SEMC Appion workshop series

September 19, 2019

PART II :
SINGLE PARTICLE ANALYSIS

SIMONS ELECTRON
MICROSCOPY CENTER

New York Structural
Biology Center

NMR
CoMD/
NMR

X-ray
NYX
@NSLS-II

Protein Production
COMPPÅ

photo by Swapnil Bhatkar

Schedule

September 19, 2019 10am-2pm

A practical workshop that continues the single-particle analysis workflow from the Part 1 workshop. The topics include Appion advanced features, moving into commonly used packages and validation methods.

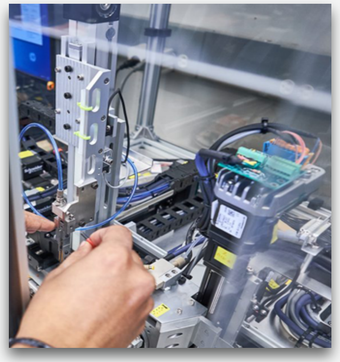
- **1. Workshop intro**
  - Overview of SPA processing and web tools
  - Advanced Appion features
  - *Photo*
- **2. Going from Appion to other packages**
  - RELION
  - cryoSPARC
- ***3. Validation & additional tools*** *[if time allows]*
  - FSC & 3D-FSC / local res & ResMap / other methods
- **4. Closing** *[Q&A with SEMC staff]*

What is Appion?

Appion is a "pipeline" for processing and analysis of EM images.

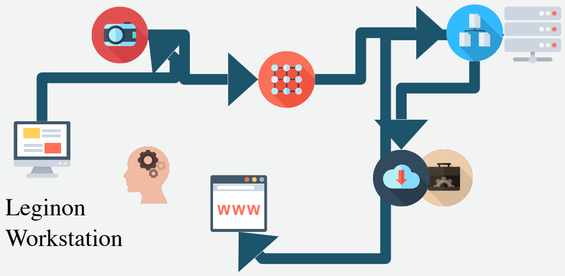
NRAMM

The overall mission of NRAMM is to develop, test and apply technology for automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.



Automated sample prep

Automated image acquisition



Automated image processing

Acknowledgements

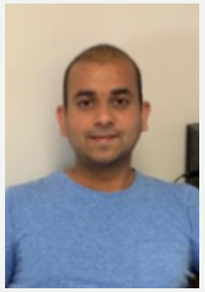
Some of this work was performed at the Simons Electron Microscopy Center at the New York Structural Biology Center that is supported by a grant from the Simons Foundation (Grant Number: SF349247) with additional support from: NIH S10 OD019994-01, the Agouron Institute (Grant Number: F00316), NIH S10 RR029300-01, NIH S10 RR017291-01, NYSTAR, and NIH C06 RR017528-01-CEM.

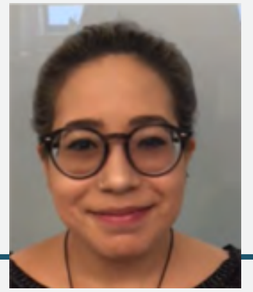
SEMC operations team

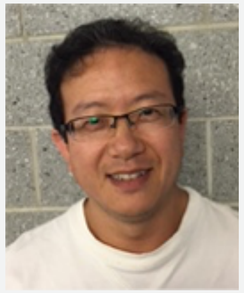
Directors

E M G @nysbc.org

Staff

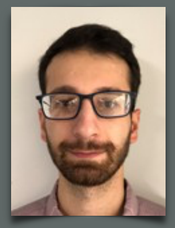






SEMC Appion development team

Swapnil Bahktar



Shaker Krit

Anchi Cheng

Sargis Dallakyan

Alex Noble

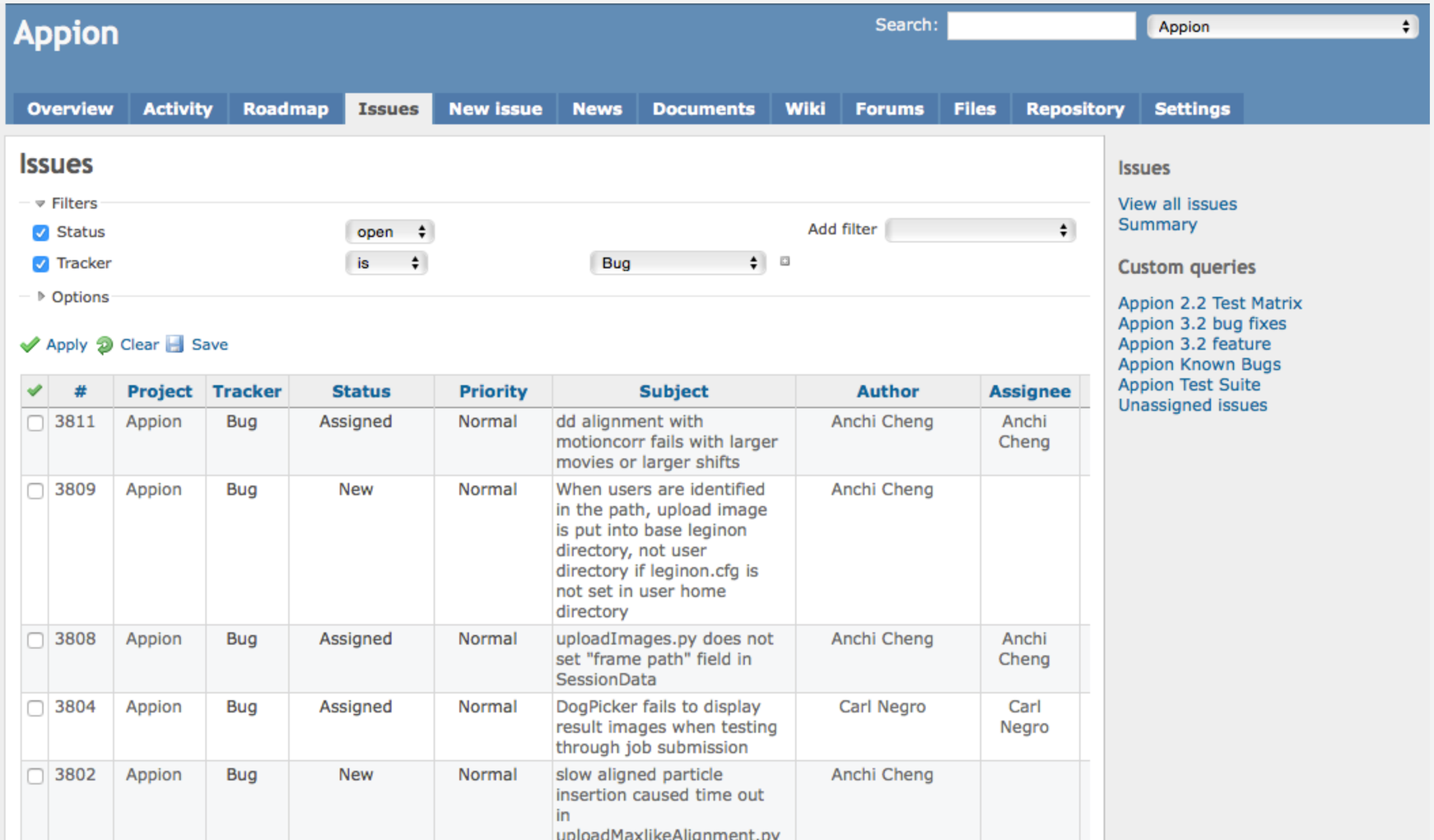
Tomography

SEMC Training

Type	Frequency	Title	Description
feedback	yearly	General user meeting	State of the center
knowledge	yearly	SEMC EM Course	Theory behind EM *Graduate level course*
computation	quarterly	SEMC Appion workshop	Data processing workshops
access	monthly	SEMC New User Orientation	Facility use and Safety Training Sample preparation [neg-stain & cryo] *Leginon intro/use of screening microscopes*
troubleshooting	weekly	User Project Discussion Meetings	*Appointments: Tue @3pm, Thurs @3pm & @3:30pm*
instrumentation	daily	Advanced Leginon use / 1-1 training appointments	Training for independent use of the microscopes and other SEMC resources

appion.org

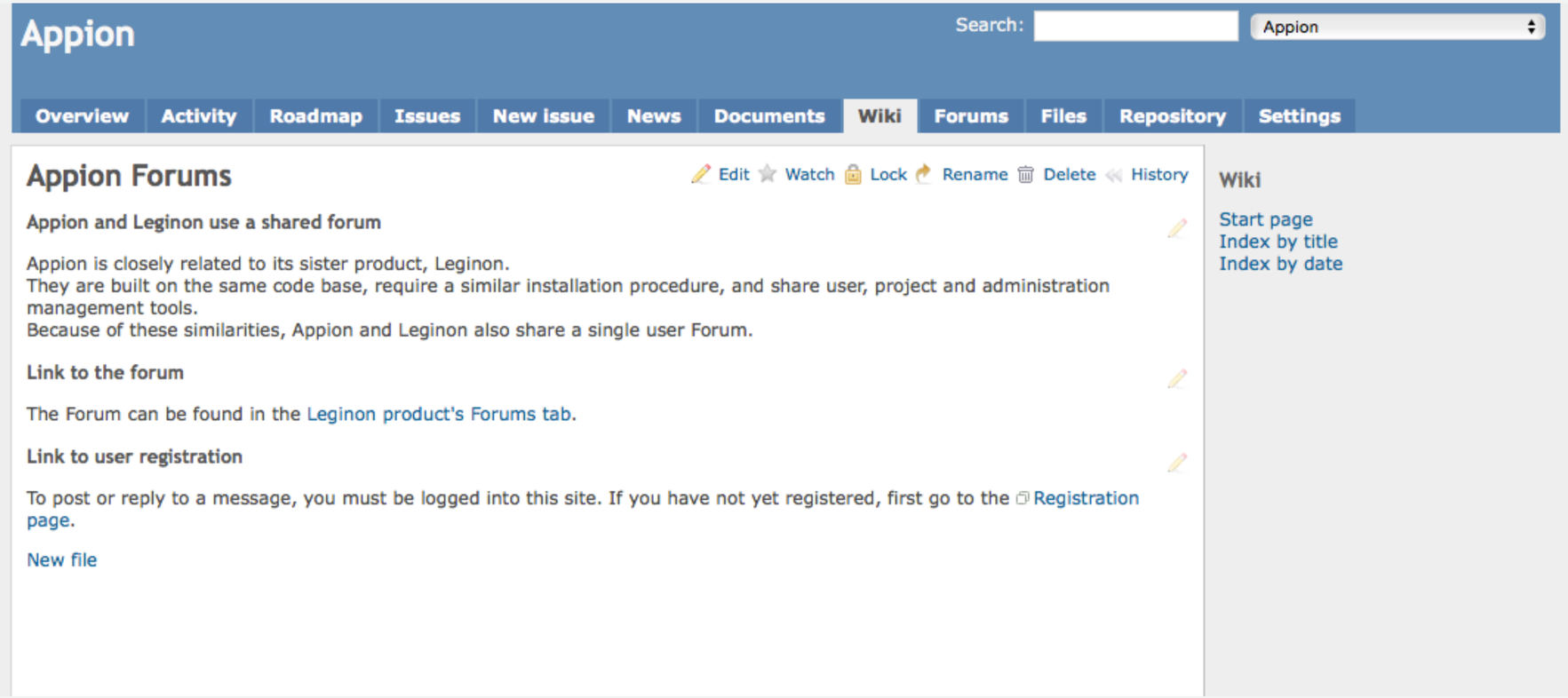
http://emg.nysbc.org/redmine/projects/nysbc-semc-user-group/issues



#	Project	Tracker	Status	Priority	Subject	Author	Assignee
3811	Appion	Bug	Assigned	Normal	dd alignment with motioncorr fails with larger movies or larger shifts	Anchi Cheng	Anchi Cheng
3809	Appion	Bug	New	Normal	When users are identified in the path, upload image is put into base leginon directory, not user directory if leginon.cfg is not set in user home directory	Anchi Cheng	
3808	Appion	Bug	Assigned	Normal	uploadImages.py does not set "frame path" field in SessionData	Anchi Cheng	Anchi Cheng
3804	Appion	Bug	Assigned	Normal	DogPicker fails to display result images when testing through job submission	Carl Negro	Carl Negro
3802	Appion	Bug	New	Normal	slow aligned particle insertion caused time out in uploadMaxlikeAlignment.py	Anchi Cheng	

appion.org

Register an account



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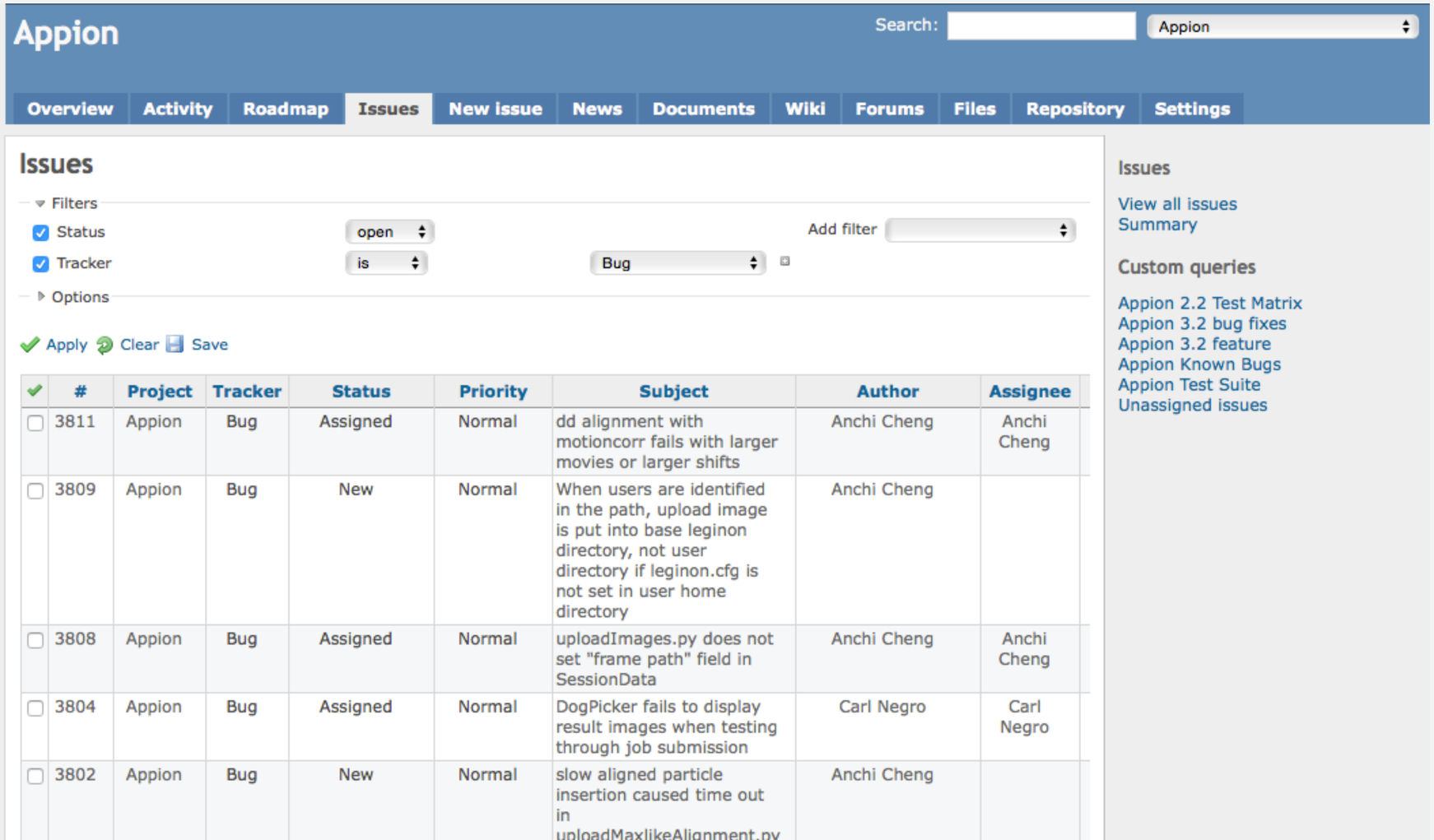

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Appion workshops

Single particle

Tomography

Part I

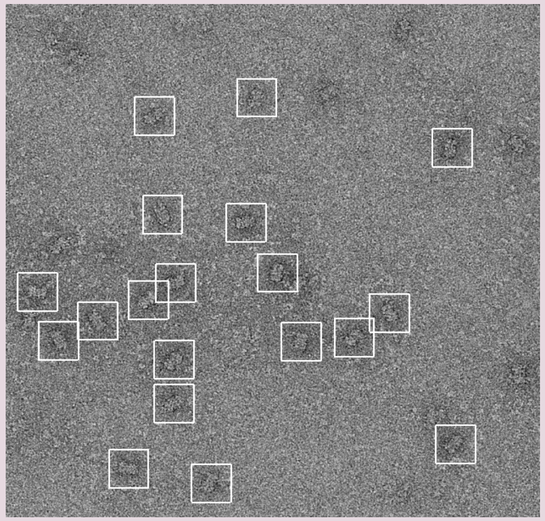
Part II

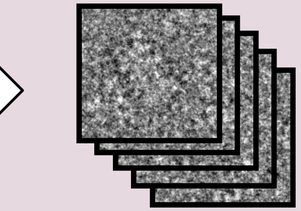
Part III

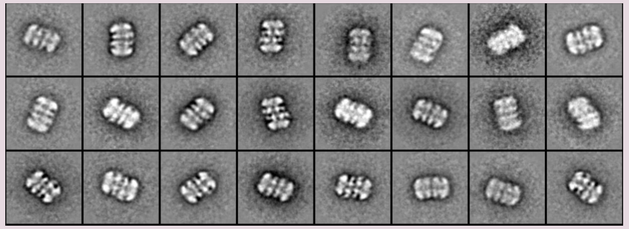
Tools to generate a particle stack and an initial model from micrographs.

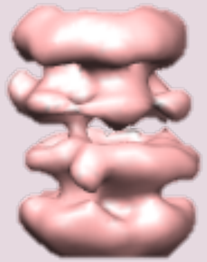
Advanced Appion tools, 3D tools and validation.

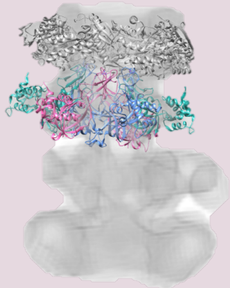
Tomography tools.











Data generation at SEMC

EM instrumentation

FEI Titan Krios#1 / #2 / #3
Falcon3 x3 | K2 x3

FEI Tecnai F20
DE20 | TVIPS 4K CMOS

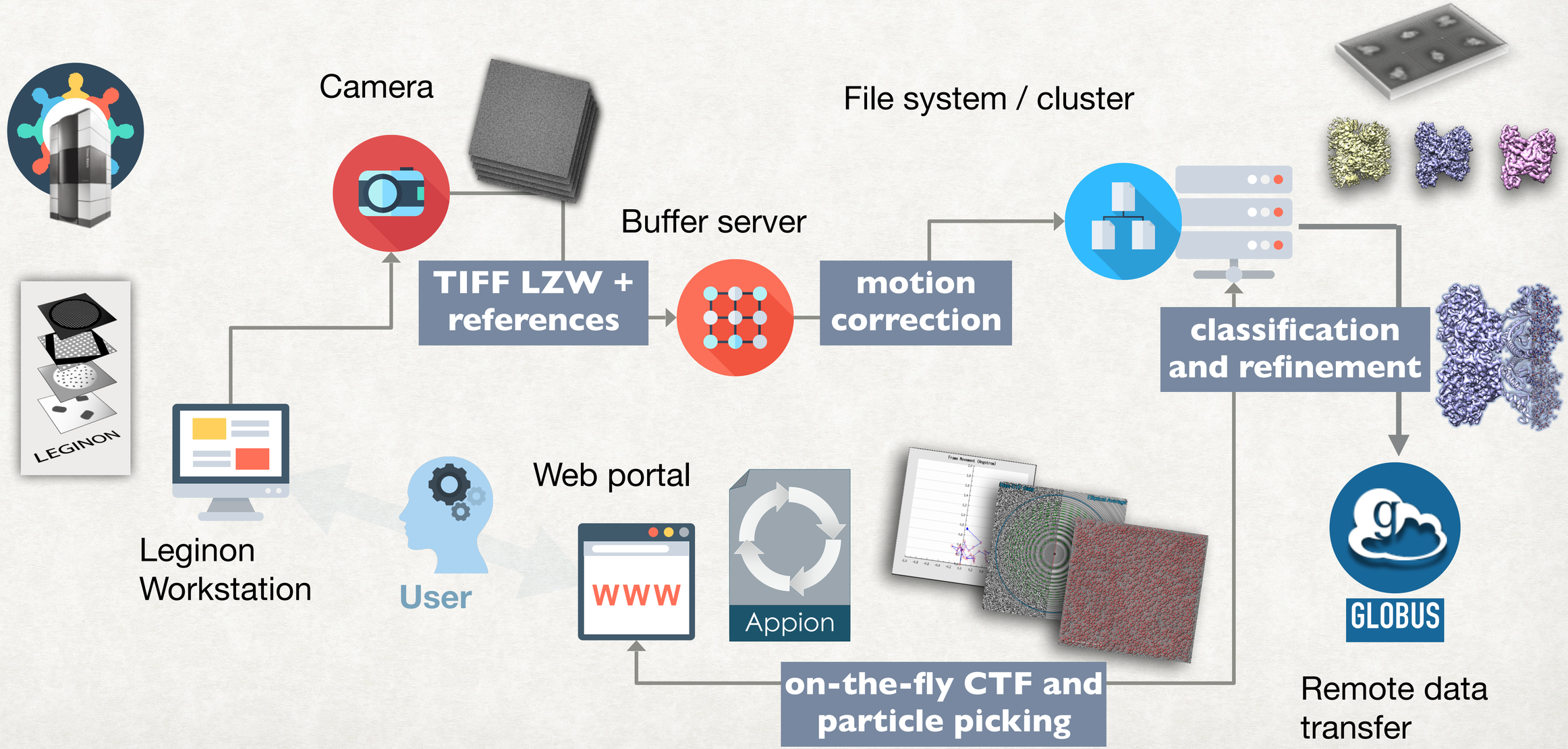
FEI Tecnai Biotwin
TVIPS 4K CMOS

JEOL 1230
Gatan US4000 CCD

FEI Helios 650
Quorum cryostage

Appion

DATA PIPELINE



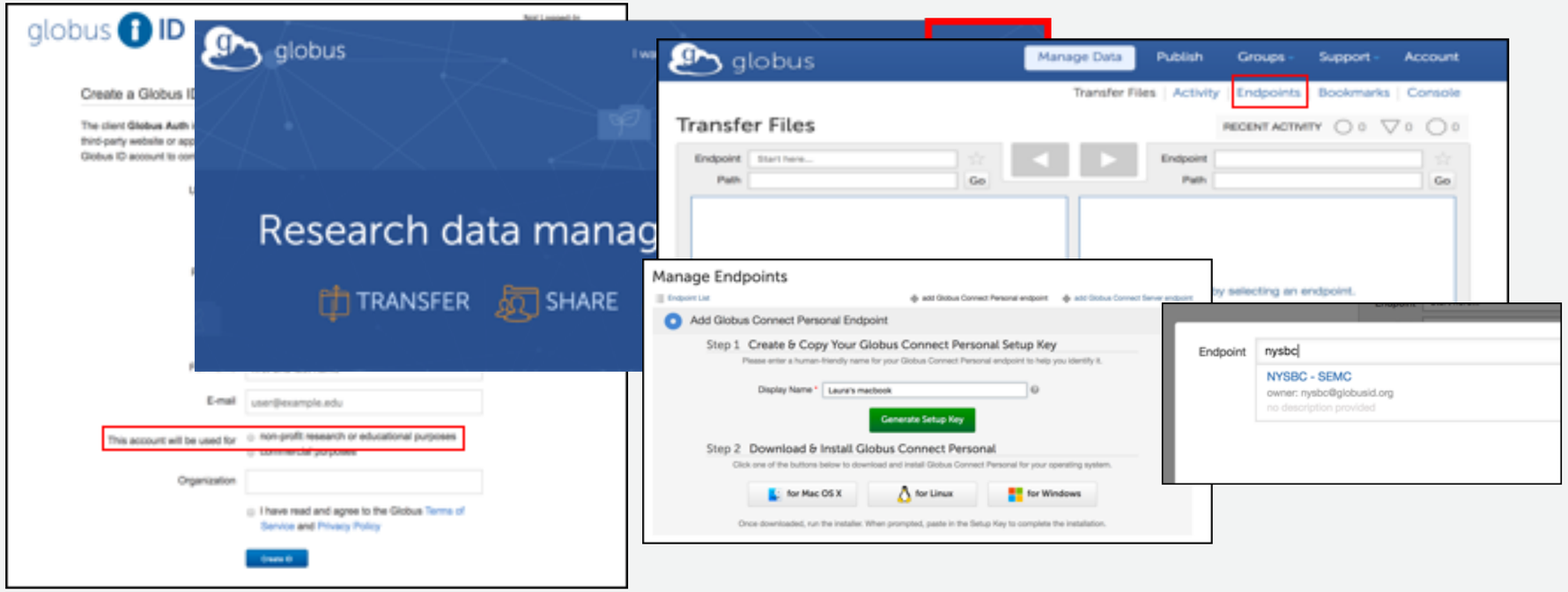
Remote data transfer

Remote data transfer

Create an account at globusid.org

Login at globus.org

Transfer files



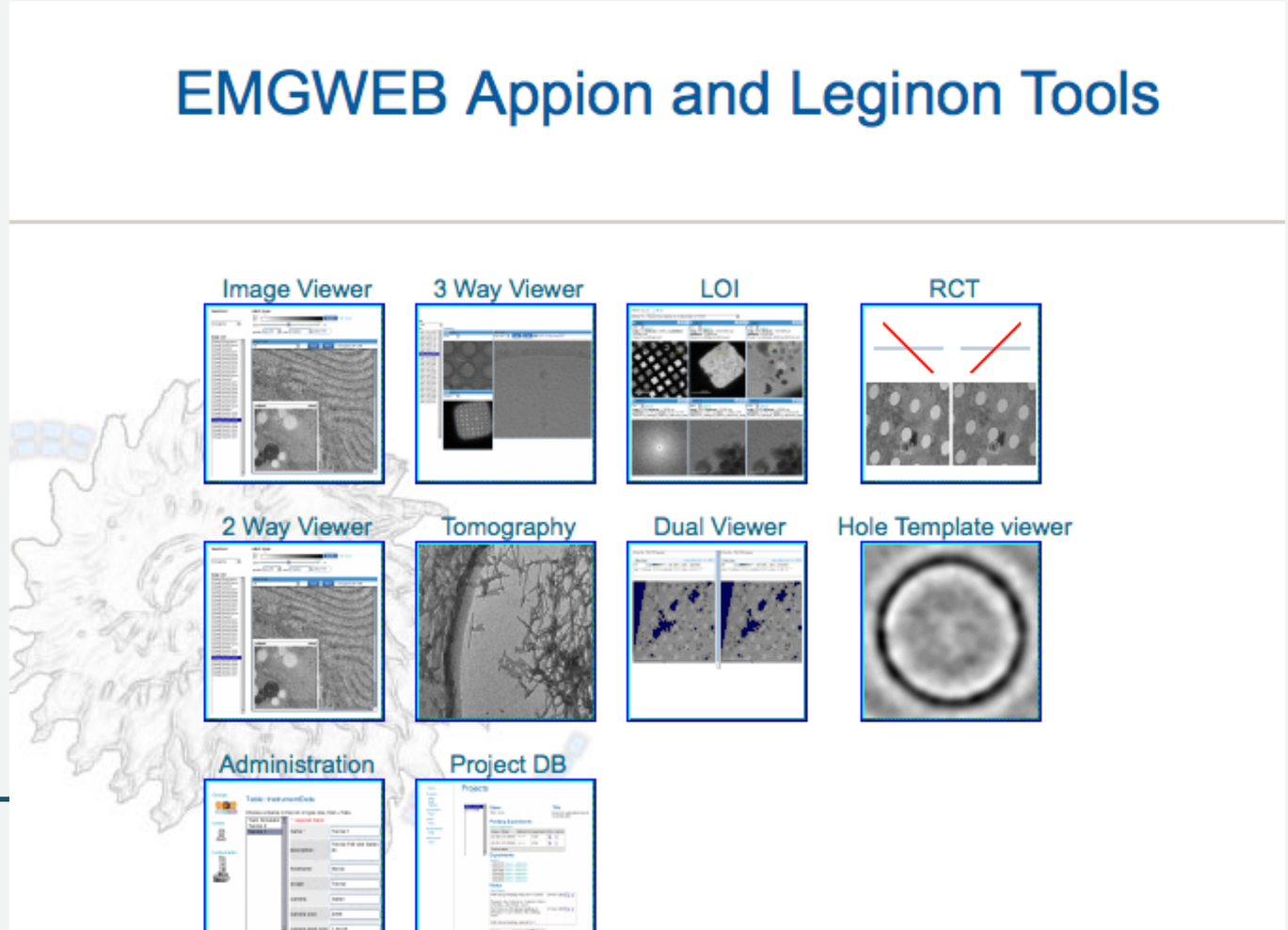
Start your web browsers

emgweb.nysbc.org

Login: test
Password: appion2019

Appion & Leginon webtools

emgweb.nysbc.org



Example: Single-particle workflow

During EM session

After EM session

Leginon session

Setting up workflow

Data acquisition

Workflow Optimization *if needed*

Appion session

Frame alignment

CTF estimation

Particle picking

Micrograph/ Particle curating

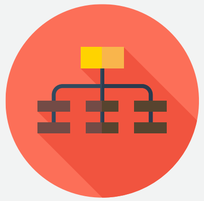
SEMC computing

Micrograph/ Particle sorting

Initial 2D classification

Initial model generation

3D refinement



Home institution computing/cluster

2D classification

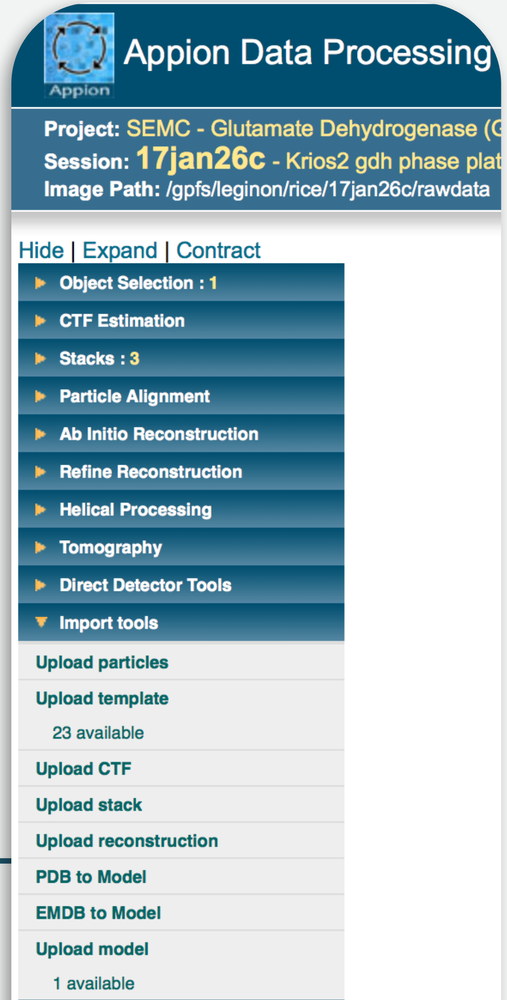
3D classification

3D refinement

Model building

Processing workflow

During EM session



Appion session

Frame alignment

↓

CTF estimation

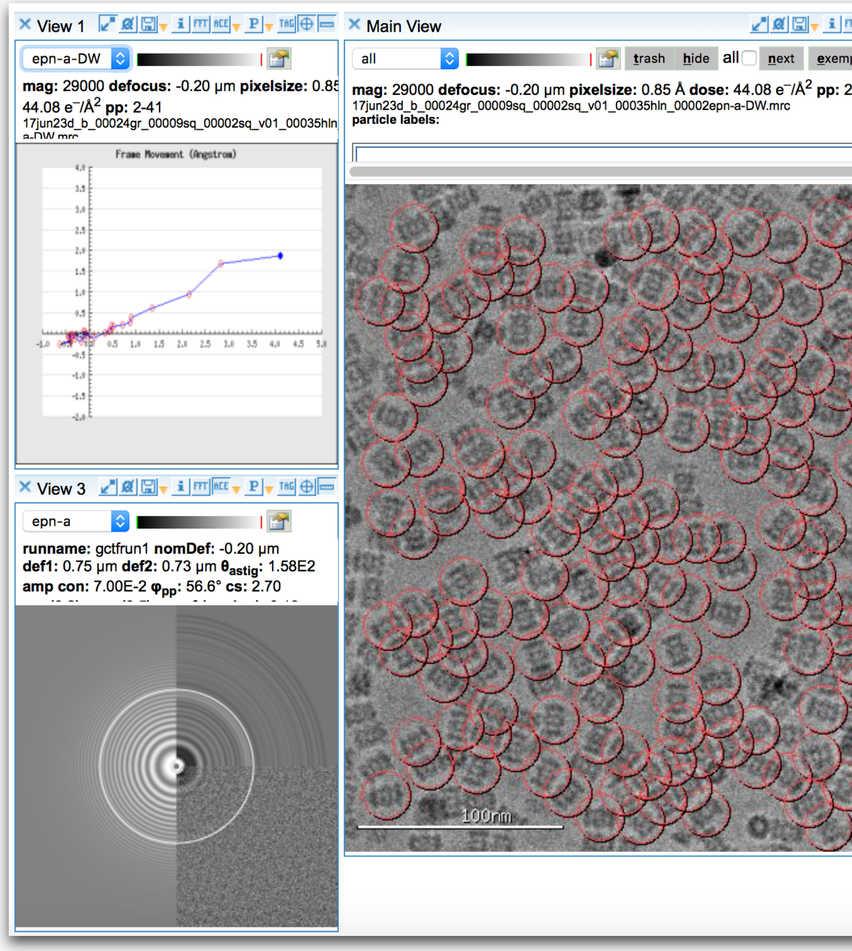
↓

Particle picking

↓

Micrograph/
Particle curating

3/4 users who use Leginon also have Appion sessions



Who does the processing workflow?

After EM session

Appion web interface

SEMC computing

Micrograph/
Particle sorting

↓

Initial 2D classification

↓

Initial model
generation

Home institution computing/cluster

2D classification

↓

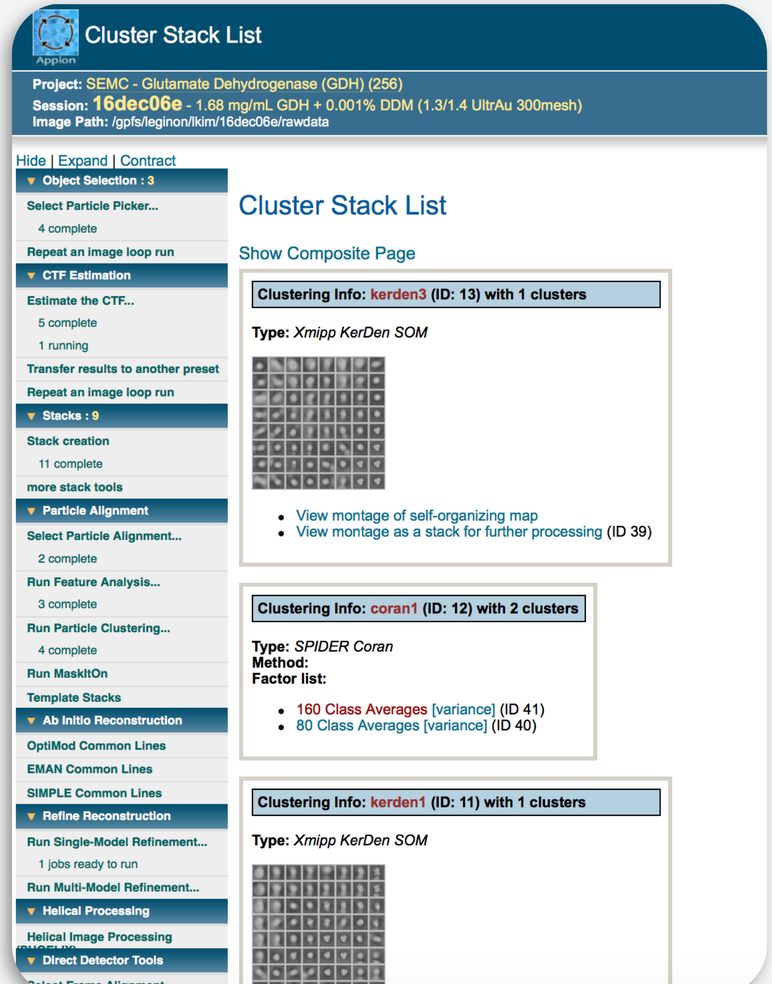
3D classification

↓

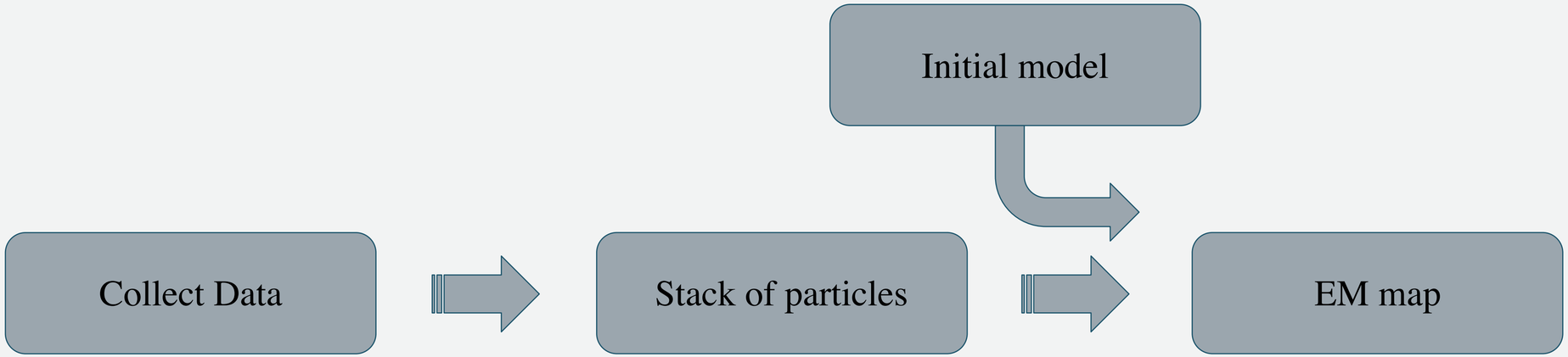
3D refinement

↓

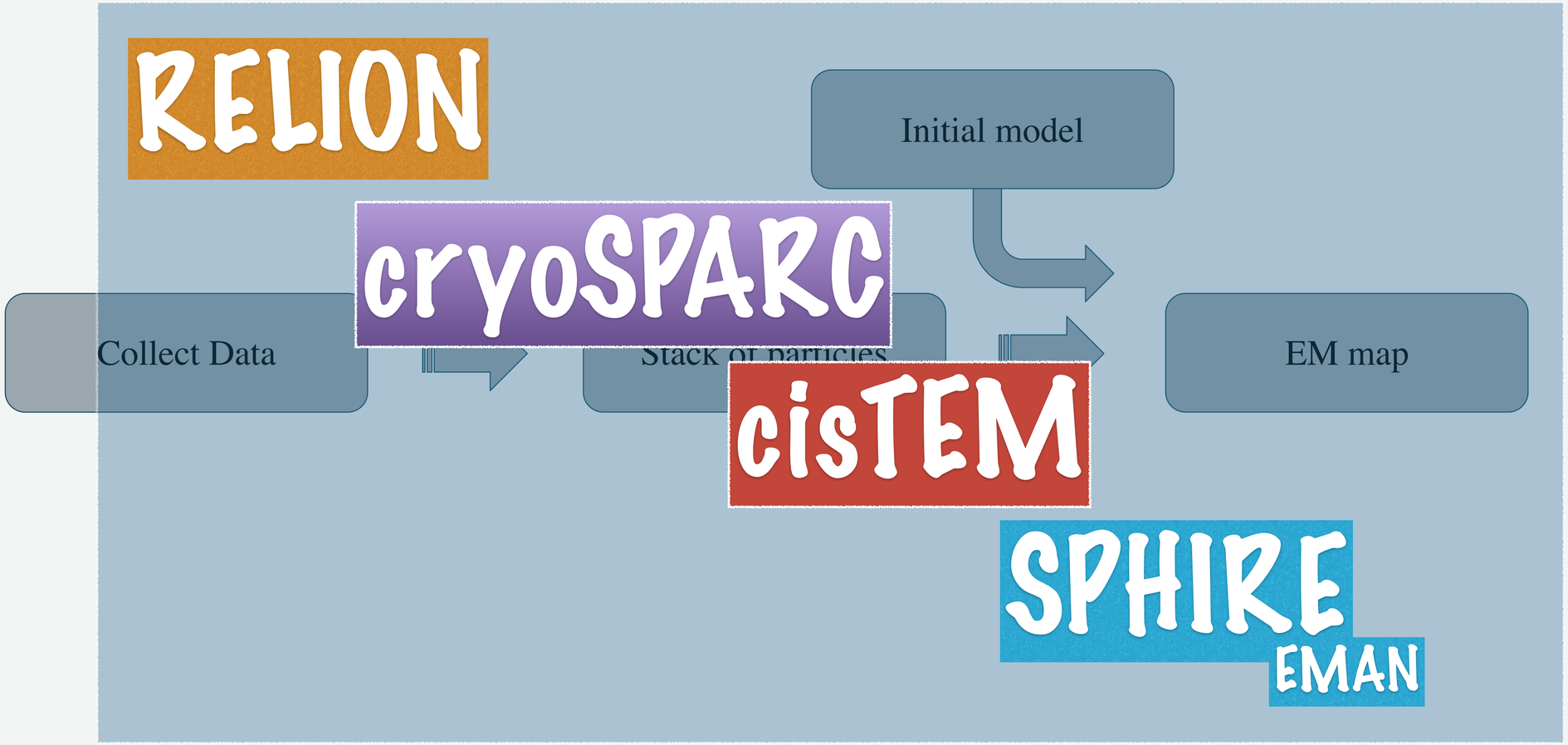
Model building



Single particle analysis

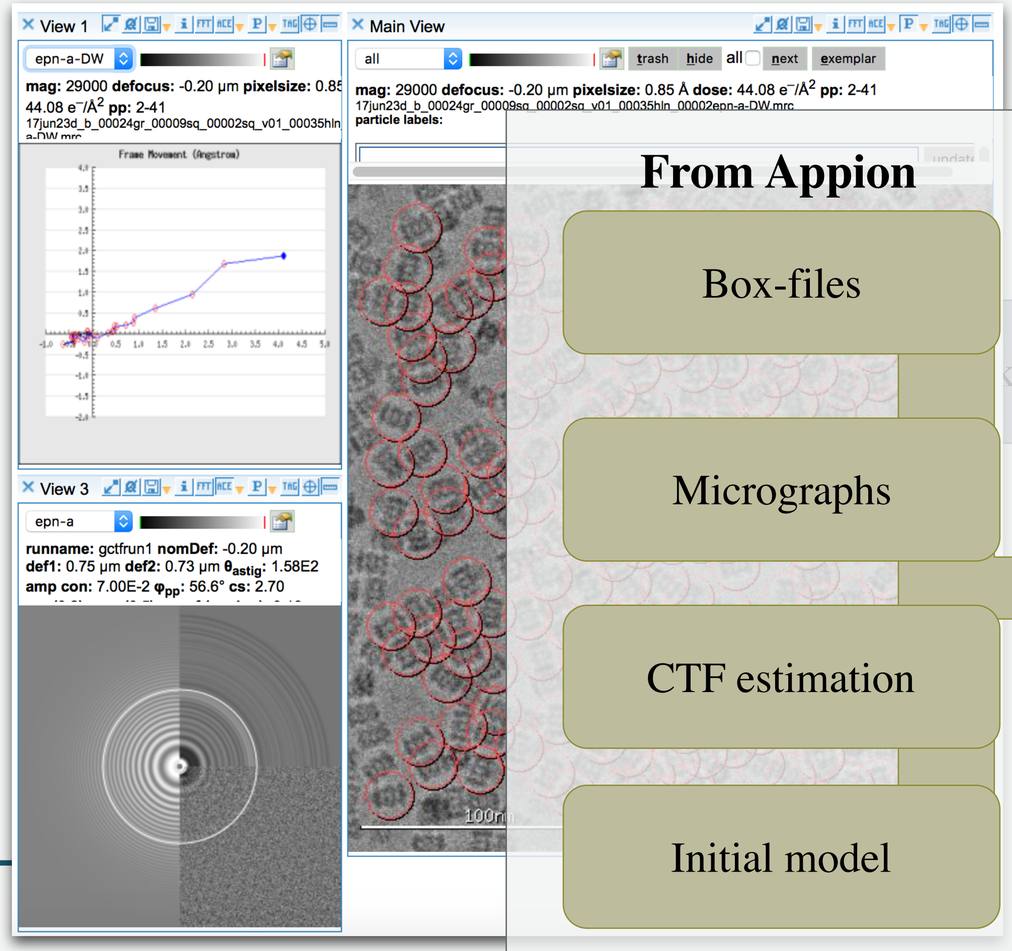


Single particle analysis



Single particle analysis

3/4 users who use Leginon also have Appion sessions



From Appion

- Box-files
- Micrographs
- CTF estimation
- Initial model

RELION/cryoSPARC

- Initial model
- Particle extraction
- 2D classification
- 3D refinement
- 3D classification
- EM map

Appion part I

- Frame alignment
- CTF estimation
- Particle picking
- Micrograph/ Particle curating
- Initial 2D classification
- Initial model generation

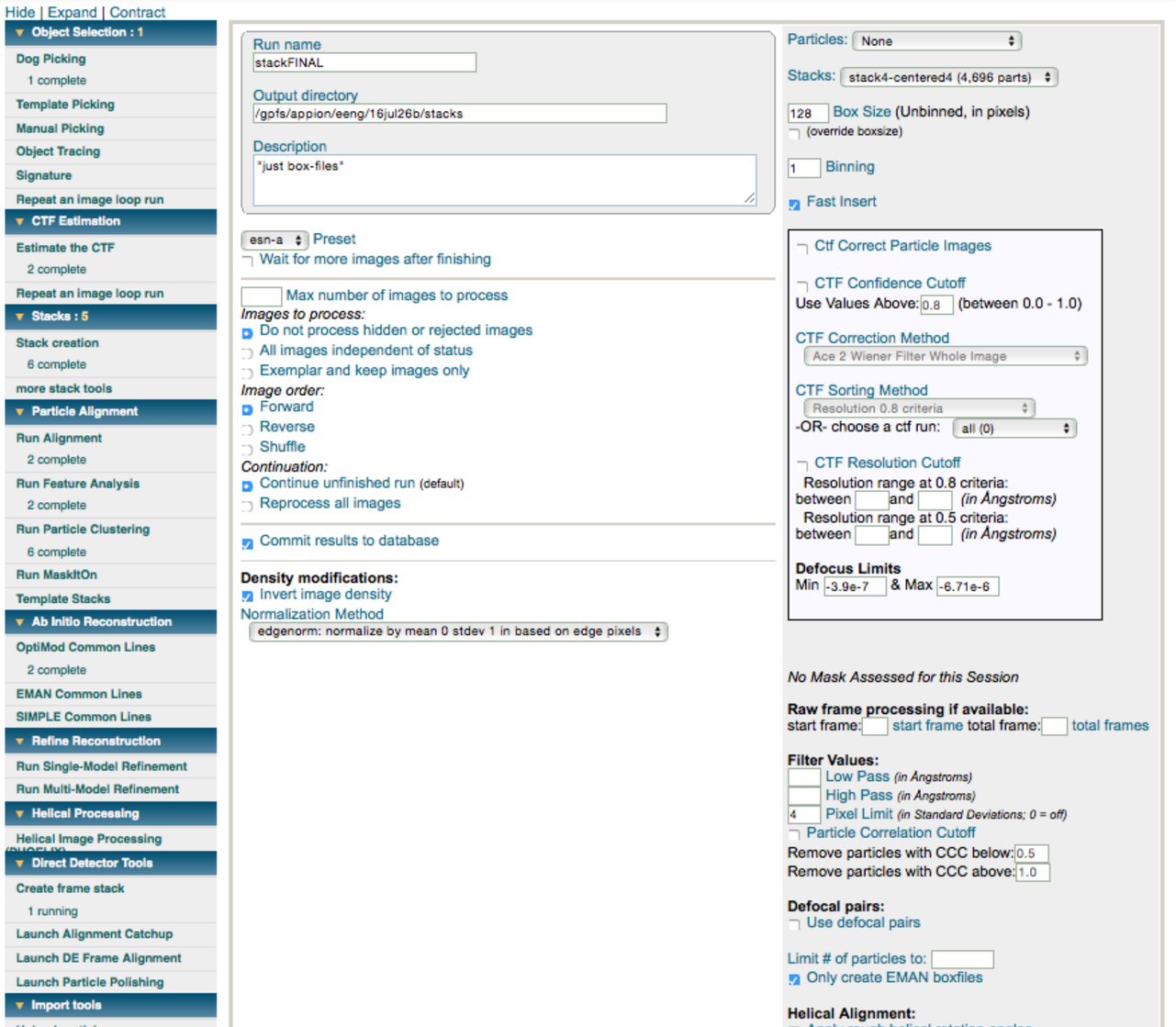
Appion part II

- 2D classification (particle sorting)
- *ab initio* model generation
- 3D classification (conformation sorting)
- 3D refinement

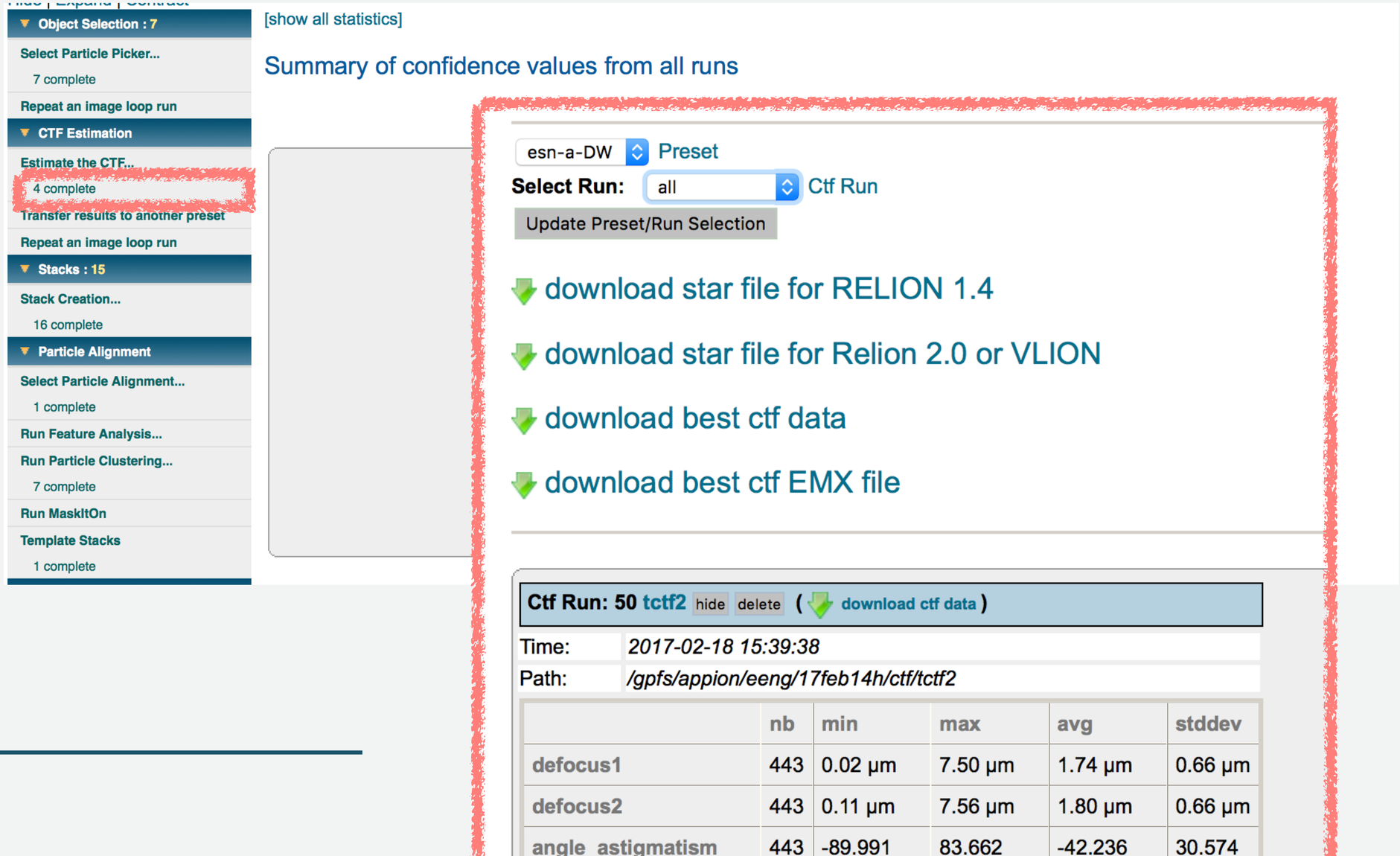
Going from Appion to RELION/cryoSPARC

Raw frames	/gpfs/frames/[username]/[sessionname]/rawdata/
Aligned Movies	/gpfs/leginon/[username]/[sessionname]/rawdata/
Particles	/gpfs/appion/[username]/[sessionname]/stacks/
CTF Estimation	/gpfs/appion/[username]/[sessionname]/CTF/

Selecting particles – box-files



Selecting CTF



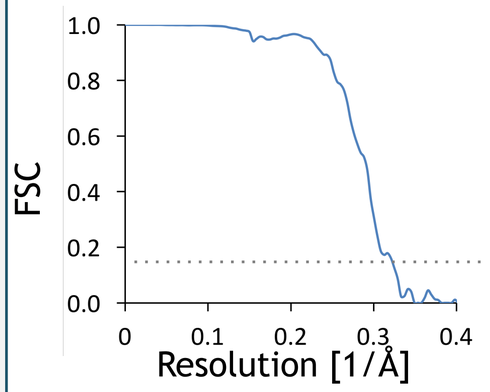
Test set

Sample: *Glutamate Dehydrogenase (GDH)*

Reconstruction: Relion 2 3D refine

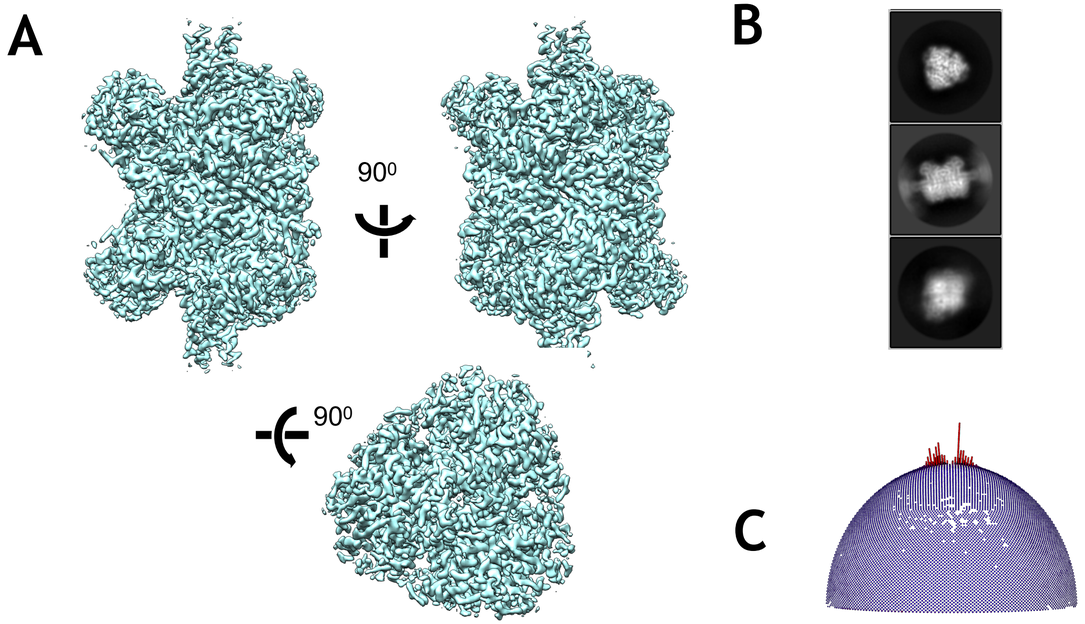
Fourier shell correlation curve

Refine3D $FSC_{0.143}$=3.12Å



Gold-standard FSC curve for the GDH reconstruction.

CryoEM reconstruction of GDH at 3.1Å resolution



(**A**) Isosurface representations of the GDHmap. (**B**) Representative 2D class-averages (box size 28x28nm). (**C**) Angular distribution plot.

Imaging statistics: 20 eV slit, stage shift

17feb14h experiment : 528 esn images, 523 esn-a-DW images used

443 *enn-a-DW images used from Appion*

16,191 particles = 3.16Å (using a CTF conf 0.8 >8Å CTF cut off)

Imaging parameters:

Grid loaded: 17feb13

Session: 17feb14h

Pixel size: 0.43Å superres

bin2 - 0.854905Å/px

Camera: K2 counting, 400 ms - 32 frames

Camera settings: 12,800 ms exp

Dose: 35.73 $e^-/Å^2$

Photo time

Appion part I

- Frame alignment
- CTF estimation
- Particle picking
- Micrograph/ Particle curating
- Initial 2D classification
- Initial model generation

Appion part II

- 2D classification (particle sorting)
- *ab initio* model generation
- 3D classification (conformation sorting)
- 3D refinement

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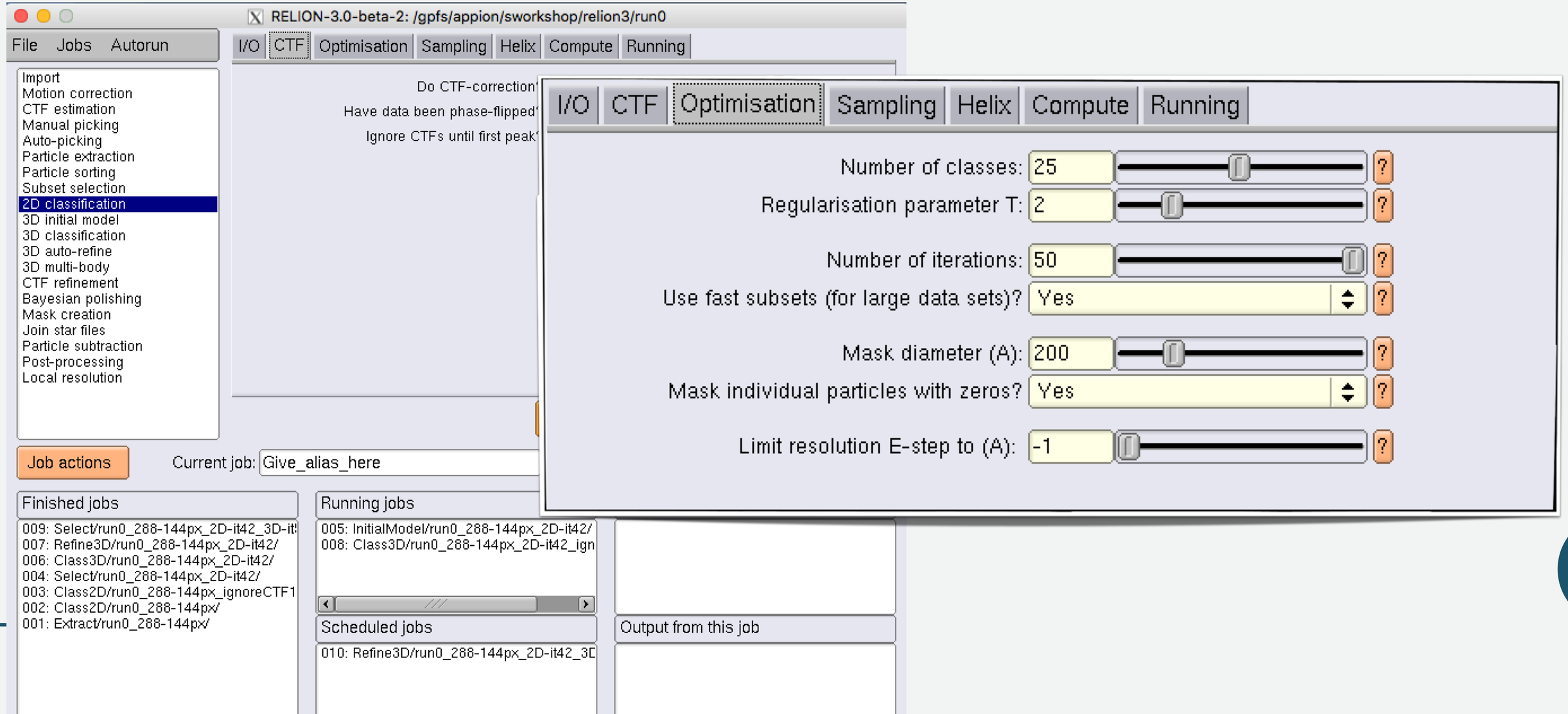
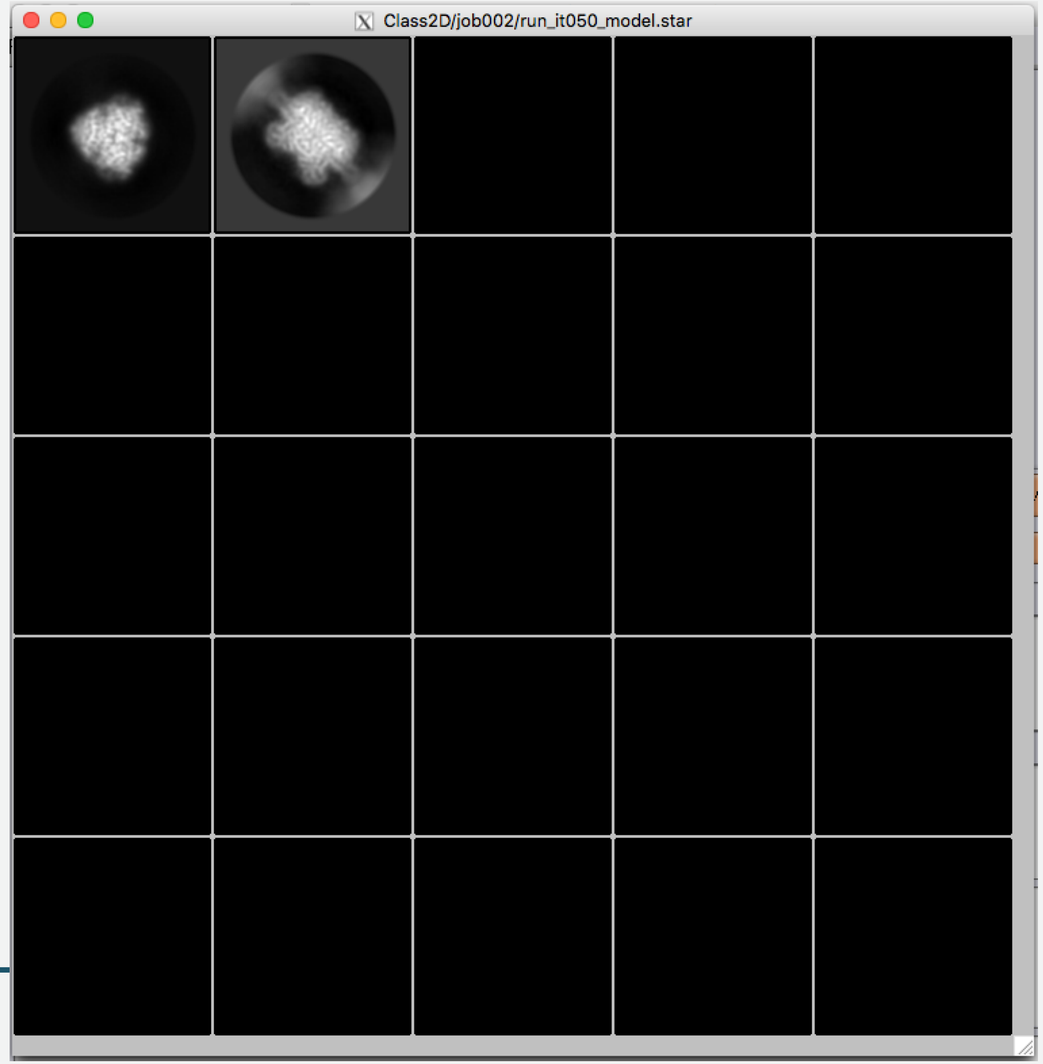
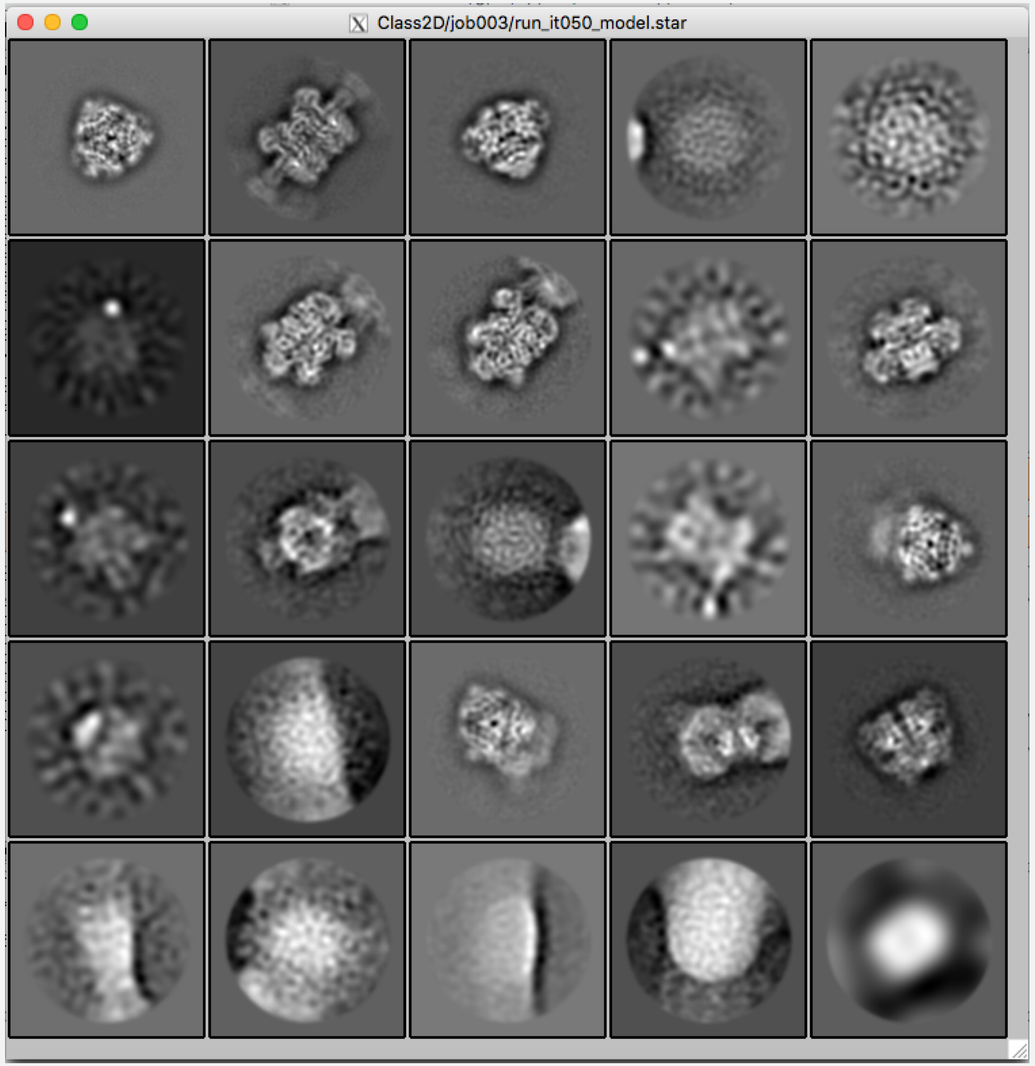
- **4. Closing** *[Q&A with SEMC staff]*

2D classification

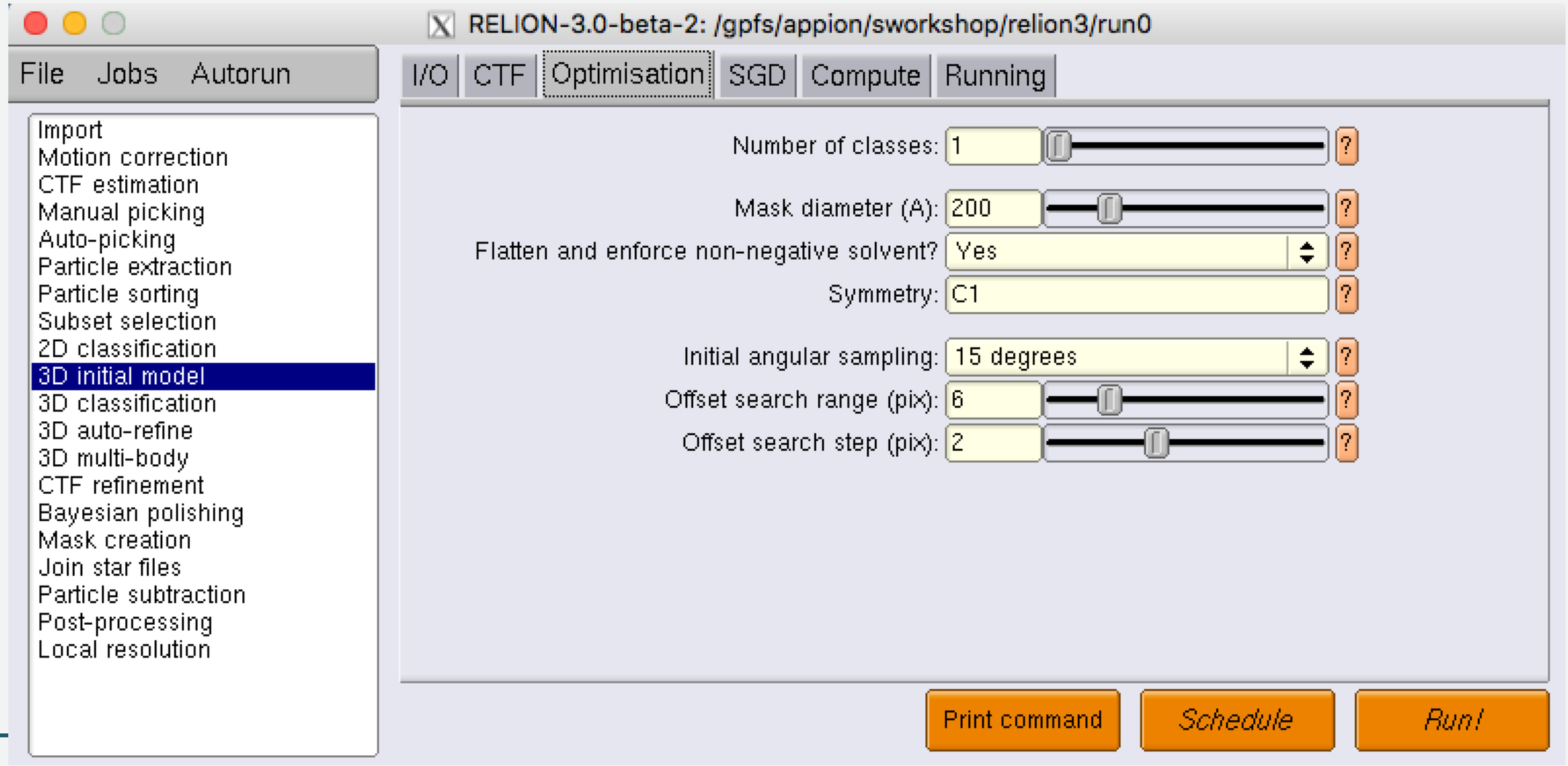


2D classification



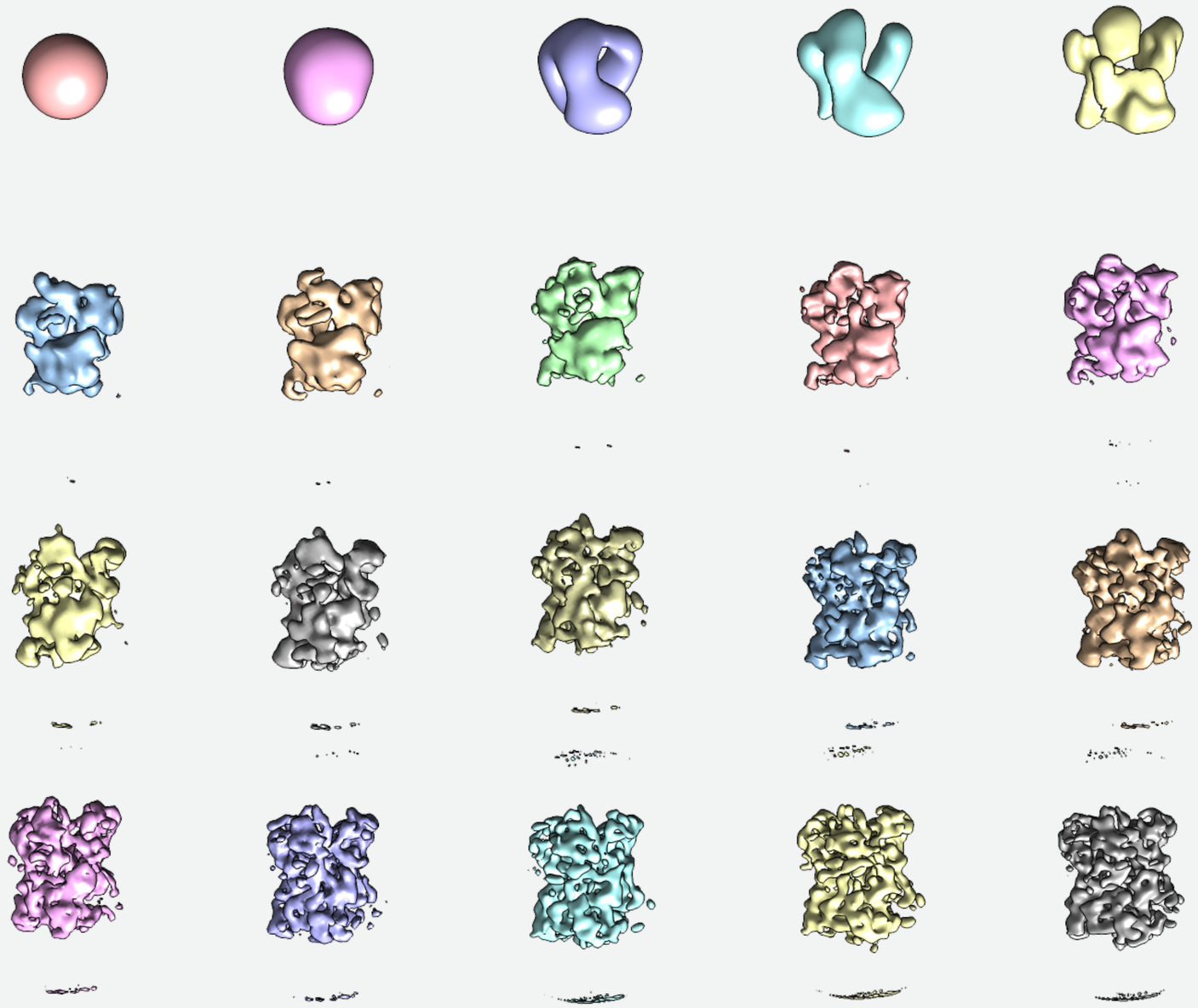


Initial model

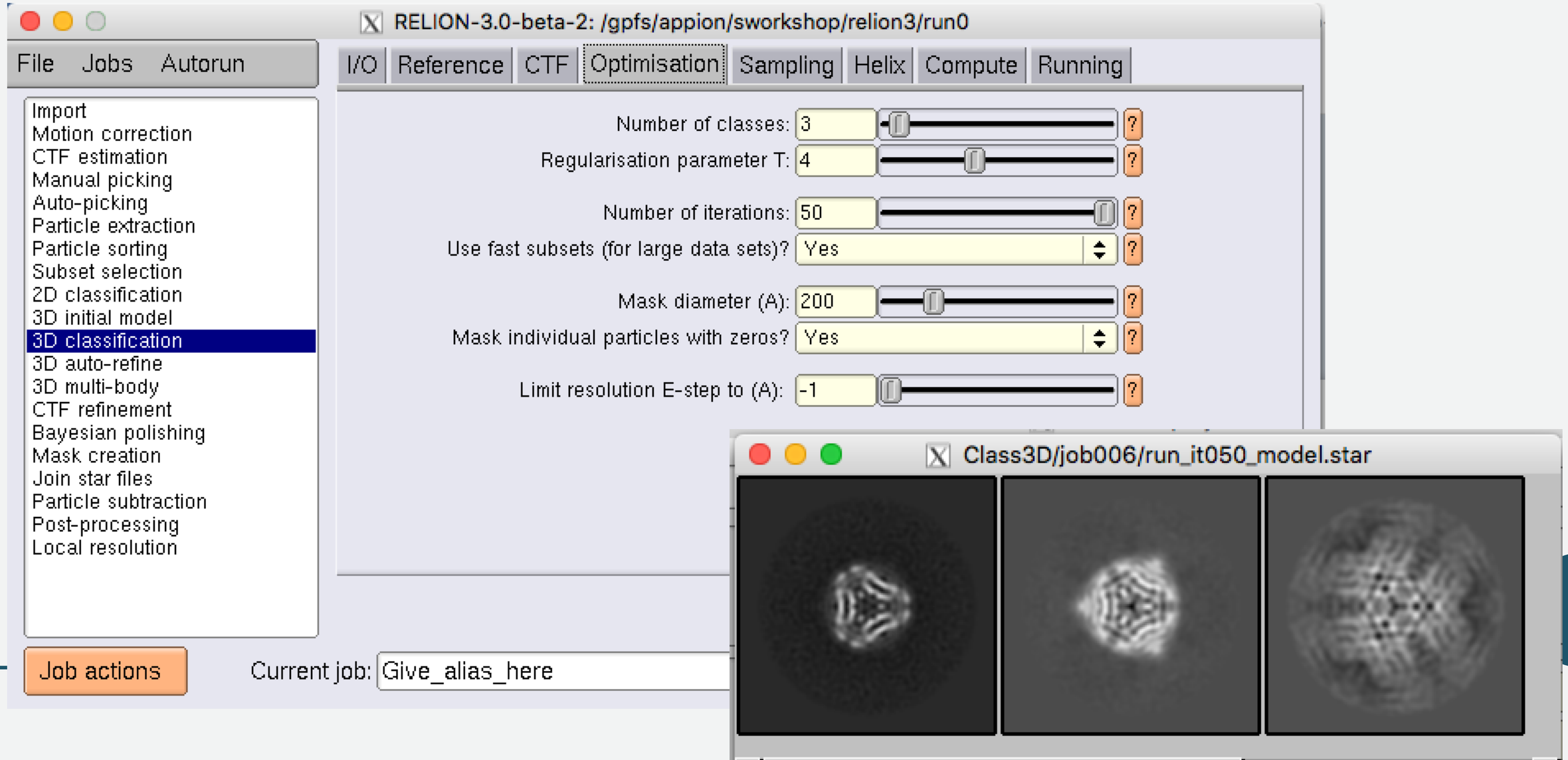


Initial model

Stochastic Gradient Descent algorithm for initial 3D model generation



3D classification



RELION 3 workflow

Workshop workflow : Real time demo test set – 17feb14h (~1/2 day run)

Particle extraction

Extract/job000 folder: 16,191 particles
(144 px box size – bin4 @ 1.70981 Å/px)

2D classification

16,124 particles
(used 10 classes from K=25, 12 iterations)

3D classification

12,131 particles
(used 2 classes from K=3, 7 iterations)

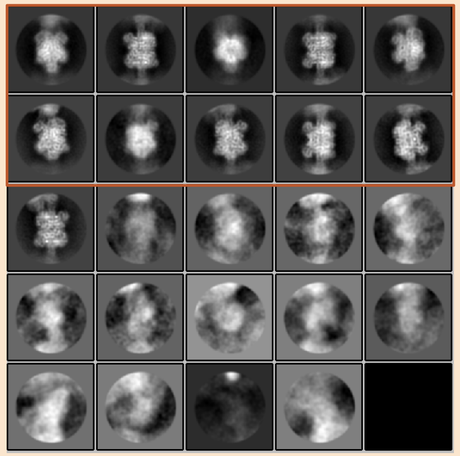
3D refine

3.7473Å res
(17 iterations, ~1 hr on semcgpu01)

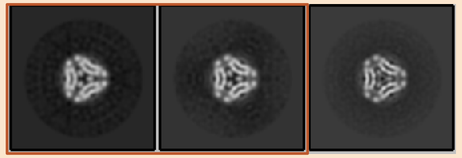
Postprocessing

3.41962Å res
(using 0.02 mask, 3 px ext, 3 px soft mask)

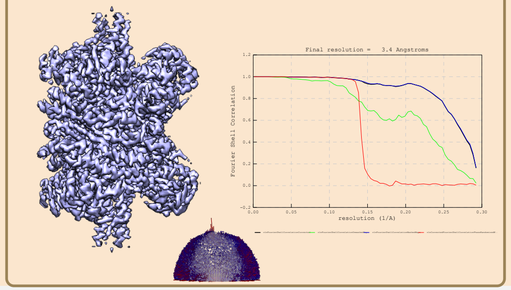
2D classification



3D classification



3D refinement



Questions?