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#1

jahull

FSC plot suffers from high resolution noise

I am working on a 4MDa ~400x230x230Angstrom, D5 virus which is elongated and mostly hollow. The map generated after CTF correction looks good (nucleotides, and aromatics are clearly distinguished), but the FSC plot appears to have high resolution artifact (attached as FSC_plot.png). If I continue local refinement the high resolution noise appears to get worse, then nonsensible.

I found an old forum post where somebody had a simmilar issue and used calculate_fsc with a mask to improve the FSC quality. I tried to do the same but found minimal improvement in the FSC plots. The calculate_fsc output is provided in the attached my_statistics.txt

I wrote out my half-maps and used the FSC validation server <https://www.ebi.ac.uk/pdbe/emdb/validation/fsc/results/> and got a reasonably believable unmasked resolution of 3.6 Angstroms (attached as FSC_plot_pdb_server.png).

Any thoughts on if some measures can be taken to avoid this behavior by cisTEM?

File:



FSC_plot.png



my_statistics.txt



FSC_plot_pdb_server.png

timgrant

Hi,

Hi,

Is it possible that you have multiple copies of particles in your refinement package?

Also, what molecular weight did you enter when creating the refinement package? If you entered 400 kDa instead of 4000 kDa for example, you may see this kind of thing.

Tim

jahull

I had to fidget with the

I had to fidget with the autopicker quite a bit to get it to find particles but it didn't appear to double count them when I previewed the micrographs. The molecular wieght entered was 4000kDa.

timgrant

Hmm, What is the max

Hmm, What is the max resolution used in the refinement?

Tim

jahull

I set maximum to 2 Angstroms

I set maximum to 2 Angstroms (nyquist).

timgrant

You are talking about the Hi

You are talking about the Hi-Res limit? That should always be quite a bit lower than your actual resolution, or it will lead to overfitting.

What did you use for your initial-resolution limit during the auto-refine?

Tim

jahull

Sorry I meant minimum, I set

Sorry I meant minimum, I set the initial low resolution to 20 angstroms for autorefinement. I lowered it to 10 angstroms when I did CTF estimation.

timgrant

hmm, but was it ever set to 2

hmm, but was it ever set to 2 Angstroms?

What was the highest (lowest number) it got to after the auto-refinement. You can see this by going to the refinement results panel, going to your last round of auto-refinement, clicking on job details and quoting the high-res limit number.

Tim

jahull

The autorefinement reached a

The autorefinement reached a high res. limit of 5.09 angstroms.

timgrant

And does the FSC still look

And does the FSC still look bad at that point?

Tim

jahull

No, the autorefinement looked

No, the autorefinement looked fine.

timgrant

Probably you increased the

Probably you increased the high-res limit beyond your actual resolution and what you see is overfitting.

In most cases, you shouldn't increase the limit much beyond what the auto-refinement tells you. I would re-run the autorefinement starting with your best reference, and an initial resolution of 8 angstroms and see what you get.

Tim

jahull

I think I understand now. Is

I think I understand now. Is the high resolution limit in manual refinement the same as the minimum refinement limit in autorefinement? If so then yes, I would want to raise it above 5.09 angstrom and start again.

jahull

As an update, your

As an update, your recommendation to set the high resolution estimate to 8 angstroms removed the high resolution noise observed. Thank you very much for your time in helping to resolve this issue.

JA Hull

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