

Single particle analysis (SPA) using CryoSPARC

Gaya P. Yadav

gaya.yadav@ag.tamu.edu

BCBP Cryo-EM Research Center (BCRC)
College Station, TX, USA

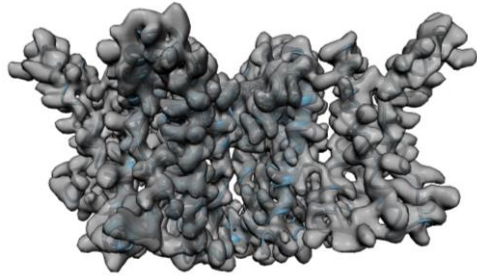
04/14/2023

Contents

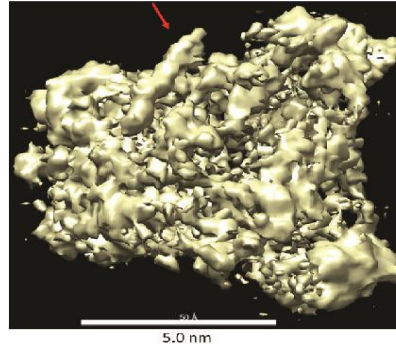
1. Our expertise in Cryo-EM.
2. Overview of BCBP Cryo-EM Research Center (BCRC).
3. Capabilities we have at BCRC.
4. Cryo-EM history, pros & cons.
5. Sample data processing using CryoSPARC

Our expertise in cryo-EM at BCRC

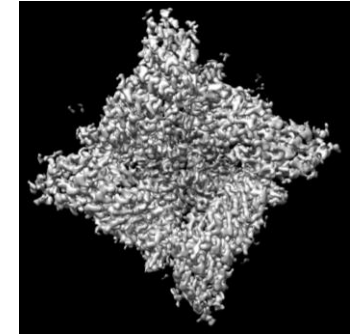
➤ Single particle analysis of Membrane proteins



Pot. channel (3.58Å, K2, 300kV)

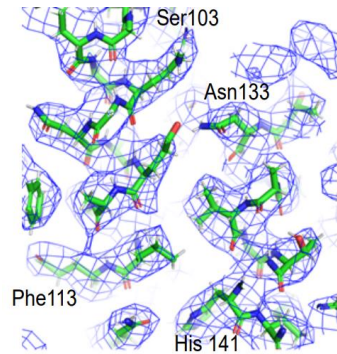


Cl⁻ channel (5.64Å), Falcon 4, 200kV

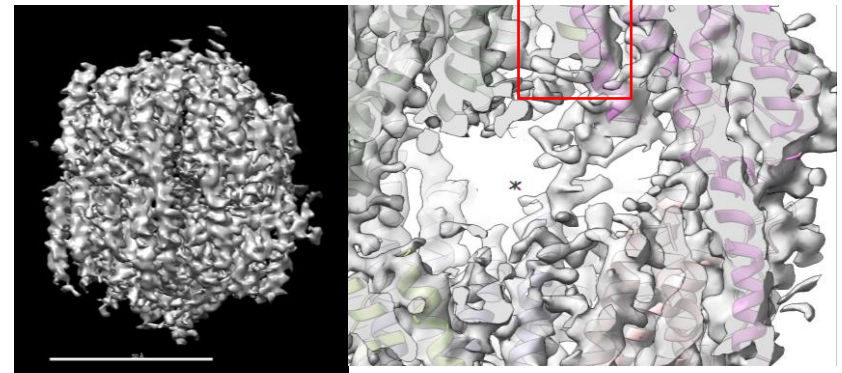
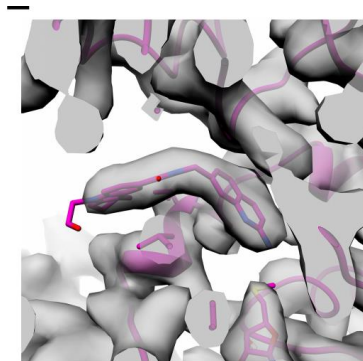


TRP channel (3.22Å),
K3, 300 kV

➤ Single particle analysis of soluble proteins & SBDD.

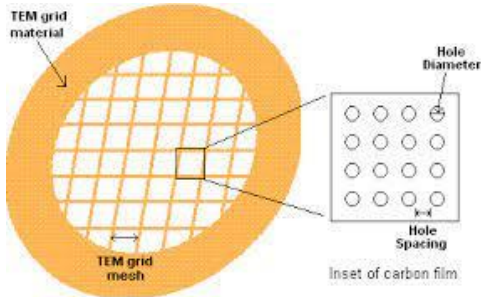


PRMT5:MEP50-11-2F (3.13Å), K2, 300 kV



C3PO-ssRNA complex (3.94Å), Falcon IV , 200kV

BCRC overview: high end equipment



Holey TEM grid



Glow-discharge

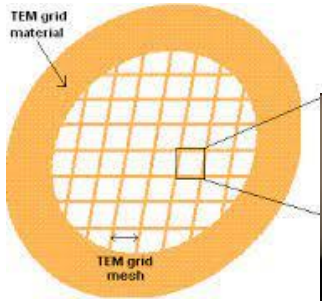


Vitrobot Mark IV



Titan Krios G4

BCRC overview: high end equipment



Holey TEM grid



Vitrobot Mark IV



Glow-discharge



Titan Krios G4

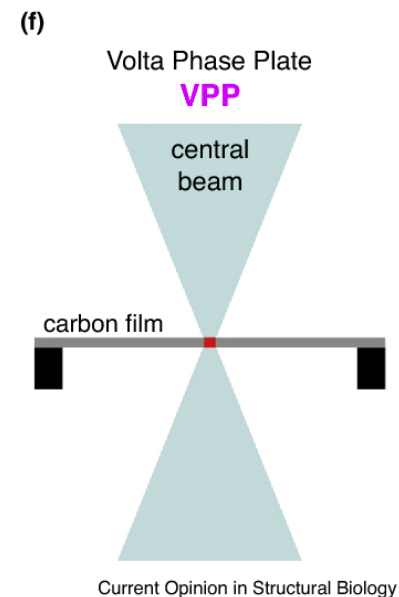
BCRC overview: DED & Energy filters



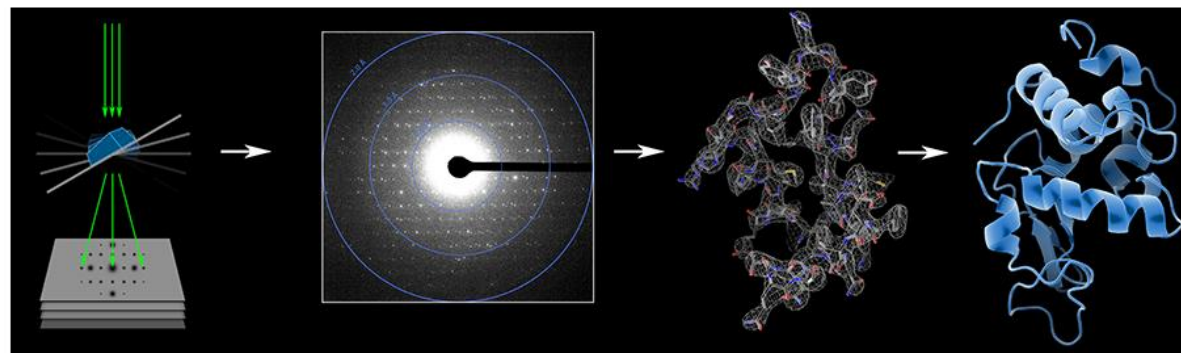
K3 DED Camera



BioContinuum Imaging Filter



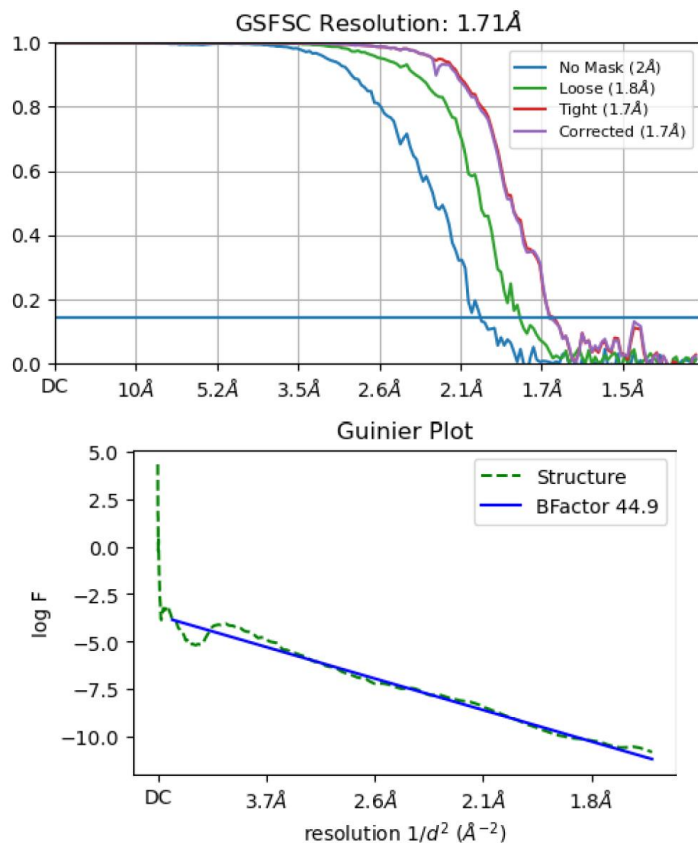
Ceta-D Camera



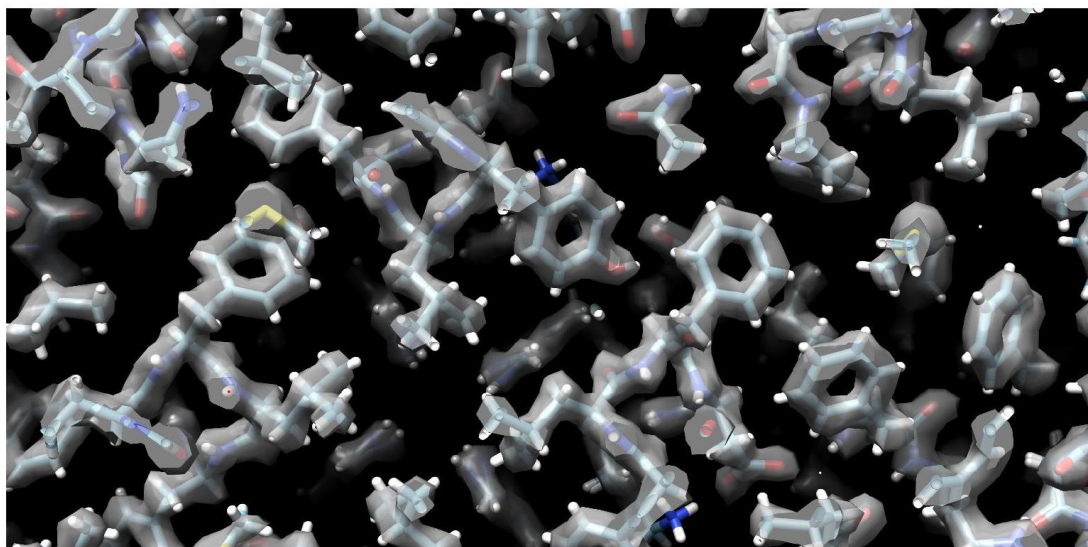
Micro-ED workflow

BCRC overview: Test results

Fourier Shell Correlation and B-factor

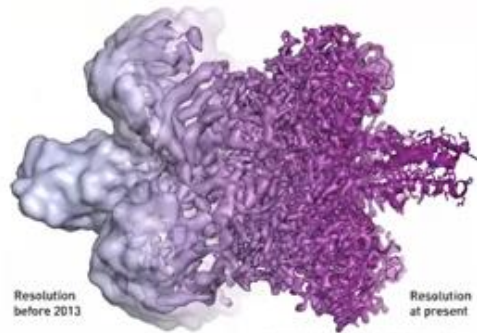


Atomic Structure Docking



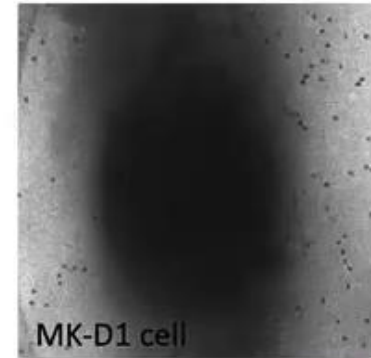
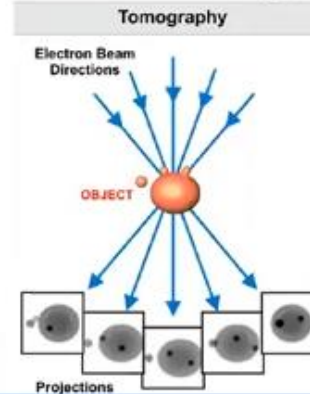
Capabilities we have at BCRC

Single Particle Analysis

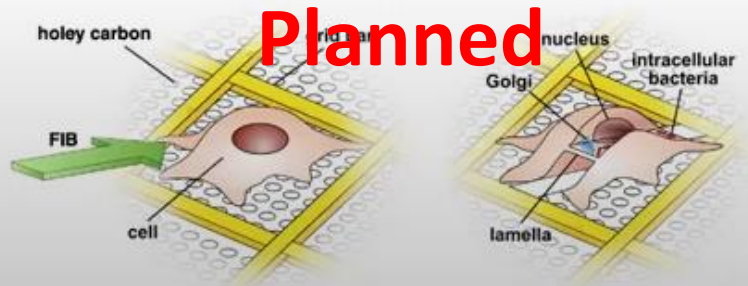


glutamate dehydrogenase, 334 kDa, 1.8 Å

Tomography (cryo-ET)



FIB-Milling

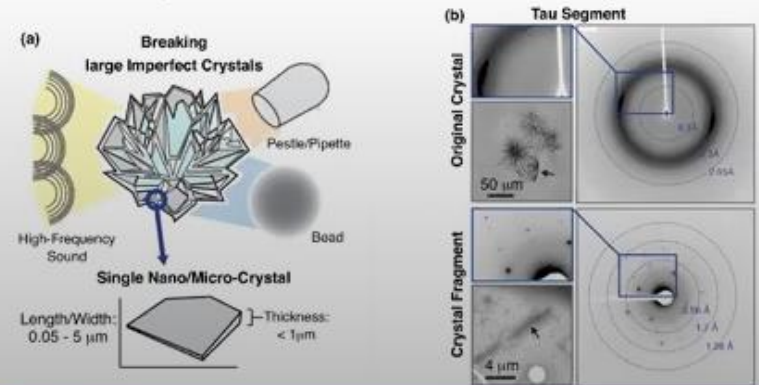


Planned

identifying a target cell for milling

milling lamellae of ~200 nm thickness

Microcrystal electron diffraction (MicroED)



Capabilities we have at BCRC

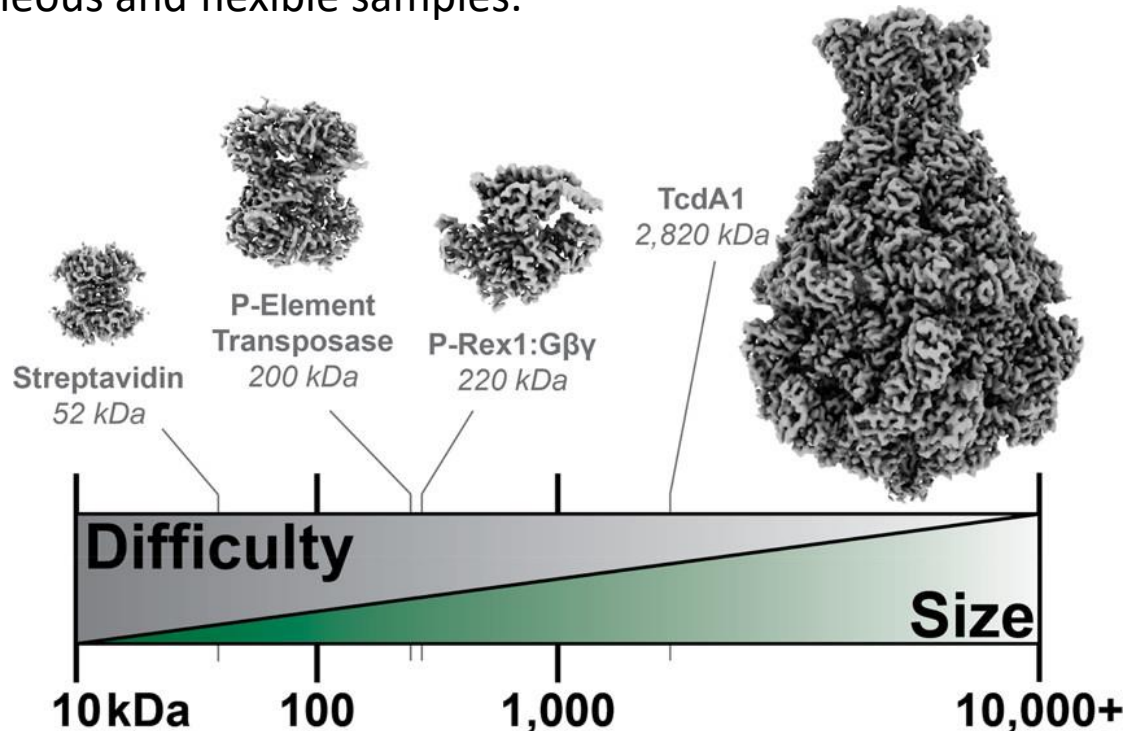
1. Sample vitrification, grid screening, and data collection for Single particle analysis.
2. Data collection for Cryo-ET.
3. Grid vitrification and data collections for micro-ED.
4. Other services such as cryo-EM data processing, refinement, and model building.
5. Training in sample vitrification, data collections, and cryo-EM data processing.

Cryo-EM: A game-changer for structural biology

- No fixing or staining is required (native state of the sample).
- No need to grow crystals (X-ray crystallography).
- A small amount of sample (vs X-ray crystallography and NMR).
- Possible applicable for heterogeneous and flexible samples.

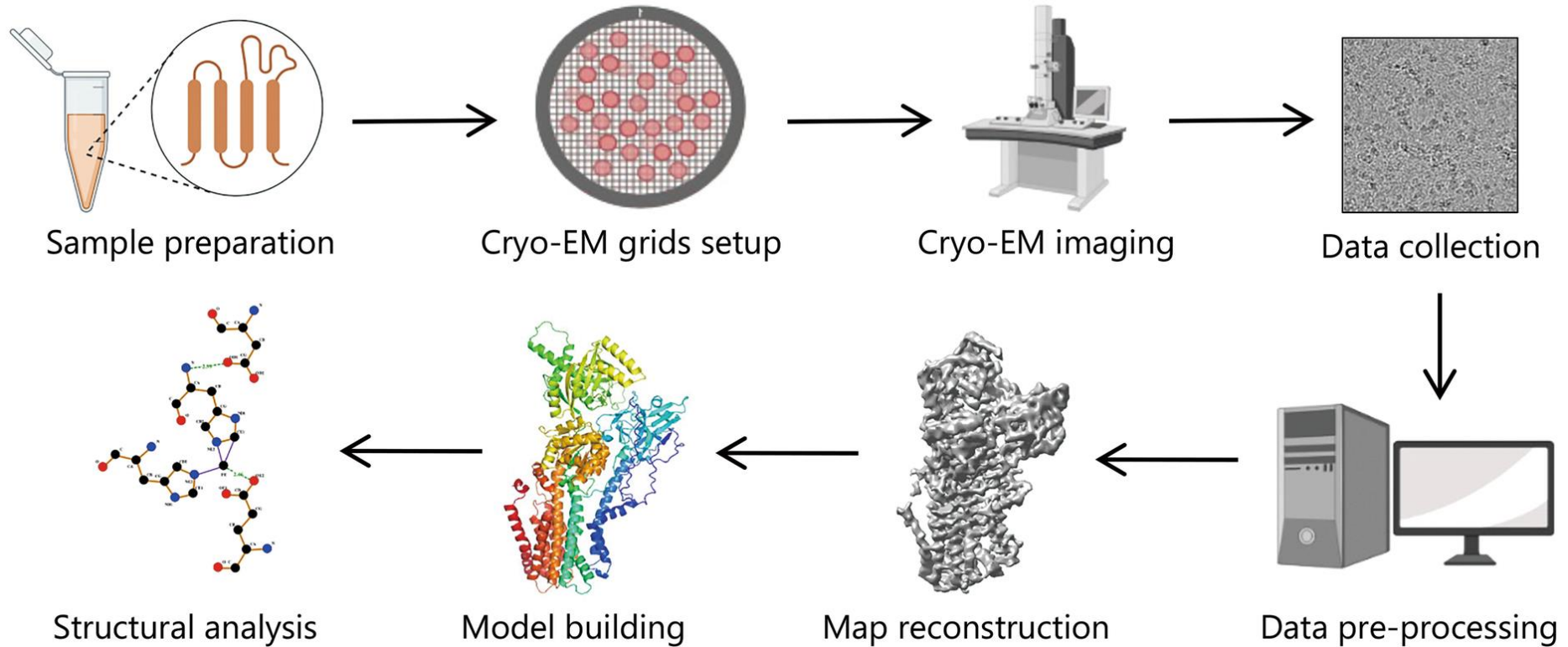
Limitations:

- Size of the complex: ~100 kDa or larger preferred.
- Sample orientation: should be randomly oriented.
- Ice thickness: ice should be just thin enough to hold particles.
- Electron dose limitation.



J. Chem. Inf. Model. 2020, 60, 5, 2458-2469

Cryo-EM: Workflow



Zhu, KF., et. al. *MMR* 10, 10 (2023)

Cryo-EM: data processing software

- RELION: https://www3.mrc-lmb.cam.ac.uk/relion/index.php/Main_Page
- cisTEM: <https://cistem.org/>
- cryoSPARC: <https://cryosparc.com/>
- EMAN2: <https://blake.bcm.edu/emanwiki/EMAN2>
- IMOD: <https://bio3d.colorado.edu/imod/>
- Scipion: <https://scipion.i2pc.es/>

cryoSPARC: Single particle analysis

CryoSPARC hierarchy

Project-specific sample



CryoSPARC: movies pre-processing

1. Import Movies: Brings raw movie frames into the program
2. Patch motion correction: Aligns movie frames to account for sample and stage movement and produces an aligned average or micrograph, deblur movies
3. Patch CTF estimation: (After motion correction) attempts to measure additional parameters that vary from one micrograph to another - astigmatism, defocus, estimated resolution, etc.

CryoSPARC: importing movies

Importing tutorial movies:

- ApoFerritin $\sim 2.3\text{\AA}$ resolution (BCRC workflow validation)
 - First 25 movies
- Accelerating voltage for the microscope: 300kV
- Spherical aberration = 2.7
- Calibrated Pixel size = 0.326 (super-resolution)
- Total accumulated dose = $42\text{ e-}/\text{\AA}^2$

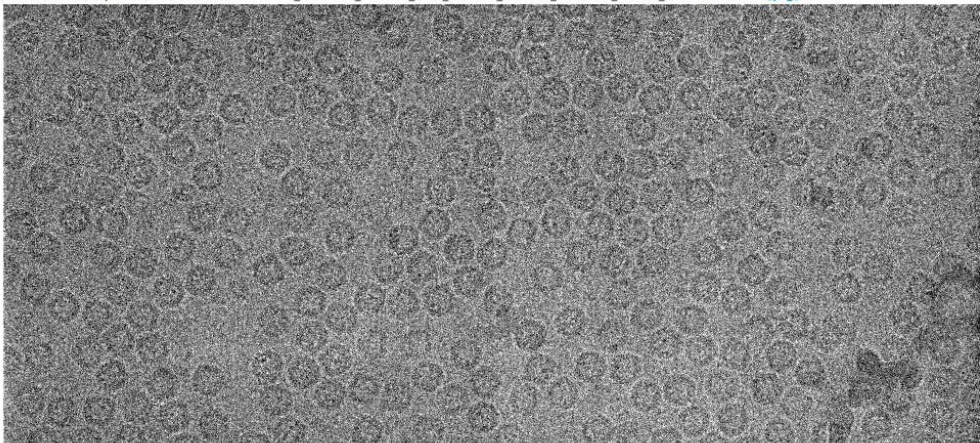
CryoSPARC: importing movies

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags


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> [CPU: 211.5 MB] =====
> [CPU: 211.5 MB] Loaded 25 movies.
> [CPU: 211.5 MB] Common fields:
> [CPU: 211.5 MB]     mscope_params/accel_kv : {300.0}
> [CPU: 211.5 MB]     mscope_params/cs_mm : {2.7}
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> [CPU: 211.5 MB]     movie_blob/shape : [ 40 8184 11520]
> [CPU: 211.5 MB]     movie_blob/is_gain_corrected : {0}
> [CPU: 211.5 MB] =====
> [CPU: 211.5 MB] Making example plots. Exposures will be displayed without defect correction.
> [CPU: 211.5 MB] Reading file...
```

Raw data J34/imported/005268782221562727002_FoilHole_3967163_Data_3960642_3960644_20221004_215755_fractions.tiff [png]



Outputs

imported_movies



exposure Count: 25

failed_movies

exposure Count: 0

J34 (Import Movies) COMPLETED

New Job J34

Enter a description.

CREATED BY admin
LAST ACCESSED BY admin
LAST ACCESSED AT Wed, Apr 12, 2023 2:06 PM
INTERACTIVE No
CREATED Sat, Apr 8 2023 11:16:52 PM
QUEUED Sat, Apr 8 2023 11:17:56 PM
LAUNCHED Sat, Apr 8 2023 11:17:57 PM
STARTED Sat, Apr 8 2023 11:17:58 PM
COMPLETED Sat, Apr 8 2023 11:18:36 PM
CHILDREN J35
SIZE 2.06 MB

ACTIONS

Queue Job

Link Job

Move Job

Kill Job

Clear Job

Clear Intermediate Results

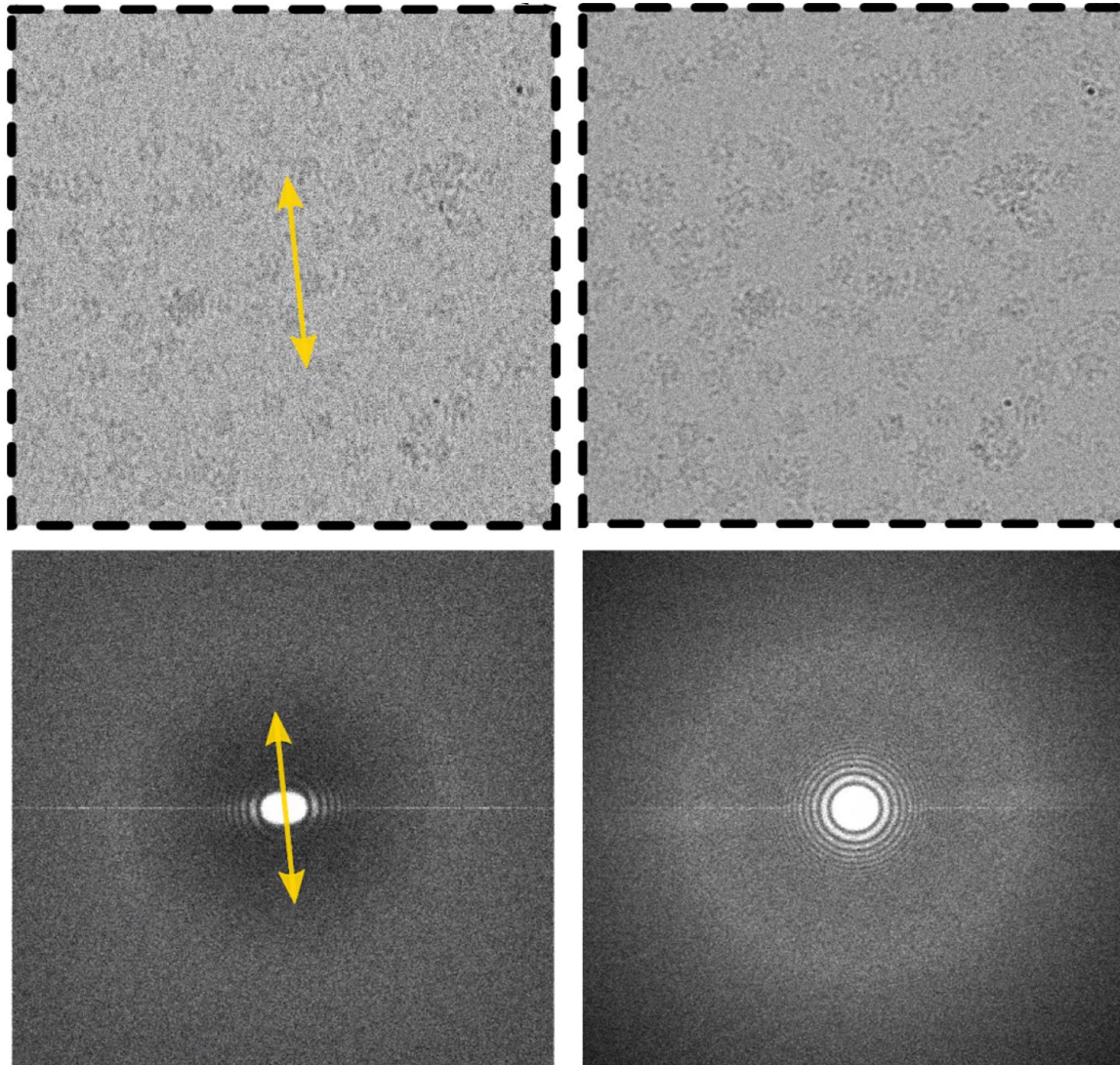
Export Job

Clone Job

Mark Job as Complete

Delete Job

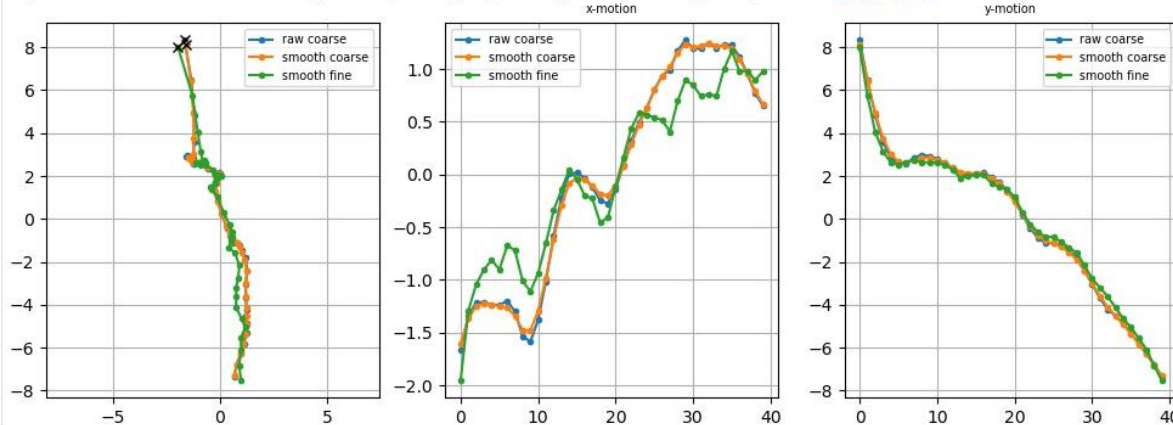
CryoSPARC: Motion correction



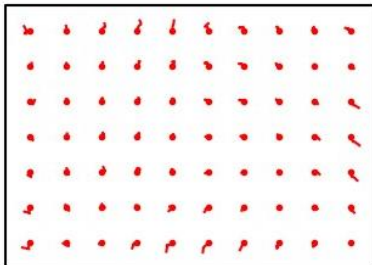
CryoSPARC: Motion correction

> [CPU: 232.2 MB] -----
> [CPU: 232.2 MB] Processed 0 of 25 movies in 24.31s

Rigid motion for 005268782221562727002_FoilHole_3967163_Data_3960642_3960644_20221004_215755_fractions [png] [pdf]



Patch motion for 005268782221562727002_FoilHole_3967163_Data_3960642_3960644_20221004_215755_fractions [png] [pdf]



Outputs

micrographs

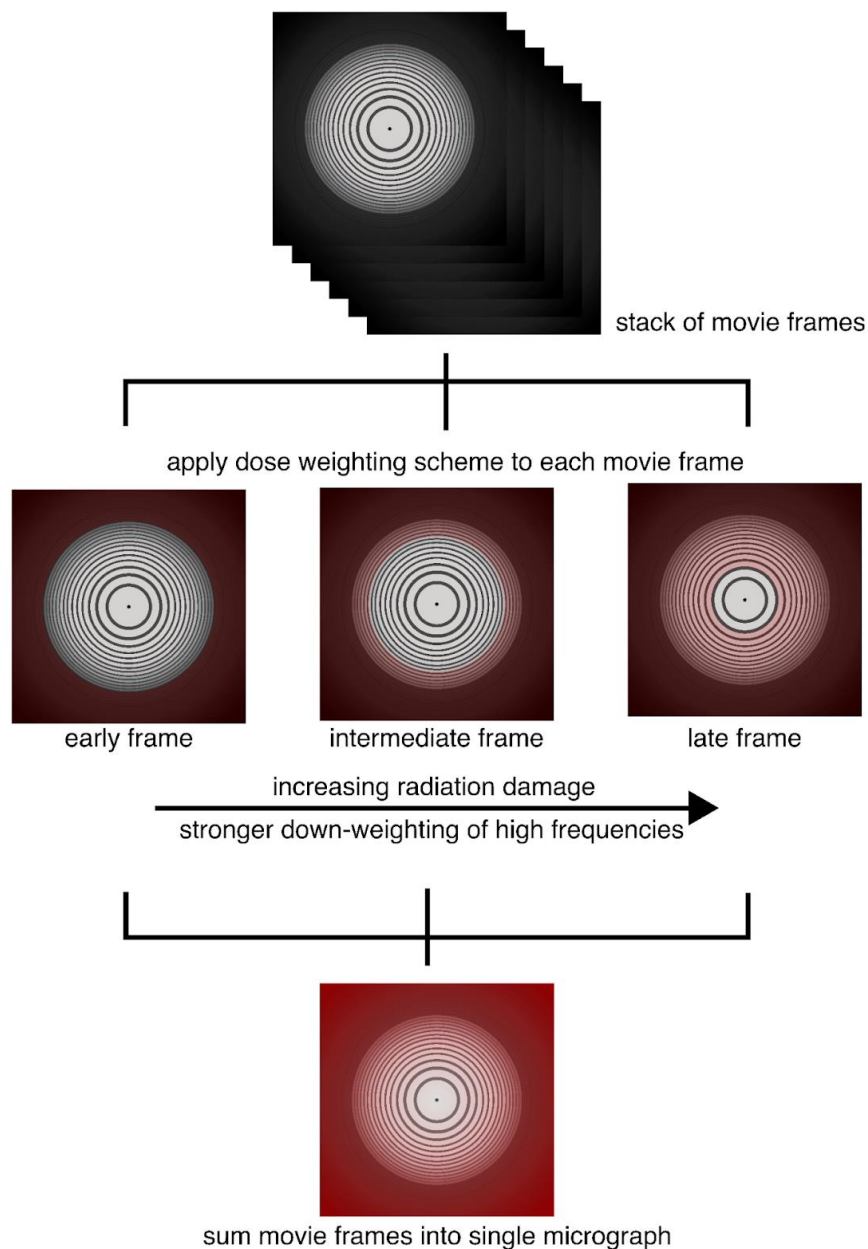


exposure Count: 25

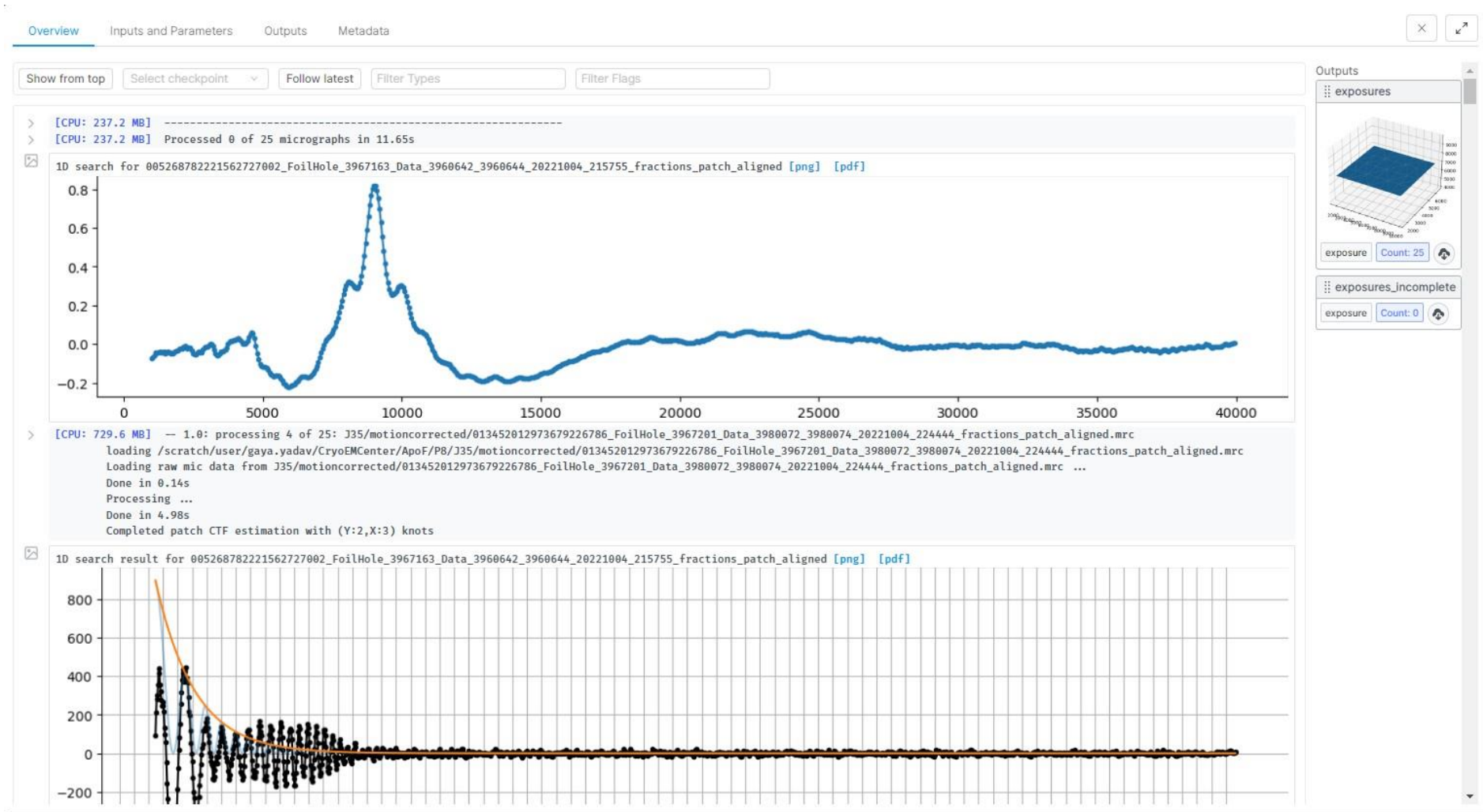
micrographs_incomplete

exposure Count: 0

CryoSPARC: Motion correction-dose weighting

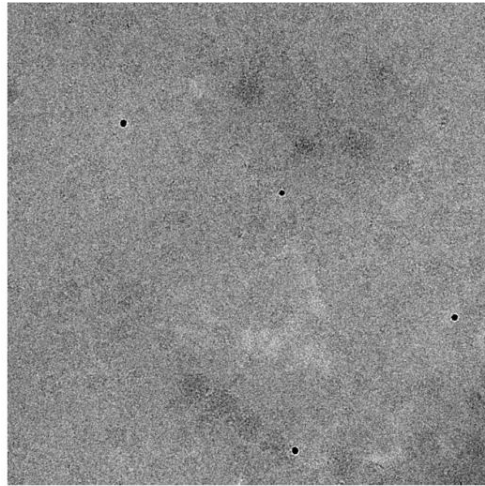


CryoSPARC: CTF estimation

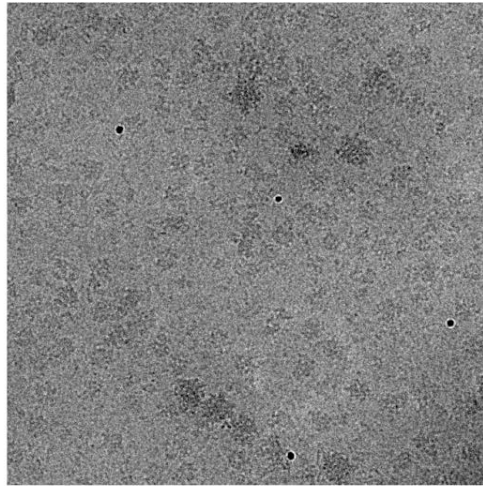


CryoSPARC: CTF estimation

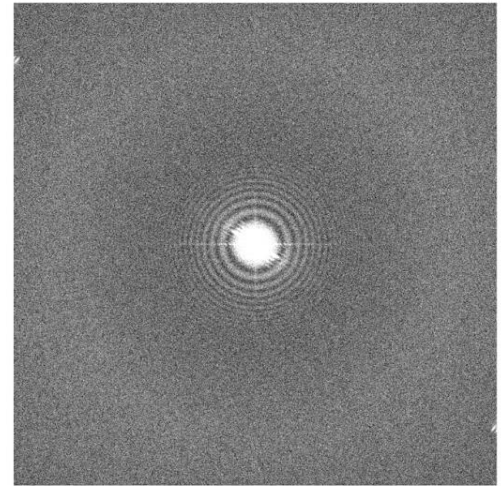
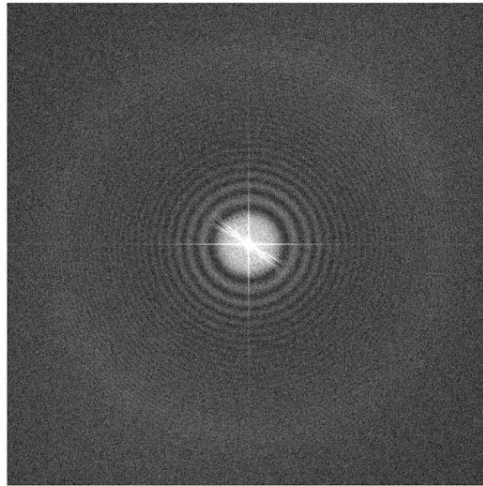
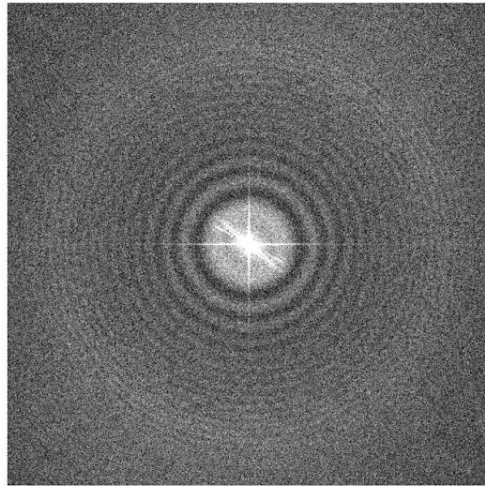
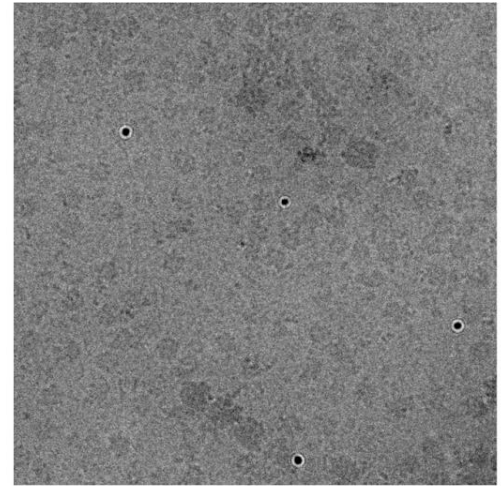
-0.75 μm



-1.5 μm



-3.0 μm



CryoSPARC: Particles picking and extraction

Particle extraction & Box size

- Select a box size that is at least double the diameter of the particle.
- Controls how much of the micrograph is cropped around each particle location
 - Larger sizes capture the most high-resolution signal that is spread out spatially due to the effect of defocus (CTF) in the microscope.
 - Larger box sizes significantly increase computation expense in further processing.

$$Box[\text{\AA}] = \frac{MaxDefocus[\text{\AA}]}{25 * BestPossibleResolution[\text{\AA}]}$$

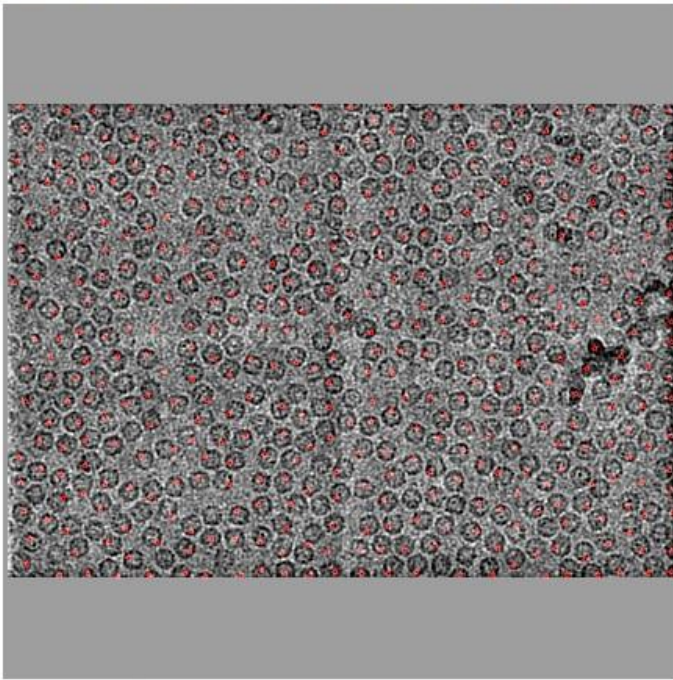
CryoSPARC: Particles picking-blob

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

> [CPU: 1.08 GB] Completed 0 of 25 : J35/motioncorrected/005268782221562727002_FoilHole_3967163_Data_3960642_3960644_20221004_215755_fractions_patch_aligned_doseweighted.mrc
Picked 424 particles in 1.51s (2.36s total)

Micrograph J35/motioncorrected/005268782221562727002_FoilHole_3967163_Data_3960642_3960644_20221004_215755_fractions_patch_aligned_doseweighted.mrc [png]



Not very accurate

> [CPU: 1.09 GB] Completed 1 of 25 : J35/motioncorrected/007159286402847523006_FoilHole_3967179_Data_3980072_3980074_20221004_221617_fractions_patch_aligned_doseweighted.mrc
Picked 407 particles in 0.46s (2.94s total)

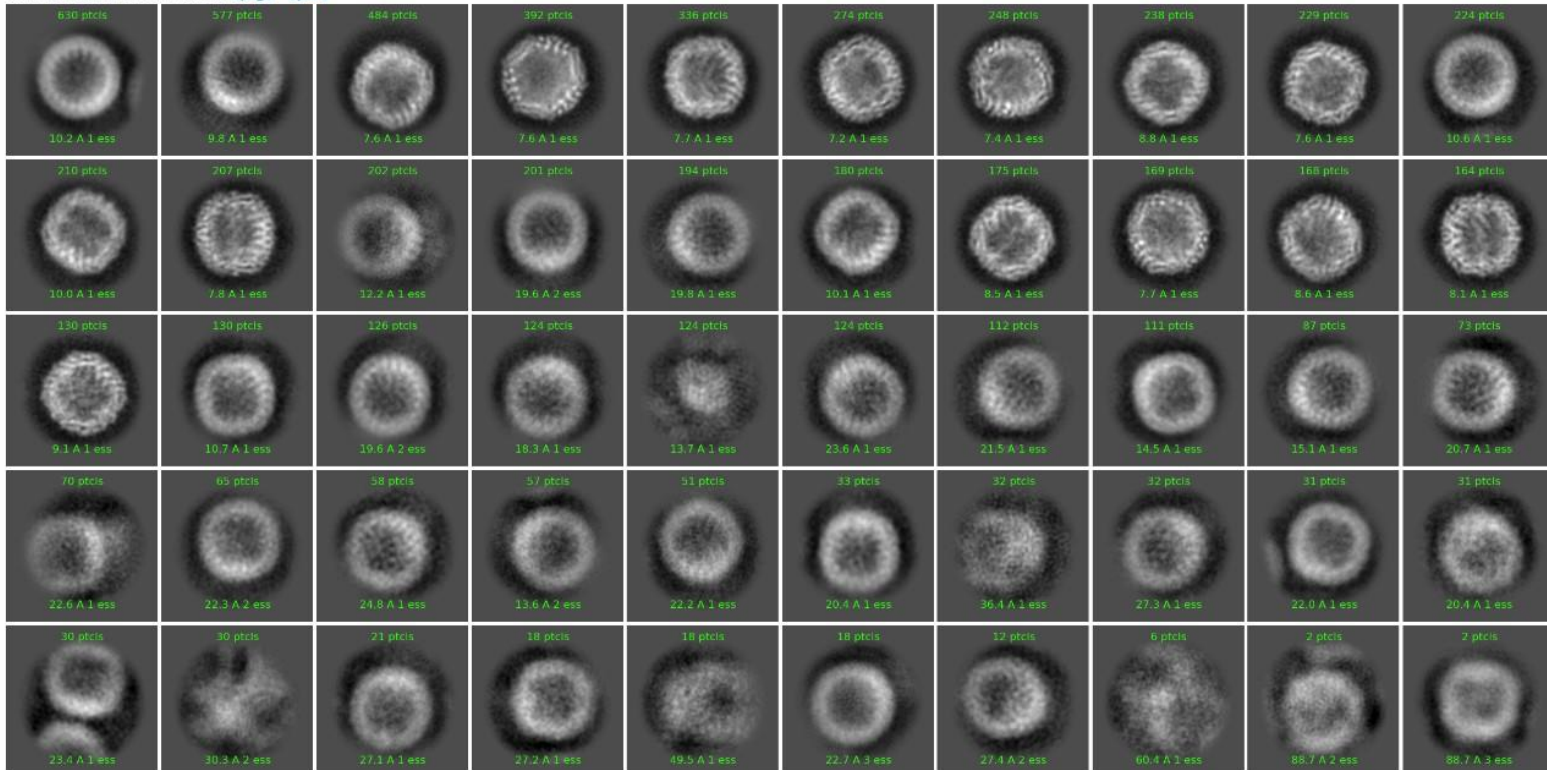
CryoSPARC: 2D classification

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

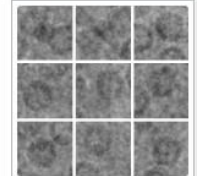
> [CPU: 2.88 GB] Iteration 24
> [CPU: 2.88 GB] — Effective number of classes per image: min 1.00 | 25-pct 1.00 | median 1.00 | 75-pct 1.14 | max 7.61
> [CPU: 2.88 GB] — Probability of best class per image: min 0.10 | 25-pct 0.93 | median 1.00 | 75-pct 1.00 | max 1.00

2D classes for iteration 24 [png] [pdf]



Outputs

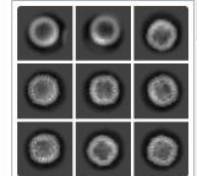
particles



particle Count: 7260



class_averages



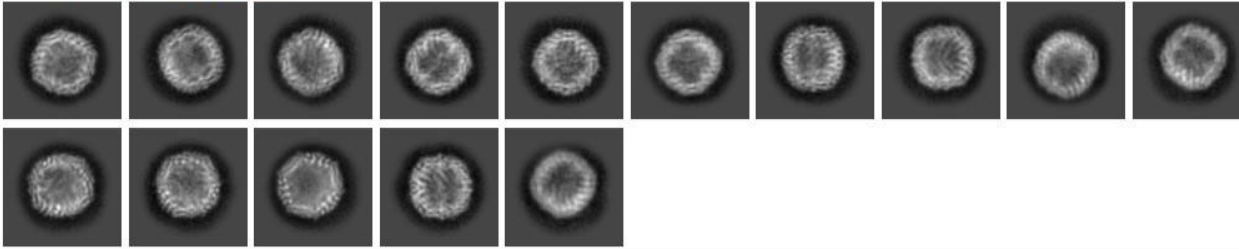
template Count: 50

CryoSPARC: 2D selection

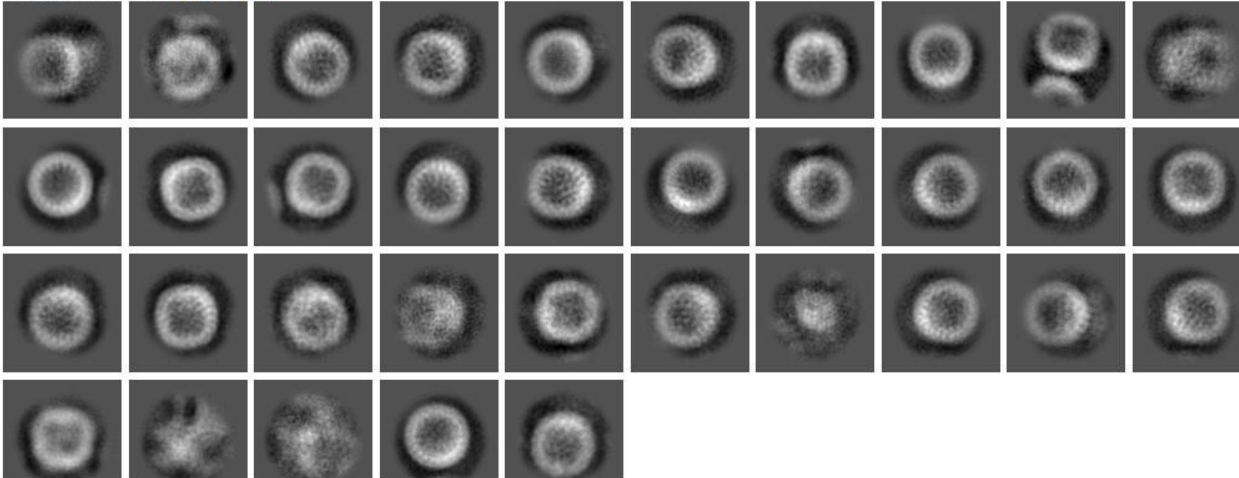
Overview Inputs and Parameters Outputs Metadata

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> [CPU: 188.9 MB] *****
> [CPU: 188.9 MB] Loaded info for 50 classes
> [CPU: 198.6 MB] Loaded info for 7260 particles
> [CPU: 199.3 MB] Interactive job running on port 34066
> [CPU: 199.7 MB] Outputting selection..
> [CPU: 199.7 MB] Templates selected : 15
> [CPU: 199.7 MB] Templates excluded : 35
```

Selected 15 classes: [png] [pdf]



Excluded 35 classes: [png] [pdf]



templates_selected

template Count: 15

particles_excluded

particle Count: 3656

templates_excluded

template Count: 35

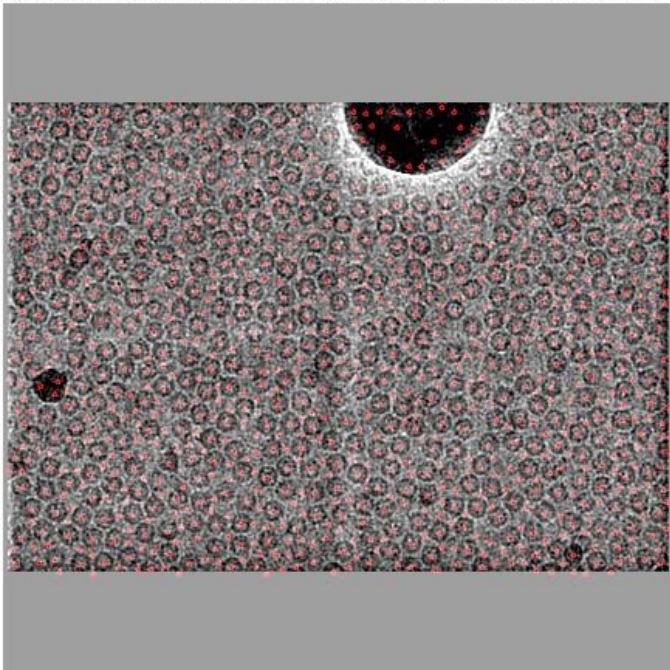
CryoSPARC: Particles picking-template

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

> [CPU: 1.13 GB] Completed 0 of 25 : J11/motioncorrected/001949740237399492928_FoilHole_3967145_Data_3960642_3960644_20221004_213659_fractions_patch_aligned_doseweighted.mrc
Picked 1069 particles in 4.70s (5.92s total)

Micrograph J11/motioncorrected/001949740237399492928_FoilHole_3967145_Data_3960642_3960644_20221004_213659_fractions_patch_aligned_doseweighted.mrc [png]



More accurate and centered

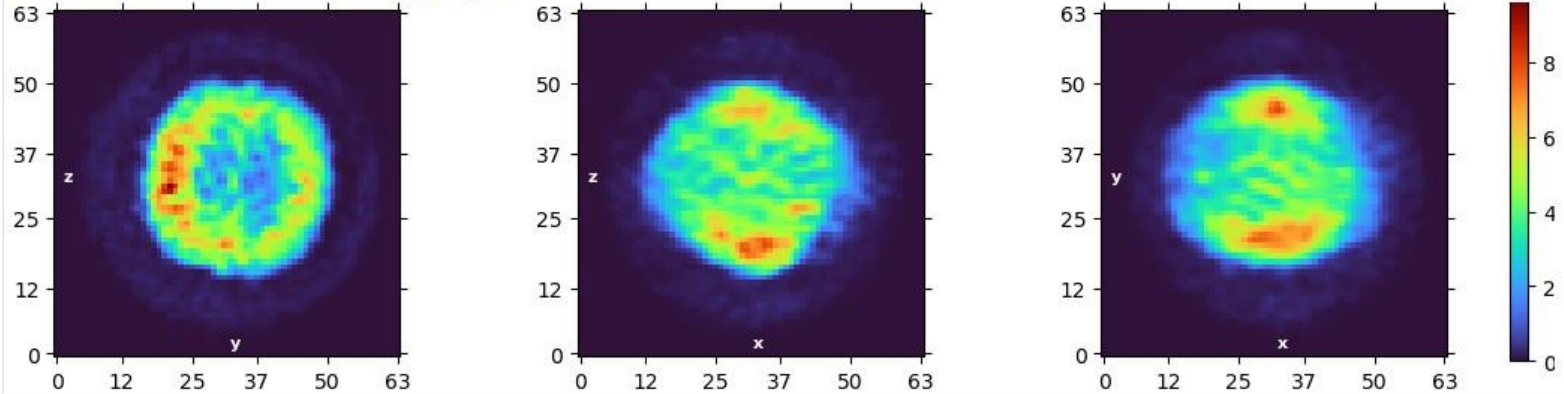
> [CPU: 1.15 GB] Completed 1 of 25 : J11/motioncorrected/001688604709404481497_FoilHole_3967145_Data_3980072_3980074_20221004_213647_fractions_patch_aligned_doseweighted.mrc
Picked 1105 particles in 0.96s (7.00s total)

CryoSPARC: ab-initio

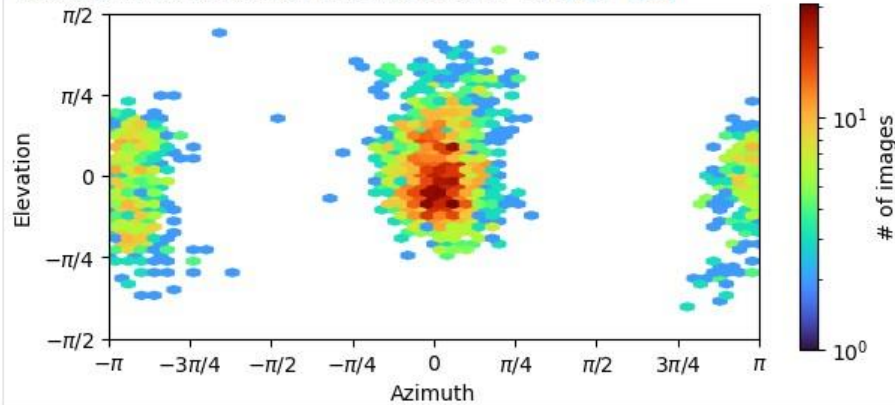
Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

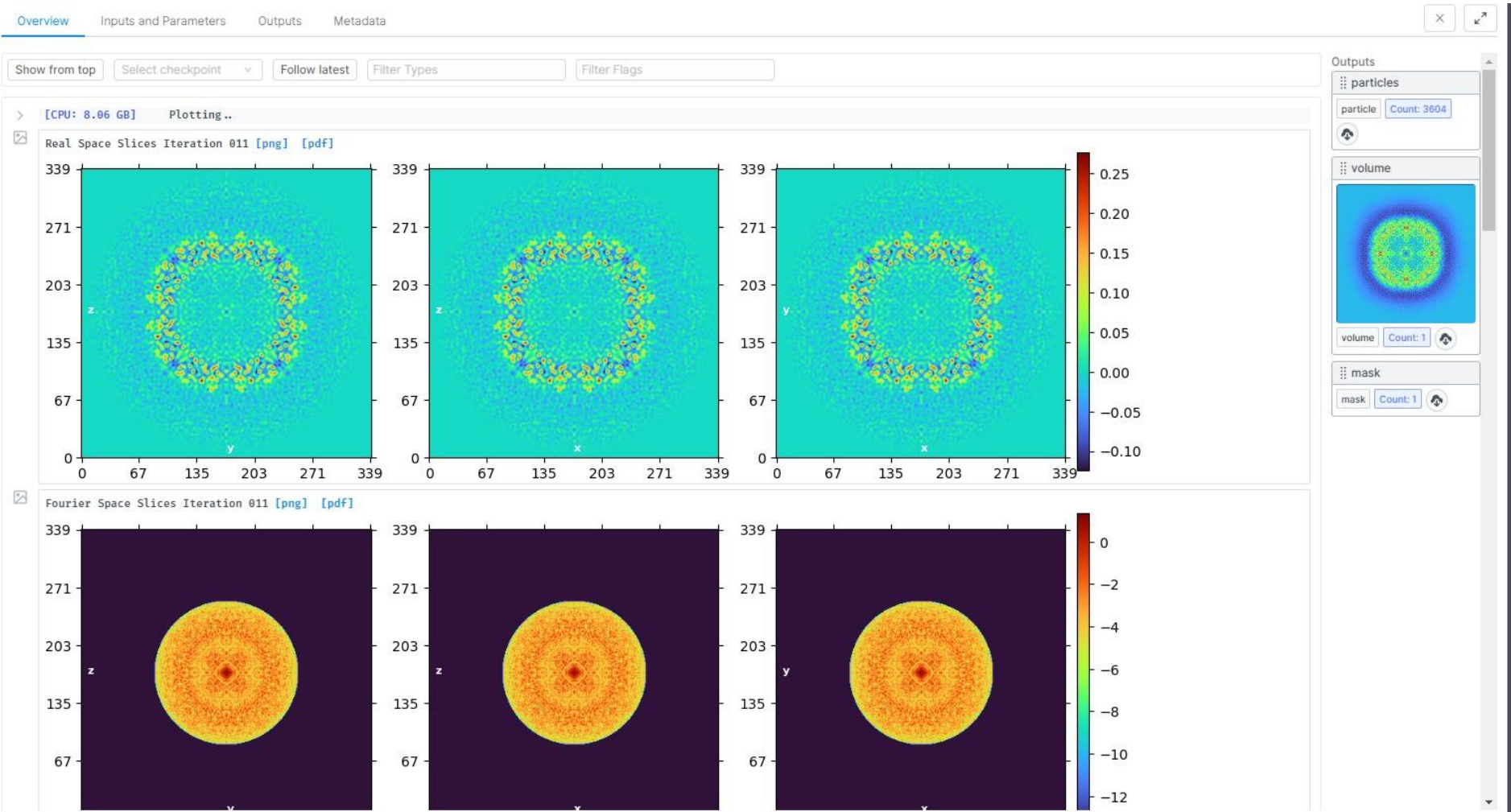
Structure for Class 000 Iteration 710 [png] [pdf]



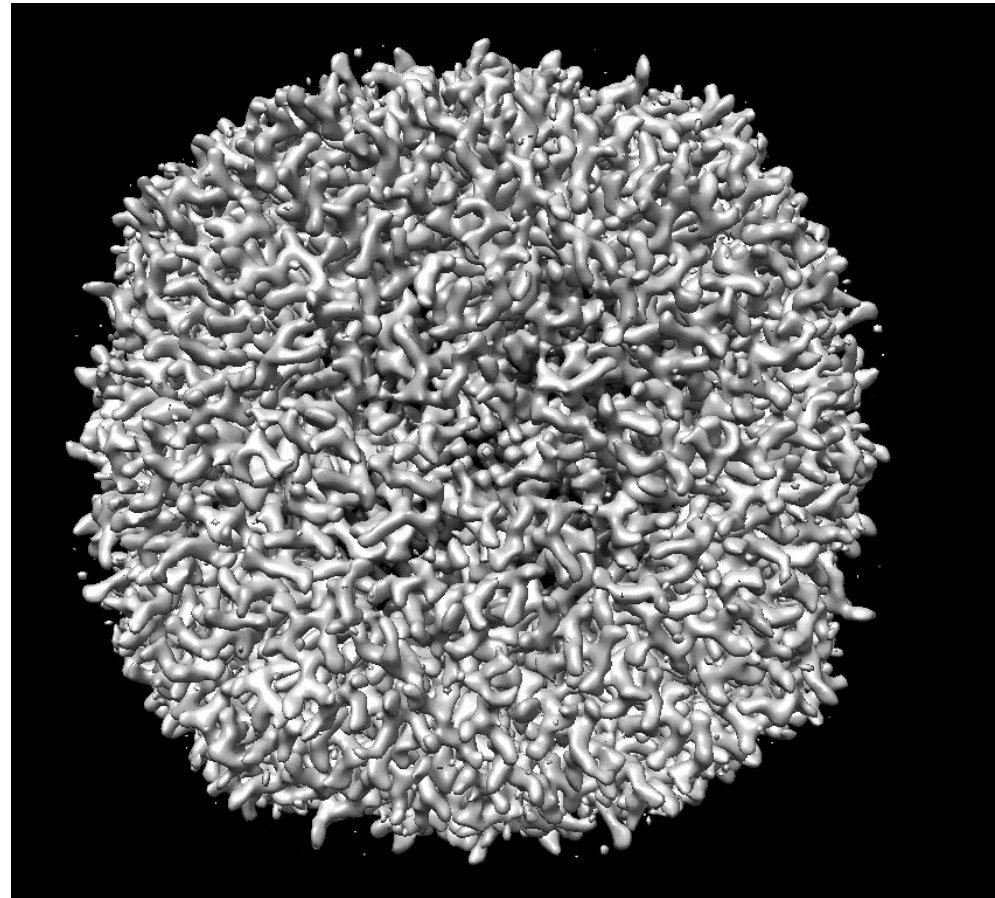
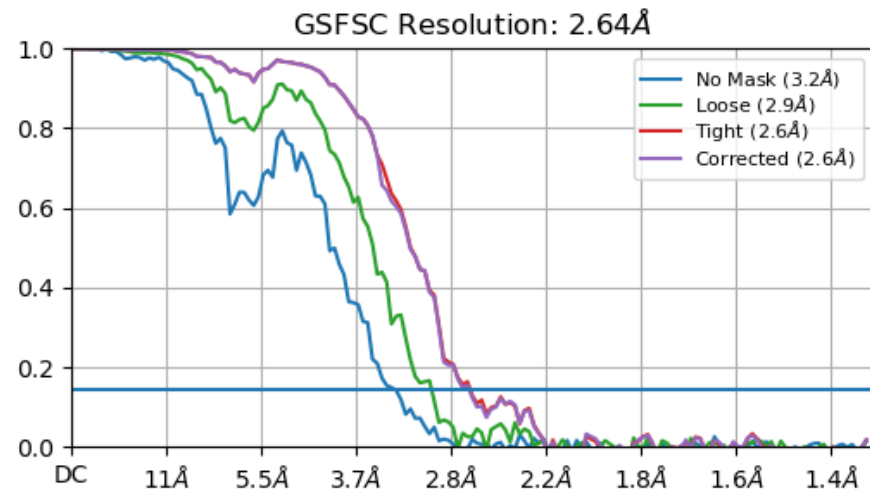
Viewing Direction Distribution Class 000 Iteration 710 [png] [pdf]



CryoSPARC: Refinement and sharpening



CryoSPARC: map visualization



Thank you

