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# movie alignment problems

Wed, 05/09/2018 - 14:59

#1



Evan

movie alignment problems

Dear all,

I'm having a problem aligning frames where it seems like movie alignment is working but the FFT of the aligned sum shows a very strong central cross (attached). I've messed with the values for "mask central cross" and "B-Factor" in the GUI, but I'm still getting a very strong central cross in the FFT. Furthermore, I've noticed that for each stack the movie alignment always picks up large shifts every 10 frames, which I don't think reflect real specimen movement.

I thought that these problems might be due to the gain correction because I was not sure if the intermediate ".raw" frames that are output from our old version of EPU were gain corrected. However, when I give cisTEM the gain reference image that was collected just before the acquisition the motion correction doesn't work and the summed frames look exactly like the input gain reference (attached).

I'm wondering if this is a fundamental issue with my data or if I am just somehow messing up the data that I am feeding into cisTEM due to ignorance. Any feedback would be appreciated.

Evan


Camera: Falcon II

Frames: Output as intermediate frames in .raw format from EPU and then converted to .mrc stacks using raw2mrc

Gain reference: Collected before data acquisition using the falcon reference image manager

**File:**

 [movie\\_alignment\\_no\\_gain.png](#)

 [Movie\\_alignment\\_gain\\_ref.png](#)





timgrant

Hi Evan,

Hi Evan,

Are you correcting for magnification distortion in these images?

Cheers,

Tim



Wed, 05/09/2018 - 15:26 (Reply to #2)



Evan

Hi Tim,

Hi Tim,

No, I have not been doing magnification distortion correction on these images.

Thanks for the quick response!

Evan





Evan

I determined that the

I determined that the movement every 10 frames was due to my useage of raw2mrc. EPU outputs the intermediate frames with the naming convention:

frame\_0.raw, frame\_1.raw ... frame\_38.raw, frame 40.raw

raw2mrc requires the frames be named:

frame\_00.raw, frame\_01.raw ... frame\_38.raw, frame 40.raw

raw2mrc was putting the frames in the wrong order. So at least the the movement every 10 frames is resolved.







timgrant

Hi Evan,

Hi Evan,

Good you figured out the weird movement. The cross suggests you have some kind of line artifact in your images. This could be gain reference, or it could be some kind of edge. If you look at the very edge of your images, do you see some kind of line? If it is a fairly minor line artifact, it probably shouldn't affect your processing and you can likely just ignore it.

Cheers,

Tim

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