Auto refinement vs manual refinement

Sat, 05/02/2020 - 21:01 #1

Alicia

Auto refinement vs manual refinement

Hi,

I am new to cisTEM and have got confused about the differences between auto refine and manual refine. Does auto refinement always start from a global search followed by local refinement for the N best matching projections for each cycle? And the percentage of particles used for each cycle and the total number of cycles are automatically determined by the program? I have seen manual refinement allows me to choose local search and no. of cycles to run, but what's the percentage of particles should I use? If I put 100%, will 100% of the particles be used for each refinement cycle? In the documentation it says "A refinement should always be finished with a few cycles using 100% of the data", so if I use less than 100%, should I run a few more cycles using 100%?

Also I have seen from previous post that for 3D classification, it's better to run a global refinement with a single class, followed by ~50 rounds of local refinement for desired number of classes simply because it is much faster. But in my practice I didn't really see it's faster... Is that because I used 100% of the particles for local refinement?

Thanks,

Alicia

timgrant

Hi,

Hi,

There is nothing that the autorefinement does that cannot be reproduced by the manual refinement, it just tries to set a lof of the settings for you, to save time. One of the most important things it does is set the high resolution limit somewhat conservatively, if you are not careful it is easy to bias your data by setting this value at too high a resolution when using the manual refinement panel.

Yes if you put 100% it will use all of hte particles, if you put a smaller percentage, at each round a random subset of the particles will be taken at each round (This is done automatically by the auto refine panel).

local refinement should always be significantly faster than global alignment, so it's kind of weird if it wasn't?

My work flow is to use 1 3D class and run an autorefinement from 20A. If that leads to a nice looking result, and the resolution is 6A or better I will run another auto-refinement starting at 8A. Sometimes after this I will use the manual refinement panel to tweak things, but often it won't make much difference.

For 3D classification I create a new refinement package from my refined data with the best parametes, setting the number of 3D classes to what I want. Then I will run ~50 rounds of local refinement in the manual refinement panel, usually with a high resolution limit of ~8A. If there are a lot of particles, I will use a subset % of the particles for this intial 50 rounds, then do a few rounds at the end with 100%.

Thanks.

Tim

Mon, 05/04/2020 - 18:06 (Reply to #2)

Alicia

Thanks much Tim! So auto

Thanks much Tim! So auto refinement always starts from a global search for each round, right? I didn't see much speed difference for the first a few rounds between auto refine and manual refine, but the job is still ongoing, so it may show a big difference for the subsequence rounds.

Also another question, can I run multiple different jobs in the same GUI? I have two different datasets for the same protein sample and I am running 3D refinement now for one dataset, could I upload the other dataset and start running movie alignemnt etc at the same time? Will they interfere with each other?

Thanks,

Alicia

Tue, 05/05/2020 - 11:40 (Reply to #3)

timgrant

Hi Alicia,

Hi Alicia,

The auto refinement is a global search on the first round, then in subsequent rounds it is a mixture of local and global, basically there is a random change of a particle being globally aligned, with particles that haven't been globally aligned for a while more likely.

In theory you can run multiple jobs, but this is not well tested, and it may lead to increased instability.

Tim