Single-Particle Image Processing using cryoSPARC

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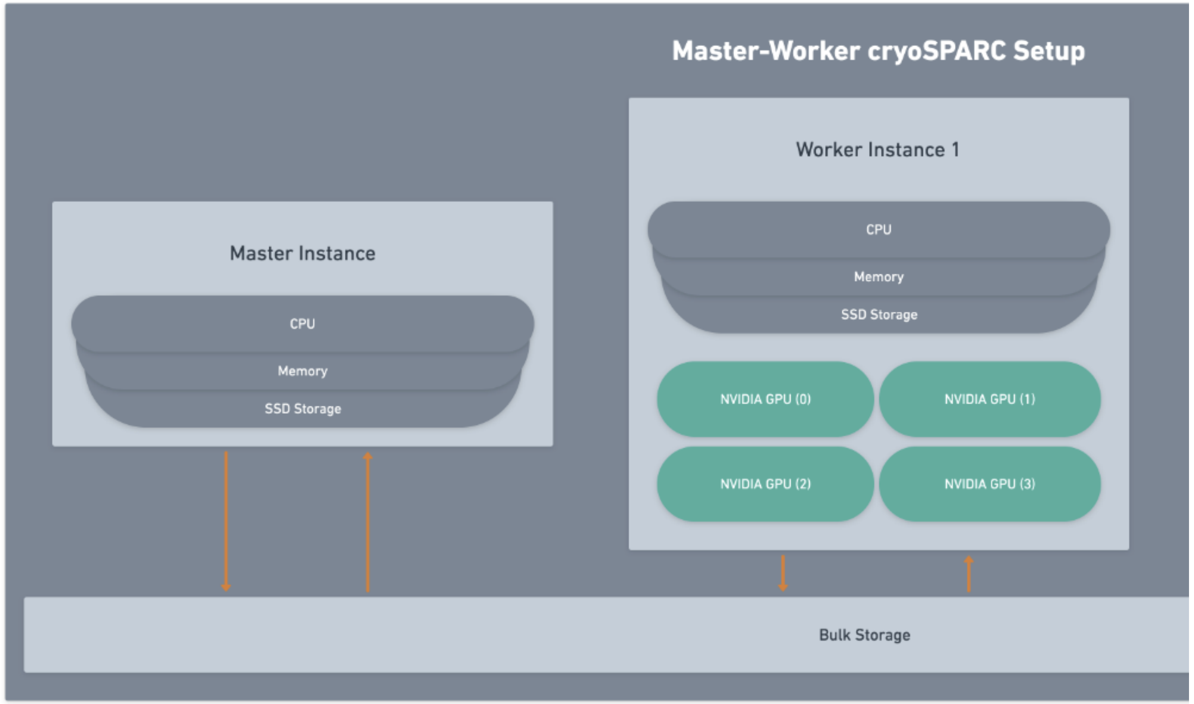
cryoSPARC

- A streamlined end-to-end single particle cryo-EM workflow
- Rapidly solve high-resolution structures
- A browser-based user interface and command line tools
- master-worker setup, with all nodes have access to the same file system
- Workers need GPU resource

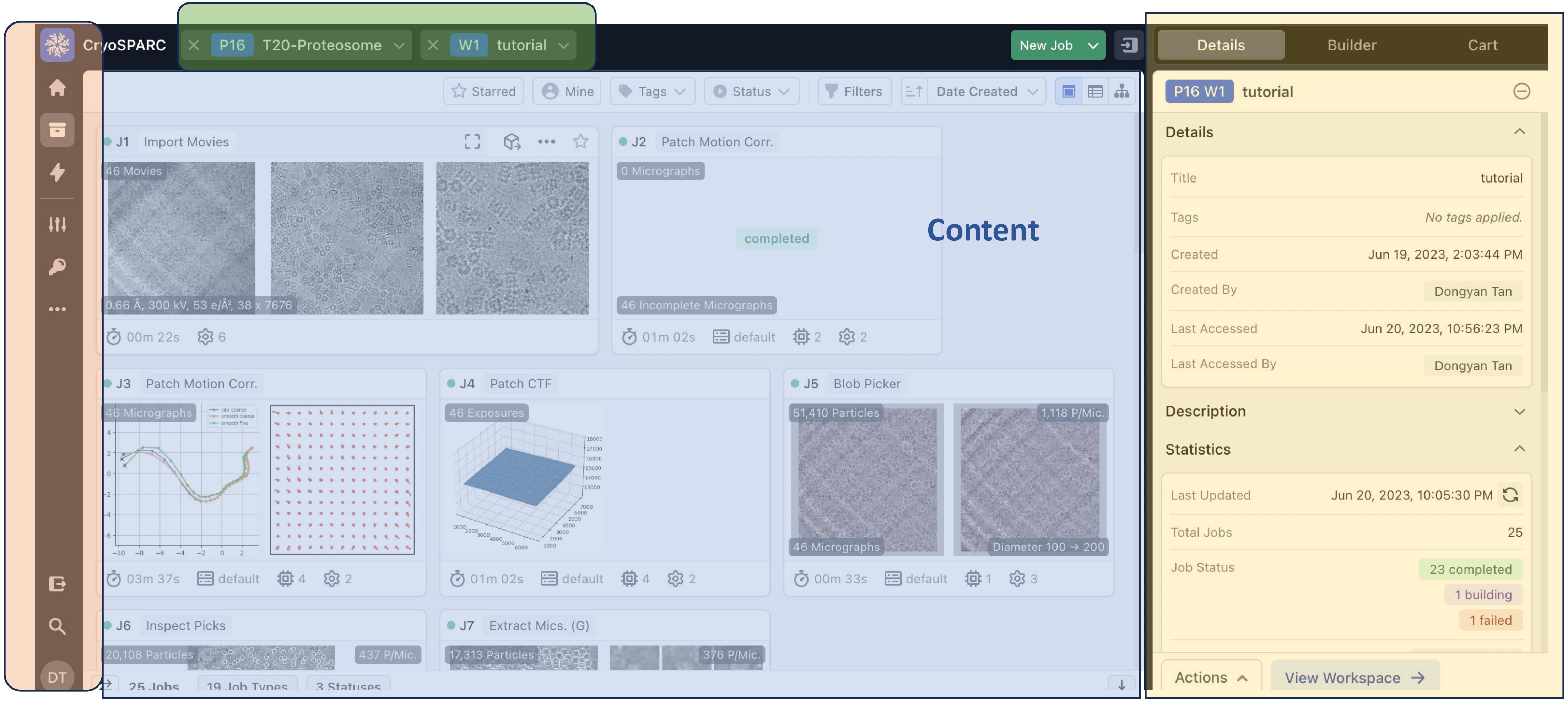


CryoSPARC Web Interface: Dashboard

Navigation bar

Navigation control

Side bar



Content

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

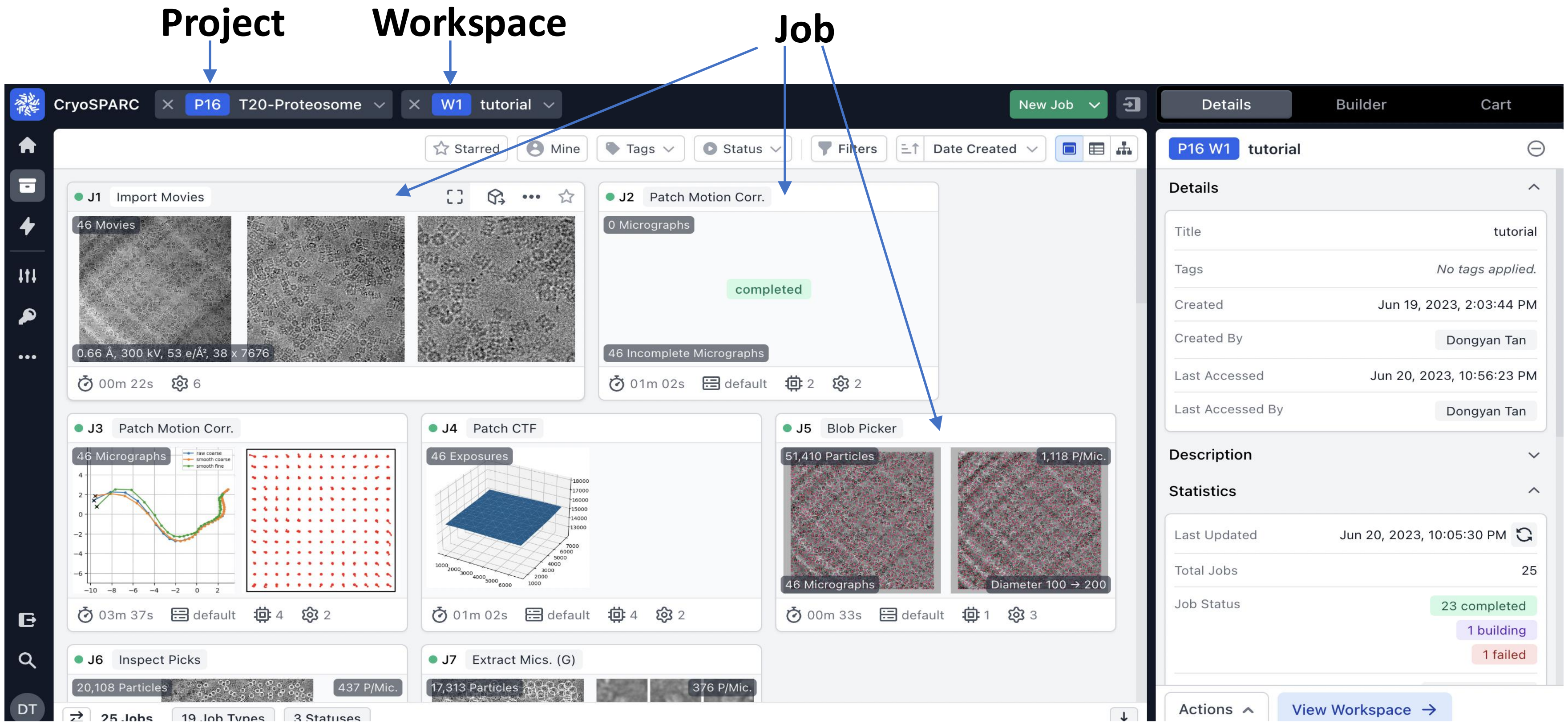
Last Updated	Jun 20, 2023, 10:05:30 PM
Total Jobs	25
Job Status	23 completed 1 building 1 failed

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 (e.g. datasets collected on different dates using the same specimen)

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



Project

Workspace

Job

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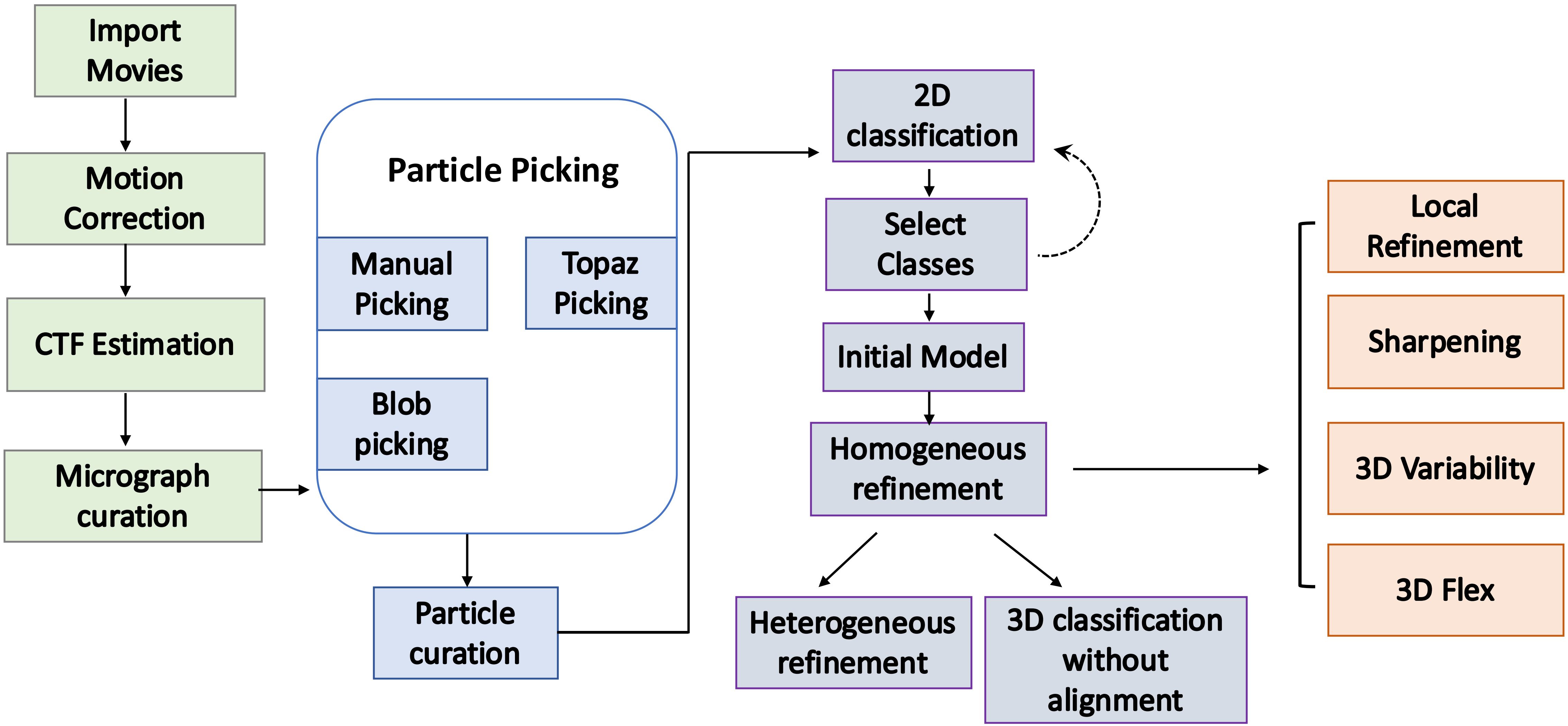
Workflow of Image Processing in cryoSPARC



Pre-process

Import Movies

Motion Correction

CTF Estimation

Micrograph curation

Particle Picking

Manual Picking

Topaz Picking

Blob picking

Particle curation

Process

2D classification

Select Classes

Initial Model

Homogeneous refinement

Heterogeneous refinement

3D classification without alignment

Post-process

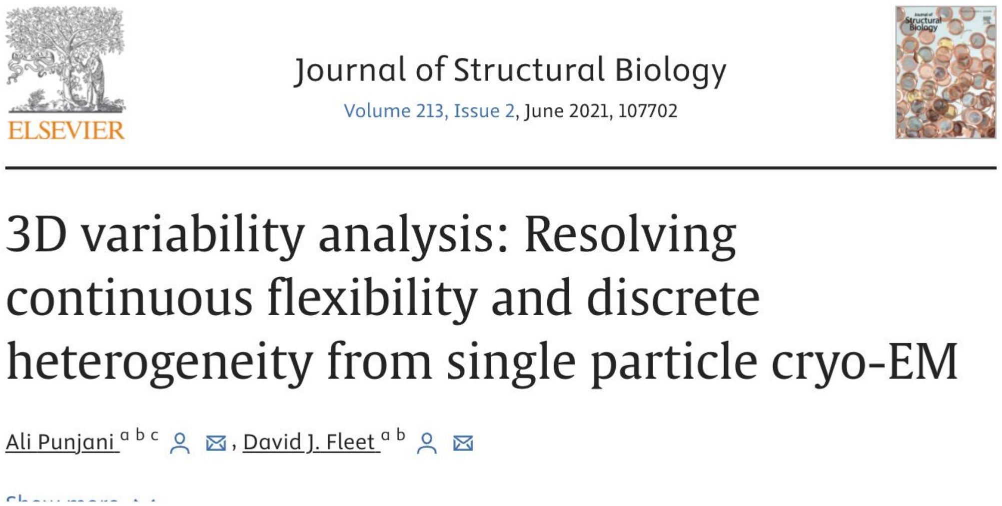
Local Refinement

Sharpening

3D Variability

3D Flex

Exploring Heterogeneity



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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]

nature methods

Article | Open access | Published: 11 May 2023

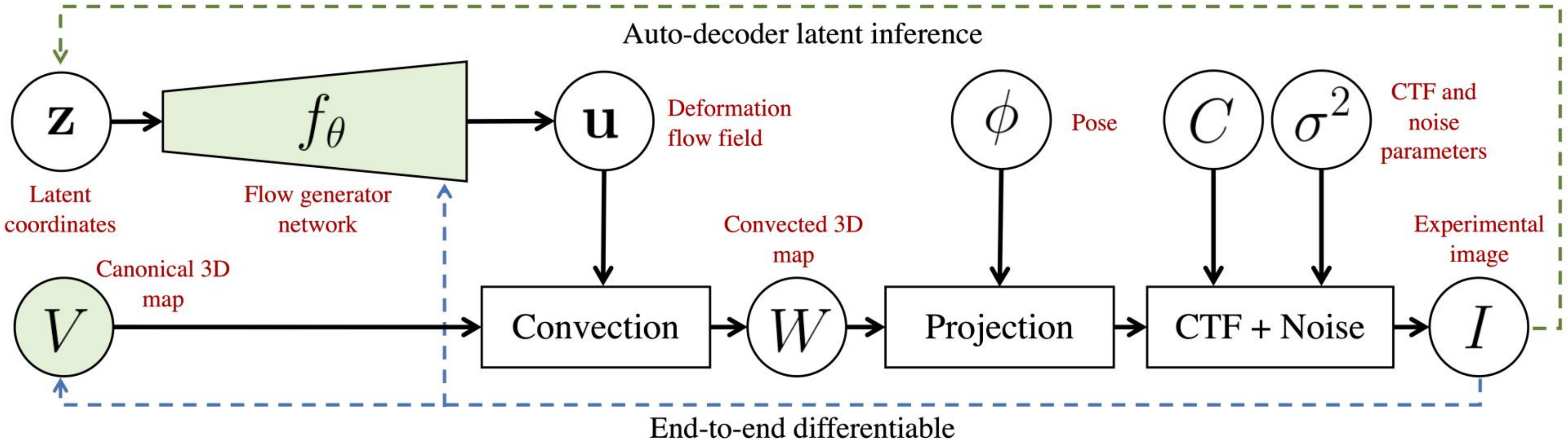
3DFlex: determining structure and motion of flexible proteins from cryo-EM

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


Auto-decoder latent inference

$\mathbf{z}$ Latent coordinates

f_θ Flow generator network

$\mathbf{u}$ Deformation flow field

ϕ Pose

C σ^2 CTF and noise parameters

V Canonical 3D map

Convection

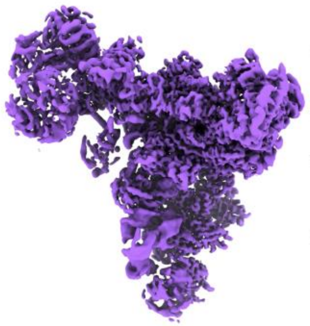
W Convected 3D map

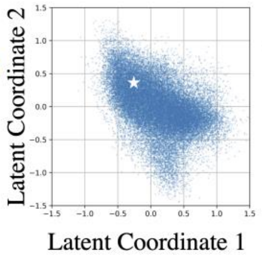
Projection

CTF + Noise

I Experimental image

End-to-end differentiable

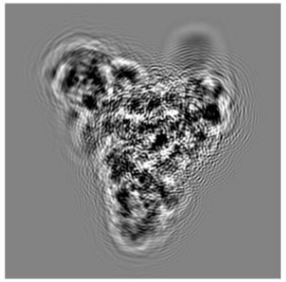

Canonical 3D map

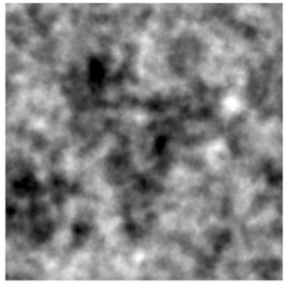


Latent space


Deformation flow field


Convected 3D map


Projected, CTF corrupted image


Experimental image

Adapted from https://guide.cryosparc.com/

The same workflow applies to other software packages

- Micrographs and particle images should be directly imported into the other software.

- metadata file used in RELION : STAR file, e.g. particles.star
Metadata file used in cryoSPARC: cs file, e.g. particles.cs

- convert the particle.cs file to a STAR file using csparc2star.py in pyem

Convert .cs file to .star file

Steps to convert the cryoSPARC metadata file (.cs) into RELION readable format (.star).

1. Download new pyem from GitHub.

2. go to the “output” tab on cryosparc browser. there should be an option to Export files from jobs.
3. This will write a .cs and .csg file that contains all the important information for a particle set (e.g. /cryosparc/PXX/groups/exports/JXXXXXX.cs or similar).
4. Use csparc2star.py software from pyem by Danial Asarnow to do the conversion.

5. The new particle star file is ready to be used in RELION.

Test Data Set: nucleosome

- Subset of 30 movies in TIFF format
- Data collected at a 300kV Krios microscope
- Pixel size: 1.08 Å
- Total Dosage : 50 e/ $Å^2$
- 210 KDa DNA-protein complex