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Fri, 12/13/2019 - 19:43

#1

btn1234

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I'm working with a small protein (57 kDa) in detergent and found it necessary to take movies of 54 frames with 120 e/A^2 total dose to have sufficient contrast for particle picking and 2D classification (at least, that worked better than smaller total doses). I'm now wanting to use the existing particles/assignments but with new images from movies processed with omission of the heavily exposed frames to see if I can get better resolution. Is there a suggested way to do that with cisTEM?

timgrant

Hi,

The movies should be optimally filtered for exposure, so "in theory" you shouldn't gain anything from removing frames.

However, if you want to try this, you can. You have to do another movie alignment, but select the range of frames that you want (this is in expert options). Once it is finished, when you go to Results->Align Movies it will show two alignment jobs, and the latest run should be selected. Whatever job is selected is the one that is used when extracting, so now create your refinement package in the same way you did before, and it should use the frame range you specified.

You will have to re-run the refinement by default, If you have alignment parameters you want to use, the only way to do it is to create the new refinement package, then take note of the particle stack filename. Go to the Results->Refinement results for the refinement you want to keep, then click the button above the FSC that looks like a clipboard. Click save on the pop-up window and save the parameters somewhere. You can then go the Assets->Refinement packages, and select import. Select Frealign, and choose your new partice stack and exported parameters.

I hope this makes sense.

Tim

Sat, 12/14/2019 - 05:32 <u>(Reply to #2)</u>

btn1234

Hi Tim,

Thank you for such prompt and clear advice. I will give it a try.

Tracy

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