

# Kinesin-5 cryoEM density pipeline

## Requirements:

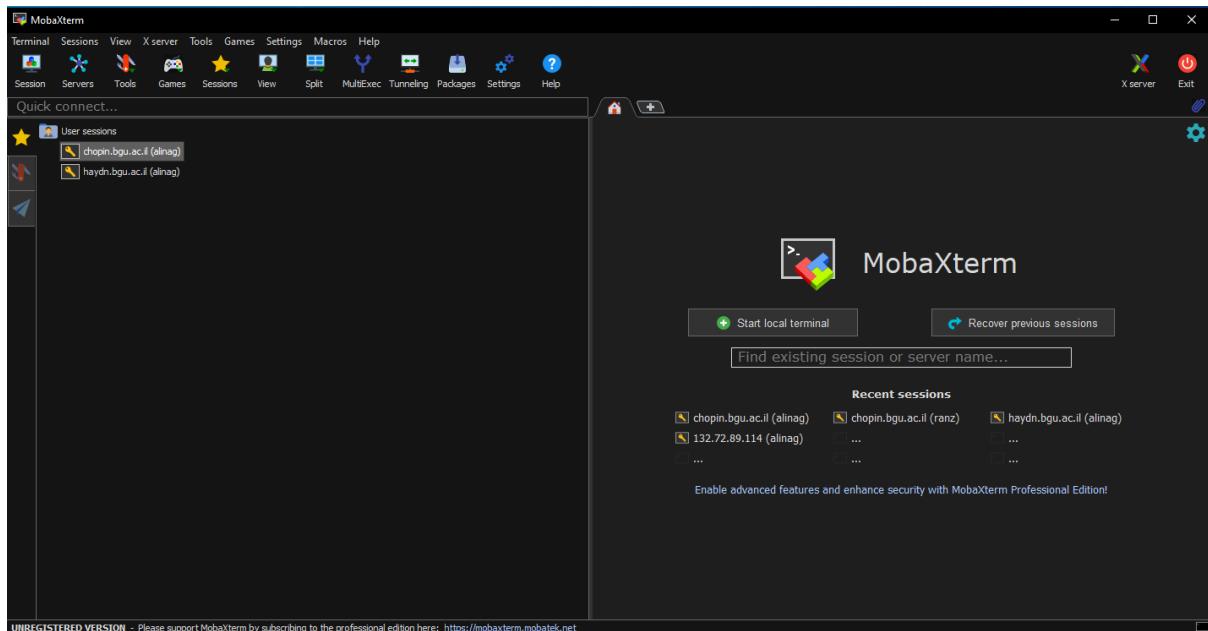
1. User and password for Chopin computer ([chopin.bgu.ac.il](mailto:chopin.bgu.ac.il)) - contact [barouchy@bgu.ac.il](mailto:barouchy@bgu.ac.il)
2. If accessing Chopin from a computer within the campus, also you will need to have connection approval (אישור חיבור) between your computer and Chopin (Get your IP address and contact [eran@bgu.ac.il](mailto:eran@bgu.ac.il) or [Infosec@bgu.ac.il](mailto: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:

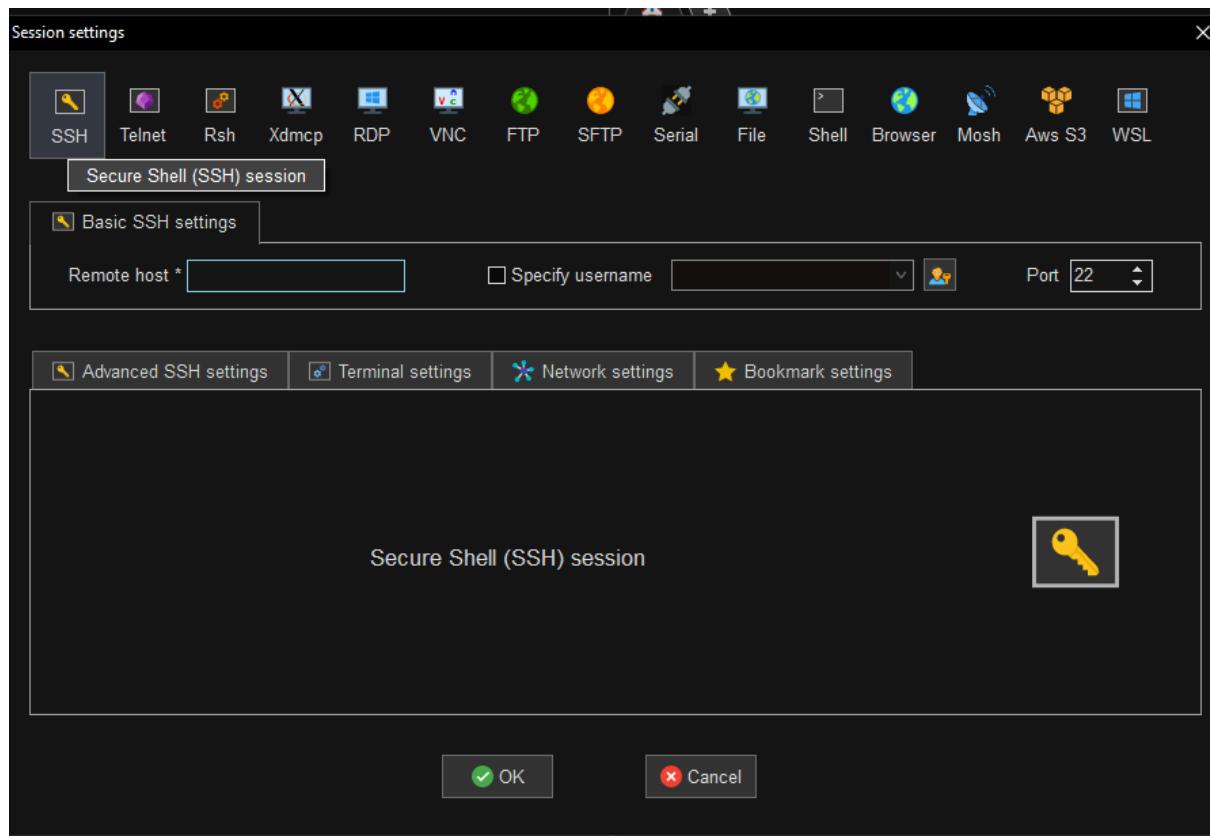
1. Refine a map containing MTs + kinesin
2. Protofilament refinement using Garret's Python code.
3. Refine kinesin only map

## MobaXterm:

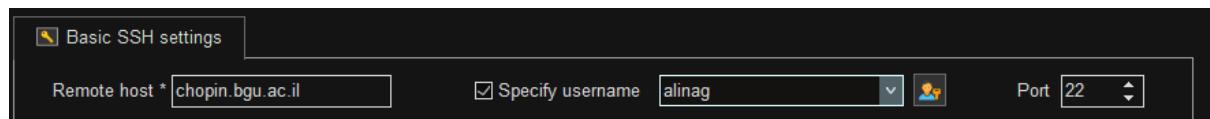
- a. To access chopin download and install MobaXterm



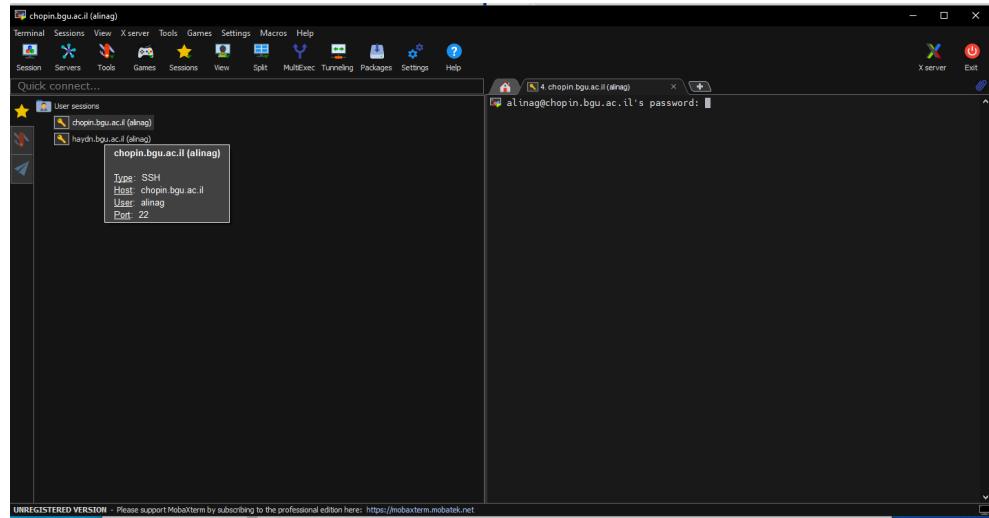
- b. To create a session, click on Session (top left button) and choose SSH



- 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-Li  
nux-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 below) FROM THE FOLDER CONTAINING YOUR DATA**

#### **II.1.1 Open RELION:**

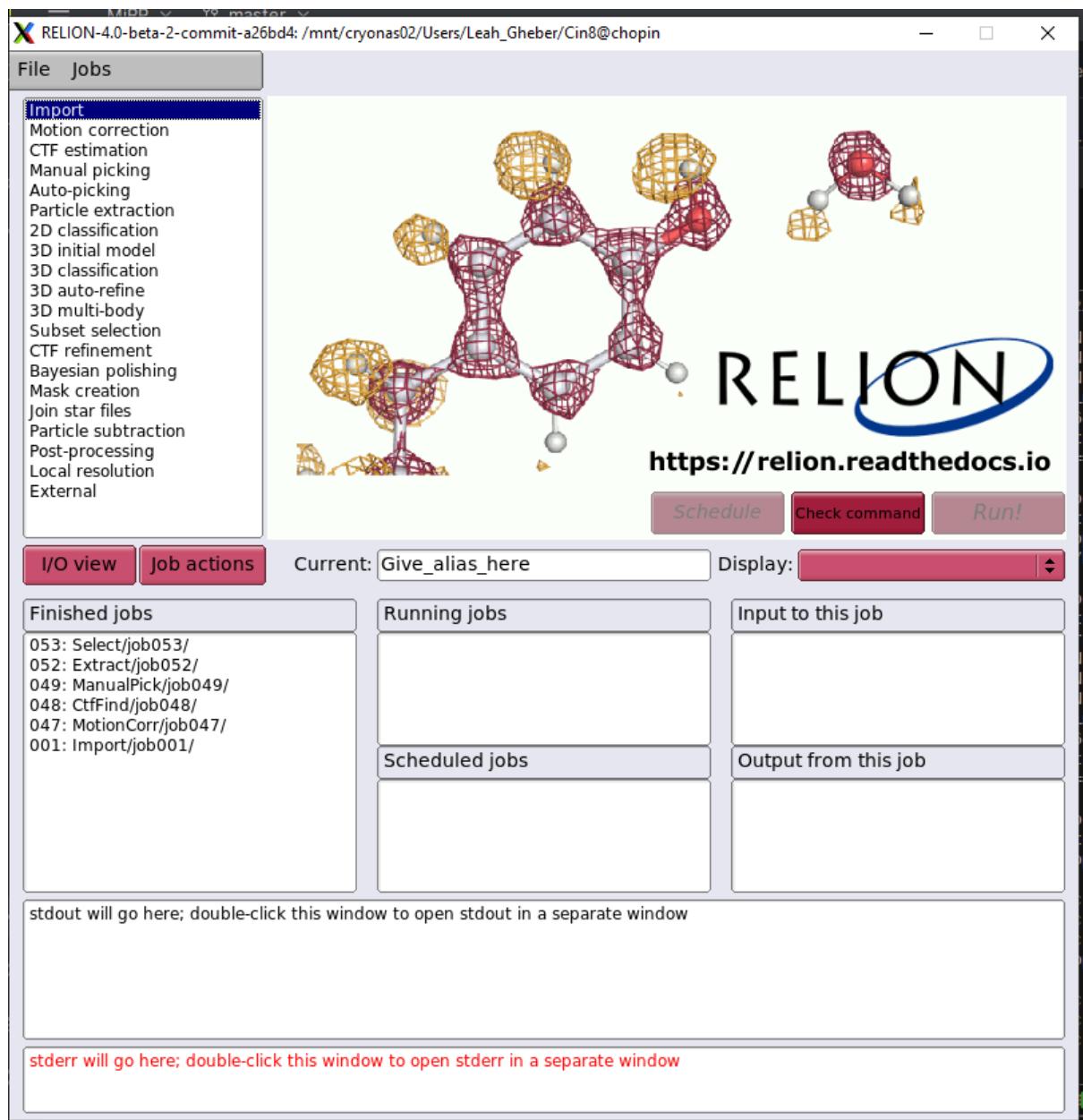
- a. Run the command from the folder containing the data:

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

Or

```
relion
```

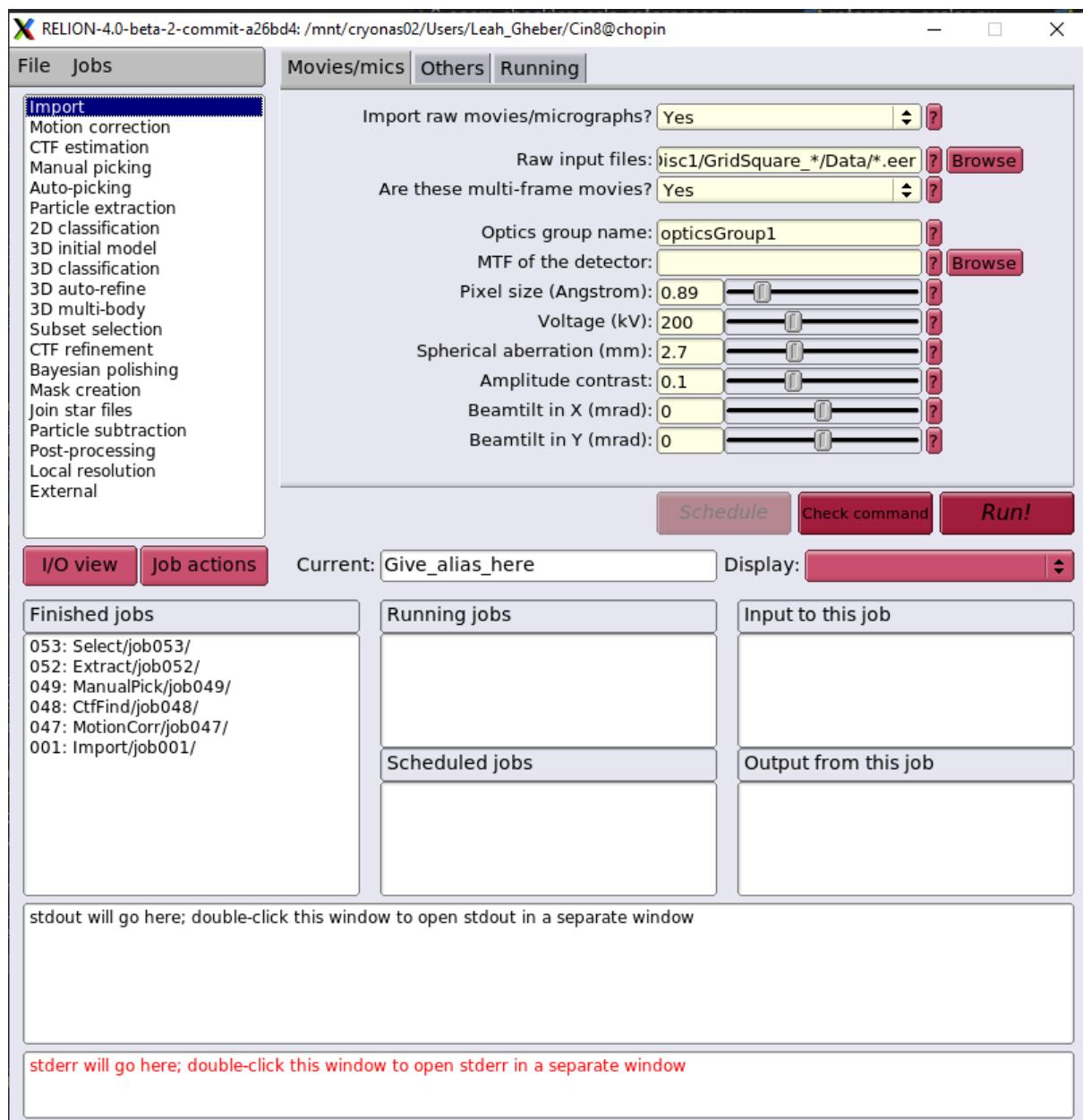
The RELION interface (GUI) will appear



\* 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:**

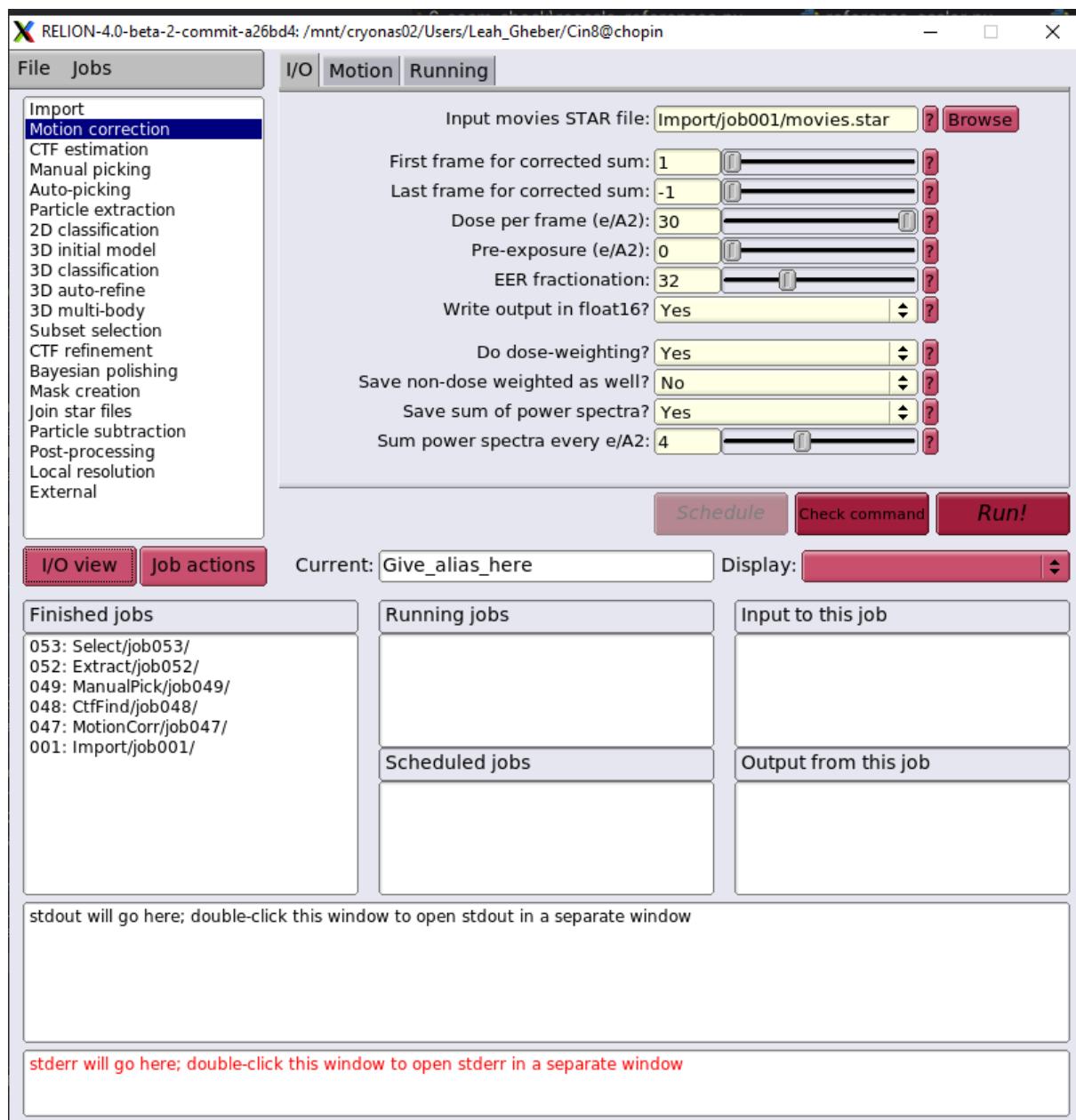
- a. Select import from the job menu in the left menu in RELION



\* Use syntax \* to import all the relevant images

### **II.1.3 Motion correction:**

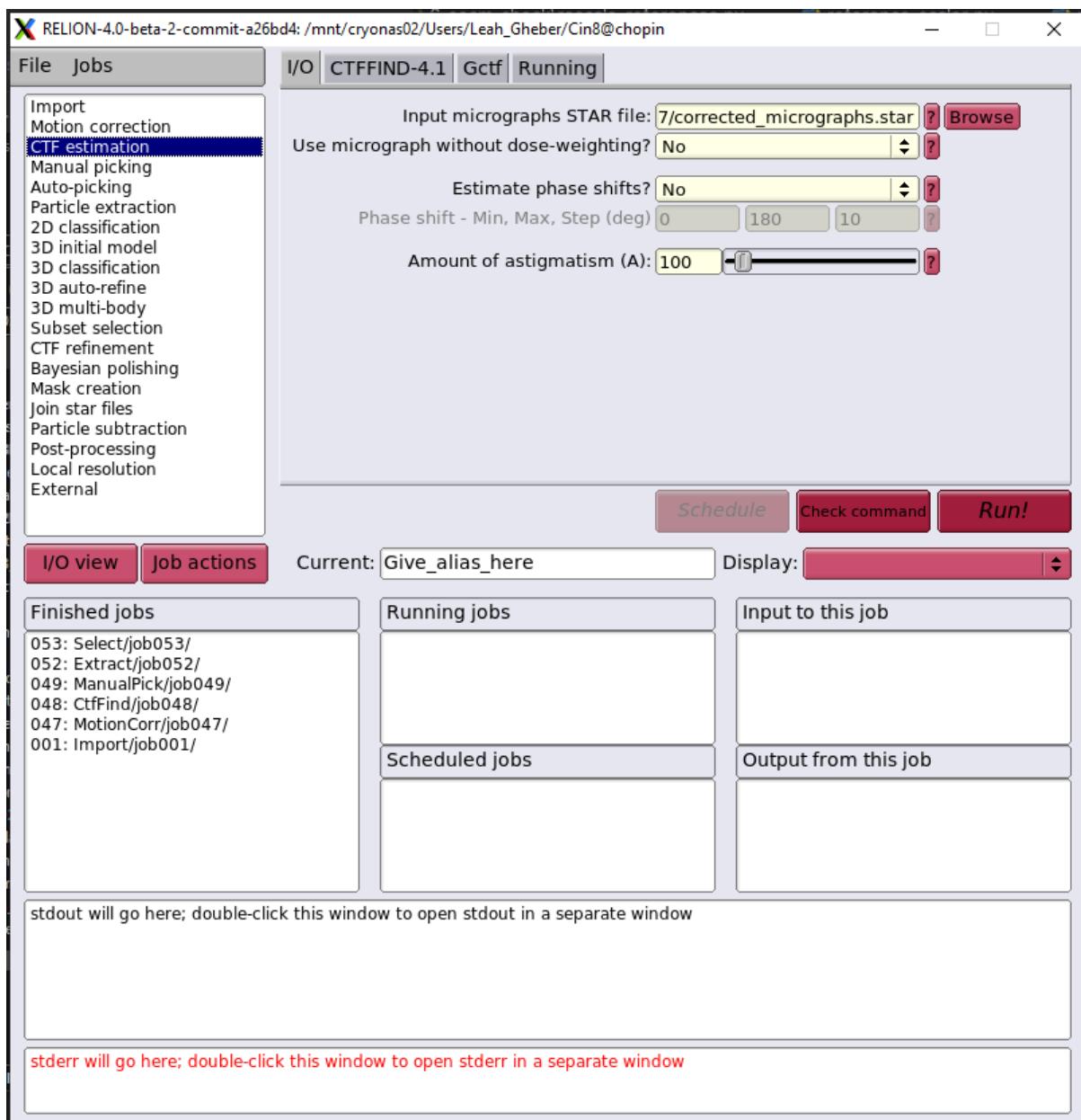
- Select Motion correction



- 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

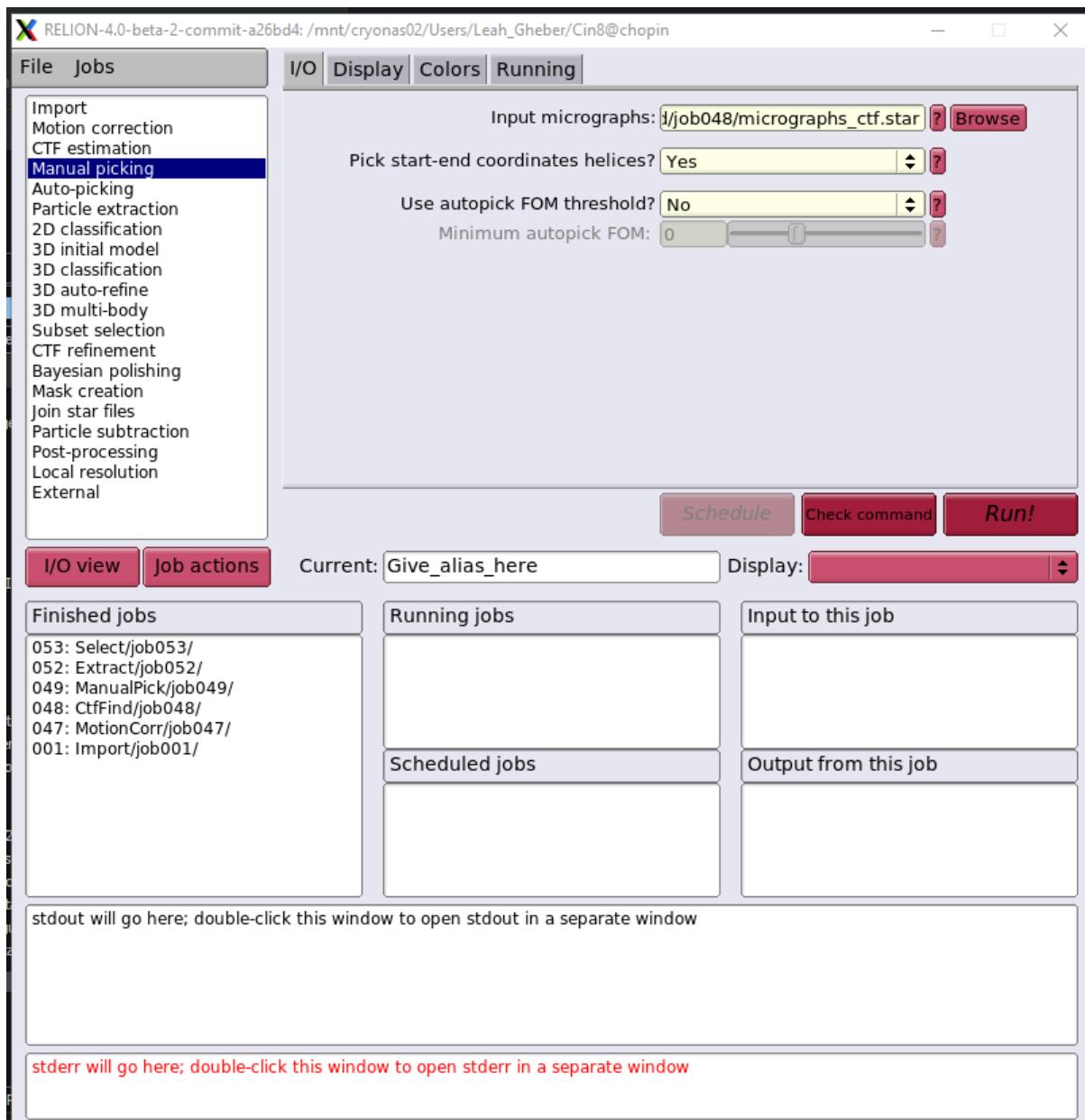


- The CTF executable is located in /home/ctffind/bin/ctffind
- You will also need more information from Ran
- This step will take around 8 hrs

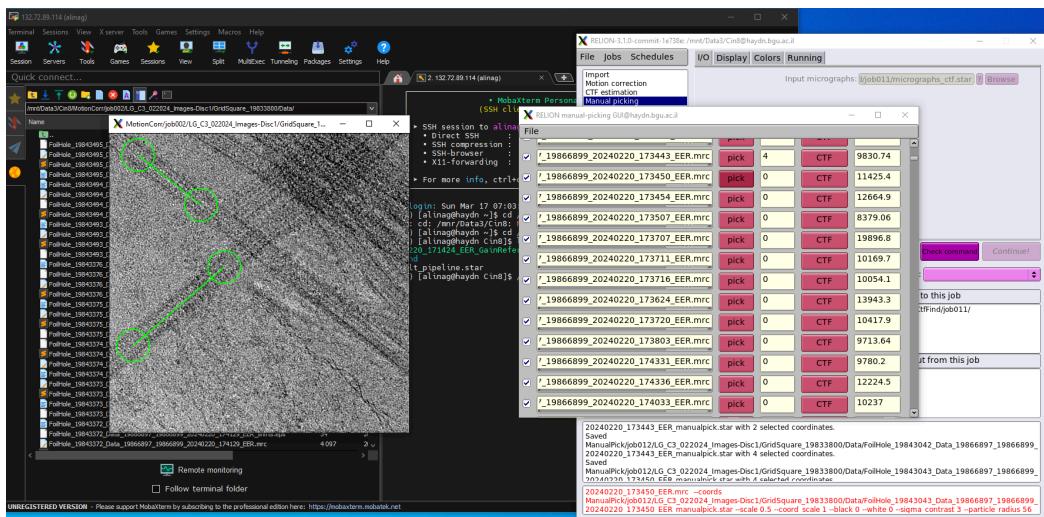
## **II.2 Particle picking, extraction and subset selection**

### **II.2.1 Manual picking:**

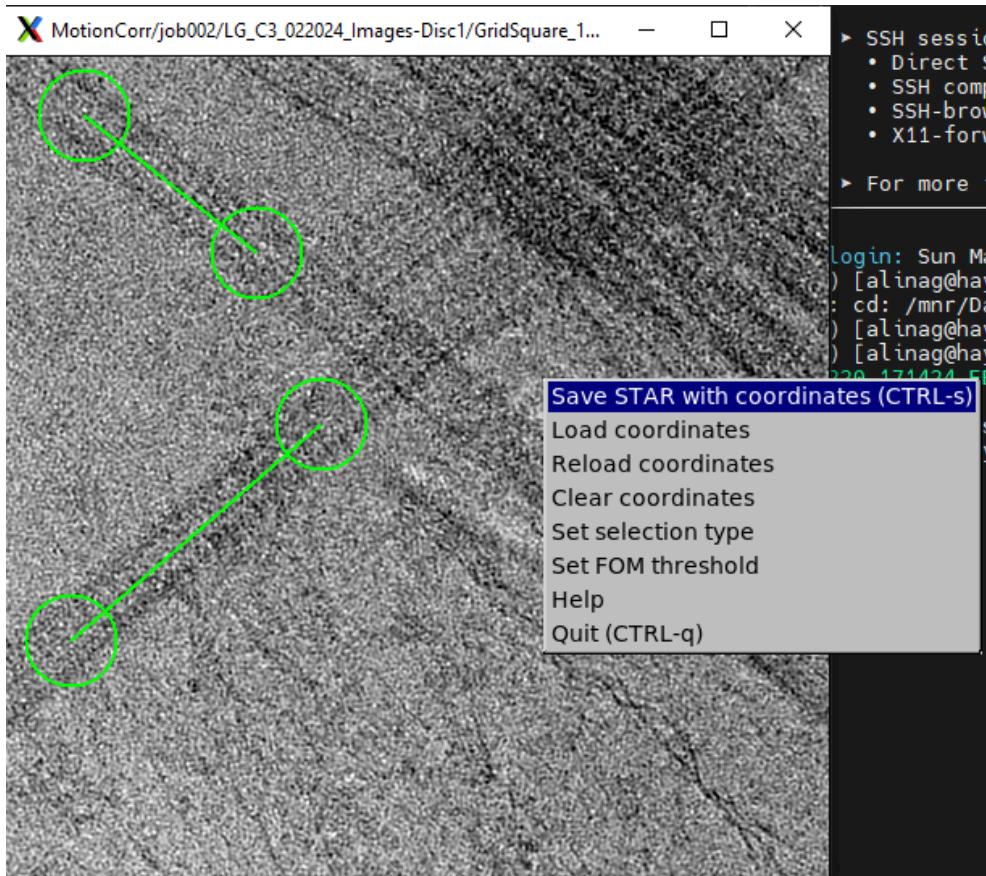
- Select Manual picking



- 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.)



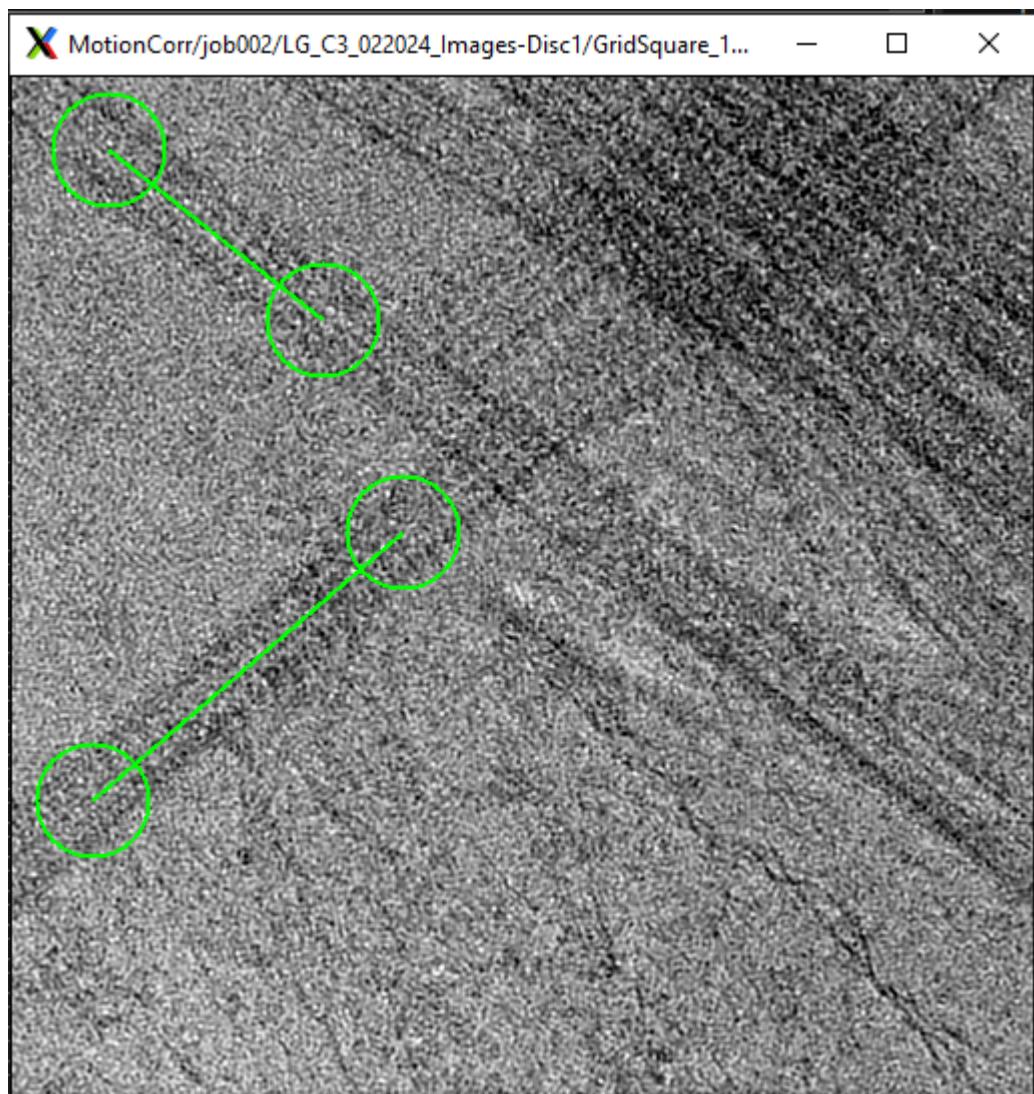
- e. To save the particles press **Ctrl+s** or right click with the mouse and select **Save STAR with coordinates**



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



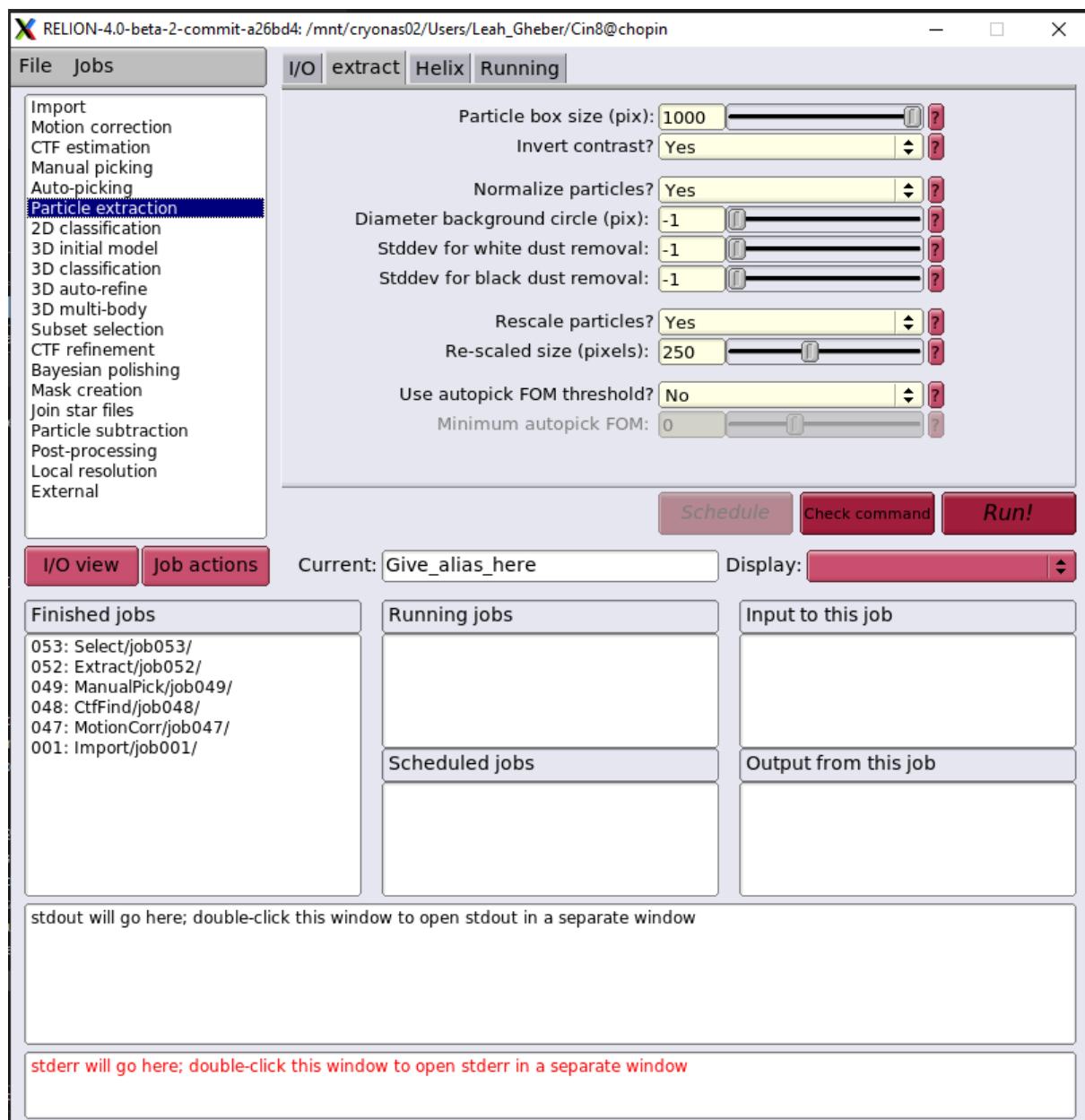
- f. Make sure not to select overlapping MTs or sections with poor quality:  
Example:



g. sdf

### **II.2.2 Particle extraction:**

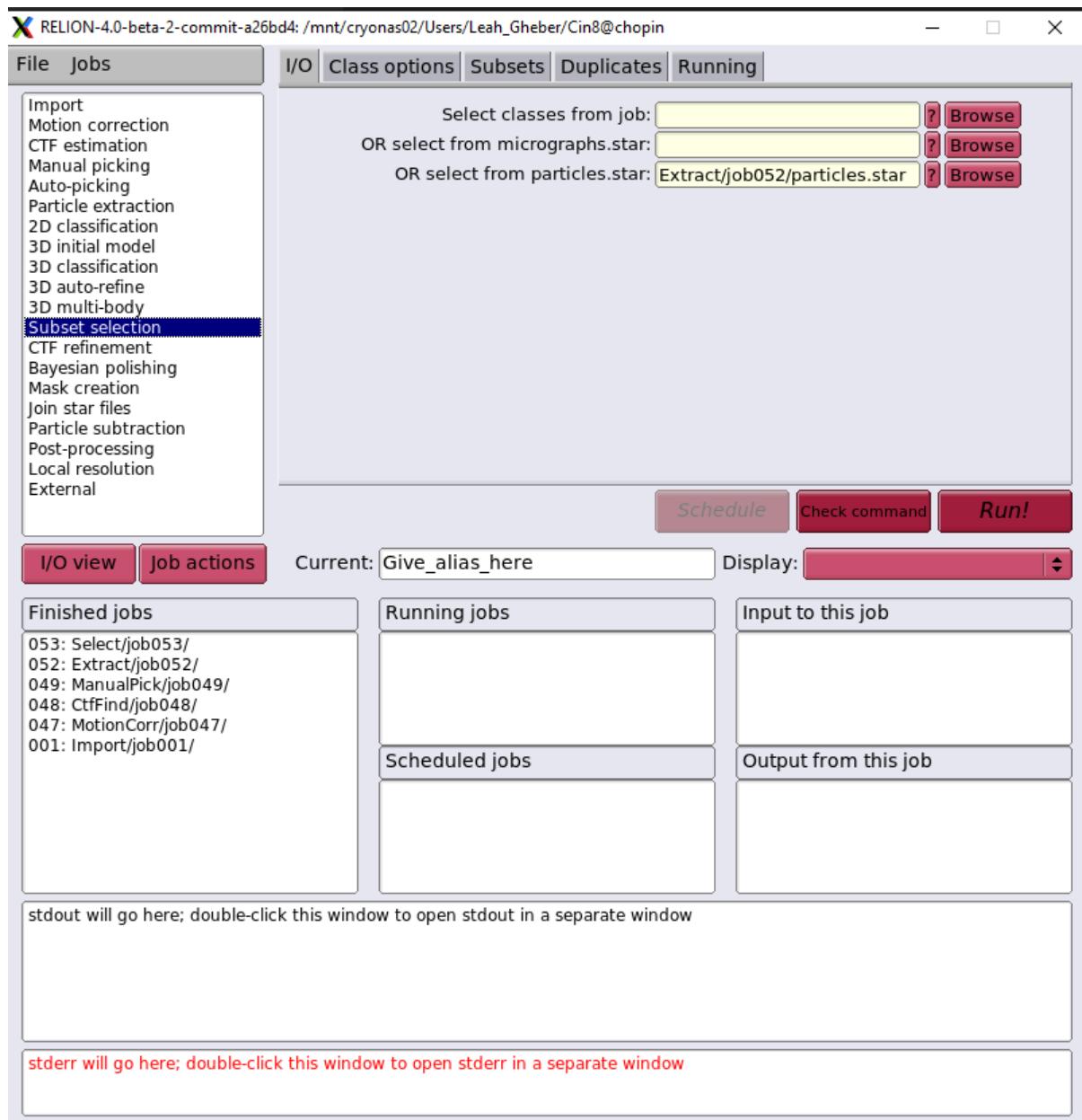
- a. Select Particle extraction



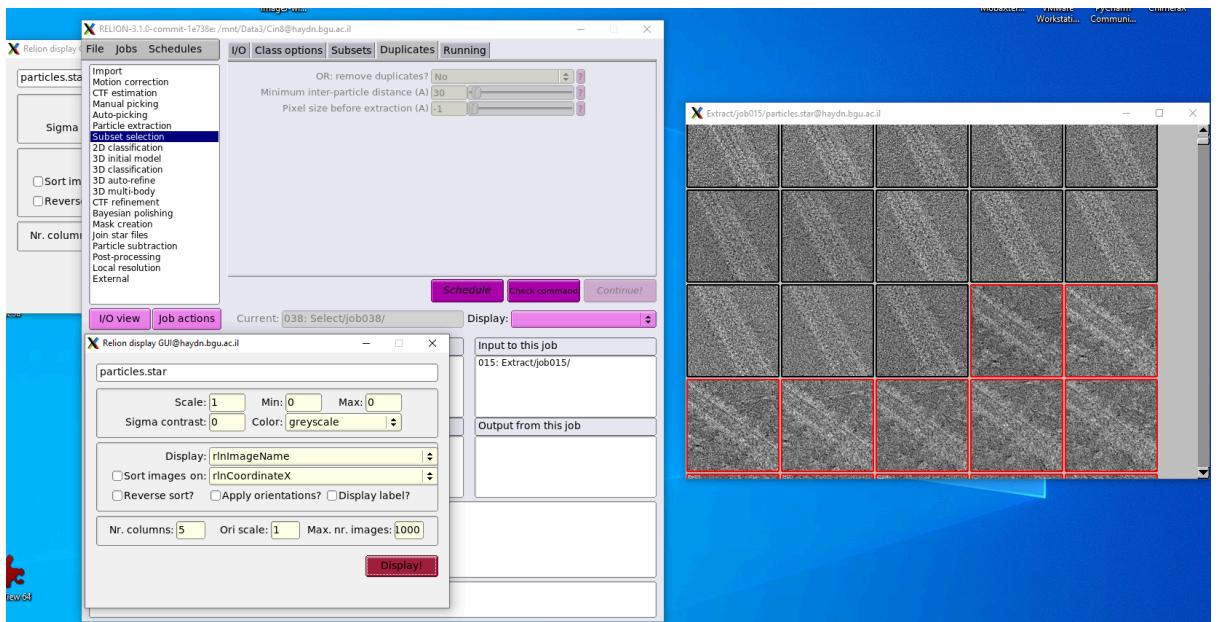
- b. The most important parameters are particle box size and rescaled size - this will determine the binning

### **II.2.3 Subset selection:**

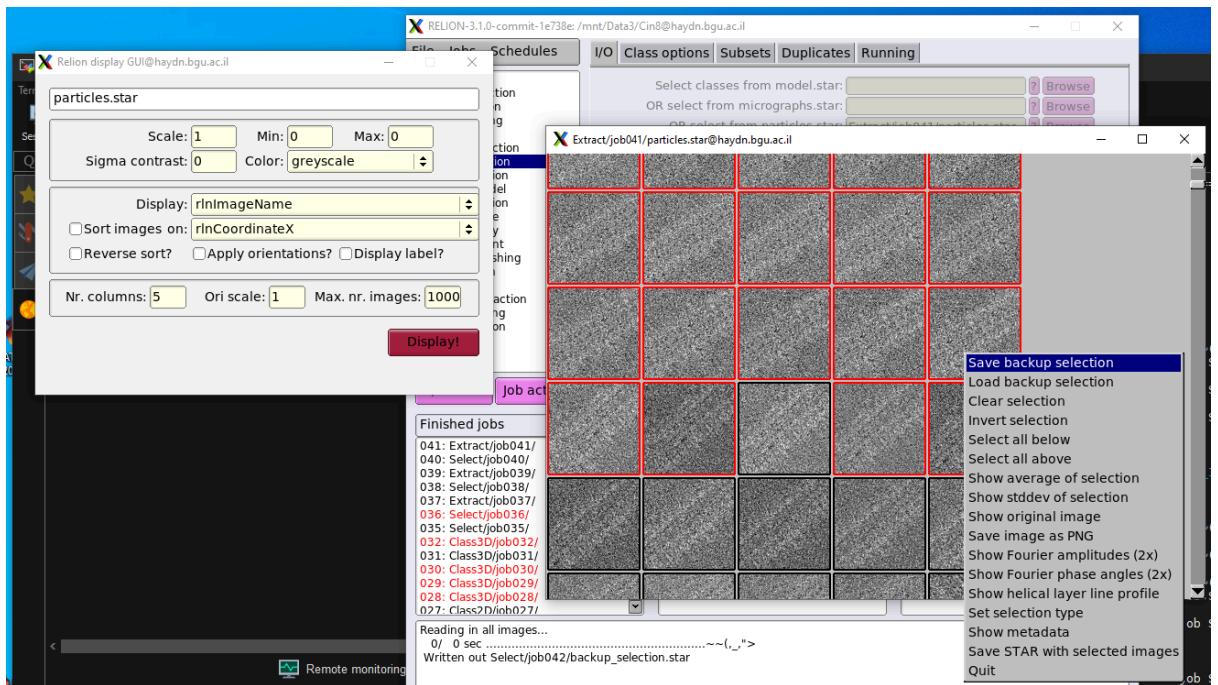
- a. Select Subset selection



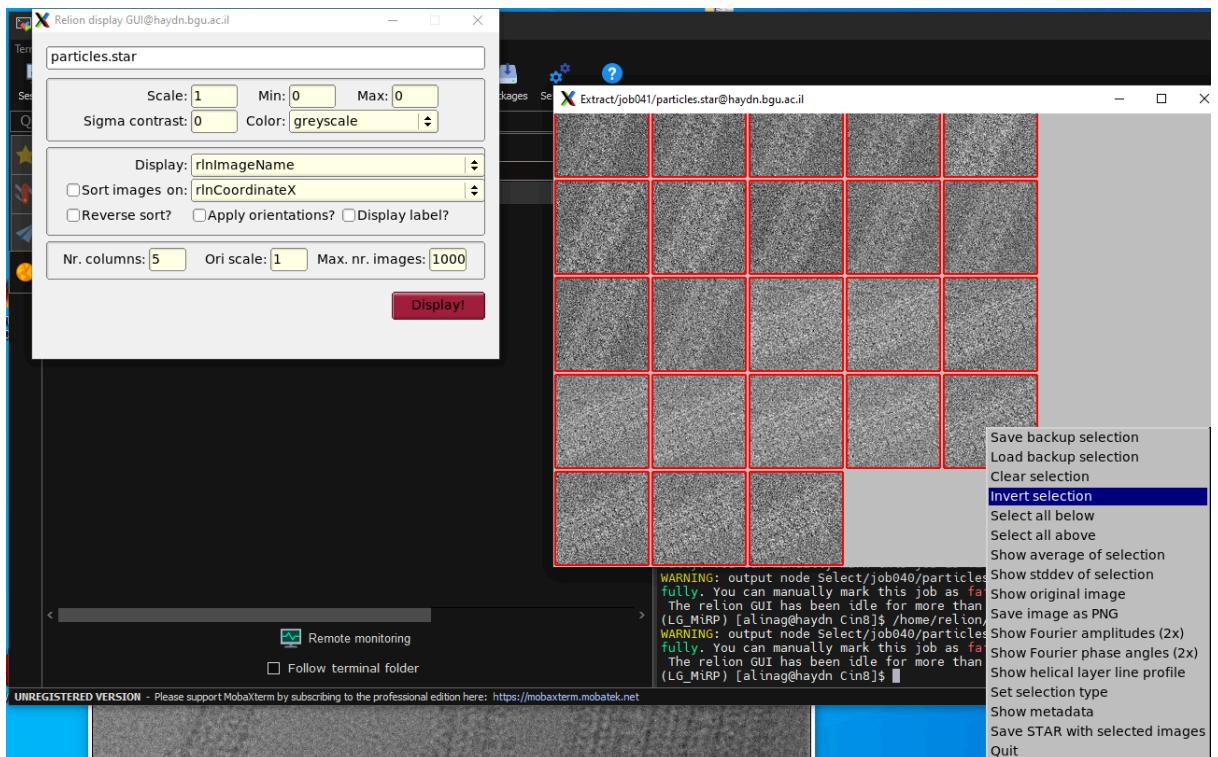
- b. Use the particles.star file from Particle extraction
- c. Start selecting particles



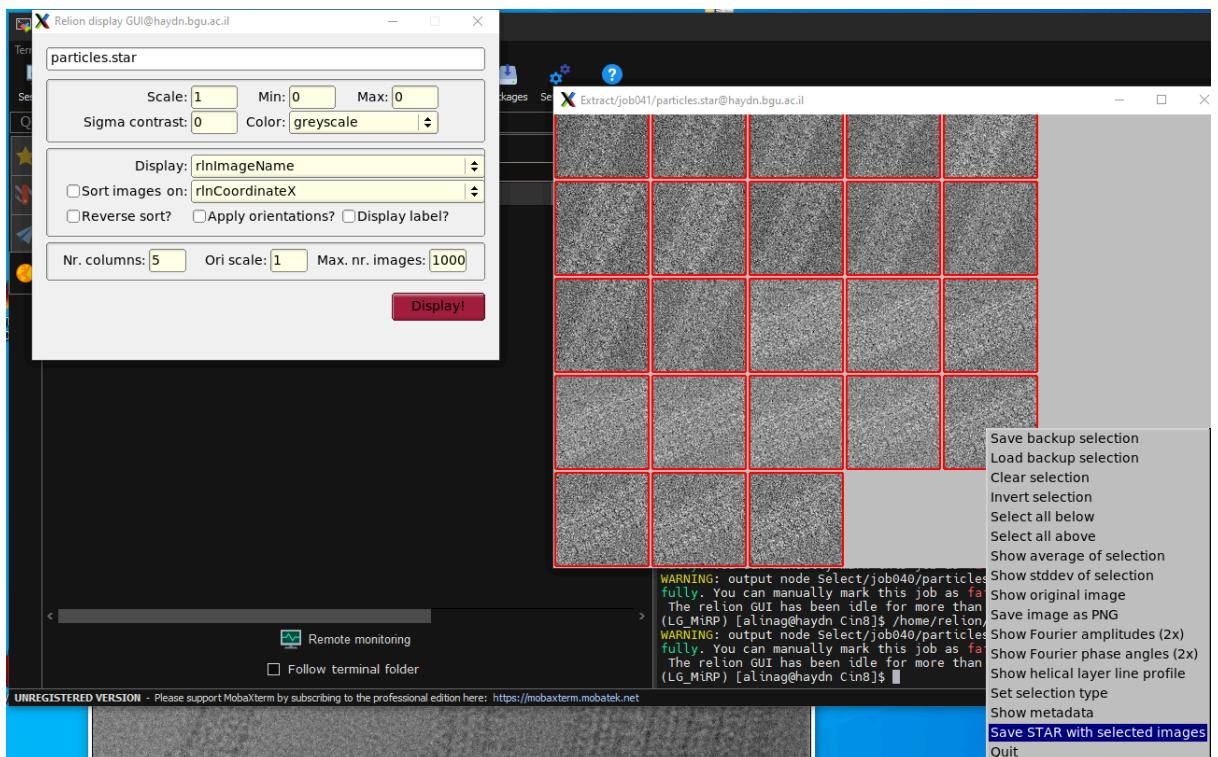
- d. To save your progress right click with the mouse and select Save backup selection



- 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
```

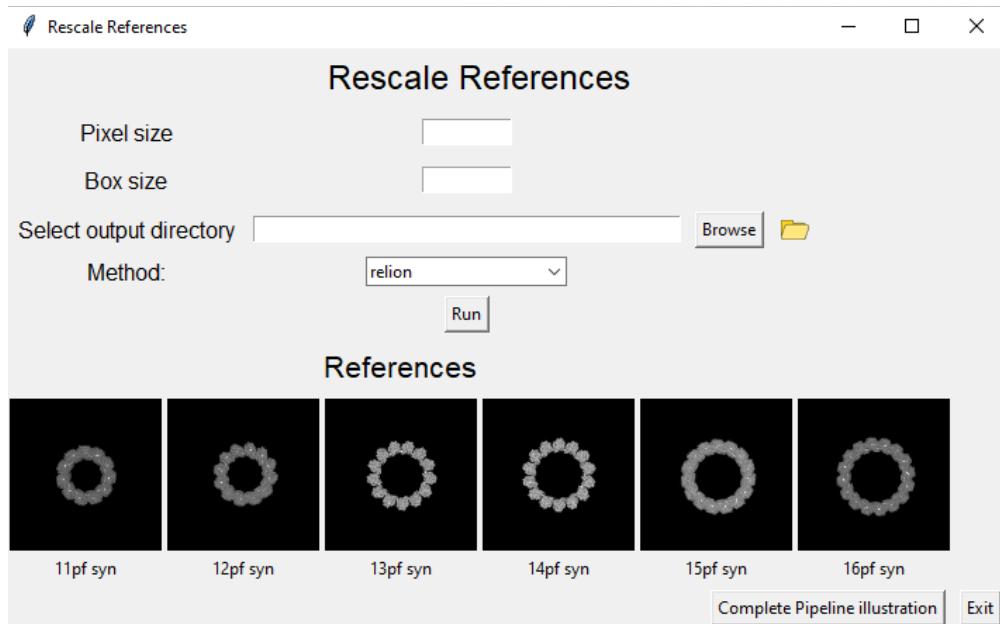
- d. Go to 3\_protofilament\_number\_sorting folder

```
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
```

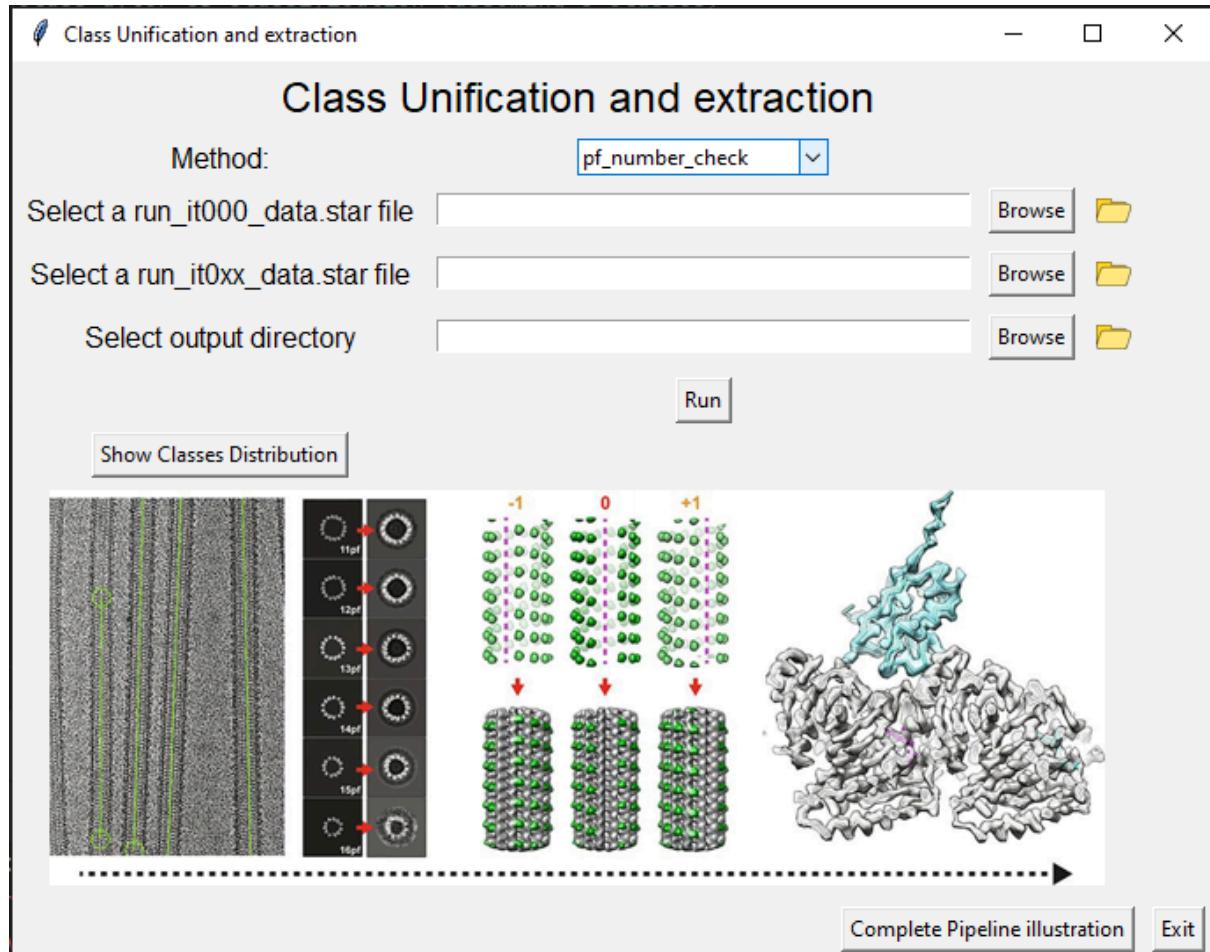
- d. Navigate to the folder 3\_protofilament\_number\_sorting

```
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
```

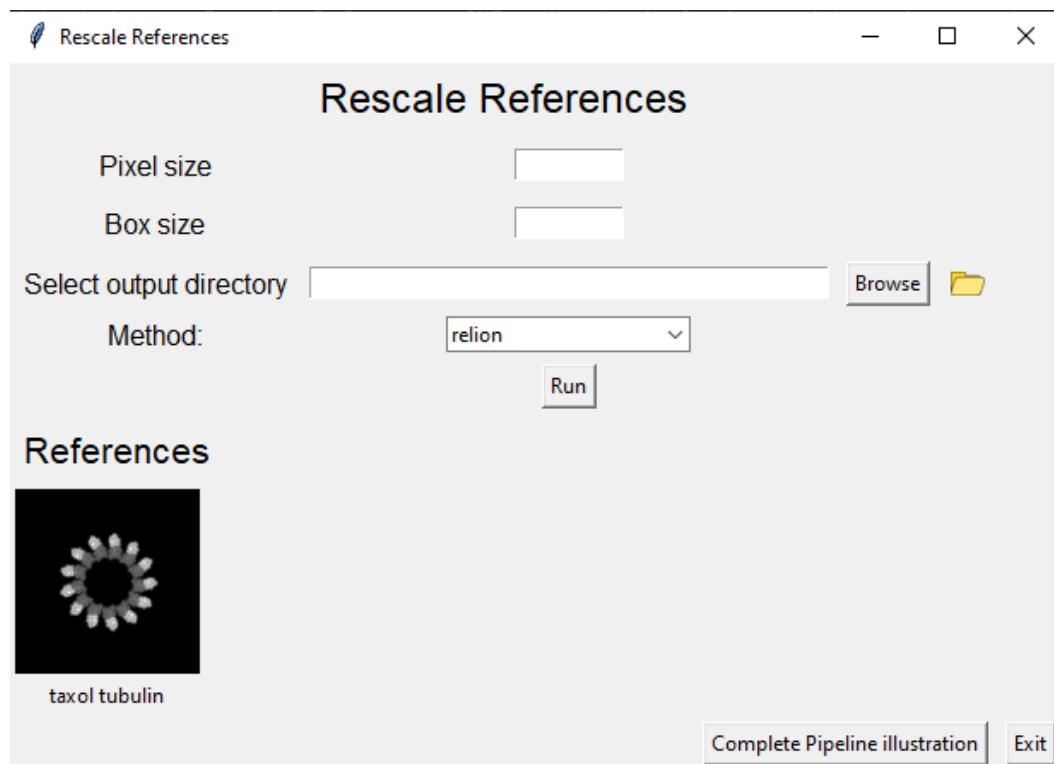
- d. Go to 4\_initial\_seam\_assignment folder

```
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:**

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
```

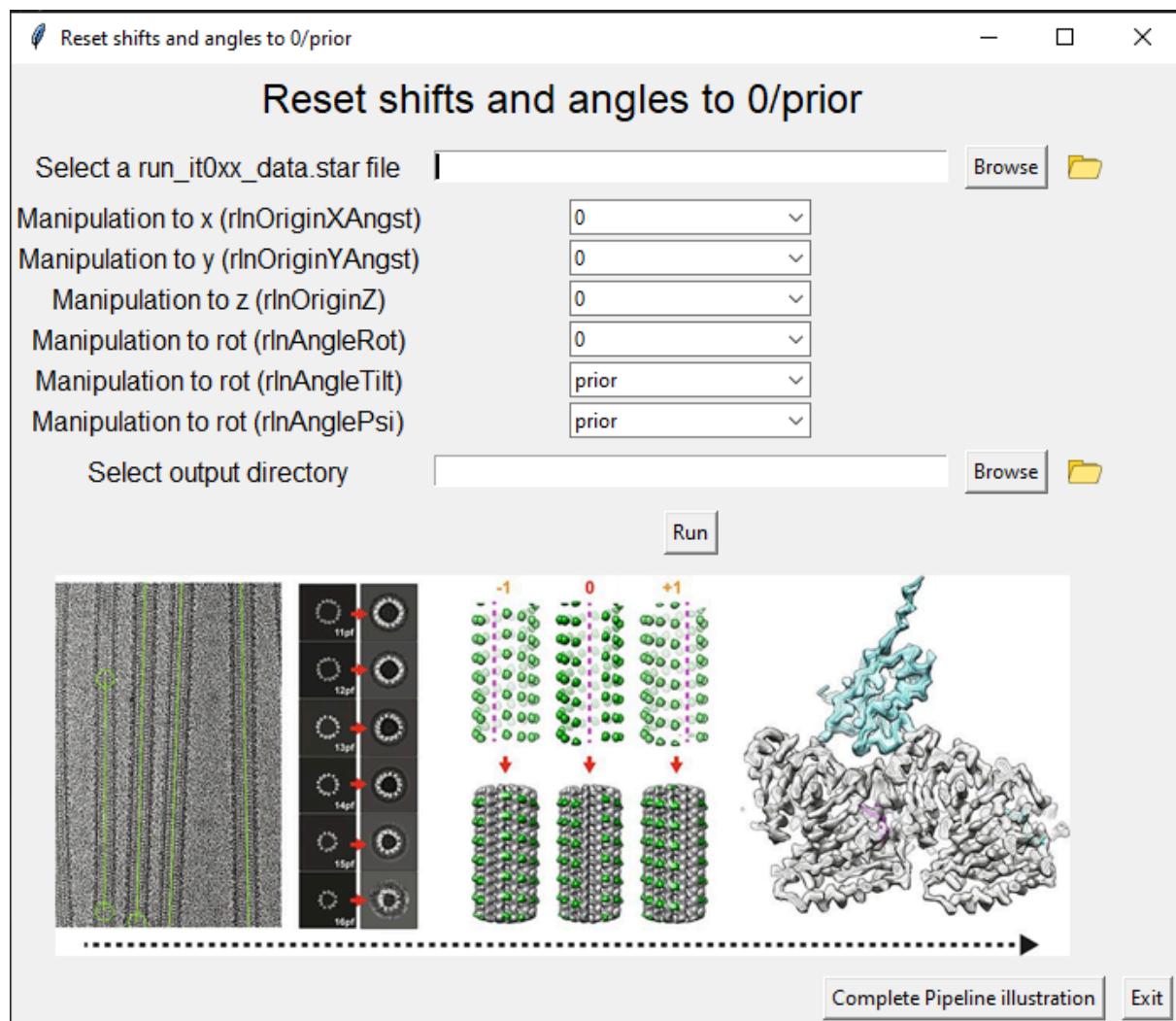
- d. Go to 4\_initial\_seam\_assignment folder

```
cd 4_initial_seam_assignment
```

- e. Run 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.

## **Protofilament refinement**

