Local defocus refinement

Tue, 05/29/2018 - 14:21 #1

tarek

Local defocus refinement

Dear all,

I just tried the defocus refinement on a ribosomal dataset and observed a considerable increase in nominal resolution.

How is the defocus estimated? Is based on cross-correlation (compare TF CRF in spider) or do you use a different approach?

Did you ever compare the results from frealignX with the local ctf estimation by gctf or ctffind?

Best,

T.

timgrant

Hi,

Hi,

The defocus search is done as a brute force search (min, max and step are defined in the GUI). for each search position the relevant CTF is applied to the projection and the score calculated. The defocus which maximises the score is taken as the optimized defocus.

I'm not aware of any direct comparisons having been done.

Cheers,

Tim

Fri, 06/08/2018 - 04:43 (Reply to #2)

tarek

many thanks Tim!

many thanks Tim!
just tried again with the last frealign (standalone) and confirmed that it didn't work before cisTEM (in my hands).

what is not clear to me yet: how is the order between reference projection, ctf application, alignment and score calculation?
How many cycles of ctf correction would you recommend?

timgrant

Hi,

Hi,

I would process your data as best as you can, then do the CTF refinement at the end. One round is likely going to be enough, but you can do a couplel to see if anything changes. When doing the CTF refinement, I would recommend not refining the alignment parameters at the sarme time, so I would untick ALL of the boxes at the top of the expert options panel. You will also need fairly high resolution to discrimate, 4A or so.

In the refinement, the projection with the current angles is calculated and a brute force scan of defocus values around the current best defocus is done. For each one the CTF is applied and the score calculated, the defocus with the best score is kept.

Cheers,

Tim