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#1

donaldb

Strange FSCs in Refinement

I have been getting some unusual artefactual FSCs when refining a number of different datasets. The FSC reaches a 0.143 threshold at a value which appears consistent with features in the map. There are then some large oscillations in the value after that point, reaching values up to ~0.4 near to nyquist. See attached picture.

Could you suggest what might be happening here and if there are any ways to alleviate the problem?

File:



[cisTEM_FSC.png](#)



[FSC_from_calculate_fsc.png](#)

niko

The FSC is calculated using

The FSC is calculated using half volumes masked with a spherical mask, followed by a scaling procedure to estimate the FSC that would have been obtained with a tight mask (see [publication on cisTEM](#)). If the particle density only occupies a small part of the spherical mask volume, the FSC may be strongly affected by the noise in the solvent, and the noise in the FSC curve is further amplified by the scaling to estimate the tight-mask FSC. Is your particle elongated and therefore requires a large mask radius that results in a spherical mask with a large amount of solvent?

donaldb

Niko,

Niko,

Thanks for the speedy response.

My particle certainly is elongated and does occupy a relatively small volume within the mask. When I calculate half-maps using the generate 3D function and calculate a masked FSC it looks fine.

The amount of noise generated (correlation of up to 0.4 at nyquist) does seem very high and is not something I come across refining the same particles in other packages. The unmasked FSCs simply underestimates the overall resolution without large spikes in signal at high values.

Is there anything I can practically do to minimise these artefacts other than calculating half maps and plotting FSCs elsewhere?

niko

You can use the `calculate_fsc`

You can use the `calculate_fsc` command-line program in the `cisTEM` bin folder to recalculate the FSC from the half volumes using a custom mask. The FSC will still be scaled (Part_FSC) but if the custom mask contains less solvent, the scaling factor will be smaller and the FSC curve will be less noisy.

donaldb

Thanks for clarifying that.

Thanks for clarifying that. The FSCs calculated from the half maps are fine but the resolution plotted by the cisTEM FSC method is a bit overinflated because of the influence of the large fluctuations producing artefacts.

Would it be possible for future versions to implement an alternative FSC plotting for cases such as this using a user provided mask or using the automasking used in the refinement, which is excellent?

It would also be useful if there was an option to automatically write out half maps during the refinement protocols rather than having to generate them separately.

niko

Thanks for the suggestions.

Thanks for the suggestions. Could you post the new FSC curve you obtained with `calculate_fsc`? It would be interesting to compare. Thanks.

donaldb

I have attached the FSC from

I have attached the FSC from `calculate_fsc` to my first post. I used my refinement mask with a soft edge added.

niko

Great, thanks! In case you

Great, thanks! In case you need to use it again, `calculate_fsc` adds a soft edge automatically. The two FSC curves seem to agree reasonably well, apart from the noise fluctuations, which I agree can be misleading. We are planning to allow user-defined masks, and we will also add automatic masking with a tighter, but still generous mask for FSC calculation.

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