

Single-Particle cryo-EM Image Processing

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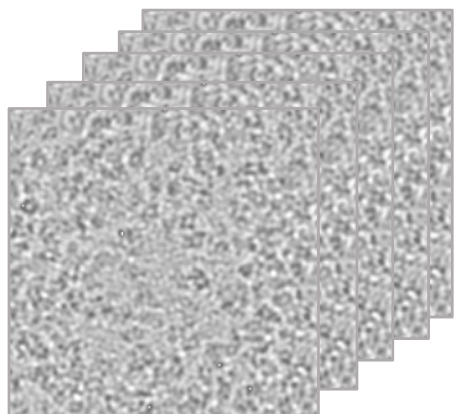
➤ Overview of single-particle data processing workflow

➤ Demo using cryoSPARC

➤ RELION

➤ Q&A.

Data/Movie

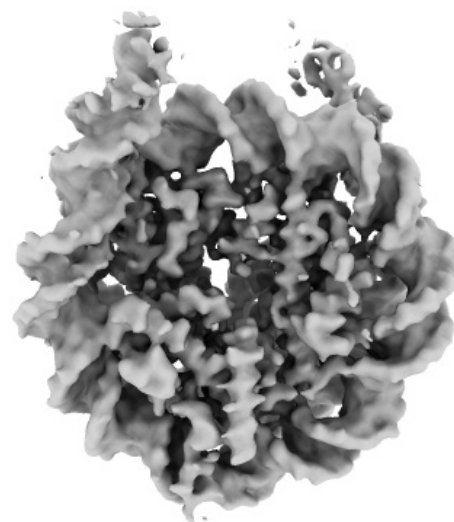


**Image
processing**



**Relion,
cryoSPARC,
cisTEM,
EMAN et al.**

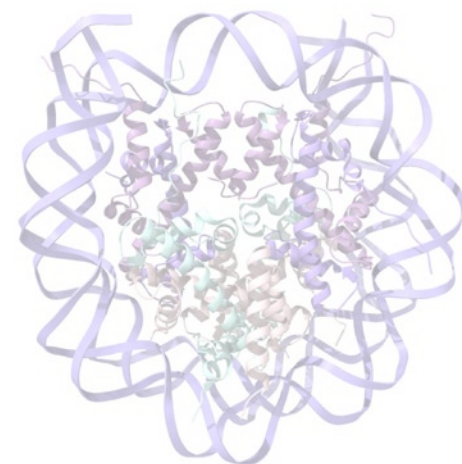
**high-resolution
density map**



**Model
building**

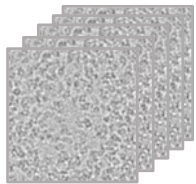


Atomic coordinate

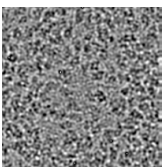


Pre-process

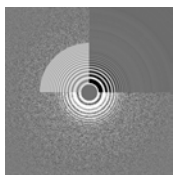
1. Movie/Frames



2. Align and average movies into micrographs

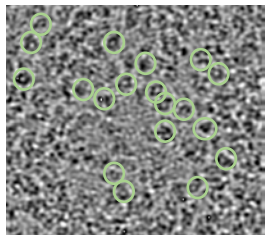


3. Defocus determination

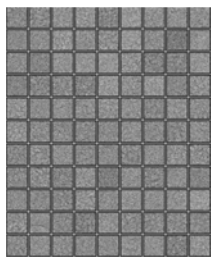


Process

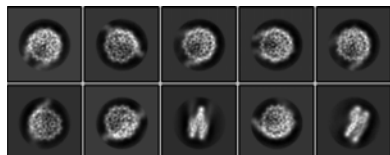
4. Particle picking



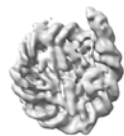
5. Extraction & normalization of particles



6. 2D Classification



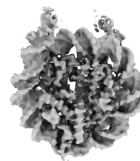
7. Initial Model



8. 3D Classification



9. Consensus Refinement



Post-Process

10. Postprocessing

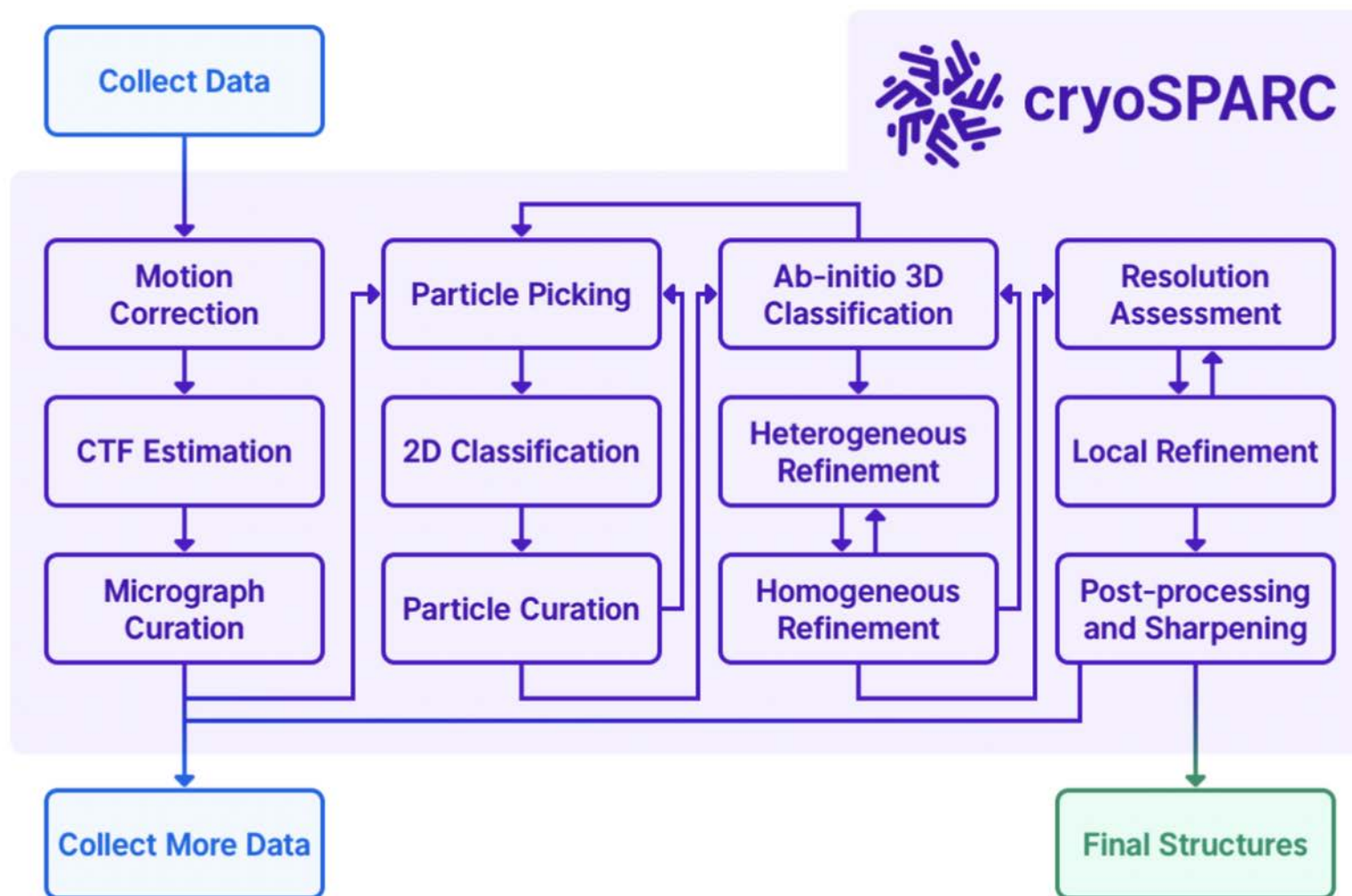
- Signal subtraction
- Focus refinement
- CTF refinement
- Bayesian Polishing
- Sharpening

Typical Workflow of Image Processing in cryoSPARC

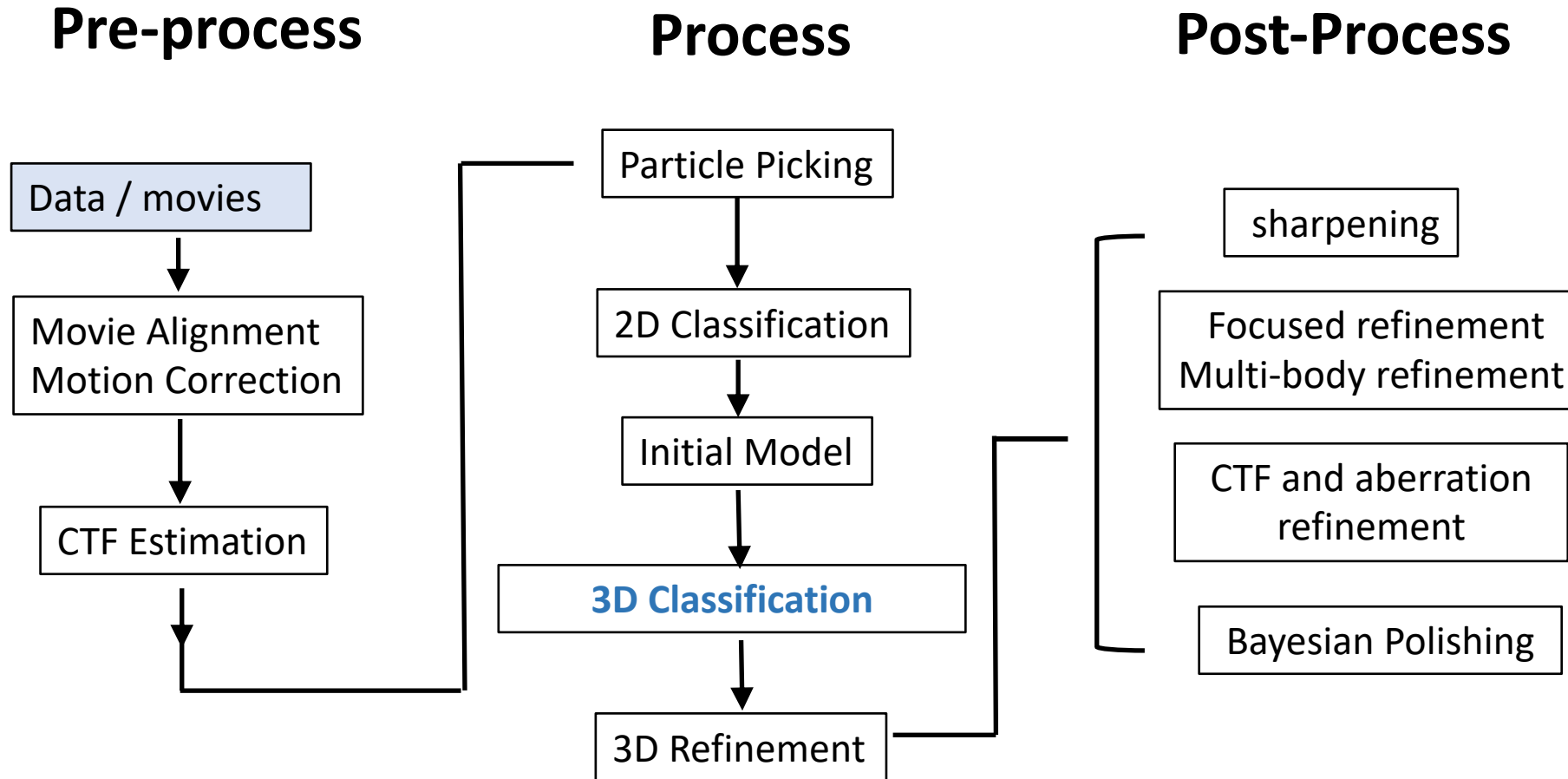
Pre-process

Process

Post-Process



Typical Workflow of Image Processing in RELION



Organization: Projects, Workspaces, Jobs

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

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

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

CryoSPARC Web Interface: Dashboard

Navigation bar

Navigation control

Side bar

The screenshot displays the CryoSPARC web interface dashboard. At the top, there is a navigation bar with tabs for 'P16 T20-Proteosome' and 'W1 tutorial', and a 'New Job' button. Below the navigation bar is a navigation control area with filters for 'Starred', 'Mine', 'Tags', 'Status', 'Filters', and 'Date Created'. The main content area is divided into several job panels:

- J1 Import Movies:** Shows 46 Movies with a resolution of 0.66 Å, 300 kV, 53 e/Å², and 38 x 7676 pixels. It includes a timer of 00m 22s and 6 settings.
- J2 Patch Motion Corr.:** Shows 0 Micrographs and is marked as 'completed'. It includes a timer of 01m 02s, 'default' settings, and 2 settings.
- J3 Patch Motion Corr.:** Shows 46 Micrographs with a graph of 'raw curve', 'smooth curve', and 'smooth fit'. It includes a timer of 03m 37s, 'default' settings, and 4 settings.
- J4 Patch CTF:** Shows 46 Exposures with a 3D surface plot. It includes a timer of 01m 02s, 'default' settings, and 4 settings.
- J5 Blob Picker:** Shows 51,410 Particles and 1,118 P/Mic. It includes a timer of 00m 33s, 'default' settings, and 3 settings.
- J6 Inspect Picks:** Shows 20,108 Particles and 437 P/Mic.
- J7 Extract Mics. (G):** Shows 17,913 Particles and 376 P/Mic.

The sidebar on the right 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:** (Collapsed)
- Statistics:** Last Updated: Jun 20, 2023, 10:05:30 PM; Total Jobs: 25; Job Status: 23 completed (1 building, 1 failed).
- Actions:** View Workspace →

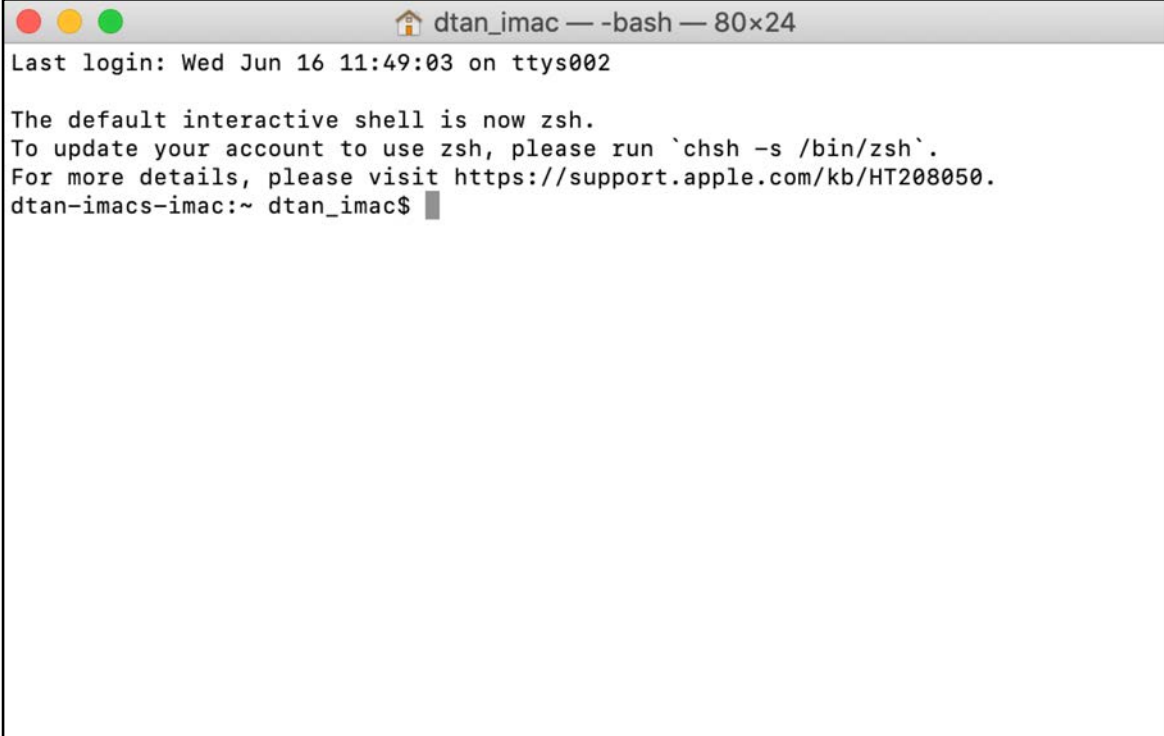
- **Movie are image stacks (files with extension like .mrc, .tiff, .spi, or .eer)**
- **Metadata (text files with extension .star in RELION and .csv in cryoSPARC)**

Tutorial Data Set: T20S Proteasome (the [EMPIAR-10025](#) dataset)

- Subset of 22 movies in MRC format
- Data collected at a 300kV microscope
- Pixel size: 0.6575 Å
- Total Dosage : 53 e/ Å²
- Stable and homogeneous complex
- 750 kDa protein complex with D7 symmetry

Practical: Create project, organize, import data

1. Make a project director.
2. Create a folder to store all the raw data/movies (or their symbolic links) under the project directory

A terminal window titled "dtan_imac — -bash — 80x24" with a home icon. The window shows the following text:

```
Last login: Wed Jun 16 11:49:03 on ttys002

The default interactive shell is now zsh.
To update your account to use zsh, please run `chsh -s /bin/zsh`.
For more details, please visit https://support.apple.com/kb/HT208050.
dtan-imacs-imac:~ dtan_imac$
```

RELION 4.0 GUI

The screenshot shows the RELION 4.0 GUI interface. The window title is "RELION-4.0-beta-2: /data1/HP1-zNuc/combined_polish". The menu bar includes "File", "Jobs", "I/O", "Reference", "CTF", "Optimisation", "Auto-sampling", "Helix", "Compute", and "Running".

Jobs: A list of job actions is shown on the left, with "3D auto-refine" selected. An arrow points to this list with the label "Jobs".

Parameters to set up the job/run: The main area contains input fields for "Input images STAR file", "Continue from here", "Reference map", and "Reference mask (optional)", each with a "Browse" button. An arrow points to these fields with the label "Parameters to set up the job/run".

Buttons: "Schedule", "Check command", and "Continue!" buttons are located below the parameter fields.

Job Selection: Below the buttons are "I/O view" and "Job actions" buttons. An arrow points to the "Job actions" button with the label "Set alias of the job".

Current Job: The "Current:" field shows "018: Refine3D/subtract-J17/". To its right is a "Display:" dropdown menu. An arrow points to this dropdown with the label "Display with dropdown menu to inspect the results".

Job Status: The bottom section shows the status of the current job: "Auto-refine: Refinement has converged, stopping now...". An arrow points to this text with the label "Job status".

Errors: The bottom-most section is a log area. An arrow points to it with the label "Errors will show up here".

Job Lists: The interface includes sections for "Finished jobs", "Running jobs", and "Scheduled jobs". The "Finished jobs" list contains entries like "020: PostProcess/job020/" and "018: Refine3D/subtract-J17/".

Input and Output: There are sections for "Input to this job" (showing "017: Subtract/job017/" and "016: MaskCreate/job016/") and "Output from this job" (showing "020: PostProcess/job020/" and "019: MaskCreate/job019/").