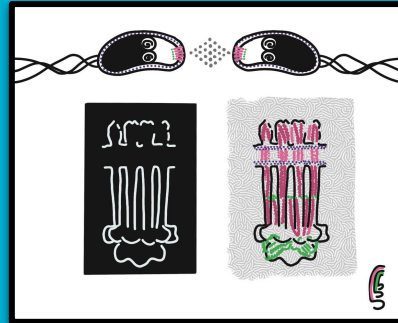
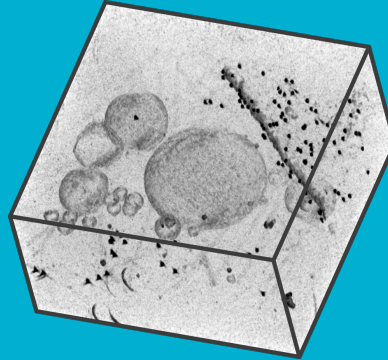


A Hitchhikers Guide to Structural Cryo-ET (in Warp 2.0 & friends)

Alister Burt

Dimitry Tegunov 's group
@ Genentech

01/04/25

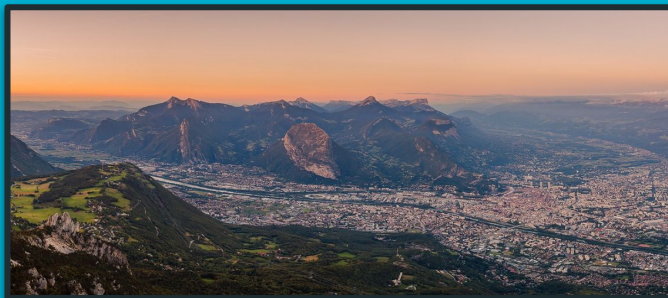


quick intro

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?*

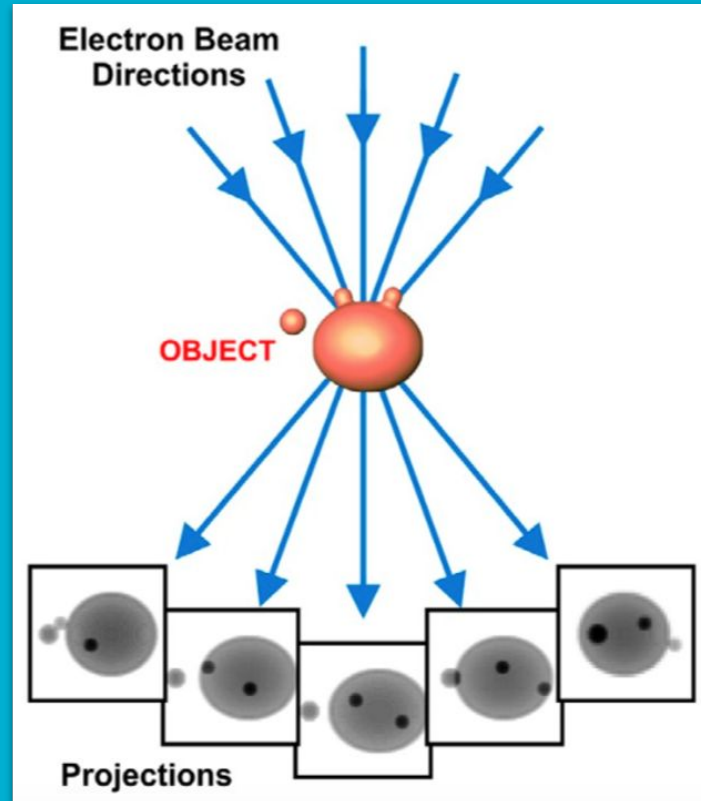
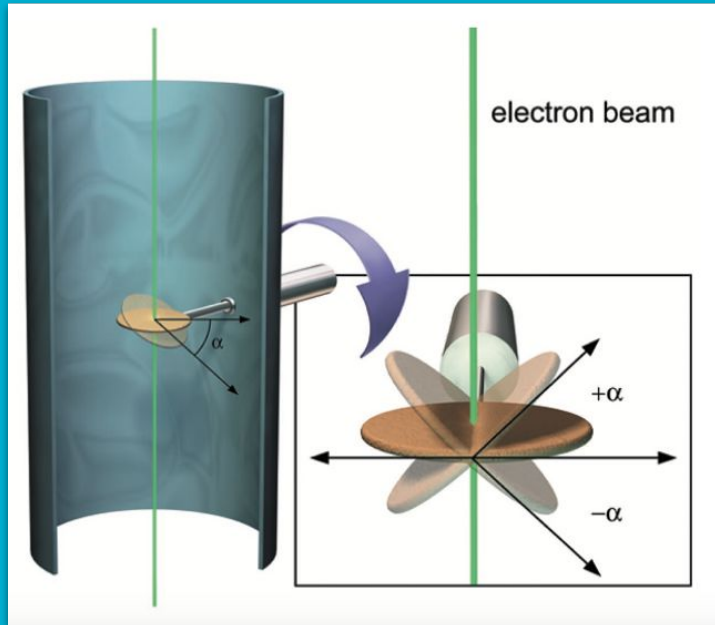
focus on Warp 2.0

sneak peak of Relay

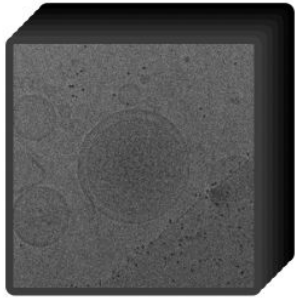
illustrated examples

a fun example where thinking was useful!

Cryo-Electron Tomography (cryo-ET)

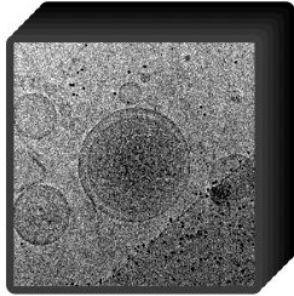


let's get on the same page...



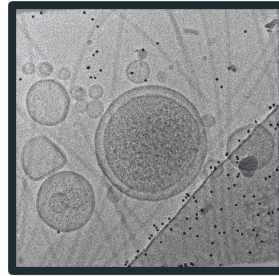
multi-frame
micrographs

dose-fractionated images
each is 2D + time



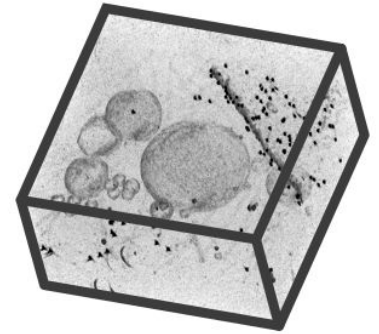
tilt-images

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



tilt-series

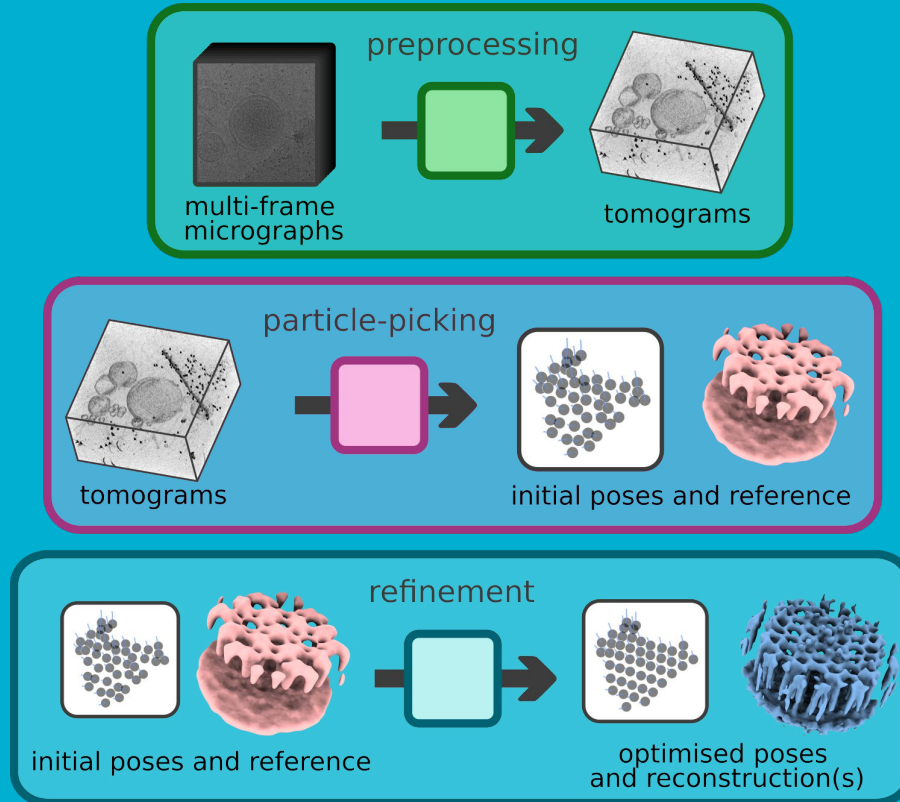
stack of 2D tilt-images with
~same field of view



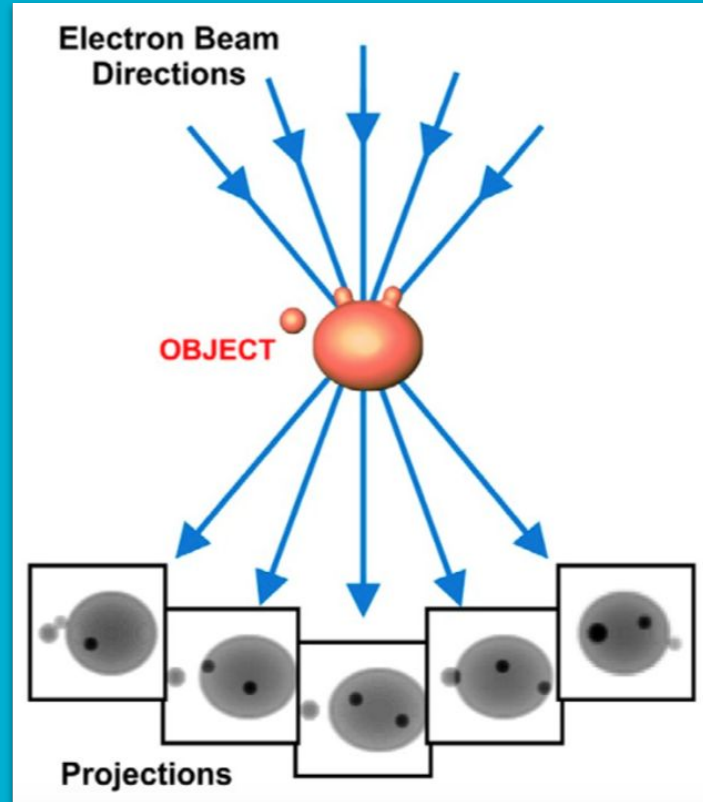
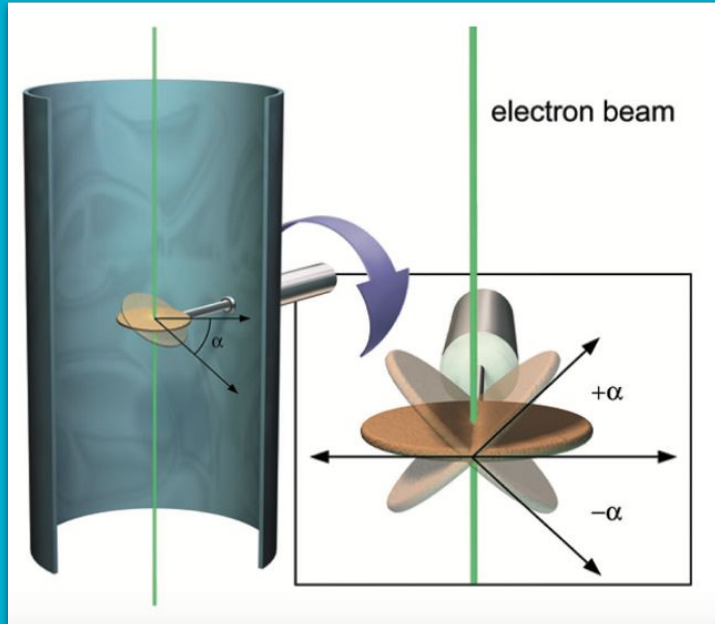
tomograms

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

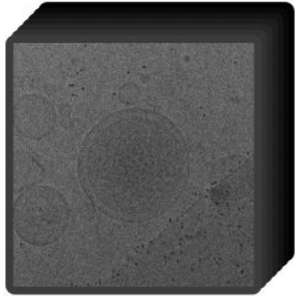
What does a structural cryo-ET workflow look like?



Cryo-Electron Tomography (cryo-ET)

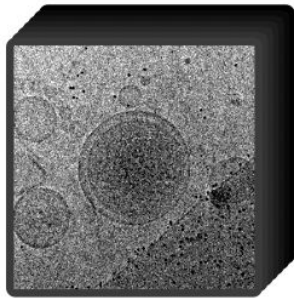


let's get on the same page...



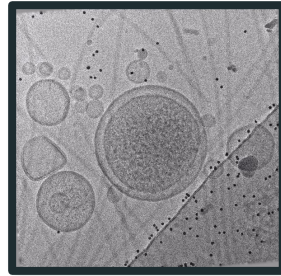
multi-frame
micrographs

dose-fractionated images
each is 2D + time



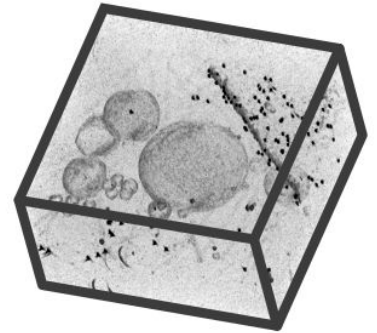
tilt-images

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



tilt-series

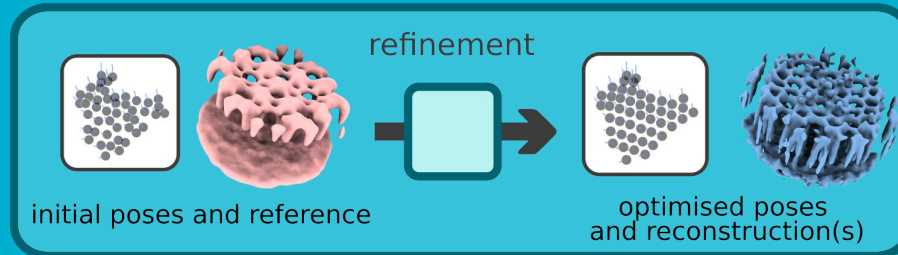
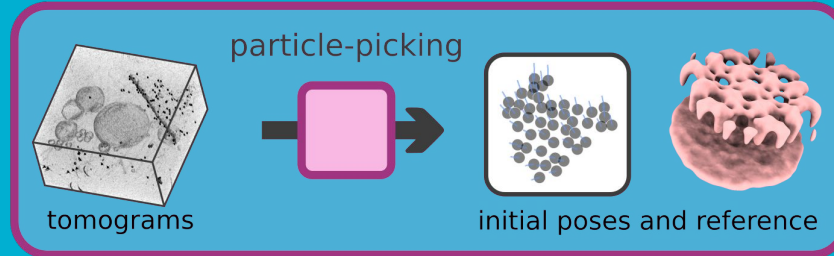
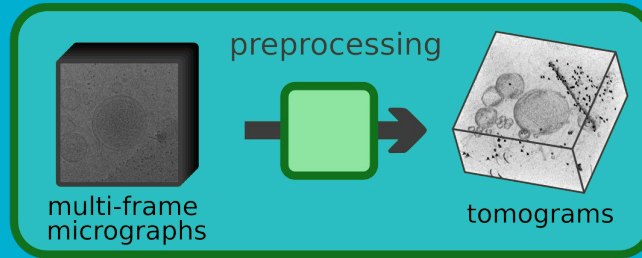
stack of tilt-images with
~same field of view



tomograms

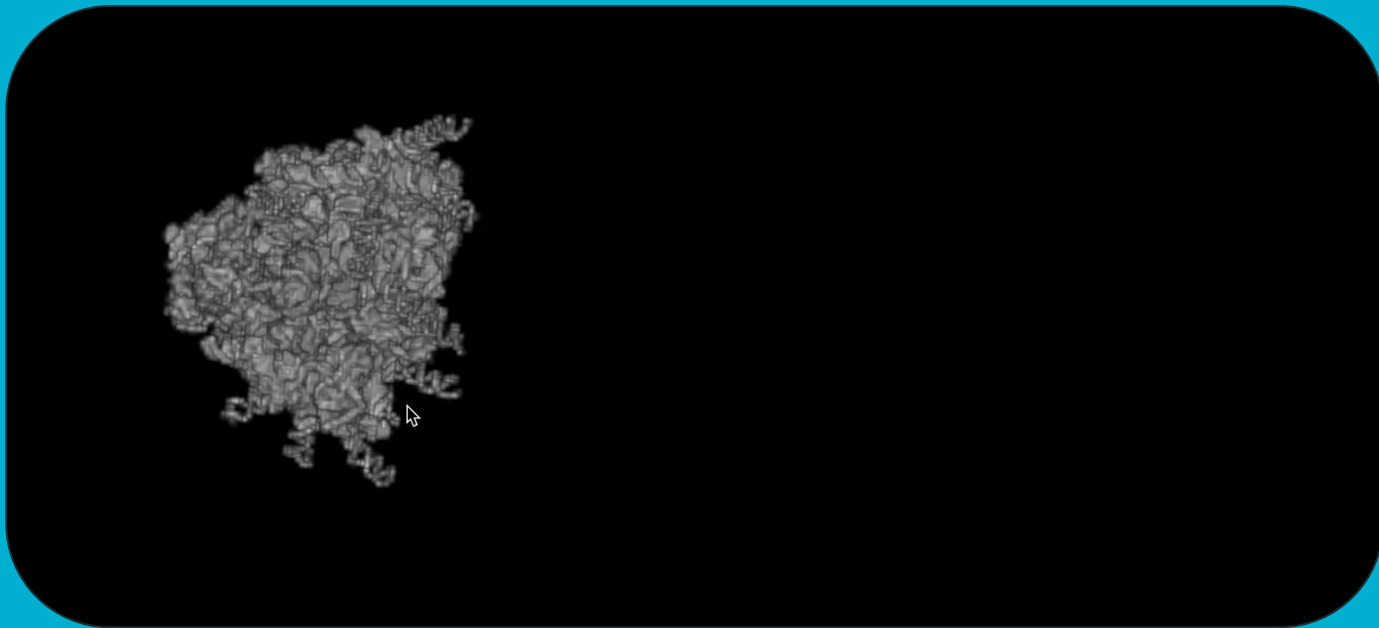
3D reconstructions
typically made using images
from a tilt-series

What does a structural cryo-ET workflow look like?



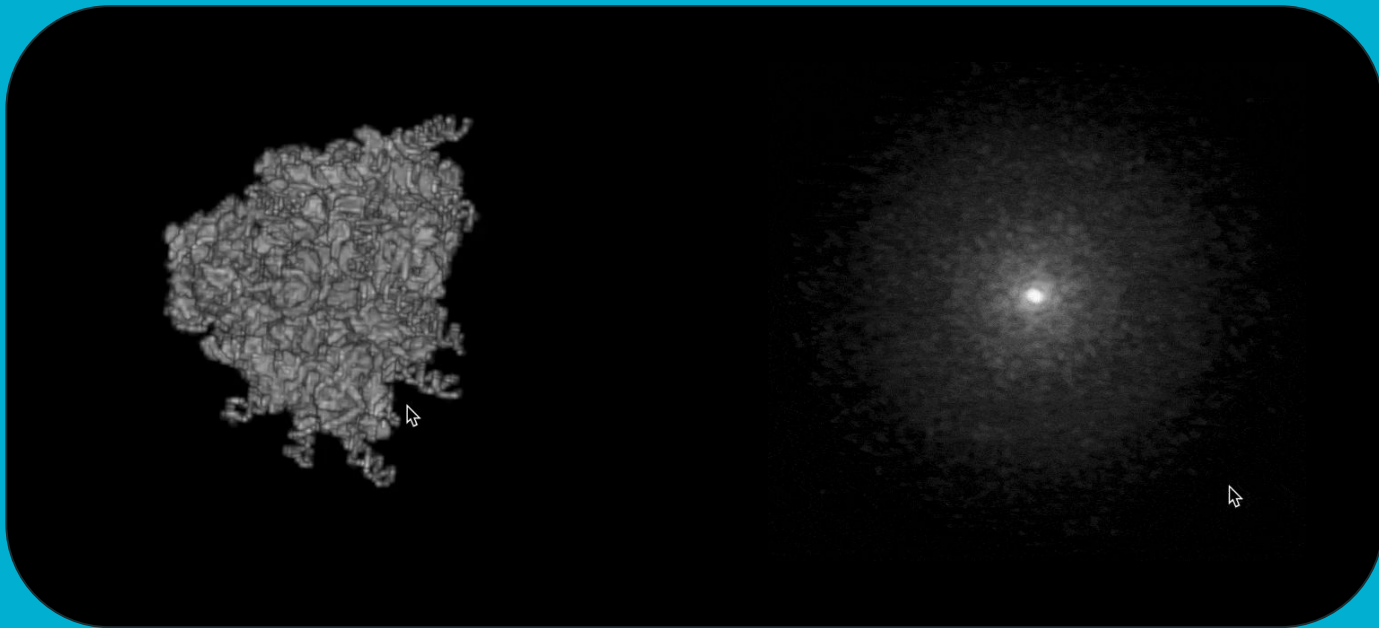
tilt series processing is a superset of frame series processing

3D reconstruction from projection images



3D object

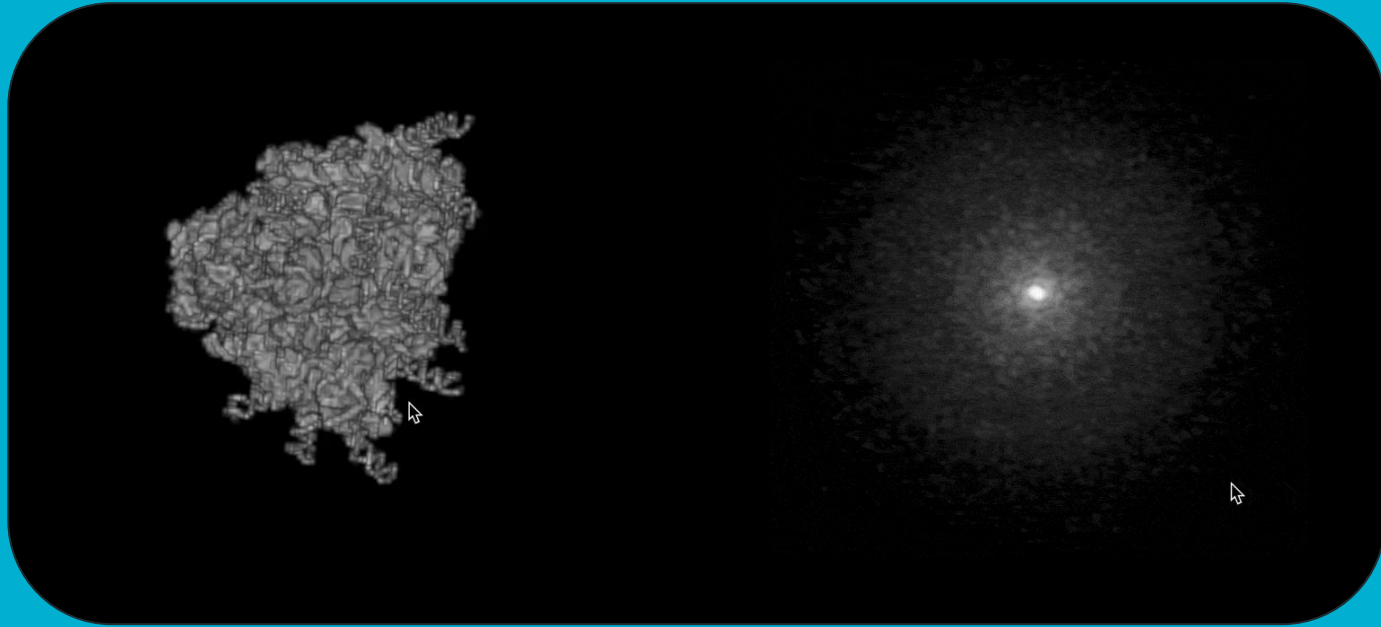
3D reconstruction from projection images



3D object

3D Fourier transform
(power spectrum)

3D reconstruction from projection images



3D object

FFT

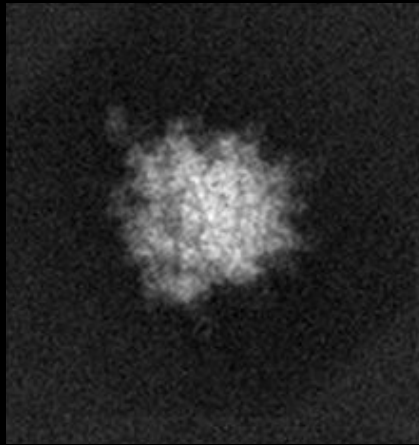
3D Fourier transform
(power spectrum)

3D reconstruction from projection images

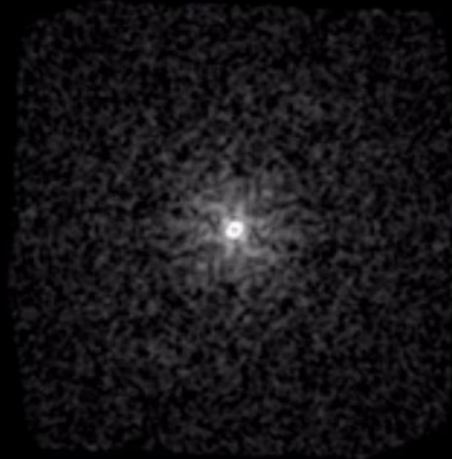


central slice of
3D Fourier transform
(power spectrum)

3D reconstruction from projection images

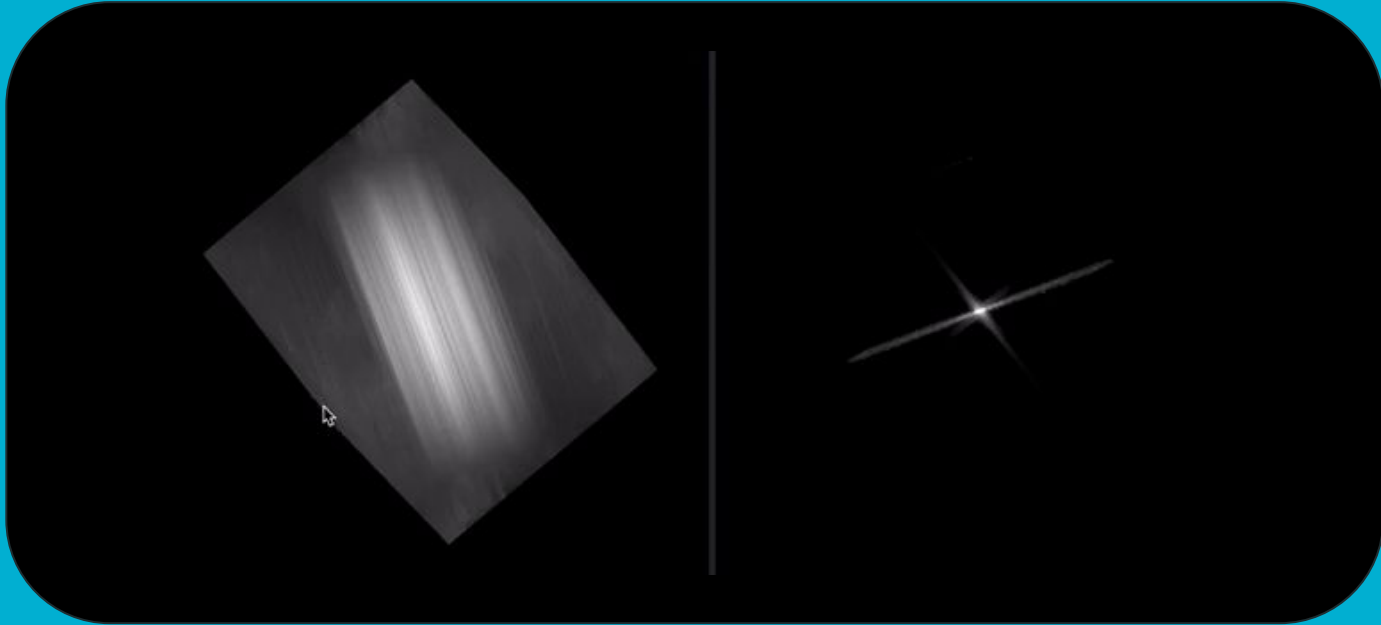


2D projection image



central slice of
3D Fourier transform
(power spectrum)

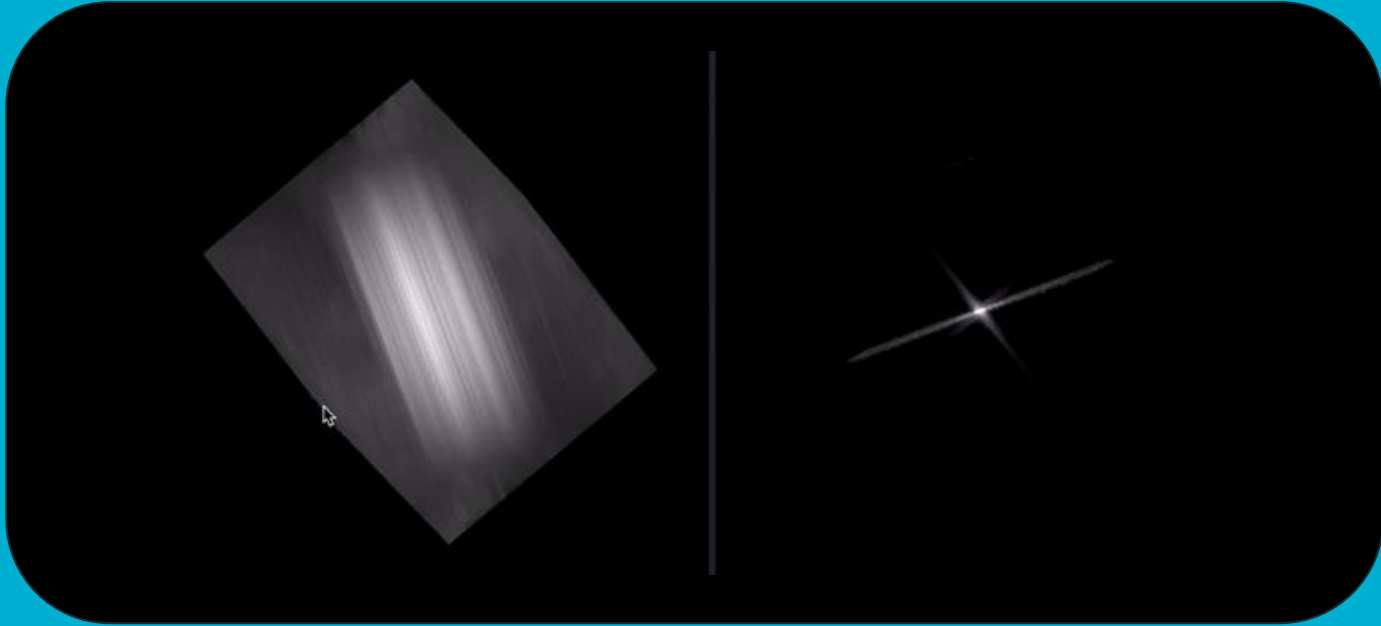
3D reconstruction from projection images



3D object
(real space)

central slice of
3D Fourier transform
(power spectrum)

3D reconstruction from projection images

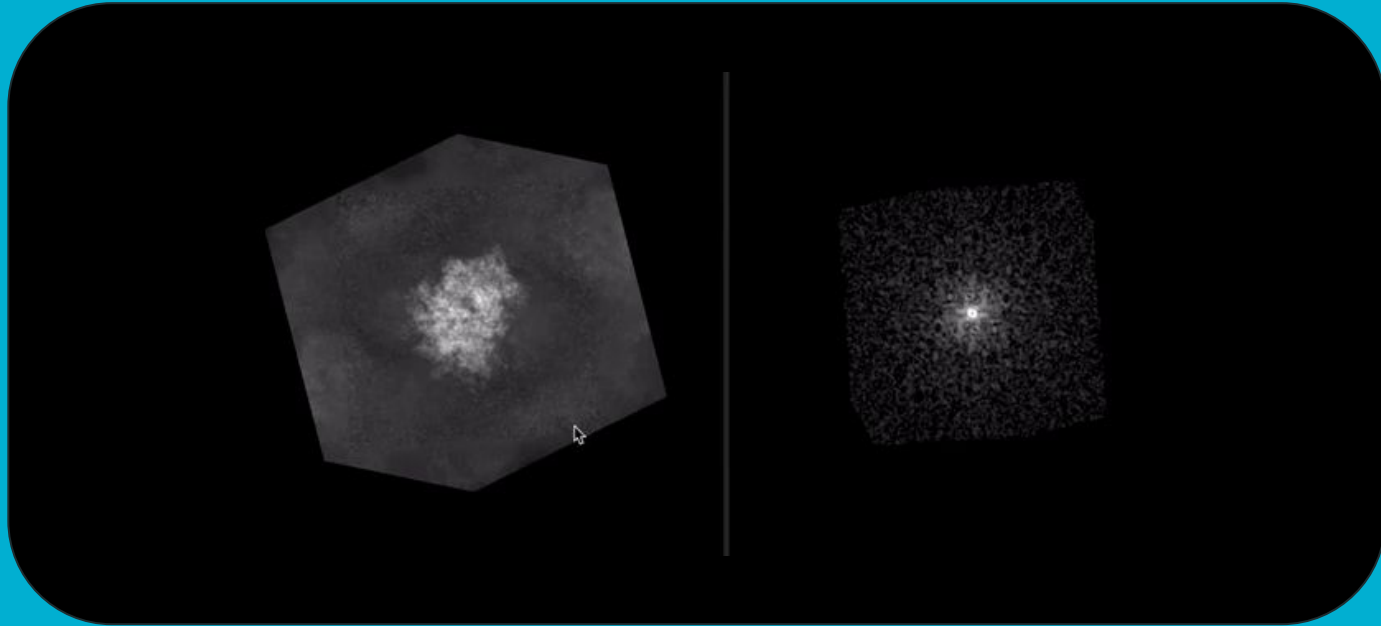


3D object
(real space)

FFT
inverse FFT

central slice of
3D Fourier transform
(power spectrum)

3D reconstruction from projection images



3D object
(real space)

inverse FFT

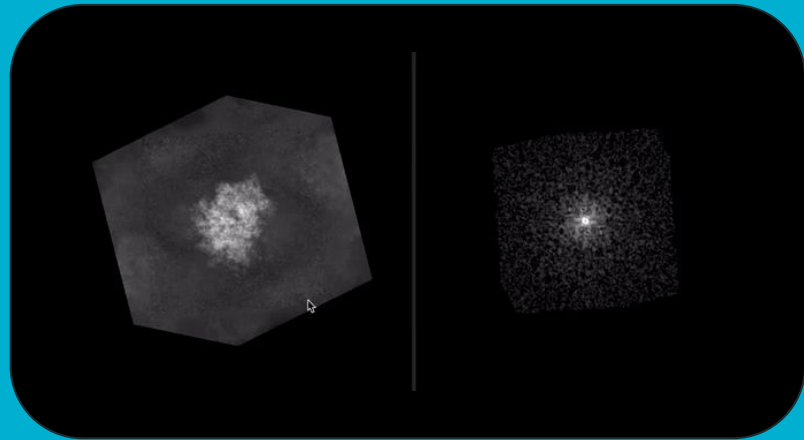
central slices of
3D Fourier transform
(power spectrum)

What is single particle analysis?

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

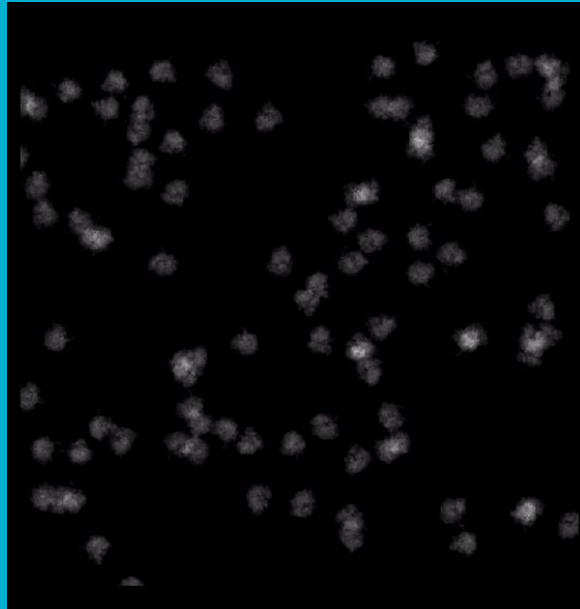
- 2D shifts
- orientations
- defocus

This allows us to build up the 3D
Fourier transform



now let's do it for tomography

tilt series



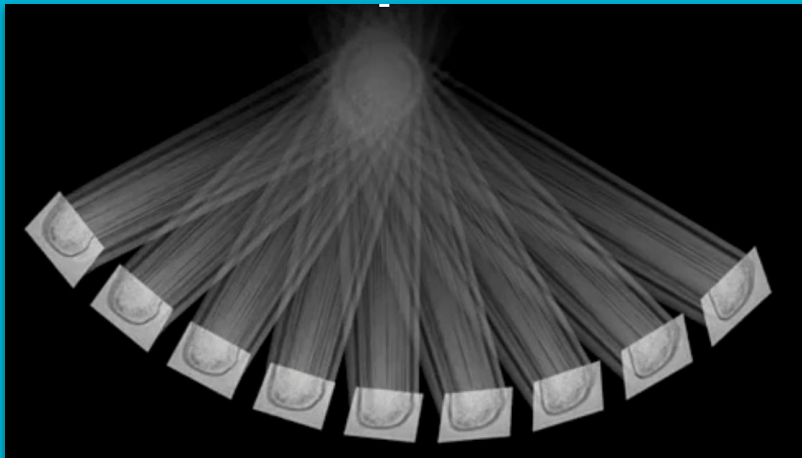
plus minus 60 degrees

3 degree increments

41 images

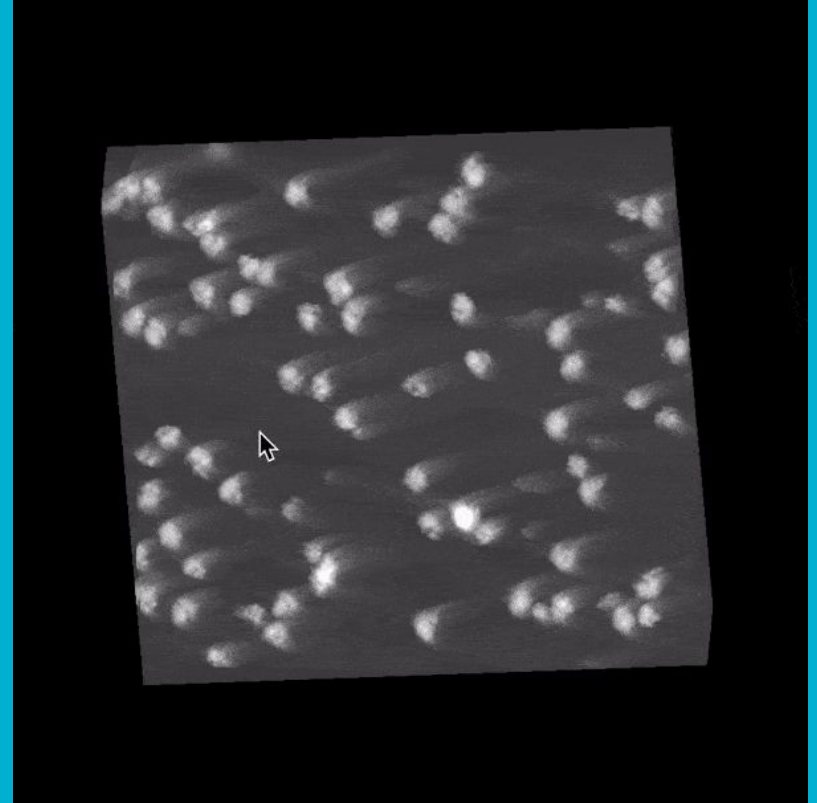
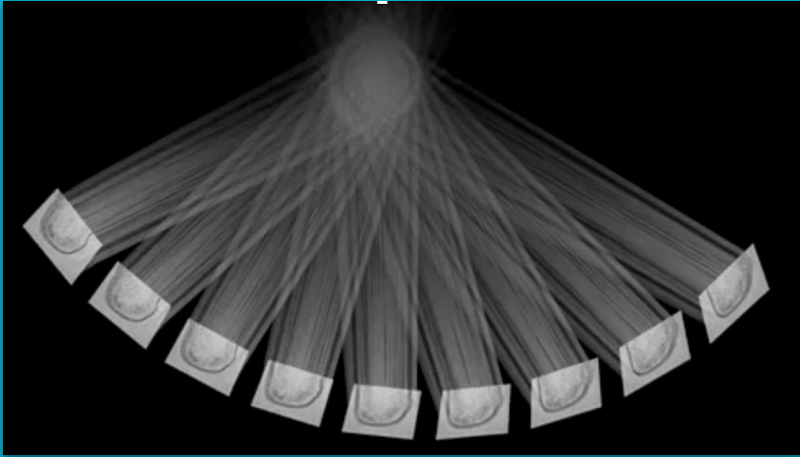
remember this is a stack of 2D images, not a 3D image

tilt series to tomograms



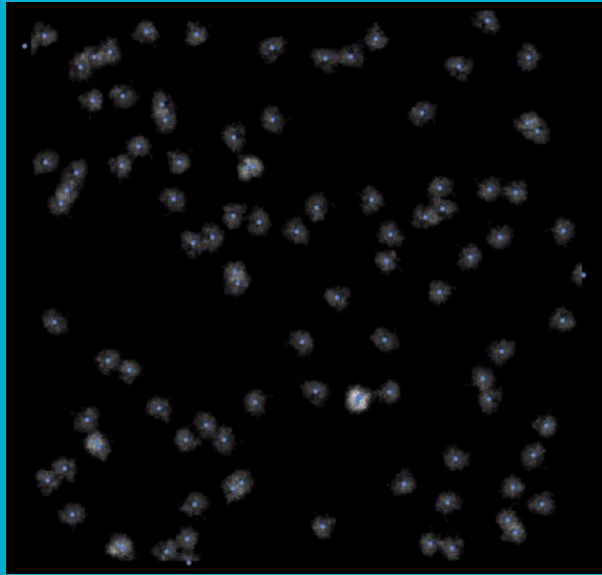
reconstruction typically
performed in real space
because images are large

tilt series to tomograms

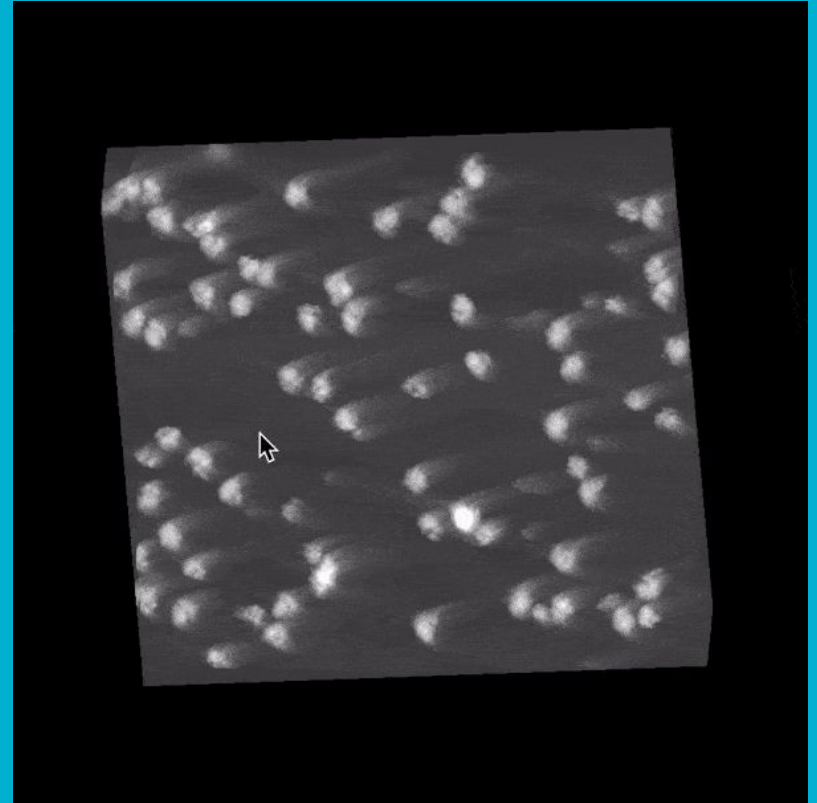


localise objects of interest in 3D

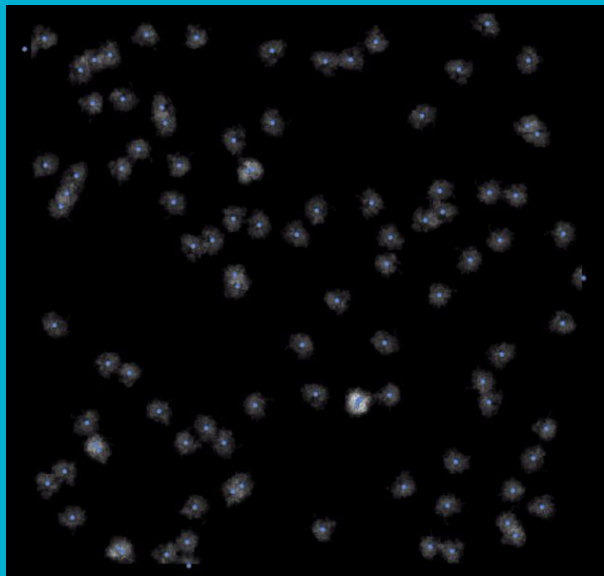
tilt series to tomograms



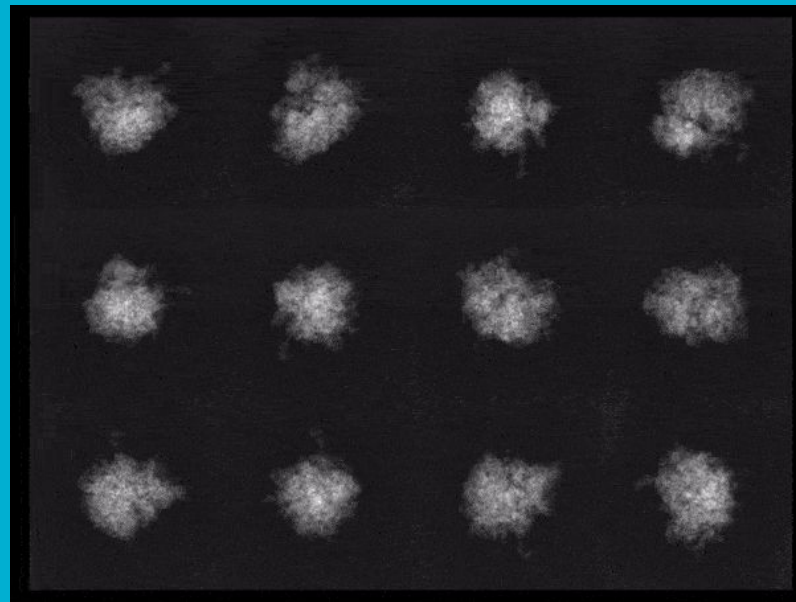
because we know the projection geometry
we can find our objects of interest in 2D



tilt series to particle tilt series

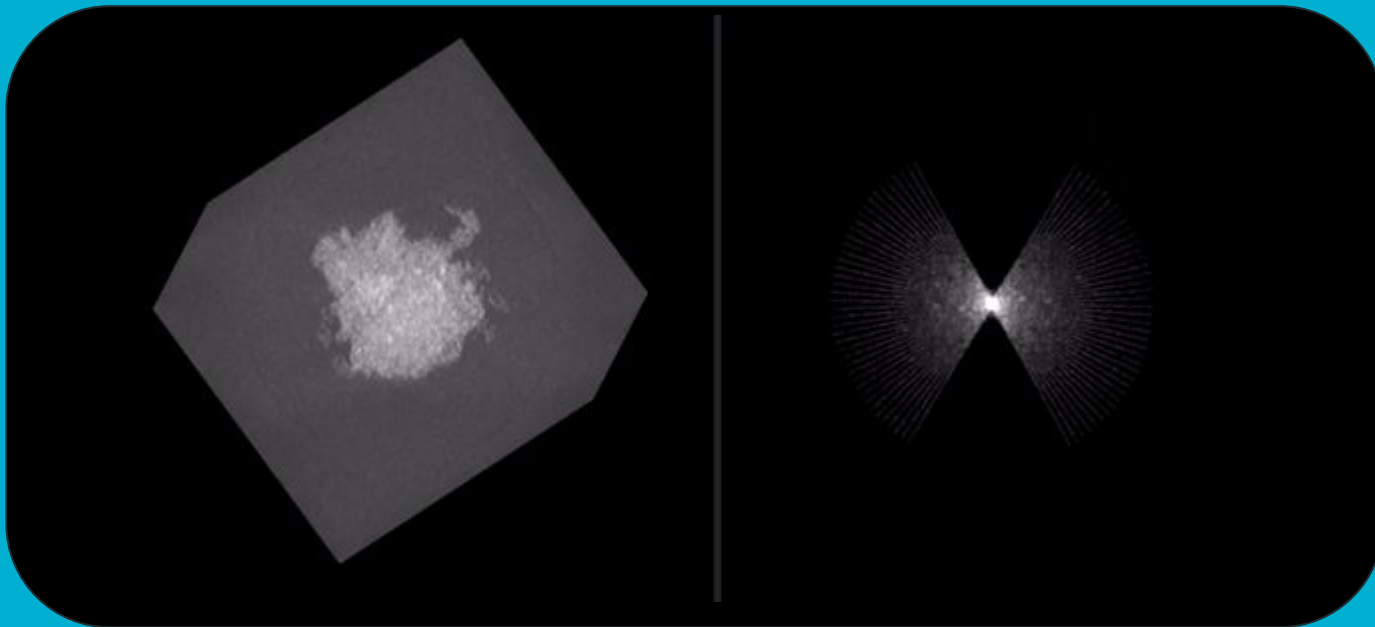


because we know the projection geometry
we can project 3D points down to 2D



this lets us extract mini tilt series
centered on each particle

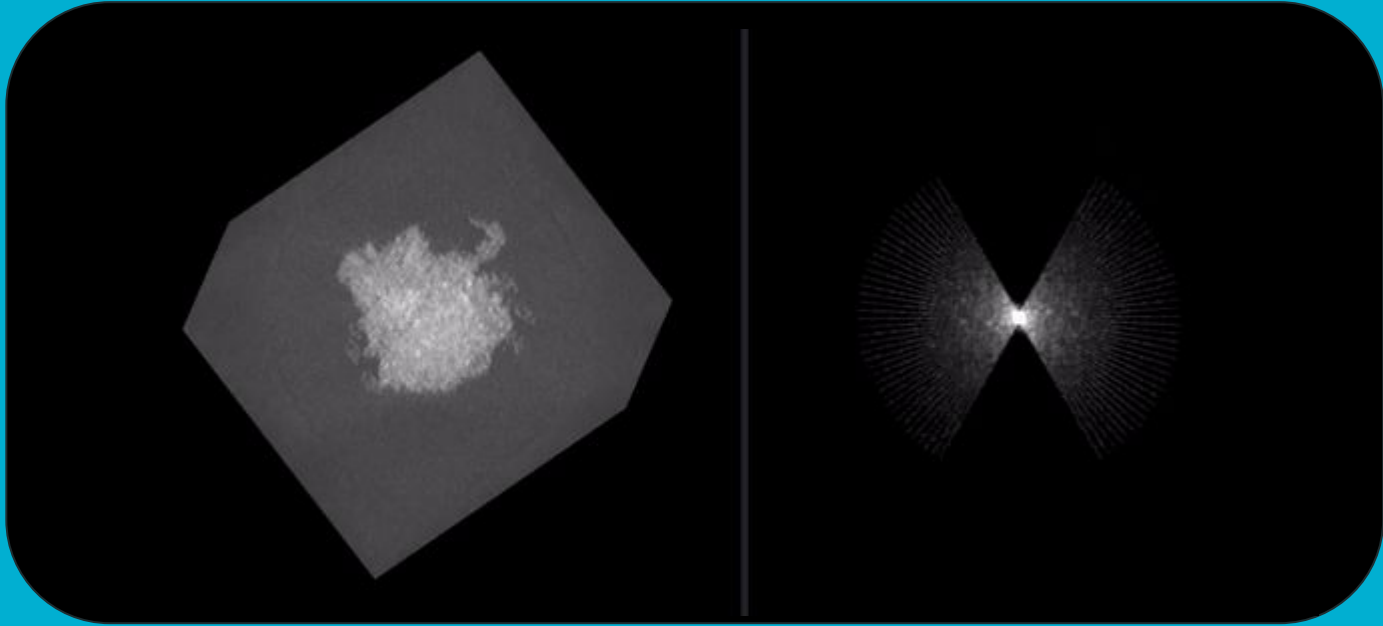
subtomograms $\sim$ particle tilt series
(but it's easier to deal with the CTF properly on 2D data)



3D reconstruction
from particle tilt series

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

3D reconstruction from particle tilt series



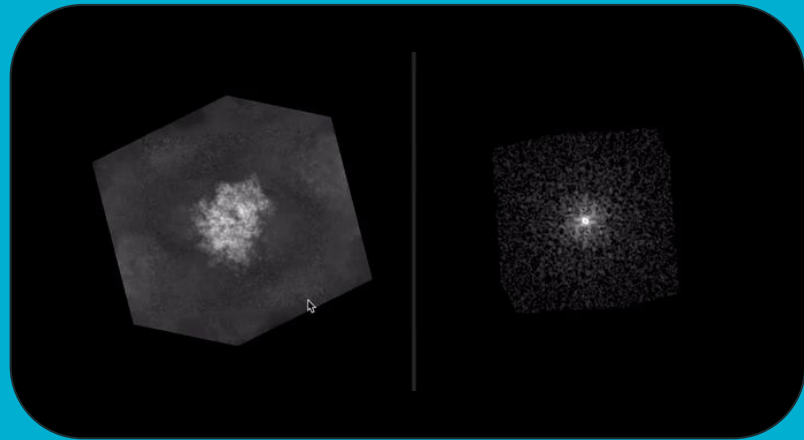
each particle contributes 41 central slices to the 3D FT

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

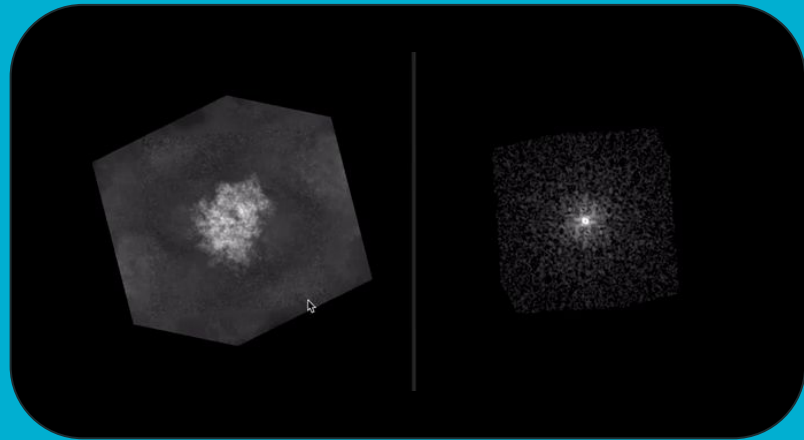


What is single particle analysis?

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

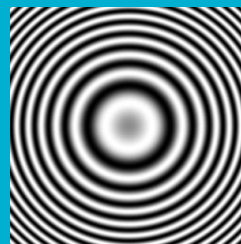
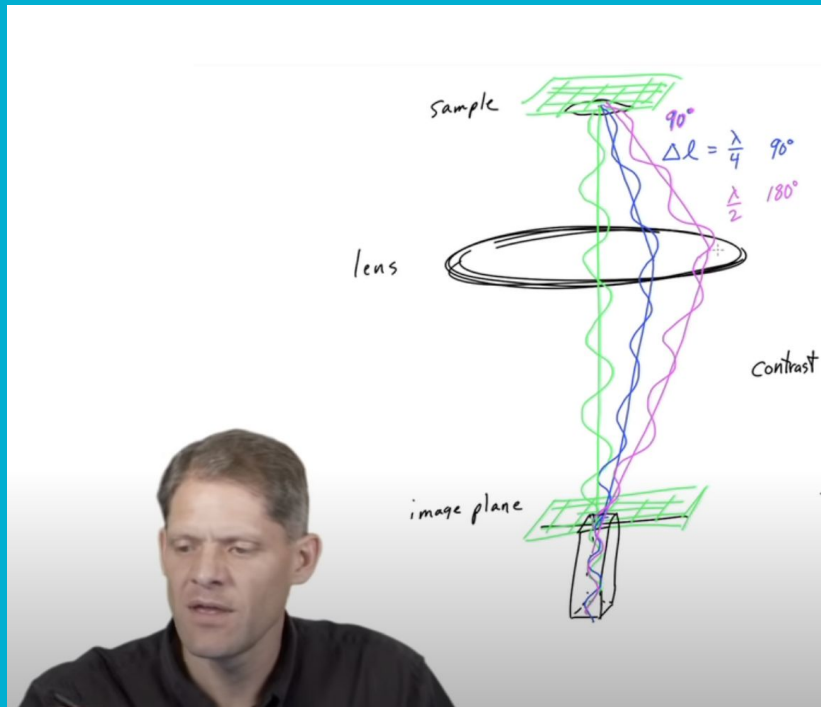


tilt series processing is a superset of frame
series processing

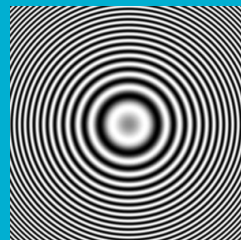
I hope I've convinced you

the CTF in tomograms

CTF is changed a lot by defocus

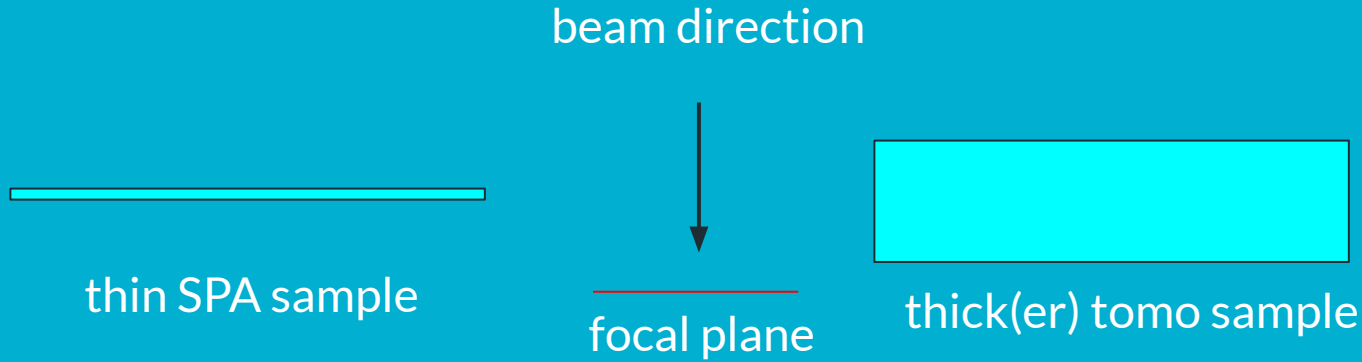


1 μm defocus
2 Å/px

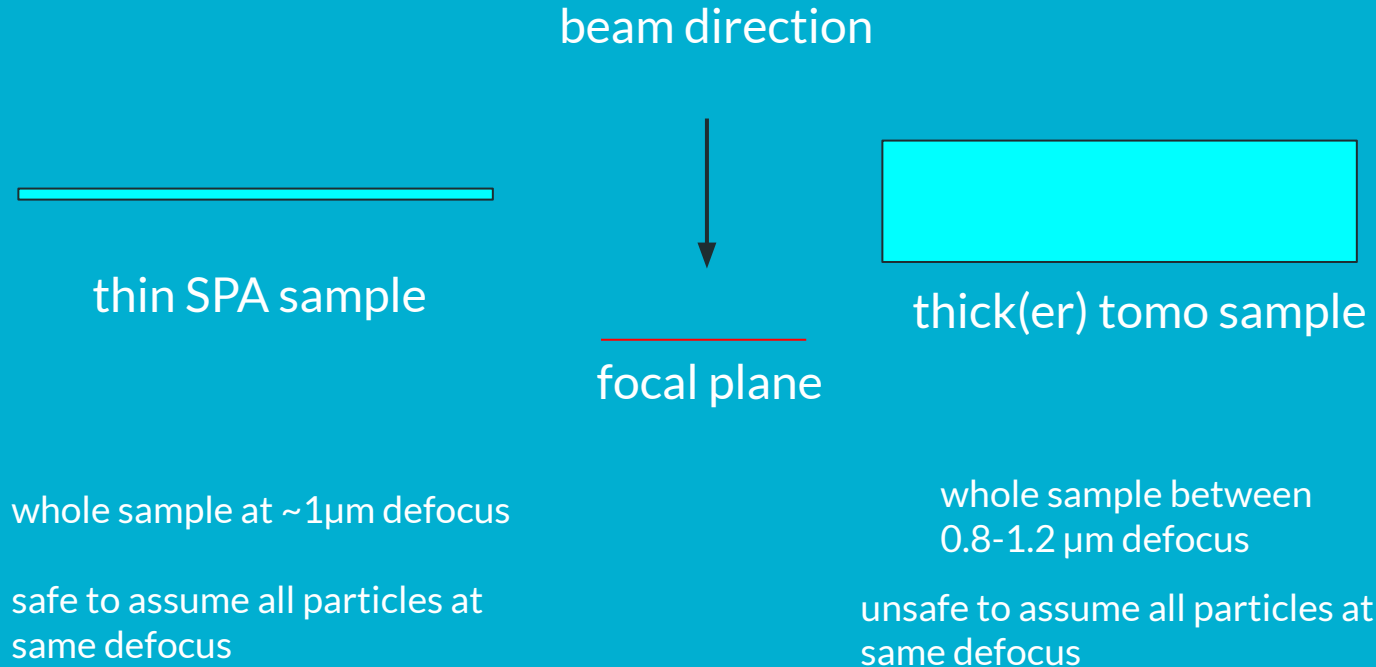


2 μm defocus
2 Å/px

sample thickness matters

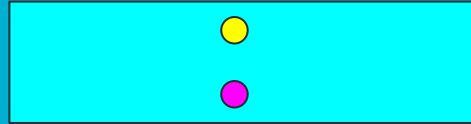


sample thickness matters



sample thickness matters

beam direction



0 degrees

focal plane

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

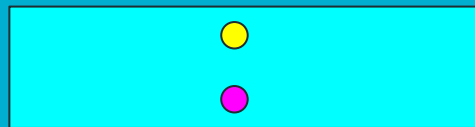
G J Jensen ¹, R D Kornberg

sample thickness matters

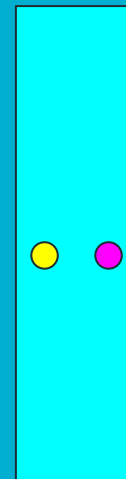
beam direction



focal plane



0 degrees



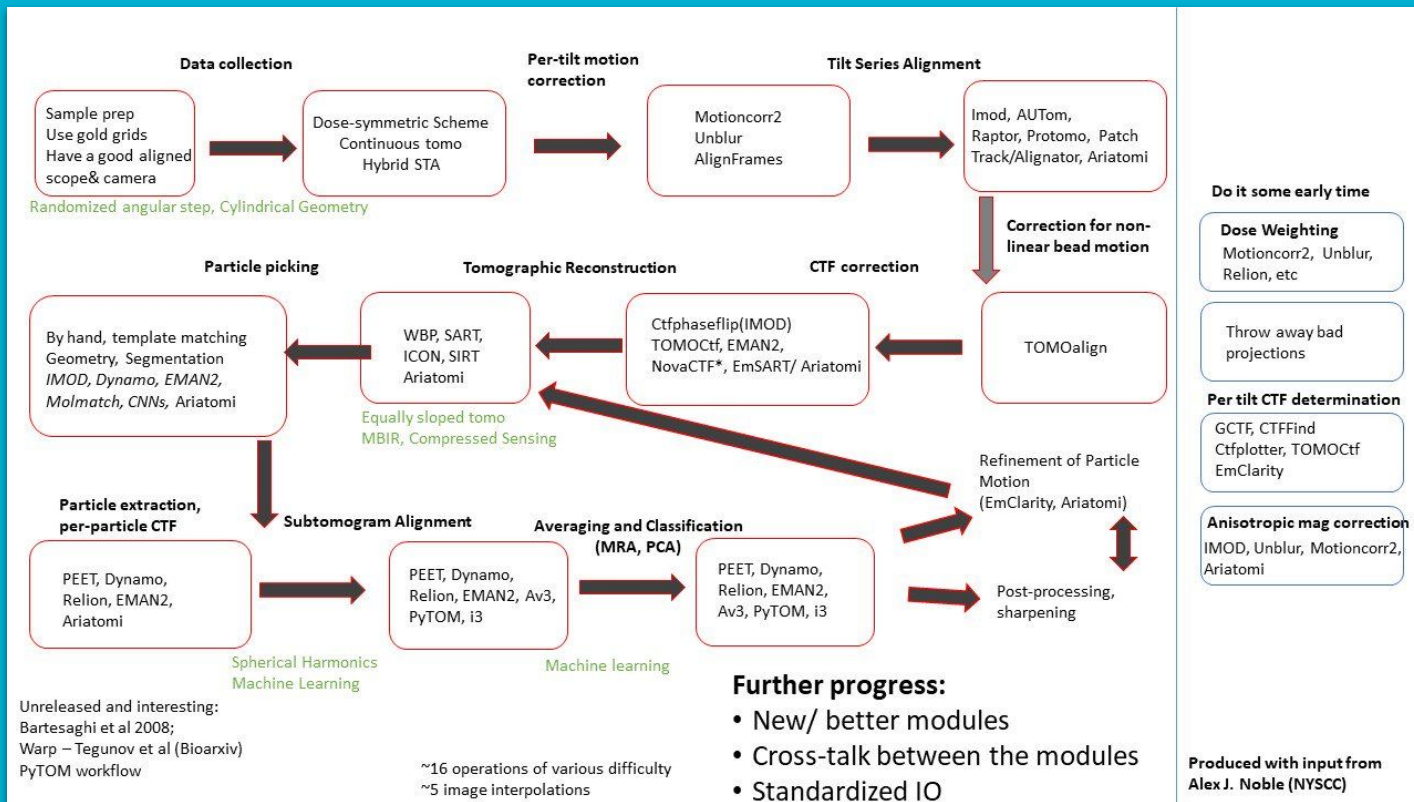
90 degrees

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

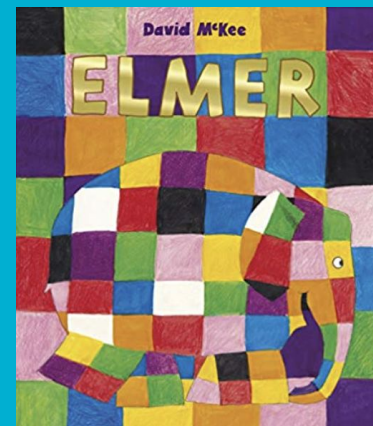
Beata Turoňová^a, Florian K.M. Schur^a, William Wan^a, John A.G. Briggs^{a,b}  

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

things are improving...

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

trend is towards 'complete pipelines'...

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

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

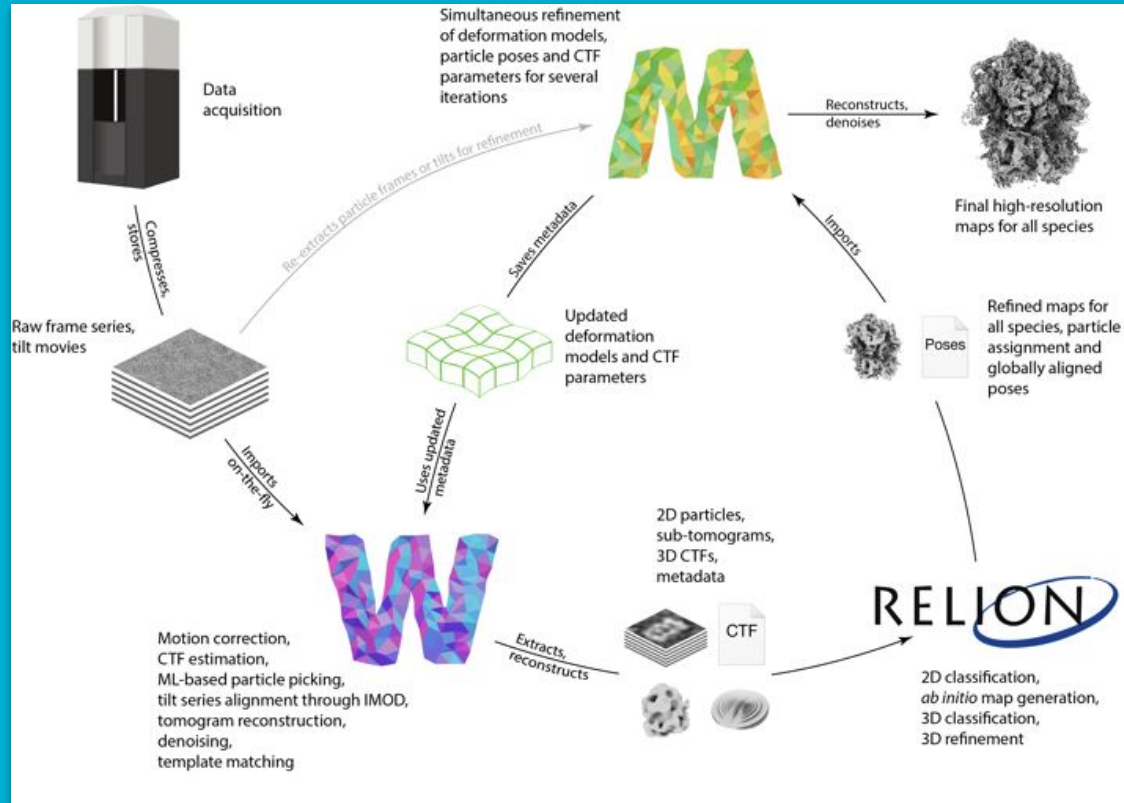
if they aren't you should probably be doing single particle

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

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



Warp/M had a great GUI

DURCHLICHTELEKTRONENMIKROSKOPIEBILDATENENTZERRUNGWERKZEUG

Raw Data

SAVE SETTINGS LOAD SETTINGS

Input

Input: G:\vpp\libox\10153\upload\...*.tif

Pixel XY: 0.5450/0.5450 Å, $\angle$ 0.0°

Bin: 1.00x (1.0900 Å/px)

Dose: 1.00 e/Å²/frame

Preprocessing

☒ Correct gain using: G:\vpp\libox\10150\gain_trans_flip.mrc

☒ CTF

Window: 512 px Range: 0.10–0.65 Ny ☐ Use Movie Sum

Voltage: 300 kV Cc: 2.70 mm Cc: 2.70 mm

Amplitude: 0.10 Ill. Aperture: 30 µrad ΔE: 0.70 eV

Defocus: 0.3–4.0 µm ☒ Phase Shift ☐ Model Ice Ring

☒ Motion

Consider 0.04–0.25 Ny, weight with B = -600 Å²

Models

Defocus: 5 × 5 × 1 Motion: 5 × 5 × 20

☐ Pick Particles

Use BoxNet2Mask_20180531

Expect 260 Å, give particles; use scores above 0.70

Maintain a minimum distance of 20 Å from ⚙

☒ Extract 360 px boxes, 1.0900 Å/px, ☒ invert, ☒ normalize

Output

Skip first 2, last 0 frames,

☐ Average

☐ Deconvolved average (strength = 1.00, falloff = 1.00)

☐ Aligned stack, collapse every 1 frames

Overview Fourier Space Real Space

EXPORT MICROGRAPH LIST ADJUST PARTICLE DEFOCUS EXPORT PARTICLES IMPORT PARTICLE COORDINATES MATCH TEMPLATE EXPORT BOXNET SAMPLES

Processing Status

90 222 3

Astigmatism (use up to 3.0 σ)

Defocus (use 0.35–6.00 µm)

Phase shift (use 0.00–1.00 π)

Estimated resolution (use better than 4.9 Å)

Average motion per frame in first 1/4 (use up to 1.5 Å)

Number of particles in BoxNet2Mask_20180514: 20510 overall, 15158 good (use at least 1)

(use up to 10 %)

START PROCESSING

but they were locked in a Windows cage



Warp 2.0

now on linux!

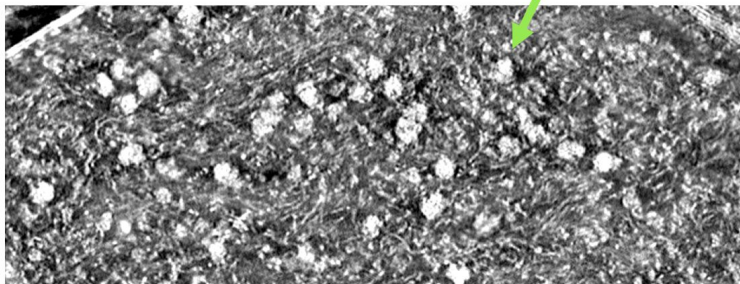
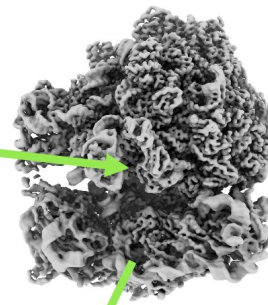
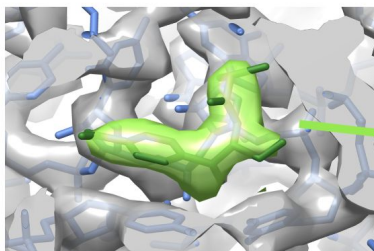


```
conda install warp -c warpem -c nvidia/label/cuda-11.7.0 -c pytorch -c conda-forge
```

M's first significant result

Small-molecule drug
resolved at 3.5 Å...

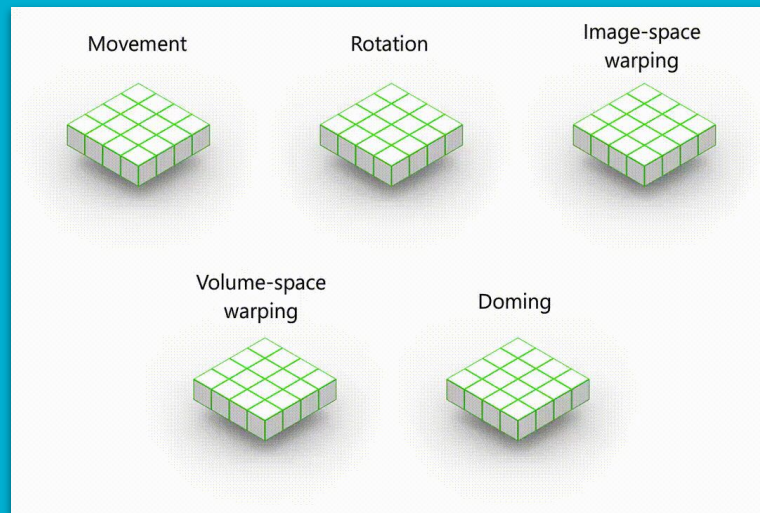
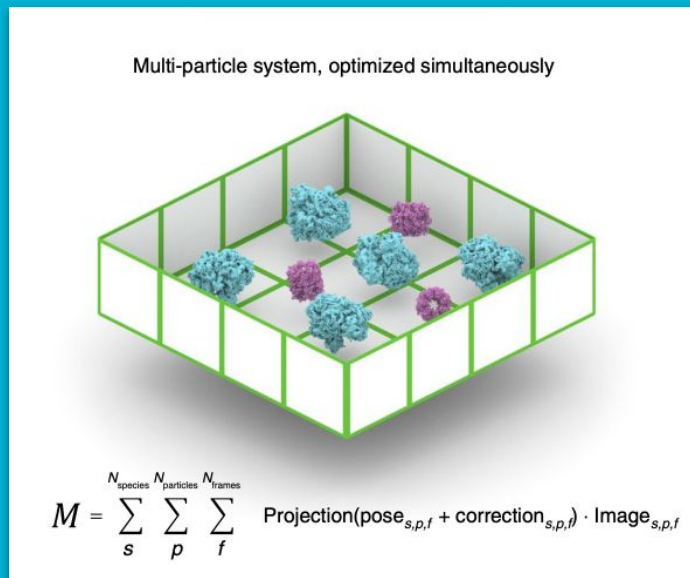
... inside a 70S ribosome...



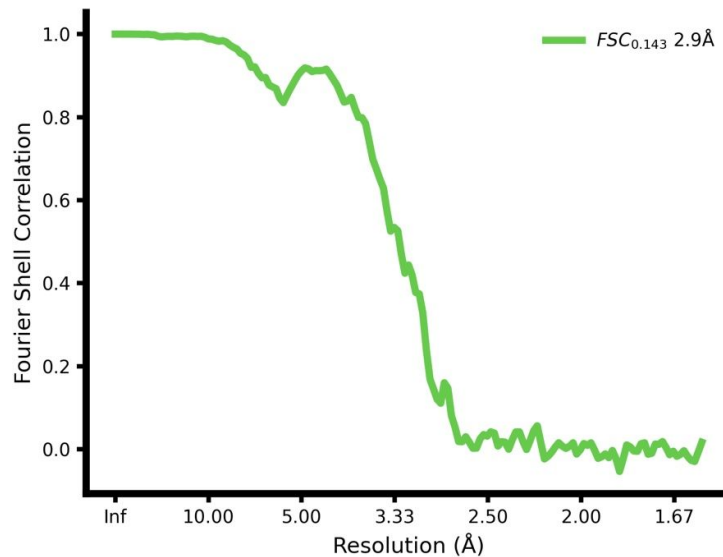
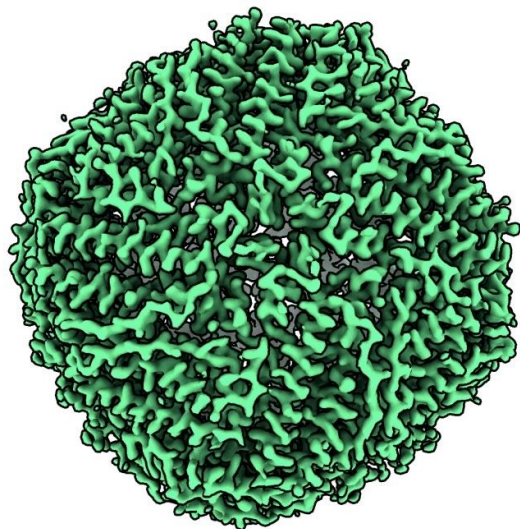
... inside a 150 nm thick *M. pneumoniae* cell

Tegunov *et al.*, Nature Methods 2021

Mmmodel the whole system



benchmarked on linux with 5-TS from EMPIAR-10491

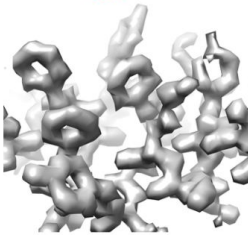


looks good! 🚀

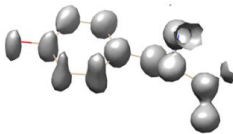
Warp & M continue to hold resolution records in SPA and Tomo in 2024 (?)



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



Documentation for users who like to read 🧐

 Quick Start: Tilt Series

 Search

 warpem/warp
v2.0.0dev15 ⭐ 27 🗑 5

 Home

 User Guides

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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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Tilt Series: Check Defocus Handedness

Tilt Series: CTF Estimation

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Template Matching

Export Particles

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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



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

- 54 J18 Tomogram Reconstruction Finished 11 h ago
- 54 J17 CTF estimation Finished 4 d ago
- 54 J16 Align with Etomo Finished 6 d ago
- 54 J15 Deselect tilts Finished 7 d ago
- 54 J14 Tilt-series data set Finished 7 d ago

< Mar '25



P2: Third Project with a very long name that will certainly...

Created 2025-01-09

- 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

< Mar '25



P3: Pooo...

Created 2025-01-18

No jobs yet



P0: My Project

Created 2024-08-27

- 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

< Mar '25



P1: Second Project

Created 2024-11-14

- S1 J7 Clone 2 of bla Building for 62 d 15h
- S1 J6 Clone of bla Building for 62 d 15h
- S1 J5 bla Building for 62 d 15h
- S1 J4 bla Building for 66 d 21h
- S1 J3 Post-processing Building for 67 d 14h

< Mar '25



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







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



V1: Key



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

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



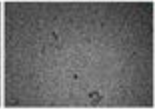
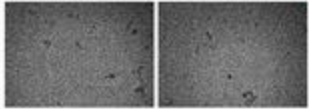
S1: Key



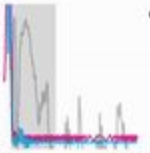
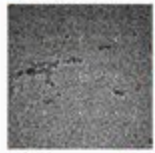
V1: View 1



J1 Frame-series data set



Finished in 00:07



05:24

86/267

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 `{{ command }}`

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

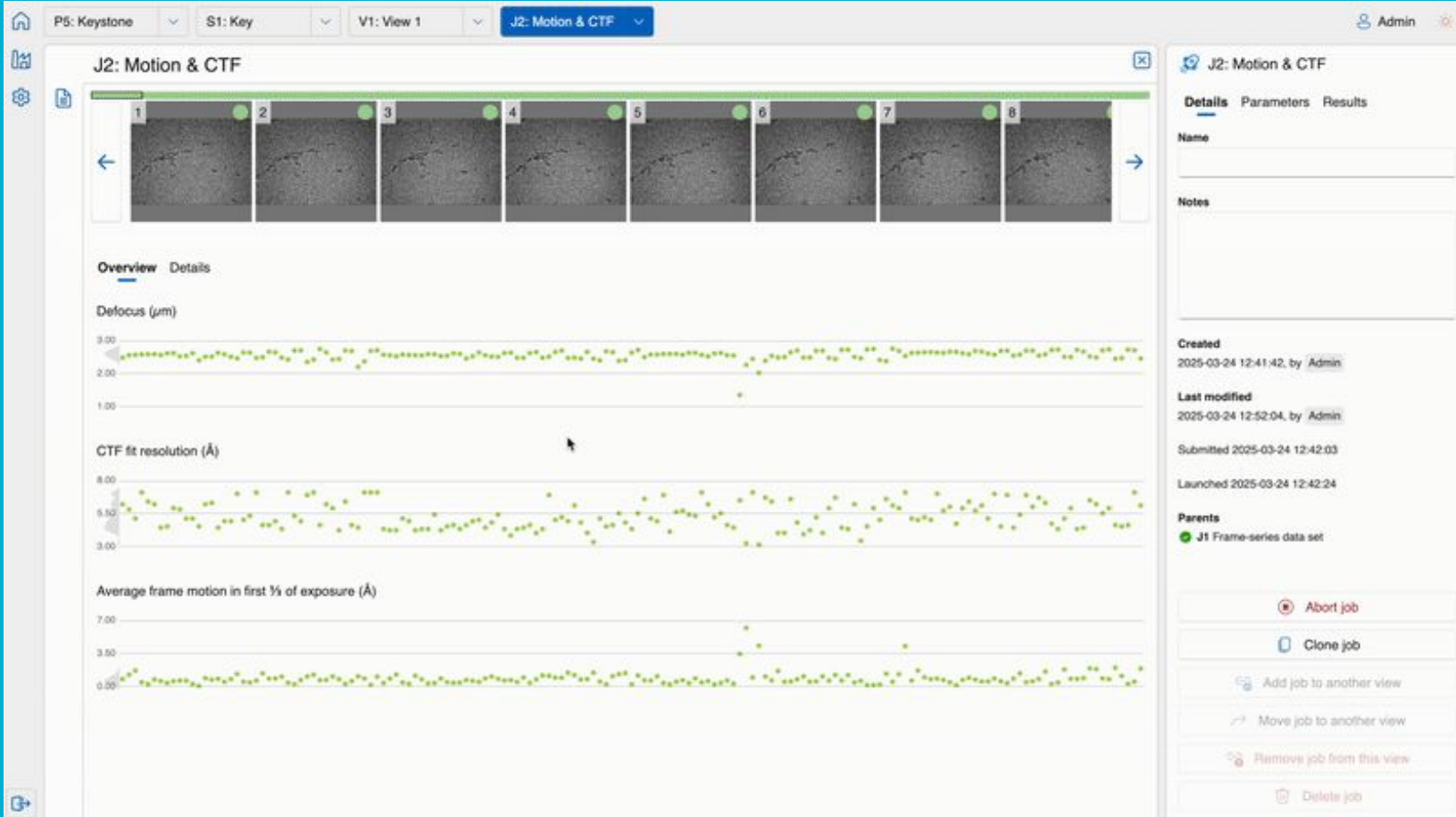
Last modified

2025-03-24 11:59, by Admin

2 jobs



Delete view





P5: Keystone



S1: Key



V1: View 1



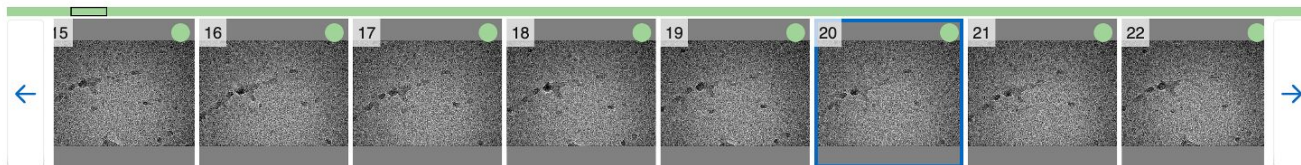
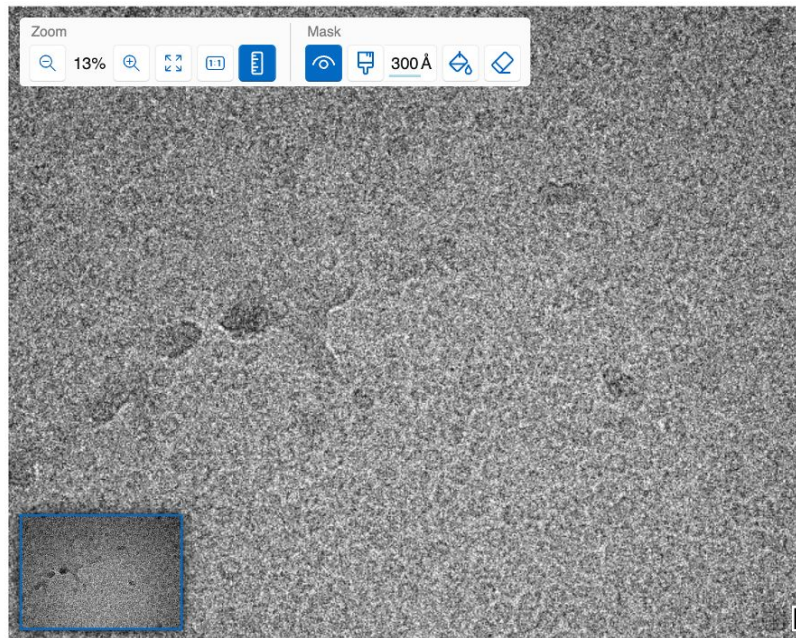
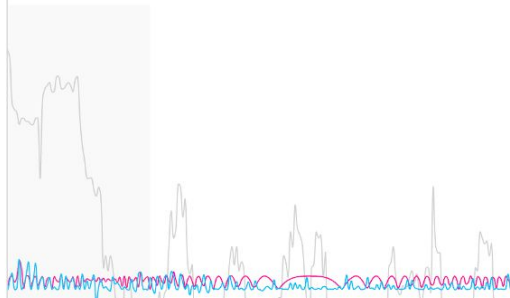
J2: Motion & CTF



Admin



J2: Motion & CTF

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

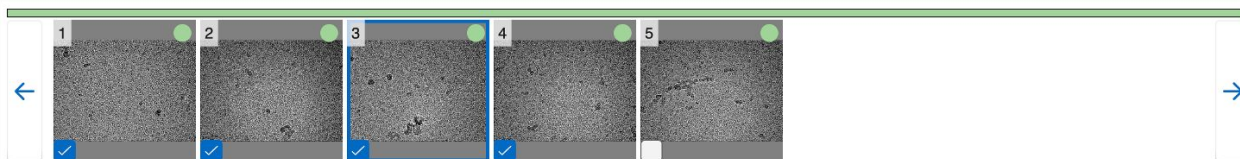
V1: View 1

J14: Tilt-series data set

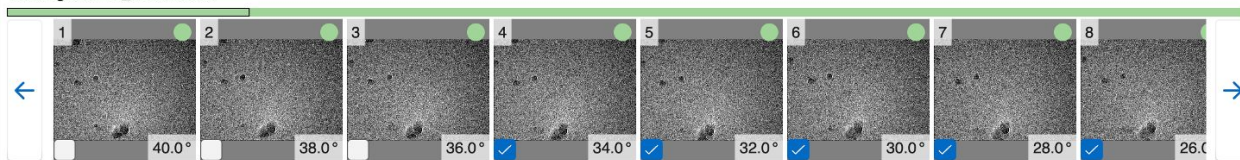
Admin



J14: Tilt-series data set



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

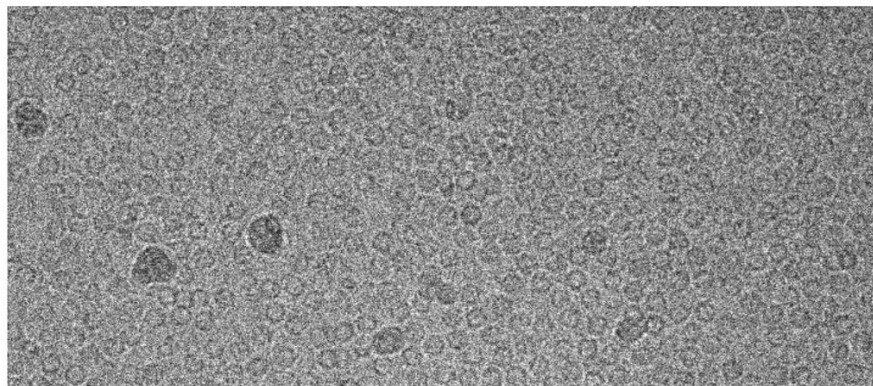
Tilt angle (°)



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



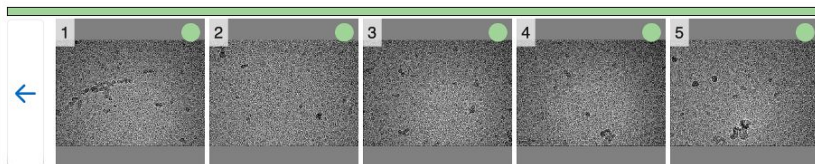
J16: Align with Etomo



Admin



J16: Align with Etomo



Overview

Tilt series details

Number of tilts

42.0

41.0

40.0

Min and max tilt angle (°)

50.0

5.00

-40.0

Tilt axis angle (°)

-84.6

-85.6

-86.6

Min, average, and max shift in X (Å)

200

100

0.00

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

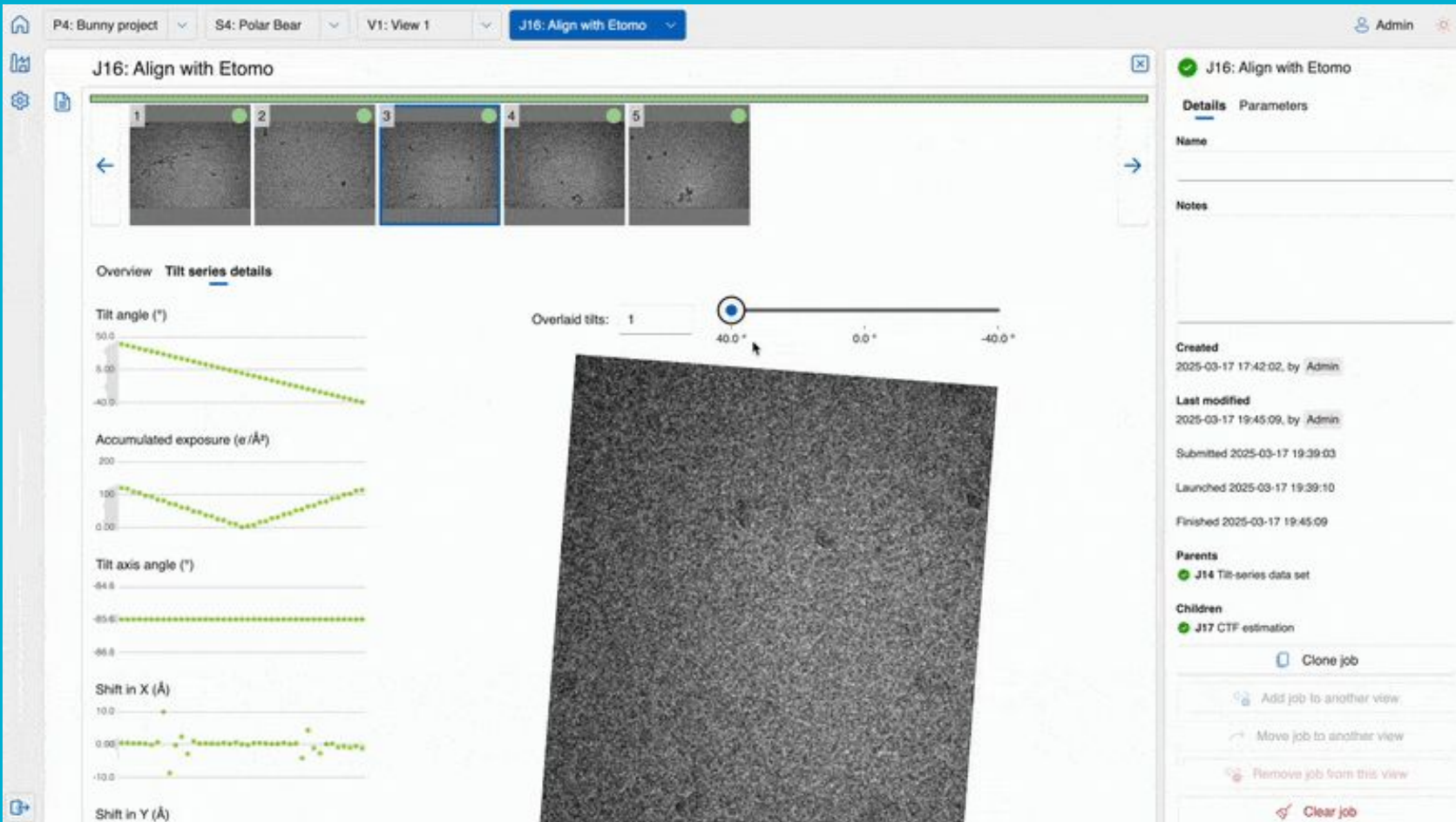
Clone job

Add job to another view

Move job to another view

Remove job from this view

Clear job



J18: Tomogram Reconstruction



✓ 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

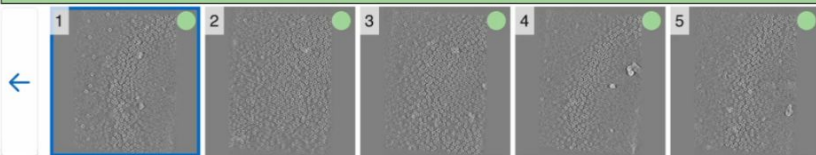
 Add job to another view

 Move job to another view

 Remove job from this view

 Clear job

 Delete job



Coordinates

162

237

42

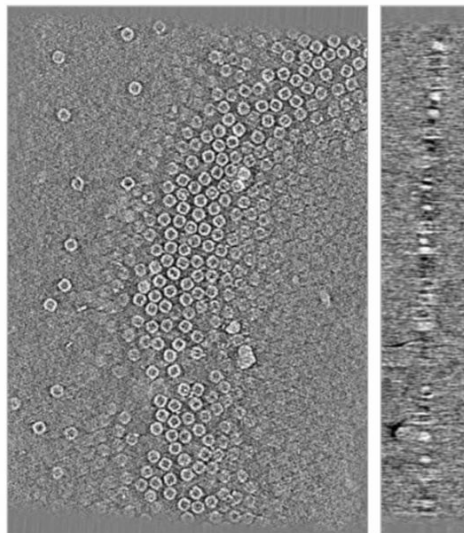
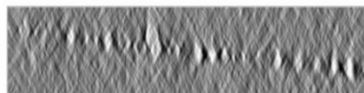
Zoom

95%

Particles

100 Å

200 Å



But tomography is still hard...



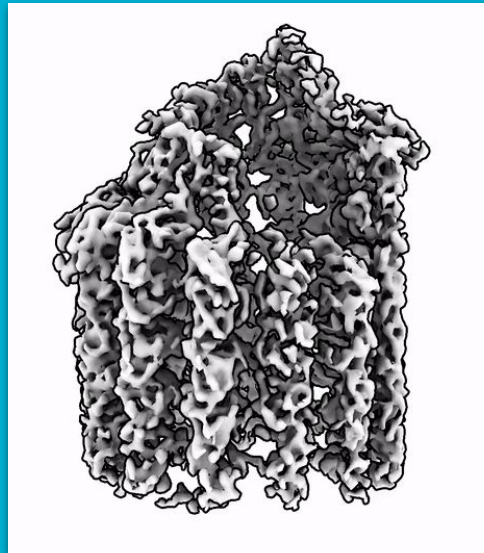
slide from Dimitry

illustrated examples

Consensus reconstruction of the native γ -tubulin ring complex

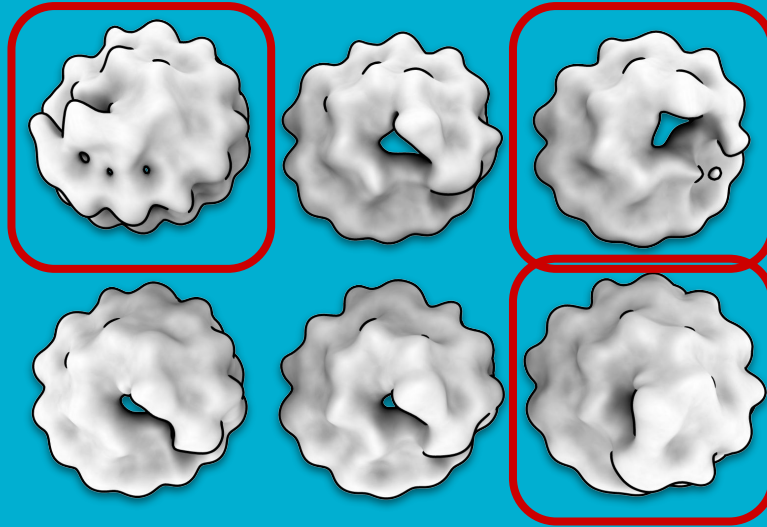


Tom Dendooven, Stan Yatskevich & John Kilmartin

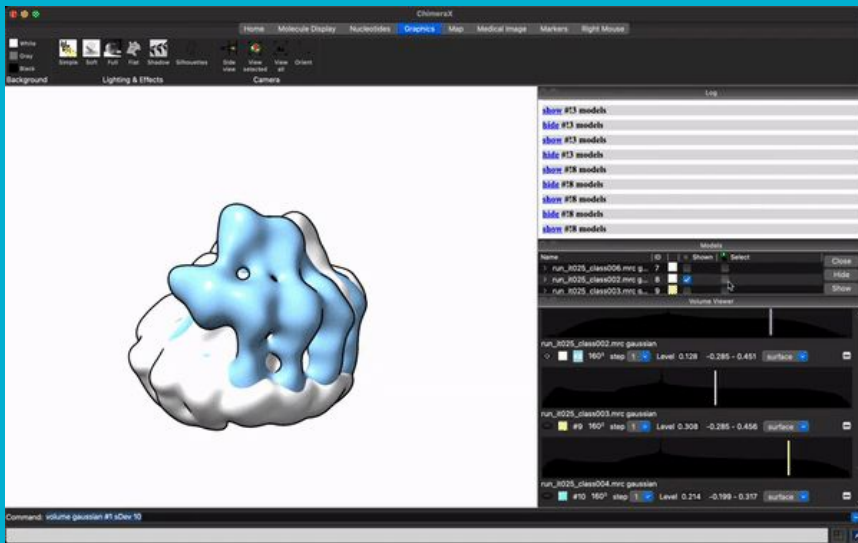


~11 Å from 8000 particles
Warp -> Dynamo -> RELION -> M
co-refined with microtubules which plateau at ~6 Å

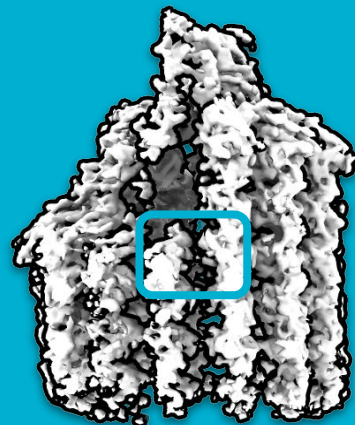
Misaligned subsets of γ -TuRC particles are identified by 3D classification



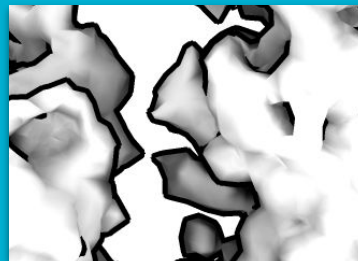
Manual registration improves density at the microtubule seam formation interface



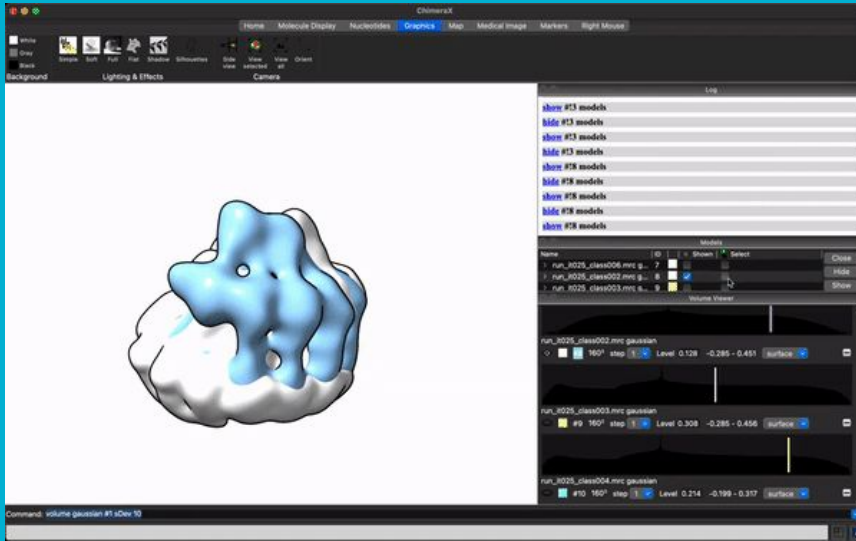
per class transformations defined in ChimeraX and applied on relevant particle subsets



before registration



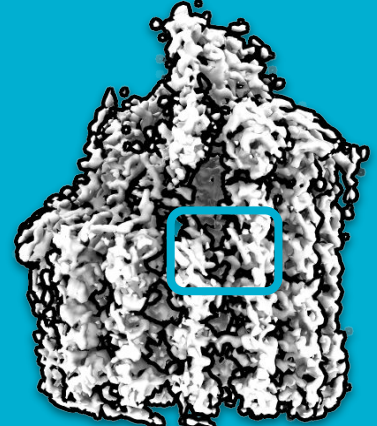
Manual registration improves density at the ring closing interface



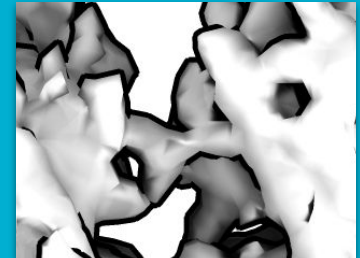
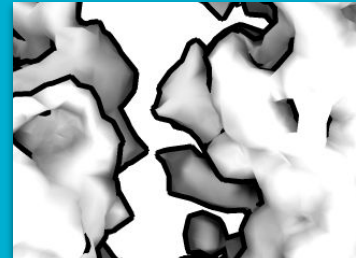
per class transformations defined in ChimeraX and applied on relevant particle subsets

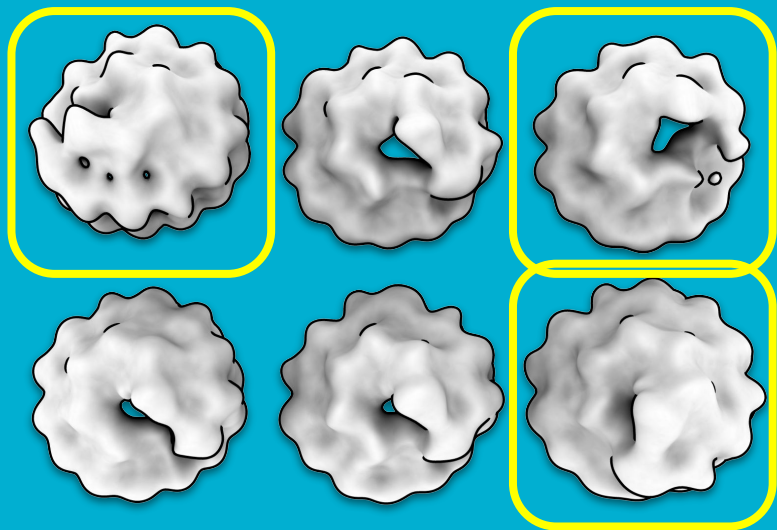


before registration

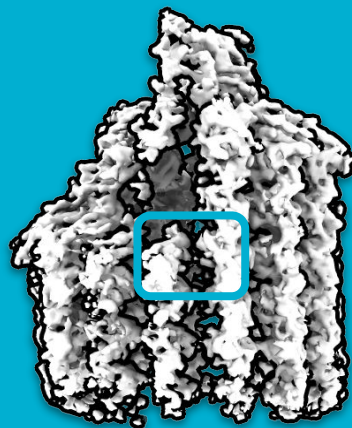


after registration

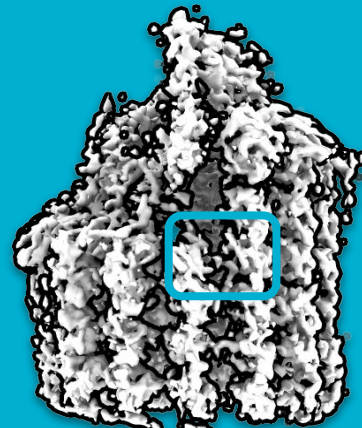
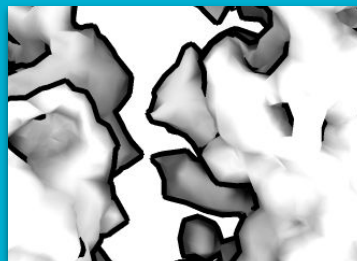




identify misaligned subsets of data
Class3D in RELION



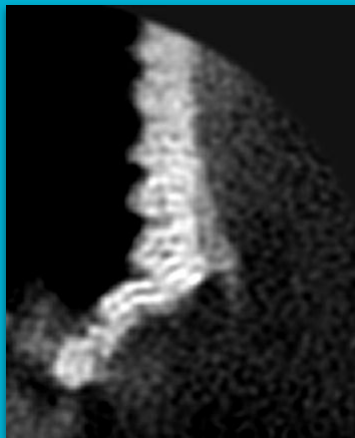
before registration



after registration

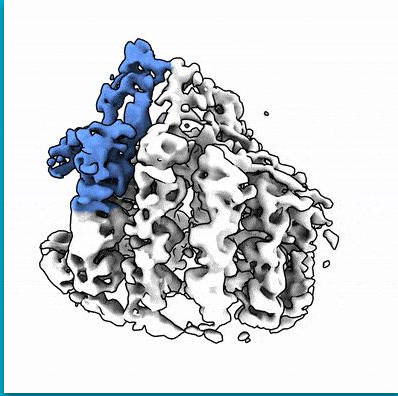


Identification of an interesting density bridging γ -TuSC and the microtubule protofilament

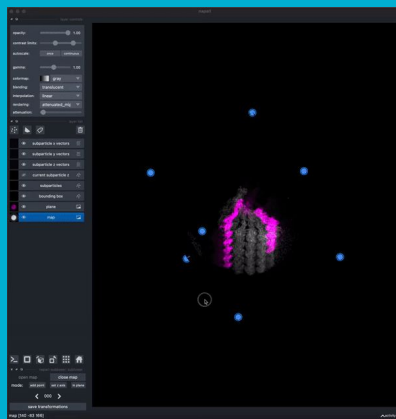
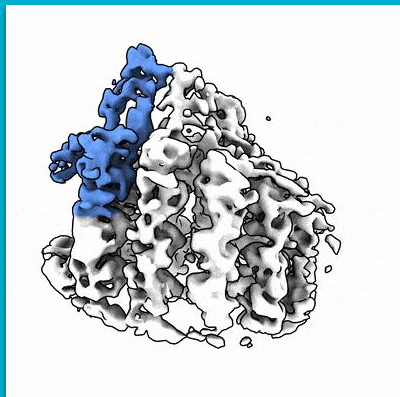


unidentified potentially interesting density
bridging γ -TuSC and microtubule protofilament

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

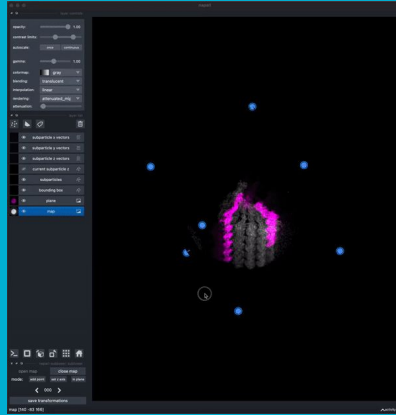
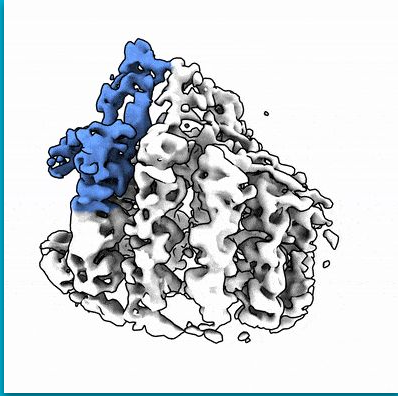


Leveraging multiplicity of γ -TuSC subcomplex improves density in regions of interest



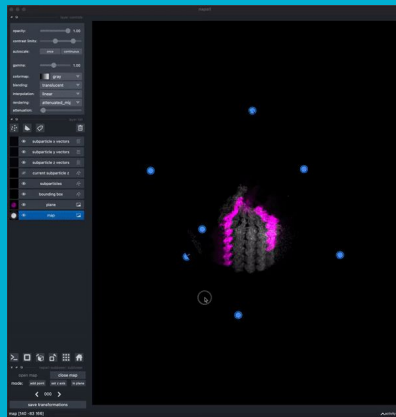
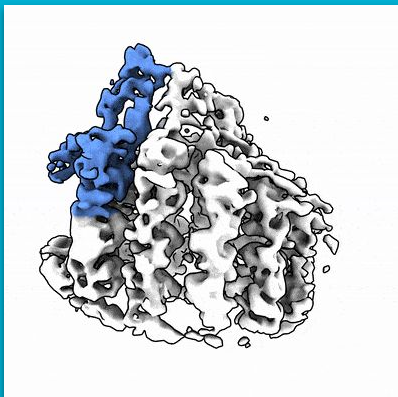
napari-subboxer

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



napari-subboxer

~7 Å reconstruction from
7 x 8000 particles



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 Rasochilov, John V. Kilmarin  & David Barford 

thanks for listening!
(cue the workshop start)