Joint Group Meeting

(Loic Carrique, postdoc in Grimes group)

wellcome trust

UNIVERSITY OF OXFORD

EMBO course resume

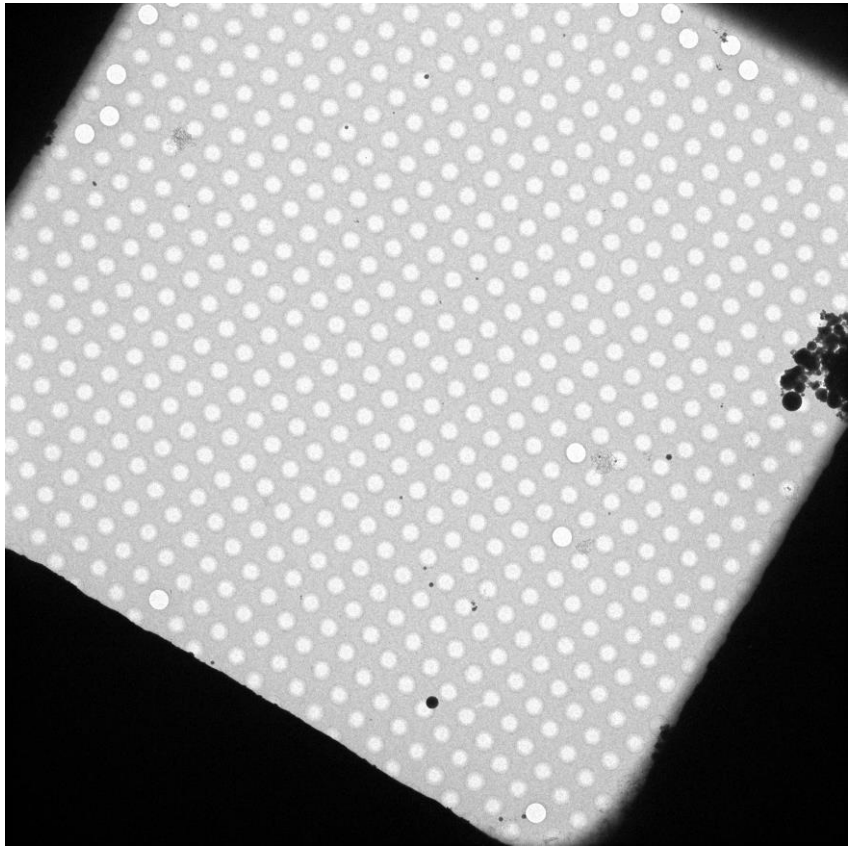
Lecture 2 Basic concepts of Fourier analysis **Giulia Zanetti, BBK** (pdf)	Lecture 3 Concepts of convolution, point spread, and cross-correlation **Elena Orlova, BBK** (pdf)	Lunch	Practical 1 Images and transforms, filtering, CTF, diffraction spacings, filter design, EMAN 2 Initial steps in processing, Scipion/motion corr/dose fractionation (eman)(scipion) **Helen Saibil, BBK ; Jose Miguel de la Rosa Trevin, Marta Carroni, Stockholm, Guendalina Marini, Scott Gardner BBK**	
Lecture 4 Principles of TEM image formation **Chris Russo, MRC-LMB** (pdf)	Lecture 5 Contrast transfer & CTF correction (phase contrast) (eBIC) **Dan Clare, Diamond** (pdf)	Lunch	**13:30-14:00 B62** Lecture **Bart Marzec, JEOL** Think Cold: Pushing the SPA Resolution Limit with CryoARM 300	Practical 2 Scipion preprocessing pipeline cont'd: CTF find4, phase contrast, Gautomatch, particle picking, examples of good and bad images, effects of dose and filtering **Jose Miguel de la Rosa Trevin, Marta Carroni, Stockholm. Dan Clare, Diamond** (scipion)
Lecture 6 Tomography and CLEM **Kay Grünewald, Hamburg** (pdf)	Lecture 7 Subtomogram averaging, 3D CTF correction **John Briggs, MRC-LMB** (pdf)	Lunch	**13:30-14:00 B62** Lecture **Andreas Förster, Dectris** Micro ED (pdf)	Practical 3 Tomography and segmentation **Daven Vasishtan, Oxford. Mauro Maiorca, Guendalina Marini, Josh Hutchings, Claudine Bisson, BBK** (imod)(segmentation)
Lecture 8 FIB milling /phase plate imaging **Julia Mahamid, EMBL-HD** (pdf)	Lecture 9 detectors, noise, frame alignment principles, particle detection **Helen Saibil, BBK** (pdf)	Lunch	**13:30-14:00 B62** Lecture **Ieva Drulyte, Thermo Fisher** Micro ED: EPU-D results and perspectives (pdf)	Practical 4 Subtomogram averaging and 3D CTF correction, Dynamo **Daniel Castano, Giulia Zanetti, Daven Vasishtan, Josh Hutchings, Claudine Bisson** (dynamo)
Lecture 10 Single particle analysis, reconstruction principles **John Rubinstein, Toronto** (pdf)	Lecture 11 Using symmetry, viruses, helices, extracting asymmetric parts **Doryen Bubeck, IC** (pdf) (mov) (ppt)	Lunch	Practical 5 Single particle analysis: cryosparc: initial model by SGD, 2D & 3D classification, refinement. Map inspection with Chimera **John Rubinstein, Ali Punjani, Marina Ivanova, Scott Gardner, BBK** (cryosparc) (pdf)	

Why collect with a large pixel size is an advantage for screening

Lecture 12 Single particle (Bayesian methods, particle classification (pdf,ppt) **Sjors Scheres, MRC-LMB**	Lecture 13 Microscopes and automated data collection **Bridget Carragher, NYSBC** (pdf)	Lunch	**13:30-14 :00 B62** Lecture **Paul Mooney, Gatan** Direct electron detectors (pdf)	Practical 6 Relion advanced topics in single particle analysis: 3D refinement with focussed classification, signal subtraction, postprocessing, sharpening, local filtering **Sjors Scheres, Kat Toropova, Giulia Zanetti, Scott Gardner** (relion)
Lecture 14 Validation of 3D EM reconstructions, post processing **Peter Rosenthal, Crick** (pdf)	Lecture 15 Interpretation of maps, docking of atomic structures **Maya Topf, BBK** (pdf)	Lunch	Practical 7 Fitting of structures, flexible fitting (Flex-EM), model validation (TEMPy) (pdf) **Tom Burnley, Maya Topf, Colin Palmer, Agnel Praveen Joseph, Sony Malhotra, Tristan Cragnolini, Aaron Sweeney**	
Lecture 16 High resolution refinement, model assessment **Arjen Jakobi, Delft** (pdf)	Lecture 17 Cryo EM in the pharmaceutical industry **Claudio Ciferri - Genentech** (pdf) **Ilaria Ferlenghi, GSK**	Lunch	Practical 8 local sharpening (LocScale), de novo structure building (CCP-EM, REFMAC) (locscale,refmac,buccaneer) **Arjen Jakobi, Tom Burnley, Colin Palmer, Sony Malhotra, Tristan Cragnolini, Aaron Sweeney**	
Lecture 18 EMDB Data deposition, EMPIAR, eBIC **Gerard Kleywegt** (pdf)**& Osman Salih, EMDB** (pdf)	Guest lecture 6 Future prospects for cryo EM **Richard Henderson, MRC LMB** (pdf)	Lunch	Course review and departure	

How to get good grids for Single Particles Analysis (SPA)

- **Which type of grids use?**
  - ✓ Gold/copper Quantifoil 200 mesh, R2/1
- **These settings are working for Strubi VitroRobot (II)**
  - ✓ 3s blotting, 3.5 µL sample, 20°C, blotting force -25



What to expect from one day on a Glacios microscope

- **How many grids can we check per 24h Glacios session:**
  - ✓ Most of the time it's 2 x 12 grids
  - ✓ This correspond to 6 – 12 different samples/conditions

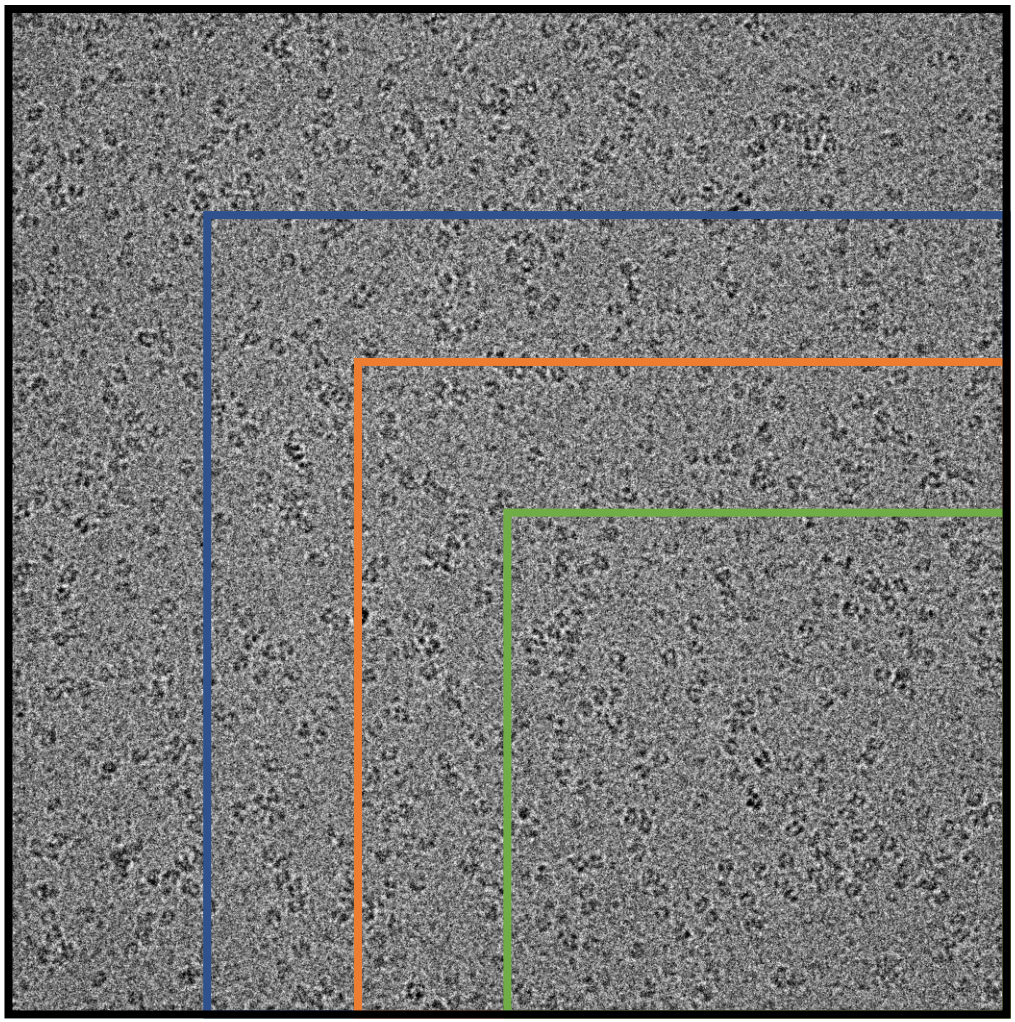
- **What we are checking when we screen**:
  - ✓ If there is sample on the grid
  - ✓ If the sample is going into the holes
  - ✓ If the distribution is correct or can be improve

- **Yes, but real data are better to check sample quality!!!**

How many dataset can I get?

Why collect with a large pixel size is an advantage for screening

- ✓ Low mag (bigger pixel size) decrease the SNR in your particles
- ✓ Resolution is limited to two time the pixel size (Nyquist)
- ✓ But low mag mean bigger field of view!



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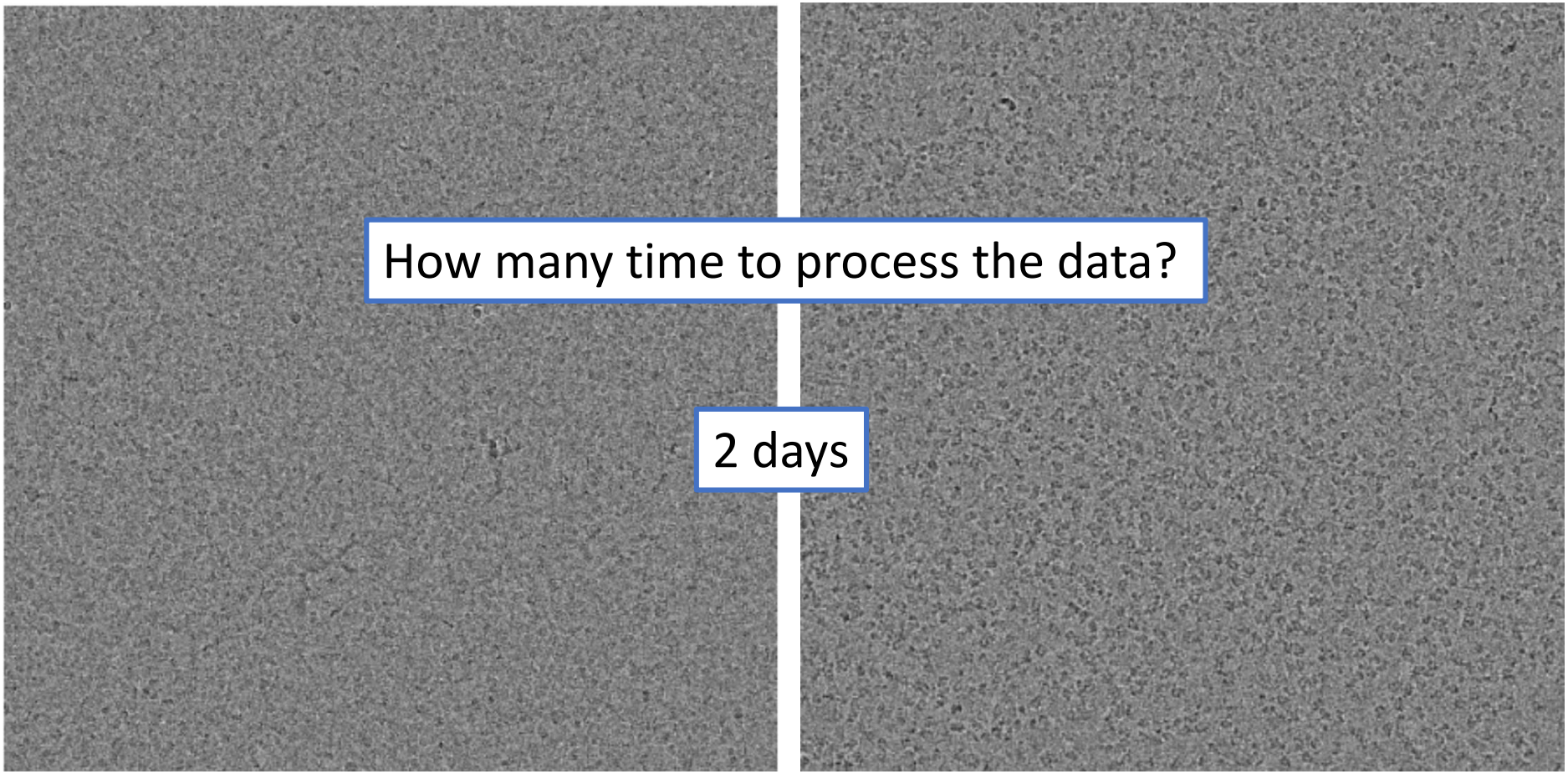
- **What we expect from a screening dataset:**
  - ✓ Have an idea of sample quality (Complex assembly, particles orientations, binding partner, conformational changes…)
  - ✓ Get an initial map at reasonable resolution (Between 4Å and 10Å) that could answer these questions

What is the minimal amount of data that I need to do that?

Glacios data collection parameters

- ✓ Glacios microscope, Falcon III detector, linear mode and 92,000x mag (Pixel size 1.6 $Å^2$)
- ✓ Total dose ~60 $e^-/Å^2$, 2s exposure, defocus range -1 to -2.5 µm → ~100 movies/h
- ✓ 1400 movies o/n for this dataset (Stage shift 10s, Fast beta, autofocus every 15 µm)

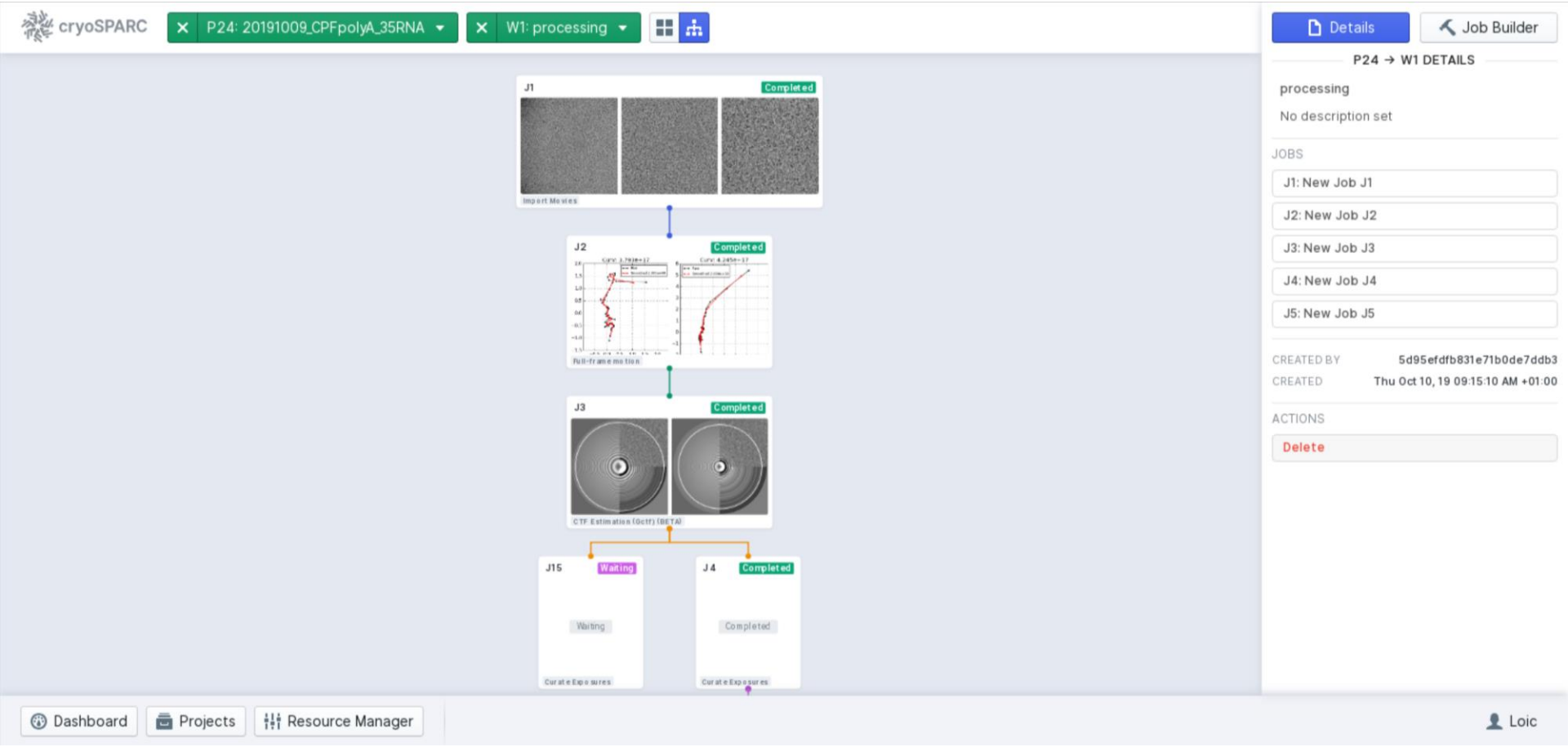


Aligned movie, Full frames
-1 µm defocus

Aligned movie, Full frames
-2.2 µm defocus

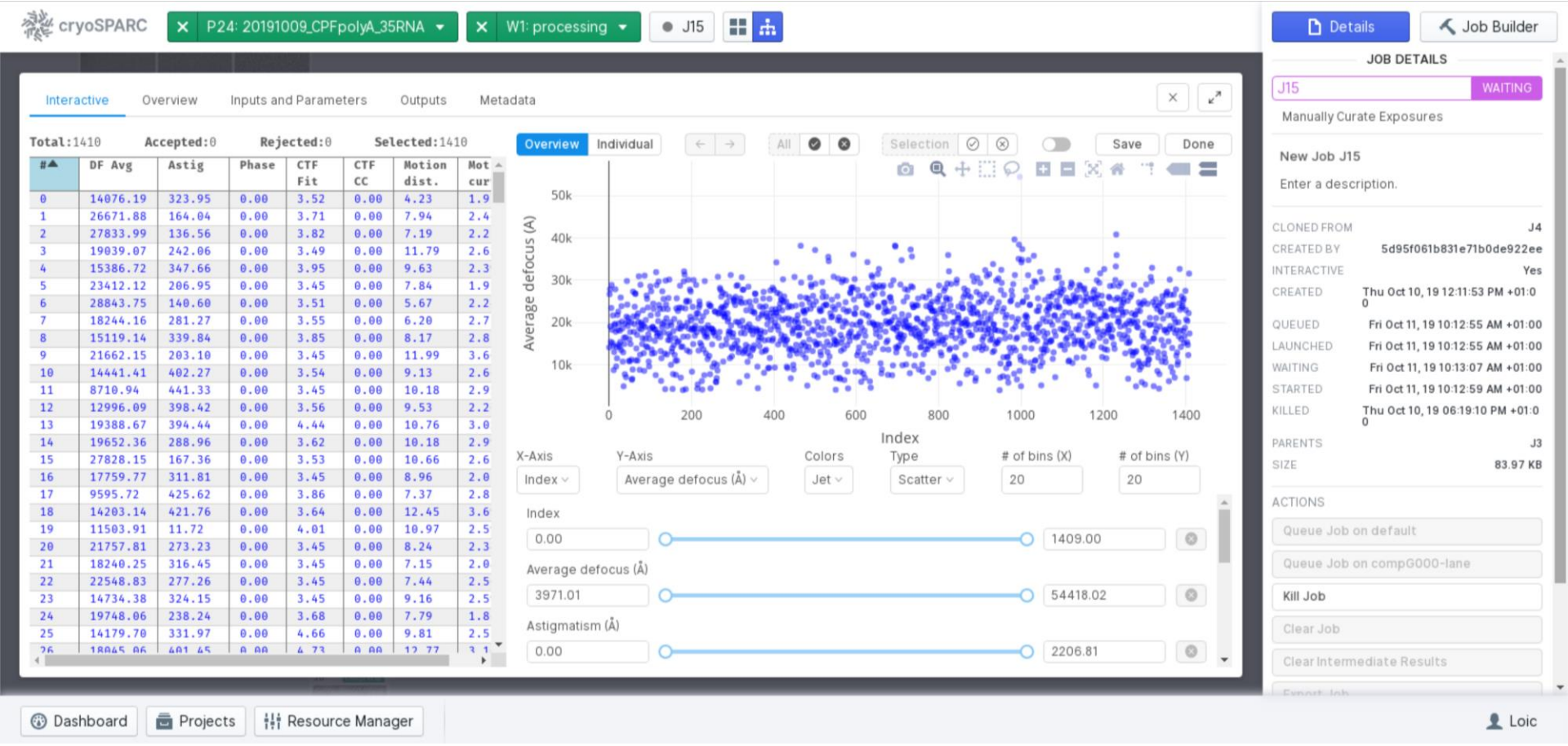
Cryosparc V2 – Motion correction and CTF estimation

- ✓ Motion Correction with Motioncor2 or CS full frame motion correction
- ✓ CTF estimation using GCTF and EquiPhase Averaging (EPA)
- ✓ Manual curation of micrographs



Cryosparc V2 – Manual curation

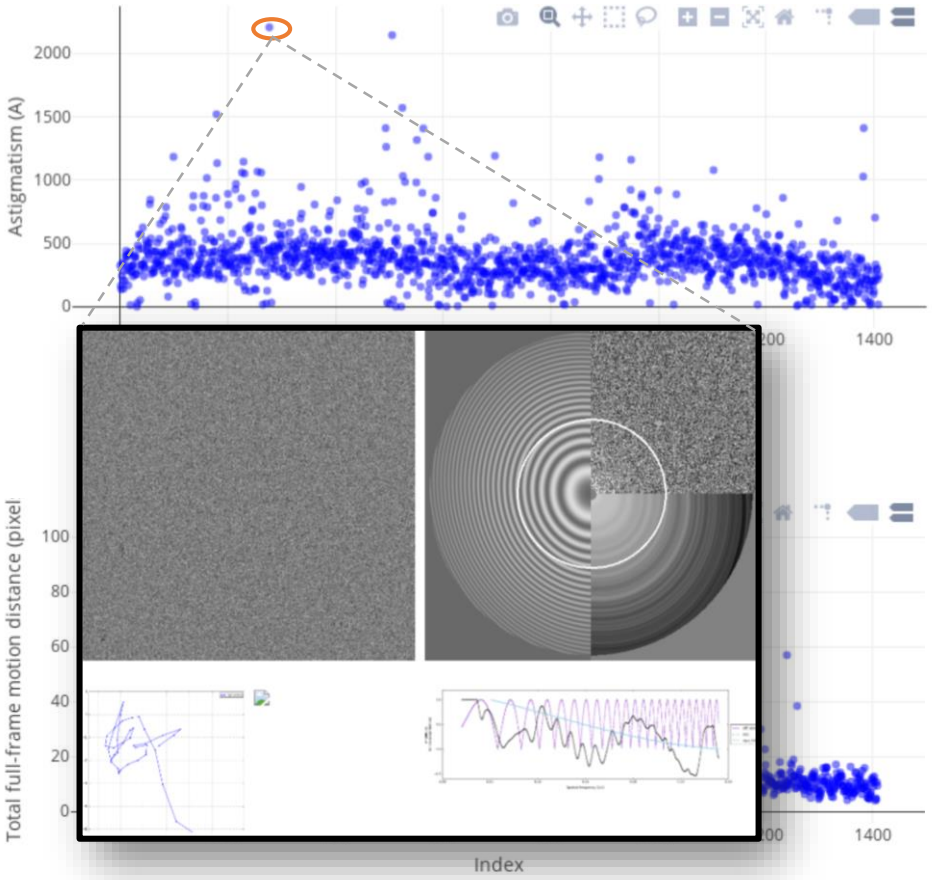
- ✓ Allow to easily assess data quality based on metric (motion, defocus, CTF…)
- ✓ Check that no problems occurred during data collection
- ✓ Discard bad micrographs



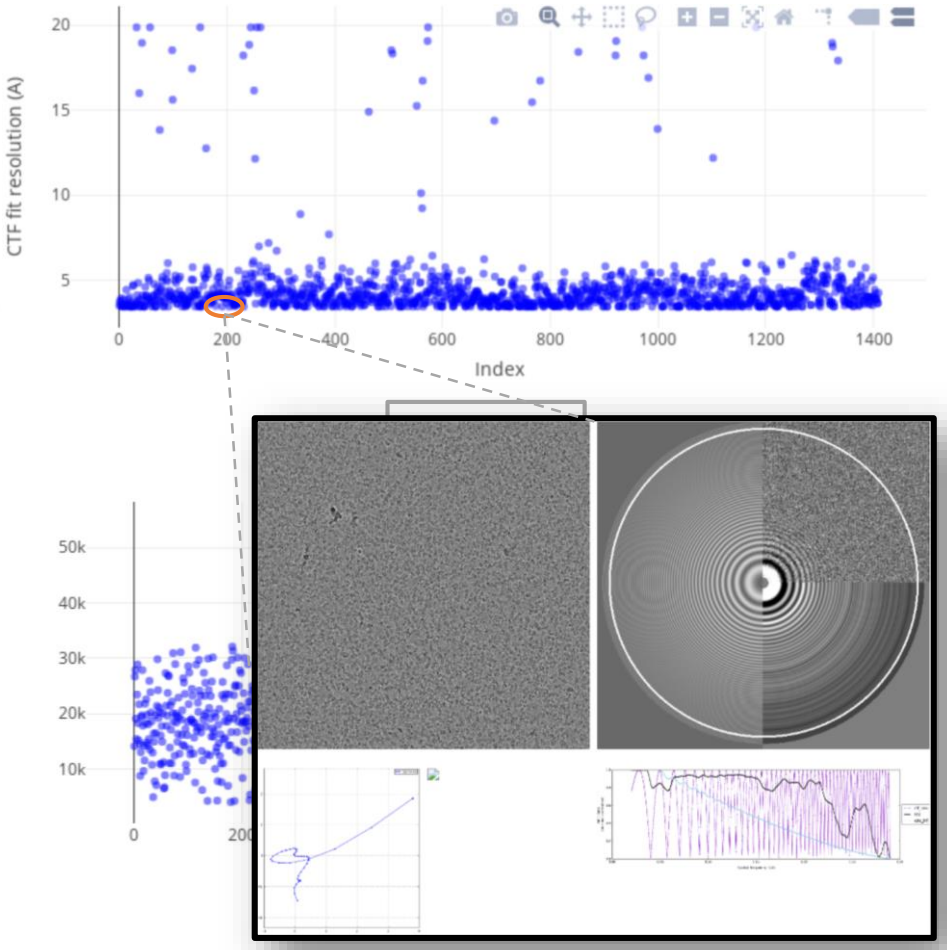
Cryosparc V2 – Manual curation

- ✓ Plots of the different metrics

Astigmatism

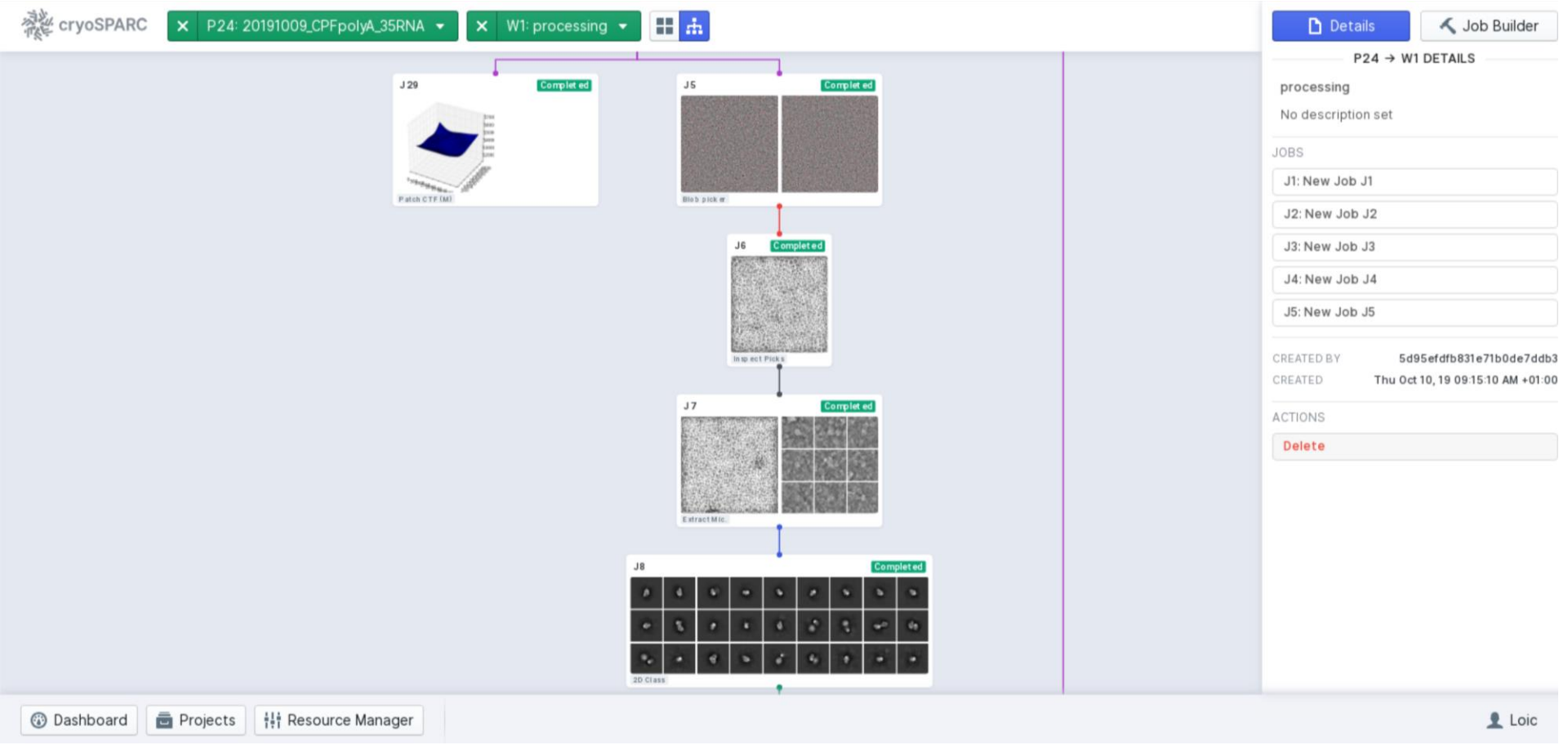


CTF estimated Max Resolution



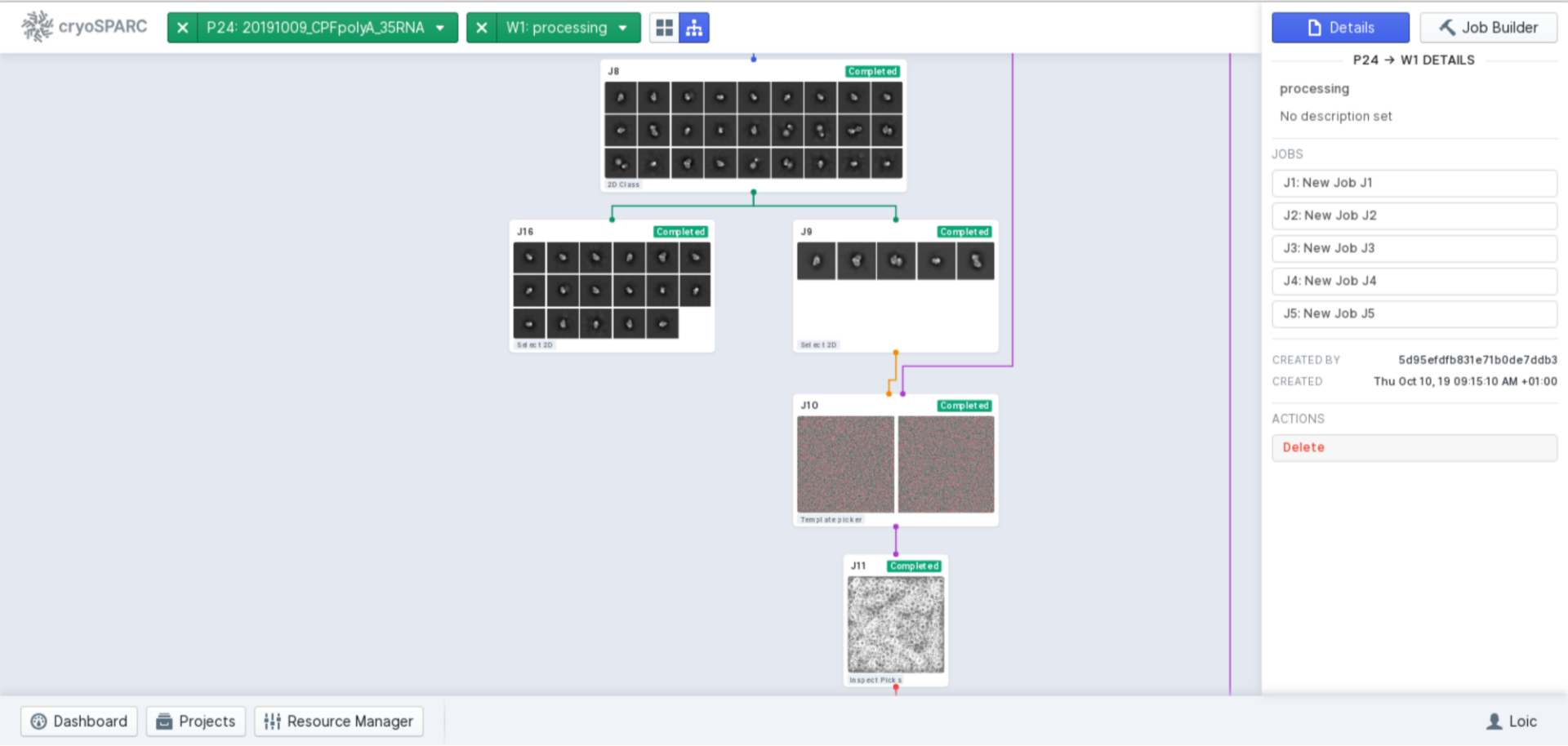
Cryosparc V2 – Particles picking Step 1

- ✓ Blob picker with a wild range of sizes to capture most of the views
- ✓ Particles extraction at bin 2 or bin 4 (Depending on the pixel size used)
- ✓ 2D classification with 50 classes (Just to get the main views)



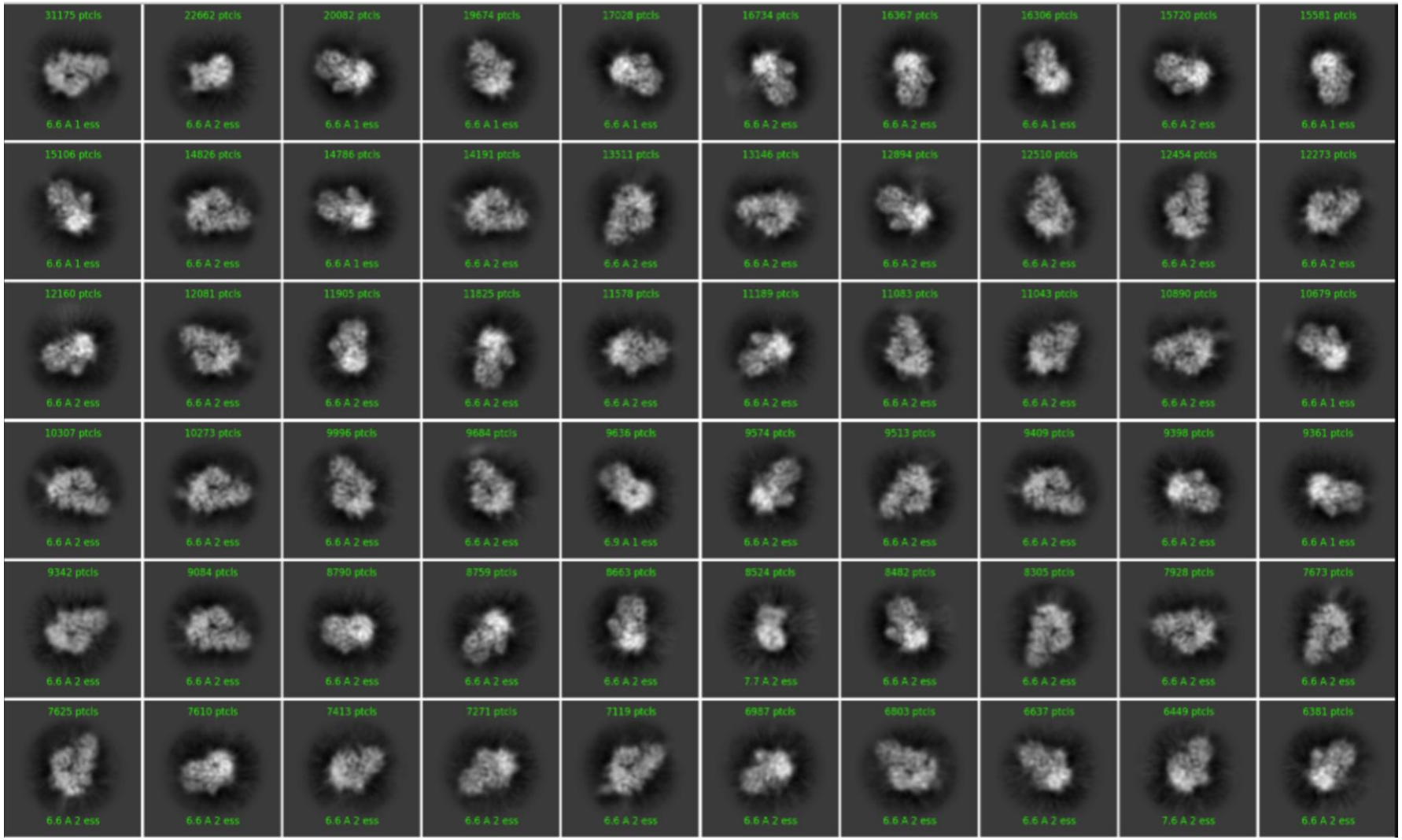
Cryosparc V2 – Particles picking Step 2

- ✓ Template picking based on previous selected 2D classes
- ✓ Particles extraction at bin 2 or bin 4
- ✓ 2D classification with 50 classes (To remove all the junks classes)
- ✓ 2nd 2D classification with 200 classes and high uncertainty score



Cryosparc V2 – 2D classifications

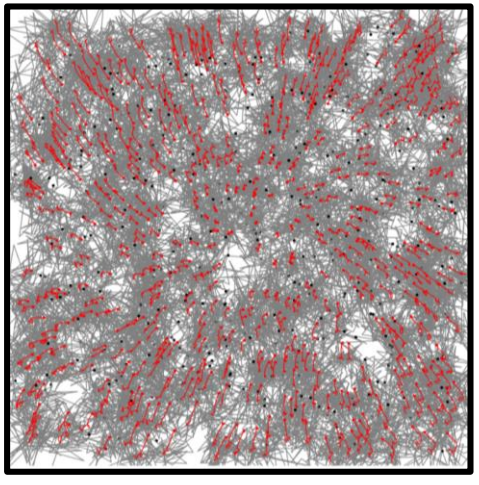
- ✓ Around 1,000,000 particles selected after 2D classification



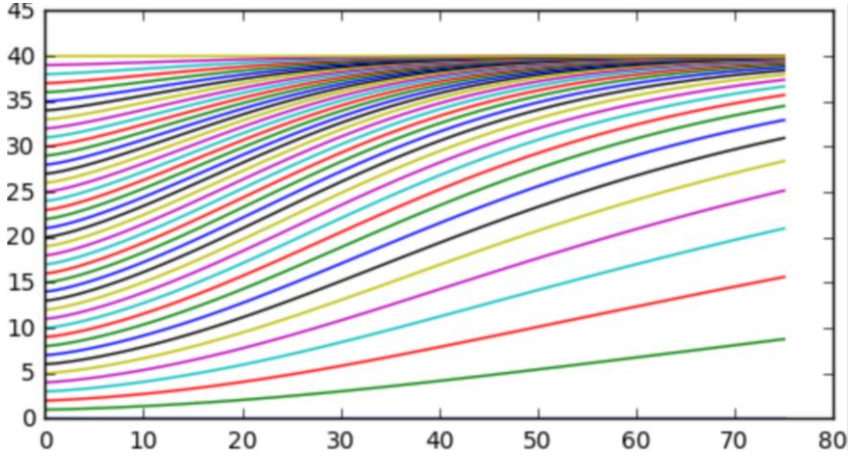
Cryosparc V2 – Local motion correction and CTF

- ✓ Local motion correction is tracking the motion of each individual particles
- ✓ It can also re-extract particles unbinned or in a new box size at the same time

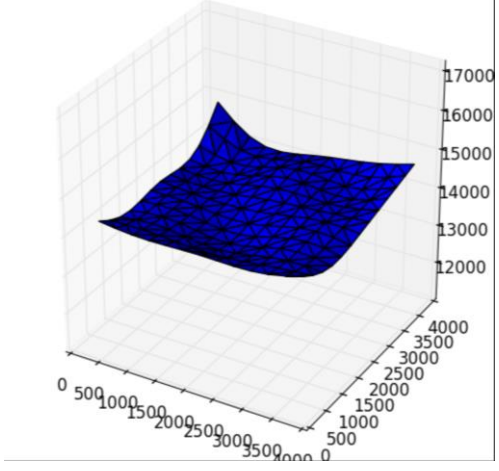
Local Motion



Exposure weights

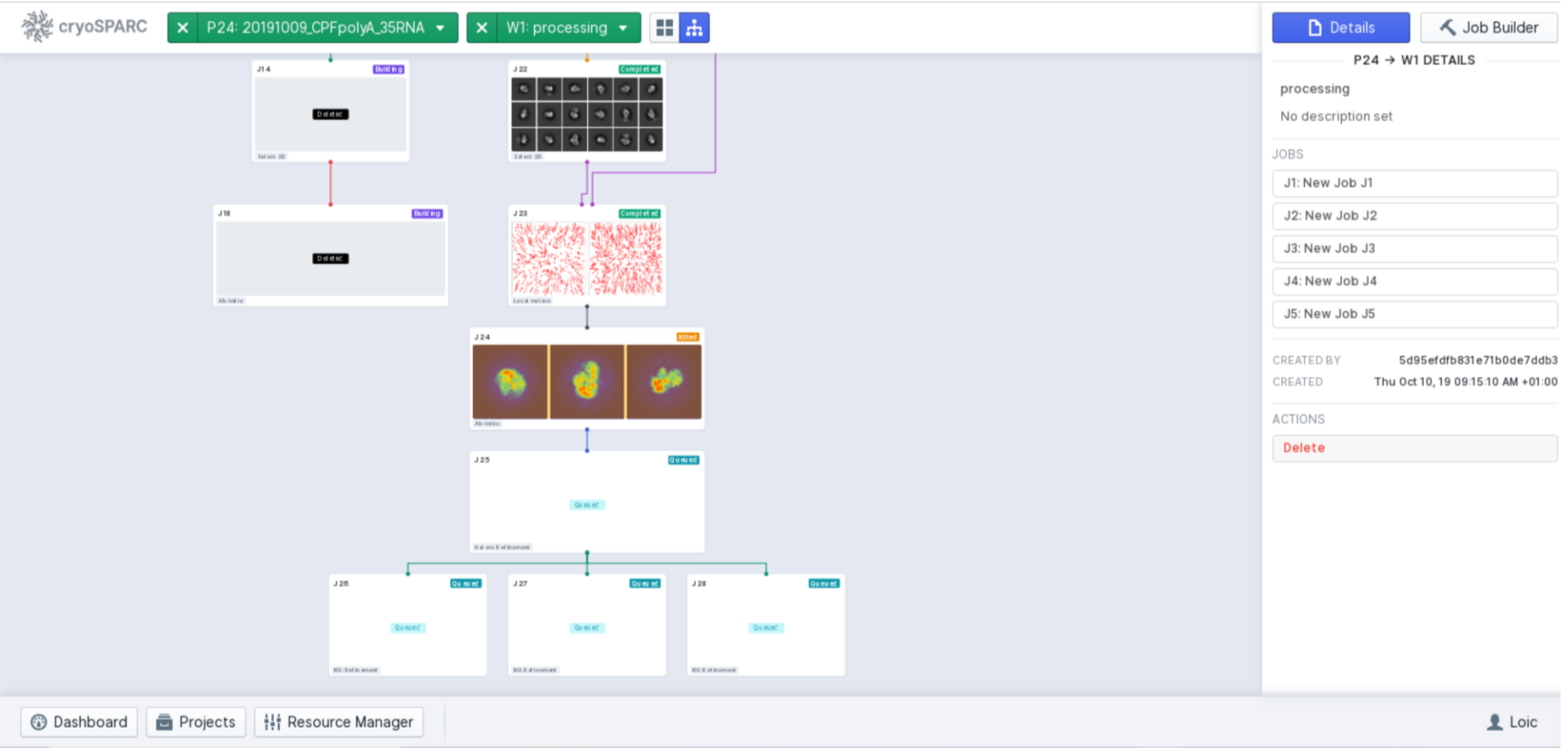


- ✓ Patch CTF estimation is estimating local variation of defocus across your micrograph



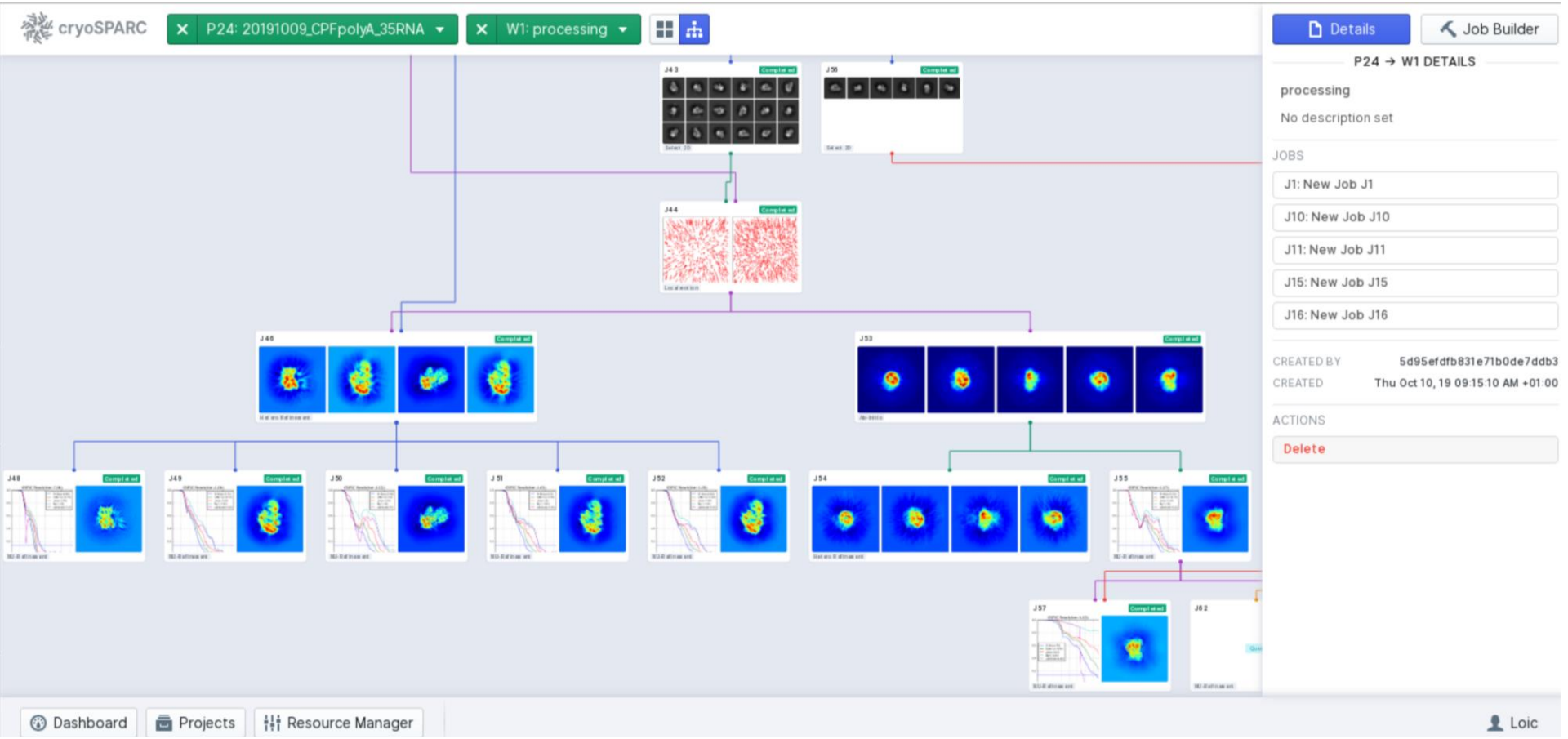
Cryosparc V2 – Ab initio model(s)

- ✓ SGD based model is one of the standard in SPA to get initial model
- ✓ 3 Ab initio models generated in parallel give good results as initial 3D classification
- ✓ Heterogenous refinement of these model is an extra step



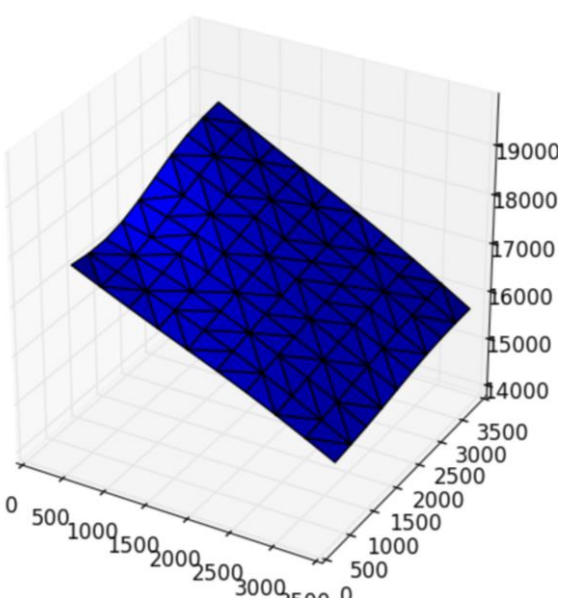
Cryosparc V2 – Homogenous Refinement

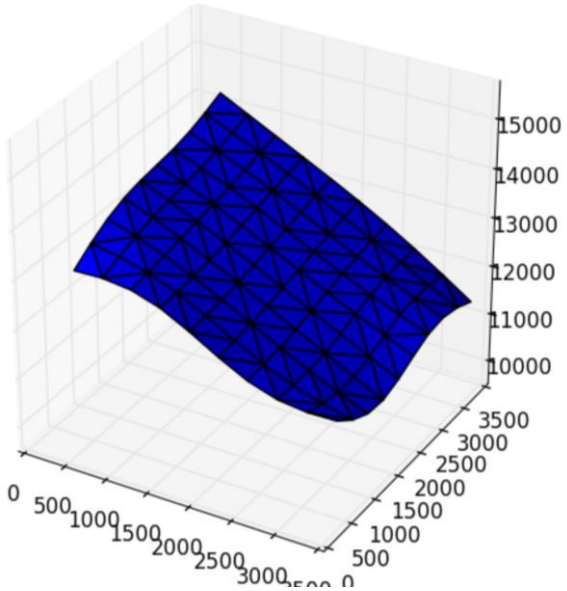
- ✓ Each map is then refined independently
- ✓ Global sharpening, local resolution estimation and local filtering

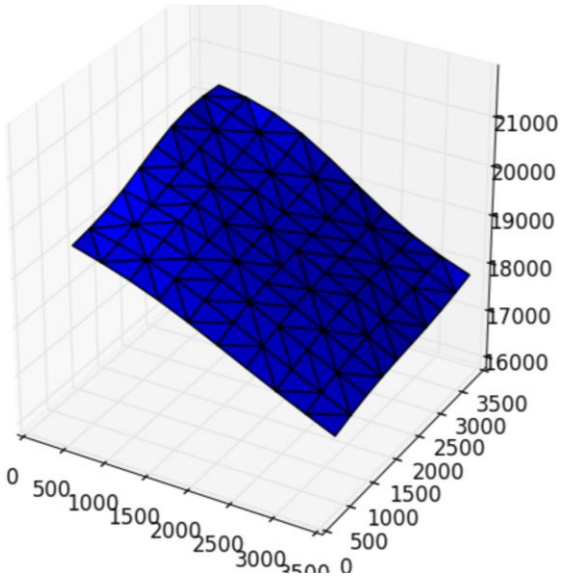


Cryosparc V2 – Good to process tilt dataset

✓ Dataset collected using a tilt angle of 30°







- **<u>Cryosparc is bad for:</u>**
  - ✓ Focus classification
  - ✓ Symmetry mismatch
  - ✓ Virus particles with icosahedral symmetry

✓ Using a script (PyEM from D. Asarnow, UCSF) you can transfer your data to relion

Relion 3.1

- ✓ New fancy pink colour
- ✓ Bayesian polishing
- ✓ CTF refinement
- ✓ Beam tilt correction
- ✓ High-order aberration correction
- ✓ Merge dataset with ≠ pixel size
- ✓ Refinement with particles that have ≠ box size and/or pixel size
- ✓ Magnification anisotropy correction
- ✓ ….