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Sun, 08/26/2018 - 15:19

#1

anaa2

Determining pixel size

Hi,

What is the best way of determining the true pixel size of a cryo-EM map? I know it sounds like a silly question, I've been fitting in a homologous structure into the map, and adjusting the pixel size of the map until the cross-correlation between the map and model is the highest. Is this an incorrect way of doing this?

Best wishes,

Noor

timgrant

Hi Noor,

Hi Noor,

I have done this also, but with crystal structures I know are basically identical to my reconstruction. In that case, I believe this is an ok way to calibrate the pixel size. If your homologous structure is not identical, then the difference between the structures may cause an error in your calibration.

Cheers,

Tim

Mon, 08/27/2018 - 12:11 (Reply to #2)

anaa2

Hi Tim,

Hi Tim,

I am calibrating to an EM structure (model not map) which is identical to the structure I am working on. Is that okay? Or could there be a propagation error in determining pixel size, when the initial structural model used for calibration was refined against an EM map with a pixel size that's not correct? Thank you in advance for all your help.

Best wishes,

Noor

timgrant

Hi Noor,

Hi Noor,

This will only work if the initial structure had the correct pixel size. In my experience a lot of EM structures will have some slight error in the pixel size. If the EM structure you use has an error, your estimate will obviously also have (at least) that error.

Tim

Mon, 08/27/2018 - 14:59 (Reply to #4)

anaa2

Thank you, Tim, your help is

Thank you, Tim, your help is much appreciated.

anaa2

Hi Tim,

Hi Tim,

I recently found that having my pixel size slightly wrong resulted in weird looking FSC curves (same as https://www2.mrc-lmb.cam.ac.uk/reliion/index.php?title=Pixel_size_issues). Is there a way to use CTFfind to help calibrate the pixel size? Thank you in advance.

Best wishes,

Noor

timgrant

Hi Noor,

Hi Noor,

Your FSC will look weird if you estimate the CTF with one pixel size, then reconstruct with another.

If you are importing, just make sure that you import with the pixel size that you use for CTF estimation, and it should be ok.

Cheers,

Tim

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