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# How to run 3D classification correctly and get particles from 3D classification

Thu, 11/15/2018 - 02:52

#1



Muchun

## How to run 3D classification correctly and get particles from 3D classification

Hi,

I wanted to run 3D classification using cisTEM. Because I'm a green hand on TEM image processing and I haven't used FREALIGN. I tried to change "Number of classes for 3D refinement" to 3 when I created a new refinement package after reading the cisTEM tutorial, then I imported it for both Ab-Initio Refine and Auto Refine. As a result, I can see three FSC curves and three panels of projection sections, and the top of panel show a percentage. In my opinion, this percentage maybe mean the number of particles in one 3D class. When I want to get particles from one class, I can't find anything in the ../Assets/Parameter. Meanwhile, there isn't a new option like "Create From 3D Class" when I went back to create new refinement package.

So, I wander whether I run 3D classification correctly or what parameter or option I should add and how to get particles from 3D classification.

Thanks.

Muchun





timgrant

Hi Muchun,

Hi Muchun,

3D classification indeed begins by creating a refinement package with multiple classes as you have done. You can do ab-initio, but if you already have a model this is probably unnecessary. In the "auto-refine" workflow, you would create your refinement package then choose the consensus refinement as your initial model and run an auto-refine. What probably works better is to use the manual refine panel. First refine all your data in one class to reach your best consensus refinement (You probably have already done that). Then, create a new refinement package, however instead of selecting "New refinement package" in the wizard, select the refinement package that contains your consensus refinement as the template. Select your best refinement as the input parameters, and you can keep everything else the same except the number of classes - change this to the desired number of classes. Leave the references as "Generate from input parameters". Then go to the manual refine panel, make sure local search is selected, put the number of rounds ~50 and the resolution to something like 8A (assuming your consensus refinement is better than 8A). Then click run.

After a 3D classification, if you want to extract the particles from one class, you create a new refinement package, again instead of selecting "New refinement package" select the refinement package you performed your classification on as the template, then keep going through the questions. At one point, the wizard will say "Carry over all particles?" If you select No here, you can choose one or more classes to take particles from.

Thanks!

Tim





















Tue, 11/20/2018 - 01:38 (Reply to #4)



Muchun



