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Wed, 02/27/2019 - 16:48

#1

rddey80

using earlier 2D class average to re-pick particles

Dear Members,

I have a good ab-initio 3D map generated from a refinement package, which is created from a number of selected 2D class averages (all done in cisTEM). Is it possible to use the 3D ab-initio map or the 2D class averages as a template to re-pick particles in cisTEM? If not, can I switch to relion to do the same? In that case, could you please guide how to do that? I mean, which file to export?

Thanks for your time and help,

Raj

timgrant

Hi Raj,

Hi Raj,

You cannot use references to pick in cisTEM. Did your picking not work well?

You can export your refinement package to relion .star file and particle stack, which you can then use to import to Relion.

Thanks!

Tim

Thu, 02/28/2019 - 11:48 (Reply to #2)

rddey80

Hi Tim,

Hi Tim,

particle picking worked well in cistem. after autorefinement final map generated at 7A res, but I can see less features than that observed in earlier ab-initio model building step. Any idea or suggestion? I have 70% known model(x-ray structure). How can I use that in this pipeline? Any suggestion would be of great help.

Thanks for your time,

Raj

timgrant

Hi Raj,

Hi Raj,

Have you tried sharpening the map?

Tim

Fri, 03/01/2019 - 15:14 (Reply to #4)

rddey80

Hi Tim,

Hi Tim,

Regarding sharpening in cisTEM, what values I should give for 'Resolution Cut-Off (Å)', 'Filter Edge-Width (Å)', and 'SSNR Scale Factor'? Could you please help deciding these parameters. Another question, should I run Generate 3D before Sharpening 3D?

Many thanks for your time and help.

Regards,

Raj

rddey80

Hi Tim,

Hi Tim,

I exported the refinement package that creates .star and .stack files for relion. But, after 2d classification in relion, it looks ugly. Not even close to what I have seen in cisTEM. This is a phase plate data-set. ctf estimation, particle picking, 2d classification, ab-initio model building and auto-ref were done in cisTEM. The final map is about 8Å. I am interested to see how relion can work with this data. For this reason I exported ref-pkg from cistem and run relion 2D classification. I selected 'yes' for the question Do CTF-correction? under CTF tab in Relion. Also, used 7 in 'Limit resolution E-step to (Å)' under Optimisation tab in Relion. Could you please tell if I have done anything wrong?

Thanks for your time and help.

Raja

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