

Kinesin-5 cryoEM density pipeline

Requirements:

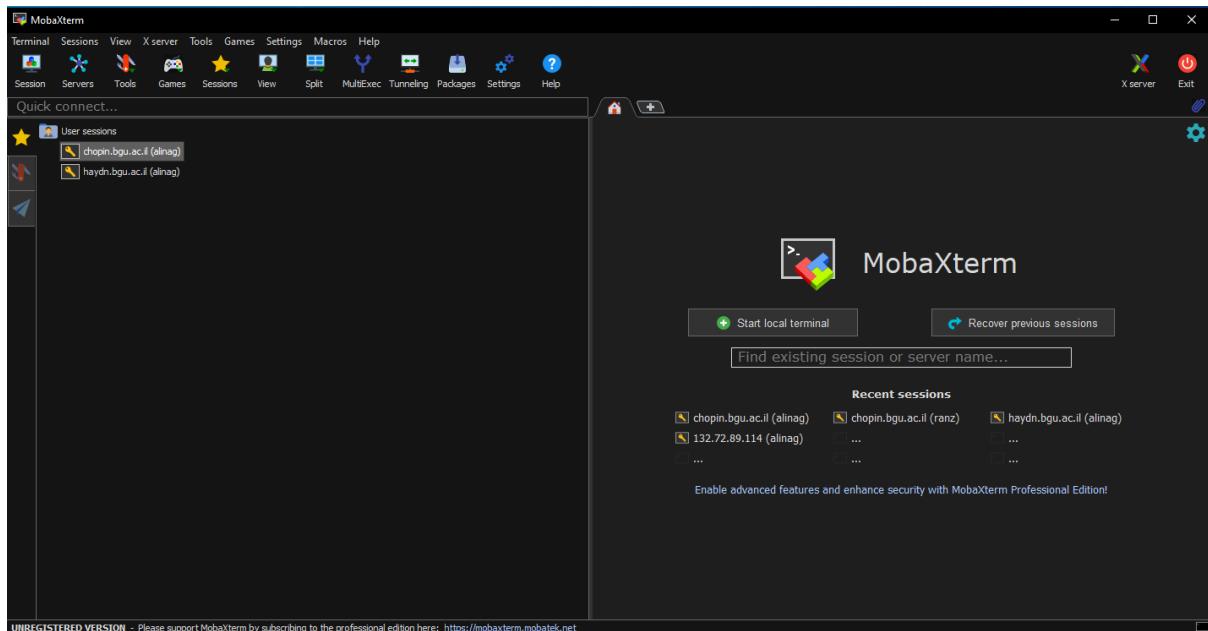
1. User and password for Chopin computer (chopin.bgu.ac.il) - contact barouchy@bgu.ac.il
2. If accessing Chopin from a computer within the campus, also you will need to have connection approval (אישור חיבור) between your computer and Chopin (Get your IP address and contact eran@bgu.ac.il or Infosec@bgu.ac.il).
3. Basic knowledge in Linux [Basic navigation commands: `cd`, `mkdir`, `ls`, `*`].
4. [MobaXterm](#) (SSH remote connect).
5. Anaconda (installation protocol is in section I)
6. LG_MiRP [[GitHub](#)] (contains the required 3D references)

TL:DR:

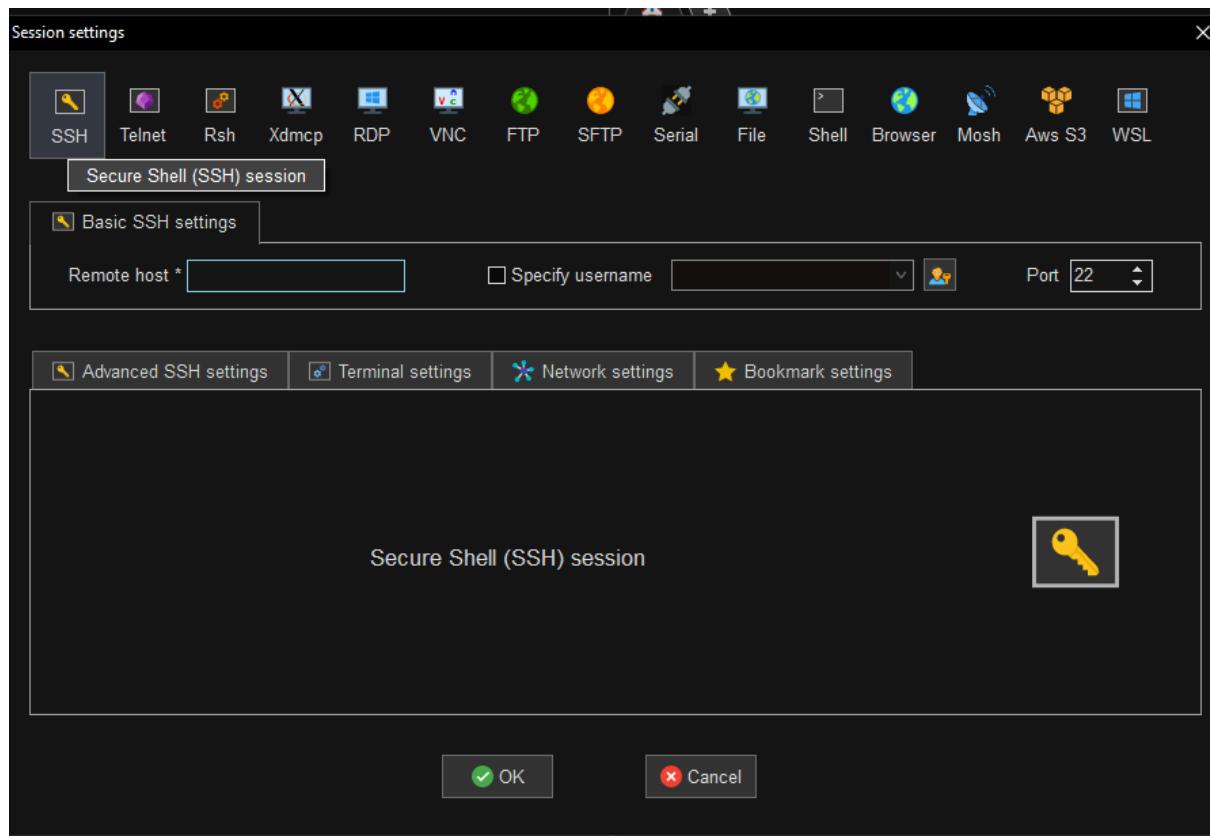
1. Refine a map containing MTs + kinesin
2. Protofilament refinement using Garret's Python code.
3. Refine kinesin only map

MobaXterm:

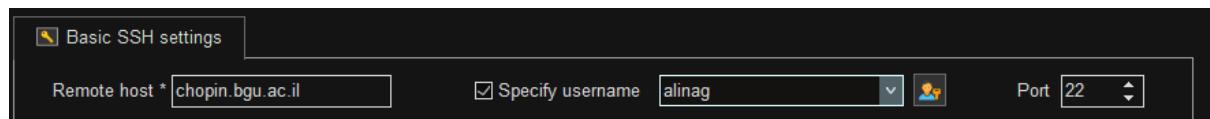
- a. To access chopin download and install MobaXterm



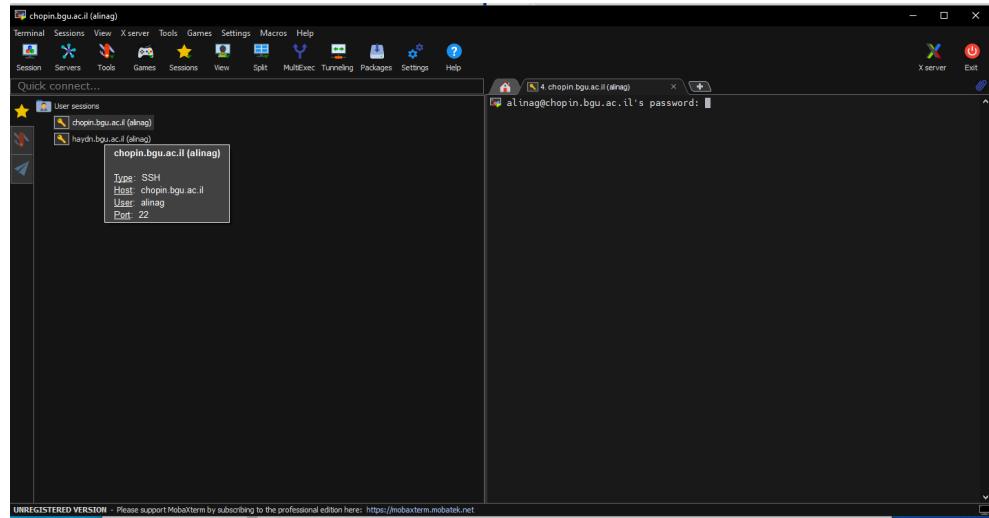
- b. To create a session, click on Session (top left button) and choose SSH



- c. Enter the host name, activate “Specify username” and enter your username



- d. Click 'OK' and enter the password you got from Yehuda
- e. To change password run:
`passwd`
- f. Enter your existing password and a new password
- g. From now on, to open a session click the session on the left and enter your new password.



Anaconda installation:

I. Install Anaconda and set up the environment:

I.1 Download the latest version:

This step might not be relevant since Anaconda is already installed on Chopin.

- Create a new folder named "tmp"

```
cd /tmp
```

- Download Anaconda

```
curl -O  
https://repo.anaconda.com/archive/Anaconda3-2023.09-0-Linux-x86_64.sh
```

I.2 Install:

This step might not be relevant since Anaconda is already installed on Chopin.

- Run the line:

```
bash Anaconda3-2023.09-0-Linux-x86_64.sh
```

- Activate the base environment:

```
source ~/.bashrc
```

- c. See what environments are set and which is active

```
(base) [alinag@haydn Anaconda]$ conda env list
# conda environments:
#
base * /home/alinag/anaconda3
```

I.3 Set up environment for pf refinement:

- a. The environment.yml file looks like this:

```
name: LG_MiRP
dependencies:
  - python=3.10
  - pip
  - cycler
  - openmpi
  - mpi4py
  - matplotlib
  - tkinter
  - numpy
  - pandas
  - olefile
  - Pillow
  - pyparsing
  - python-dateutil
  - scipy
  - six
  - pytz
  - tqdm
  - pip:
      - mrcfile
      - starfile
      - tensorflow
```

I.4 Create an environment using this file:

- a. Go to MiRP folder
- b. Use this command inorder to set up the environment

```
conda env create -f environment.yml
```

- c. Activate the environment

```
conda activate LG_MiRP
```

- d. Setup the environment

```
python setup.py develop
```

I.5 To turn off the environment:

***Relion requires that the environment will be turned off**

- a. Run the line

```
conda deactivate
```

- b. Or turn off all environments:

```
conda config --set auto_activate_base False  
source ~/.bashrc
```

MiRP: Microtubule RELION-based Pipeline for cryo-EM image processing (based on [Carolyn Moores protocol](#))

II.1 Data preparation, import, motion correction and CTF estimation in RELION

TO INITIATE A NEW PROJECT OPEN RELION (see bellow) FROM THE FOLDER CONTAINING YOUR DATA

II.1.1 Open RELION:

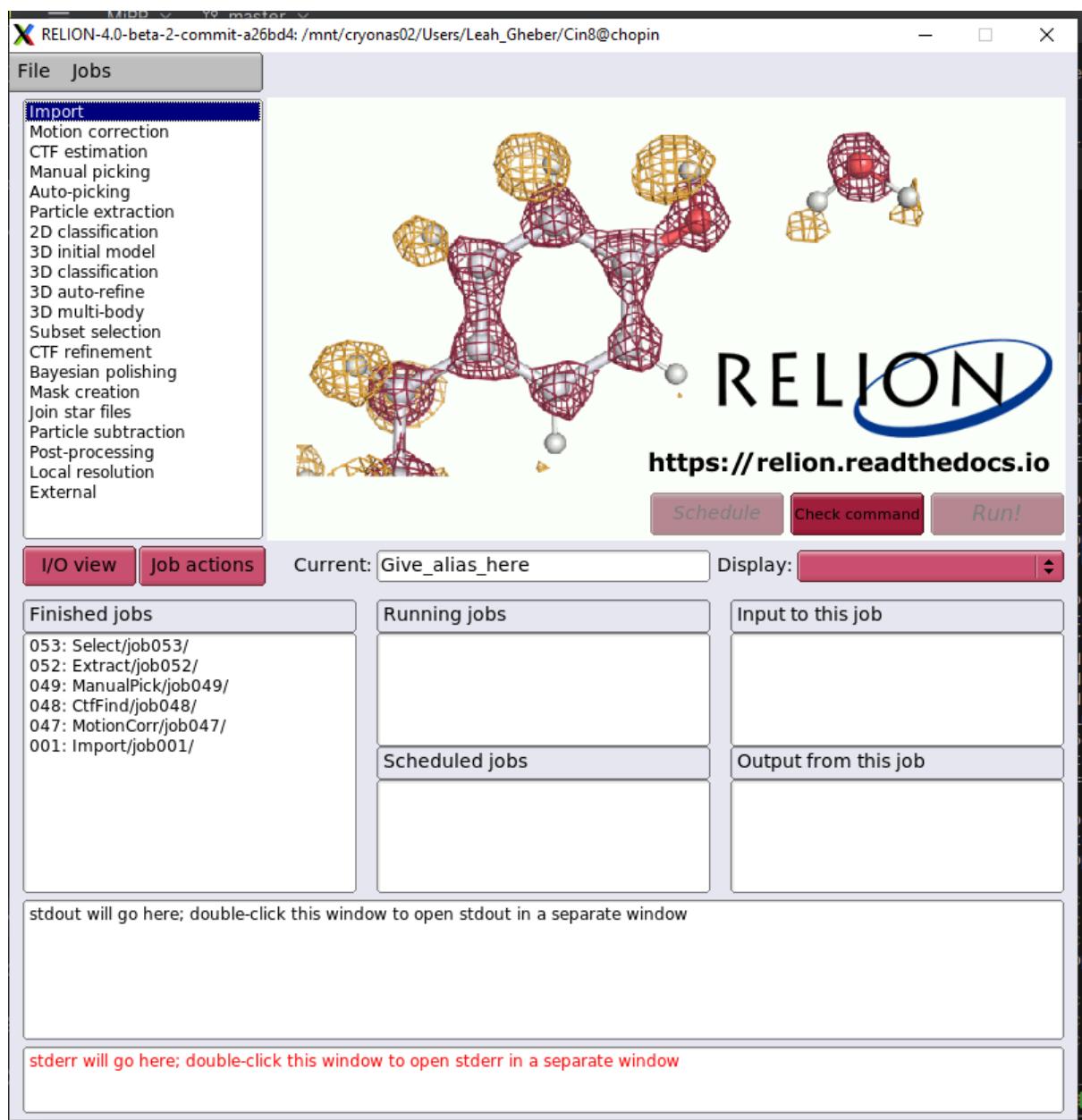
- a. Run the command from the folder containing the data:

```
/home/relion/relion
```

Or

```
relion
```

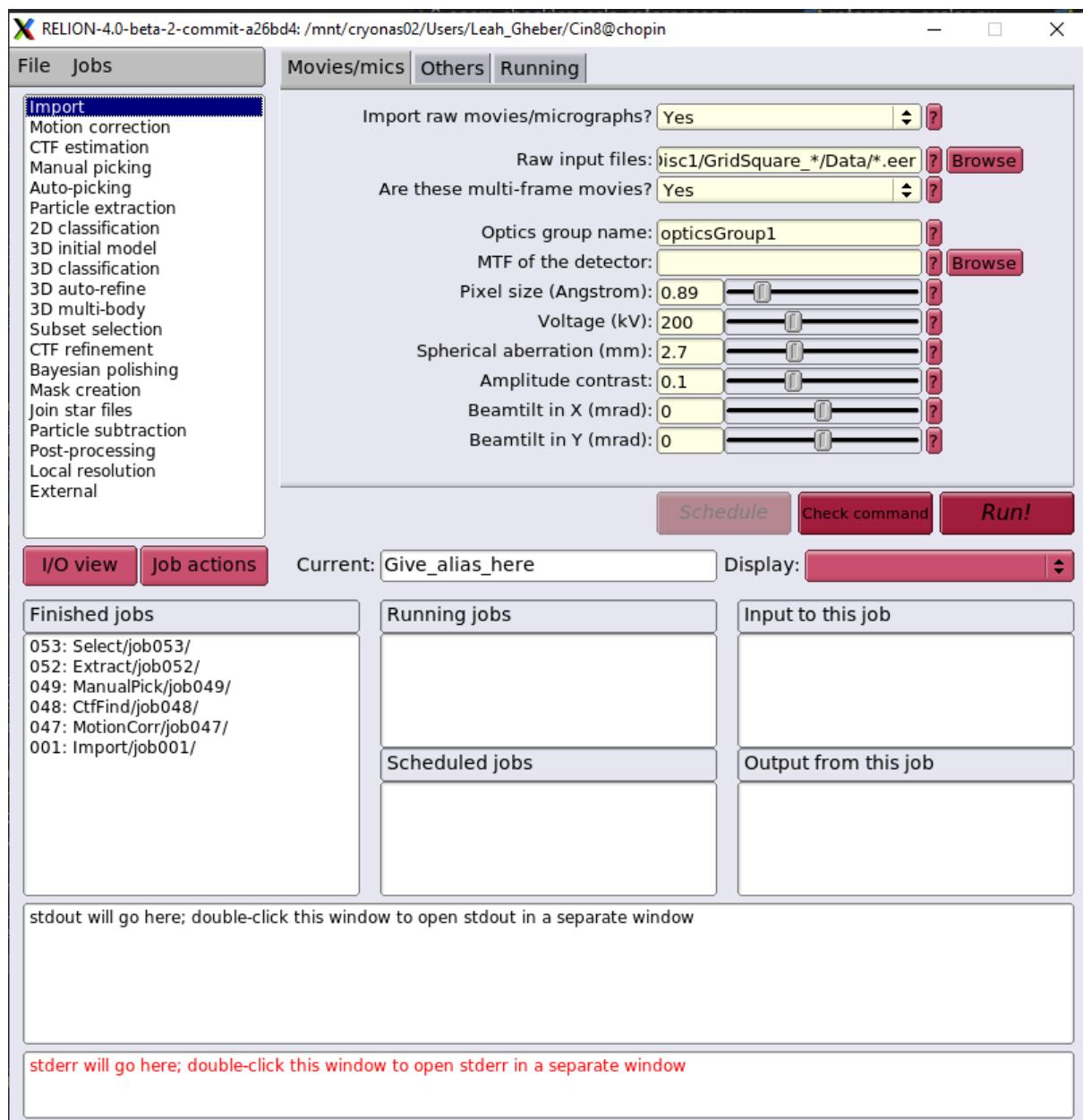
The RELION interface (GUI) will appear



* This screenshot already contains some jobs (bottom left) usually it's blank when a new project is initiated

II.1.2 Import the data to RELION:

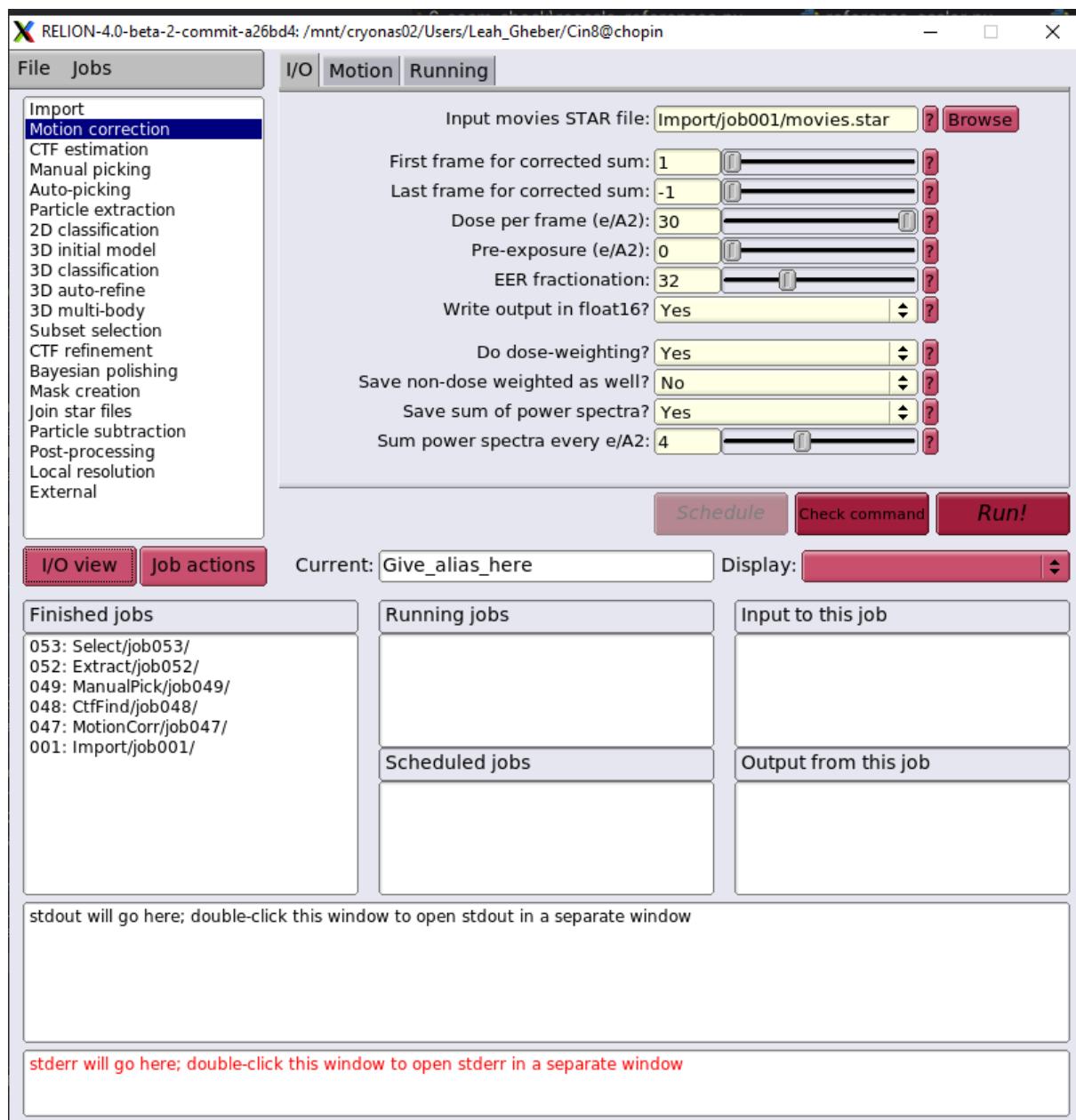
- a. Select import from the job menu in the left menu in RELION



* Use syntax * to import all the relevant images

II.1.3 Motion correction:

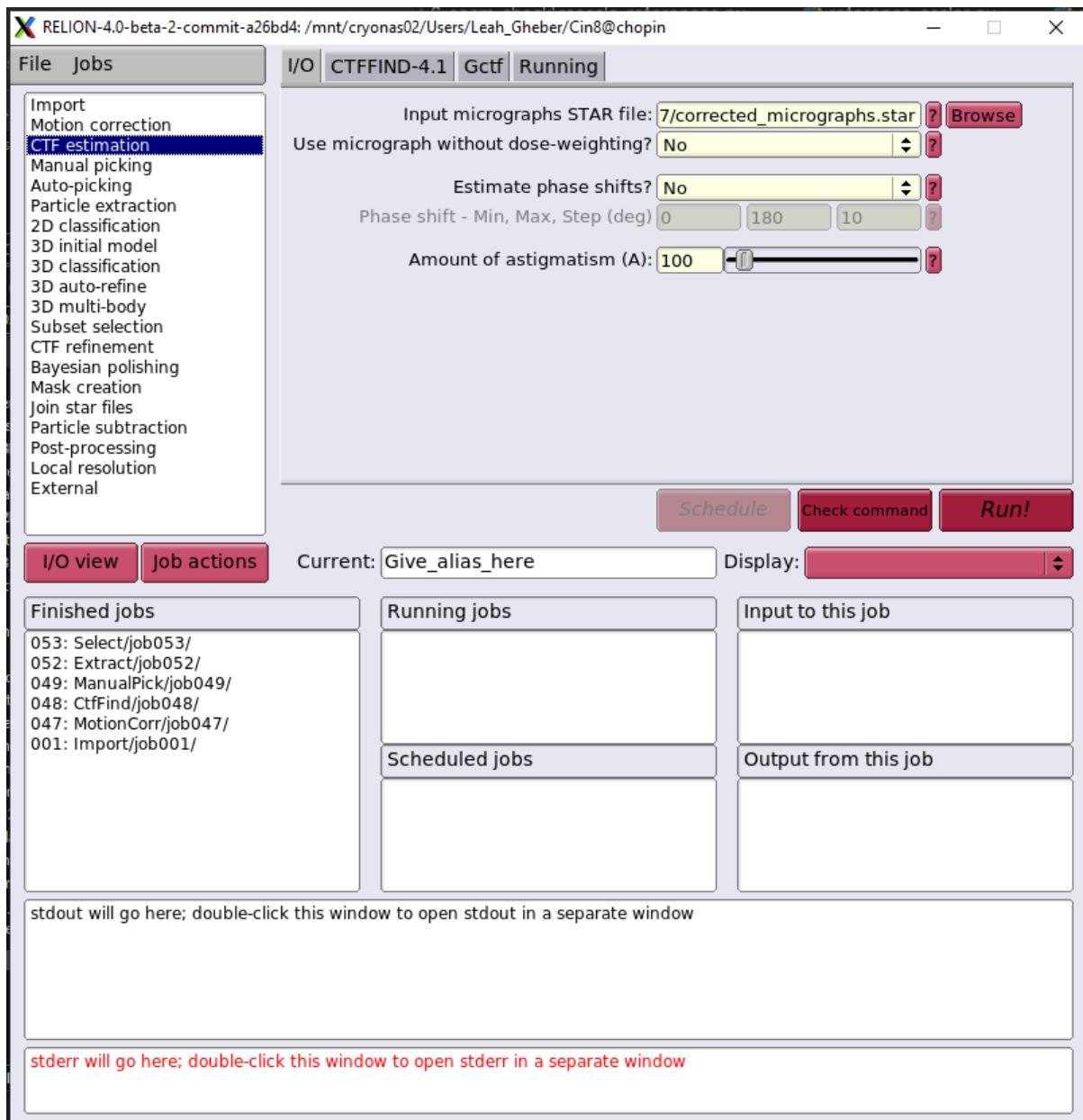
- a. Select Motion correction



- Fill the required fields according to the information from Ran
- Make sure the binning factor is 1
- This will might take some time (around 24hrs)

II.1.4 CTF estimation:

- Select CTF estimation

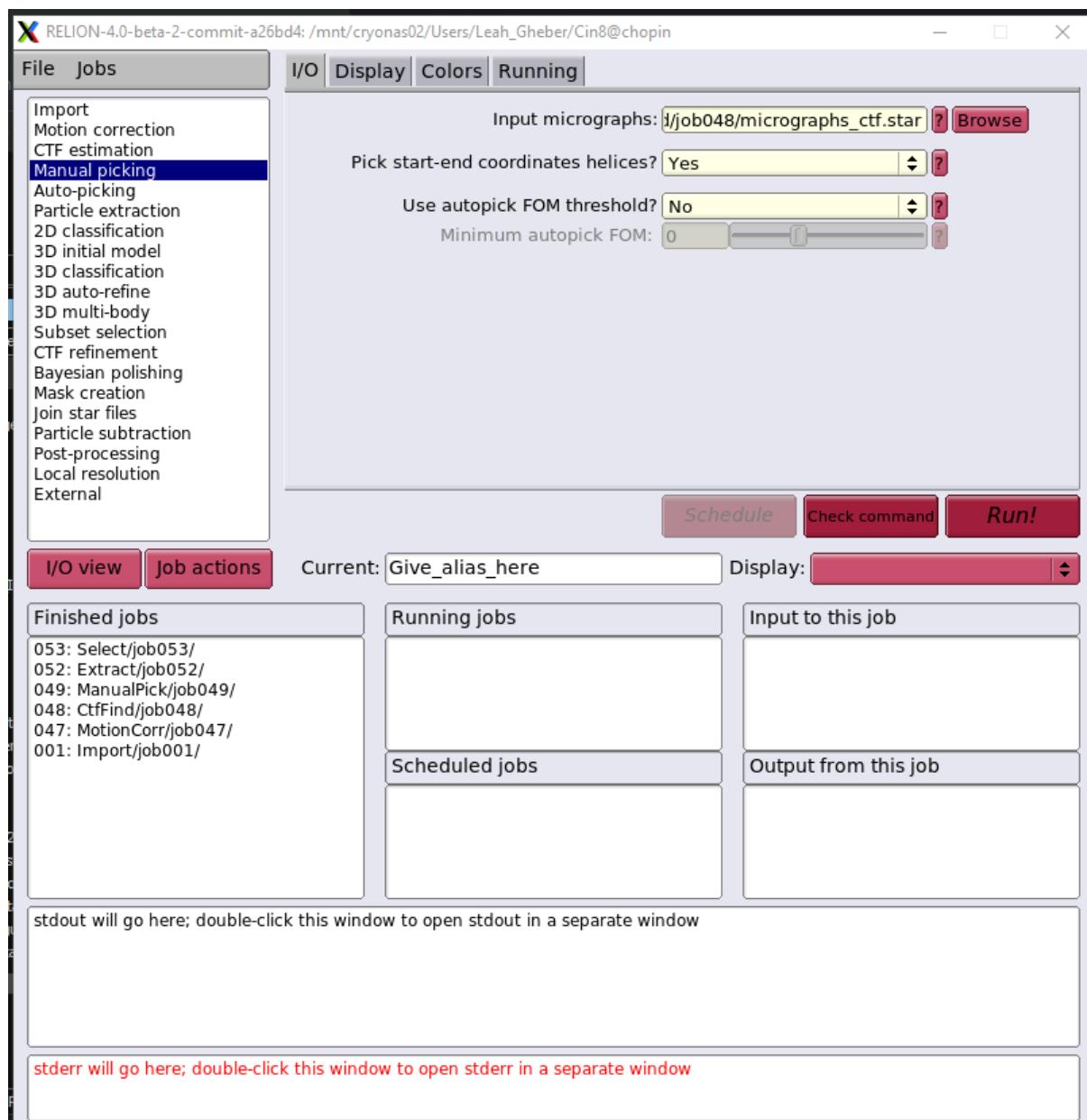


- The CTF executable is located in /home/ctffind/bin/ctffind
- You will also need more information from Ran
- This step will take around 8 hrs

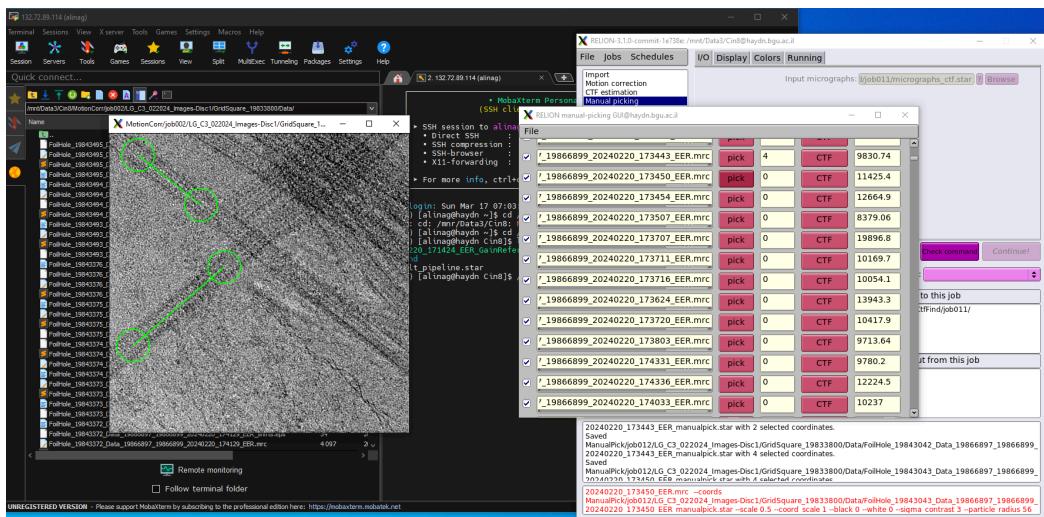
II.2 Particle picking, extraction and subset selection

II.2.1 Manual picking:

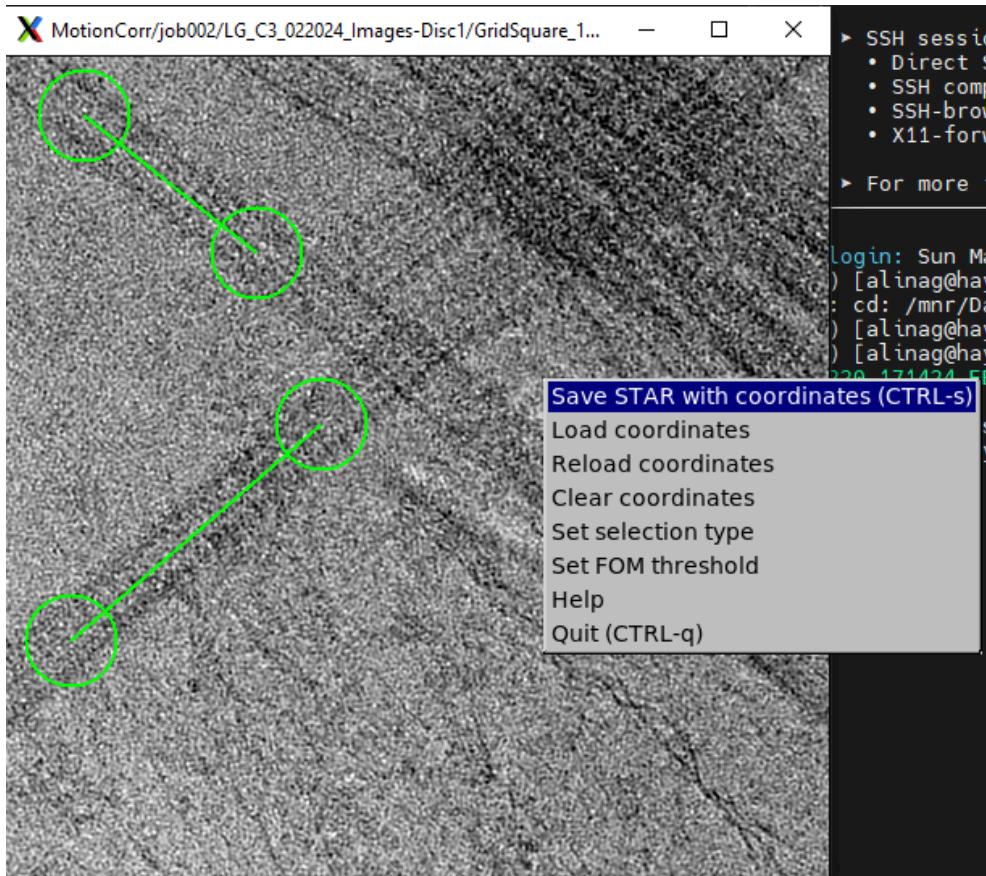
- Select Manual picking



- b. Particle diameter 400A
- c. Pick start-end coordinates helices? Yes
- d. Start picking particles
(Click on pick and then click with the mouse on the start and end of the MTs you choose.)



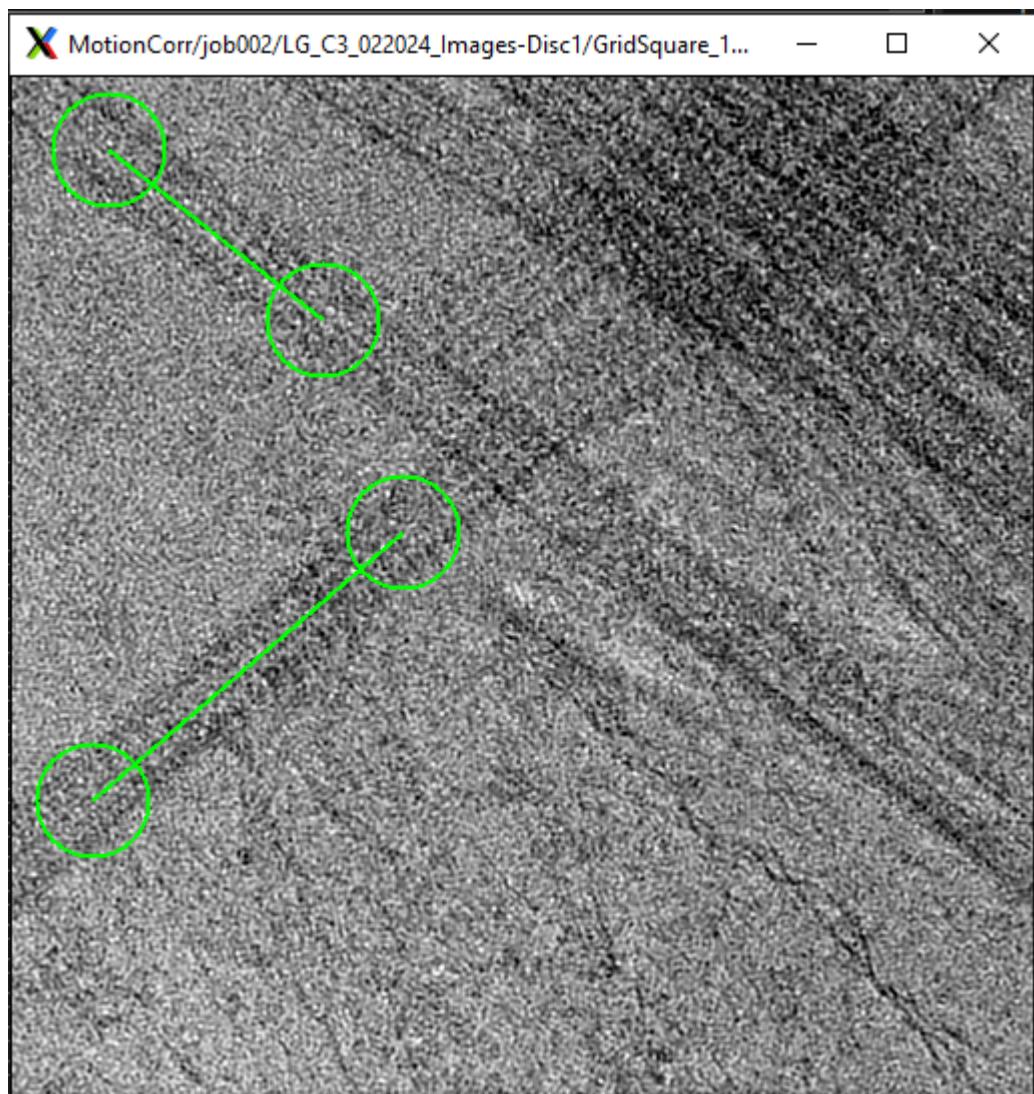
- e. To save the particles press **Ctrl+s** or right click with the mouse and select **Save STAR with coordinates**



This will fill the box right next to “pick” button:



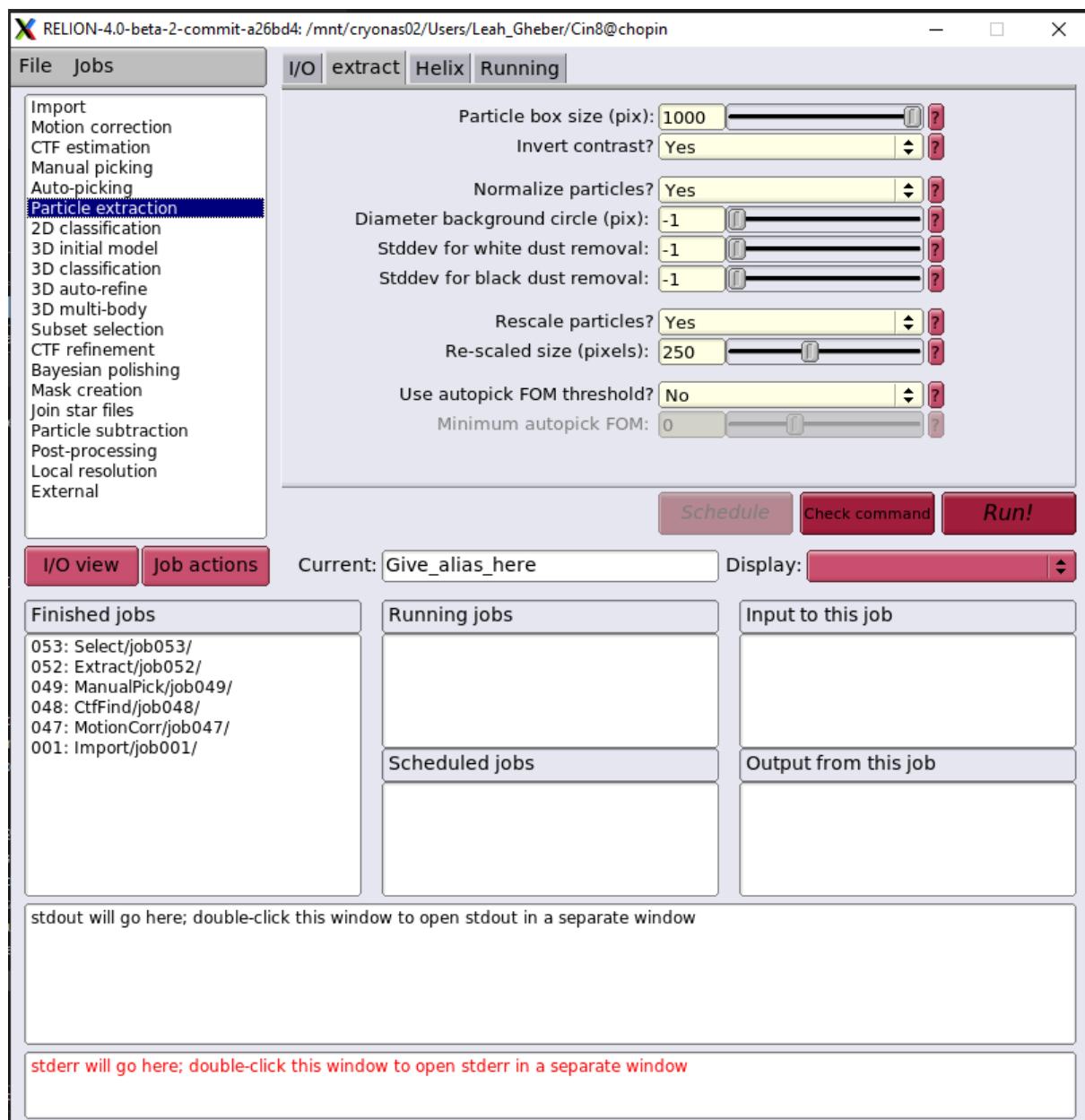
- f. Make sure not to select overlapping MTs or sections with poor quality:
Example:



g. sdf

II.2.2 Particle extraction:

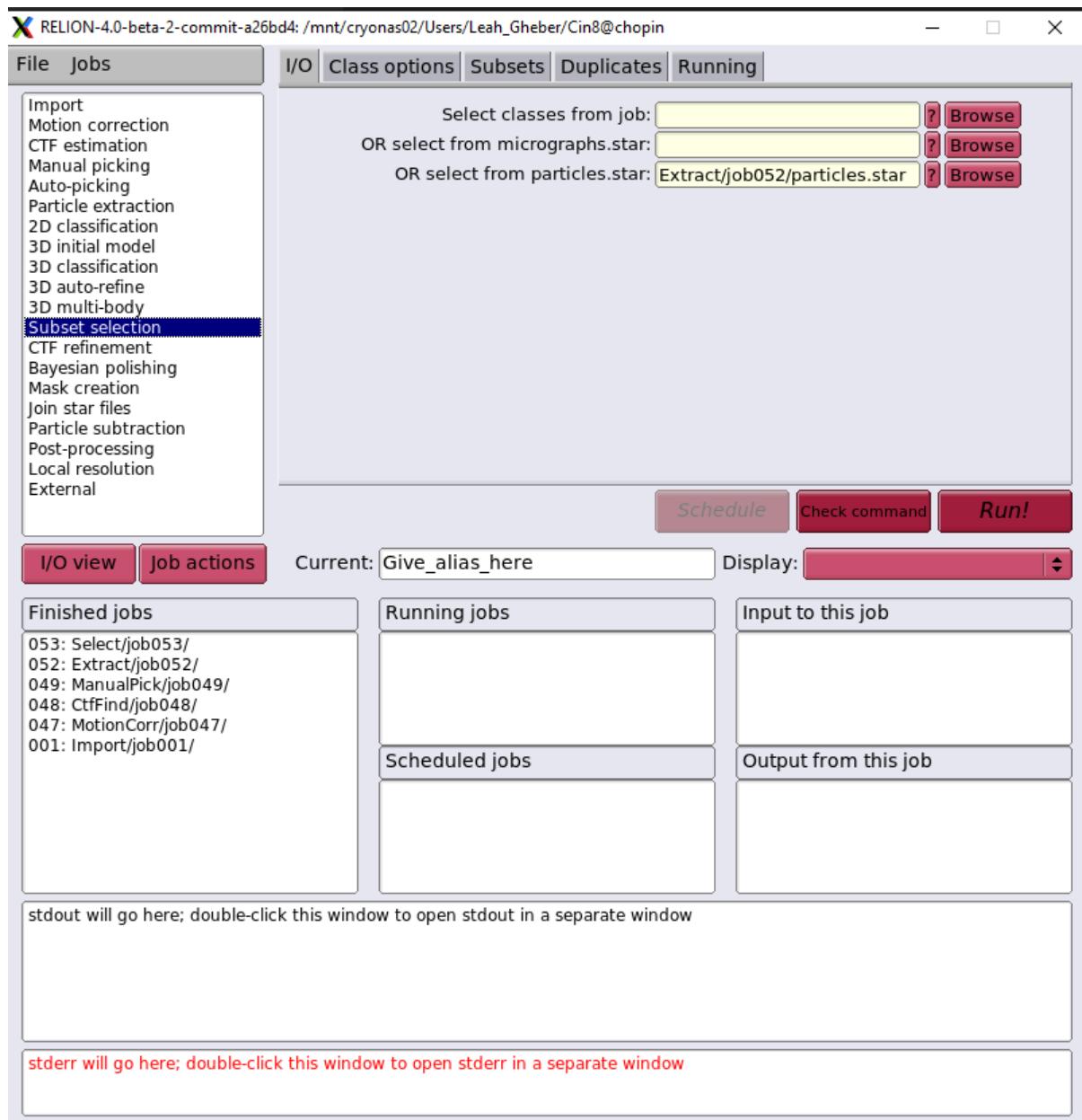
- a. Select Particle extraction



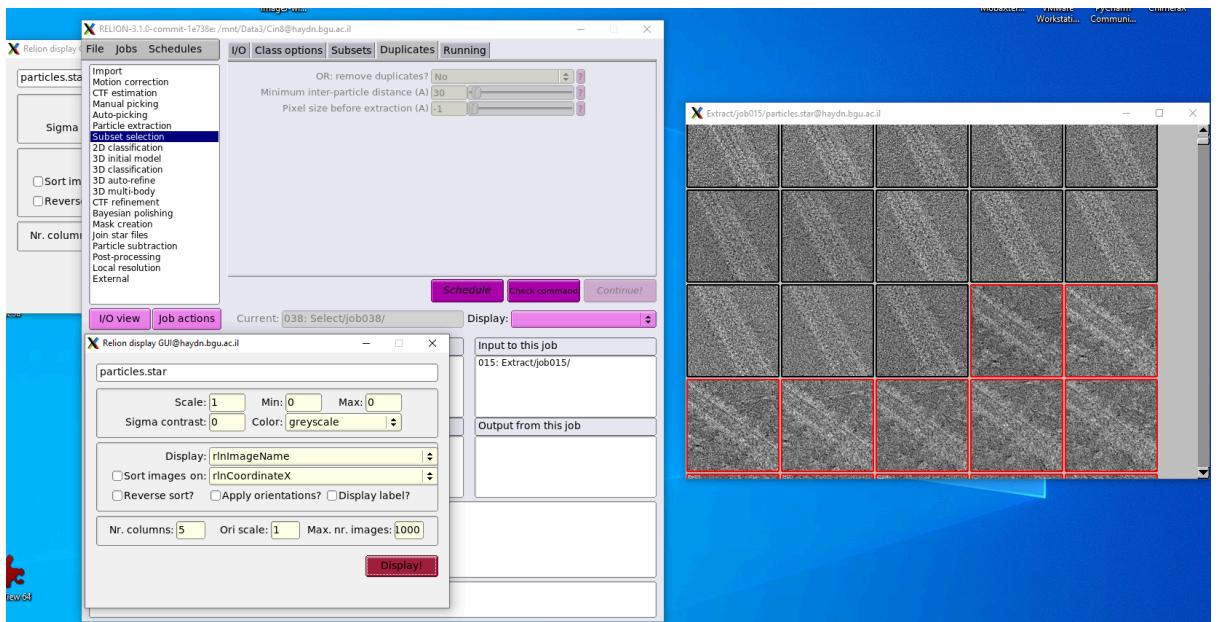
- b. The most important parameters are particle box size and rescaled size - this will determine the binning

II.2.3 Subset selection:

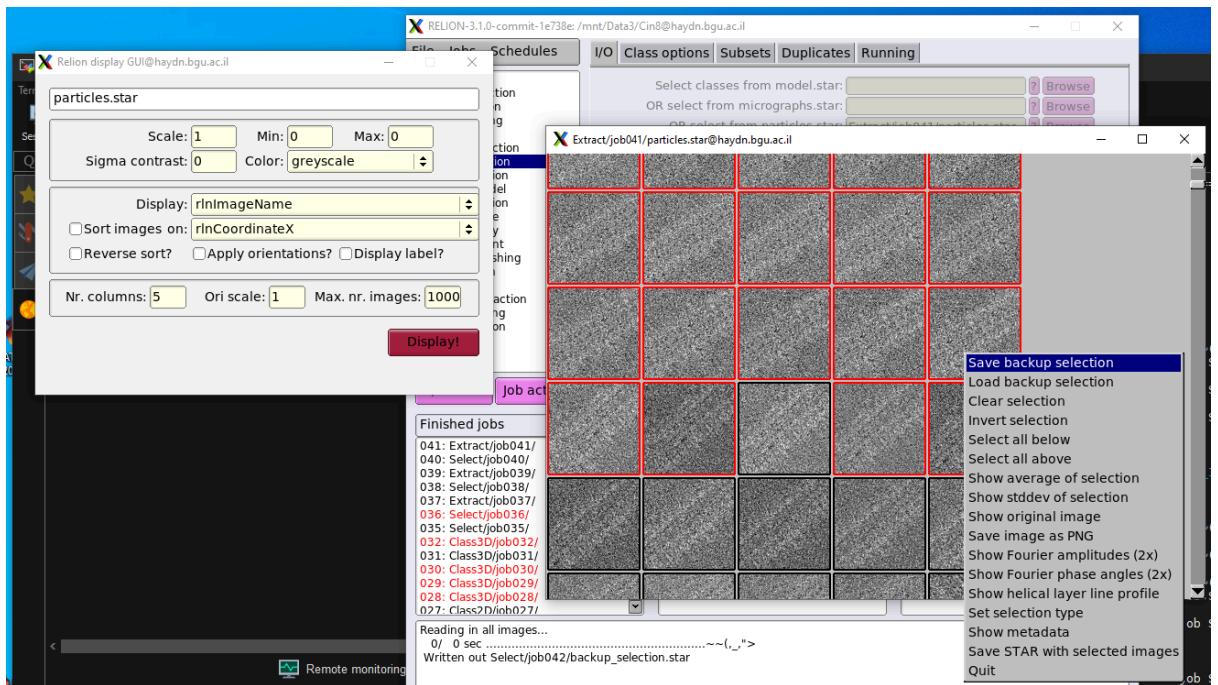
- a. Select Subset selection



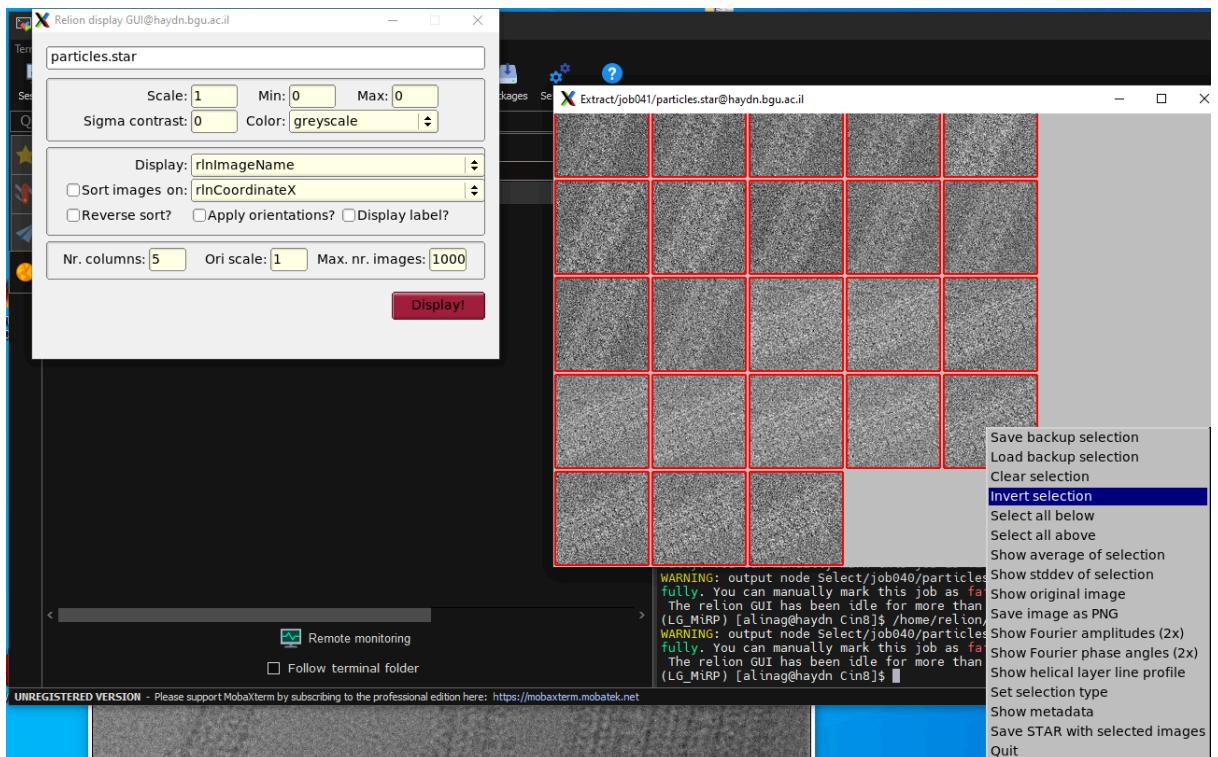
- b. Use the particles.star file from Particle extraction
- c. Start selecting particles



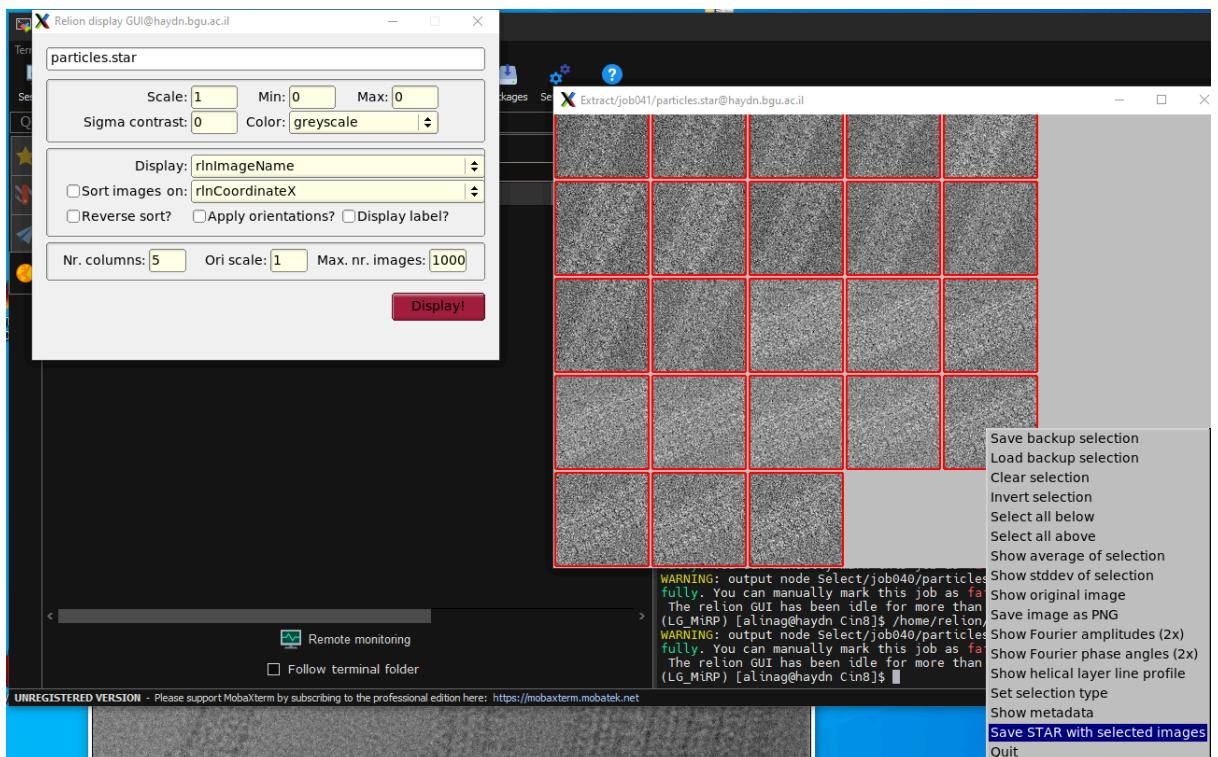
- d. To save your progress right click with the mouse and select Save backup selection



- e. Finally, incase you selected back particles, right click with the mouse and select Invert selection



- Otherwise, right click with the mouse and select Save STAR with selected images



II.3 Protofilament number sorting: Reference rescale, 1st 3D Classification, Class unification

II.3.1 Reference rescale:

- a. Go to the MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- b. Activate the environment

```
conda activate LG_MiRP
```

- c. Setup the environment

```
python setup.py develop
```

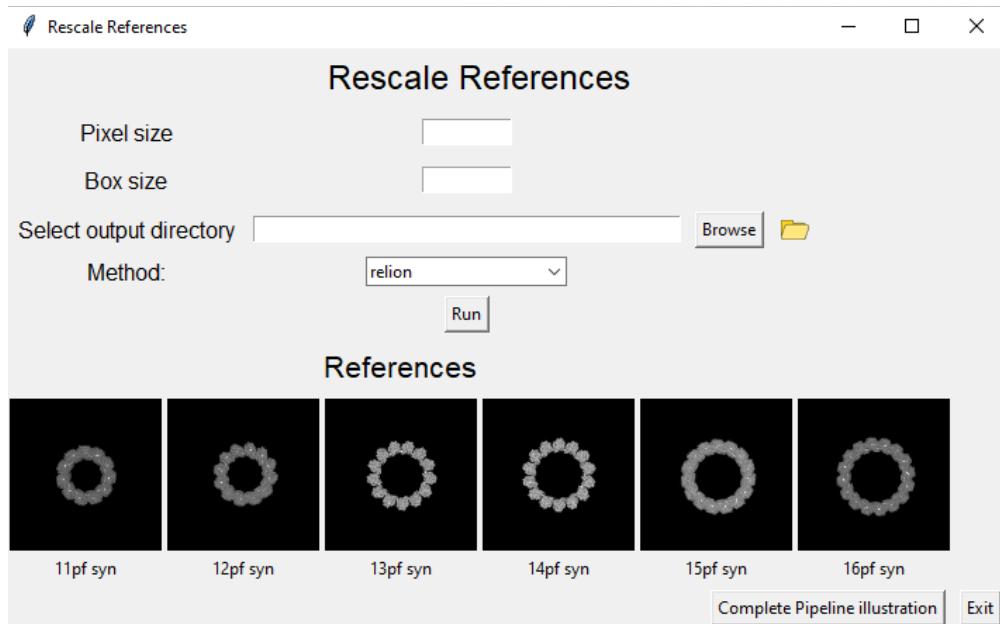
- d. Go to 3_protofilament_number_sorting folder

```
cd 3_protofilament_number_sorting
```

- e. Run rescale_references

```
python rescale_references.py
```

- f. You will get a window similar to the window bellow:



* The references below show references of microtubules with different protofilament number

- g. Enter the desired pixel size and the box size (according to the scaling of the images in the extract particles step)

- h. Select an output folder (if a folder named new_references already exists, it will delete the contents of this folder)
- i. Select a method - relion or scipy
- j. Press run
- k. The resulting file location should be printed in the terminal

II.3.2 1st 3D Classification:

- a. Deactivate conda

```
conda deactivate
```

- b. Change working directory to your project and run relion
- c.
- d.

II.3.3 1st 3D Classification:

- a. activate conda

```
conda activate LG_MiRP
```

- b. Navigate to MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- c. And activate LG_MiRP

```
python setup.py develop
```

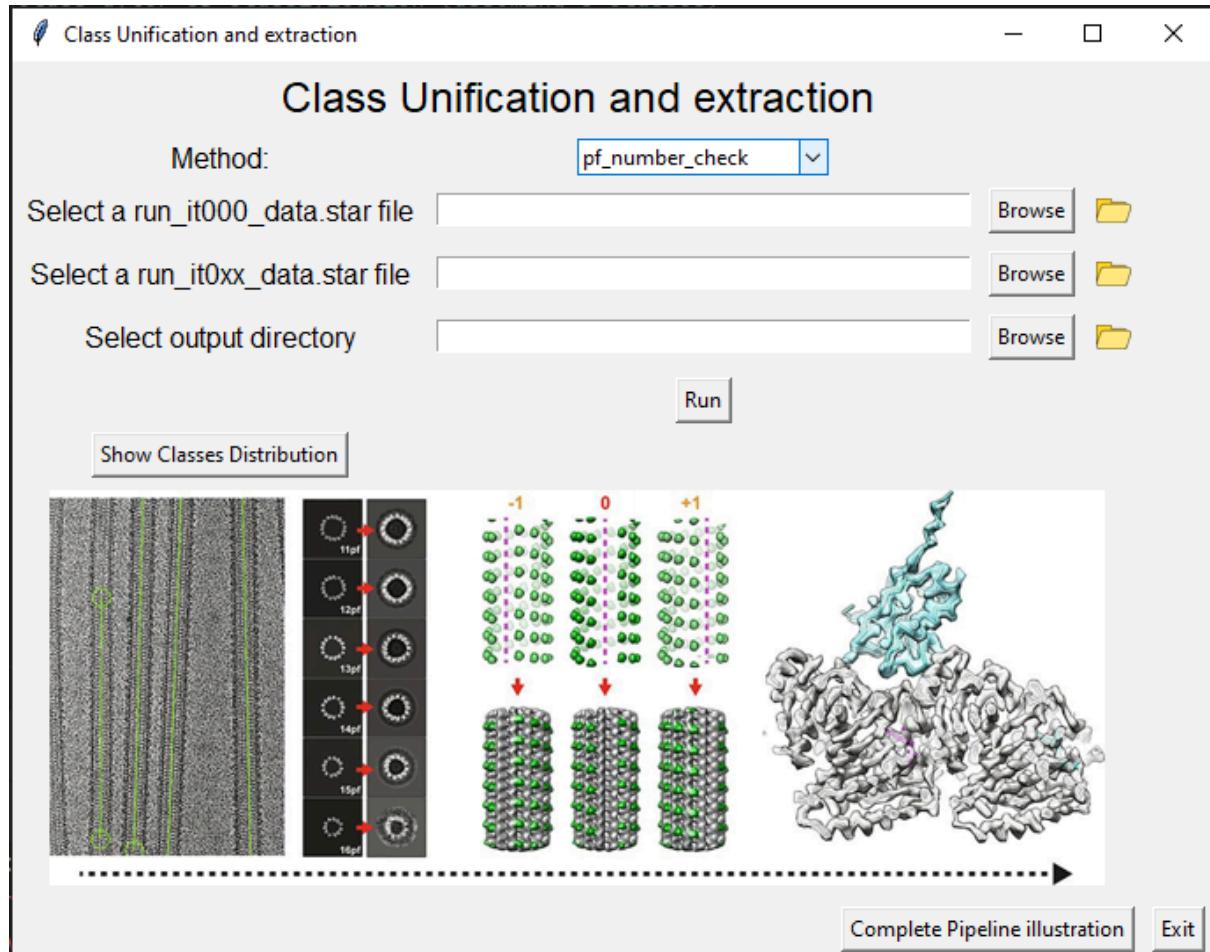
- d. Navigate to the folder 3_protofilament_number_sorting

```
cd 3_protofilament_number_sorting
```

- e. Run the command:

```
python class_unify_and_extract.py
```

- f. You should get a window like this:



- g. Select pf_number_check in the method
- h. Select run_it000_data.star file which is located in the Class3D folder from the previous step
- i. Select run_it0xx_data.star file which is located in the Class3D folder from the previous step (xx should be the highest number - meaning the last iteration)
- j. Select an output directory and press run
- k. This will produce six star files with particles belonging to each class in the desired output location.
- l. (optional) You can view the distribution of classes by clicking on the Show Classes Distribution button.

II.4 Initial seam assignment: Reference rescale, 1st 3D Auto-refine, angles and shifts reset, 2nd 3D Auto-refine, angle smoothing, reset shifts, 3rd 3D Auto-refine, shifts smoothing and 4th 3D Auto-refine.

II.4.1 Reference rescale:

For the next step you will need to choose the class that corresponds to a tubulin with 13 protofilaments (at the moment the pipeline is able to process only MT with 13 protofilaments). In the Class3D folder (of your successful job) you will have .mrc files of the different classes. You can open these files in Chimera or Fiji to look at the results directly.

- a. Go to the MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- b. Activate the environment

```
conda activate LG_MiRP
```

- c. Setup the environment

```
python setup.py develop
```

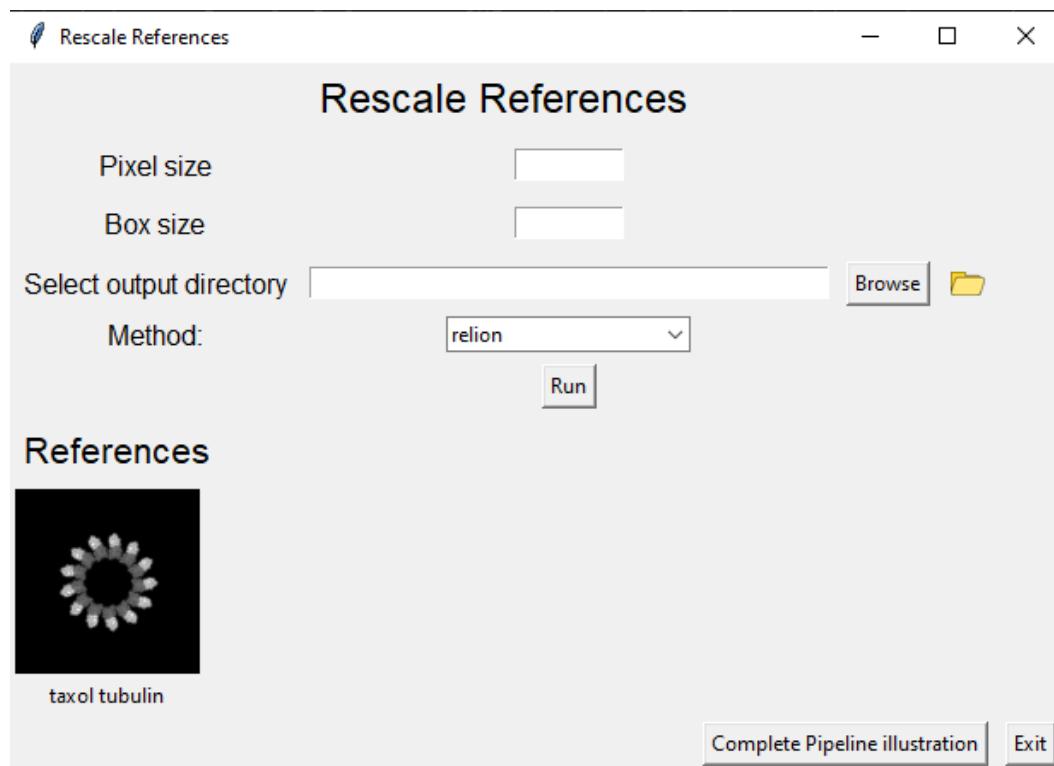
- d. Go to 4_initial_seam_assignment folder

```
cd 4_initial_seam_assignment
```

- e. Run rescale_references

```
python rescale_references.py
```

- f. You will get a window similar to the window bellow:



* The references below show references of kinesin bound to a microtubule with 13 protofilaments

- g. Enter the desired pixel size and the box size (according to the scaling of the images in the extract particles step, pixel size and box size can be found in the run_it0xx_data_class_x.star file)
- h. Select an output folder (if a folder named new_references already exists, it will delete the contents of this folder)
 - i. Select a method - relion or scipy
 - j. Press run
- k. The resulting file locations should be printed in the terminal

II.4.2 1st 3D Auto-refine:

- a. Deactivate conda

```
conda deactivate
```

- b. Change working directory to your project and run relion
- c.
- d.

II.4.3 Angles and shifts reset:

- a. Go to the MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- b. Activate the environment

```
conda activate LG_MiRP
```

- c. Setup the environment

```
python setup.py develop
```

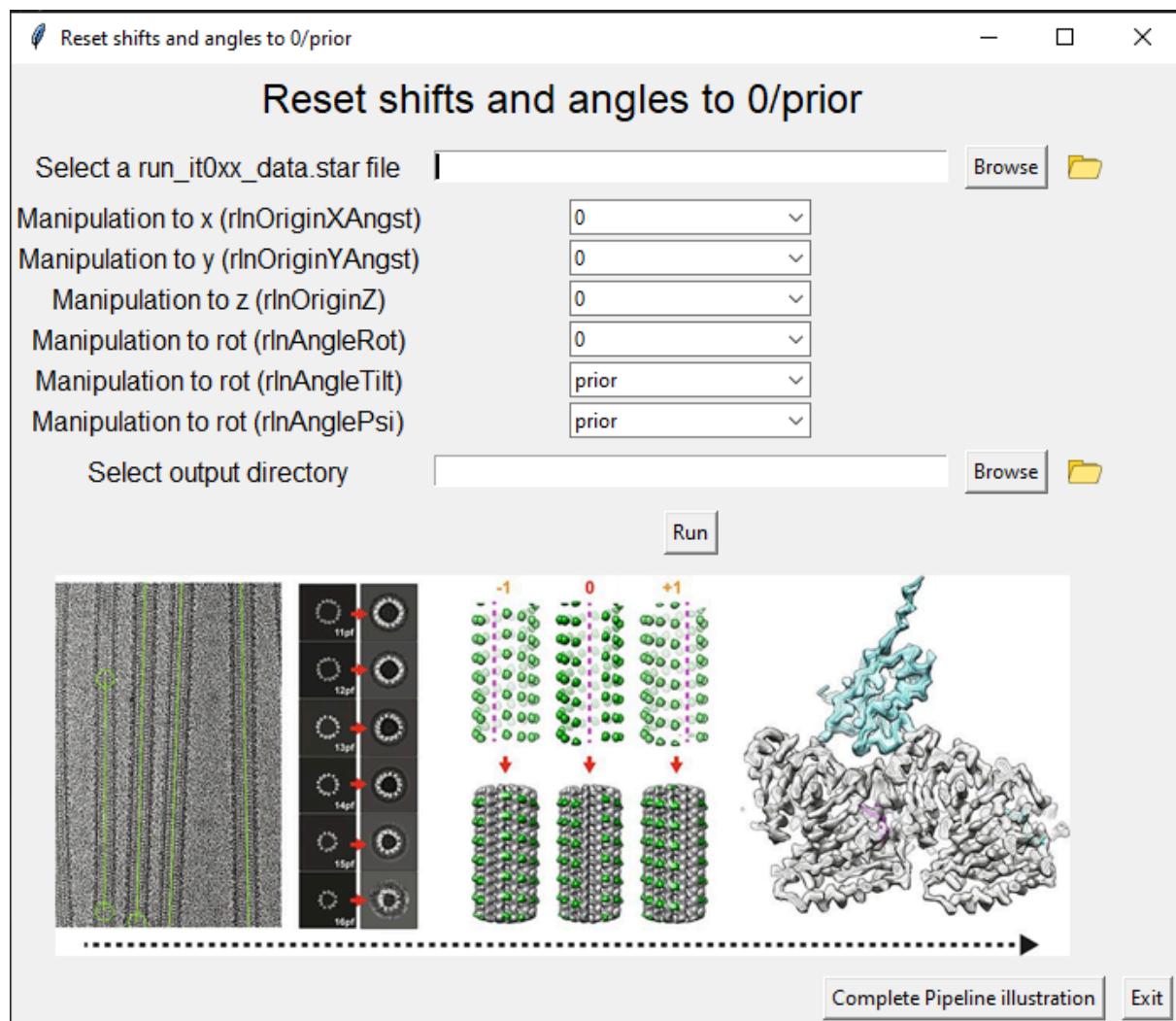
- d. Go to 4_initial_seam_assignment folder

```
cd 4_initial_seam_assignment
```

- e. Run reset_shifts_angles.py

```
python reset_shifts_angles.py
```

- f. You will get a window similar to the window bellow:



- g. Choose the last run_it0xx_data.star from the successful Auto-refine job from the previous step (located in Refine3D folder)
- h. Don't change the manipulation parameters
- i. Select an output folder
- j. Press the Run button.
- k. The resulting file location should be printed in the terminal

II.4.4 2nd 3D Auto-refine:

- a. Deactivate conda

```
conda deactivate
```

- b. Change working directory to your project and run relion

c.

d.

II.4.5 Angles smoothing:

- a. Go to the MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- b. Activate the environment

```
conda activate LG_MiRP
```

- c. Setup the environment

```
python setup.py develop
```

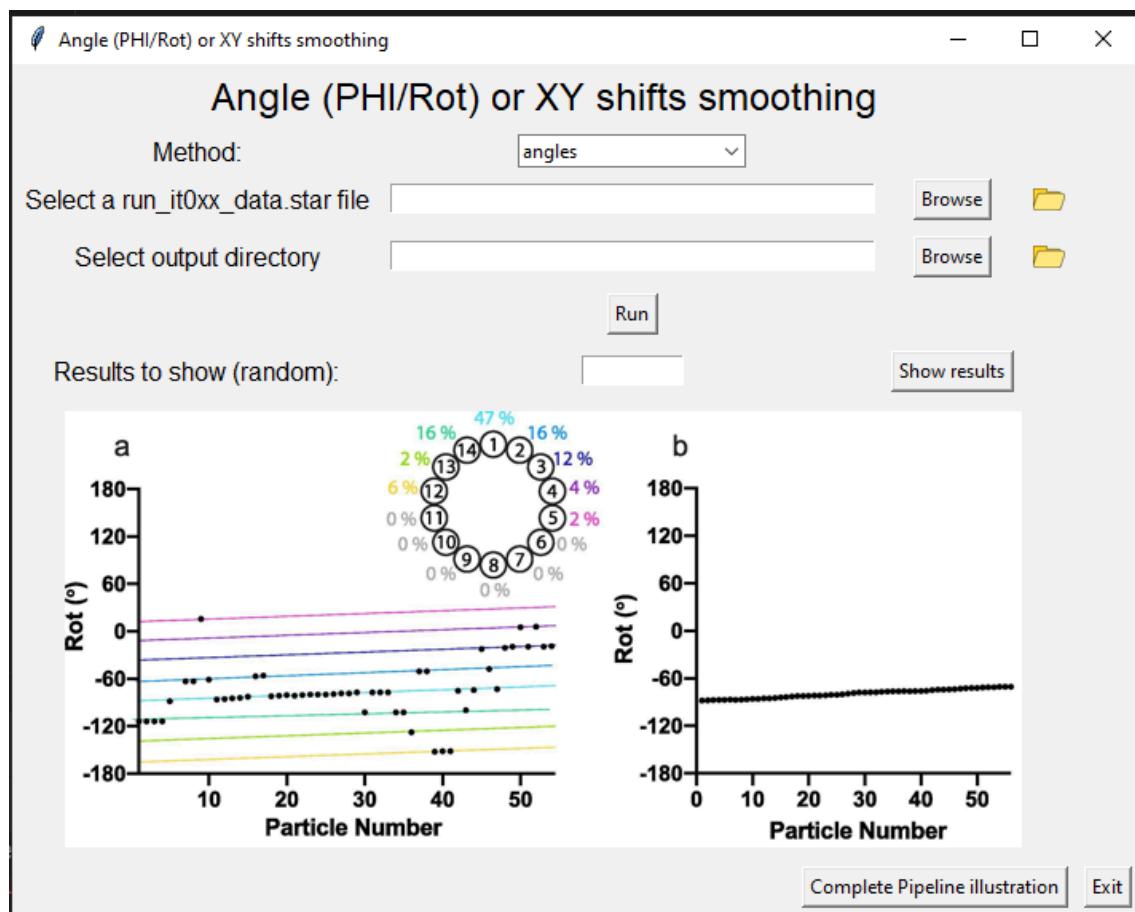
- d. Go to 4_initial_seam_assignment folder

```
cd 4_initial_seam_assignment
```

- e. Run reset_shifts_angles.py

```
python reset_shifts_angles.py
```

- f. You will get a window similar to the window bellow:



- g. In method choose angles
- h. Choose the last run_it0xx_data.star from the successful Auto-refine job from the previous step (located in Refine3D folder)
- i. Select an output folder
- j. Press the Run button.
- k. The resulting file location should be printed in the terminal
- l. (optional) Select a number of random plots to show (The Rot/Phi angle should look smooth line in the image at the bottom of the GUI)

II.4.6 Keep angles (Rot/Phi), reset shifts:

- a. Go to the MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- b. Activate the environment

```
conda activate LG_MiRP
```

- c. Setup the environment

```
python setup.py develop
```

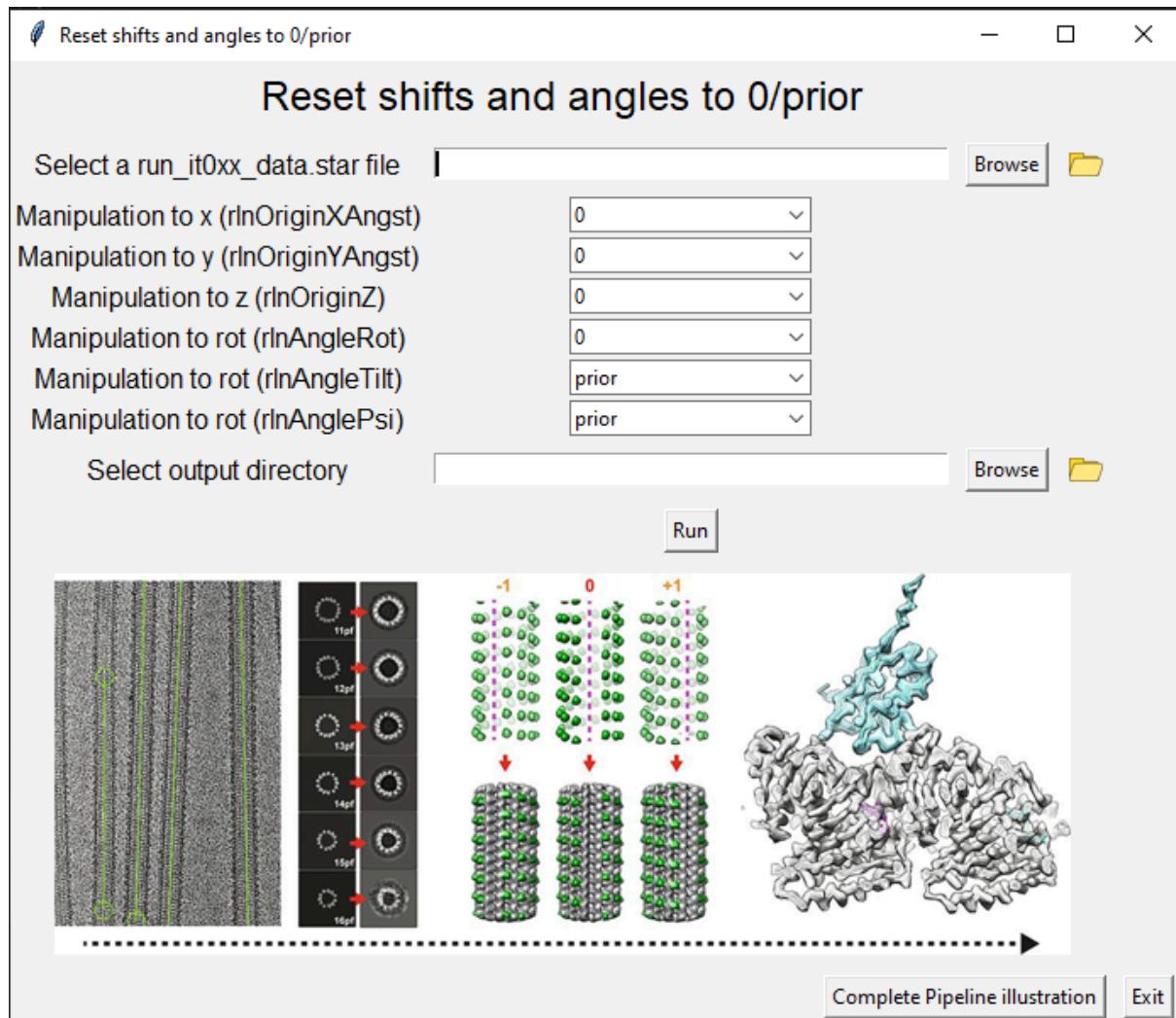
- d. Go to 4_initial_seam_assignment folder

```
cd 4_initial_seam_assignment
```

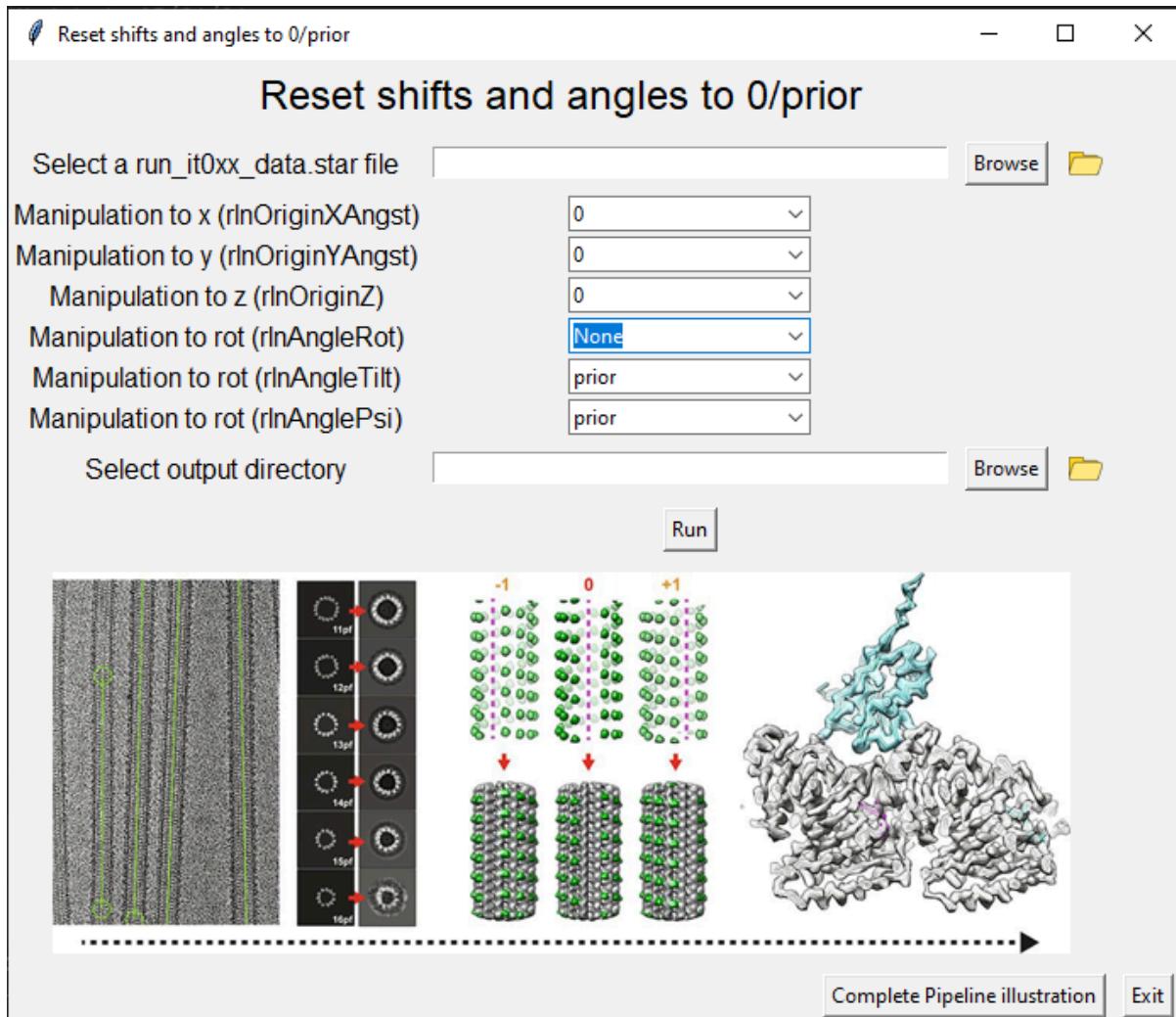
- e. Run reset_shifts_angles.py

```
python reset_shifts_angles.py
```

- f. You will get a window similar to the window bellow:



- g. Choose the last run_it0xx_data.star from the successful Auto-refine job from the previous step (located in Refine3D folder)
- h. Change the rot (rlnAngleRot) to None like this:



- i. Select an output folder
- j. Press the Run button.
- k. The resulting file location should be printed in the terminal

II.4.7 3rd 3D Auto-refine:

- a. Deactivate conda

```
conda deactivate
```

- b. Change working directory to your project and run relion

c.

d.

II.4.8 Angles smoothing:

- a. Go to the MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- b. Activate the environment

```
conda activate LG_MiRP
```

- c. Setup the environment

```
python setup.py develop
```

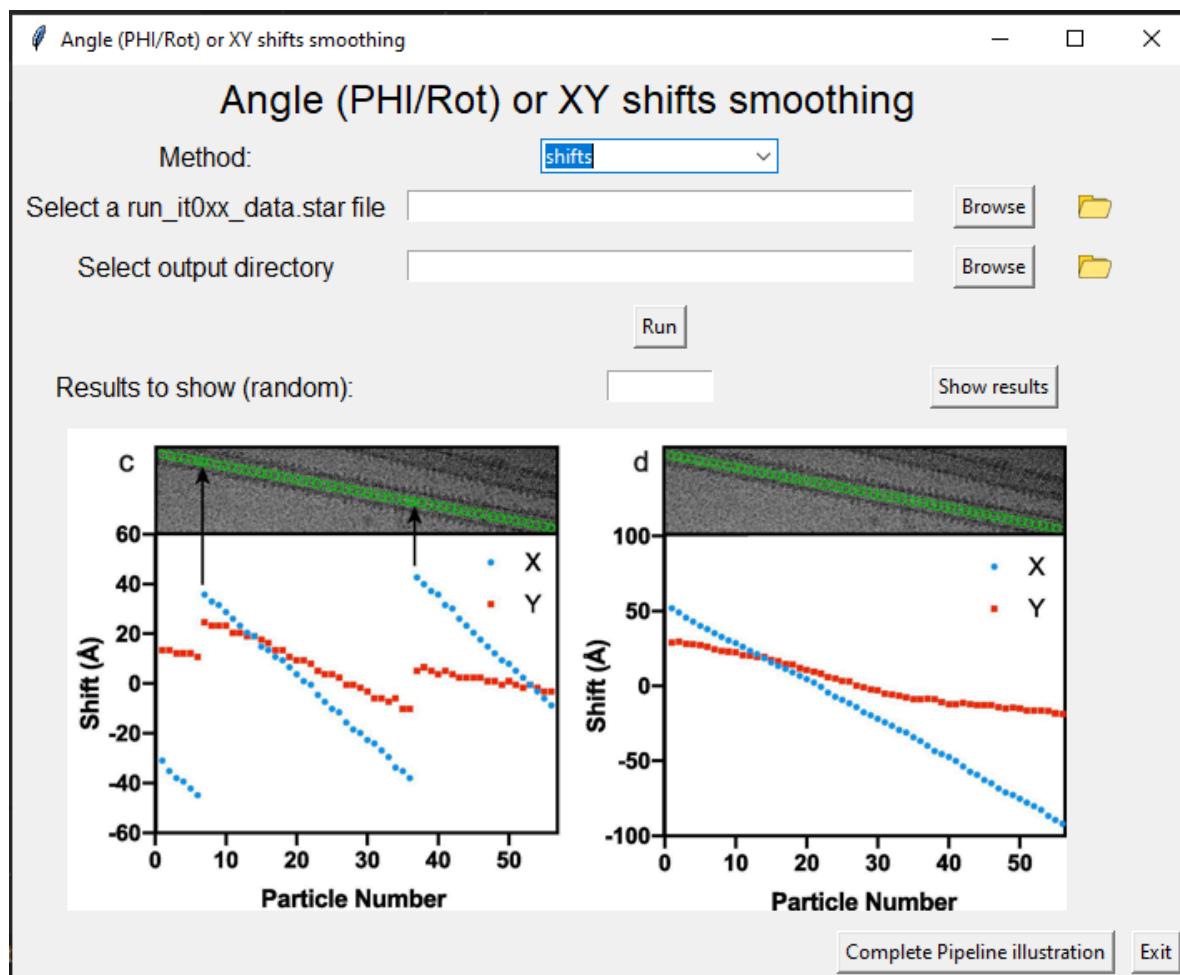
- d. Go to 4_initial_seam_assignment folder

```
cd 4_initial_seam_assignment
```

- e. Run reset_shifts_angles.py

```
python reset_shifts_angles.py
```

- f. You will get a window similar to the window bellow:



- g. In method choose shifts
- m. Choose the last run_it0xx_data.star from the successful Auto-refine job from the previous step (located in Refine3D folder)
- n. Select an output folder
- o. Press the Run button.
- p. The resulting file location should be printed in the terminal
- q. (optional) Select a number of random plots to show (The X/Y shifts should look like smooth lines like in the image at the bottom of the GUI)

II.4.9 4th 3D Auto-refine:

- a. Deactivate conda

```
conda deactivate
```

- b. Change working directory to your project and run relion
- c.
- d.

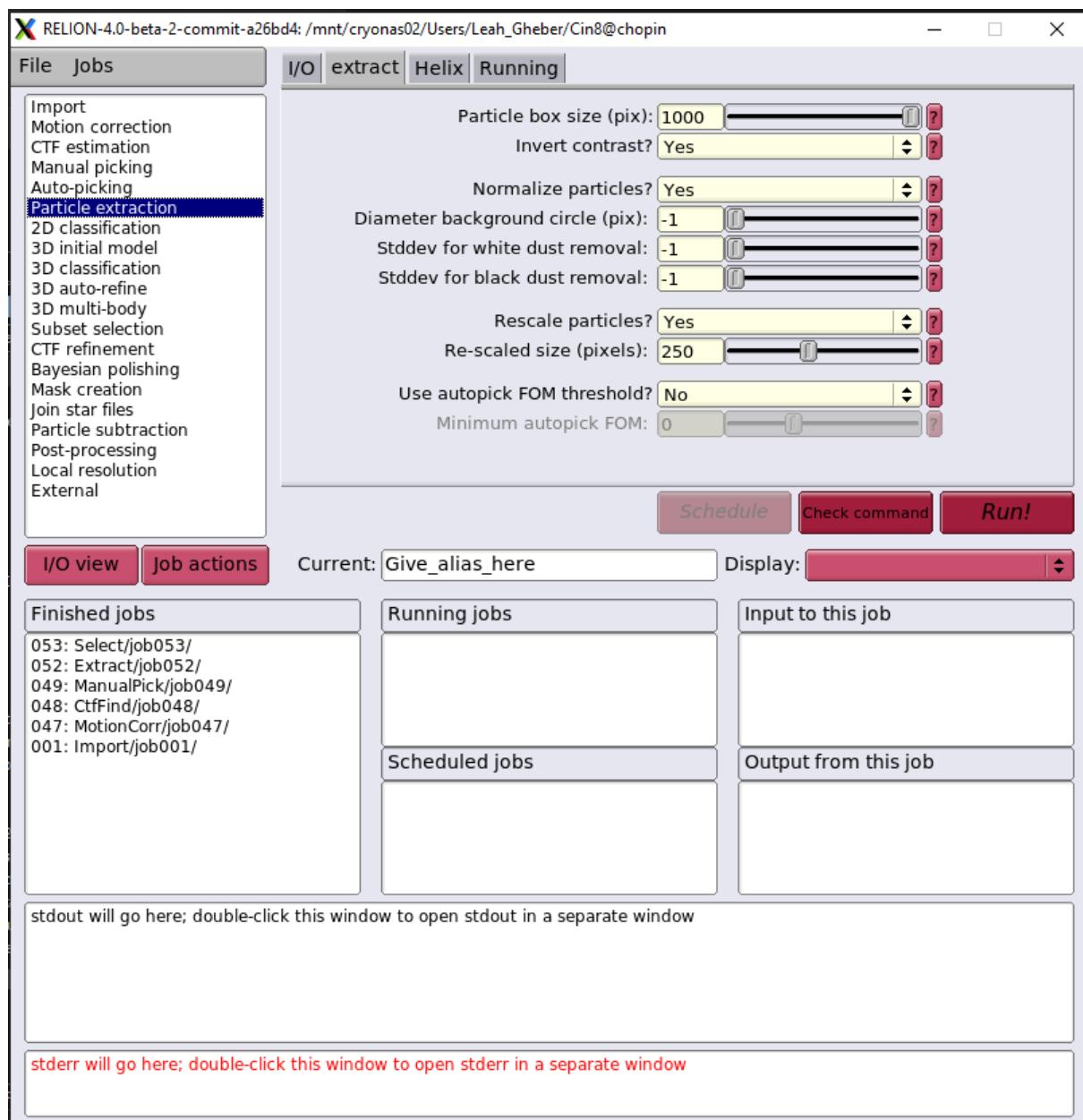
II.5 Seam check: Re-extract particles, Reference rescale, Generate mask, final 3D Auto-refine.

II.5.1 Re-extract particles:

- a. Deactivate conda

```
conda deactivate
```

- b. Change working directory to your project and run relion
- c. Select Particle extraction



- d. In the I/O tab select re-extract particles
- e. As a “Refined particles STAR, select the output .STAR file from the previous step.
- f. In the extract tab set “Rescale particles?” as No
- g.

II.5.2 Reference rescale:

- a. Go to the MiRP directory (for example)

```
cd /home/alinag/MiRP
```

- b. Activate the environment

```
conda activate LG_MiRP
```

- c. Setup the environment

```
python setup.py develop
```

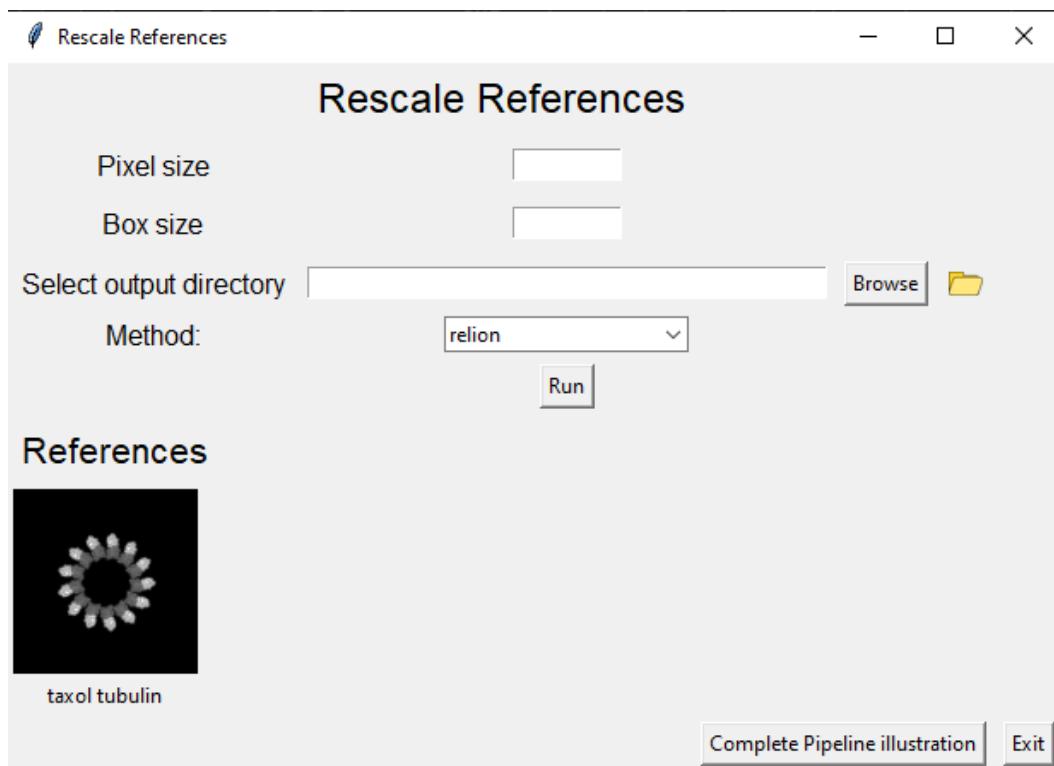
- d. Go to 5_seam_check folder

```
cd 5_seam_check
```

- e. Run rescale_references

```
python rescale_references.py
```

- f. You will get a window similar to the window bellow (but with a lot more references - 26 to be exact):



* The references below show references of microtubules with 13 protofilaments at different orientations of the seam.

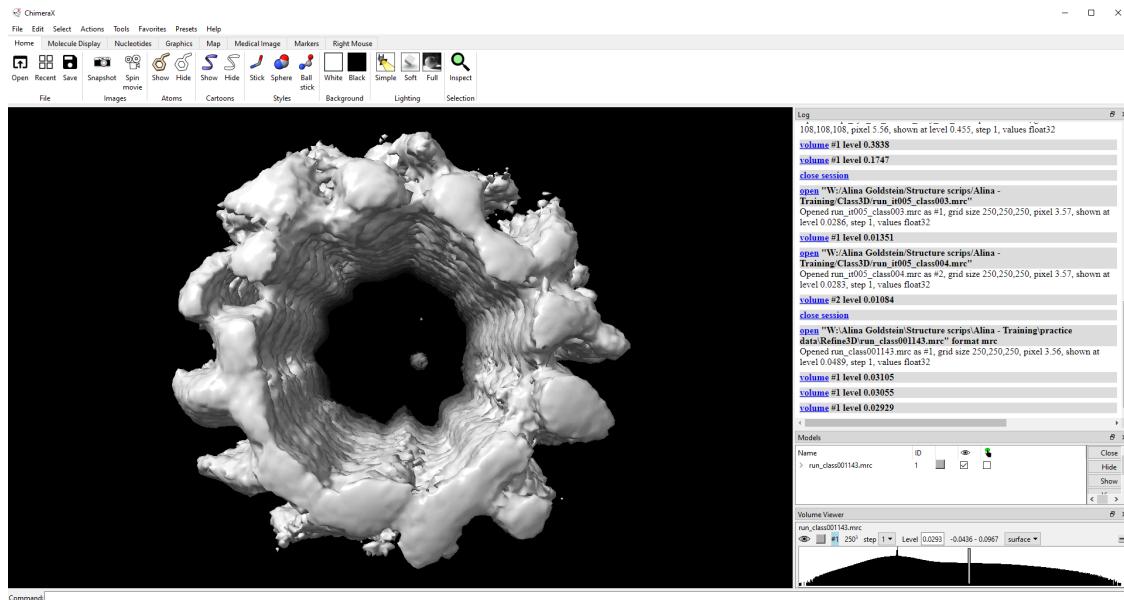
- g. Enter the desired pixel size and the box size (according to the scaling of the images in the extract particles step, pixel size and box size can be found in the re-extracted particles.star file)

- h. Select an output folder (if a folder named new_references already exists, it will delete the contents of this folder)
- i. Select a method - relion or scipy
- j. Press run
- k. The resulting file locations should be printed in the terminal

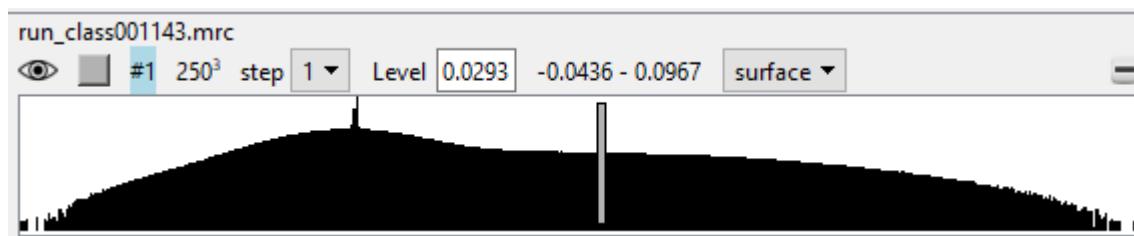
II.5.3 Create a mask:

For the next step you will need to set the mask threshold. To do so, copy the output run_class001.mrc from the 4th auto-refine job to your computer (you can do it by dragging it from MobaXterm to your computer, and open it in ChimeraX.

It will look something like this:



On the bottom right, there is a slider named Volume Viewer. Set a threshold that looks like a MT with attached kinesin without too much noise and look at the Level value:



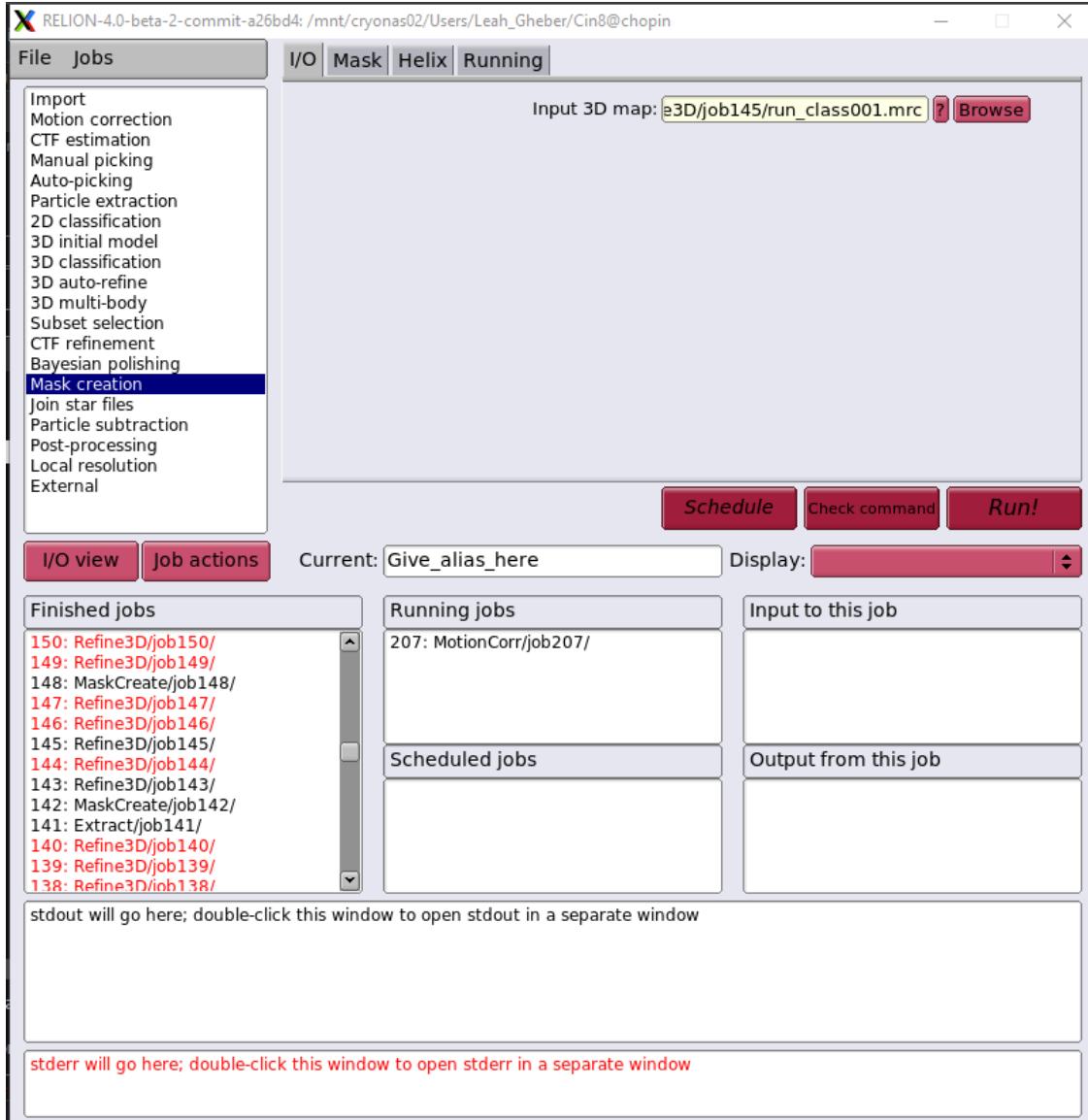
Set the threshold in RELION according to the level (in this example I'll use 0.03 as a threshold)

- Deactivate conda

```
conda deactivate
```

- Change working directory to your project and run relion

- Select Mask creation job



- In the I/O tab, select the output run_class001.mrc file
- In the Mask tab set the “Initial binarisation threshold” the threshold level you choose in ChimeraX
- In the Helix tab, set “Mask a 3D helix?” as No
- Press the “Run!” button

II.5.4 Final (5th) 3D Auto-refine:

- a. Deactivate conda

```
conda deactivate
```

- b. Change working directory to your project and run relion
- c.
- d.

Protofilament refinement