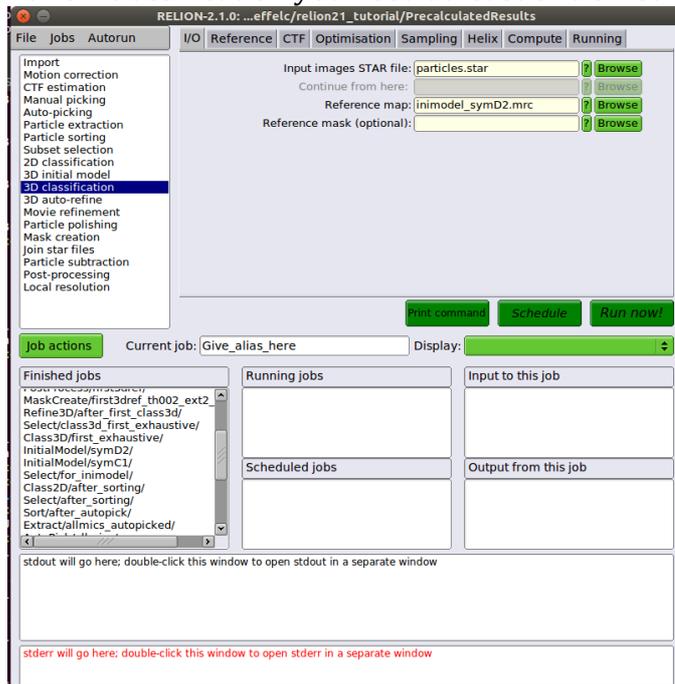
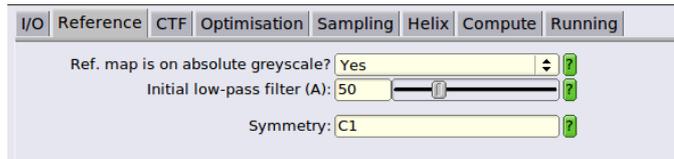


3D classification in Relion 2.1

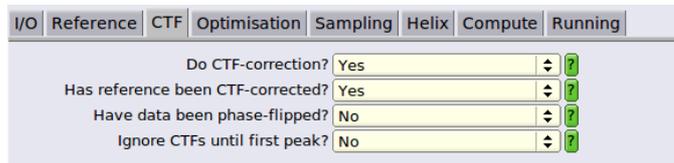
For 3D classification you need at least one 3D structure and particles as an input.



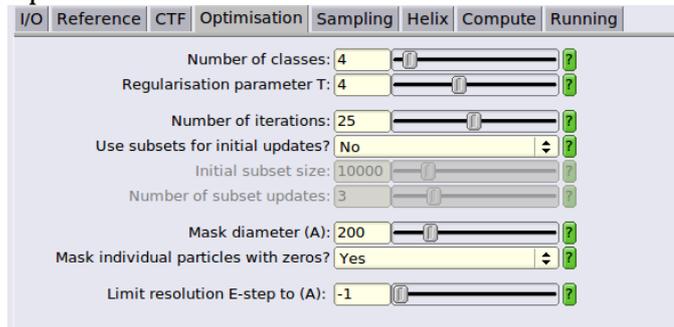
Reference tab:



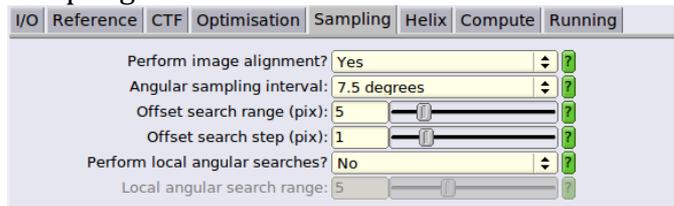
CTF tab:



Optimisation tab:



Sampling tab:



Helix tab:

I/O	Reference	CTF	Optimisation	Sampling	Helix	Compute	Running
Do helical reconstruction? <input type="text" value="No"/>							
Tube diameter - inner, outer (Å):		<input type="text" value="-1"/>	<input type="text" value="-1"/>				
Angular search range - tilt, psi (deg):		<input type="text" value="15"/>	<input type="text" value="10"/>				
Apply helical symmetry? <input type="text" value="Yes"/>							
Number of asymmetrical units:		<input type="text" value="1"/>					
Initial twist (deg), rise (Å):		<input type="text" value="0"/>	<input type="text" value="0"/>				
Central Z length (%):		<input type="text" value="30"/>					
Do local searches of symmetry? <input type="text" value="No"/>							
Twist search - Min, Max, Step (deg):		<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>			
Rise search - Min, Max, Step (Å):		<input type="text" value="0"/>	<input type="text" value="0"/>	<input type="text" value="0"/>			
Range factor of local averaging:		<input type="text" value="-1"/>					

Compute tab:

I/O	Reference	CTF	Optimisation	Sampling	Helix	Compute	Running
Use parallel disc I/O? <input type="text" value="Yes"/>							
Number of pooled particles:		<input type="text" value="3"/>					
Pre-read all particles into RAM? <input type="text" value="Yes"/>							
Copy particles to scratch directory: <input type="text" value=""/>							
Combine iterations through disc? <input type="text" value="No"/>							
Use GPU acceleration? <input type="text" value="No"/>							
Which GPUs to use:		<input type="text" value="0:1:2:3"/>					

Running tab:

I/O	Reference	CTF	Optimisation	Sampling	Helix	Compute	Running
Number of MPI procs:		<input type="text" value="1"/>					
Number of threads:		<input type="text" value="1"/>					
Submit to queue? <input type="text" value="No"/>							
Queue name:		<input type="text" value="openmpi"/>					
Queue submit command:		<input type="text" value="qsub"/>					
Standard submission script:		<input type="text" value="elion-prerelease/bin/qsub.csh"/>	<input type="button" value="Browse"/>				
Minimum dedicated cores per node:		<input type="text" value="24"/>					
Additional arguments:		<input type="text" value=""/>					

Now you could press run.

If the calculations take too long you can instead copy the precalculated run to your running directory and use these results:

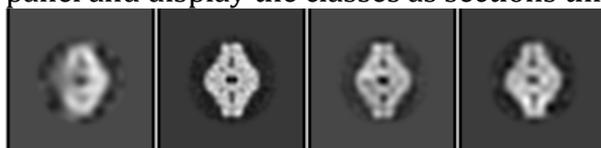
```
cp -r PrecalculatedResults/Class3D/job018 Class3D/job018
```

Analysing the results and selection of the best 3D classes

There are several ways to analyze your 3D classes:

1. The Relion gui:

Choose the **Class3D/job018/run_it025_model.star** file in the **Subset selection** panel and display the classes as sections through the middle of the structures:



You can select the best classes and save the particles corresponding to these classes for your further calculations.

2. the statistics in your run_it025_model.star file:

in the terminal type

more Class3D/job018/run_it025_model.star

data_model_general

```
_rlnReferenceDimensionality      3
_rlnDataDimensionality           2
_rlnOriginalImageSize           100
_rlnCurrentResolution            14.750000
_rlnCurrentImageSize             68
_rlnPaddingFactor                2.000000
_rlnIsHelix                      0
_rlnFourierSpaceInterpolator     1
_rlnMinRadiusNnInterpolation    10
_rlnPixelSize                    3.540000
_rlnNrClasses                    4
_rlnNrBodies                     1
_rlnNrGroups                     15
_rlnTau2FudgeFactor             4.000000
_rlnNormCorrectionAverage        0.510559
_rlnSigmaOffsets                 1.833866
_rlnOrientationalPriorMode       0
_rlnSigmaPriorRotAngle           0.000000
_rlnSigmaPriorTiltAngle          0.000000
_rlnSigmaPriorPsiAngle           0.000000
_rlnLogLikelihood                1.347089e+08
_rlnAveragePmax                  0.901647
```

data_model_classes

loop_

```
_rlnReferenceImage #1
_rlnClassDistribution #2
_rlnAccuracyRotations #3
_rlnAccuracyTranslations #4
_rlnEstimatedResolution #5
_rlnOverallFourierCompleteness #6
Class3D/job018/run_it025_class001.mrc 0.080344 3.815000 0.767000 27.230769 0.999997
Class3D/job018/run_it025_class002.mrc 0.507154 2.412000 0.442000 14.750000 0.999020
Class3D/job018/run_it025_class003.mrc 0.234911 3.080000 0.561000 19.666667 0.999275
Class3D/job018/run_it025_class004.mrc 0.177591 3.305000 0.603000 25.285714 0.999998
```

the yellow marked column shows you how the particles are distributed between the classes. The remaining columns tell you something about the accuracy of alignment of the particles in the different classes, the estimated resolution of the classes and their 3D completeness (preferential orientation).

Also the value of `_rlnLogLikelihood` should become smaller each iteration.

3. using Chimera

Open the 4 classes in chimera using the command line:

chimera Class3D/job018/run_it025_class*.mrc

You can estimate which classes are the best in the easiest way by looking at them.