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Wed, 07/22/2020 - 08:19   **#1**

xtalee

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Dear Tim,

Attached is FSC curves from the auto refine, showing an inflated curve. I've tried with setting the "search range X/Y" from default value (39) to something small (e.g., 20, 10, 5), but the same issue occured. The particles were auto-picked in cryoSPARC and imported into cisTEM for ab-initio reconstruction, which looks ok and almost same with cryoSPARC's. It's not a helical reconstruction although the particle is a bit elongated (~260A largest dimension vs. ~120A smallest dimension). The circular mask doesn't cut the density in the refinement panel.

Thank you in advance for your advice and I look forward to hearing from you.

John

File:
🖼 Screenshot from 2020-07-22 12-09-29.png
🖼 Screen Shot 2020-07-21 at 8.43.39 PM.png

timgrant

Hi John,

It definitiely looks weird. What did you set as your molecular weight?
Also, if there were quite a few duplicate picks it could lead to a problem
such as you are seeing.

Tim

Thu, 07/23/2020 - 04:43 <u>(Reply to #2)</u>

xtalee

Hi Tim

Thank you very much for your comment. I set ~520 kDa as a molecular weight when imported particles into cisTEM. Either increasing (e.g., ~800 kDa) or decreasing (e.g., ~250 kDa) molecular weight ended up with the same issue. I did double check and there are no duplicate picks.

Kind regards,

John

timgrant

Hi John,

This is weird, normally this kind of artifact occurs from the same data being included in both half maps. If not because of duplicate picks, because the same movie was included multiple times and picked multiple times, or because very close picks are being moved such that it is really use the same particle twice.

Did you import parameters from cryoSPARC as well, or were they all got through cisTEM?

Tim

Source URL:https://cistem.org/over-refinement-inflated-fsc