Leginon tomography data collection

Updated at 7/30/2019

This document covers Leginon tomography application on Titan Krios with K3 camera at FSU BSIR. Since the basic setting and concept are very similar to that in single particle, only the unique settings for tomography is addressed here, although the whole data collection workflow still remains in this document.

Before you start this document for practice, please make sure you have gone through *Leginon single particle protocol*, except for the Hole_Targeting and Exposure_Targeting since they are not implemented in the tomography application.

The unique setting of tomography is highlighted in red.

1 Create a session

1.1 Start Leginon clients

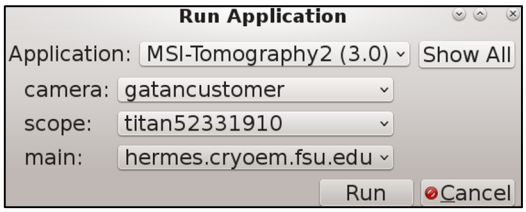
1.2 Switch to your account

1.3 Launch Leginon

1.4 Start Leginon GUI to create a new session

1.5 Choose Leginon application

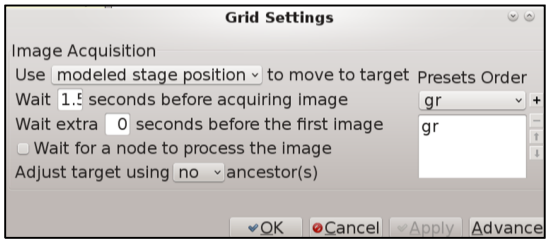
- Leginon GUI Menu -> Application
  Application: MSI-Tomography2 (3.0)
  Main: hermes.cryoem.fsu.edu
  Camera: gatancustomer
  Scope: titan52331910

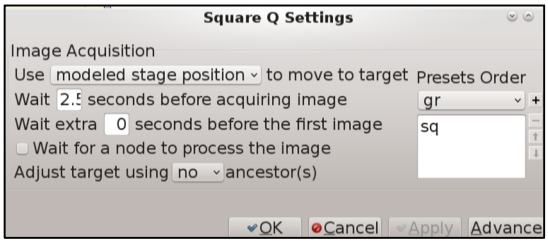


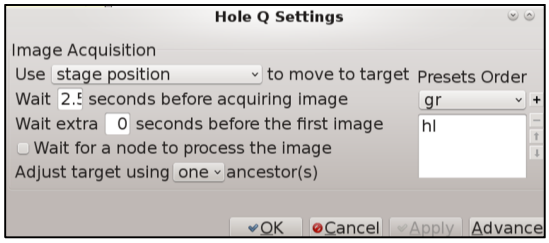
2 Leginon setup

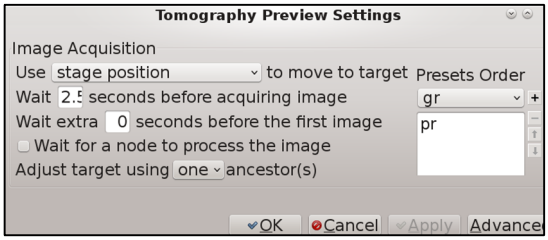
2.1 Pre-setup

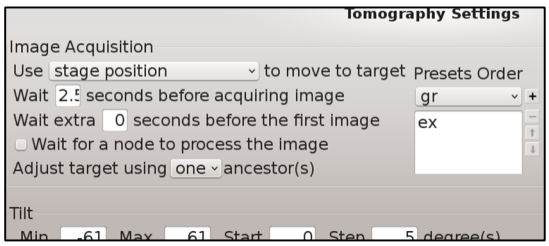
- Tomography application has a unique node and preset for preview, which is optional but very useful in case when you want to preview gold beads concentration or sample details before you set up for data collection. Three steps are needed to make it work: (a) Load or create a new preview preset as 'pr', which usually is the same mag as 'ex', but with very short exposure time (~100ms), high defocus (-6μm), and bin8 for camera. (b) Tomography_Preview node setting -> set 'pr' as its preset. (c) During Tomography_Targting add preview, and usually have it overlapping with 'ex' acquisition. You will see details later.
- Grid node -> Settings: Use 'modeled stage position', set 'gr' as it's only preset on the list.
- Square_Q node -> Settings: Use 'modeled stage position', set 'sq' as it's only preset on the list.
- Hole_Q node -> Settings: Use 'stage position', set 'hl' as it's only preset on the list.
- Tomography_Preview node -> Settings: Use 'stage position', set 'pr' as it's only preset on the list.
- Tomography node -> Settings: Use 'stage position', set 'ex' or 'ec' as it's only preset on the list. The reason is to minimize the image shift accumulation since anyway image shift has been applied to each tilting to ensure imaging on the same object.



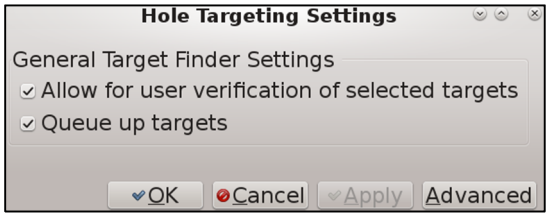


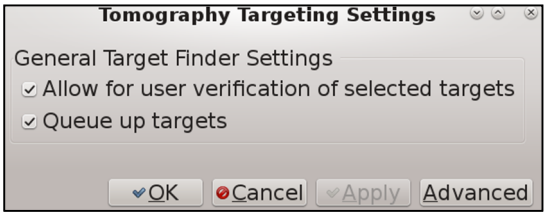




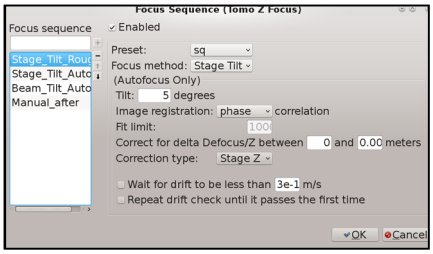


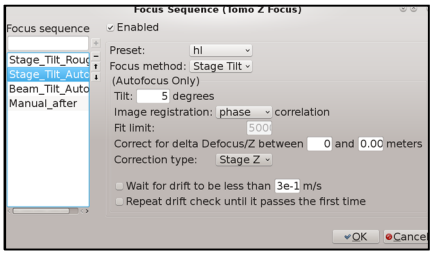
- Hole_Targeting node -> Settings: check 'Allow for user verification of selected targets' and 'Queue up targets'.
- Tomography_Targting node -> Settings: check 'Allow for user verification of selected targets' and 'Queue up targets'.



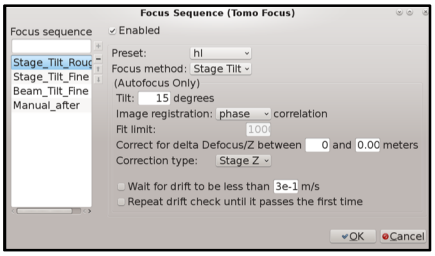


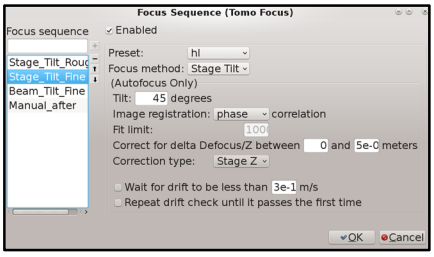
- Z_Focus node -> Focus Sequence  : enable Stage_Tilt_Rough and Stage_Tilt_Auto, disable all others.
  - Stage_Tilt_Rough: Preset: sq
    Focus method: Stage Tilt
    Tilt: 5 degree
    Image registration cross correlation
    Between 0 and 0.0004 meters
    Correction type: Stage Z
    Uncheck 'wait for drift'
  - Stage_Tilt_Auto: Preset: hl
    Focus method: Stage Tilt
    Tilt: 3 degree
    Image registration cross correlation
    Between 0 and 0.0001 meters
    Correction type: Stage Z
    Uncheck 'wait for drift'

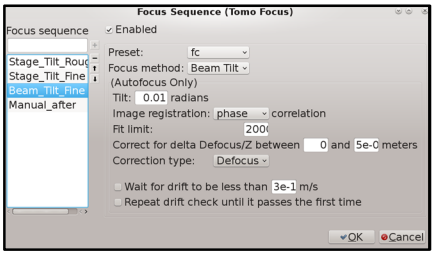




- Focus node -> Focus Sequence: enable Stage_Tilt_Rough, Stage_Tilt_Fine, and Beam_Tilt_Fine. The reason we do Z height again is (a) all tomography targets are queued up before data collection starts, so the stage have to travel back to the current target and then redoing Z height is necessary, (b) tomography needs very accurate Z height, that is why here two big stage tilt (15° and 45°) are set up at hole mag for Z height determination.
  - Stage_Tilt_Rough: Preset: hl
    Focus method: Stage Tilt
    Tilt: 15 degree
    Image registration phase correlation
    Between 0 and 0.0001 meters
    Correction type: Stage Z
    Uncheck 'wait for drift'
  - Stage_Tilt_Fine: Preset: hl
    Focus method: Stage Tilt
    Tilt: 45 degree
    Image registration phase correlation
    Between 0 and 5e-05 meters
    Correction type: Stage Z
    Uncheck 'wait for drift'
  - Beam_Tilt_Fine: Preset: fc
    Focus method: Beam Tilt
    Tilt: 0.01 radians
    Image registration phase correlation
    Fit limit: 2000
    Between 0 and 5e-05 meters
    Correction type: Defocus
    Uncheck 'wait for drift'
- All above settings only need to be done once.







2.2 Load presets

- Presets_manager node -> Import presets from another session -> Instrument TEM: EF-Krios; Digital camera: GatanK3 -> Find someone's presets in the past xx days on the left panel -> select all presets from the right panel, usually including gr, sq, hl, fc, fa, ex (or ec) -> Import.
- Preview preset 'pr' can be simply copied from ex (or ec), and changed to short exposure time (100ms), high defocus (-6µm), and high binning of camera (bin8).
- Edit presets as the example below.

Preset	Mag	Defocus	SP	Intensity	EF	Camera	Exposure	Purpose
gr	135	0	8	0.00096	0 eV	1440X1023 bin8	1001 ms	atlas
sq	275	0	8	0.00038	0 eV	1008X1008 bin8	1001 ms	Square
hl	4800	-8e-05	8	1.322e-05	40 eV	1008X1008 bin8	1001 ms	Hole
fc	33000	-2e-06	7	2.86e-06	20 eV	1008x1008 bin8	1001 ms	focusing
fa	33000	-2e-06	7	2.86e-06	20 eV	1008x1008 bin8	1001 ms	Melting ice
ex	33000	-3e-06	7	2.86e-06	20 eV	5760x4092 bin2	760 ms	Data
pr	33000	-6e-06	7	2.86e-06	20 eV	1008x1008 bin8	100 ms	preview

2.3 Check if Hermes communicates with Titan and camera

2.4 Preset alignment

2.5 Collect atlas

2.6 Pick up two squares: one 'empty' and one 'feature'

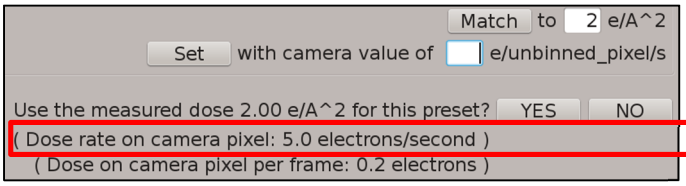
3 Camera gain preparation through DigitalMicrography

4 Determine Exposure beam for data collection

4.1 Rough setup beam for data collection

4.2 Fine determination of beam

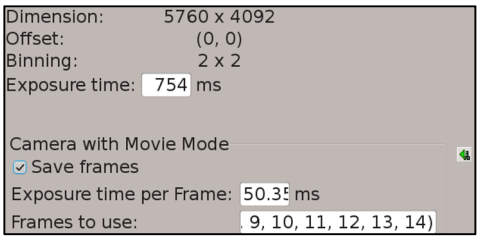
- Here is an example of total dose 2e/Å^2 for one tilting image at Mag 33K and pixel 2.74 Å.
- Presets_Manager node -> ex -> Acquire dose image for the selected preset
- Determine dose rate: Change spot size and Intensity to reach dose rate between 15~20. The example below is 20 e/p/s.



*5.0 *4 ~= 20 e/pixel/second*

*0.25*4 = 1 e/frame/pixel; 0.2 here indeed is 0.25*

- Determine exposure time: match dose to 2e/ Å^2, Just click the Match button then the exposure time of ex preset will be automatically updated (754ms).
- Determine frame number: 2e/Å^2 = 2e/ (1 pixel/2.74)^2 = 15e/pixel, which means that every frame will have 1e/frame/pixel if asking for 15 frames.
- Calculate exposure time per frame. Total exposure time/frame number=754/15=50.3ms



- Fill calculated Exposure time per frame to ex preset. Acquire dose image again, and then the frame number will be automatically calculated and filled in (0…...14).
- Have 'save frame' checked to save frames
- Have defocus range setup for ex or ex preset at 'Random Defocus Range', usually -3.0~-6.0µm defocus. The defocus value here is for each tilt series, but not for each tilting image.

5 Objective astigmatism and coma-free

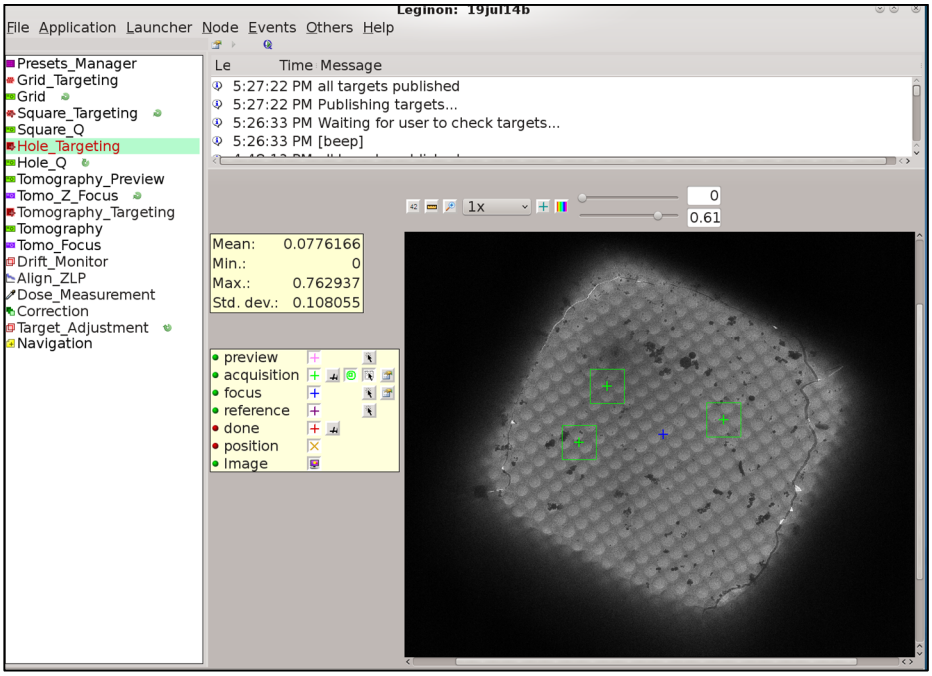
6 Insert Objective aperture

7 Square targeting

- Reference target can be selected in Square_Targeting, Hole_Targeting, or Tomography_Targting node. The reference target should be of either a broken square or an empty hole if no broken square can be found. The Reference target will be used if 'Align zero loss peak' or 'Measure dose' is activated, otherwise no need to pick any reference target.

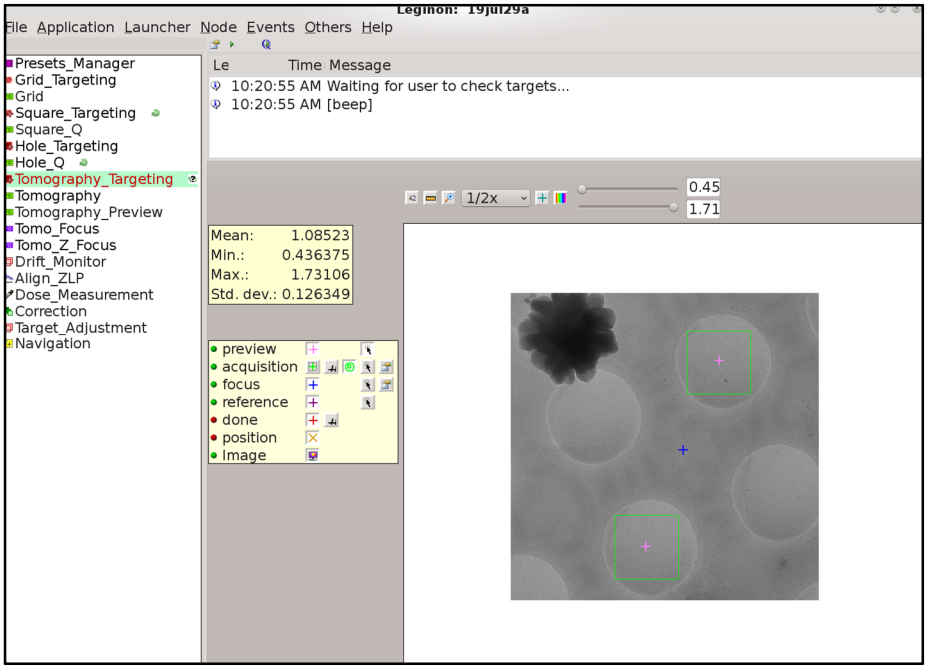
8 Hole targeting

- Manually add multiple acquisitions and one focus for Z focus.

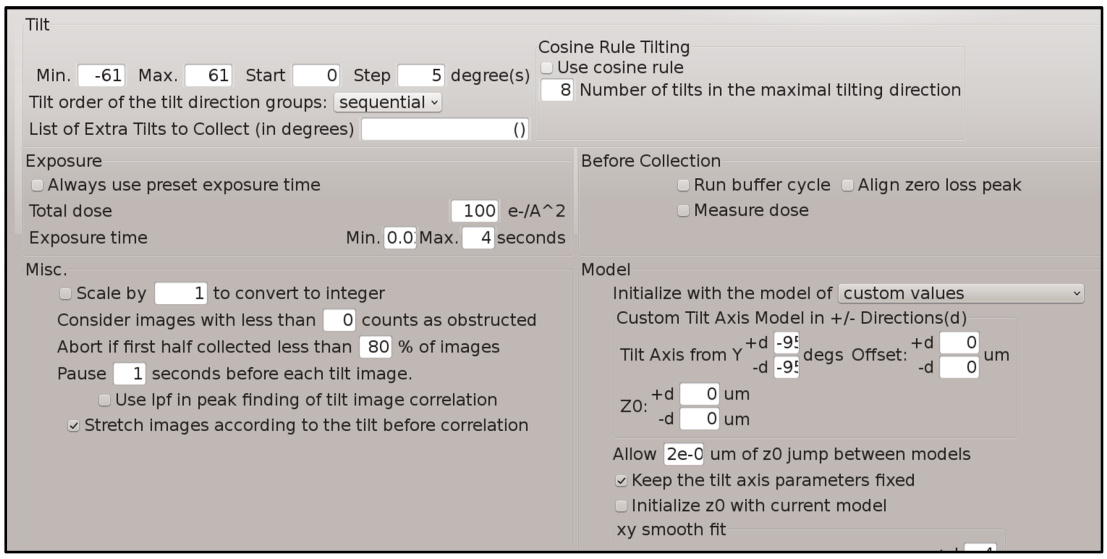


9 Exposure targeting

- Manually add acquisition and focus. You can also add preview (set it overlapping with acquisition) if needed.
- Preview targets are processed before focus and acquisition targets, and published at Tomography_Preview node. After preview images are reviewed, user can go back to Tomography_Targting node to include or exclude those targets.



10 Tomography settings



- Tilt Min. and Max.: always provides 1 degree larger than you need. For example you will have 60 degree collected only when you put max as 61 degree. You will only collect till 58 degree if you set 60 as max.
- Step >0: start from ‘start’ tilt stepping into ‘Max’ then from ‘start’ tilting stepping to ‘Min’
  Step <0: start from ‘start’ tilt stepping into ‘Min’ then from ‘start’ tilting stepping to ‘Max’
- Tilt order of the tilt direction groups:
  - Sequential: 0,2,4,…..,60,-0,-2,-4,……,-60
  - Alternate: 0,2,-2,-4,4,6,-6,-8,……
  - Swing: -60,-58,…...,-2,0,-2,…..,58,60

- Cosine Rule tilting: it accepts the Min and Max tilt range but the angular interval will be determined by cosine rule, which uses smaller interval at higher tilt angles, bigger interval at lower angles. The image number on each half side is determined by the following 'Number of tilts in the maximal tilting direction'.
- Exposure: (two strategies below, you can choose either one for data collection)
  - Always use preset exposure time: use fixed dose from ex for each tilting image
  - Total dose xx e/A^2: use higher dose on higher tilting angle to balance image contrast within one tilt series. The dose rate is imported from preset ex which should have been set up for dose image. Here is an example for total dose 100e/$Å^2$, covering from -60° to 60° with 2° interval using sequential method.

Image index	Tilt (2° interval)	Dose (e/Å²)	Frame number
#1 ~ #13	0° ~ 24°	1.33	10
#14 ~ #18	26° ~ 34°	1.47	11
#19 ~ #21	36° ~ 40°	1.60	12
#22 ~ #23	42° ~ 44°	1.74	13
#24 ~ #25	46° ~ 48°	1.87	14
#26 ~ #27	50° ~ 52°	2.00	15
#28	54°	2.14	16
#29	56°	2.27	17
#30	58°	2.4	18
#31	60°	2.54	19
#32 ~ #44	-0° ~ -24°	1.33	10
#45 ~ #49	-26° ~ -34°	1.47	11
#50 ~ #52	-36° ~ -40°	1.60	12
#53 ~ #54	-42° ~ -44°	1.74	13
#55 ~ #56	-46° ~ -48°	1.87	14
#56 ~ #57	-50° ~ -52°	2.00	15
#58	-54°	2.14	16
#59	-56°	2.27	17
#60	-58°	2.4	18
#61	-60°	2.54	19

- Exposure time: make sure your exposure time for each tilting image is limited in this range.
- Scale by xx to convert to integer: uncheck. This setting is for CCD camera.
- Consider images with less than 0 counts as obstructed. This is useful to abort the tilt series collection when tilting to high angle and beam is blocked. But since K3 counts has been modified to very small value in Leginon (divided by 1000), so here put either 0, or a very small value.
- Abort if first half collected less than 80% of images: This is to abort the tilt series when the tracking is way off when it comes back to 0 degree prior to the second half collection.
- Use lpf in peak finding of tilt image correlation: uncheck: use low pass filter for cross correlation
- Stretch images according to the tilt before correlation: checked: This is always checked to obtain good cross correlation between two neighboring tilting images.
- Model: have 'keep the tilt axis parameter fixed' checked; Initial with the model of 'Custom Values'. The tilt axis are -95° for both + and – directions (This value is calculate from Protomo for Mag 33k; Other Mags should be very similar to this one).

11 Tomography model learning

- Only authorized user can do model learning, otherwise don't touch it since everyone shares the same model.
- There is no such an ideal compuStage on EM that its rotation axis just crosses the camera center and Z height stays constant during tilting. It is the reason that some software does tracking (find the same imaging object at low mag) and focus (compensate the Z height change) on each tilt

angle during tilt series collection. Instead, Leginon doesn't do any tracking or focus during tilt series collection, but fully relies on the model which stores the compuStage behaviors and should have been calibrated before use.

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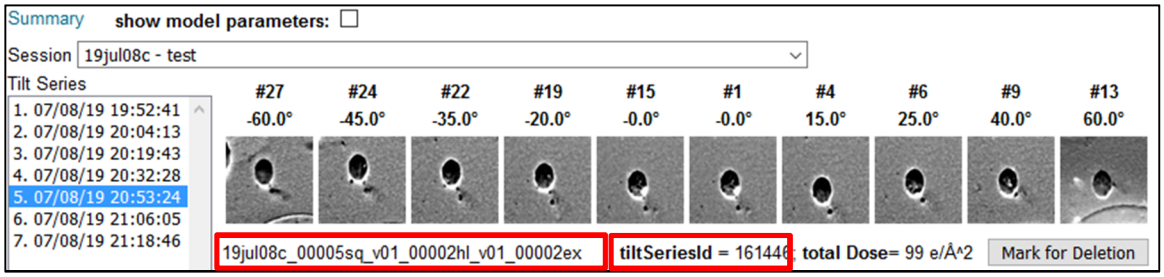
12 Transfer frame data and pre-process

- Transfer frame from K3 computer to GPFS (necessary)
- Run motionCor2, but not with dose weighting.
- Compose each tilt series into a stack
  - Find the tilt series ID: Login Appion -> Tomography -> select your session -> select the tilt series -> find image name and tilt series ID (19jul08c_00005sq_v01_00002hl_v01_00002ex and 161446)



  - *load module myamidev*
  - *eman2tomoprep.py --tiltid=161446 --seriesname=19jul08c_00005sq_v01_00002hl_v01_00002 --alignprefix=ex-a*
  - Assume you have done motionCor2 and the output prefix is *ex-a*
  - The tilt series and tilt angle file will be saved in one individual folder.
- Retrieve dose information through Protomo
  - Appion -> image viewer -> processing
  - Tomography (protomo2) -> Align Tilt-Series -> Protomo 2.4.1
  - Select one tilt series -> Click 'Just show Command'
  - Replace the 'CoarseAlign' to 'Batch' on the website address bar, return
  - File Preparation -> File Preparation -> 'No' Dose Compensation Type -> Click 'Just show Command' -> run the command in terminal
  - *cd /gpfs/research/secm4/appiondata/session/protomo_alignments/tiltseries0003/*
  - *cp series0003.tlt series0003000.tlt*
  - *cp /gpfs/research/secm4/appiondata/how_to_get_dose/par.param tiltseries0003.param*
  - *cp /gpfs/research/secm4/appiondata/how_to_get_dose/stack-dose.sh .*
  - Modify the script correspondingly and run it.
  - The dose file 'full_dose_lp_list.txt' is located within stack folder and stores tilt angle at the 1st column and dose at the 2nd column.
  - An alternative way is to manually record the dose information from each individual tilt image in Appion -> Image Viewer.

13 Clean up after data collection is done

- Close Leginon GUI
- Exit your account from the Linux terminal by typing *exit*
- Close column valve on EM GUI

- Turn turbo on at Autoloader panel on EM GUI
- Remove Objective aperture at Aperture panel on EM GUI
- Insert Flu camera
- Unload the grid after Turbo is ready
- Undock the cassette
- Fill in the paper log book and the electronic log book as well.