# 2D classification- blank classes

Tue, 11/06/2018 - 06:50 #1

ubjath01

2D classification- blank classes

Hi,

1. I am new to cisTEM- I was wondering if anyone has any information on why for a 2D classification job I get blank images for 2D classes from iteration 1 onwards (see attached)?

2. Am I correct to say that if you import a 'package' from relion, the star file does not need to have the correct links to particles- as cisTEM works on the order of particles in the large particle  stack instead (i.e line 327 would refer to slice 327 in the particle stack etc)?

Cheers,

Joe

**File:**
Round0.png
Round1_onwards.png

timgrant

Hi Joe,

Hi Joe,

Yes, when you import a package it just reads the first line of the star file and uses that info for the first particle in the stack etc.

What are your defocus values?  In the Assets->Refnement package, when you click on your imported refinement package and look at the imported defocus values, are they weird? E.g. are they all 0.0, or all very similar?

Cheers,

Tim

Tue, 11/06/2018 - 11:12 (Reply to #2)

ubjath01

# 2D classification- blank classes

Hi Tim,

Thanks for your quick reply.

Defocus, defocus angle and phase shift looks ooks fairly normal for phase plate data... see below for first 20 particles.

Clearly something is wrong as there is lttile change in the parameters from round1 to round 20. Also if I am right that OCC is the class membership in the .par files, then it appears that essentially no classification has occured.

Cheers,

Joe

ROUND1 (classification_input_par_1.par)

| C | PSI | THETA | PHI | SHX | SHY | MAG | FILM | DF1 | DF2 | ANGAST | PSHIFT | OCC | LogP | SIGMA | SCORE | CHANGE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 2 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 3 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 4 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 6 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 7 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 8 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 9 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | 24.16 | 1.49 | 100.00 | 0 | 10.0000 | 0.00 | 0.00 |
| 13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 | | | | | | | |

24.16 1.49 100.00 0 10.0000 0.00 0.00
14 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 10.0000 0.00 0.00
15 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 10.0000 0.00 0.00
16 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 10.0000 0.00 0.00
17 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 10.0000 0.00 0.00
18 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 10.0000 0.00 0.00
19 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 10.0000 0.00 0.00
20 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 10.0000 0.00 0.00

ROUND 20 (classification_input_par_20.par)

C PSI THETA PHI SHX SHY MAG FILM DF1
DF2 ANGAST PSHIFT OCC LogP SIGMA SCORE CHANGE
1 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
2 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
3 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
4 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
5 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
6 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
7 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
8 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
9 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
10 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
11 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 100.0000 0.00 0.00
12 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 77.1605 0.00 0.00
13 0.00 0.00 0.00 0.00 0.00 0 0 4687.5 4083.9
24.16 1.49 100.00 0 77.1605 0.00 0.00

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 |
| 24.16 | 1.49 | 100.00 | 0 | 77.1605 | 0.00 | 0.00 | | | |
| 15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 |
| 24.16 | 1.49 | 100.00 | 0 | 100.0000 | 0.00 | 0.00 | | | |
| 16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 |
| 24.16 | 1.49 | 100.00 | 0 | 100.0000 | 0.00 | 0.00 | | | |
| 17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 |
| 24.16 | 1.49 | 100.00 | 0 | 77.1605 | 0.00 | 0.00 | | | |
| 18 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 |
| 24.16 | 1.49 | 100.00 | 0 | 100.0000 | 0.00 | 0.00 | | | |
| 19 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 |
| 24.16 | 1.49 | 100.00 | 0 | 100.0000 | 0.00 | 0.00 | | | |
| 20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0 | 0 | 4687.5 | 4083.9 |
| 24.16 | 1.49 | 100.00 | 0 | 100.0000 | 0.00 | 0.00 | | | |

Tue, 11/06/2018 - 15:45 (Reply to #3)

niko

Please check that all your

Please check that all your parameters, including pixel size and mask size/particle diameter, are set correctly.

Wed, 11/07/2018 - 07:23 (Reply to #4)

ubjath01

Found the problem

Hi Tim,

I've identified the problem- a symptom of me not paying enough attention- I realise now that it is the mask RADIUS which is required in cisTEM rather than the mask DIAMETER as in relion. The classification is running fine now with a mask not bigger than the box!

Thanks for your help,

Joe