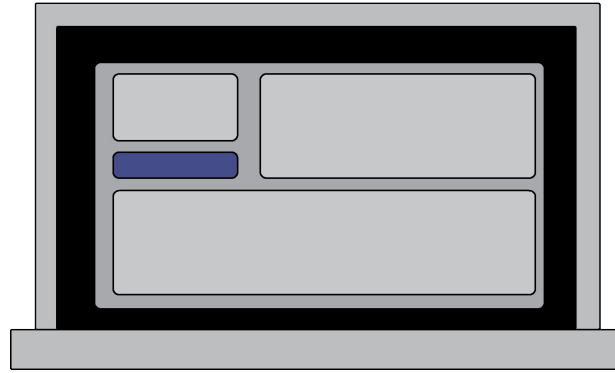


# Introduction to the RELION Simulator



cryoEDU

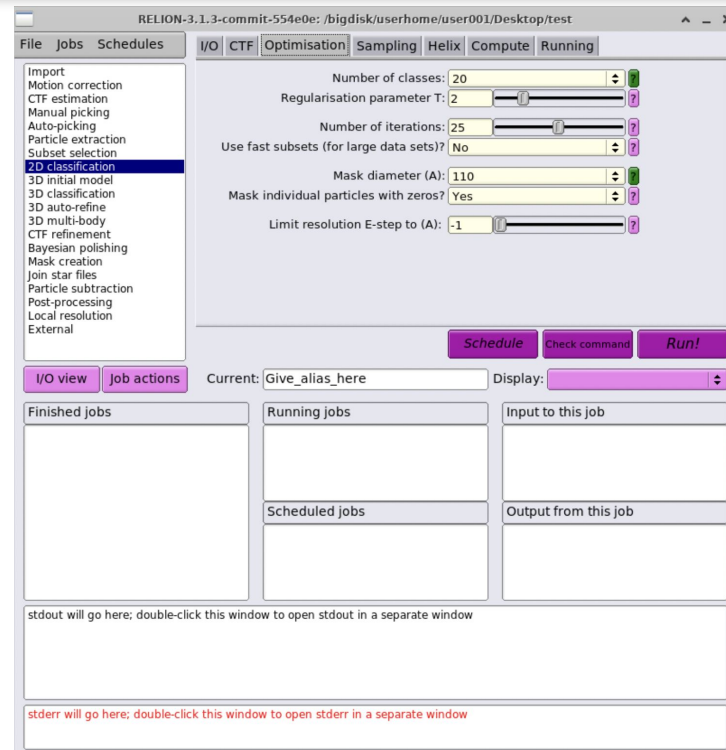


1. Logging onto and opening the RELION simulator on a cloud desktop environment
2. Practical Guide to the RELION simulator

# 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



# How to use the simulator



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

RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Desktop/test

File Jobs Schedules

Movies/mics Others Running

Import

Motion correction

CTF estimation

Manual picking

Auto-picking

Particle extraction

Subset selection

2D classification

3D initial model

3D classification

3D auto-refine

3D multi-body

CTF refinement

Bayesian polishing

Mask creation

Join star files

Particle subtraction

Post-processing

Local resolution

External

Import raw movies/micrographs? Yes

Raw input files: Micrographs/\*.tif **Browse**

Are these multi-frame movies? Yes

Optics group name: opticsGroup1

MTF of the detector: **Browse**

Pixel size (Angstrom): 0.455

Voltage (kV): 200

Spherical aberration (mm): 2.7

Amplitude contrast: 0.1

Beamtilt in X (mrad): 0

Beamtilt in Y (mrad): 0

Schedule Check command Run!

Current: Give\_alias\_here Display:

RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Desktop/test

File Jobs Schedules

I/O CTF Optimisation Sampling Helix Compute Running

Import

Motion correction

CTF estimation

Manual picking

Auto-picking

Particle extraction

Subset selection

2D classification

3D initial model

3D classification

3D auto-refine

3D multi-body

CTF refinement

Bayesian polishing

Mask creation

Join star files

Particle subtraction

Post-processing

Local resolution

External

Number of classes: 20

Regularisation parameter T: 2

Number of iterations: 25

Use fast subsets (for large data sets)? No

Mask diameter (A): 110

Mask individual particles with zeros? Yes

Limit resolution E-step to (A): -1

Schedule Check command Run!

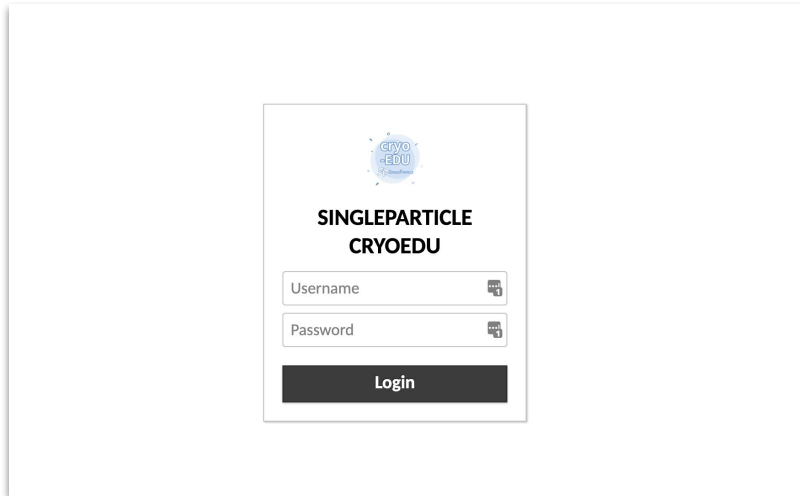
Current: Give\_alias\_here Display:


# 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>



  
**SINGLEPARTICLE  
CRYOEDU**

Username

Password

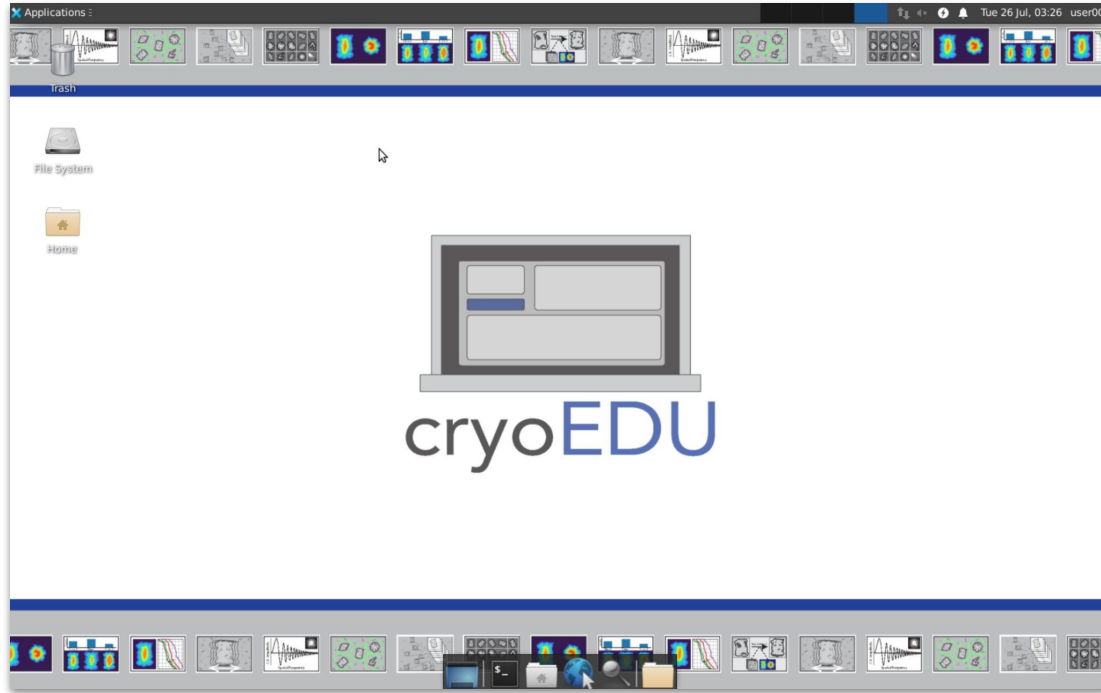
**Login**

*Username & password  
provided separately*

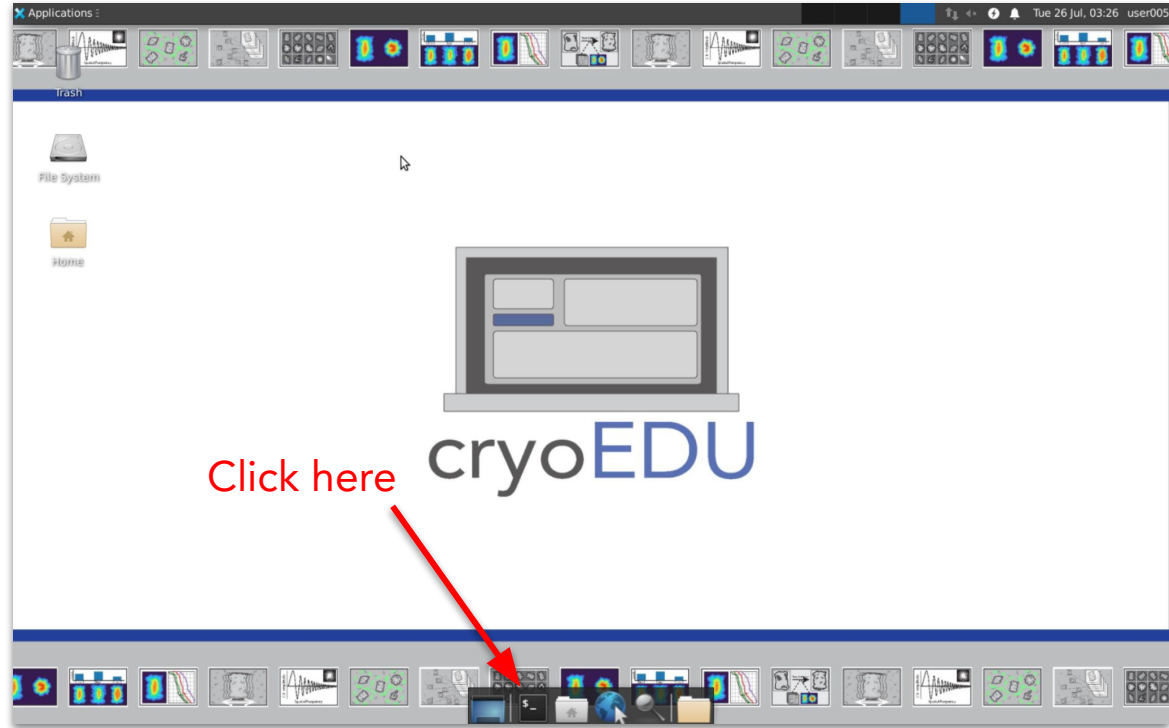
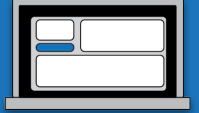
# The cloud desktop



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

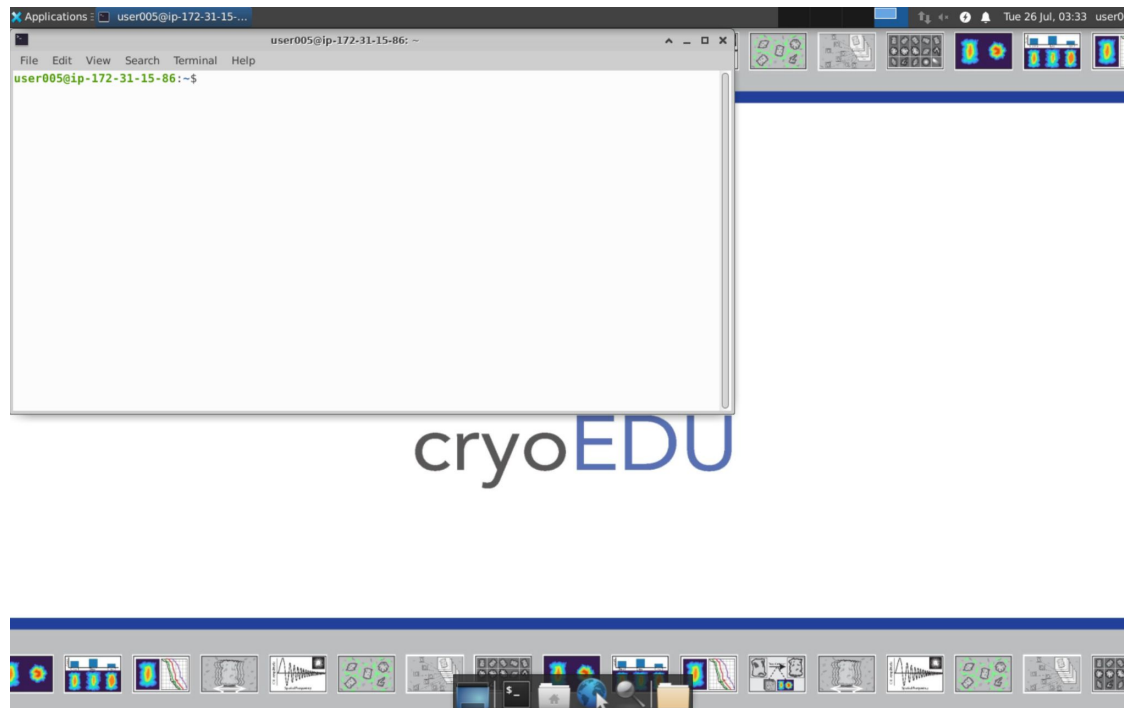


# Opening the terminal



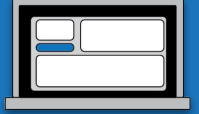
Click here

# 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



```
user005@ip-172-31-15-86: ~  
File Edit View Search Terminal Help  
user005@ip-172-31-15-86:~$ ls  
Desktop Downloads Pictures Templates gny mike-test mike-test-250722 slurm-client  
Documents Music Public Videos lw mike-test-240722 relion-test  
user005@ip-172-31-15-86:~$ mkdir Tutorial  
user005@ip-172-31-15-86:~$ ls  
Desktop Downloads Pictures Templates Videos lw mike-test-240722 relion-test  
Documents Music Public Tutorial gny mike-test mike-test-250722 slurm-client  
user005@ip-172-31-15-86:~$
```

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*

```
user001@ip-172-31-15-86: ~/Tutorial
File Edit View Search Terminal Help
user001@ip-172-31-15-86:~$ ls
Desktop  Downloads  Pictures  Templates  Videos      slurm-client
Documents Music      Public    Tutorial   relion-test
user001@ip-172-31-15-86:~$ cd T
bash: cd: T: No such file or directory
user001@ip-172-31-15-86:~$ cd Tutorial/
user001@ip-172-31-15-86:~/Tutorial$ ls
AutoPick  CtfFind  Import  MotionCorr  default_pipeline.star  movies_sel  track.txt
user001@ip-172-31-15-86:~/Tutorial$ source /etc/profile.d/singleparticle.sh ←
user001@ip-172-31-15-86:~/Tutorial$
```

# Import dataset



Import dataset:

*ln -s /bigdisk/sptrain/fulldataset/movies\_sel*

```
user005@ip-172-31-15-86: ~
File Edit View Search Terminal Help
user005@ip-172-31-15-86:~$ ls
Desktop  Extract  Public  Videos  mike-test      relion-test
Documents Music    Templates gny      mike-test-240722 slurm-client
Downloads Pictures Tutorial lw        mike-test-250722
user005@ip-172-31-15-86:~$ rmdir -r Extract
rmdir: invalid option -- 'r'
Try 'rmdir --help' for more information.
user005@ip-172-31-15-86:~$ rmdir r Extract
rmdir: failed to remove 'r': No such file or directory
rmdir: failed to remove 'Extract': Not a directory
user005@ip-172-31-15-86:~$ rm -r Extract
user005@ip-172-31-15-86:~$ ls
Desktop  Downloads  Pictures  Templates  Videos  lw        mike-test-240722  relion-test
Documents Music      Public   Tutorial   gny      mike-test  mike-test-250722  slurm-client
user005@ip-172-31-15-86:~$ ln -s /bigdisk/sptrain/fulldataset/movies_sel
user005@ip-172-31-15-86:~$
```

# Dataset for the cryoEDU RELION simulator



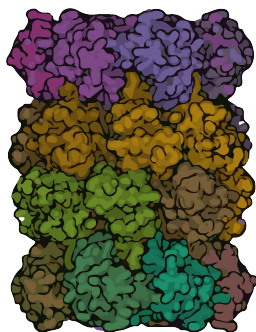
Published: 09 October 2017

## 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](#)

10k Accesses | 90 Citations | 47 Altmetric | [Metrics](#)



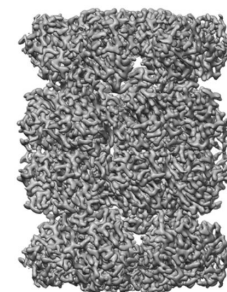
## EMPIAR-10185

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](https://doi.org/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](https://doi.org/10.6019/EMPIAR-10185)  
**Experimental metadata:** [Download xml](#)

**Contains:**  
 micrographs



# Start up RELION!



user005@ip-172-31-15-86:~/Tutorial\$ relion

RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user005/Tutorial

File Jobs Schedules

- Import
- Motion correction
- CTF estimation
- Manual picking
- Auto-picking
- Particle extraction
- Subset selection
- 2D classification
- 3D initial model
- 3D classification
- 3D auto-refine
- 3D multi-body
- CTF refinement
- Bayesian polishing
- Mask creation
- Join star files
- Particle subtraction
- Post-processing
- Local resolution
- External

SciLifeLab

MRC Laboratory of Molecular Biology www.mrc-lmb.cam.ac.uk/relion

Schedule Check command Run!

I/O view job actions Current: Give\_alias\_here Display: [v]

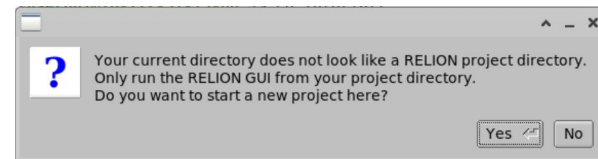
Finished jobs	Running jobs	Input to this job

stdout will go here; double-click this window to open stdout in a separate window

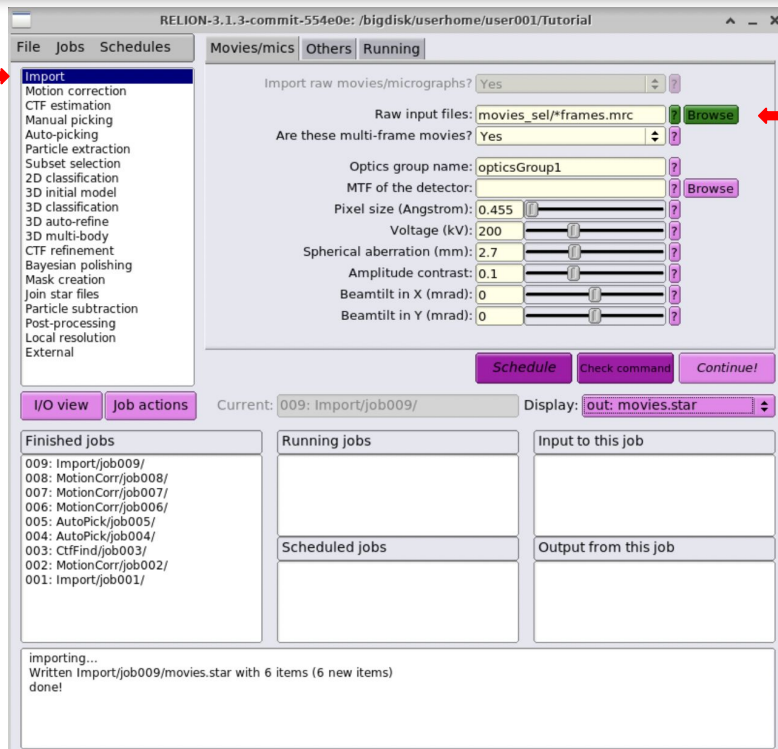
stderr will go here; double-click this window to open stderr in a separate window

In your directory:  
*relion*

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

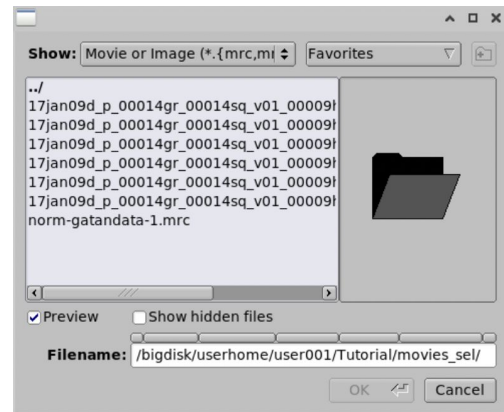


# Import movies



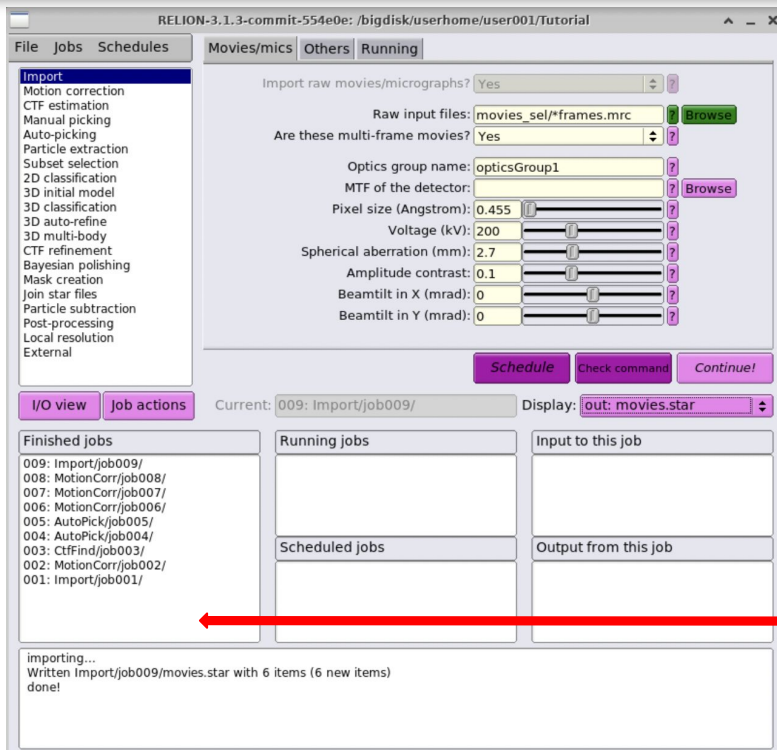
Hit the *Import* tab on the left

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$
```

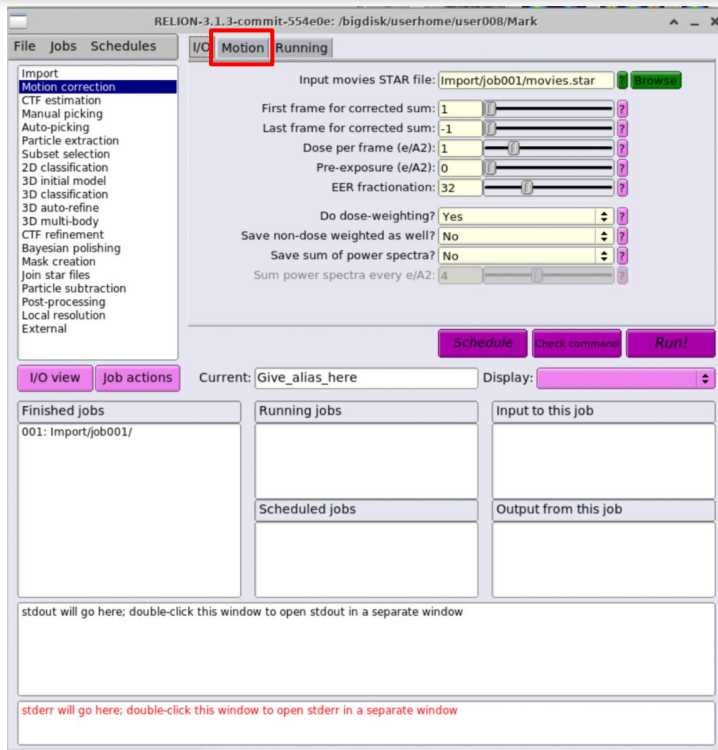
Directories created for each job type

```
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



## 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

```
user001@ip-172-31-15-86: ~/Tutorial/MotionCorr/job002
File Edit View Search Terminal Help
# version 30001

data_optics

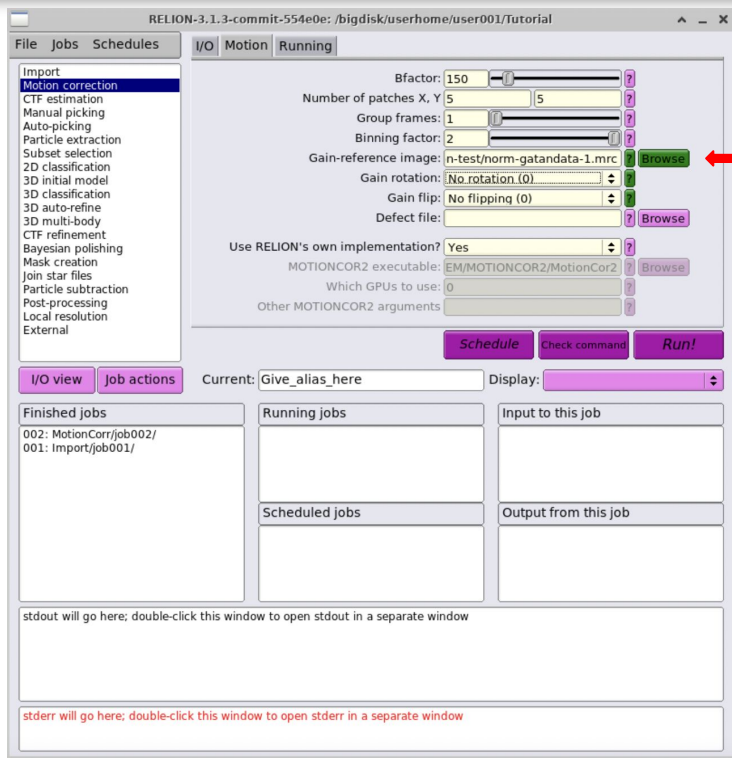
loop
_rlnOpticsGroupName #1
_rlnOpticsGroup #2
_rlnMicrographOriginalPixelSize #3
_rlnVoltage #4
_rlnSphericalAberration #5
_rlnAmplitudeContrast #6
_rlnMicrographPixelSize #7
opticsGroup1      1      0.455000      200.000000      2.700000      0.100000      0.910000

# version 30001

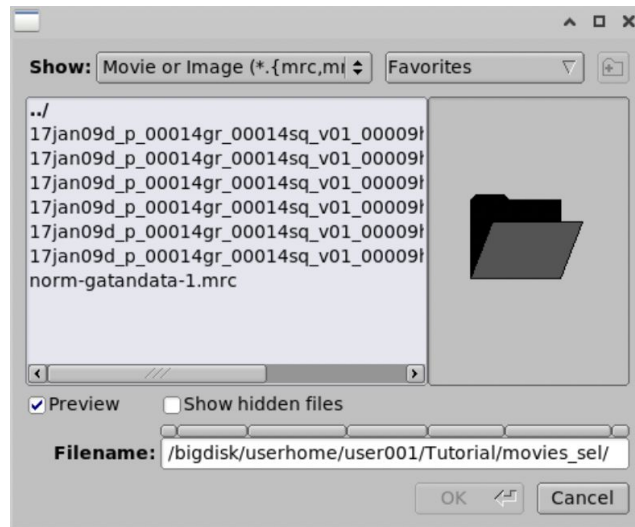
data_micrographs

loop
_rlnMicrographName #1
_rlnMicrographMetadata #2
_rlnOpticsGroup #3
_rlnAccumMotionTotal #4
_rlnAccumMotionEarly #5
_rlnAccumMotionLate #6
MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00002edhi_frames.mrc MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00002edhi_frames.star      1      4
3.188346      5.990562      37.197784
MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00003edhi_frames.mrc MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00003edhi_frames.star      1      3
0.648989      4.210024      26.438965
MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00004edhi_frames.mrc MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00004edhi_frames.star      1      2
6.976145      1.480148      25.495997
MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00005edhi_frames.mrc MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00005edhi_frames.star      1      3
3.052950      2.152053      30.906897
MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00007edhi_frames.mrc MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00007edhi_frames.star      1      2
9.836353      2.630926      27.205427
MotionCorr/job002/movies_sel/17jan09d_p_00014gr_00014sq_v01_00009hl16_00008edhi_frames.mrc MotionCorr/job002/
```

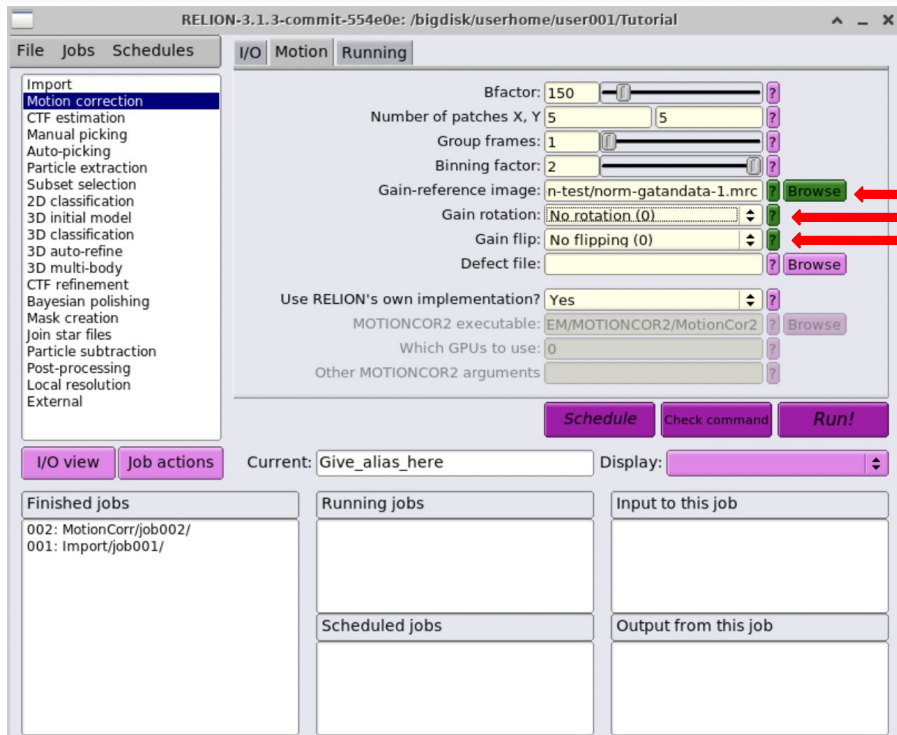
# Motion correction



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



The screenshot shows the RELION-3.1.3 interface with the 'Motion' tab selected. The 'Motion correction' job is highlighted in the left sidebar. The main panel displays configuration parameters for motion correction, including Bfactor (150), Number of patches X, Y (5, 5), Group frames (1), Binning factor (2), Gain-reference image, Gain rotation, Gain flip, Defect file, and MOTIONCOR2 executable. Below the configuration are buttons for 'Schedule', 'Check command', and 'Continue!'. At the bottom, there are panels for 'Finished jobs', 'Running jobs', 'Scheduled jobs', 'Input', and 'Output from this job'. A red arrow points to the 'Display' button, which is currently set to 'out: corrected\_micrographs.star'. Another red arrow points to the 'Finished jobs' panel, which lists several jobs, including '002: MotionCorr/job002/'.

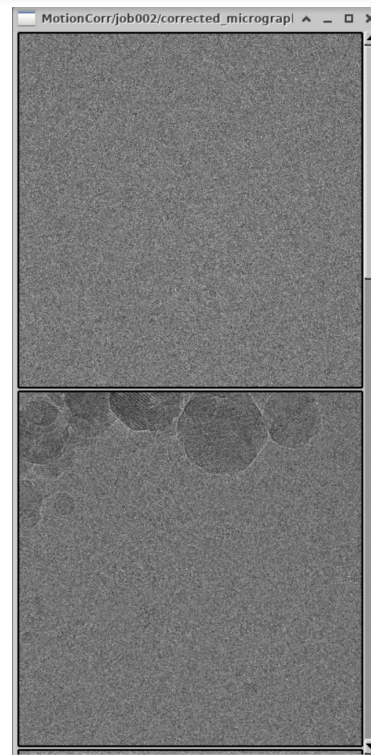
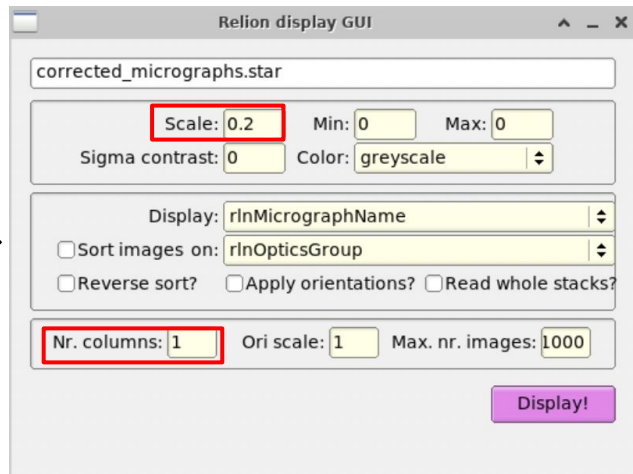
Select the job whose results you want to view in the *Finished jobs* panel

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

# Visualizing/Assessing motion correction results



Display: out: corrected\_micrographs.star  
out: logfile.pdf

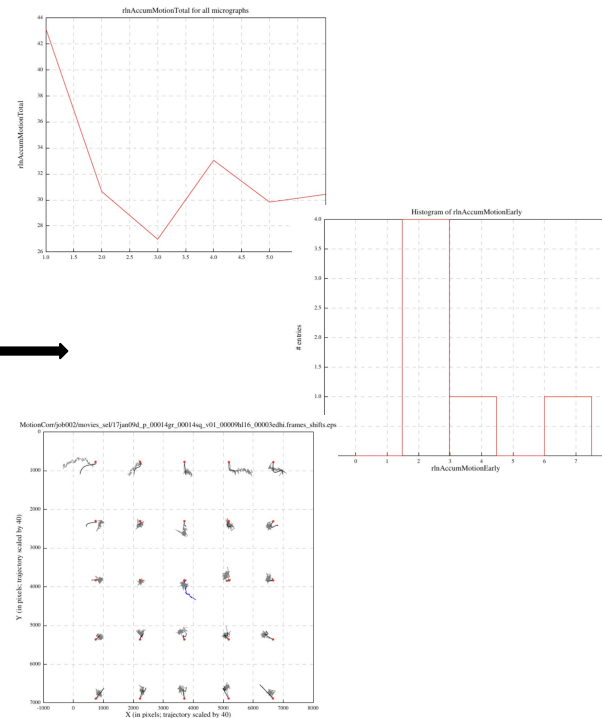
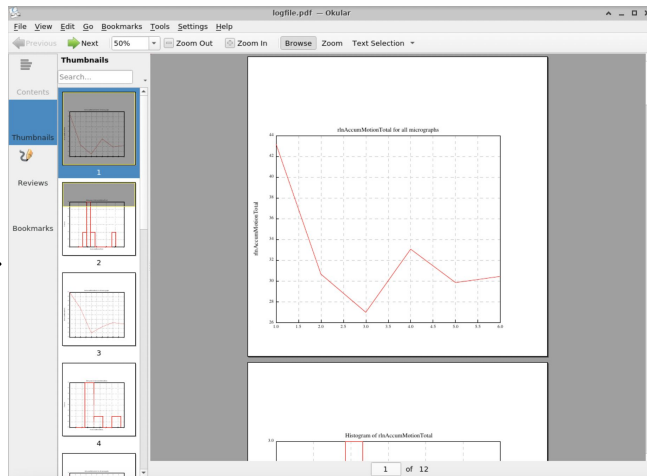


Change *Scale* and *Nr. columns* to optimize visualization

# Visualizing/Assessing motion correction results



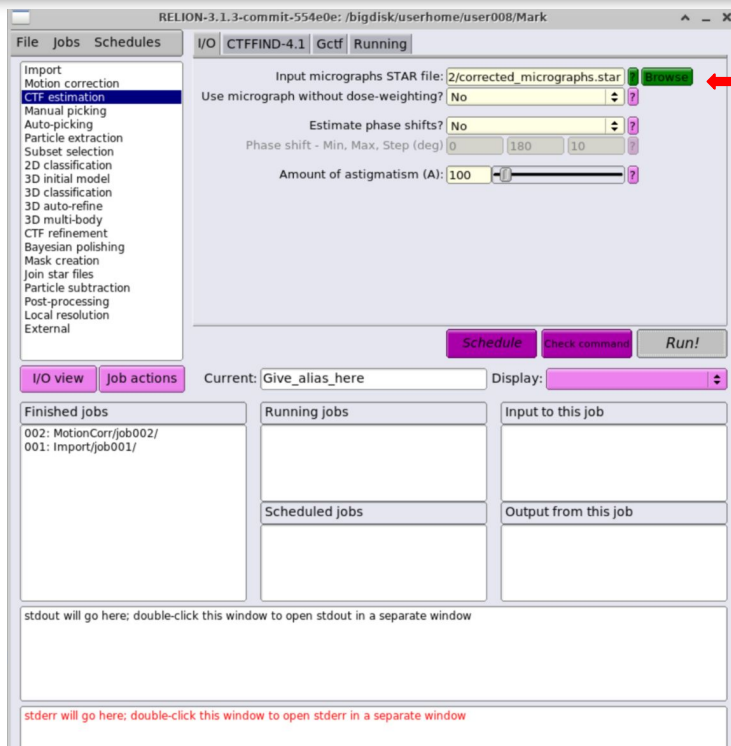
Display: out: corrected\_micrographs.star  
out: logfile.pdf



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



RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial

File Jobs Schedules

I/O CTFIND-4.1 Gctf Running

Input micrographs STAR file: 2/corrected\_micrographs.star

Use micrograph without dose-weighting? No

Estimate phase shifts? No

Phase shift - Min, Max, Step (deg) 0 180 10

Amount of astigmatism (A): 100

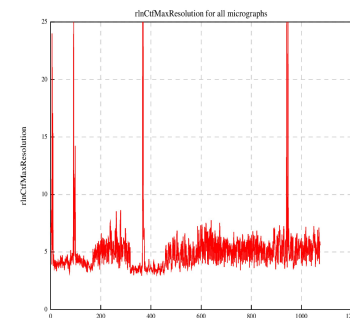
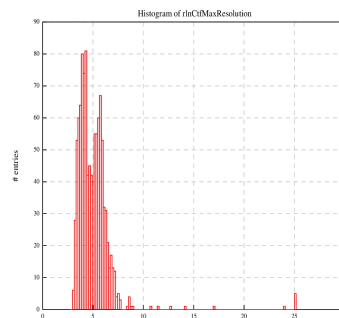
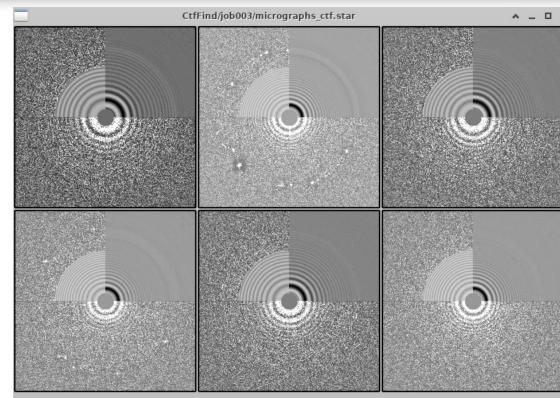
I/O view job actions Current: 003: CtfFind/job003/ Display: out: micrographs\_ctf.star

in: corrected\_micrographs.star  
out: micrographs\_ctf.star  
out: logfile.pdf

Finished jobs	Running jobs	Input files
008: MotionCorr/job008/ 007: MotionCorr/job007/ 006: MotionCorr/job006/ 005: AutoPick/job005/ 004: AutoPick/job004/ 003: CtfFind/job003/ 002: MotionCorr/job002/ 001: Import/job001/		002: M...

Scheduled jobs	Output from this job
	004: AutoPick/job004/ 005: AutoPick/job005/

\* MotionCorr/job002/movies\_sel/17jan09d\_p\_00014gr\_00014sq\_v01\_00009h16\_00008edhi\_frames.mrc  
Estimating CTF parameters using Niko Grigorieff's CTFIND ...  
5/ 5 sec .....~(,\_">  
Generating logfile.pdf ...  
0/ 0 sec .....~(,\_">  
Done! Written out: CtfFind/job003/micrographs\_ctf.star and CtfFind/job003/logfile.pdf

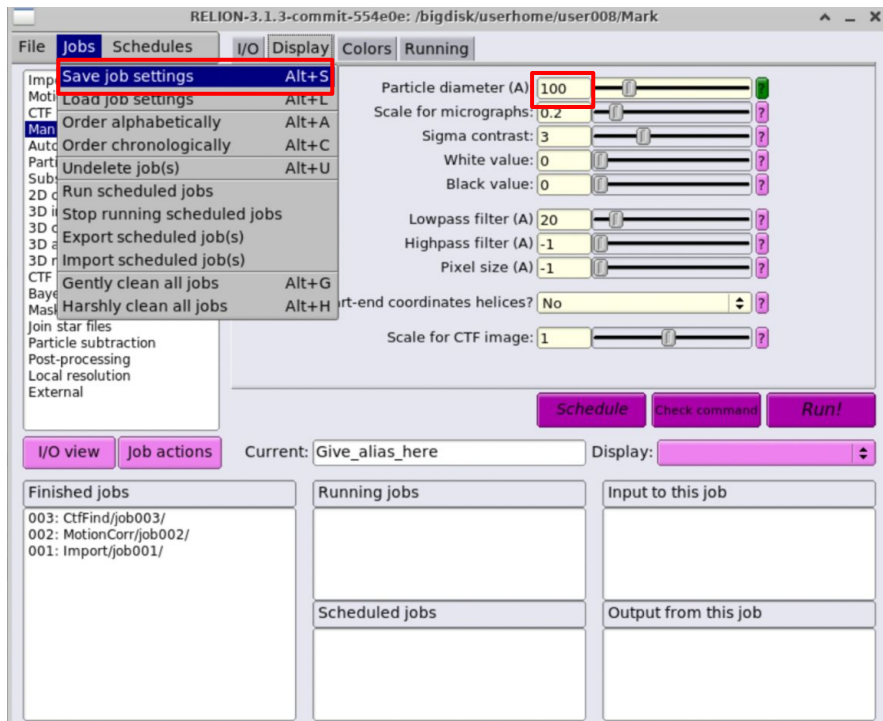


Data shown from  
a much larger  
dataset

Break!



# Manual picking settings



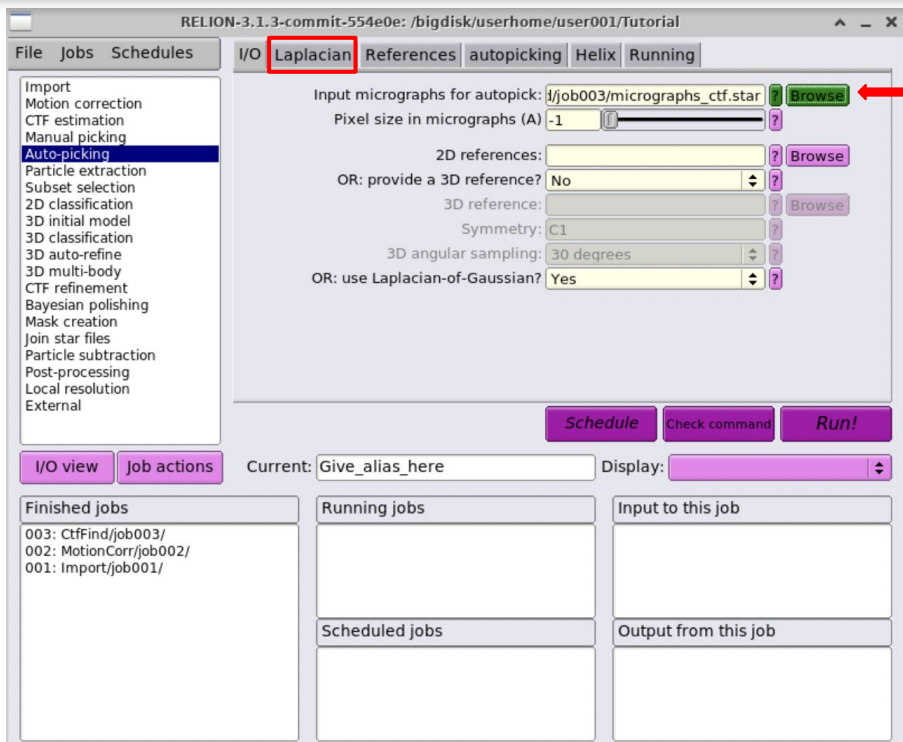
To ensure Autopick results display...

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



RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial

File Jobs Schedules I/O Laplacian References autopicking Helix Running

Import  
Motion correction  
CTF estimation  
Manual picking  
**Auto-picking**  
Particle extraction  
Subset selection  
2D classification  
3D initial model  
3D classification  
3D auto-refine  
3D multi-body  
CTF refinement  
Bayesian polishing  
Mask creation  
Join star files  
Particle subtraction  
Post-processing  
Local resolution  
External

Min. diameter for LoG filter (A) 120  
Max. diameter for LoG filter (A) 180  
Are the particles white? No  
Maximum resolution to consider (A) 20  
Adjust default threshold (stddev): -1  
Upper threshold (stddev): 4

Schedule Check command Run!

I/O view Job actions Current: Give\_alias\_here Display:

Finished jobs  
003: CtfFind/job003/  
002: MotionCorr/job002/  
001: Import/job001/

Running jobs

Input to this job

Scheduled jobs

Output from this job

Test out different parameters to optimize autopicking!

*Run!*

Computational demand  
circumvented by  
precalculated results

# Visualizing/Assessing Autopicking results



RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Tutorial

File Jobs Schedules I/O Laplacian References autopicking Helix Running

Import  
Motion correction  
CTF estimation  
Manual picking  
**Autopicking**  
Particle extraction  
Subset selection  
2D classification  
2D initial model  
3D classification  
3D auto-refine  
3D multi-body  
CTF refinement  
Bayesian polishing  
Mask creation  
Join star files  
Particle subtraction  
Post-processing  
Local resolution  
External

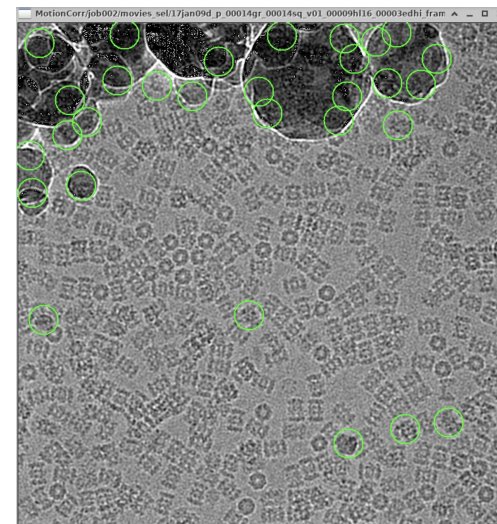
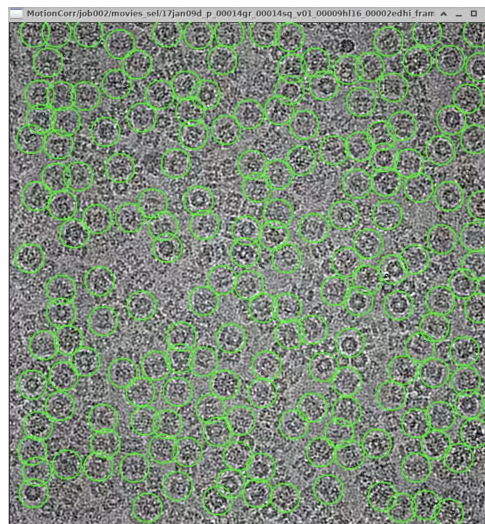
Input micrographs for autopick: /job003/micrographs\_ctf.star   
Pixel size in micrographs (Å) -1   
2D references:    
OR: provide a 3D reference? No   
3D reference:    
Symmetry: C1   
3D angular sampling: 30 degrees   
OR: use Laplacian-of-Gaussian? Yes

Current: 005: AutoPick/job005/ Display: in: micrographs\_ctf.star  
out: coords\_suffix\_autopick.star  
out: logfile.pdf

Finished jobs	Running jobs	Input to this job
008: MotionCorr/job008/ 007: MotionCorr/job007/ 006: MotionCorr/job006/ 005: AutoPick/job005/ 004: AutoPick/job004/ 003: CtfFind/job003/ 002: MotionCorr/job002/ 001: Import/job001/		003: CtfFind/job003/

Scheduled jobs	Output from this job

3/ 3 sec .....~{~>  
Generating logfile.pdf ...  
0/ 0 sec .....~{~>  
Total number of particles from 6 micrographs is 1415  
i.e. on average there were 236 particles per micrograph  
Done! Written: AutoPick/job005/logfile.pdf



Changes to particle diameters and thresholding significantly alter particle picking



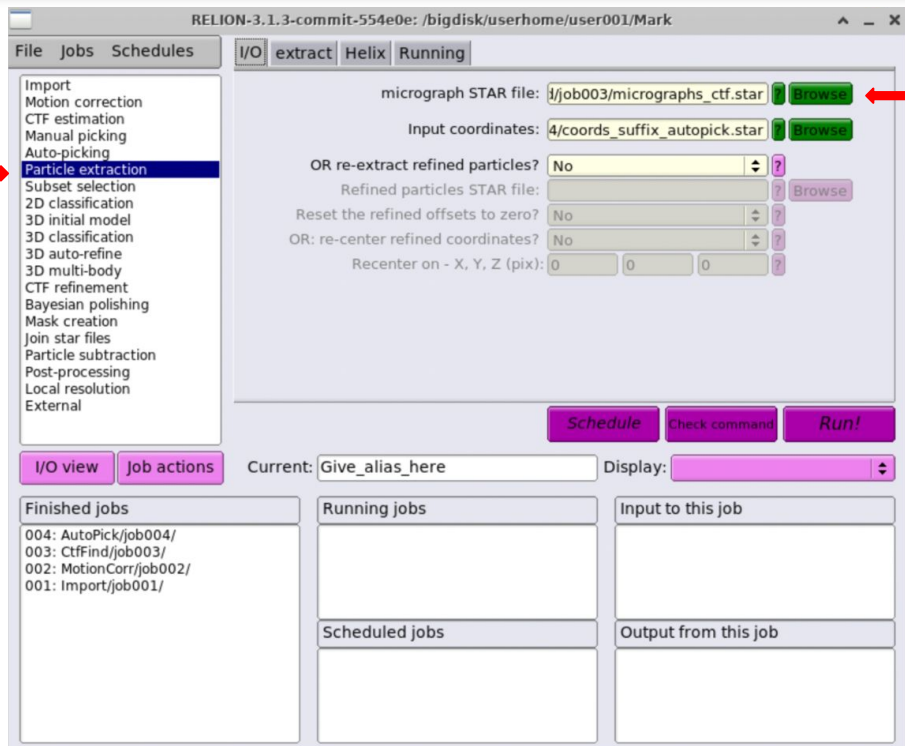
Try out different parameters from

- Motion correction
- CTF estimation
- Autopicking

To optimize your particle picks!

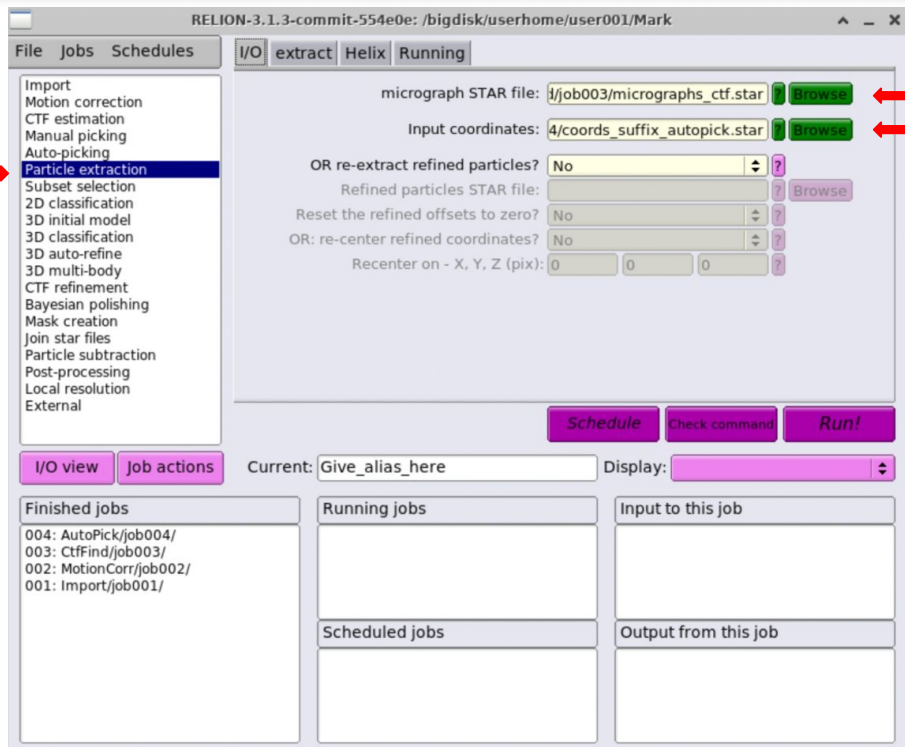


# Particle extraction



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



RELION-3.1.3-commit-554e0e: /bigdisk/userhome/user001/Mark

File Jobs Schedules I/O extract Helix Running

Import  
Motion correction  
CTF estimation  
Manual picking  
Auto-picking  
**Particle extraction**  
Subset selection  
2D classification  
3D initial model  
3D classification  
3D auto-refine  
3D multi-body  
CTF refinement  
Bayesian polishing  
Mask creation  
Join star files  
Particle subtraction  
Post-processing  
Local resolution  
External

Particle box size (pix): 300  
Invert contrast? Yes  
Normalize particles? Yes  
Diameter background circle (pix): -1  
Stddev for white dust removal: -1  
Stddev for black dust removal: -1  
Rescale particles? Yes  
Re-scaled size (pixels): 76

Schedule Check command Run!

I/O view Job actions Current: Give\_alias\_here Display:

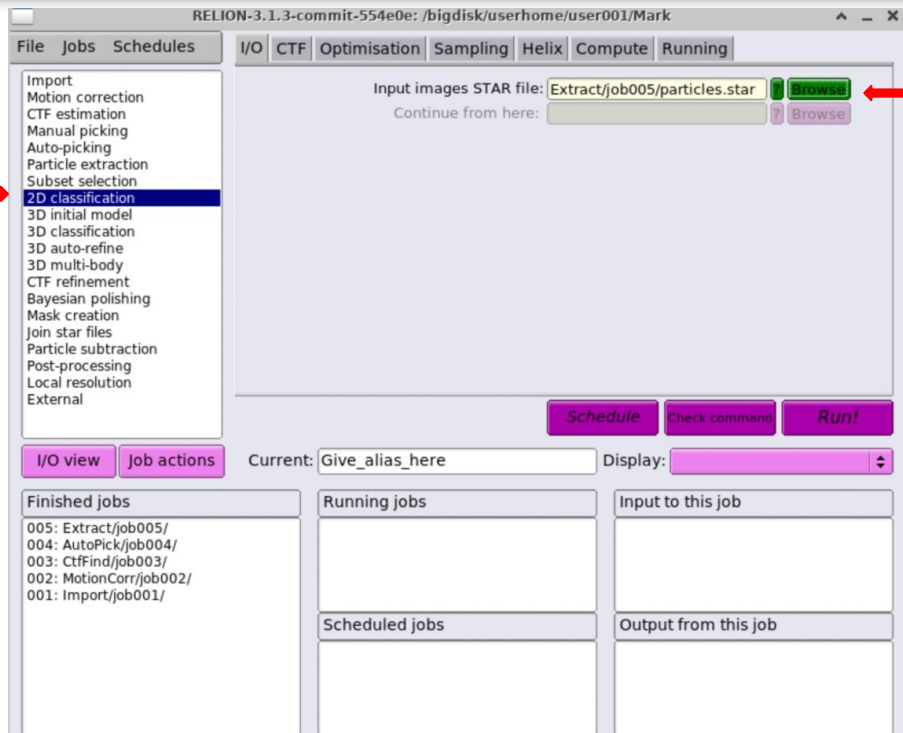
Finished jobs	Running jobs	Input to this job
004: AutoPick/job004/ 003: CtfFind/job003/ 002: MotionCorr/job002/ 001: Import/job001/		

Scheduled jobs	Output from this job

Test out different extraction box and scaling parameters!

*Run!*

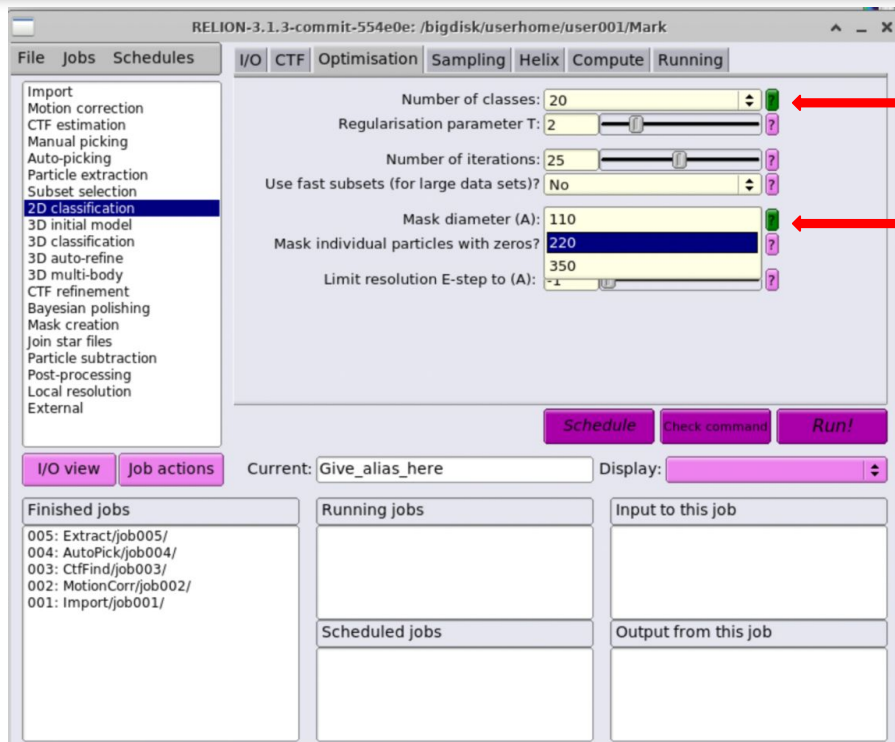
# 2-D Classification



STAR file from particle extraction:  
*particles.star*

Navigate to the *Optimisation* tab

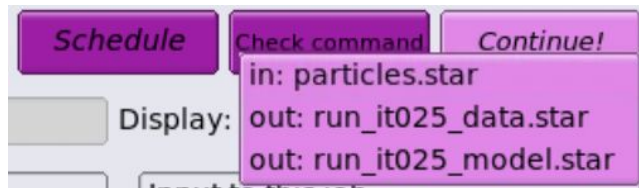
# 2-D Classification



Test out different 2-D  
classification parameters!

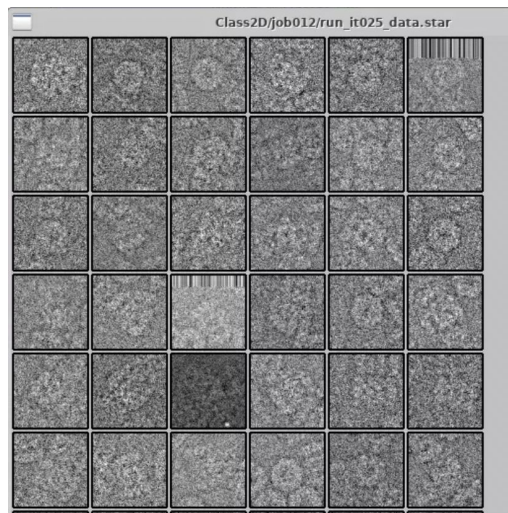
*Run!*

# Visualizing/Assessing 2-D classification results



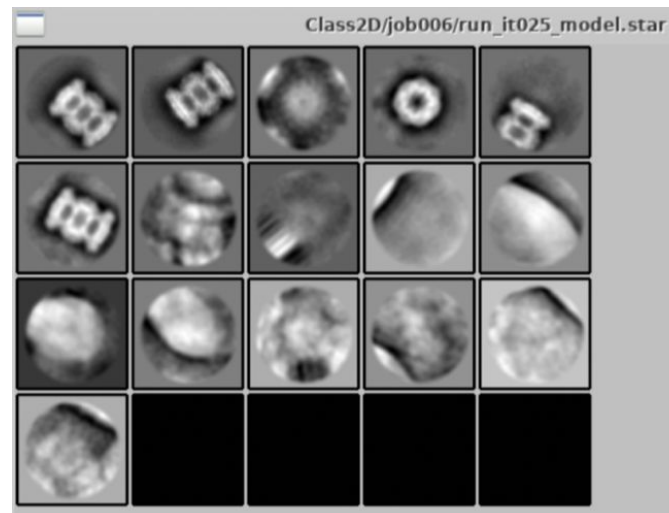
*data.star*

Individual  
particles



*model.star*

Class  
averages





## More precalculated results coming soon!

### Modules

cryoEM

<p><b>Motion correction &amp; CTF estimation</b></p> <p><b>Motion correction</b></p> <ul style="list-style-type: none"><li>- Gain reference applied: <b>Yes/No</b></li><li>- Patch-based motion correction: <b>1x1, 5x5, 10x10 patches</b></li></ul> <p><b>CTF estimation</b></p> <ul style="list-style-type: none"><li>- Pixel size: <b>Input correct or incorrect value</b></li><li>- Resolution limits during estimation: <b>[5-30Å],[3-20Å],[10-50Å]</b></li><li>- Defocus search range: <b>[0.5-5.0µm], [2.0-4.0µm]</b></li></ul> <p><b>Results</b></p> <ul style="list-style-type: none"><li>- 2D classification results using K=100, Tau=2, Diam =200Å</li></ul> <p><b>Total combinations = 72</b></p>	<p><b>Particle picking</b></p> <p><b>Template-based auto picking</b></p> <ul style="list-style-type: none"><li>- Picking threshold: <b>0.001, 0.05, 0.1</b></li><li>- Interparticle distance: <b>-1, &lt;diameter, &gt;diameter</b></li><li>- Low pass filter templates: <b>-1, 20, 100Å</b></li><li>- Rotational sampling: <b>1, 5, 30 degrees</b></li><li>- Maximum standard deviation noise: <b>-1, 0.8, 1.1</b></li></ul> <p><b>Results</b></p> <ul style="list-style-type: none"><li>- 2D classification results using K=100, Tau=2, Diam =200Å</li></ul> <p><b>Total combinations = 243</b></p>	<p><b>2D classification</b></p> <ul style="list-style-type: none"><li>- Number of classes: <b>3, 10, 25, 50, 100</b></li><li>- Mask diam.: <b>&lt;diameter, =diameter, &gt;diameter</b></li><li>- CTF correction: <b>No, Yes, ignore CTFs until first peak</b></li><li>- Mask particles with zeros: <b>Yes/No</b></li><li>- Tau fudge: <b>1, 2, 8, 24</b></li></ul> <p><b>Results</b></p> <ul style="list-style-type: none"><li>- 2D classification results</li></ul> <p><b>Total combinations = 480</b></p>
<p><b>3D classification</b></p> <ul style="list-style-type: none"><li>- Number of classes: <b>2, 5</b></li><li>- Mask diameter: <b>&lt;diameter, =diameter, &gt;diameter</b></li><li>- CTF correction: <b>No, Yes</b></li><li>- Initial low pass filter of reference: <b>-1, 60, 200Å</b></li><li>- Tau fudge: <b>1, 4, 10</b></li><li>- Symmetry: <b>C1, Correct, Incorrect</b></li><li>- Sampling: <b>3.7, 7.5, 30 degrees</b></li></ul> <p><b>Results</b></p> <ul style="list-style-type: none"><li>- 3D classification results</li></ul> <p><b>Total combinations = 972</b></p>	<p><b>3D refinement</b></p> <ul style="list-style-type: none"><li>- Different input stacks: <b>No cleaning, Missing views, Box size too small</b></li><li>- Symmetry: <b>C1, Incorrect, Correct</b></li><li>- Symmetry axis: <b>On axis, off axis</b></li><li>- Starting volume: <b>Correct or not</b></li></ul> <p><b>Results</b></p> <ul style="list-style-type: none"><li>- 3D refinement results</li></ul> <p><b>Total combinations = 60</b></p>	<p><b>CTF Refinement</b></p> <ul style="list-style-type: none"><li>- Anisotropic magnification: <b>Yes/No</b></li><li>- CTF parameterfitting:<ul style="list-style-type: none"><li>- Defocus: <b>Yes/No; micrograph, particle</b></li><li>- Astigmatism: <b>Yes/No; micrograph, particle</b></li><li>- B-factor: <b>Yes/No; micrograph, particle</b></li><li>- Phase shift: <b>Yes/No; micrograph, particle</b></li></ul></li><li>- Beam tilt: <b>Yes/No; +/- Trefoil</b></li><li>- 4th order aberrations: <b>Yes/No</b></li></ul> <p><b>Results</b></p> <ul style="list-style-type: none"><li>- 3D refinement results from selected CTF refined parameters</li></ul> <p><b>Total combinations = 488</b></p>