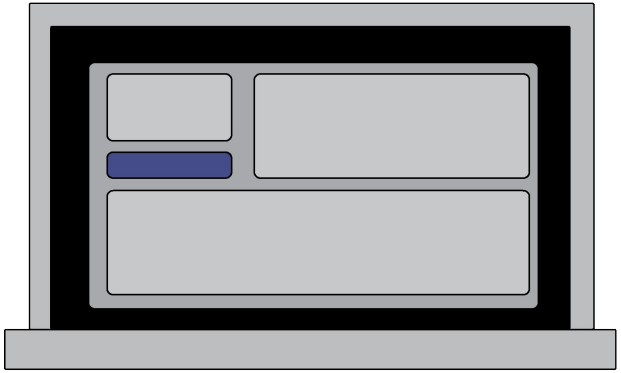
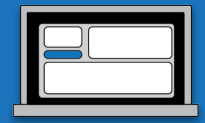
Introduction to the RELION Simulator



cryoEDU

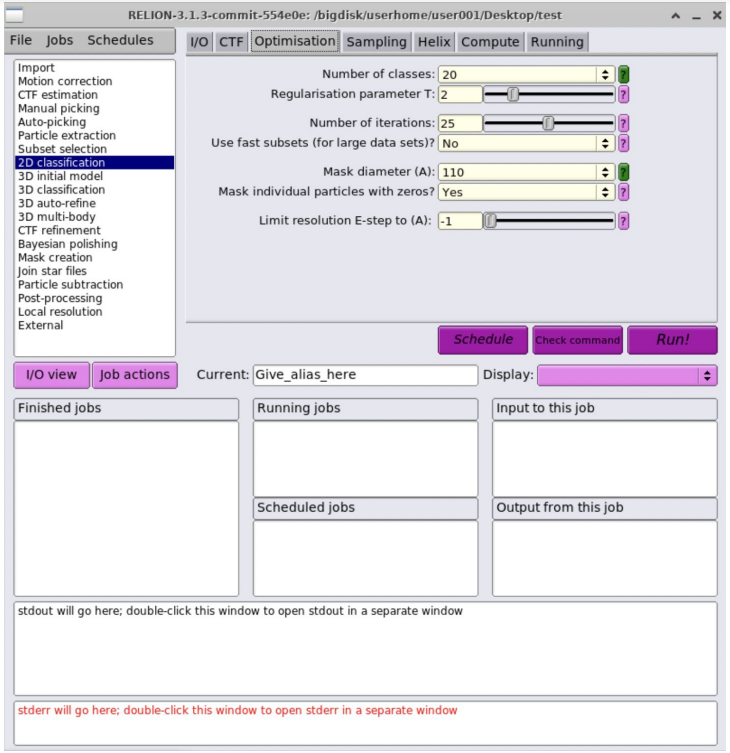
Outline



1. Logging onto and opening the RELION simulator on a cloud desktop environment
2. Practical Guide to the RELION simulator

RELION in a nutshell

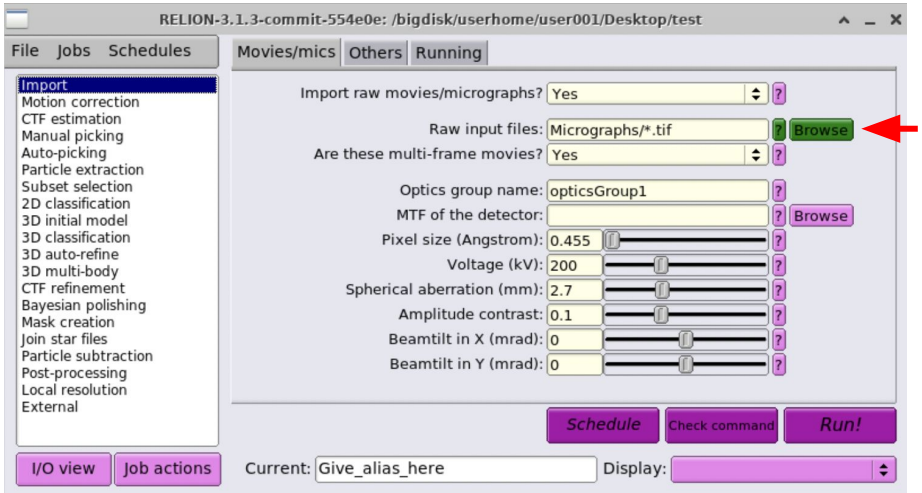
- RELION is a graphical user interface (GUI) that takes user input parameters and runs the job on the command line.
- RELION outputs data into automatically named & numbered directories
- The output and error from jobs is display in the ‘stdout’ and ‘stderr’ windows
- The simulator does *not* take into account any of the ‘Running’ tab

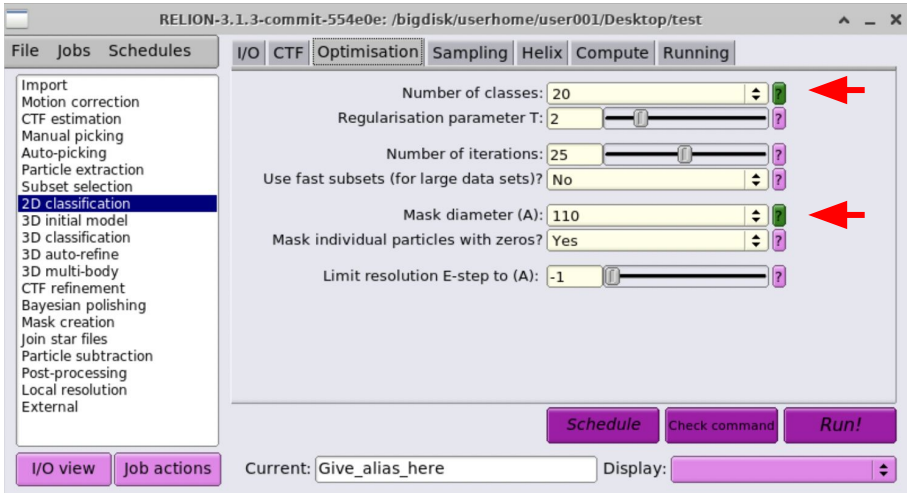


https://relion.readthedocs.io/en/release-3.1/

How to use the simulator

With RELION open, you will modify all parameters that are **GREEN**

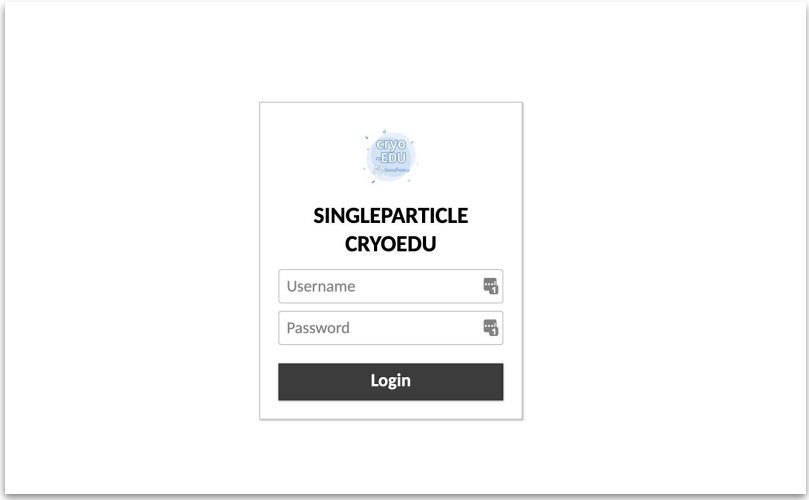




Accessing the cloud desktop from web browser

Open a web browser (ideally Chrome) and navigate to this URL:

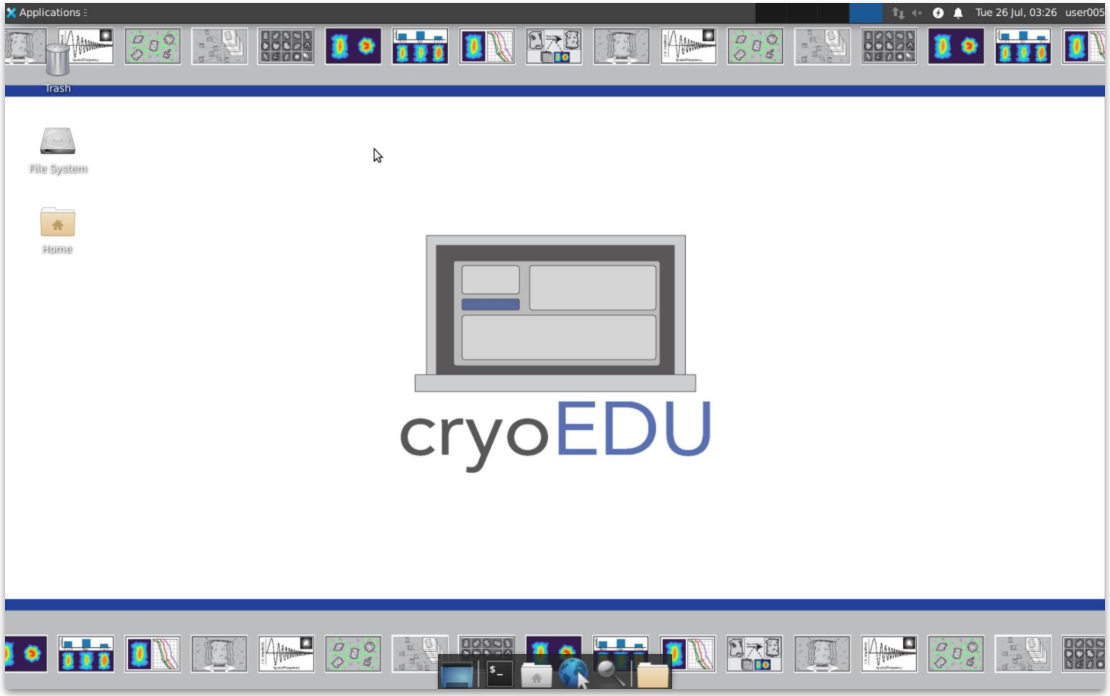
http://100.20.93.108:8080/cryoedu



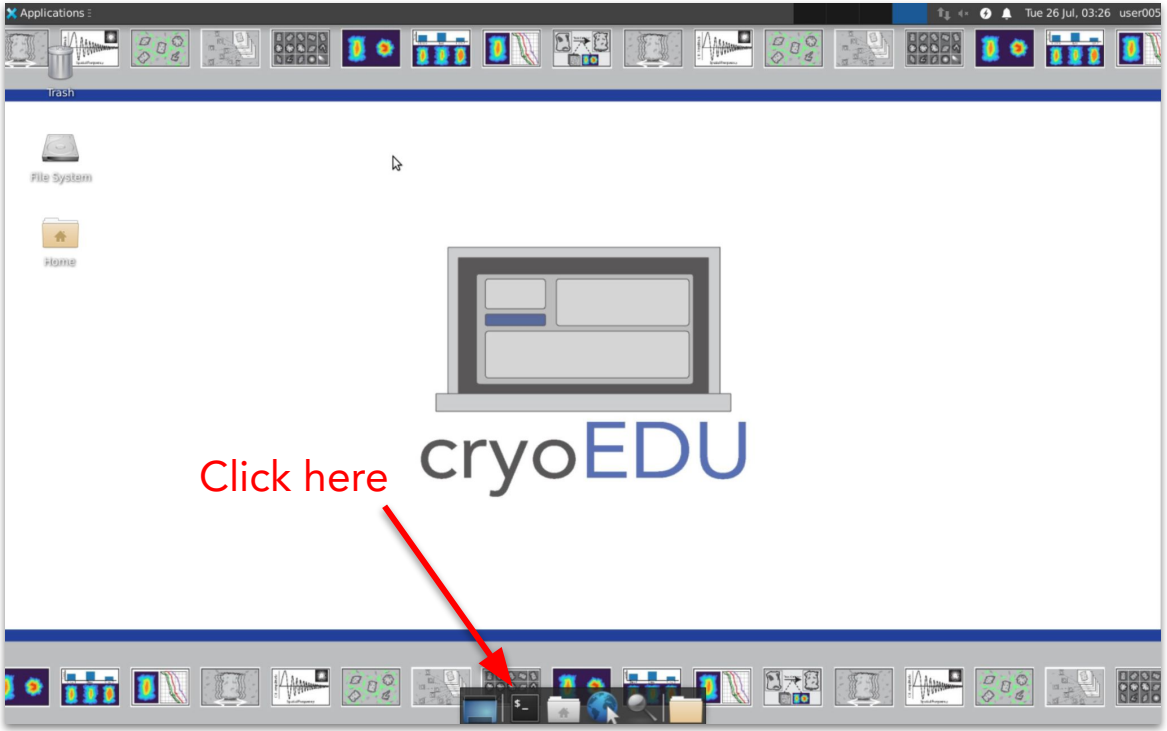
Username & password provided separately

The cloud desktop

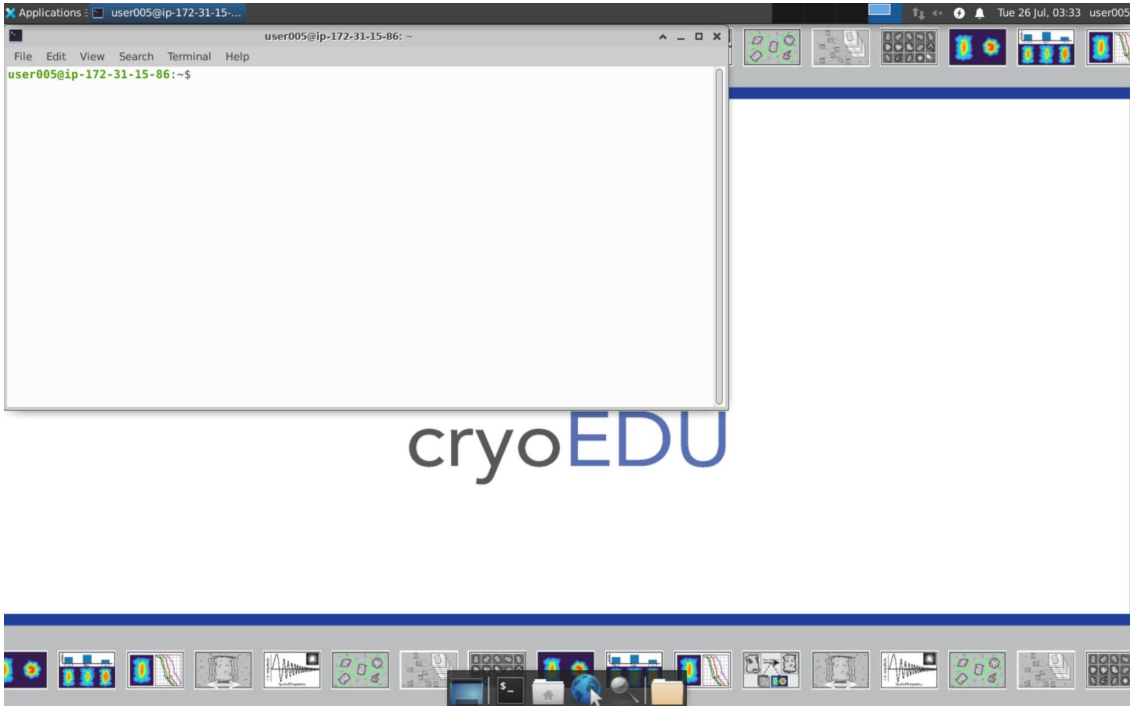
After logging in, you'll see the following desktop:



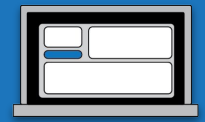
Opening the terminal



Opening the terminal



Navigate to your home directory



```
user008@ip-172-31-15-86:/$ pwd
/
user008@ip-172-31-15-86:/$ cd
user008@ip-172-31-15-86:~$ pwd
/bigdisk/userhome/user008
user008@ip-172-31-15-86:~$
```

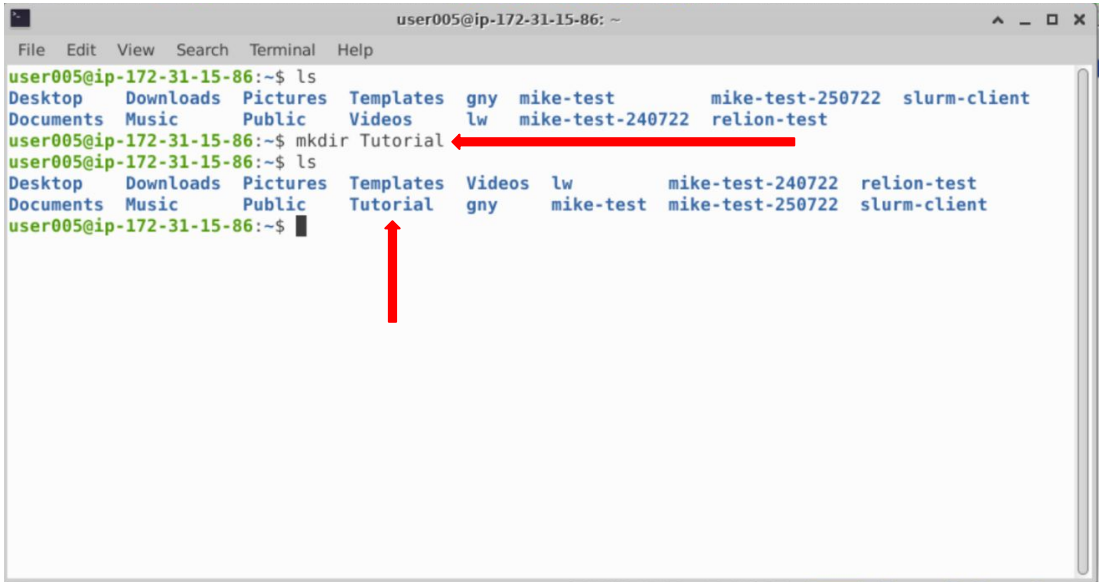
Check to see which directory you're in:

pwd

If you are in the top directory, enter:

cd

Making a directory



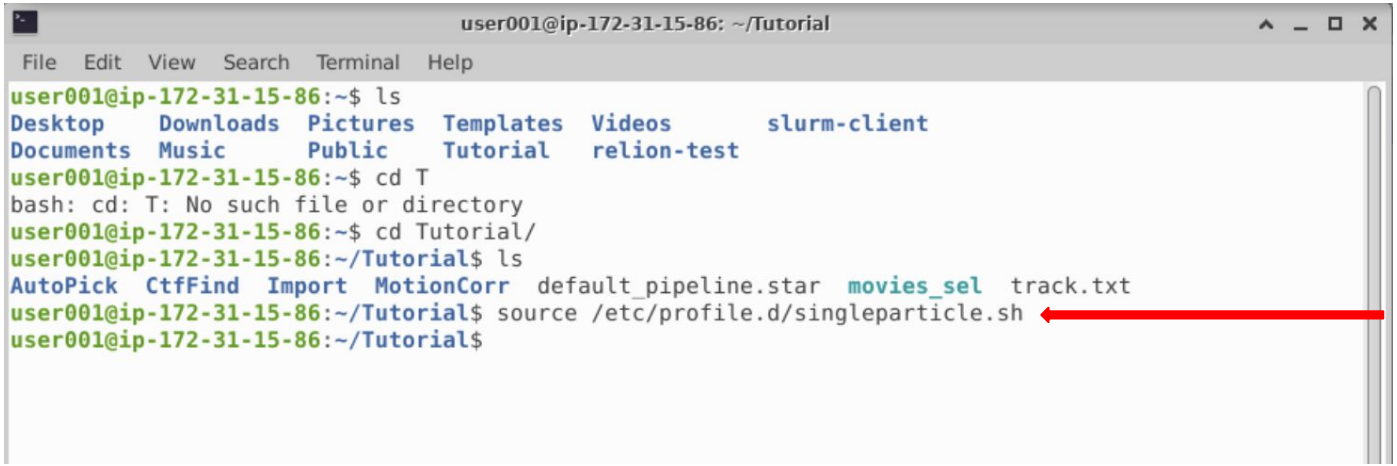
In the command line:
mkdir [name of directory]

Navigate to your directory:
cd [name of directory]

Create a source environment to display PDFs

Create a source environment:

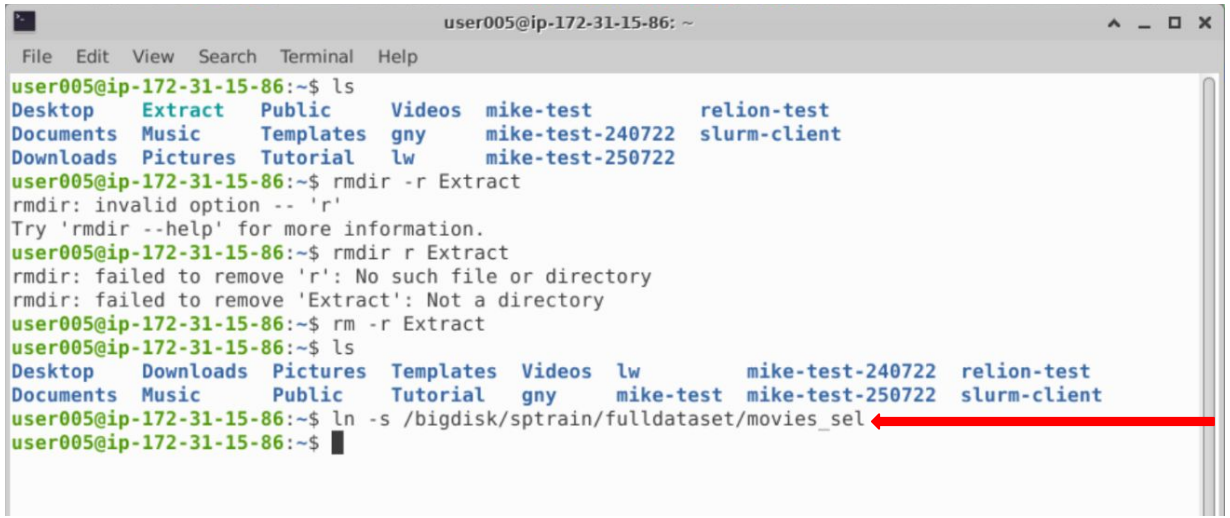
source /etc/profile.d/singleparticle.sh



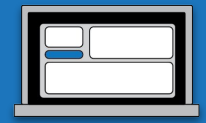
Import dataset

Import dataset:

ln -s /bigdisk/sptrain/fulldataset/movies_sel



Dataset for the cryoEDU RELION simulator

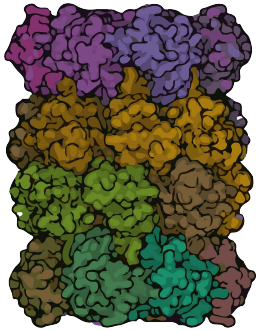


Published: 09 October 2017

Achieving better-than-3-Å resolution by single-particle cryo-EM at 200 keV

Mark A Herzik Jr, Mengyu Wu & Gabriel C Lander

Nature Methods **14**, 1075–1078 (2017) | Cite this article



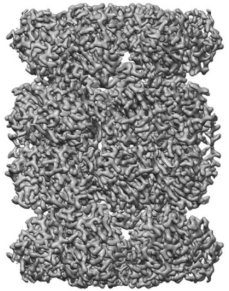
EMPIAR-10185

T. acidophilum 20S proteasome core movies obtained using Talos Arctica operating at 200 kV equipped with a K2 – stage position used for exposure target navigation

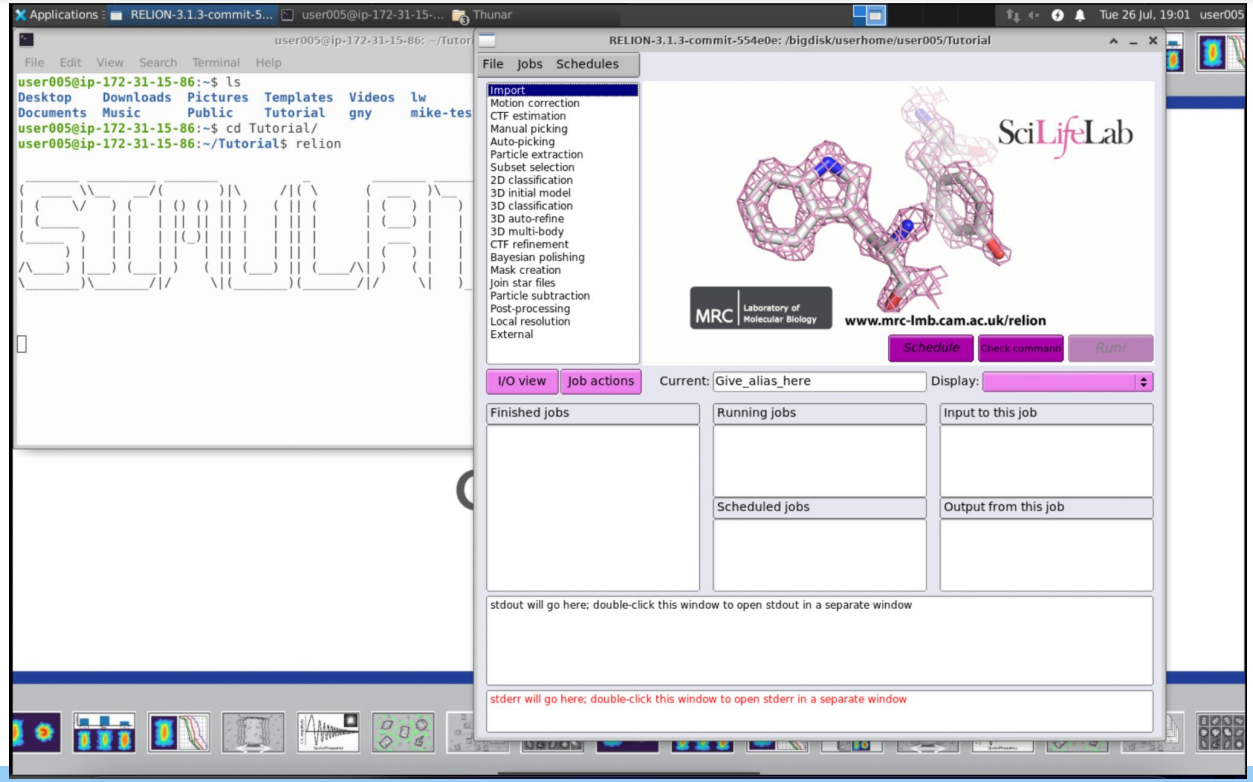
Publication:	Achieving better-than-3-Å resolution by single-particle cryo-EM at 200 keV Herzik Jr MA, Wu M, Lander GC *Nat. Methods* **14** 1075-1078 (2017) PMID: 28991891 DOI: 10.1038/nmeth.4461
Related PDB entry:	5vy3
Related EMDB entry:	EMD-8741 (3.1Å)
Deposited:	2018-05-03
Released:	2018-05-11
Last modified:	2018-05-11
Dataset size:	1.1 TB
Dataset DOI:	10.6019/EMPIAR-10185
Experimental metadata:	Download xml

Contains:

micrographs

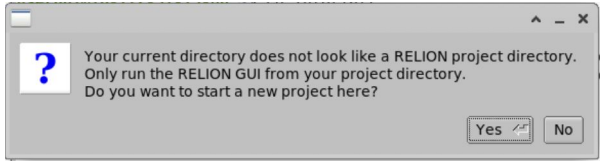


Start up RELION!

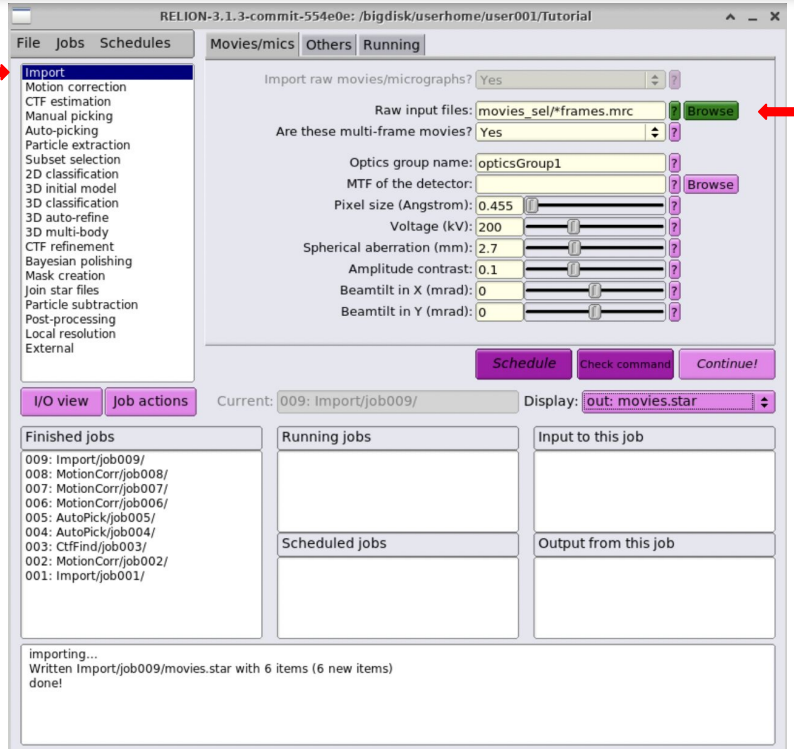


In your directory: *relion*

If the following pop-up appears hit yes to start a new project

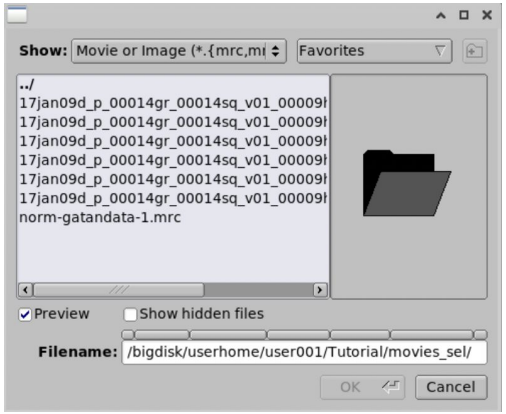


Import movies



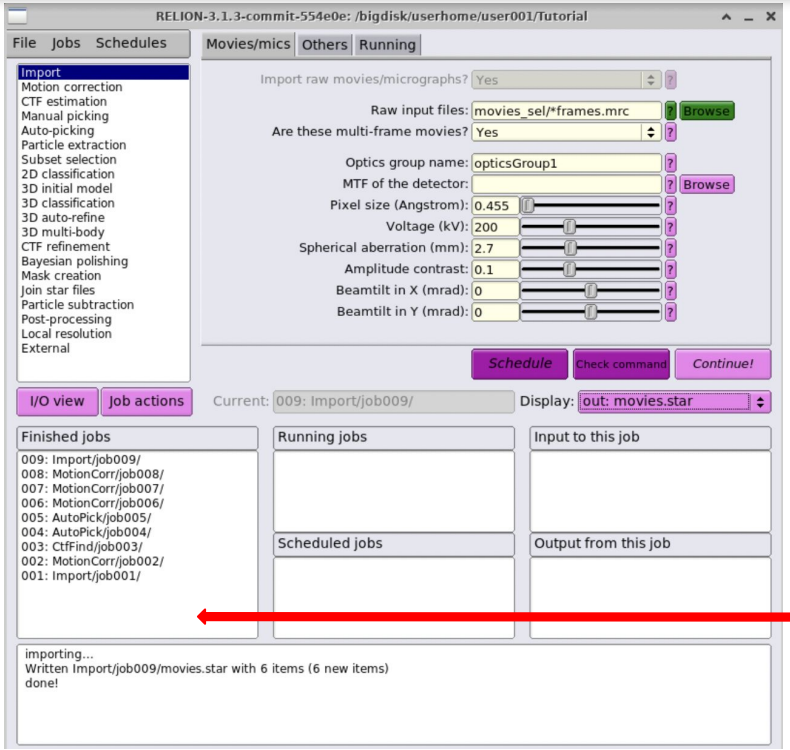
Hit the *Import* tab on the left

Hit the green browse button



Navigate to an image file and hit OK

Import movies



To grab all the movies, change the *Raw input files* field to:

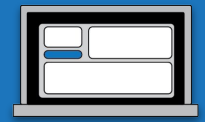
*movies_sel/*frames.mrc*

*: Allows targeting of *all* files with a given suffix

Run!

Completed jobs will be shown on the left

File structure within the simulator



```
user005@ip-172-31-15-86:~/Tutorial$ ls
Import  MotionCorr  default_pipeline.star  movies_sel  track.txt
user005@ip-172-31-15-86:~/Tutorial$
```

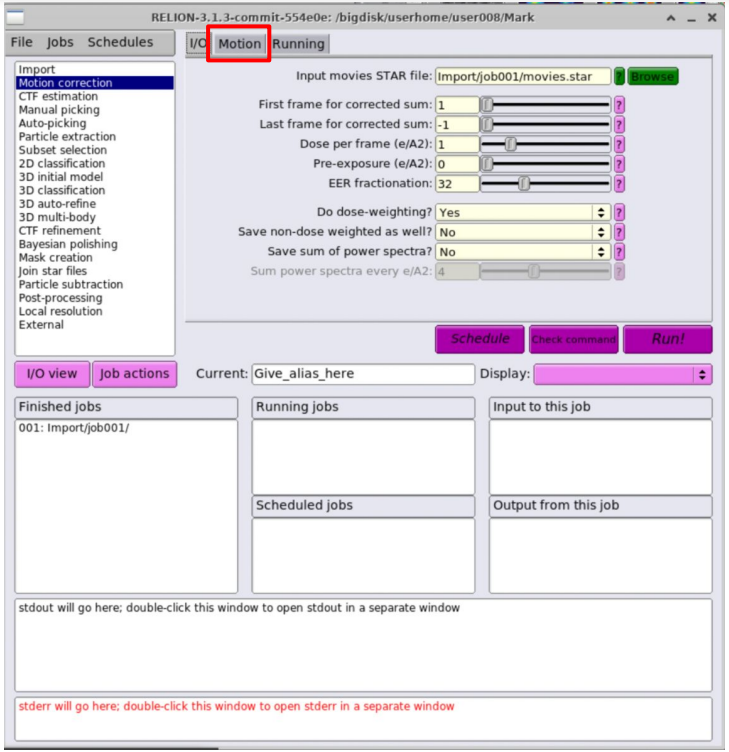
Directories created for each job type

```
user001@ip-172-31-15-86:~/Tutorial$ ls
Import  MotionCorr  default_pipeline.star  movies_sel  track.txt
user001@ip-172-31-15-86:~/Tutorial$ cd Import/
user001@ip-172-31-15-86:~/Tutorial/Import$ ls
job001
user001@ip-172-31-15-86:~/Tutorial/Import$ cd job001/
user001@ip-172-31-15-86:~/Tutorial/Import/job001$ ls
RELION_JOB_EXIT_SUCCESS  job.star           note.txt  scheduler_stderrclass.txt
default_pipeline.star    job_pipeline.star  run.err   scheduler_stdoutclass.txt
done.txt                 movies.star        run.out
user001@ip-172-31-15-86:~/Tutorial/Import/job001$
```

Directory created for each job

Metadata contained within each job

Motion correction



Navigate to the *Motion Correction* job

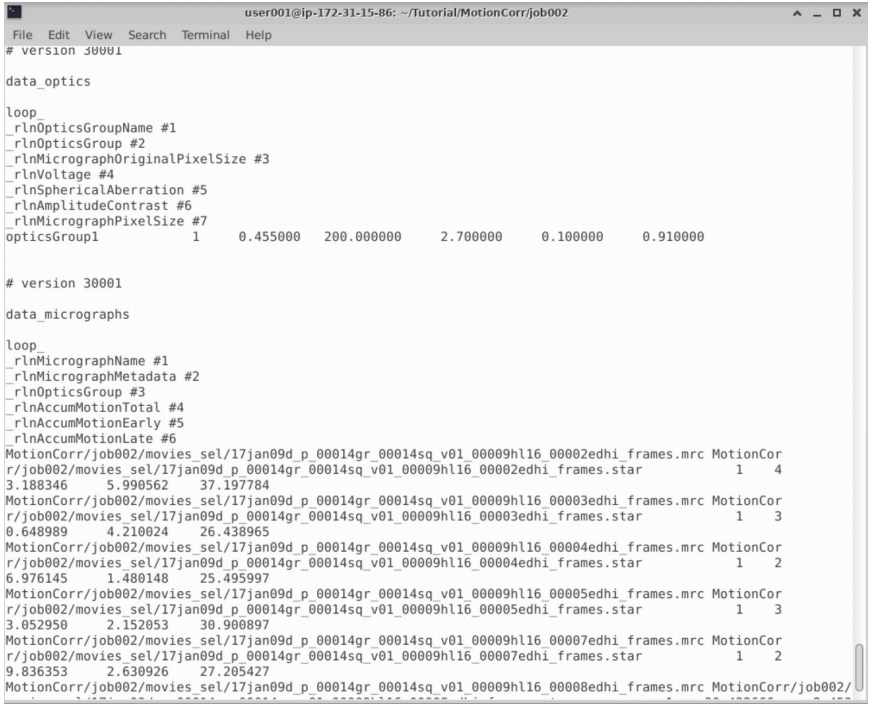
Input movies STAR file:
Import/job001/movies.star

Navigate to the *Motion* tab

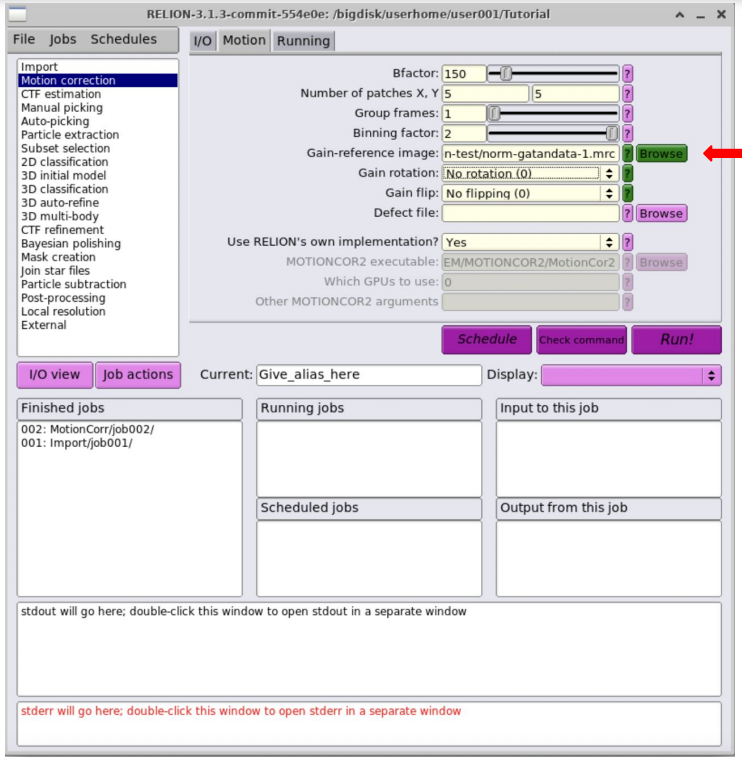
STAR Files

Self-defining Text Archive and Retrieval (STAR)

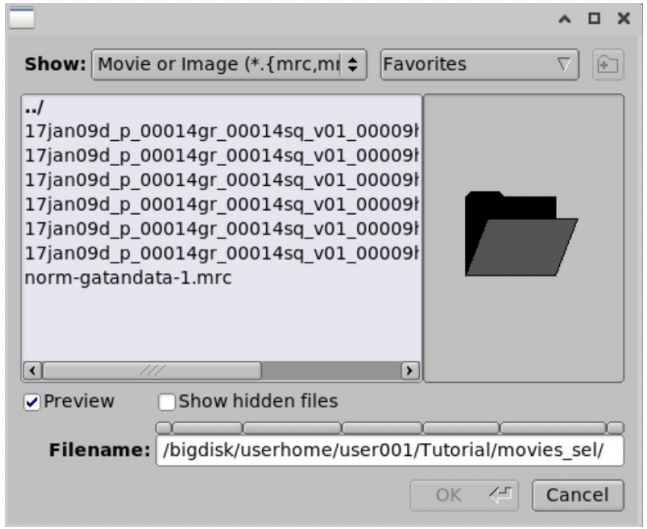
- First proposed in 1991
- Text-based file format for structured data
- Widely used in molecular-structural sciences but not exclusive



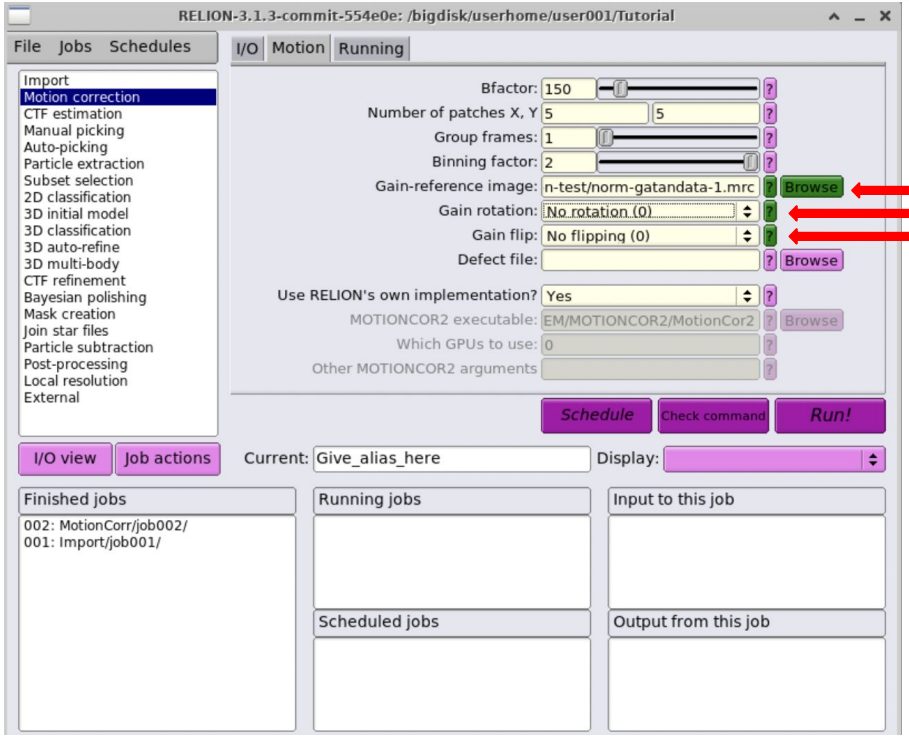
Motion correction



Gain-reference image:
movies_sel/norm-gatandata-1.mrc



Motion correction

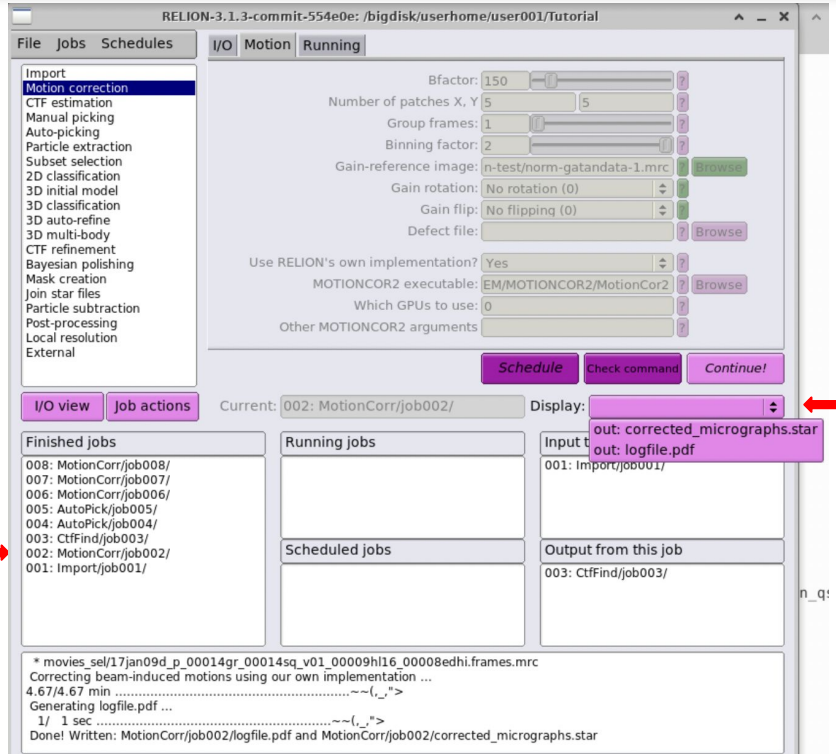


Try different gain orientations and visualize effect on motion correction

Computational demand circumvented by precalculated results

Run!

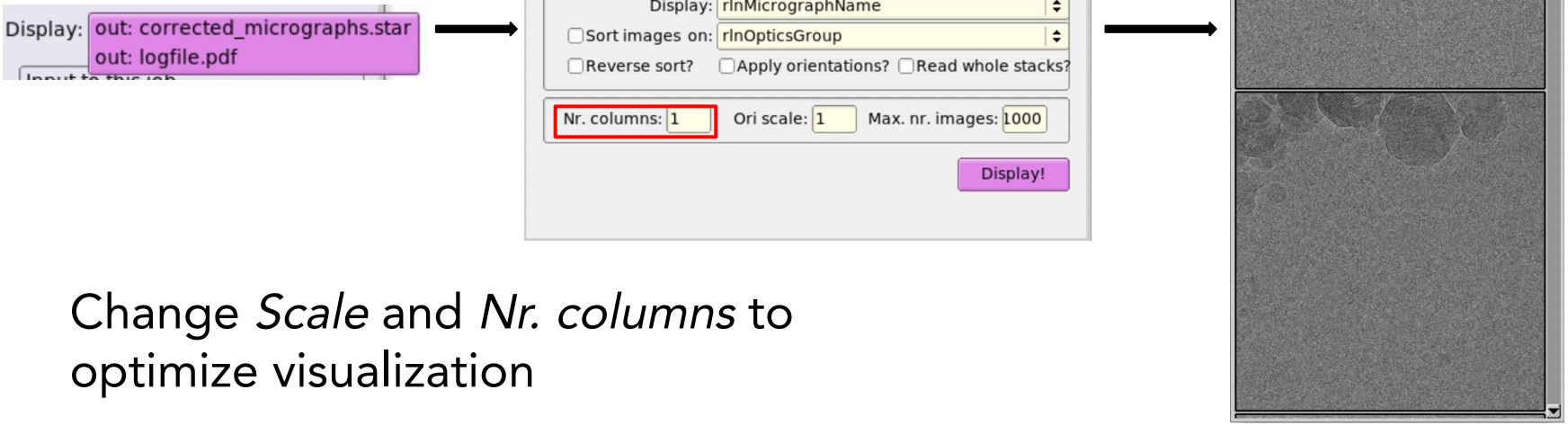
Visualizing/Assessing motion correction results



Select the job whose results you want to view in the *Finished jobs* panel

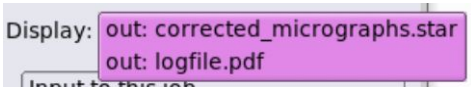
Hitting *Display* will show a drop-down menu of viewable results

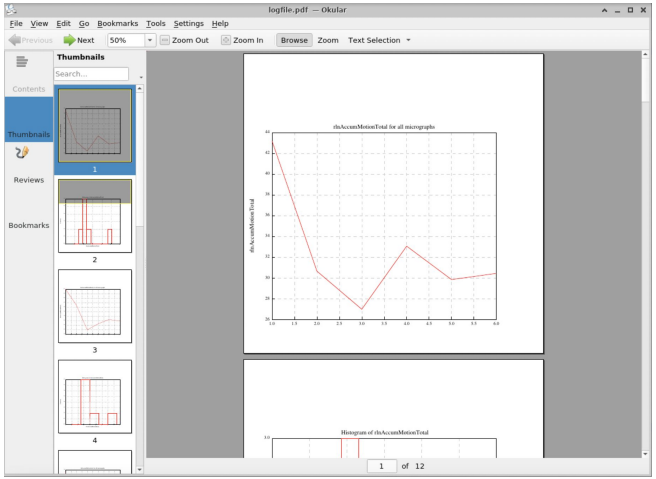
Visualizing/Assessing motion correction results

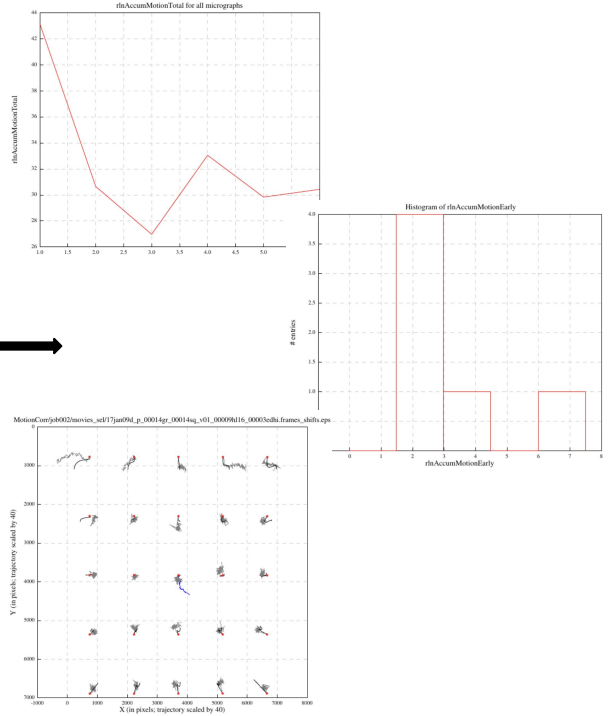


Change *Scale* and *Nr. columns* to optimize visualization

Visualizing/Assessing motion correction results

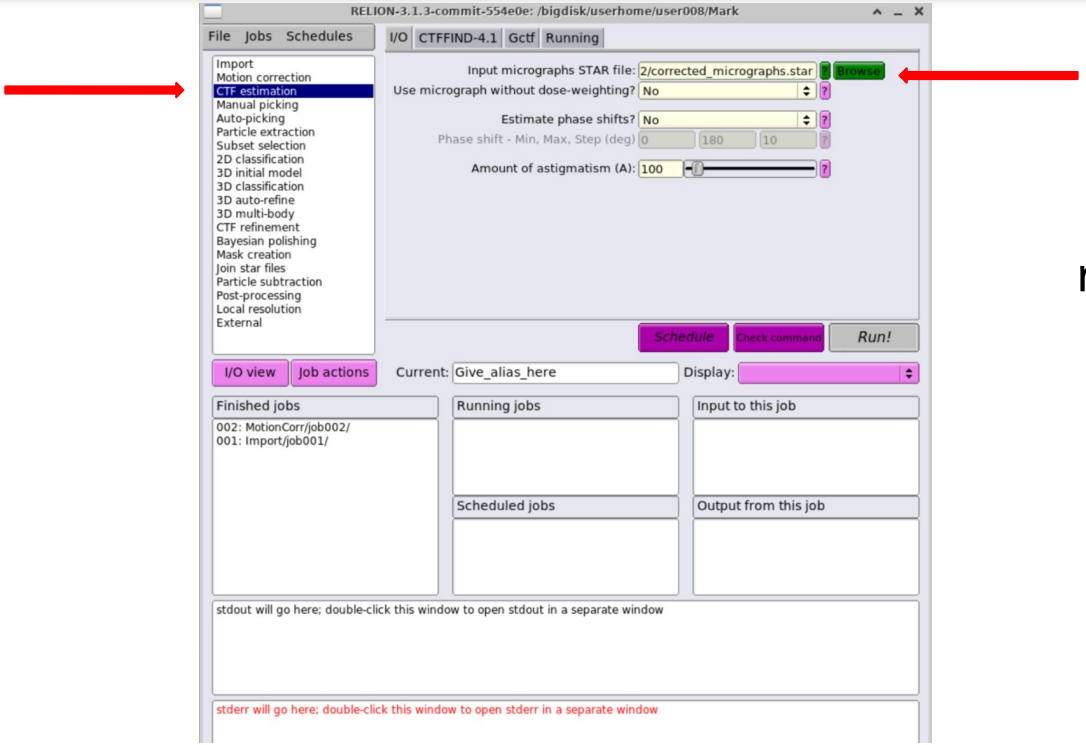






Log files provide useful quantitative information on motion

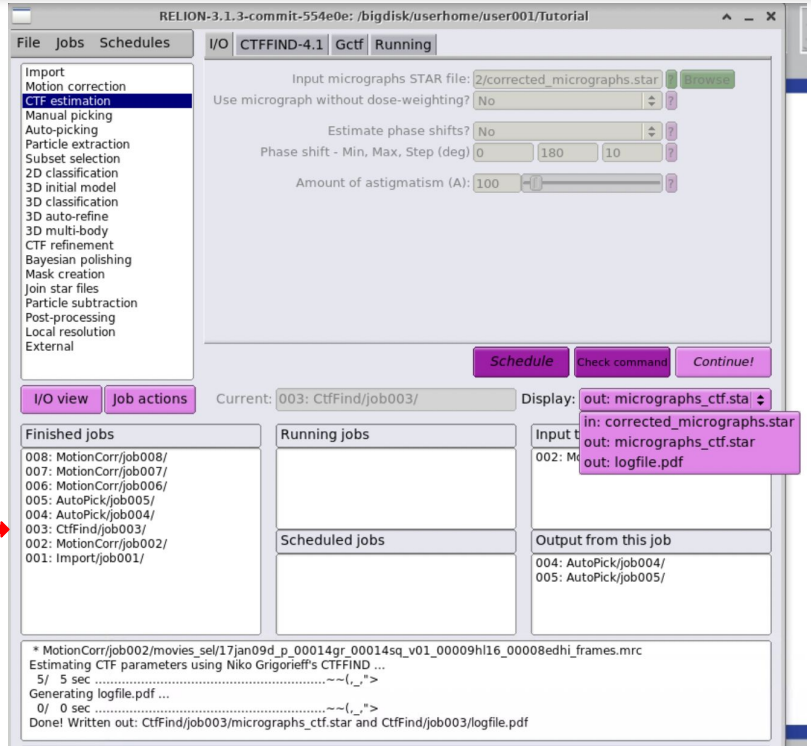
CTF estimation

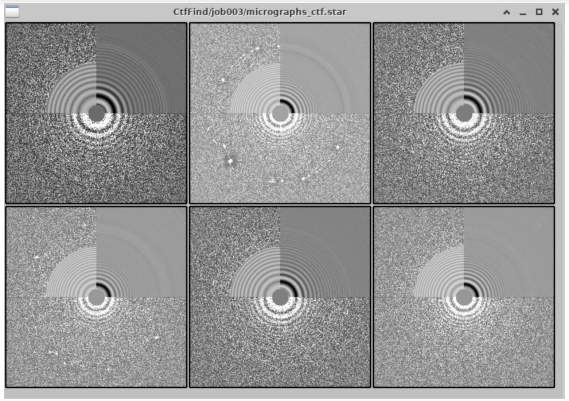


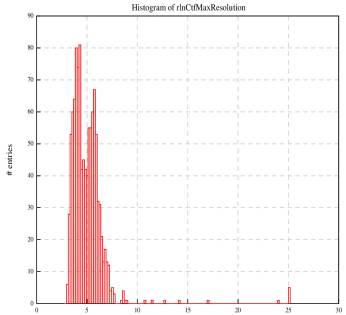
Select the STAR file from your motion corrected micrographs: *corrected _micrographs.star*

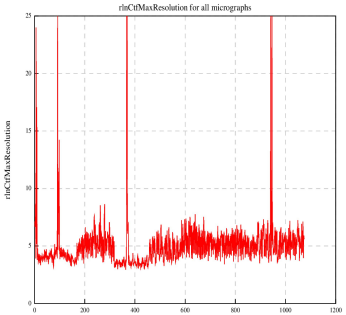
Run!

Visualizing/Assessing CTF estimation results



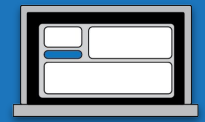






Data shown from a much larger dataset

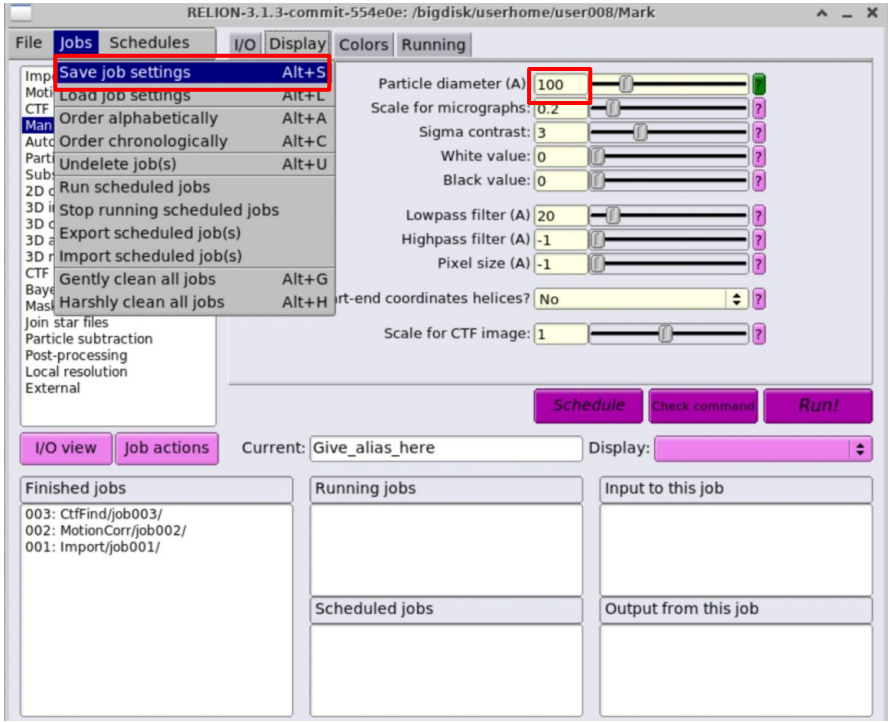
Break!



MiTeGen

www.mitegen.com

Manual picking settings



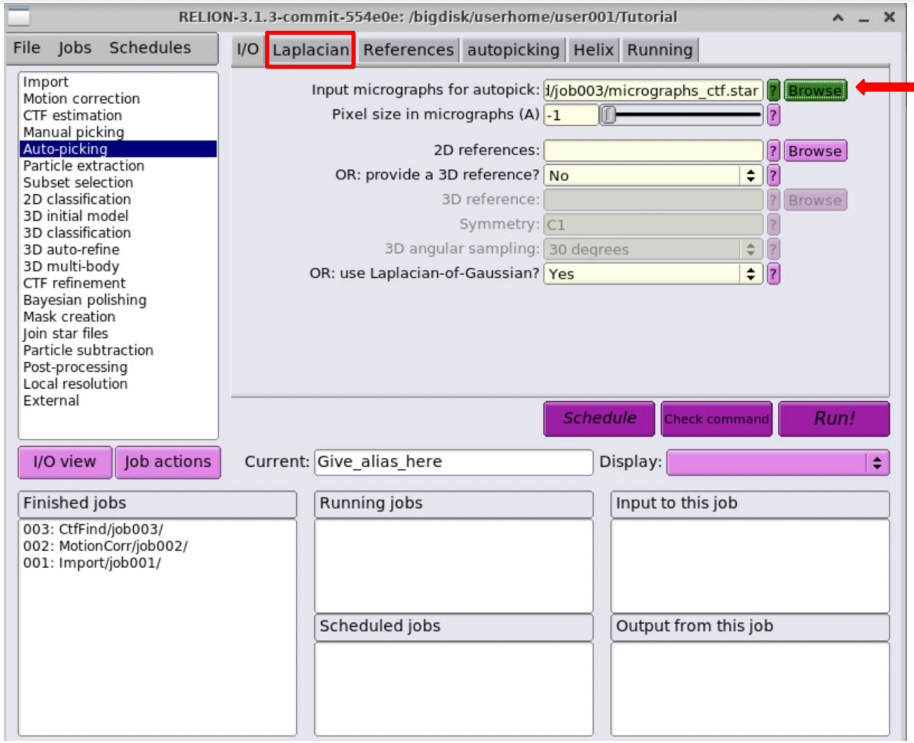
To ensure Autopick results display…

Navigate to the *Manual picking* job

Particle diameter (Å): 100
Jobs → Save job settings

No need to Run job

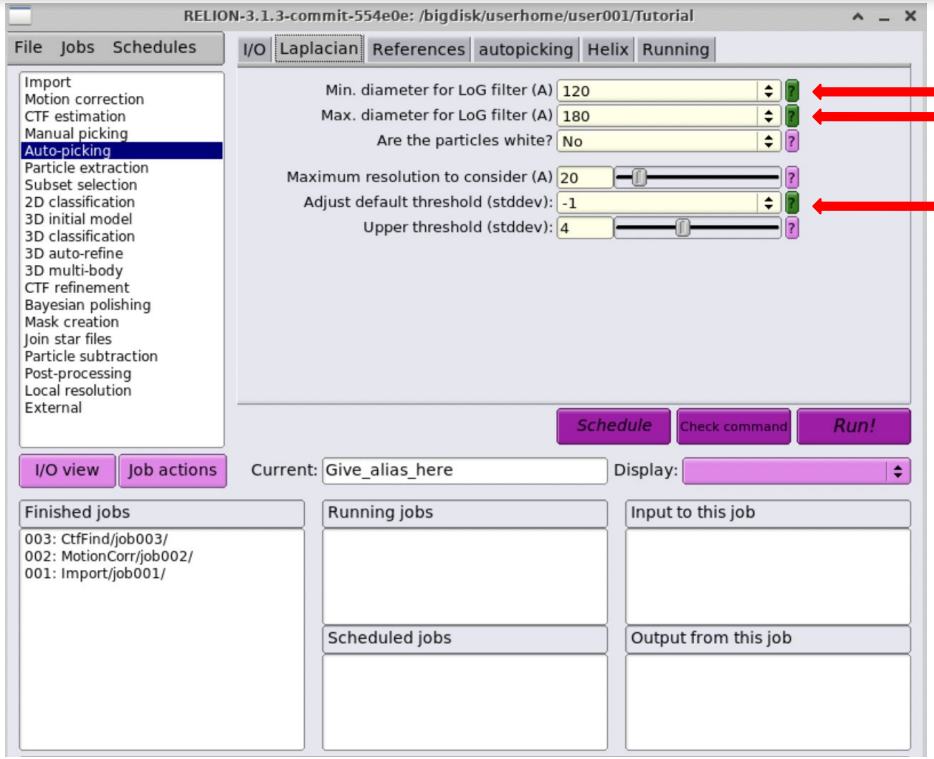
Autopicking



Select the STAR file from your CTF estimation job: *micrographs_ctf.star*

Navigate to the *Laplacian* tab

Autopicking

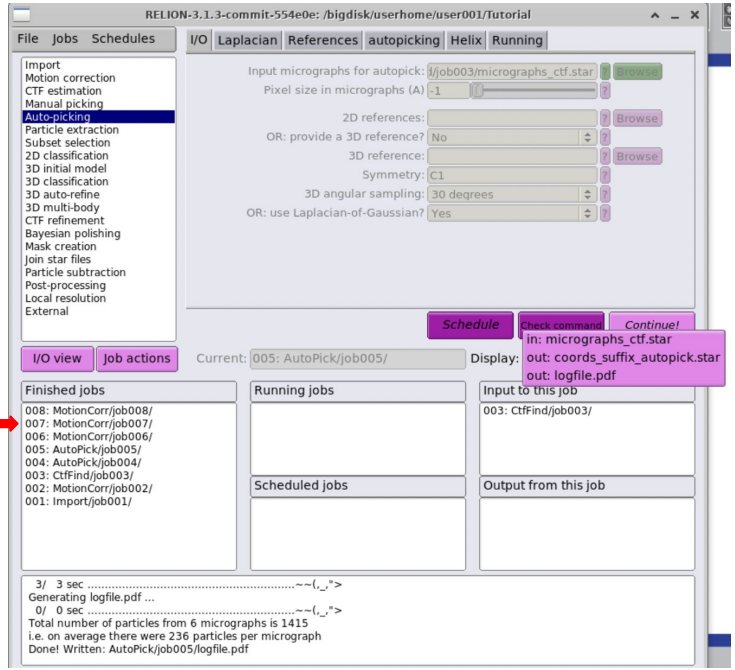


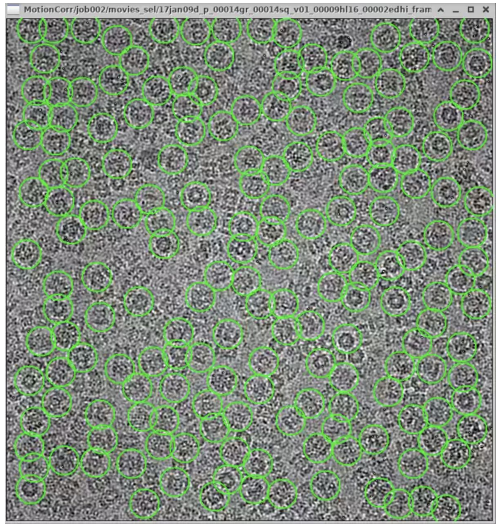
Test out different parameters to optimize autopicking!

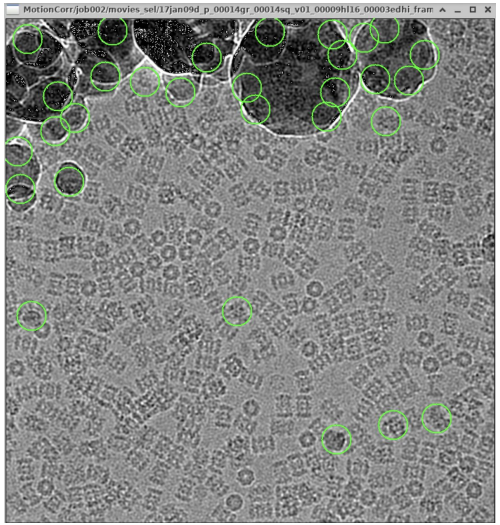
Run!

Computational demand circumvented by precalculated results

Visualizing/Assessing Autopicking results

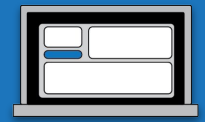






Changes to particle diameters and thresholding significantly alter particle picking

Lunch Break!

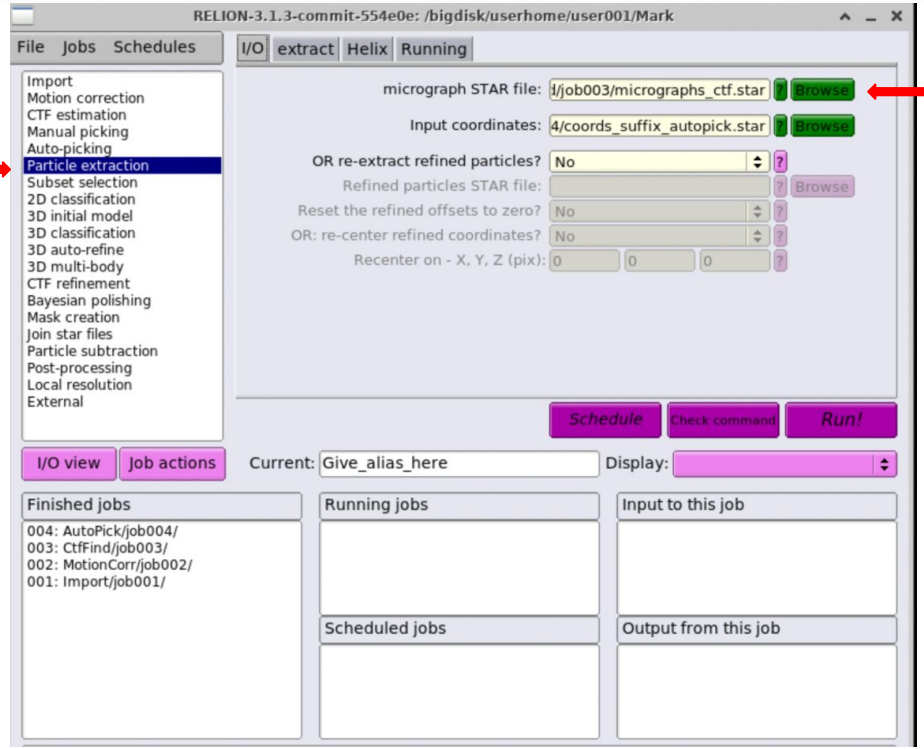


Try out different parameters from

- Motion correction
- CTF estimation
- Autopicking

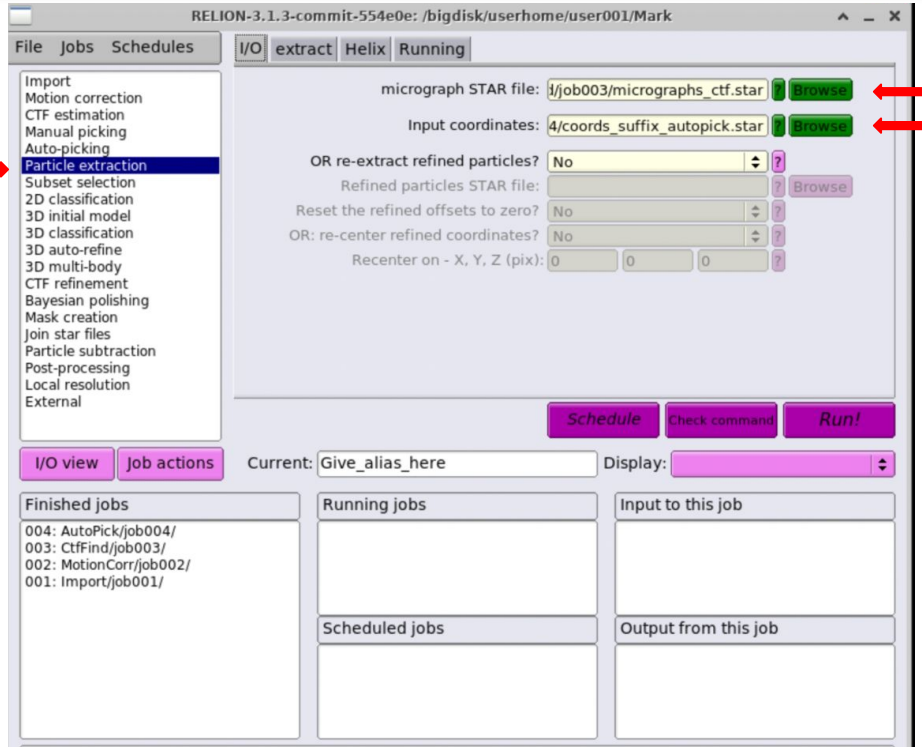
To optimize your particle picks!

Particle extraction



STAR file from your CTF estimation: *micrographs_ctf.star*

Particle extraction

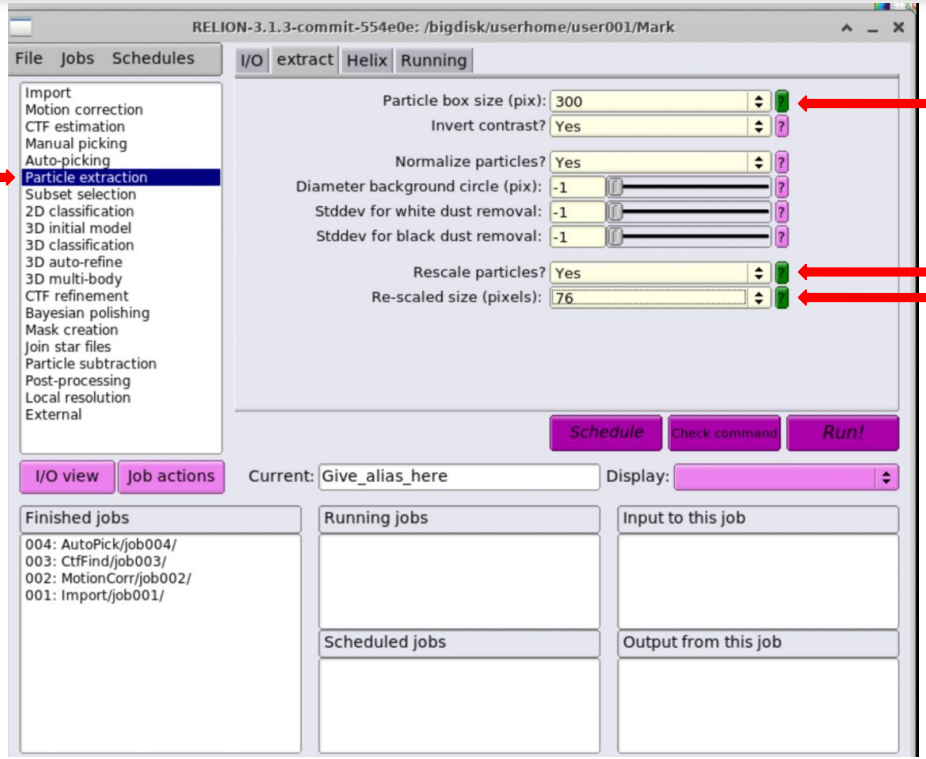


STAR file from your CTF estimation:
micrographs_ctf.star

Input coordinates:
coords_suffix_autopick.star

Navigate to the *extract* tab

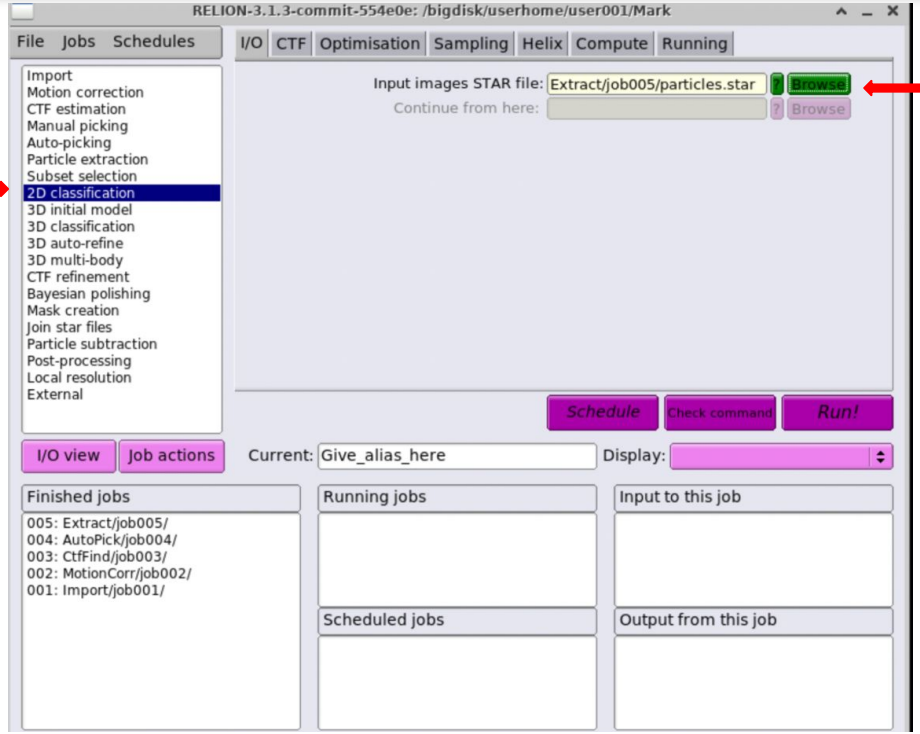
Particle extraction



Test out different extraction box and scaling parameters!

Run!

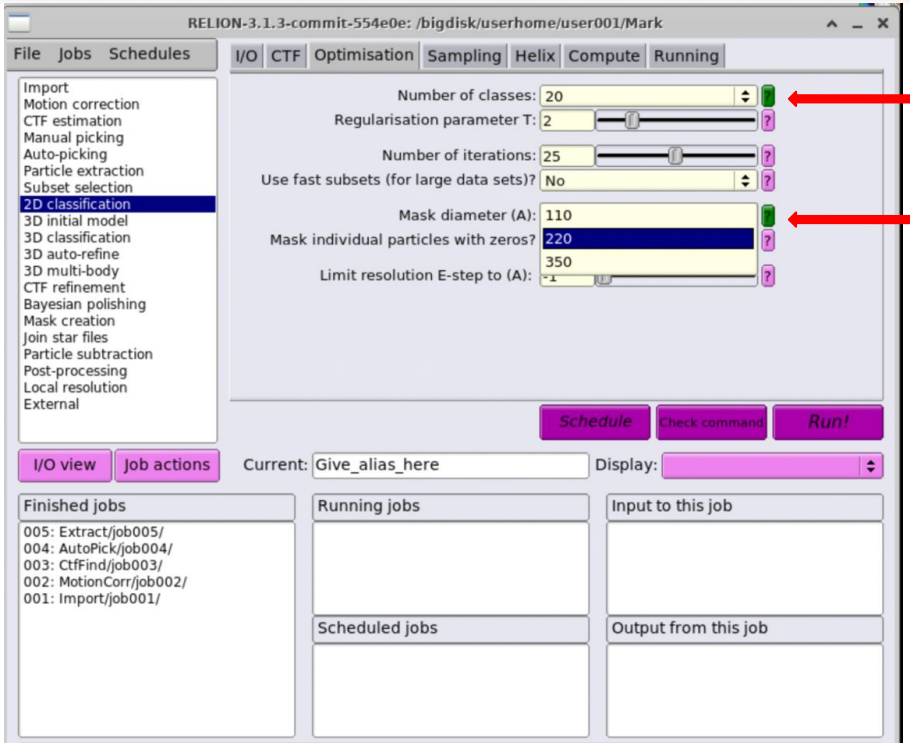
2-D Classification



STAR file from particle extraction: *particles.star*

Navigate to the *Optimisation* tab

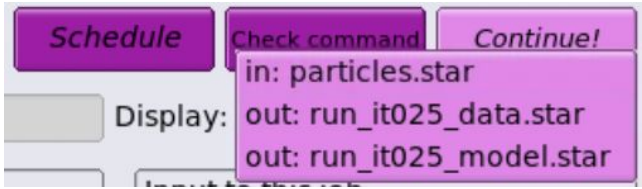
2-D Classification



Test out different 2-D classification parameters!

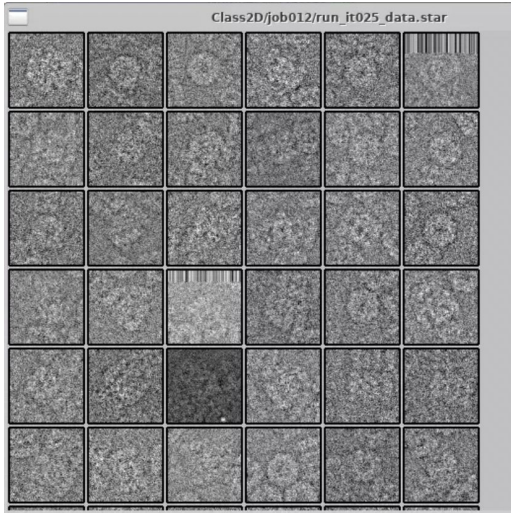
Run!

Visualizing/Assessing 2-D classification results



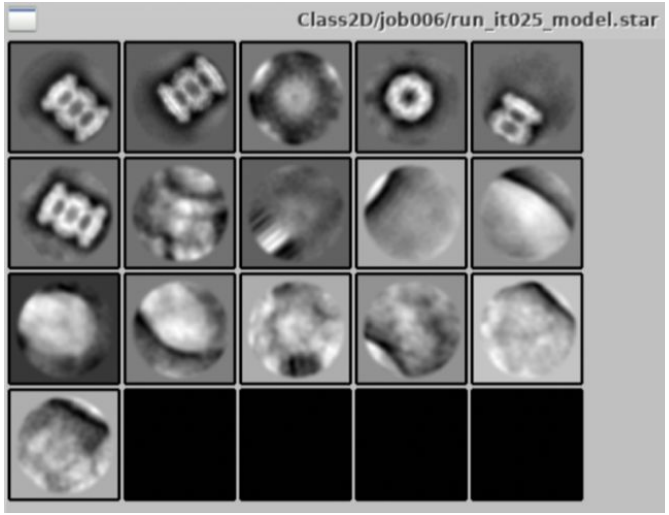
data.star

Individual particles



model.star

Class averages



Go forth and simulate!

More precalculated results coming soon!