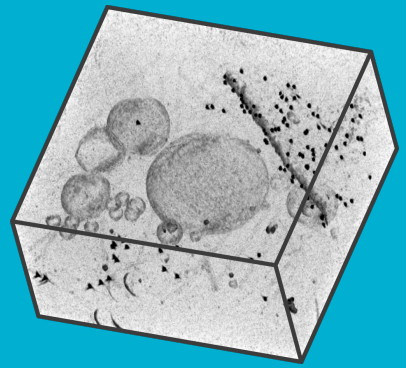
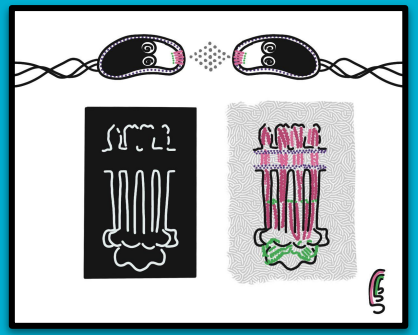
A Hitchhikers Guide to Structural Cryo-ET (in Warp 2.0 & friends)

Alister Burt

Dimitry Tegunov 's group
@ Genentech

01/04/25





Genentech CRYO-EM

quick intro

name: Alister Burt

how I got here:

- PhD in Grenoble 🇫🇷 with Irina Gutsche
- MRC-LMB Cambridge 🎓 with David Barford
- Genentech 🧬 with Dimitry Tegunov

scientific interests

- cryo-ET/averaging
- **enabling** cool biological discoveries

technical interests

- scientific visualization
- python/open source

Cryo-EM Team

Alexis Rohou
Director / Sr.Pr.Sci.

Matt Johnson
Pr.Sys.Spec.

Caleigh Azumaya
Sr.Sys.Spec.

Przemek Dutka
Sr.Sys.Spec.

Dimitry Tegunov
Pr. Scientist

Alister Burt
Comp. Sci. 3

Established in 2015
by Claudio Ciferri

The Scopes

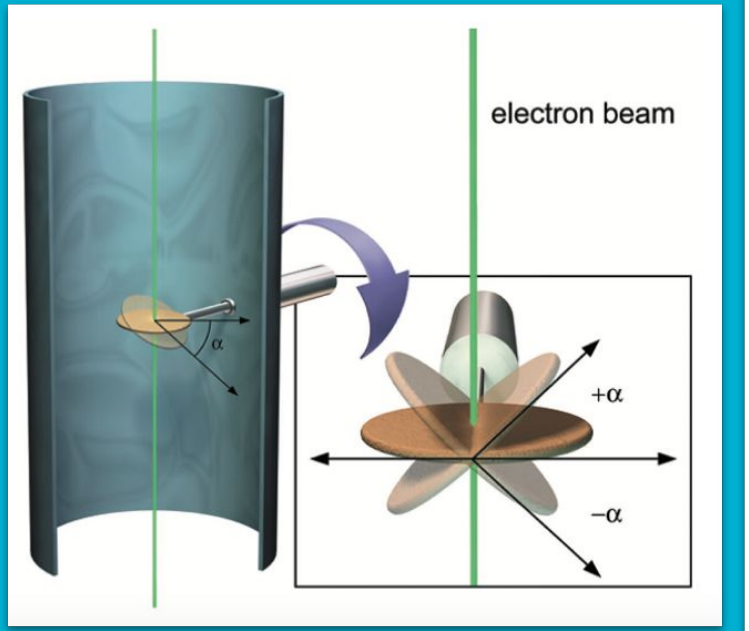
Talk Overview

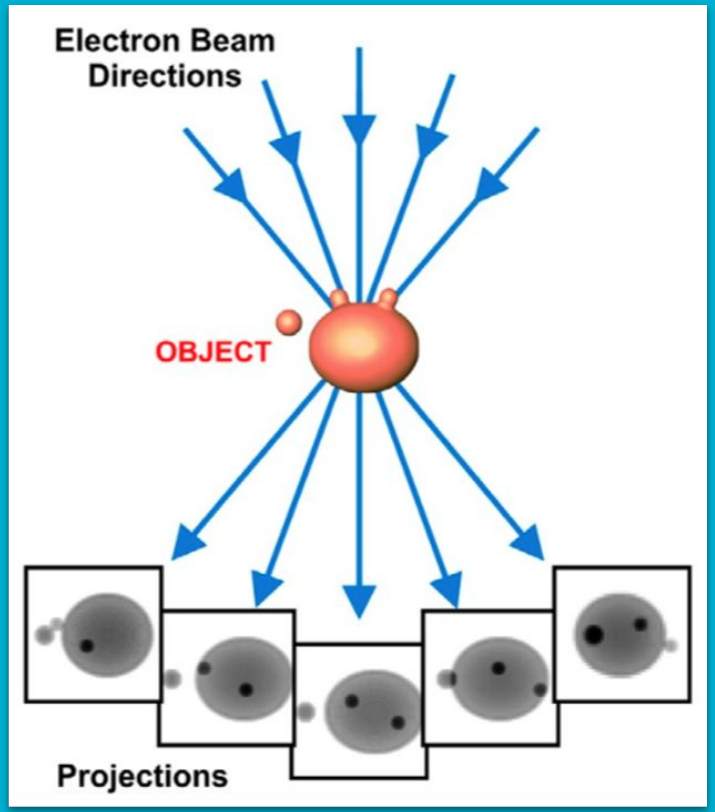
structural cryo-ET
theory: from micrographs to maps

software workflows
*how do I actually *do* cryo-ET processing?*
focus on Warp 2.0
sneak peak of Relay

illustrated examples
a fun example where thinking was useful!

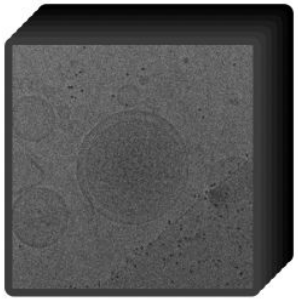
Cryo-Electron Tomography (cryo-ET)





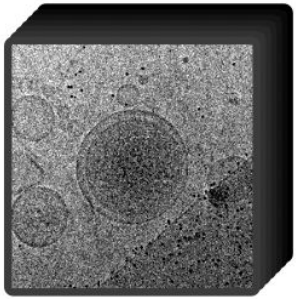
Lučić *et al. JCB (2013)*

let’s get on the same page…



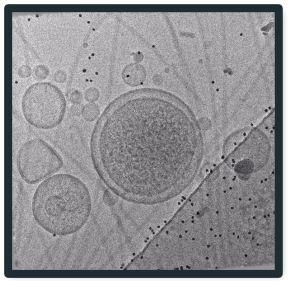
multi-frame micrographs

dose-fractionated images each is 2D + time



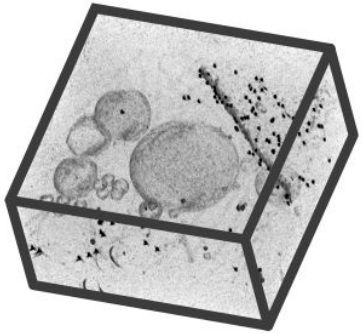
tilt-images

individual 2D images with higher SNR than frames from multi-frame micrographs



tilt-series

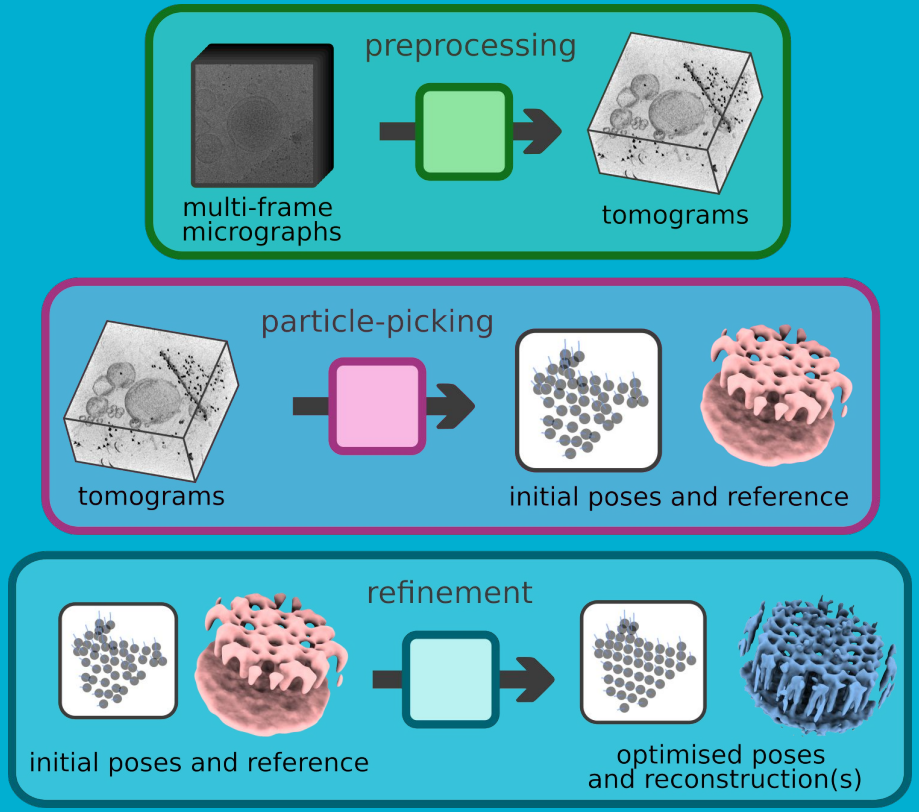
stack of 2D tilt-images with ~same field of view



tomograms

3D reconstructions typically made using images from a tilt-series

What does a structural cryo-ET workflow look like?



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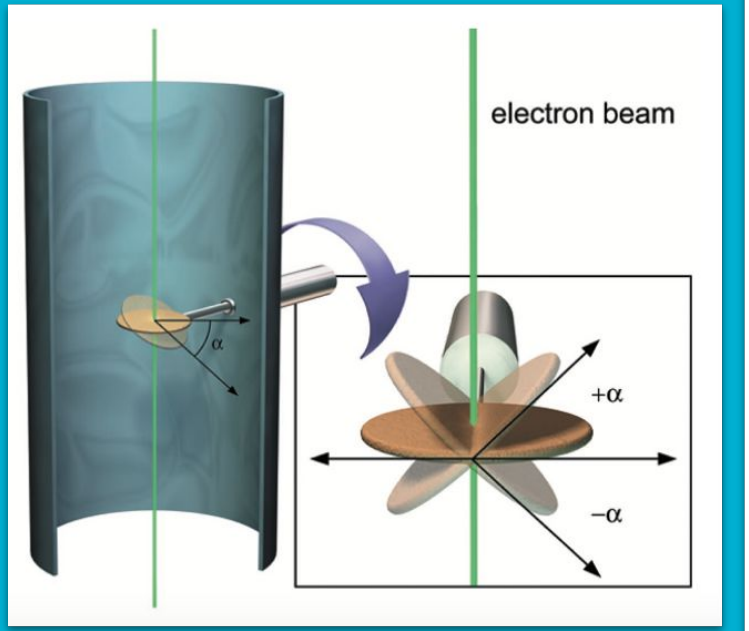

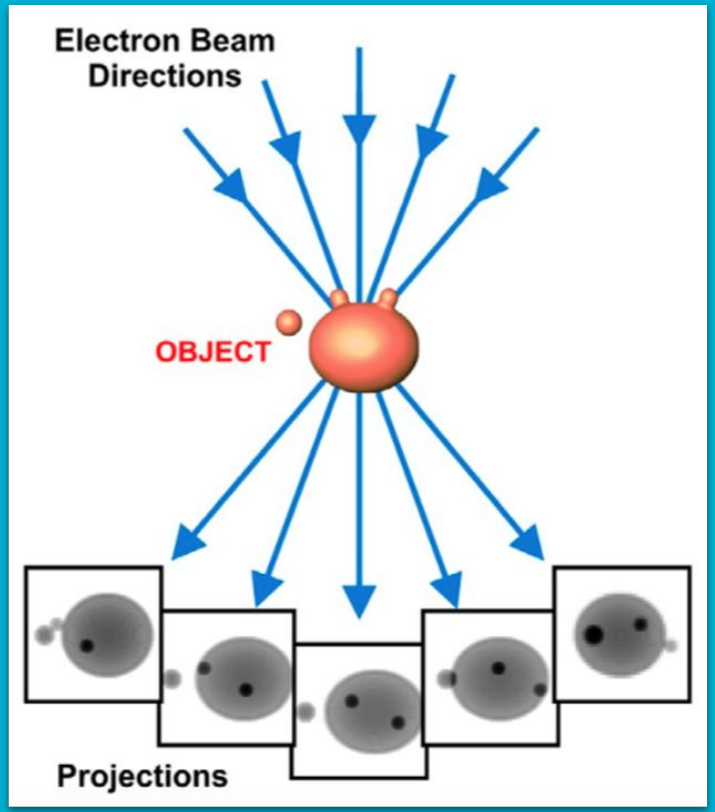

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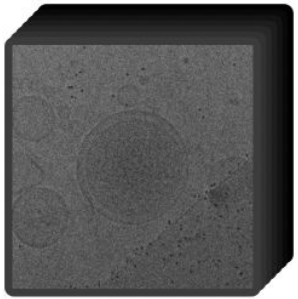

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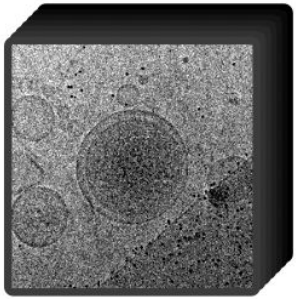

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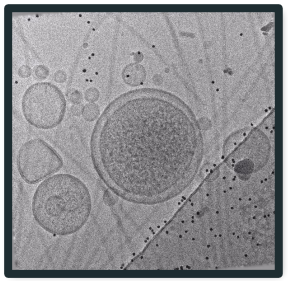

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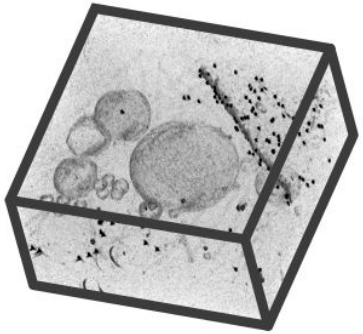

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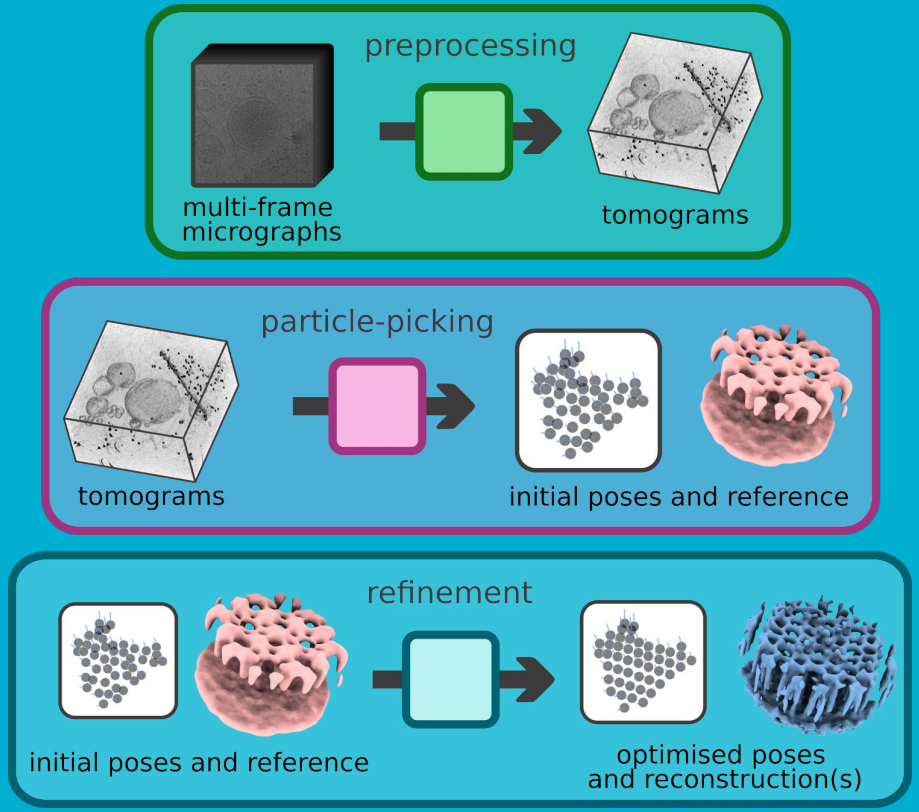
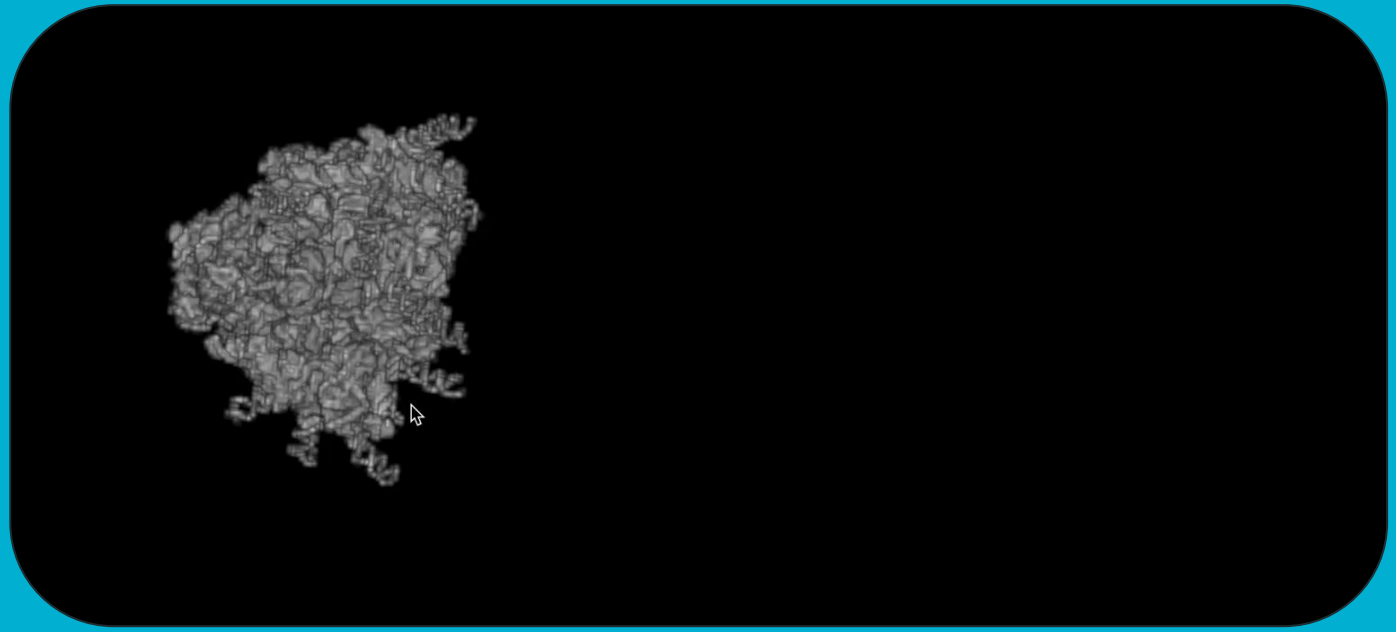


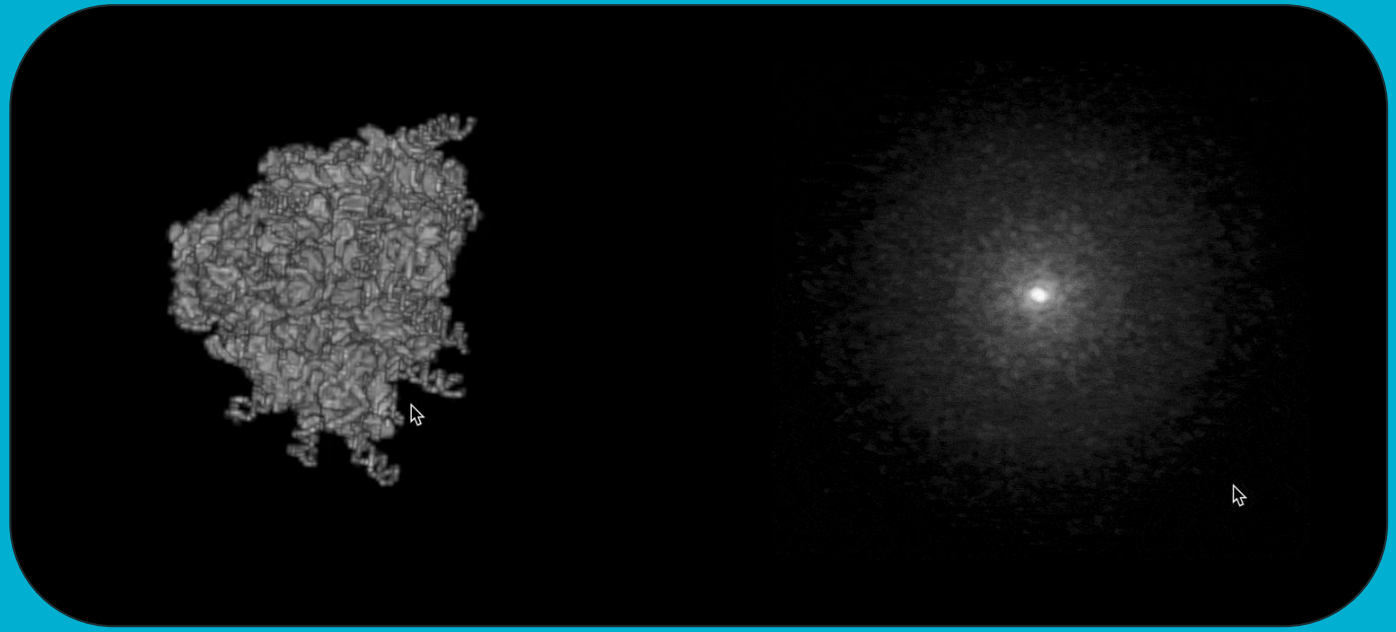
tilt series processing is a superset of frame series processing

3D reconstruction from projection images



3D object

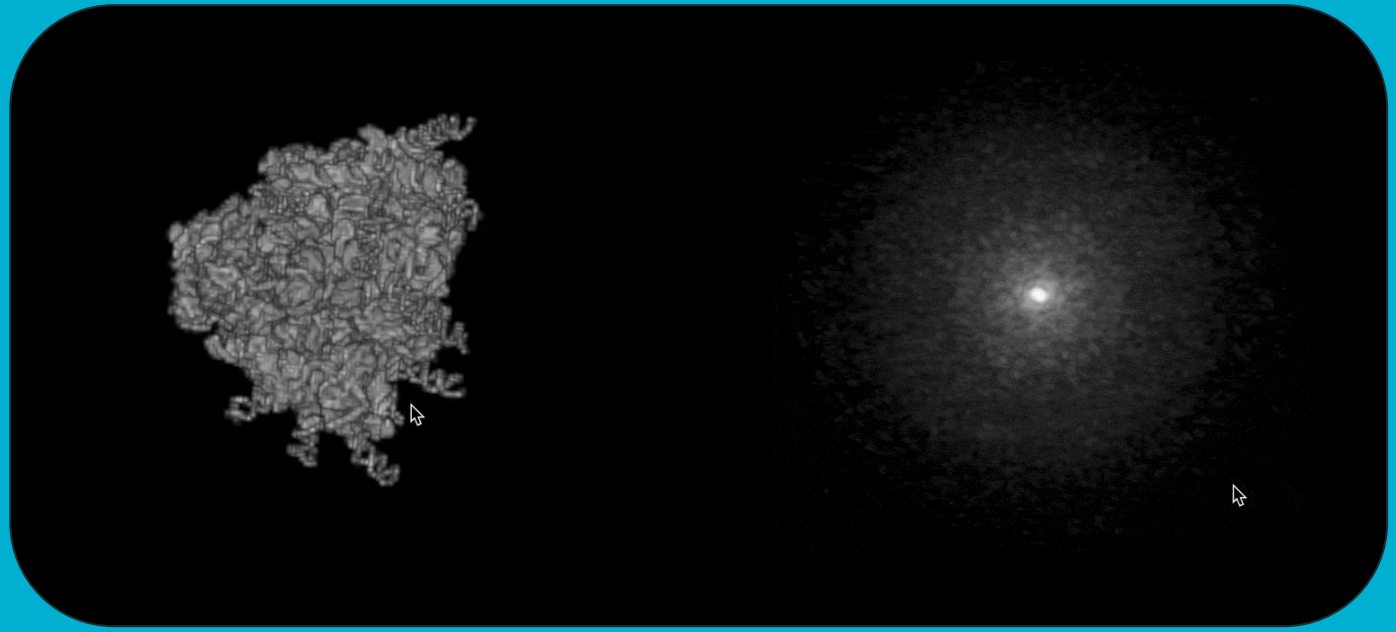
3D reconstruction from projection images



3D object

3D Fourier transform
(power spectrum)

3D reconstruction from projection images



3D object

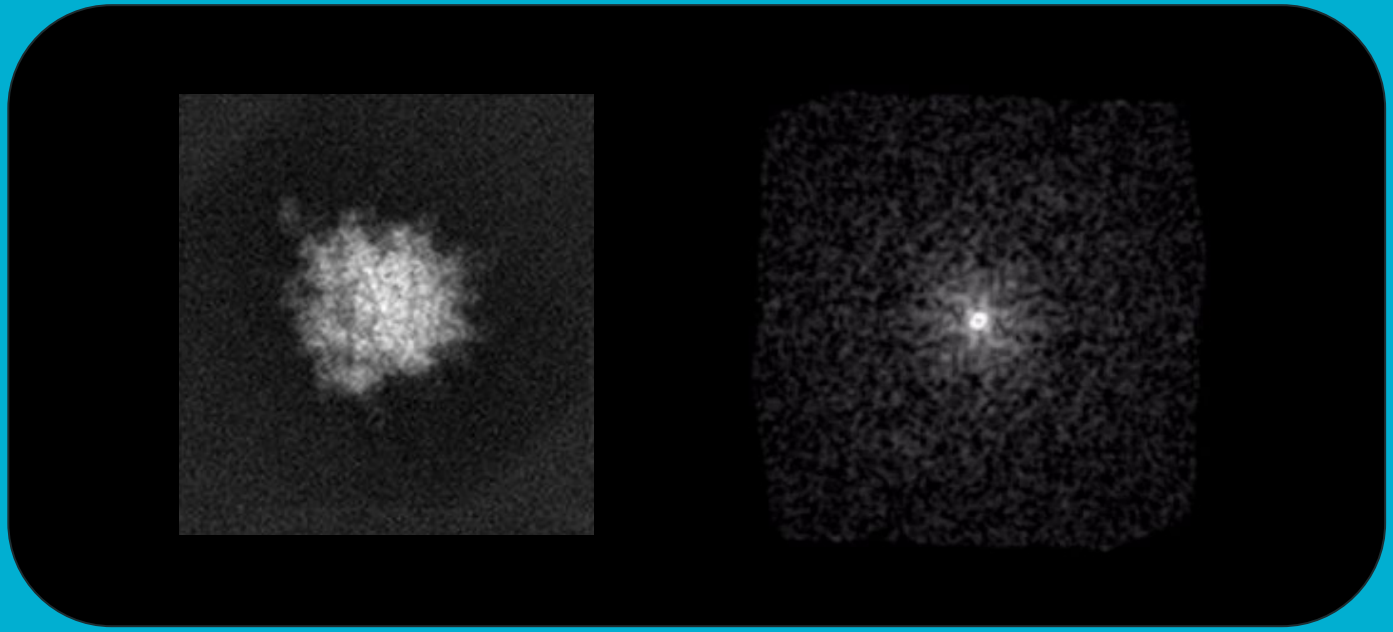
FFT

3D Fourier transform
(power spectrum)

3D reconstruction from projection images

central slice of
3D Fourier transform
(power spectrum)

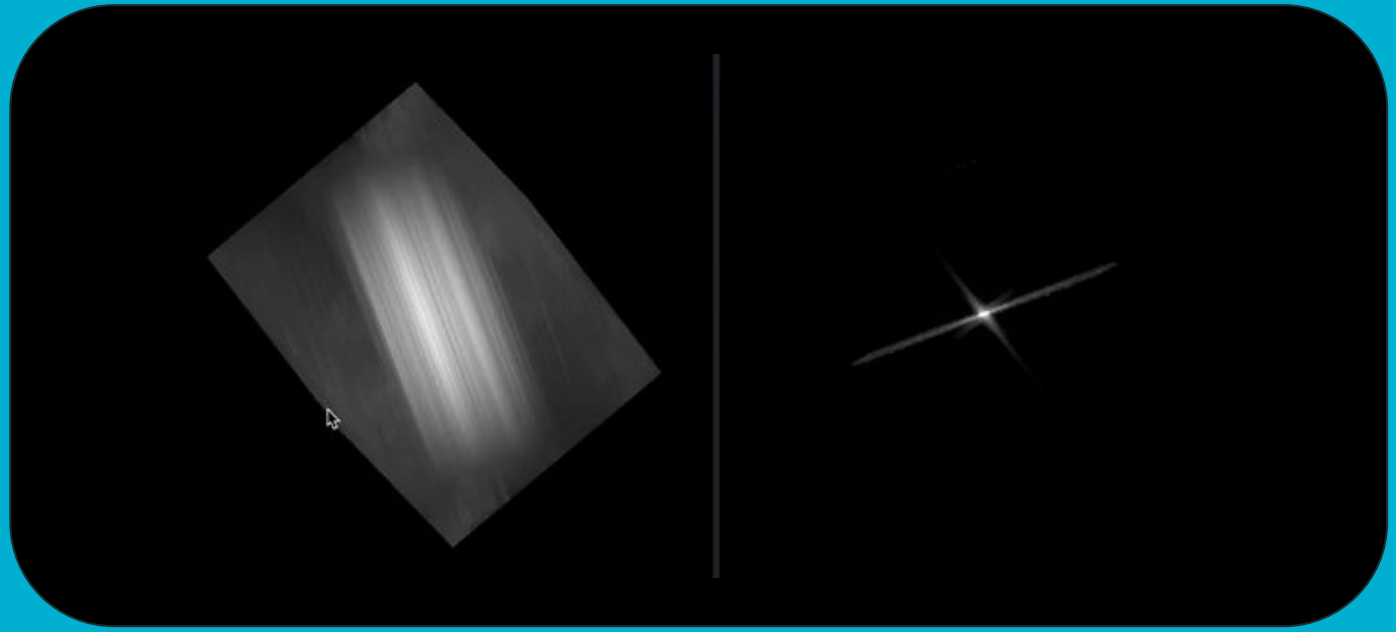
3D reconstruction from projection images



2D projection image

central slice of
3D Fourier transform
(power spectrum)

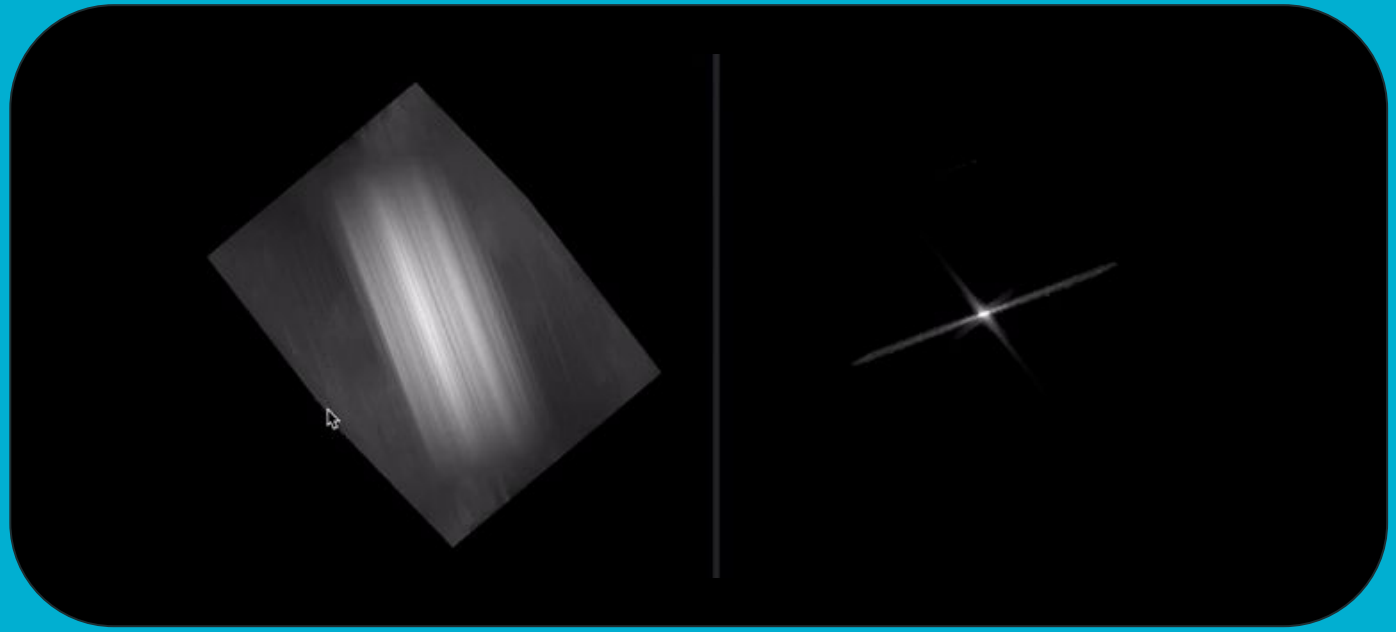
3D reconstruction from projection images



3D object
(real space)

central slice of
3D Fourier transform
(power spectrum)

3D reconstruction from projection images



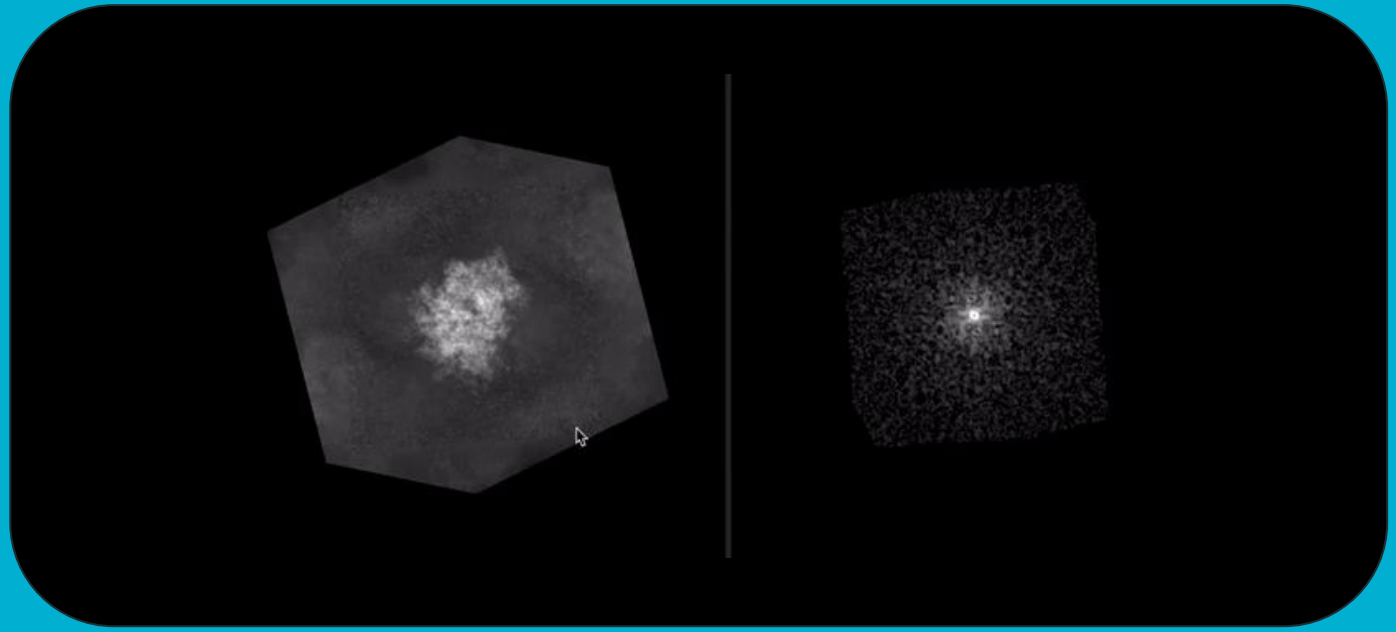
3D object
(real space)

FFT

inverse FFT

central slice of
3D Fourier transform
(power spectrum)

3D reconstruction from projection images



3D object
(real space)

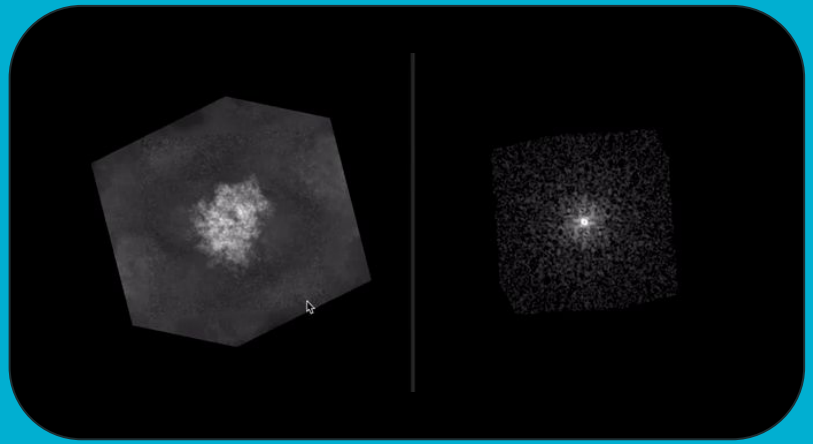
inverse FFT

central slices of
3D Fourier transform
(power spectrum)

What is single particle analysis?

SPA is a process for determining the following parameters for each particle image

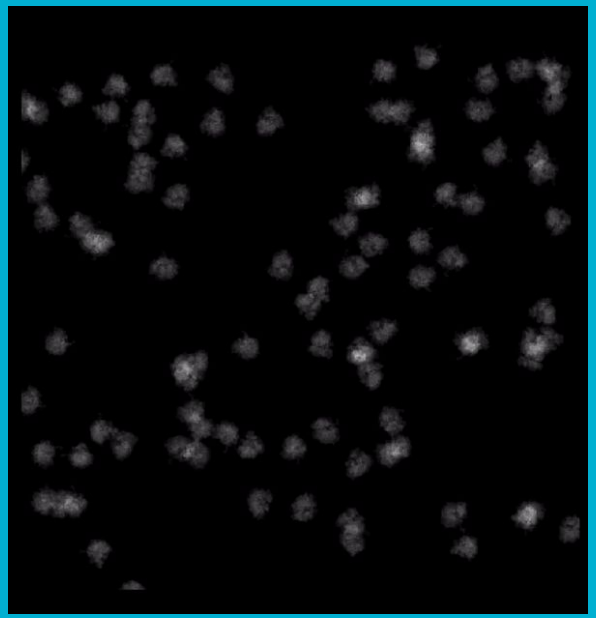
- 2D shifts
- orientations
- defocus



This allows us to build up the 3D Fourier transform

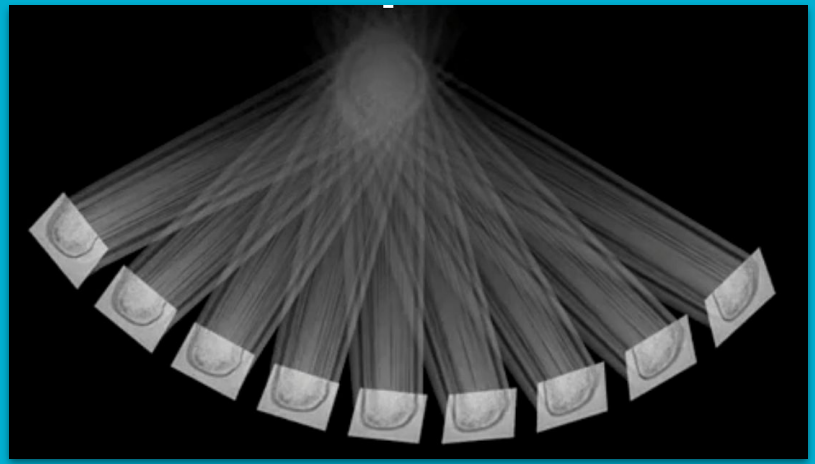
now let’s do it for tomography

tilt series



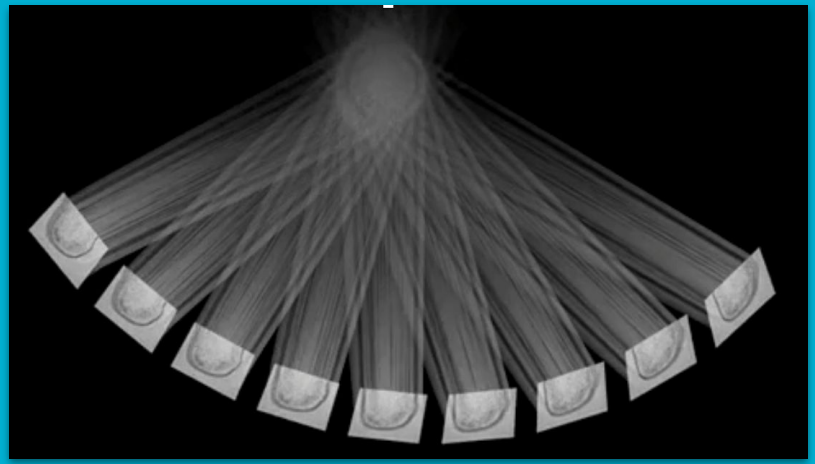
plus minus 60 degrees
3 degree increments
41 images
remember this is a stack of 2D images, not a 3D image

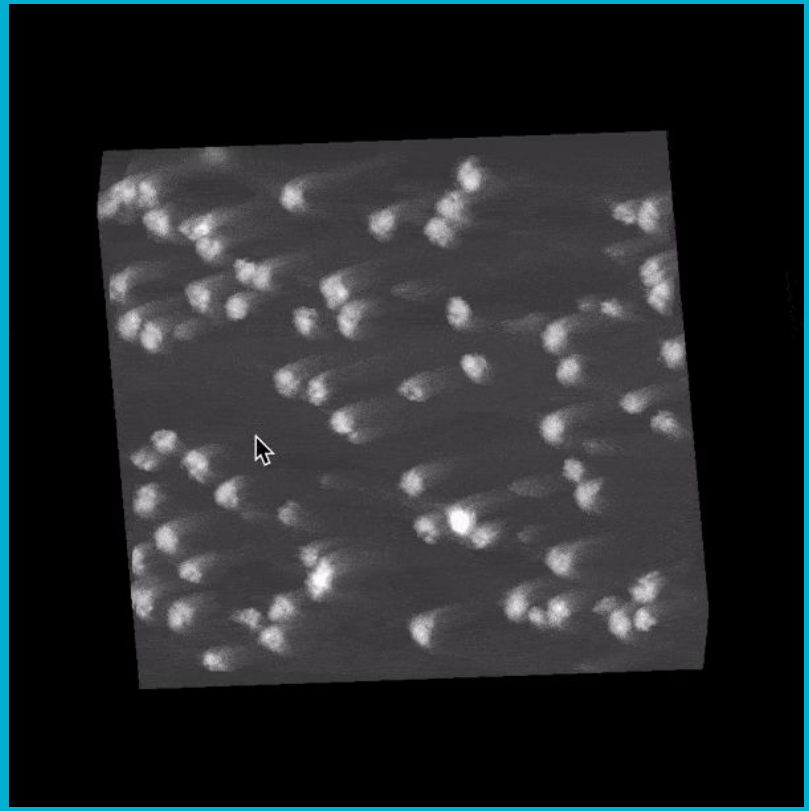
tilt series to tomograms



reconstruction typically
performed in real space
because images are large

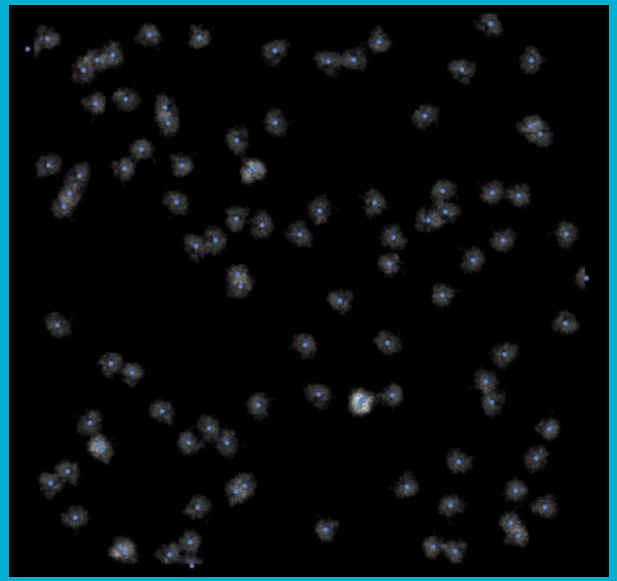
tilt series to tomograms

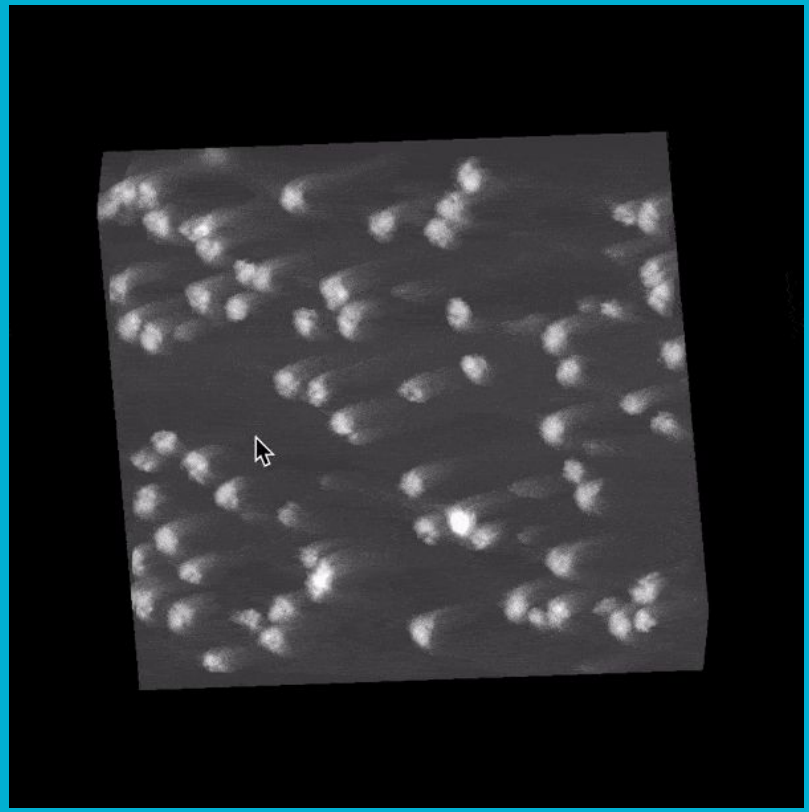




localise objects of interest in 3D

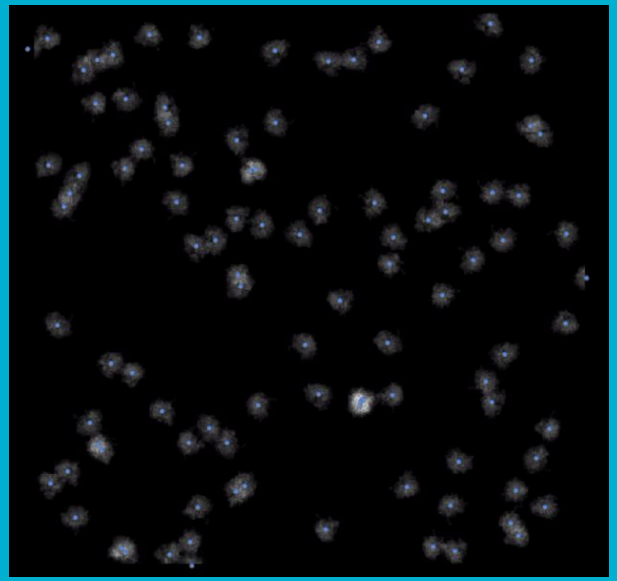
tilt series to tomograms



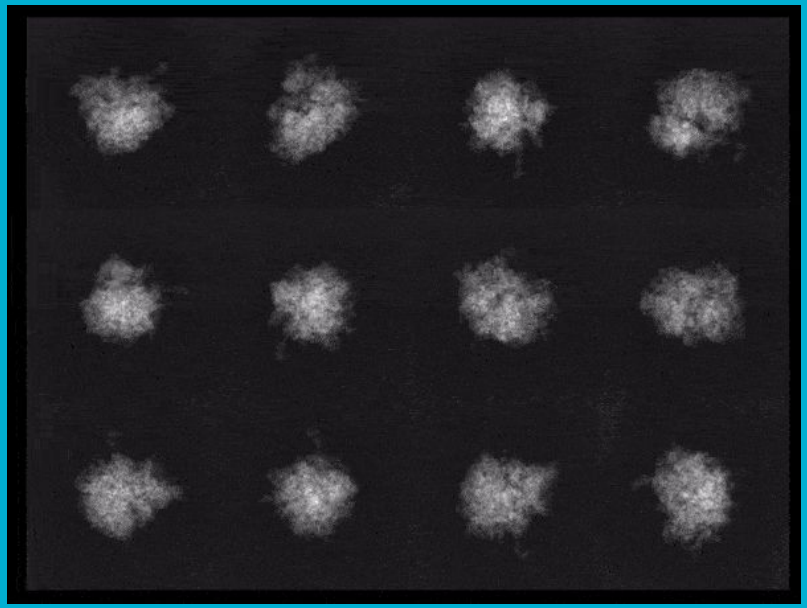


because we know the projection geometry
we can find our objects of interest in 2D

tilt series to particle tilt series

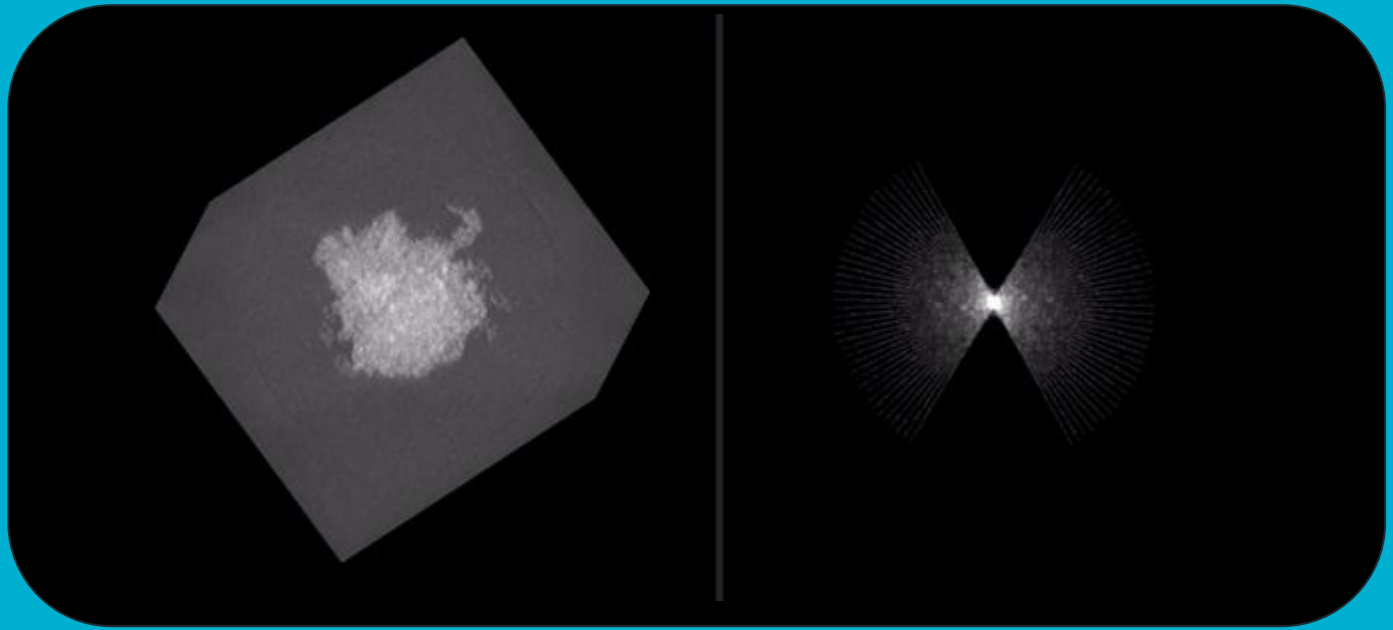


because we know the projection geometry
we can project 3D points down to 2D



this lets us extract mini tilt series
centered on each particle

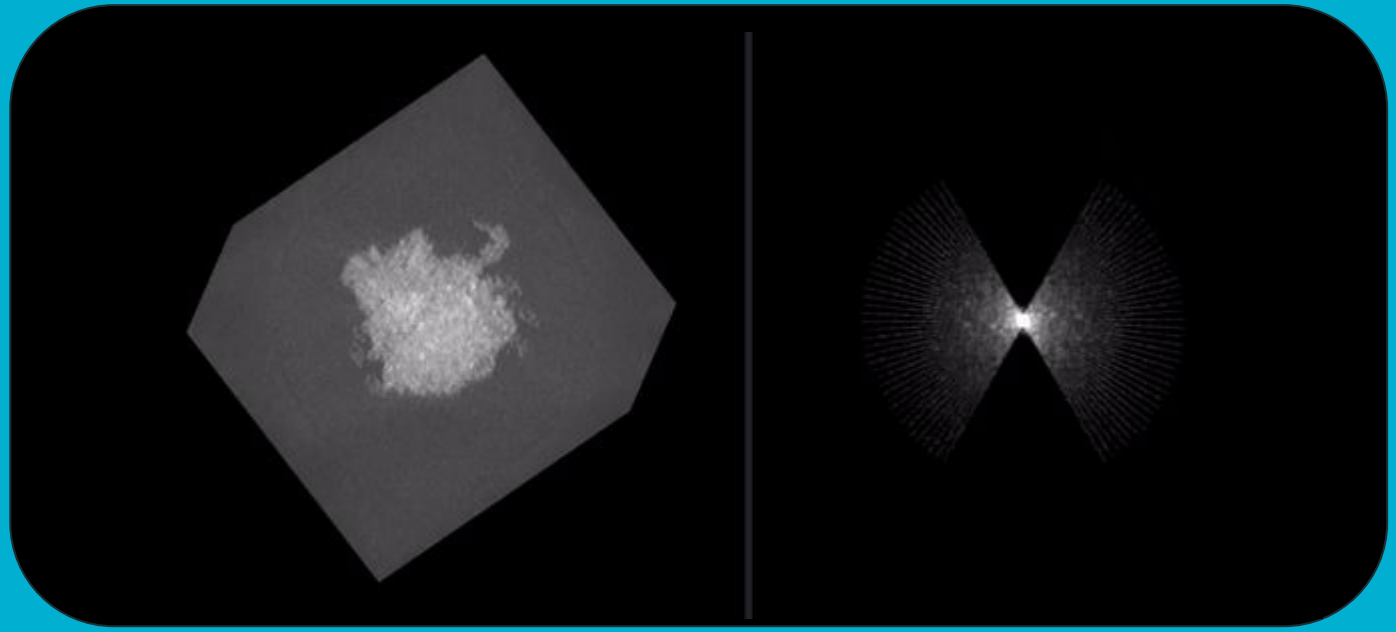
subtomograms ~= particle tilt series
(but it's easier to deal with the CTF properly on 2D data)



3D reconstruction
from particle tilt series

particle tilt image FTs inserted as
central slices
(power spectrum)

3D reconstruction from particle tilt series

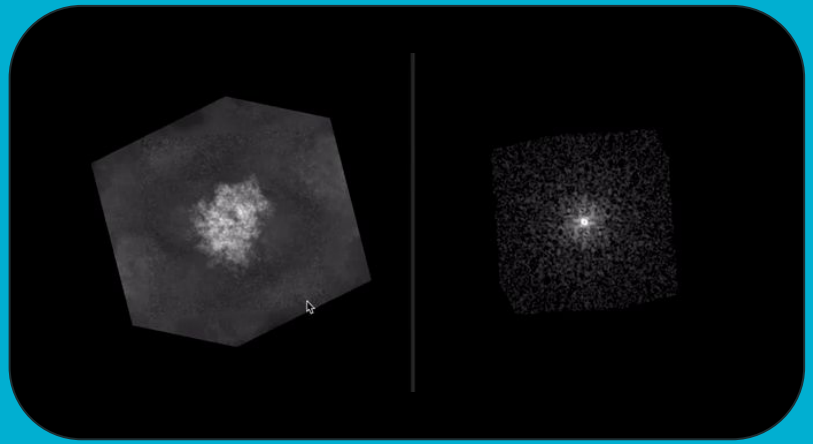


each particle contributes 41 central slices to the 3D FT

What is ‘subtomogram averaging’?

STA is a process for determining the following parameters for each particle tilt series

- 3D shifts
- orientations
- defocus



This allows us to build up the 3D Fourier transform

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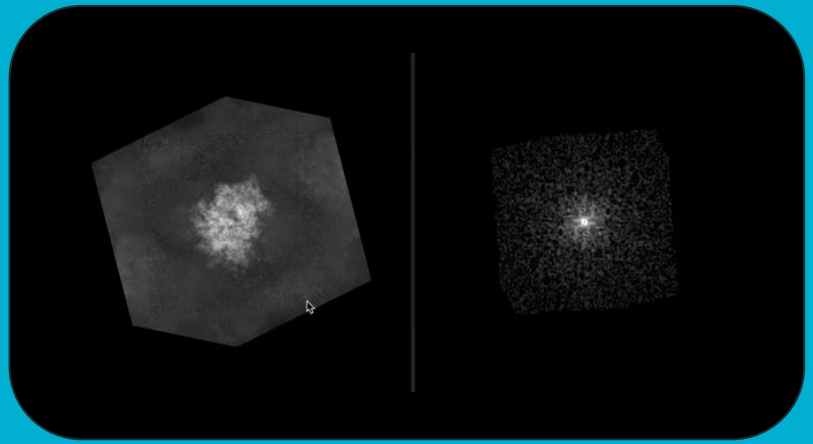


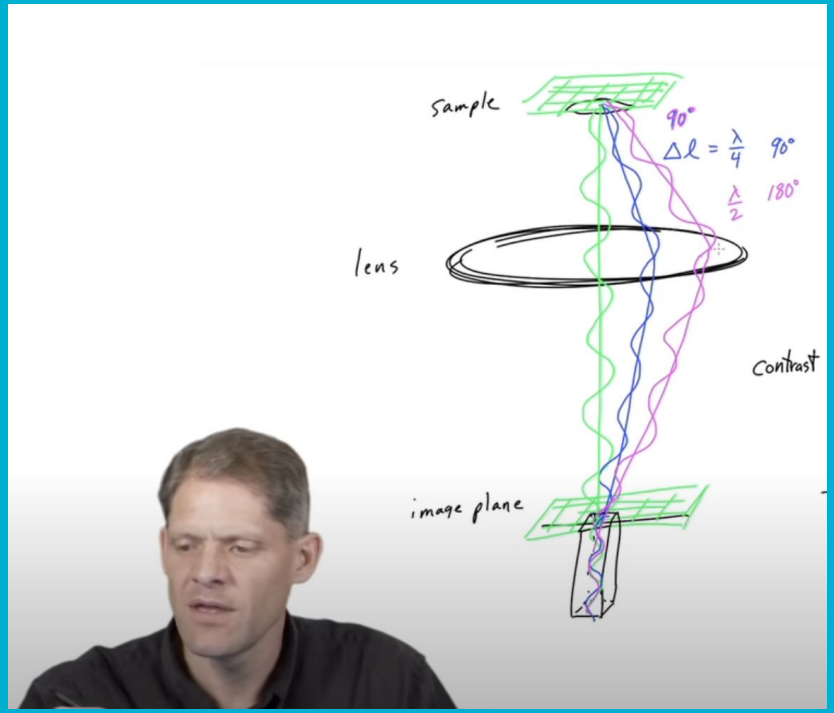
This allows us to build up the 3D Fourier transform

tilt series processing is a superset of frame series processing

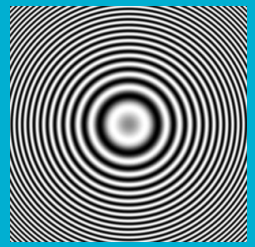
I hope I've convinced you

the CTF in tomograms

CTF is changed a lot by defocus

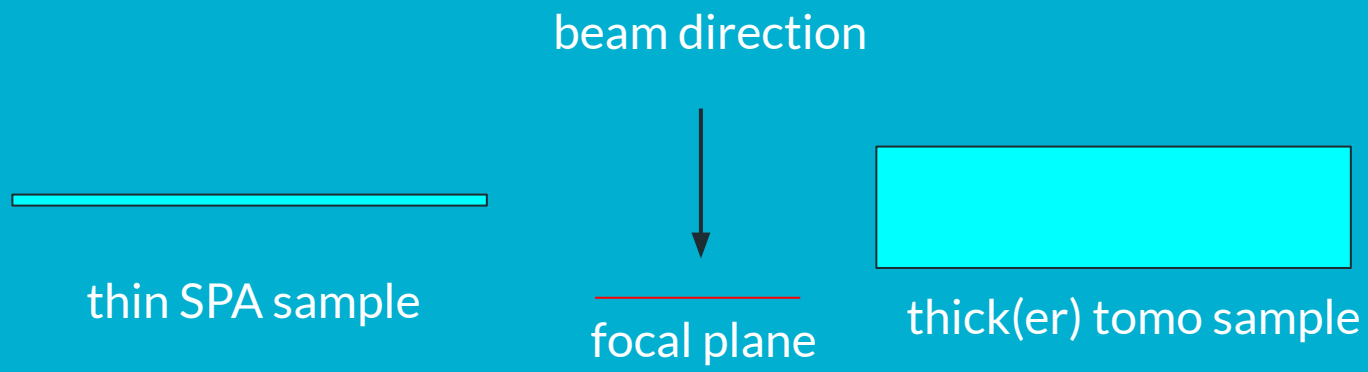


1µm defocus
2 Å/px



2µm defocus
2 Å/px

sample thickness matters



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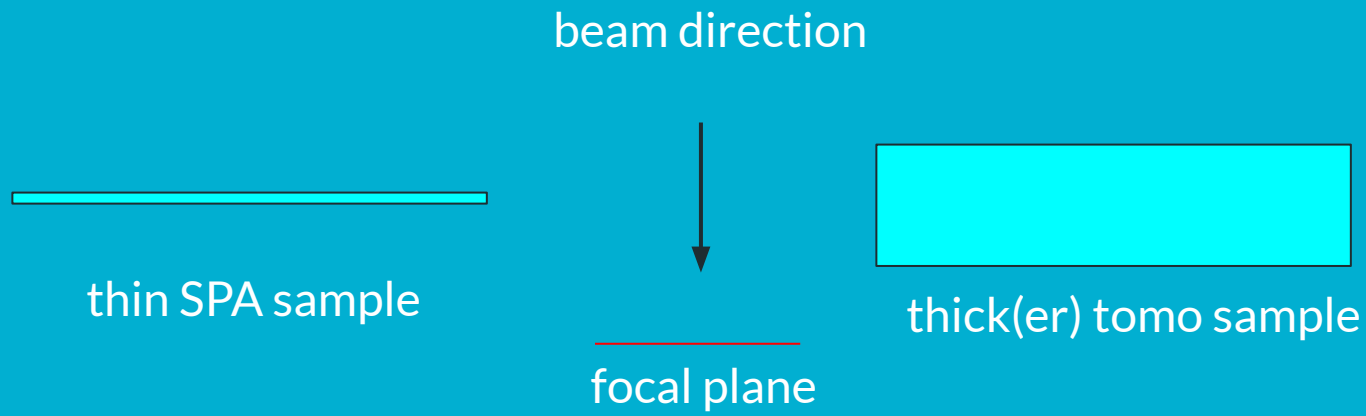


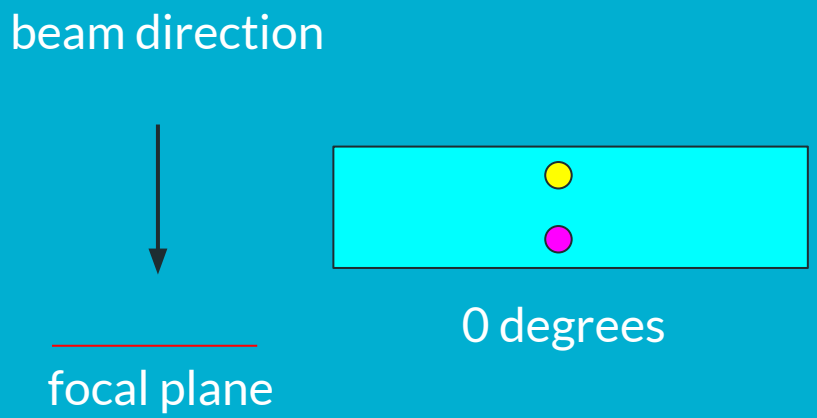
whole sample at ~1µm defocus

safe to assume all particles at same defocus

whole sample between 0.8-1.2 µm defocus

unsafe to assume all particles at same defocus

sample thickness matters



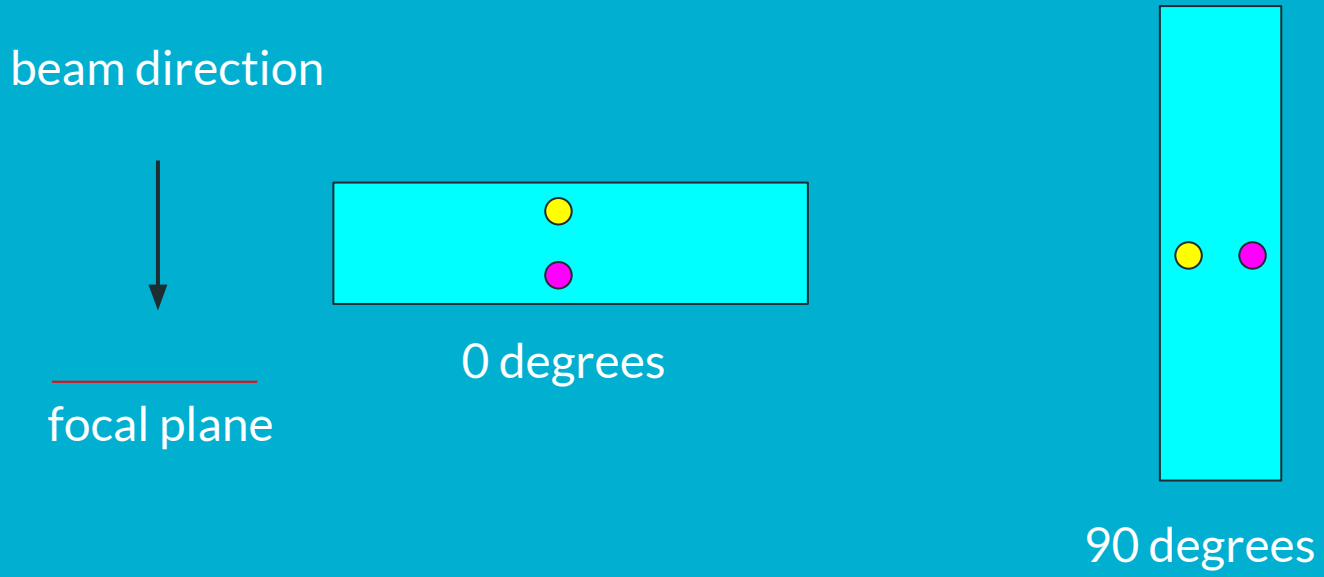
different defoci for particles at different distances from focal plane

> Ultramicroscopy. 2000 Jul;84(1-2):57-64. doi: 10.1016/s0304-3991(00)00005-x.

Defocus-gradient corrected back-projection

G J Jensen [1], R D Kornberg

sample thickness matters

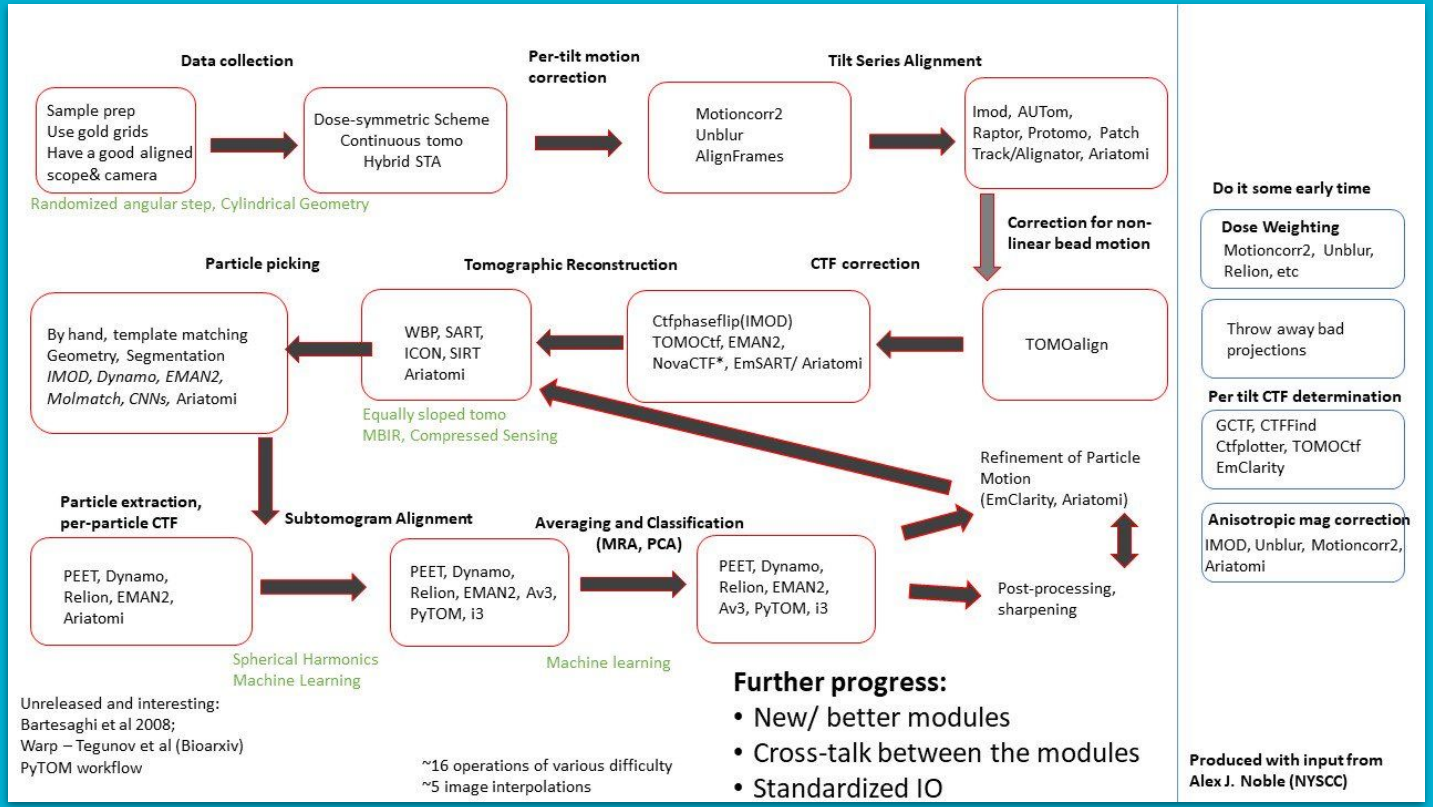


Efficient 3D-CTF correction for cryo-electron tomography using NovaCTF improves subtomogram averaging resolution to 3.4Å

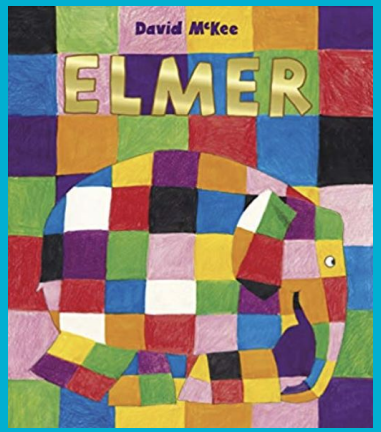
Beata Turoňová [a], Florian K.M. Schur [a], William Wan [a], John A.G. Briggs [a b]

Software Workflows

the patchwork of cryo-ET software



Ca. 2018



Kendra Leigh &
Misha Kurdryashev

things are improving…

- Warp + M
- RELION 5
- STOPGAP
- EMAN
- Dynamo
- IMOD/PEET
- nextpyp
- scipion

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trend is towards ‘complete pipelines’...

- is that what we want?
- is that realistic?

tomography (and tomography projects) are, by definition, complicated…

if they aren't you should probably be doing single particle

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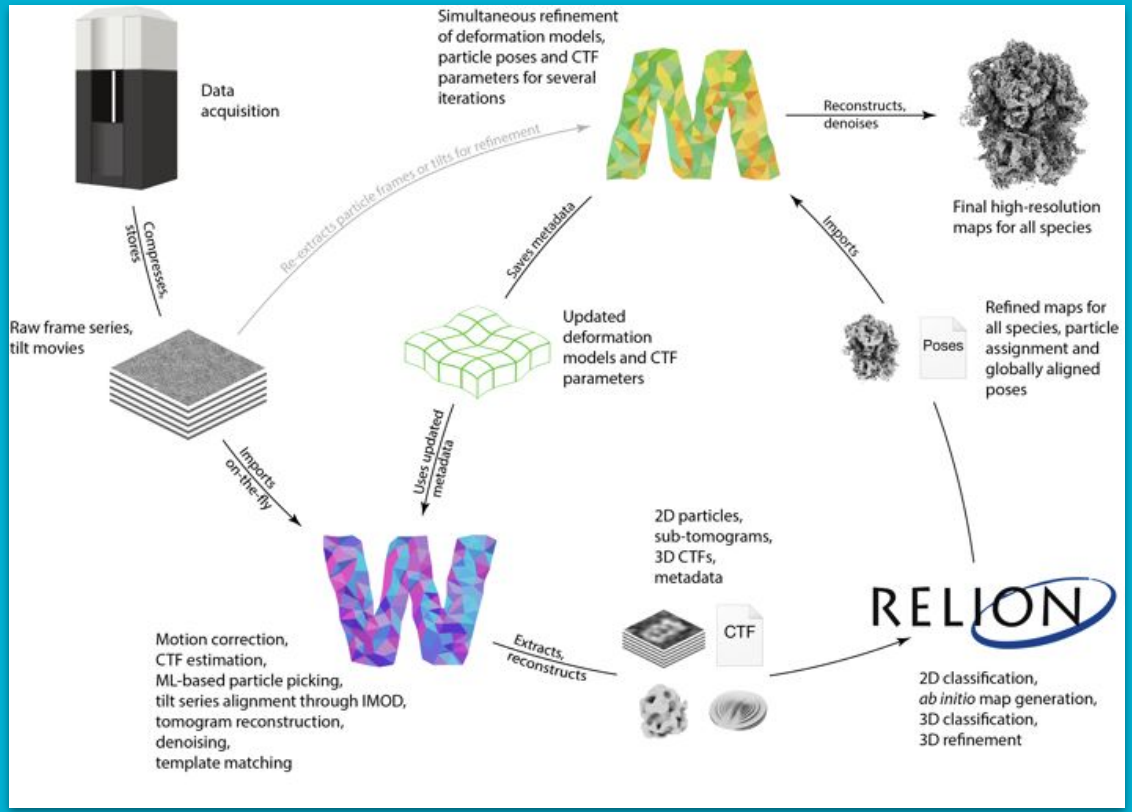
if they aren't you should probably be doing single particle

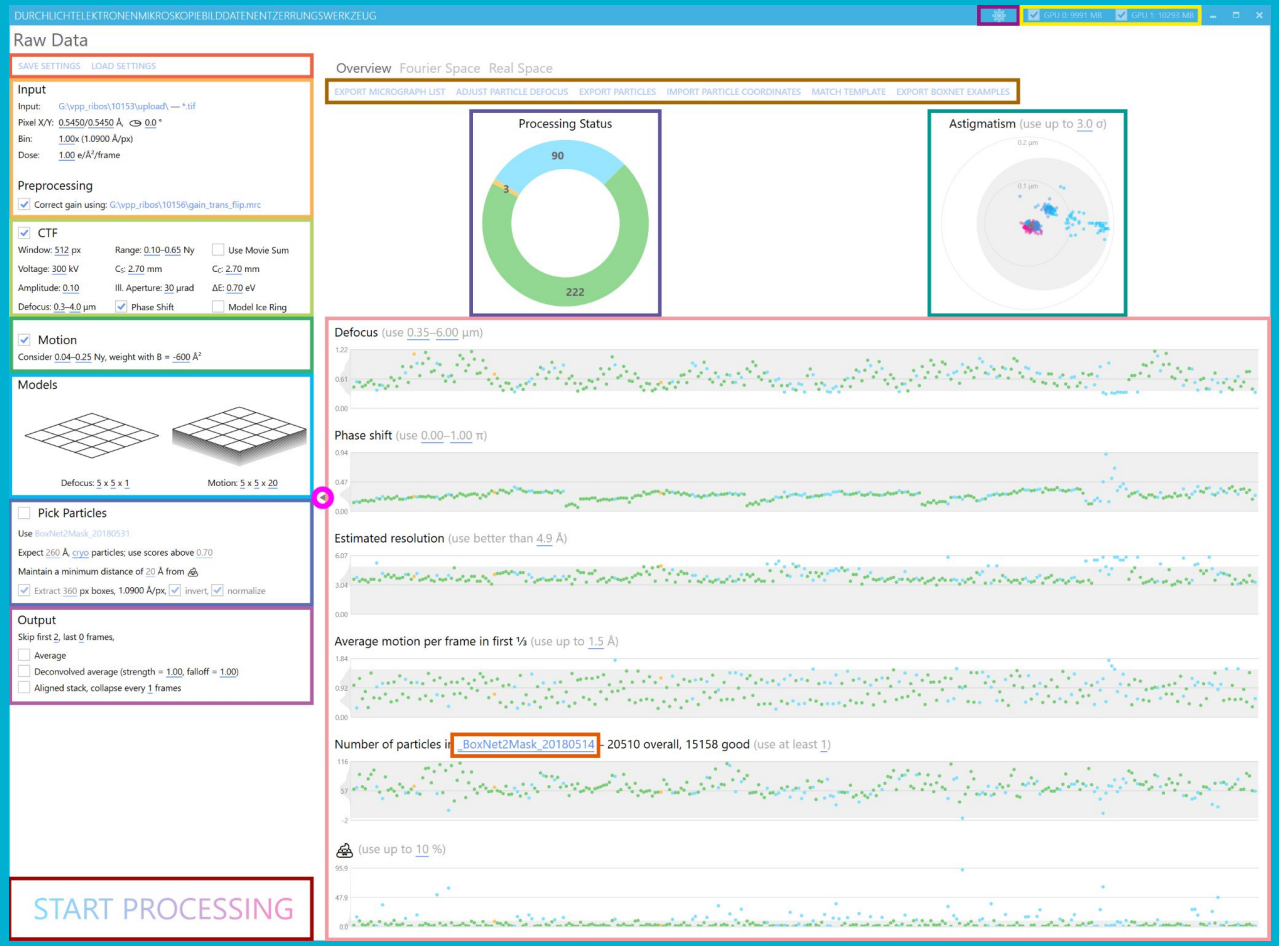
one software package is unlikely to solve *all* of your problems

the Warp + M workflow



slide from Dimitry

Warp/M had a great GUI



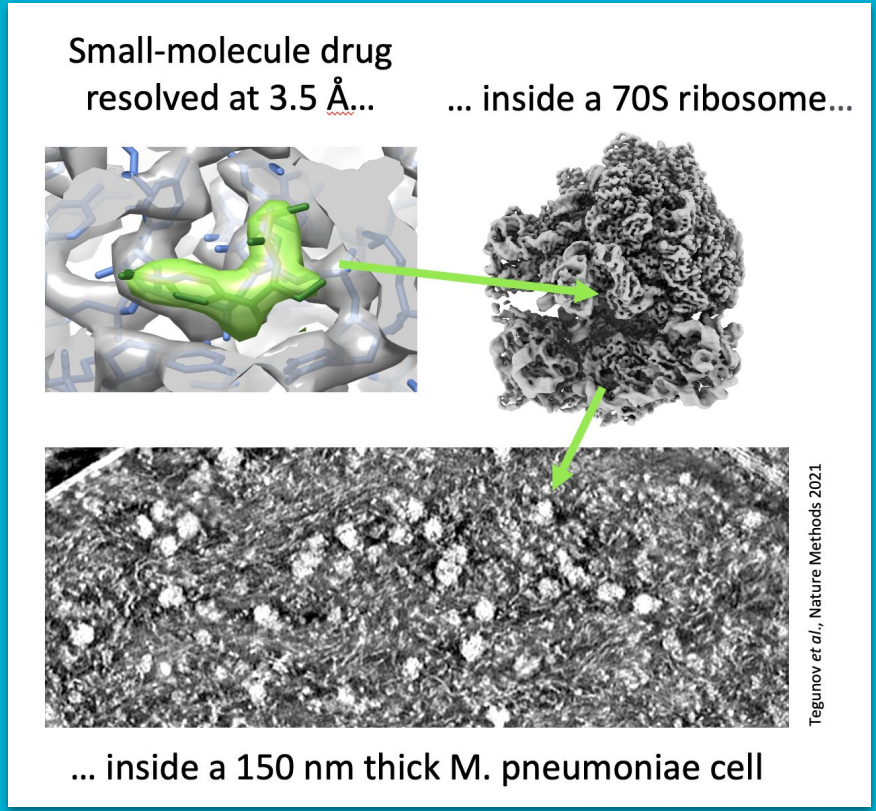
but they were locked in a Windows cage

Warp 2.0

now on linux!

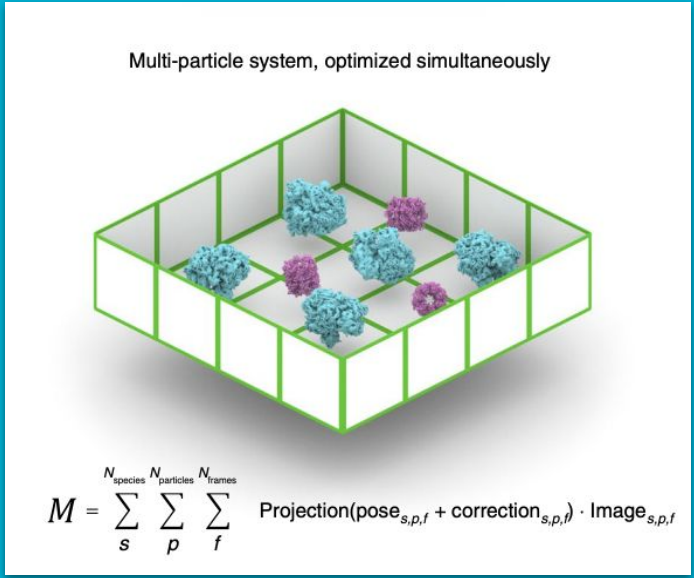
```
conda install warp -c warpem -c nvidia/label/cuda-11.7.0 -c pytorch -c conda-forge
```

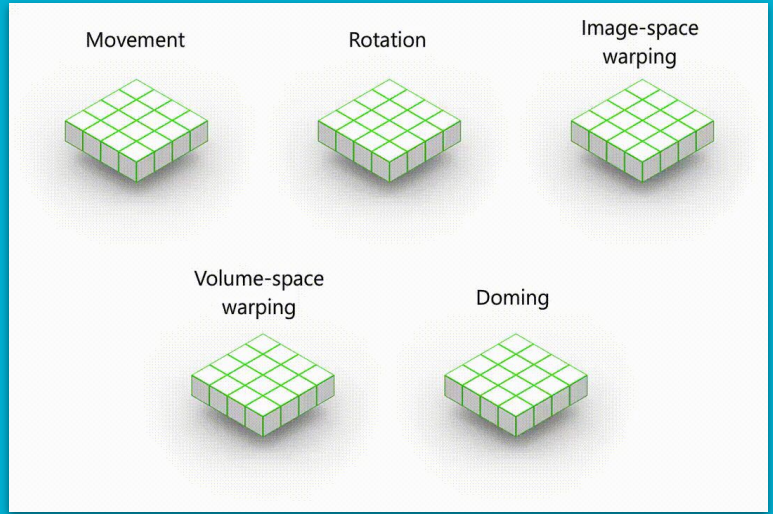
M's first significant result



slide from Dimitry

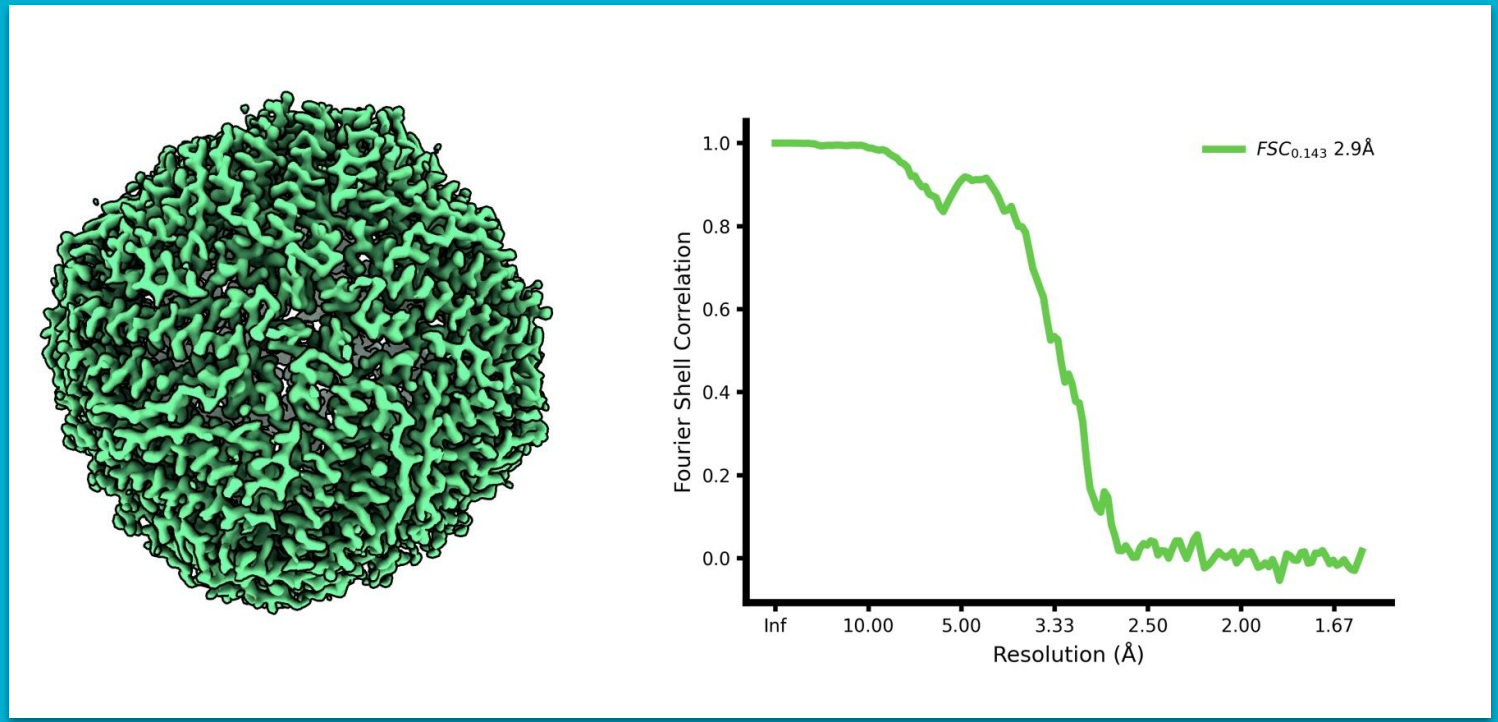
Mmodel the whole system





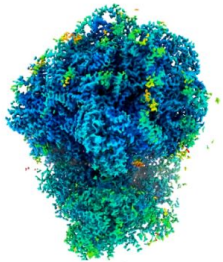
Tegunov *et al.*, *Nature Methods* (2021)

benchmarked on linux with 5-TS from EMPIAR-10491

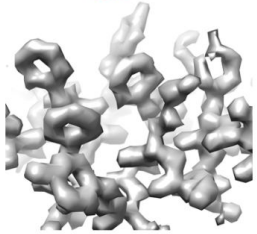


looks good! 🚀

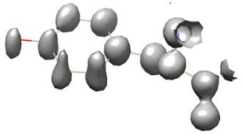
Warp & M continue to hold resolution records in SPA and Tomo in 2024 (?)



In situ tilt series, 80S ribosome: 2.5 Å
Huaipeng Xing & Martin Beck's team



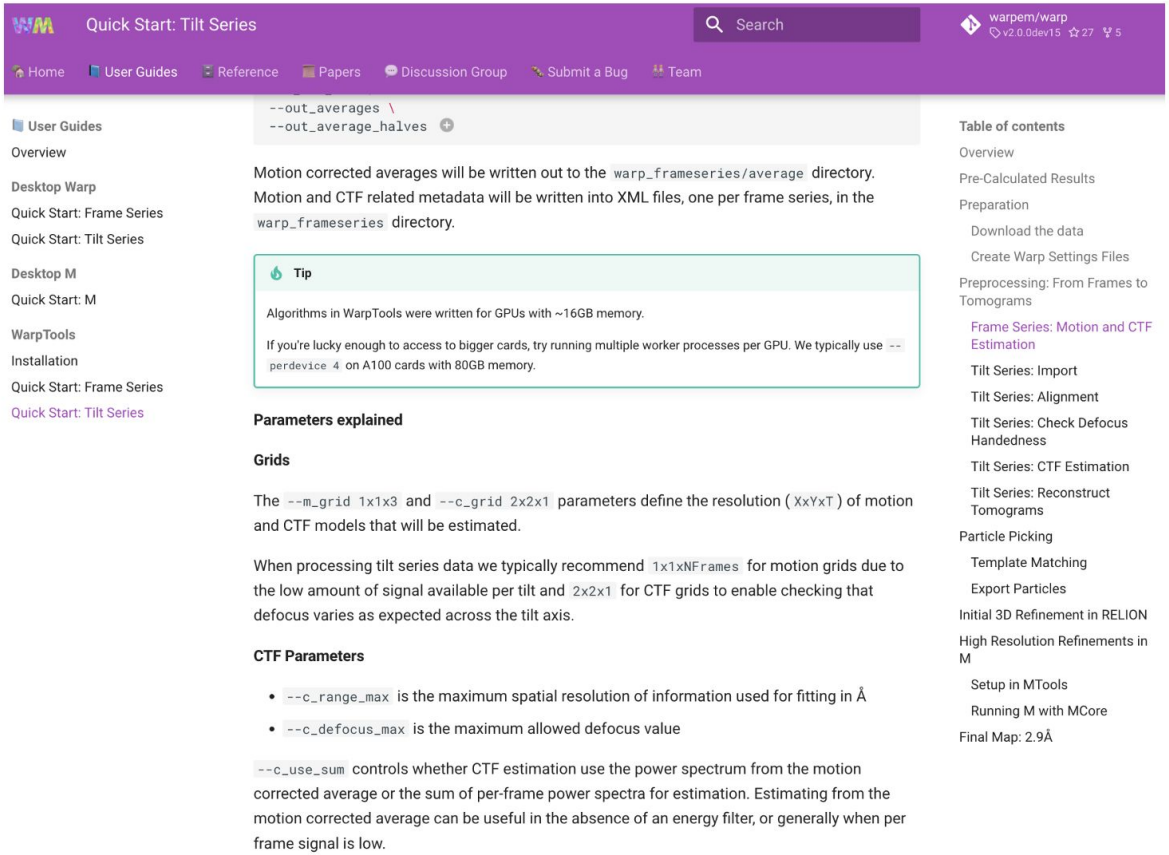
In vitro apoferritin tilt series: 1.5 Å
Martin Obr & Abhay Kotecha's team



In vitro apoferritin frame series: 0.99 Å
My reprocessing of Holger Stark's EMPIAR-10591

slide from Dimitry

Documentation for users who like to read 🤓



Quick Start: Tilt Series

Search

warpem/warp
v2.0.0dev15 ☆27 ⑂5

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User Guides
Overview

Desktop Warp
Quick Start: Frame Series
Quick Start: Tilt Series

Desktop M
Quick Start: M

WarpTools
Installation
Quick Start: Frame Series
Quick Start: Tilt Series

```
--out_averages \
--out_average_halves
```

Motion corrected averages will be written out to the `warp_frameseries/average` directory. Motion and CTF related metadata will be written into XML files, one per frame series, in the `warp_frameseries` directory.

> **Tip**
>
> Algorithms in WarpTools were written for GPUs with ~16GB memory.
>
> If you're lucky enough to access to bigger cards, try running multiple worker processes per GPU. We typically use `--perdevice 4` on A100 cards with 80GB memory.

Parameters explained

Grids

The `--m_grid 1x1x3` and `--c_grid 2x2x1` parameters define the resolution (`XxYxT`) of motion and CTF models that will be estimated.

When processing tilt series data we typically recommend `1x1xNFrames` for motion grids due to the low amount of signal available per tilt and `2x2x1` for CTF grids to enable checking that defocus varies as expected across the tilt axis.

CTF Parameters

- `--c_range_max` is the maximum spatial resolution of information used for fitting in Å
- `--c_defocus_max` is the maximum allowed defocus value

`--c_use_sum` controls whether CTF estimation use the power spectrum from the motion corrected average or the sum of per-frame power spectra for estimation. Estimating from the motion corrected average can be useful in the absence of an energy filter, or generally when per frame signal is low.

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Pre-Calculated Results
Preparation
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- Create Warp Settings Files

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- Frame Series: Motion and CTF Estimation
- Tilt Series: Import
- Tilt Series: Alignment
- Tilt Series: Check Defocus Handedness
- Tilt Series: CTF Estimation
- Tilt Series: Reconstruct Tomograms

Particle Picking
- Template Matching
- Export Particles

Initial 3D Refinement in RELION
High Resolution Refinements in M
- Setup in MTools
- Running M with MCore

Final Map: 2.9Å

Workshops for users who don’t like to read 😌

Shyamal Mosalaganti
@s_mosalaganti

Follow

Day1 of cryoET image processing workshop. Warp/Relion/M masterclass from @AlisterBurt

6:12 PM · Jun 10, 2024 · **7,947** Views

3 7 69 7

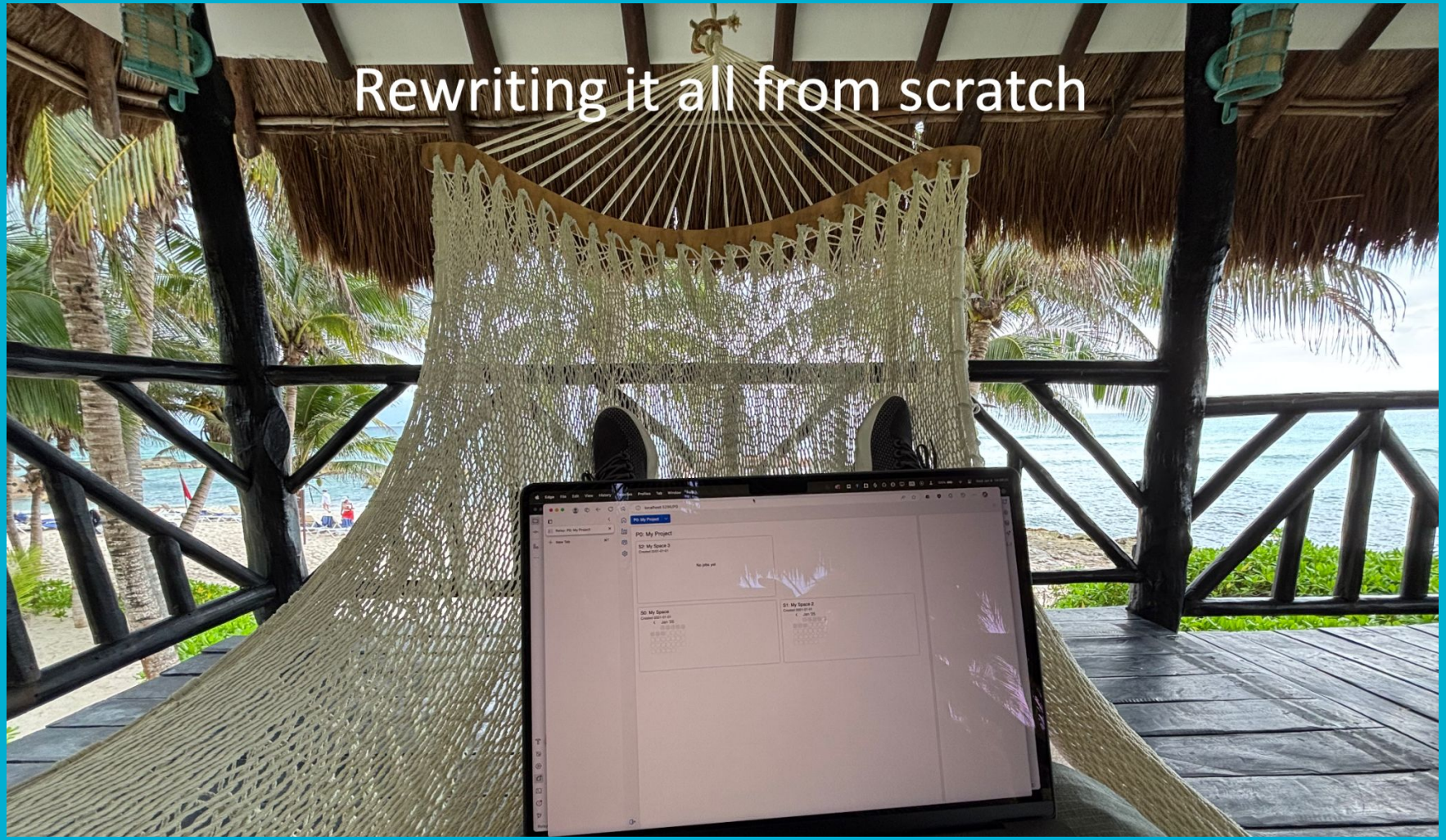
Relay

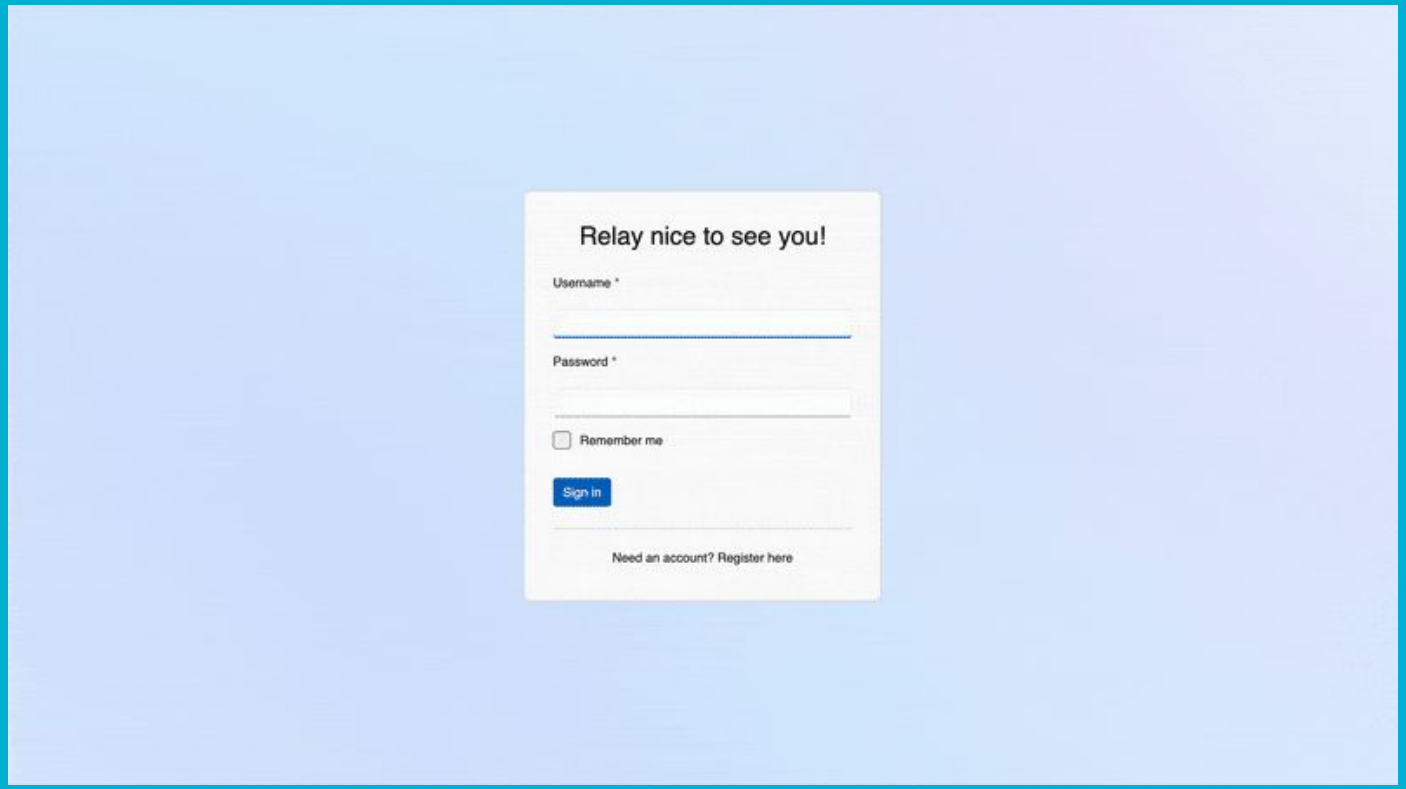
Warp, M + friends on the web!

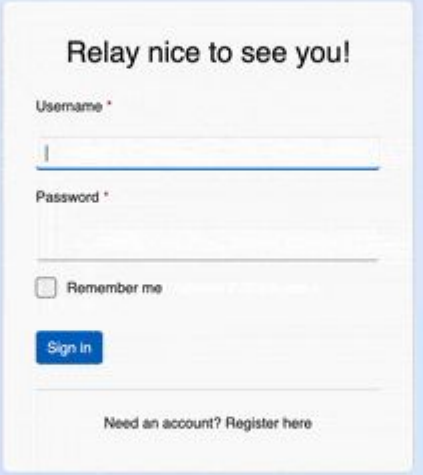
(not yet released)

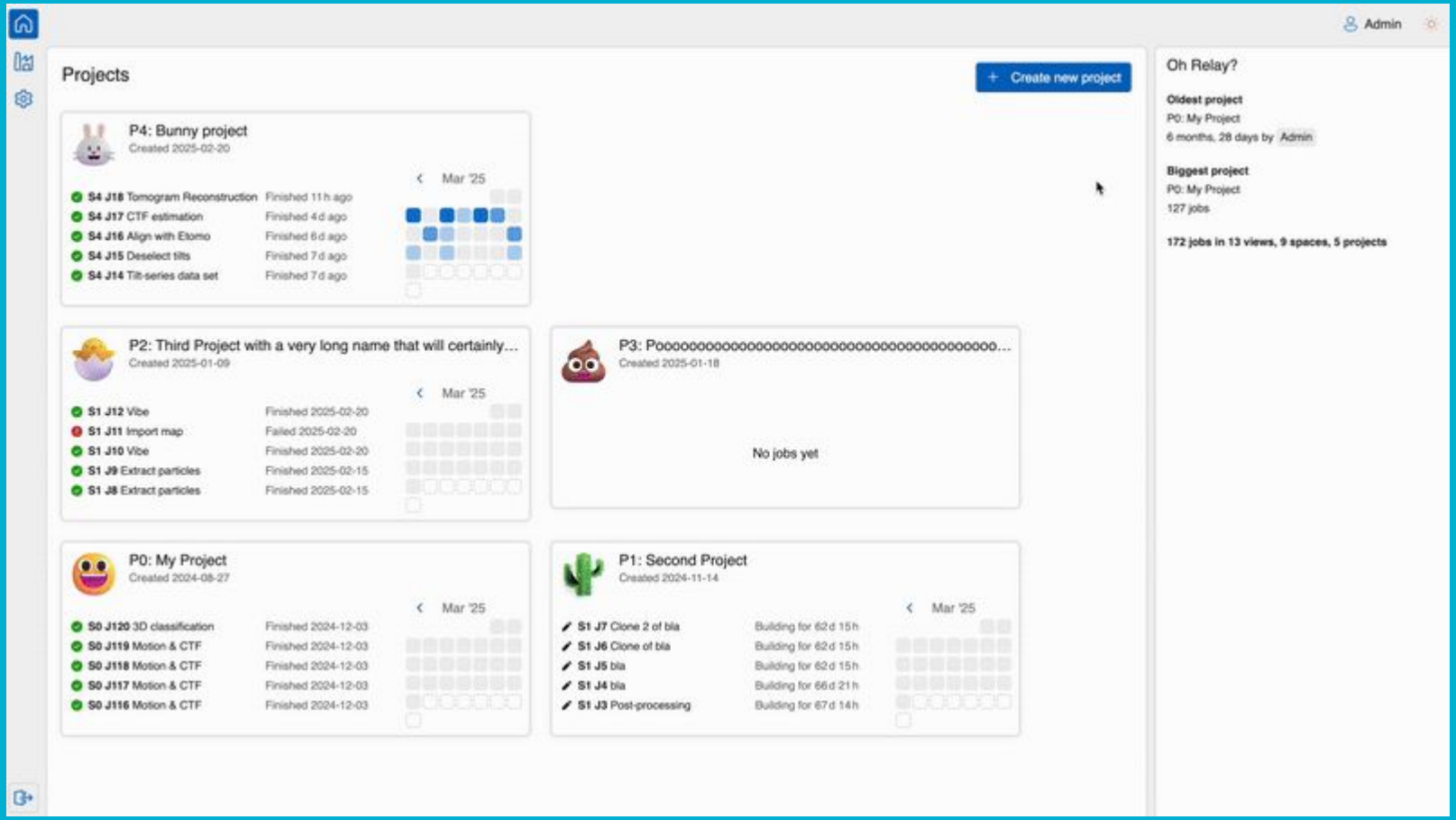
(soon, maybe?)

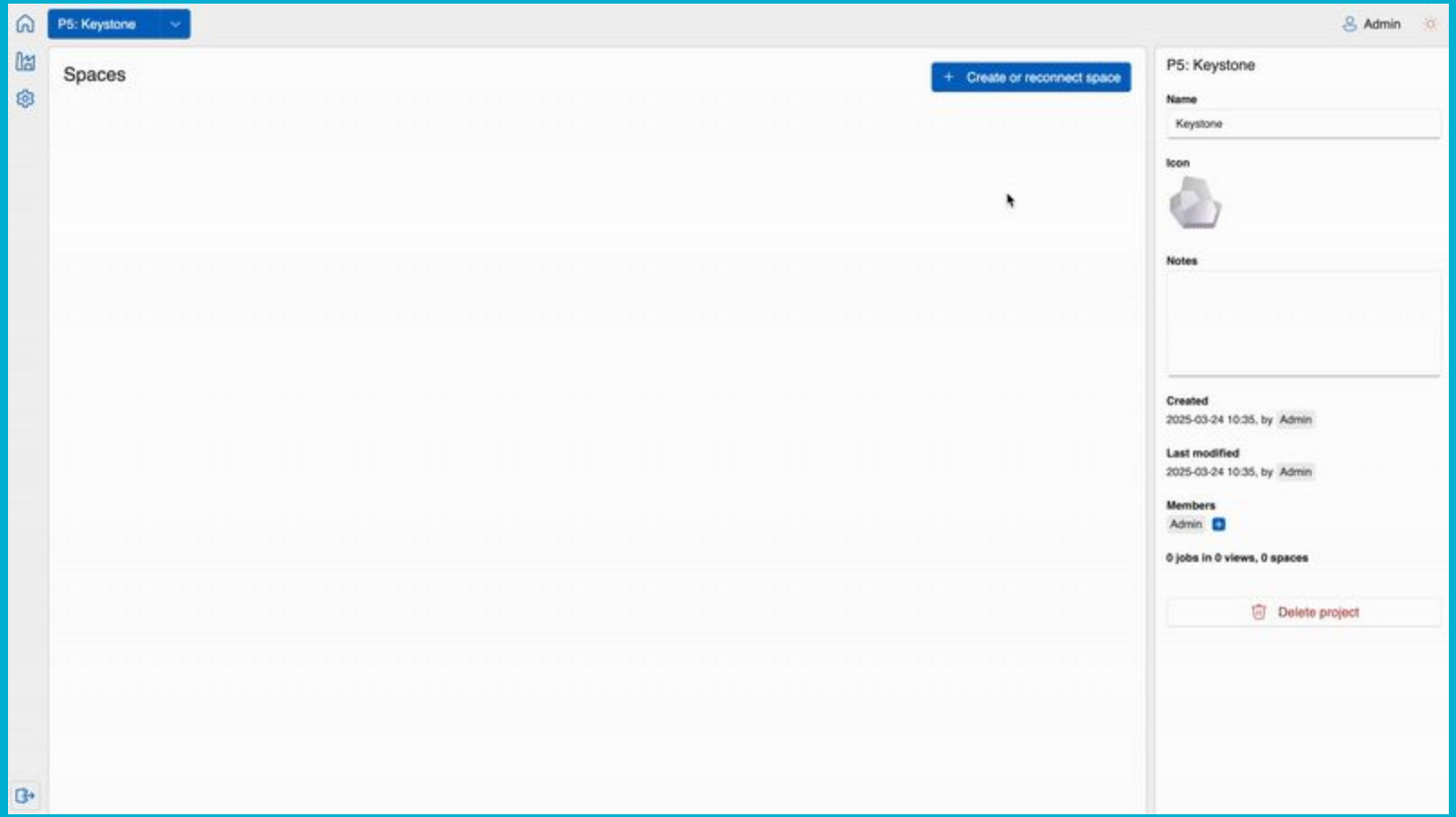
Rewriting it all from scratch

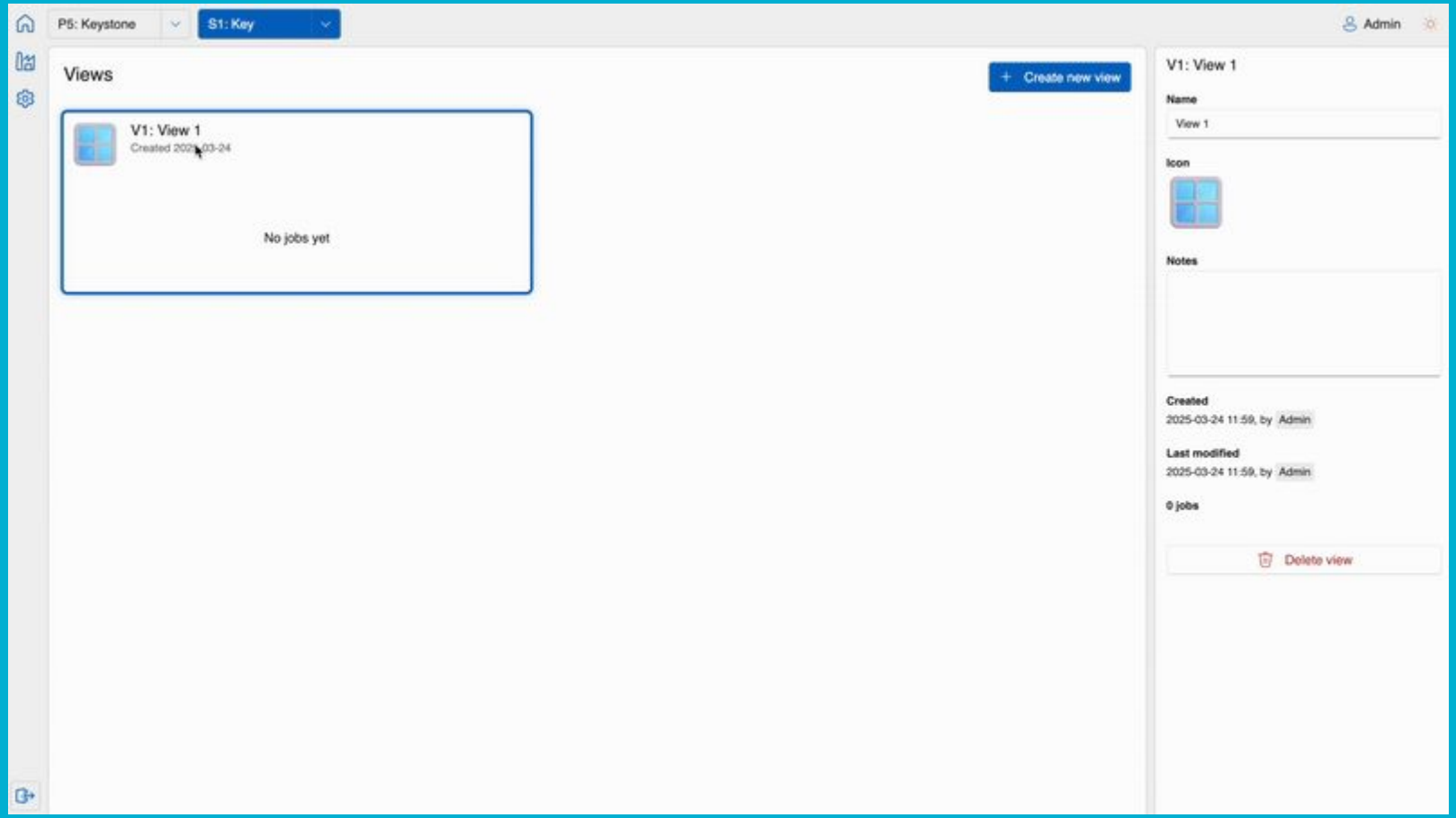


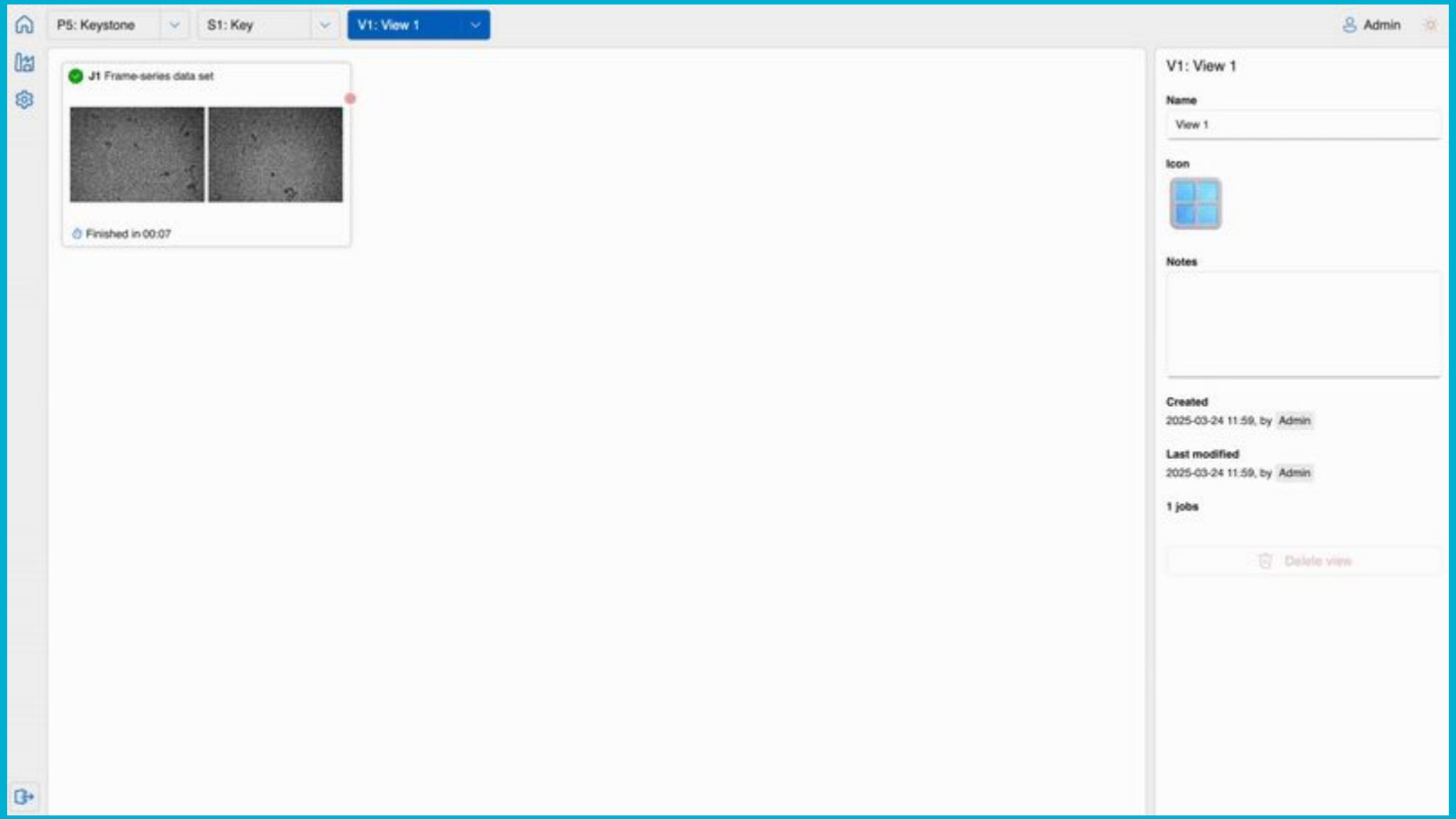

Relay nice to see you!
Username *
Password *
Remember me
Sign in
Need an account? Register here

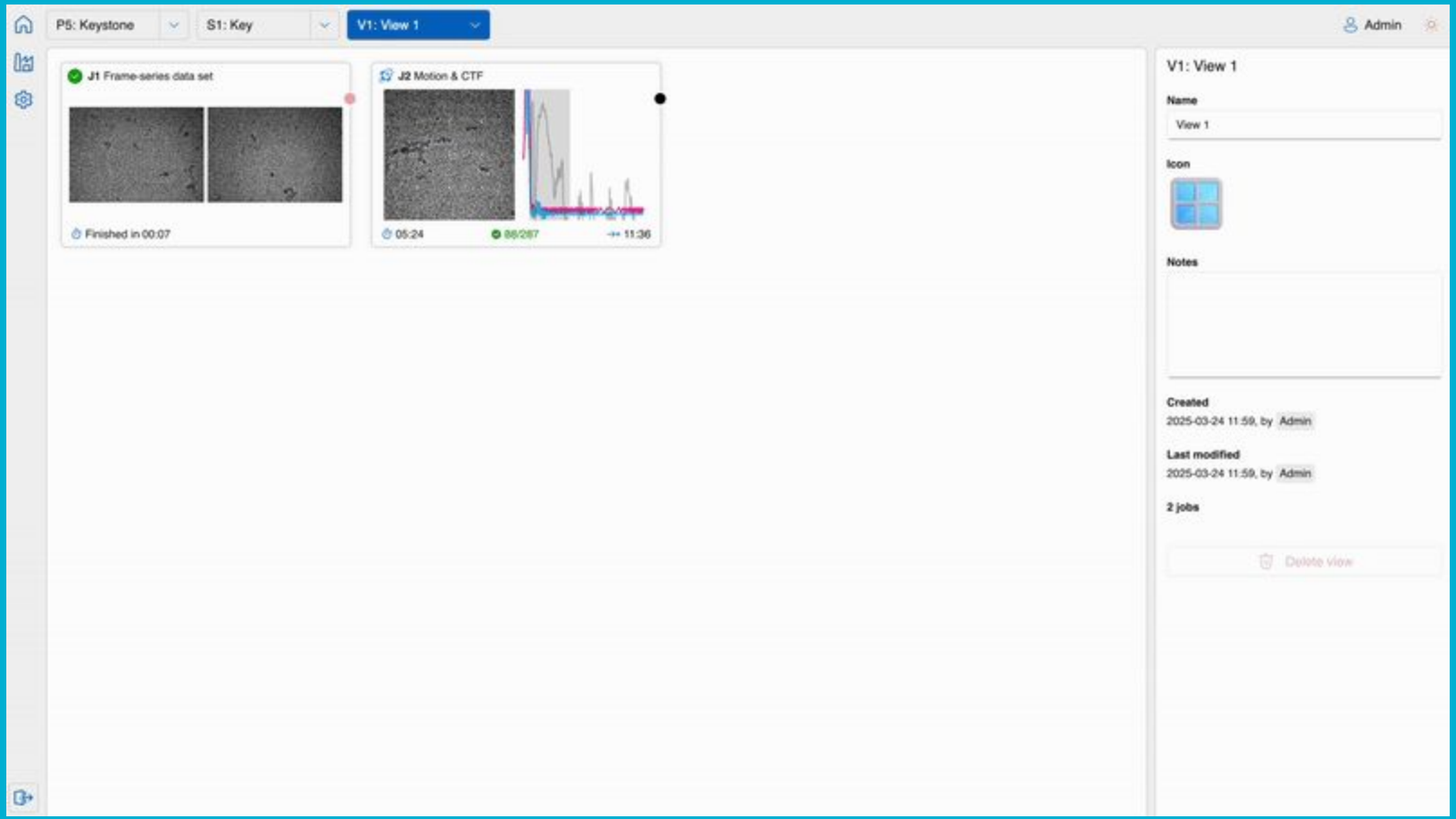

Relay nice to see you!
Username *
Password *
Remember me
Sign In
Need an account? Register here

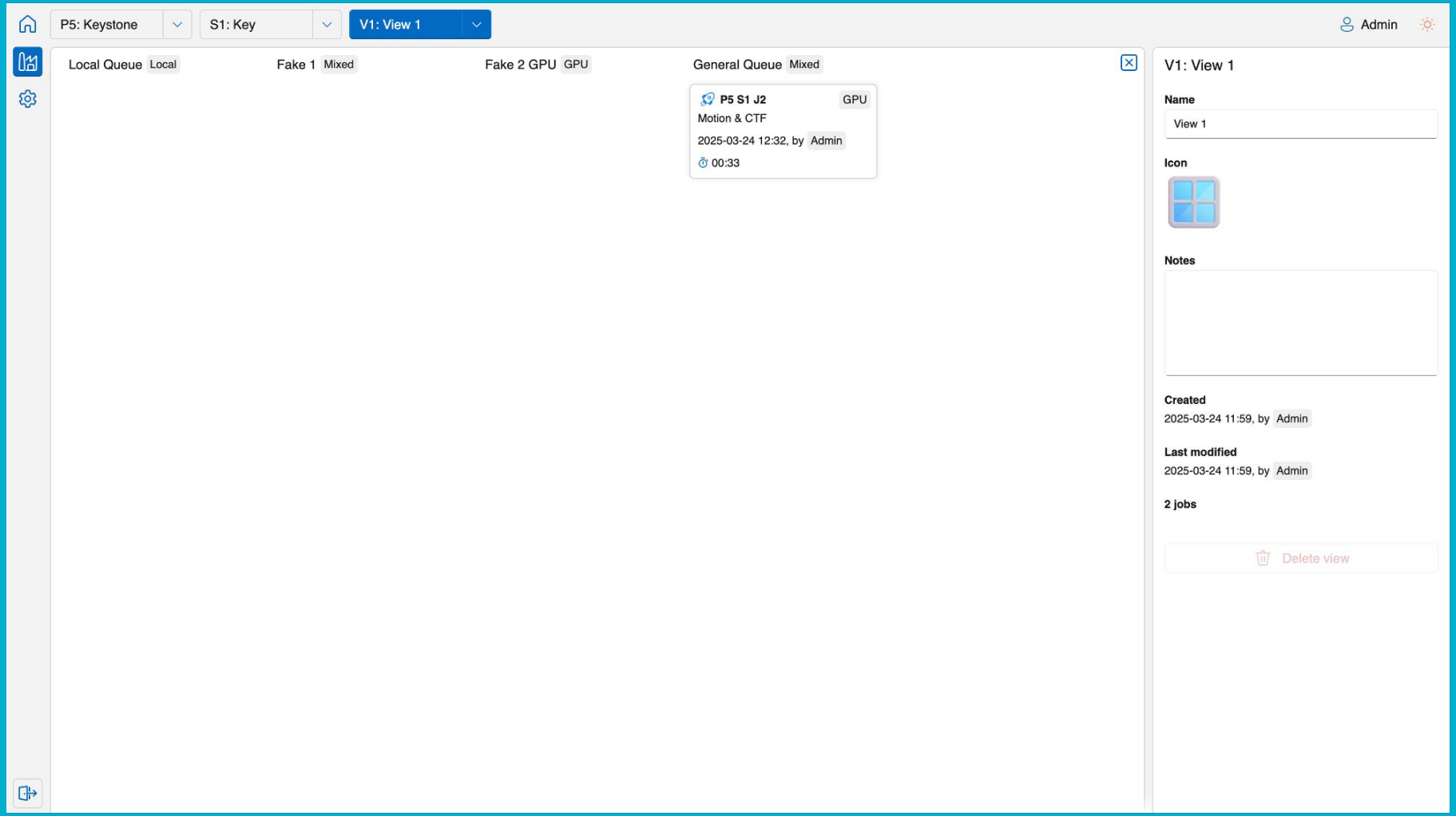

Admin
Projects
Create new project
P4: Bunny project
Created 2025-02-20
Mar '25
S4 J18 Tomogram Reconstruction Finished 11 h ago
S4 J17 CTF estimation Finished 4 d ago
S4 J16 Align with Etomo Finished 6 d ago
S4 J15 Deselect tilts Finished 7 d ago
S4 J14 Tilt-series data set Finished 7 d ago
P2: Third Project with a very long name that will certainly...
Created 2025-01-09
Mar '25
S1 J12 Vibe Finished 2025-02-20
S1 J11 Import map Failed 2025-02-20
S1 J10 Vibe Finished 2025-02-20
S1 J9 Extract particles Finished 2025-02-15
S1 J8 Extract particles Finished 2025-02-15
P3: Poooooooooooooooooooooooooooooooooooooooooo...
Created 2025-01-18
No jobs yet
P0: My Project
Created 2024-08-27
Mar '25
S0 J120 3D classification Finished 2024-12-03
S0 J119 Motion & CTF Finished 2024-12-03
S0 J118 Motion & CTF Finished 2024-12-03
S0 J117 Motion & CTF Finished 2024-12-03
S0 J116 Motion & CTF Finished 2024-12-03
P1: Second Project
Created 2024-11-14
Mar '25
S1 J7 Clone 2 of bla Building for 62 d 15 h
S1 J6 Clone of bla Building for 62 d 15 h
S1 J5 bla Building for 62 d 15 h
S1 J4 bla Building for 66 d 21 h
S1 J3 Post-processing Building for 67 d 14 h
Oh Relay?
Oldest project
P0: My Project
6 months, 28 days by Admin
Biggest project
P0: My Project
127 jobs
172 jobs in 13 views, 9 spaces, 5 projects

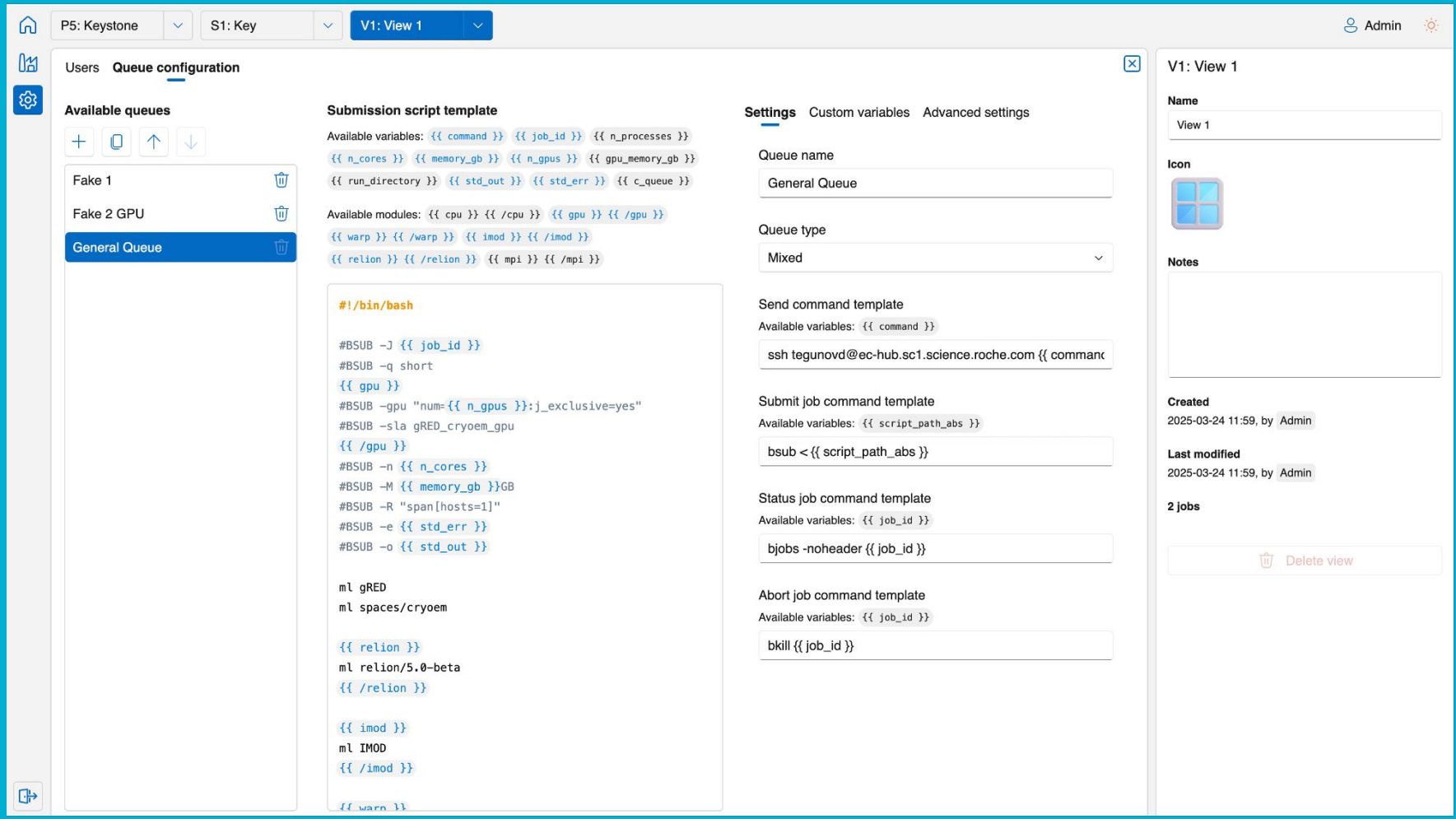

P5: Keystone
Admin
Spaces
Create or reconnect space
P5: Keystone
Name
Keystone
Icon
Notes
Created
2025-03-24 10:35, by Admin
Last modified
2025-03-24 10:35, by Admin
Members
Admin
0 jobs in 0 views, 0 spaces
Delete project

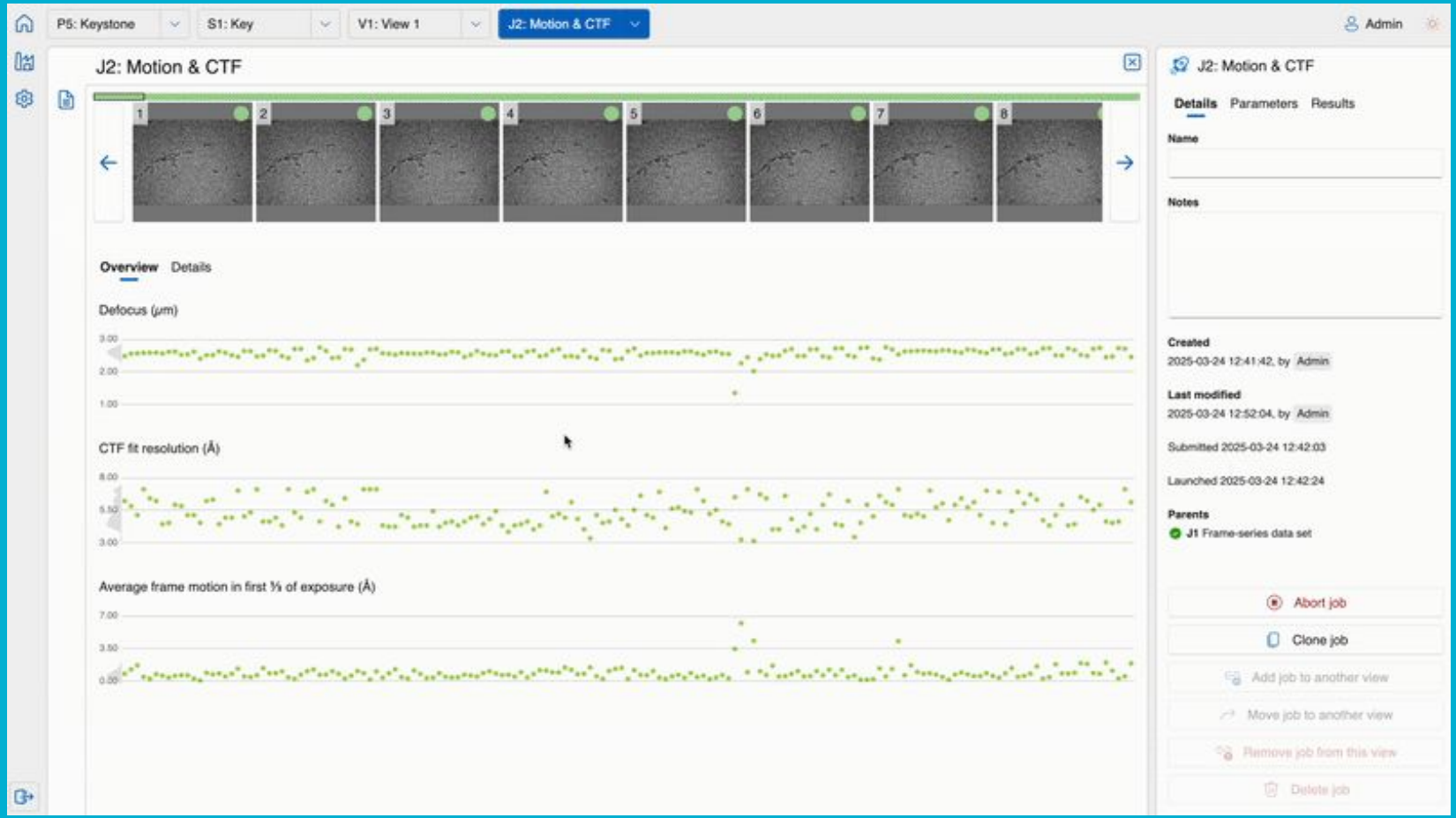

P5: Keystone
S1: Key
Admin
Views
+ Create new view
V1: View 1
Created 2025-03-24
No jobs yet
V1: View 1
Name
View 1
Icon
Notes
Created
2025-03-24 11:59, by Admin
Last modified
2025-03-24 11:59, by Admin
0 jobs
Delete view

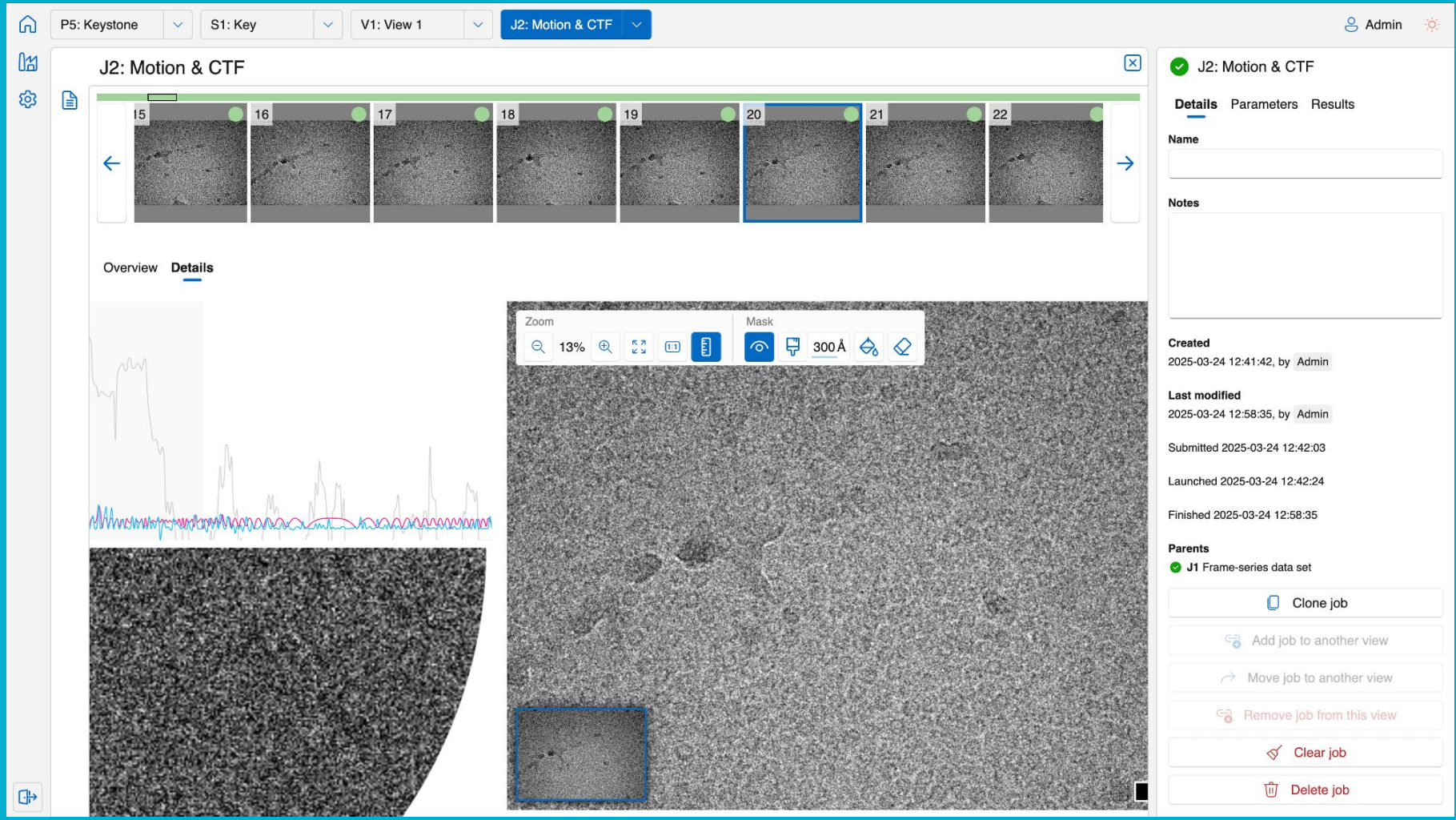

P5: Keystone
S1: Key
V1: View 1
Admin
J1 Frame-series data set
Finished in 00:07
V1: View 1
Name
View 1
Icon
Notes
Created
2025-03-24 11:59, by Admin
Last modified
2025-03-24 11:59, by Admin
1 jobs
Delete view

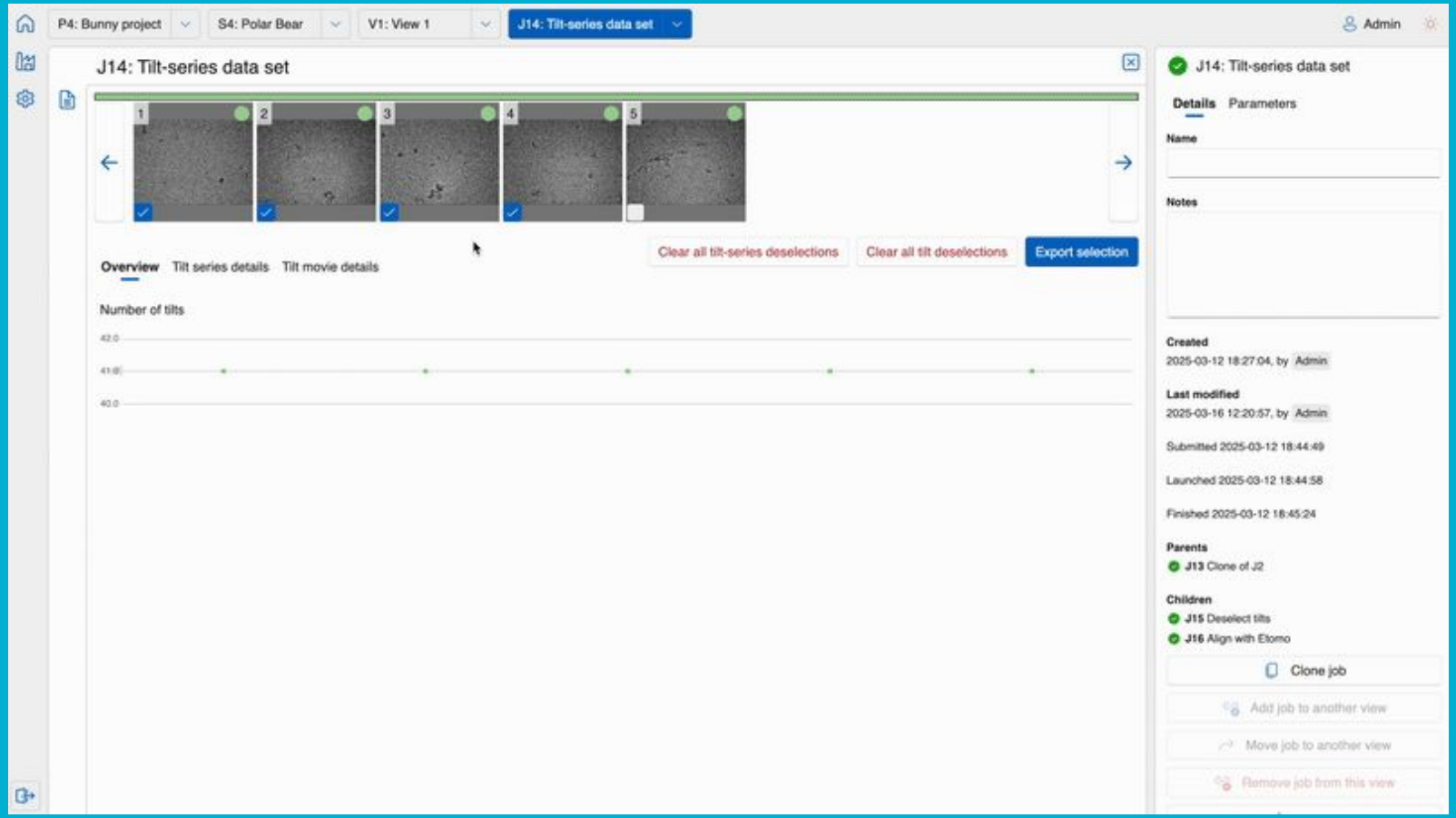

P5: Keystone
S1: Key
V1: View 1
Admin
J1 Frame-series data set
Finished in 00:07
J2 Motion & CTF
05:24
86/287
11:36
V1: View 1
Name
View 1
Icon
Notes
Created
2025-03-24 11:59, by Admin
Last modified
2025-03-24 11:59, by Admin
2 jobs
Delete view

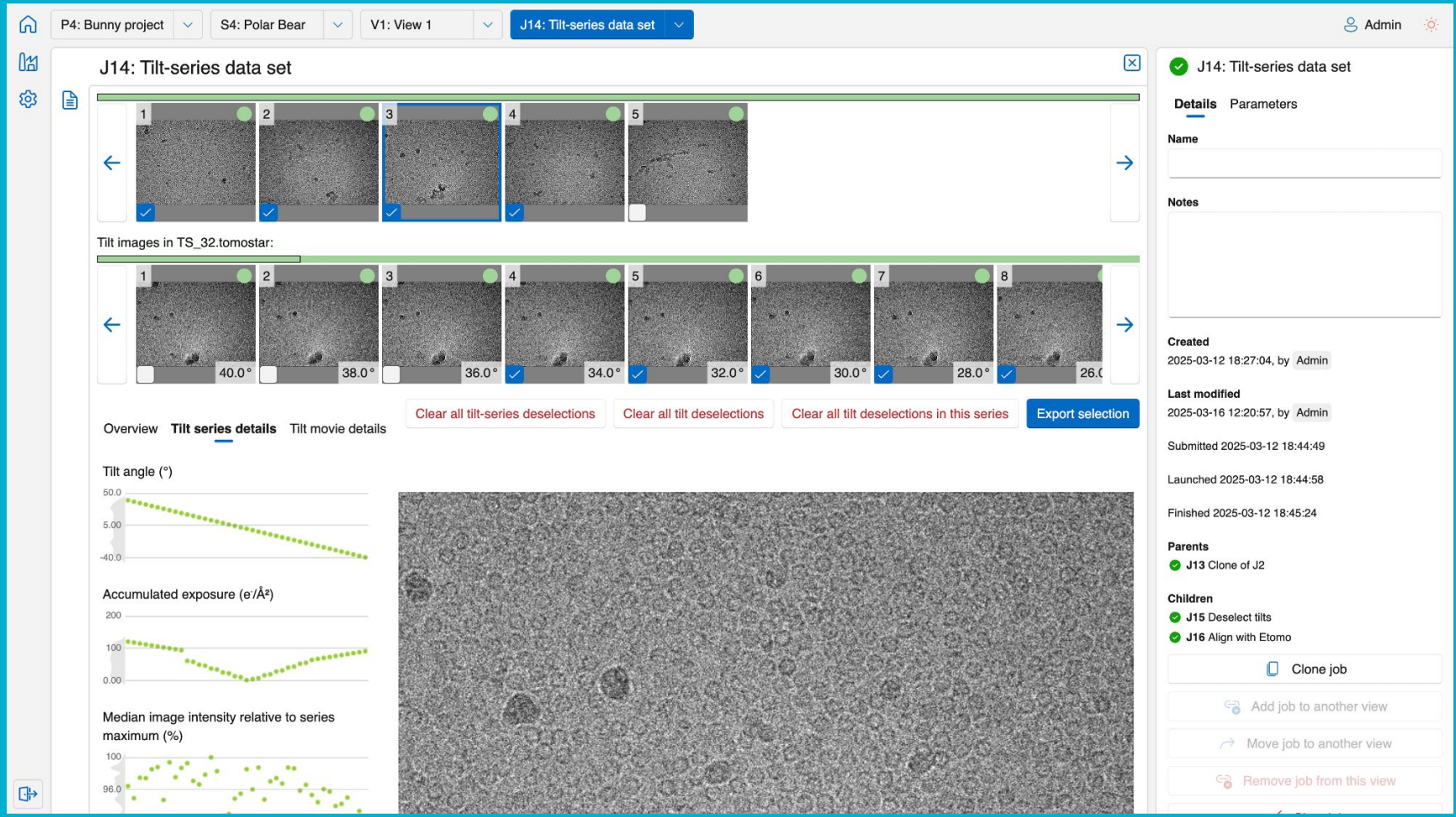

P5: Keystone
S1: Key
V1: View 1
Admin
Local Queue Local
Fake 1 Mixed
Fake 2 GPU GPU
General Queue Mixed
P5 S1 J2
GPU
Motion & CTF
2025-03-24 12:32, by Admin
00:33
V1: View 1
Name
View 1
Icon
Notes
Created
2025-03-24 11:59, by Admin
Last modified
2025-03-24 11:59, by Admin
2 jobs
Delete view


P5: Keystone
S1: Key
V1: View 1
Admin
Users
Queue configuration
Available queues
Fake 1
Fake 2 GPU
General Queue
Submission script template
Available variables: {{ command }} {{ job_id }} {{ n_processes }} {{ n_cores }} {{ memory_gb }} {{ n_gpus }} {{ gpu_memory_gb }} {{ run_directory }} {{ std_out }} {{ std_err }} {{ c_queue }}
Available modules: {{ cpu }} {{ /cpu }} {{ gpu }} {{ /gpu }} {{ warp }} {{ /warp }} {{ imod }} {{ /imod }} {{ relion }} {{ /relion }} {{ mpi }} {{ /mpi }}
#!/bin/bash
#BSUB -J {{ job_id }}
#BSUB -q short
{{ gpu }}
#BSUB -gpu "num={{ n_gpus }}:j_exclusive=yes"
#BSUB -sla gRED_cryoem_gpu
{{ /gpu }}
#BSUB -n {{ n_cores }}
#BSUB -M {{ memory_gb }}GB
#BSUB -R "span[hosts=1]"
#BSUB -e {{ std_err }}
#BSUB -o {{ std_out }}
ml gRED
ml spaces/cryoem
{{ relion }}
ml relion/5.0-beta
{{ /relion }}
{{ imod }}
ml IMOD
{{ /imod }}
{{ warp }}
Settings
Custom variables
Advanced settings
Queue name
General Queue
Queue type
Mixed
Send command template
Available variables: {{ command }}
ssh tegunovd@ec-hub.sc1.science.roche.com {{ commanc
Submit job command template
Available variables: {{ script_path_abs }}
bsub < {{ script_path_abs }}
Status job command template
Available variables: {{ job_id }}
bjobs -noheader {{ job_id }}
Abort job command template
Available variables: {{ job_id }}
bkill {{ job_id }}
V1: View 1
Name
View 1
Icon
Notes
Created
2025-03-24 11:59, by Admin
Last modified
2025-03-24 11:59, by Admin
2 jobs
Delete view


P5: Keystone
S1: Key
V1: View 1
J2: Motion & CTF
Admin
J2: Motion & CTF
1
2
3
4
5
6
7
8
Overview
Details
Defocus (μm)
3.00
2.00
1.00
CTF fit resolution (Å)
8.00
5.50
3.00
Average frame motion in first ⅓ of exposure (Å)
7.00
3.50
0.00
J2: Motion & CTF
Details
Parameters
Results
Name
Notes
Created
2025-03-24 12:41:42, by Admin
Last modified
2025-03-24 12:52:04, by Admin
Submitted 2025-03-24 12:42:03
Launched 2025-03-24 12:42:24
Parents
J1 Frame-series data set
Abort job
Clone job
Add job to another view
Move job to another view
Remove job from this view
Delete job

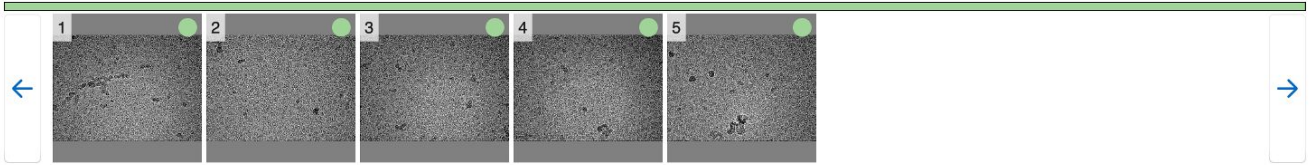

P5: Keystone
S1: Key
V1: View 1
J2: Motion & CTF
Admin
J2: Motion & CTF
15
16
17
18
19
20
21
22
Overview
Details
Zoom
13%
Mask
300 Å
J2: Motion & CTF
Details
Parameters
Results
Name
Notes
Created
2025-03-24 12:41:42, by Admin
Last modified
2025-03-24 12:58:35, by Admin
Submitted 2025-03-24 12:42:03
Launched 2025-03-24 12:42:24
Finished 2025-03-24 12:58:35
Parents
J1 Frame-series data set
Clone job
Add job to another view
Move job to another view
Remove job from this view
Clear job
Delete job


P4: Bunny project
S4: Polar Bear
V1: View 1
J14: Tilt-series data set
Admin
J14: Tilt-series data set
1
2
3
4
5
Overview
Tilt series details
Tilt movie details
Clear all tilt-series deselections
Clear all tilt deselections
Export selection
Number of tilts
42.0
41.0
40.0
J14: Tilt-series data set
Details
Parameters
Name
Notes
Created
2025-03-12 18:27:04, by Admin
Last modified
2025-03-16 12:20:57, by Admin
Submitted 2025-03-12 18:44:49
Launched 2025-03-12 18:44:58
Finished 2025-03-12 18:45:24
Parents
J13 Clone of J2
Children
J15 Deselect tilts
J16 Align with Etomo
Clone job
Add job to another view
Move job to another view
Remove job from this view


P4: Bunny project
S4: Polar Bear
V1: View 1
J14: Tilt-series data set
Admin
J14: Tilt-series data set
Tilt images in TS_32.tomostar:
Overview
Tilt series details
Tilt movie details
Clear all tilt-series deselections
Clear all tilt deselections
Clear all tilt deselections in this series
Export selection
Tilt angle (°)
Accumulated exposure (e⁻/Å²)
Median image intensity relative to series maximum (%)
J14: Tilt-series data set
Details
Parameters
Name
Notes
Created
2025-03-12 18:27:04, by Admin
Last modified
2025-03-16 12:20:57, by Admin
Submitted 2025-03-12 18:44:49
Launched 2025-03-12 18:44:58
Finished 2025-03-12 18:45:24
Parents
J13 Clone of J2
Children
J15 Deselect tilts
J16 Align with Etomo
Clone job
Add job to another view
Move job to another view
Remove job from this view

P4: Bunny project | S4: Polar Bear | V1: View 1 | J16: Align with Etomo | Admin

J16: Align with Etomo



Overview Tilt series details

Number of tilts

Min and max tilt angle (°)

Tilt axis angle (°)

Min, average, and max shift in X (Å)

Min, average, and max shift in Y (Å)

J16: Align with Etomo

Details Parameters

Name

Notes

Created
2025-03-17 17:42:02, by Admin

Last modified
2025-03-17 19:45:09, by Admin

Submitted 2025-03-17 19:39:03

Launched 2025-03-17 19:39:10

Finished 2025-03-17 19:45:09

Parents
J14 Tilt-series data set

Children
J17 CTF estimation

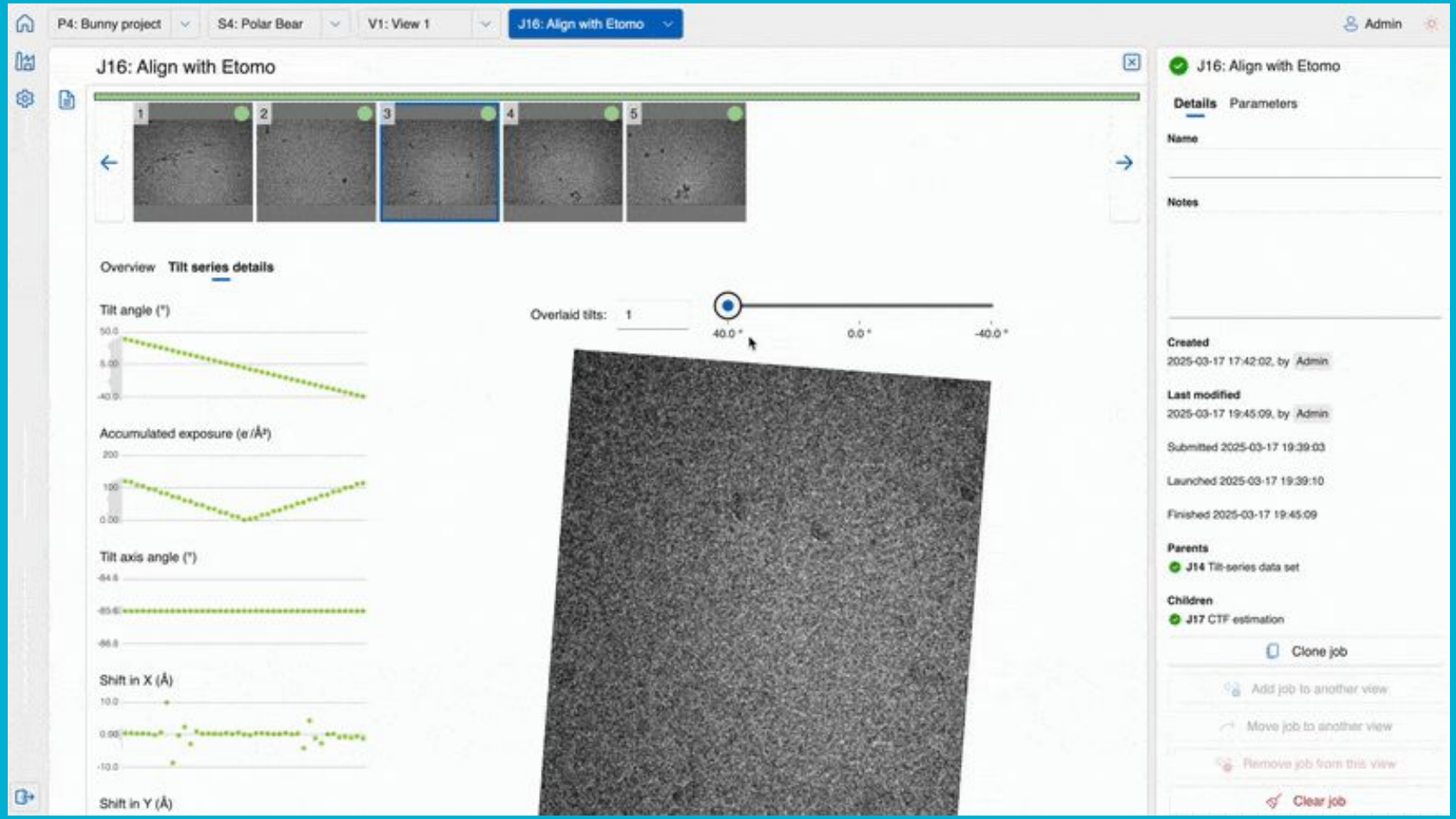
Clone job

Add job to another view

Move job to another view

Remove job from this view

Clear job


P4: Bunny project
S4: Polar Bear
V1: View 1
J16: Align with Etomo
Admin
J16: Align with Etomo
1
2
3
4
5
Overview
Tilt series details
Tilt angle (°)
Accumulated exposure (e⁻/Å²)
Tilt axis angle (°)
Shift in X (Å)
Shift in Y (Å)
Overlaid tilts: 1
40.0 °
0.0 °
-40.0 °
J16: Align with Etomo
Details
Parameters
Name
Notes
Created
2025-03-17 17:42:02, by Admin
Last modified
2025-03-17 19:45:09, by Admin
Submitted 2025-03-17 19:39:03
Launched 2025-03-17 19:39:10
Finished 2025-03-17 19:45:09
Parents
J14 Tilt-series data set
Children
J17 CTF estimation
Clone job
Add job to another view
Move job to another view
Remove job from this view
Clear job

P4: Bunny project | S4: Polar Bear | V1: View 1 | J18: Tomogram Reco… | Admin

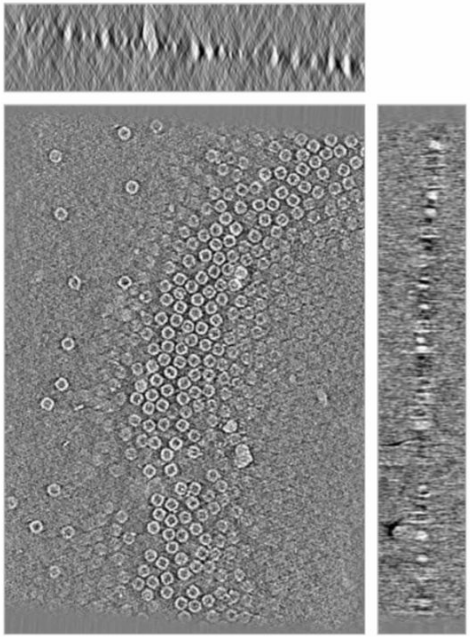
J18: Tomogram Reconstruction

1 2 3 4 5

Coordinates: 162 237 40

Zoom: 95%

Particles: 100 Å 200 Å



J18: Tomogram Reconstruction

Details | Parameters

Name

Notes

Created
2025-03-23 22:44:08, by Admin

Last modified
2025-03-23 23:06:49, by Admin

Submitted 2025-03-23 23:05:21

Launched 2025-03-23 23:05:49

Finished 2025-03-23 23:06:49

Parents
J17 CTF estimation

Clone job

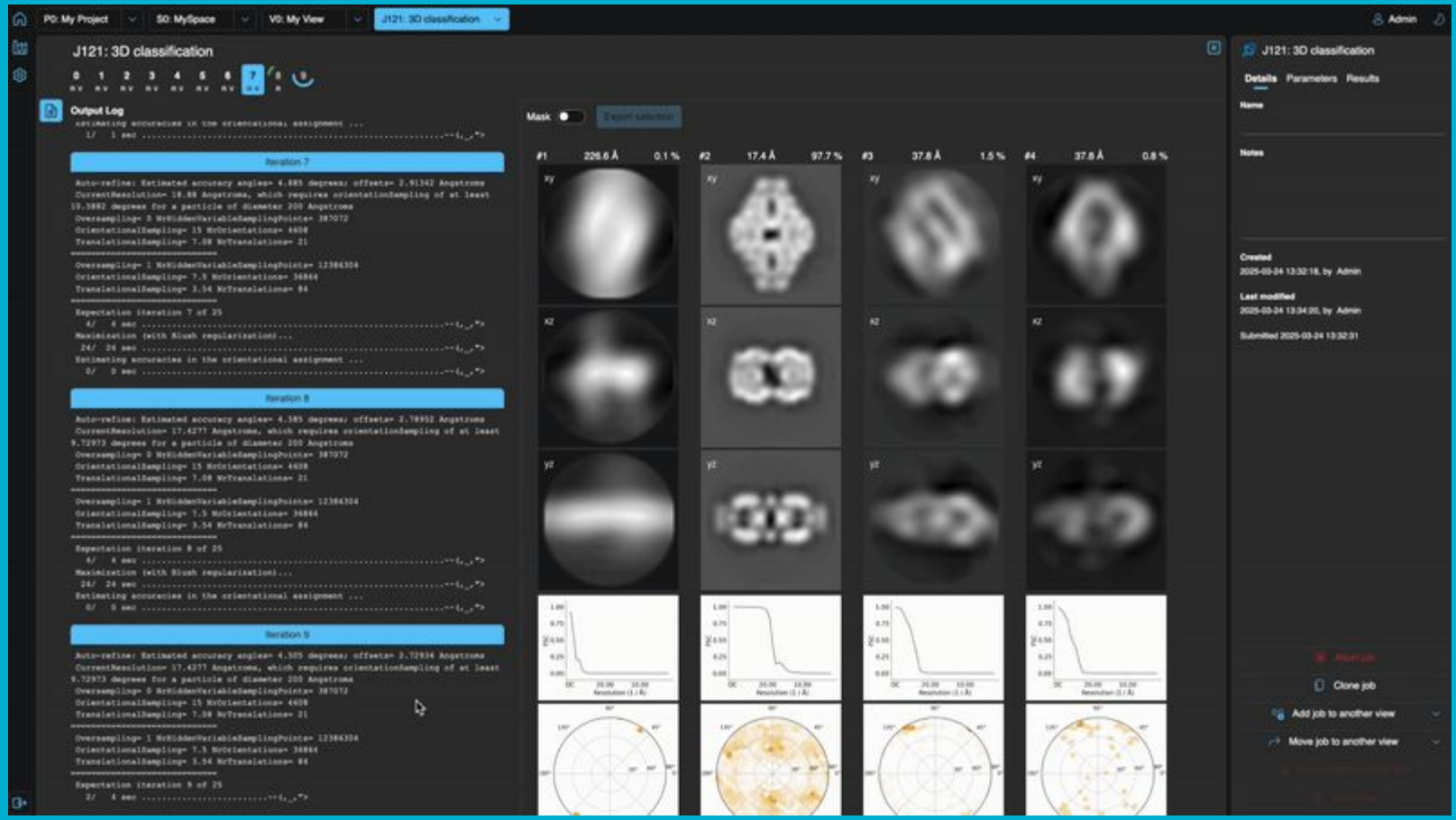
Add job to another view

Move job to another view

Remove job from this view

Clear job

Delete job


P0: My Project
S0: MySpace
V0: My View
J121: 3D classification
Admin
J121: 3D classification
Output Log
Iteration 7
Iteration 8
Iteration 9
Mask
#1 226.6 Å 0.1 %
#2 17.4 Å 97.7 %
#3 37.8 Å 1.5 %
#4 37.8 Å 0.8 %
xy
xz
yz
J121: 3D classification
Details
Parameters
Results
Name
Notes
Created
2025-03-24 13:32:18, by Admin
Last modified
2025-03-24 13:34:20, by Admin
Submitted 2025-03-24 13:32:31
Clone job
Add job to another view
Move job to another view

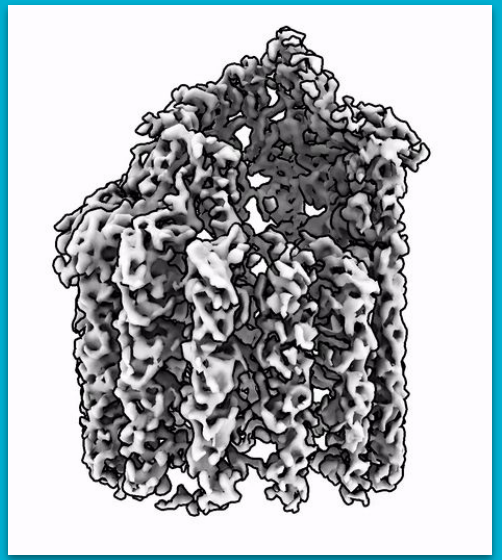
But tomography is still hard…

slide from Dimitry

illustrated examples

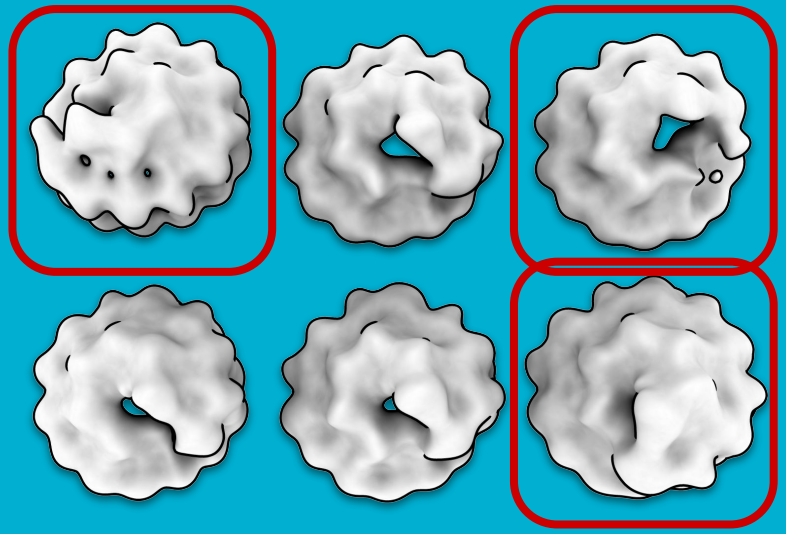
Consensus reconstruction of the native γ-tubulin ring complex

Tom Dendooven, Stan Yatskevich & John Kilmartin

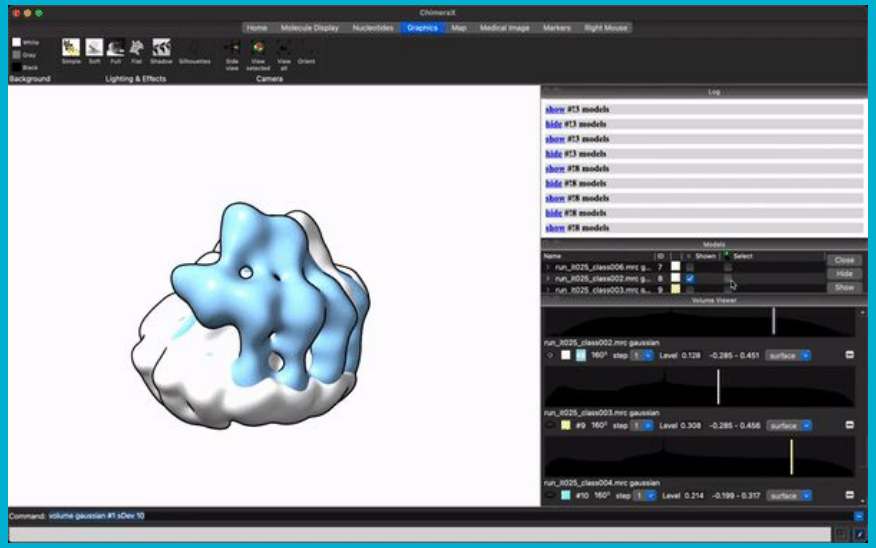


~11 Å from 8000 particles
Warp -> Dynamo -> RELION -> M
co-refined with microtubules which plateau at ~6 Å

Misaligned subsets of γ-TuRC particles are identified by 3D classification

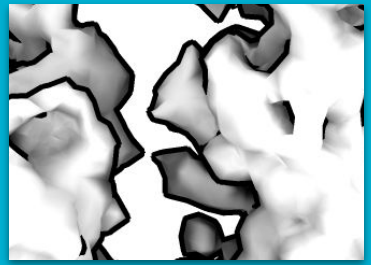


Manual registration improves density at the microtubule seam formation interface

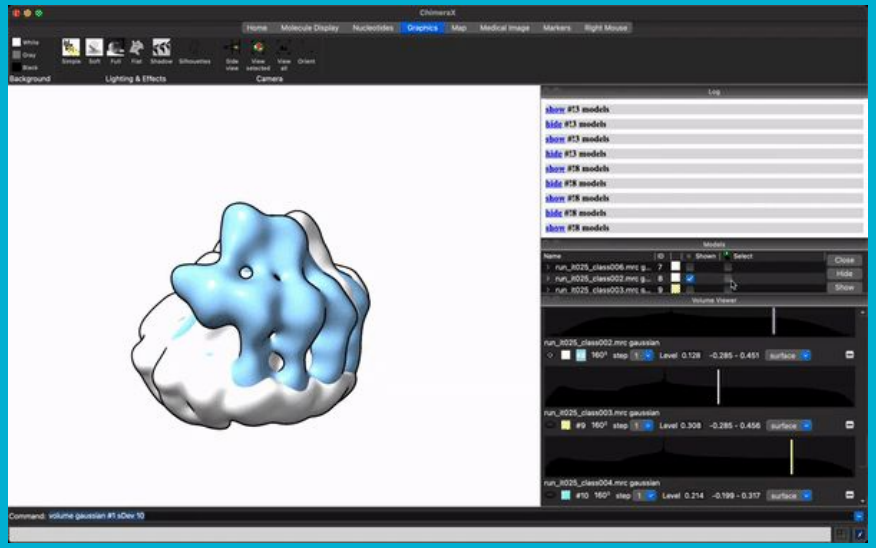


per class transformations defined in ChimeraX and applied on relevant particle subsets

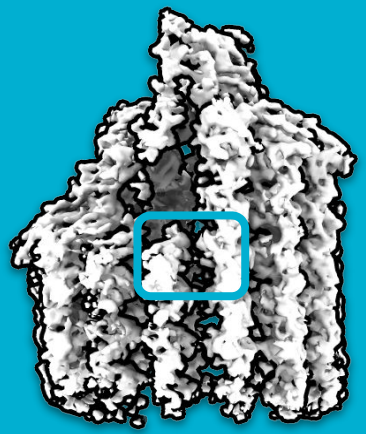
before registration



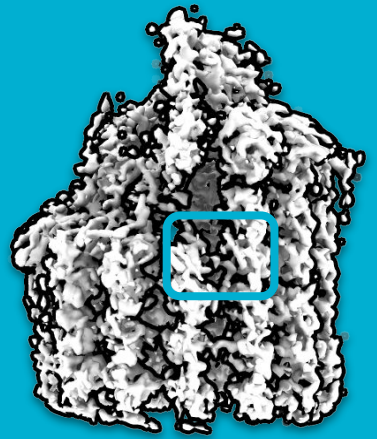
Manual registration improves density at the ring closing interface



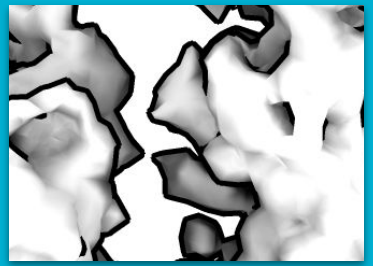
per class transformations defined in ChimeraX and applied on relevant particle subsets

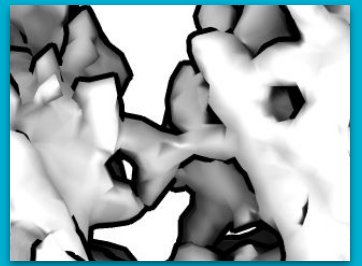


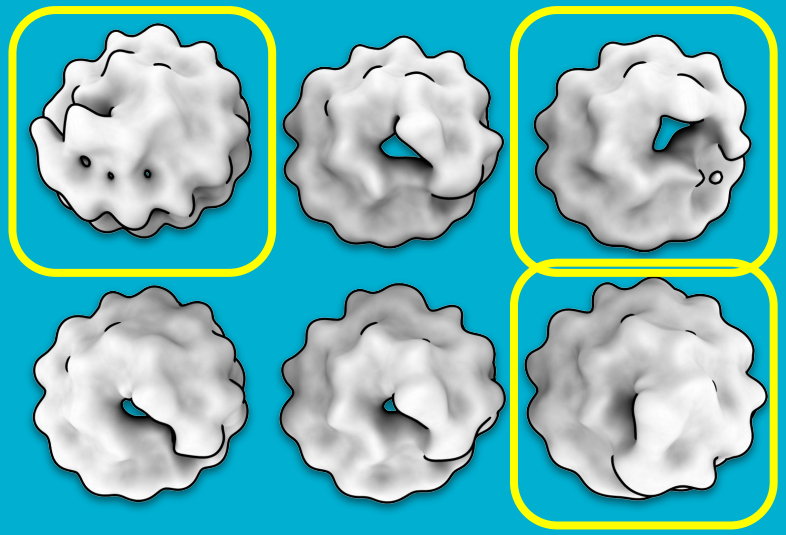
before registration



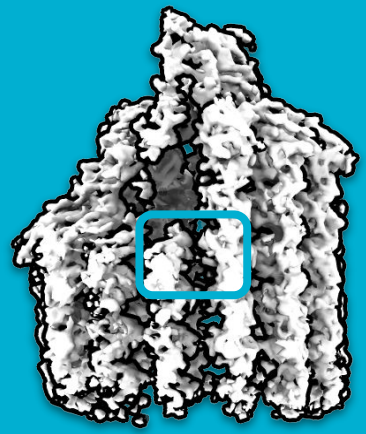
after registration

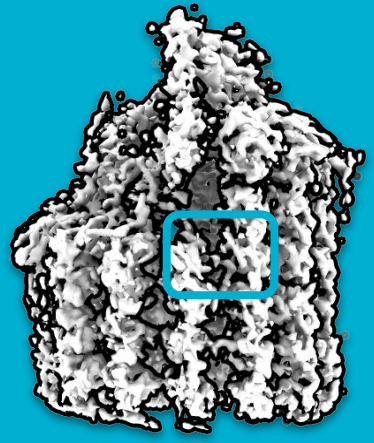






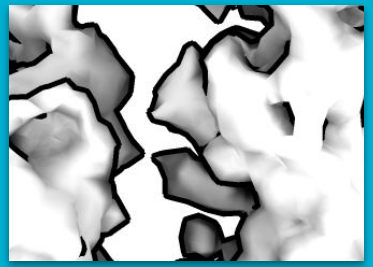
identify misaligned subsets of data
Class3D in RELION

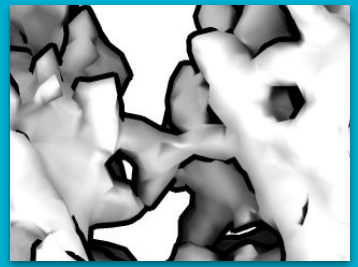




before registration

after registration

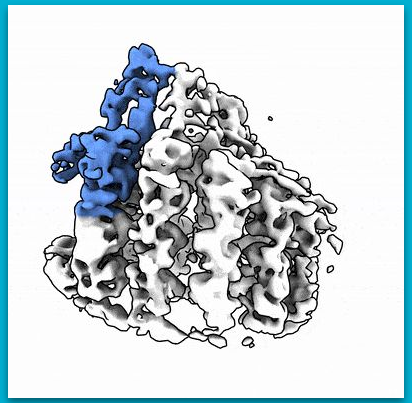




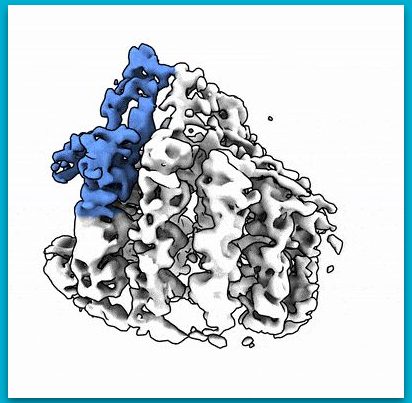
Identification of an interesting density bridging γ-TuSC and the microtubule protofilament

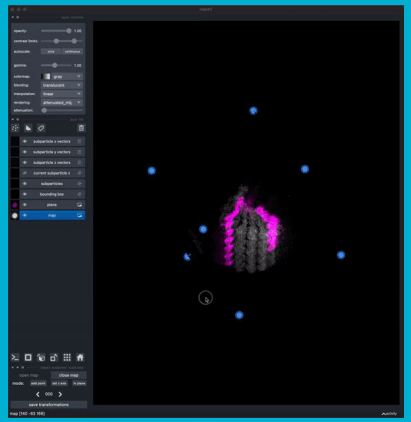
unidentified potentially interesting density
bridging γ-TuSC and microtubule protofilament

Leveraging multiplicity of γ-TuSC subcomplex improves density in regions of interest



Leveraging multiplicity of γ-TuSC subcomplex improves density in regions of interest

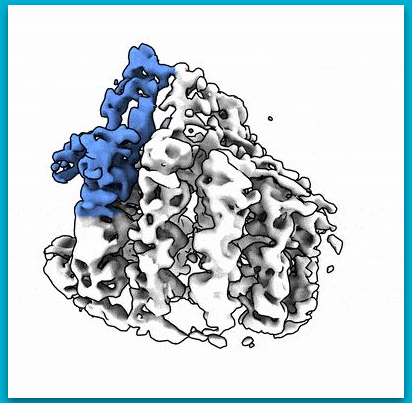


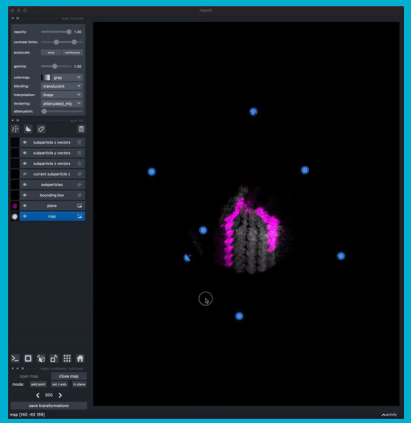


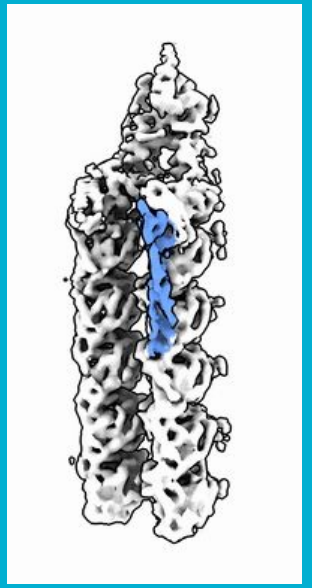
napari-subboxer

c.f. Localized reconstruction, Ilca *et al.* 2015

Leveraging multiplicity of the γ-TuSC subcomplex improves density in regions of interest



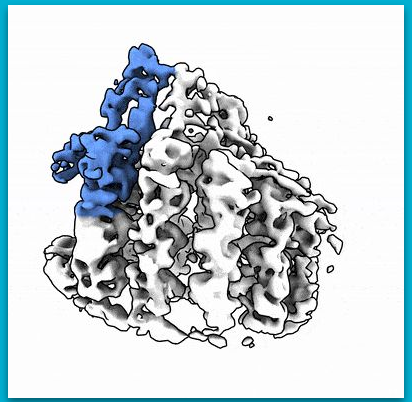


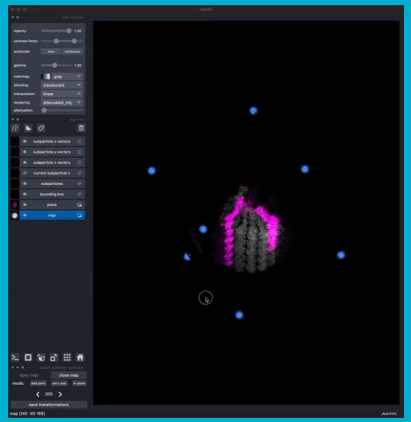


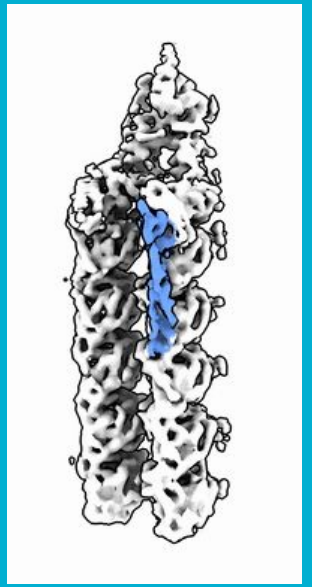
napari-subboxer

~7 Å reconstruction from
7 x 8000 particles

c.f. Localized reconstruction, Ilca *et al.* 2015







napari-subboxer

~7 Å reconstruction from
7 x 8000 particles

Article | Open access | Published: 12 April 2024

Structure of the native γ-tubulin ring complex capping spindle microtubules

Tom Dendooven, Stanislau Yatskevich, Alister Burt, Zhuo A. Chen, Dom Bellini, Juri Rappsilber, John V. Kilmartin & David Barford

thanks for listening!

(cue the workshop start)