3D classification in Relion 2.1

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

File Jobs Autorun	I/O	Reference	CTF	Optimisation	Sampling	, Helix	Compute	Running
Import Motion correction CTF estimation Manual picking Particle extraction Particle sorting Subset selection 2D classification 3D initial model DD classification 3D auto-refine Movie refinement Particle polishing Mask creation Join star file subtraction Particle polishing Particle subtraction Post-processing		Re	Input Co ferenc	images STAR ntinue from he Reference m e mask (optior	file: (particle re: inimod aap: (inimod aal):	el_symD	2.mrc	? Browse ? Browse ? Browse ? Browse ? Browse
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Reference tab:

I/O Reference CTF Optimisation Sampling Helix Compute Running

Ref. map is on absolute greyscale?	Yes 🗘 ?
Initial low-pass filter (A):	50 7
Symmetry:	C1 ?

CTF tab:

I/O	Reference	CTF	Optimisation	Sampling	Helix	Compute	Running	

Do CTF-correction?	Yes	÷	
Has reference been CTF-corrected?	Yes	\$?
Have data been phase-flipped?	No	\$?
Ignore CTFs until first peak?	No	\$?

Optimisation tab:

I/O Reference CTF Optimisation Sampling Helix Compute Running

Number of classes: Regularisation parameter T:	
Number of iterations:	25
Use subsets for initial updates?	No \$?
Initial subset size:	10000 [] ?
Number of subset updates:	3 []]
Mask diameter (A): Mask individual particles with zeros?	200 7 Yes \$7
Limit resolution E-step to (A):	-1 0 7

Sampling tab: 1/0 Reference CTF Optimisation Sampling Helix Compute Running

Perform image alignment?	Yes 🔷	?
Angular sampling interval:	7.5 degrees	?
Offset search range (pix):	5 -	?
Offset search step (pix):	1 0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-	?
Perform local angular searches?	No	?
Local angular search range:	5 []	?

Helix tab:

0 Reference CTF Optimisation Sampling Helix Compute Running
Do helical reconstruction? No
Tube diameter - inner, outer (A): -1 -1 7
Angular search range - tilt, psi (deg): 15
Apply helical symmetry? Yes 🗘 ?
Number of asymmetrical units: 1
Initial twist (deg), rise (A): 0 0 ?
Central Z length (%): 30
Do local searches of symmetry? No 🗘 👔
Twist search - Min, Max, Step (deg): 0 0 0 7
Rise search - Min, Max, Step (A): 0 0 0
Range factor of local averaging: -1
ompute tab:
0 Reference CTF Optimisation Sampling Helix Compute Running
Number of pooled particles: 3
Pre-read all particles into RAM? Yes
Copy particles to scratch directory:
Copy particles to scratch directory:
Copy particles to scratch directory:
Copy particles to scratch directory: Combine iterations through disc? No Use GPU acceleration? No Which GPUs to use: 0:1:2:3
Copy particles to scratch directory: Combine iterations through disc? No Use GPU acceleration? No Which GPUs to use: 0:1:2:3
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Copy particles to scratch directory: Combine iterations through disc? No Use GPU acceleration? No Which GPUs to use: 0:1:2:3 (Unning tab: /O Reference CTF Optimisation Sampling Helix Compute Running Number of MPI procs: 1
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Copy particles to scratch directory: Combine iterations through disc? No Use GPU acceleration? No Use GPU acceleration? No Which GPUs to use: 0:1:2:3 Which GPUs to use: 0:1:2:3 Number of HPI procs: 1 Number of MPI procs: 1 Number of threads: 1 Submit to queue? No Queue submit command: qsub Standard submission script: 6100, proceedaace bindraub or b The script of the script: 6100, proceedaace bindraub or b The script of the script: 6100, proceedaace bindraub or b The script of the script: 6100, proceedaace bindraub or b The script of the script: 6100, proceedaace bindraub or b The script of the script: 6100, proceedaace bindraub or b The script of the script
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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

_rInReferenceDimensionality	3
_rInDataDimensionality	2
_rlnOriginalImageSize	100
_rlnCurrentResolution	14.750000
_rlnCurrentImageSize	68
_rInPaddingFactor	2.000000
_rlnIsHelix	0
_rInFourierSpaceInterpolator	1
_rInMinRadiusNnInterpolatio	n 10
_rlnPixelSize	3.540000
_rlnNrClasses	4
_rlnNrBodies	1
_rlnNrGroups	15
_rlnTau2FudgeFactor	4.000000
_rlnNormCorrectionAverage	0.510559
_rInSigmaOffsets	1.833866
_rlnOrientationalPriorMode	0
_rlnSigmaPriorRotAngle	0.000000
_rlnSigmaPriorTiltAngle	0.000000
_rInSigmaPriorPsiAngle	0.000000
_rlnLogLikelihood	1.347089e+08
_rInAveragePmax	0.901647
data_model_classes	
loop_	
_rlnReferenceImage #1	
_rlnClassDistribution #2	
rInAccuracyRotations #3	

_rinAccuracyRotations #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 rinLogLikelihood 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.