



Single-Particle Data Analysis using cryoSPARC

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cryoSPARC

- A streamlined end-to-end single particle cryo-EM workflow
- Rapidly solve high-resolution structures
- SSH into one of the ports according to the instruction provided.
- A browser-based user interface and command line tools
- Work best on Google Chrome, may also work on Firefox and Safari.

Organization: Projects, Workspaces, Jobs

Project: a new sample on which you have collected data.

Workspace: created inside a project. Each project can contain multiple workspaces.

Job: One unit of processing work. Many jobs within each workspace.

P22 LBMS_tutorial

J16 3D Var J15 Homo Refine



about 2 hours ago about 2 hours ago

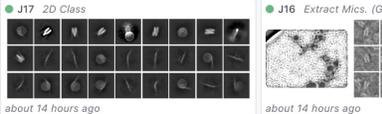
Created by Dongyan Tan on Apr 10, 2024

J17 NU-Refine J12 Manual Picker

View 1 Workspace No Sessions

P21 BSB580-Kai

J17 2D Class J16 Extract Mics. (G)



about 14 hours ago about 14 hours ago

Created by Dongyan Tan on Apr 2, 2024

View 1 Workspace No Sessions

P20 coVariant-nuc_BSB580

J27 Flex Generate J26 3D Var Display J25 Flex Reconstruct



2 months ago 2 months ago 2 months ago

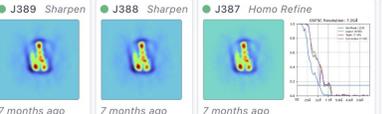
Created by Dongyan Tan on Apr 2, 2024

J4 Manual Picker J6 Inspect Picks

View 1 Workspace No Sessions

P18 native nucl 20230921

J389 Sharpen J388 Sharpen J387 Homo Refine



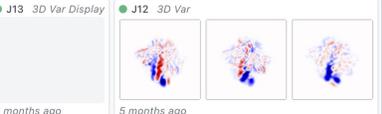
7 months ago 7 months ago 7 months ago

Created by Vladyslava Sokolova on Oct 24, 2023

View 1 Workspace No Sessions

P15 I

J13 3D Var Display J12 3D Var



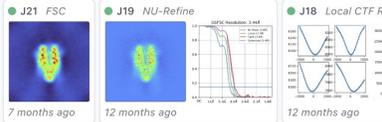
5 months ago 5 months ago

Created by Dongyan Tan on Aug 21, 2023

View 1 Workspace No Sessions

P13

J21 FSC J19 NU-Refine J18 Local CTF Ref.



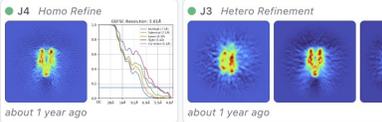
7 months ago 12 months ago 12 months ago

Created by Dongyan Tan on Jun 14, 2023

View 1 Workspace No Sessions

P12

J4 Homo Refine J3 Hetero Refinement



about 1 year ago about 1 year ago

Created by Dongyan Tan on May 28, 2023

View 1 Workspace No Sessions

P11

J36 Hetero Refinement J35 Ab-Initio



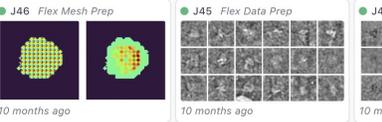
9 months ago 9 months ago

Created by Vladyslava Sokolova on Oct 3, 2022

View 1 Workspace No Sessions

P10

J46 Flex Mesh Prep J45 Flex Data Prep J4



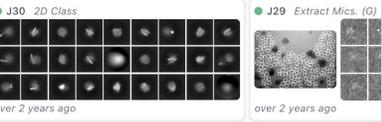
10 months ago 10 months ago 10 months ago

Created by Dongyan Tan on Feb 18, 2022

View 2 Workspaces No Sessions

P9

J30 2D Class J29 Extract Mics. (G)



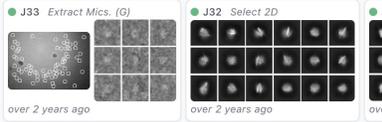
over 2 years ago over 2 years ago

Created by Dongyan Tan on Nov 8, 2021

View 1 Workspace No Sessions

P8

J33 Extract Mics. (G) J32 Select 2D J3



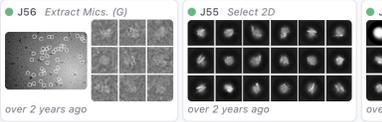
over 2 years ago over 2 years ago over 2 years ago

Created by Dongyan Tan on Nov 7, 2021

View 1 Workspace No Sessions

P7

J56 Extract Mics. (G) J55 Select 2D J5



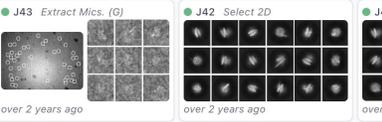
over 2 years ago over 2 years ago over 2 years ago

Created by Dongyan Tan on Oct 16, 2021

View 1 Workspace No Sessions

P6

J43 Extract Mics. (G) J42 Select 2D J4



over 2 years ago over 2 years ago over 2 years ago

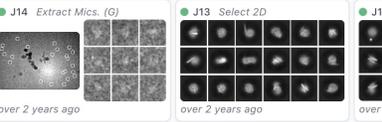
Created by Dongyan Tan on Oct 6, 2021

using Relion motioncor micrographs done in HPC

View 1 Workspace No Sessions

P5

J14 Extract Mics. (G) J13 Select 2D J1



over 2 years ago over 2 years ago over 2 years ago

Created by Dongyan Tan on Oct 2, 2021

View 1 Workspace No Sessions

P4

J6 CTF Estimation (CTFFIND4) J5 Import Micrographs



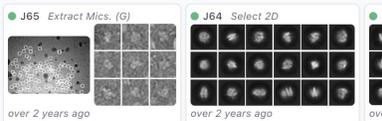
over 2 years ago over 2 years ago

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View 1 Workspace No Sessions

P3

J65 Extract Mics. (G) J64 Select 2D J6



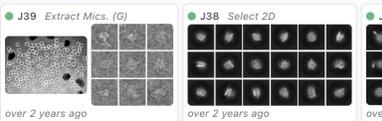
over 2 years ago over 2 years ago over 2 years ago

Created by Dongyan Tan on Sep 17, 2021

View 1 Workspace No Sessions

P2

J39 Extract Mics. (G) J38 Select 2D J3



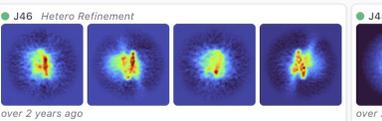
over 2 years ago over 2 years ago over 2 years ago

Created by Dongyan Tan on Sep 16, 2021

View 1 Workspace No Sessions

P1

J46 Hetero Refinement J45



over 2 years ago over 2 years ago

Created by Dongyan Tan on Sep 13, 2021

View 1 Workspace No Sessions

Organization: Projects, Workspaces, Jobs

Card View

Project **Workspace** **Job**

CryoSPARC × P16 T20-Proteosome × W1 tutorial New Job

Starred Mine Tags Status Filters Date Created

J1 Import Movies 46 Movies 0.66 Å, 300 kV, 53 e/Å², 38 x 7676 00m 22s 6

J2 Patch Motion Corr. 0 Micrographs completed 46 Incomplete Micrographs 01m 02s default 2 2

J3 Patch Motion Corr. 46 Micrographs 03m 37s default 4 2

J4 Patch CTF 46 Exposures 01m 02s default 4 2

J5 Blob Picker 51,410 Particles 1,118 P/Mic. 46 Micrographs Diameter 100 → 200 00m 33s default 1 3

J6 Inspect Picks 20,108 Particles 437 P/Mic.

J7 Extract Mics. (G) 17,313 Particles 376 P/Mic.

25 Jobs 19 Job Types 3 Statuses

Details Builder Cart

P16 W1 tutorial

Details

Title tutorial

Tags No tags applied.

Created Jun 19, 2023, 2:03:44 PM

Created By Dongyan Tan

Last Accessed Jun 20, 2023, 10:56:23 PM

Last Accessed By Dongyan Tan

Description

Statistics

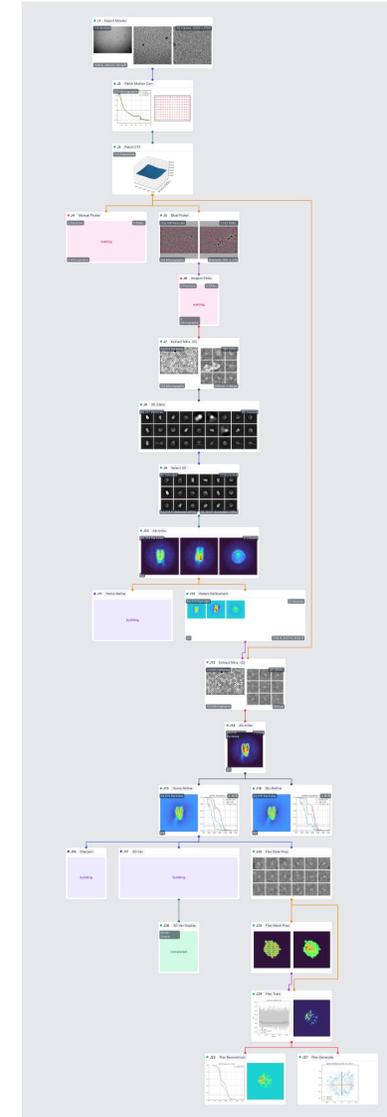
Last Updated Jun 20, 2023, 10:05:30 PM

Total Jobs 25

Job Status 23 completed 1 building 1 failed

Actions View Workspace

Tree View



CryoSPARC Web Interface: Dashboard

Navigation bar

Navigation control

Side bar

The screenshot displays the CryoSPARC Web Interface Dashboard. The interface is divided into several key sections:

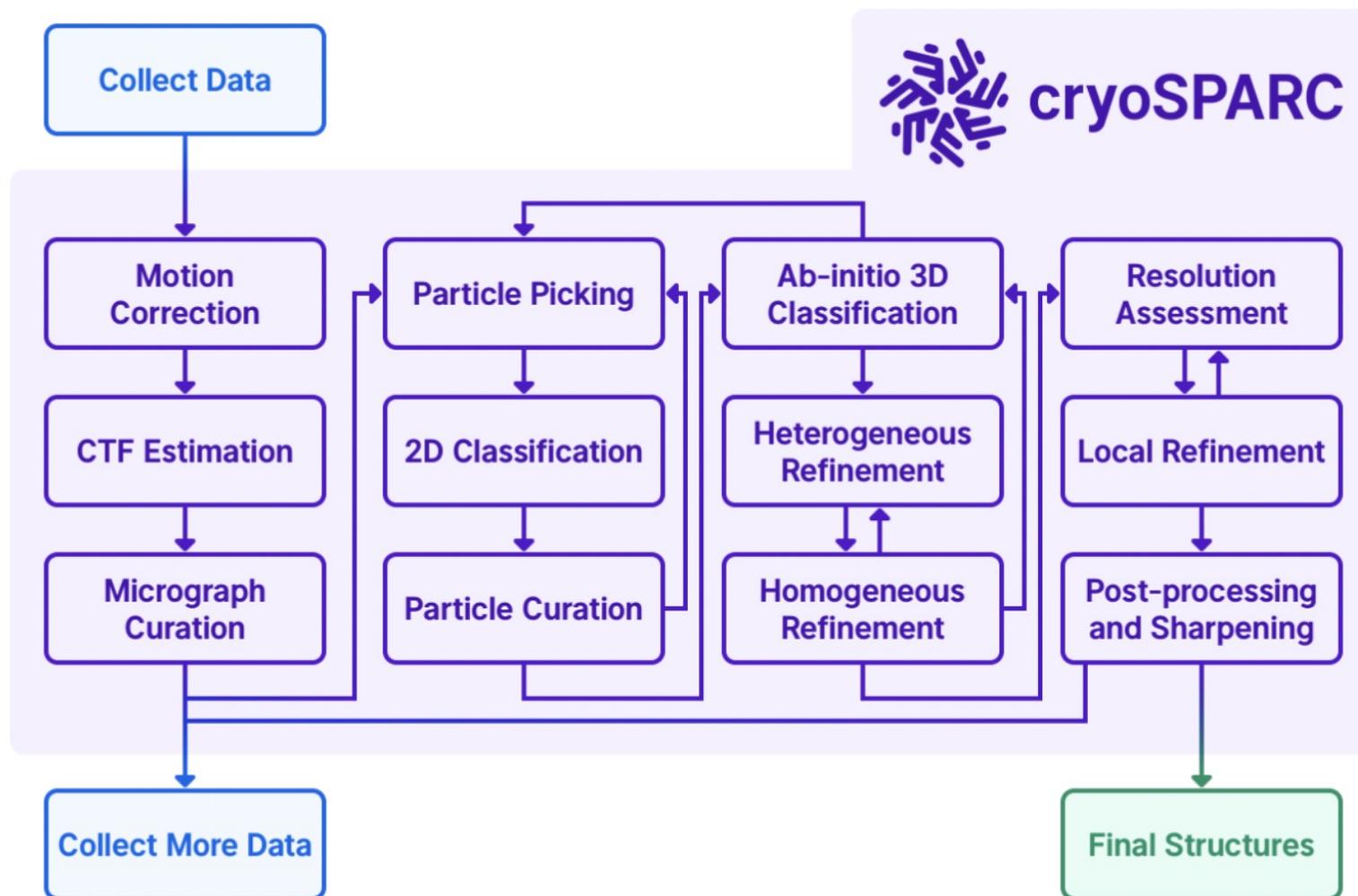
- Navigation bar:** Located at the top, it includes the CryoSPARC logo, a breadcrumb trail (P16 T20-Proteosome > W1 tutorial), and a 'New Job' button.
- Navigation control:** A green bar at the top center containing filters for 'Starred', 'Mine', 'Tags', 'Status', 'Filters', and 'Date Created'.
- Content Area:** The main workspace showing a workflow of jobs (J1 to J7).
 - J1 Import Movies:** Shows 46 movies with a resolution of 0.66 Å, 300 kV, 53 e/Å², and 38 x 7676 pixels. Status: 00m 22s.
 - J2 Patch Motion Corr.:** Shows 0 micrographs and is marked as 'completed'. Status: 01m 02s.
 - J3 Patch Motion Corr.:** Shows 46 micrographs with a graph of FWHM vs. defocus. Status: 03m 37s.
 - J4 Patch CTF:** Shows 46 exposures with a 3D surface plot. Status: 01m 02s.
 - J5 Blob Picker:** Shows 51,410 particles and 1,118 P/Mic. Status: 00m 33s.
 - J6 Inspect Picks:** Shows 20,108 particles and 437 P/Mic.
 - J7 Extract Mics. (G):** Shows 17,313 particles and 376 P/Mic.
- Side bar:** Located on the right, it provides details for the selected job 'P16 W1 tutorial'.
 - Details:** Title (tutorial), Tags (No tags applied), Created (Jun 19, 2023, 2:03:44 PM), Created By (Dongyan Tan), Last Accessed (Jun 20, 2023, 10:56:23 PM), Last Accessed By (Dongyan Tan).
 - Description:** (Collapsible section).
 - Statistics:** Last Updated (Jun 20, 2023, 10:05:30 PM), Total Jobs (25), Job Status (23 completed, 1 building, 1 failed).
 - Actions:** View Workspace (arrow icon).

Typical Workflow of Image Processing in cryoSPARC

Pre-process

Process

Post-Process



Tutorial Data Set: nucleosome

- Subset of 26 movies in TIFF format
- Data collected at a 300kV microscope
- Pixel size: 1.08 Å
- Total Dosage : 50 e/ Å²
- Stable 200 KDa DNA-protein complex

Exploring Heterogeneity



Journal of Structural Biology

Volume 213, Issue 2, June 2021, 107702



3D variability analysis: Resolving continuous flexibility and discrete heterogeneity from single particle cryo-EM

Ali Punjani^{a b c}  , David J. Fleet^{a b}  

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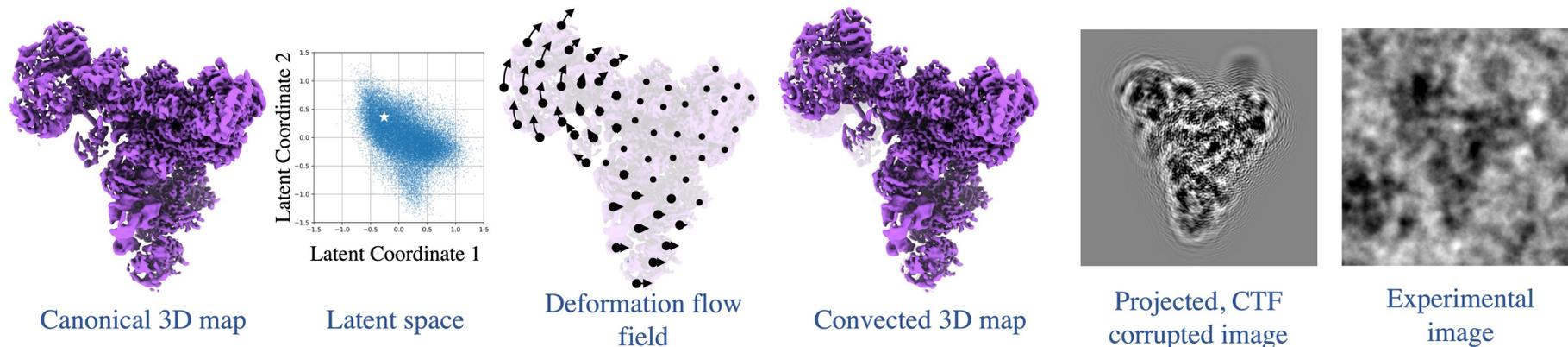
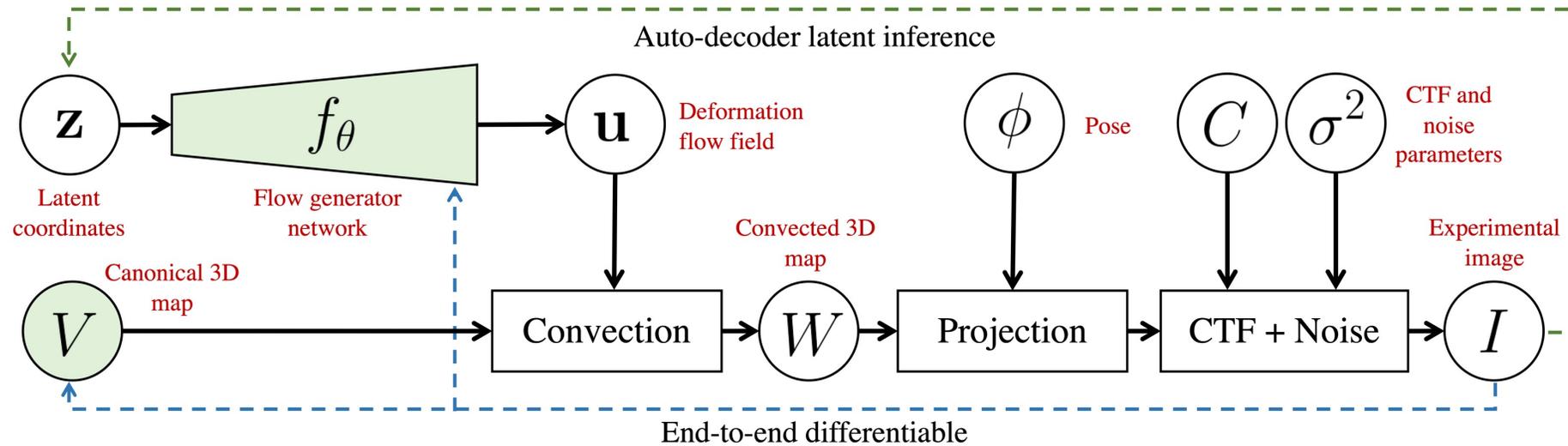
3DFlex: determining structure and motion of flexible proteins from cryo-EM

[Ali Punjani](#)  & [David J. Fleet](#) 

[Nature Methods](#) **20**, 860–870 (2023) | [Cite this article](#)

3DFlex Refinement

A advanced motion-based deep generative model for continuous heterogeneity



3DFlex Refinement

5 Steps

- 3D Flex Data Prep
- 3D Flex Mesh Prep – create a mesh
- 3D Flex Training – to model the movement of the particles
- 3D Flex Reconstruction

Limitation

- Compositional heterogeneity.
- Intricate motions such as side chain or loop motion
- Intermediate states with no data
- Interpretation of latent space, how it relates to a physically meaningful notion of energy