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[Home](#) > manual refine

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## manual refine

Thu, 12/14/2017 - 05:30

#1



qcxy8886

manual refine

hi,

thank you for releasing this excellent fast software.

for my structrue, previously by frealign 9, it can be solved to 5A. I tried the manual refine in cisTEM. The resolution is pretty good, 4.4A. But on the map, there are some stripes. It means the potential overfitting. In frealign9, I can close FBOOST function. Here, this function changed to signed CC resolution limit. What the resolution should I change to avoid the overfitting? What else options I should use to avoid the overfitting?

thank you very much.

best regards,

chao





timgrant

Hi Chao,

Hi Chao,

What high resolution limit are you setting? Do you have auto masking set to yes? And are the stripes all over your reconstruction, or just in some places?

Thanks,

Tim







qcxy8886

hi Tim,

hi Tim,

i tried signed cc resolution to 3A and 4A. They all look similar.

the stripes are not at all construction. there are two places obviously. Because it is a membrane protein. i didn't use automask. i tried to delete the micelle to do refinement. At the top of the micelle, there are some stripes. Do you think the micelle which I didn't delete completely would affect the stripes?

best regards,

chao





timgrant

Hi Chao,

Hi Chao,

What was your alignment resolution though? not the CC resolution. 3A/4A sounds high for a 4.4A structure?

Tim







qcxy8886

Hi Tim,

Hi Tim,

the alignment resolution is 8Å. I first refine it in relation to 5Å, then lowpass to 8Å to be the initial model.

best regards,

chao





timgrant

Aah I see, well in that case

Aah I see, well in that case your Signed CC setting will not have an effect. The Signed CC resolution specifies what resolution the signed CC should be used until, after that the unsigned will be used. In your case, if you specify an alignment resolution of 8A, and a signed CC limit of 3 or 4A then the signed CC limit will have no effect as you are not using frequencies after 8A anyway. For example, if you put your signed CC resolution to 10A, then the unsigned would be used from 10A-8A.

Micelles do indeed often show bad overfitting. The way we typically overcome this, is to use a mask, but with filtering instead of true masking. If you start with a mask that is quite tight to the protein, and does not contain the micelle, import this and select it as the active mask in the manual refinement panel. Then in expert options under masking - set the "outside weight" to 1.0. Change "Low-pass filter outside of mask" to YES, and then set the resolution to something like 20 or 30A. This leaves low resolution signal in the reference from the micelle which can actually be useful for alignment, but removes high resolution signal and should therefore prevent overfitting within the micelle.

Cheers,

Tim







qcxy8886

Hi Tim,

Hi Tim,

Does cisTEM have the function to produce a mask? I tried to delete the detergent and import the mask produced by relion. But the result is not good. There are always some detergent stripes left.

Best regards,

chao





timgrant

There isn't currently a true

There isn't currently a true mask producing function in cisTEM - this will hopefully be added in a future version. The reason your relion mask isn't working is because it is not tight enough. Tight masks are bad, but in the case I'm suggesting nothing is truly masked, it is only filtered, so tight masks are not too bad. One option is to play with the relion settings if that is allowed to produce a tighter mask, filter less, use a smaller or zero cosine edge for example.

Here is how I make my masks, maybe it is helpful.

1. Take your reconstruction and open in chimera - in chimera turn on "hide dust" and adjust the threshold until your structure remains, but the noise and micelle are gone (hopefully this is possible, you may need to play with the sharpening) - you want the lowest value where this is true, record this number.
2. Run make\_size\_map, it is a command line program included with cisTEM. when it asks for a binarisation threshold enter the value you figured out above. This creates a new map where the values for each voxel are replaced with the number of connected voxels. You should now be able to binarise this program such that it keeps on the density for your protein. You cannot do this in cisTEM currently, you will need to use another program such as EMAN, IMAGIC, TIGRIS etc.. You could use this directly as a very tight mask, but i prefer to dilate it a little bit (maybe a couple of voxels), you can do this by filtering it and rebinarising it for example.

Actually - rereading your post It sounds like you have a map where you have erased the micelle. You can probably make a good mask from this just by binarising it at the level required to remove the background and keep your protein (you can check this in chimera). Again, you need to do this binarisation in another program.

Cheers,

Tim







qcxy8886

Hi Tim,

Hi Tim,

Thank you very much for the detailed explanation. I just have a last question. I tried to use EMAN2 to binarise the map produced by map\_size\_map, but I could not find the right command. How did you do it?

Best regards,

Chao





timgrant

proc3d should do it :-

proc3d should do it :-

proc3d input\_map.mrc output\_map.mrc bin=threshold\_value.

Cheers,

Tim



Sun, 01/14/2018 - 09:45 (Reply to #10)



qcxy8886

Hi Tim,

Hi Tim,

I tried your method and it works very good. The detergent micelle has no overfitting features now. There is a question I am still confused. When I open the map which produce by `make_size_map`( or the mask map after binarise) and the em map which I used to produce mask( or the map produced by `cistem`), the centers of these two maps are not in the same position. Did `cisTEM` automaticlly adjust the center of the mask map to match the referece map? Or I have to to adjust the center by myself, and how to do it?

Best regards,

Chao

Sun, 01/14/2018 - 10:16 [\(Reply to #11\)](#)



timgrant

Hi Chao,

Hi Chao,

If you open the maps in a program like chimera or pymol or other surface renderer, then the co-ordinate of the centre of the map will be taken into account (this is written into the MRC header). In cisTEM all of this information is ignored and the maps are just considered pixel by pixel. Thus if you open the two maps in the display that comes with cisTEM (go to Assets->Volumes select the two volumes and click display) you should see that in this slice representation the two line up.

I hope this answers your question?

Tim







timgrant

Hi Chao,

Hi Chao,

If you open the maps in a program like chimera or pymol or other surface renderer, then the co-ordinate of the centre of the map will be taken into account (this is written into the MRC header). In cisTEM all of this information is ignored and the maps are just considered pixel by pixel. Thus if you open the two maps in the display that comes with cisTEM (go to Assets->Volumes select the two volumes and click display) you should see that in this slice representation the two line up.

I hope this answers your question?

Tim

Mon, 01/15/2018 - 17:20 (Reply to #13)



qcxy8886

hi Tim,

hi Tim,

yes. previous i open with chimera. Now in cistem, they looks in the same center. thank you very much.

best regards,

chao



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