

CiCLE 单粒子解析リモート講習会

2021/06/24-25



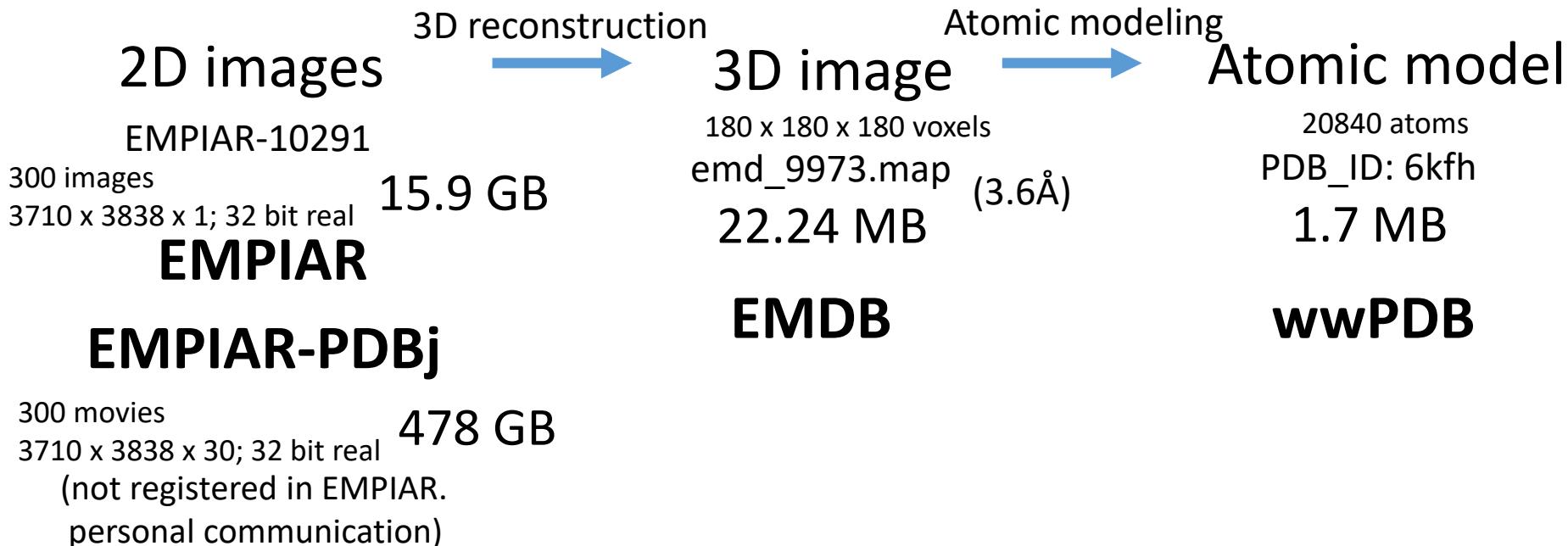
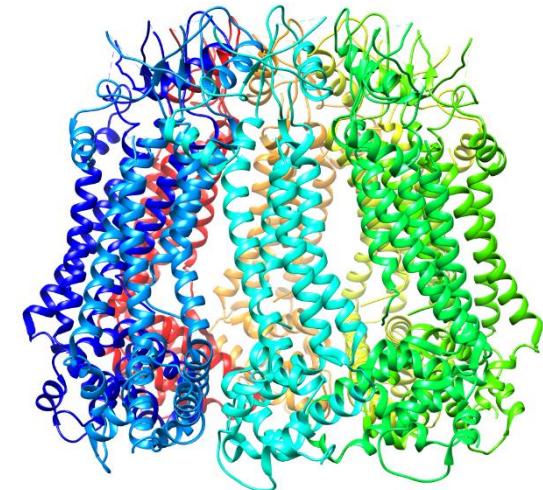
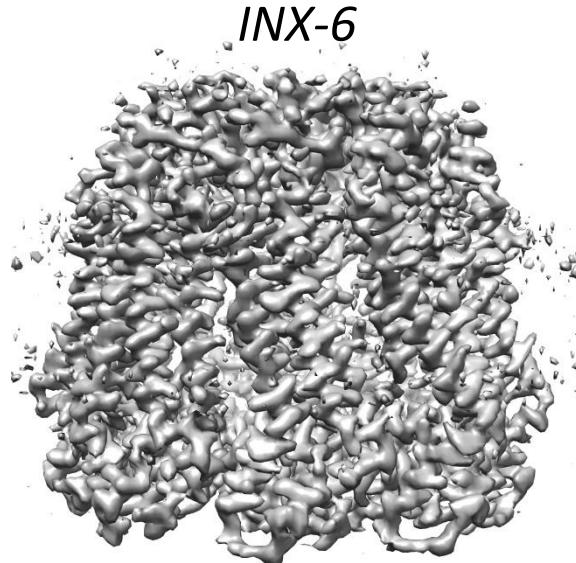
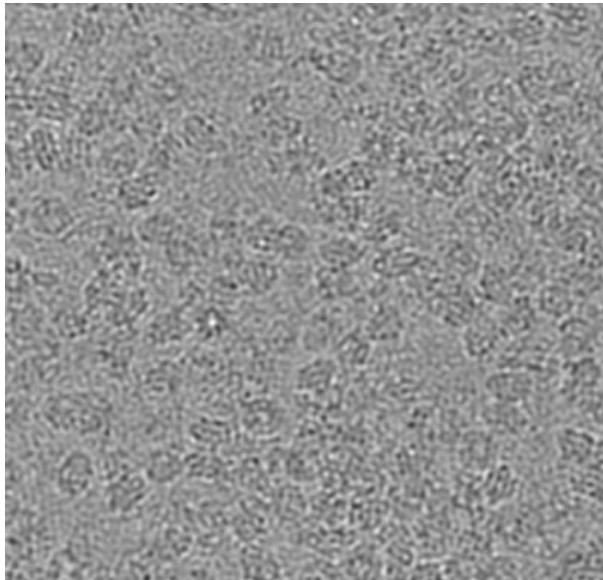
Tutorial of cryoSPARC : Innexin-6

Takeshi Kawabata (Protein Research Foundation, Osaka University)
川端 猛 (蛋白質研究奨励会・大阪大学)

2021/06/24-25



Data processing for EM Single Particle Analysis



Summary of innexin-6 EM data

Batuujin Burendei, Ruriko Shinozaki, Masakatsu Watanabe, Tohru Terada, Kazutoshi Tani,*, Yoshinori Fujiyoshi and Atsunori Oshima.
Cryo-EM structures of undocked innexin-6 hemichannels in phospholipids. Science Advances 12 Feb 2020: Vol. 6, no. 7, eaax3157
DOI: 10.1126/sciadv.aax3157

EMPIAR	EMDB	Resolution	PDB	molecules	Details of EMPIAR	Data size of EMPIAR entry
10289	9971	3.8 Å	6kff	WT INX-6 in a nanodisc	Motion corrected 2D projection images of innexin-6 gap junction hemichannels in nanodiscs.	49.5 GB. 933 *.mrc files. Each mrc files has 3710 x 3838 x 1 pixels.
10290	9972	3.8 Å	6kfg	WT INX-6 in a detergent	Motion corrected 2D projection images of innexin-6 gap junction hemichannels in detergent.	26.4 GB. 497 *.mrc files. Each mrc files has 3710 x 3838 x 1 pixels.
10291	9973	3.6 Å	6kfh	WT INX-6ΔN in a nanodisc	Motion corrected 2D projection images of N-terminal deleted innexin-6 gap junction hemichannels in nanodisc.	15.9 GB. 300 *.mrc files. Each mrc files has 3710 x 3838 x 1 pixels.

- EMPIARに登録されているのはMotion Correction後の静止画像のみ
- undocked hemichannelはEMPIAR, EMDB, PDBで公開。Docked hemichannelは、EMDB (9570, 9571), PDB(5h1q, 5h1r)で公開されているが、EMPIARにはデータがない。

JEM-3000SFF(JEOL)

The data were collected using a JEM-3000SFF (JEOL) electron microscope at 300 kV equipped with a K2 summit direct electron detector camera (Gatan).

Burendei, B., Shinozaki, R., Watanabe, M., Terada, T., Tani, K., Fujiyoshi, Y., Oshima, A. Sci Adv, 6:eaax3157-eaax3157, 2020



Flagellar filament @9Å (1995)
Flagellar filament @4.5Å (2003)

JEOL JEM-3000SFF (G3)
Liq-He cooled specimen stage
First FEG (Field Emission Gun)

+

Gatan社製K2 Summit直接検出型カメラ



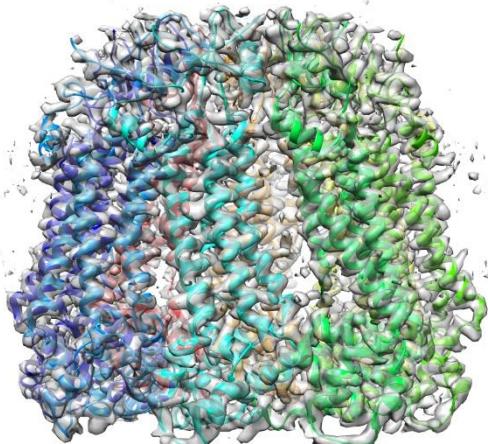
GATAN

Innixin-6 : docked and undocked states

Structural component of the gap junctions.

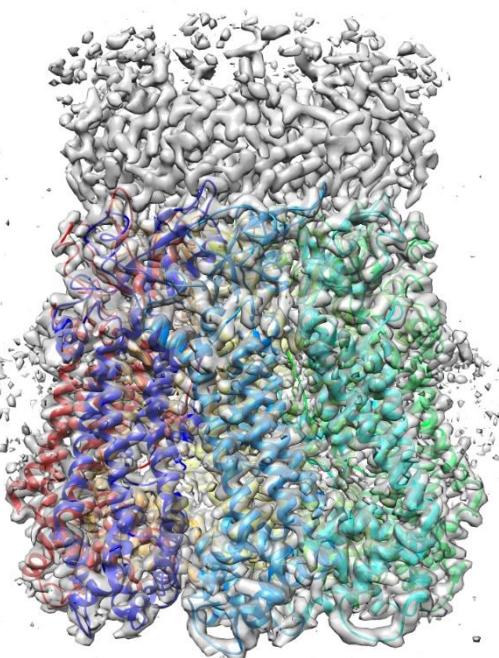
Undocked innixin-6
hemichannel

EMPIAR-10291



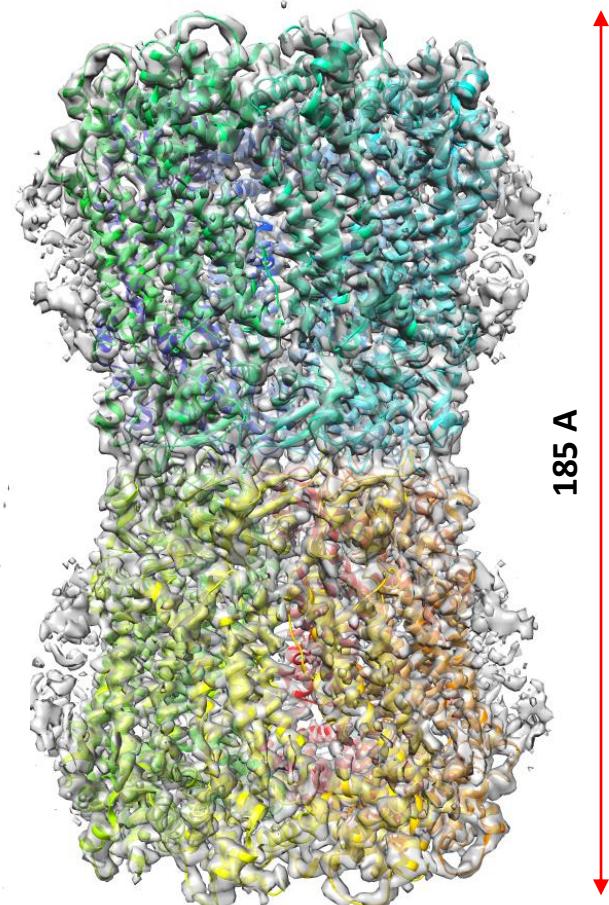
EMD-9973/PDB_ID:6kfh

Burendei, B., Shinozaki, R., Watanabe,
M., Terada, T., Tani, K., Fujiyoshi, Y., Oshima, A.
Sci Adv, 6:eaax3157-eaax3157, 2020



EMD-9570/PDB_ID:5h1q

Docked innixin-6



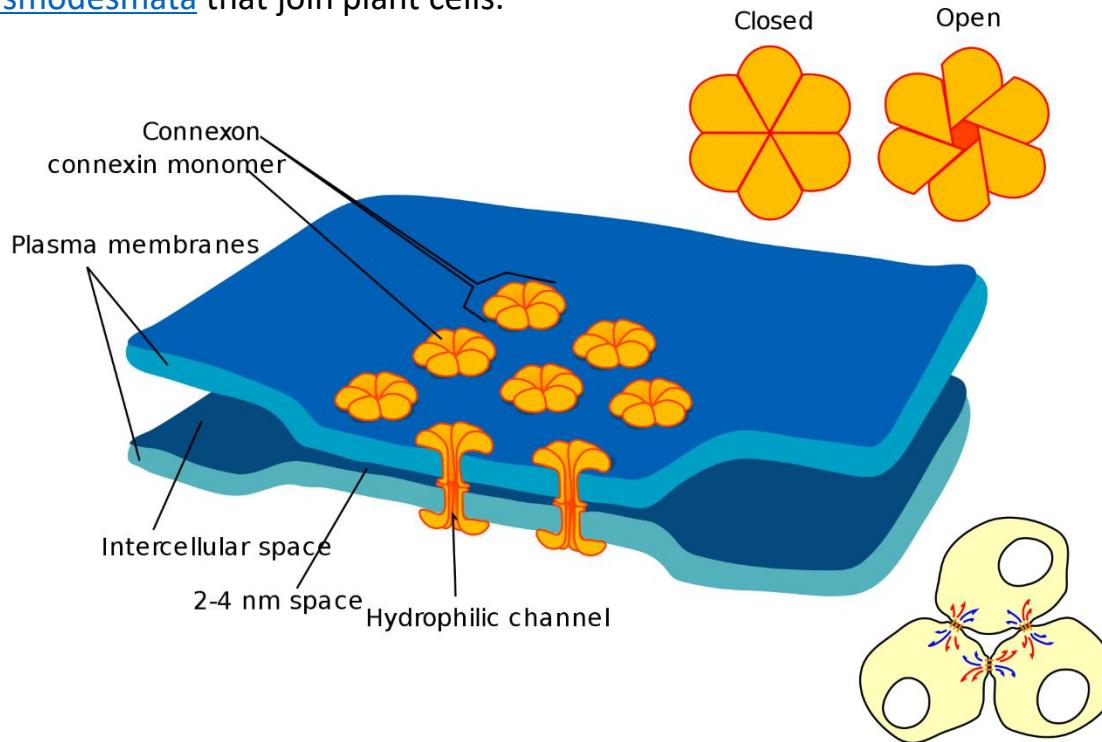
EMD-9571/PDB_ID:5h1r

Oshima, A., Tani, K., Fujiyoshi, Y. Nat Commun, 7:13681-13681,

ギャップ結合 (gap junction)

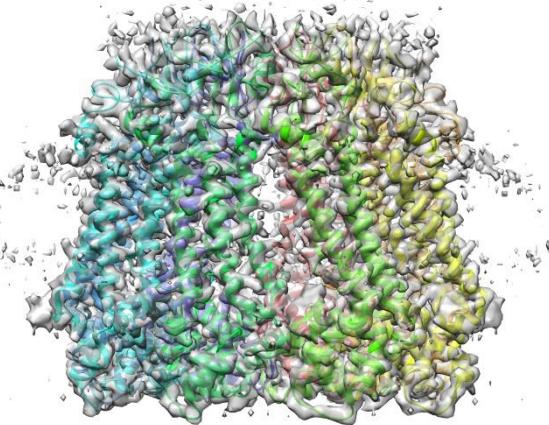
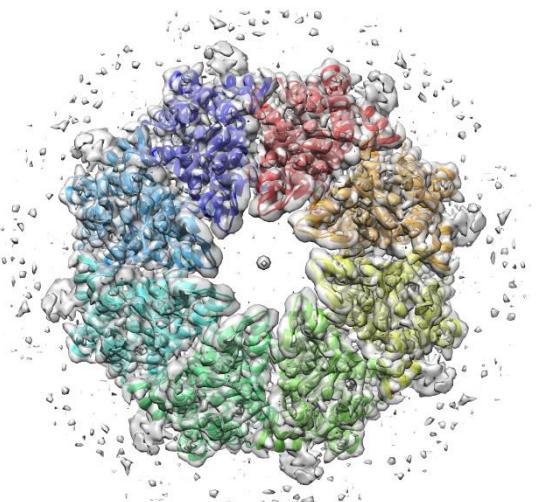
ギャップ結合(ギャップけつごう、英: Gap junction)は、隣り合う上皮細胞をつなぎ、水溶性の小さいイオンや分子を通過させる細胞間結合のこと。並んだ2つの細胞の細胞膜にはコネクソンと呼ばれるタンパク複合体の末端が複数並んでおり、橋渡し構造をなしている。このコネクソンがチャネルとなり、ここを通って無機イオンや小さい水溶性分子が隣接細胞の細胞質から細胞質へと直接移動することができる。また、細胞同士を電気的に結合するため、心筋組織などの興奮伝播にも関わっている。

Gap junctions are a specialized intercellular connection between a multitude of animal cell-types. They directly connect the cytoplasm of two cells, which allows various molecules, ions and electrical impulses to directly pass through a regulated gate between cells. One gap junction channel is composed of two connexons (or hemichannels), which connect across the intercellular space. Gap junctions are analogous to the plasmodesmata that join plant cells.

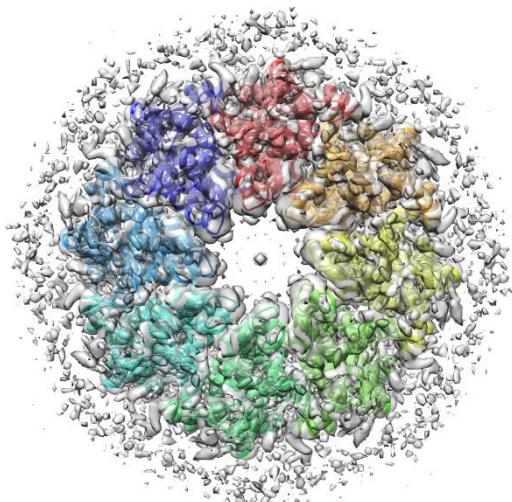


Wikipediaからの引用

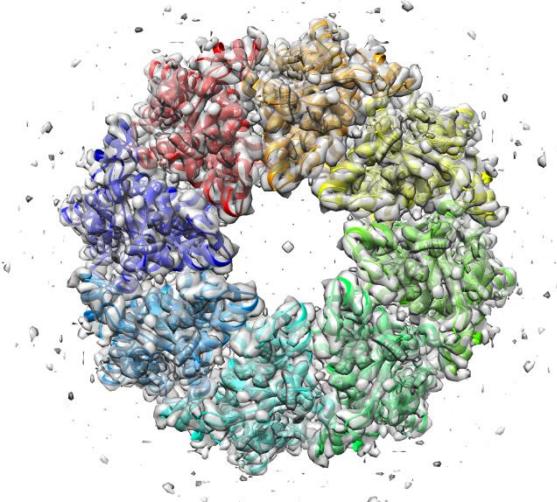
Undocked innexin-6 hemichannel



EMD-9971/PDB_ID:6kff
EMPIAR-10289
WT INX-6 in a nanodisc



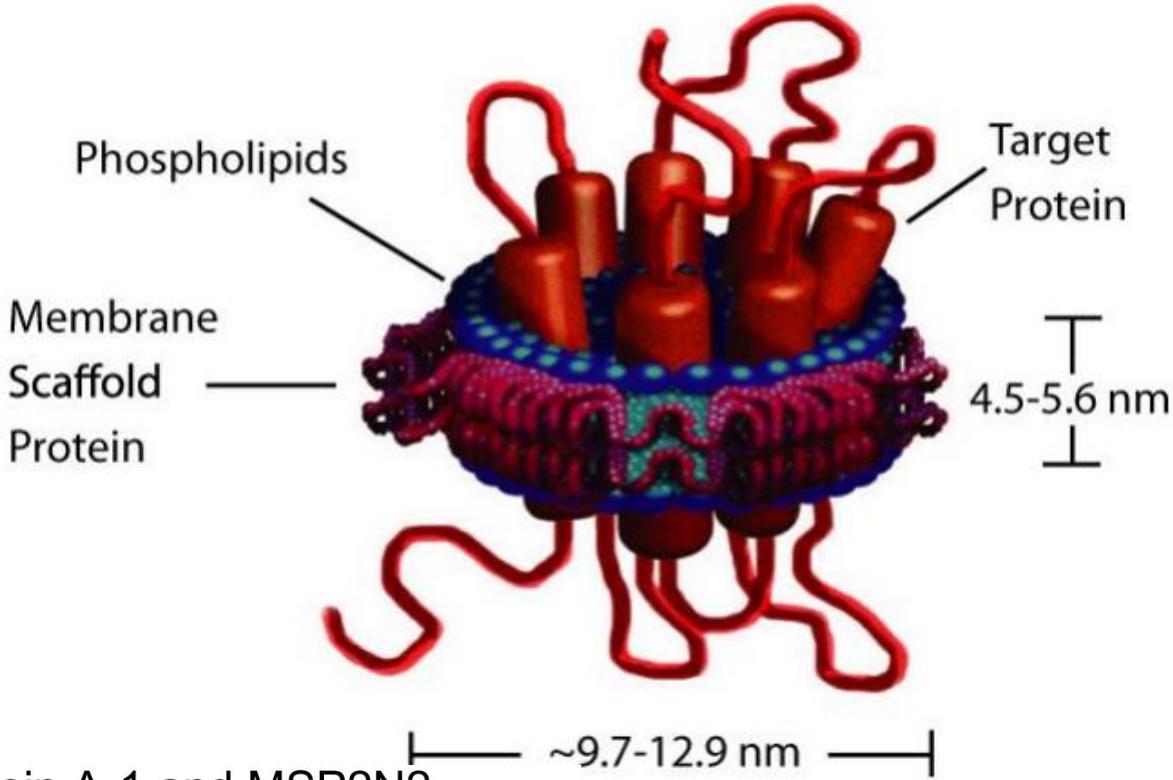
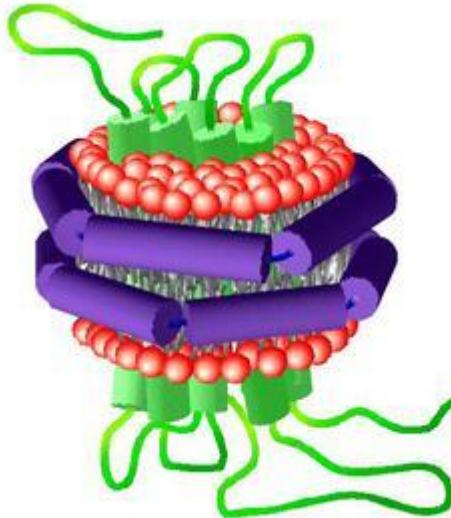
EMD-9972/PDB_ID:6kgf
EMPIAR-10290
WT INX-6 in a detergent



EMD-9973/PDB_ID:6kfh
EMPIAR-10291
WT INX-6 Δ N in a nanodisc

nanodisc

Example of a Nanodisc containing a 7-transmembrane protein

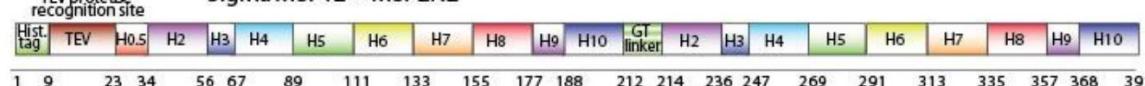


Protein Maps of Apolipoprotein A-1 and MSP2N2

Apolipoprotein A1



Sigma MSP12 = MSP2N2



PDB_ID: 1av1
Apolipoprotein A-1
(APOA1_HUMAN)

To investigate the structure in a lipid bilayer, we reconstituted undocked WT INX-6 hemichannels in nanodiscs using the membrane scaffold protein 2N2 (**MSP2N2**) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), as confirmed by Coomassie brilliant blue-stained gel and negatively stained electron micrographs (fig S1A).

Burendei, B., Shinozaki, R., Watanabe, M., Terada, T., Tani, K., Fujiyoshi, Y., Oshima, A.,
Sci Adv, 6:eaax3157-eaax3157, 2020

EMPIAR-10289
WT INX-6 in nanodisc
INX6hemiNano001

Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

These long circles
are not proteins.
micelle ? Nanodiscs ?

EMPIAR-10290
WT INX-6 in detergent
INX6hemiDet002

Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

EMPIAR-10291
WT INX-6 Δ N in nanodisc
INX6hemiDet001

Pixel_width: 1.232 Å

After CtfFind,
SigmaContrast:3
Lowpass filter 10 Å

Download tutorial data

1) Googled by “EMPIAR PDBj”

Google

EMPIAR PDBj

すべて 地図 動画 ニュース 画像 もっと見る

約 709,000 件 (0.31 秒)

empiar.pdbj.org このページを訳す

EMPIAR-PDBj - Electron Microscopy Public Image Archive

EMPIAR is an archive for 2D image data related to EMDB. It includes micrographs, p and tilt-series.

2) Click [Workshop] at the header

EMPIAR PDBj

Electron Microscopy Public Image Archive

EMPIAR home Deposition REST API FAQ About EMPIAR **Workshop** Feedback

EMPIAR, the Electron Microscopy Public Image Archive, is a public resource for raw, 2D electron microscopy images. Here, you can browse, upload, and download and reprocess the thousands of raw, 2D images used to build a 3D model of a protein complex.

<https://empiar.pdbj.org/workshop>

Quick links

EMPIAR@EBI
EMDB@EBI

3) Download “EMPIAR-10291_50mic.tar” (2.7 GB)

EMPIAR PDBj

Electron Microscopy Public Image Archive

EMPIAR home Deposition REST API FAQ About EMPIAR Workshop

Documents and data for EMPIAR workshop

Analyzing 2D image data stored in EMPIAR often requires large computational costs and detailed knowledge about single particle analysis. For beginners for single particle analysis, we open the tutorial documents and data used for workshops organized by PDBj.

Innxin-6 from EMPIAR-10291

The images stored as [EMPIAR-10291](#) collected on JEOL JEM-3000SFF(G3), provides a high resolution (3.6 angstrom) 3D density map for the membrane protein innixin-6. This data set contains 300 motion-corrected still micrographs (15.9 GB). To reduce computation costs, we prepared 100 micrographs and 50 micrographs data set. Both datasets provides a map with a reasonably high resolution (3.96 angstrom). We thank an original author, Prof. Atsushi Oshima to advice us to analyze their data.

ftp directory of EMPIAR-10291

- [EMPIAR-10291_100mic.tar \(5.4 GB\)](#)
- [EMPIAR-10291_50mic.tar \(2.7 GB\)](#)
- [EMPIAR-10291_100mic_precalculated_results.tar \(14 GB\)](#)
- [EMPIAR-10291_50mic_precalculated_results.tar \(8.5 GB\)](#)
- [Tutorial document for preprocessing \(in Japanese\)](#)
- [Tutorial document for 2D_3D_classification \(in Japanese\)](#)

Apo-ferritin from EMPIAR-10248

The images stored as [EMPIAR-10248](#) collected on JEOL CRYO ARM 300, provides a very high resolution (3.6 angstrom) 3D density map for the ferritin protein. This data set contains 300 micrographs (15.9 GB).

	EMPIAR-10291	EMPIAR-10291_100mic	EMPIAR-10291_50mic
Filesize	15.9 GB	5.4 GB	2.7 GB
Images	300 micrographs	100 micrographs	50 micrographs
3Dmap	EMD-9973		
Resolution	3.6 angstrom	3.96 angstrom	3.96 angstrom

Mizzon G, Romero-Brey I, Santarella-Mellwig R, Schorl M, Boermel M, Mocer K, Beckwith MS, Templin RM, Gross V, Pape C, Tischer C, Frankish J, Horvat NK, Laketa V, Stanifer M, Boulant S, Ruggieri A, Chatel-Chaix L, Schwab Y, Bartenschlager R. (2020)

[See all citations](#)

cryoSPARC プログラム

- トロント大学 計算機科学科で2017年に最初の版が公開・出版
- 現在、Vector InstituteとStructura Biotechnology Inc.の二つの企業によって、開発・配布・販売
- アカデミックな利用は無料だが、商用目的は有料
- ソースは非公開
- GPU、複数コア、複数ノードによる並列計算環境を想定
- Webサーバから、計算ジョブを投入するインターフェース

Structura Biotechnology Inc.

The screenshot shows the homepage of the Structura Biotechnology website. At the top left is a small logo of a snowflake-like molecular structure. The main title "STRUCTURA BIOTECHNOLOGY" is displayed in large, bold, blue capital letters. Below the title is a brief description: "We are unlocking the potential of cryo-electron microscopy (cryo-EM) as a high-throughput structure determination technique for life sciences and drug discovery." A "Learn More" button is located at the bottom left. To the right of the text is a 3D molecular model of a complex, yellowish-green structure.

<https://structura.bio>

Our Team



Ali Punjani

CO-FOUNDER & CEO



Saara Virani

CHIEF OPERATING OFFICER



Suhail Dawood

CHIEF PRODUCT OFFICER



Stephan Arulthasan

SENIOR SOFTWARE ENGINEER



Nick Frasser

SOFTWARE ENGINEER



Harris Snyder

SCIENTIFIC COMPUTING
ENGINEER



Kelly Barber

FULL-STACK DEVELOPER



Michael McLean

SCIENTIFIC DEVELOPER
INTERN



cryoSPARC computational platform for single particle cryo-EM



Ali Punjani



500+ institutions
700+ citations since 2017

2017
v0.1 - v0.6

- Novel algorithms for ab-initio reconstruction and high speed refinement
- Fast 2D classification, 3D heterogeneity
- Fluid web-UI for particle datasets

2018
v2.0 - v2.5

- Fastest full pipeline for single particle EM
- Non-uniform refinement methods, preprocessing, and postprocessing
- Integrated complete web interface

2019
v2.6 - v2.9 + CryoSPARC Live

- First real-time processing solution with 3D streaming refinement during collection
- New 3D Variability methods for discrete and continuous heterogeneity

CryoSPARC Team



Ali Punjani



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Virani



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Dawood



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Marcus Brubaker

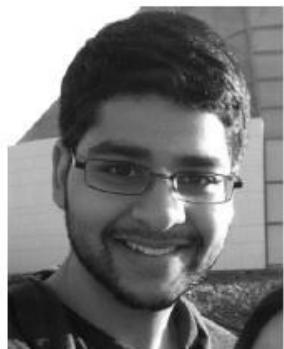
York University



Articles of cryoSPARC

Articles	Comment
Punjani A, Rubinstein JL, Fleet DJ, Brubaker MA. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. <i>Nat Methods</i> . 2017 Mar;14(3):290-296. doi: 10.1038/nmeth.4169.	Stochastic Gradient Descent
Punjani A, Brubaker MA, Fleet DJ. Building Proteins in a Day: Efficient 3D Molecular Structure Estimation with Electron Cryomicroscopy. <i>IEEE Transactions on Pattern Analysis and Machine Intelligence</i> , 2017, vol. 39, no. 4, 706-718, 1 April 2017, doi: 10.1109/TPAMI.2016.2627573.	
Punjani A, Zhang H, Fleet DJ. Non-uniform refinement: adaptive regularization improves single-particle cryo-EM reconstruction. <i>Nat Methods</i> . 2020 Dec;17(12):1214-1221. doi: 10.1038/s41592-020-00990-8.	Non-uniform refinement
Punjani A, Fleet DJ. 3D variability analysis: Resolving continuous flexibility and discrete heterogeneity from single particle cryo-EM. <i>J Struct Biol</i> . 2021 Feb 11;213(2):107702. doi: 10.1016/j.jsb.2021.107702	3D variability analysis
Punjani A, Fleet DJ. 3D Flexible Refinement: Structure and Motion of Flexible Proteins from Cryo-EM. <i>bioRxiv</i> 2021 doi: https://doi.org/10.1101/2021/04.22.440893	3D flexible refinement

Who are they ?



Ali Punjani received the BASc degree in aerospace engineering from the University of Toronto, Canada, in 2012 and the MS degree in computer science from the University of California, Berkeley. He is working toward the PhD degree at the University of Toronto, where he works on computer vision and machine learning. His research interests include deep learning, optimization methods, and computational biology. He has also worked on large-scale data visualization, robotics, and autonomous control. He received the NSERC Canada Graduate Scholarship. He is a member of the IEEE.



Marcus A. Brubaker received the PhD degree in computer science from the University of Toronto, in 2011. From 2011 to 2016 he was a postdoctoral fellow with TTI-Chicago and University of Toronto. He is currently an assistant professor with York University, Toronto, Canada, and consults with Cadre Research Labs. His research interests span statistics, machine learning, and computer vision and includes applications in computational biology, robotics and forensics. He is a member of the IEEE.



David J. Fleet received the PhD degree in computer science from the University of Toronto, in 1991. Following 8 years on faculty with Queen's University, and then 5 years with the Palo Alto Research Center (PARC), he joined the University of Toronto as a professor of computer science. He is senior fellow of the Canadian Institute for Advanced Research. His research interests include computer vision, image processing, visual perception, and visual neuroscience. He has published numerous research articles on a broad range of topics, including optical flow, motion perception and human stereopsis, image-based tracking, 3D hand and human pose tracking, latent variable models, physics-based models for motion analysis, and large-scale image retrieval. He received the Alfred P. Sloan Research Fellowship in 1996. He has won several research awards, including the 2010 Koenderink Prize for his work with Michael Black and Hedvig Sidenbladh on human pose tracking. He has served as area chair for numerous computer vision and machine learning conference. He was program co-chair for the CVPR 2003 and ECCV 2014. He has been an associate editor and associate editor-in-chief of the *IEEE Transactions on Pattern Analysis and Machine Intelligence*, and currently serves on the TPAMI Advisory Board. He is a member of the IEEE.

Punjani A, Brubaker MA, Fleet DJ. Building Proteins in a Day: Efficient 3D Molecular Structure Estimation with Electron Cryomicroscopy. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, 2017, vol. 39, no. 4, 706-718, 1 April 2017, doi: 10.1109/TPAMI.2016.2627573.

Patent ?

Declaration of Competing Interest

A.P. is CEO of Stuctura Biotechnology Inc. which builds the *cryoSPARC* software package, distributed freely for academic non-profit use with software licenses available for commercial use. D.J.F. is an advisor to Stuctura Biotechnology Inc. The novel aspects of the method presented are described in a provisional patent application.

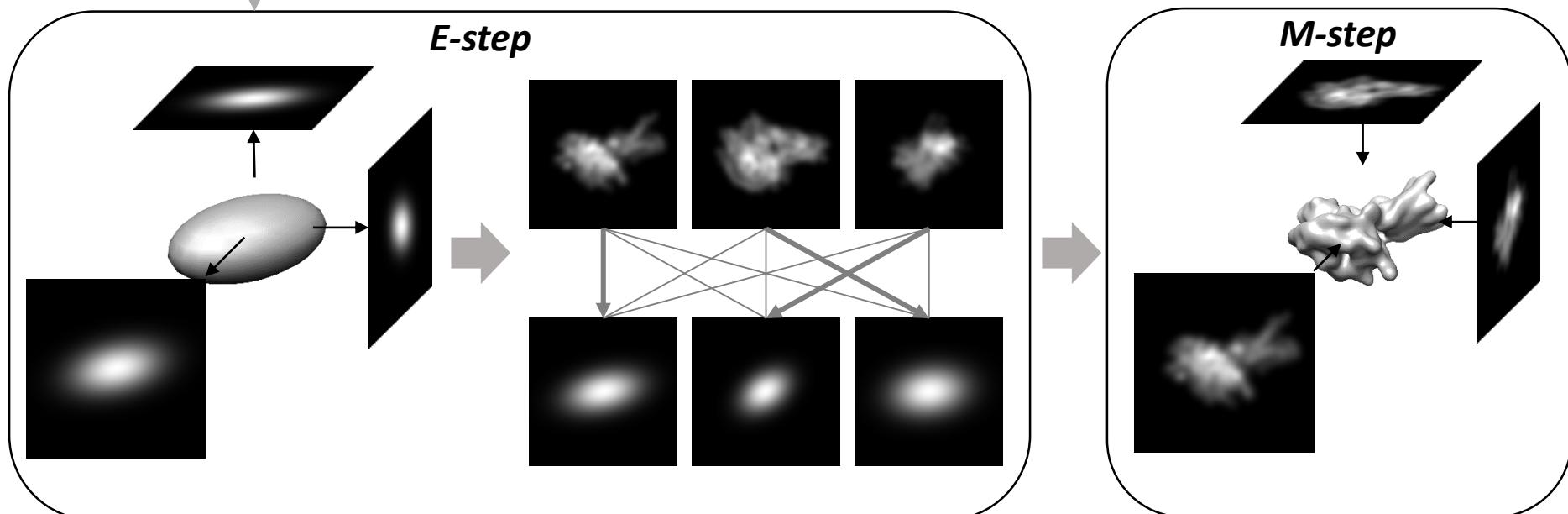
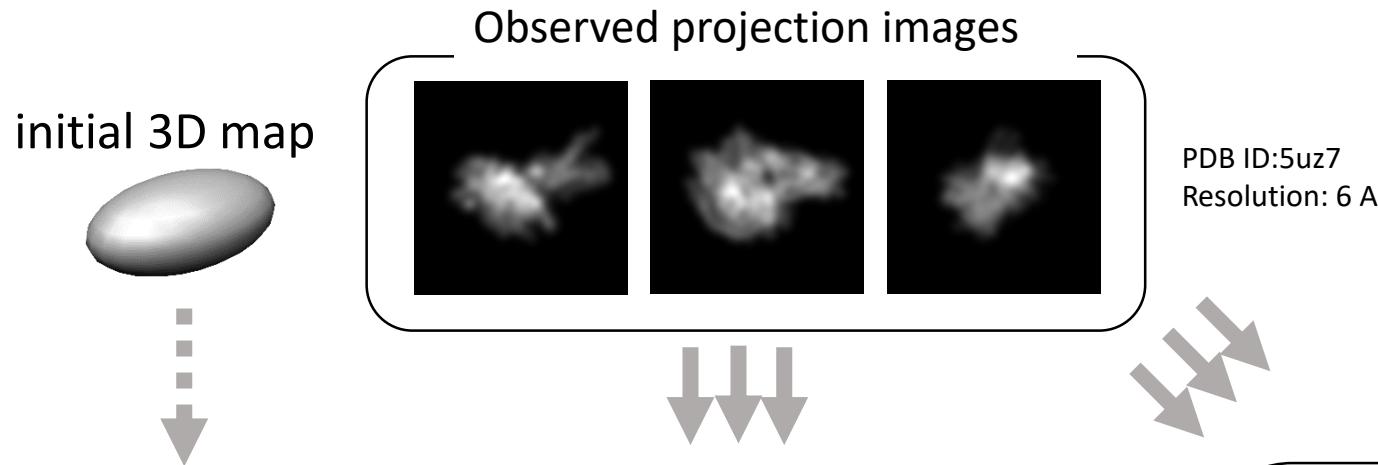
Punjani A, Fleet DJ. 3D variability analysis: Resolving continuous flexibility and discrete heterogeneity from single particle cryo-EM. J Struct Biol. 2021 Feb 11;213(2):107702. doi: 10.1016/j.jsb.2021.107702

Competing Interests

The novel aspects of the method presented are described in a provisional patent application.

Punjani A, Fleet DJ. 3D Flexible Refinement: Structure and Motion of Flexible Proteins from Cryo-EM. bioRxiv 2021 doi: <https://doi.org/10.1101/2021/04.22.440893>

Algorithm for 3D reconstruction



(1) 2D projections are generated from 3D map.

(2) Choose the closest projection for each observed projection to estimate its orientation

(3) 3D reconstruction is performed using estimated orientations

3D reconstruction of cryoSPARC

- **3D Refinement** : based on Relion-like Bayes estimation framework

Input : Prepare an initial 3D map

E-step: Estimate projection orientation for each 2D particle image

M-step: Reconstruct 3D map from 2D particles images
using the estimated orientations, in Fourier space.

- E-step : Efficient search of projection orientations
using Branch and Bound
- M-step : the standard EM algorithm may be employed.

- **Ab initio reconstruction:**

M-step: gradient-based algorithm is employed, not EM algorithm. EM \Rightarrow
Furthermore, SGD(Stochastic Gradient Descent) has been introduced.

*SGD: gradient is obtained from a part of particle images, to avoid a trapping into local optimum.

Stochastic Gradient Descent for M-step

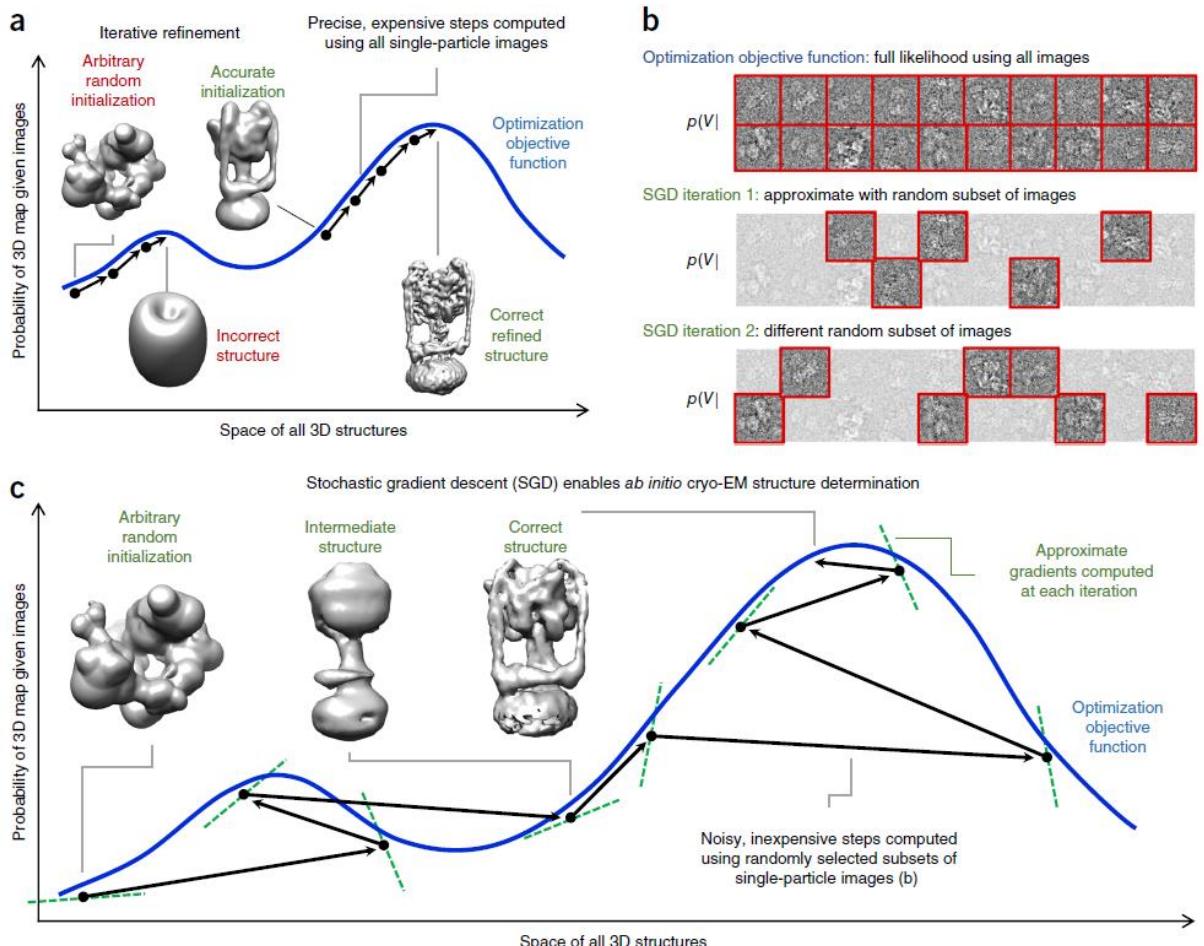


Figure 1 | Stochastic gradient descent for cryo-EM map calculation. **(a)** Iterative refinement methods are sensitive to initialization. An arbitrary initialization far from the correct 3D map will be refined into an incorrect structure that attains a locally optimal probability within the space of all 3D maps. An accurate initialization will be refined to the correct structure. Iterative refinement uses all single-particle images in a data set to compute each step. **(b)** Random selection of particle images in the SGD algorithm. At each iteration, a different small random selection of images is used to approximate the true optimization objective. Each iteration may use a different number of images. **(c)** Stochastic gradient descent (SGD) algorithm enables *ab initio* structure determination through insensitivity to initialization. An arbitrary computer-generated random initialization is incrementally improved by many noisy steps. Each step is based on the gradient of the approximated objective function obtained by random selection in **b**. These

Branch-and-bound for E-step

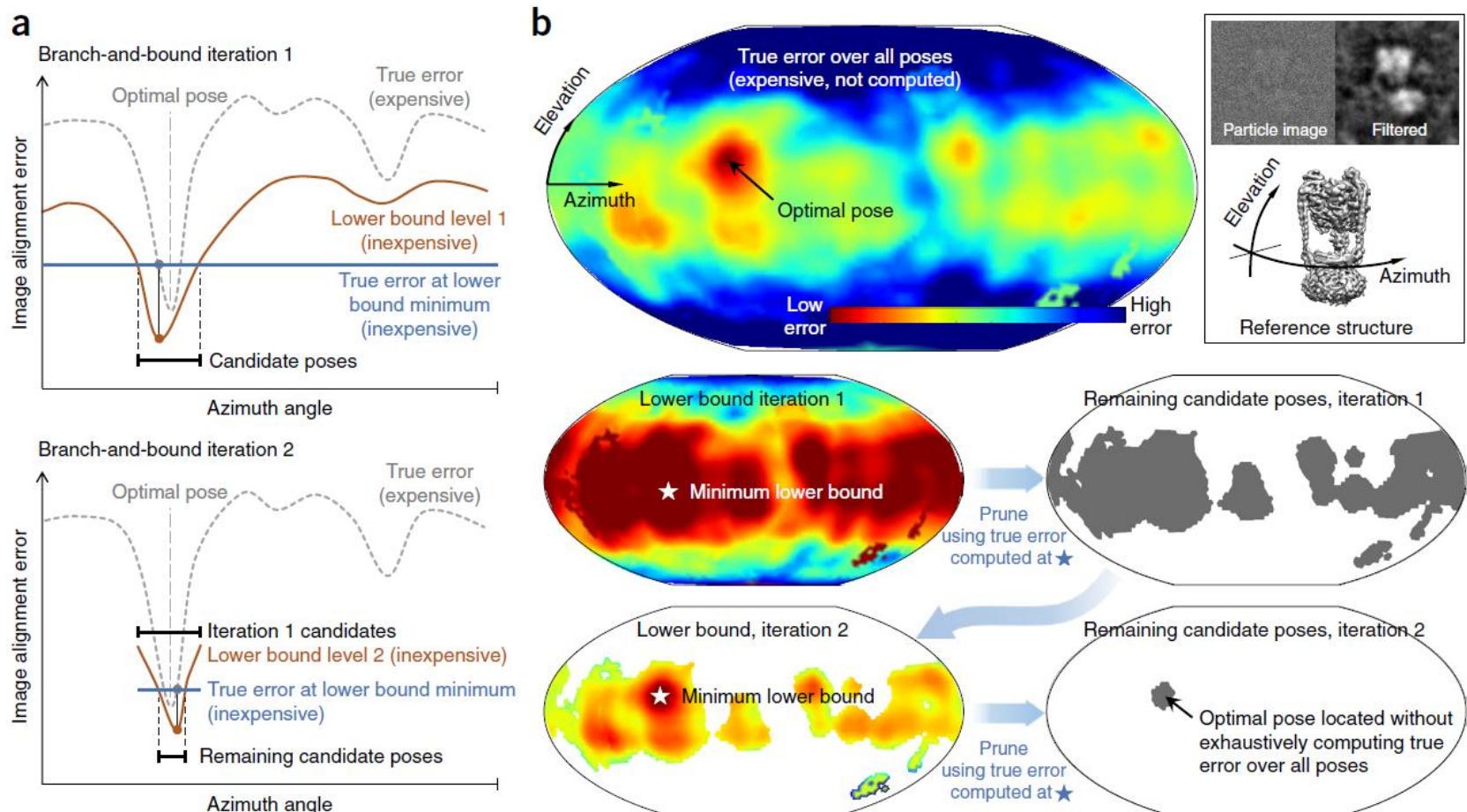


Figure 3 | The branch-and-bound approach to high-resolution cryo-EM map refinement. **(a)** Two iterations of a simplified 1D representation of the branch-and-bound approach. Candidate poses are iteratively eliminated by evaluation of an inexpensive lower bound over all poses, and the true error function at the minimum of the lower bound. **(b)** For cryo-EM images, the true error function over all poses (top left) for an individual particle (top right) is never evaluated. Instead, the entire lower bound is computed (middle left), the true error is calculated at the minimum of the bound, and all poses where the lower bound exceeds this calculated error are eliminated (middle right). A tighter lower bound is evaluated and the process repeated until the optimum pose is identified (bottom left and right).

Hardware required for Relion/cryoSPARC

I am buying new GPUs, what do you recommend to run RELION on?

Our collaborator in Stockholm, Erik Lindahl, has made a useful [blog with GPU hardware recommendations](#). Briefly, you'll need an NVIDIA GPU with [a CUDA compute ability of at least 3.5](#), but you don't need the expensive double-precision NVIDIA cards, i.e. the high-end gamer cards will also do, but do see Erik's blog for details! Note that 3D auto-refine will benefit from 2 GPUs, while 2D and 3D classification can be run just as well with 1 GPU. Apart from your GPUs you'll need a decent amount of RAM on the CPU (at least 64Gb), and you may also benefit from a fast (e.g. a 400Gb SSD!) scratch disk, especially if your working directories will be mounted over the network connecting multiple machines.

OS : Linux。GPU用のライブラリとしてCUDAが必要。

GPU の種類: NVIDIA社製。ゲーム用の安価なカードでかまわない。GeForce 1080など。高価な倍精度のGPU(例えば、Tesla)は必要ない。

GPUの枚数: 2D分類は1枚でも動く。3D auto-refineは2枚以上必要。

主メモリ: できるだけ多く。最低でも64Gbyte。

ディスク: scratch領域にSSDがあるとよい。

CPU: CPUだけしか動かないタスクもあるので、コア数も多いほうがよい。



- ・事前にインストールが必要なライブラリ
 - ・CUDA (GPU計算のためのライブラリ)
 - ・openmpi (ジョブ並列用のライブラリ)
- ・以下のライブラリは無ければ、relionのコンパイラがインストール時にローカルにインストールする。
 - ・FFTW (高速フーリエ変換のライブラリ)
 - ・FLTK (GUIのライブラリ)



NVIDIA GeForce 1080 Ti



NVIDIA GeForce RTX™ 2080 Ti

This tutorial assumes 2 persons share 1 Linux machine with 4GPUs.

ライセンス IDの取得

(1) non-profit academic useに限り無料

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CryoSPARC™ and cryoSPARC Live™ are available free of charge for [non-profit academic use](#). Please fill out the form to request a license.

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First name

Last name

Institution name

Lab name

Email

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I'm a Researcher at a for-profit educational/research institution

I'm a Researcher or Manager at a centralized cryo-EM microscope facility providing data collection and/or processing services to academic or industry users

I'm a Researcher or Manager at a contract research organization

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I am downloading cryoSPARC™ for non-profit academic use

[Request academic license](#)

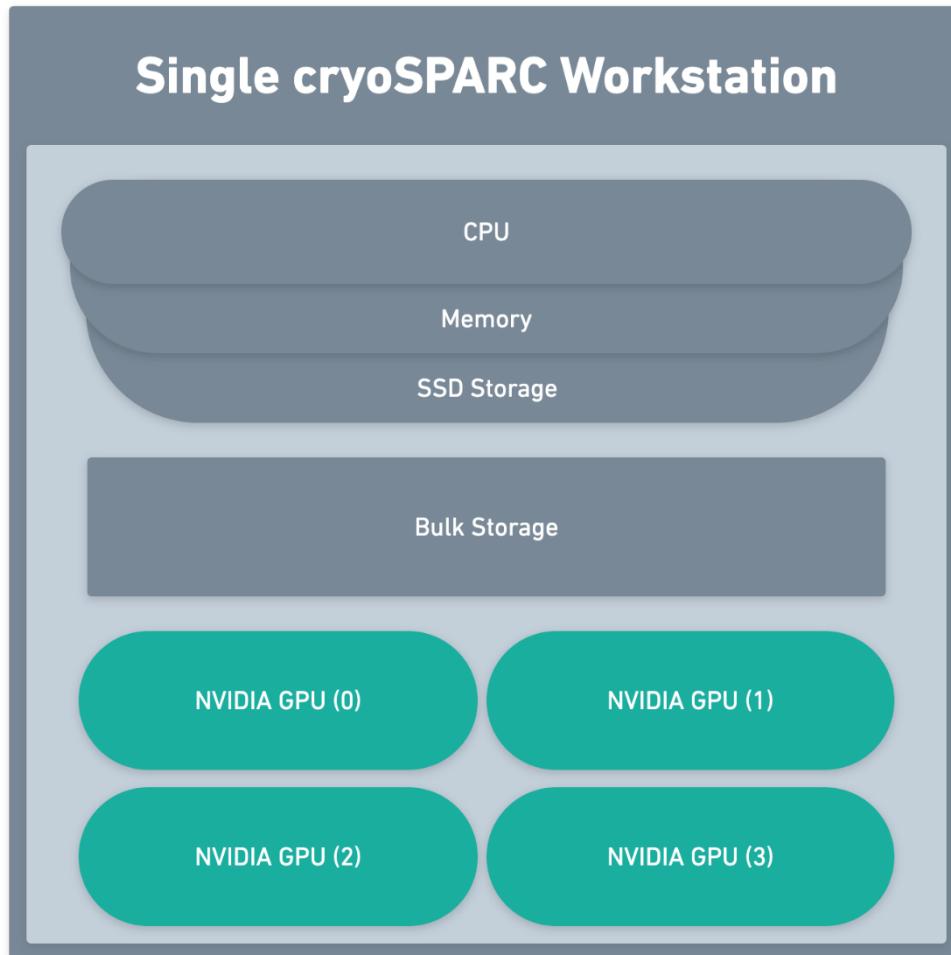
(2) 申請すると24時間以内に登録メールアドレスにライセンスIDが送付される

(3) 登録メールアドレスとして、@gmail.comや@yahoo.co.jpを入れると即座にPlease enter a valid institution/company email addressというエラーで棄却。@docomo.ne.jpでは受け付けられるがライセンスIDが送付されてこない....

(4) 一つのライセンスIDでは、一つのmasterノードしかインストール・実行できない。

(5) 研究室などで、複数の人が共有で使用する場合は、Master-workersかClusterの様式でセットアップする必要

Install: Single Workstation Setup



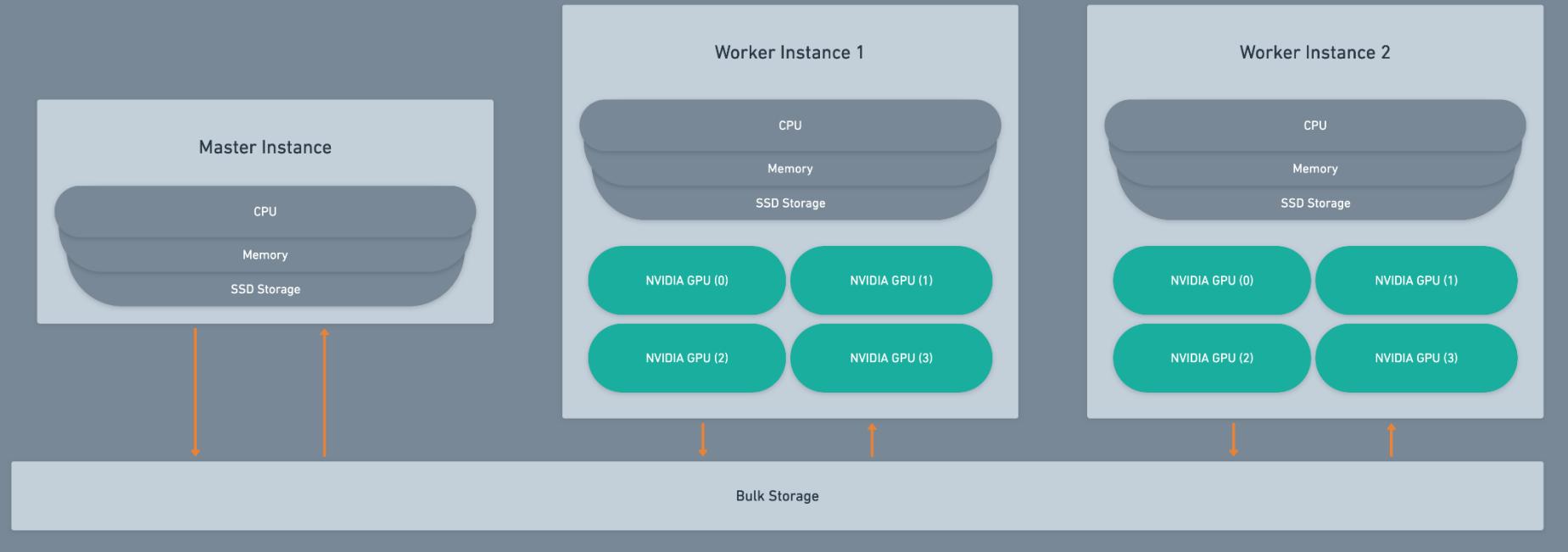
Simplest setup.

Master and worker can run on the same machine.

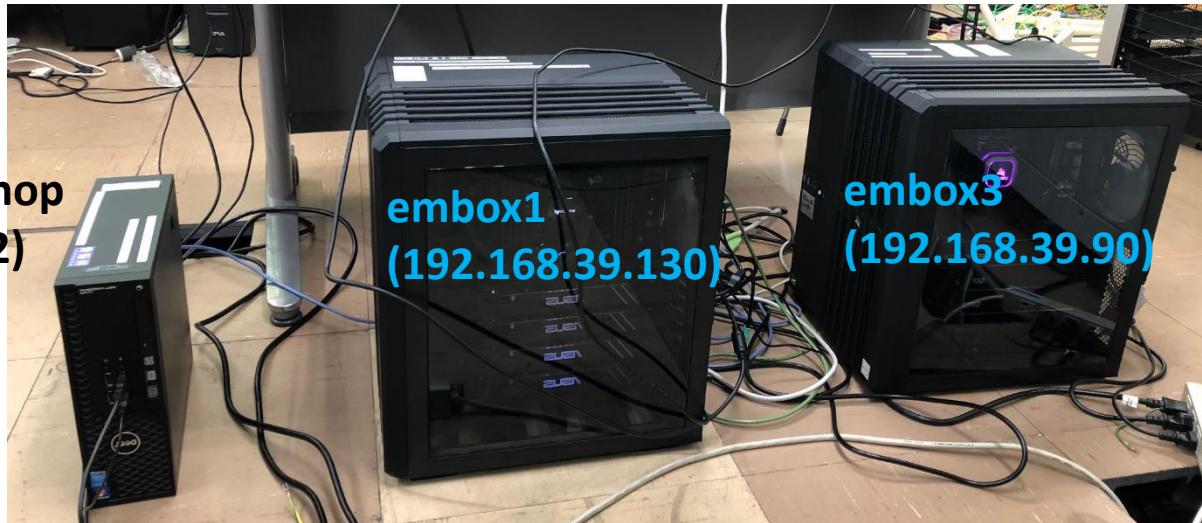


Install: Master Workers Setup

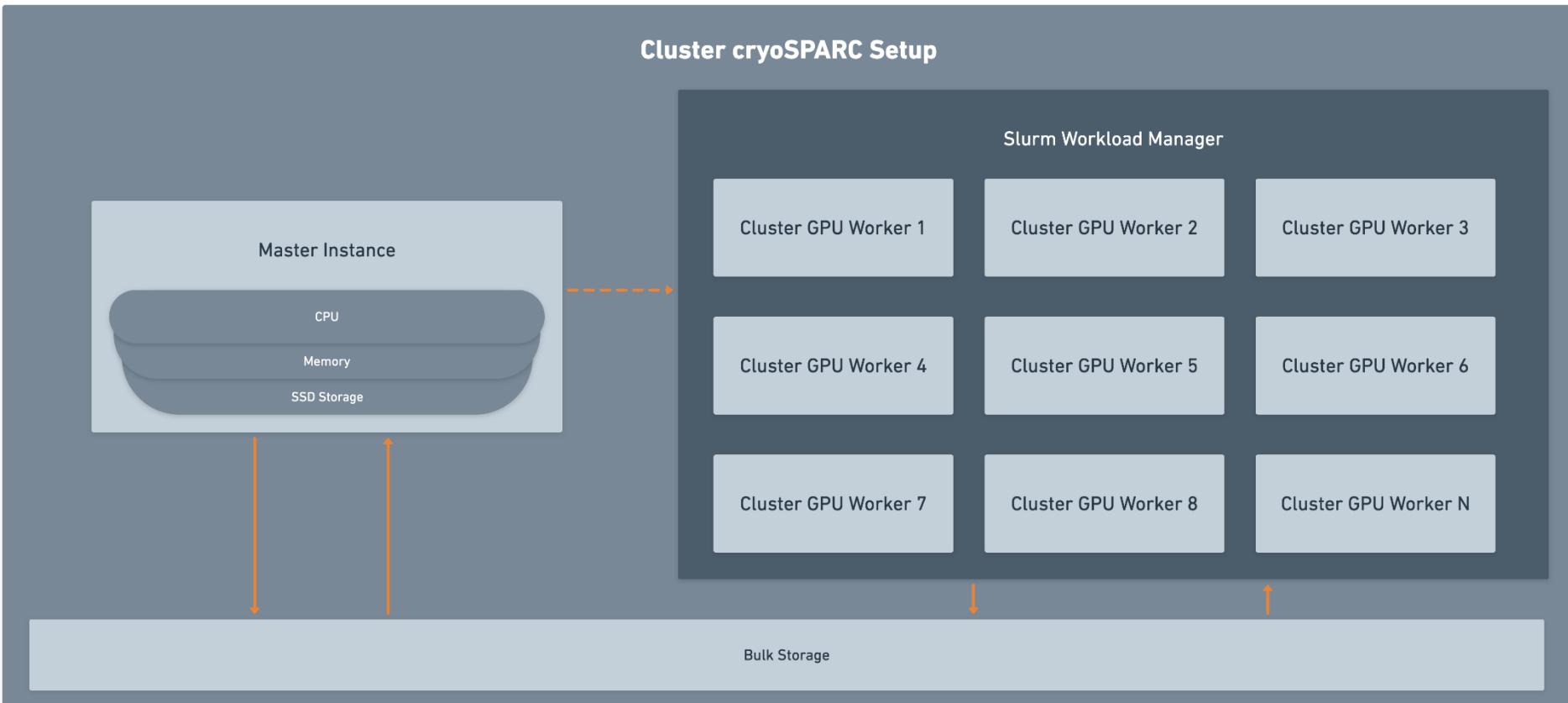
Master-Worker cryoSPARC Setup



master-workshop
(192.168.39.72)



Install: Cluster Setup



Hardware Requirements

Master node

Component	Minimum Requirement	Recommended
CPU	4+ cores	8+ cores at 2.8GHz+
RAM	16GB+	32GB DDR4
Fast Local Storage	Not Required	Not Required
GPU	Not Required	Not Required
Network	1Gbps link to storage servers	10Gbps link to storage servers

Worker node

Component	Minimum Requirement	Recommended
CPU	2+ cores per GPU	4 cores per GPU
CPU Memory Bandwidth	50+ GB/s	100+ GB/s
RAM	32GB+ per GPU	64GB DDR4 per GPU
Fast Local Storage	1TB SSD	2TB PCIe SSD
GPU	1+ NVIDIA GPU with CC 3.5+ , 11GB+ VRAM	1+ NVIDIA Tesla V100, RTX2080Ti, RTX3090, etc
Network	1Gbps link to storage servers	10Gbps link to storage servers

Software Requirements

Component	Requirement
Operating System	Modern Linux OS (Ubuntu)
Shell	Bash
User Account	cryosparcuser
Software	CUDA ≥10 (worker nodes only) GCC
Filesystem	Shared file system across all nodes

(1) OSはUbuntuを強く推奨。CentOS7ではエラーの報告があるらしい。

(2) Masterノードのインストール・使用ごとに1つのライセンスが必要。

Master-worker setupの場合の設定について

- (1)Master, workersで共通のアカウント **cryosparcuser**を設定
- (2) **cryosparcuser** はmasterからworkersへパスワード無しでsshログイン可能とする
- (3) ポート番号39000-39010が開放されていること(ubuntuはデフォルトが開放状態)
- (4) master, workers間で読み書きのできる共有のファイルシステム

CryoSPARCのユーザーアカウント

- (1) 実行にはcryoSPARCのアカウントが必要。Unixアカウントとは別。
- (2) cryoSPARCのアカウントは Emailアドレスとパスワード
- (3) どのcryoSPARCのアカウントで操作しても、Unixからみると、**cryosparcuser**というUnixアカウントが実行しているように見える。
- (4) データのアップロードにcryoSPARCにUnixのアカウントも必要。計算結果のダウンロードはWebからできるが、入力画像のアップロードはWebからできないので、WebマシンにUnixアカウントでログインして入力電顕画像のアップロード作業する必要がある。

今回の講習会用マシンの設定

(1) 所内用: Master + 2 Workers 所内から <http://192.168.39.72:39000>



アカウント:

guest01@master-workshop

guest02@master-workshop

guest03@master-workshop

guest04@master-workshop

guest10@master-workshop

←計算済みの結果が見れます

※4人のアシスタントの方だけに
使っていただきます。

(2) 所外のリモート参加者用: Single Workstation



所内から <http://ciclews.pdbj.org>

アカウント:

guest01@embox2

guest02@embox2

guest03@embox2

guest04@embox2

パスワード(共通):

embox2remote

guest10@embox2 ←計算済みの結果が見れます

※80人以上の人がある場合、可能性があるので、くれぐれもジョブを実行しないでください。

リモート受講者用の計算途中結果のデータ提供

講習会のWEBページ <https://pdbj.org/news/workshop20210624>

<スケジュール>

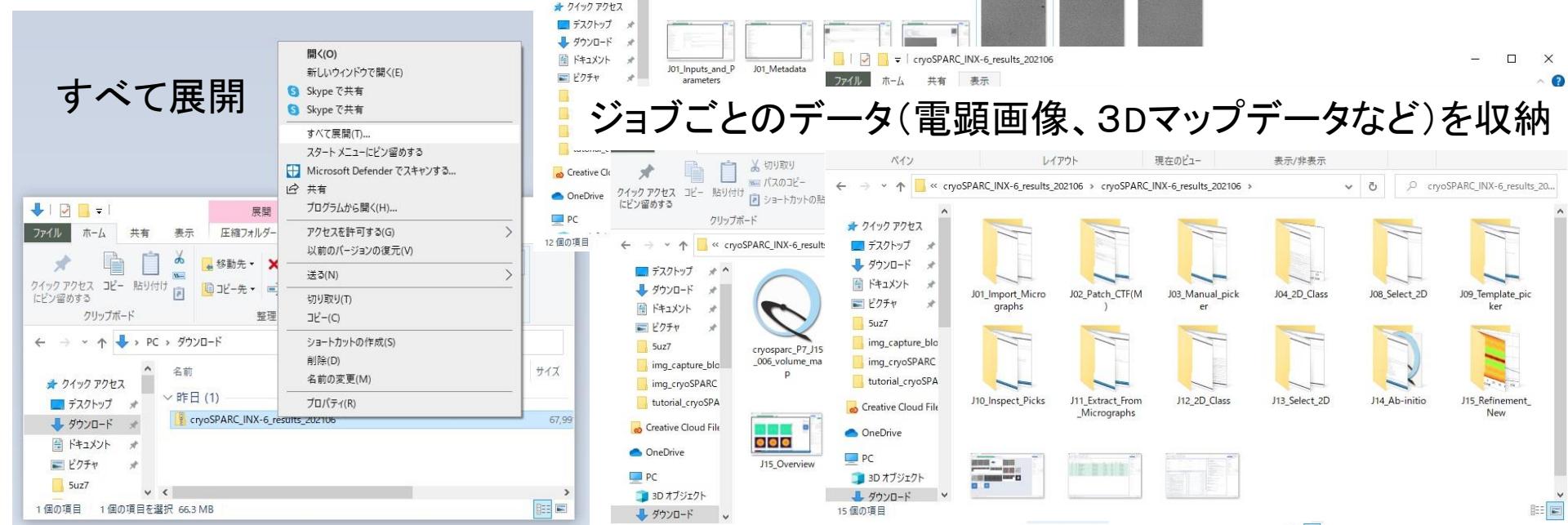
2021年6月24日(木)

13:00 - 15:00	講義「単粒子解析の原理」加藤 貴之
15:10 - 16:00	講義「日本電子の低温電子顕微鏡の紹介」牧野 文信 [講義資料]
16:10 - 18:00	実習「cryoSPARCによる電顕画像処理」川端 猛、加藤 貴之 [講義資料] [パソコン用の講習会データ(cryoSPARC_INX-6_results_202106.zip)(67MB)]

2021年6月25日(金)

13:00 - 15:30	実習「cryoSPARCによる三次元再構成」
15:40 - 18:00	実習「Cootによる原子モデルリング」

すべて展開



Job history

#	Project	Job	Type	Elapsed
1	P7	J16	Sharpening Tools	08 sec.
2	P7	J15	Homogeneous Refinement	05 min. 20 sec.
3	P7	J14	Ab-Initio Reconstruction	05 min. 38 sec.
4	P7	J13	Select 2D classes	11 min. 35 sec.
5	P7	J12	2D Classification	06 min. 31 sec.
6	P7	J11	Extract from Micrographs	01 min. 40 sec.
7	P7	J10	Inspect particle picks	33 min. 52 sec.
8	P7	J9	Template picker	01 min. 06 sec.
9	P7	J8	Select 2D classes	08 min. 58 sec.
10	P7	J4	2D Classification	01 min. 03 sec.
11	P7	J3	Manual picker	33 min. 17 sec.
12	P7	J2	Patch CTF estimation (multi	02 min. 06 sec.
13	P7	J1	Import Micrographs	05 sec.

Day 2 ?

Day 1 ?

Comparison with Relion 3.1

cryoSPARC v3.2.0	Comp. Time	Relion 3.1	Comp. Time
J2:Patch CTF estimation	02 min 06 sec	CTF estimation	< 1min
J4:2D classification (100particles)[1GPU]	01 min 03 sec	2D classification (341 particles) [1GPU]	1 min
J9:Template picker	01 min 06 sec	Auto-picking, reference-based [1GPU]	2 min
J12:2D classification (27680 particles; 50 class) [2 GPUs]	06 min 31 sec	2D classification (38244 particles; 20 class) [2GPUs]	23 min
J14: Ab-initio reconstruction(128^3)[1GPU]	05 min 38 sec	3D Initial model (64^3)[1GPU]	10 min
J15: Homogeneous Refinement (180^3 ;1class) [1GPU]	05 min 20 sec	3D classification (64^3 ;4classes) [2GPUs]	12 min
		3D refinement (180^3 ;1class) [2GPUs]	25 min

Comp.Time by Single and Four users

	Single user	Four users			
cryoSPARC v3.2.0	guest10	guest01	guest02	guest03	guest04
machines	embox3	embox3		embox1	
J2:Patch CTF estimation	02:06	02:08	02:07	02:21	02:20
J4:2D classification (100particles)[1GPU]	01:03	01:06	01:15	01:03	01:18
J9:Template picker	01:06	01:03	02:30	02:25	01:54
J12:2D classification (27680 particles; 50 class) [2 GPUs]	06:31	11:26	11:33	14:56	14:34
J14: Ab-initio reconstruction(128^3)[1GPU]	05:38	09:30	09:34	04:48	04:54
J15: Homogeneous Refinement (180^3 ;1class) [1GPU]	05:20	06:33	08:08	05:23	05:10

*2D class and Template picker job with 4 users takes ~2 times time than those with 1 user.
This may due to DISC I/O; 4 users access to one shared_disc in the master node.

Step 0: Log-in

The screenshot shows the cryoSPARC web interface. On the left, there's a sidebar with 'Dashboard' and 'cryoSPARC' tabs, and a search bar with the IP address '192.168.184.143:61000'. Below the search bar are buttons for 'Projects' and 'Workspaces'. The main area has a 'Statistics' section with tables for 'Projects', 'Workspaces', 'Jobs', and 'Completed Jobs' across 'This week', 'This month', and 'Total' periods. It also includes a 'Change Log' section for version v3.2.0. A central modal window titled 'Log In | cryoSPARC' is open, showing fields for 'Email' (filled with 'guest01@master-workshop') and 'Password'. Below the fields are 'Reset password' and 'New account' links, and a large blue 'Log In' button. To the right of the modal, there's a 'Links' section with 'CryoSPARC Guide' and 'Tutorials and Case Studies' sections. At the bottom, there are links to 'EMPIAR' and 'EMDB'.

Dashboard | cryoSPARC

セキュリティ保護なし | 192.168.184.143:61000

cryoSPARC Projects Workspaces

Statistics

	This week	This month	Total
Projects	0	1	4
Workspaces	0		
Jobs	0		
Completed Jobs	0		

Change Log

v3.2.0 Mar 29, 2024

- NEW** Added option for sorting jobs by date and title. Projects and workspaces can be sorted by title as well
- NEW** Check Particles job for verifying data integrity of particle stacks [Patch 21051]
- NEW** Computational batch size parameter in Heterogeneous Refinement job [Patch 21051]
- NEW** cryosparc changeport command and related utilities for changing the base port [Patch 21060]
- UPDATE** Substantially improved performance in iterative particle-processing jobs (2D Classification, Refinement) when particles are stored on some types of filesystems (including FSx for Lustre), resolving cases where some users found that jobs run in v3.1 were slower than in v2.15
- UPDATE** Deep Particle Picking jobs (Deep Picker Train, Deep Picker Inference) now require CUDA Toolkit 11+
- UPDATE** The 'Metadata' tab within the job preview dialog is more performant and features expandable sections and colour highlighting based on data type
- UPDATE** Import Particles no longer ignores the `rImagePixelSize` column while trying to import a particle `.star` file
- UPDATE** CryoSPARC processes are now less likely to become orphaned due to more robust supervisor management configuration
- UPDATE** Removed unused cubLAS dependency that caused multiple GPU contexts on all GPUs in a system to be created even when using only a single GPU
- UPDATE** GPU status info is now updated when a worker is connected for the first time, or when a worker connection is updated
- UPDATE** New option to work around bug in CUDA on CentOS 7 that causes `cudaMemcpy` errors in multiple libtuner. To engage this add

Log In | cryoSPARC

セキュリティ保護なし | 192.168.1... Email guest01@master-workshop Password

Reset password New account Log In

CryoSPARC Guide

Tutorials and Case Studies

EMPIAR Electron Microscopy Public Image Archive

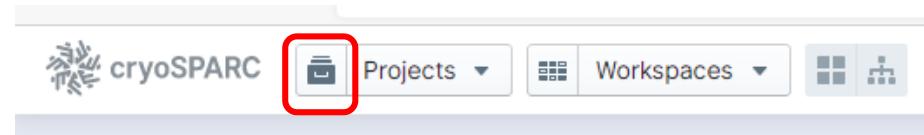
EMDB Electron Microscopy Data Bank

Dashboard Projects Resource Manager

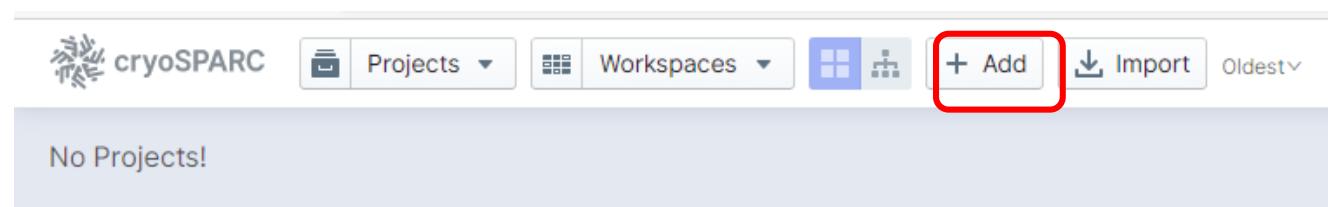
guest05

Step 1: Create a Project

(1) Click [Projects] icon



(2) Click [+ Add] button



(3) Input “Title” and “Directory in which to create new project directory”

New Project

* Title
EMPIAR-10291_50mic

* Directory in which to create new project directory:
/shared_data SSD4TB/cryosparc_projects/guest05

Select a location where the project directory will be stored. All files associated with this project will be stored in this directory.

/shared_data SSD4TB/cryosparc_projects/guest10

Description

Cancel Create

(4)



Project directory must exist.
Project directory must be in the shared directory.

Step 2: Create a Workspace

(1) Click your Project number



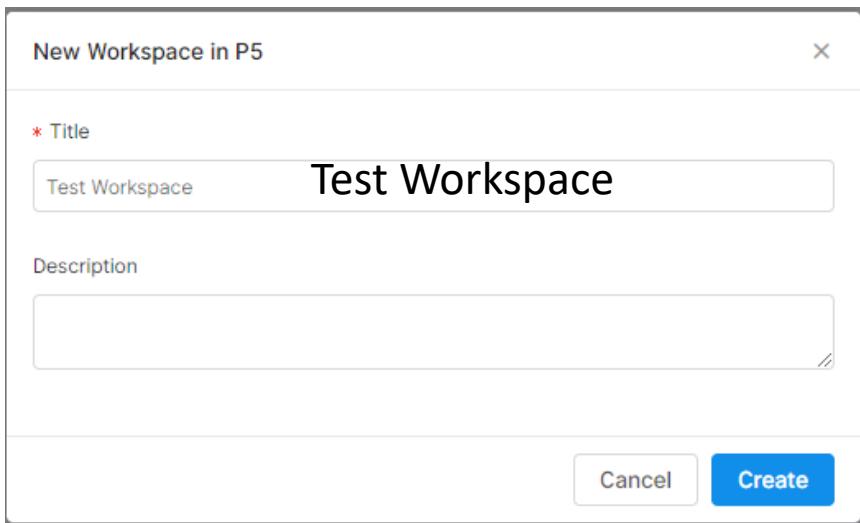
P7: EMPIAR-10291_50mic
Created in a few seconds

(2) Click [+ Add]



cryoSPARC P7: EMPIAR-10291_50mic Workspaces + Add Oldest

(3) Input Title of a new Workspace



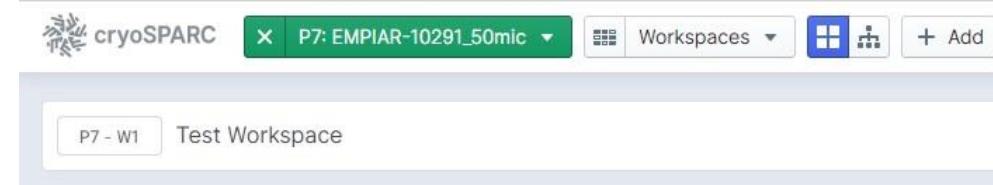
New Workspace in P5

* Title
Test Workspace

Description

Cancel Create

(4) A new workspace is shown.



P7: EMPIAR-10291_50mic Workspaces + Add

P7 - W1 Test Workspace

Download Tutorial Data by Command

※この作業は今回は不要です。masterサーバの

/shared_data_SD4TB/tutorial_data/EMPIAR-10291_50mic
にコピー済みです。

(1) Log-in to the “master” server.

(2) Change directory to your project directory

```
$ cd /shared_data_SSD4TB/cryosparc_projects/guest01
```

(3) Change directory to your project directory

```
$ wget ftp://empiar.pdbj.org/pub/empiar/workshop/10291/00001/EMPIAR-  
10291_50mic.tar
```

(4) Extract the tar file

```
$ tar xvf EMPIAR-10291_50mic.tar
```

Step 4: J1:Import Micrographs

(1) Click a Workspace

P7 - W1 Test Workspace

(2) Choose [Job Builder]

Details Job Builder

P5 → W1 DETAILS

Test Workspace
No description set

Details Job Builder

Import Micrographs

/shared_data_SSD4TB/tutorial_data/EMPIAR-10291_50mic/data/*.mrc

NOTE: file extension, such as *.mrc, *.tif, has to be added.

(4) Input “Micrographs data path” and other parameters. then click [Queue]

Micrographs

Micrographs data path: /shared_data_SSD4TB/tutorial_data/EMPIAR-10291_50mic/data/*.mrc

Pixel size (Å): 1.232

Accelerating Voltage (kV): 300

Spherical Aberration (mm): 1.6

Total exposure dose (e/Å²): 35.0

Negative Stain Data:

Phase Plate Data:

Override Exposure Group ID: NONE

Skip Header Check:

Output Constant CTF:

Compute settings

Number of CPUs to parallelize during header check... 4

Cancel Queue

Queue P5 → J1 (import_micrographs)

* Lane

Lane default (node) (node)

embox2 CPUs: 24 | RAM: 128 GB | GPUs: 4

embox3 CPUs: 28 | RAM: 128 GB | GPUs: 4

Description

Enter a description.

* Run Job in

Current Workspace (W1)

New Workspace

Create

(5) Click [Create]

Step 4: J1:Import Micrographs

J1 (P7: W1) | cryoSPARC +

セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J1

cryoSPARC P7: EMPIAR-10291_50mic W1: Test Workspace J1

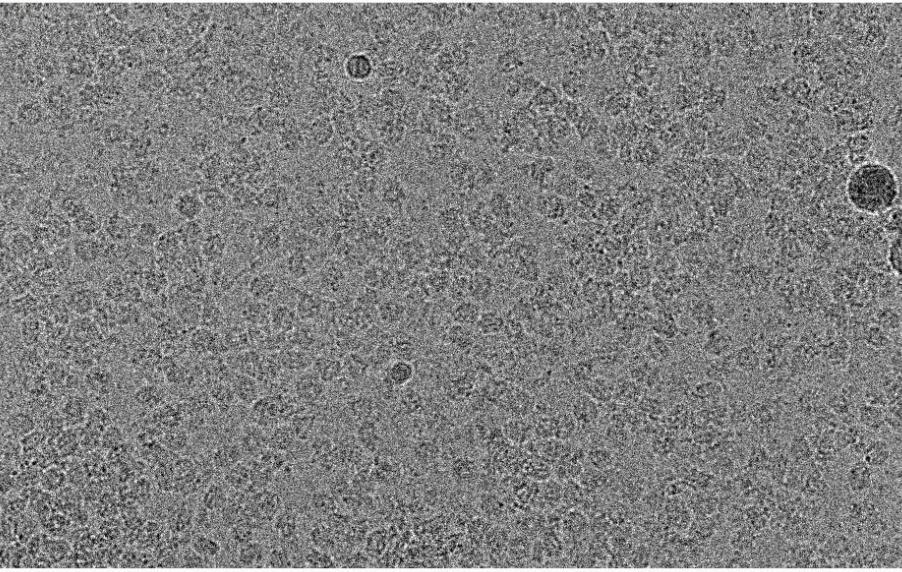
Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

```
> [CPU: 242.3 MB] =====
> [CPU: 242.3 MB] Loaded 50 micrographs.
> [CPU: 242.3 MB] Common fields:
> [CPU: 242.3 MB]     mscope_params/accel_kv : {300.0}
> [CPU: 242.3 MB]     mscope_params/cs_mm : {1.6}
> [CPU: 242.3 MB]     mscope_params/total_dose_e_per_A2 : {35.0}
> [CPU: 242.3 MB]     mscope_params/exp_group_id : {0}
> [CPU: 242.3 MB]     mscope_params/phase_plate : {0}
> [CPU: 242.3 MB]     mscope_params/neg_stain : {0}
> [CPU: 242.3 MB]     micrograph_blob/psize_A : {1.232}
> [CPU: 242.3 MB]     micrograph_blob/shape : [3838 3710]
> [CPU: 242.3 MB] =====
> [CPU: 242.3 MB] Making example plots. Exposures will be displayed without defect correction.
> [CPU: 242.3 MB] Reading file ...

```

Raw data J1/imported/INX6Ndelnano018.mrc [png]



Outputs

- imported_micrographs
- failed_micrographs

exposure Count: 50

exposure Count: 0

JOBDETAILS

J1 (Import Micrographs) COMPLETED

New Job J1

Enter a description.

CREATED BY guest10
LAST ACCESSED BY guest10
LAST ACCESSED AT Sat, Jun 19, 2021 4:00 PM
INTERACTIVE No
CREATED Sat, Jun 19, 2021 3:58:43 PM
QUEUED Sat, Jun 19, 2021 4:00:47 PM
LAUNCHED Sat, Jun 19, 2021 4:00:47 PM
STARTED Sat, Jun 19, 2021 4:00:48 PM
COMPLETED Sat, Jun 19, 2021 4:00:54 PM

SIZE 19.11 KB

ACTIONS

- Queue Job on default
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

Step 6: J2:CTF estimation (2.5 min with 1GPU)

The screenshot shows the cryoSPARC software interface. In the top right corner, the 'Job Builder' panel is open, displaying a list of processing steps. The 'Patch CTF estimation (multi)' step is highlighted with a red box. On the left, the main workspace shows three micrograph thumbnails under the 'Import Micrographs' section. The bottom navigation bar includes links for Dashboard, Projects, Resource Manager, and guest10.

(1) Select [Patch CTF estimation(multi)] job

This screenshot shows the cryoSPARC interface after selecting the 'Patch CTF estimation (multi)' job. The 'Job Builder' panel now displays 'J2 (Patch CTF estimation (multi))' with the status 'BUILDING'. The 'Import Micrographs' step is also listed in the builder. The 'J1' job, which was previously selected, is highlighted with a red box in the main workspace. The workspace shows three micrograph thumbnails under the 'Import Micrographs' section. The bottom navigation bar includes links for Dashboard, Projects, Resource Manager, and guest10.

(2) Select previous [Import Micrographs] job

Step 6: J2:CTF estimation (2.5 min with 1GPU)

(1) Drag and drop !

JOB BUILDER
J2 (Patch CTF estimation (multi)) BUILDING

Outputs
↳ imported_micrographs
↳ exposure (Exposure)
↳ Min: 1, Max: Infinity, Repeats: no

Inputs and Parameters
Outputs
Metadata
Overview
Show from top
Select checkpoint
Follow latest
Filter Types
Filter Flags

```
> [CPU: 242.3 MB] =====  
> [CPU: 242.3 MB] Loaded 50 micrographs.  
> [CPU: 242.3 MB] Common fields:  
> [CPU: 242.3 MB]     mscope_params/accel_kv : {300.0}  
> [CPU: 242.3 MB]     mscope_params/cs_mm : {1.6}  
> [CPU: 242.3 MB]     mscope_params/total_dose_e_per_A2 : {35.0}  
> [CPU: 242.3 MB]     mscope_params/exp_group_id : {0}  
> [CPU: 242.3 MB]     mscope_params/phase_plate : {0}  
> [CPU: 242.3 MB]     mscope_params/neg_stain : {0}  
> [CPU: 242.3 MB]     micrograph_blob/psize_A : {1.232}
```

Queue P5 → J2 (patch_ctf_estimation_multi)

* Queue Type
Queue to Lane Run on specific GPU

* Lane
Lane default (node) (node)
embox2 CPUs: 24 | RAM: 128 GB | GPUs: 4
embox3 CPUs: 28 | RAM: 128 GB | GPUs: 4

Description
Enter a description.

* Run Job in
Current Workspace (W1)
New Workspace

Create

(3) Click [Create]

(2) Set Number of GPU 1 and click [Queue]

J2 (Patch CTF estimation (multi)) BUILDING

INPUTS
exposures (exposure)
Min: 1, Max: Infinity, Repeats: no

CTF estimation
Amplitude Contrast: 0.1

Compute settings
Number of GPUs to parallelize: 1

Number of GPU:1

Queue

Step 6: J2:CTF estimation (2.5 min with 1GPU)

J2 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J2

cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace J2

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

```
> [CPU: 241.5 MB] -----  
> [CPU: 241.5 MB] Processed 0 of 50 micrographs in 5.45s  
> [CPU: 459.8 MB] -- 0.0: processing 2 of 50: J1/imported/INX6Ndelnano028.mrc  
    loading /shared_data SSD4TB/cryosparc_projects/guest10/P7/J1/imported/INX6Ndelnano028.mrc ...  
    Loading raw mic data from J1/imported/INX6Ndelnano028.mrc ...  
    Done in 0.06s  
    Processing ...  
    Done in 2.15s  
    Completed patch CTF estimation with (Y:4,X:4) knots
```

1D search for INX6Ndelnano021 [png] [pdf]

1D search result for INX6Ndelnano021 [png] [pdf]

2D patch result for INX6Ndelnano021 [png] [pdf]

Outputs

- exposures
- exposures_incomplete

exposure Count: 50

exposure Count: 0

CREATED BY guest10
LAST ACCESSED BY guest10
LAST ACCESSED AT Sat, Jun 19, 2021 4:11 PM NO
INTERACTIVE
CREATED Sat, Jun 19 2021 4:06:16 PM
QUEUED Sat, Jun 19 2021 4:09:41 PM
LAUNCHED Sat, Jun 19 2021 4:09:42 PM
STARTED Sat, Jun 19 2021 4:09:43 PM
COMPLETED Sat, Jun 19 2021 4:11:49 PM J1
PARENTS
SIZE 143.28 MB
ACTIONS Queue Job on default
Kill Job
Clear Job
Clear Intermediate Results
Export Job
Clone Job
Mark Job as Complete
Delete Job

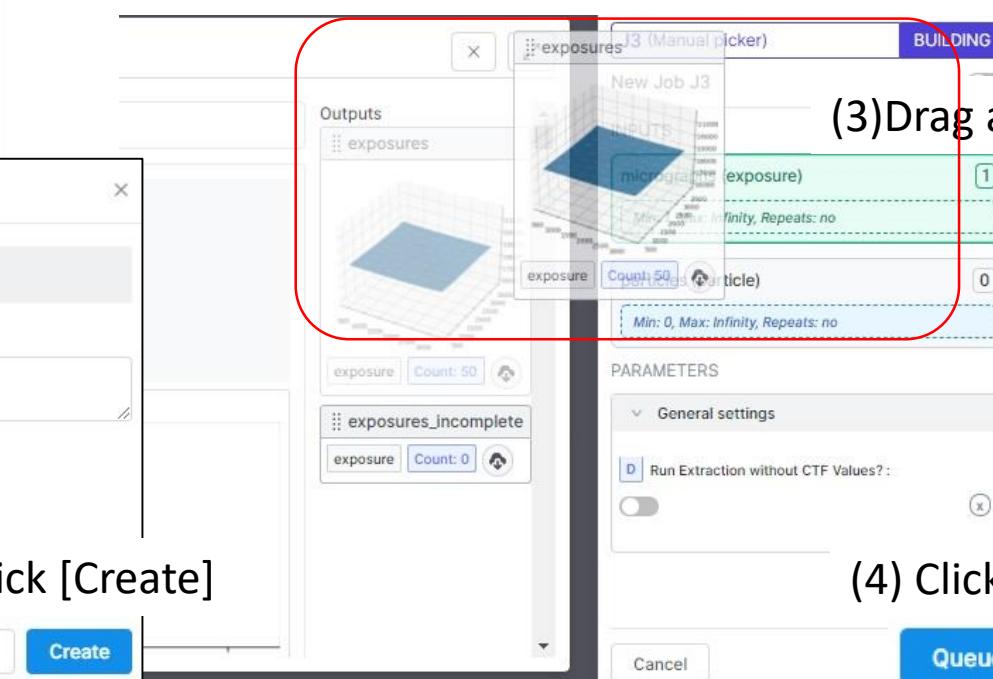
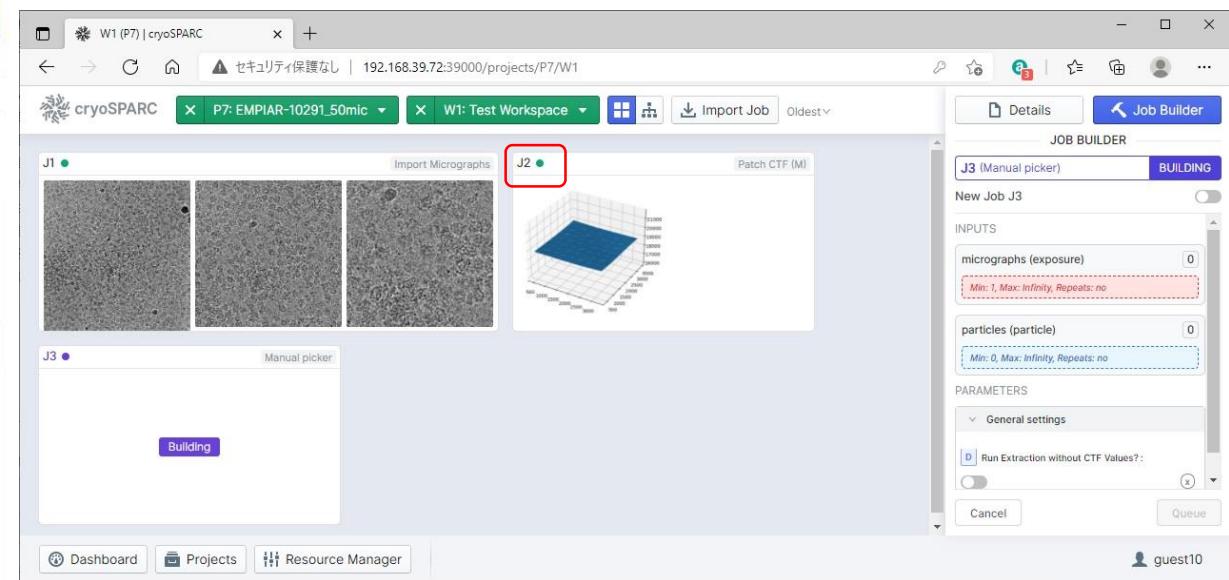
Dashboard Projects Resource Manager guest10

Step 7: J3 Manual Picker

(2) Select CTF estimation Job



(1) Choose [Manual Picker] Job



(5) Click [Create]

(4) Click [Queue]

Step 7: J3: Manual Picker

The screenshot shows the cryoSPARC software interface. At the top, there are tabs for 'P7: EMPIAR-10291_50mic' and 'W1: Test Workspace'. Below the tabs, a navigation bar includes 'Interactive' (which is highlighted with a red box), 'Overview', 'Inputs and Parameters', 'Outputs', and 'Metadata'. Under the 'Interactive' tab, there are buttons for 'Show from top', 'Select', and 'Filter Flags'. A message box displays log information: 'License is valid.', 'Running job on master node', '[CPU: 80.3 MB] Project P7 Job J3 Started', and '[CPU: 80.3 MB] Master running v3.2.0, worker running v3.2.0'. The main area shows a list of particles.

(1) Choose [Interactive]

(2) Set Low pass filter (A)=10, and Box size(px)=180

The screenshot shows the cryoSPARC interface with the 'Interactive' tab selected. A message box at the top says 'Done Picking! Extract Particles' with a count of '10'. Below it, the 'Low pass filter (A)' is set to '10' and the 'Box size (px)' is set to '180'. There is also an 'Override: Contrast Intensity Override' section with a slider. A table below lists particle details: Name (INX6Ndelnano2 96.mrc), Defocus (18821), CTF fit (3.17), and Picks (0). To the right, a grayscale micrograph shows numerous small, dark particles.

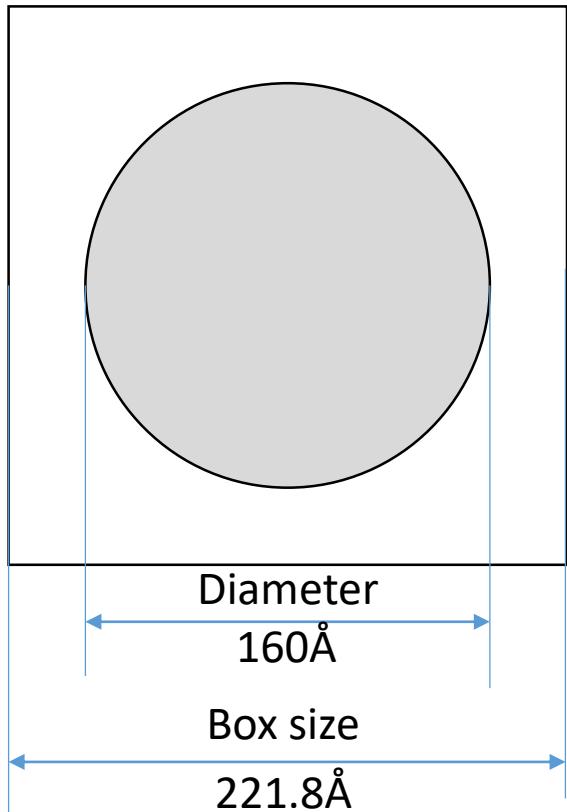
Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	0

(3) Pick particles by clicking the left button of your mouse. At least 100 particles should be picked from the first 4 micrographs. About 25 particles per one micrograph.

NOTE: Click the **right** mouse button **un-pick** a particle.

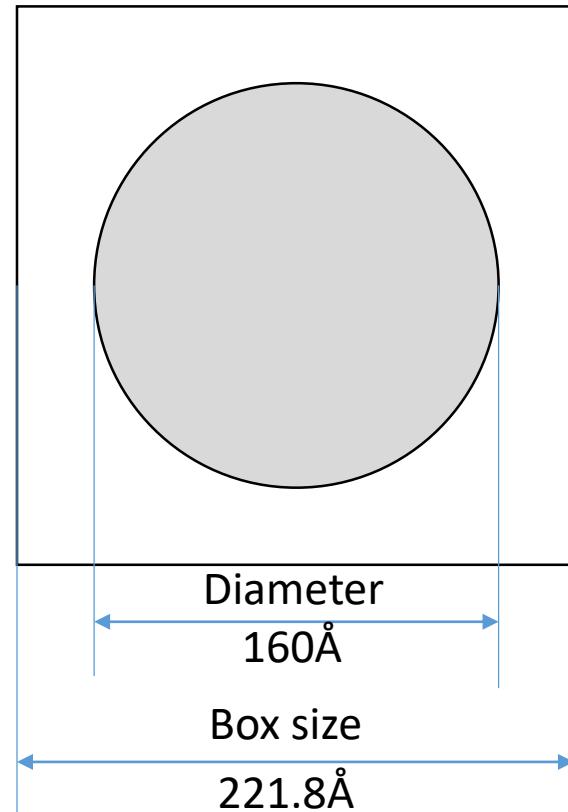
Box size of particles

Pixel size: 1.232Å



180 pixel

Pixel size: 3.465Å



To reduce
Computation costs
→
←
Just before
Refine3D

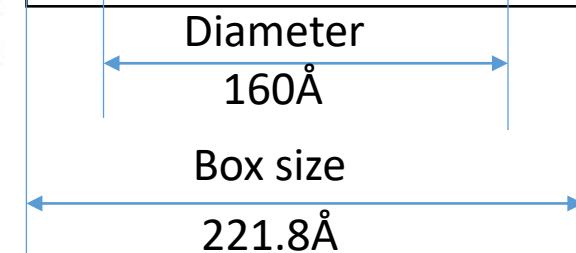
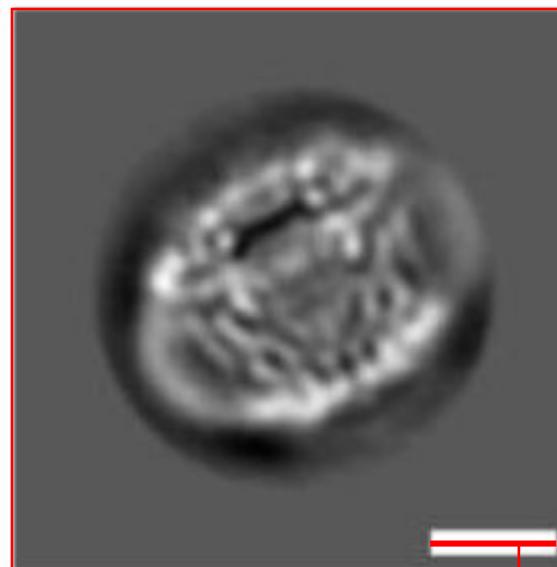
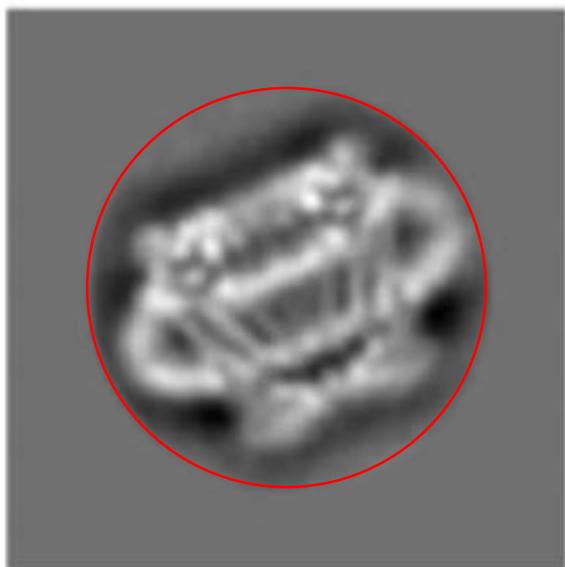
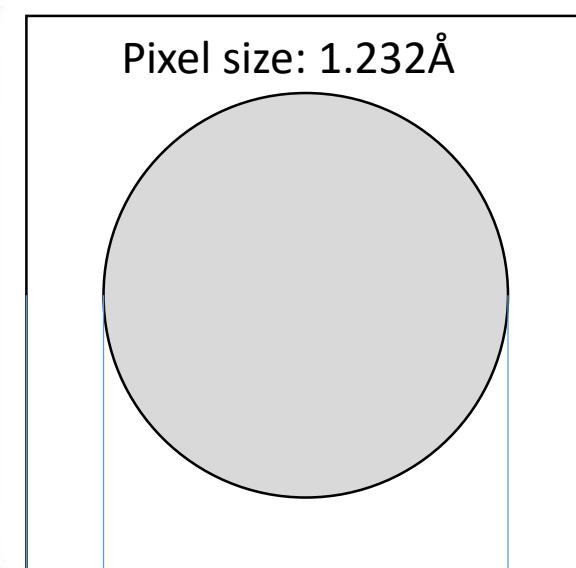
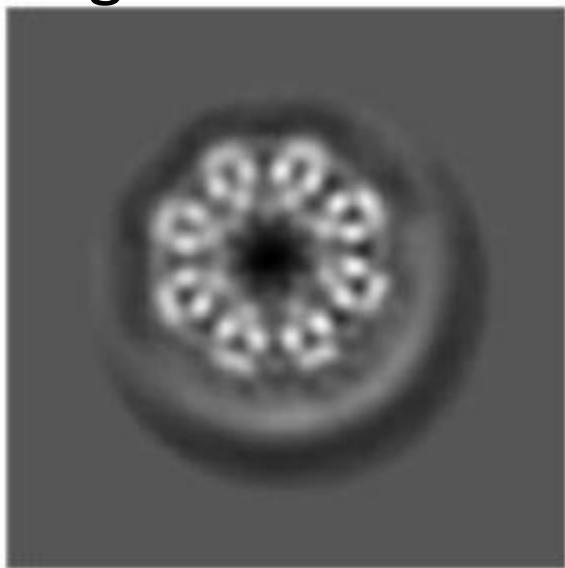
64 pixel

C

2D Class average images from the original article

Fig.S3c

Burendei et al. Science Advances. 2020. DOI: 10.1126/sciadv.aax3157



180 pixel

方形の1辺 $7.44\text{cm} = 222.75\text{\AA}$

マスク直径 $5.28\text{cm} = 158.08\text{\AA}$

1.67 cm : 50 Å

Step 7: J3:Manual Picker

J3 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J3

cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace x J3

Interactive Overview Inputs and Parameters Outputs Metadata

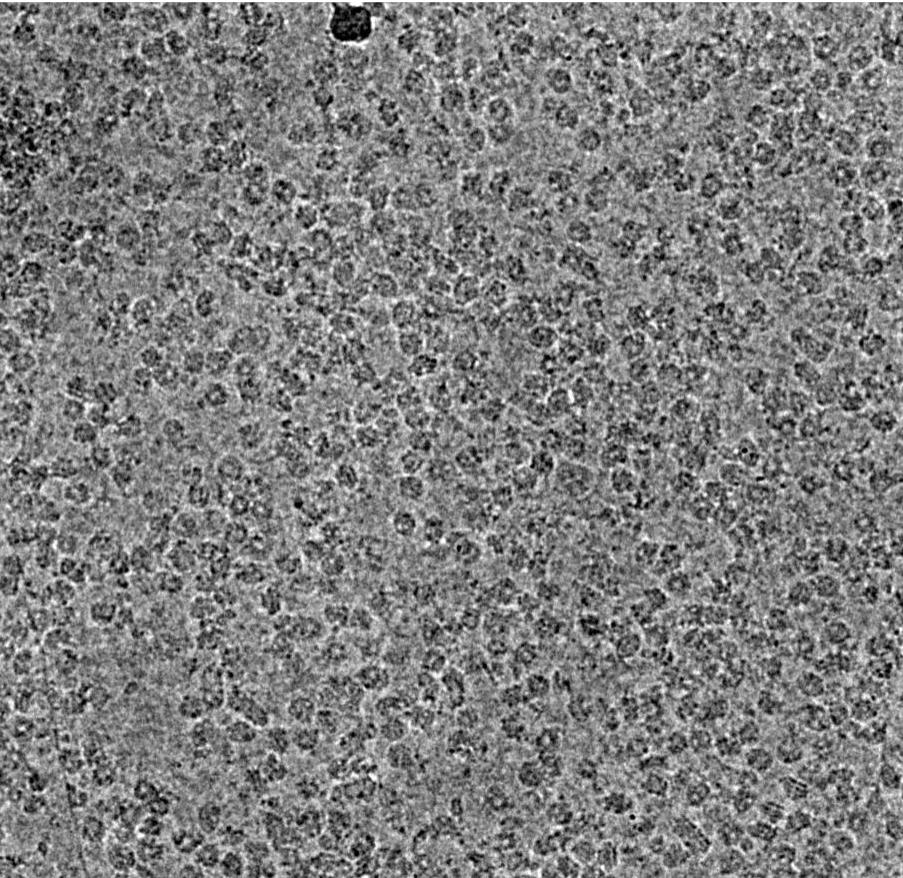
Done Picking! Extract Particles

Lowpass filter (Å): Box size (px): Show:

Override: Contrast Intensity Override:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	0
INX6Ndelnano2 61.mrc	21545	4.16	0
INX6Ndelnano2 49.mrc	20600	3.24	0
INX6Ndelnano2 48.mrc	21179	3.14	0
INX6Ndelnano2 36.mrc	20896	3.39	0
INX6Ndelnano2 33.mrc	16594	3.10	0
INX6Ndelnano2 32.mrc	15628	3.05	0
INX6Ndelnano2 22.mrc	19811	3.23	0
INX6Ndelnano2 05.mrc	14116	3.10	0
INX6Ndelnano2 01.mrc	19871	3.23	0
INX6Ndelnano1 83.mrc	21216	3.06	0
INX6Ndelnano1 80.mrc	20991	3.04	0

Picked 0 particles.



Details Job Builder

J3 (Manual picker) WAITING

New Job J3
Enter a description.

CREATED BY guest10
INTERACTIVE Yes
CREATED Sat, Jun 19 2021 4:53:10 PM
QUEUED Sat, Jun 19 2021 5:08:41 PM
LAUNCHED Sat, Jun 19 2021 5:08:41 PM
WAITING Sat, Jun 19 2021 5:08:47 PM
STARTED Sat, Jun 19 2021 5:08:42 PM
PARENTS J2

SIZE 0 Bytes

ACTIONS

- Queue Job
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

Step 7: J3:Manual Picker

J3 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J3

cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace x J3

Interactive Overview Inputs and Parameters Outputs Metadata

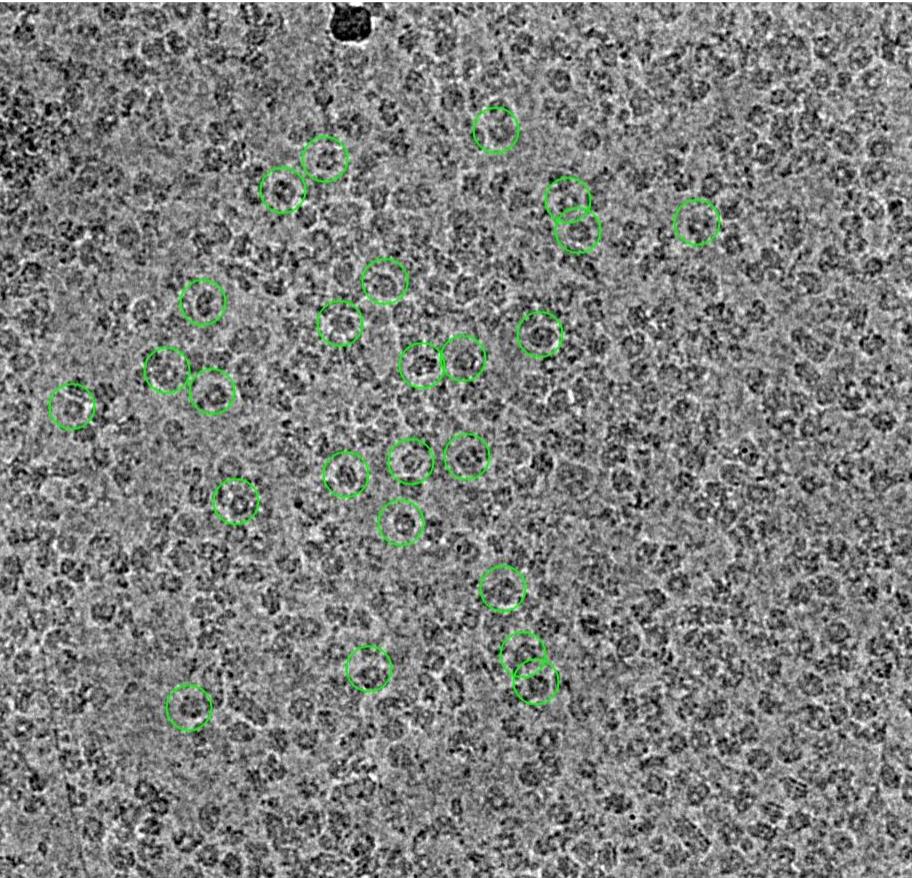
Done Picking! Extract Particles

Lowpass filter (Å): Box size (px): Show:

Override: Contrast Intensity Override:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	25
INX6Ndelnano2 61.mrc	21545	4.16	0
INX6Ndelnano2 49.mrc	20600	3.24	0
INX6Ndelnano2 48.mrc	21179	3.14	0
INX6Ndelnano2 36.mrc	20896	3.39	0
INX6Ndelnano2 33.mrc	16594	3.10	0
INX6Ndelnano2 32.mrc	15628	3.05	0
INX6Ndelnano2 22.mrc	19811	3.23	0
INX6Ndelnano2 05.mrc	14116	3.10	0
INX6Ndelnano2 01.mrc	19871	3.23	0
INX6Ndelnano1 83.mrc	21216	3.06	0
INX6Ndelnano1 80.mrc	20991	3.04	0

Picked 25 particles.



Details Job Builder

J3 (Manual picker) WAITING

New Job J3

Enter a description.

CREATED BY guest10 Yes

INTERACTIVE Sat, Jun 19 2021 4:53:10 PM

CREATED Sat, Jun 19 2021 5:08:41 PM

QUEUED Sat, Jun 19 2021 5:08:41 PM

LAUNCHED Sat, Jun 19 2021 5:08:47 PM

WAITING Sat, Jun 19 2021 5:08:47 PM

STARTED Sat, Jun 19 2021 5:08:42 PM

PARENTS J2

SIZE 0 Bytes

ACTIONS

- Queue Job
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

Step 7: J3:Manual Picker

J3 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J3

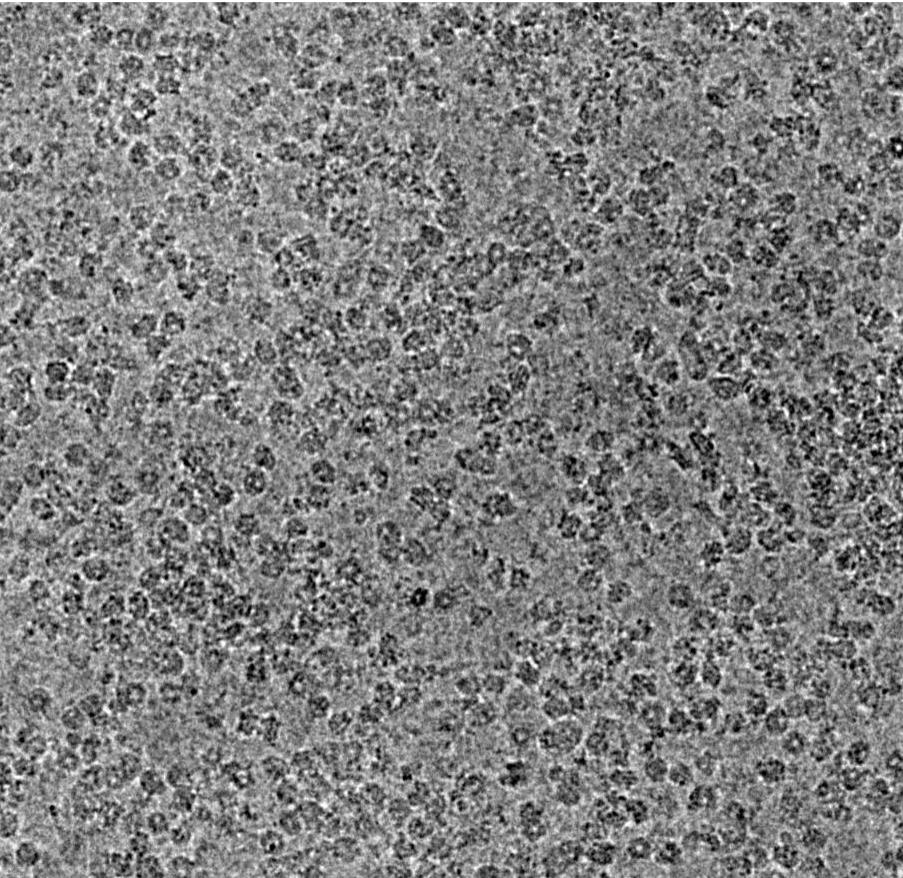
cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace x J3

Interactive Overview Inputs and Parameters Outputs Metadata

Done Picking! Extract Particles
Lowpass filter (Å): Box size (px): Show:
180 180

Override: Contrast Intensity Override:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	0
INX6Ndelnano2 61.mrc	21545	4.16	0
INX6Ndelnano2 49.mrc	20600	3.24	0
INX6Ndelnano2 48.mrc	21179	3.14	0
INX6Ndelnano2 36.mrc	20896	3.39	0
INX6Ndelnano2 33.mrc	16594	3.10	0
INX6Ndelnano2 32.mrc	15628	3.05	0
INX6Ndelnano2 22.mrc	19811	3.23	0
INX6Ndelnano2 05.mrc	14116	3.10	0
INX6Ndelnano2 01.mrc	19871	3.23	0
INX6Ndelnano1 83.mrc	21216	3.06	0
INX6Ndelnano1 80.mrc	20991	3.04	0
Picked 0 particles.			



Details Job Builder

J3 (Manual picker) WAITING

New Job J3
Enter a description.

CREATED BY	guest10
INTERACTIVE	Yes
CREATED	Sat, Jun 19 2021 4:53:10 PM
QUEUED	Sat, Jun 19 2021 5:08:41 PM
LAUNCHED	Sat, Jun 19 2021 5:08:41 PM
WAITING	Sat, Jun 19 2021 5:08:47 PM
STARTED	Sat, Jun 19 2021 5:08:42 PM
PARENTS	J2
SIZE	0 Bytes

ACTIONS

- Queue Job
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

Step 7: Particle Picking

J3 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J3

cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace J3

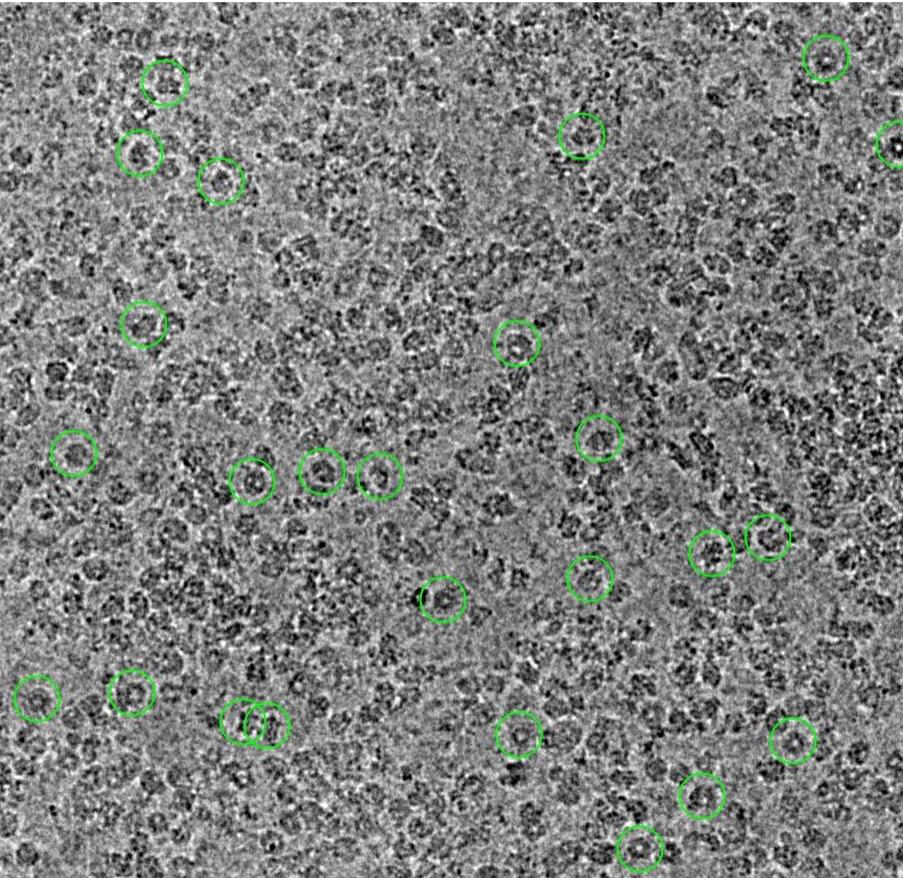
Interactive Overview Inputs and Parameters Outputs Metadata

Done Picking! Extract Particles
Lowpass filter (Å): Box size (px): Show:
180 180

Override: Contrast Intensity Override:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	25
INX6Ndelnano2 61.mrc	21545	4.16	25
INX6Ndelnano2 49.mrc	20600	3.24	0
INX6Ndelnano2 48.mrc	21179	3.14	0
INX6Ndelnano2 36.mrc	20896	3.39	0
INX6Ndelnano2 33.mrc	16594	3.10	0
INX6Ndelnano2 32.mrc	15628	3.05	0
INX6Ndelnano2 22.mrc	19811	3.23	0
INX6Ndelnano2 05.mrc	14116	3.10	0
INX6Ndelnano2 01.mrc	19871	3.23	0
INX6Ndelnano1 83.mrc	21216	3.06	0
INX6Ndelnano1 80.mrc	20991	3.04	0

Picked 50 particles.



Details Job Builder

J3 (Manual picker) WAITING

New Job J3
Enter a description.

CREATED BY	guest10
INTERACTIVE	Yes
CREATED	Sat, Jun 19 2021 4:53:10 PM
QUEUED	Sat, Jun 19 2021 5:08:41 PM
LAUNCHED	Sat, Jun 19 2021 5:08:47 PM
WAITING	Sat, Jun 19 2021 5:08:47 PM
STARTED	Sat, Jun 19 2021 5:08:42 PM
PARENTS	J2

SIZE 0 Bytes

ACTIONS

- Queue Job
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

Dashboard Projects Resource Manager guest10

Step 7: J3:Manual Picker

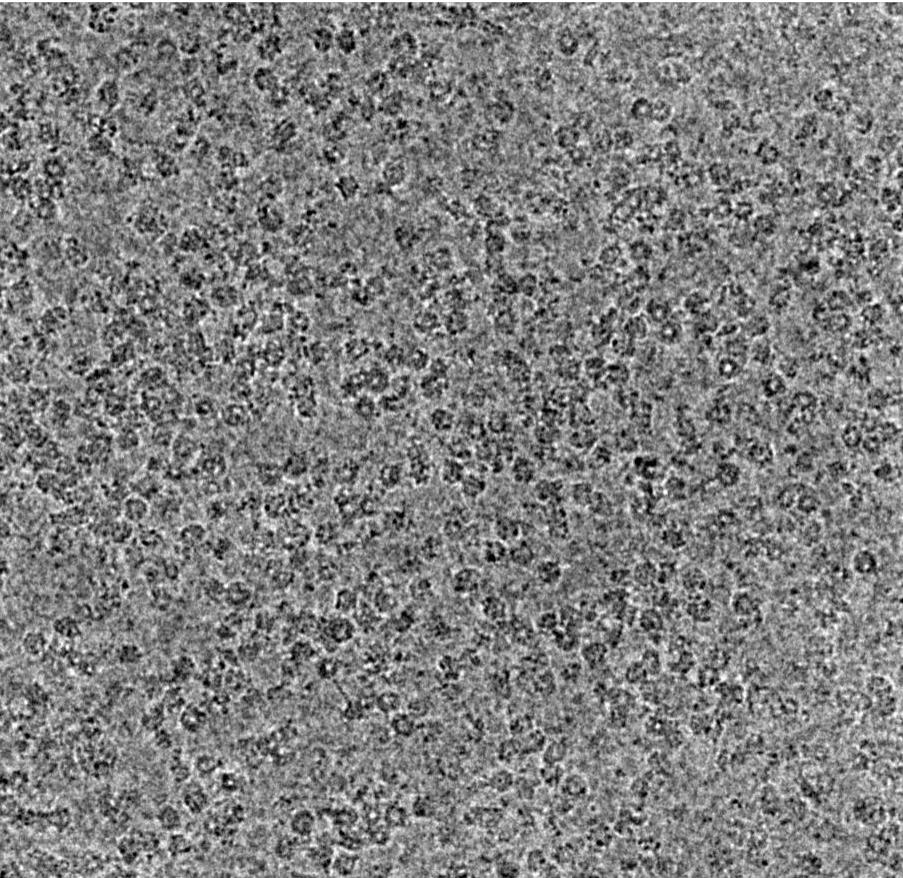
J3 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J3

cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace x J3

Interactive Overview Inputs and Parameters Outputs Metadata

Done Picking! Extract Particles
Lowpass filter (Å): Box size (px): Show:
180
Override: Contrast Intensity Override:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	0
INX6Ndelnano2 61.mrc	21545	4.16	0
INX6Ndelnano2 49.mrc	20600	3.24	0
INX6Ndelnano2 48.mrc	21179	3.14	0
INX6Ndelnano2 36.mrc	20896	3.39	0
INX6Ndelnano2 33.mrc	16594	3.10	0
INX6Ndelnano2 32.mrc	15628	3.05	0
INX6Ndelnano2 22.mrc	19811	3.23	0
INX6Ndelnano2 05.mrc	14116	3.10	0
INX6Ndelnano2 01.mrc	19871	3.23	0
INX6Ndelnano1 83.mrc	21216	3.06	0
INX6Ndelnano1 80.mrc	20991	3.04	0
Picked 0 particles.			



Details Job Builder

J3 (Manual picker) WAITING

New Job J3
Enter a description.

CREATED BY guest10
INTERACTIVE Yes
CREATED Sat, Jun 19 2021 4:53:10 PM
QUEUED Sat, Jun 19 2021 5:08:41 PM
LAUNCHED Sat, Jun 19 2021 5:08:41 PM
WAITING Sat, Jun 19 2021 5:08:47 PM
STARTED Sat, Jun 19 2021 5:08:42 PM
PARENTS J2

SIZE 0 Bytes

ACTIONS
Queue Job
Kill Job
Clear Job
Clear Intermediate Results
Export Job
Clone Job
Mark Job as Complete
Delete Job

Step 7: J3:Manual Picker

J3 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J3

cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace x J3

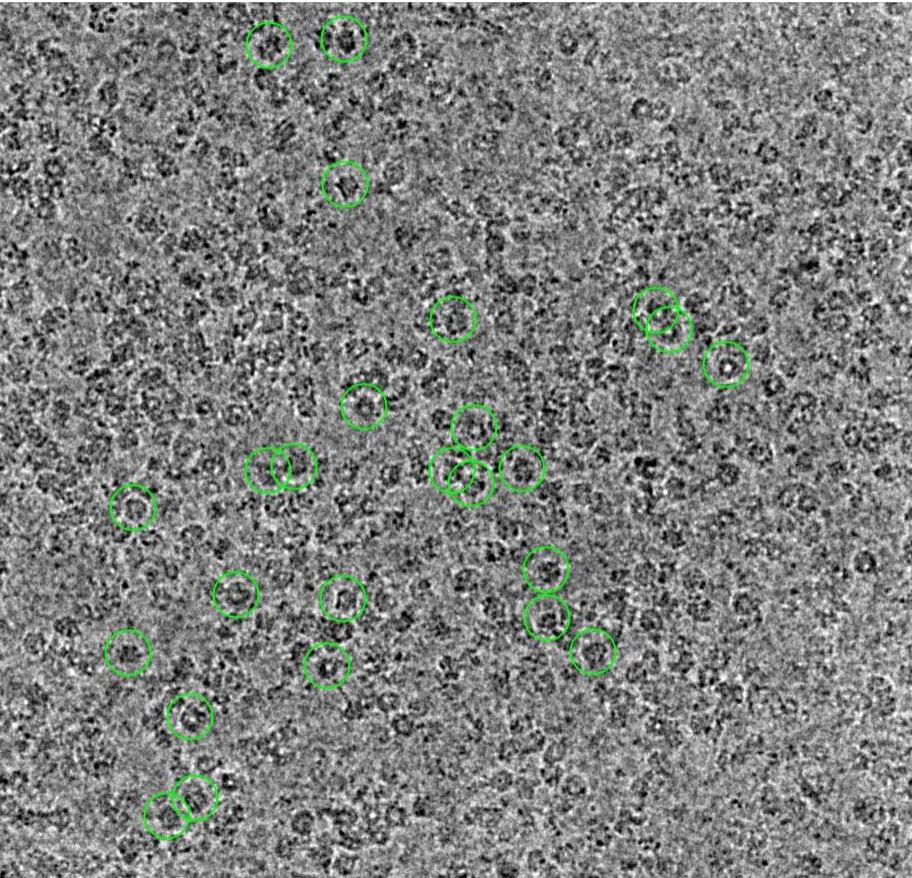
Interactive Overview Inputs and Parameters Outputs Metadata

Done Picking! Extract Particles
Lowpass filter (Å): Box size (px): Show:
180 180

Override: Contrast Intensity Override:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	25
INX6Ndelnano2 61.mrc	21545	4.16	25
INX6Ndelnano2 49.mrc	20600	3.24	25
INX6Ndelnano2 48.mrc	21179	3.14	0
INX6Ndelnano2 36.mrc	20896	3.39	0
INX6Ndelnano2 33.mrc	16594	3.10	0
INX6Ndelnano2 32.mrc	15628	3.05	0
INX6Ndelnano2 22.mrc	19811	3.23	0
INX6Ndelnano2 05.mrc	14116	3.10	0
INX6Ndelnano2 01.mrc	19871	3.23	0
INX6Ndelnano1 83.mrc	21216	3.06	0
INX6Ndelnano1 80.mrc	20991	3.04	0

Picked 75 particles.



Details Job Builder

J3 (Manual picker) WAITING

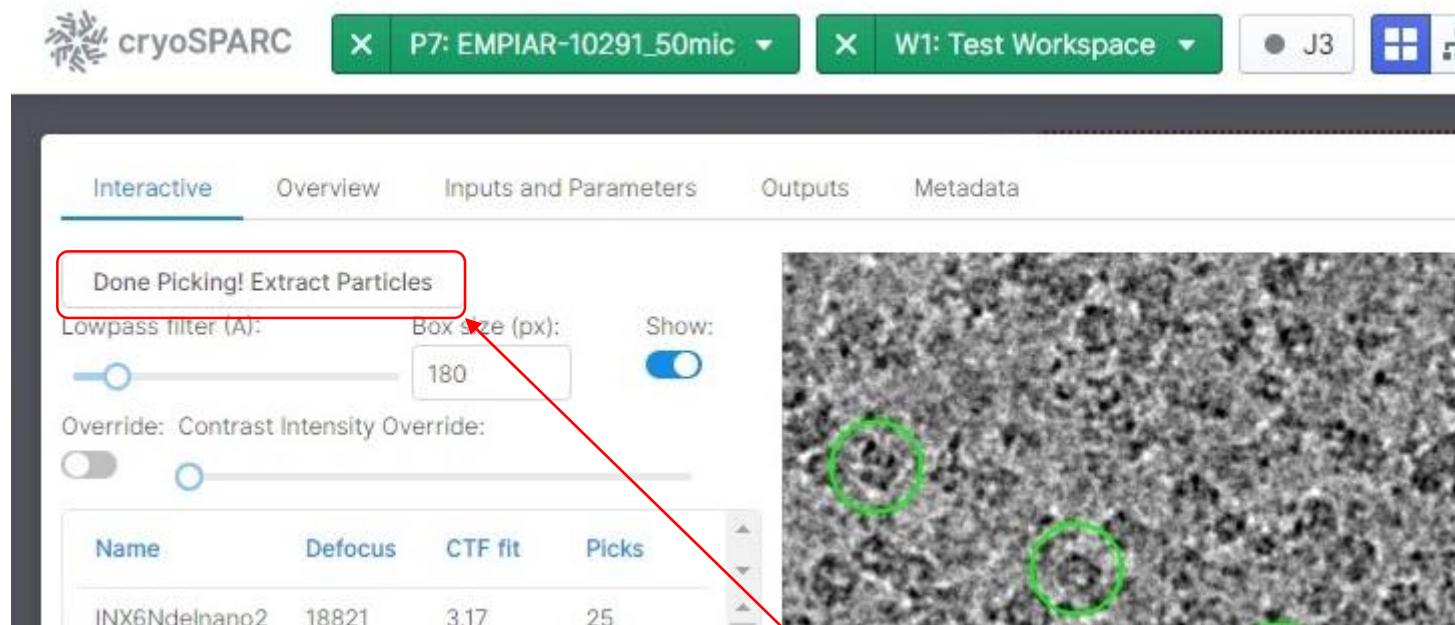
New Job J3
Enter a description.

CREATED BY guest10
INTERACTIVE Yes
CREATED Sat, Jun 19 2021 4:53:10 PM
QUEUED Sat, Jun 19 2021 5:08:41 PM
LAUNCHED Sat, Jun 19 2021 5:08:41 PM
WAITING Sat, Jun 19 2021 5:08:47 PM
STARTED Sat, Jun 19 2021 5:08:42 PM
PARENTS J2

SIZE 0 Bytes

ACTIONS
Queue Job
Kill Job
Clear Job
Clear Intermediate Results
Export Job
Clone Job
Mark Job as Complete
Delete Job

Step 7: J3:Manual Picker



(1) After finishing picking, click **[Done Picking! Extract Particles]**

Step 7: J3:Manual Picker

J3 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J3

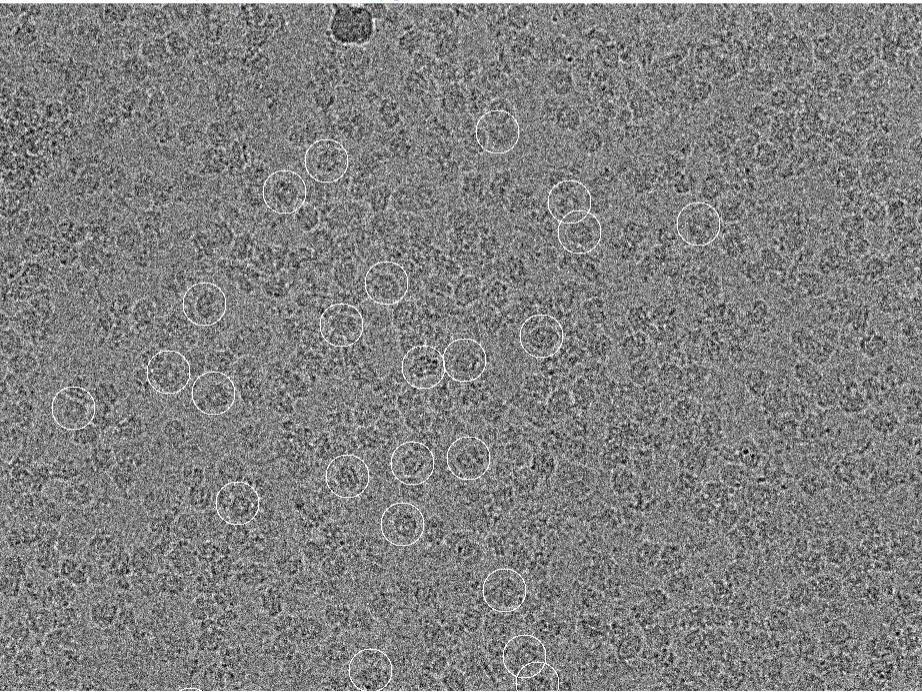
cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace x J3

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

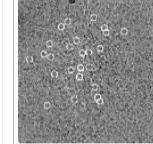
```
> [CPU: 346.2 MB] -----  
> [CPU: 346.2 MB] Processed 0 of 4 micrographs in 0.01s  
> [CPU: 346.2 MB] Extracting from J1/imported/INX6Ndelnano296.mrc : 25 particles (0 rejected near edges)  
> [CPU: 346.2 MB] Loading micrograph..  
> [CPU: 390.8 MB] Computed scale constant of 1.00000  
> [CPU: 390.8 MB] Computed overall normalizer of 4.42692  
> [CPU: 390.8 MB] Writing out particles..
```

Extracted coordinates on J1/imported/INX6Ndelnano296.mrc [png]



Outputs

micrographs



exposure Count: 4

particles



particle Count: 100

ACTIONS

- Queue Job
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

CREATED BY guest10
INTERACTIVE Yes
CREATED Sat, Jun 19 2021 4:53:10 PM
QUEUED Sat, Jun 19 2021 5:08:41 PM
LAUNCHED Sat, Jun 19 2021 5:08:41 PM
WAITING Sat, Jun 19 2021 5:08:47 PM
STARTED Sat, Jun 19 2021 5:08:42 PM
COMPLETED Sat, Jun 19 2021 5:42:00 PM
PARENTS J2
SIZE 12.42 MB

JOB DETAILS

J3 (Manual picker) COMPLETED

New Job J3
Enter a description.

Step 8: Template Picking – J4:2D classification

Details

Job Builder

(1) Choose [2D Classification] Job



Particle Curation (5)

2D Classification

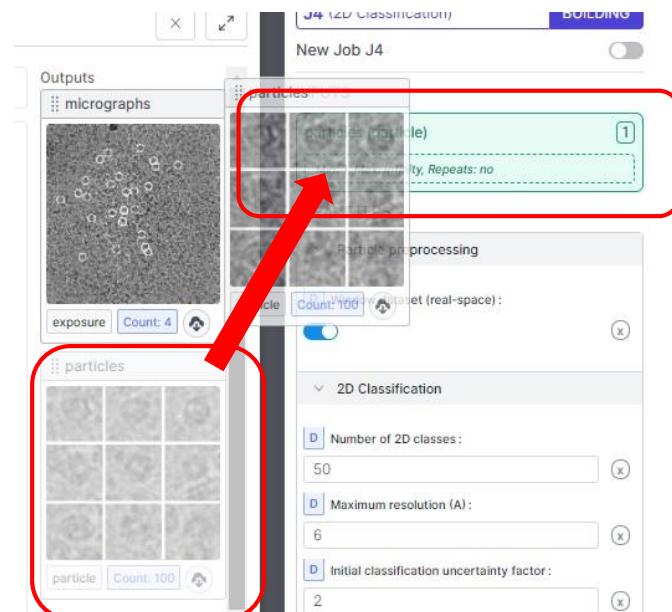
Select 2D classes

Class Probability Filter

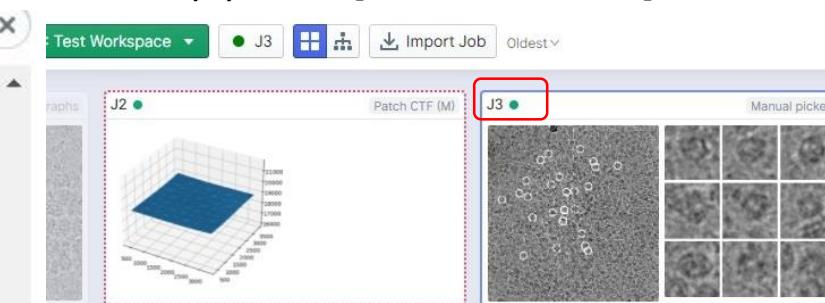
Rebalance 2D Classes (BETA)

Create Templates

(3) Drag “particles” of Manual Picker and Drop



(2) Click [Manual Picker] Job



(4) Setup parameters

INPUTS

particles (particle)

Min: 1, Max: Infinity, Repeats: no

Group 1 → J3.particles

PARAMETERS

Particle preprocessing

Window dataset (real-space):



2D Classification

Number of 2D classes:

10

Maximum resolution (A):

6

Initial classification uncertainty factor:

2

Circular mask diameter (A):

NONE

Re-center 2D classes:



Show plots from intermediate steps:



Random Seeds

Compute settings

Cache particle images on SSD:



Number of GPUs to parallelize:

1

Cancel

Create

(5) Click [Create]

Queue

Step 8: Template Picking – J4:2D classification [1min with 1GPU]

J4 (P7: W1) | cryoSPARC +

セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J4

cryoSPARC P7: EMPIAR-10291_50mic W1: Test Workspace J4

JOB DETAILS

J4 (2D Classification) COMPLETED

New Job J4

Enter a description.

CREATED BY guest1000

INTERACTIVE N

CREATED Sat, Jun 19 2021 5:51:41 PM

QUEUED Sat, Jun 19 2021 6:08:35 PM

LAUNCHED Sat, Jun 19 2021 6:08:37 PM

STARTED Sat, Jun 19 2021 6:08:37 PM

COMPLETED Sat, Jun 19 2021 6:09:40 PM

PARENTS

SIZE 13.75 MB

ACTIONS

- Queue Job on default
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types

Outputs

particles Count: 100

class_averages Count: 10

Inputs and Parameters

Outputs

Metadata

Effective number of classes per image: min 1.00 | 25-pct 1.00 | median 1.00 | 75-pct 1.00 | max 1.00

Probability of best class per image: min 1.00 | 25-pct 1.00 | median 1.00 | 75-pct 1.00 | max 1.00

2D classes for iteration 20 [png] [pdf]

Noise Model for iteration 20 [png] [pdf]

Effectiveness of assigned classes for iteration 20 [png] [pdf]

Probability of best class for iteration 20 [png] [pdf]

Dashboard Projects Resource Manager

guest1000

Step 8: Template Picking – J8:Select 2D classes

(1) Choose [Select 2D classes] Job

JOB RUII DFR

Particle Curation (5)

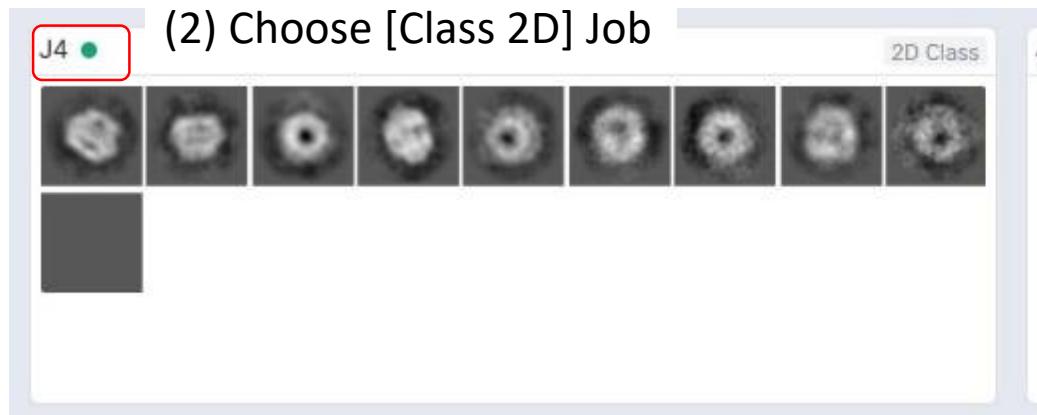
2D Classification

Select 2D classes (highlighted with a red box)

Class Probability Filter

Rebalance 2D Classes (BETA)

Create Templates



(3) Drag “particles” of Class2D and drop to “particles”

Drag “class averages” of Class2D and drop to “templates”

particles

class_averages

INPUTS

particles (particle) 0
Min: 0, Max: 1, Repeats: no

templates (template) 0
Min: 1, Max: 1, Repeats: no

General Settings

Settings

Classes where resolution better than : NONE

Classes where particle count higher than : NONE

INPUTS

particles (particle) 1
Min: 0, Max: 1, Repeats: no

Group 1 → J4.particles

templates (template) 1
Min: 1, Max: 1, Repeats: no

Group 1 → J4.class_averages

PARAMETERS

General Settings

Auto Thresholds

Classes where resolution better than : NONE

Classes where particle count higher than : NONE

Queue P7 → J5 (select_2D)

This interactive job will run on the master node

Description

Enter a description.

* Run Job in

Current Workspace (W1) (radio button selected)

New Workspace

Cancel Create (highlighted with a red box)

(4) Click [Queue]

Cancel Queue (highlighted with a red box)

(5) Click [Create]

Step 8: Template Picking – J8:Select 2D classes

(1) Click [Interactive]

The screenshot shows the top navigation bar with tabs for 'Interactive' (highlighted with a red box), 'Overview', 'Inputs and Parameters', 'Outputs', and 'Metadata'. Below the tabs are several buttons: 'Show from top', 'Select checkpoint', 'Follow latest', 'Filter Types', and 'Filter Flags'. The main area is currently empty.

(2) Select 2-4 good averages for the templates

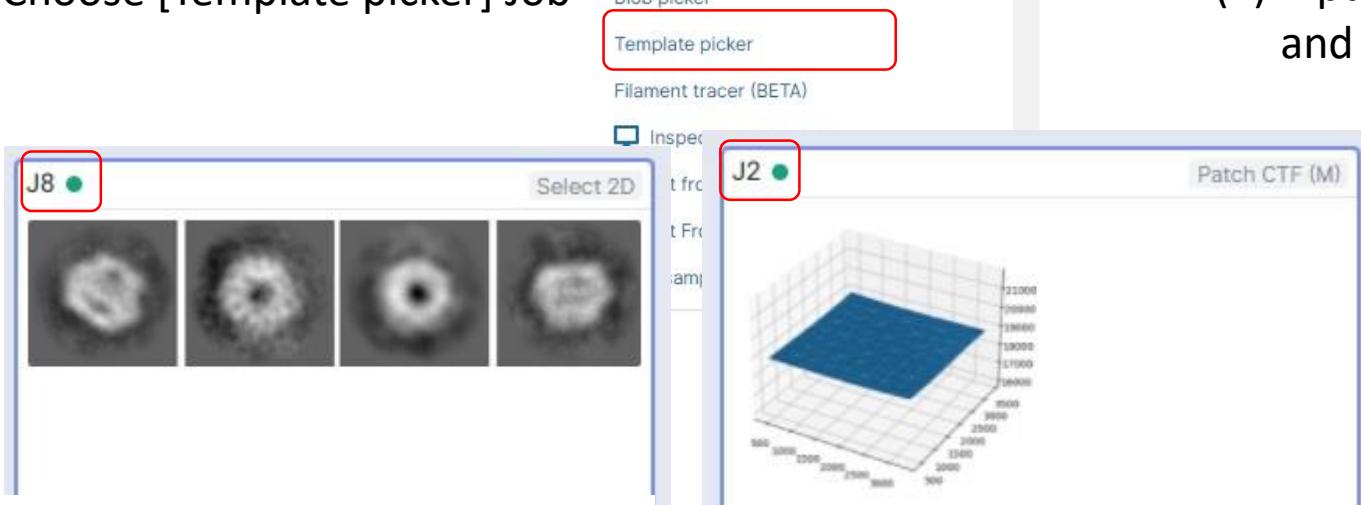
(3) Click [Done]

The screenshot shows the 'Interactive' tab selected. At the top, there are filters for '# of particles', 'Resolution', and 'ECA'. Below these are buttons for 'Select All', 'Select None', and 'Invert Selection', with status indicators 'Selected: 60' and 'Total: 100'. On the right, a green 'Done' button is highlighted with a red box. The main area displays ten 2D class averages, each with its name below it: Class 8, Class 1, Class 7, Class 4, Class 3, Class 6, Class 5, Class 9, Class 2, and Class 0. The classes are arranged in two rows of five. The first three classes are highlighted with blue selection boxes, while the others are grey. Each class entry includes its name, particle count, resolution, and ECA value.

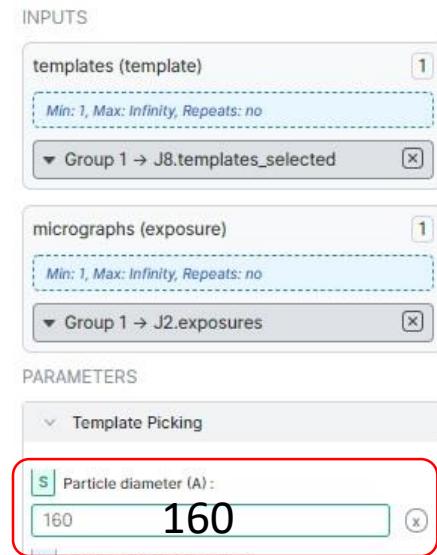
# of particles	Resolution	ECA	
19 Particles 30 Å	1.0 ECA	19 Particles 24 Å	1.0 ECA
13 Particles 35 Å	1.0 ECA	10 Particles 37 Å	1.0 ECA
10 Particles 33 Å	1.0 ECA	9 Particles 45 Å	1.0 ECA
9 Particles 89 Å	1.0 ECA	7 Particles 89 Å	1.0 ECA
4 Particles 89 Å	1.0 ECA	0 Particles 0 Å	0.0 ECA

Step 8: Template Picking – J9:Template Picker [1min]

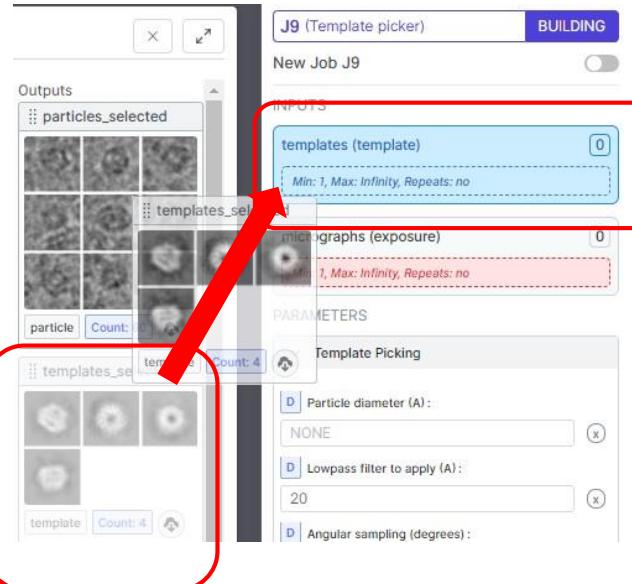
(1) Choose [Template picker] Job



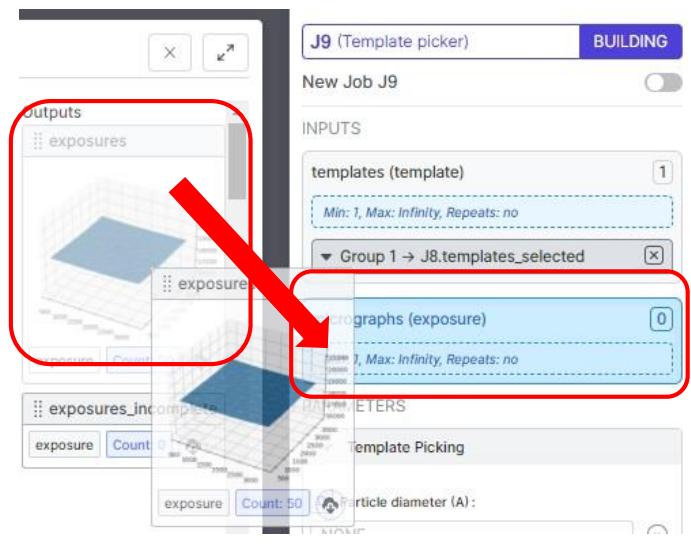
(4) Input Particle diameter and click [Queue] and [Create]



(2) Drag “templates_selected” of Select 2D and Drop to templates



(3) Drag “exposures” of Patch CTF(M) and Drop to micrographs



Cancel Queue

Step 8: Template Picking – J9:Template Picker [1min]

J9 (P7: W1) | cryoSPARC

セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J9

cryoSPARC

P7: EMPIAR-10291_50mic W1: Test Workspace J9

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

[CPU: 764.7 MB] Completed 0 of 50 : J1/imported/INX6Ndelnano010.mrc
Picked 1233 particles in 4.34s (5.04s total)

Micrograph J1/imported/INX6Ndelnano010.mrc [png]

[CPU: 801.0 MB] Completed 1 of 50 : J1/imported/INX6Ndelnano021.mrc
Picked 1239 particles in 1.07s (6.29s total)

Micrograph J1/imported/INX6Ndelnano021.mrc [png]

Outputs

particles Count: 60528

micrographs Count: 50

exposure Count: 50

WORKSPACES

Go to J9 in W2

JOBDATA

New Job J9
Enter a description.

CREATED BY guest10
LAST ACCESSED BY guest10
LAST ACCESSED AT Sat, Jun 19, 2021 8:18 PM
INTERACTIVE No
CREATED Sat, Jun 19 2021 7:42:27 PM
QUEUED Sat, Jun 19 2021 8:03:59 PM
LAUNCHED Sat, Jun 19 2021 8:04:00 PM
STARTED Sat, Jun 19 2021 8:04:01 PM
COMPLETED Sat, Jun 19 2021 8:05:07 PM
PARENTS J2, J8
SIZE 11.31 MB

ACTIONS

Queue Job on default
Move Job
Kill Job
Clear Job
Clear Intermediate Results
Export Job
Clone Job
Mark Job as Complete
Unlink Job from Workspace
Delete Job

Step 9: J10:Inspect Picks

Job Builder

JOB BUILDER

Search...

Particle Picking (8)

Manual picker

Blob picker

Template picker

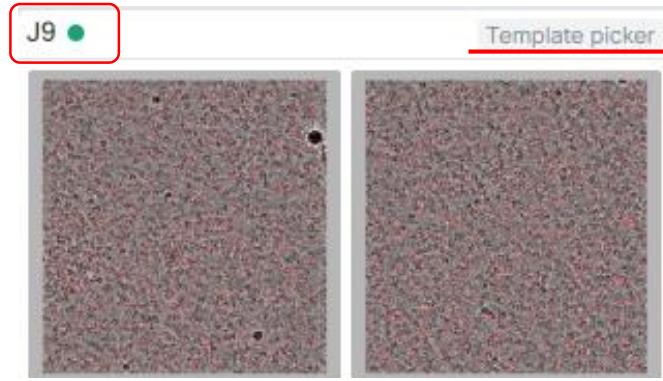
Filament tracer (BETA)

Inspect particle picks

Extract from Micrographs

Extract From Micrographs (CPU)

Downsample Particles



(2) Drag “particles” of Template picker and Drop to “particles”

J10 (Inspect particle picks) BUILDING

Inputs

particles

micrographs

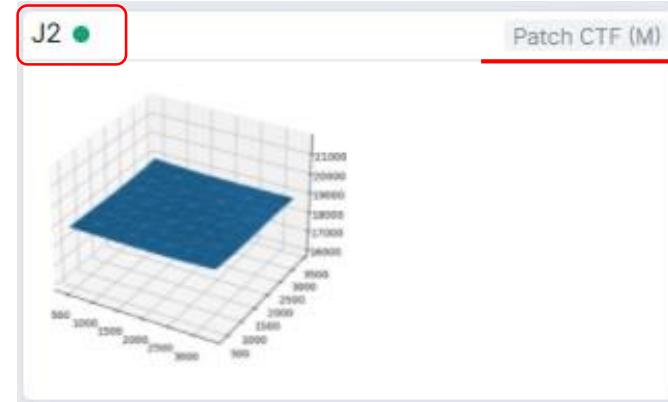
Outputs

particles (particle)

exposures (exposure)

parameters

Number of mics. to plot: 10



(3) Drag “exposures” of Patch CTF(M) and Drop to “micrographs”

J10 (Inspect particle picks) BUILDING

New Job J10

INPUTS

particles (particle)

exposures

outputs

exposures (exposure)

exposures_incomplete

exposure

outputs

(4) Click [Queue] and [Create]

Queue

Create

Step 9: J10:Inspect Picks

The screenshot shows the cryoSPARC interface for inspecting particle picks. The top navigation bar includes tabs for 'Interactive' (highlighted with a red box), 'Overview', 'Inputs and Parameters', 'Outputs', and 'Metadata'. Below the tabs are several filter and search tools: 'Show from list', 'Select checkpoint', 'Follow latest', 'Filter Types', and 'Filter Flags'. The main workspace displays a grayscale micrograph with numerous green particles outlined by a grid. A tooltip 'Box size (px): 180' points to a parameter in the control panel. The control panel also features a 'Picking! Output Locations' section with a 'Locations filter (A)' set to 10, and a 'Box size (px)' input field set to 180. Other controls include 'Use denised' and 'SHOW PICKS'. On the left, a heatmap shows Power Score vs. NCC Score. At the bottom, a table lists particle parameters: Name, Defocus, CTF fit, and Picks. The table shows four entries: INX6Ndelnano2_96.mrc (Defocus: 18821, CTF fit: 3.17, Picks: 1197), INX6Ndelnano2_61.mrc (Defocus: 21545, CTF fit: 4.16, Picks: 1220), INX6Ndelnano2_49.mrc (Defocus: 20600, CTF fit: 3.24, Picks: 1213), and INX6Ndelnano2_21179 (Defocus: 21179, CTF fit: 3.14, Picks: 1227).

Interactive Overview Inputs and Parameters Outputs Metadata

Show from list Select checkpoint Follow latest Filter Types Filter Flags

J10 (P7: W1) | cryoSPARC x +

セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J10

Low pass filter (A) = 10

Picking! Output Locations

Locations filter (A): 10

Box size (px): 180

Box size (px): 180

Power Score

NCC Score

NCC threshold: Power threshold:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2_96.mrc	18821	3.17	1197
INX6Ndelnano2_61.mrc	21545	4.16	1220
INX6Ndelnano2_49.mrc	20600	3.24	1213
INX6Ndelnano2_21179	21179	3.14	1227

Step 9: J10:Inspect Picks

The screenshot shows the cryoSPARC interface for inspecting picks. The top navigation bar includes tabs for "Interactive" (highlighted with a red box), "Overview", "Inputs and Parameters", "Outputs", and "Metadata". Below the tabs, there are buttons for "Show from top", "Select checkpoint", "Follow dataset", and "Filter Type". The main workspace displays a grayscale micrograph with numerous green circles overlaid, representing detected particles. On the left, a control panel shows a heatmap of Power Score vs. NCC Score. Key parameters visible include:

- Low pass filter (A) = 10
- Box size (px): 180
- Use denoised:
- Show Picks:
- NCC th: 0.33 (highlighted with a red box)
- NCC Score: Power threshold: (highlighted with a red box)

Below the control panel is a table of particle statistics:

Name	Defocus	CTF fit	Picks
INX6Ndelnano2 96.mrc	18821	3.17	666
INX6Ndelnano2 61.mrc	21545	4.16	585
INX6Ndelnano2 49.mrc	20600	3.24	458
INX6Ndelnano2 21179	21179	3.14	411

Annotations with arrows point to specific controls and the final output button:

- (1) Set NCC threshold: 0.33
- (2) Power threshold: 700
- (3) Click [Done Picking ! Output Locations]

On the right side of the interface, a vertical sidebar displays logs or status messages.

Step 10: J11:Extract from Micrographs

Particle Picking (8)

Manual picker

(1) Choose [Extract from Micrographs]

Template picker

Filament tracer (BETA)

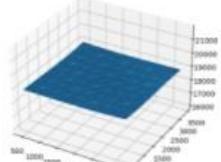
Inspect particle picks

Extract from Micrographs

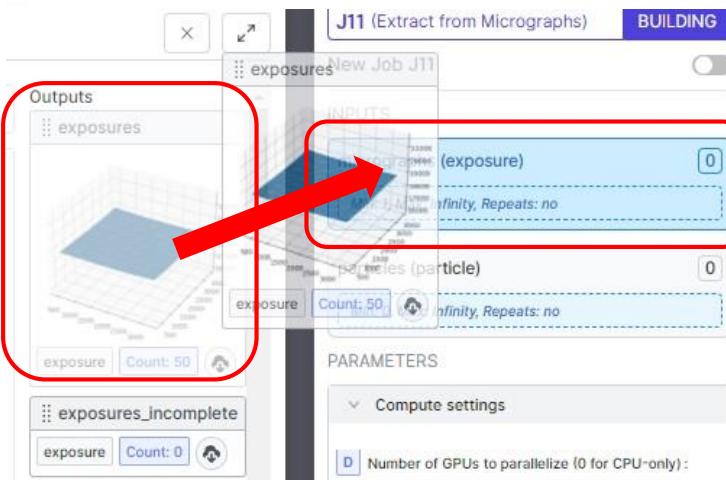
Extract From Micrographs (CPU)

J2

Patch CTF (M)



(2) Drag “exposures” of Patch CTF(M) and Drop to “micrographs”



J11 (Extract from Micrographs) BUILDING

New Job J11

INPUTS

outputs

exposures

exposure (exposure) Count: 0 Min: 0, Max: Infinity, Repeats: no

particles (particle) Count: 0 Min: 0, Max: Infinity, Repeats: no

PARAMETERS

Compute settings

D Number of GPUs to parallelize (0 for CPU-only): 1

Particle Extraction

S Extraction box size (pix): 180

D Fourier crop to box size (pix): NONE

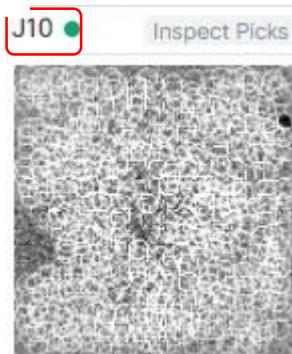
D Recenter using aligned shifts: ON

D Number of mics to extract: NONE

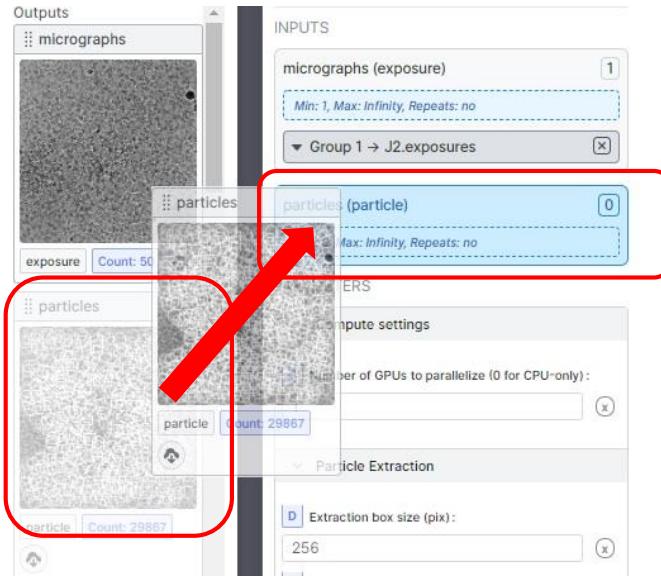
D Flip mic. in x before extract?: OFF

D Flip mic. in y before extract?: OFF

Cancel Queue Create



(3) Drag “particles” of Inspect Particles and Drop to “particles”



J10 Inspect Picks

Outputs

micrographs

exposures

exposure (exposure) Count: 50 Min: 1, Max: Infinity, Repeats: no

particles (particle) Count: 0 Min: 0, Max: Infinity, Repeats: no

Inputs

micrographs (exposure)

exposure (exposure) Count: 50 Min: 1, Max: Infinity, Repeats: no

Group 1 → J2.exposures

particles (particle)

exposure (exposure) Count: 29867 Min: 0, Max: Infinity, Repeats: no

Group 1 → J10.particles

PARAMETERS

Compute settings

D Number of GPUs to parallelize (0 for CPU-only): 1

Particle Extraction

S Extraction box size (pix): 180

D Fourier crop to box size (pix): NONE

D Recenter using aligned shifts: ON

D Number of mics to extract: NONE

D Flip mic. in x before extract?: OFF

D Flip mic. in y before extract?: OFF

Cancel Queue Create

(4) Input Extraction box size(pic) and click [Queue] and [Create]

particles (particle) 1

Min: 0, Max: Infinity, Repeats: no

Group 1 → J10.particles

PARAMETERS

Compute settings

D Number of GPUs to parallelize (0 for CPU-only): 1

Particle Extraction

S Extraction box size (pix): 180

D Fourier crop to box size (pix): NONE

D Recenter using aligned shifts: ON

D Number of mics to extract: NONE

D Flip mic. in x before extract?: OFF

D Flip mic. in y before extract?: OFF

Cancel Queue Create

Step 10: J11:Extract from Micrographs

J11 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J11

cryoSPARC x P7: EMPIAR-10291_50mc x W1: Test Workspace x J11

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types

> [CPU: 446.6 MB] -----
> [CPU: 446.6 MB] Processed 50 of 50 micrographs in 93.86s
Lowpass Filtered Images: [png] [pdf]
[CPU: 456.4 MB] (50 of 50) Finished processing micrograph 49.
[CPU: 456.4 MB] === Completed. Extracted 27680 particles in 94.85s.
[CPU: 271.5 MB] -----
[CPU: 271.5 MB] Compiling job outputs...
[CPU: 271.5 MB] Passing through outputs for output group micrographs from input group micrographs
[CPU: 271.5 MB] This job outputted results ['micrograph_blob']
[CPU: 271.5 MB] Loaded output dset with 50 items
[CPU: 271.5 MB] Passthrough results ['mscope_params', 'ctf', 'ctf_stats', 'micrograph_blob_non_dw']
[CPU: 271.5 MB] Loaded passthrough dset with 50 items
[CPU: 271.6 MB] Intersection of output and passthrough has 50 items
[CPU: 271.6 MB] Passing through outputs for output group particles from input group particles
[CPU: 275.0 MB] This job outputted results ['blob']
[CPU: 275.0 MB] Loaded output dset with 27680 items
[CPU: 275.0 MB] Passthrough results ['location', 'pick_stats', 'ctf']
[CPU: 273.8 MB] Loaded passthrough dset with 29867 items
[CPU: 278.7 MB] Intersection of output and passthrough has 27680 items
[CPU: 277.4 MB] Checking outputs for output group micrographs
[CPU: 277.4 MB] Checking outputs for output group particles
[CPU: 282.3 MB] Updating job size...
[CPU: 282.3 MB] Exporting job and creating csg files...
[CPU: 282.3 MB] *****
[CPU: 282.3 MB] Job complete. Total time 95.79s

Outputs
micrographs
exposure Count: 50
particles
particle Count: 27680

27680 particles

JOBDETAILS
J11 (Extract from Micrographs) COMPLETED
New Job J11
Enter a description.
WORKSPACES
Go to J11 in W3
CREATED BY guest10
LAST ACCESSED BY guest10
LAST ACCESSED AT Sun, Jun 20, 2021 12:11:43 PM
INTERACTIVE
CREATED Sun, Jun 20 2021 12:11:43 PM
QUEUED Sun, Jun 20 2021 12:22:13 PM
LAUNCHED Sun, Jun 20 2021 12:22:13 PM
STARTED Sun, Jun 20 2021 12:22:14 PM
COMPLETED Sun, Jun 20 2021 12:23:55 PM
PARENTS J10, J12
SIZE 3.35 GB
ACTIONS
Queue Job on default
Link Job v
Move Job v
Kill Job
Clear Job
Clear Intermediate Results
Export Job
Clone Job
Mark Job as Complete
Unlink Job from Workspace
Delete Job

You are looking at the new notification system! To clear this notification, open the Notification Manager tab in the Resource Manager and click 'clear' next to this notification. x

Dashboard Projects Resource Manager guest10

Step 11: J12:2D Classification [6.5 min with 2GPUs]

(1) Choose [2D Classification] job

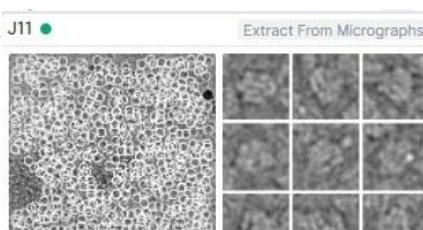
Particle Curation (5)

2D Classification

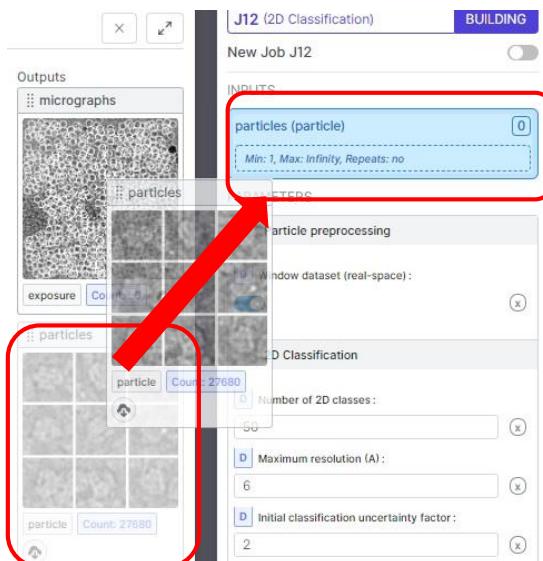
Select 2D classes

Class Probability Filter

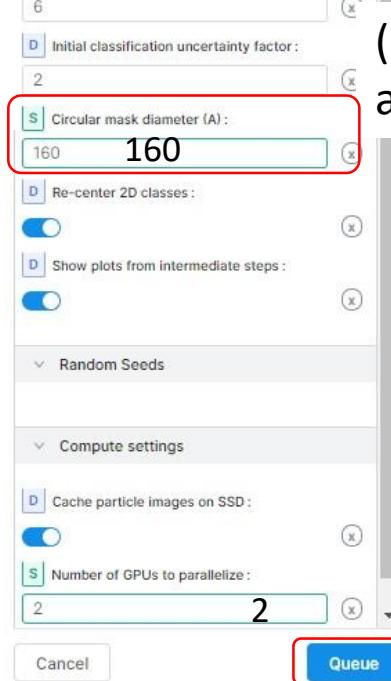
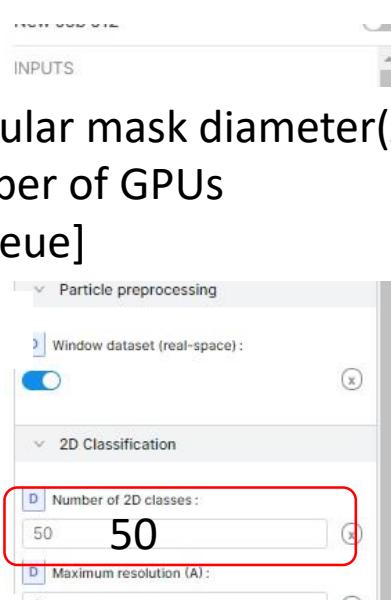
(2) Click [ExtractFromMicrographs]



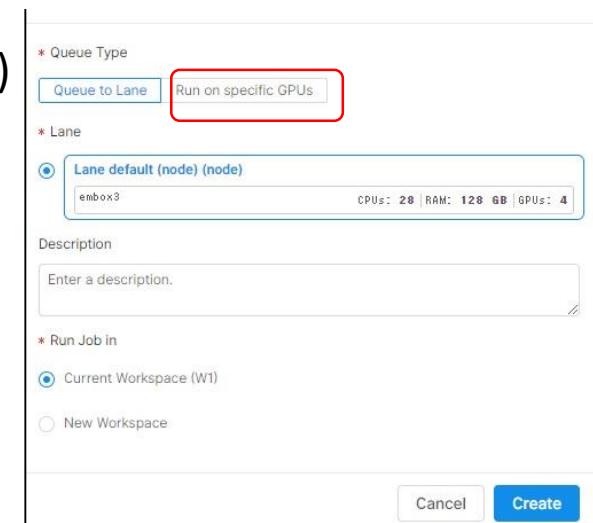
(3) Drag “particles” of [Extract From Micrograph] and Drop to “particles”



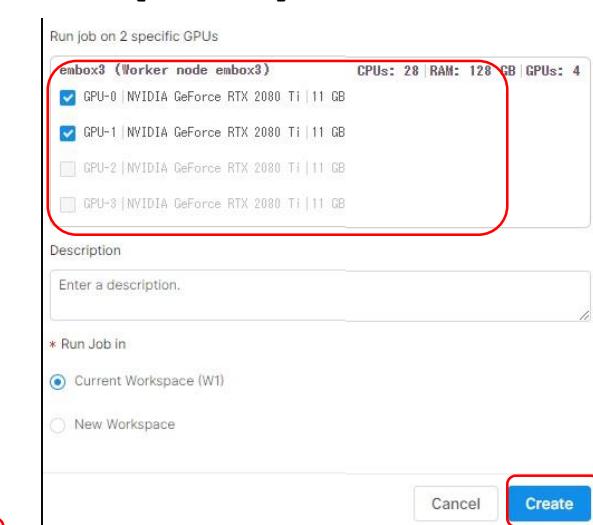
(4) Input Circular mask diameter(A)
and Number of GPUs
and click [Queue]



(5) click [Run on specific GPUs]



(6) Check two GPUs assigned for you
and click [Create]



Step 11: J12:2D Classification [6.5 min with 2GPUs]

J12 (P7: W1) | cryoSPARC +

セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J12

cryoSPARC P7: EMPIAR-10291-50mic W1: Test Workspace J12

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types Filter Flags

[CPU: 2.61 GB] Iteration 20
[CPU: 2.61 GB] — Effective number of classes per image: min 1.00 | 25-pct 1.00 | median 1.04 | 75-pct 1.65 | max 13.80
[CPU: 2.61 GB] — Probability of best class per image: min 0.06 | 25-pct 0.75 | median 0.98 | 75-pct 1.00 | max 1.00

20 classes for iteration 20 [png] [pdf]

Class	Count	Size
1	2669	7.3 Å 1.ess
2	1850	6.8 Å 1.ess
3	1532	7.4 Å 1.ess
4	1502	7.0 Å 1.ess
5	1458	6.5 Å 1.ess
6	1416	7.1 Å 1.ess
7	1384	7.3 Å 1.ess
8	1187	7.7 Å 1.ess
9	1111	7.5 Å 1.ess
10	1096	7.0 Å 1.ess
11	947	7.4 Å 1.ess
12	852	9.6 Å 1.ess
13	713	7.4 Å 1.ess
14	672	12.3 Å 1.ess
15	611	20.1 Å 1.ess
16	539	8.5 Å 1.ess
17	533	8.8 Å 1.ess
18	431	13.4 Å 2.ess
19	415	11.4 Å 2.ess
20	389	17.5 Å 2.ess
21	387	9.1 Å 1.ess
22	385	12.9 Å 2.ess
23	373	9.1 Å 2.ess
24	343	21.2 Å 2.ess
25	311	19.5 Å 2.ess
26	310	10.8 Å 2.ess
27	306	20.0 Å 2.ess
28	296	19.9 Å 2.ess
29	288	9.3 Å 2.ess
30	285	13.7 Å 2.ess
31	284	15.5 Å 2.ess
32	268	13.5 Å 2.ess
33	256	13.2 Å 1.ess
34	236	19.5 Å 1.ess
35	235	19.0 Å 1.ess
36	220	16.5 Å 1.ess
37	210	18.7 Å 2.ess
38	186	19.9 Å 1.ess
39	184	20.2 Å 2.ess
40	147	19.3 Å 1.ess
41	141	19.9 Å 2.ess
42	138	20.5 Å 1.ess
43	122	22.5 Å 2.ess
44	100	19.4 Å 1.ess
45	84	17.0 Å 1.ess
46	68	22.0 Å 2.ess
47	67	20.5 Å 2.ess
48	61	19.5 Å 2.ess
49	53	23.0 Å 1.ess
50	29	24.0 Å 2.ess

Noise Model for iteration 20 [png] [pdf]

current sigma current noise

You are looking at the new notification system! To clear this notification, open the Notification Manager tab in the Resource Manager and click 'clear' next to this notification.

Dashboard Projects Resource Manager

Details Job Builder

J12 (2D Classification) COMPLETED

New Job J12

Enter a description.

CREATED BY guest10

LAST ACCESSED BY guest10

LAST ACCESSED AT Sun, Jun 20, 2021 12:53 PM

INTERACTIVE No

CREATED Sun, Jun 20 2021 12:37:09 PM

QUEUED Sun, Jun 20 2021 12:53:18 PM

LAUNCHED Sun, Jun 20 2021 12:53:18 PM

STARTED Sun, Jun 20 2021 12:53:19 PM

COMPLETED Sun, Jun 20 2021 12:59:50 PM

PARENTS J11

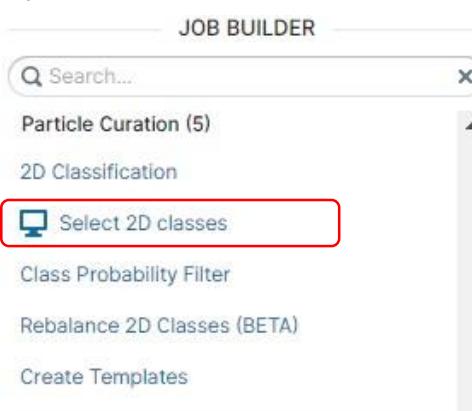
SIZE 197.02 MB

ACTIONS

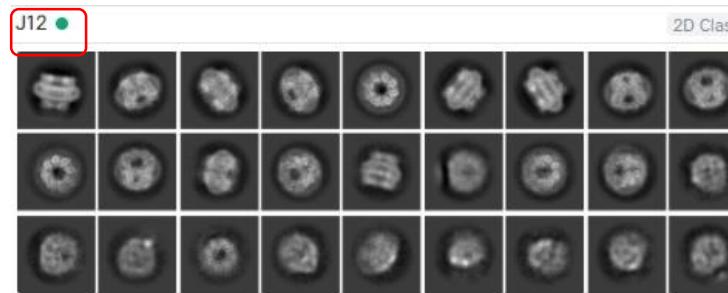
- Queue Job on default
- Link Job
- Move Job
- Kill Job
- Clear Job
- Clear Intermediate Results
- Export Job
- Clone Job
- Mark Job as Complete
- Delete Job

Step 12: J13:Select 2D classes

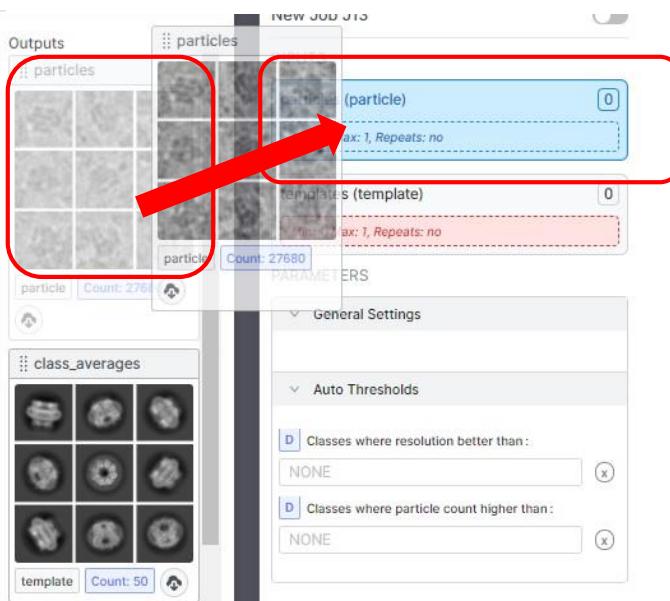
(1) Choose [Select 2D classes]



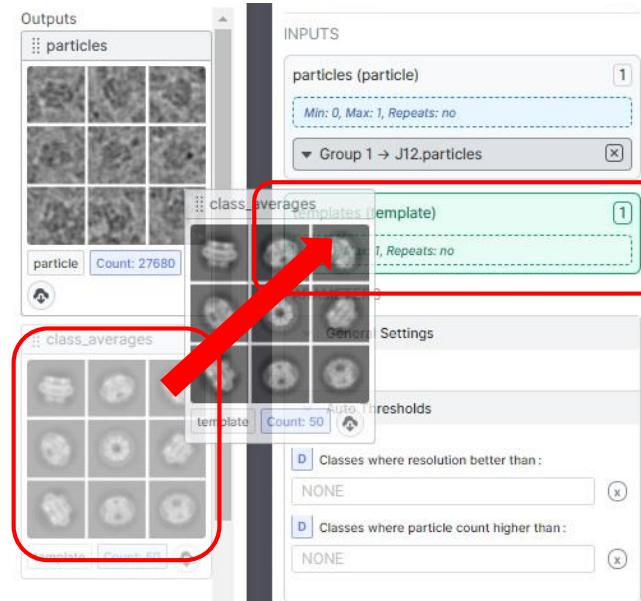
(2) Choose [2D class] job



(3) Drag “particles” of [2D Class] and Drop to “particles”



(4) Drag “class averages” of [2D Class] and Drop to “templates”



(5) Click [Queue] and [Create]

Queue

Create

Step 12: J13:Select 2D classes

cryoSPARC

X P7: EMPIAR-10291_50mic

X W1: Test Workspace

J13



Interactive

Overview

Inputs and Parameters

Outputs

Metadata

J13 (P7: W1) | cryoSPARC +

セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J13

cryoSPARC X P7: EMPIAR-10291_50mic X W1: Test Workspace J13

Interactive Overview Inputs and Parameters Outputs Metadata

of particles Resolution ECA

Select All Select None Invert Selection

Selected: 0 Total: 27680 Done

# of particles	Resolution	ECA	# of particles	Resolution	ECA	# of particles	Resolution	ECA	# of particles	Resolution	ECA
2669 Particles 7 Å 1.1 ECA	1850 Particles 7 Å 1.2 ECA	1532 Particles 7 Å 1.3 ECA	1502 Particles 7 Å 1.3 ECA	1458 Particles 6 Å 1.1 ECA	1416 Particles 7 Å 1.4 ECA	1384 Particles 7 Å 1.2 ECA	1187 Particles 8 Å 1.4 ECA	1111 Particles 7 Å 1.4 ECA	1096 Particles 7 Å 1.3 ECA	947 Particles 7 Å 1.4 ECA	
Class 1	Class 48	Class 17	Class 45	Class 0	Class 35	Class 46	Class 19	Class 49	Class 44	Class 24	
852 Particles 10 Å 1.7 ECA	713 Particles 7 Å 1.4 ECA	672 Particles 12 Å 1.6 ECA	611 Particles 20 Å 1.9 ECA	539 Particles 8 Å 1.5 ECA	533 Particles 9 Å 1.6 ECA	431 Particles 13 Å 2.1 ECA	415 Particles 11 Å 2.1 ECA	389 Particles 18 Å 2.2 ECA	387 Particles 9 Å 1.9 ECA	385 Particles 13 Å 2.2 ECA	
Class 43	Class 26	Class 20	Class 12	Class 40	Class 14	Class 38	Class 22	Class 7	Class 42	Class 5	
373 Particles 9 Å 2.0 ECA	343 Particles 21 Å 2.1 ECA	311 Particles 20 Å 2.0 ECA	310 Particles 20 Å 2.1 ECA	306 Particles 20 Å 2.2 ECA	296 Particles 20 Å 2.1 ECA	288 Particles 9 Å 2.0 ECA	285 Particles 14 Å 2.3 ECA	284 Particles 16 Å 2.1 ECA	268 Particles 13 Å 2.1 ECA	256 Particles 13 Å 2.1 ECA	
Class 18	Class 34	Class 2	Class 28	Class 15	Class 6	Class 37	Class 23	Class 33	Class 11	Class 32	
236 Particles 20 Å 1.8 ECA	235 Particles 19 Å 1.8 ECA	220 Particles 17 Å 1.8 ECA	210 Particles 19 Å 2.0 ECA	186 Particles 20 Å 2.0 ECA	184 Particles 20 Å 2.4 ECA	147 Particles 19 Å 1.8 ECA	141 Particles 20 Å 2.5 ECA	138 Particles 21 Å 2.1 ECA	122 Particles 22 Å 2.2 ECA	100 Particles 19 Å 1.7 ECA	
Class 10	Class 4	Class 3	Class 31	Class 39	Class 41	Class 36	Class 21	Class 9	Class 29	Class 27	
84 Particles 17 Å 1.8 ECA	68 Particles 22 Å 2.6 ECA	67 Particles 21 Å 2.2 ECA	61 Particles 20 Å 3.0 ECA	53 Particles 23 Å 2.0 ECA	29 Particles 25 Å 1.9 ECA						

JOB DETAILS

J13 (Select 2D classes) WAITING

New Job J13
Enter a description.

CREATED BY guest10
INTERACTIVE Yes
CREATED Sun, Jun 20 2021 2:12:28 PM
QUEUED Sun, Jun 20 2021 2:21:18 PM
LAUNCHED Sun, Jun 20 2021 2:21:19 PM
WAITING Sun, Jun 20 2021 2:21:23 PM
STARTED Sun, Jun 20 2021 2:21:23 PM
PARENTS J12
SIZE 0 Bytes
ACTIONS Queue Job
Link Job
Move Job
Kill Job
Clear Job
Clear Intermediate Results
Export Job
Clone Job
Mark Job as Complete
Delete Job

Step 12: J13:Select 2D classes

cryoSPARC

X P7: EMPIAR-10291_50mic

X W1: Test Workspace

J13



Interactive

Overview

Inputs and Parameters

Outputs

Metadata

(1) Select “good” classes, considering Nparticles and resolution.

Interactive Overview Inputs and Parameters Outputs Metadata

of particles Resolution ECA

Select All Select None Invert Selection

Selected: 16152 Total: 27680

# of particles	Resolution	ECA								
2669 Particles 7 Å 1.1 ECA	1850 Particles 7 Å 1.2 ECA	1532 Particles 7 Å 1.3 ECA	1502 Particles 7 Å 1.3 ECA	1458 Particles 6 Å 1.1 ECA	1416 Particles 7 Å 1.4 ECA	1384 Particles 7 Å 1.2 ECA	1187 Particles 8 Å 1.4 ECA	1111 Particles 7 Å 1.4 ECA	1096 Particles 7 Å 1.3 ECA	947 Particles 7 Å 1.4 ECA
Class 1	Class 48	Class 17	Class 45	Class 0	Class 35	Class 46	Class 19	Class 49	Class 44	Class 24
852 Particles 10 Å 1.7 ECA	713 Particles 7 Å 1.4 ECA	672 Particles 12 Å 1.6 ECA	611 Particles 20 Å 1.9 ECA	539 Particles 8 Å 1.5 ECA	533 Particles 9 Å 1.6 ECA	431 Particles 13 Å 2.1 ECA	415 Particles 11 Å 2.1 ECA	389 Particles 18 Å 2.2 ECA	387 Particles 9 Å 1.9 ECA	385 Particles 13 Å 2.2 ECA
Class 43	Class 26	Class 20	Class 12	Class 40	Class 14	Class 38	Class 22	Class 7	Class 42	Class 5
373 Particles 9 Å 2.0 ECA	343 Particles 21 Å 2.1 ECA	311 Particles 20 Å 2.0 ECA	310 Particles 20 Å 2.1 ECA	306 Particles 20 Å 2.2 ECA	296 Particles 20 Å 2.1 ECA	288 Particles 9 Å 2.0 ECA	285 Particles 14 Å 2.3 ECA	284 Particles 16 Å 2.1 ECA	268 Particles 13 Å 2.1 ECA	256 Particles 13 Å 2.1 ECA
Class 18	Class 34	Class 2	Class 28	Class 15	Class 6	Class 37	Class 23	Class 33	Class 11	Class 32
236 Particles 20 Å 1.8 ECA	235 Particles 19 Å 1.8 ECA	220 Particles 17 Å 1.8 ECA	210 Particles 19 Å 2.0 ECA	186 Particles 20 Å 2.0 ECA	184 Particles 20 Å 2.4 ECA	147 Particles 19 Å 1.8 ECA	141 Particles 20 Å 2.5 ECA	138 Particles 21 Å 2.1 ECA	122 Particles 22 Å 2.2 ECA	100 Particles 19 Å 1.7 ECA
Class 10	Class 4	Class 3	Class 31	Class 39	Class 41	Class 36	Class 21	Class 9	Class 29	Class 27
84 Particles 17 Å 1.8 ECA	68 Particles 22 Å 2.6 ECA	67 Particles 21 Å 2.2 ECA	61 Particles 20 Å 3.0 ECA	53 Particles 23 Å 2.0 ECA	29 Particles 25 Å 1.9 ECA					

In this case, I selected 11 classes.
It means 16152 particles among 27680 particles.

Details Job Builder

JOB DETAILS

J13 (Select 2D classes) WAITING

New Job J13

Enter a description.

CREATED BY guest10

INTERACTIVE Yes

CREATED Sun, Jun 20 2021 2:12:28 PM

QUEUED Sun, Jun 20 2021 2:21:18 PM

LAUNCHED Sun, Jun 20 2021 2:21:19 PM

WAITING Sun, Jun 20 2021 2:21:23 PM

STARTED Sun, Jun 20 2021 2:21:19 PM

PARENTS J12

SIZE 0 Bytes

Done

(2) Click [Done]

Link Job Move Job

Kill Job Clear Job

Clear Intermediate Results Export Job

Clone Job Mark Job as Complete

Delete Job

Step 13: J14:Ab-initio Reconstruction

JOB BUILDER

(1) Choose [Ab-initio Reconstruction]

Ab-Initio Reconstruction

3D Refinement (6)

Homogeneous Refinement (Legacy)

Homogeneous Refinement

Heterogeneous Refinement

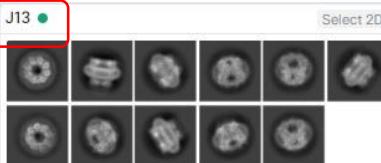
Non-uniform Refinement (Legacy)

Non-uniform Refinement (NEW!)

Homogeneous Reconstruction Only

(2) Choose [Select 2D] job

J13



(3) Drag “particles_selected” of [Select 2D] and Drop to “particles”

J14 (Ab-Initio Reconstruction) BUILDING
New Job J14

Outputs
particles_selected
particle (particle)
Count: 16152
tax: Infinity, Repeats: no

particle
Count: 16152

particle preprocessing
Window dataset (real-space):

Ab-Initio reconstruction
Number of Ab-Initio classes: 1
Num particles to use: NONE
Maximum resolution (Angstroms): 12
Initial resolution (Angstroms): 35
Class similarity: 0.1
Show plots from intermediate steps:

Random Seeds
Compute settings

templates_selected
Count: 11

particles_excluded

J14 (Ab-Initio Reconstruction)

New Job J14

INDI ITC

particle
Min: 1, Max: Infinity, Repeats: no

Group 1 → J13.particles_selected

PARAMETERS

Particle preprocessing

Window dataset (real-space):

Ab-Initio reconstruction

Number of Ab-Initio classes: 1

Num particles to use: NONE

Maximum resolution (Angstroms): 12

Initial resolution (Angstroms): 35

Class similarity: 0.1

Show plots from intermediate steps:

Random Seeds

Compute settings

(5) click [Run on specific GPUs]

Queue to Lane Run on specific GPU

* Lane

Lane default (node) (node)

embox3

CPUs: 28 | RAM: 128 GB | GPUs: 4

Description

Enter a description.

* Run Job in

Current Workspace (W1)

New Workspace

Queue P7 → J14 (homo_abinit)

(6) Check one GPU assigned for you and click [Create]

embox3 (Worker node embox3) CPUs: 28 | RAM: 128 GB | GPUs: 4

GPU-0 | NVIDIA GeForce RTX 2080 Ti | 11 GB

GPU-1 | NVIDIA GeForce RTX 2080 Ti | 11 GB

GPU-2 | NVIDIA GeForce RTX 2080 Ti | 11 GB

GPU-3 | NVIDIA GeForce RTX 2080 Ti | 11 GB

Description

Enter a description.

* Run Job in

Current Workspace (W1)

New Workspace

(4) Click [Queue]

Cancel

Queue

Cancel

Create

Step 13: J14:Ab-initio Reconstruction [5.5 min with 1GPU]

J14 (P7: W1) | cryoSPARC

セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J14

cryoSPARC P7: EMPIAR-10291_50mic W1: Test Workspace J14

(1) select [Outputs] tab

Overview Inputs and Parameters Outputs Metadata

Show from top Select checkpoint Follow latest Filter Types

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

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

Noise Model Iteration 710 [png] [pdf]

You are looking at the new notification system! To clear this notification, open the Notification Manager tab in the Resource Manager and click 'clear' next to this notification.

Details Job Builder

J14 (Ab-Initio Reconstruction) COMPLETED

New Job J14

Enter a description.

CREATED BY guest
LAST ACCESSED BY guest
LAST ACCESSED AT Sun, Jun 20, 2021 2:50:00 PM
INTERACTIVE
CREATED Sun, Jun 20 2021 2:40:19 PM
QUEUED Sun, Jun 20 2021 2:51:04 PM
LAUNCHED Sun, Jun 20 2021 2:51:05 PM
STARTED Sun, Jun 20 2021 2:51:43 PM
COMPLETED Sun, Jun 20 2021 2:56:43 PM
PARENTS
SIZE 83.89
ACTIONS Queue Job on default
Link Job Move Job
Kill Job Clear Job
Clear Intermediate Results
Export Job Clone Job
Mark Job as Complete
Delete Job

Dashboard Projects Resource Manager guest10

Step 13: J14:Ab-initio Reconstruction [5.5 min with 1GPU]

J14 (P7: W1) | cryoSPARC x + セキュリティ保護なし | 192.168.39.72:39000/projects/P7/W1/J14

cryoSPARC x P7: EMPIAR-10291_50mic x W1: Test Workspace J14

Overview Inputs and Parameters Outputs Metadata

No Image Available

GROUP TYPE particle
TOTAL ITEMS 16152
ACTIONS Export

blob J14.particles_all_classes.blob
VERSIONS alignments_class_0 J14.particles_all_classes.alignments_class_0
VERSIONS location (passthrough) J14.particles_all_classes.location

ctf J14.particles_all_classes.ctf
VERSIONS alignments2D (passthrough) J14.particles_all_classes.alignments2D
alignments2D (passthrough) J14.particles_all_classes.alignments2D

pick_stats (passthrough) J14.particles_all_classes.pick_stats
particle.pick_stats

particles_class_0

No Image Available

GROUP TYPE particle
TOTAL ITEMS 16152
ACTIONS Export

blob J14.particles_class_0.blob
VERSIONS alignments3D J14.particles_class_0.alignments3D
VERSIONS location (passthrough) J14.particles_class_0.location

ctf J14.particles_class_0.ctf
VERSIONS alignments2D (passthrough) J14.particles_class_0.alignments2D
alignments2D (passthrough) J14.particles_class_0.alignments2D

pick_stats (passthrough) J14.particles_class_0.pick_stats
particle.pick_stats

volume_class_0

map J14.volume_class_0.map
VERSIONS

Download output

(1) Download “J*.volume_class_0.map”

Details Job Builder

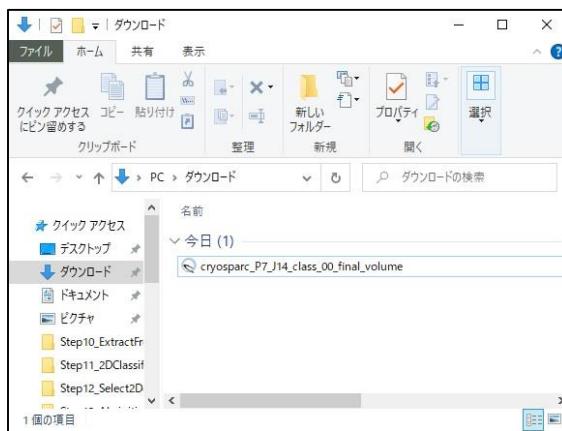
J14 (Ab-Initio Reconstruction) COMPLETE

New Job J14
Enter a description.

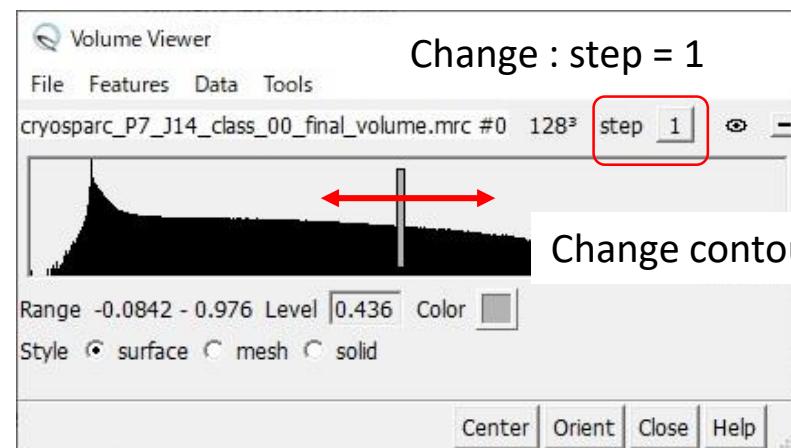
CREATED BY gue
LAST ACCESSED BY gue
LAST ACCESSED AT Sun, Jun 20, 2021 3:07 AM
INTERACTIVE
CREATED Sun, Jun 20 2021 2:40:19
QUEUED Sun, Jun 20 2021 2:51:04
LAUNCHED Sun, Jun 20 2021 2:51:04
STARTED Sun, Jun 20 2021 2:51:05
COMPLETED Sun, Jun 20 2021 2:56:43
PARENTS
SIZE 83.89
ACTIONS Queue Job on default
Link Job
Move Job
Kill Job
Clear Job
Clear Intermediate Results
Export Job
Clone Job
Mark Job as Complete
Delete Job

Step 13: J14:Ab-initio Reconstruction [5.5 min with 1GPU]

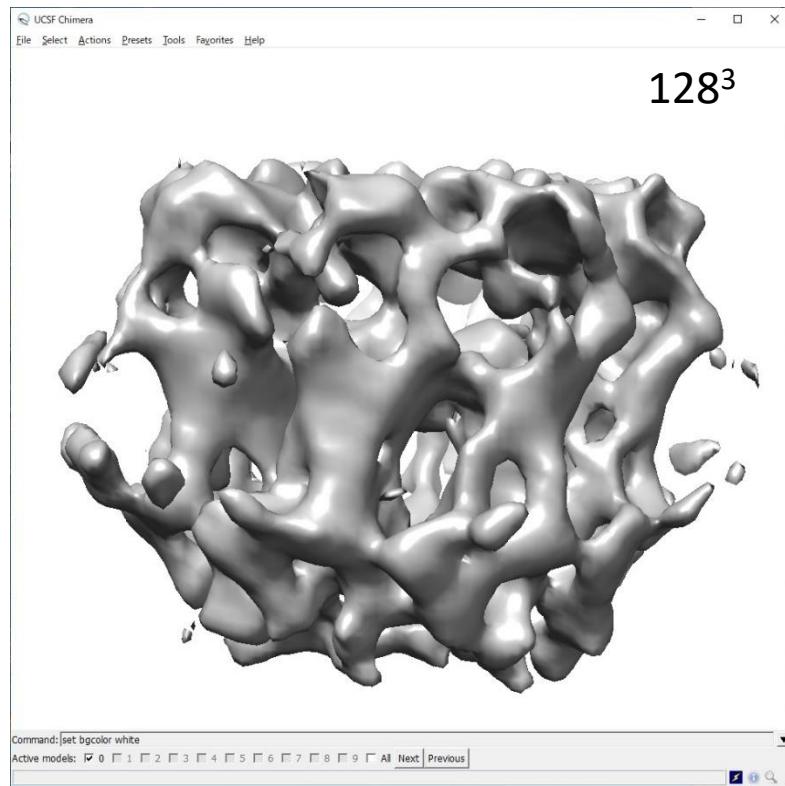
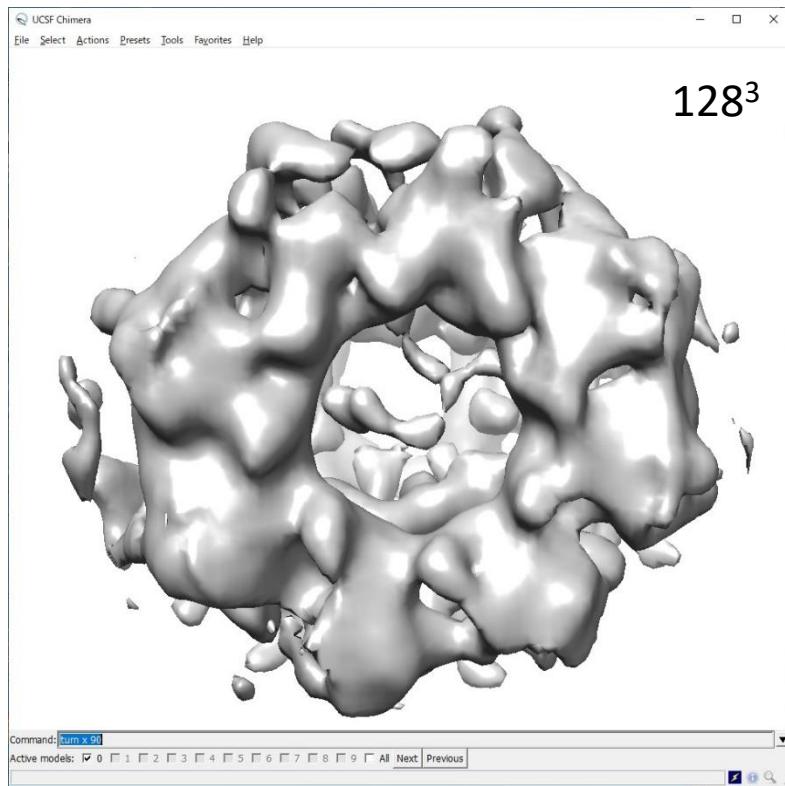
[Tools]->[Volume Data]->[Volume Viewer]



[File]->[Open...]



Change contour Level

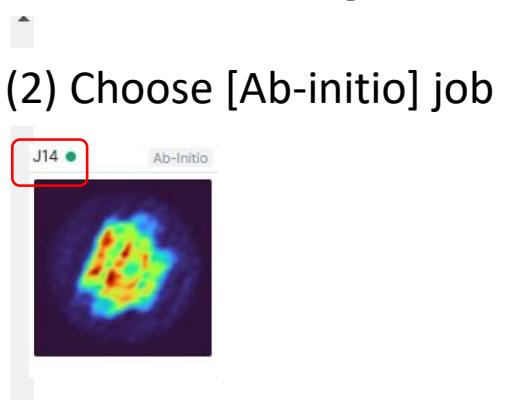


Step 14: J15:Homogeneous Refinement

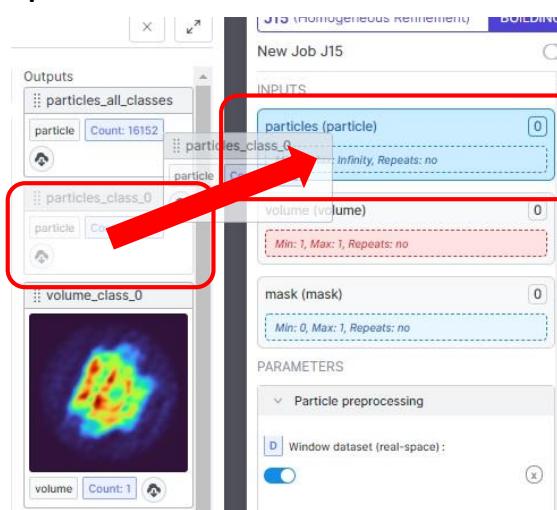
JOB BUILDER

(1) Choose [Homogeneous Refinement]

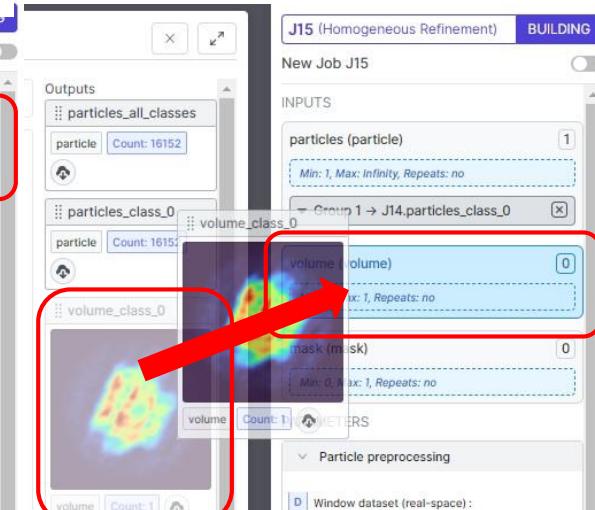
The Job Builder interface shows the '3D Refinement' section. Under 'Homogeneous Refinement', 'Homogeneous Refinement' is selected and highlighted with a red box. Other options like 'Heterogeneous Refinement' and 'Non-uniform Refinement (Legacy)' are also listed.



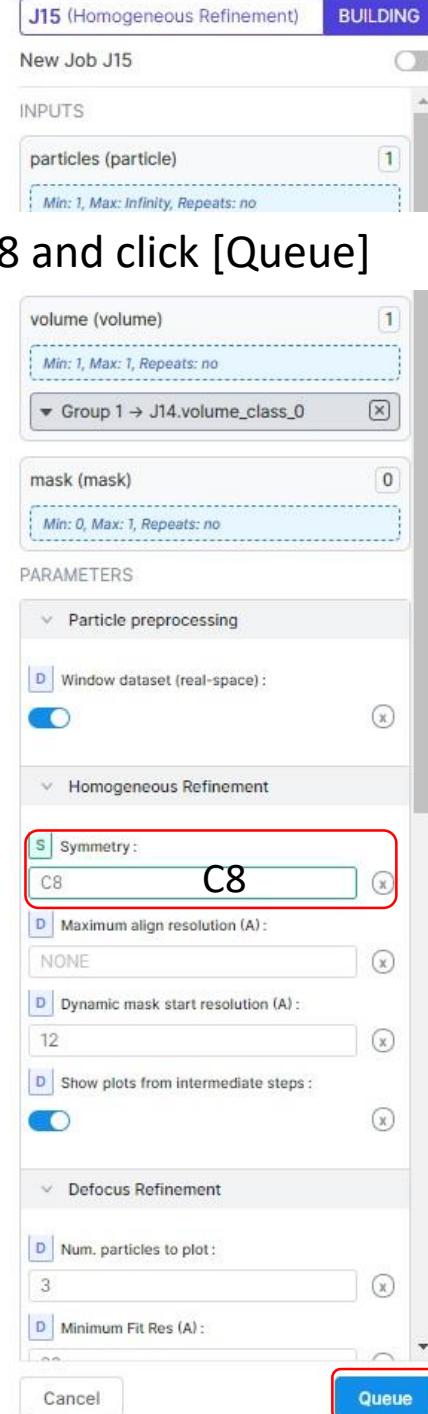
(3) Drag “particles_class0” of [Ab-initio] and Drop to “particles”



(4) Drag “volume_class0” of [Ab-initio] and Drop to “volume”



(5) Input Symmetry:C8 and click [Queue]



Step 14: J15:Homogeneous Refinement [5.5 min with 1GPU]

(1) click [Run on specific GPUs]

Queue P7 → J15 (homo_refine_new)

* Queue Type

Queue to Lane Run on specific GPU

* Lane

Lane default (node) (node)
embox3 CPUs: 28 | RAM: 128 GB | GPUs: 4

Description

Enter a description.

* Run Job in

Current Workspace (W1) New Workspace

(2) Check one GPU assigned for you and click [Create]

Queue P7 → J15 (homo_refine_new)

* Queue Type

Queue to Lane Run on specific GPU

Run job on a specific GPU

embox3 (Worker node embox3) CPUs: 28 | RAM: 128 GB | GPUs: 4

GPU-0 | NVIDIA GeForce RTX 2080 Ti | 11 GB
 GPU-1 | NVIDIA GeForce RTX 2080 Ti | 11 GB.
 GPU-2 | NVIDIA GeForce RTX 2080 Ti | 11 GB.
 GPU-3 | NVIDIA GeForce RTX 2080 Ti | 11 GB.

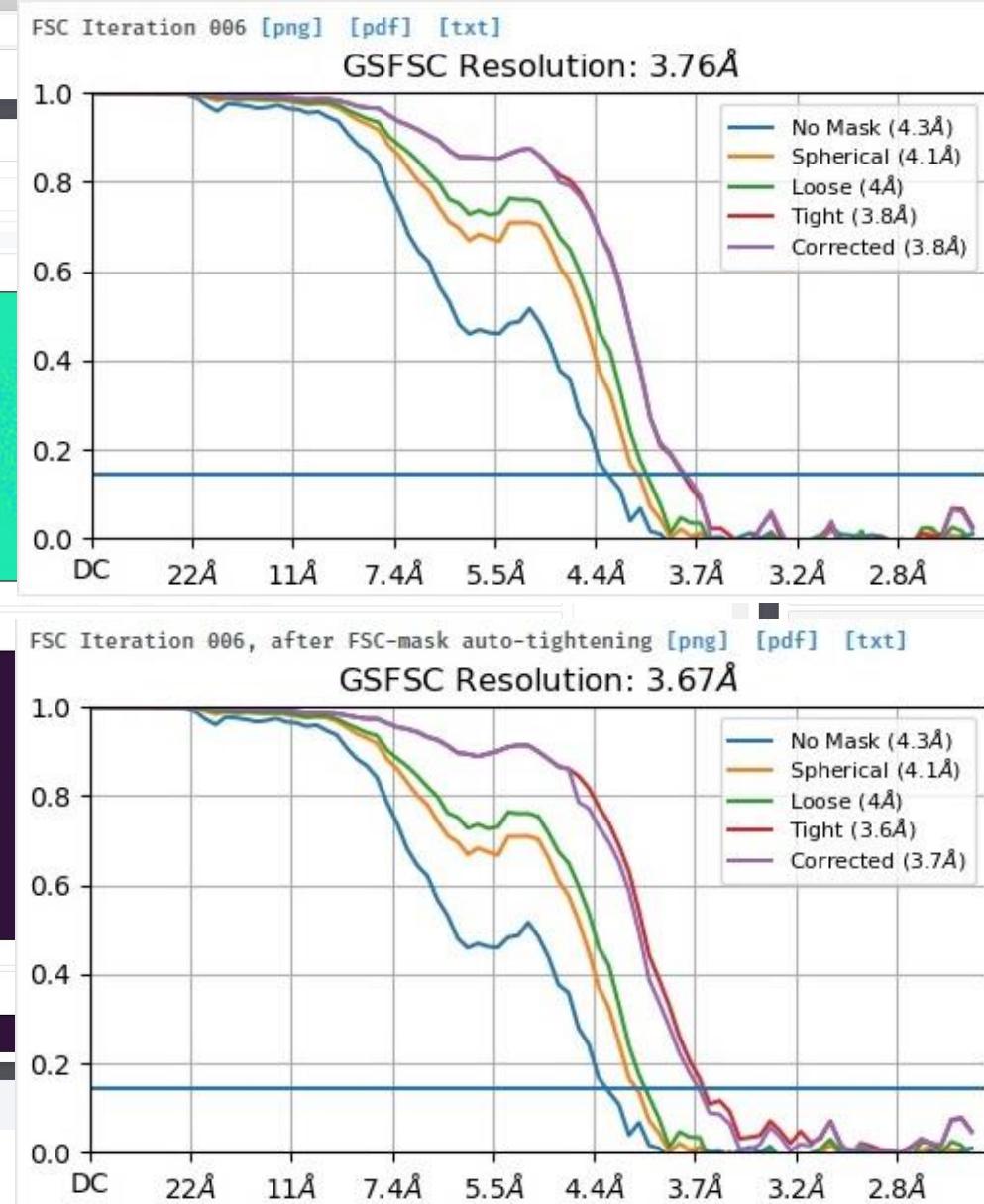
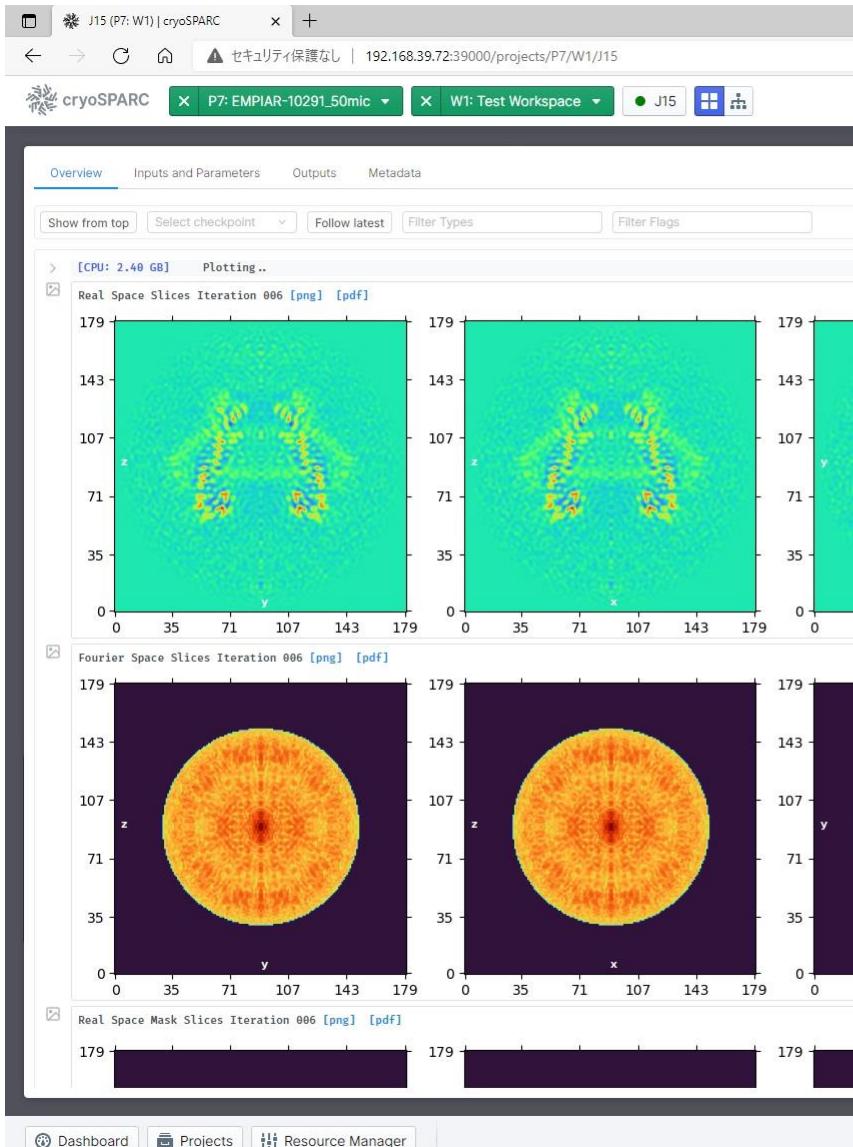
Description

Enter a description.

* Run Job in

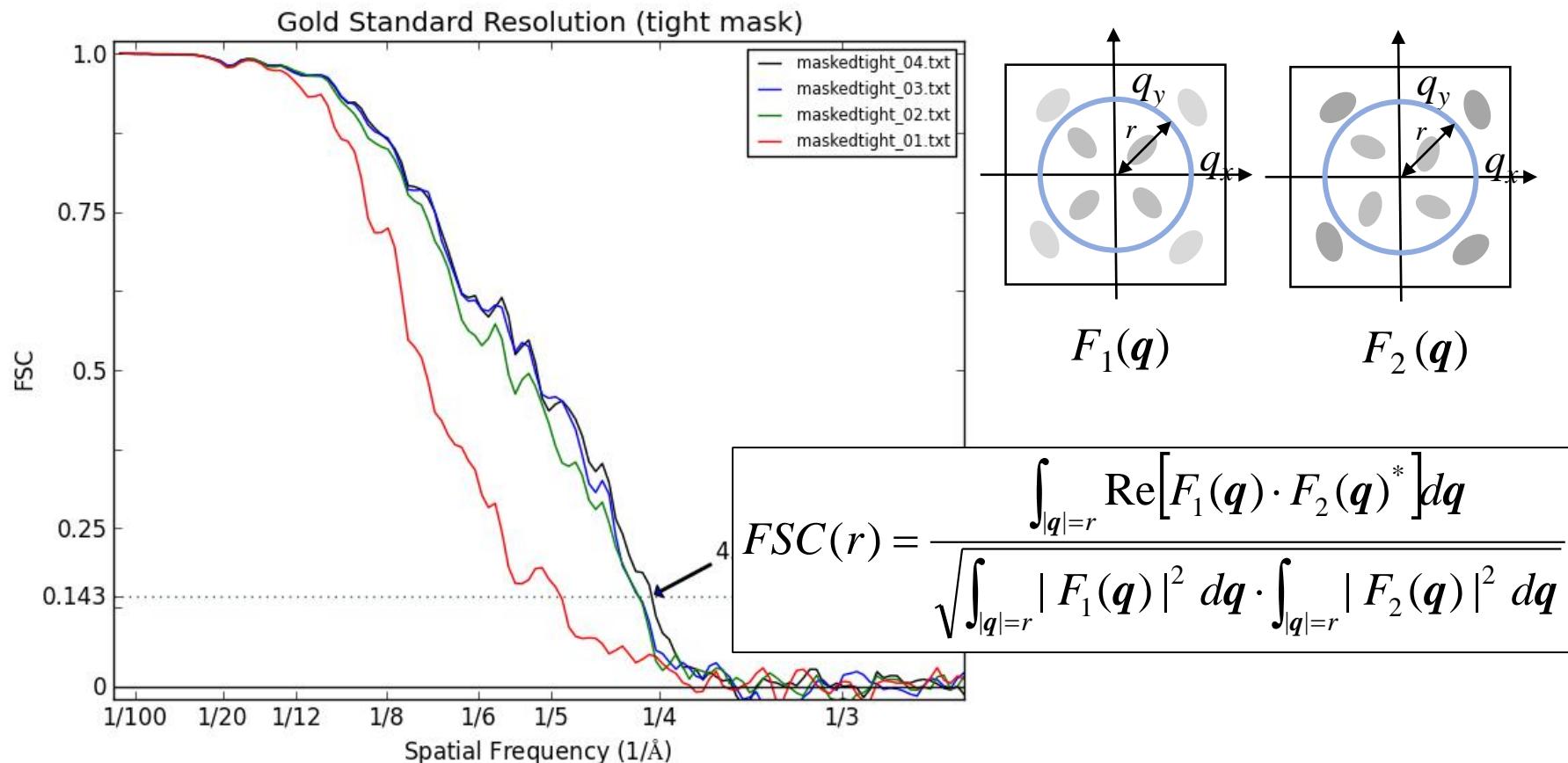
Current Workspace (W1) New Workspace

Step 14: J15:Homogeneous Refinement [5.5 min with 1GPU]



Resolution from Fourier Shell Correlation (FSC)

- 1) Divide 2D particle images into two sets, and reconstruct two 3D maps $f_1(x)$ and $f_2(x)$. Their Fourier transferred maps are called $F_1(\mathbf{q})$ and $F_2(\mathbf{q})$.
- 2) Calculate correlation $FSC(r)$ on a spherical shell with spatial frequency $r=|\mathbf{q}|$.
- 3) Plot $FSC(r)$ versus spatial frequency r . Resolution is defined spatial frequency with $FSC(r) = 0.143$ ($=1/7$).



FSC curves in cryoSPARC

Apunjani cryoSPARC Team
Mar '17

<https://discuss.cryosparc.com/t/tight-corrected-and-loose-gsfsc-curves/201/4>

Here is a short explanation of the different FSC curves in cryoSPARC:

#FSC calculations in CryoSPARC

##No Mask:

This is the raw FSC calculated between two independent half-maps reconstructed from the data. There is no masking applied, so both the structure and solvent are included in this FSC.

##Spherical:

This is the FSC calculated after applying a soft spherical mask to both half maps. The outer radius of the soft sphere is equal to half the volume box-size (i.e. the sphere extends to the faces of the box in all directions). The inner radius is 85 percent of the outer radius. Between inner and outer radii, a soft cosine edge transitions from a mask value of one to a value of zero.

##Loose:

This is the FSC calculated after applying a soft solvent mask to both half maps. The loose mask is calculated as follows. First, the density map is thresholded at 50% of the maximum density value. The resulting volume is dilated to create a soft mask. Voxels in the mask that are within 25 angstroms of the thresholded region receive a mask value of 1.0. Voxels between 25 and 40 angstroms fall off with a soft cosine edge, and voxels outside 40 angstroms receive a value of 0.0.

##Tight:

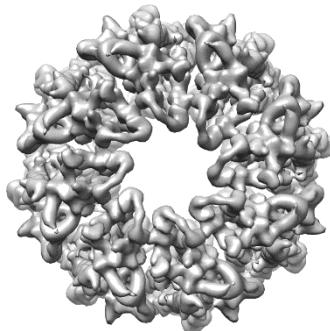
This is the same as the loose mask, except the dilation distances are 6 angstroms for the value 1.0 distance and 12 angstroms for the value 0.0 distance.

##Corrected:

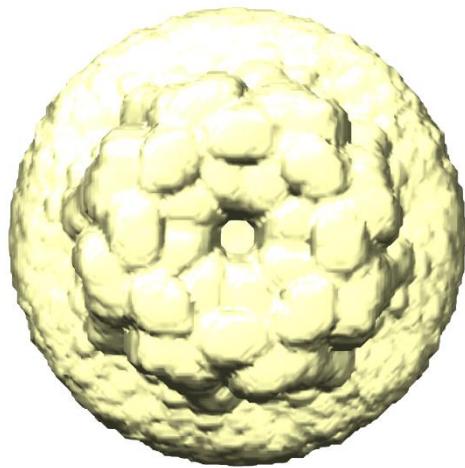
This is the FSC curve calculated using the tight mask with correction by noise substitution [1]. The two half maps have their phases randomized beyond a certain resolution, then the tight mask is applied to both, and an FSC is calculated. This FSC is used along with the original FSC before phase randomization to compute the corrected FSC as in [1]. This accounts for correlation effects induced by masking. The resolution at which phase randomization begins is the resolution at which the no-mask FSC drops below the FSC = 0.143 criterion.

1.Chen, S. et al. High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy. Ultramicroscopy 135, 24–35 (2013).

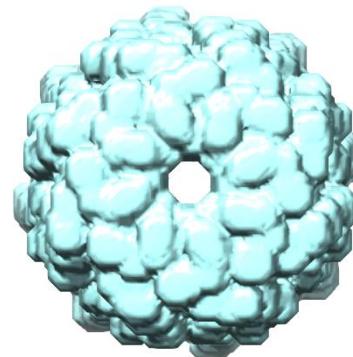
Mask maps



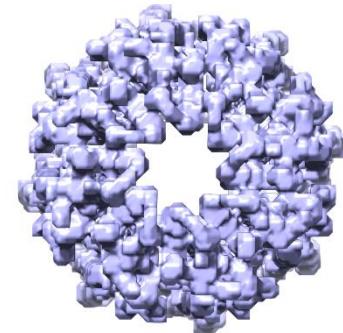
volume_map



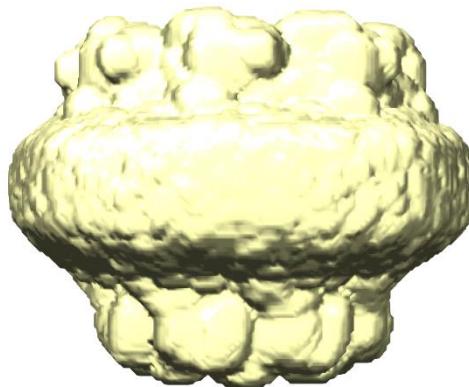
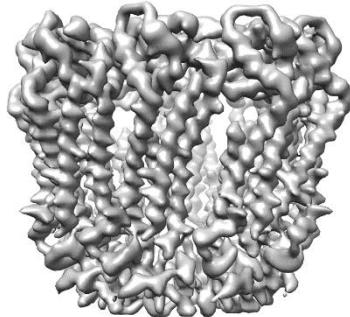
volume_mask_refine



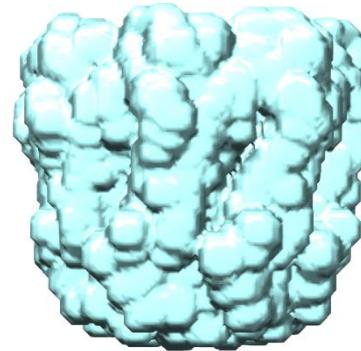
volume_mask_fsc



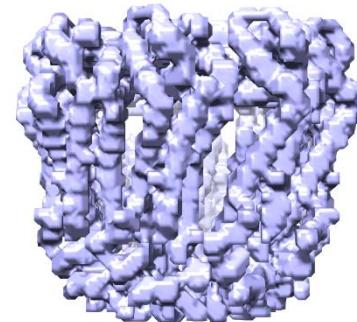
volume_mask_fsc_auto



Loose ?



Tight ?



Tight ?

Step 14: J15:Homogeneous Refinement [5.5 min with 1GPU]



P7: EMPIAR-10291_50mic

W1: Test Workspace

J15



Overview

Inputs and Parameters

Outputs

Metadata

(1) select [Outputs] tab

Overview

Inputs and Parameters

Outputs

Metadata

particles

No Image Available

GROUP TYPE
particle

TOTAL ITEMS
16152

ACTIONS

Export

alignments3D
J15.particles.alignments3D

particle.alignments3D



blob (passthrough)

J15.particles.blob

particle.blob



location (passthrough)

J15.particles.location

particle.location



ctf

J15.particles.ctf

Group 1

particle.ctf



alignments2D (passthrough)

J15.particles.alignments2D

particle.alignments2D



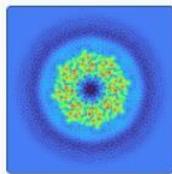
pick_stats (passthrough)

J15.particles.pick_stats

particle.pick_stats



volume



GROUP TYPE
volume

TOTAL ITEMS
1

ACTIONS

Export

map

J15.volume.map

volume.blob



VERSIONS

map_half_A

J15.volume.map_half_A

volume.blob



VERSIONS

mask_refine

J15.volume.mask_refine

volume.blob



VERSIONS

mask_fsc

J15.volume.mask_fsc

volume.blob



VERSIONS

precision

J15.volume.precision

volume.blob



VERSIONS

mask

No Image Available

GROUP TYPE
mask

TOTAL ITEMS
1

ACTIONS

mask_refine

J15.mask.mask_refine

volume.blob



Group 2

volume.blob



volume.blob



volume.blob



volume.blob

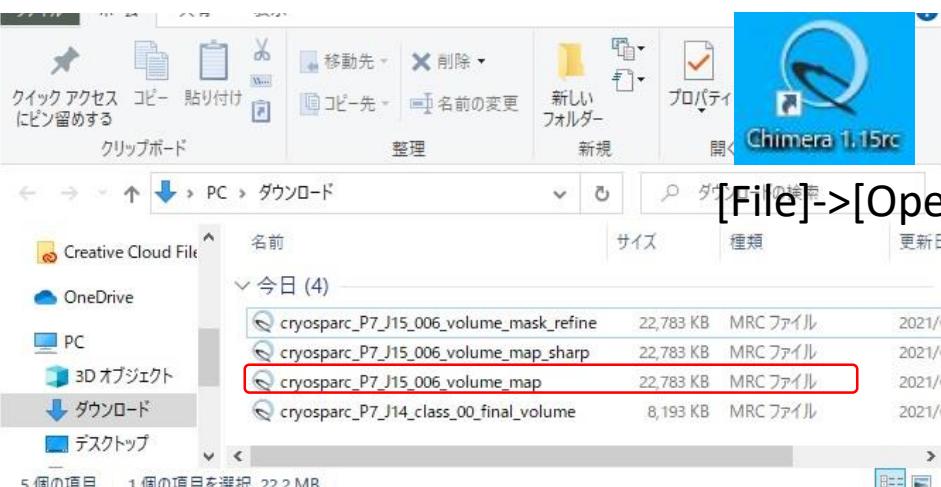


Download output

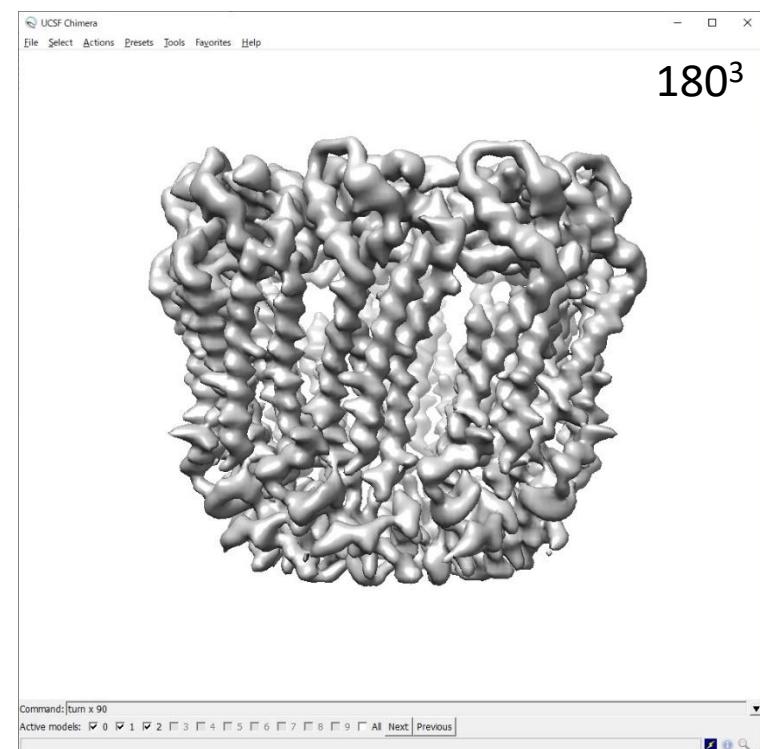
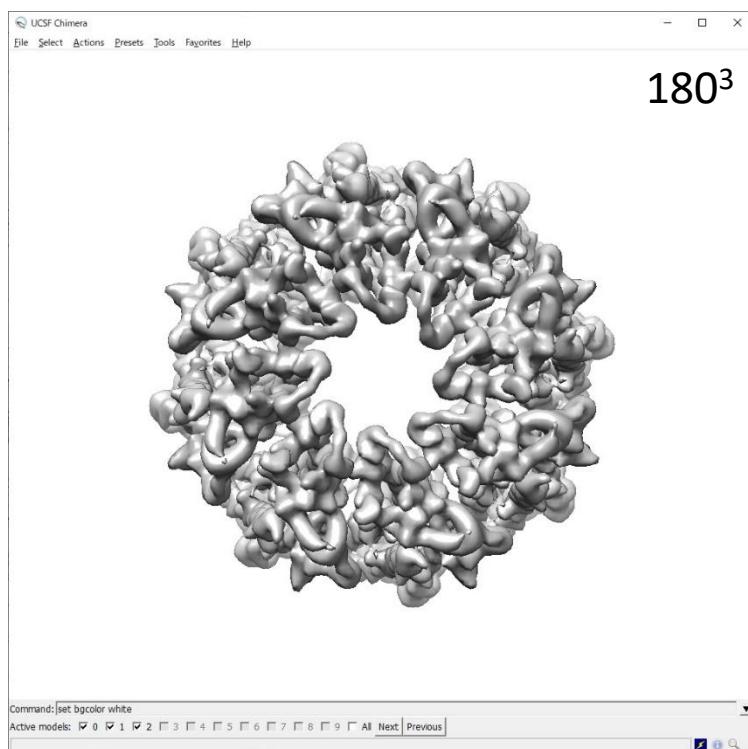
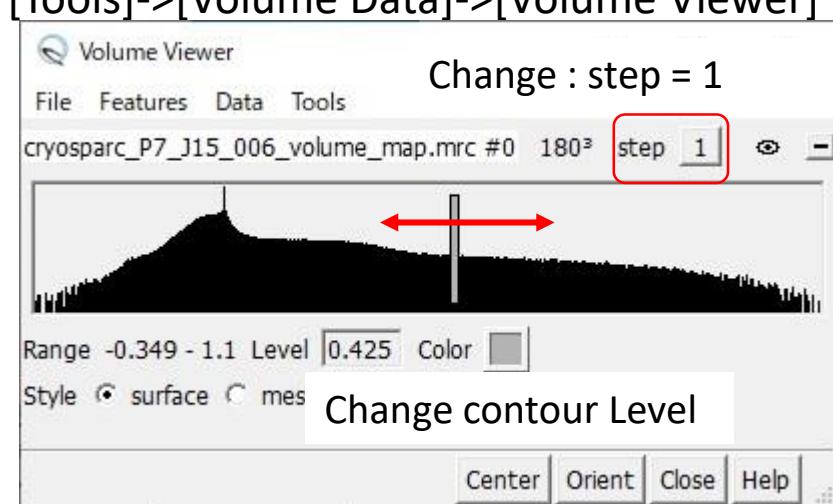
(2) Download "J*.volume_map"

Step 14: Homogeneous Refinement [5.5 min with 1GPU]

[Tools]->[Volume Data]->[Volume Viewer]

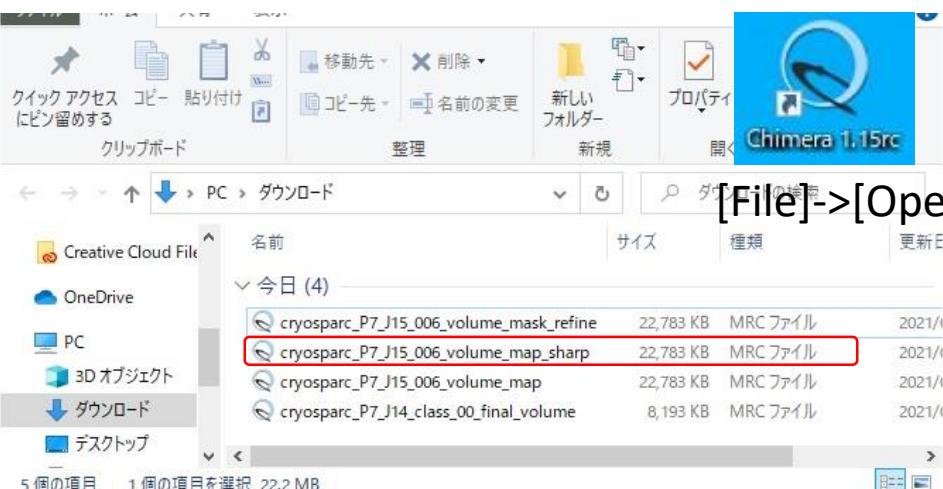


[File]->[Open...]

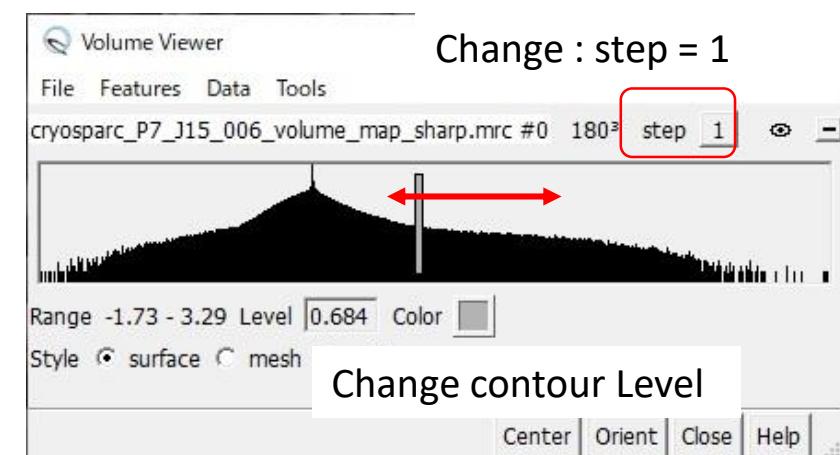


Step 14: Homogeneous Refinement [5.5 min with 1GPU]

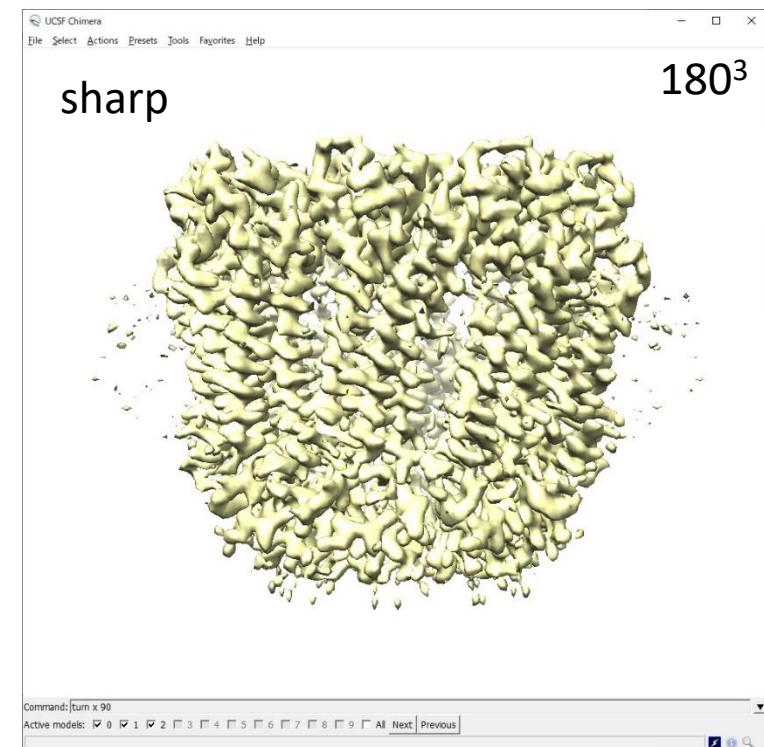
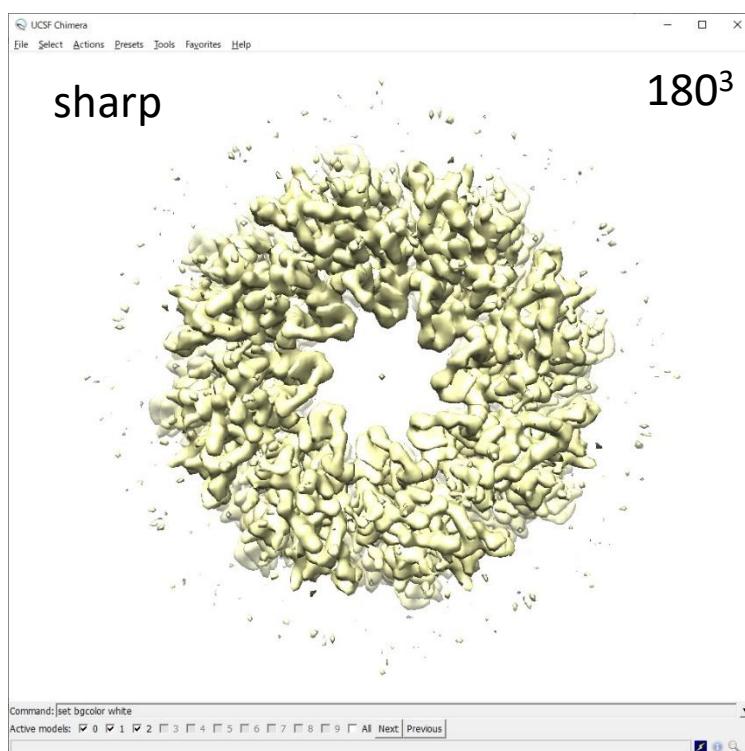
[Tools]->[Volume Data]->[Volume Viewer]



[File]->[Open...]

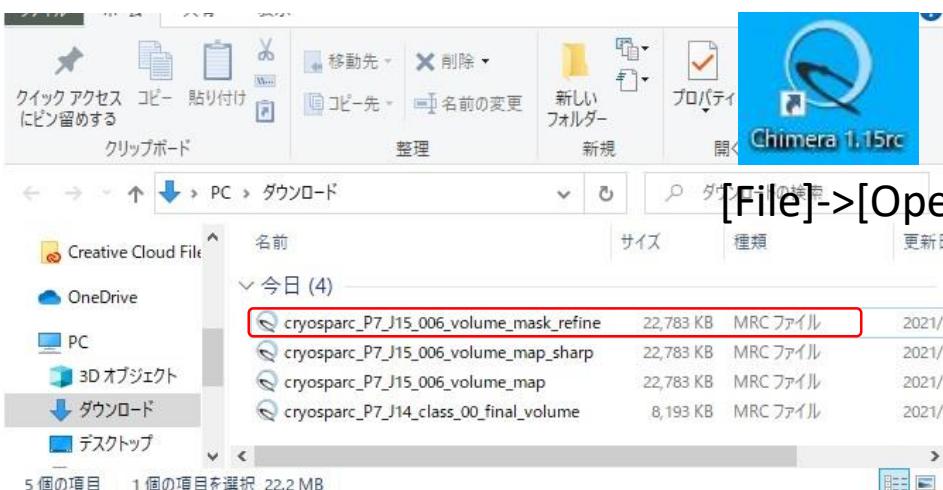


Change contour Level

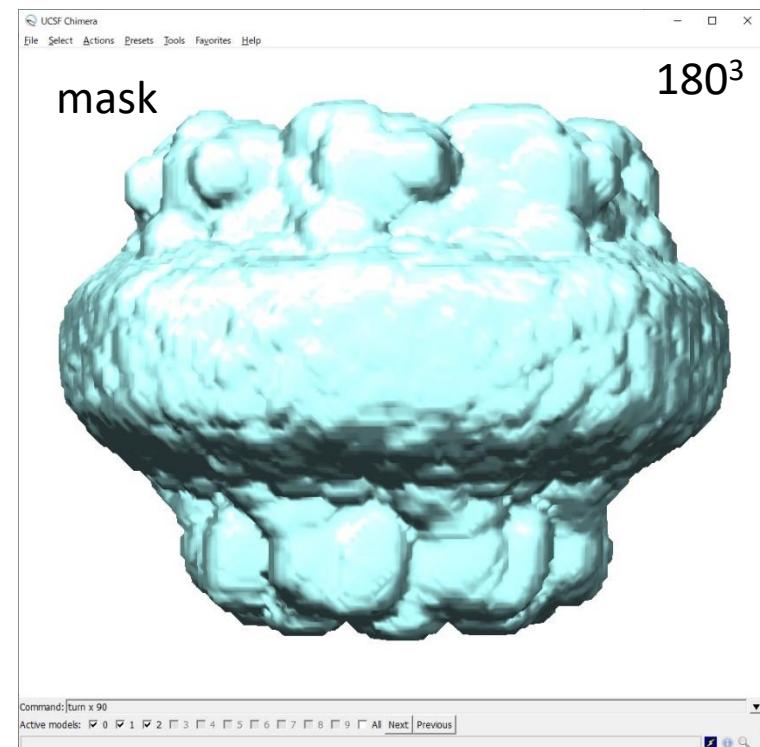
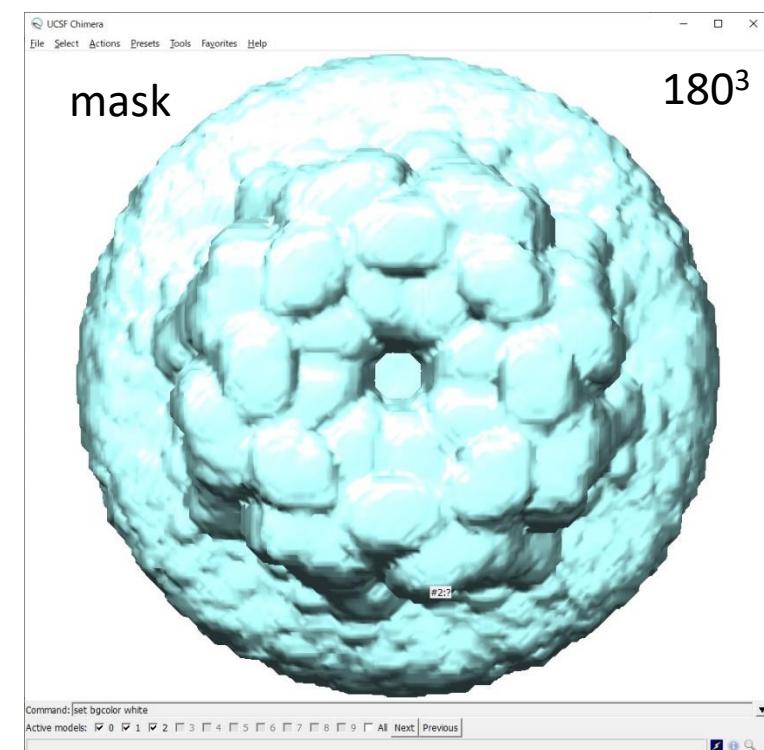
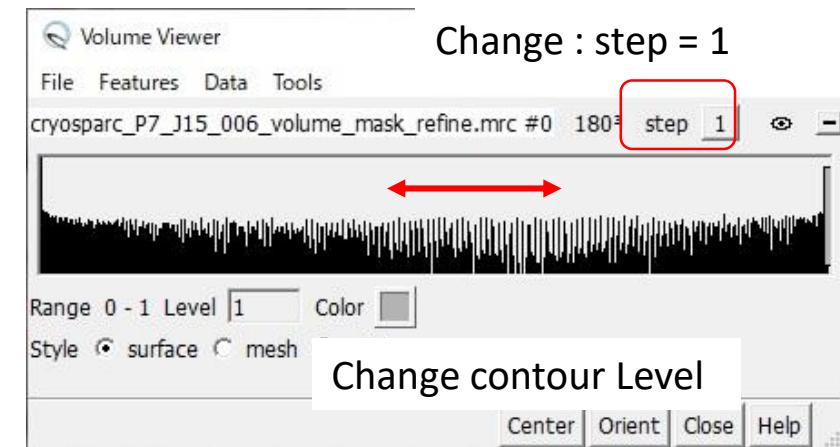


Step 14: Homogeneous Refinement [5.5 min with 1GPU]

[Tools]->[Volume Data]->[Volume Viewer]



[File]->[Open...]



Acknowledgements

We thank following people:

- **Developers for cryoSPARC**
- **Prof. Atsunori Oshima** for providing his EM data and helpful advices.