

Single-Particle cryo-EM Image Processing

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- Overview of single-particle data processing workflow
- Demo using RELION
- Demo using cryoSPARC
- Q&A.

Data/Movie

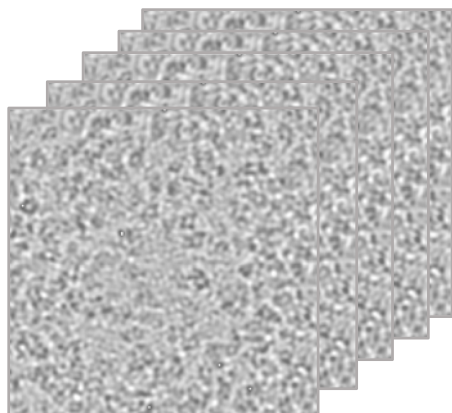
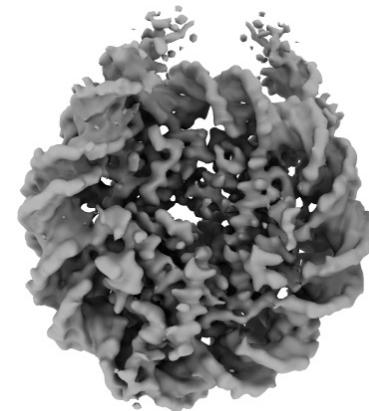


Image processing

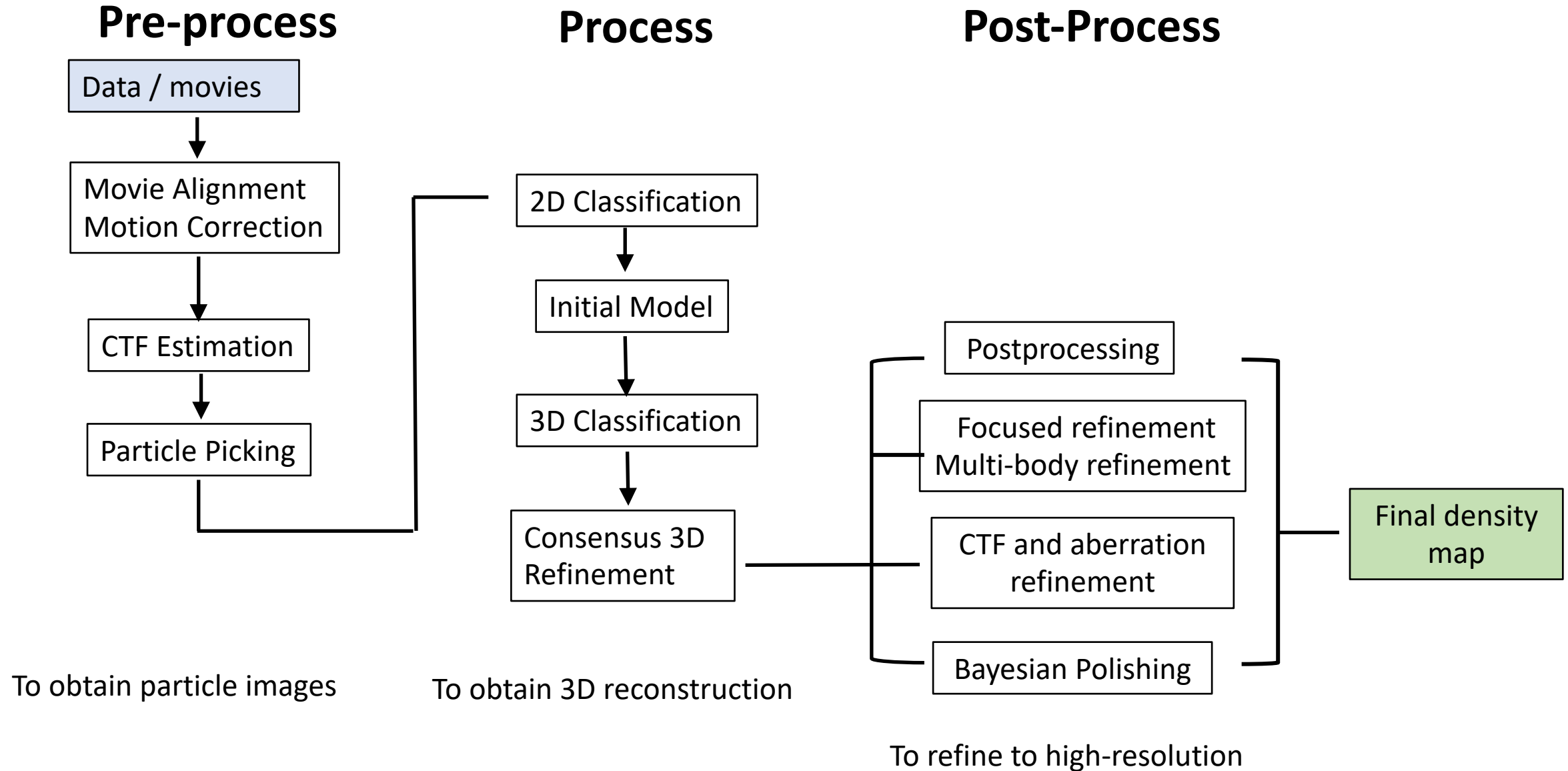


**Relion, cryoSPARC,
cisTEM, EMAN et al.**

**atomic resolution
density map**



Overall Workflow of Image Processing

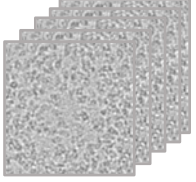


Pre-process

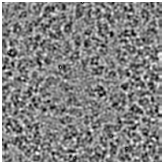
Process

Post-Process

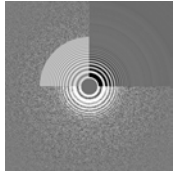
1. Movie/Frames



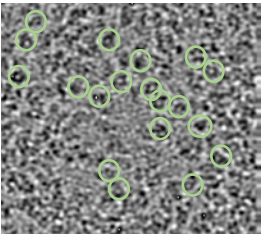
2. Aligned & averaged movie



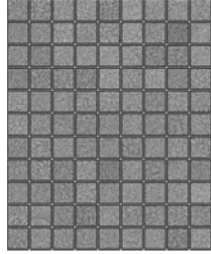
3. Defocus determination & CTF correction



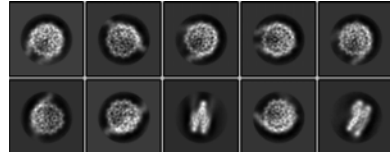
4. Particle picking



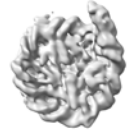
5. Extraction & normalization of particles



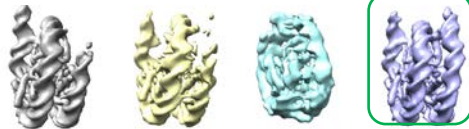
6. 2D Classification



7. Initial Model



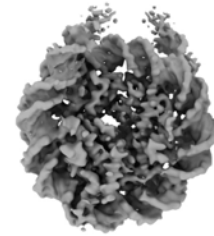
8. 3D Classification



9. Consensus Refinement



10. Postprocessing



CTF refinement,
Bayesian Polishing,
focus refinement

- **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: beta-galactosidase

- Subset of 24 movies in TIFF format
- Data collected at a 200kV microscope
- Pixel size: 0.885 Å
- Dose rate: 1.277 e/ Å²
- Stable and homogeneous complex
- 465 kDa protein complex with D2 symmetry

Relion 4.0 tutorial

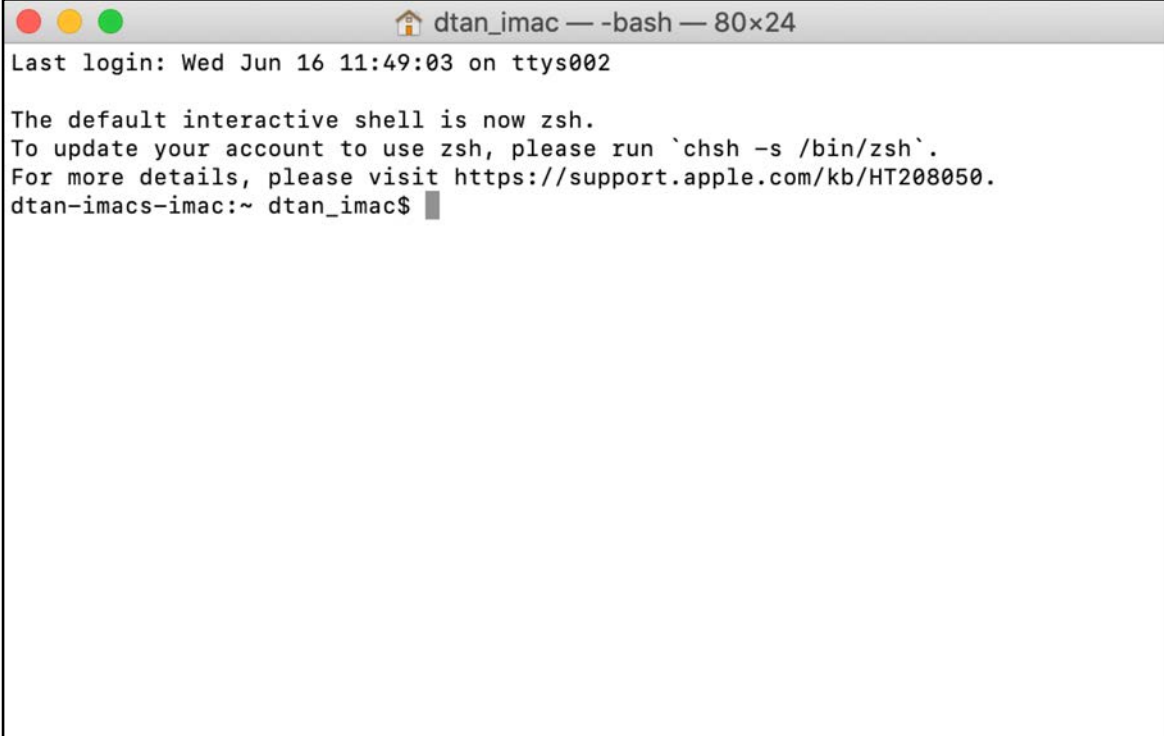
https://relion.readthedocs.io/en/latest/SPA_tutorial/index.html

cryoSPARC tutorial

<https://guide.cryosparc.com/processing-data/cryo-em-data-processing-in-cryosparc-introductory-tutorial>

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



```
dtan_imac — -bash — 80x24
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-illac:~ 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. The "Current:" field shows "018: Refine3D/subtract-J17/". An arrow points to the "Job actions" button with the label "Set alias of the job".

Display with dropdown menu to inspect the results: The "Display:" field has a dropdown menu. An arrow points to it 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 will show up here: Below the job status is a large text area for logs. An arrow points to this area 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: The "Input to this job" section lists "017: Subtract/job017/" and "016: MaskCreate/job016/". The "Output from this job" section lists "020: PostProcess/job020/" and "019: MaskCreate/job019/".

cryoSPARC Dashboard

Web browser

The screenshot shows the cryoSPARC web dashboard in a browser window. The address bar displays 'localhost:8080/projects/P6/W2'. The interface includes a top navigation bar with project names 'P6: Z-mononuc' and 'W2: Znuc_11a13_combined'. The main area is divided into several panels: 'J9' and 'J10' for 'Import Particles' (showing grids of micrographs), 'J11' through 'J14' for 'Import Volumes' (showing 2D maps), 'J15' for 'Hetero Refinement', and 'J16' for 'Refinement' (showing a GSFSC resolution plot). A right-hand sidebar titled 'JOB BUILDER' contains a list of functions under categories like 'WORKFLOWS', 'IMPORTS', 'MOTION CORRECTION', and 'CTF ESTIMATION'. An arrow points to the 'Import Micrographs' option in the 'IMPORTS' section. The bottom of the dashboard features navigation tabs for 'Dashboard', 'Projects', and 'Resource Manager', along with a 'New update available: v3.2.0' notification and a user profile for 'Dongyan Tan'.

Jobs and functions

cryoSPARC user interface pages are served by a web server, which is responsible for displaying datasets, experiments, streaming real time results, user accounts, and updating.