

isSPA Manual

version: 1.2

Authors: Yuanhao Cheng

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cryoSPARC

1. Do “Import Movies, Patch Motion Correction, Patch CTF Estimation, Manually Curate Exposures” in cryoSPARC

2. Activate pyem environment, and convert .cs file to .star file:

```
csparc2star.py      CS-test-data/J3/exposures_ctf_estimated.cs      CS-test-data  
/J3/J3_passthrough_exposures.cs                      CS-test-  
data/J4/J4_passthrough_exposures_accepted.cs micrograph_ctf.star
```

3. Enter the directory of cryoSPARC project: `cd CS-test-data`

4. Start GUI: `isSPA.py &`

5. Click “Import parameters from RELION” and select `micrograph_ctf.star`.

6. Select an appropriate 3D Template by clicking “select”.

7. (Optional) If you have the corresponding FSC file (STAR format), select it.

8. Choose Protein Mass range, and input Diameter.

9. Confirm the No. of images to be processed.

10. Modify the output file name.

11. (Optional) Click Save Config and save the parameters you have inputted in the GUI, for your convenience.

12. Click Start Processing, and calculation will start automatically.

13. (Optional) Click Display Particles and check the picking results. Select the file that ends with “merge_31.star”.

14. Use RELION to do Particle Extraction, 3D classification and Subset selection

a) Reference: Initial low-pass filter (A): 30-60

b) Sampling: Perform image alignment? No!!!

15. You can also do 3D Classification (without alignment) in cryoSPARC by providing solvent mask and focus mask.

RELION

1. Start GUI: [isSPA.py &](#)
2. Click “Start RELION” and use RELION to do “Import, Motion Correction, CTF estimation”.
3. (Optional) Select micrographs with mrc_picker.py.
16. In the isSPA GUI, click “Import parameters from RELION” and select micrograph_ctf.star in the CtfFind directory.
4. The following steps are the same as those in cryoSPARC step 6.