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Sat, 08/04/2018 - 10:42

#1

hzhan3

Movie alignment failed when add directory

Hi all,

I am new to cisTEM and recently collected dose-fractionation tilts at Janelia. When I added Directory to import movies for alignment, some tilts showed big shifts and blurry but when I just imported that blurry tilt only, and ran the same parameters, it turned out fine... Does that mean I should do one by one for tilt series?

For my image condition: 1) 2.93 e/A² and 5 frames, because we would like to keep min dose for each frame to give density; 2) we did 3 degree increment from -60 to 60; 3) pixel size is 1.3 at super-res mode and binned by 2, 2.6Å/pixel. 4) applied gain reference when importing.

Thanks for this amazing software!

Best,
Hong

File:



[Screenshot from 2018-08-04 09-38-47.png](#)



[Screenshot from 2018-08-04 09-39-04.png](#)

timgrant

Hi Hong,

Hi Hong,

Are you trying to use cisTEM to process tomograms? It doesn't really support that at the moment.

If you just want to use the movie alignment you can do, but each tilt would have to be imported as a separate movie, otherwise it will align all the frames to the sum of all the frames - which is definitely not what you want. If you just want to use it for aligning movies for tilts - you may be better off using the command line version of unblur.

Thanks!

Tim

hzhan3

Hi Tim,

Hi Tim,

Thanks!! I only used it for aligning movies for now... Thanks! that is what I thought... In the command line, unblur seems only support mrc files?

Thanks Tim!

Hong

timgrant

Are you running the unblur

Are you running the unblur executable from the cisTEM bin directory? It should support tif.

Tim

hzhan3

I tried from the unblur not

I tried from the unblur not from cisTEM bin directory. I will try the unblur from cisTEM bin directory. Thanks Tim!

hzhan3

Hi Tim, unblur from cisTEM is

Hi Tim, unblur from cisTEM is good to do movie alignment. one stupid question... How can I apply gain reference? I don't see there is an option when I use unblur... Or I should use clip function to apply the gain reference first?

Thanks!!!

Hong

```
Input stack filename [my_movie.mrc]      :
232_51.0_Jul26_22.36.37.tif
Output aligned sum [my_aligned_sum.mrc]   :
232_51.0_aligned_sum.mrc
Pixel size of images (A) [1.0]           : 1.3
Output binning factor [1]                : 2
Apply Exposure filter? [yes]             : yes
Acceleration voltage (kV) [300.0]        : 300
Exposure per frame (e/A^2) [1.0]         : 0.586
Pre-exposure amount (e/A^2) [0.0]        :
Set Expert Options? [no]                 :
Correct Magnification Distortion? [no]    :
image #0 = 0.574013, 1.064871
image #1 = 0.396857, -0.953290
image #2 = 0.000000, 0.000000
image #3 = -0.358658, 0.305641
image #4 = -0.753628, -0.196185
```


timgrant

Hi Hong,

Hi Hong,

Select yes for Set Expert Options, it will give you more options, you can accept the defaults for them all - but say NO to input is already gain corrected, you can then give a gain reference.

Tim

hzhan3

Thanks, Tim !!!!! sorry for

Thanks, Tim !!!!! sorry for the stupid questions.

Hong

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