High-resolution 3D refinement in RELION 2.1 (3D auto-refine)

If you do not have the selected particles after 3D Classification, copy them from the precalculated results to the working directory:

#>cp -r PrecalculatedResults/Select/job019 Select/job019

Then select your particles star and the best map from the 3D classification step in the gui:



170 Reference CIF Optimisation Huto-sampling Helix Compute Running
Ref. map is on absolute greyscale? Ye:
Initial low-pass filter (A): 40
Symmetry: D2

CTF tab:

I/0	Reference	CTF	Optimisation	Auto-sampling	Helix	Compute	Running		
			Do CTF-	correction? Ye	s			¢	2
	Has	refe	rence been CTF	-corrected? Ye	\$			¢Ì	i
		Have	data been pha	ase−flipped? <mark>No</mark>				¢	1
		Igno	re CTFs until	first peak? <mark>No</mark>				\$	2
Opti	misation	tab:							
I/0	Reference	CTF	Optimisation	Auto-sampling	Helix	Compute	Running		
			Maali da		0				
	Maek in	diuid	nask ui Val particles	with zeros2 Ve	×[<u>-</u>
	hask in	01010	uar par creres						
		Us	e solvent-flat	tened FSCs? No	I			¢	?
Auto	-sampling	g tal):						
I/0	Reference	CTF	Optimisation	Auto-sampling	Helix	Compute	Running		
	Initial angular sampling: 7.5 degree: Initial offset range (pix): 5 Initial offset step (pix): 1 Local searches from auto-sampling: 1.8 degrees \$								
	Loca	I l sea	nitial offset rches from aut	step (pix): 1 co-sampling: 1.	8 degree	-0			2
Helix	Loca k tab:	I l sea	nitial offset rches from aut	step (pix): 1	8 degre	-0		- (2
Helix I/0	Loca (tab: Reference	I l sea	nitial offset rches from aut Optimisation	step (pix): 1 co-sampling: 1. Auto-sampling	8 degree	es Compute	Running	- (2
Helix I/0	Loca (tab: Reference	I l sea CTF	nitial offset rches from aut Optimisation Do helical reco	step (pix): 1 co-sampling: 1. Auto-sampling	8 degree Helix	es Compute	Running		?
Helix I/0	Loca (tab: Reference Tut	I l sea CTF	nitial offset rches from aut Optimisation Do helical reco ameter - inner,	step (pix): 1 co-sampling: 1. Auto-sampling onstruction? No , outer (A): -1	8 degree	es Compute	Running		2
Heli) I/0	Loca (tab: Reference Tut Angular s	I sea CTF De dia search	nitial offset rches from aut Optimisation Do helical reco ameter - inner, n range - tilt,	step (pix): 1 co-sampling: 1. Auto-sampling onstruction? No , outer (A): -1 , psi (deg): 15	8 degree	Compute	Running		? ? ? ? ?
Helix I/0	Loca (tab: Reference Tub Angular (I sea CTF J search Numh Init:	nitial offset rches from aut Optimisation Do helical reco ameter - inner, n range - tilt, Apply helica per of asymmetr ial twist (deg) Central Z	step (pix): 1 co-sampling: 1. Auto-sampling onstruction? No , outer (A): -1 , psi (deg): 15 al symmetry? Ya rical units: 1), rise (A): 0 length (%): 30	8 degred Helix	Compute	Running		? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Helix I/0	Loca (tab: Reference Tut Angular :	I sea CTF De dia search Numb Init: Do lo	nitial offset rches from aut Optimisation Do helical reco ameter - inner, n range - tilt, Apply helica per of asymmetr ial twist (deg) Central Z	step (pix): 1 co-sampling: 1. Auto-sampling onstruction? No , outer (A): -1 , psi (deg): 15 al symmetry? Yo rical units: 1), rise (A): 0 length (%): 30 of symmetry? No	Helix	Compute	Running		? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ? ?
Helix	Loca (tab: Reference Tub Angular :	I sea CTF I be dia search Init: Do lo search	nitial offset rches from aut Optimisation Do helical reco ameter - inner, h range - tilt, Apply helica per of asymmetr ial twist (deg) Central Z ocal searches o ch - Min, Max,	step (pix): 1 co-sampling: 1. Auto-sampling onstruction? No , outer (A): -1 , psi (deg): 15 al symmetry? Yo rical units: 1), rise (A): 0 length (%): 30 of symmetry? No Step (deg): 0	Helix	Compute	Running		? ?
Helix I/0	Loca (tab: Reference Tub Angular : Twist Ris	I sea CTF De dia search Init: Do lo searc	nitial offset rches from aut Optimisation Do helical reco ameter - inner, h range - tilt, Apply helica ber of asymmetr ial twist (deg) Central Z cocal searches o ch - Min, Max, arch - Min, Max	step (pix): 1 co-sampling: 1. Auto-sampling: 1. Auto-sampling onstruction? No , outer (A): -1 , psi (deg): 15 al symmetry? Ye rical units: 1), rise (A): 0 length (%): 30 of symmetry? No Step (deg): 0 x, Step (A): 0	8 degree	Compute Compute	Running		

Compute tab:

I/O Reference CTF Optimisation Auto-sampli	ng Helix Compute Running
Use parallel disc I/O? Number of pooled particles: Pre-read all particles into RAM? Copy particles to scratch directory: Combine iterations through disc?	Yes \$? 3 • • ? Yes \$? No \$?
Use GPU acceleration? Which GPUs to use: Running tab:	No \$?
I/O Reference CTE Optimisation Auto-sample	ing Helix Compute Punning
	ing herry compare mainting
Number of MPI procs: Number of threads:	1 []
Number of MPI procs: Number of threads: Submit to queue?	1 []? 1 []? No \$?
Number of MPI procs: Number of threads: Submit to queue? Queue name: Oueue submit command:	1 ? 1 ? 1 ? No \$? openmpi ? nsub ?
Number of MPI procs: Number of threads: Submit to queue? Queue name: Queue submit command: Standard submission script:	1 ? 1 ? 1 ? No \$? openmpi ? qsub ? 34-linux/vlion/none/bin/qsub.csh ?
Number of MPI procs: Number of threads: Submit to queue? Queue name: Queue submit command: Standard submission script: Minimum dedicated cores per node:	1 ? 1 ? 1 ? No \$? openmpi ? qsub ? 34-linux/vlion/none/bin/qsub.csh ? 1 ?

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/Refine3D/job020 Class3D/job020

Analysing the results :

#>chimera Class3D/job020/run_class1.mrc Class3D/job020/run_class1_angdist.bild



As we can see by varying the threshold at the Volume Viewer window, there are noisy regions that can be easily masked out. Let's do it. To create a mask:

	RELION-2.1.0: /ssd/reliontuto/relion21_tutorial/betagal	+ = ×
File Jobs Autorun	I/O Mask Helix Running	
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Particle sorting Subset selection 2D classification 3D initial model 3D classification 3D auto-refine Movie refinement Particle polishing Mask creation Join star files Particle subtraction Post-processing Local resolution	Input 3D map: Refine3D/job020/run_class001.mrd ? Br	DWSE
	Print command Schedule	Run now!
Job actions Current	nt job: Give_alias_here Display:	\$
Finished jobs	Running jobs Input to this job	
PostProcess/job023/ MaskCreate/job022/ Refine3D/job021/ Class3D/job019/ Class3D/job018/ InitialModel/job017/ InitialModel/job016/ Select/job015/ Class2D/job014/ Extract/allmics_autopicked/ AutoPick/job012/ AutoPick/job011/ Select/templates4autopick/ Class2D/manualnick/ Stdout will go here; double-co	Scheduled jobs Output from this job Click this window to open stdout in a separate window	
Mask tab:		
I/O Mask Helix Runn	ing	
	Lowpass filter map (A) 15 - 7 Pixel size (A) 3.54 - 7	
Initial	binarisation threshold: 0.02 - 1 - 1 - 2 - 2	
Extend binar	y map this many pixels: 2	
Add a soft-ed	lge of this many pixels: 3 [[]	
Helix tab:		
I/O Mask Helix Runn	ling	
	Mask a 3D helix? No 🔷 🖓	
	Central Z length (%): 30	

Running tab:

I/O Mask Helix Running	
Submit to queue?	No \$?
Queue name:	openmpi ?
Queue submit command:	qsub ?
Standard submission script:	34-linux/vlion/none/bin/qsub.csh ? Browse
Minimum dedicated cores per node:	1 7
Additional arguments:	2

Run...

To check the obtained	mask, select Display out: mask.mrc
	Relion display GUI 🛛 🔶 💶 🗙
mask.mrc	
Scale:	1 Black value: 0
Sigma contrast:	5 White value: 0
Nr. columns: 10	Ori scale: 1 Max. nr. images: 1000
	Display!

MaskCreate/job022/mask.mrc									3	
					ų	4	4	\$	*	
*	•	+	•							1
							•	+	-	
*	*	*	**	*	4					

Now, let's apply the mask above to obtain a higher resolution map:

	RELION-2.1.0: /ssd/reliontuto/relion21_tutorial/betagal	+ = ×
File Jobs Autorun	I/O Sharpen Filter Running	
Import Motion correction CTF estimation Manual picking Auto-picking Particle extraction Particle sorting Subset selection 2D classification 3D initial model 3D classification 3D auto-refine Movie refinement Particle polishing Mask creation Join star files Particle subtraction Post-processing Local resolution	One of the 2 unfiltered half-maps:)20/run_half1_class001_unfil.mrc) Brow Solvent mask: MaskCreate/job021/mask.mrc	use
Job actions Current	Print command Schedule Ru t job: Give_alias_here Display:	un now!
Finished jobs	Running jobs Input to this job	
PostProcess/job023/ MaskCreate/job022/ Refine3D/job021/ Class3D/job019/ Class3D/job018/ InitialModel/job016/ Select/job015/ Class2D/job015/ Class2D/job014/ Extract/allnics_autopicked/ AutoPick/job012/ AutoPick/job012/ Select/job010/ Select/job010/ Select/job010/ Select/job010/	Scheduled jobs	
stdout will go here; double-c	lick this window to open stdout in a separate window lick this window to open stderr in a separate window	

Sharpen tab:

I/O Sharpen Filter Running
MTF of the detector (STAR file) mtf_falcon2_300kV.star Prowse
Estimate B-factor automatically? Yes 🔷 😫 💡
Lowest resolution for auto-B fit (A): 10
Use your own B-factor?No 🔷 😫
User-provided B-factor: -1000
Filter tab:
I/O Sharpen Filter Running
Skip FSC-weighting? No 2 ? Ad-hoc low-pass filter (A): 5 ?

Running tab:

I/O Mask Helix Running	
Submit to queue?	No \$?
Queue name:	openmpi ?
Queue submit command:	qsub ?
Standard submission script:	34-linux/vlion/none/bin/qsub.csh ? Browse
Minimum dedicated cores per node:	1 7
Additional arguments:	[

Now the map should look better:

#>chimera PostProcess/job022/postprocess_masked.mrc

