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

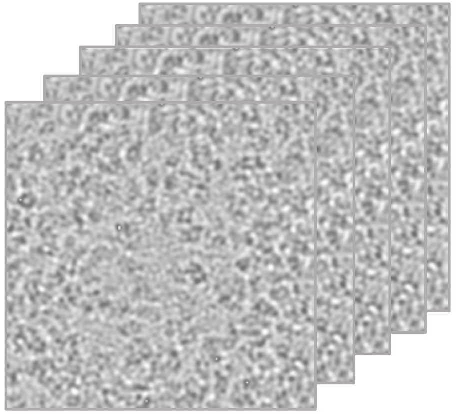
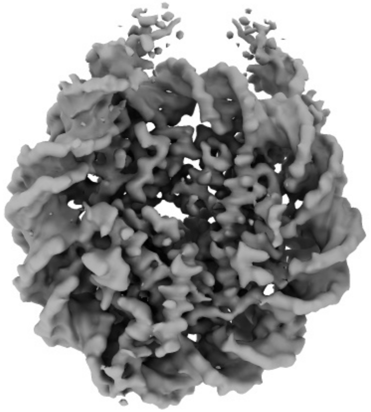


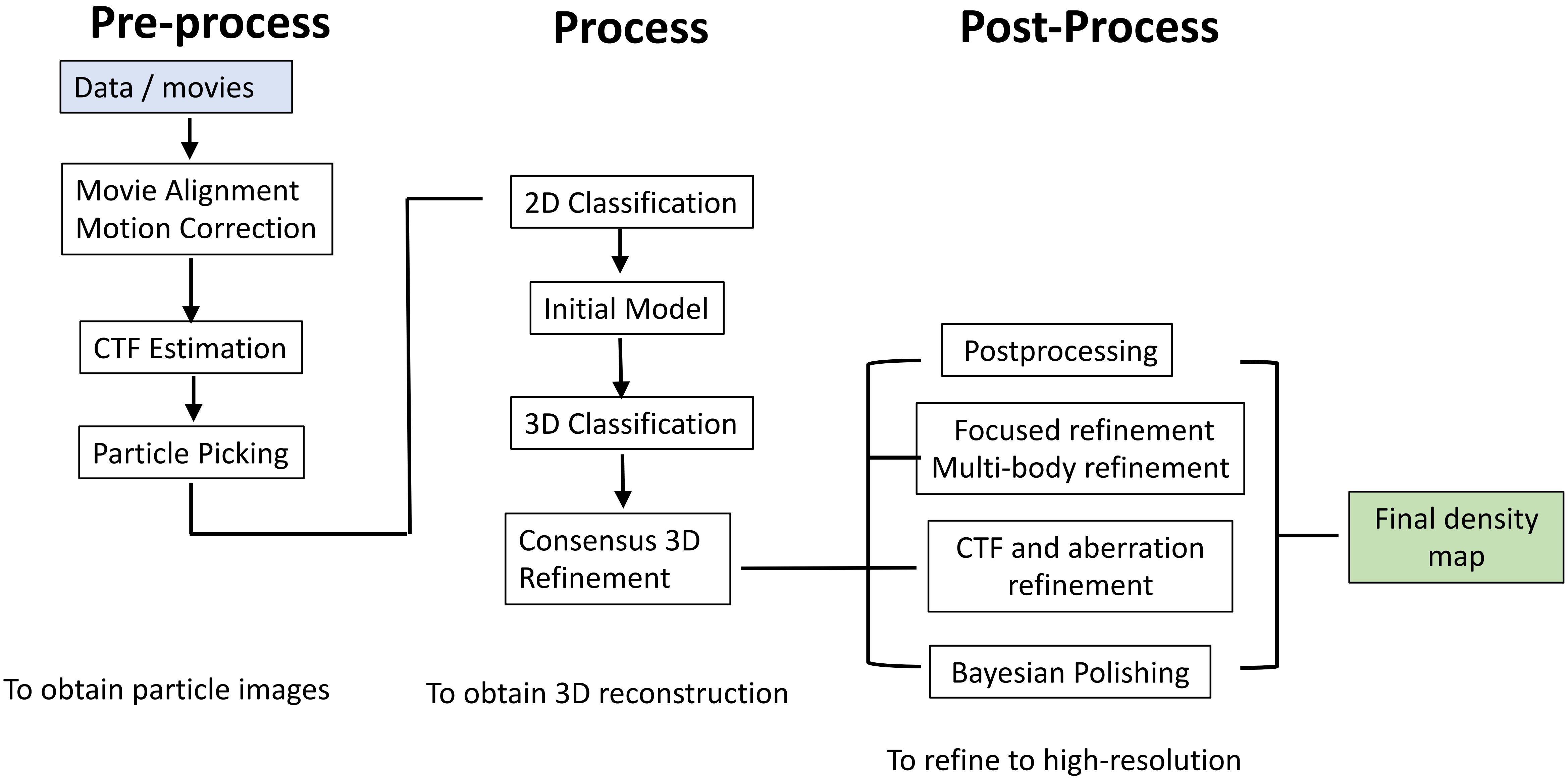
Image processing

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

atomic resolution
density map



Overall Workflow of Image Processing



Pre-process

Data / movies

Movie Alignment
Motion Correction

CTF Estimation

Particle Picking

To obtain particle images

Process

2D Classification

Initial Model

3D Classification

Consensus 3D Refinement

To obtain 3D reconstruction

Post-Process

Postprocessing

Focused refinement
Multi-body refinement

CTF and aberration refinement

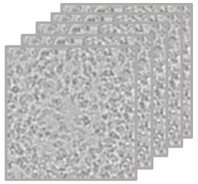
Bayesian Polishing

Final density map

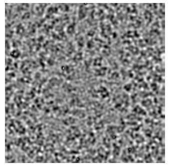
To refine to high-resolution

Pre-process

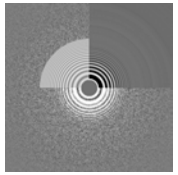
1. Movie/Frames



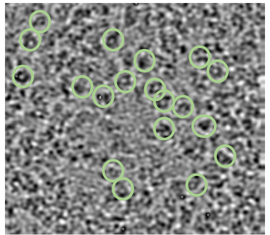
2. Aligned & averaged movie



3. Defocus determination & CTF correction

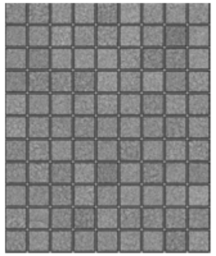


4. Particle picking

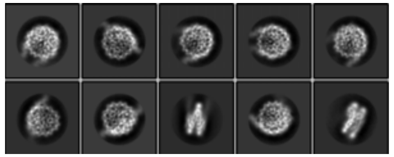


Process

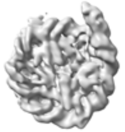
5. Extraction & normalization of particles



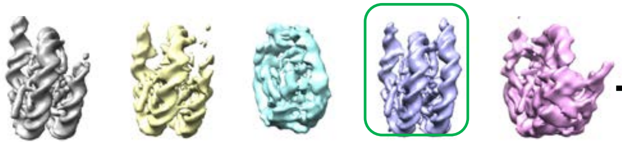
6. 2D Classification



7. Initial Model



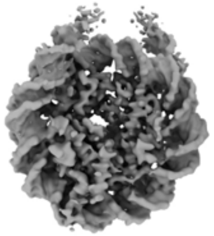
8. 3D Classification



9. Consensus Refinement

Post-Process

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/ $Å^2$
- 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

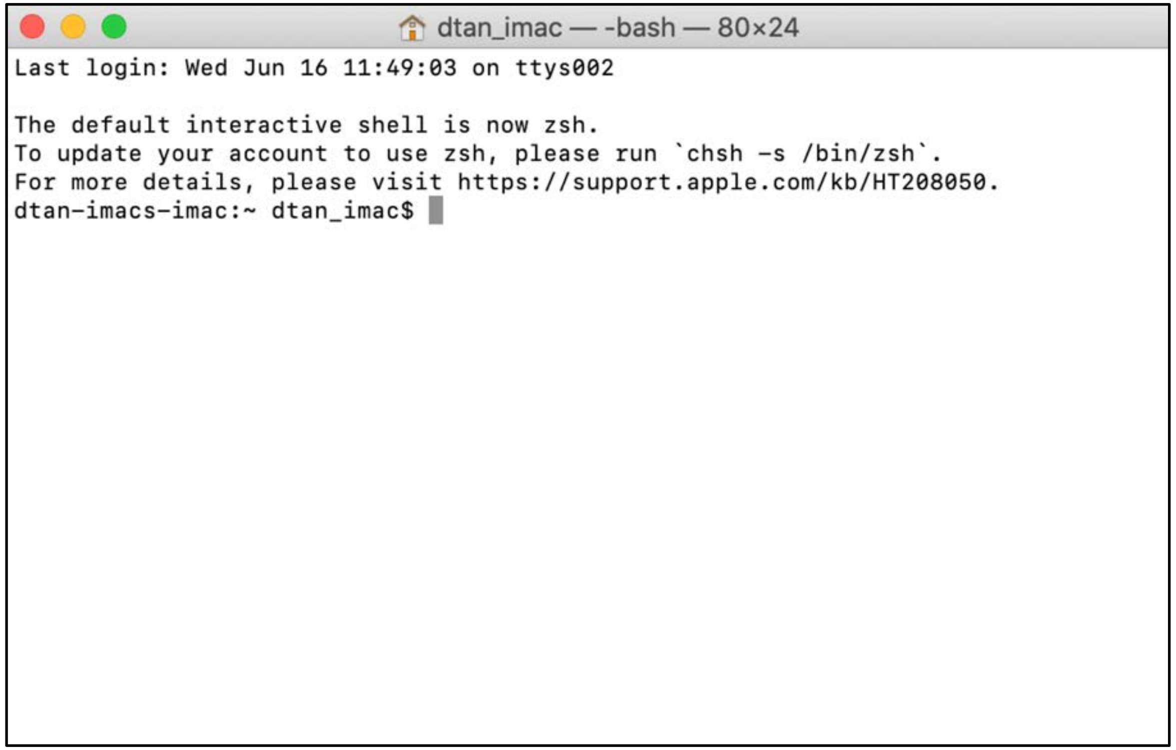
cryoSPARC tutorial

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.

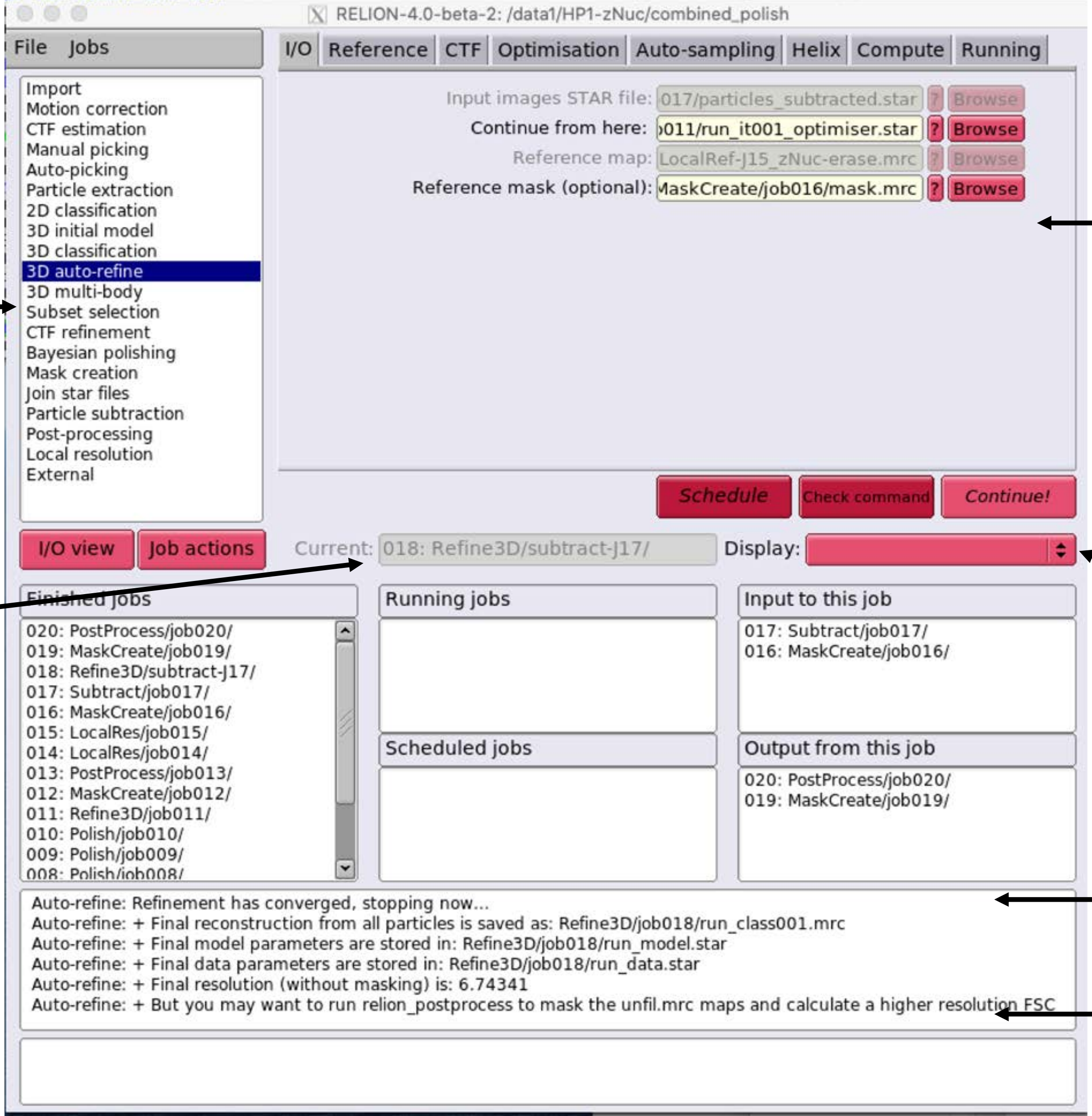
2. Create a folder to store all the raw data/movies (or their symbolic links) under the project directory



```
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



Input images STAR file: 017/particles_subtracted.star

Continue from here:)011/run_it001_optimiser.star

Reference map: LocalRef-J15_zNuc-erase.mrc

Reference mask (optional): MaskCreate/job016/mask.mrc

Current: 018: Refine3D/subtract-J17/

Finished jobs

020: PostProcess/job020/
019: MaskCreate/job019/
018: Refine3D/subtract-J17/
017: Subtract/job017/
016: MaskCreate/job016/
015: LocalRes/job015/
014: LocalRes/job014/
013: PostProcess/job013/
012: MaskCreate/job012/
011: Refine3D/job011/
010: Polish/job010/
009: Polish/job009/
008: Polish/job008/

Input to this job

017: Subtract/job017/
016: MaskCreate/job016/

Output from this job

020: PostProcess/job020/
019: MaskCreate/job019/

```
Auto-refine: Refinement has converged, stopping now...
Auto-refine: + Final reconstruction from all particles is saved as: Refine3D/job018/run_class001.mrc
Auto-refine: + Final model parameters are stored in: Refine3D/job018/run_model.star
Auto-refine: + Final data parameters are stored in: Refine3D/job018/run_data.star
Auto-refine: + Final resolution (without masking) is: 6.74341
Auto-refine: + But you may want to run relion_postprocess to mask the unfil.mrc maps and calculate a higher resolution FSC
```

Parameters to set up the job/run

Jobs

Set alias of the job

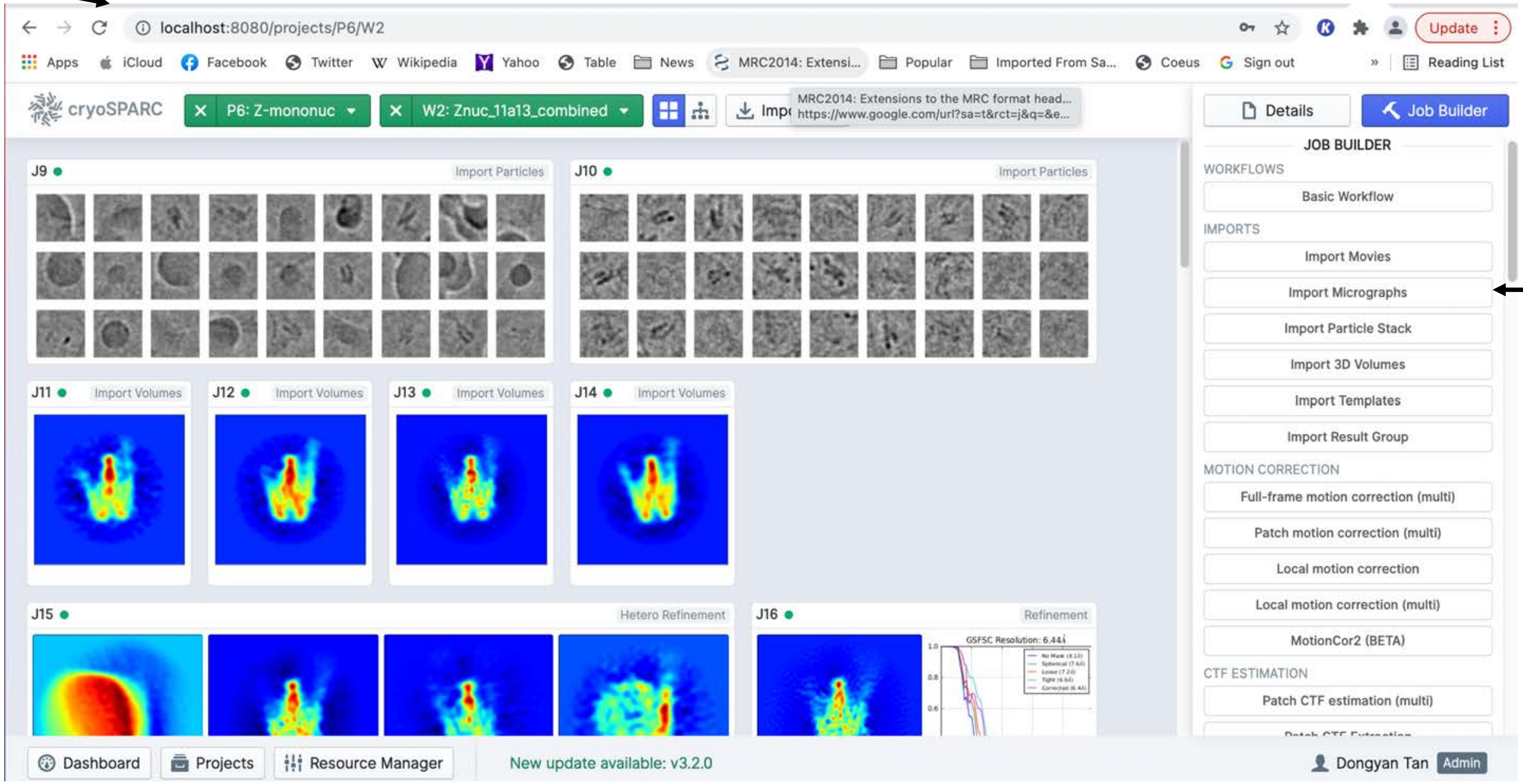
Display with dropdown menu to inspect the results

Job status

Errors will show up here

cryoSPAC Dashboard

Web browser



New update available: v3.2.0

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.