

Stony Brook **Medicine**



# Single-Particle Image Processing using cryoSPARC

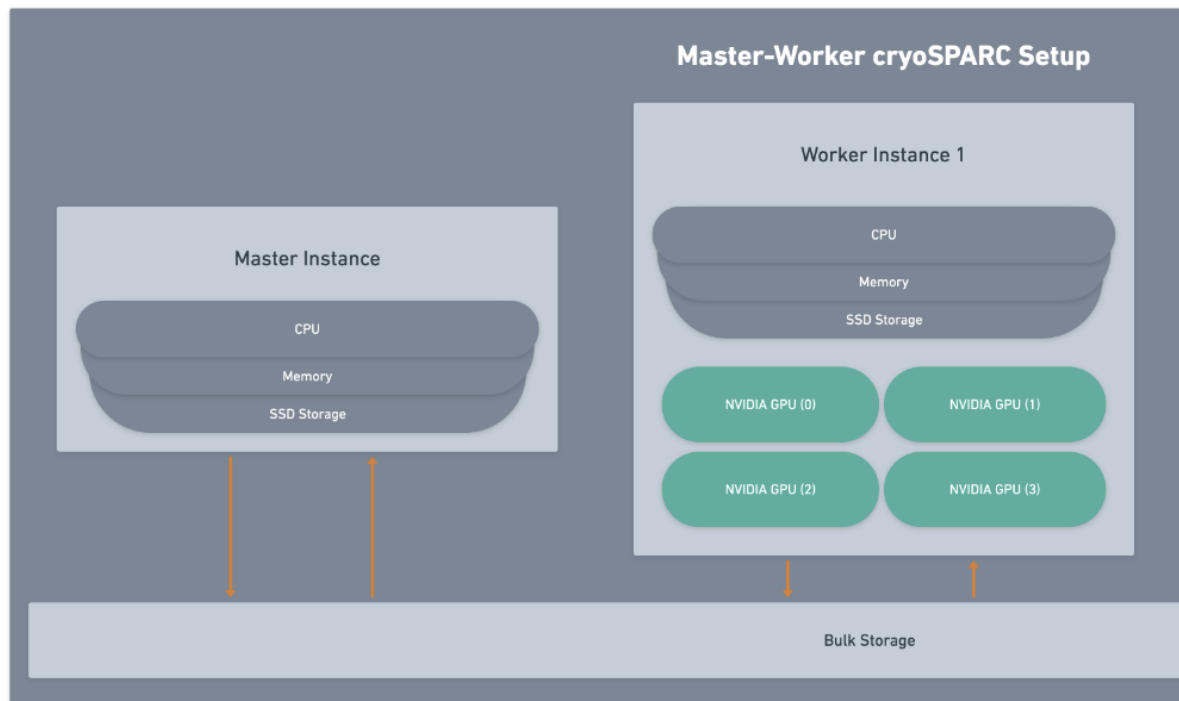
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# cryoSPARC

- 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

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

- Navigation bar:** Located at the top, it includes the CryoSPARC logo, tabs for 'P16 T20-Proteosome' and 'W1 tutorial', a 'New Job' button, and a search icon.
- Navigation control:** A green bar at the top of the main content area, containing filters for 'Starred', 'Mine', 'Tags', 'Status', 'Filters', 'Date Created', and view options (list, grid, people).
- Content:** The main workspace area, labeled 'Content' in blue text. It contains several job panels:
  - J1 Import Movies:** Shows 46 Movies with a thumbnail and technical details: 0.66 Å, 300 kV, 53 e/Å², 38 x 7676. It has a timer of 00m 22s and 6 settings.
  - J2 Patch Motion Corr.:** Shows 0 Micrographs and is marked 'completed'. It has a timer of 01m 02s and default settings.
  - J3 Patch Motion Corr.:** Shows 46 Micrographs with a graph of 'Peak Center', 'Smooth Center', and 'Smooth Fine'. It has a timer of 03m 37s and 4 settings.
  - J4 Patch CTF:** Shows 46 Exposures with a 3D surface plot. It has a timer of 01m 02s and 4 settings.
  - J5 Blob Picker:** Shows 51,410 Particles with a thumbnail and 'Diameter 100 → 200'. It has a timer of 00m 33s and 3 settings.
  - J6 Inspect Picks:** Shows 20,108 Particles with a thumbnail and '437 P/Mic.'.
  - J7 Extract Mics. (G):** Shows 17,313 Particles with a thumbnail and '376 P/Mic.'.
- Side bar:** Located on the right, it contains tabs for 'Details', 'Builder', and 'Cart'. The 'Details' tab is active, showing information for 'P16 W1 tutorial':
  - 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:** (collapsed)
  - Statistics:** (expanded)
    - Last Updated:** Jun 20, 2023, 10:05:30 PM
    - Total Jobs:** 25
    - Job Status:** 23 completed, 1 building, 1 failed
  - Actions:** (expanded) View Workspace →

# 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.

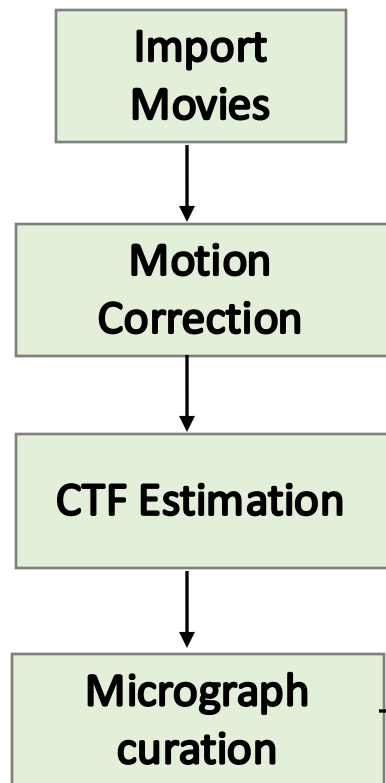
The screenshot displays the CryoSPARC web interface. At the top, the breadcrumb navigation shows 'CryoSPARC' > 'P16 T20-Proteosome' (labeled as the **Project**) > 'W1 tutorial' (labeled as the **Workspace**). Below this, the main workspace contains several job cards (labeled as **Jobs**):

- J1 Import Movies:** Shows 46 Movies, a micrograph, and parameters: 0.66 Å, 300 kV, 53 e/Å², 38 x 7676. Status: 00m 22s, 6 settings.
- J2 Patch Motion Corr.:** Shows 0 Micrographs, a 'completed' status, and parameters: 01m 02s, default, 2 settings.
- J3 Patch Motion Corr.:** Shows 46 Micrographs, a graph, and parameters: 03m 37s, default, 4 settings.
- J4 Patch CTF:** Shows 46 Exposures, a 3D plot, and parameters: 01m 02s, default, 4 settings.
- J5 Blob Picker:** Shows 51,410 Particles, 46 Micrographs, and parameters: 00m 33s, default, 3 settings.
- J6 Inspect Picks:** Shows 20,108 Particles, 437 P/Mic.
- J7 Extract Mics. (G):** Shows 17,313 Particles, 376 P/Mic.

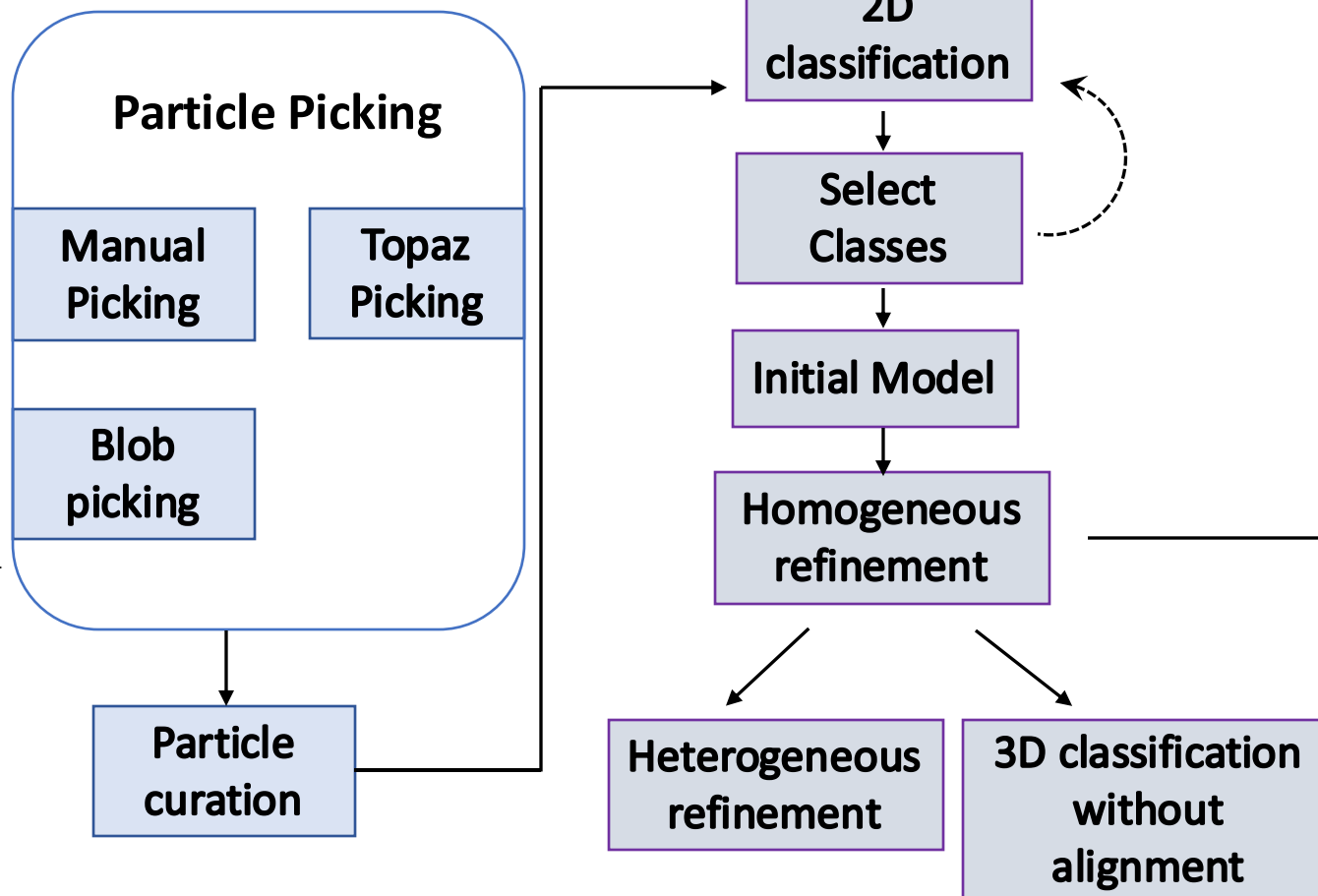
On the right side, the 'Details' panel for the 'P16 W1 tutorial' workspace is visible, showing metadata such as Title, Tags, Created date, and Job Status (23 completed, 1 building, 1 failed). At the bottom, a summary bar indicates '25 Jobs', '19 Job Types', and '3 Statuses'.

# Workflow of Image Processing in cryoSPARC

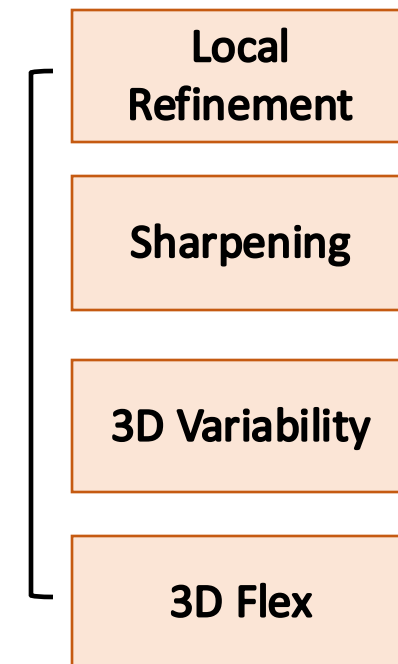
## Pre-process



## Process



## Post-process



# 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](#)<sup>a b c</sup>  , [David J. Fleet](#)<sup>a b</sup>  

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

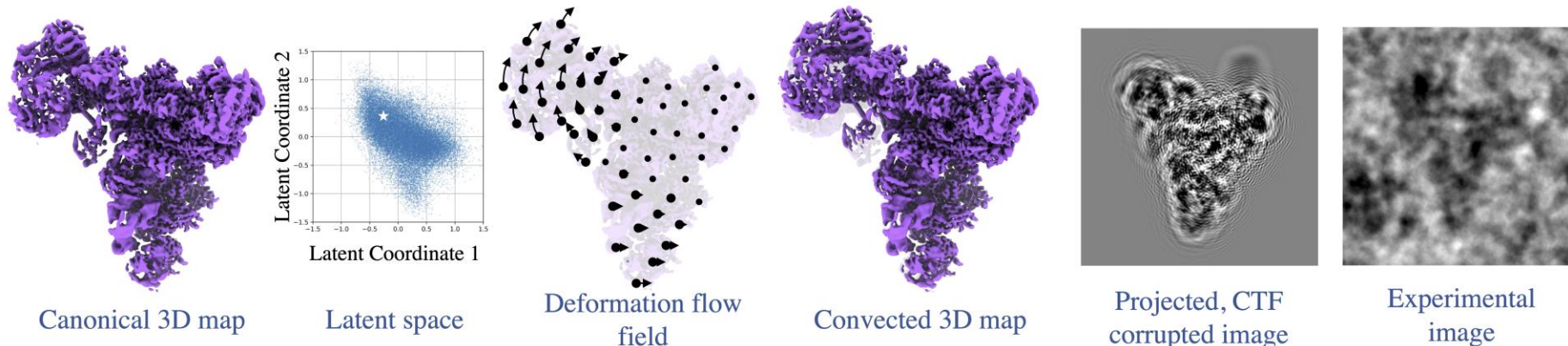
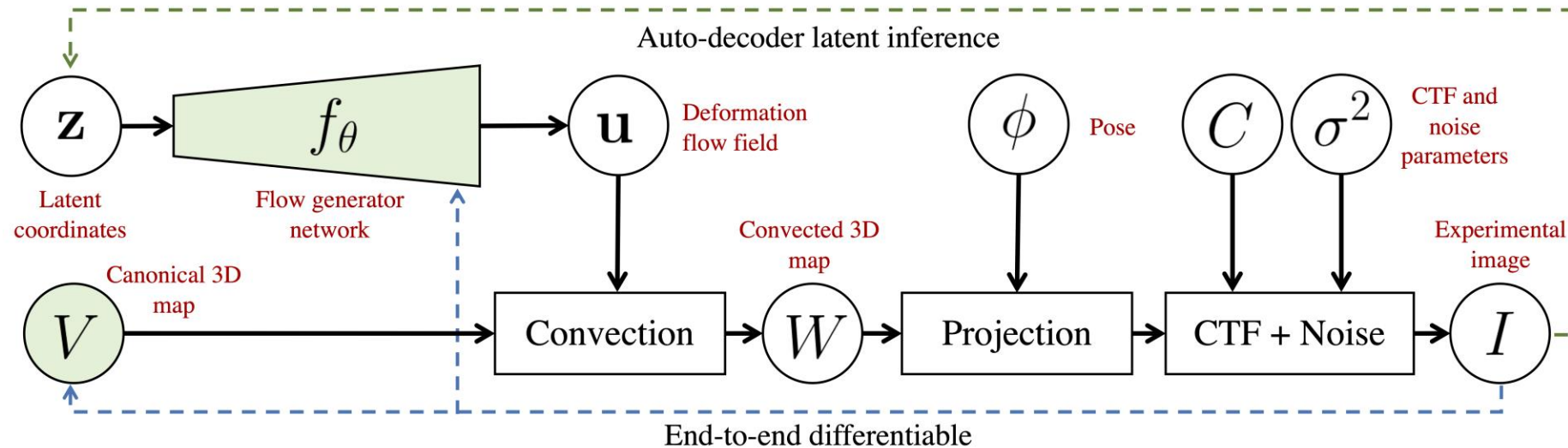
[Ali Punjani](#)  & [David J. Fleet](#) 

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



# 3DFlex Refinement

A advanced motion-based deep generative model for continuous heterogeneity



# 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<sup>2</sup>
- 210 KDa DNA-protein complex