Do I need to clean the particles before 2D classification?

Tue, 09/11/2018 - 18:50 #1

Yifan

Do I need to clean the particles before 2D classification?

Hi, I am very new in Cryo-EM area.

Recently, I have collected Cryo-EM dataset with around 2,000 images. Every image contains 50-100 particles.

Then, I used cisTEM to do the auto-picking of the particles and directly went to 2D classification. From the 2D results, I picked up the good classes and will do ab-initio.

However, at this moment, my colleagues told me that I should manually clean the particles on every image after auto picking. They said that it will affect the final resolution if I don't do this cleaning step.

It will take me several days to clean particles on the 2,000 images, and you can imagine that this work is really boring.

So I would like to ask you, in your case, do you do manually particle cleaning before 2D classification?

Thanks.

Yifan

niko

You should inspect your

You should inspect your picked particles and decide if manual cleaning is really necessary. If there is a lot of "junk" (things that you think are not particles) it may be worth trying to adjust the picking parameters to improve the picking results. If this does not help, manual cleaning may be your only option. If you do not have much junk, manual cleaning is probably not needed. In any case, processing is quite fast and you could try how far you get. If your resolution is not sufficient you can always go back and manually clean your dataset.

Wed, 09/12/2018 - 16:41 (Reply to #2)

Yifan

Thank you so much for your

Thank you so much for your answer, niko. Now I understand much better. I also asked one professor from Japan. I paste his reply here (hopefully he will not mind):

No, no, you do not need to clean the picked particles manually.

Nobody does do such a thing.

But everyone around me has reached <3A resolution (i.e. 2.Xangstrom resolution) using various samples.

It is better to repeat 2D classification and 3D classification rather than manual discarding bad particles.

In addition, at the first round of 2D classification, you do not need to set the strict threshold.

Thu, 10/11/2018 - 14:22 (Reply to #3)

kaoweichun

Apparent bad particles from Class Members

Adding to this, I also find that the strategy to use 2D classification to remove obvious junk classes is a more efficient and systematic approach. Although some 2D calsses are predominantly comprised by outisers and can be easily excluded, I can still find obvious ice particles from the class member view in the classes I would like to proceed. However, further 2D classification cannot get rid of these probably because they have similar perspective as the biological molecule in thar particular orientation. Is there any way to delete them from the Class Member view?

Thanks,

WCK