

Relion Workshop – AutoPicking, Extraction, and 2D-Classification

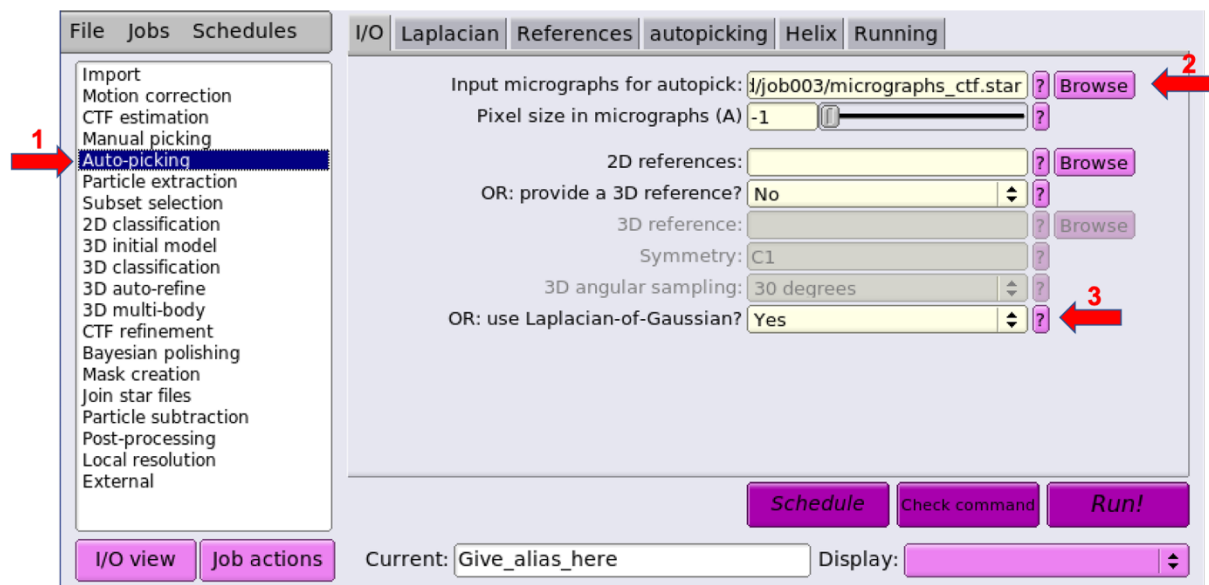
Start the RELION GUI from the terminal with the command

```
relion &
```

The first three jobs in the project (collectively known as pre-processing) have already been run in the tutorial project directory. This tutorial covers the next three steps in a reconstruction.

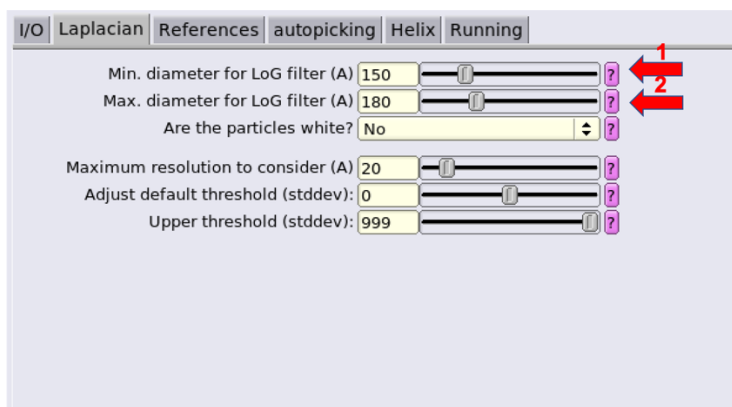
A) AutoPicking

There are multiple methods for automatically picking particles. The Laplacian of Gaussian (LoG) method used here does not require any references. Other methods identify particles using 2D or 3D references, or particles can be picked manually.



- 1) Select the Auto-picking job type
- 2) Input the file generated by the CTF estimation job (`CtfFind/job003/micrographs_ctf.star`)
- 3) Select the Laplacian-of-Gaussian method

Set the LoG parameters



Switch to the Laplacian tab

- 1) Set the minimum diameter to 150
- 2) Set the maximum diameter to 180

This gives the algorithm a rough idea of the size of the particles it is identifying

Set the running parameters

The 'Running' tab in the RELION GUI shows the following configuration parameters:

- Number of MPI procs: 8 (indicated by a red arrow)
- Submit to queue?: No
- Queue name: scarf
- Queue submit command: sbatch
- Standard submission script: job_scripts/slurm_template.sh (with a 'Browse' button)
- Minimum dedicated cores per node: 1
- Additional arguments: (empty field)

Switch to the Running tab

1) Set the number of MPI processes to 8

Then press Run. The AutoPicking job will be created as: AutoPick/job004

Examine the results

The screenshot shows the RELION GUI with the 'Auto-picking' option selected in the left sidebar. The 'Running' tab is active, showing job details for '004: AutoPick/job004/'. The 'Display' dropdown menu is open, showing the following options:

- in: micrographs_ctf.star
- out: coords_suffix_autopick.star (indicated by a red arrow)
- out: logfile.pdf

Display the results of the AutoPicking Job

1) Select cords_suffix_autopick.star in the display menu

The 'RELION manual-picking GUI' shows a list of micrographs for selection:

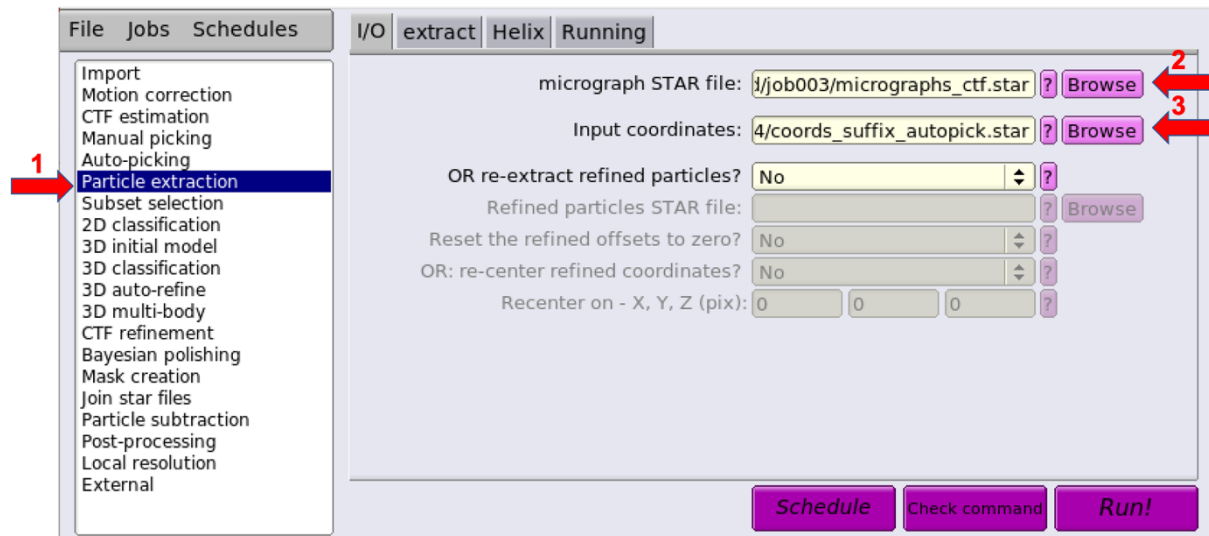
File	pick	227	CTF	13070.4
✓ vives/20170629_00046_framelimage.mrc	pick	227	CTF	13070.4
✓ vives/20170629_00049_framelimage.mrc	pick	227	CTF	13100.3

A red arrow points to the 'pick' button for the first micrograph.

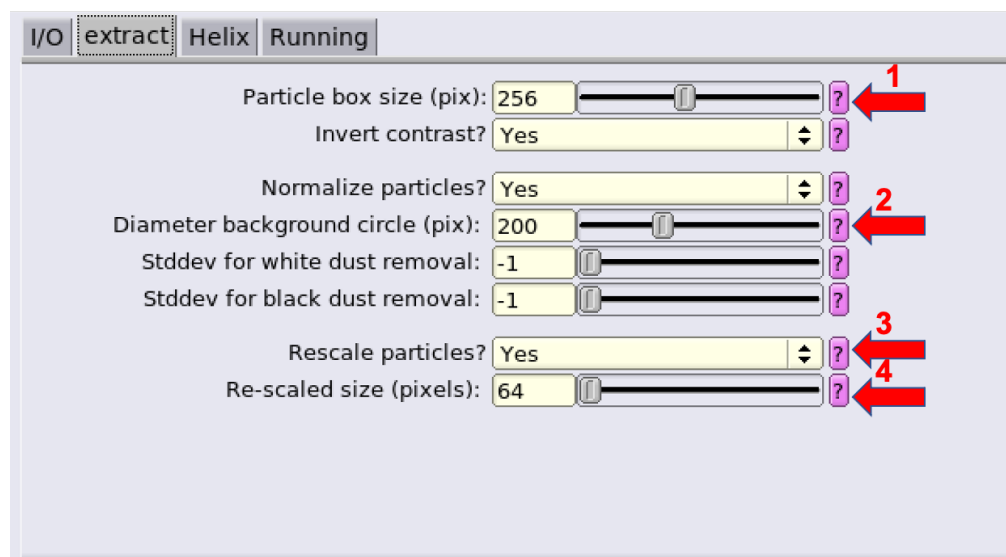
In the display window select 'pick' for one of the micrographs

B) Particle Extraction

Next the selected particles will be extracted as small individual images



Set the extraction parameters



Go to the Extract tab

- 1) Set the particle box size to 246
- 2) Set the diameter for the background circle to 200

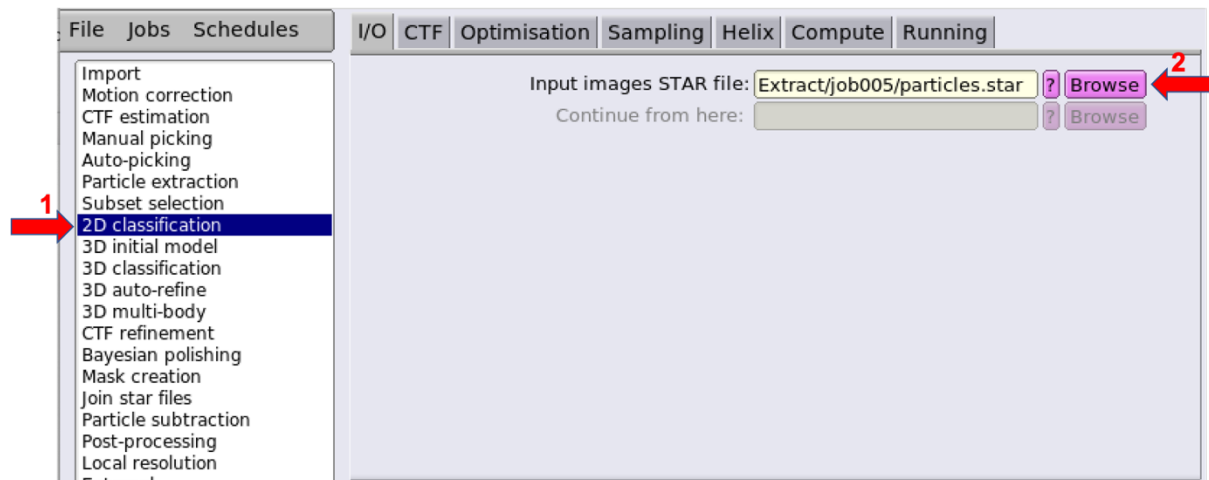
We will bin (shrink) the extracted particles to make the subsequent jobs run more quickly

- 3) Select yes to rescale the particles
- 4) Set the re-scaled size to 64

Go to the Running tab and set the number of MPI processes to 8 as in the previous job
Then run the job. It will run as: Extract/job005

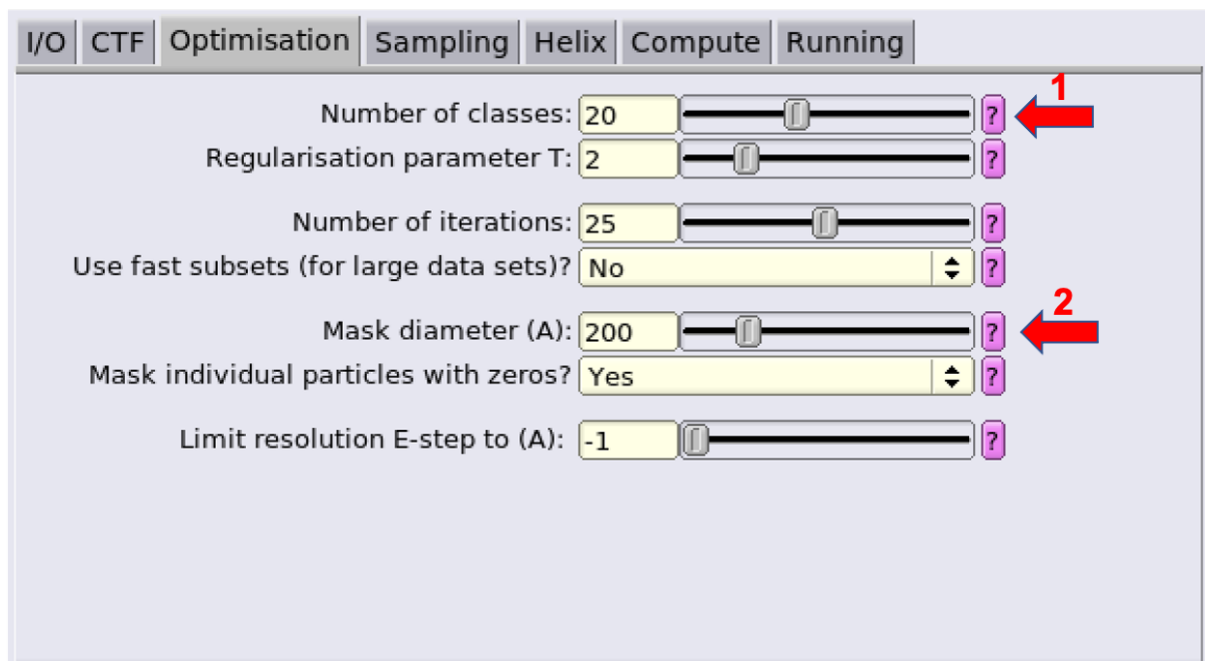
C) 2-Dimensional Classification

2D Classification will align and classify the particles generating class averages with improved signal to noise



- 1) Select the 2D Classification job type
- 2) Set the input to the particles generated by the extract job (Extract/job005/particles.star)

Set the classification parameters



Go to the Optimization tab

- 1) Set the number of classes to 20
- 2) Set the mask diameter to 200

Set the running parameters

The screenshot shows the 'Running' tab of a software interface. The top navigation bar includes tabs for I/O, CTF, Optimisation, Sampling, Helix, Compute, and Running. The 'Running' tab is active. Below the tabs, there are several parameters for job configuration:

- Number of MPI procs: 8 (with a slider and a help icon ?)
- Number of threads: 1 (with a slider and a help icon ?)
- Submit to queue? No (with a dropdown arrow and a help icon ?)
- Queue name: scarf (with a help icon ?)
- Queue submit command: sbatch (with a help icon ?)
- Standard submission script: /ub_scripts/slurm_template.sh (with a help icon ? and a 'Browse' button)
- Minimum dedicated cores per node: 1 (with a slider and a help icon ?)
- Additional arguments: (with a text input field and a help icon ?)

Red arrows labeled 1 and 2 point to the 'Number of MPI procs' and 'Number of threads' sliders respectively.

Go to the Running tab

- 1) Set the number of MPI processes to 8
- 2) Set the number of threads to 1

Run the 2D Classification job and view the results

Then run the Class2D job. It will run as: Class2D/job006

View the results of the Class2D job using the Display pulldown menu. Choose run_it025_model.star