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Basic tutorial - How to use quick_tomo

QuickTomo by Alessio d'Acapito
Tutorial by Alice Decombe

Get all your files from the QuickTomo GitHub (https://github.com/alessiodacapito/quick_tomo)
This includes

- This tutorial
- A scipion workflow (.json file)
- The required Aretomo executable
- The quicktomo script

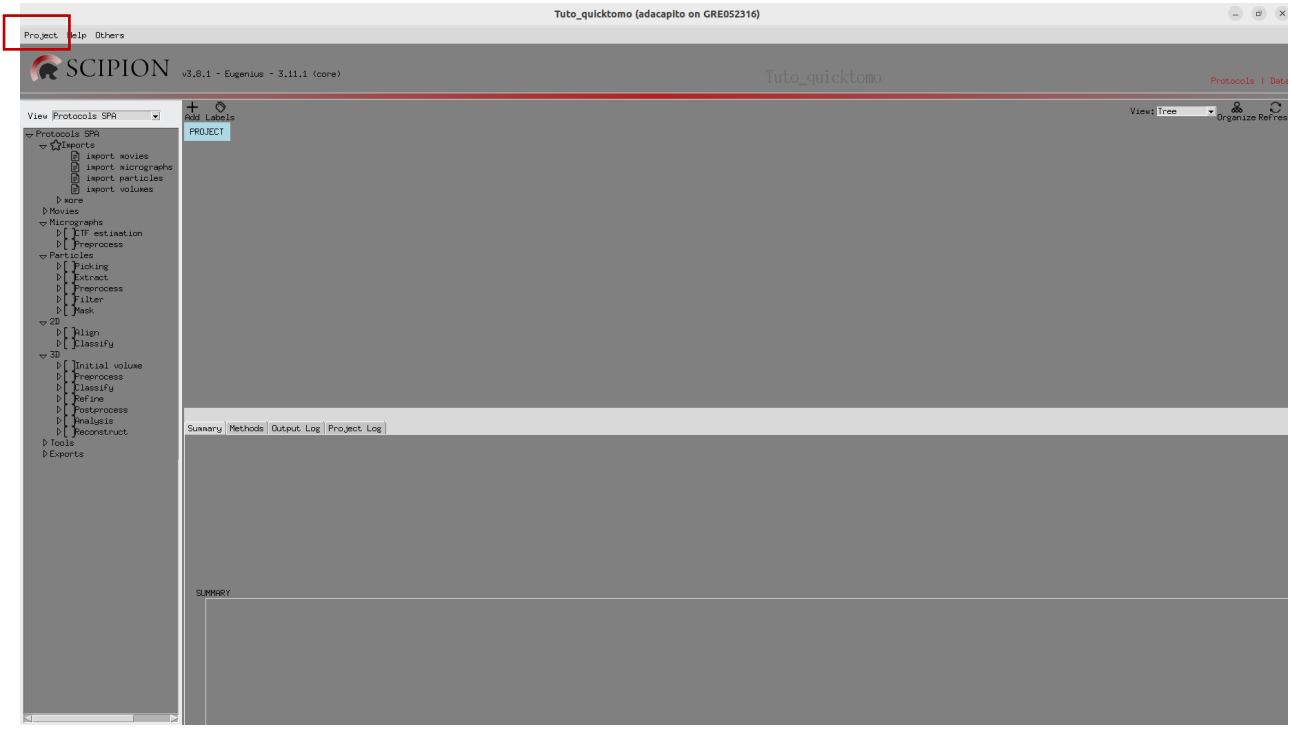
Place everything in a quick_tomo directory of your choice, preferably on your home.

Requirements for QuickTomo are

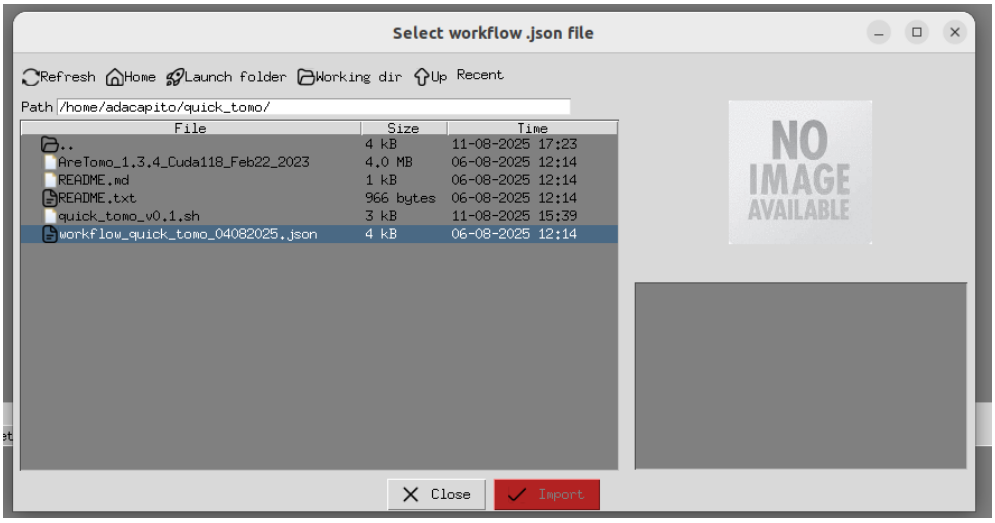
- A running installation of Scipion with at least motioncorr2, Ctffind and imod plugins installed. This is available on the cluster or through the tomography GPU machine (contact Benoit Gallet)
- At least one GPU and cuda version > 10
- Access to your data

Launch scipion. Depending on your installation the command may vary. For a SBGrid installation just type scipion.

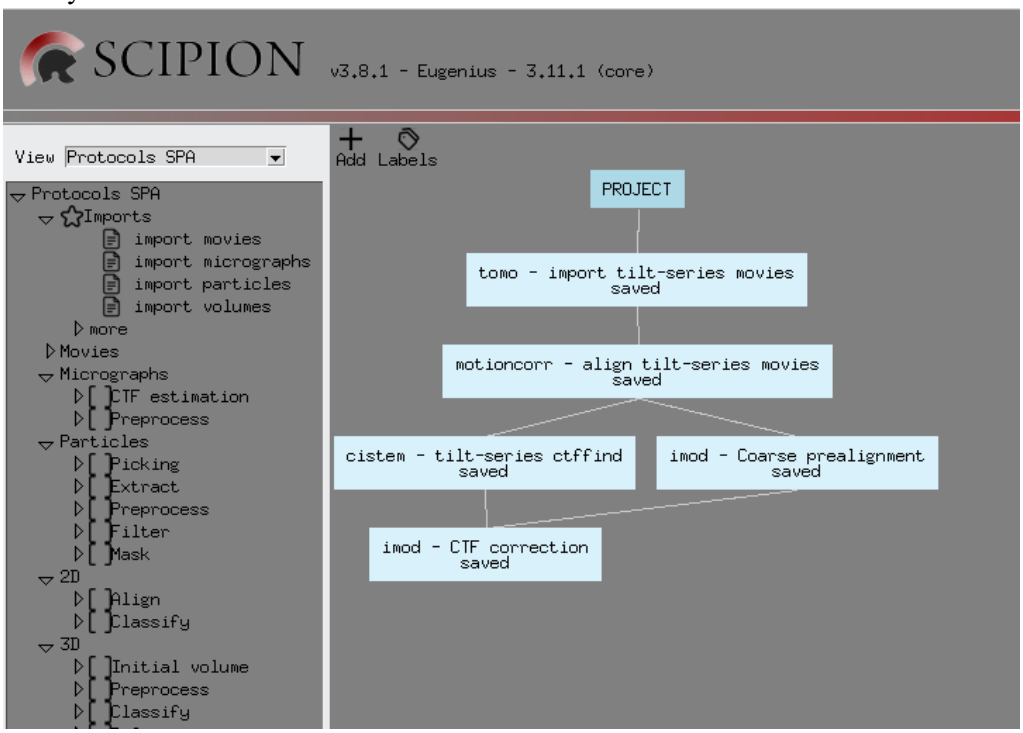
You just opened scipion. Create your project by giving it a name and choosing the right location. Make sure you remember this location you will need it for later. Then you get this screen:



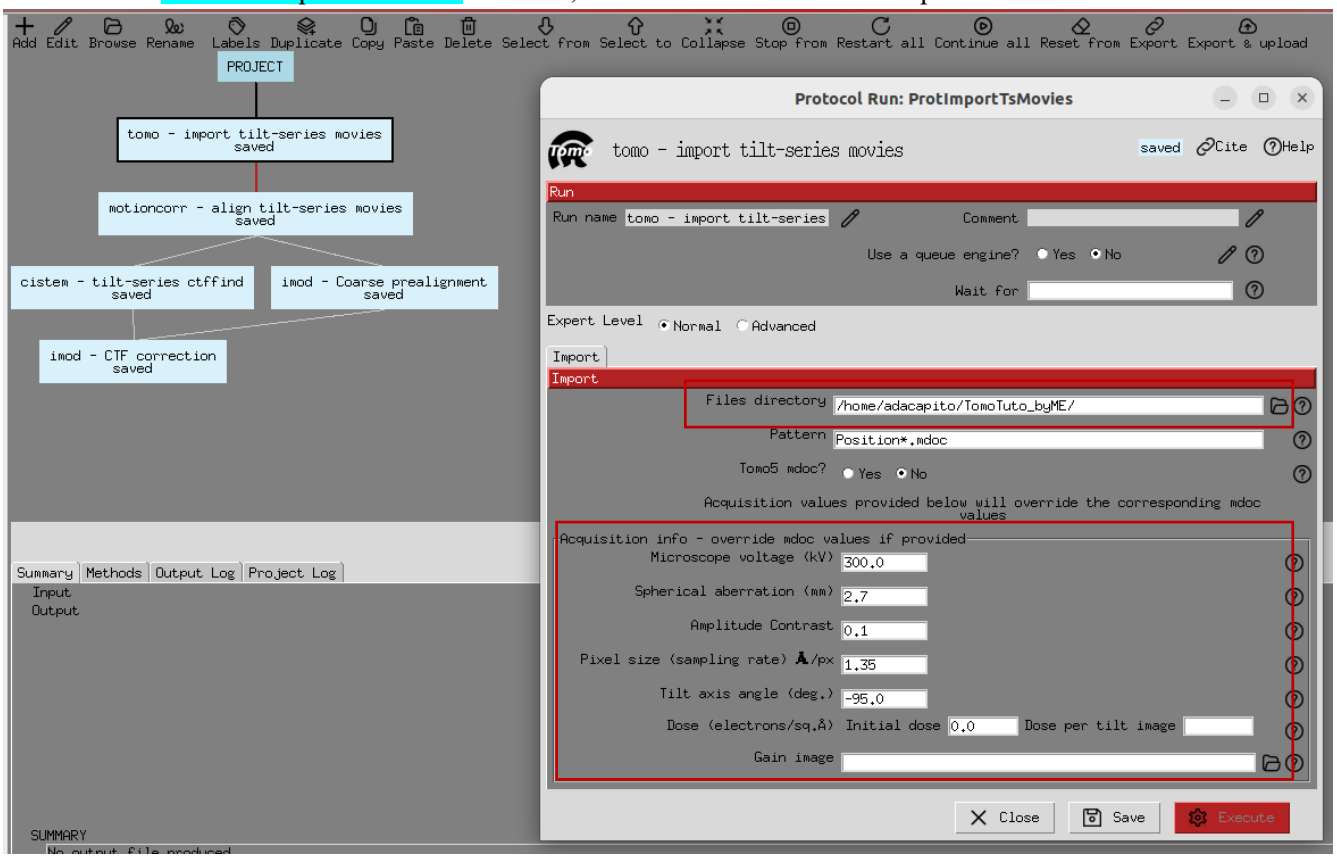
To start the preprocessing, just import the Scipion workflow that comes with QuickTomo. This will set up all the steps you need and most of the processing options.
Click on Project → Import workflow



Import the .json file that comes with quick_tomo.
Now you have this tree:



Double click on tomo-import tilt-series and wait, it can takes few seconds to open.



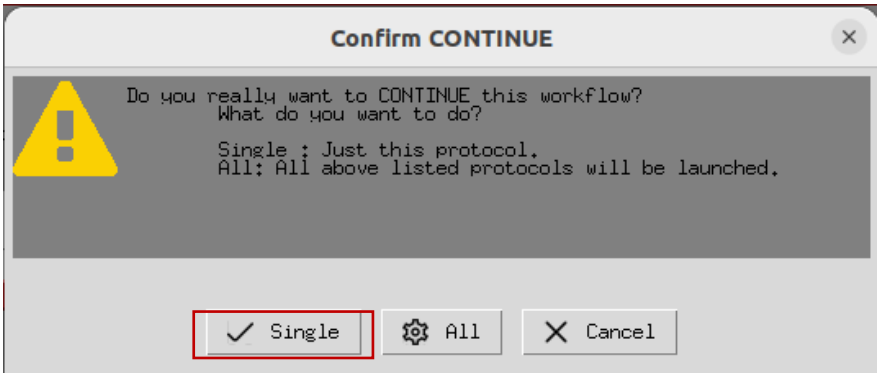
There, fill in the location of your raw data (.tiff), they should be in the same directory as the .mdoc files. Fill up carefully all the parameters specific to your data collection

- Voltage, 300kV for Krios, 200kV for Glacios
- pixel size
- tilt axis angle (specific to the microscope, ask your local contact for this info), but usually -95.0 works
- Gain ref (.gain file given by your local contact)

All parameters you leave empty will be filled up based on the mdoc metadata. It is good practice to fill up all the parameters that you know to avoid errors that could be present in the mdoc files.

IMPORTANT : always tick no to “tomo 5 mdoc?”

Once everything is filled up hit Execute. Click on Single when the following window opens (and do so for the following jobs, don’t click on All):

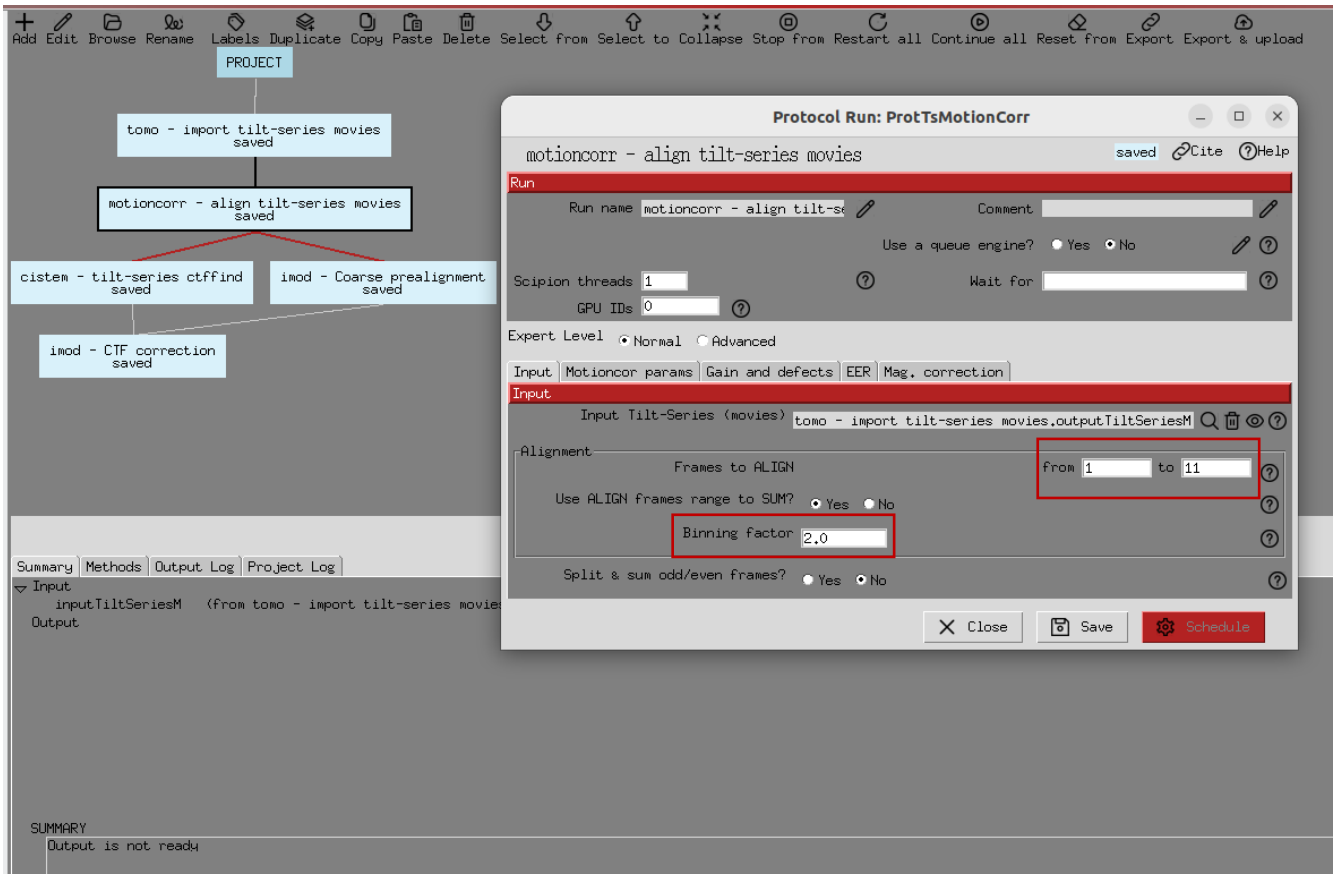


When it's done the job will appear in green. You can refresh the screen sometimes (button at the top right of the Scipion screen).

If a job doesn't start, usually what you can do is: right click on the job, Duplicate your job, then Delete the old one and check if it is working.

Now, let's start motion correction.

Open the job by double clicking on motiocorr.



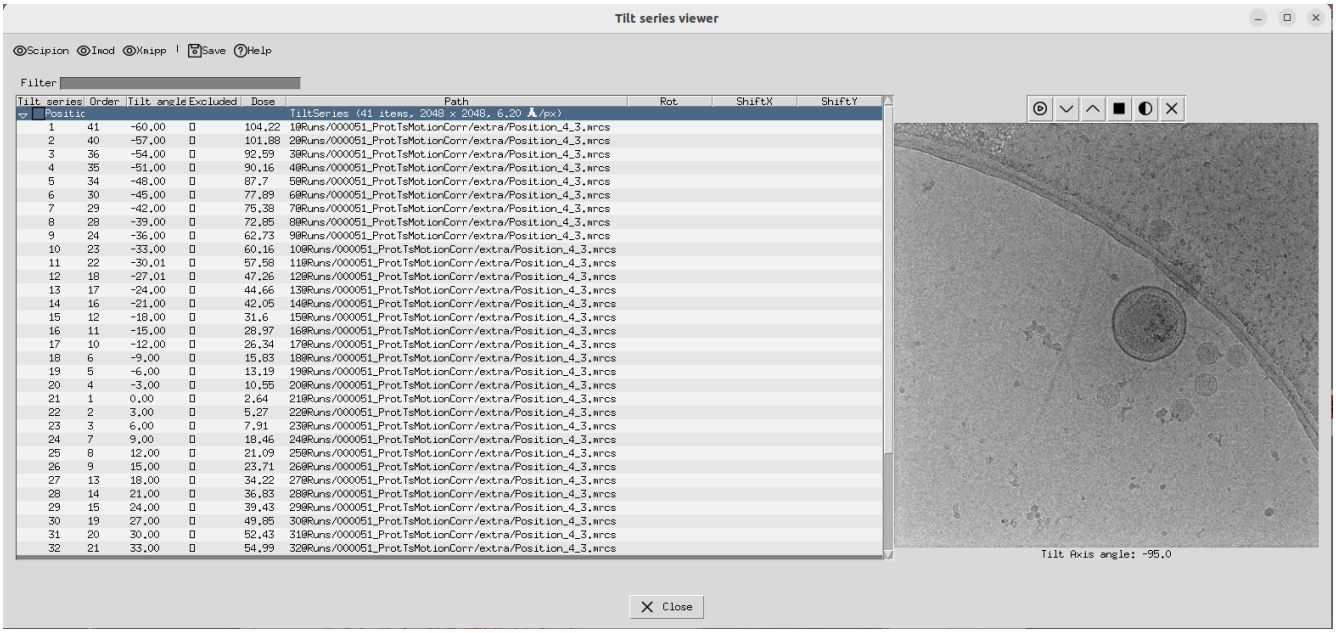
Let the binning factor on 2, it is good practice in tomography to bin by two at this step as we don't need (and will not reach here) high resolutions.

Change Frames to ALIGN from 1 to 0 for the program to take into account all the frames.

Execute. This process takes time.

When it's done (green panel) it's time for manually checking for bad tilt series and dark images. This step is crucial for the tomography image processing and should not be skipped for any reason.

Click on Analyze Results (middle right of the main screen).
A window opens, click on the eye icon after "Display full frame aligned tilt series". This leads you to this screen:



There you can check every position and every frame in it. Good practice is to exclude single tilts that are abnormally shifted as well, as this will help for automated alignment of the tilts.

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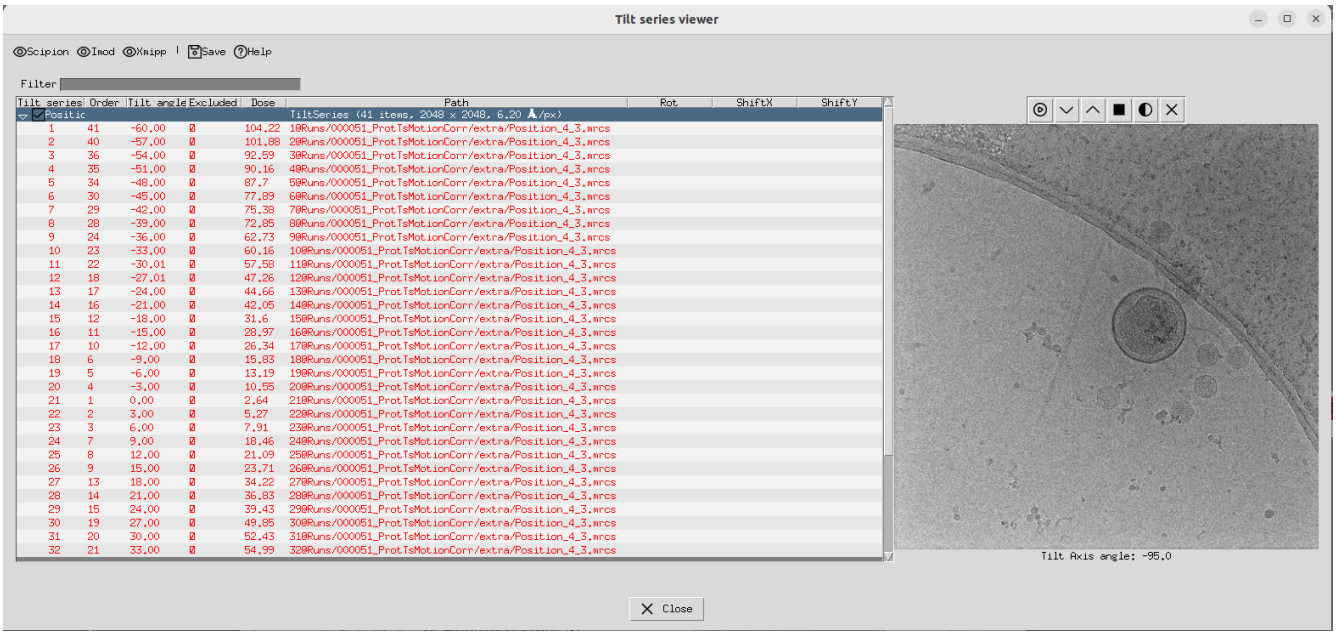
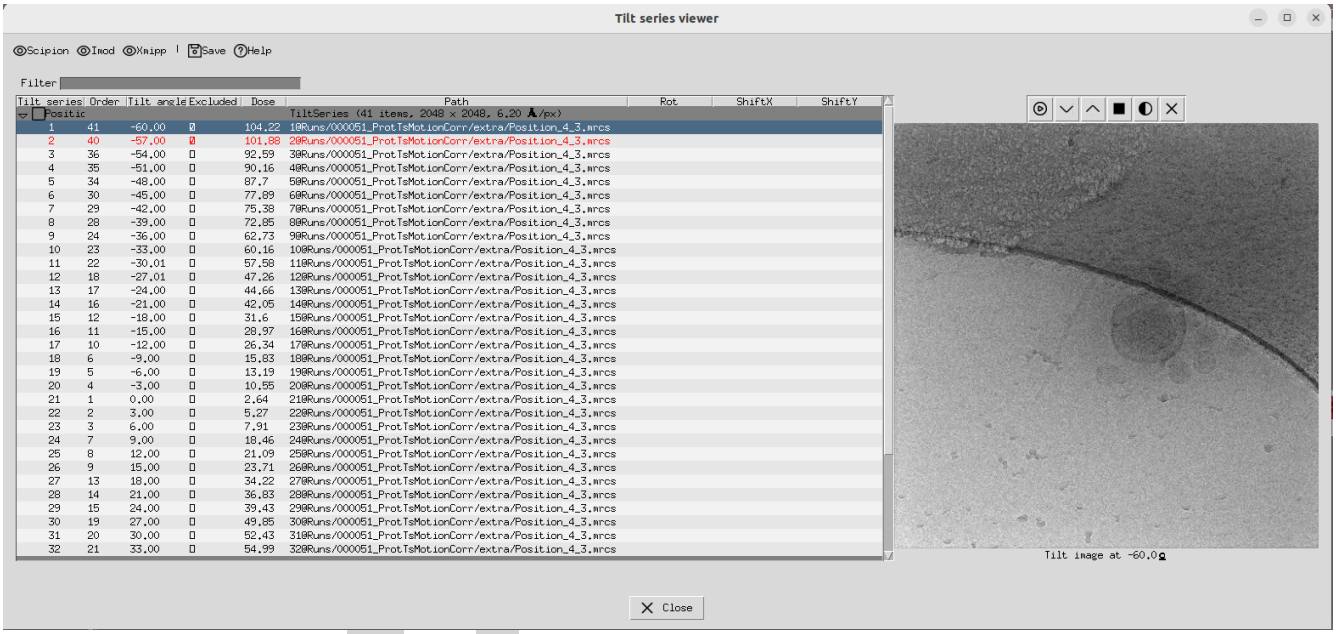
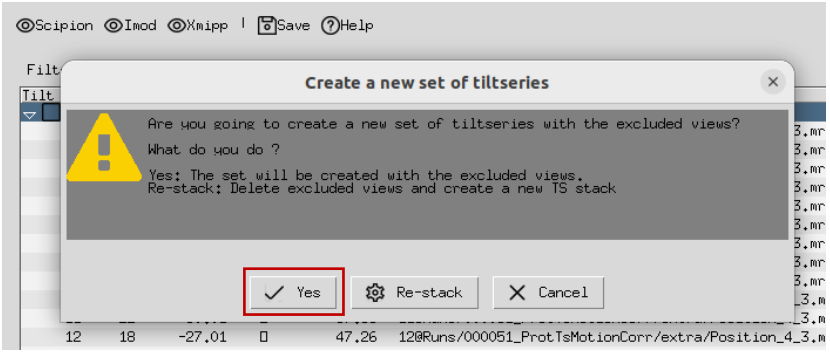
To exclude a full tomogram tick in the box near the position:



To exclude one frame or more tick the “Excluded” box:



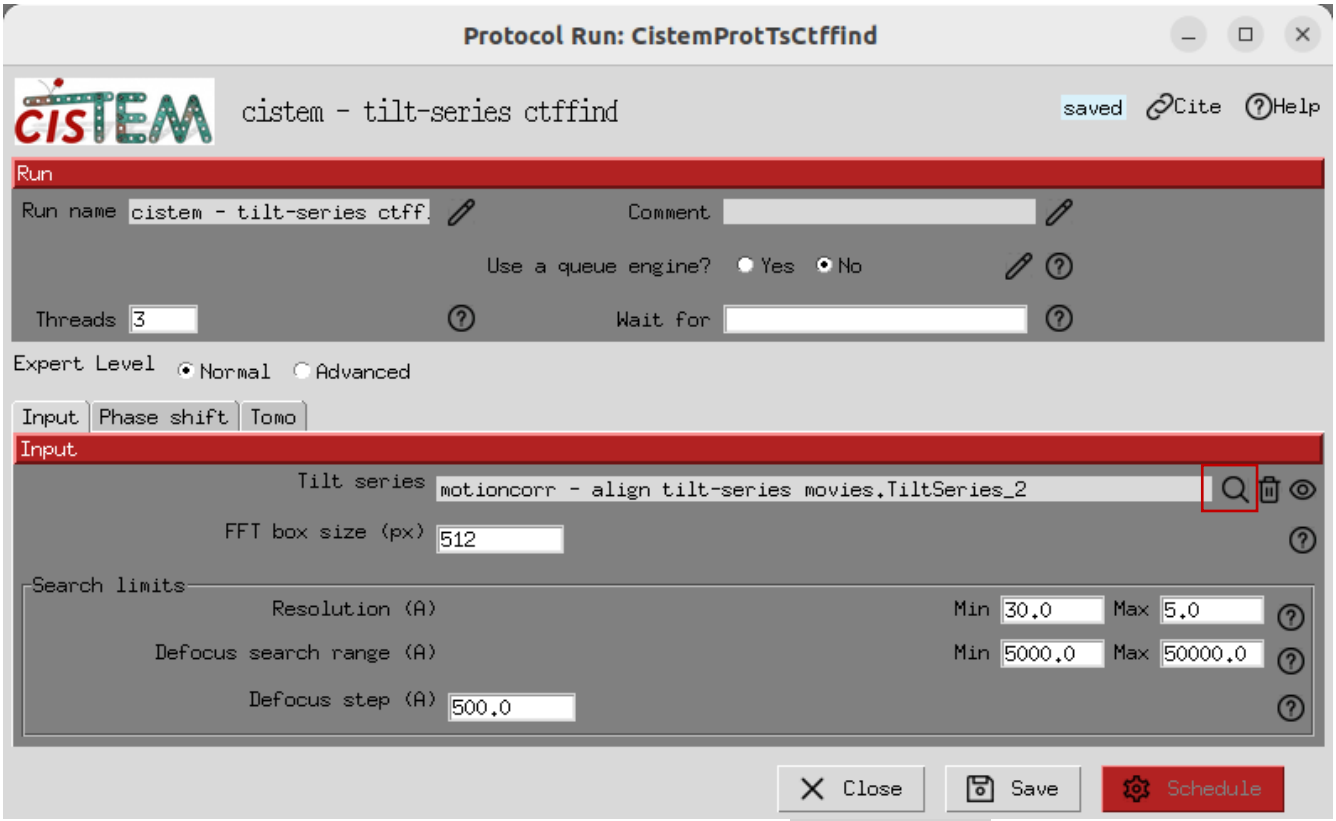
When you are done, click on Save, then Yes.



At this point, be sure you have a message in red saying the files are being written one by one, then close both windows. This will save a second set of tilt series that is called TiltSeries_2. Check in the log that this set was created and has indeed the number of tilt series that you expect.
If the file does not exist, close and:

- Wait for a while, take a coffe and check again
- Resfresh the screen and check
- Close and relaunch your terminal/scipion and check
- Reboot your computer and check
- Try again to sort out your tomograms and write again the files with excluded tomograms…

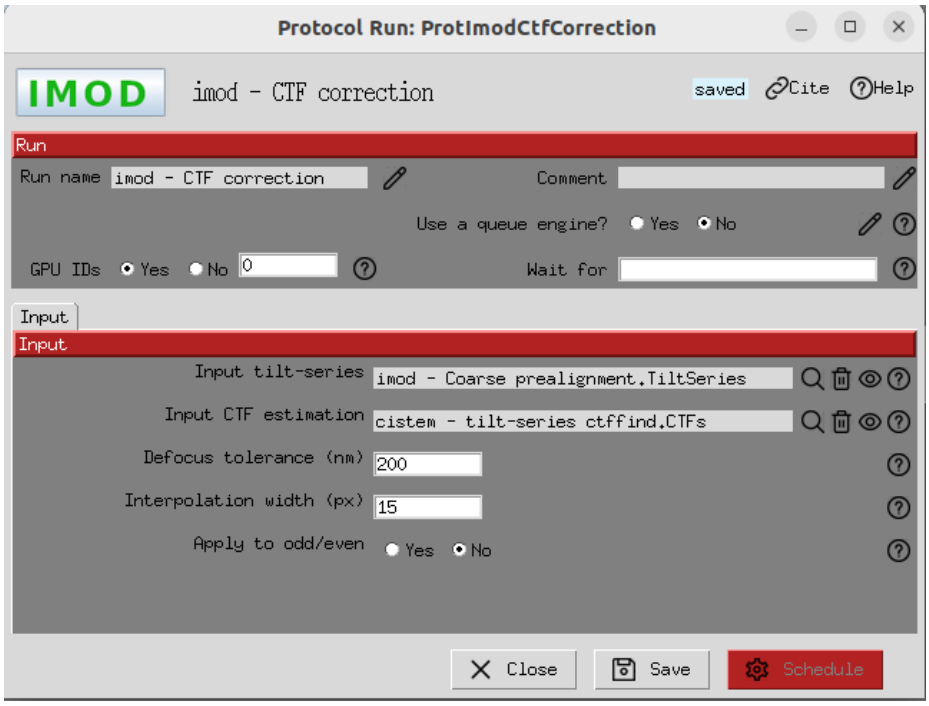
Double click on cistem-tilt-series-ctffind:



Make sure that the TiltSeries_2 series file exists by clicking on the magnifying glass.
Execute.

Double click on imod-Coarse prealignment and leave everything as it is and Execute. This step will pre-align your tilt series. This is a non-mandatory step that could be skipped but usually improves a lot the final result.

When the two last jobs are complete, run the last one: imod-CTF correction.



Execute without changing the presets.

When everything is green, your data is done preprocessing and you are ready to run quick_tomo !

On a terminal window, go to your quick_tomo directory and launch the quick_tomo script.

```
(base) adacapito@GRE052316:~/quick_tomo$ ./quick_tomo_v0.1.sh
```

Now you have questions to answer:

```
(base) adacapito@GRE052316:~/quick_tomo$ ./quick_tomo_v0.1.sh
Enter the full path to your Scipion project: /ibshome/aldecombe/Tomography/ReconTomo-9nA-mc-20250811
Enter the path to the output directory (where reconstructed tomograms will be saved): /ibshome/aldecombe/Tomography/ReconTomo_quicktomo
Chose your binnig factor by taking into account if you have already binned at motioncorr:2
```

1. Enter the path of the folder of your scipion project that you have of course saved from before
2. Enter the path and name of the folder that is going to be created and where you want your reconstructed tomograms to be. This can be wherever you want.
3. Binning factor: we recommend to bin 2 times at this step, the files are less heavy, plus the contrast is usually good. As we binned 2 times in the motiocorr, the resulting binning will be 4.

What quick_tomo does:

- It uses AreTomo
- The basic command of AreTomo is like this:

AreTomo_1.3.4_Cuda115_Feb22_2023
-inmrc ~/TomoTuto_byME/tototest/Runs/000230_ProtImodCtfCorrection/extra/Position_69/Position_69.mrcs
#takes tomograms in your scipion project that are ctf corrected
-outmrc position_69_aretomo.mrc
#gives an .mrc file for every reconstructed tomogram with a new name
#then it needs the following parameters:
-AngFile ~/TomoTuto_byME/tototest/Runs/000230_ProtImodCtfCorrection/extra/Position_69/Position_69.tlt
-AlignZ 250 # AlignZ can be critical, usually 250 works well

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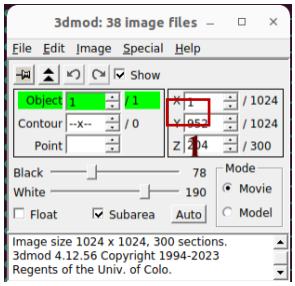
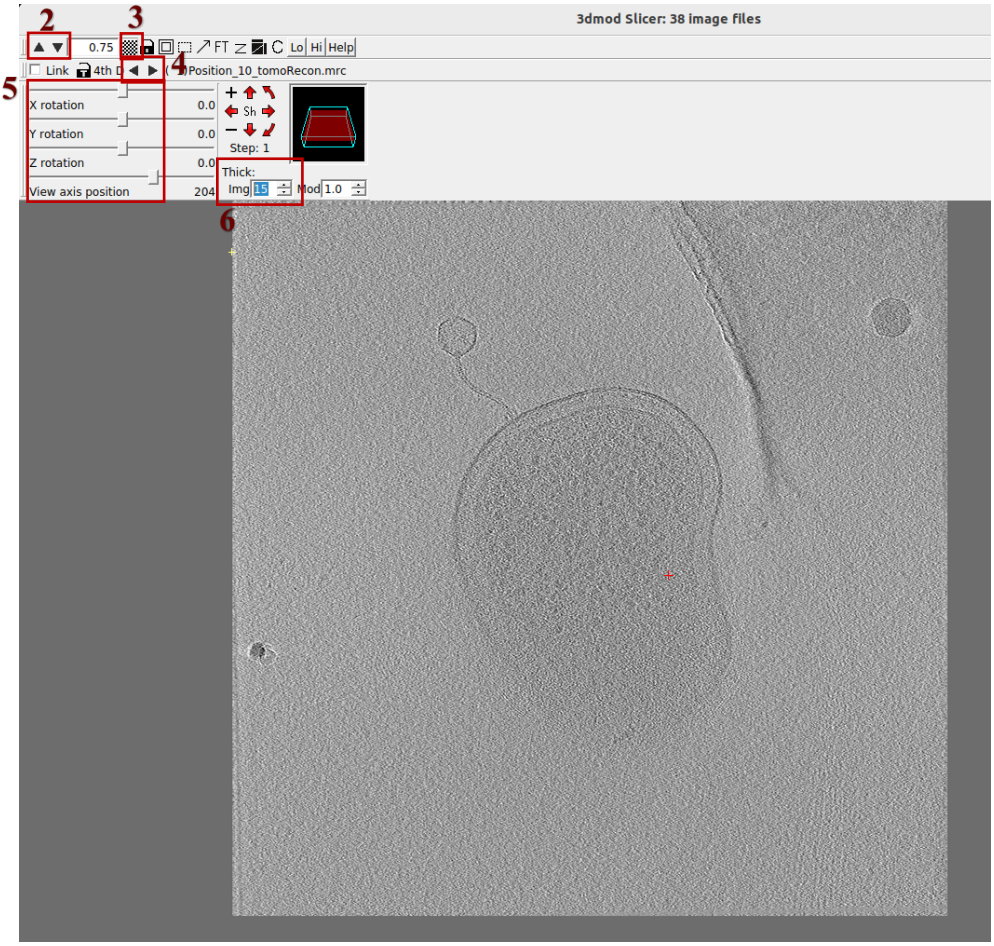
-VolZ 1400 # VolZ should be adjusted by changing the code if needed (tomogram that is to thick = lots of noise = lot's of wasted storage space)
-OutBin 4 #you chose the binning factor with quick_tomo
-Gpu 0 #ask which of your GPU to run (by default it's 0)
-FlipVol 1
-Wbp 1
-DarkTol 0
→Then you get xx.mrc files = your reconstructed tomograms.

The secret sauce here is the value for AlignZ that should work on most of the samples. This value worked fine for all the data we tested so far.

Check your tomograms with your favorite image processor. If you don't know where to start we recommend 3dmod (if you are new, you can start here https://bio3d.colorado.edu/imod/doc/3dmodguide.html), but other software as Napari (get the BLIK plugin for tomography data handling https://napari-hub.org/plugins/blik.html) or bshow work as well.





Here you go ! enjoy your tomograms !