



RELIONv3 tutorial v0.1

Appion Part II

September 19, 2019

Tutorial setup:

Requirements:

A terminal for ssh with X-display

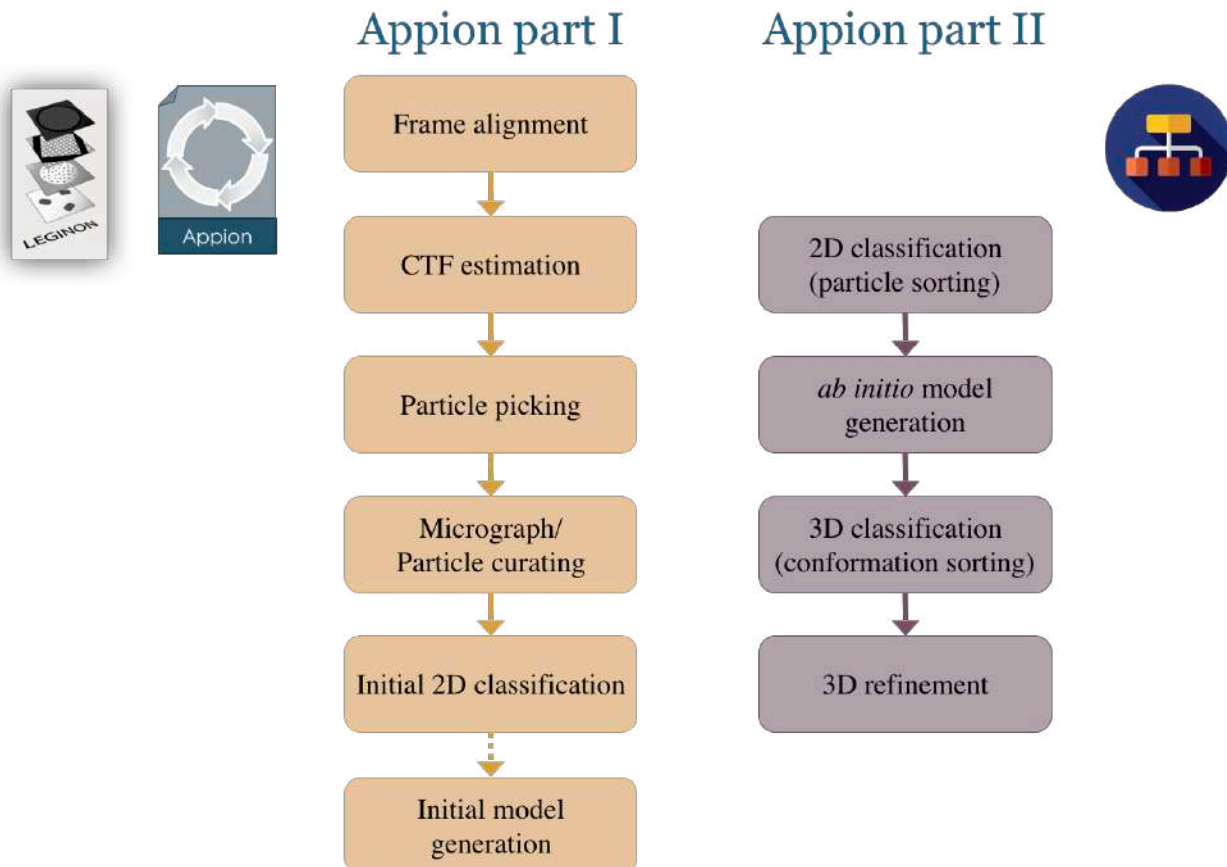
• If you're using a Mac, download XQuartz for X11 forwarding to use the graphical interface. Download link: <https://dl.bintray.com/xquartz/downloads/XQuartz-2.7.11.dmg>

• If you're using windows, download third party tool 'MobaXterm' to remotely login to the linux server
Download link: <https://mobaxterm.mobatek.net/download-home-edition.html> .

Recommended:

UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) for visualization.

Workflow goals:



This tutorial assumes that students have taken “Appion part I: Basics and 2D processing” where the pre-processing pipeline has been covered and will focus “Appion part II: 3D reconstruction/refinement”.

Given standard SEMC pre-processing workflow within minutes of the last acquired image during data collection the user should have: 1) curated aligned micrographs, 2) CTF estimation, and 3) particle picks. If the user has also utilized skills learned in Appion part I they may also have: 4) initial particle curation and 5) an initial model.

RELION in the cloud:

Accessing RELION on the Cloud

1. What is RELION?
 - RELION (REgularised LIkelihood Optimisation) is a software package commonly used for computing high-resolution Cryo-EM structures (<http://www2.mrc-lmb.cam.ac.uk/relion>). We have installed it on the Amazon web services cloud for this practical.
 - To read more about running cryo-EM software on the cloud, go here: <http://cryoem-tools.cloud/>
2. To access, download “Appion-2.pem” key.
3. Login using the following credentials in a terminal.
ssh -i "Appion-2.pem" ubuntu@[see sheet for ip address] -XY
4. Once you sign in, you will be on the main page.

```
Welcome to Ubuntu 16.04.6 LTS (GNU/Linux 4.4.0-1092-aws x86_64)

* Documentation:  https://help.ubuntu.com
* Management:    https://landscape.canonical.com
* Support:       https://ubuntu.com/advantage

Get cloud support with Ubuntu Advantage Cloud Guest:
http://www.ubuntu.com/business/services/cloud

69 packages can be updated.
0 updates are security updates.

New release '18.04.2 LTS' available.
Run 'do-release-upgrade' to upgrade to it.

Last login: Tue Sep 17 21:43:20 2019 from 38.96.169.131
ubuntu@ip-172-31-3-232:~$
```

-
- The default path will be /home/ubuntu/

```
ubuntu@ip-172-31-3-232:~$ pwd
/home/ubuntu
ubuntu@ip-172-31-3-232:~$ ls
appion1-T20S
appion2-dataset
appion2-group0
appion2-movies
cuda-repo-ubuntu1604-10-1-local-10.1.105-418.39_1.0-1_amd64.deb
default_pipeline.star
EMPIAR
NVIDIA-Linux-x86_64-410.104.run
relion-3.0_beta
test
ubuntu@ip-172-31-3-232:~$
```

- You will have several folders:
 - appion1-T20S (data from Appion part 1)
 - appion2-dataset (data for this tutorial)
 - appion2-group0 (reference data with jobs already pre-run)
 - appion2-movies (an advanced dataset with movies to test additional features)
 - EMPIAR (T20S dataset EMPAIR-10025)

Making Your Project and Dataset

- Today, we will processing bovine GDH (a 334 kDa D3 symmetry protein). Our goal is to compute a reconstruction of this protein while going over the basic workflow for single particle 3D construction. Note, upon familiarization with this workflow time will be allotted to test different workflows and/or the full single-particle pipeline.
- From Leginon/Appion users have access to:
 - frames folder with raw TIFF LZW images
 - legion folder with the data found on emgweb.nysbc.org
 - appion folder with CTF estimation and object selection information.

In particular for this test example the aligned summed images were downloaded from the legion folder, through the web interface box-files were generated from stack creation and a star file with micrograph list and CTF information was downloaded from the Leginon-Appion webtools.

- The standard test dataset will be located in "appion2-dataset".

```
ubuntu@ip-172-31-3-232:~$ ls appion2-dataset/
boxes          gdh_1670A_144px.mrc  micrographs
gdh_0035A_288px.mrc  K2_300kV.star       micrographs_ctf-appion.star
```

- It contains box files, GDH reference reconstructions at 0.835Å/px at 288px box size and 1.67Å/px at 144 px box size, K2 MTF file, micrographs folder and CTF star file.
- Create a new directory to work in. In this instance you will have a little over 100 Gb of space to work with.
 - mkdir [your directory of choice] (in this case I named it appion2-test)
 - cd [your directory of choice]

```
ubuntu@ip-172-31-3-232:~$ mkdir appion2-test
ubuntu@ip-172-31-3-232:~$ cd appion2-test/
ubuntu@ip-172-31-3-232:~/appion2-test$
```

- Create a folder for your micrographs and link your micrographs in that folder.
 - mkdir micrographs
 - cd micrographs
 - ln -s /home/ubuntu/appion2-dataset/micrographs/*mrc .

```
ubuntu@ip-172-31-3-232:~/appion2-test$ mkdir micrographs
ubuntu@ip-172-31-3-232:~/appion2-test$ cd micrographs/
ubuntu@ip-172-31-3-232:~/appion2-test/micrographs$ ln -s /home/ubuntu/appion2-d
ataset/micrographs/*mrc .
```

- Now link your box files into a folder called micrographs
 - ln -s /home/ubuntu/appion2-dataset/boxfiles/ micrographs

```
ubuntu@ip-172-31-3-232:~/appion2-test/micrographs$ ln -s /home/ubuntu/appion2-d
ataset/boxes/ micrographs
```

- Check to make sure files are in the newly created folders

```
ubuntu@ip-172-31-3-232:~/appion2-test/micrographs$ ls
17feb14h_gdh_au_00010gr_00005sq_v02_00002hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00003hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00004hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00005hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00006hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00008hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00010hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00011hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00012hln_00002esn-a-DW.mrc
17feb14h_gdh_au_00010gr_00005sq_v02_00013hln_00002esn-a-DW.mrc
ubuntu@ip-172-31-3-232:~/appion2-test/micrographs$ ls micrographs
17feb14h_gdh_au_00010gr_00005sq_v02_00003hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00004hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00005hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00006hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00008hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00010hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00011hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00015hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00017hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00021hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00026hln_00002esn-a-DW.box
17feb14h_gdh_au_00010gr_00005sq_v02_00031hln_00002esn-a-DW.box
```

- Go back to the main directory, copy the micrographs_ctf-appion.star into that folder.

```

ubuntu@ip-172-31-3-232:~/appion2-test/micrographs$ cd ..
ubuntu@ip-172-31-3-232:~/appion2-test$ cp /home/ubuntu/appion2-dataset/micrographs_ctf-appion.star .
ubuntu@ip-172-31-3-232:~/appion2-test$ ls
micrographs  micrographs_ctf-appion.star
ubuntu@ip-172-31-3-232:~/appion2-test$

```

12. Look at the .star file using a text editor, e.g. vi.

```

data_
loop
  _rlnMicrographName #1
  _rlnCtfImage #2
  _rlnDefocusU #3
  _rlnDefocusV #4
  _rlnDefocusAngle #5
  _rlnVoltage #6
  _rlnSphericalAberration #7
  _rlnAmplitudeContrast #8
  _rlnMagnification #9
  _rlnDetectorPixelSize #10
  _rlnCtfFigureOfMerit #11
  _rlnPhaseShift #12
micrographs/17feb14h_gdh_au_0001igr_00003sq_v02_00003hln_00002esn-a-DW.mrc micr
oggraphs/17feb14h_gdh_au_0001igr_00003sq_v02_00003hln_00002esn-a-DW.ctf.mrc 1790
5.492188 18973.392578 -66.831846 300.000000 0.001000 0.070000 10000.000000 0.88
4778 0.938309 0.000000
micrographs/17feb14h_gdh_au_0001igr_00003sq_v02_00004hln_00002esn-a-DW.mrc micr
oggraphs/17feb14h_gdh_au_0001igr_00003sq_v02_00004hln_00002esn-a-DW.ctf.mrc 1980
0.667969 21999.589644 -69.799248 300.000000 0.001000 0.070000 10000.000000 0.88
4778 0.919894 0.000000
0
0
"micrographs_ctf-appion.star" 459L, 115308C 1,0-1 Top

```

- These particle stacks have a list file with CTF estimation (.star file). (Note, the pixel size was calibrated subsequently by scaling the EM reconstruction to a pdb of the crystal structure and it actually should be 1.71Å/px at bin2.)

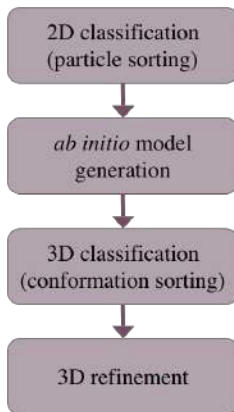
13. Copy the remaining files in the dataset into the main directory.

```

ubuntu@ip-172-31-3-232:~/appion2-test$ cp /home/ubuntu/appion2-dataset/boxes/K2_300kV.star
gdh_0835A_288px.mrc micrographs/
gdh_1670A_144px.mrc micrographs_ctf-appion.star
ubuntu@ip-172-31-3-232:~/appion2-test$ cp /home/ubuntu/appion2-dataset/*mrc .
ubuntu@ip-172-31-3-232:~/appion2-test$ cp /home/ubuntu/appion2-dataset/K2_300kV.star .

```

[Example SPA workflow for this tutorial]



We are going to focus on this type of single particle analysis (SPA) workflow for the tutorial. By making use of Appion we enter into the workflow from particle extraction. In terms of RELION it would be roughly these tasks:

Particle curation

- 1) Particle extraction
- 2) 2D Classification
- 3) Select 2D classes

3D initial model/classification

- 4) Ab-Initio Reconstruction (optional)
- 5) 3D classification

3D refinement

- 6) 3D refinement

Your directory structure should look something like this:

```

ubuntu@ip-172-31-3-232:~/appion2-test$ ls
gdh_0835A_288px.mrc K2_300kV.star micrographs_ctf-appion.star
gdh_1670A_144px.mrc micrographs
ubuntu@ip-172-31-3-232:~/appion2-test$ relion

```

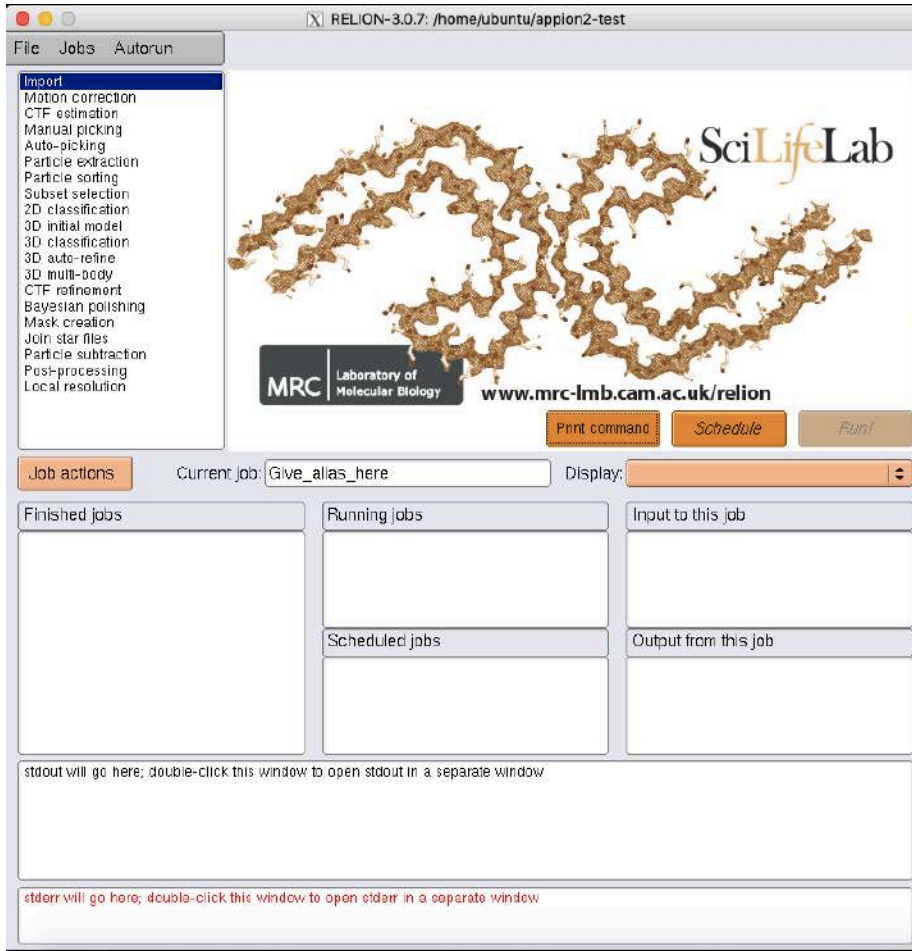
Start relion by typing "relion" in the terminal:

```

ubuntu@ip-172-31-3-232:~/appion2-test$ ls
gdh_0835A_288px.mrc K2_300kV.star micrographs_ctf-appion.star
gdh_1670A_144px.mrc micrographs
ubuntu@ip-172-31-3-232:~/appion2-test$ relion
Only run the relion GUI from your ProjectDirectory. Do you want to start a new
project here [y/n]? y

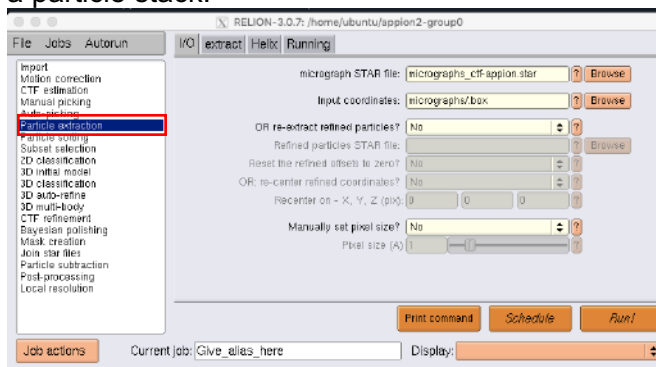
```

The main relion window should look like this:



Particle extraction

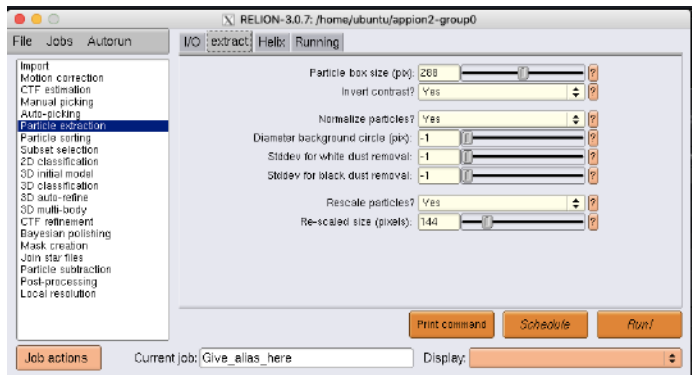
14. Create a particle stack.



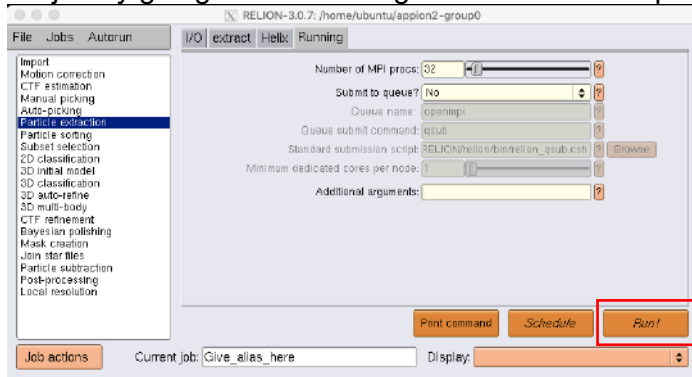
-
-
- Select a particle extraction job.
- Find the micrograph STAR file and type the path of the input coordinates, in this case micrographs/.box.

15. We will be binning the data for efficient use of time.

- Go to the Extract tab.
- The original box size was 288px and we will bin by 2 for a final box size of 144px.



16. Set up the job by going to the Running tab. We have 32 cpus in this instance.



17. Run the job by clicking the “Run!” button.

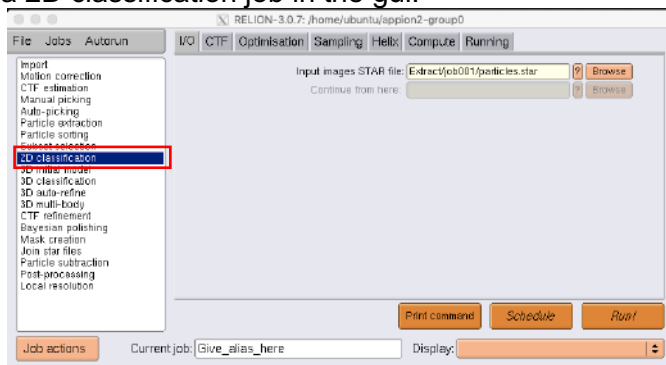
18. When the job finishes in the log file you will see that it has extracted several particles.

```
Extracting particles from the micrographs ...
2.02/2.02 min .....~(.,>
Joining metadata of all particles from 443 micrographs in one STAR file...
Written out STAR file with 59663 particles in Extract/job001/particles.star
The new pixel size of the extracted particles are 1.76956 Angstrom/pixel.
Done preprocessing!
```

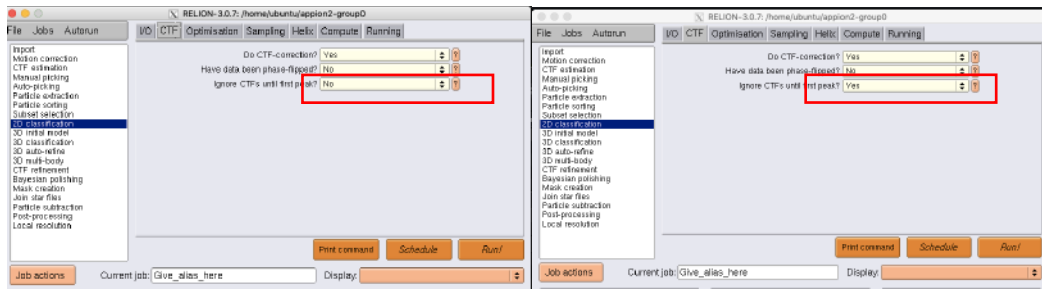
-
- For organizational purposes you can add an alias and notes to jobs.

2D classification

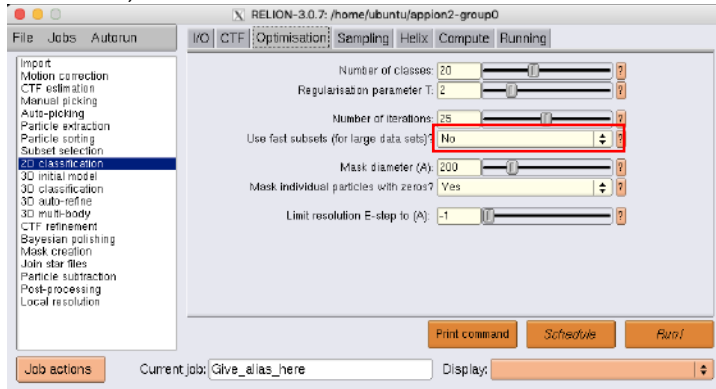
19. Select a 2D classification job in the gui.



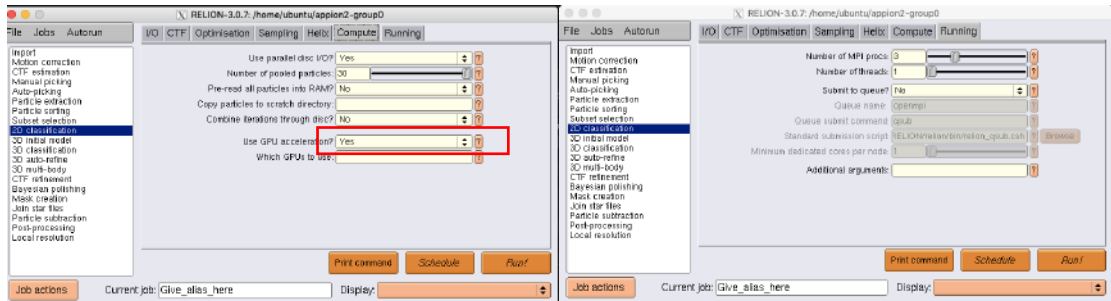
- - Find the previously run Particle extraction job as the input images STAR file.
20. Under the CTF tab do CTF correction. See what happens if you ignore CTF's until first peak Yes or No.
- Choose one:



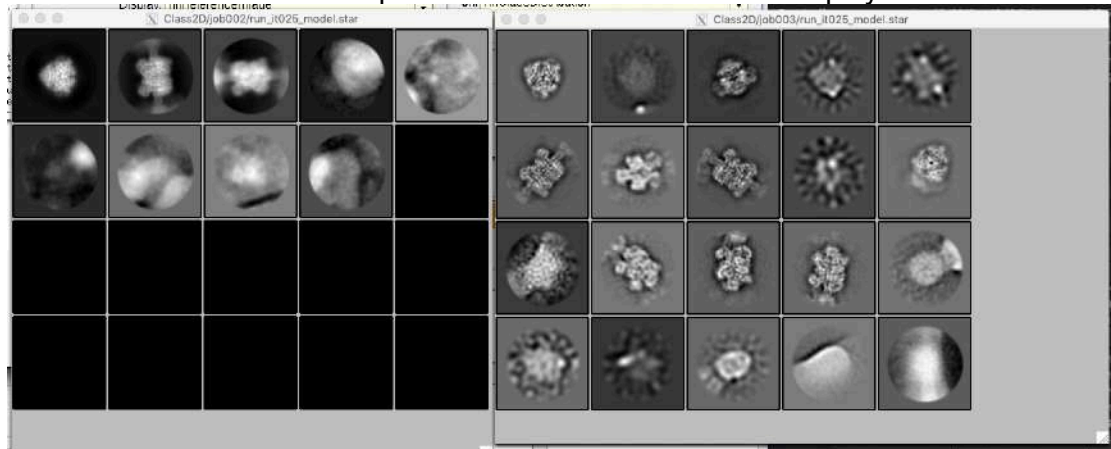
-
- In the optimization tab we will be using certain parameters to ensure that the job completes in a timely manner. In addition to binning, we will use 10-20 classes, no more than 25 iterations, and set use fast subsets as Yes.



-
- Under the Compute tab we will be using GPU acceleration. This instance has access to 2 GPUs. In the Running tab select 3 MPI procs as 1 will be the master and the 2 available cards will be the slave.



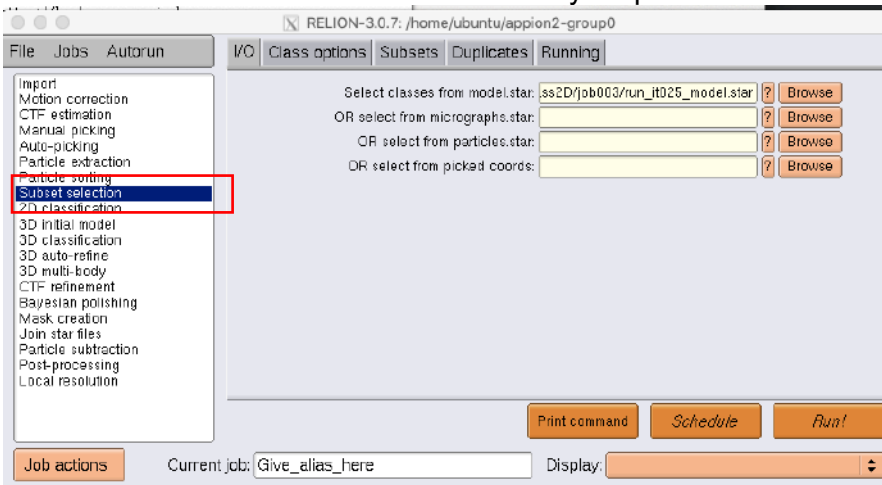
-
- Depending on if you ignored the first CTF peak you may see the following results. To view the final 2D classification output select the model.star file in Display.



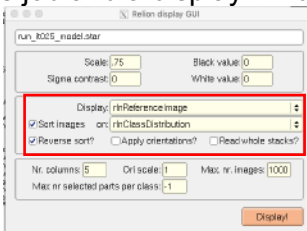
-

Select 2D classes

21. Select subset selection to choose 2D classes from your previous 2D Classification run.

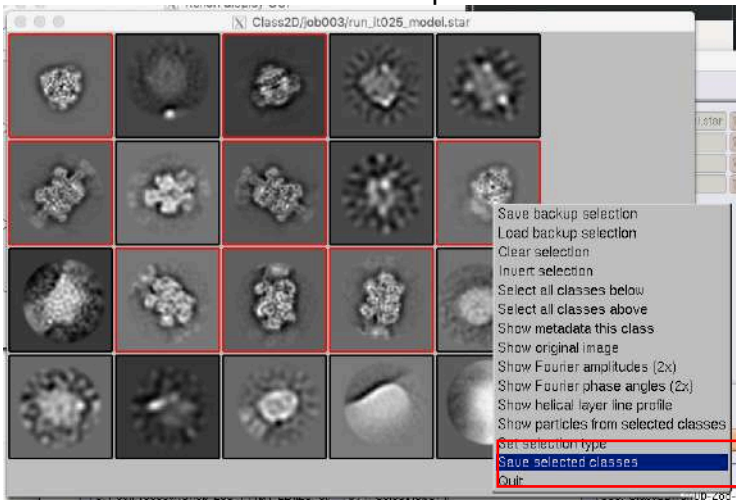


22. Run this job and a display window will open up.



- Depending on your screen size you may want to scale the images. For ease of display you may sort images by a particular criteria.

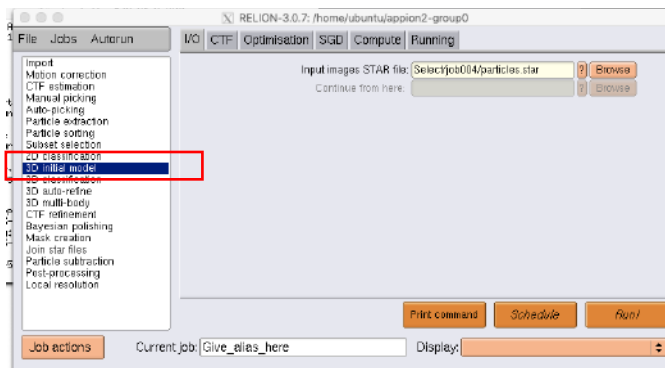
23. In a moment an interactive window will open to allow selection of classes.



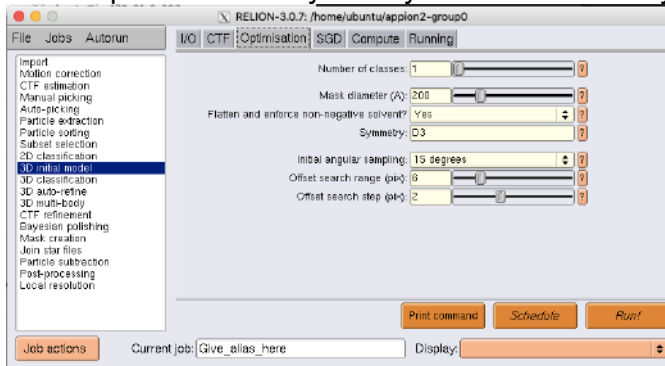
- Select desired classes, which will be highlighted, and right click to bring up a menu to save selected classes.

Ab-initio reconstruction (optional)

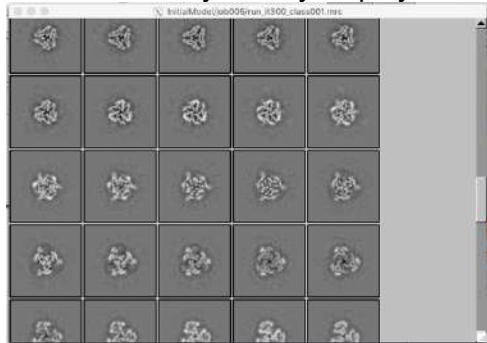
24. Create an *ab initio* structure from the curated particle set with 3D initial model job.



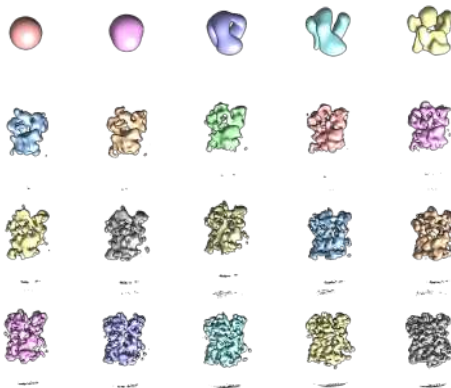
-
- Similar to before add the appropriate particle stack to your job.
- In the Optimisation tab you may choose to utilize symmetry.



-
- 25. When the run finishes you may display the resulting model.



-
- 26. For the tutorial you do not have sftp access to download the models, but if you were display the various iterations you would see the following assuming you chose D3 symmetry.



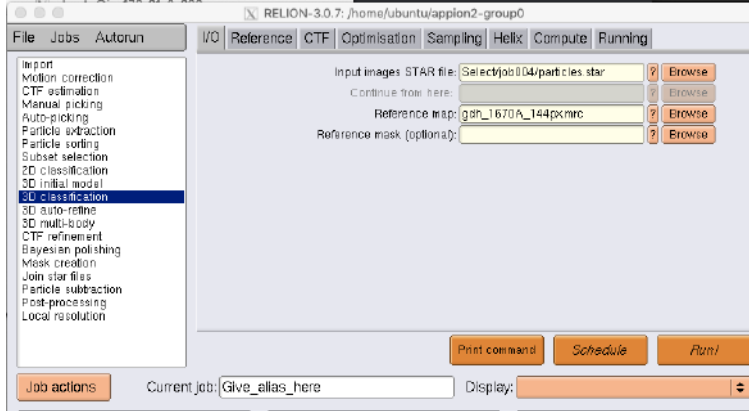
-

3D classification

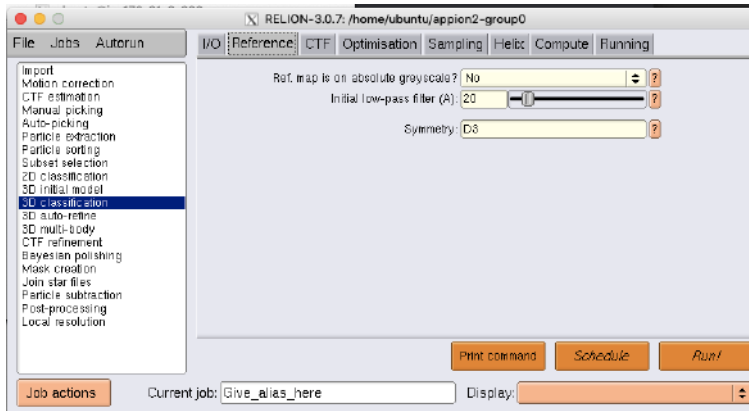
27. This test dataset has primarily one conformation, but in most datasets of macromolecular complexes there are multiple conformations, and/or varying state of completeness. One way to sort out these differences is to use 3D classification, however initial models are required. Since we have models via *ab initio* modelling we can potentially better curate the dataset with 3D models. This tutorial also contains an initial model if you skipped the *ab initio* model step.

```
ubuntu@ip-172-31-3-232:~$ ls appion2-dataset/  
boxes gdh_1670A_144px.mrc micrographs  
gdh_0835A_288px.mrc K2_300kV.star micrographs_ctf-appion.star
```

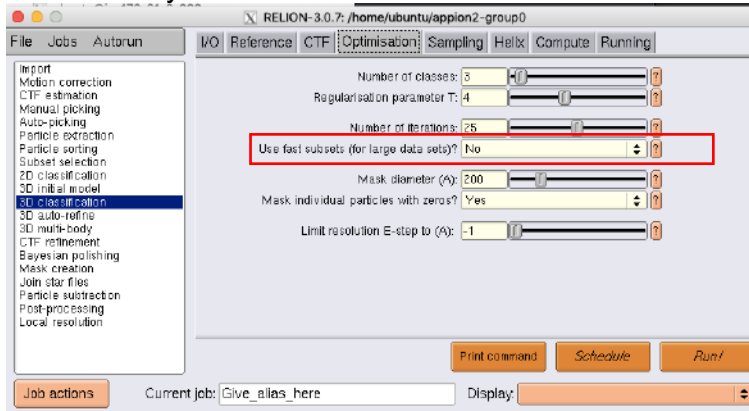
28. Choose a 3D classification job, select a STAR file and reference map.



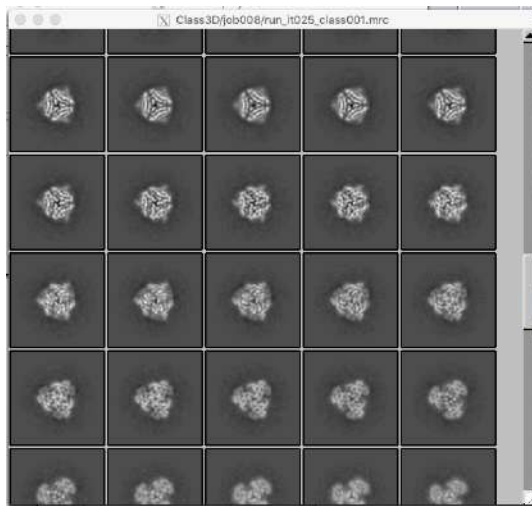
You may choose to either run this job with C1, C3 or D3 symmetry, and also low pass filter the model.



For efficiency do not use more than 3 classes and use fast subsets.



You may see the various classes or particular model in display.

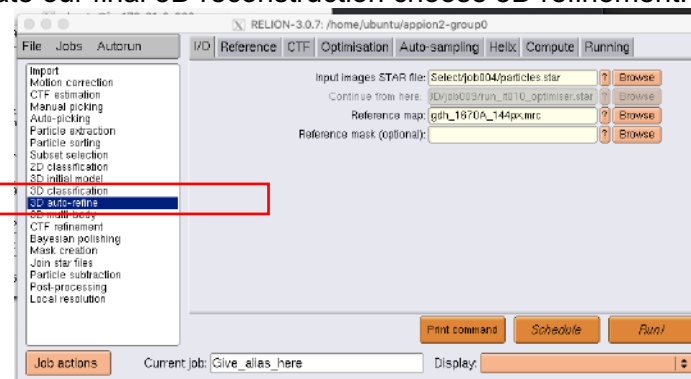


29. Start another subset selection job to select and save a particular class, or you may choose to passthrough all the particles.

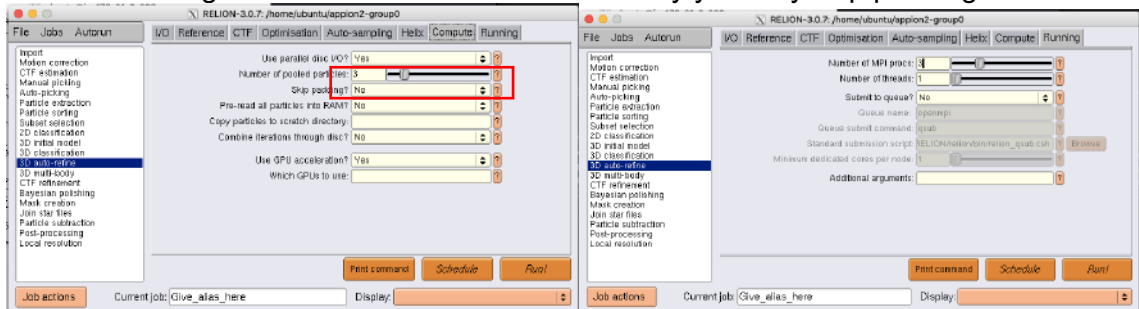


3D refinement

30. To create our final 3D reconstruction choose 3D refinement.



- Add a particle stack and volume for the refinement. Multiple particle stacks would have to be combined to create 1 star file. Only 1 reference map may be used for 3D refinement. Again you may choose to use symmetry.
- We will be using GPU acceleration and for efficiency you may skip padding.



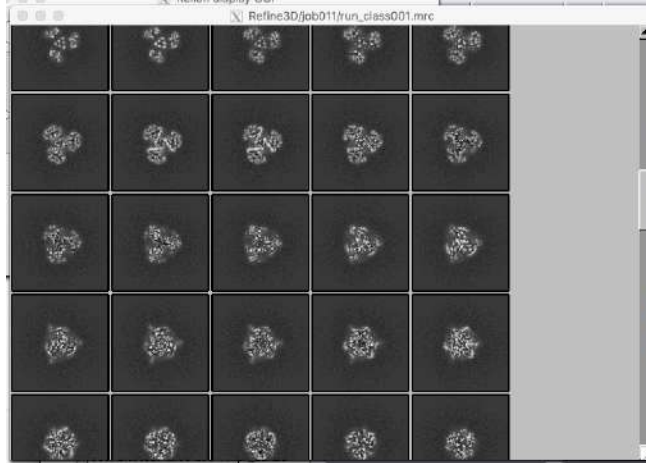
31. You may start the run and this will take a while.

32. Submit the job and monitor the run.

- Analyse the feedback to ensure the quality of the reconstruction.

```
Auto-refine: Refinement has converged, stopping now...
Auto-refine: + Final reconstruction from all particles is saved as: Refine3D/job011/run_class001.mrc
Auto-refine: + Final model parameters are stored in: Refine3D/job011/run_model.star
Auto-refine: + Final data parameters are stored in: Refine3D/job011/run_data.star
Auto-refine: + Final resolution (without masking) is: 3.7473
Auto-refine: + But you may want to run relion_postprocess to mask the unfil.mrc maps and calculate a higher resolution FSC
```

-
- 33. When the job finishes you may display the volume.



-
-

Additional tasks (if time allows)

34. Run postprocessing to sharpen your map.
35. Run any of the previous tasks with C1, C3 or D3 symmetry.
36. Try a different combination of particles or a different curation criteria to create a substack.
37. Try a different workflow.
38. Try additional tasks, such as ResLog Analysis, ThreeDFSC, etc...
39. Repeat the process with another dataset/movies.