

# S2C2 CryoEM Image Processing Workshop June 10-12, 2020

## cryoSPARC Instructions

### Day 1 Practical Instructions

#### Import and Motion correction

1. Create a new project
2.
  1. Use your name in the project title, this will be used for all your processing
  2. Select  
  
`/scratch/training/projects`  
  
as the project directory
  3. **Set project level SSD parameter to “Disabled by default”**
3. Create a new workspace
4.
  1. Call it “T20S”
5. Import Movie data
6.
  1. Create a new “Import Movies” job
  2. Set the movie data path to  
`/scratch/training/SLAC_workshop_data/1_T20S/movies/*.tif`
  3. Set the gain reference path to  
  
`/scratch/training/SLAC_workshop_data/1_T20S/movies/norm-amibox05-0.mrc`
  4. Pixel size 0.6575 ;  
  
Accelerating voltage 300 ;  
  
Spherical aberration 2.7 ;  
  
Exposure dose 53
7. Create a “Patch motion correction” job
8. Connect the outputs of your import job to the new job
9.
  1. Drag and drop into the job builder
10. Ensure parameters are correct
11.
  1. We will use defaults
12. Run the job
- 13.

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1. Click “Queue”
2. Select the default lane
14. Inspect the job to see it running
15.
  1. Press “spacebar” or click on the job number on the job card
  2. Look at the “Overview” tab

#### CTF Estimation

1. Create a “Patch CTF estimation” job
2.
  1. Connect the outputs of your motion correction job to the new job
  2. Run the job

#### Data Curation

1. Create an “Exposure curation” job
2.
  1. Connect the output of CTF estimation to this job

#### Particle picking

1. Create a blob picking job
2.
  1. Connect the outputs of CTF estimation to this job
  2. Number of mics to process: 5 ;  
  
Minimum diameter: 100 Å ;  
  
Maximum diameter: 200 Å ;
3. Inspect particle picks job
4.
  1. Connect output of blob picking
  2. Interactive job
  3. Threshold NCC score ~0.22; power score 600 – 1000
5. Extract from micrographs job
6.
  1. Connect output of inspect picks
  2. Box size 440; Fourier-crop size 256
7. 2D classification with 20 classes
8.
  1. Connect output of Extract from micrographs

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2. If the job does not run with a message about SSD caching, manually turn off the “Cache particles on SSD” parameter
9. Select 2D
10.
  1. Connect the output of 2D classification
  2. Select one top and one side view
11. Template picking job
12.
  1. Connect the output of Select 2D (templates)
  2. Connect the output of CTF estimation (micrographs)
  3. 200 Å particle diameter
13. Inspect particle picks
14.
  1. Connect the output of template picking
  2. Threshold NCC score ~0.34 ; power score 1100 – 1700
15. Extract from micrographs
16.
  1. Connect the output of inspect picks
  2. Box size 440; Fourier-crop size 256

#### 2D classification

1. Create a 2D classification job with 50 classes
2.
  1. Connect the output of Extract from micrographs
3. Create a 2D classification job with 100 classes
4.
  1. By cloning the previous 2D class job and changing the number of classes parameter

#### Ab-initio reconstruction

1. Select 2D classes
2.
  1. Connect the output of 2D classification
  2. Select all good classes
3. Create a 1 class ab-initio reconstruction job
4.
  1. Connect the output of Select 2D
5. Create a 2 class ab-initio reconstruction job
6.
  1. Clone from previous job

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### Refinement

1. Homogeneous refinement (NEW!) job
2.
  1. Connect the output of ab-initio reconstruction (1 class) both particles and volume
  2. Change symmetry to D7