2D classification of samples on carbon film

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fullerj
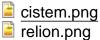
2D classification of samples on carbon film

I've been attempting to process a small dataset of ribosomes sitting on a thin continuous amorphous carbon film in CisTEM. CisTEM's particle picker still does a great job picking particles despite the increased background noise that comes with the carbon film, but 2D classification results in a set of classes that look uniformly like fuzzy spheres. Importing the same particle coordinates into Relion results in 2D classes that look recognizably like ribosomes, using essentially the default settings in Relion. However, I'm still interesting in using CisTEM to process this and similar future datasets.

I've tried varying amplitude contrast (from 0.07 to 0.15), the starting and final resolution (from 40-60 A and 8 to 25 A, respectively), the low pass filter (from 300 to 700 A), and various particle mask radii (from tight to generous), and smoothing factor (from 0.1 to 1.0), and -- at least individually -- none of these seem to change the results much.

I'll attach a sample of a 2D class average from CisTEM (all classes come out looking like this) vs Relion (using the same particle set) to this post.

What settings would you recommend for 2D classification of particles on thin carbon films?

File:
🖼 cistem.png
🖼 relion.png

timgrant

Hi,

We have processed a number of ribosome datasets on thin carbon film using the defaults without problem, so I'm not sure what the issue could be here.

Are they all very similar defoci? This could be an issue.

The only thing I can think of is to run more rounds, we have had it take maybe 50 or 100 rounds to get all the class averages very good, but there should definitely be some nice ones after 20 rounds.

Tim

Source URL:https://cistem.org/2d-classification-samples-carbon-film?page=0