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[Home](#) > global search or local refinement in manual refine

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Mon, 04/22/2019 - 12:48

#1

lbchenj

global search or local refinement in manual refine

Hi,

I'm puzzled with the selection between global search and local refinement in manual refine.

(1) If I'd like to do global 3D classification, should I choose global search or local refinement? One published paper in your lab (http://grigoriefflab.janelia.org/sites/default/files/Zhang_Ultramicroscopy2019.pdf) suggested to use global search, but you also suggested to choose local refinement shown in your previous post (

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

).

(2) If I'd like to push resolution subsequent to autorefine, should I choose global search or local refinement in the further manual refine? No detailed clarification on this point is present in the manual (*If time permits, users are strongly encouraged to try to improve their reconstructions using Manual Refine. For example, starting from the last result of auto-refinement, try to run a few more (3-5) refinement cycles using a slightly higher resolution limit. For example, if auto-refinement used a resolution limit of 5.0 Å in the last cycle, try manual refine with a Hi-Res Limit of 4.5 Å. Be careful not to come too close to your current estimated resolution! Remember the FSC is only unbiased beyond the Hi-Res Limit*). It seems that autorefine only involves global search and refinement.

Looking forwards to your response and thanks for your time!

Best,

Bin

timgrant

Hi Bin,

Hi Bin,

1. In the case where you think you already have a pretty good alignment for your images from a previous refinement, and where you expect the angles not to change very much in the classification (this is the case most of the time), then you should use local refinement for the classification simply because it is much faster. In general my strategy for classification is to get a consensus reconstruction, then create a new refinement package with the desired number of classes based on this consensus refinement, then to run ~50 rounds of local refinement with ~8Å resolution limit. If this does not work, you may need to play with some variables, but it should work in most cases.

2. For high resolution data, I like to have done a global refinement at at least 8Å resolution, then I will switch to local refinement. My general processing strategy will be to run an auto-refine starting at ~20Å. Then, assuming the result is better than 8Å resolution, to run another auto-refine starting from 8Å resolution, using the result of the initial auto-refine as the reference. After this, I may also try to improve things using manual refinement - particularly via CTF refinement, which may give some small improvement, particularly at the very high resolution end.

Hope this is helpful!

Tim

Tue, 04/23/2019 - 14:32 (Reply to #2)

lbchenj

Hi Tim,

Hi Tim,

That's great! Thanks so much for the valuable information.

Best,

Bin

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