1) <https://www.beechwoodhotel.com/>
- 2) <https://www.marriott.com/hotels/travel/bosri-residence-inn-worcester/?scid=bb1a189a-fec3-4d19-a255-54ba596febe2>

1.1.3 ... display the screened data on my own computer locally?

The data for screening is usually at three kinds.

1. Low Mag Montage (LMM) map (grid atlas), usually taken at mag from ~50X to ~150X. The data itself is in MRC 16-bit sign integer format. It is a MRC stack file containing about ~62 pieces if at 46X.
2. Medium Mag Montage (LMM) maps (square atlas) or some single shots. This is usually taken at lower range of M on Talos, such as 1750X. It is also MRC stack file.
3. High mag shots, usually taken at 22,000X or 28,000X. It is MRC stack file, each section is from an exposure. If K2 camera frame mode is used, the section is usually a single image from aligned movie stacks in-fly.

Note: The MRC files we saved here usually have filename extension as **mrc**, **st** (for stack file) and **map**. Regardless the extensions, they are all simply MRC files.

All the three kinds of data can be easily viewed using IMOD. For Windows, if you don't want to install full IMOD package, a stripped version of display portion called "Windows3dmod" can be installed - from <http://bio3d.colorado.edu/ftp/Windows3dmod/>. For all the other platforms including Windows, a complete IMOD software package is available. IMOD User Guide can be found in <http://bio3d.colorado.edu/imod/doc/guide.html>.

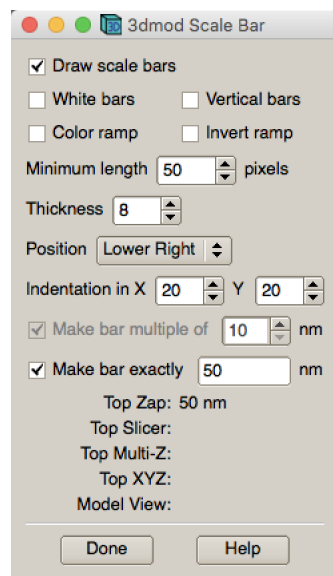
Note:

- Since September 2017, most of screening images are also saved into JPG format at the same time when MRC files are saved. This gives you a quick feedback for your sample conditions. The small file size makes it easy for us to upload to DropBox to share with users.
 - After March 2018, all JPG files saved are true JPG instead of JPG compressed TIFF as before. They can be opened by almost any standard image software including ImageJ and Photoshop.
-

1.1.4 ... insert a scale bar into the screened image?

We now normally send screened data with JPG files with scale bar in the images. If not, you can insert the scale bar following steps below:

1. Open a MRC or JPG image with IMOD.
2. IMOD - Edit - Scale Bar ...



3. With proper scale bar displayed, press SHIFT+S. It will save a jpeg file with scale bar inserted.
4. Click “Done” to dismiss the setup window.

1.1.5 ... mount the data hard drive I received from you?

The hard drive for data should be in its original filesystem which is normally Windows NTFS. The interface on the hard drive should be USB3. It is in my mind that the drive should be most compatible possible.

If you plug the hard drive onto a Windows or Mac computer, the volume should automatically show up. And you can copy data out from the volume directly.

If you want to mount the hard drive directly onto a Linux computer, you have to connect and mount it manually.

After plugging the hard drive to USB port (USB3 preferred) on Linux computer, you should be able to see lines similar to these from *dmesg* command output on Linux computer.

```
[334449.716558] usb 4-1: new SuperSpeed USB device number 2 using xhci_hcd
[334449.728460] usb 4-1: New USB device found, idVendor=0bc2, idProduct=ab34
[334449.728482] usb 4-1: New USB device strings: Mfr=2, Product=3, SerialNumber=1
[334449.728485] usb 4-1: Product: Backup+ Desk
[334449.728487] usb 4-1: Manufacturer: Seagate
[334449.728489] usb 4-1: SerialNumber: NA7H29DX
[334449.749996] usbcore: registered new interface driver usb-storage
[334449.752139] scsi host6: uas
[334449.752539] scsi 6:0:0:0: Direct-Access      Seagate  Backup+ Desk      040B PQ: 0_
↪ANSI: 6
[334449.752586] usbcore: registered new interface driver uas
[334449.768013] sd 6:0:0:0: [sdc] Spinning up disk...
[334449.768023] sd 6:0:0:0: Attached scsi generic sg3 type 0
```

From this, you can see the logic volume is assigned to *sd*c.

On RedHat/RHEL7, CentOS 7 and Scientific Linux 7 and possibly later versions of Linux flavors, the NTFS filesystem is directly supported. For older version of Linux, you might have to install *ntfs-3g* package first. Therefore, you can mount the volume easily with a mounting command as below.

```
$ sudo mount -t ntfs /dev/sdc2 /mnt
```

This command should not give you errors. After the command, you should be able to see the volume is mounted using *df* output

```
/dev/sdc2          4883638268  1418392 4882219876    1% /mnt
```

and you should see a few more lines in *dmesg* output like this:

```
[334450.768547] .....ready
[334465.784580] sd 6:0:0:0: [sd] 9767541167 512-byte logical blocks: (5.00 TB/4.54
↪TiB)
[334465.784585] sd 6:0:0:0: [sd] 2048-byte physical blocks
[334465.817288] sd 6:0:0:0: [sd] Write Protect is off
[334465.817294] sd 6:0:0:0: [sd] Mode Sense: 4f 00 00 00
[334465.817451] sd 6:0:0:0: [sd] Write cache: enabled, read cache: enabled, doesn't
↪support DPO or FUA
[334466.214227]  sdc: sdc1 sdc2
[334466.215286] sd 6:0:0:0: [sd] Attached SCSI disk
[334626.393838]  sdc: sdc1 sdc2
```

1.1.6 ... know the image conditions of the data collected on your system?

1. From *Frames.md* file. This is a metadata file to record all the conditions for each frame stack file collected. It contains the most complete information including total dose, stage positions, frame dose, frame numbers and navigator label for this exposure.

A typical section of *Frames.md* file is as following:

```
[FrameSet = 0]
TiltAngle = 0.00249969
StagePosition = 24.2868 -41.113
StageZ = -29.8365
Magnification = 105000
Intensity = 0.114429
ExposureDose = 34.9645
PixelSpacing = 0.694291
SpotSize = 8
Defocus = -3.33245
ImageShift = -2.22045e-016 -7.77156e-016
RotationAngle = -94.0936
ExposureTime = 7
Binning = 0.5
CameraIndex = 1
DividedBy2 = 0
MagIndex = 31
CountsPerElectron = 35.3
TargetDefocus = -2.2
SubFramePath = X:\Anna_20171223\1015B_g1_0000_Dec23_13.20.21.tif
NumSubFrames = 35
```

(continues on next page)

(continued from previous page)

```
FrameDosesAndNumber = 0.99898 35
DateTime = 23-Dec-17 13:20:32
NavigatorLabel = 84-1
```

You might be interested only in total dose, pixelsize, frame dose and frame numbers etc., but it contains fairly complete information.

2. From *Setup.png* - an image file. This is snapshot for Camera Setup Dialog window and with frame data setup window. This image shows total dose, dose rate on camera, frame numbers, frame time etc..
3. From image header. You can get header information for MRC and TIFF image stack by an IMOD program *header*:

```
$ header image-stack.mrc
```

1.1.7 ... use the defect file for MotionCor2?

According to SerialEM helpfile - http://bio3d.colorado.edu/SerialEM/hlp/html/about_camera.htm, here are the step to convert defect map that MotionCor2 needs.

Finally, if you want to run MotionCor2 directly on the un-normalized data, you should give it a defect map file as well as the gain reference file. You can make a defect map from the text file with 'clip defect' in IMOD 4.10.7 or higher:

```
clip defect -D defects...txt fileWithFrames defects...mrc
```

where the "fileWithFrames" is used only to set the size of the output and can be any file of the right X and Y size. To make a compressed TIFF file, which will be much smaller, use:

```
clip defect -D defects...txt -f tif fileWithFrames defects...tif
```

1.2 What is ...

1.2.1 ... service and charging details about your screening service?

Screening result usually includes

1. Low Mag Montage (LMM) maps at a LM range such as 34X for entire grid atlas
2. Medium Mag Montage (MMM) maps at about 2000X for a few promising meshes
3. final mag shots for 10-20 holes. We also provide JPEG format too for convenient cloud reviewing.

For academic, the screening mostly happens on Talos. We split Talos 24 hours into two session - daytime and evening. If you have more than 8 grids, we will simply regard as daytime session. Less than 6, will be charged by per grid, which is less expensive than being regarded as a daytime session. So you may only screen 2-3 grids, for example, and we won't charge by session in that case. We are very flexible at this and take the cost of customer into consideration.

The most common style to screen is to load 8-9 grids and start to screen, by 5 or 6PM of that day, one of the good conditions hopefully is located and we extend into full day (24 hours) session by collecting on the good grid into next morning 9 AM. We could get as many as 2000 - 4000 movie stacks and possibly even more. These are high quality shots on Gatan K3 camera. People get about 3A resolution structures on our Talos with similar setup.

Please feel free to ask if you have any question, at any time.

1.2.2 ... the data I will receive from your facility after imaging?

You received mainly three or four folders as below:

rawTIFF folder:

- *.tiff - compressed raw image stack containing multiple image frames, not gain normalized
- *.pcm - IMOD python command file for frame alignment using IMOD program
- *.mdoc - adoc file that contains all the imaging condition information
- *.log - it is log file of aligning result (after run pcm file to align frames)

alignedMRC folder:

- *_ali.mrc files - those are aligned of multiple movie frames, they are single images not movie frames

alignedJPG folder:

- JPG images of the aligned MRCs together with power spectrum. These are for visually checking image quality and sample condition.

SerialEM-Maps folder:

- all the control files for SerialEM software running for the session, it contains useful information including map montages and navigator files etc.. It is part of the session data, very useful to track where images are acquired, ice conditions etc., but less useful for end user to process the data.

1.2.3 ... the Cs value I should use for CTF calculation for Talos and Krios?

2.7mm for both Talos Arctica and Titan Krios.

1.2.4 ... the method I can get my data after collected at your facility?

Mass Chan Med School has firewall and VPN in place. There is no way to “pull” data from our storage without establishing VPN first. However, outbound traffic - “push” is possible. There are a few ways we can send data to you.

- At Harvard Medical School, some labs ask SBGrids folks to setup a DropBox like account for their lab. With specific command, we can push data directly from our storage to HMS special DropBox location.
- If you setup a user account on a Linux box for us, we can transfer data via sftp or via rsync over SFTP protocol. We can also use your personal account without knowing your password but using SSH keys. We provide our public key to you and you put it in `./ssh/known_hosts`, and we can establish connection using our private key at our end. You can remove that line to disable the possibility of connection.
- The data can be also sent to you after copying onto a portable HDD drive with USB3 interface. Default NTFS filesystem coming with most of the HDD is usually working fine.
- AWS. Cloud is becoming reasonable and attractive way to store and compute data. If you setup AWS S3 bucket, and share with us the keyID and secret key, we can upload onto AWS S3 bucket easily. The overall speed is not super fast, but fairly decent and faster than most of SFTP transfer to regional institutions. For large filesize like a typical raw TIFF stack file about 300-400 MB, it can reach about ~68 MB/s. For external institutions who already have AWS, we recommend to use this way.
- Google Cloud Services. All needed is a key file to upload files into google bucket. “-m” option can make transfer speed as fast as ~460 MiB/s on our setup.

1.2.5 ... What is difference between dose and dose rate? What condition should I use?

They are two different things, but related by the magnification of microscope.

Normally, dose means the total electrons hitting the specimen in a unit area. It usually has unit like $e^-/\text{\AA}^2$.

Dose rate means how strong the beam is, it is how many electrons hitting in one physical pixel area on the detector sensor for a unit time period. It usually has a unit like **primary electrons/unbinned pixel/second**. A proper dose rate is required for optimal performance of a camera.

Under a giving beam condition, your dose rate is fixed, you can change exposure time to obtain target total dose on specimen. Therefore, we should always determine the proper dose rate first.

TRAINING CONTENTS AND MATERIALS

2.1 Cryo-EM Training - Basic (level I & II)

(Each session is set 2 - 2.5 hours)

Author Chen Xu

Contact <chen.xu@umassmed.edu>

Date 2016-07-27

Goal The goal of the training we offer is to help a new user coming to EM field to learn some basic knowledge and skill of Cryo-EM. Hopefully, at the end of these two levels of training sessions, you will be able to screen your negative stained specimen and cryo grids on a TEM scope without too much difficult.

Requirement You are not required to have experience with TEM operation. Certainly, we could progress faster if you have some basic knowledge about a TEM. The only requirement to you, the fresh trainee, is to have a spirit of commitment. You need to be determined to learn this new technique. If you think to have your data collected while in your training session and have your project done half way, you should not attend the training session that we offer. You will be wasting your own time, let alone ours. Usually, if an user doesn't take good note during training session and doesn't come back to practise on microscope, that is a good hint that the user is *not* serious at it. Operating a transmission electron microscope is very much like driving a car - you need to be serious and to practise if you really want to learn it!

This document lists the training contents that these two-level, 8 sessions will cover.

2.1.1 Level I - Basic TEM Operation (4 sessions)

Session I - 1

- Prepare continuous carbon film grid
- Introduction to TEM; brief history; compare to X-ray as beam source
- Basic TEM Operation on Tecnai G2 system
- User Interface & Vacuum system

Session I - 2

- Specimen rod insertion and retraction
- Tuning microscope (Eucentricity, gun alignment, filament saturation, beam-tilt pivot points, rotation center)
- C2 aperture centering, C2 lens stigmatism; Obj. aperture centering, Obj lens stigmatism
- Correct side of beam spreading
- Demo and introduce CTF concept

Session I - 3

- Negative Stain sample preparation
- Refreshing microscope tuning procedure
- Introduce digital cameras - hardware component, background preparation, pixelsize at levels of detector and specimen
- Introduce DQE, and latest status of direct electron detectors
- Negative stain specimen imaging using CCD

Session I - 4

- Supervising practice of users for negative stain and scope operation
- Assist users' operation and answer questions
- Demo and explain cryo-cycle procedure to condition the column

2.1.2 Level II - Cryo TEM Operation (4 sessions)

Session II - 1

- Prepare side-entry cryo holder, explain dry pumping station - warm-up & zeolite cycle
- Demo and explain FEI Virobot operation
- Explain holey grid pre-treatment - bake, wash & electron beam irradiation
- Demo full procedure for Cryo freezing EM grids
- explain the Ethane tank operation

Session II - 2

- Low Dose TEM operation, explain low dose concept and setup
- Introduce and explain shutter control on TEM system
- CCD imaging with Tecnai Low Dose software

Session II - 3

- Cryo grid loading to cryo holder on transfer station
- Cryo holder insertion & retraction, explain airlock pumping mechanism
- Demo and explain vacuum condition and how to quiet LN2 bubbling
- Go through whole process from sample loading to Low Dose imaging
- Explain ice condition

Session II - 4

- Supervising Cryo Session for user practice
- Debugging ice condition, cryo-cycle
- Supervising warm-up and baking cryo holder

2.2 SerialEM Training - Basic, Tomography, Single Particle, Advanced

(Each session is set 2 - 2.5 hours)

Author Chen Xu

Contact <chen.xu@umassmed.edu>

Date 2016-08-12

Goal This is to provide hands-on training on SerialEM. I will teach basic functions of the program. And I will teach how to use the powerful program for electron tomography data collection, and for single particle application as well.

Requirement You are required to have basic knowledge for TEM operation, preferred for Tecnai/Titan/Talos system. You should be able to operate scope independently to get a properly focused image. You are *not* required, however, to have pre-knowledge of SerialEM itself.

This document lists the training contents that are covered in the four categories - basics, tomography, single particle and advanced topic.

Note: If you have any thought and suggestion to improvement the training, I love to hear them.

2.2.1 SerialEM - Basics (3 sessions)

Session 1

- Introduction to SerialEM, launch and exit the program. Explain system files and user setting file.
- Explain how SerialEM controls microscope and camera, and its relationship with microscope and camera control software interfaces.
- Explain SerialEM interface and layout - control panels and menus
- Camera setup, how to acquire an image from SerialEM interface
- Demo basic function such as Eucentricity (and shift beam to tilting axis)

Session 2

- Refresh Tune-scope procedure
- Prepare gain reference file in SerialEM
- Explain image buffers and how to save buffer image into file, explain MRC stack and modes
- Image Shift and Stage Shift
- Eucentricity, Autofocus and montaging
- Explain pixelsize and dose/dose rate.

Session 3

- Introduce Navigator - navigater items: map, point and polygon
- Demo full grid montage, Medium Map Montage (Using Image Shift and Stage Shift)
- Realign To Nav Item, demo and explain
- demo acquire map or image at multiple points ...
- introduce script/macro

2.2.2 SerialEM - Tomography (5 sessions)

Session 1

- demo and explain how to collect a tilting series
- explain cooking resin specimen, defocus and other parameters (mag, binning etc.)
- explain proper sample preparation for platic sections (thickness, gold beads etc.)

Session 2

- Supervise user to acquire MMM maps and collect a tilting series, answer questions and comment on the condition used.
- Setup montage tilting series
- Setup batch mode for multiple locations

Session 3

- demo and explain dual axis tomography data collection
- demo how to rotate grid 90 degree and find the same location (registration transformation)

Session 4

- Low dose mode setup for Cryo Tomography applications
- refresh cryo sample and holder handling

Session 5

- LMM, MMM in low dose mode
- bi-directional tilting series collection
- batch mode for cryo data collection.

2.2.3 SerialEM - Single Particle (5 sessions)

Session 1

- positioning X,Y, Image Shift and Stage Shift, backlash
- dragging to a new position, with Script/Macro
- positioning for preselected multiple location using `RealignToItem` and `ZeroIS`.
- positioning Z, using stage and using tilted beam image pair
- demo and explain scripts `Z_byG` and `Z_byV`
- demo center beam using keyboard and script

Session 2

- demo simple script `LD`, and explain actions
- refine hole centering using template
- draw grid point - manual picking and using hole finder
- introduce script `LD-group` and explain the ideas

Session 3

- K2/3 specific - image format(MRC, TIFF), Compression, 4-bit special for Super-res frames
- Asynchronize mode for K2/3 imaging, separate gain reference from raw image frame stack
- Consideration for dose - total dose, dose per frame, frame time
- In-fly frame aligning option

Session 4

- go through whole single particle procedure
- LMM, LD setup, MMM with “Z_byV2”, draw grid point, prepare hole template
- run LD-group

Session 5

- supervising user practise session to go through all the steps
- answer question
- explain script command to limit defocus changing range

2.2.4 SerialEM - Advanced Topics (3 sessions)

Session 1

- SerialEM installation and Calibration

Session 2

- Setup multiple accounts
- Setup multiple system files
- Setup executables for production and tests
- Setup Dummy instance to pick target holes while main instance is busy collecting

Session 3

- Explain SerialEM scripting interface and major functions
- Explain example script to control LN₂refilling and obtain K2/3 hardware dark background
- Setup email alert system
- in-fly aligning frames using standalone GPU server computer

INSTRUCTIONS, MANUALS AND PROTOCOLS

3.1 CM120 Operation Instruction

Author Chen Xu

Contact <chen.xu@umassmed.edu>

Date-created Aug 8, 2016

Date-Updated Oct 3, 2021

Abstract This document tries to list steps and procedures for a typical daily operation on CM120. You can use it as guidebook to help you when you are sitting with CM120, especially if you are a new user. I here assume you are already familiar with scope interface, what the knobs and buttons do etc.. You might use the section titles as quick bullet points, but the explanation inside each sections are supposed to be useful and informative.

If you have suggestion how to improve this document to make it more useful, please feel free to let me know. Thank you!

Note: all the turning knobs on the panel and push buttons are marked like `button`.

3.1.1 Check log book to see if there have been any problems

It is always a good idea to check the LOG book. You can find useful information listed there, such as:

- If there have been any recent problems with the scope.
- The conditions that were used last, especially the filament saturation.
- Any special note that last user wants you to know.

3.1.2 Check vacuum status

The status must be Ready before you can operate the scope. If not, you should ask for help and report it to the manager. Usually, if the vacuum is not ready, it is due to one of the following reasons:

- The scope is malfunctioning.
- The air pressure is not within a good range(the building compressed air may be down?).
- The cooling water is off.

Vacuum being Ready means:

- Top line of vacuum page shows **ready**.
- $P3 < 50$, $IGP < 26$ (normally, they are shown 0, 5).
- LEDs for UVAC and HiVAC are lit.

3.1.3 Add Liquid Nitrogen to the BIG anti-contaminator dewar

LN2 in the cold trap dewar is necessary for fast vacuum recovery. It is very useful, especially when you need to change grids and/or do a cryo session. However, if you are working with a negative stain or plastic section - a dry grid, the scope can still run without LN2 cold trap. Unless you are running automatic, long overnight session, you should always use it.

Tip: Generally, when you first put LN2 dewar to its stand, you want to be SLOW, or the strong evaporation will make LN2 spilling.

3.1.4 Apply High Tension

Turn High Tension (H.T.) on if it is off by pressing the `H.T.` button on the panel. From `Parameter` page, set it to 120kV or the voltage you want.

3.1.5 Turn filament on

It is recommended to turn the filament on while on the `configuration` page where the actual filament current number is shown and the `limit` can be checked(highlighted).

3.1.6 Check saturation and gun tilt, and then saturate the filament

The procedure is as following:

- Desaturate the filament about 2-3 clicks.
- Press `Align` button, highlight `Gun Tilt`.
- Adjust the `multi-function X, Y` to get the best shape for the tip and maximum intensity as well.
- Bring the filament current to saturation.

3.1.7 Check C2 aperture mechanical position and C2 stigmatism

Steps to adjust the C2 aperture mechanical position are:

- Make sure the C2 aperture is in.
- At around 5kX, cross the beam first and center beam using track ball on the left panel, then spread the beam until its diameter is close to 5cm ring on the screen. Adjust mechanical screws X, Y on C2 aperture to center the beam.
- Repeat this process 2-3 times.
- At high mag ~30kX or higher, change the Intensity of beam through the crossover and adjust the screws X, Y to make the beam symmetric when spreading out.

There are two ways to check and adjust the C2 stigmatism:

- When the filament is desaturated, press Stig button and select Cond, then adjust Multi-function X, Y so that the details in the filament shadowing image can be clearest and sharpest.
- When changing the beam intensity, use the Multi-function X, Y to adjust the beam into symmetric and round shape, i.e. not elliptical.

Note: For this purpose, don't pay attention to the beam shape when beam is exactly at cross point, as that more reflects the shape of the crystal tip, rather than stigmatism. You want to make beam *round* when spread out.

3.1.8 Check specimen holder & load grid

Important: This is important. If you see any problem with any of the holders, report it to the manager immediately. Otherwise, you could be the one held accountable for the damage.

Several details about the holder must be checked carefully before use:

1. Overall shape is good, and there is not obvious damage.
2. Make sure there is no crack or any other damage on the O-ring. If you do see a damage, like a cut etc., ask manager to replace it for you. Check if there any dirt or fibril on the O-ring. You might want to clean it gently with alcohol and slightly re-grease it. Do not over-grease. The main function of the grease is to lubricate.
3. Gently secure the specimen grid on its position. Use the tool pin to open and close the clamping device.

3.1.9 Insert specimen holder into column

Warning: Be careful! Only at this stage, you might damage the scope or specimen holder mechanically. Be sure that you understand what you are doing. Should you feel any confusion about this procedure, please stop and ask for help.

Note: For the sake of filament crystal, it is REQUIRED to turn down the filament to 10 before inserting the specimen rod. That way, in case IGP shoots high, there will be no substantial damage to the LaB6 tip crystal. In general, filament should be kept at 10 or completely off until IGP recovers to below 26.

The procedure to insert a room temperature specimen rod is below:

1. define airlock pumping time as 60 seconds, from Vacuum - Cryo page.
2. Reset stage tilt angle to 0 if it is not.
3. Insert rod in, with the Pin at 3’oclock position.
4. As soon as it reach the end, rotate rod CLOCKWISE with some pushing force so that the pin slides into the locking groove at 5’oclock position. You should feel the rod goes “in” about 8mm.
5. Wait until the red LED on the stage disappears. Dismiss the “non-standard” flushing message on the screen by pressing `Reset` button at lower left corner of the screen display.
6. Turn rod Count-Clockwise until pin is at 12’oclock position, while watching IGP reading. You should adjust your rotating speed to keep $IGP < 40$.

3.1.10 Adjust the specimen height to the eucentric height

Eucentricity is a fixed reference point in a scope. It is the intercross point of stage axis and column axis. We want to observe our specimen grid at this height level so that the actual magnification doesn’t differ much from day to day. And scope is designed to perform better when specimen is at such height. It is good to have the habit to always adjust specimen to eucentric height after rod insertion. On CM120, the procedure is as following:

1. Have beam seen on large screen, at ~3000X, and find a feature on the grid.
2. Rotate stage back and forth by pressing `CompuStage - A-wobbler`.
3. Adjust joystick Z to minimize the shifting of the feature.

3.1.11 Check Beam Tilt Pivot Point X, Y

Note: The prerequisites for Pivot-Point is specimen being at eucentric height and objective being preceisly at focus level. **The order is important here.**

1. Make sure the specimen is at eucentric height.
2. Take out Obj. aperture.
3. Press button `Align - Direct Alignment - Beam Tilt Pivot X`.
4. Merge image feature by adjusting `Focus` knob. This is to precisely focus the image.
5. Merge beam using `Multi-fcuntion` knobs.
6. Repeat the last two steps for Beam Tilt Pivot Y.
7. Press `Align` button again to exit.

3.1.12 Check voltage and current rotation centers

This step is to align the beam to make it parallel to the axis of the column. The purpose of this step is to make beam to hit specimen perpendicularly. A coma is not a good thing, as it generates some phase error to the data.

The procedure is below.

1. Press `Align` button and select `Rotation Center`.
2. Select `Voltage` or `Current` from the same page.
3. Adjust the `Multi-function Knobs` to let the wobbling be symmetrical around the center of the beam. The feature at very center of the large screen has minimum shift.

If you perform this with `Obj` aperture in, then re-check the centering of objective aperture.

The step size button on `Focus` is used to control the amplitude of the beam wobbling.

The steps used here only give “roughly” parallel beam to the axis. If you need very accurate “0” tilt beam, a different alignment procedure - Coma-Free is needed.

3.1.13 Put in objective aperture and center it

It is important to know what size of the `obj.` aperture you are using. You don’t want to use too small size to actual cut off useful high resolution signal. Meantime, you don’t want to use the aperture size too large, as the non-usable high resolution beam becomes noise to your image. This reduces signal to noise ration unnecessarily.

The position of the aperture could affect the `obj.` lens stigmatism. Therefore, you want to do this step before you finally check `Obj` lens stigmatism.

Here are the steps to insert and center Objective lens aperture:

1. Make sure the large screen is down, to prevent CCD from damage.
2. Switch to diffraction mode by pressing the diffraction `D` button.
3. Adjust camera length to ~1m using magnification knob.
4. Adjust the `Intensity` and `Defocus` knobs to see the shape edge of the `obj.` aperture.
5. Adjust the related mechanical screws on aperture holder to choose the proper size of the aperture and center it to the central beam on diffraction pattern.
6. Switch back to image mode by pressing `D` button again.

3.1.14 Check Objective Lens stigmatism

The `obj.` stigmatism should be corrected as much as possible, and it should be checked for every netative stain low-dose image that you are taking, as staining material might change field in local area. This is a bit hard by hand. Even with latest version of SerialEW, this can be done by software, it is still not easy and time efficient. However, slight stigmatized image is not critically bad, as it can be corrected as part of CTF correction computationally.

Here are steps to correct `Obj` lens stigmatism, manually:

1. Go to a relatively high mag., such as 100,000X, and focus the image.
2. If possible, acquire continous CCD image with live FFT so Thon rings can be seen.
3. Press the `Stig` button, highlight `Obj`, and select proper stepsize.
4. Adjust the stigmatism using `Multi-function X, Y` until it becomes minimum at all defocus levels. (It shows up more at close to focus.)

3.1.15 Low-Dose Setup

Magnification setup for three modes

Here are some typical magnification setup for Low-Dose condition.

Table 1: A Typical Low-Dose Setup

Mode	Mag. (X)
Search	3000
Focus	60,000
Record	60,000

One might use diffraction mode to `Search`, which usually gives better contrast but the “image” might be distorted. The final magnification depends on target pixel size on image. If possible, use the same magnification for both `Focus` and `Record` to eliminate the defocus offset between the two magnifications. The off-axis distance is usually about 1.5 - 2.0 microns.

Align an identified area under `Exposure` and `Search`

This step is to insure that what you see under low mag. (`Search` mode) will be the same area you get under imaging mag. Here is how I do it:

1. At `Exposure` mode, MECHANICALLY drive an identified spot to the center of the screen.
2. At `Search` mode (and usually in `Diffraction` mode also), using the `Multi-function` knobs to backtrack the identified spot at the center of the screen (electronically). This uses `Image Shift` or `Diffraction Shift` (when `Search` mode is set up in `Diff` mode) to “shift” image without actually moving the stage position.

Tip: You can use a corner of a mesh as the identified spot for a negative stain specimen or to use an ice burn mark in the cryo case.

3.1.16 Finishing Up

When you are done with your session, perform finishing up procedure.

- Specimen rod out.
- Reset Stage Position, X, Y, Z and A.
- Filament 0.
- H.T. OFF.
- Cryo-cycle, normally for 2-3 hours.
- Data display OFF.
- Display OFF.
- Log your session on logbook.

3.2 CCD Cameras on CM120

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Date_Created July 30, 2016

Last_Updated Oct. 3, 2021

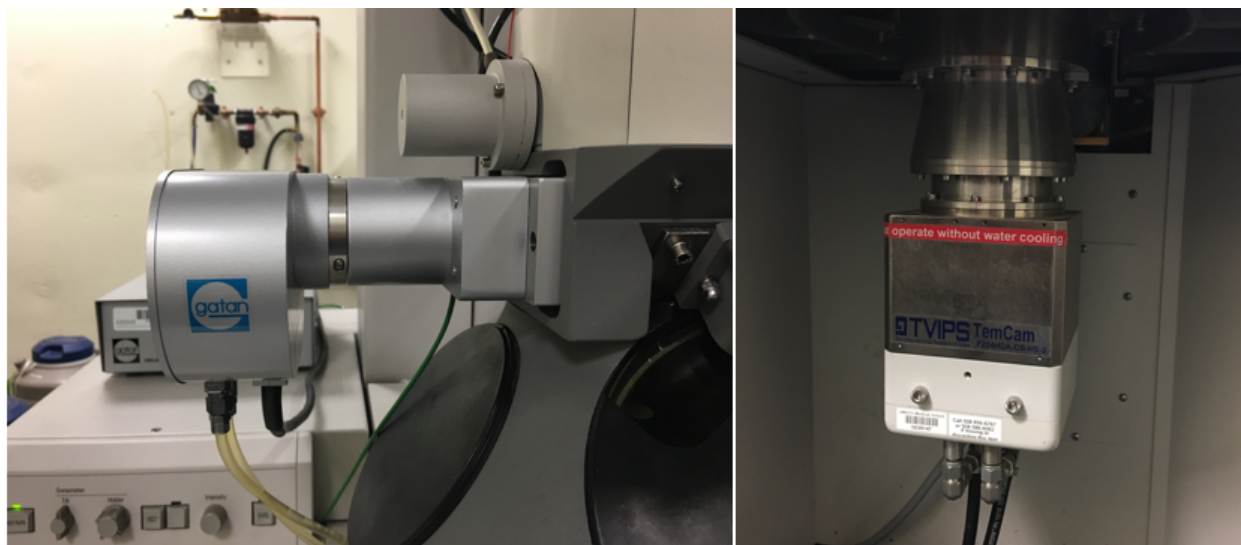
Abstract We no longer use film for TEM exposure anymore! Instead, we use digital camera. There are basically two types of digital cameras available on the market for TEM applications - Charge-Coupled Device (CCD) and Direct Electron Detector. On CM120, there are two cameras installed - Gatan Orius model 832 and Tietz TemCam 224-HD. They both are CCD type of cameras. In this document, I give information about basic properties of the cameras, how to use them with CM120 and specific technical note for why we need use them in such way.

It also lists pixelsize information for both cameras so you can decide which magnification you want to use for your final image. I also try to show you how to use SerialEM to control them.

3.2.1 Some Basic Properties of the Two Cameras

Here are images for the two cameras on the CM120.

Fig.1 Camera Heads of Gatan Orius & Tietz 224HD



The table below lists some basic properties of these two cameras.

Table.1 Some basic properties

Table 2: **Table.1 Some basic properties**

Property	Gatan Orius 832	Tietz TemCam 224-HD
Format	3768 x 2672	2048 x 2048
Physical PixelSize (m)	9.0	24.0
Digitization (bit)	14	16
Light Coupling Mechanism	Fiber Optic	Fiber Optic
Mounting Position	sided-mount	bottom-mount

3.2.2 Shutter Control - Background Information

Shutter control usually means TEM shutter control by hardware or software of digital camera. The purpose, in most cases, is to have no beam on camera except exposure period. Not all camera requires having TEM shutter control to get an image. For cryoEM applications where accurate dose is to be concerned, it makes a lot of sense to have shutter control. Fortunately, most of high-end digital cameras for TEM have TEM shutter control support.

The shutter discussed here is not any kind of mechanical shutter. Instead, it is electronic. If an alignment coil in TEM column is put to a “bad” value, then the beam disappears due to misalignment. We call it shutter closed. And we call it open when the good alignment coil current is restored. Therefore, this kind of electronic shutter can open and close very quickly.

An exposure on film requires no beam before film is pushed in to final location and stabilized; and it opens shutter to let beam shine on film to expose and then shutter is closed again when exposure finishes. On FEI microscope with Low Dose functionality, one can even define pre-expose time. In this case, two shutters are required to work together - first shutter above specimen opens to have beam pre-expose on sample for certain period of time, then second shutter below specimen opens to start exposure on film. Usually, the two alignment coils to be controlled as upper and lower shutters are Gun Upper coil and a Film Shutter coil next to projector lens. All this is done within FEI software control for when and which shutter is open or close. We usually call this internal shutter control.

To image with a digital camera, especially for a beam sensitive specimen, shutter control is therefore also needed. If configured correctly, when camera is inserted and large screen of TEM is lifted, the beam should be blanked. This shutter control is achieved by changing the same alignment coils using shutter cable(s) from camera controller. The shutter cable directly connects to TEM hardware so that when large screen is lifted, an extra voltage is sent to TEM coil to “screw up” a good alignment. Thus, beam is blanked. If we need to pre-expose our sample for whatever reason, then two cables will be needed - one for pre-specimen and one for post-specimen. This control is done by using external hardware. We normally call this “external” shutter control as it is not via TEM software.

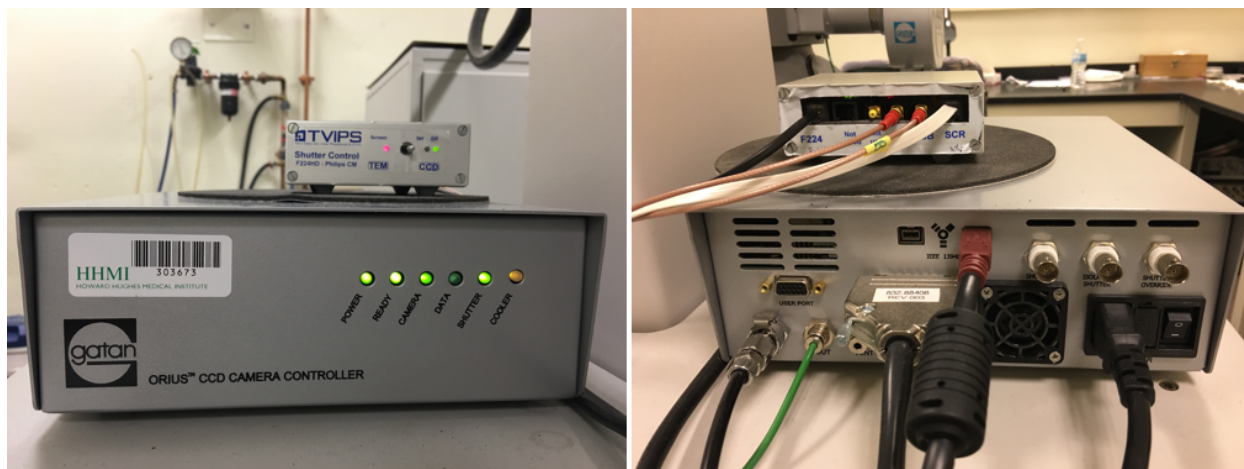
For convenience, we usually call the shutter of Gun Upper Coil “the beam blanker”; and Film Shutter Coil “the film shutter”. On modern models of FEI/TFS TEMs, there might be dedicated beam blanker coil for this purpose.

3.2.3 Shutter Control Setup for Orius 831 and TemCam 224HD

The Orius controller for 35mm port sided-mount camera has no shutter cable from the controller. Therefore, it doesn’t control the beam at all. It is possible to add the shutter control cable to the system, but for acquisition of an image, the TEM shutter control is optional for this camera. Of course, as you might imagine, this is not ideal for cryoEM application.

The picture below is the front and back of Orius camera controller, and Tietz camera shutterbox as well (small one on the top). You can see there is no cable hooked on Orius controller for shutter control.

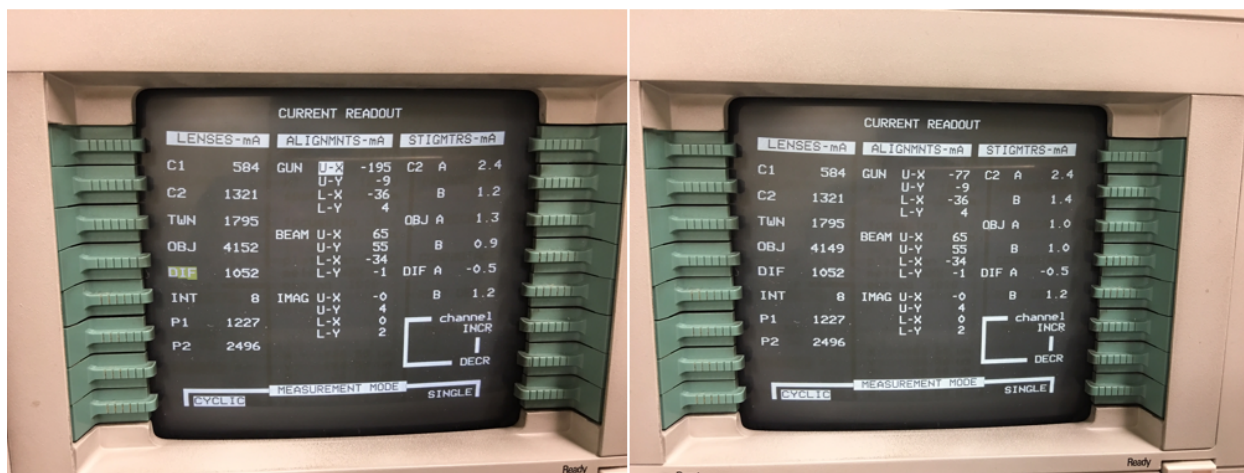
Figure 2. Front and Back of controller of Orius and shutterbox of TemCam 224-HD



For TemCam 224HD camera, there are shutter control cables from the controller box, shown in the image above. This is little box on the top of Orius controller. As explained in previous section, the two shutter control cables (in red) are directly connected to TEM hardware. And there is a flat ribbon-like cable, is also connected to TEM hardware. That is to control signal with large screen of TEM being Up or Down. To be more specific, when large screen is lifted Up, the signal from this “Screen” cable to controller box triggers a 5V signal being sent to scope via the two shutter control cables, so beam is blanked with large screen position as a switch.

The two cables with red connectors are labeled “BB” and “SH”, for Beam Blanker and Shutter respectively. BB cable is connected to Gun Upper Coil as explained in previous section. It can be confirmed from scope “CURRENT READOUT” page. Below is two screen snapshots for large screen being at Down and Up. You can see the Gun Upper Coil current values in these two situations. One can easily see the Gun U-X is changed from -77 to -195 when screen is lifted.

Figure 3. Gun Coil Current Changes When Large Screen Up (left) and Down (right)



When you lift the screen, you should also see beam disappearing suddenly.

Note: SH shutter is hooked to the film shutter coil, which is not among the coil currents displayed here. It is indeed working. One can confirm this by disconnecting BB cable - the beam still disappears when large screen is UP. According to the vendor, this shutterbox can not provide pre-exposure function. One will have to upgrade to shutterbox II for that.

Note: The dip switch on Tietz camera control box has to flip to right “CCD”. If it is on “TEM”, the shutter control

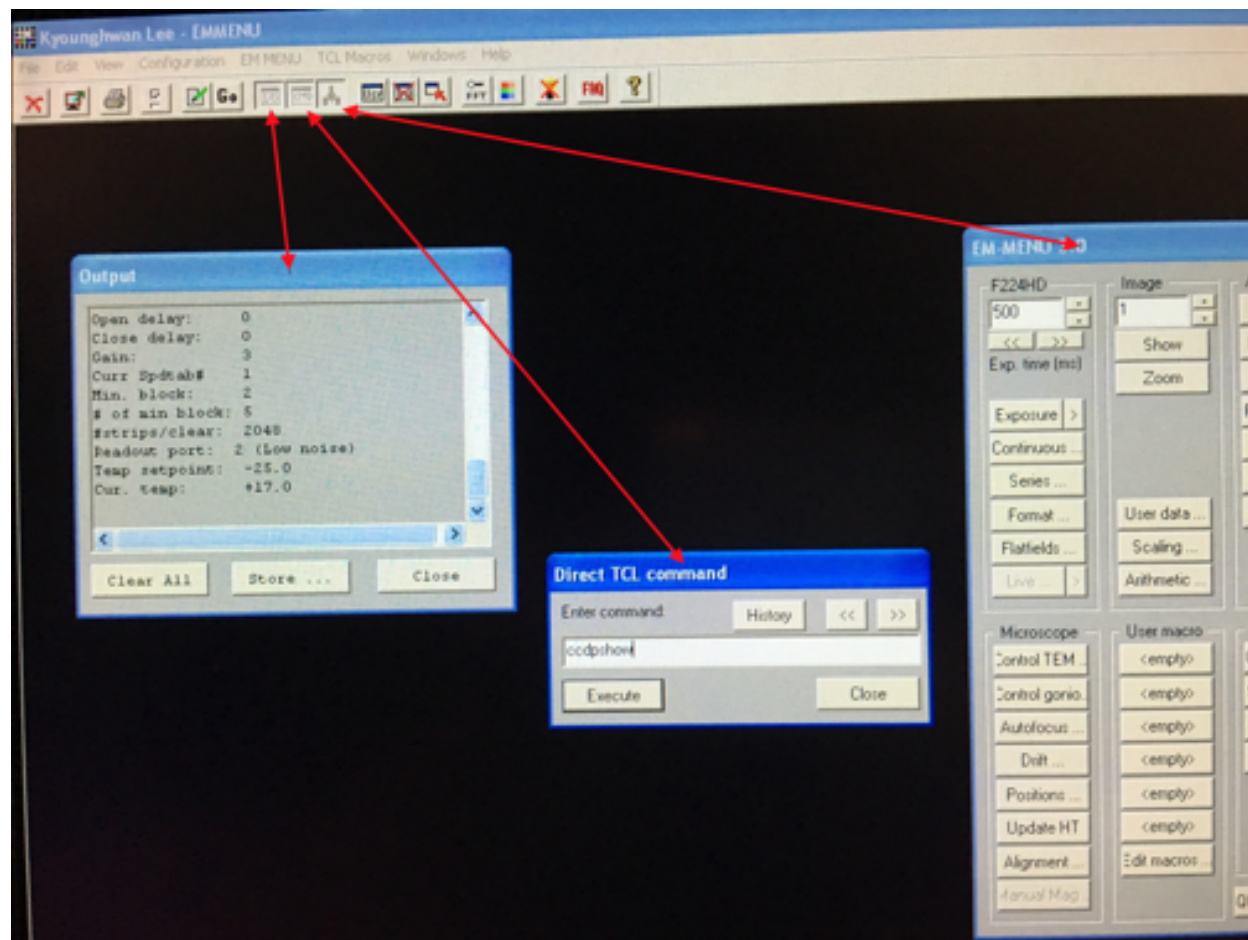
by Tietz CCD is completely off.

3.2.4 A Note about the Temperature

These both CCD cameras are water cooled. More strictly speaking, the dissipated heat from electronic cooling has to be taken away by water. Therefore, we must have cooling water running normally to run the CCD cameras. The set working temperature for Orius is 10°C, and it is -25°C for Tietz TemCam 224HD. The advantage to have lower temperature for CCD imaging is low thermal noise. In general, it is advisable to let CCD be cooled long enough before using the camera. In most labs, the CCD camera is simply left ON all the time until camera chamber is vented.

The temperature can be checked for each camera using their softwares. For Orius, from DigitalMicograph, one can check menu Camera - Temperature. On Tietz camera, one has to start up TCL interface and also EMMENU from TCL. After that, one has to open result log window and command window. From command window, type “ccdpslow” and then hit [Execute]. The temperature will show in result window.

Figure 4. command “ccdpslow” to get temperature for 224HD



3.2.5 Control both cameras using SerialEM

Each camera has its own controlling software. It is DigitaMicrograph (DM) for Orius and EM-MENU for Tietz. In order to have pixel size information, both software tries to communicate with CM120 via a single COM port. If you run start both softwares, one of them will complain for not being able to connect to COM port. You can still take images using either one, just you might not get scale bar for one camera.

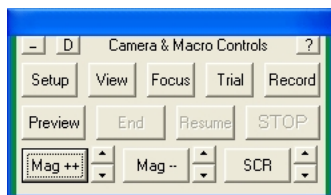
For image data that will be processed later, the scale bar is not that important, but the pixelsize is. And we don't have to always use its own native software to control CCD camera to get an image. One of the good alternative is SerialEM.

SerialEM is a program for TEM data collection for both single particle and tomography applications. It communicates to TEM and digital camera. Currently, it doesn't support CM120 without any hacking due to TEM software, but it controls both cameras we have just fine. One of the advantages with SerialEM is to be able to directly save data as stack file and MRC as image format.

SerialEM controls Gatan camera by a plugin dll file put in Gatan's plugin folder. In this case, DM has to be running. When controlling Tietz camera, it directly talks to the Tietz's registered dll modules. In this case, no Tietz software needs to be started. Therefore, you start DM first and then SerialEM and you are ready to go!

Since SerialEM doesn't talk to CM120 computer, we have to use fake mag and screen position to match the real situation on scope. This way, the image file saved on SerialEM will have valid and correctly pixelsize information in MRC header.

Figure 5. Camera Setup Window where one can change mag and screen position to match scope



To use it, you click on the three macro buttons Mag++, Mag-- and SCR to match current microscope state. That's it.

3.2.6 Electron Dose

A proper dose on sample for an exposure is important. If the total dose is too low, the signal to noise ratio is low. If the total dose is too high, the sample gets radiation damage during exposure. With the latest CMOS direct electron detector, we might be able to record a single exposure into many separable frames, but the idea for dose and radiation damage still holds.

On a real system, if we know the value how many counts generated from one primary electron under a specific accelerating voltage, and we can calculate the total dose (e/A^2) from the mean value of the counts in an image.

SerialEM is setup so that it reports the dose on specimen on the top of the image display, for every and each image.

3.2.7 Pixelsize Calibration for Both Cameras

The table below list pixelsize at specimen level for different scope magnifications. Please note that with large screen position as Up and Down, the CM120 TEM interface displays different mag values. There is no difference in term of lens current in this case - this is due to the recording media being at different "collecting" level along the projection. ScreenDown mag means at screen level and ScreenUp means at film level. We run Orius with screen Down and Tietz with Screen Up.

The magnifications display in TEM interface are nominal. For actual magnification or pixelsize at specimen for a CCD camera, careful calibration is needed. Below is the calibration table for both cameras.

Table 3: **Table.2 Pixelsizes**

Mag_Screen UP/DOWN (X)	Pixelsize_Orius(nm) bin=1	Pixelsize_Tietz(nm) bin=1
35 / 31	1134	464
45 / 40	882	361
60 / 54	661	270
80 / 71	496	203
110 / 97	361	148
150 / 135	265	108
190 / 170	209	85.4
260 / 230	153	62.4
340 / 300	117	47.7
450 / 400	87.3	36.1
600 / 530	66.1	27.0
800 / 710	49.6	20.3
1000 / 880	39.7	16.2
1400 / 1259	28.4	11.6
1800 / 1040	22.3	9.11
2300 / 2050	17.7	7.22
3000 / 2650	13.1	5.22
3800 / 3400	10.3	4.18
5000 / 4400	8.03	3.27
6300 / 5600	6.09	2.47
8000 / 7100	4.84	1.96
10000 / 8800	3.94	1.59
13000 / 11500	3.01	1.23
17000 / 15000	2.34	0.948
22000 / 19500	1.94	0.836
28000 / 25000	1.50	0.648
35000 / 31000	1.18	0.508
45000 / 40000	0.909	0.392
60000 / 53000	0.647	0.279
75000 / 66000	0.523	0.225
100000 / 88000	0.404	0.164
125000 / 110000	0.323	0.131
160000 / 140000	0.253	0.102
200000 / 175000	0.202	0.082
260000 / 230000	0.155	0.063
340000 / 300000	0.119	0.048
430000 / 380000	0.094	0.038
580000 / 510000	0.070	0.028
750000 / 660000	0.054	0.022

3.3 Making Graphene Oxide Grid

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Date-created 2018-3-10

Last-updated 2018-8-21

Abstract This protocol is based on MRC LMB one. We modify the protocol based on Xudong Wu's method at Harvard Medical School.

3.3.1 Materials and Equipment Used:

1. GO Solution (Sigma:763705, 2 mg/ml)
2. ddH₂O
3. Tweezers
4. Pipette (1 µl, 3 µl and 20 µl)
5. Tabletop centrifuge
6. Parafilm
7. Whatman filter paper No.1

3.3.2 Procedure:

1. Dilute GO solution 10x to 0.2 mg/mL with ddH₂O
2. Sonicate for 15s to break up aggregates
3. Spin for 30 seconds at 300 rcf to remove aggregates
4. Prepare a flat and clean working area (e.g. with parafilm)
5. Further dilute GO solution 10x to 0.02 mg/mL with ddH₂O
6. Glow discharge grids with carbon side up (easiGlow (PELCO, Discharge System); Quantifoil Cu 300 R1.2/1.3 120 seconds at 0.2 mBar and 15 mA)
7. Take up grids with anti-capillary tweezers
8. Place 1µl of the diluted GO solution (4) on the carbon side of the grid
9. Wait for the grid to dry out

Note:

1. This method is good for high coverage of GO, but maybe get multiple layers of GO on the grid.
 2. Discharging conditions are varied between grids and discharge system.
-

3.4 Single Particle Data Collection Using SerialEM

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Date created Oct 18, 2016

Last updated Apr 13, 2021

Abstract This document is to list step-by-step operations to perform single particle data collection using SerialEM. I often receive requests to provide script/macro for single particle data collection using SerialEM as control program. It is not very easy to explain that script/macro itself is only the small portion of whole operation steps. I realized that a brief but detailed protocol for whole process is perhaps more useful, specially for novice cryoEM users. It should be useful for more experience users as well as a quick checklist in case some step is forgotten. I wrote something similar at Brandeis EM webpage, but here I rewrite this to reflect newer hardware of microscope and camera, and with updated SerialEM scripts/macros.

A Krios with K2 Summit camera and FEI Ceta camera is the base hardware setup for this protocol.

Note: This doc is a working progress. If you have comment and suggestion, please let me know. Thank you!

3.4.1 Check Scope Condition and Perform Tuning

Before you commit large dataset time, it is always a good idea to check scope condition to make sure everything is good. Calm down and be patient! Here are a few things I usually check.

- Check Gun Lens, Extracting Voltage, High Tension are set at correct values.
- Stare for a few seconds at the focused beam at the highest SA mag, to see if the beam has good shape and there is no shaking or jumping.
- From Direct Alignment, do gun tilt, beam tilt PP, Coma-Free alignment if needed.
- Check Thon Ring at roughly the same condition (mag, dose) as your image condition. Make sure there is no obvious frequency cutoff, and Thon Ring reaches the resolution as in good condition.

3.4.2 Prepare Cameras

For K2 camera, perform full procedure to prepare backgrounds from DM interface. This include software and hardware backgrounds. The hardware background file is for processor to use, while the software gain reference files sit in K2 computer for final software image correction. I was told the software gain reference was more stable than hardware background, but not sure this is still the case. Any way, just perform the full procedure following DM steps.

- After preparing camera, take a single shot with proper dose rate (~5-10 e/pix/s) for 1 second with no specimen and do an FFT. The FFT should show clean background without strong center cross or lines. - For Ceta camera, do the same from FEI user interface.

3.4.3 Make Low Mag Montage (LMM) Map or Grid Atlas

It saves time with large area detector. Therefore, Ceta camera is probably better for this step.

- Select Ceta from Camera Control Setup
- Insert/load your cryo grids
- Set mag at ~87X, retract Obj Aperture
- Spread beam to cover whole Ceta camera area
- Start SerialEM if not yet
- Select Ceta from SerialEM camera control setup *and* FEI Camera ocx.
- Setup camera condition from SerialEM: Record (e.g. bin=4, exposure=0.4); a Record image gives proper counts (~2000)
- Navigator menu -> Open
- Navigator menu -> Montaging & Grids -> Setup Full Montage; define montage file to open
- Montage Control Panel -> Start
- Click “Yes” to make final overview of montage into a map
- Close the montage file

Tip: If you are not happy with the aligning of the pieces, you may check and uncheck boxes like “Treat sloopy...” and reload the map.

3.4.4 Setup Low Dose Condition

You should have known how to setup Low Dose condition already. Here are some tips.

- Turn on Low Dose Mode from SerialEM Low Dose control panel
- Setup R beam first so that dose on detector and on specimen are all good.
- Defocus offset 100um for View is usually a good start.
- Always cycle “area” (low dose mode) in one directional looped fashion, i.e., V-F-T-R-V...
- Using the same spotszise for all the areas (low dose modes) is a good idea.

3.4.5 Make Medium Mag Montage Maps

- select K2 camera from Camera Control Setup (from now on)
- add a polygon (a mesh) in LMM map
- add points for good meshes at center
- add one landmark such as a dirt point in LMM map
- take the landmark into View image (you may use FlowCam to move that feature into middle first.)
- while landmark point being current (highlighted), left click on the landmark in View image, a green cross will appear
- Navigator menu -> Shift to Marker -> Yes (this will change all the coordinates for all the navigator items)

- highlighting polygon item on navigator window, so it is currently selected
- Navigator menu -> Montaging & Grids -> Setup Polygon Montage -> Check “using View ...” in the dialog window -> define montage filename.
- Add flag “A” to all the interested mesh point items
- Navigator menu -> Acquire At Points ... -> Check “Eucentric Rough” in Pre-action and “Acquire Montage Map” in main action
- When finished, the MMM maps should be added to Navigator windows. You perhaps can close the montage file now.

3.4.6 Draw Grids Points for Each Mesh

For each of the MMM map, do the following steps to add group points.

- add a polygon item to exclude bad area
- add 5 point items to define grid geometry
- make any of the 5 items in the group is currently selected
- Navigator menu -> Montaging & Grids -> Set Group Size (10um is a good start)
- Navigator menu -> Montaging & Grids -> Check “Divide point into Groups”
- Navigator menu -> Montaging & Grids -> Add Grid Points -> give polygon item number -> Flag “A” for all

3.4.7 Test Main Script to Run

Lets load the script to script editor and try to run it.

Listing 1: LD-Group.txt

```
1 ScriptName LD-Group
2
3 ## when to perform CenterBeam and AutoFocus, and defocus range
4 groupOption = 1           # 1 = at group head, 0 = at every item
5 defLow = -1.0
6 defHigh = -2.5
7 step = 0.1
8
9 ## Drift control
10 driftControl = 1          # 1 = yes, 0 = no
11 limit = 3.0              # Angstroms
12
13 ## X,Y positioning
14 templateOption = 1        # 1 = to use a fixed ref, 0 = use dynamic one
15 refBuffer = P            # reference buffer for template image
16
17 ## Multi or single shot
18 multiShot = 1            # 1 = multishit, 0 = single shot
19
20 ##### no edit below #####
21 RealignToNavItem 0
22 ResetImageShift 2
23 If $templateOption == 1
24     Echo --- assuming you have a template image in buffer $refBuffer ---
```

(continues on next page)

(continued from previous page)

```

25 Else
26     Copy A $refBuffer          # use dynamic ref (whole image itself)
27 Endif
28 AcquireToMatchBuffer $refBuffer
29 AlignTo $refBuffer 0 1
30
31 ## turn ON drift protection if it's off so Autofocus can report drift
32 ReportUserSetting DriftProtection DP
33 If $DP == 0
34     SetUserSetting DriftProtection 1 1
35 Endif
36
37 ## center beam & focus
38 ReportGroupStatus gs          # 1 = group head, 0 = individual, 2 = group member
39 If $groupOption == 0
40     #AutoCenterBeam
41     CallFunction CycleTargetDefocus $defLow $defHigh $step
42     AutoFocus
43 Else
44     If $gs == 1 OR $gs == 0
45         #AutoCenterBeam
46         CallFunction CycleTargetDefocus $defLow $defHigh $step
47         AutoFocus
48     Else
49         Echo    group member, skip focusing...
50     Endif
51 Endif
52
53 ## drift                      # if reported drift is high, call drift control
54 If $driftControl == 1
55     ReportFocusDrift FD
56     If $FD > 0.09              # 0.09 reported here is close to real 2.0A/s.
57         CallFunction Drift $limit
58     Endif
59 Endif
60
61 ## shot
62 AdjustBeamTiltforIS          # needed for single shot, so leave it here regardless
63 If multiShot == 1
64     MultipleRecords
65 Elseif multiShot == 0
66     Record
67 Endif
68
69 ## post-exposure
70 RefineZLP 30                  # refine ZLP every 30 minutes
71
72 ##### Functions
73 Function CycleTargetDefocus 3 0 defLow defHigh step
74 Echo ==> Running CycleTargetDefocus ...
75 Echo --- Range and Step (um) => [ $defLow, $defHigh ], [ $step ] ---
76
77 delta = -1 * $step
78 SuppressReports
79 ReportTargetDefocus tarFocus
80 If $tarFocus > $defLow OR $tarFocus < $defHigh
81     SetTargetDefocus $defLow

```

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```

82 Else
83     IncTargetDefocus $delta
84     ChangeFocus $delta
85 Endif
86
87 ReportTargetDefocus
88 EndFunction
89
90 #####
91 Function Drift 1 0 crit
92 # A function to measure drift rate, if good, skip to the end of loop.
93 # Otherwise, exit execution -- i.e. skip the point.
94 Echo ==> Running Drift $crit (A) ...
95
96 shot = F
97 interval = 4
98 times = 10
99
100 period = $interval + 1
101 $shot
102 Delay $interval
103 Loop $times index
104     $shot
105     AlignTo B
106     ReportAlignShift
107     ClearAlignment
108     dx = $repVal3
109     dy = $repVal4
110     dist = sqrt $dx * $dx + $dy * $dy
111     rate = $dist / $period * 10
112     echo Rate = $rate A/sec
113     echo -----
114
115     If $rate < $crit
116         echo Drift is low enough after shot $index
117         break
118     ElseIf $index < $times
119         Delay $interval
120     Else
121         echo Drift never got below $crit: Skipping ...
122         exit
123     Endif
124 EndLoop
125 EndFunction

```

This script calls two functions - CycleTargetDefocus and Drift. This is a standalone script. Some other functions can found [here on github.com](#).

If running with python support, the code looks something like this:

Listing 2: LD-Group-Python.txt

```

1 #!Python
2 #ScriptName LD-Group-Python
3 import seriallem
4 from math import sqrt
5

```

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```

6  ### user defined parameters
7  # when to do focus and center beam, set range
8  groupOption = 1          # 1 = only group head, 0 = every item
9  defLow, defHigh, step = -1.0, -2.5, 0.1
10
11 # drift control, limit
12 driftControl = 1          # 1 = yes, 0 = no
13 limit = 3.0
14
15 # for X,Y position
16 templateOption = 1        # 1 = use fixed ref, 0 = dynamic one (whole image)
17 refBuffer = 'P'
18
19 # multi or single shot
20 multiShot = 1             # 1 = multi, 0 = single
21
22 ##### no editing below #####
23
24 ### Functions
25 def CycleTargetDefocus(defLow, defHigh, step):
26     print(' ---> running CycleTargetDefocus ...')
27     print(' --- defLow, defHigh, step = ', defLow, defHigh, step, '---')
28     seriallem.SuppressReports()
29     tarFocus = seriallem.ReportTargetDefocus() # float
30     if tarFocus > defLow or tarFocus < defHigh:
31         seriallem.SetTargetDefocus()
32     else:
33         seriallem.IncTargetDefocus(-step)
34         seriallem.ChangeFocus(-step)
35     seriallem.ReportTargetDefocus()
36
37 def Drift(crit):
38     print(' ---> Running Drift ', crit, 'A ...')
39     shot = seriallem.Focus # without () -> alias to function_
40     ↪reference itself
41     interval = 4
42     times = 10
43     period = interval + 1
44     #
45     shot()
46     seriallem.Delay(interval)
47     for index in range(1, times+1):
48         shot()
49         seriallem.AlignTo('B', 0, 1)
50         aliShift = seriallem.ReportAlignShift()
51         dx, dy = aliShift[2], aliShift[3]
52         rate = sqrt(dx*dx + dy*dy)/period*10
53         print(' Rate =', rate, 'A/sec')
54         if rate < crit:
55             print('Drift is low enough after shot ', index)
56             break
57         elif index < times:
58             seriallem.Delay(interval)
59         else:
60             print('Drift never got below ', crit, 'skipping...')
61             seriallem.Exit()

```

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```

62  ### main
63
64  seriallem.RealignToNavItem(0)
65  seriallem.ResetImageShift(2)
66  if templateOption == 1:
67      print(' --- assuming a template image in buffer', refBuffer, '---')
68  elif templateOption == 0:
69      seriallem.Copy('A', refBuffer)
70  else:
71      print('templateOption needs to be 0 or 1, please fix it and continue')
72      seriallem.exit()
73  seriallem.AcquireToMatchBuffer(refBuffer)
74  seriallem.AlignTo(refBuffer, 0, 1)
75
76  # turn on Autofocus drift protection so it reports drift rate
77  DP = seriallem.ReportUserSetting('DriftProtection')    # float
78  if DP == 0.:
79      seriallem.SetUserSetting('DriftProtection', 1, 1)
80
81  # center beam and defocus
82  gs = seriallem.ReportGroupStatus()    # tuple
83  gs = gs[0]    # now a float
84  if groupOption == 0:
85      seriallem.AutoCenterBeam()
86      CycleTargetDefocus(defLow, defHigh, step)
87      seriallem.Autofocus()
88  else:
89      if gs == 1. or gs == 0.:
90          seriallem.AutoCenterBeam()
91          CycleTargetDefocus(defLow, defHigh, step)
92          seriallem.AutoCenterBeam()
93      else:
94          print('  group member, skip focusing ...')
95
96  # drift
97  if driftControl == 1:
98      FD = seriallem.ReportFocusDrift()
99      if FD > 0.09:
100         Drift(limit)
101
102  # shot
103  seriallem.AdjustBeamTiltforIS()    # keep this line
104  if multiShot == 1:
105      seriallem.MultipleRecords()
106  elif multiShot == 0:
107      seriallem.Record()
108
109  # post-expose
110  seriallem.RefineZLP(30)    # if GIF exists
111

```

This is a good time to test running this script on one of the point items in navigator window, to make sure it runs fine.

3.4.8 Final Checking

Now we should check to make sure all the conditions are good for batch data collections for hours and days.

- Low Dose beams lined up for all the modes (area is the term SerialEM uses)
- Record beam has proper intensity
- Objective aperture is inserted and centered
- Objective Stigmation is good
- Thon ring with R beam on carbon area shows good scope condition
- Total exposure time, frame time, total frame number, binning, output file options, frame saving folder etc. are all good.

3.4.9 Run it!

Navigator -> Acquire at Points... -> Run Script “LD-Group” in Main action -> OK.

3.5 Post Processing K2/K3 Frames from SerialEM Data Collection

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Date_Created 2017-02-13

Last_Updated 2022-02-18

Abstract At UMASS Cryo-EM Facility, we use SerialEM to collect data for both single particle and tomography applications. And we do that on both Talos Arctica and Titan Krios with K2 cameras.

For single particle, usually we save frames in compressed TIFF format without gain normalized (select Dark Subtracted in camera setup window). One of the advantages of doing this is to reduce data size. For Super-resolution frames, the raw frame data is in unsigned 4-bit. Pixel values are in the range of 0 - 15. For weak beam, there are a lot of zeros there too. With lossless compression methods, such data can be compressed into much smaller filesize without losing image information. Therefore, instead of applying gain normalized reference to all the frames, we leave the raw data compressed and saved to the disk and we later do post-processing to recover the full information of the image data.

In this doc, the procedures to do post processing are presented here for your reference.

3.5.1 For K2 camera on Talos Arctica

The DM camera configuration for camera orientation setup for K2 camera on Talos Arctica is 270 degree rotation and Flip along Y. The idea is that with a proper orientation setup, the image from camera is at the same orientation as on FluCam. This is initial condition for SerialEM setup.

However, when saving frames from single particle data collection, this orientation might not always be needed. As long as all the data is saved the same way for the entire session, it is fine with and without this orientation applied to all the frames before saving. This option is from a check box “Save frames without rotation/flip to standard orientation” in K2 Frame File Option dialog window.

If you saved frame as un-normalized TIFF, and you need to recover the image stack to a MRC format and apply gain reference file and mask out defects, here are steps.

1. check out the orientation from header of the file.

```
$header YURI_B1_G1-SuperRes_636_Feb05_10.42.09.tif

RO image file on unit   1 : YURI_B1_G1-SuperRes_636_Feb05_10.42.09.tif      Size=
↪805815 K

          This is a TIFF file.

Number of columns, rows, sections .....   7676   7420    80
Map mode .....                          0   (byte)
Start cols, rows, sects, grid x,y,z ...   0     0     0   7676   7420    80
Pixel spacing (Angstroms).....          0.8714    0.8714    0.8714
Cell angles .....                      90.000   90.000   90.000
Fast, medium, slow axes .....           X     Y     Z
Origin on x,y,z .....                  0.000    0.000    0.000
Minimum density .....                  0.0000
Maximum density .....                  15.000
Mean density .....                     7.5000
tilt angles (original,current) .....     0.0   0.0   0.0   0.0   0.0   0.0
Space group, # extra bytes, idtype, lens .           0           0           0           0

  1 Titles :
SerialEMCCD: Dose frac. image, scaled by 1.00  r/f 0
```

The last parameter in title line shows the orientation of imaging. Here is 0 - no rotation and no flip. In this case, Gatan gain reference file doesn't need to do any rotation and flip. We simply convert it into MRC format.

2. Convert Gatan gain reference .dm4 into MRC format.

```
$dm2mrc GatanGainRef.dm4 GatanGainRef.mrc
```

3. Use "clip" to apply gain reference and deal with defects all in a single command line (later IMOD can take tiff file format as input directly). I quote a section from SerialEM helpfile here:

<helpfile>

Once you have the reference in the right orientation, you can use the program 'clip' in IMOD to apply gain normalization (and defect correction with version 4.8.6 or higher). In the following, 'scalingFactor' is the regular scaling factor applied to summed images, 'fileWithFrames' is the data file to normalize, 'gainReference.mrc' is the reoriented gain reference, and 'normalizedFrames.mrc' is the desired output file. The alternatives for GMS 2.3.0 or lower are:

Counting mode, not packed: The data need to be scaled to preserve precision after normalization. The command is

```
clip mult -n scalingFactor  fileWithFrames.mrc  gainReference.mrc ↪
↪normalizedFrames.mrc
```

Super-resolution mode, not packed: The data need to be scaled to preserve precision after normalization. To have the same scaling by 16 that the plugin would apply, the command is

```
clip mult -n 16  fileWithFrames  gainReference.mrc  normalizedFrames.mrc
```

but if you want to apply the regular scaling factor, the output will need to be integers and the command is

```
clip mult -n scalingFactor  -m 1  fileWithFrames  gainReference.mrc ↪
↪normalizedFrames.mrc
```

Counting mode, packed as bytes: The data need to be scaled to preserve precision and output as integers to preserve the range. The command is

```
clip mult -n scalingFactor -m 1 fileWithFrames gainReference.mrc ↵
↪normalizedFrames.mrc
```

Super-resolution mode, packed as 4-bit numbers: By default, the data will be scaled by 16 when unpacking with normalization, so the command to get this scaling is just

```
clip unpack fileWithFrames gainReference.mrc normalizedFrames.mrc
```

but if you want to apply the regular scaling factor, the output will need to be integers and the command is

```
clip unpack -n scalingFactor -m 1 fileWithFrames gainReference.mrc ↵
↪normalizedFrames.mrc
```

It is also possible to remove extreme values from the data at the same time with the ‘-h’ and ‘-l’ options. For example, adding ‘-h 6 -l 1’ after the ‘unpack’ will replace all values above 6 with 1.

To apply defect correction to files from GMS 2.3.1 or higher, add ‘-D defects...txt’ before ‘fileWithFrames’ in the appropriate command, where ‘defects...txt’ is the file saved by the plugin.

In IMOD version 4.8.41 or higher, all programs can read 4-bit files directly. The ‘clip unpack’ command has thus been changed so that it can be used for normalizing any kind of data, and it can also be invoked as either ‘clip unpack’ or ‘clip norm’. A command that works for all of the above cases is

```
clip norm -n scalingFactor -m 1 fileWithFrames gainReference.mrc ↵
↪normalizedFrames.mrc
```

Where the default scaling factor is 16, extreme values can be removed with ‘-l’ and ‘-h’ options, and ‘-D defects...txt’ would be added for files from GMS 2.3.1 or higher. With IMOD 4.9.2/4.10.1 or higher, you can add ‘-R -l’ and use the DM reference directly instead of a rotated reference.

For K3 frames, you should specify a scaling factor of 32.

</helpfile>

3.6 Align Movie Frames with SerialEM and IMOD Programs

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Date Created Dec 17, 2017

Last Updated Feb 18, 2022

Abstract IMOD can align movie frames nicely and very quickly. In late versions of IMOD, the program *AlignFrames* also utilizes GPU and can efficiently read in compressed TIFF frame images and decompress them, apply gain reference to normalize image frames, deal with defects and align all the frames - all at once.

To align small movie stack in-fly during tilting series data collection might be among motivations that David M developed this. One can easily see how useful and nice it is that every tilt is aligned for small movie stacks and return to SerialEM automatically in the background. It could save user huge amount of processing time unless you want to redo the movie alignment again yourself. For this purpose, the same function and code of IMOD program are also included into SerialEM program so that the alignment can be done during data collection.

I personally find this in-fly aligning capability extremely useful for single particle applications too. With SerialEM setup properly, I can get aligned image for an exposure directly. This not only provides feedback immediately, but also does it without changing low dose imaging conditions. I don't have to change back and forth the low dose record exposure time, counting to Linear mode etc..

Framewatcher, an IMOD python script program, makes it very easy to align all the movie frames in a changing directory. No need to bother with cron job and file lock etc.. It watches for any unprocessed image stack in the directory and align them for you.

In this document, I try to tell you how I use them.

3.6.1 Alignframes

This program takes many options as command line arguments. For details, please read the man page with example usages <http://bio3d.colorado.edu/imod/betaDoc/man/alignframes.html>.

As usual, the long command line can be run with a command file. Here is an example of python command file YURI_B1_G1-SuperRes_2967_Feb04_01.14.57.pcm.

Listing 3: An example pcm file

```
1 $alignframes -StandardInput
2 UseGPU 1
3 StartingEndingFrames 3 42
4 MemoryLimitGB 20.0
5 PairwiseFrames 20
6 GroupSize 1
7 AlignAndSumBinning 6 1
8 AntialiasFilter 4
9 RefineAlignment 2
10 StopIterationsAtShift 0.100000
11 ShiftLimit 20
12 MinForSplineSmoothing 0
13 FilterRadius2 0.060000
14 FilterSigma2 0.008574
15 VaryFilter 0.060000
16 ModeToOutput 2
17 InputFile YURI_B1_G1-SuperRes_2967_Feb04_01.14.57.tif
18 OutputImageFile YURI_B1_G1-SuperRes_2967_Feb04_01.14.57.ali.mrc
19 ScalingOfSum 37.549999
20 CameraDefectFile defects_YURI_B1_G1-SuperRes_358_Feb01_07.52.46.txt
21 GainReferenceFile SuperRef_YURI_B1_G1-SuperRes_001_Jan31_15.48.35.dm4
22 RotationAndFlip -1
23 DebugOutput 10
```

One can run this command file like this:

```
$subm YURI_B1_G1-SuperRes_2967_Feb04_01.14.57.pcm
```

3.6.2 Framewatcher

framewatcher is a python script to run *alignframes* at batch process. One feature I like a lot is that it can watch a growing directory and process new coming frame files. For details usage, please refer man page <http://bio3d.colorado.edu/imod/betaDoc/man/framewatcher.html>.

If frame stack files are with their command file *.pcm, then one can just run it by issuing command in the directory:

```
$framewatcher
```

This will start to align all the frame files in the same direcotry, until you do Ctrl_C.

If there is no *.pcm existed for each file, and you just want to align them using the same parameters, then you can do that using a master pcm file to take care all the files you wanted to align. Here is an example of master.pcm:

Listing 4: A master pcm file

```
1 $alignframes -StandardInput
2 UseGPU 1
3 StartingEndingFrames 3 42
4 MemoryLimitGB 20.0
5 PairwiseFrames 20
6 GroupSize 1
7 AlignAndSumBinning 6 1
8 AntialiasFilter 4
9 RefineAlignment 2
10 StopIterationsAtShift 0.100000
11 ShiftLimit 20
12 MinForSplineSmoothing 0
13 FilterRadius2 0.060000
14 FilterSigma2 0.008574
15 VaryFilter 0.060000
16 ModeToOutput 2
17 InputFile
18 OutputImageFile
19 ScalingOfSum 37.549999
20 CameraDefectFile defects_YURI_B1_G1-SuperRes_358_Feb01_07.52.46.txt
21 GainReferenceFile SuperRef_YURI_B1_G1-SuperRes_001_Jan31_15.48.35.dm4
22 RotationAndFlip -1
23 DebugOutput 10
```

As you can see, this is the same as individual pcm file, except without InputFile and OutputImageFile defined in the command file. In this case, you tell the program to use this master.pcm file:

```
$framewatcher -m master.pcm
```

The program will go through all the individual files and generate their individual pcm file based on master.pcm and align each one.

Since *framewatcher* can flexibly define output location, we can utilize it to save all the raw files and as well as aligned result files into a network drive from local SSD drive. Sometimes, directly saving on network drive and also aligning frames there could cause slowdown of SerialEM data collection. This works as a neat way to empty X or Y drive on K2 computer, they will never fill. For example, following command will move all the new files saved by SerialEM and aligned files on X drive to the network drive Z.

```
$framewatcher -w X:\MyData -o Z:\Storage\MyData -pr Z:\Storage\MyData
```

framewatcher can also output aligned sum together with power spectrum into a single image in JPEG format. This is ideal to send to remote user who wants to check image quality during data collection session. The file is small and can

be opened with any image viewer.

```
$framewatcher -w X:\MyData -po 1024 -o Z:\Storage\MyData -pr Z:\Storage\MyData
```

You can even simply move all the raw files without aligning them.

```
$framewatcher -w X:\MyData -noc -pr Z:\Storage\MyData
```

Interestingly, *framewatcher* will also copy (not move) Gatan gain reference file and Defect file to Z drive too.

If you also want pcm file to move together with raw file, you can use “-after” option:

```
$framewatcher -w X:\MyData -noc -pr Z:\Storage\MyData -after 'mv ${rootName}.pcm %  
↪{processedDir} '
```

You can even do *ctffind* and plot the curve using the “-after” option, if you installed Albert’s *ctffindPlot* program. The command is like this:

```
framewatcher -gpu 0 -bin 2 -po 1024 -dtotal 46.6 -after 'ctffindPlot ${outputFile} '
```

From November 23, *alignframes* and *framewatcher* also have options to do dose weighting. This is still in alpha version, but perhaps will be IMOD main production soon. Here I demo a couple of options to use with *framewatcher*:

```
$framewatcher -w X:\MyData -po 1024 -dtotal 39.8 -Vt 200 -o Z:\Storage\MyData -pr Z:\  
↪Storage\MyData
```

where the total dose on sample is 39.8 electrons/A², accelerating voltage is 200kV.

Here is another example of using option “-after” - to copy the jpg files into a DropBox folder which can be shared with remote user conveniently.

```
framewatcher -gpu 0 -bin 2 \  
-o /cygdrive/y/Test/alignedMRC \  
-pr /cygdrive/y/Test/rawTIFF \  
-po 1024 \  
-thumb /cygdrive/y/Test/alignedJPG \  
-volt 300 -dtotal 48.5 -thread 8 \  
-after 'cp ${reducedDir}/${rootName}_powpair.jpg "/cygdrive/e/Dropbox (UMass_  
↪Medical School)/Test" '
```

Note: Very often, people get confused by the terms “dose” and “dose rate”, partially because there seems to have no *official* definition here. As per my understanding, “dose” means electron dose on specimen and usually has unit electron/A², while “dose rate” means beam intensity level for detector and usually has unit electron/unbinned pixel/second. Dose rate is a reference value for the performance of a detector. In the case of K2 Summit counting or super-resolution mode, this value is usually chosen between 5 - 10. Much higher than 10, the performance of K2 camera is likely to be worse. Once this value is fixed under current microscope conditions, we select exposure time and frame time etc. to satisfy the total dose on the sample and frame dose (also on sample) within the frame time for movie alignment purpose.

3.6.3 Using GPU

To my understanding, the code for *alignframes* is optimized to utilize GPU and parallelization as well. Reading in and decompressing TIFF stack file is also very efficient. On my linux box with Xeon(R) CPU E5-2650 v3, with 256GB memory and Nvidia M4000 GPU, it aligns a 50 Super-resolution frame file in about 22 seconds with GPU option.

3.6.4 On K2 Computer

Since K2 computer comes with pretty high-end hardware, it could be used to align the frames in background. All I had to do is to install a decent GPU card. I replaced the ATI video card that comes with the K2 box and install a M4000 GPU card in with 8GB memory on the card. One advantage for this card is that it is single slot high, not like most Nvidia cards which occupies two PCI slot space. This makes the replacement simple and easy.

Now, after installing IMOD with Cygwin, I align all the movie frames right off the K2 computer box.

3.6.5 Align using SerialEM directly

Beside aligning frames at the background separately with IMOD, we can also use SerialEM plugin to align the frames directly. From camera setup page of SerialEM interface, you can define to let SerialEM Plugin to align the frames. Slightly different from using IMOD which aligns as separate process, SerialEM Plugin aligns all the frames from an exposure and returns the aligned average to SerialEM main instance. This is very handy for us to obtain sample information quickly and conveniently.

3.7 Monitor Data Collection In The Fly

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Date-Created 2018-08-02

Last-Updated 2024-01-10

Abstract We already routinely align movie frames during data collection so we can check images from time to time. We did most directly using K2 camera computers. This works very nicely. However, there are still a couple things we feel missing. 1) we need to see the defocus range and phase shift values computed and plotted out. 2) we need to do this with no delay and without slowing down the data collection itself.

We decided to align movies and perform CTF determination using a dedicated workstation with dual GPU. Our Summer Student, Albert Xu, made this completely automatic. During data collection, a plot is showing on web browser and refreshing itself.

This document is mainly for ourselves as a check list. Hopefully, it can also be useful for your setup.

For Albert's *ctffindPlot* project, please see <https://github.com/alberttxu/ctffindPlot>.

3.7.1 Setup for Shipping, alignframes and CTffindPlot

1. Ship raw data from K2 local SSD to storage tank.

Assuming storage tank is CIFS mounted onto K2 computer, as W:, and we have a new folder call ChenXu_20180802. We create a folder on local ssd drive X: usually using the same folder name. We collect everything off camera onto this local SSD folder X:\ChenXu_20180802 first including all LMM, MMM maps etc. and raw TIFF data as well. We use IMOD program *framewatcher* to ship the raw data, pcm parameter files, defect file and gain reference file to storage.

From cygwin shell terminal on K2 computer, go into local folder X:\ChenXu_20180802 and do this:

```
$ framewatcher -nocom -pr W:\ChenXu_20180802
```

From today - Sept 09, 2018 the latest package (currently in nightly builds) supports multiple processed folders so the collected files can be shipped into several folders. This is very useful to align them parallelly by running *alignframes* from inside of each folder separately. A example is below:

```
$ framewatcher -nocom -pr W:\ChenXu_20180802\tmp1 -pr W:\ChenXu_20180802\tmp2 -pr W:\ChenXu_20180802\tmp3 -pr W:\ChenXu_20180802\tmp4
```

This will move all the raw files onto storage location, so local SSD never fills.

2. ssh login GPU computer as you and su to “guest”, make new folders and align movies

```
$ ssh xuchen@gpu
[xuchen@gpu ~]$ su - guest
[guest@gpu ~]$ cd /mnt/Titan/ChenXu_20180802
[guest@gpu ChenXu_20180802]$ mkdir rawTIFF alignedMRC alignedJPG
[guest@gpu ChenXu_20180802]$ framewatcher -gpu 0 -bin 2 -po 1024 -pr rawTIFF -thumb_
alignedJPG -dtotal 46.5
```

This will move raw data files (TIFF, dm4, defect, pcm) into *rawTIFF* and *_powpair.jpg* into *alignedJPG*. You can also add an option “-o alignedMRC” to move all the aligned MRC files into that folder *alignedMRC*.

As mentioned above, one can also run a few jobs of *framewatcher* from multiple directories separately, so speed thing up, with or without a GPU card. You can manually run the command from tmp1, tmp2, tmp3 and tmp4. You can also ask a simple shell script to do that. The only disadvantage might that you have to “kill” when you need to stop *framewatcher* because you cannot do Ctrl_C from shell interactively in this case.

```
#!/bin/bash

for dir in tmp{1..4} ;
do
    cd $dir
    framewatcher -bin 2 -po 1024 -o ../ -pr ../rawTIFF -thumb ../alignedJPG -thr 4 -
    dtotal 46.5 &
    cd ..
done
```

3. Copy and edit ctffind parameter file (as “guest”, in the same folder; we usually create a new terminal from tmux by “Ctrl_B C”).

```
[guest@gpu ChenXu_20180802]$ cp /usr/local/ctffindplot/test/ctffindoptions.txt .
[guest@gpu ChenXu_20180802]$ vim ctffindoptions.txt
```

edit to fit your situation. The file looks like this:

```
ctffind << EOF
(filename)
(basefilename)_ali_output.mrc
1.059
300.0
2.70
0.07
512
30.0
5.0
5000.0
50000.0
100.0
no
no
no
yes
0.0
3.15
0.5
no
EOF
```

Laterly, testctffind of IMOD package can also do a very good job. You may not get into hassle to compile ctffind on Windows by using IMOD installed.

You run a bash script from cygwin windows (assuming you have IMOD installed):

```
# set parameters here
volt=200
sph=2.7
dmin="5000"
dmax="50000"
dstep="400"
minres=30
maxres=5
testctffind -v $volt -sph $sph -dstep $dstep -dmi $dmin -dma $dmax -rmi $minres -rma
↪$maxres -pha (filename) > testctffind_output.txt
```

4. plot

```
[guest@gpu ChenXu_20180802]$ ctffindPlot
```

This will generate a plot and continuously update a file called *ctf_plot.png* which can be loaded into a web browser and let it refresh periodically. All the aligned MRC files will be moved into *alignedMRC* by the plot program after done.

For convenience, there are a few parameter files for common conditions which you can directly use with option “-t”.

```
[guest@gpu ChenXu_20180802]$ ctffindPlot -t /usr/local/ctffindPlot/ctffind_params/
↪Titan_130k_NoVPP.txt
```

3.7.2 End Results - CTF plotting and JPG of aligned image & power Spectrum

During the collection, the both CTF plotings and aligned image with power spectrum can be viewed immediately. No delay.

Fig.1 CTF plot

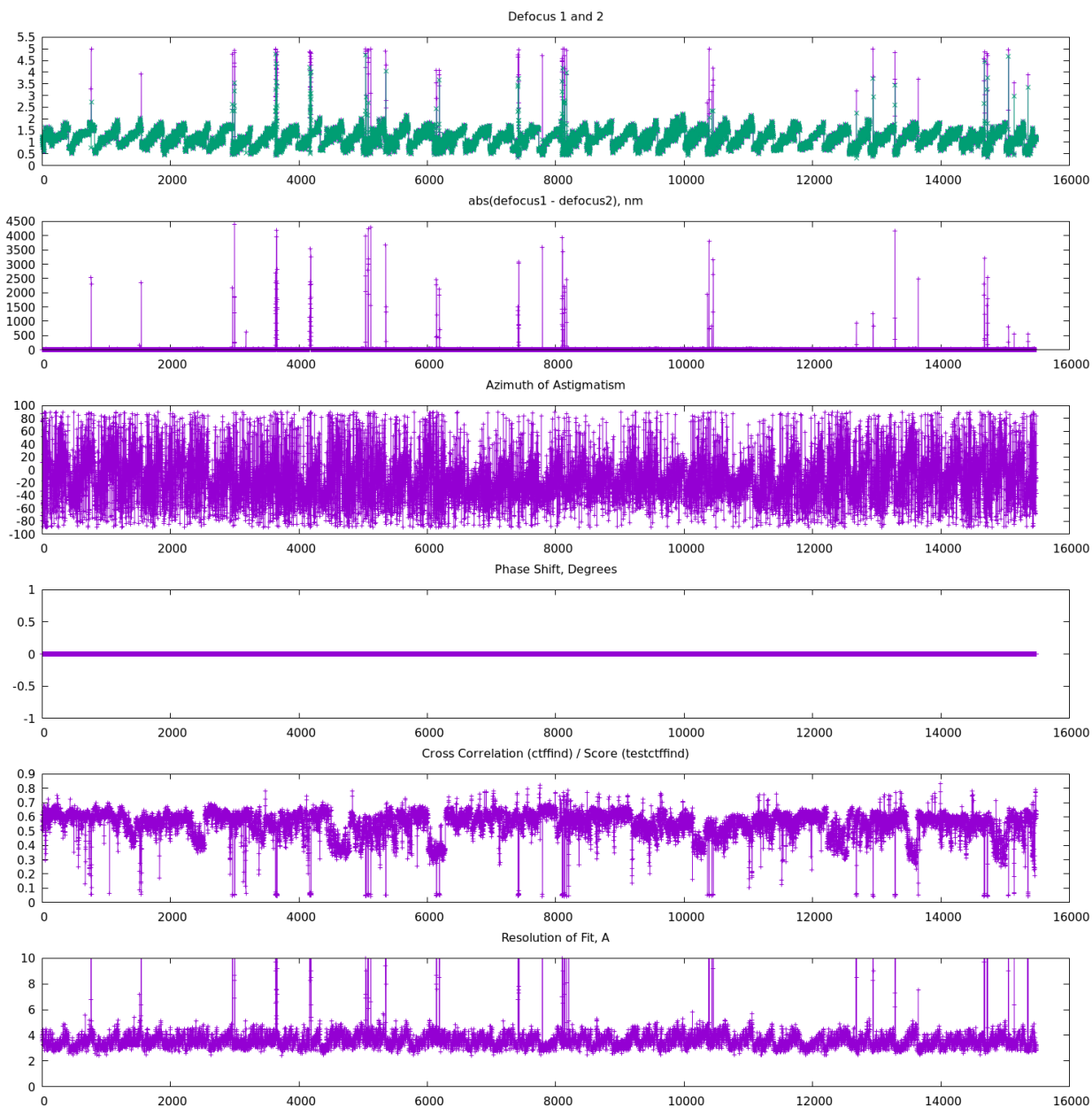
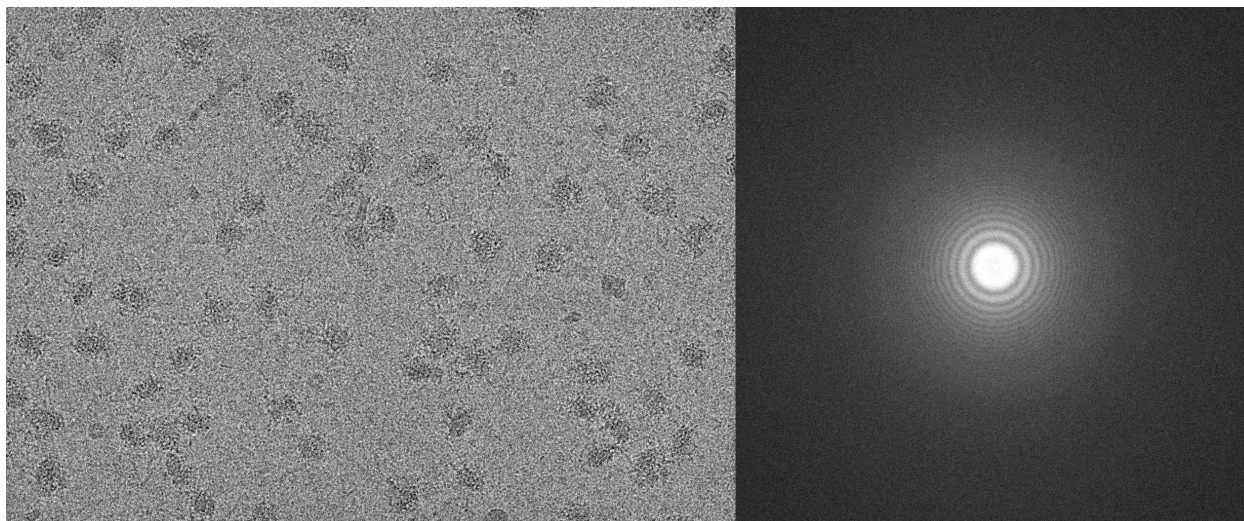


Fig.2 Aligned Image and its power spectrum



3.8 Pixelsize and Distortion Info on K2/3 Cameras

Author Chen Xu

Contact <chen.xu@umassmed.edu>

Date_Created 2017-2-23

Last_Updated 2019-3-28

Abstract We have K2 on Talos Arctica and GIF/K2 on Krios.

This doc lists pixelsize information for K2 cameras so you can decide which magnification you want to use for your final image. I also try to give information about image distortion on these cameras.

3.8.1 On Talos Arctica

According to FEI document http://www.fei.co.jp/_documents/DS0189-10-2014_Talos_Arctica_WEB.pdf, the Cs value for Talos Arctica is 2.7mm.

Below are pixelsizes on K2 for a few magnifications.

Table.1 Pixelsize (Å) of K2 camera on Talos

Magnifications (X)	Counted (Å)	Super resolution (Å)
17,500	2.425	1.213
22,000	1.888	0.944
28,000	1.485	0.742
36,000	1.169	0.584
45,000	0.914	0.457
57,000	0.719	0.360

Also the distortion information at these few mags. This mag distortion is believed due to stretch on projection lens system. The measurement and correction programs are used and available from <http://grigoriefflab.janelia.org/magdistortion>.

Table.2 Mag Distortion Parameters for K2 camera

(Note: this is for images saved by SerialEM directly. So this is after rotation and flip applied. Please do not use this with raw frame saved by plugin without rotation and flip, as it will have different but related values.)

Magnifications (X)	Dist.Angle(degree)	Major Scale	Minor Scale	Total Distortion (%)
11,000				
17,500				
22,000	65.9	1.016	1.000	1.56
28,000	24.5	1.015	1.000	1.51
36,000	22.5	1.015	1.000	1.51
45,000	21.3	1.016	1.000	1.56
57,000	22.6	1.017	1.000	1.66

3.8.2 On Titan Krios

Table.3 Pixelsize (Å) of GIF K2 camera on Titan Krios - BEFORE Sept. 27, 2017

Magnifications (X)	Counted (Å)	Super resolution (Å)
26,000	4.505	2.253
33,000	3.588	1.794
42,000	2.939	1.470
53,000	2.274	1.137
64,000	1.863	0.931
81,000	1.485	0.742
105,000	1.170	0.585
130,000	0.910	0.455
165,000	0.720	0.360
215,000	0.569	0.279

On September 2017, Gatan serviced GIF/K2 system on Krios and they removed a housing block between column and GIF, so the pixelsize changed. The new set of values is below in Table. 4.

Table.4 Pixelsize (Å) of GIF K2 camera on Titan Krios - AFTER Sept. 27, 2017

Magnifications (X)	Counted (Å)	Super resolution (Å)
64,000	2.147	1.087
81,000	1.714	0.857
105,000	1.353	0.677
130,000	1.059	0.529
165,000	0.832	0.416
215,000	0.654	0.327
275,000	0.516	0.258

From March 10, 2019, K3 was upgraded on Krios to replace the K2. GIF alignment has changes too. The pixel sizes changed a lot.

Table.5 Pixelsize (Å) of GIF K3 camera on Titan Krios - AFTER March 10, 2019

Magnifications (X)	Counted (Å)	Super resolution (Å)
81,000	1.060	0.530
105,000	0.830	0.415
130,000	0.650	0.325

3.9 Relion and cisTEM Docker Services

Author Albert Xu

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Date-created 2019-01-08

Last-updated 2019-02-14

Abstract This is a quick information how to use Relion3 and cisTEM dockers on two Linux workstations in CryoEM Core Facility. One of the potential advantages to use docker is to avoid CUDA version conflict.

Some of the dockerfile information can be found at https://github.com/alberttxu/cryoem_dockerfiles.

3.9.1 Relions

1. on Eagle

```
ssh -Y relion3@172.18.8.143 -p 30003
password = *****
```

2. on Falcon

```
ssh -Y relion3@172.18.8.136 -p 30003
password = *****
```

3.9.2 cisTEM

1. on Eagle

```
ssh -Y cisTEM@172.18.8.143 -p 30001
password = *****
```

2. on Falcon

```
ssh -Y cisTEM@172.18.8.136 -p 30001
password = *****
```

Note: Let other people know if your are using either eagle or falcon. For each machine there is only one account for Relion3 and one for cisTEM.

3.10 cisTEM detaching and re-attaching sessions

Author Albert Xu

Contact <albert.t.xu@gmail.com>

Date-created 2018-06-13

Last-updated 2018-06-13

Abstract In cisTEM when you close the main window, all running jobs are terminated. Xpra is a window server that saves the state of graphical applications so that you can detach from your cisTEM session and have it run in the background.

<https://xpra.org/> “Xpra is an open-source multi-platform persistent remote display server and client for forwarding applications and desktop screens. ... it allows you to run programs, usually on a remote host, direct their display to your local machine, and then to disconnect from these programs and reconnect from the same or another machine, without losing any state.”

3.10.1 Installing Xpra on Linux

1. Download the Xpra repository information into your system’s package manager folder.

For our Centos 7 system,

```
sudo bash -c "curl https://xpra.org/repos/CentOS/xpra.repo > /etc/yum.repos.d/xpra.  
↪repo"
```

Xpra also supports Fedora, Debian, and Ubuntu. For Debian and Ubuntu, the package manager folder is /etc/apt/sources.list.d

2. Install

For Centos and Fedora,

```
sudo yum install xpra
```

For Debian and Ubuntu,

```
sudo apt install xpra
```

3.10.2 Usage Examples

1. Log into the cisTEM computer from your remote computer. You will need less strict X forwarding with the -Y option.

```
ssh -Y username@ipaddress
```

2. Start a cisTEM process using xpra. You will need to choose a sessionID number. I arbitrarily chose 100.

```
xpra start :100 --start-child=cisTEM
```

Hit enter one more time, and now the session has been created in the background. You will have to attach to it.

3. Attach to the session. From the cisTEM computer,

```
xpra attach :100
```

and the cisTEM window should open. If there is only one session, you don’t need the :100

4. Detach from the session. From the command line, hit Ctrl-C and the window will disappear.

Note: when re-attaching: Usually remote connections from outside the local network are laggy. Fortunately, Xpra has compressions to lessen the amount of bandwidth. To enable compression when reattaching, do


```
xpra attach --encoding=rgb --compress=1
```

This is the recommended way from Xpra.org.

3.11 Adding a Scalebar to Images

Author Christna Ouch

Contact <Christna.Ouch@umassmed.edu>

Date_Created Jan 02, 2020

Last_Updated Apr 08, 2021

3.11.1 Overview

Stack file images taken with SerialEM can be processed and labeled using Imod and ImageMagick.

I have written a simple shell script that will add a scale bar to the lower right-hand side of an image. This script first converts your stack file into a series of tifs using mrc2tif (Imod), scales the image by 50%, normalizes, adds a scale bar and converts to jpeg using ImageMagick.

3.11.2 Prerequisites

This program requires that you have both Imod and Imagemagick installed on your computer.

On linux, you can use your package manager to install Imagemagick. Download and run the install packages Imod.

On Windows, you can use the cygwin environment to install both Imod and ImageMagick. Follow the instructions on Imod's website to install cygwin and Imod. After the installation, rerun Cygwin installer, then select the internet as a source. Type in magick into the search bar and install the ImageMagick package.

After installation, both Imod and ImageMagick should be installed. Check that each program is installed by running 'convert -version' and 'imod'.

3.11.3 Script Download

Download the script from: https://github.com/ouchc/sphinx-emdocs/blob/ouchc/scripts/labeling_script_new_v4.zip

Extract the sh-script to the directory with your stack files.

3.11.4 Editing the Script

The script has a few fields that need to be updated for your particular microscope setup. Edit the top of the file for the magnifications and pixel size. For this case, we have three magnifications, 1250x, 17.5k and 45k with pixel sizes 32.91, 2.27 and 0.87 angstroms respectively. Edit these to match your microscope setup. It is important that these values match the pixel sizes for your images for each mag. Verify it by running 'header' command on the stack file.

```

RO image file on unit   1 : btx2-g2-Car7-R3-45k-edge.st      Size=      690529 K

Number of columns, rows, sections .....    5760    4092    15
Map mode .....    1    (16-bit integer)
Start cols, rows, sects, grid x,y,z ...    0    0    0    5760    4092    15
Pixel spacing (Angstroms).....    0.8700    0.8700    0.8700
Cell angles .....    90.000    90.000    90.000
Fast, medium, slow axes .....    X    Y    Z
Origin on x,y,z .....    0.000    0.000    0.000
Minimum density .....    0.0000
Maximum density .....    6621.0
Mean density .....    922.64
tilt angles (original,current) .....    0.0    0.0    0.0    0.0    0.0
Space group, # extra bytes, idtype, lens .    0    3072    0    0

```

This script captures the pixel size from the header and generates a scale bar based on that pixel size.

The other parameters to edit is `scale_length`, `scale_label` and `scale_label_offset`. The length is in nanometers and the offset is used to center the scalebar label. Adjust this value to center the label. Alternatively, set the `scale_bar_label_offset` to 0 to left-justify the label. The `scale_x2` parameter defines the right-most position of the scale-bar. The current value of 2750 works well for K3 micrographs binned 2x. For K2 data or other cameras, you may need to reduce this value so that it is within the bounds of the bin2 image. Adjust `y_anchor` and `y_text` to position the scalebar and label respectively. The options for the header are shown below.

```

# Magnification #1
mag1="1750"
apix1="32.91"
scale_length_1="1000"
scale_label_1="1000 nm"
scale_label_offset_1="10"

# Magnification #2
mag2="17.5k"
apix2="2.277"
scale_length_2="100"
scale_label_2="100 nm"
scale_label_offset_2="55"

# Magnification #3
mag3="45k"
apix3="0.87"
scale_length_3="50"
scale_label_3="50 nm"
scale_label_offset_3="100"

# Scale bar location options
# We will use coordinate 2750,1900 as an anchor point for the right-most
position on our scale bar. labeling will be relative to that point.
scale_x2="2750"
y_anchor="1900"

# Fonts will be positioned at a y value of 1940
y_text="1940"

```

3.11.5 Running the Script

Place the script in the same directory with all of your stack files that need to be labeled. These stack files need to have a .st extension to be recognized by the script. Open a linux terminal or Cygwin and cd to that directory. Run the edited script. This script will create subdirectories using the mag variables defined in the header.

Note: When editing the script in windows, hidden newline characters may be added to the file. These newline characters prevent the script from running. Please run this command in cygwin to remove these hidden characters. Replace input with your original script and output for the fixed script

Command syntax:

```
tr -d '\r' <input >output
```

Example:

```
tr -d '\r' <labeling_script_new_v4.sh >labeling_script_new_v4-fixed.sh
```

Use a coding editor or notepad (do not use wordpad or office) to make future changes to the file.

3.12 Setup Simple Lab Electronic Logbook

Author Chen Xu

Contact <Chen.Xu@umassmed.edu>

Date_Created Feb 24, 2022

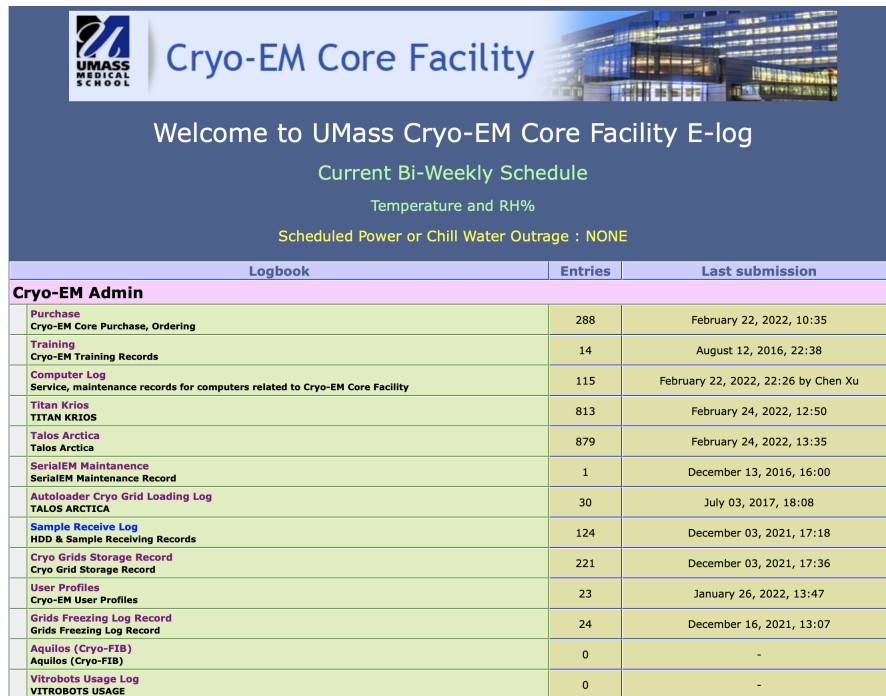
Last_Updated Feb 24, 2022

Abstract It is desirable to have very simple electronic log books for a scientific research laboratory. For an electron microscope lab, it would be very nice to be able to record all the imaging conditions, grid information etc. and all that. And it would be even better if such program can be easily configurable.

After much searching, we found a little program called **ELOG**. It was originally developed by high energy physics people for their experiment log. We found this fit our needs very well. I share some of the information here with you.

3.12.1 Elog

The program can be found in <https://elog.psi.ch/elog/>. It is open source and can be installed on Linux, Windows, MacOS and FreeBSD. It doesn't rely on any existing web server. Instead, it comes with its own web server **elogd** running as a daemon style. It has a built-in very simple database. Here is a snapshot of the program main interface for our intranet access.



Logbook	Entries	Last submission
Cryo-EM Admin		
Purchase Cryo-EM Core Purchase, Ordering	288	February 22, 2022, 10:35
Training Cryo-EM Training Records	14	August 12, 2016, 22:38
Computer Log Service, maintenance records for computers related to Cryo-EM Core Facility	115	February 22, 2022, 22:26 by Chen Xu
Titan Krios TITAN KRIOS	813	February 24, 2022, 12:50
Talos Arctica Talos Arctica	879	February 24, 2022, 13:35
SerialEM Maintenance SerialEM Maintenance Record	1	December 13, 2016, 16:00
Autoloader Cryo Grid Loading Log TALOS ARCTICA	30	July 03, 2017, 18:08
Sample Receive Log HDD & Sample Receiving Records	124	December 03, 2021, 17:18
Cryo Grids Storage Record Cryo Grid Storage Record	221	December 03, 2021, 17:36
User Profiles Cryo-EM User Profiles	23	January 26, 2022, 13:47
Grids Freezing Log Record Grids Freezing Log Record	24	December 16, 2021, 13:07
Aquilos (Cryo-FIB) Aquilos (Cryo-FIB)	0	-
VitroBots Usage Log VITROBOTS USAGE	0	-

You can see it has multiple e-logbooks you can access. Each log book has its own config file and you can directly edit it from web interface. After saving the change, the **elogd** takes it in automatically.

3.12.2 Config

Here is one example entry page to enter all the information for a Talos session.

Cryo-EM Admin

Purchase Training Computer Log Titan Krios Talos Arctica SerialEM Maintenance Autoloader Cryo Grid Loading Log Sample Receive Log Cryo Grids Storage Record User Profiles Grids Freezing Log Record Aquilos (Cryo-FIB) Vitrobot Usage Log

Talos Arctica

Submit Save Preview Delete

Fields marked with * are required

Entry time: February 24, 2022, 13:47

Operator: - please select -

Customer*:

Group: - please select - Add Group

Customer Category*: - please select -

Date of Loading: Year:

For Scope: ☒ Titan Krios ☒ Talos Arctica ☐ Not Sure yet

Capule:

Cassette:

Storage Location:

Car 12:

Car 11:

Car 10:

Car 9:

Car 8:

Car 7:

Car 6:

Car 5:

Car 4:

Car 3:

Car 2:

Car 1:

Date on Scope*: Year:

Time Length:

Instrument Hours for Billing*:

Session Subject*: ☒ Screening ☐ SPA ☐ Tomo ☐ micro-ED ☐ Maintenance ☐ FEI Service ☐ Gatan Service ☐ Info ☐ Training for Cryo Sample Prep ☐ Operation Instruction

No of Grids Screened*:

Specimen:

Billable Staff Hours*:

No of C-Ring/Clip used:

Total C-Ring/Clip Charge:

Extra Charge:

Flat Staff Fee:

Vitrobot Usage(hr):

Quote#:

PO#:

Invoice: ☒ Yes ☐ No

Invoice No.:

Exposures Processed:

H.T.(kV): ☒ 200 ☐ 100 ☐ 80

Gun lens: ☒ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8

Extr.Voltage: ☒ 4000 ☐ 4100 ☐ 4150 ☐ 4200 ☐ 4250* ☐ 4300 ☐ 4350 ☐ 4400

Emission(uA):

C1 aperture: ☒ 1 ☐ 2 ☐ 3 ☐ 4

C2 aperture(um): ☒ 150 ☐ 70 ☐ 50 ☐ 30

Obj. aperture(um): ☒ 70 ☐ 100 ☐ Ph1 ☐ Ph2 ☐ Ph3 ☐ Ph4 ☐ Ph5 ☐ Ph6

Camera Used: ☒ Ceta ☐ K2 ☐ K3

Camera Condition: ☒ OK ☐ problematic (provide detail using comment space below)

Movies Collected:

Frames per Exposure:

Exposure Time:

Frame Time:

Magnification:

Spotsize: ☒ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 ☐ 11

C2 Lens(%):

Dose Rate:

Total Dose:

Multi-Hole-Shot:

Cryo-cycle started?: ☒ Yes ☐ No

Cryo-cycle length(hrs.):

Optional comment:

Suppress Email notification

Attachment 1: no file selected

Drop attachments here...

Submit Save Preview Delete

ELDG V3.1.4-unknown

Below is the config file for this page shown above.

```

Restrict edit = 1

; look and feel
Guest menu commands = Back, Find, Login, Help
Guest find menu commands = Find, Login, Help
Date format = %a, %b %d, %Y
;Time format = %A, %B %d, %Y, %H:%M
Time format = %B %d, %Y, %H:%M
Display mode = summary

Theme = default
Comment = Talos Arctica

; Attributes
Attributes = Operator, Customer, Group, Customer Category, Date of Loading, For Scope,
↳ Capsule, Cassette, Storage Location, Car 12, Car 11, Car 10, Car 9, Car 8, Car 7,
↳ Car 6, Car 5, Car 4, Car 3, Car 2, Car 1, Date on Scope, Time Length, Instrument,
↳ Hours for Billing, Session Subject, No of Grids Screened, Specimen, Billable Staff,
↳ Hours, No of C-Ring/Clip used, Total C-Ring/Clip Charge, Extra Charge, Flat Staff,
↳ Fee, Vitrobot Usage(hr), Quote#, PO#, Invoice, Invoice No., Exposures Processed, H.
↳ T.(kV), Gun lens, Extr.Voltage, Emission(uA), C1 aperture, C2 aperture(um), Obj.
↳ aperture(um), Camera Used, Camera Condition, Movies Collected, Frames per Exposure,
↳ Exposure Time, Frame Time, Magnification, Spotsize, C2 Lens(%), Dose Rate, Total,
↳ Dose, Multi-Hole-Shot, Cryo-cycle started?, Cryo-cycle length(hrs.)

Options Operator = KangKang, Chen, Kyoungwan, Albert, Christna, Jeng-Yih, ZhongWu,
↳ Customer
Group = group1, group2

Extendable Options = Group
Options Customer Category = Internal Academic, External Academic, Industry Partner,
↳ Industry, not sure
Options Time Length =
Options Instrument Hours for Billing =
MOptions Session Subject = Screening, SPA, Tomo, micro-ED, Maintenance, FEI Service,
↳ Gatan Service, Info, Training for Cryo Sample Prep, Operation Instruction
Option Billable Staff Hours =
Option No of Grids Screened =
ROptions For Scope = Titan Krios, Talos Arctica, Not Sure yet
ROptions Invoice = Yes, No
ROptions H.T.(kV) = 200, 100, 80
ROptions Gun lens = 1, 2, 3, 4, 5, 6, 7, 8
ROptions Extr.Voltage = 4000, 4100, 4150, 4200, 4250*, 4300, 4350, 4400
ROptions Column vacuum crash = 0, 1, 2, 3, more
ROptions C1 aperture = 1, 2, 3, 4
ROptions C2 aperture(um) = 150, 70, 50, 30
ROptions Obj. aperture(um) = 70, 100, Ph1, Ph2, Ph3, Ph4, Ph5, Ph6
MOptions Camera Used = Ceta, K2, K3
ROptions Camera Condition = OK, problematic (provide detail using comment space below)
;ROptions Filter Used? = Yes, No
;ROptions Slit Width = 20, 25, 30, 35, 40
ROptions Spotsize = 5, 6, 7, 8, 9, 10, 11
ROptions Cryo-cycle started? = Yes, No
Options Cryo-cycle length(hrs.) =
ROptions Staff Flat Fee = Yes, No

```

(continues on next page)

(continued from previous page)

```

; Required Attributes
Required Attributes = Date on Scope, Customer, Group Head (PI), Customer Category,
↳Instrument Hours for Billing, Session Subject, No of Grids Screened, Billable
↳Staff Hours
; extendable options
;Extendable Options = Hours
;Extendable Options = Session Subject
;Extendable Options = Extr.Voltage
; comment of Attribute
; Comment Session Subject = E.g. Practice, C-complex data collection etc.

; flags
Message comment = <i>Optional comment:</i>
List display = ID, Date on Scope, Customer, Group, Customer Category, Session Subject,
↳ Instrument Hours for Billing, No of Grids Screened, No of C-Ring/Clip used, Extra
↳Charge, Flat Staff Fee, Billable Staff Hours, Vitrobot Usage(hr), Quote#, PO#,
↳Invoice, Invoice No.
Type Date on Scope = date
Type Date of Loading = date
Menu commands = List, New, Edit, Delete, Find, Last Day, Logout, Help
;Preset Name = $long_name
HTML default = 2
; disable attachment
Enable attachments = 1
Show attachments = 0

; Combine attributes into singles lines
;; using Format, kind of messy
;Format Date on Scope = 1

Page Title = UMass CryoEM Log - $Session Subject / $Name
Reverse sort = 1
Quick filter = Date, Customer, Session Subject

```

Note: you can see a single, long line is needed for after “=”.

3.12.3 Install on FreeBSD

There are available install packages for Windows and Linux OS. This makes things easier. For other system, you can build it from source code. We have it running on a Linux workstation where we use for our processing. I also have a backup elog running on **TrueNAS** system in a jail.

After unpacking from the source code, one can simply compile it with *gmake* in main directory.

```
gmake
```

If it compiles successfully, you can then just copy the *elogd* to a location like `/usr/local/sbin`. To make it running automatically after computer starts, we need a rc script as below.

```
#!/bin/sh

# PROVIDE: xuchen
```

(continues on next page)

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```
. /etc/rc.subr

name="elogd"
start_cmd="${name}_start"
stop_cmd="${name}_stop"

elogd_start() {
    echo "elogd starting"
    # your commands here
    /usr/local/bin/sudo -u elog /usr/local/sbin/elogd -D -c /usr/local/elog/elogd.cfg -
    ↪d /usr/local/elog/logbooks
}

elogd_stop() {
    echo "qssite stopping"
    # your commands here
}

run_rc_command "$1"
```

And also we need an entry in `/etc/rc.conf` like this.

```
elogd_enable="YES"
```

After jail starting, the **elogd** runs at the background and you can point your web browser to jail's IP with port 8080 which is default for elog running at.

SERIALEM NOTES

4.1 SerialEM Note: Installation and Calibration

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Date_Created 2017-11-12

Last_Updated 2022-10-06

Abstract When I helped a few sites to install and calibrate SerialEM, I had impression that most new users felt this process was very hard. I felt the same way when I initially learned to install and calibrate SerialEM by myself. I even got frustrated and had to call David for a few times. When I think back about all the troubles I had to install and calibrate SerialEM, I believe I would have an easier time if I had a brief guideline document for what steps to follow in order, and what to do in each step. The helpfile from SerialEM is very complete to provide almost all information needed, but it is perhasp a lot to read and not clear where to start for a beginner.

I wanted to list some steps here to guide you through this initial installation and calibration phase. It is like a crash list. For more detailed information, you should always find it from helpfile.

4.1.1 Installation

Here are steps to follow.

Step 1

- Ask David for the initial system file. Normally, you would fill out a “questionnaire” available at the ftp server - <http://bio3d.colorado.edu/ftp/SerialEM/questionnaire.txt> and send it to David. David or Guenter Resch will then create a framework file on the same ftp server for you to download. This framework file is a zip file, you can download it to local like Desktop and unzip it by double clicking on the file. Beside a sub-folder “Admin” created under “C:\ProgramData\SerialEM”, the most important file in the framework is one initial system file called “SerialEMproperties.txt”. You must have this file to get started. Please refer to the SerialEM webpage for the latest information regarding this.

Note: If on Windows the Folder Option -> View -> “Hide extensions for known types” is enabled, the property filename shown will be “SerialEMproperties”. If you change it to “SerialEMproperties.txt”, it actually is “SerialEM-properties.txt.txt” which won’t be read in as a valid property file.

Step 2

- Make sure your camera computer and microscope computers are on the same local network. For example, K2/3 computer can be configured to have a network interface with IP address 192.168.1.2, and FEI scope with 192.168.1.1. And they should be able to *ping* each other. - You might be confused by Gatan's DM already being able to communicate with scope, as it can detect magnification change of scope. However, this DM connection to scope is usually via serial port by a direct serial cable. SerialEM uses standard TCP/IP to communicate to a remote computer and therefore requires a standard network setup in place. - If you configured the local network, you should have DM communicate to scope (via Gatan's RemoteTEM) using TCP/IP too. It is a lot more robust. But this is not a concern for SerialEM operation here.

Step 3

- Decide which computer to install SerialEM. In theory, you can install SerialEM on either computer - camera or microscope. For K2/3 camera, SerialEM should be normally installed on the K2/3 computer, as K2/3 image returning to SerialEM locally is usually faster than via network.

Step 4

- Decide which type of executable to use. SerialEM builds for both 32 and 64-bit platforms. Unless you have to run it on a Windows XP, you should choose 64-bit.

Step 5

- Download SerialEM software. You should start with the latest release version from ftp server at <http://bio3d.colorado.edu/ftp/SerialEM/> and save it somewhere local like Desktop.

Step 6

- Unzip the installation package file downloaded. You can double click on this file, it will unzip the program into C:\Program Files\SerialEM. The folder "SerialEM" will be created automatically if there isn't one already. The new package content will be unzipped into a new sub-folder, e.g. SerialEM_3-6-13_64.

Step 7

- Quit Gatan DM if it is running.

Step 8

- Right click on a file called *install.bat* in the package folder C:\Program Files\SerialEM\SerialEM_3-6-13_64 and select 'Run as Administrator'. This will copy some files into upper folder which is C:\Program Files\SerialEM, register DM plugin file and copy it to the Gatan plugin folder at C:\ProgramData\Gatan\Plugin.

Step 9

- Manually copy a file called *FEI-SEMServer.exe* from C:\Program Files\SerialEM on K2/3 computer to C:\Program Files\SerialEM on scope computer. This is a bridging program to control scope by passing the scope function calls between SerialEM main program on remote K2/3 computer and the scope scripting interface. Run the program by double clicking on it(it needs to run or SerialEM cannot control scope). This is 32-bit application, runs on both 32 and 64-bit Windows platforms. So there is only one such executable to run on Windows 7, XP or 2000 Windows OS.

Step 10

- On K2/3 computer, Edit *SerialEMproperties.txt* file in folder C:\ProgramData\SerialEM to have proper lines in general property area to define network properties.

```
#GatanServerIP 192.168.1.2
GatanServerIP 127.0.0.1
GatanServerPort 48890
SocketServerIP 1 192.168.1.1
SocketServerPort 1 48892
```

Step 11

- On K2/3 computer where SerialEM is to be installed, define a system environment variable SERIAL-CCD_PORT with the value 48890 or other selected port number, as described in the section in helpfile.
- If everything goes well, you should be able to start SerialEM and it should connect to “see” both scope and DM. Congratulations!

4.1.2 Calibration

Although most of calibration results will be written into another system file *SerialEMcalibraions.txt* when you save the calibration from Calibreton menu, there are a few places you need to manually edit the *SerialEMproperties.txt* to take in the calibration results. These include pixelsize and tilting axis angle - they are more like instrument parameters.

For pixelsize calibration, it is best to use standard 2160 line waffle grid. For all other calibration like Image Shift and Stage Shift, it would make things a lot easier to use a non-periodic sample. Please see the *NOTE* at the end of this document.

Tip: Since μP and nP modes give very different beams, it is required to perform calibration for **both** modes for some basic items:

- Beam Crossover
- Beam Intensity
- Spot Intensity
- Beam Shift
- Autofocus
- Electron Dose
- Standard Focus

See https://bio3d.colorado.edu/SerialEM/betaHlp/html/setting_up_serial-em.htm#nanoprobe

Step 0

- Determine camera orientation configuration. Make sure the image orientation from camera shot agree with that of on large screen or FluCam. If it doesn't, try to adjust the camera orientation of Gatan K2/3 camera from Camera - Configuration. You can use beamstop to help. You should add a property entry to reflect the DM configuration so SerialEM takes care of it even someone might have changed DM configuration.

```
DMRotationAndFlip 7
```

Step 1

- Edit property file to define the camera configuration information about orientation determined by step 0. SerialEM will return to main display with proper orientation. This is initial starting point for all the calibrations.

```
RotationAndFlip 7
```

Step 2

- SerialEM - Calibration - List Mag. Scope will go through all the mags and list them on log window, from lowest to highest. Check it with what are in *SerialEMproperties.txt*, update that if needed.

Step 3

- Load standard waffle grating grid (TedPella Prod.# 607, http://www.tedpella.com/calibration_html/TEM_STEM_Test_Specimens.htm#_607).

Step 4

- Start with lowest magnification above LM range. On Talos, it is 1250X. At close to Eucentricity, and close to eucentric focus.

Step 5

- Take a T shot with 2x binning on a K2/3 camera, make sure the counts are neither too low nor too high.

Step 6

- Take a T shot, then Calibration - Pixel Size - Find Pixel Size. The log window shows both mag index and pixel size. Edit *SerialEMproperties.txt* to add a line like below in K2/3 camera property section.

```
# MagIndex DeltaRotation (999 not measured) SolvedRotation (999 not measured)
↪Pixel size (nm, 0 not measured)
RotationAndPixel 17 999 999 3.396
```

Here, 17 is mag index for 1250X, and 3.396 is pixel size in nm just calibrated.

Step 7

- You might want to change to a grid without repeating features, please see “note” at the end of this document.
- Calibration - Image & Stage Shift - IS from Scratch.

Step 8

- Calibration - Image & Stage Shift - Stage Shift.

Step 9

- Calibration - Administrator, turn it on.

Step 10

- Calibration - Save Calibration.

Step 11

- Take the tilting axis value (e.g. 86.1) from step 8 - stage shift calibration, edit it into the 2nd “999” in *SerialEM-properties.txt* like below.

```
RotationAndPixel 17 999 86.1 3.396
```

Note: The pixel size and tilting axis can just be done for a couple of switching mags such as the lowest M and the highest LM. SerialEM uses these couple of calibrations and all the Image Shift calibration to interpolate to obtain the pixelsizes and tilting axis angles for all other magnifications. This is very cute. HOWEVER, this is for a scope to have consistent image shift for all the mags. For some scopes this might not be true. Thus it is not a bad idea at all to just have pixel size calibrated for all the important mags you will use.

When mag gets high, you can use montage overview images to calibrate pixel size. With Image Shift already calibrated well, montaging using Image Shift can be pretty fast. So in the end you also have lines like below from menu Calibration - Pixel Size - List Relative Rotations

```
RotationAndPixel 18 0.22 999 2.602 # 1600, p=4164
RotationAndPixel 19 -0.29 999 2.006 # 2000, p=4012
RotationAndPixel 20 -1.29 999 1.571 # 2600, p=4084
RotationAndPixel 21 -0.25 999 1.22 # 3400, p=4149
RotationAndPixel 22 0.08 999 0.954 # 4300, p=4102
RotationAndPixel 24 999 999 0.6203 # 6700, p=4156
RotationAndPixel 25 0.02 999 0.4912 # 8500, p=4176
RotationAndPixel 26 0.58 999 0.3799 # 11000, p=4179
RotationAndPixel 27 -0.05 999 0.2992 # 13500, p=4039
RotationAndPixel 28 -0.58 999 0.2383 # 17500, p=4170
RotationAndPixel 29 3.37 999 0.1849 # 22000, p=4069
RotationAndPixel 30 -0.11 999 0.1456 # 28000, p=4077
RotationAndPixel 31 -0.09 999 0.1141 # 36000, p=4108
#RotationAndPixel 32 -0.06 999 0.09005 # 45000, p=4052
RotationAndPixel 32 -0.06 999 0.087 # from KK's pixel refine
RotationAndPixel 33 0.02 999 0.07141 # 57000, p=4071
```

Step 12

- Increase Mag by 1 click and do Calibration - Image & Stage Shift - Image Shift

Step 13

- Repeat above step to cover all the magnification till the highest to be used such as 100kX.

Step 14

- Decrease Mag by 1 click and do Calibration - Image & Stage Shift - Image Shift

Step 15

- Repeat above step to cover all magnification till the lowest to use like 46X.

Step 16

- At about 20kX, do Autofocus calibration (only need to do at single mag).

Step 17

- Beam Crossover calibration

Step 18

- Start with most used spotsize like 7, do Beam Intensity calibration

Step 19

- repeat Beam Intensity Calibration for all other spot sizes likely to be used: 3, 4, 5, 6, 8, 9.

Step 20

- At one mag like 5000X, using spot size 9, do Beam Shift Calibration (only need to do at single mag).

Step 21

- Usually, people use the lowest M mag for Low Dose View beam and with large defocus offset such as -200 or -300 microns. You need to calibrate High-Defocus Mag for this View mag. This will make stage shifts still good for such large defocus, as they are interpolated for the defocus offset.

Note:

- Calibrations needed to be done for *both* μ P and nP mode include: *beam crossover*, *beam intensity*, *beam shift* and *autofocus*.

- Waffle grating grid is good and handy for pixel size calibration, but it is not ideal for Image Shift and Stage Shift calibrations, as the waffle pattern might screw up the correlation in the calibration procedures. I found the normal Quantifoil grid with some 10nm Au particles absorbed onto can be very good for normal calibration purpose. I glow discharge a Quantifoil grid and add 1 μ l deca-gold solution on the grid and let it dry.
- I found that standard **PtIr** grid for TFS to perform Thon Ring test also works very well for calibration purpose.
- Most of SerialEM actions are cross-correlation based, including calibrating. Therefore, a clean and recent preparation of camera gain reference file is desired, because it will help to have less screw-up due to fixed noise pattern dominating the cross-correlation.

4.2 SerialEM Note: Make All LMM Maps Automatically

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Date_Created Apr 3, 2018

Last_Updated Feb 18, 2022

Abstract We found that it was extremely useful to be able to make Low Mag Montage (LMM) maps for all the grids in autoloader cassette automatically. Since it can take a while for multiple grids, you should give yourself a good break while scope is busy working without feeling guilty.

4.2.1 Procedure

Here are steps to follow.

1. Dock the cassette. After temperature in the autoloader recovers, do *Inventory*.
2. Setup image condition. I do it inside or SerialEM Low-Dose mode. I use **Search** area for the job. On our Krios with GIF/K2, I set mag for **Search** as 220X (I cannot go lower as wish, because some hardware piece in lower portion of column will start to cut into image.).
3. Setup proper exposure and binning for **Search** parameter from camera control panel. I usually use binning 2, exposure 1 seconds, and in Linear mode (mP mode, Spotsize 8).
4. Take a *Search* shot, make sure the count value is proper, no beam/aperture edge in the image.
5. Navigator - Montaging & Grids - Setup Full Montage. Make sure **Search** is checked in the montage setup dialog window. Define a file like LMM.map.
6. Edit script **Cars** to reflect cartridge and sample information, like below:

Listing 1: Cars.txt

```

1 ScriptName Cars
2
3 # script of cartridges information
4
5 rootDir = X:\Munan_20180402
6 cat = { 2 3 4 5 6 7 }
7 name = { 56-g1 56-g2 56-g3 56-g4 54-g2 54-g4 }
8
9 SetDirectory $rootDir
10 ReportDirectory

```

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```
11
12 If $#cat != $#name
13     Echo      >>> Arrays "cat" and "name" have different length, exit ...
14     Exit
15 else
16     echo      cat = { $cat }
17     echo name = { $name }
18 Endif
```

Here you define folder location, cartridge #, and sample names. The map filename will have the info in it, such as LMM-Car2-56-g1.st.

7. Now run the Script **LMMCars** as below:

Listing 2: LMMCars.txt

```
1 ScriptName LMMCars
2 # LMM for multiple cartridges, assumes the montage file opened.
3
4 #####
5 # navigator must be open
6 #####
7 Call Cars
8
9 ##### No editing Below #####
10 CallFunction LMMCars
11 ## in the end, rise mag to settle temp & Close the valves
12 #GoToLowDoseArea V
13 SetColumnOrGunValve 0
14
15 #####
16 Function LMMCars 0 0
17
18 Loop $#cat index
19     LoadCartridge $cat[$index]
20     #SetNavRegistration $cat[$index]
21     SetColumnOrGunValve 1
22     MoveStageTo 0 0
23     OpenNewMontage 0 0 LMM-Car$cat[$index]-$name[$index].st
24     Montage
25     NewMap
26     CloseFile
27 EndLoop
28
29 EndFunction
```


4.2.2 Convert LMM maps into JPEG format

For easy display and small file size, we usually convert all the maps in MRC format to JPEG.

- Set Bin Overview to 1 on Montage control panel (default is usually higher than 1 with montage from command)
- Load the map file, the overview will be displayed in a specific buffer such as Q
- Run a small script

Listing 3: LMM->JPEG.txt

```
1 ScriptName LMM->JPEG
2 # convert to JPEG format for easy display
3
4 SetDirectory X:\Munan_20180402
5
6 # reduced image for good JPEG density range, reduced one will be in A
7 ReduceImage Q 2
8 SaveToOtherFile A JPEG JPEG LMM-Car2-56-g1.jpeg
```

Note:

- The JPEG image generated from above script is *true* JPEG file, not a JPG compressed TIFF file as before. Compressed JPG cannot be displayed properly by Photoshop and ImageJ, although preview, paint and web browser can show them nicely.
 - You can also convert MMM maps and single shot MRC image the same way.
-

4.3 SerialEM Note: Setup Dummy Instance

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Date_Created Dec 16, 2017

Last_Updated Apr 14, 2021

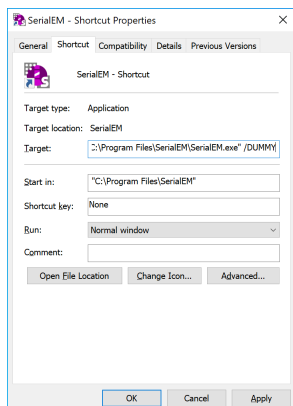
Abstract Dummy instance of SerialEM can be very useful in two cases: 1) to be used on the same computer while main instance of SerialEM is busy collecting data; 2) can be used on a remote computer, e.g., a home computer to pick particles or test scripting etc.. Here I list what is needed to setup dummy instance in these two cases.

4.3.1 On the same computer

Since SerialEM is installed and working, this is very simple.

1. make another alias (shortcut) from main instance icon.
2. edit new shortcut's property to add "/DUMMY" at the end of the Target line, as below.

Fig.1 Property Widows for Dummy Instance



4.3.2 On a remote computer

1. install SerialEM onto a remote computer, as described in [Installation and Calibration](#).
2. get property and calibration files from a working scope and put them in the default location C:\ProgramData\SerialEM, on a Windows 7 or 10 system.
3. make a shortcut from the executable and edit the property of the shortcut to add “/DUMMY” to the end of Target line, as above.
4. repeat the last two steps for a different scope.

4.3.3 Make Multiple Dummies For Different Scopes

If we want to make multiple dummies on the same computer, say, one for Talos and one for Krios, we modify above procedure slightly different.

1. put two sets of systems files (property and calibration files) to two folders, such as C:\ProgramData\SerialEM\Krios and C:\ProgramData\SerialEM\Talos.
2. Edit both property files to insert two lines into each one.

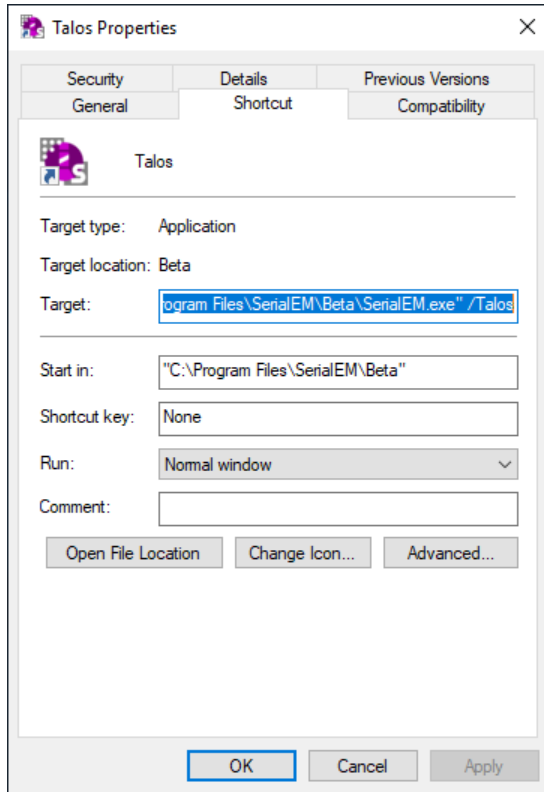
Listing 4: /DUMMY equivalent properties

```

1 NoScope      1
2 NoCameras    1

```

3. Instead of /DUMMY, the /Krios or /Talos is used in the Target line.

Fig.2 Property Widows for Talos Dummy Instance

4.3.4 On a Mac computer

Thanks to Pranav Shah <p.shah.lab@gmail.com> who shared with me how he setup SerialEM on a Mac computer using Wine. It turns out fairly straightforward, as long as you know which wine to install. The nice tutorial [Installing Wine on Mac](#) written by David Baumgold is easy enough to follow.

Note: Wine support is not quite as good as on a real Windows system. I have found the best solution to run SerialEM on a Mac computer is via a virtual machine. I have briefly tried a couple of them, and Parallels seems to be the best VM program. It even supports Mac with M1 chip, to run a Windows on ARM. SerialEM runs well on it. It is not free though.

4.4 SerialEM Note: Setup LD with Mix of μ P and nP Modes

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Date-created 2018-03-31

Last-updated 2020-09-21

Abstract There are cases and situations that people want to use nanoprobe(nP) mode , but nP is not comfortable for lower mag range such as first a few magnifications just above LM, because the beam doesn't spread wide enough to cover entire camera area. This forces us to use μ P for View and nP for rest of LD areas, namely F, T and R etc..

However, most people find it hard to setup LD conditions with the mix of nP and μ P modes. I had frustrated time doing so too. This is, I think, mainly because nP and μ P don't share the same origins for beam shift and defocus (and beam tilt too) - they have their one origins. In SerialEM, all the LD conditions are linked together. Therefore, the seprate origins of focus and beam shift for μ P and nP modes give extra hard time setting up LD in this mix use of nP and μ P.

SerialEM already has a way to deal with this problem. I hope this doc makes it clearer and easier to follow practically.

4.4.1 Procedure Setting Up LD with μ P and nP

In my case, I use μ P for View area with -300 microns focus offset. All other areas - F, T and R are with nP. I usually use the same spot size for everything.

0. Before LD is turned on, make sure beam is centered for both μ P and nP beam. I usually use Direct Alignments to do this with μ P and nP beam. That is, turn μ P on, Directly Alignments - Beam Shift (multi-function to center) - done. Repeat with nP mode.
1. Turn on SerialEM LD.
2. Lower Down large screen or insert screen.
3. From Task - Specialized Options, make sure the "Adjust Focus on Probe Mode Change" is NOT checked.
4. Set View Defocus Offset to 0 using dial Up-Down button on SerialEM LD Control Panel.
5. Select R area (radio button) on LD control panel.
6. On microscope right panel, press "Eucentric Focus".
7. Reset Defocus (L2 button on our current setup for soft buttons, yours could be different), this makes defocus display 0.
8. Select V area (radio button) on LD control panel.
9. wait 6-7 seconds to allow scope to switch to this mag and μ P mode.
10. On microscope right panel, press "Eucentric Focus".
11. Reset Defocus (L2 button on our current setup for soft buttons, yours could be different), this makes defocus display 0.
12. Set View Defocus Offset to target value (e.g., -300) using dial Up-Down button on SerialEM LD Control Panel.
13. From Task - Specialized Options, make sure the "Adjust Focus on Probe Mode Change" is **NOW** checked.

That's it.

4.5 SerialEM Note: More About Z Height

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Date Created Dec 12, 2017

Last Updated Apr 08, 2021

Abstract This is the first of a series of SerialEM notes I have wanted to write for a while. An application of using SerialEM, even a simple one, could be very useful and “handy” for a SerialEM user. I try to give more explanation for what I did, rather than to just present plain lines of codes (yes, SerialEM scripting code) so that it can be helpful for a SerialEM user, especially a new comer to understand better how SerialEM works.

Quickly and accurately moving specimen to eucentric height is a frequently needed task. Everything is going to be easier if specimen is at eucentric height and objective lens at eucentric focus. I wrote a little document before how to use tilted-beam method to do this using SerialEM “[SerialEM HowTo: Positioning Z](#)”. In this note, I give you an improved version and hopefully it is easier to use and more robust too.

4.5.1 Background Information

SerialEM has built-in task function to do eucentricity using stage-tilt method. It is robust, but slower than beam-tilt method. Beam-tilt method is opposite to autofocus function. When specimen is at eucentric height and objective lens at eucentric focus, the tilted beams produce precise overlapped image pair. This is how autofocus works. This principle can also be used to adjust specimen height, because if we preset objective at eucentric focus, cross-correlation between two tilted beam images also provides information how much specimen is away from eucentricity. Specifically, beam-tilt method used here does a few things:

- it sets scope objective lens to eucentric focus value
- it measures the defocus value for current specimen height using tilted-beam image pair,
- it then changes stage position to that reported value but in opposite direction,
- it moves stage to determined direction and distance,
- and it iterates until the reported defocus value is close enough to zero.

The beam-tilt method works very nicely most of time and it is pretty quick. However, there are couples of things making it less perfect. First, the signal becomes very weak when stage is already close eucentricity. We all know the contrast is the lowest when focus matches z height. We can use focus offset to increase the contrast, but non-linearity property causes some inaccuracy. The calibrated standard focus value could also change a little with time and scope condition. All these together makes it less robust.

When we use SerialEM Low-Dose mode, we often give large focus offset such as -200 microns to View area (I call it View beam) to make the View image good contrast. If we can use this large defocused View beam to obtain tilt-beam pairs for measuring defocus value accurately, that would be ideal.

4.5.2 Z_byV2 Function

The function code is below.

Listing 5: Function Z_byV2

```
1 Function Z_byV2 2 0 iter offset
2 Echo ==> Running Z_byV2 ...
3
4 # for defocus offset of V in Low Dose, save it
5 GoToLowDoseArea V
6 SaveFocus
7
8 ## set objective lens
9 SetStandardFocus 0
10 ChangeFocus $offset
11
12 ## Adjust Z
13 Loop $iter
14     Autofocus -1 2
15     ReportAutofocus
16     If ABS $repVal1 < 0.3
17         Echo Close enough, break...
18         Break
19     Endif
20     Z = -1 * 0.72 * $repVal1
21     Z = ROUND $Z 2
22     MoveStage 0 0 $Z
23     echo Z has moved --> $Z microns
24 EndLoop
25
26 ## restore the defocus set in V originally
27 RestoreFocus
28 EndFunction
```

The real difference between this and previous version *Z_byV* is an additional line inserted after *SetEucentricFocus*:

```
SetStandardFocus 0
ChangeFocus $offset
```

These two lines will set objective lens to a specific strength; the large defocus offset also offers good contrast. A special thing here is to measure defocus value using a LD_View beam. The advantage of this command is to use high contrast image.

```
Autofocus -1 2
```

This function should be called in script like this way:

```
CallFunction MyFuncs::Z_byV2 3 -288.32
```

Obviously, the -288.32 is to pass to variable *\$offset* in the function, and 3 to iteration variable *\$iter*.

Now question is how to determine this offset value for accurate Z height for and under current scope condition.

4.5.3 Find the Offset Value using Script FindOffset

If we found the good “offset” value, it will be good for some time, at least this session. So this like a short term calibration. Here is how to find it:

- Adjust specimen to Eucentricity, using FEI interface tool or SerialEM task function
- run script as below.

Listing 6: FindOffset

```

1 ScriptName FindOffset
2
3 # script to find proper offset value to run Z_byV2
4 # assume specimen is ON the eucentricity
5
6 ## Eucentric Z
7 ##
8 #Eucentricity 3
9 ReportStageXYZ
10 Z0 = $repVal3
11 #Z0 = 187.81
12
13 SetCameraArea V H
14 ReportUserSetting AutofocusBeamTilt BT
15 echo BT = $BT
16 SetUserSetting AutofocusBeamTilt 1.6
17
18 ## now find the offset
19 # for initial offset, get a close value from current setting
20 ReportUserSetting LowDoseViewDefocus
21 offset = 0.72 * $repVal1 # or
22 # offset = -153 # some starting value from previous run
23
24 Loop 10
25     CallFunction MyFuncs::Z_byV2 1 $offset
26     ReportStageXYZ
27     Z = $repVal3
28     diffZ = $Z - $Z0
29     echo $diffZ
30     If ABS $diffZ < 1
31         offset = ROUND $offset 2
32         echo >>> Found "offset" is $offset
33         echo >>> run "Z_byZ2 $offset"
34         Break
35     Else
36         offset = $offset + $diffZ
37     Endif
38 EndLoop
39
40 NewArray X 1 11
41 NewArray Y 1 11
42
43 temp_offset = $offset - 10
44
45 Loop 11 i
46     CallFunction MyFuncs::Z_byV2 1 $temp_offset
47     ReportStageXYZ

```

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```

48     Z = $repVal3
49     diffZ = $Z - $Z0
50     Y[$i] = $diffZ
51     X[$i] = $temp_offset
52     temp_offset = $temp_offset + 2
53 EndLoop
54
55 LinearFitToVars X Y
56 echo $repVal1 $repVal2 $repVal3 $repVal4
57
58 real_offset = - $repVal3 / $repVal2
59 echo =====> $real_offset
60
61 SetUserSetting AutofocusBeamTilt $BT
62 RestoreCameraSet

```

It uses function `Z_byV2` to see which offset value to recover the Z height determined early by other method. It first find an *offset* value that recovers Z height within 1um(you can define 0.5), then it uses a fitting method to refine this value to make it more accurate. If this script runs and gives offset value as -153.51, then you should use the function with this value.

Note: This offset value changes when V beam size changes. Therefore, it makes sense to do this “calibration” of finding offset value after all the Low Dose area conditions are set and fixed. With the “good” offset value that gives good results, the program works very reliably, if the V beam doesn’t change. For example, on our Krios, the V beam (called Low Dose area V) illumination area stays the same, the script works very well.

```
CallFunction MyFuncs::Z_byV2 3 -153.51
```

It will move stage position to Eucentric Z height, almost magically!

4.5.4 Note about Damping Factor

You might have noticed I used 0.72 in the value of Z movement:

```
Z = -1 * 0.72 * $repVal1
```

This is to compensate the non-linear behavior of autofocus measurement, with the condition of large defocus offset used. For example, when the stage Z position is -100 microns off from the eucentric height, the autofocus measurement gives something like -136 microns. Therefore, using a proper damping factor ($100 / 136 \sim 0.73$ here) can make the Z movement more accurately to the target. Since this is a non-linear behavior, this damping factor changes with Z. For example, when Z is off very little, say 5 micron, the factor can be larger like 0.85. One would naturally try to find the curve so to use a more accurate damping factor value in interpolating fashion dynamically. However, if you think about backlash of stage movement, it is the best to avoid any overshoot. Using a single, slightly smaller value could help to keep stage move with backlash corrected when iterating a few times. 0.72 is found to be a good number in our situation.

What exactly the damping factor value should you use? I suggest you move your stage 200 microns away, and you calculate the the ratio of 200 to autofocus measurement value `$repVal1` after `ReportAutofocus` (damping factor = $200 / \$repVal1$) and use the result.

If setting correctly, even your stage is more than 150 microns away, calling the function with three rounds of iteration can bring the stage to eucentric height close to within 0.5 microns in a few seconds. Amazing to me.

Note: Since 4.0 Testing, eucentricity task using titled beam has been built in SerialEM task. Please see SerialEM helpfile [Eucentricity by Focus Setup Dialog](#).

4.6 SerialEM Note: Speed, Speed and Speed

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Created 2017-12-26

Updated 2022-02-19

Abstract Speed of data collection is important, specially for a facility running 24/7 and for whole year long. If we can save a few seconds for an exposure, which might not sound much, it becomes a lot accumulated in a year time. We could collect more data, help more users if we are more efficient.

Thanks to the author and developer, David Mastronade, SerialEM is always under active development and improvement. I believe efficiency has been one of the goals for development. For example, positioning routine - Realign earlier always run two rounds: first round to align to the center of a map or a center of a piece of a map; and second round to align to the actual target. Later version of SerialEM can skip the first round of center align if it is done recently. This saves huge amount of time for single particle application.

In this document, I want to mention a few tips which you might or might not be aware of. Some of the things are related to newly added features of SerialEM. Some are from my personal experience. It would make me happy if they can also save you a few seconds.

4.6.1 Minimize Mag Switch

Switching between mags takes time. You can definitely feel the slowness of mag switching, say between 1250X and 130kX. You might think of turning off some lens normalization via FEI interface, but I always worry the stability of the system might suffer. I am not trying to save time from there.

However, I found that I could save time from this positioning actions:

```
RealignToNavItem 1
Copy A P
ResetImageShift

View
AlignTo P
ResetImageShift
```

This little script uses the last image of Realign routine which has some image shift in it, as reference to do another round of aligning and *ResetImageShift* to get rid of image shift. It seems to be flawless and it is actually working. But I noticed the scope switched from View mag to Record mag for short period of time and then switch to View mag again during the actions. There is an extra switch there! At first, I was very puzzled, then I realized that I had been using a wrong command!

The problem is caused by the argument 1 in command line:

```
RealignToNavItem 1
```

The argument “1” here means scope will resume to the state before realigning routine. And that state is high, record mag from exposure of last navigator point. Therefore, with above script, scope switch to View mag to perform realign function and then it switches back to record mag. It then switches to View mag again when at line of

```
View
```

If I put “0” as argument for “RealignToNavItem” like here:

```
RealignToNavItem 0
```

then scope stays in View mag. It at least saves 5 seconds!

4.6.2 Order of Actions

When we use “Acquire at points . . .” to collect single particle data, the default action of control mechanism is to move stage to the new item’s stage position. And then it starts to run the actual collecting script like “LD”. If the first action in the “LD” script is `RealignToNavItem`, the scope changes to the map mag, usually is View mag. Therefore, there are two physical actions here involved - stage move and mag switch.

For whatever reason, before stage movement finishes, scope can not do anything. Since “`RealignToNavItem`” will also introduce stage movement, if we ask `RealignToNavItem` to take care of mag switching and stage movement, it can move stage while mag switching is happening. This can initiate two actions at the same time; therefore, saves time.

This is new feature added not long ago. In late versions, there is a check box “Skip initial stage move” in “Navigator Acquire Dialog” window for this very purpose.

4.6.3 Using Beam Tilt for Z Height Change

We all know how important is to have Z height close enough to eucentricity. If there is 10 micron off, then everything won’t work quite right. SerialEM’s built-in function “Eucentricity” is a robust function, straightforward to use. However, it takes some time to run due to stage tilting and settling time required. I wrote two scripts (functions) “`Z_byG`” and “`Z_byV`” to use beam tilting pair for the same job. They do not use stage tilt and takes less images, therefore, it runs faster. You do have to get calibration done for Standard Focus value though.

In single particle data collection, sometimes, we have to make MMM maps from many meshes. The very first thing we do after getting to the center of a mesh is to fix the eucentricity height before map is collected. Using beam tilting method, it can save bit of time in this process.

From my own experience, doing the eucentricity using beam tilting method even works fairly well in low range of magnifications. It seems to be accurate enough for parallel beam capable scope like Krios.

4.6.4 Relaxing Stage After Moving to Target

For high quality movie stacks, even we use short frame time, the stage drift rate is still needed to be monitored. Some people use longer frame time due to worry the signal within frame being too weak for frame aligning later. In this case, drift control needs to be in place seriously, as stage naturally drifts and it can have different speeds at different time.

SerialEM can ask stage to move with backlash retained or imposed. After such movement, relaxing stage stress by moving backwards a small distance can help stage settle down much faster, at least to a normal behaviour stage. This feature has been implemented into SerialEM now. I have found it saves us huge amount of time for our routine data collection. I strongly recommend to upgrade to later version for this reason.

The feature is used this way:

```
ResetImageShift 2
```

2 means moving stage with backlash imposed or retained, and moving backward 25nm distance in the end. This small distance doesn't actually move the stage location, but helps relax the stage mechanical stress. You can also ask to move backwards a different distance by adding 2nd argument to the command, like below.

```
ResetImageShift 2 50
```

This will move 50nm, rather than 25nm as default.

Moving stage with backlash imposed takes extra time itself. Therefore, we don't want to move stage always using this way, but the final movement to the target. Here is a portion of a function called "AlignToBuffer" I wrote.

```
## align
Loop $iter ind
  $shot
  # still need crop, for Camera which doesn't do flexible sub-size like FEI cameras
  ImageProperties A
  XA = $reportedValue1
  YA = $reportedValue2
  If $XA > $XP OR $YA > $YP
    echo CallFunction MyFunc::CropImageAToBuffer $buffer
    CallFunction MyFuncs::CropImageAToBuffer $buffer
  Endif
  AlignTo $buffer
  If $ind == $iter          # last round of loop, relax stage
    ResetImageShift 2
  Else
    ResetImageShift
  Endif
Endif
EndLoop
```

Here, I asked stage to relax only at final round of iteration. If you use this function, you should update it to include this nice feature.

Alternatively, we can also directly move stage backwards after ResetImageShift. The idea is if we know the align shift in last image that is to be cleared out by ResetImageShift command, we know the directions of stage movement and we just stage backwards a little. This again demonstrates the powerfulness of flexibility of SerialEM scripting!

```
AlignTo $buffer          # comment out this line if last action is RealignToNavItem
ResetImageShift

## relax
# report shift in buffer A from last round of Align
# move stage 0.025um in opposite directions
ReportAlignShift
shiftX = $repVal5
shiftY = $repVal6

# just in case it got a blank image so no shift found
If $shiftX == 0 OR $shiftY == 0
  signX = 0
  signY = 0
Else
  signX = $shiftX / ABS ( $shiftX )
  signY = $shiftY / ABS ( $shiftY )
Endif
```

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```

moveX = -1 * $signX * 0.025
moveY = -1 * $signY * 0.025
echo Relaxing ...
MoveStage $moveX $moveY

```

This relaxing portion can be put into a function so the script can be neater.

```

AlignTo $buffer      # comment out this line if last action is RealignToNavItem
ResetImageShift
CallFunction Relax

Function Relax 0 0
## relax
# report shift in buffer A from last round of Align
# move stage 0.025um in opposite directions
ReportAlignShift
shiftX = $repVal5
shiftY = $repVal6

# just in case it got a blank image so no shift found
If $shiftX == 0 OR $shiftY == 0
    signX = 0
    signY = 0
Else
    signX = $shiftX / ABS ( $shiftX )
    signY = $shiftY / ABS ( $shiftY )
Endif

moveX = -1 * $signX * 0.025
moveY = -1 * $signY * 0.025
echo Relaxing ...
MoveStage $moveX $moveY
EndFunction

```

And if you allow final position with a little Image Shift (very OK with coma compensation in place), then this part positioning can be accurate and simple:

```

AlignTo $buffer      # comment out this line if last action is RealignToNavItem
ResetImageShift
CallFunction Relax
AlignTo $buffer      # final round of align to buffer, so position is accurate

```

4.6.5 Using Compression on K2 Data

Most people collect single particle data with K2 camera using Super-resolution mode. One of the “hidden” advantages is that the Super-res raw frame data is in 4-bit unsigned integer type, and there are lot of zero’s there. Such data can be compressed very efficiently and losslessly using mature compression algorithms. Unfortunately, MRC is not a file format that can directly use those algorithm libraries for compression. TIFF is.

SerialEM implemented this compression feature in. It gives options not to apply gain reference before saving and to use compressed TIFF as saved data format. This might not sound a big deal, but the minimal size of lossless compressed raw dataset makes huge difference for a facility that runs constantly. The small dataset file size is not only beneficial for long term storage, but also makes it a lot faster to transfer and copy off. Network behaves very differently for a lot of 400MB datasets from a lot of 10GB datasets.

Personally, I recommend to use compressed TIFF and without gain normalization applied for data saving format.

4.6.6 Using Local HDD or SSD

It is usually fine to save the frame data directly onto a large size data storage network system. In our systems, a CIFS mount initiates a network drive on K2 computer so that we can directly save to that. However, in the case that the storage system is busy doing some other tasks such as transferring data to customers, being used by local image processing programs etc., directly saving to network drive could take extra time than saving onto local SSD drive on K2 computer.

In our experience, it is best to save raw data on local SSD or HDD first, and then align frames using *framewatcher* (IMOD program) on-the-fly and let the *framewatcher* move the processed raw frames and aligned output average to network drive. This way, not only the local SSD drive will never be filled, but also the network activities on the LAN are spread out more evenly. Data collection won't slow down at all due to network performance.

4.6.7 Using Multishot

Multi-shot method is perhaps the most efficient way for single particle data collection. It can speed up quite a bit. Please refer a separate note - **Tackle the Coma**.

4.7 SerialEM Note: Tackle the Coma

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Date-created 2018-3-11

Last-updated 2022-2-19

Abstract For high resolution data, coma is always a concern or something we don't want to miss or ignore. With image-beam shift, even on a carefully aligned, coma-free scope, there might always be some coma induced by the shift. On the other hand, if we can collect CryoEM data with some image shift, that would increase the efficiency a lot. The question is how much worse the data becomes with certain amount of image shift in the shots. A more important question is if we can have a way to correct coma that is induced by the image-beam shift.

In this note, I try to explain how to assess coma induced by the shift, more or less quantitatively and how to correct the coma using currently available functions in SerialEM.

This is very fresh, work in progress, both in SerialEM and this document.

4.7.1 Background

The coma we discuss here is axial coma. It is how much the incident direction of electron beam is off from perfect optical axis. This small angle makes electron beam hitting on specimen not perpendicularly. The effect might not be easily seen visually and directly from a typical single particle electron image, but it is very real. If you look at inorganic material lattice image in electron microscope, a tiny alpha angle change will make the lattice image no longer even and symmetric. For single particle image, this introduces a phase error because we assume all the images are taken with perpendicular incident beam. It is hard or almost impossible to correct this error from all the images taken with coma, at least for single particle images. Therefore, if we can eliminate this error experimentally, that would be a good thing to do.

Align scope to minimize coma

There are tools from microscope operating software interface. For example, in FEI Tecnai/Talos/Titan interface, there are Rotation Center and Coma-Free alignment you can use to minimize this instrument parameter. You might call this manual alignment.

There are also separate tools (programs) to align the scope for coma-free purpose in more automated fashion. FEI has Auto-CTF, Legicon uses Zemlin plateau method to correct coma; SerialEM also has its own built-in functions to do coma-free alignment. In SerialEM, the two functions are called [BTID Coma-free Alignment](#) and [Coma-free alignment by CTF](#). One uses beam tilt induced displacement (BTID) and other uses fitted CTF information. CTF method is quick and accurate, but it does require clear Thon rings to fit. It gives options to use full 9-piece panel (Zemlin plateau) or 5-piece method. They work fairly well to my eyes.

4.7.2 Linearity relationship between Image Shift and Induced Beam Tilt

With the built-in tools to correct and measure coma, it is possible to study the behavior of beam tilt induced by image shift. On a well aligned scope, image shift still introduces extra beam tilt, because the beam is no longer on axis anymore, and alignment for beam shift pivot points perhaps is never accurate enough. This is known, but the relationship between the them was not clear.

With the latest version, we can run following SerialEM script (this is modified from the David Mastronade's original one) to learn the behaviors.

```
ScriptName BTvsIS
extent = { 0.5 1.0 1.5 2.0 2.5 3.0 }
FixComaByCTF
Loop $#extent ind
  ReportImageShift xbase ybase
  SetImageShift $xbase + $extent[$ind] $ybase
  FixComaByCTF 1 1
  ReportComaTiltNeeded xpxplus ypxplus
  SetImageShift $xbase - $extent[$ind] $ybase
  FixComaByCTF 1 1
  ReportComaTiltNeeded xpxminus ypxminus
  SetImageShift $xbase $ybase + $extent[$ind]
  FixComaByCTF 1 1
  ReportComaTiltNeeded xpyplus ypyplus
  SetImageShift $xbase $ybase - $extent[$ind]
  FixComaByCTF 1 1
  ReportComaTiltNeeded xpyminus ypyminus
  SetImageShift $xbase $ybase
  xpx = ($xpxplus - $xpxminus) / (2 * $extent[$ind])
  ypx = ($ypxplus - $ypxminus) / (2 * $extent[$ind])
  xpy = ($xpyplus - $xpyminus) / (2 * $extent[$ind])
  ypy = ($ypyplus - $ypyminus) / (2 * $extent[$ind])
  echo extent = $extent[$ind] matrix = $xpx $ypx $xpy $ypy
EndLoop
```

The results obtained indicate linearity relationship between extra beam tilt (coma needed to be corrected) and image shift amount. And we also found that for fixed condition, especially beam size, the bi-linear matrix remained remarkably consistent. This provides a base for automatic correction of coma induced by image shift.

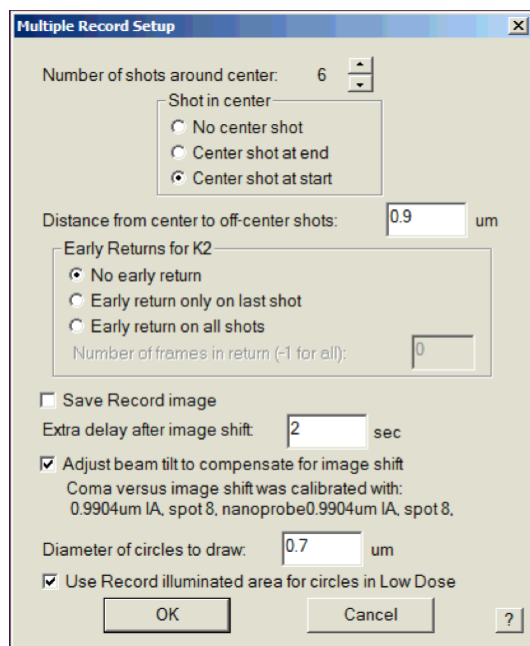
Note: The linear matrix depends on scope alignment, specially Beam-Shift pivot points. We also found that it is sensitive to beam size.

On FEI microscope, image shift and beam shift are linked. The “action” of image shift results in image shifting below Obj lens AND beam shift above obj lens.

4.7.3 Procedure to correct the coma induced by image-beam shift in SerialEM

1. decide LD image conditions specially beam size (C2% or IA).
2. perform coma-free correction routine, SerialEM - Focus/Tune - Coma-free alignment by CTF
3. calibrate the linear matrix for current image condition, SerialEM - Calibration - Coma vs. Image Shift.
4. save the SerialEM setting file. (yes, this calibrated matrix is saved in setting file.)
5. Setup multi-shot condition from SerialEM - Navigator - Montaging & Grids - Set Multi-shot Parameters. . . , and make sure the check box “adjust beam tilt to compensate . . .” is checked, as shown below.

Fig.1 Setup Multi-shot and Beam Tilt Compensation



Note: There is a script command to retrieve the calibrated Coma vs ImageShift Matrix

```
ReportComaVsISmatrix xpx xpy ypx ypy
```

It is very safe to change beam tilt this way, as beam tilt will always get restored to its original value after being corrected for this specific image shift. The exception is SerialEM program crash during the multi-shot routine finishes. If that happens, which is very rare, then one only needs to perform SerialEM - Focus/Tune - Coma-free alignment by CTF after restarting SerialEM. The matrix saved in the setting file should be still good as long as your beam size remains the same.

As always, for details please read the helpfile related sections. Two of them are:

- ‘Coma vs. Image Shift command (Calibration - Focus & Tuning sub-menu)

<http://bio3d.colorado.edu/SerialEM/hlp/html/menu_calibration.htm#hid_focustuning_comavs>`_ - Multiple Record Setup Dialog

4.8 SerialEM Note: Utilize JPG File format

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Created Apr 29, 2018

Updated Apr 20, 2021

Abstract SerialEM supports MRC and TIF file formats. Although SerialEM doesn't directly save JPG file from graphic interface as default, it does support JPG file format.

JPG file format can be very convenient for sharing and viewing due to its small file size. When we send screening results to users via DropBox, we also upload all the JPG files for LMM, MMM maps and single record shots. These JPG files take very little disc space and can be directly viewed on the web browsers. People love this feature.

In this doc, two scripts or functions were introduced to conveniently save maps and single shots to JPG format. It is nice to be able to do it in-the-fly with SerialEM imaging.

4.8.1 Save Map Overview to JPG

The function to save maps to JPG is below.

Listing 7: MapToJPG

```
1  Function MapToJPG 0 0
2
3  # SerialEM Script to convert map overview to a jpg image.
4  # it works on currently selected map item and should work for "Acquire at points..."
5  # for multiple map items.
6
7  # skip non-map item
8  ReportNavItem
9  If $RepVal5 != 2          # if not a map item
10     Echo -> Not a map item, exit!
11     Exit
12 EndIf
13
14 # load map overview into Q unbinned
15 SetUserSetting BufferToReadInto 16 # Q is 16th in alphabet, if A is 0.
16 SetUserSetting LoadMapsUnbinned 1
17 LoadNavMap
18
19 # make a jpeg image
20 ReduceImage Q 2          # loading buffer Q, factor 2 to make JPG image density_
↪range more pleasant
21 SaveToOtherFile A JPG JPG $navNote.jpg
22 EndFunction
```

One of tricks here is to load map into a buffer **unbinned** before saving to JPG. When we make montage maps using script to open montage files, the default binning for overview display is usually not 1. To take advantage of full resolution of the map, we load it unbinned. The other trick is to define a temporary loading (Read-in) buffer so the read-in buffer setup in **Buffer Control** panel becomes irrelevant.

This function can be used by inserting a line right below line `NewMap` in your script like here:


```
NewMap
CallFunction MyFuncs::MapToJPG
```

It can also be used standalone as a script for multiple maps using “Acquire at points...”.

```
ScriptName MapToJPG
CallFunction MyFuncs::MapToJPG
```

This function and its script work on **current** item of map. However, if you run on a point item and create a map from this, the above function or script won’t work on this in-the-fly situation because **current** item is the very point item instead of newly created map. For this, one needs to use a slight different one as below.

Listing 8: NewMapToJPG

```
1 Function NewMapToJPG 0 0
2 #
3 # SerialEM Script to convert last map item to a jpg image.
4 # It uses Note string as part of jpg filename.
5 # it works on an item which creates a map and should work for "Acquire at points..."
6 # as post-action.
7
8 # skip non-map item
9 ReportOtherItem -1      # last item - supposedly the newly created map.
10 If $RepVal15 != 2      # if not a map item
11   Echo -> Not a map item, exit ...
12   Exit
13 EndIf
14
15 # load map overview into Q unbinned
16 SetUserSetting BufferToReadInto 16  # Q is 16th in alphabet, if A is 0.
17 SetUserSetting LoadMapsUnbinned 1
18 LoadOtherMap -1        # last item on the nav list
19
20 # make a jpeg image
21 ReduceImage Q 2        # assuming loading buffer is Q, and reduce 2 to make JPG_
22 ↪image density range more pleasant
23 SaveToOtherFile A JPG JPG $navNote.jpg
24 EndFunction
```

The trick here is to Report and Load the last item in the nav list which is the newly created map.

4.8.2 Save Single Shots to JPG

We can also save every single shot to JPG format along with MRC images. The MRC file is required to be opened. The JPG filename contains root name of the MRC file and section numbers.

Listing 9: AToJPG

```
1 Function AToJPG 0 0
2
3 # SerialEM Script to save image in buffer A to a jpg image.
4 # It reduces image in A by 2 for comfortable JPG density range. It
5 # takes current filename and Z into jpg filename. Therefore, MRC file
6 # is required to be opened.
7
8 ReportCurrentFilename 1
```

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```

9 root = $RepVal1
10 ReportFileZsize
11 z = $RepVal1
12
13 ReduceImage A 2
14 SaveToOtherFile A JPG JPG $root-$z.jpg

```

It can be used after saving MRC image for each exposure, like below:

```

Record
Save
CallFunction MyFuncs::AToJPG

```

4.9 SerialEM Note: Setup Email on K2 Computer

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Created 2018-05-05

Updated 2018-05-05

Abstract SerialEM can send email; this feature has been there for a long time. I did not feel this was a must have so did not pay much attention to it. Now, we run scope 24/7 and like to get notification when something is wrong so we won't lose too much time.

Although setting up a standalone email server might not be the easist thing, setting up a SMTP server and relay service on K2 computer is not that hard. This is partially because K2 computer is running a server operating system - Windows 2008 R2.

In this doc, I list what I have done to setup email notification on our SerialEM system.

4.9.1 Setup SMTP and Relay Service

I followed instructions in an on-line article [Setup and Configure SMTP Server on Windows Server 2008 R2](#). It has images for each step and very easy to follow.

4.9.2 On SerialEM Part

There are a few steps we have to do on SerialEM side.

1. add two property lines in property file:

```

# SMTPserver
SMTPServer 127.0.0.1
SendMailFrom admin@talos-k2.cryoem.umassmed.edu

```

2. define email address to receive the notification. This from Tilt Series Menu - Set Email Address.
3. insert a command line in main collection script for single partile data collection. Location of this line doesn't matter.

ErrorBoxSendEmail Script Stopped!

4. check “Send email at end” in Acquire at points ... dialog window.

4.10 SerialEM Note: K3 is installed on Talos

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Date_Created Oct 25, 2018

Last_Updated Apr 09, 2021

Abstract We have upgraded from K2 to K3 on Talos Arctica. Overall, we have had more positive experience than negative one. We are now collecting the first benchmark data, just one week after K3 installation was completed. Of course, we are using SerialEM on it.

I wanted to share our experience here. Hope it helps people to prepare for their own installation.

4.10.1 Installation Went Smooth

The installation was fairly smooth. The engineers had installed K3 at other sites before so they have quite some experience already. Total installation only took three full days. We had two K3 systems in crates - one for Talos and one for Krios. Therefore, one unique advantage we had was an extra set of identical hardware components to swap test when needed.

There were two failed hardware components in the original package for Talos, one was processing board and the other was MIB box and cable. Once these two faulty hardware components were replaced with ones in Krios K3 crates, the system came up nicely.

One can imagine, without spare hardware parts to trial and test, the installation could have taken a lot longer. We were lucky.

4.10.2 SerialEM Control

It was pretty easy to get good control of K3 camera based on previous K2 camera setup. There are only a few things we needed to redo for SerialEM calibration.

1. Camera Timing and Shutter Dead Time.
2. Image Shift calibration for all the mags to be used.
3. Pixelsizes at mag index 4, 16 and 17. They are 46X(LM), 2300X(LM) and 1250X (M). *Note: This is only to get SerialEM going, more precise measurement of pixelsize at final image mags will need to be done carefully using different methods, for high resolution image processing.*
4. Stage Shift calibration for mag index 4, 16 and 17. They are 46X(LM), 2300X(LM) and 1250X (M). Double check tilting axis angles from this step too.

The K3 camera section of properties is below:

Listing 10: K3 Properties

```

1 CameraProperties 1
2 Name K3
3 K2Type 3
4 DMGainReferenceName K3-18140113 Gain Ref. x1.m0.dm4
5 # THESE 5 WILL NEED CHANGING IF CAMERA ORIENTATION CHANGES
6 CameraSizeX 5760
7 CameraSizeY 4092
8 SizeCheckSwapped 1
9 RotationAndFlip 0
10 DMRotationAndFlip 0
11 #UsableArea 0 0 3712 3840 # top left bottom right!
12 UseSocket 0
13 MakesUnsignedImages 1
14 XMustBeMultipleOf 4
15 YMustBeMultipleOf 2
16 FourPortReadout 0
17 Binnings 1 2 3 4 5 6 8
18 BasicCorrections 49
19 HotPixelsAreImodCoords 1
20 #DarkXRayAbsoluteCriterion 20
21 #DarkXRaySDCriterion 15
22 #DarkXRayRequireBothCriteria 1
23 MaximumXRayDiameter 6
24 BeamBlankShutter 0
25 OnlyOneShutter 1
26 StartupDelay 1.195
27 ExtraBeamTime 0.10
28 BuiltInSettling 0.0
29 ShutterDeadTime 0.00
30 MinimumDriftSettling 0.05
31 MinimumBlankedExposure 0.35
32 ExtraUnblankTime 0.012
33 ExtraOpenShutterTime 0.12
34 Retractable 1
35 InsertionDelay 5.0
36 RetractionDelay 3.0
37 GIF 0
38 Order 2
39 FilmToCameraMagnification 1.31 # orig=1.342
40 PixelSizeInMicrons 5.0
41 #CountsPerElectron #37.55 not needed for K3 # measured at 3.15 e/p/s
42 ExtraRotation 0.
43 # MagIndex DeltaRotation (999 not measured) SolvedRotation (999 not measured)
44 # Pixel size (nm, 0 not measured
45 RotationAndPixel 3 999 -94.4 0
46 RotationAndPixel 16 999 -94.4 1.74 #k2=1.797
47 RotationAndPixel 17 999 90.9 3.291 #k2=3.396
48 #
49 EndCameraProperties

```

4.10.3 Shutter Control

There are a number of things one should pay attention to, in my opinion. The shutter control is the top 1 on the list.

Shutter control. This is perhaps the most important thing you do not want to miss. If shutter control is not working properly, you might have sample burned without notice. Normally, if shutter control is not working, you will have hard time preparing gain reference. So you might notice it. However, since we are not required to prepare gain reference often in daily bases, if it stops working, you might or might not notice it promptly. You might still get image, but your sample might not be protected as it should be.

With properly working shutter, the beam will get blanked if following conditions are all met:

1. Hardware components are communicating with each other normally.
2. DM is running and K3 camera is in inserted position.
3. Software configuration in DM interface - Camera Configuration has set properly as idle state for shutter one “Pre-specimen” to be closed. There is normally only single shutter cable from Gatan MIB box - shutter 1 connecting to FEI shutter router “CSU” box at one of the channels. This is a BNC connector. In our case, it connects to Channel C - *Blanker*. Make sure it is the blanker, as the other one on CSU channel “shutter” means below specimen.
4. large screen of scope is in raised position (large screen is a switch to trigger sending or retracting 5V signal through the shutter cable.).
5. In FEI scope “CCD/TV Camera” interface, make sure the fake camera name assigned for K2/K3 (Falcon in our case) is selected from the list and “insert” button is in yellow color. Click on it if this is not. This is to tell FEI CSU shutter router to let Channel C take control electronically, not to mechanically insert K3 camera, as K3 is not fully integrated into FEI TIA system. This is a standalone camera in that sense. In fact, newer version of FEI software no longer requires to add a fake camera onto camera list. Instead, there is a large button “Standalone Camera” to be clicked to do the same.

In our case, when all above conditions are met, the green LED “shutter” indicator on K3 power supply unit should be on. The “Blanker” orange color LED indicator on Channel C will be lit when idle. It blinks when a shot is taken from DM or SerialEM. If you take an exposure for 3 seconds, the LED will disappear for 3 seconds. The two images below show Gatan Power Supply unit and FEI CSU unit:

Fig.1 Gatan K3 Camera Power Supply Unit (click for full size image)



Fig.2 FEI Shutter Router Unit (CSU) (click for full size image)

Please note: at least in our case, during an exposure, there is nothing change to reflect shutter status from either CCD/TV camera interface or FEI's Jave program "Shutter Blanker Monitor". This is probably due to Gatan camera being an "external" camera.

To make absolutely sure the shutter is working properly, it is better to check it with burn marker method. You lift large screen and wait for sometime and take an image of ice sample or plastic sample in a lower mag, and you check if you see any sign of burn marker. If no burn marker seen, that would indicate the beam is blanked without a shot is taken.

4.10.4 Other things to Watch

I listed a few more other things here that I also paid attention to.

1. Camera mounting orientation. This is not critical but can give you an easier life later. Our camera is mounted in the way that camera insertion is toward autoloader. Then there is no need to configure camera rotation and flip in DM configuration.
2. There is no exsiting fiber NIC available (like the Spare port on K2 computer) for us to use. However, there is a Ethernet NIC on the motherboard you can use. I prefer to have fiber NIC for faster data transfer so I added one PCI-E 8X 10GbE network card into the main computer. It sits in the very first PCI-E slot from the top. I literually get ~1GB/s real data transfer speed, from SSD Raid X drive to my storage via CIFS. Reverse direction - from storage to local SSD is about 600+ MB/s.
3. I pre-odered extended 40 meter long data cable bundle, that includes 5 fiber bundles and one Cat6 cable. It also needs a long USB cable to connect to FEI computer for COM port communication for remoteTEM running on FEI scope for scope function calls. This one is easy to miss. I am using remote KVM system for the USB signal. The one we bought is [this one](#), it does support 4k resolution, but refreshing frequency drops to 30Hz.
4. Only at starting computer, we hear huge jet engine kind of laud sounds. After it is running, it is still noisy not too bad. I heard some lab were testing to use soundproof rack to host the computer. If this is no concern for vibration, then it would be better to locate the K3 computer and soundproof rack in the scope room. I would like that a lot. Not sure how much more heat load this one gives compared to previously K2 computer plus its processors though. I have a feeling that this soundproof server rack should work - <https://www.rackmountsolutions.net/12u-ucoustic-soundproof-server-rack/>, but haven't tested anything myself yet. Hope to hear from people about their experience.

5. There is Nvidia card K2200 for monitor display. That one doesn't have HDMI port, only two DisplayPort ports. If you need to buy KVM for remote AV/USB purpose, make sure to buy the unit that supports DisplayPort directly. DP to HDMI converter might not give 4K resolution that 32 inch Dell 4K monitor offers.
6. You should check water flow and air pressure gauge often for a fresh installation of K3, as they might change a bit in the beginning. We had a startup hiccup when the water is a little too low (~19 GPM). It became fine after it was raised to 24 GPM.
7. If there is any memory test error on any of the processors, one should shutdown and restart computer rather than a software reboot. Power cycle is likely needed to clear out memory errors.
8. K3 outputs more data than K2, one has to deal with storage capacity seriously if you run a scope efficiently. Otherwise, one might find that you quickly run out of data storage space. **Saving frame data with compression and without gain applied has clear advantages here!**
9. Our K3 system package came with a GP100 Nvidia card. Also there is MotionCor2 utility via DM interface. However, there is no way to access to MotionCor2 outside of DM. Fortunately, we can still utilize the powerful GPU card. If we run *framewatcher* to align ~30-40 Super-res frames, it can do as fast as ~10 seconds for one stack. This is sufficient at least for our session monitoring purpose. Very nice indeed!
10. Always remember to retract K3 camera first BEFORE you try to insert Ceta camera.
11. Sometimes on our system, when restarting DM, the communication between DM and microscope gets interrupted. A Keyspan USB Serial Adapter is used to establish the communication in our case. Unplugging and replugging the USB connection usually fixes the problem. However, it is almost impossible to do it remotely. We have found a workaround to re-activate the Keyspan USB Serial Adapter, **remotely**. 1) Exit DM first, 2) From Device Manager, find "Keyspan USB Serial Adapter", in its "Driver" tab, disable and enable it. This will reset the adapter. 3) Restarting DM. After that, communication will be OK.
12. After configuring communication between DM and Remote TEMserver using TCP/IP protocol, it has never got interrupted like before. So we abandoned the Keyspan USB adapter completely.

4.10.5 Additional Info

There is some additional information regarding the K3 camera from UTSW facility that you might find useful. Please find [the pdf file](#).

4.11 SerialEM Note: Adding Point Items by Template Matching

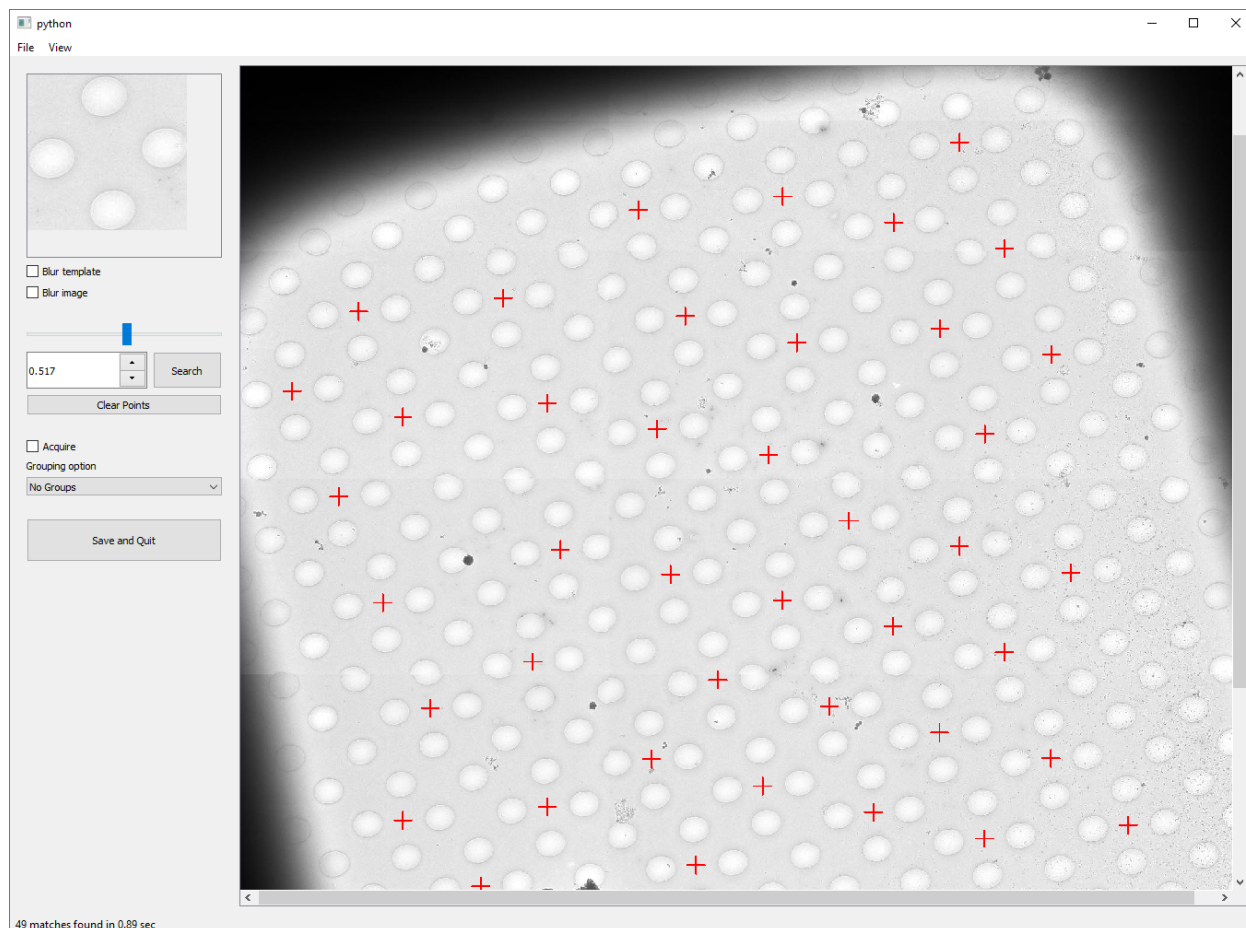
Author Albert Xu

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Date_Created 2019-07-25

Last_Updated 2019-07-25

Abstract This article describes a method of automatically picking points in SerialEM maps. Currently SerialEM does not have built-in functionality of adding points by correlation. However, SerialEM is now capable of running external Windows programs via the RunInShell script command, as well as merging in externally defined navigator items. This means that it is possible to use any external program to search for desirable points and add them to the navigator. As an example, I present a graphical tool called **semmatch** that uses cross-correlation based template matching. Hopefully in the future others will be inspired to implement their own methods and programs to improve the SerialEM user experience.



4.11.1 Install

A SerialEM script called **TemplateMatch_GUI** at the end of this article is used to launch `semmatch`. All that is required is to install `semmatch` in order to use the script. Installation instructions are on the *github* page, <https://github.com/alberttxu/semmatch>

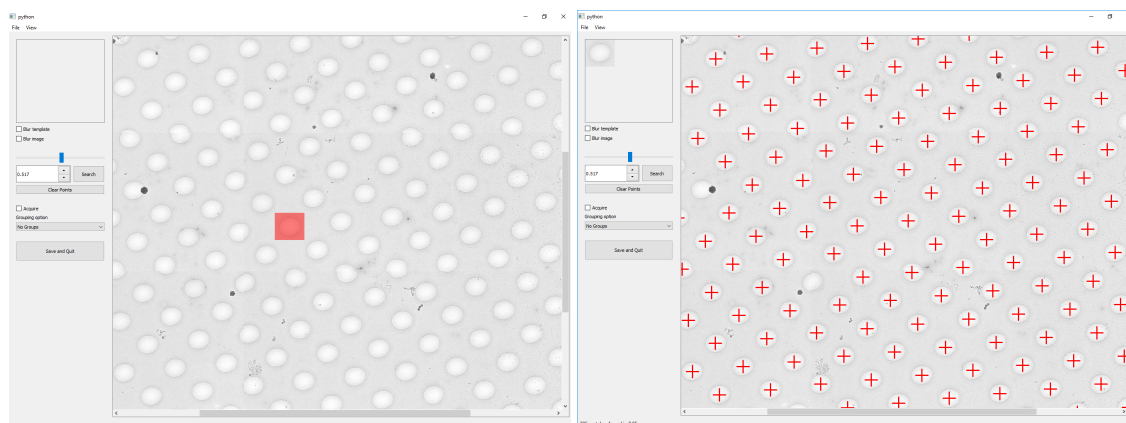
4.11.2 Usage

1. First, select a map item from the navigator. You can double click to load the image, or single click to simply highlight.

- Before running the script, you can optionally crop out a template in

SerialEM. See `TemplateMatch_GUI` for details.

2. Run `TemplateMatch_GUI`



- +/- to zoom in and out.
- To crop, just click and drag.
- Change the threshold and click search until you are satisfied with the result.
- Click Save and Quit to merge the generated points into SerialEM. To quit without saving, close the window from the top.

```
ScriptName TemplateMatch_GUI

### Before running this script:
#   (Optional) Save a template of a hole/pattern as a jpg image.
#       1. Crop a hole using ctrl+shift+drag,
#       2. Menubar->Process-> Crop Image.
#       3. Using the Edit/Run one Line prompt, run
#           SaveToOtherFile A JPG JPG T.jpg
#

# If something goes wrong, set Debug = 1
Debug = 0           # True = 1 ; False = 0

### semmatch arguments
threshold = 0.8

acquire = 1          # True = 1 ; False = 0
groupOption = 4
#   0 = no groups
#   1 = groups based on radius
#   2 = all points as one group
#   3 = specify a certain number of groups
#   4 = specify number of points per group

# names of temporary files used by semmatch
outputNav = semmatch_nav.nav
image = MMM.jpg
template = T.jpg

ReportIfNavOpen
If $reportedValue1 != 2
    Exit
Endif
ReportNavFile 1
navfile = $reportedValue1$reportedValue2
```

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```

navdir = $reportedValue3
SetDirectory $navdir

If $acquire != 1 AND $acquire != 0
    Echo acquire should be either 1 or 0
    Exit
Endif

If $Debug == 1
    debugStr = /k
ElseIf $Debug == 0
    debugStr = /c
Else
    Echo Debug should be either 1 or 0
    Exit
Endif

## load and bin MMM map
ReportNavItem
If $RepVal5 != 2      # if not a map item
    Echo Not a map item. Select a Map item from the navigator.
    Exit
Endif
MAP = $navLabel
Echo Map Label: $MAP
SetUserSetting BufferToReadInto 16
SetUserSetting LoadMapsUnbinned 1
# uncheck Montage Controls "Align pieces in overview"
ReportUserSetting MontageAlignPieces alignChecked
If $alignChecked == 1
    SetUserSetting MontageAlignPieces 0
Endif
LoadNavMap

# reduce image if larger than 2000x2000
maxdimLimit = 2000
ImageProperties Q width height
maxdim = $width
If $width < $height
    maxdim = $height
Endif

If $maxdim < $maxdimLimit
    Copy Q A
    reduction = 1
Else
    reduction = $maxdim / $maxdimLimit
    ReduceImage Q $reduction
Endif
Show Q

## make a jpeg image
SaveToOtherFile A JPG JPG $image
Echo saved $image

ReportOtherItem -1
newLabel = $navIntLabel + 1

```

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```

RunInShell cmd $debugStr " " semmatch " \
"--gui" \
"--navfile" "$navfile" \
"--reduction" "$reduction" \
"--image" "$image" \
"--template" "$template" \
"--mapLabel" "$MAP" \
"--newLabel" "$newLabel" \
"--threshold" "$threshold" \
"--groupOption" "$groupOption" \
"--output" "$outputNav" \
"--noBlurTemplate" \
"--noBlurImage" \
"--acquire" "$acquire" "

MergeNavFile $outputNav
If $alignChecked == 1
    SetUserSetting MontageAlignPieces 1
Endif
Show Q

```

4.12 SerialEM Note: Refine ZLP on Au Foil Grids

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Date-Created 2019-08-23

Last-Updated 2023-12-04

Abstract This is to introduce new implemented “Custom Time” script commands to handle refining Zero Loss Peak (ZLP) over Au foil grids.

4.12.1 Background Information

SerialEM has one script command to handle the ZLP refinement over time. Very useful. For example, if we put a line at the end of script **LD-group** like this:

```
RefineZLP 30
```

It will refine ZLP position if longer than 30 minutes since last refinement. It works very well for us, on a typical carbon based holey grid. Unfortunately, it doesn’t run well on a gold foil based grid which it is very “black” between holes. This is mainly due to large pieces of gold crystals that form the film diffract electron beams away in relatively large angle so they don’t hit the camera. SerialEM’s `refineZLP` routine works by comparing camera counts at different energy shifts. If there is clear count drop, the slit edge is detected. When with a Au foil grid, if the area but not a hole is used for such refinement, the black nature of the film causes this procedure likely to fail. When we take multi-hole exposure for 4 holes, for example, the stage position is on such “black” area.

It is possible to move to one of the multi-holes after exposure is done, but we prefer not to it for every exposure because ZLP might have been refined recently. Otherwise, time is wasted.

Therefore, we need a timer to track time and then perform two tasks in this situation after certain period. 1) move to a nearby hole, and 2) refine ZLP.

4.12.2 Timer Function Commands

This timer function is now available.

The two script commands related to the timer functions are:

```
SetCustomTime name
ReportCustomInterval name
```

Below is how to use them.

4.12.3 Use the Timer Functions

1. set timer to start counting, for example, one can simply run one-line command like this:

```
SetCustomTime ZLP
```

ZLP is the name of this timer. You can set up multiple timers with different names so they won't confuse.

2. Add this section to the end of your LD-group:

```
#SetCustomTime ZLP
ReportCustomInterval ZLP
If $repVal1 >= 30
    MoveToLastMultiHole      # move to a hole (bright area)
    RefineZLP                # refine ZLP now!
    SetCustomTime ZLP        # reset timer
Endif
```

You can use the timer function, similar to the above example, to perform various periodic tasks across the Acquire items conveniently.

4.13 SerialEM Note: Flexible Timer

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Date-created 2019-10-09

Last-updated 2023-12-04

Abstract Note about the flexible timer available for scripting and an example how to use it.

4.13.1 Background information

In SerialEM scripting, there are a few commands with timer built in. For example, command `LongOperation` can take a few specific actions with timer defined. Here is one of the example:

```
LongOperation Da 3
```

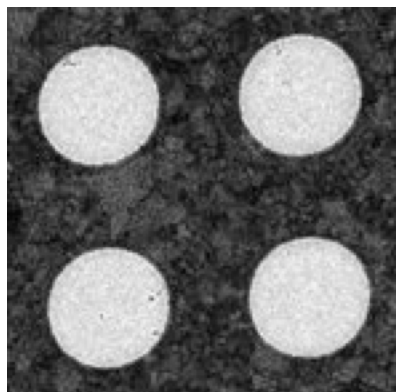
This will perform dark reference for K2/K3 camera every 3 hours. This is very handy indeed. Another example is `RefineZLP`.

```
RefineZLP 30
```

This will perform refining ZLP every 30 minutes.

However, if we want to set a timer and to do a multiple and general actions when time is up, a more flexible timer is needed. In the case shown in picture below, which is Au-foil grid, we use multiple hole mechanism to collected images in these 4 holes using beam-image shift while the stage is centered at the middle of the 4 hole pattern. When the procedure is finished, the shift is reset and Record beam would be hitting on the black Au crystals.

Fig.1 4-hole black Au crystals



It is known that this kind of black crystal film is bad for `refineZLP` routine to work properly. It needs to use a hole area to make it work. As you can see, a simple timer built in for a specific function is not sufficient here. Two actions are needed: 1) move to one of the four holes and 2) perform `RefineZLP`. It requires a more flexible timer to do this.

4.13.2 Flexible Timer

A typical timer which could handle multiple actions would be like this:

```
If time is up
  do task1
  do task2
  ...
EndIf
```

One of the script commands related to timer is `SetCustomTime`. Below is example code to perform two actions mentioned above.

```
SetCustomTime ZLP      # start timer
```

Then you can use the timer to do things based elapsed time.

```
### move to a hole and refineZLP every 30 minutes
ReportCustomInterval ZLP
if $repVal1 >= 30
  StageToLastMultiHole
  #ImageShiftToLastMultiHole
  RefineZLP           # refine now
  SetCustomTime ZLP   # reset it
Endif
###
```

4.14 SerialEM Note: More About X,Y Positioning

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Date-Created Dec 12, 2019

Last-Updated Nov 22, 2020

Abstract Robust positioning to the target position is critical for high level operation of CryoEM data collection. In this note, I like to share my own version of the latest script to perform X,Y positioning task. And I try to explain every line of the code and ideas behind them as well.

I have spent a lot of time thinking and testing about this. If you have better and different ideas, I'd love to hear from you.

4.14.1 The script lines

The script is fairly short, as shown below. It can be inserted in beginning of a single particle data collection script.

Listing 11: X,Y positioning

```

1  buffer = T
2  RealignToNavItem 0
3  ResetImageShift 2
4  #Copy A $buffer      #comment out if a fixed template in $buffer is used.
5  AcquireToMatchBuffer $buffer
6  AlignTo $buffer 0 1

```

4.14.2 Explanations Line by Line

```
buffer = T
```

This is to define which buffer will be used to store reference image, a whole image or a cropped area of an image. Buffer after N are all beyond rolling range, thus won't be pushed out by taking too many images.

```
RealignToNavItem 0
```

`RealignToNavItem` is one of the most important functions in **SerialEM**, in my opinion. It will bring the specimen stage to a valid map item. It typically uses combination of stage shift and image shift to get the job done. 0 here means to stay in the condition from which the map was created. For example, the map was generated using LD **View**, and the scope currently is at LD **R**, the scope will switch to the View mag, beam intensity etc.. After *realign* is done, it stays in View mag. Argument 1 will bring scope back to **R**, after routine finishes.

This command line will bring the specimen to the picked item position, with some image shift in the last image of the routine takes, in buffer A.

I should point out that this perhaps reflects one of the most fundamental differences between SerialEM and other data collection software - it doesn't rely on fixed template at all. As long as an item in a valid map is defined (picked), **SerialEM** will drive the stage there! For example, for a lacey grid with no regular size holes, SerialEM can go to the target positions precisely wherever they are defined as point items.

```
ResetImageShift 2
```

`ResetImageShift` is to clear out any image shift existing in the system and use stage shift to compensate. Then, there is no image shift, which means beam is straight down on the axis. However, the intrinsic inaccuracy of stage movement makes target being slightly off, more or less.

The argument 2 here means stage will clear the backlash by moving to opposite direction for 0.025 microns as default. This can be very useful to slow down the stage drifting after moving to a new location. Low drift is a very good thing since there is no way to correct drifts accumulated within a frame. This is particularly true if one has to use long frame time on some camera system.

```
#Copy A $buffer
```

If not commented out, this line will copy the last image (after realign) in buffer A to a target buffer (T in this case). If one uses a fix image, for example, a cropped hole as reference image, then it should be manually copy into T and leave this line commented out.

```
AcquireToMatchBuffer $buffer
```

This is a new command, available in 3.8 beta Dec 10, 2019 built and later. It does two things: 1) take a shot using the exact condition of what in the reference buffer for mag, beam condition, binning, exposure time etc.; 2) make the final image the same size as what in the reference buffer, by cropping if necessary. I used to have to do this in a lengthy script using two functions.

```
AlignTo $buffer 0 1
```

Simply align the image in buffer A to reference buffer. This would make the target right on again with image shift. The very last argument 1 means no trimming to any of the source image and reference image. This is needed for UltrAuFoil® Holey Gold Films grids which have very “dark” region of the film.

4.14.3 Other thoughts

1. It is helpful to use large defocus offset for map and realigning, as the contrast is significantly better. On our Krios, we use -300um for View offset (in LD).
2. If offset is more than 200um, it most likely needs High-def Mag calibration. With this, system dynamically interpolates the stage shift matrix which is calibrated using near-focus condition. This makes stage movement much more accurate and robust.
3. If possible, use whole image as “dynamic” template instead of sub-area. Using sub-area such as a single hole is a quick workaround for a grid which has periodic feature and 5-point way of picking points might be not very accurate due to local geometry variation.

4.15 FastTomo: A Hybrid Approach to Speedup Tomography Data Collection

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Date-Created May 24, 2020

Last-Updated Feb 21, 2022

Abstract Due to the imperfection of stage movement, a tomography target positon (X, Y, Z) usually keeps changing with tilting angle. In order to obtain useful data, some kind of engineering control has to be in place to ensure the target postions are within the acceptable range through the entire tilting series. This engineering control

might involve complete prediction or/and frequent tracking for X, Y and Z positions. Unfortunately, these extra control actions cost time for each tilting series collection. Here, we propose a hybrid method to make the data collection as fast as possible without sacrificing too much data quality.

We use a precalibration for each and every tilting series for Z, and we use proportional control to constantly compensate X, Y positions. We try to eliminate tracking and auto-focusing shots as much as possible.

We present a SerialEM script using this Hybrid method for all three tilting schemes: uni-directional, bi-directional and dose-symmetric. We slightly modify the original Hagen scheme of dose-symmetric with added options to 1) switch to bi-directional beyond certain defined angle and 2) change exposure time at high angle ranges after switching to bi-directional.

4.15.1 Some Brief background Information

Our tests on stage tilting behaviour mainly show two things. 1) Statistically, it is hard to get specimen height to desirable range of eucentricity. There seems to be always some error to get to eucentric height, large or small. 2) the relationship between tilt angle and X,Y,Z position is not quite linear when the specimen is not close enough to eucentric height; even at very close to eucentric position it is no longer linear at high tilt range such as above ± 45 degree.

Most of time consuming actions are the multiple tracking shots and auto-focusing procedures.

This makes us to think if we could have a way to eliminate most of, if not completely, the Focus and Tracking Shots, we might save time for tilting series data collection.

4.15.2 FastTomo Script

For Z, we found that a **SINE** function can describe the Z change fairly well. We only need a few tilt points to pre-calibrate such a **SINE** curve for each target position. Such pre-calibration does cause some time, but the prediction based on the calibrated **SINE** curve seems to be very robust and we then completely eliminate Focus shots in the collection step. The Objective lens is used to compensate.

For X,Y, we do not perform any precalibration. Instead, we just use the returned Record images to perform the proportional control. Namely, we align the feature from Record image at each tilt to its previous one and we use Image Shift to compensate. We completely eliminate Tracking T shots in the procedure except in dose-symmetric scheme which tracking shot is used only at switching angles.

The SerialEM script - FastTomo and its usage can be found from the github.com project page.

4.15.3 A Few Points to Discuss

0. This is no replacement for SerialEM controller to collect tilting series. It can save some time, by skipping most of Focus and Trial shots, if the condition is proper. For example, the eucentricity is working very well, beam center shifted to tilting axis nicely so there is no much lateral displacement, the stage is working well mechanically and the pixel size is not too small. When all these conditions are met, this script might work well and fast.

1. The same idea and method should be also applied to a FISE collection, provided the frames from each tilt can be grabbed and available for scripting.

2. A single, long exposure with shutter opening and closing during tilting series saves time for startup delay and returning time, compared to conventional way of multiple exposure one after another. But the significant time saving is from minimizing the numbers of F and T shots already! Using our script **FastTomo**, the total time for bi-directional tilting series from -51 to $+51$ degree with 3 degree step is about 6 minutes including pre-calibration for Z prediction curve.

4.16 SerialEM Note: Hidden Goodies

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Date-created Nov 21, 2020

Last-updated Feb 19, 2022

Abstract One of the amazing things about SerialEM, to me, is that I almost always find some cool functions and features which I did not use before. It could be also true for some other users, even you might not regard yourself as a novice user. If you see someone using it, you immediately get it. Otherwise, you might not realize that the cool function exists.

In this note, I want to give a few examples to show some of the hidden beauties I like a lot. And I hope you can learn them from reading this note if you don't know yet. Hopefully, the example list can grow longer with time and more exploration.

4.16.1 Example 1 - delete multiple nav items at once

This might not sound anything special, as you can use the `Delete` button on Navigator window easily, and you just click on the button when the item is highlighted, and you do this multiple time to delete a bunch of items. However, if you want to delete a series (a lot of points) items that do not belong to a single group or are only part of a group, this might become a little troublesome.

Here is a trick - to use SHIFT-D for this: you first click on the first item to be removed and press SHIFT-D, then you click on the last item and press SHIFT-D again; this will remove all the items in the range regardless their group properties.

You might find there are many hot keys available which you did not pay attention before.

https://bio3d.colorado.edu/SerialEM/betaHlp/html/about_mouse.htm

4.16.2 Example 2 - make multiple polygon montage maps at once

Your reaction might be that you already well know how to do this. Indeed, this can be done with steps below:

1. Open a polygon montage map file via menu *Navigator - Montaging & Grids - Setup Polygon Montage*.
2. Add point items to all and each of the montage positions to be acquired, and give them flag "A".
3. *Navigator - Acquire at points - Acquire Map Image or Montage*.

One of the nice key things here is that once the montage map is open and current, the program knows how to make montage for each of the positions. The montage setup dialog is only set once. Because all the items share the same file, there is no need to draw multiple polygons at different locations.

We also do this very often. We collect all the good meshes to make them into montage maps so we can pick and realign to each positions later. However, there are a couple of things we don't like with this way:

- The super-stack file for all the montages can be very large. It is not handy to look at a particular mesh off-line.
- The section # of the file is from 0,1... to the last one, they are directly linked with mesh label/numbers.

It would be really nice to make all the meshes to have their own separate files and with the mesh ID in the filenames, something like *Grid3-Mesh8.map*. This way, we can check each mesh map easily when off-line. On a windows computer with 3dmod installed, this is as simple as double clicking on the filename.

How to do that? Not so easy anymore? If you don't already know, here is how:

1. add a polygon item as you normally do.
2. make sure this polygon item is above of all and any mesh point items you already add. You do this by left mouse dragging the polygin item UP.
3. *Navigator - Options - Use Item Labels in Filenames.*
4. With polygon item highlighted, check the boxes before “Acquire (A)” and “New file at item”.
5. In the pop-up “Property of File to Open” dialog window, select “Montaged Images”. And check both boxes before “Fit montage to polygon” and “Skip montage setup dialog when fitting in future”.
6. Input in the pop-up montage setup dialog window.
7. Input your string, e.g. “Grid3-Mesh” in the filename dialog, click *Save*.
8. Now highlight the mesh item you want to make map, check “Acquire (A)” and “New file at item”.
9. repeat for all the mesh items.

After you do Navigator - Acquire at Point, you will get multiple files such as *Grid3-Mesh3.map*, *Grid3-Mesh4.map*, *Grid3-Mesh15.map* etc.. Very nice, isn’t it?

Here each mesh map will have its own filename. During collection, the filenames are opened and closed for each item. The setup step uses **heritage** function which is also quite hidden.

Tip: To obtain filename with MapID and ItemID in the filename string, here is a tip:

Listing 12: Get Map-index as part of filename

```
1 ReportNavItem
2 NavIndex = $repVall
3 pt_label = $NavLabel
4
5 NavIndexItemDrawnOn $NavIndex
6 ReportOtherItem $repVall
7 map_label = $NavLabel
8
9 filename = $map_label-$pt_label
```

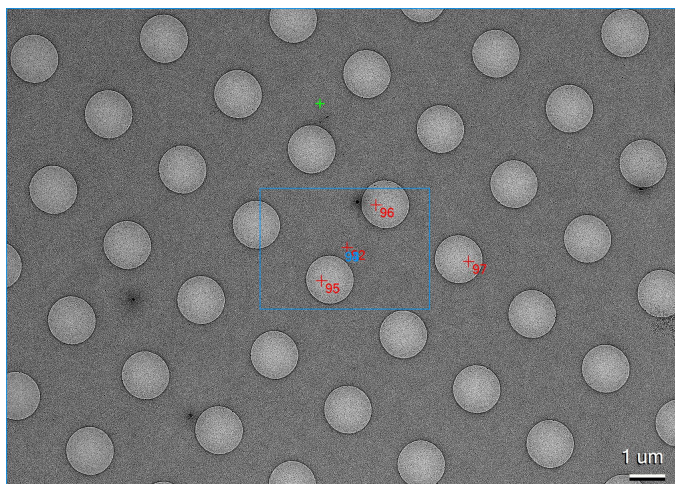
4.16.3 Example 3 - make snapshot with nav features

We already know SerialEM script has a command to make a snapshot of an image in a buffer and save it in JPG format. But that doesn’t include any nav items drawn or a scalebar. A snapshot function with possibility of including navigator items drawn and a scalebar can be very useful sometimes.

For example, in a screening session, we make a mesh montage map and pick a new point items on the map from different areas. Before or after we take all the images from these point items, it would be really cool to have a snapshot map image with all the points drawn on it. It can be very useful for us to track the image quality with ice conditions etc., because we know which image is from which area in the map.

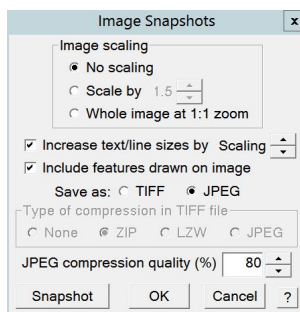
Something like an image below:

Fig.1 An example snapshot (click for full size image)



This function has been implemented fairly recently. It has a small GUI tool window and a script command for this task. You can open this tool window from *Window - Take Image Snapshot...*

Fig.2 Snapshot Tool Window (click for full size image)



There is also a command which can be used like this:

```
SnapshotToFile 1 1 1 JPG JPG snap.jpg
```

For more complete information about this little function, please check helpfile section:

https://bio3d.colorado.edu/SerialEM/betaHlp/html/hidd_screenshot.htm

and command usage description.

4.16.4 Example 4 - Run script at SerialEM program start or exit

This is a new feature in 3.9 beta. It can be very handy if you have some tasks to do when you startup and exit. For example, you might want to get into Low Dose mode and clear some persistent variable from last run. Or you have some other tasks to do when you quit SerialEM program.

You can setup it from menu *Script - Run at Program Start...* or *- Run at Program End...*, and define a script accordingly. Here I give two examples - one to define current working directory in the startup and one to make sure column and gun valves be taken care of.

Below script running at program start will pop up a file chooser to ask you define the current working directory.

Listing 13: StartUp Script

```
1 ScriptName StartUp
2 # script to run when starting SerialEM program
3
4 #SetLowDoseMode 1
5 #GoToLowDoseArea V
6
7 SetDirectory X:\
8 UserSetDirectory
9 Echo -----
10 ReportDirectory
11 Echo -----
12 OpenChooserInCurrentDir
```

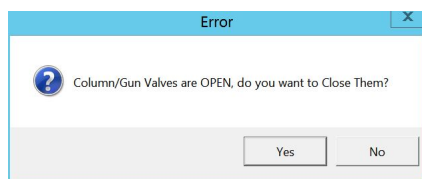
Here is my little script to run at end.

Listing 14: Ending Script

```
1 ScriptName EndingScript
2
3 # script to run when exiting SerialEM program
4
5 ReportProperty NoScope noscope # determine on Dummy or not
6 If $noscope == 0
7     ## Close Column/Gun Valves if they are OPEN
8     ReportColumnOrGunValve
9     If $repVal1 == 1 # open
10         YesNoBox Column/Gun Valves are OPEN, do you want to Close Them?
11         If $repVal1 == 1 # answer Yes
12             SetColumnOrGunValve 0
13             Echo ----- Now Valves are CLOSED! -----
14         Else
15             Echo Valves are still OPEN!
16         Endif
17     Else
18         Echo Already closed!
19     Endif
20 Endif
```

The window below will pop up when exiting SerialEM so you will never forget about this.

Fig.3 YesNo Window (click for full size image)



Clicking on Yes will close the valves and No will keep them open.

4.16.5 Example 5 - Scripting With Python

SerialEM Python Code Example

Around March 23, 2021, scripting also supports python. SerialEM native collection of commands act like a module. To enable python support, I have these lines in the property file.

Listing 15: Properties for Python Support

```
1 PathToPython 3.9 C:\Users\xuchen\AppData\Local\Programs\Python\Python39
2 PythonModulePath C:\Program Files\SerialEM\PythonModules
3 ScriptMonospaceFont Consolas
4 EnableExternalPython 1
```

Here is an example of the same CycleTargetFocus function in Python code.

Listing 16: Python Example Code

```
1 #!Python
2 #ScriptName CycleTargetDefocus
3 import seriallem
4
5 ## function
6 def CycleTargetDefocus(low, high, step):
7     print('==> running CycleTargetDefocus ...')
8     print('    ---> low, high, step is ', low, high, step)
9     tarDef = seriallem.ReportTargetDefocus()
10    if tarDef > low or tarDef < high + step:
11        seriallem.SetTargetDefocus(low)
12    else:
13        seriallem.IncTargetDefocus(-step)
14        seriallem.ChangeFocus(-step)
15
16 ## run it
17 CycleTargetDefocus(-1.0, -3.0, 0.1)
```

Calling Python Script From Regular Script

Around Apr 29, 2021, SerialEM not only got more matured at supporting Python, but also provides functionality to call regular script from a Python script and Vice Versa. The variable's value can also pass to each other. Below are two example scripts, one in Python and one in regular.

Listing 17: Regular Script

```
1 ScriptName Regular
2 a = { 1 2 3 }
3
4 echo a = $a
5 Call Python
6 Echo b = $b
```

Listing 18: Python Script

```
1 #!Python
2 #ScriptName Python
```

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(continued from previous page)

```

3 import seriallem as sem
4
5 #print('>>> running Python script ...')
6 #sem.CallFunction('Hello::ChangeMag', '', 4)
7 #sem.CallFunction('Hello::SetMagIndex', '', 17)
8
9 ## get a
10 ret = SEMArrayToInts(sem.GetVariable('a'))
11 #print(ret)
12
13 ## make reversed a into b
14 b = ret[::-1]
15 #print(b)
16
17 ## get b ready for regular
18 sem.SetVariable('b',listToSEMarray(b))

```

Running regular SerialEM script “Regular” by clicking *Run* button from the editor, the log window prints:

```

a = 1  2  3
b = 3  2  1

```

As you can see, it calls Python script “Python”. The array *a* defined in the regular script is received and convert to python list in the python script. After doing something in Python script (reverse array *a* in this case), it can get new array ready to be fetched by regular script.

Calling Regular Script and Function From Python Script

One can also do reverse - calling regular script from a Python one and passing values of list variable to from Python to regular script. Look at two scripts below:

Listing 19: Python Script

```

1 #!Python
2 #ScriptName Python
3 import seriallem as sem
4
5 a = [ 1, 2, 3, 4, 5, 6, 7 ]
6 sem.SetVariable('a',listToSEMarray(a))
7 sem.Call('Regular')

```

Listing 20: Regular Script

```

1 Echo --- running Regular Script
2 Echo $a

```

Running the “Python” script gives this in log window:

```

--- running Regular Script
1 2 3 4 5 6 7

```

Python External Control

You can also use Python Console to control SerialEM process. SerialEM acts as a complete embedded system in this case, and Python is completely external. If you run Python on a different computer from the Windows computer on which SerialEM is installed, you have to build a SerialEM Python Module on your system. Here is an example to build the SerialEM Python Module on a Mac.

```

$ hg clone http://bio3d.colorado.edu/SerialEM/PythonModule
$ cd PythonModule
$ python setup.py build

```

Below is screen output when running the python control on the same Windows computer that SerialEM is running on.

Listing 21: Python External Control - Console

```

PS C:\Users\xuchen> python
Python 3.9.2 (tags/v3.9.2:1a79785, Feb 19 2021, 13:44:55) [MSC v.1928 64 bit (AMD64)]
-> on win32
Type "help", "copyright", "credits" or "license" for more information.
>>> import sys
>>> sys.path.insert(0, 'C:\Program Files\SerialEM\PythonModules')
>>> import serialsem as sem
>>> sem.ReportTargetDefocus()
-1.600000023841858
>>> sem.ReportMag()
(59000.0, 0.0)
>>> x = sem.ReportMag()
>>> type(x)
<class 'tuple'>
>>> print(x[0])
59000.0
>>> sem.GoToLowDoseArea('R')
Traceback (most recent call last):
  File "<stdin>", line 1, in <module>
serialsem.SEMError
>>> sem.SetLowDoseMode(1)
0.0
>>> sem.GoToLowDoseArea('R')
>>> sem.ReportLowDose()
(1.0, 3.0)
>>>

```

Below is an example of an interactive Python external control from a Mac.

Listing 22: Python Console on Mac (to connect to SerialEM on Windows (192.168.1.16))

```
(base) UMWMLF8LVCJ% python
Python 3.8.5 (default, Sep 4 2020, 02:22:02)
[Clang 10.0.0 ] :: Anaconda, Inc. on darwin
Type "help", "copyright", "credits" or "license" for more information.
>>> import sys
>>> sys.path.insert(0, '/Users/xuchen/tem/SerialEM/build/lib.macosx-10.9-x86_64-3.8')
>>> import serialsem as sem
>>> sem.ConnectToSEM(48888, '192.168.1.16')
>>> sem.ReportMag()
(59000.0, 0.0)
>>> sem.GoToLowDoseArea('V')
>>> sem.ReportLowDose()
(1.0, 0.0)
>>>
```

Here is another example to run a python script on Mac to control a SerialEM running on Windows:

Listing 23: Another Python Script Example - to cycle LD areas, twice.

```
1  import sys
2  sys.path.insert(0, '/Users/xuchen/tem/SerialEM/build/lib.macosx-10.9-x86_64-3.8')
3  import serialsem as sem
4  sem.ConnectToSEM(48888, '192.168.1.16')
5  sem.ReportMag()
6  ld = sem.ReportLowDose()          # tuple
7  ld = float(ld[0])                # float
8  if ld == 0:
9      sem.SetLowDoseMode(1)
10
11  ## function to cycle LD areas, twice
12  def cycleLD():
13      for area in [ 'V', 'F', 'T', 'R' ] * 2 :
14          sem.GoToLowDoseArea(area)
15          sem.ReportMag()
16          sem.ReportLowDose()
17
18  cycleLD()
19
20  sem.ReportLowDose()
21  sem.Exit(1)
22  exit()
```

After saving to a file (CycleLD.py), we can then run it from a typical python environment normally.

```
$ python CycleLD.py
```


Embedding a Python Script in Regular Script

We know that both type of scripts can call each other. As things getting even fancier, now we can also embed a Python Script block directly in the Regular Script. Below is an example of that. Assuming there is a Python Script called “PyFuncs”, inside that there is a Python function called “CycleTargetDefocus()”, as mentioned earlier. The example below is to call that Python function directly from Regular script without a dedicated script editor for the python part. The single “hybrid” script gets the job done.

Listing 24: Embedded Python Script Example

```

1 ScriptName Regular
2
3 ArrayDef = {-1. -2.5 0.1}
4
5 PythonScript $ArrayDef[1] $ArrayDef[2] $ArrayDef[3]
6 #!/Python # cannot have indent here
7 #include PyFuncs
8
9 a = SEMargStrings # string list ["-1.", "-2.5", "0.1"] now available
10
11 # convert to float
12 for i in range(0, len(a)):
13     a[i] = float(a[i])
14
15 CycleTargetDefocus(a[0],a[1],a[2]) # defined in script "PyFuncs"
16 EndPythonScript

```

The embedded Python code is between “PythonScript” and “EndPythonScript”. More usefully, we can even pass some of the regular script variables into the Python, by placing arguments after “PythonScript” and a special Python variable “SEMargStrings”. In this case, SEMargStrings has value of a string list [“-1.”, “-2.5”, “0.1”]. We convert it into real floats so they can be given to the function.

4.17 SerialEM Note: Scriptless Control

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Date_Created 2021-09-18

Last_Updated 2021-09-18

Abstract Since 4.0, sophisticated control without scripting has become possible. In this note, I give an example with some explanation how to do this for a typical single particle data collection.

4.17.1 Background Information

There are basically two new features available in version 4.0.

1. Adjust Eucentric Height using tilted beam.
2. Flexibly arrange helping tasks and primary actions from “Acquire at Items” dialog window, without a script.

In the past, a sophisticated script is need to control pipeline of actions for single particle data collection. Typically, the actions may include eucentric height positioning, X-Y positioning, beam centering, autofocusing within a range, drift control and final image taking. It is now possible to arrange these actions without scripting in the new version.

4.17.2 The New “Acquire at Items” Dialog Window

Below is the new full dialog window.

Fig.1 Full Dialog Window - Acquire at Items (click for full size image)

Indeed, it looks quite different from previous versions. Here are a few things to mention:

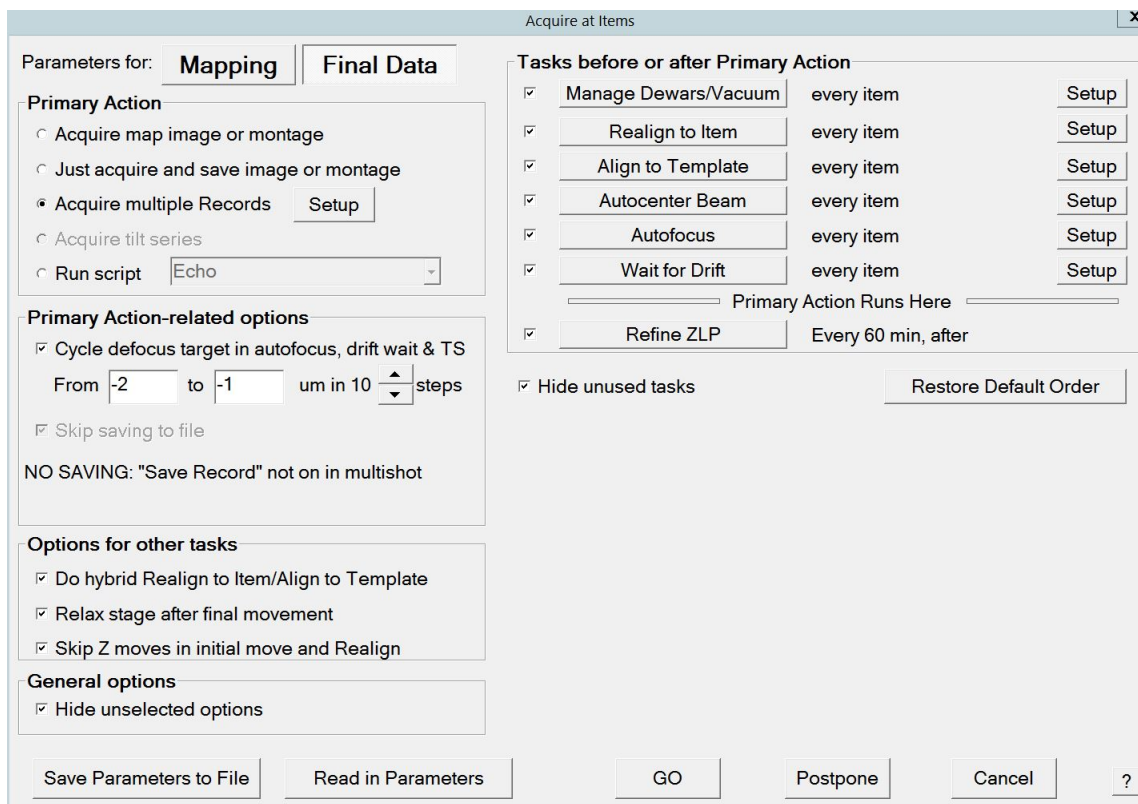
- Left side of the windows is for Primary actions and bunch of options. Right side is for helping tasks to be selected and setup to use.
- Multiple Shot is new on primary action list compared to before.
- For each task on the right side, there are three things to set.
 1. checkbox to enable/disable the task.
 2. task push button to configure order and how often the task is run.
 3. “setup” button to engage actual conditions.
- non-selected options (on left) and tasks (on right) can be hidden

respectively, to make the window neater and more focused.

4.17.3 An Example Configuration for Single Particle

If I hide the non-selected, I get a short, more focused dialog windows like below.

Fig.2 Configured Dialog Windows - an exmaple (click for full size image)



This setup can completely replace scripting for our single particle data collection pipeline.

4.17.4 A Couple of Extra Notes

1. If the “Cycle defocus...” under “Primary Action-related Options” is checked, the target defocus will change for every “Acquire at Items” cycle run. There is no longer needed to have a script for the cycling target defocus.
2. If “Realign To Item” and “Align to template” both are checked, normally, it will perform full routine of “realign to item” first, then followed by a template aligning using pre-defined template from its “setup”. If the option “Hybrid...” is also checked, the 2nd round of realign will be skipped and replaced by the template aligning. This saves a few seconds. However, when the stage is not very reliable or when the template area is fairly small, it works better without “Hybrid...” being used.

4.18 SerialEM Note: Eucentricity With Beam Tilting

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Date Created March 23, 2023

Last Updated March 23, 2023

Abstract A few years ago, I wrote a note [More About Z Height](#). It describes how to use Beam Tilt method to perform eucentric height procedure, using a script. Since then, this beam tilting method has been built in and can be accessed from menu “Tasks - Eucentricity - Eucentricity by Focus”. It is faster and more robust than using scripting, although the ideas behind them are very much the same.

In this note, I give more thorough information about how to prepare to use it and how to use in Low Dose mode.

4.18.1 System Calibrations

The procedure runs without any extra calibration. However, when one uses it in low dose mode in which View area has large defocus offset, a couple of extra calibrations help.

1. Calibration - Focus & Tuning - Extended Autofocus

This can take the defocus way over typical range used for autofocus calibration of 72 microns. For example, we do this for a range from -300 to +100 microns. This makes autofocusing measured value more accurate for high defocus condition.

The calibration result should be saved from menu Calibration - Save Calibrations.

2. A script run to get information which helps moving stage more accurately. It is called Non-reciprocity Calibration.

```
# Measures non-reciprocity between defocus measurements
# and Z changes at a series of View offsets
# Adjust Z offset list to stay within range of the extended focus
# calibration
# Make sure the beam and specimen will give good focus
# measurements at the highest offset
offsetArr = { 0 -50 -100 -150 -200 -250 -300 }
zRange = 50
backlash = 3

SuppressReports
SetLowDoseMode 1
ReportUserSetting LowDoseViewDefocus offsetOrig
startZ = -1 * ( $zRange / 2. + $backlash)
MoveStage 0 0 $startZ
MoveStage 0 0 $backlash

loop $#offsetArr ind
  SetUserSetting LowDoseViewDefocus $offsetArr[$ind]
  Autofocus -1 1
  ReportAutoFocus
  if $ind == 1
    lowerArr = $repVal1
  Else
    lowerArr = { $lowerArr $repVal1 }
```

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```

    Endif
EndLoop

MoveStage 0 0 $zRange
Loop $#offsetArr ind
    SetUserSetting LowDoseViewDefocus $offsetArr[$ind]
    Autofocus -1 1
    ReportAutoFocus
    factor = ROUND ( $zRange / ( $repVal1 - $lowerArr[$ind] ) 3)
    if $ind == 1
        calArr = { $offsetArr[$ind] $factor }
    Else
        calArr = { $calArr $offsetArr[$ind] $factor }
    Endif
EndLoop
echo Add property:
echo ZbyGFocusScalings $calArr

MoveStage 0 0 -$zRange / 2.

```

The running of the script will spit out a line like below in the log, which you should put in your system property file.

```

ZbyGFocusScalings 0 1.015 -50 0.984 -100 0.974 -150 0.975 -200 0.972 -250
↪0.982 -300 0.979

```

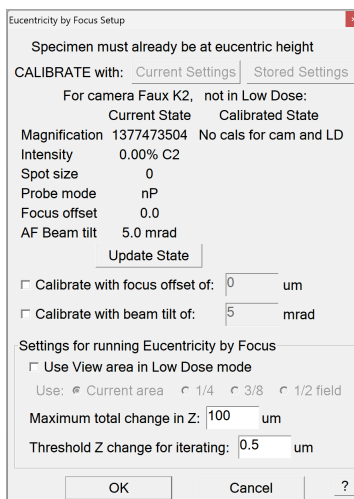
For more information about the two above calibrations, please refer the helpfile section [Extended Autofocus and Non-reciprocity Calibration for Using Eucentricity by Focus \(Priority 2\)](#).

4.18.2 Session Calibration

To use the procedure for optimal accuracy, it is recommended to do this calibration each of your session. We normally do this with Low Dose View, after View parameters (C2% and focus offset) are fixed.

This session calibration is accessed from a menu “Tasks - Eucentricity - Calibrate Focus Targets ...”. This leads to a dialog window as below.

Fig.1 Calibrate Focus Target dialog



Here are usually how we do it.

1. Bring stage to eucentricity. You can do it on any sample, and you can use stage method to get to eucentricity. You can always check how well by comparing two shots - one at 0 and other one at say 30 degree. We normally use LD view.

2. **With the specimen at eucentric height**, click on the button “Current Settings” in the dialog window shown above. In our case, we check the box in front of “Use View area in Low Dose mode”.

The calibration procedure will measure how much “defocus” from the beam tilts and that value will be used to converge to later. This value is in the memory and will be saved in your setting file. But we usually redo this in the next session, just in case small changes in a new session (day).

4.18.3 Eucentricity by Focus

With the calibrations available, we invoke this form one of the two ways:

1. from “Tasks - Eucentricity - Eucentricity by Focus”.
2. from script command

```
Eucentricity -1
```

It will measure the “defocus” at current stage position and adjust the Z height by converging to the “calibrated defocus” value done as above. It uses the exact conditions such as spotsize, intensity and OBJ% etc. for what was used in the session calibration.

That’s it! You will find this is fast and very accurate. We use it almost exclusively in our daily operations. Hope you enjoy it as we do.

4.19 SerialEM Note: Define Image Shift Vectors for Multiple Exposure

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Date Created Sept. 27, 2023

Last Updated Oct. 22, 2023

Abstract Finding image shift vectors is among the most common tasks for high-throughput multiple exposure setup.

In this note, I try to give some “under the hood” information about the procedure and some new features implemented in the latest versions that makes this easier and more dynamic for our daily operation and potentially automation too.

This is for regular pattern and on a rectangular geometry grid.

4.19.1 The Manual Way

In high-throughput, multiple exposure data collection using image shift on a regular pattern grid, it is essential to have accurate image shift pattern setup so that the beam can go to each and every hole accurately. SerialEM does this **from** determining image shift vectors from 4 corner hole positions. **It uses the IS values for 4 corner positions and derive to obtain a pair of basic image shift vectors for current conditions.** Also, once these basic two image shift vectors are determined, you can switch the pattern, say from 3 x 3 to 5 x 5 etc without needing to redo this setup procedure.

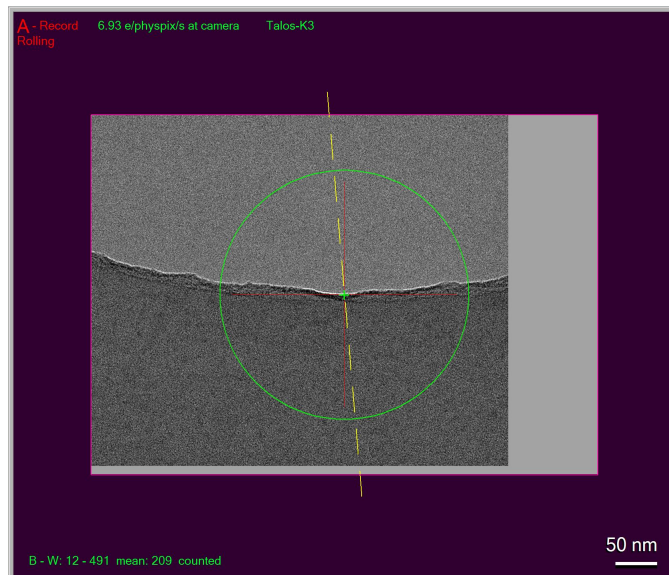
I suppose you probably are already familiar with the setup in manual way. In a brief summary, the steps are:

1. Define 3 x 3 (or similar) regular pattern from **Multiple Record Setup** dialog window.

2. Add four corner points in an image in circular fashion.
3. Click on button [**For Corners of Regular Pattern**], and use buttons [**IS to Nav Pt**] and [**Save Image Shift**] to shift to each corner point and save it.

You likely will need to take a Record shot (e.g. LD R) to touch up the shift positions before saving them. The figure below shows adjustment before saving the image shift value for that corner point.

Fig.1 Adjusted to Center before saving



Sometimes, you cannot see feature (such as a edge of partial hole) because the position is too far away in the high mag image. In an ideal world, the image shift in R mag should land on the exact location of the point you picked. But in reality, possibly due to imperfection of system calibrations, this rarely happens. You usually have to reduce R mag temporarily or use an IS montage at R to catch the feature, and you have to do this four times for each of the four corner points.

Admittedly, it is a pain, at least not very fun to me.

Despite the pain, at end of the procedure from the above steps, the precise image shift vectors are obtained. The image shift pattern based on the vectors drawn back to lower mag image (e.g. LD View) might seem off a little, as shown in Fig 5(Q) below. This is again due to imperfect calibrations so showing not being accurate. No worries, in your final collected images, the positions will be right!

As soon as the procedure is finished, there appears a line right under “Regular array of holes” in the dialog window, something like **Spacing 2.74 and 2.75um Maximum shift: 3.9um**. This indicates that the basic IS vectors for multiple exposure are available or updated. If you have never done this before, this line is not shown until you have done it. If you save setting file, there will be a line starting with “MultiShotParams” containing this very information. And in your next SerialEM session with a new grid, this information will be existed there but likely wrong. You will have to redo this for a new grid.

Oh, you probably will naturally ask: “Can we do this an easier way?” The answer is YES.

4.19.2 Using Hole Vectors

Instead of manually adding some corner points as nav items, we can directly use hole vectors. If we perform hole finding on an image with multiple holes, the hole vectors become available. These are two basic vectors to reflect hole distances in two directions and their relative orientation to the camera system. Even there might not be good holes to be included, the vectors themselves are available after hole finding. They can be used to convert to Image Shift vectors as starting point. We can do this simply by pressing the button [**Use Last Hole Vectors**].

Fig.2 Use Last Hole Vector

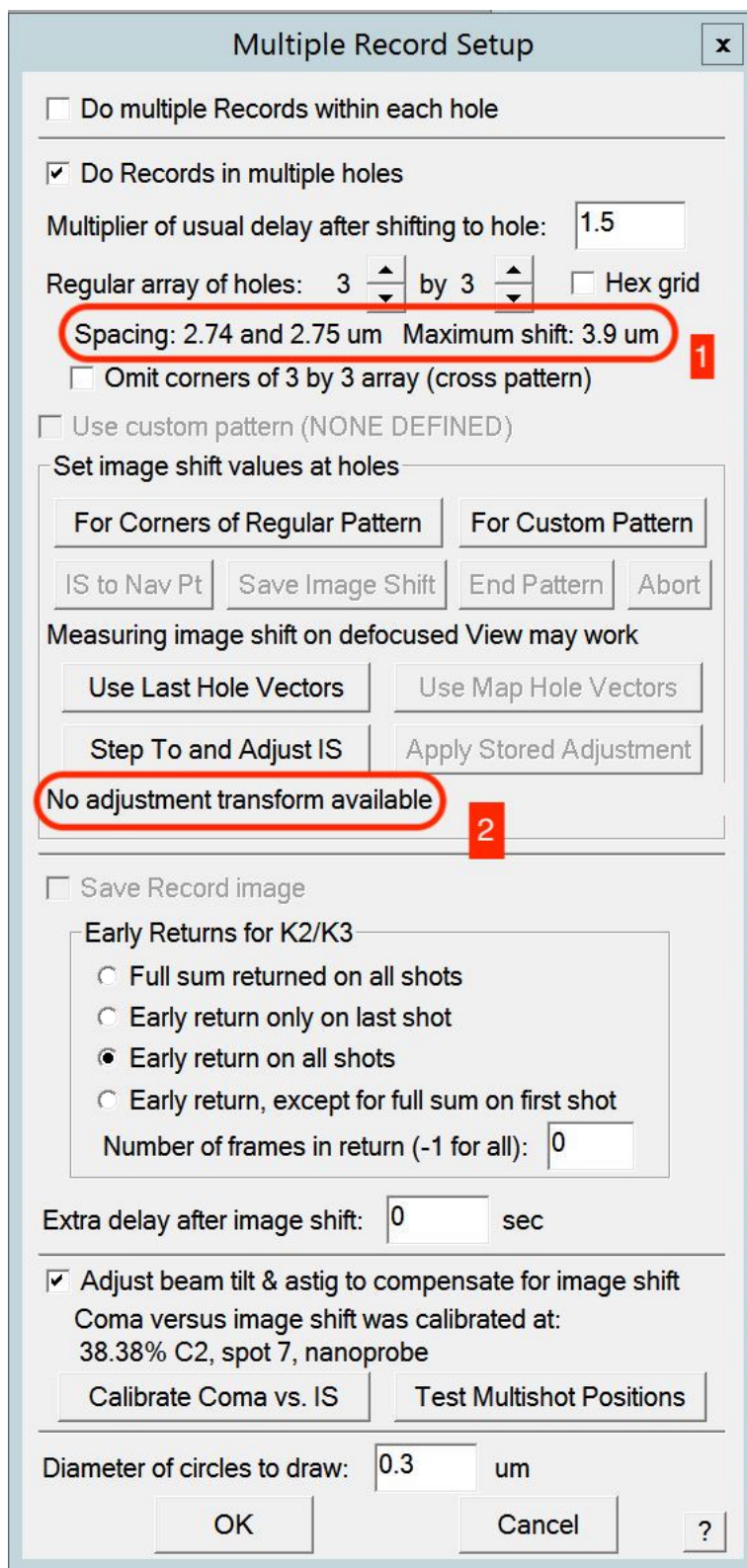


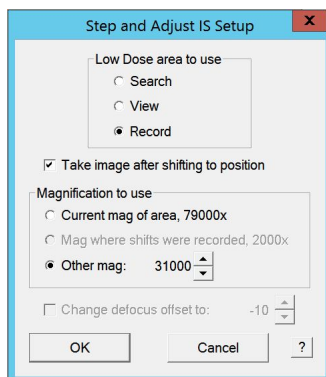
Figure 2. shows two places get updated in the dialog after pushing [**Use Last Hole Vectors**]. Shown in red outline 1, the image shift vectors are updated from the new stage hole vector conversion. And also there is an interesting line “No adjustment transform available” in red outline 2. What is that then?

4.19.3 Adjustment Transform

After the button pushed, we have image shift vectors. And we wanted to refine them by stepping to and adjust so they can be accurate. This is very much similar to previously manual shift, but we do this via a more automatic way from [**StepTo and Adjust IS**] button.

Below Figure 3. is a setup dialog from pushing [**StepTo and Adjust IS**] button.

Fig.3 Step and Adjust IS Setup



It can conveniently shift to a corner and take a shot for you to see, you can adjust the position and save the image shift value, for this corner, by pressing [**Save Image Shift**] like before. This routine steps through all four points for you. In the end, you have a new set of accurate image shift vectors!

We started with a set of image shift vectors converted from stage hole vectors initially (at LD View mag), and we ended up with final accurate set of image shift vectors (at LD R mag)! This procedure not only determines refined image shift vectors for R mag, but also **results in an adjustment transform matrix between the two!**

As soon as the procedure is finished, the adjustment transform is available (in memory), and already applied for the CURRENT one, as shown in below figure 4.

Fig.4 Adjustment transform already applied

Multiple Record Setup

☐ Do multiple Records within each hole

☒ Do Records in multiple holes

Multiplier of usual delay after shifting to hole:

Regular array of holes: 3 by 3 ☐ Hex grid

Spacing: 2.95 and 2.99 μm Maximum shift: 4.2 μm

☐ Omit corners of 3 by 3 array (cross pattern)

☐ Use custom pattern (NONE DEFINED)

Set image shift values at holes

For Corners of Regular Pattern For Custom Pattern

IS to Nav Pt Save Image Shift End Pattern Abort

Measuring image shift on defocused View may work

Use Last Hole Vectors Use Map Hole Vectors

Step To and Adjust IS Apply Stored Adjustment

Adjustment transform already applied

☐ Save Record image

Early Returns for K2/K3

☐ Full sum returned on all shots

☐ Early return only on last shot

☒ Early return on all shots

☐ Early return, except for full sum on first shot

Number of frames in return (-1 for all):

Extra delay after image shift: sec

☒ Adjust beam tilt & astig to compensate for image shift

Coma versus image shift was calibrated at:
38.38% C2, spot 7, nanoprobe

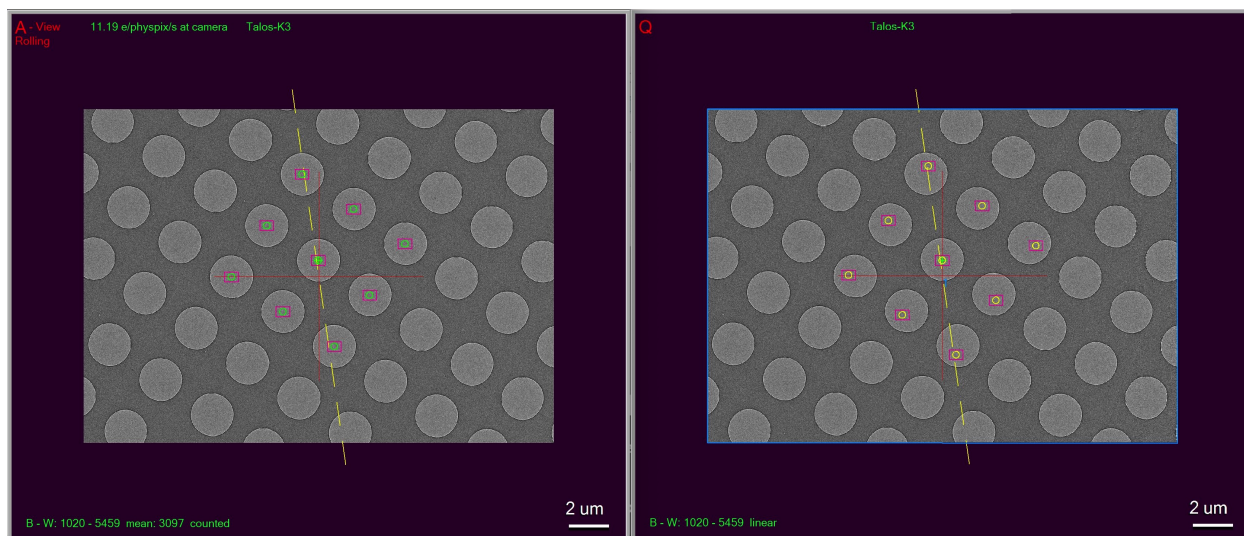
Calibrate Coma vs. IS Test Multishot Positions

Diameter of circles to draw: μm

OK Cancel ?

Below is the figure 5 showing 3 x 3 patterns before and after this adjustment transform.

Fig.5 Image Shift Patters before and after adjustment



The left image 5.(A) shows image shift pattern right after converted from stage hole vectors. The right one 5.(Q) is the pattern after adjustment is done. Note, the beam circles in the image (A) is in green, and they become yellow after adjustment. You may also notice the pattern after adjustment seems shown slightly off from hole positions. This is due to imperfection in calibrations. In ideal world, the two would be lined up perfectly. Here, the off-looking one is actually an accurate set.

4.19.4 A Smarter and More Automated Way

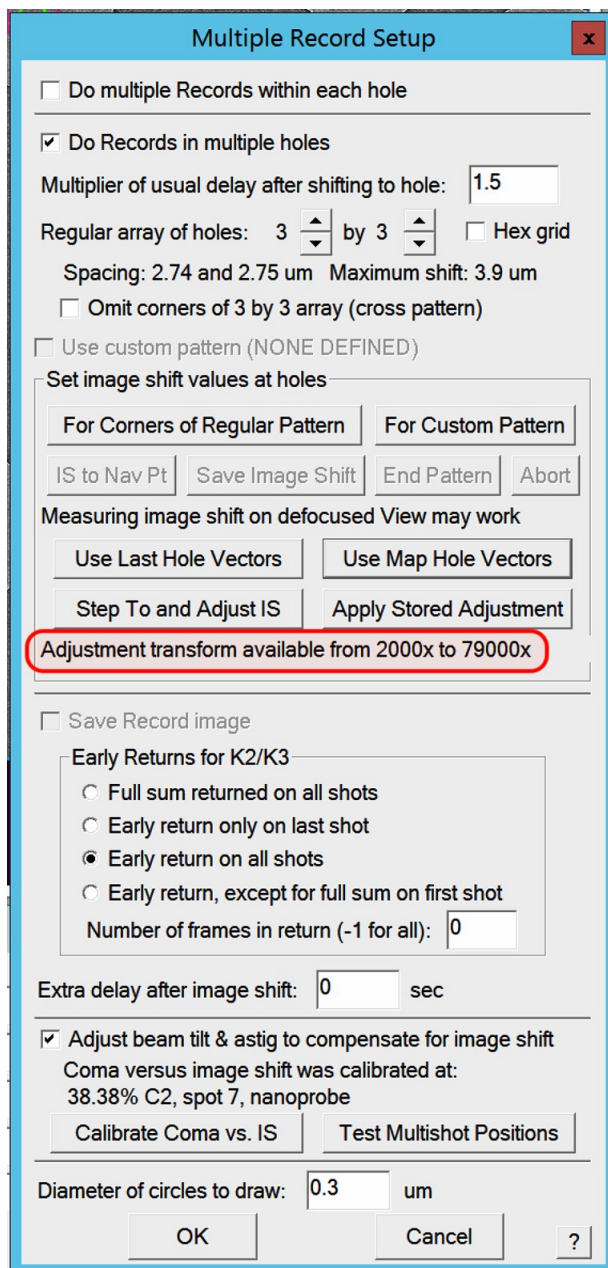
The adjustment transform matrix is also preserved in setting file.

```
HoleAdjustXform 20 0 0 20 34 1.009630 0.005789 -0.002779 1.021203
```

It is a matrix between LD View (index 20) and LD R(index 34). As long as your LD View and LD R conditions doesn't change much, this matrix works conveniently. When you start with a new grid, your old pattern is no longer valid, but this adjustment transform stays good.

So for daily operation, you find hole on the new grid first and you then [**Use Last Hole Vectors**]. As soon as that button is pushed, the message to tell you the transform is available shows up, as shown in red outline below.

Fig.6 Adjustment transform available



You can utilize the transform by pushing the button [**Apply Stored Adjustment**], your new Image Shift vectors for the pattern is updated immediately. You might want to do [**StepTo and Adjust IS**] here also to refine the IS vectors for today's condition and make sure the positions are perfect. This time, not like manually adjusting from scratch, you will find your features easily.

It is worth mentioning that if you perform hole finding on a montage map overview image, the map item will contain the hole vectors information. If you save the nav file and open it in text editor, you can see the two lines:

```
HoleISXspacing = 2.69895 -0.758684 0
HoleISYspacing = -0.768892 -2.71193 0
```

You can then click on [**Use Map Hole Vectors**] on a map item and then [**Apply Stored Adjustment**]. As you can imagine, this can work for a grid with slight geometry change across the grid meshes. It can also be used conveniently to a new grid with completely different geometry.

Once your adjustment transform is good, you can do it in script too, to have an automated workflow.

```
UseHoleVectorsForMulti 0      # 0 means LD Record mag
AdjustMultishotPattern 0
```

4.20 SerialEM Note: Running A Script During Montaging

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Date Created Feb. 24, 2024

Last Updated Feb. 24, 2024

Abstract SerialEM allows to run a script during montaging procedure. This can be handy sometimes. In this note, I will give a few cases where this can be very useful.

4.20.1 Enable it by a property

If you put the line in your property file as below:

```
MontageScriptToRun 7
```

Restarting SerialEM will have this function enabled.

Or you can even conveniently using script command to turn it on:

```
SetProperty MontageScriptToRun 7
```

And you can turn it off (disable it) by:

```
SetProperty MontageScriptToRun 0
```

When it is enabled, the script number 7 will run after the montage image acquired and before saved.

4.20.2 Case Studies

A few case studies I can think of might take advantages of this function.

Dynamic defocus adjustment in-the-fly

If we collect a montage for a large area, the specimen might not be always in flat plane, and height changes are expected. In this situation, one can easily run CTF program such as “ctfplotter” to obtain the defocus and adjust objective lens dynamically.

Collect multiple montages at the same time

If we collect a montage tilting series, say 2 x 2 image shift montage, we might also want to collect two more montages along the tilting axis so at every angle we can have three montages rather than one. This is perfectly reasonable for lamella specimen.

This is what we can do.

a. before starting the tilting series collection, open three files - two for image stack file, and one for montage file say 2 x 2. After this, the montage file is on the “top” and file number is 3, while the other normal image files opened are 1 and 2. Lets assume they have name as mon.st, f1.st and f2.st.

b. Have a script in 7th script editor like below:

```
ScriptName ToRun

ImageShiftByMicrons 3.5 0      # shift 3.5um along tilting axis
R
ImageShiftByMicrons -3.5 0     # clear IS

ImageShiftByMicrons -3.5 0     # -3.5um
R
ImageShiftByMicrons 3.5 0      # clear IS
# at this point, three images are in buffer A, B and C. C is one from
# montage shot

Save A 1
Save B 2
Copy C A      # let montager save which happens after the script finishes
```

Thus, after montage is done, there will be also two stack files for single image shots. Those two images stacks are not a montage stack, as there is no piece list info in the file header. However, with a piece list info, they have all the info to be treated as montages.

```
$ extractpieces mon.st mon.pl
```

f1.st and f2.st have the exact piece list info as mon.st.. So you can display them with the piece list info:

```
$ 3dmod -p mon.pl f1.st
```

It will work!

Colleting STEM multi-channel montages Simontaneously

When collecting with multi-channel STEM detector, one exposure will have images in the buffers for the multiple channels. For example, if there 4 channels, buffer A, B, C and D will have image in each. The goal is to save 4 montages at the same time.

One would open three normal image files, such as chan1.mrc, chan2.mrc, chan3.mrc and last one to be a new montage file say 2 x 2 with image shift, with name chan4-mon.mrc.

The “ToRun” script would be like this:

```
ScriptName ToRun
ReportFileNumber mont
Loop 3 ind
  fil = $mont - $ind  #number here, not filename for easiness.
```

(continues on next page)

(continued from previous page)

```

buf = $ind + 1      # also number, 2-4 for single image, 1 for montage
Save $buf $fil
Endloop

```

As above, three single image stack files share the same piece list info which can extract from chan4-mon.mrc.

4.21 SerialEM Note: Cross-mag Alignment

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Date Created March 7, 2024

Last Updated March 8, 2024

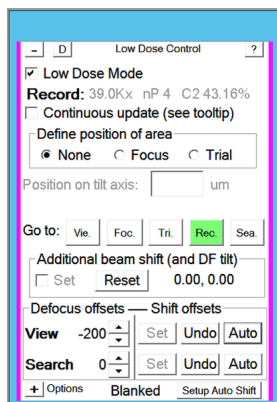
Abstract Cross-mag alignment functions have been recently implemented since around beginning of 2024. They allow one to align two images at very different magnifications, and it takes into consideration of scale and rotation.

There are a few scripting commands available related to this function. The routine should work for more general purpose when different magnifications are involved. Here I give two application examples that were built upon this cross-mag alignment functions.

4.21.1 Set Image Shift Offset for LD View or Search Automatically

The late Testing branch has a new look for Low Dose Control panel, as shown below.

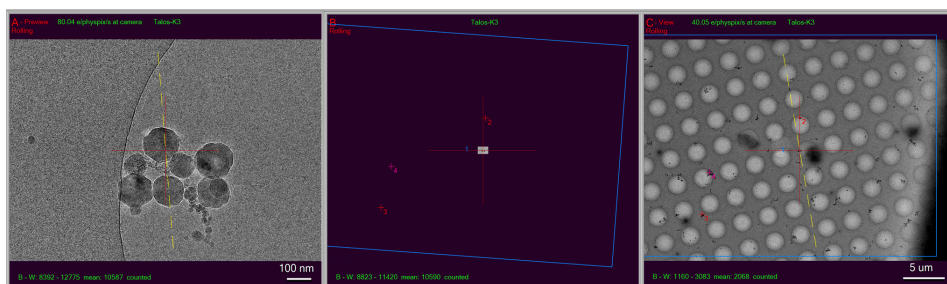
Fig.1 New Low Dose Control Panel



This new design re-arranges Offset portion. It seems to be more intuitive for offsets of Image Shift and Defocus, for both View and Search areas. Moreover, it has a `Auto` button for Image Shift offset now.

This is how it works. If you take a Record or Preview image with some clear feature in it, you click on `Auto` button. The program will transform this image to scale down to match magnification of View and align it to View image for the same feature. This results in image shift offset be set accurately.

Fig.2 Images for Preview, shrunk Preview and View



In above figure, the first image on the left - “A buffer” is from a Preview. The image in the middle is a scaled down one of it based on the View mag. You can see the FOV is small compared to a real View image which is on the right. By aligning these two images at the same magnifications / pixelsizes of View, the procedure determines IS offset for View and take it in.

4.21.2 Realign a Feature in View map to Match Preview/Record

Realign routine now can take advantage of this cross-mag alignment functionality. With this feature, Realign is able to bring the target to the center of camera at higher mag (Preview) than original map (View).

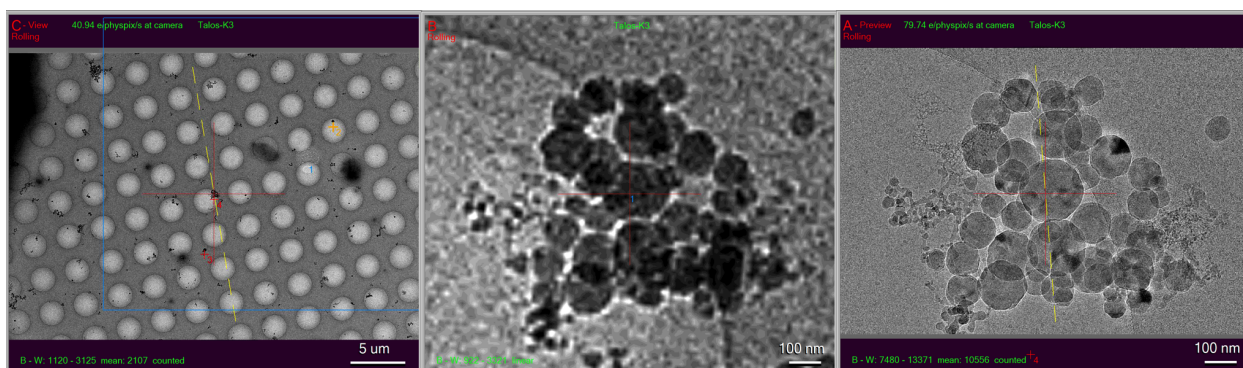
Fig.3 Option for Realign to Scaled map



This feature can be accessed from scripting command. If you run “Acquire at Items”, it can be activated from the option for Actions on the left column, as shown in above figure.

In this case, after a typical two rounds of aligning at View map, it then scaled up the portion of the map to match final higher mag (Preview or P in this case) and does the 3rd round aligning at Preview mag.

Fig.4 Scaled up Realign - ideal for initial target in TS



In above figure, the first image on the left has a feature centered after normal Realign to Item, it is the scaled up this portion of the map to match the higher target mag, as shown in the middle. The routine then aligns the real Preview image (on the right) to this transformed one in the middle. So the target is centered at Preview mag. This is ideal for initial targeting for tilting series.

As you can see, even your low dose image shift offset for View might have not been adjusted perfectly, this procedure still can bring the final mag target to the center. This is indeed very convenient!

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- `genindex`

BIBLIOGRAPHY

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