

S2C2 CryoEM Image Processing Workshop June 10-12, 2020

cryoSPARC Instructions

Day 2 Practical Instructions

For each dataset, create a new workspace inside your project. Do not create a new project.

Note your project number "PXX" - you will have to fill this in in some of the import paths below.

HA Trimer - new workspace

1. Import Movies job
2.
 1. Movies data path:

```
/scratch/training/SLAC_workshop_data/5_HAtrimer/*.bz2
```
 2. Gain reference:

```
/scratch/training/SLAC_workshop_data/5_HAtrimer/16nov14z_16nov08a_08124646_01_3838x3710_norm_0.mrc
```
 3. **Flip gain in Y**
 4. Pixel size 1.31 ; accelerating voltage 300 ; spherical aberration 2.7 ; exposure dose 82
3. Patch motion correction job - default parameters
4. Patch CTF estimation job - default parameters
5. Exposure curation job

AAA+ Unfoldase - new workspace

1. Import particles job
2.
 1. Particle meta data path:

```
/scratch/training/SLAC_workshop_data/3_AAA/particles/particles.star
```
3. 2D classification job
4.
 1. Connect imported particles
 2. Default parameters
5. 2D classification job
6.
 1. Clone previous 2D classification job
 2. Circular window diameter 150Å

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7. Select 2D classes
8.
 1. Connect input from second 2D class job (with windowing)
 2. Select all “good” classes
9. Abinitio reconstruction
10.
 1. Connect particles from select 2D job
 2. Set number of classes to 3
11. Homogeneous refinement (NEW) job
12.
 1. Connect particles and volumes for inactive state class from ab-initio reconstruction
 2. Default parameters
13. Homogeneous refinement (NEW) job
14.
 1. Connect particles and volumes for active state class from ab-initio reconstruction
 2. Set symmetry to C6

GPCR - new workspace

1. Import result group job
2.
 1. Path to csg file (NB: fill in your project number for PXX)


```
/scratch/training/projects/PXX/SLAC_workshop_data/2_GP  
CR/P26_J35_particles/P26_J35_particles_exported.csg
```
3. 2D classification job
4.
 1. Connect imported particles
 2. Default parameters
5. Select 2D classes
6.
 1. Select all “good” classes
7. Abinitio reconstruction - default parameters
8. Homogeneous refinement NEW - default parameters
9. Non-uniform refinement - default parameters

Apoferritin - new workspace

1. Import result group
2.
 1. Path to csg file: (NB: fill in your project number for PXX)

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```
/scratch/training/projects/PXX/SLAC_workshop_data/6_APOF/P18_J1175_particles/P18_J1175_particles_exported.cs  
g
```

3. Import volumes

4.

1. Volume data path:

```
/scratch/training/SLAC_workshop_data/6_APOF/map.mrc
```

5. Homogeneous refinement (NEW) job

6.

1. Connect imported particles and volume
2. Set symmetry to O
3. Turn off "Do symmetry alignment"
4. Set "Initial lowpass resolution" to 15 Å
5. Turn on "Minimize over per particle scale"

7. Homogeneous refinement (NEW) job

8.

1. Clone previous job
2. Ensure advanced params toggle is on
3. Turn on "Optimize per-particle defocus"
4. Turn on "Optimize per-group CTF params"

ATPase - new workspace

1. Import result group

2.

1. Path to csg file: (NB: fill in your project number for PXX)

```
/scratch/training/projects/PXX/SLAC_workshop_data/7_ATPase/P96_J13_particles/P96_J13_particles_exported.csg
```

3. Import Volume job

4.

1. /scratch/training/SLAC_workshop_data/7_ATPase/mask.mrc
2. Set "Type of volume" to mask

5. 3D Variability Analysis job

6.

1. Connect imported particles and mask
2. Set "Filter resolution" to 8 Å

7. 3D Variability Display job

8.

1. Connect particles and volume from 3D VA job
2. Set "Downsample to box size" to 128

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3. Set “Crop to size” to 100

On Thu, Jun 11, 2020 at 11:02 AM Polizzi, JoAnn <joannp@slac.stanford.edu> wrote:

Dear Practical Participants,

Please see below Day 1 Practical Instructions, please go through and make sure you are caught up. Ali will send along Day 2 dataset instructions this morning.

Slides should be up on website by end of day.

Best,

JoAnn

From: Ali Punjani <alipunjani@cs.toronto.edu>

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

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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

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13.

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

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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
 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)

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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

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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

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