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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 wonder 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



Mon, 11/19/2018 - 22:12 (Reply to #2)



Muchun

Hi Tim

Hi Tim,

Thanks for your reply, I have run 3D classification and as you told, it worked. Now I have a small question when I got one class particle from 3D classification, should I leave the reference as "Generate from input parameters" or input last run result of manual refine ?





timgrant

Hi Muchun,

Hi Muchun,

Do you mean when you take only one class from the the classification to do further processing? That is kind of up to you, if you do generate from parameters it will make a 3D from the current best parameters, which is probably fine. You could also select the 3D of the selected class as a reference, which will basically save you a 3D reconstruction step. In the end, it probably won't make much difference which you do, but generate from parameters has the advantage that you won't accidentally select the wrong reference, it is also a sanity check as the generated 3D should look like the class you selected.

Cheers,

Tim



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



Muchun

Hi Tim,

Hi Tim,

Thanks for your quickly reply, it's very helpful for me what you tell.

Thanks again,

Muchun

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