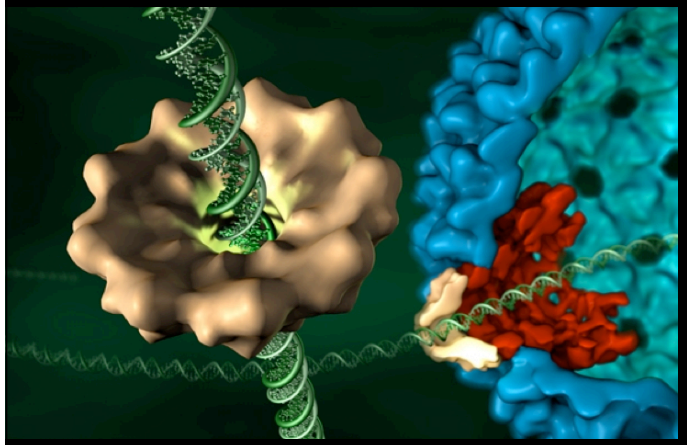
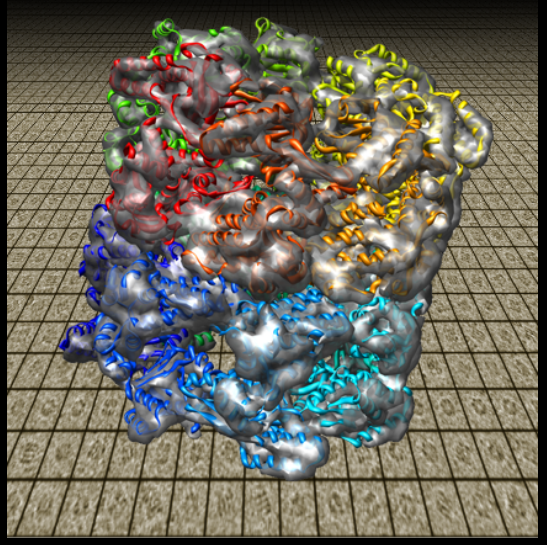
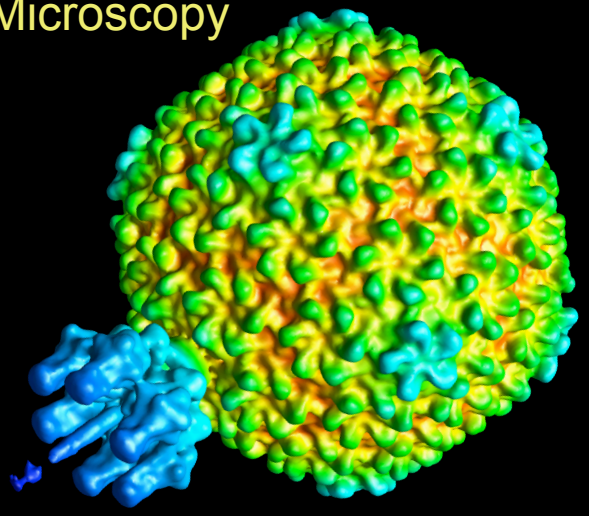
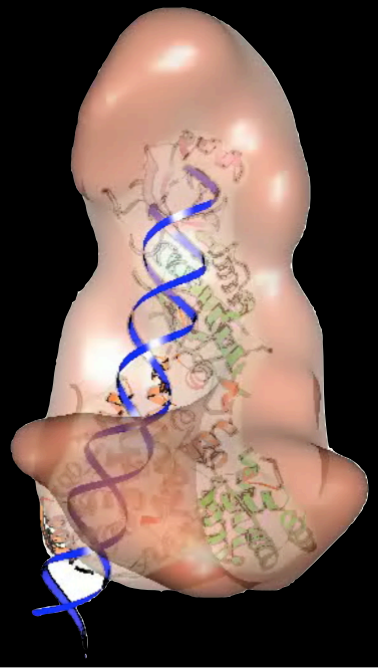
NRAMM

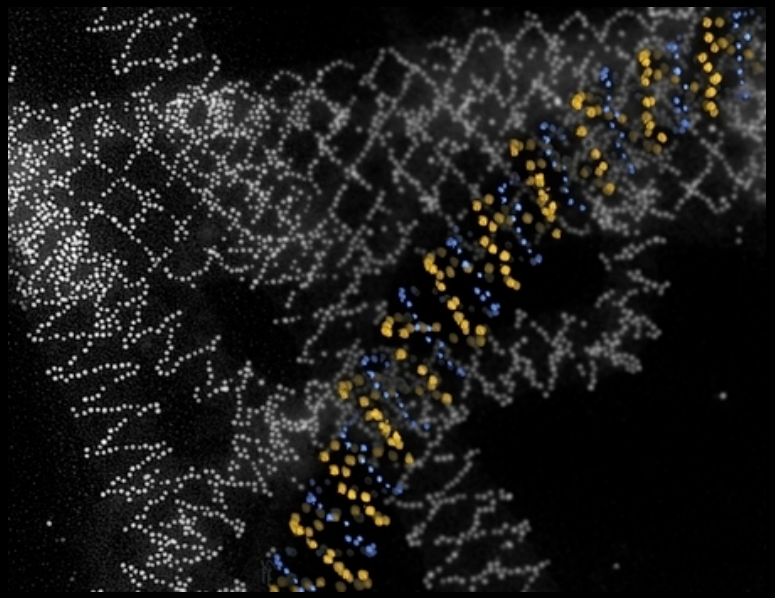
National Resource for Automated Molecular Microscopy

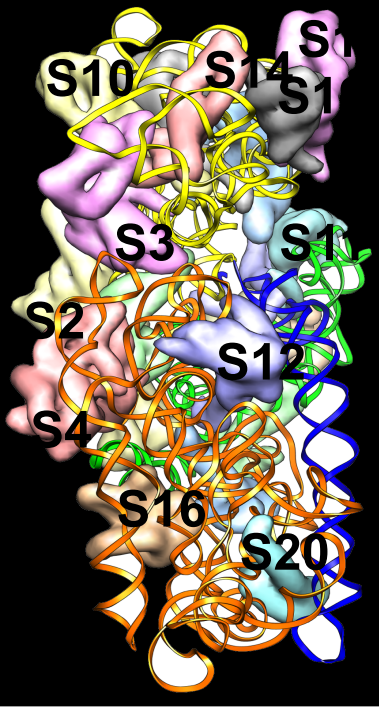






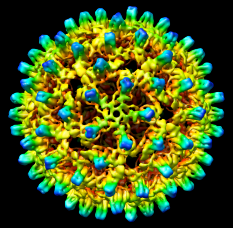




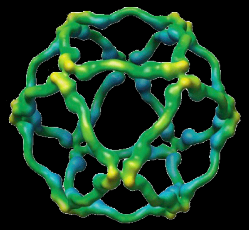


NRAMM

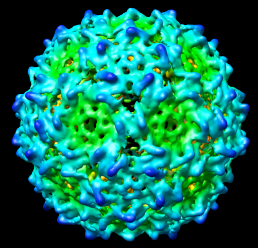
Supported by the National Center for Research Resources

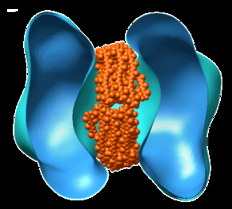


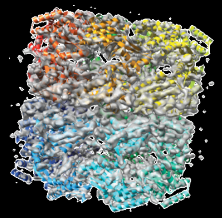
The overall mission of NRAMM is to develop, test and apply technology aimed towards automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.

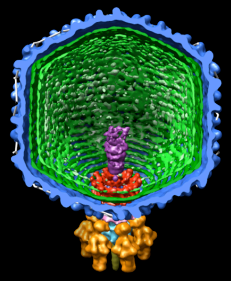


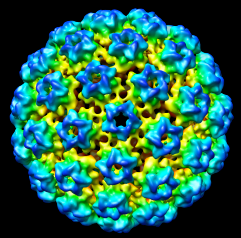
Core technology research and development
Collaboration
Service
Training
Dissemination

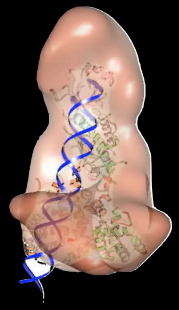


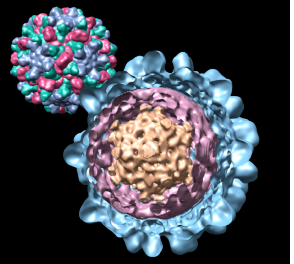






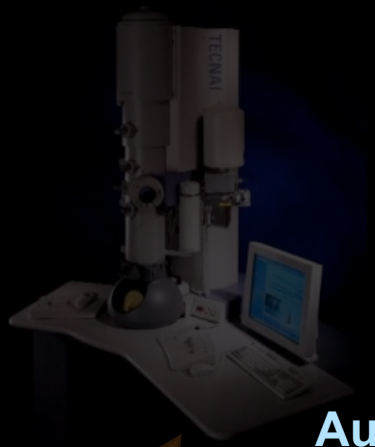






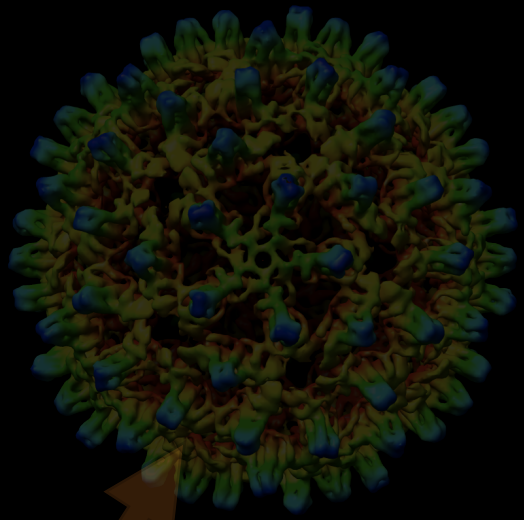
EM Pipeline at NRAMM

Sample



Automated
Data Collection
(Leginon)

3D EM Density

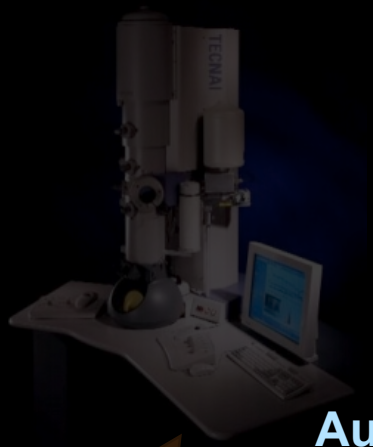


Streamlined
Processing
(Appion)

Specimen preperation

EM Pipeline at NRAMM

Sample



Automated
Data Collection
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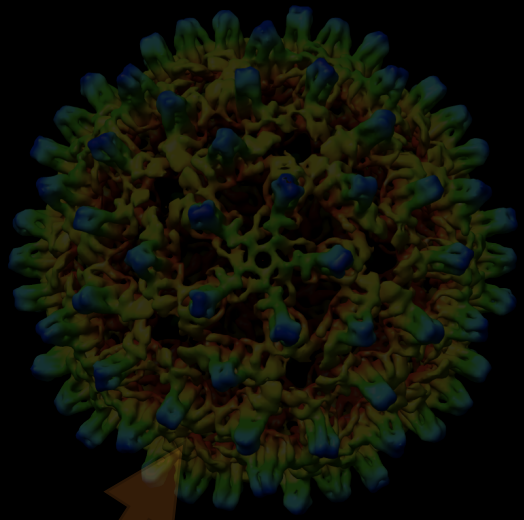
3D EM Density

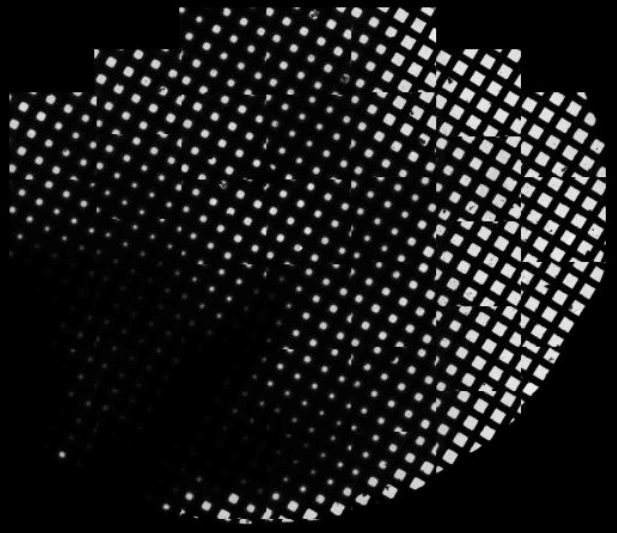


Specimen preperation

Streamlined
Processing
(Appion)

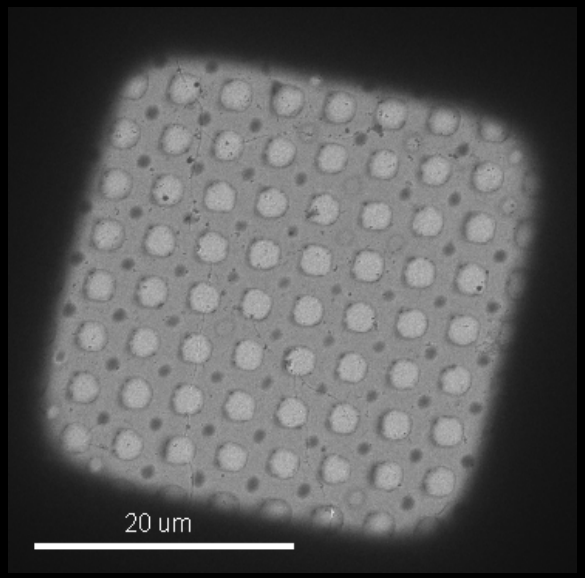
Specimen preparation

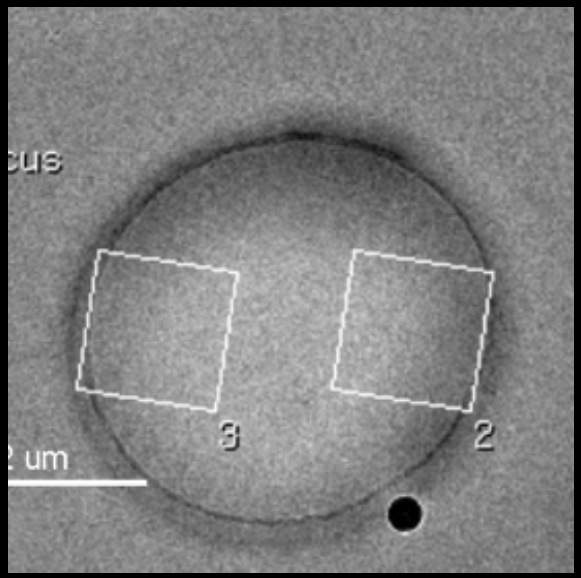
Gatan Solarus Plasma Cleaner



C-Flat grids

FEI Vitrobot



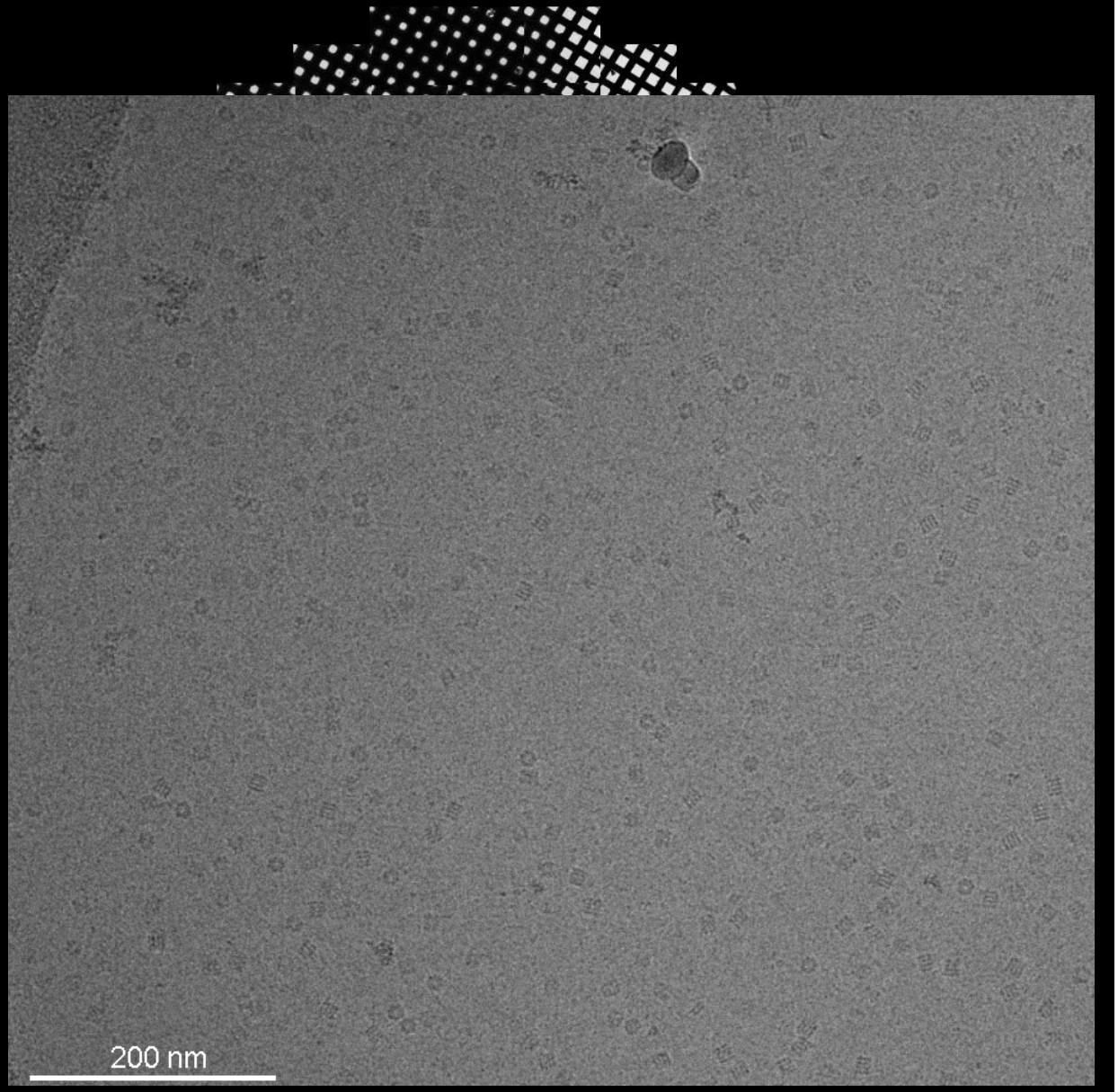


http://cimbio.scripps.edu/misc/documentation/protocol/freeze.php

Specimen preparation

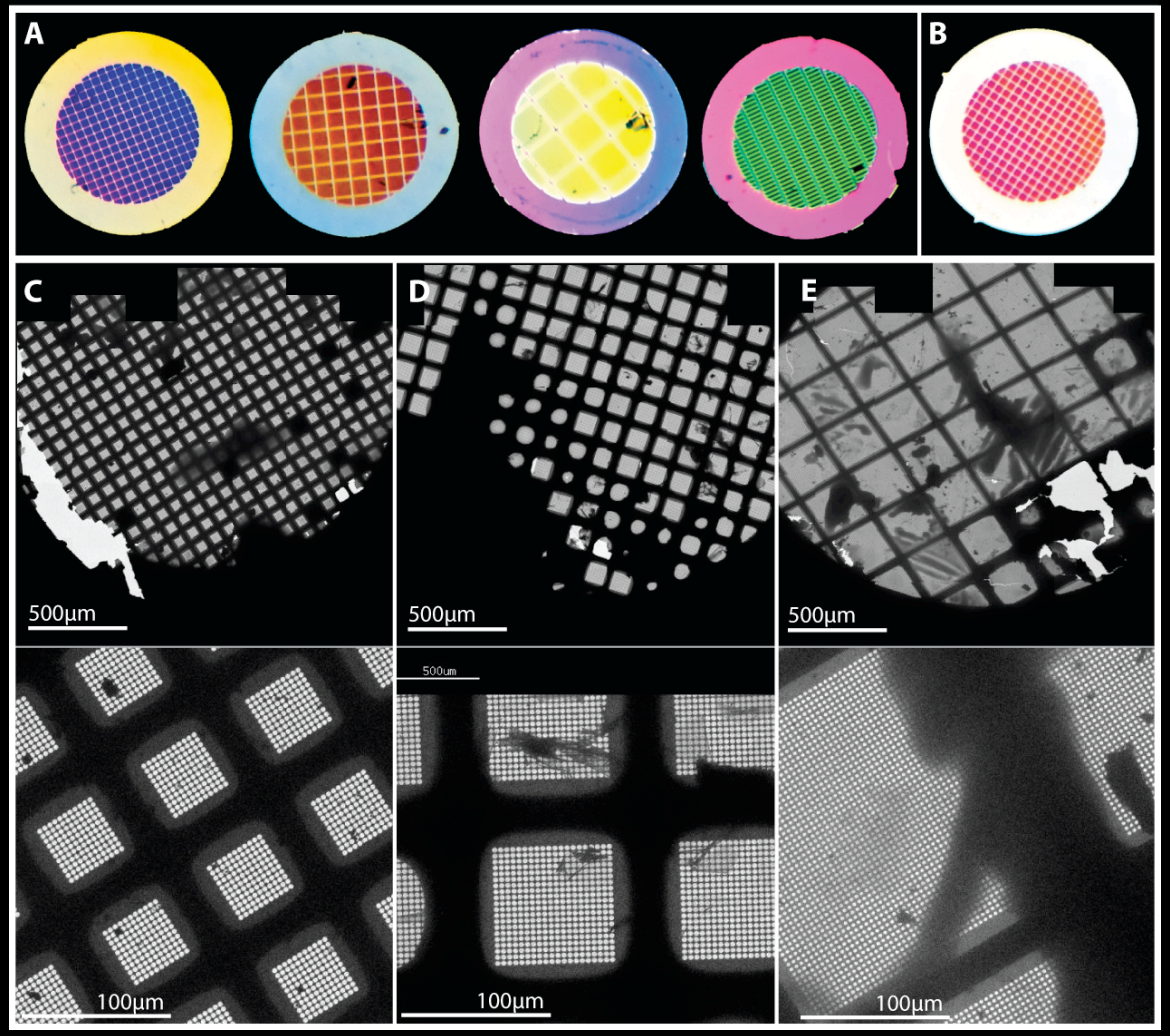
Gatan Solarus Plasma Cleaner

FEI Vitrobot



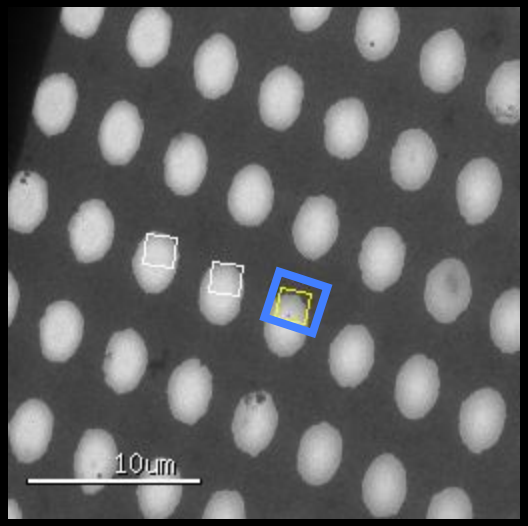
http://cimbio.scripps.edu/misc/documentation/protocol/freeze.php

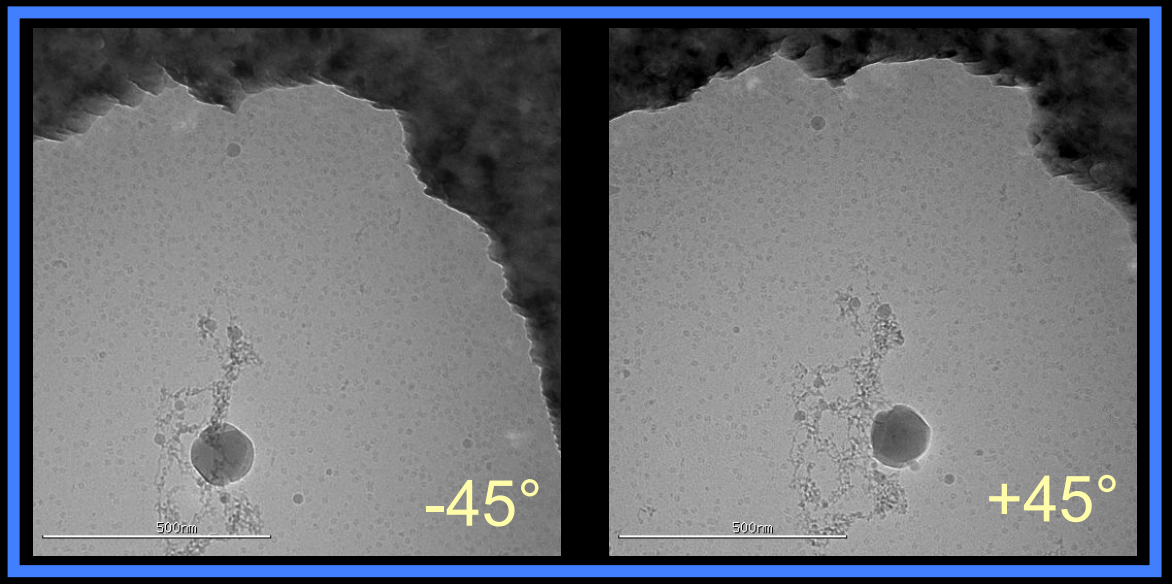
Specimen preparation
Cryomesh grids

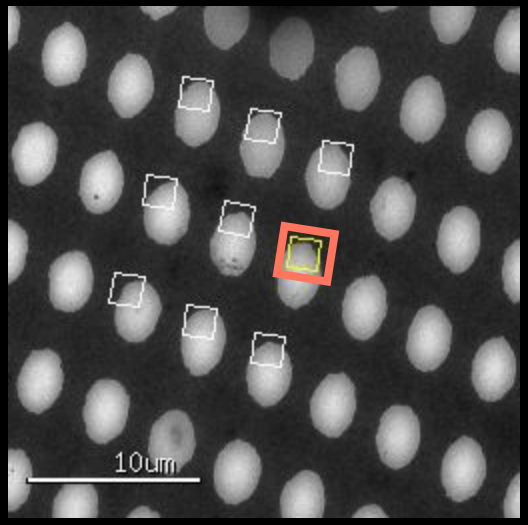


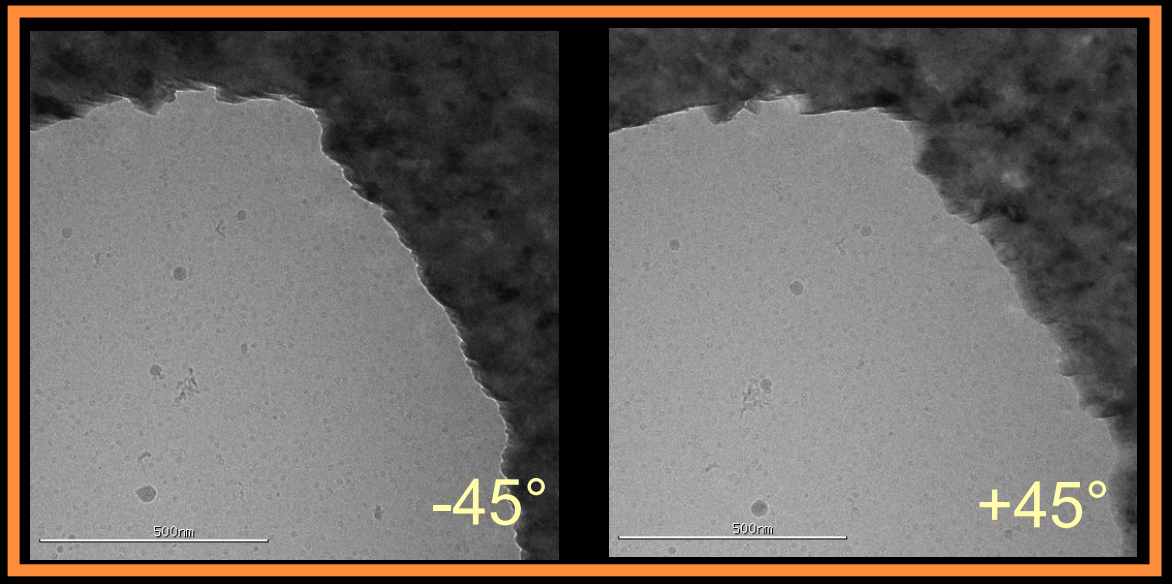
Cryomesh grids help reduce BIM/charging at high tilt

Leginon: Automated OTR tilt pair imaging on vitreous ice grids



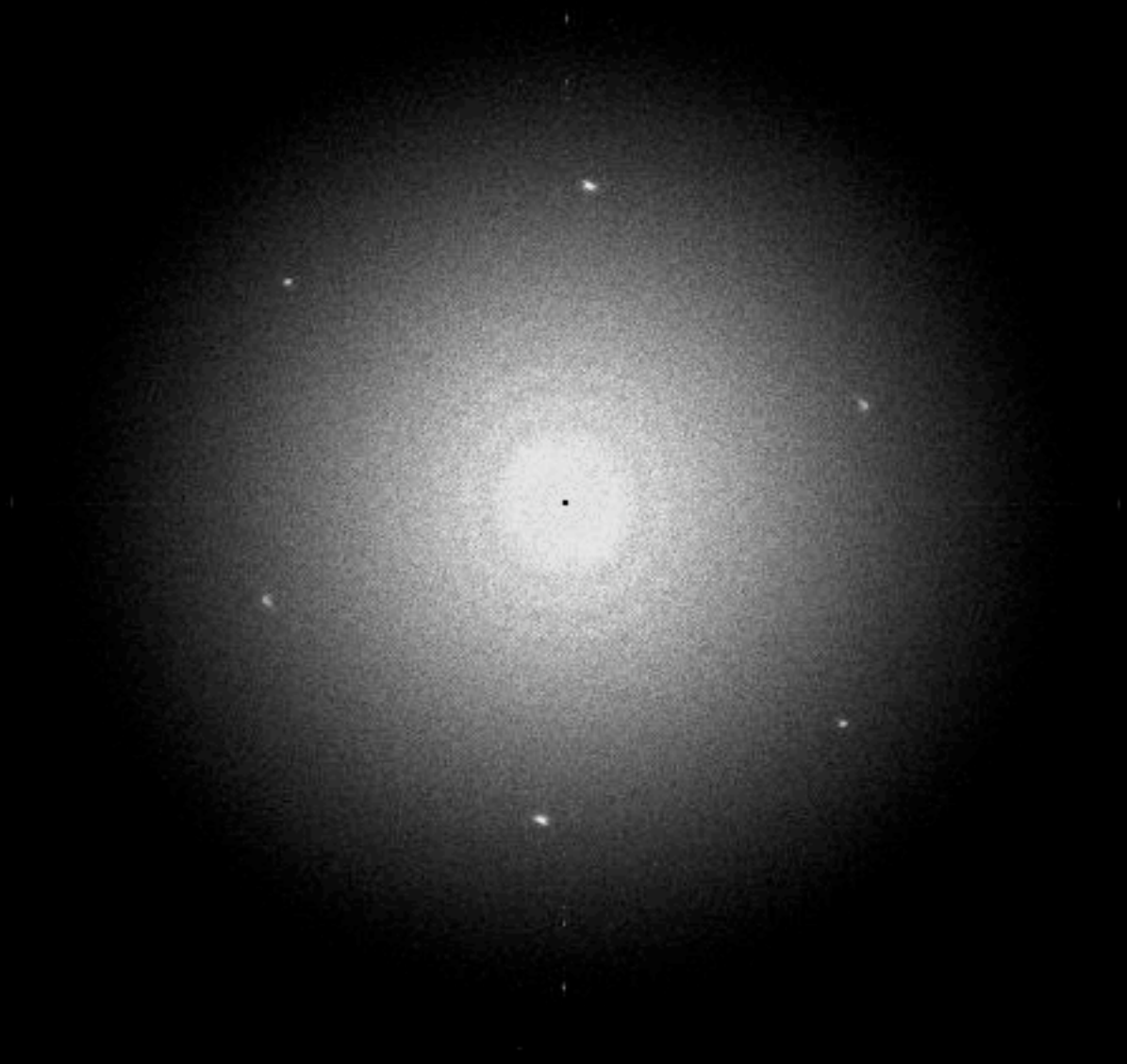






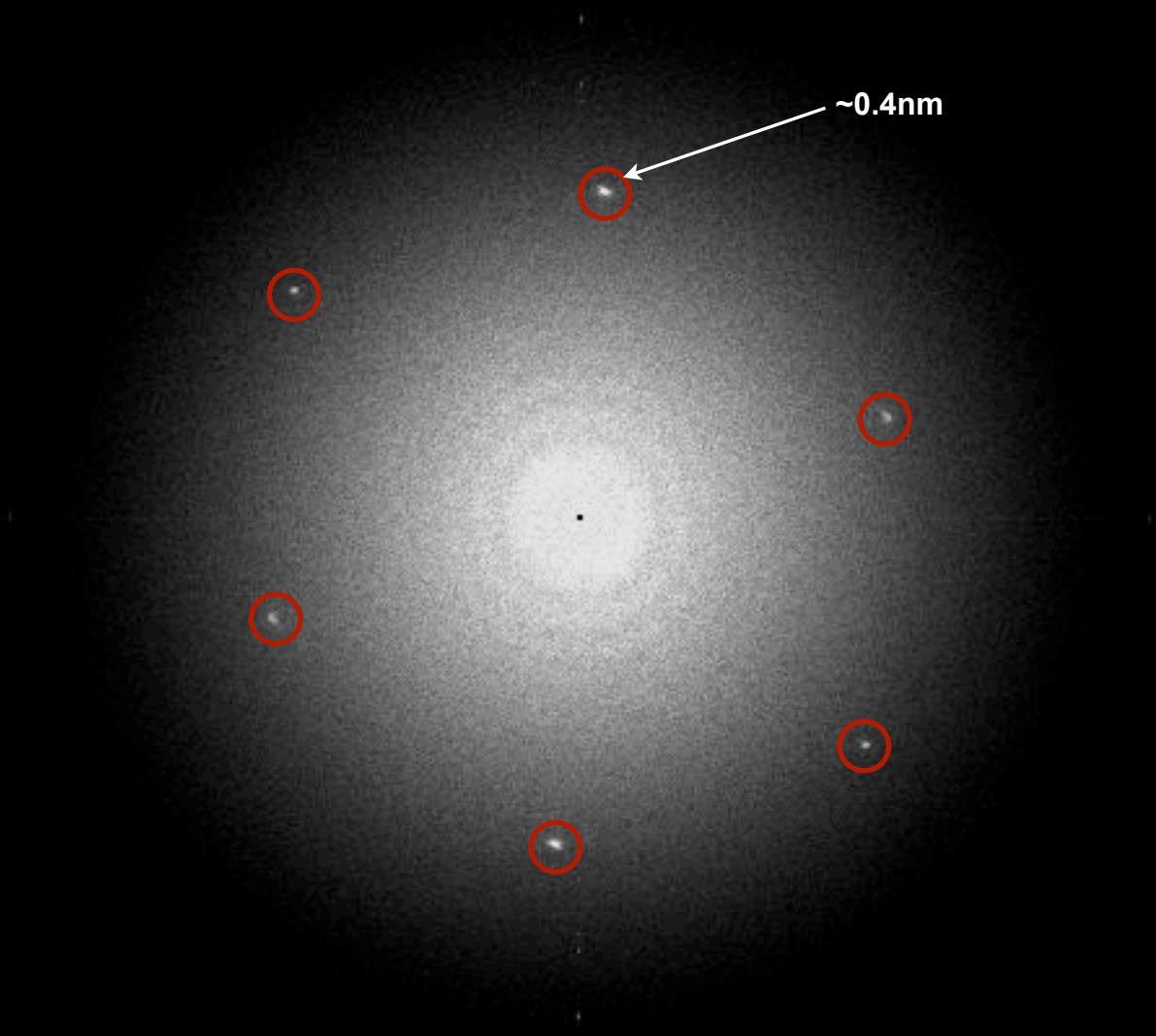
57 tilt pairs in 2 hours (1 pair every 2.2 minutes); >80% have “minimal” charging

Would cryomesh grids help in 2D crystallography?



Paraffin crystals on C over cryomesh (FFT of Image)

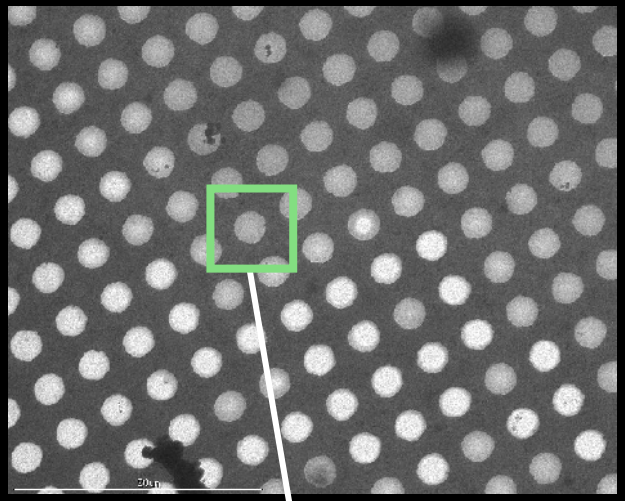
Would cryomesh grids help in 2D crystallography?

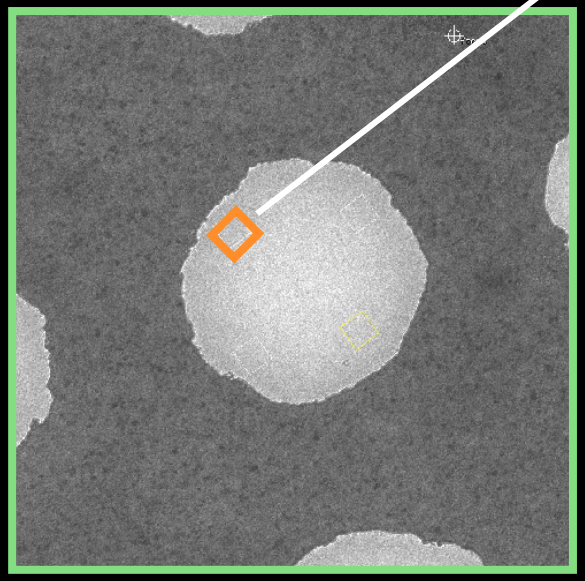


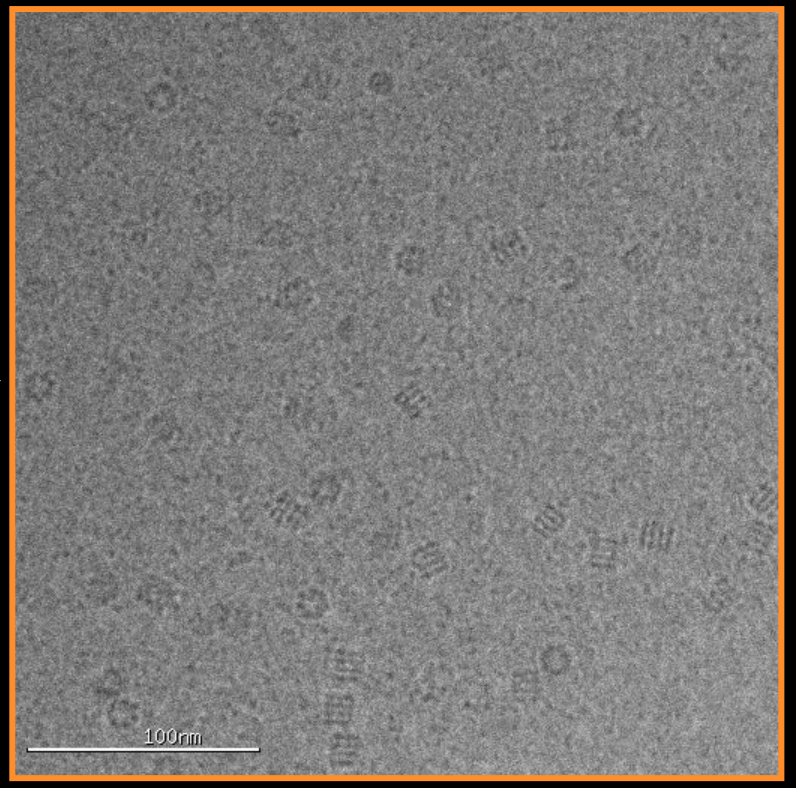
Paraffin crystals on C over cryomesh (FFT of Image)

What's next?

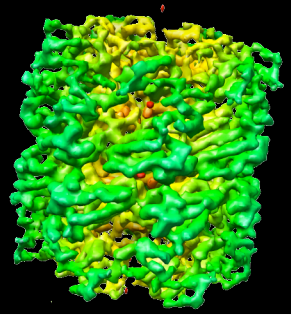
Start using cryomesh at NRAMM for "routine" data collection







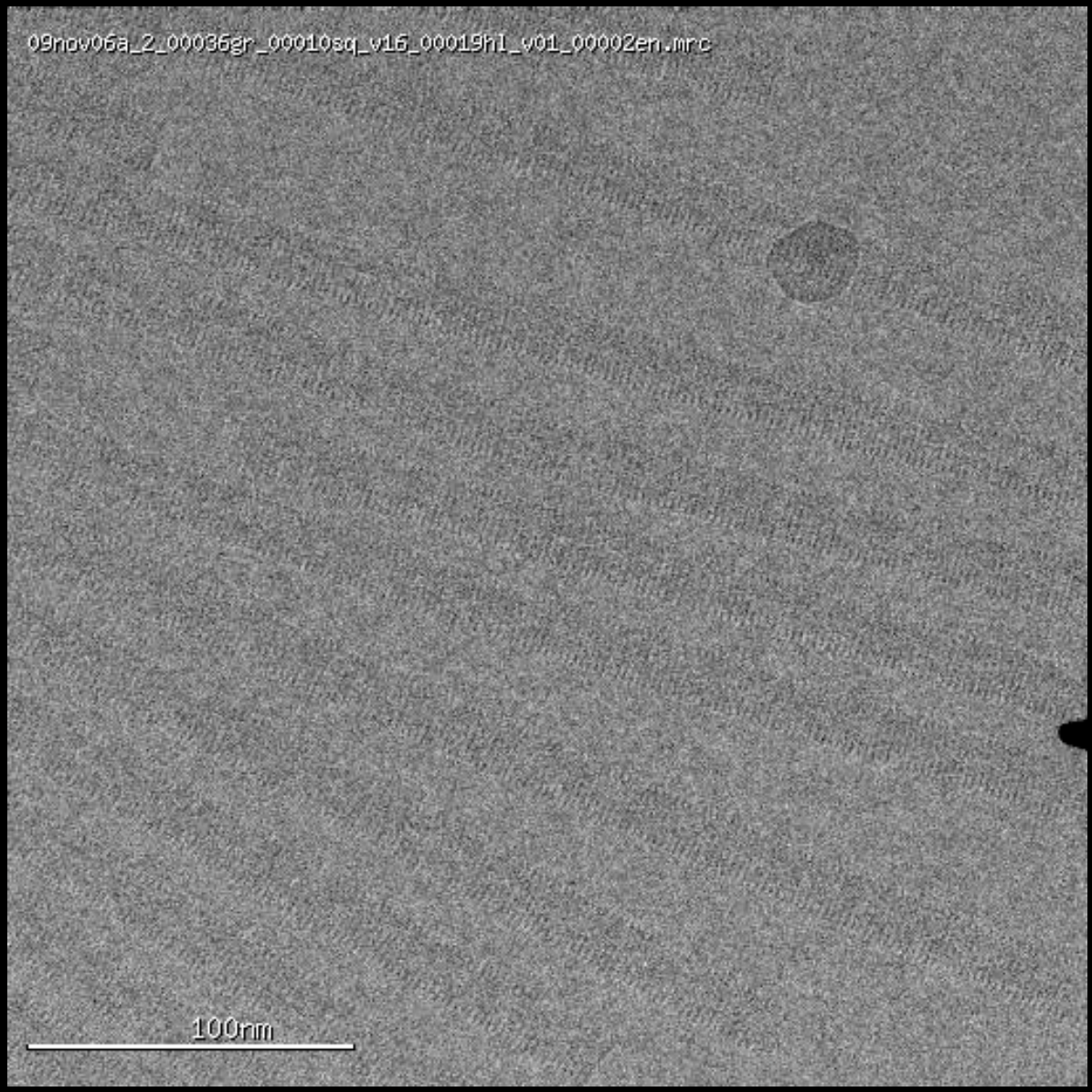
~1,000 images (mag 100,000x) acquired using Leginon; 50,000 particles selected



7.5Å resolution
38,714 particles used

What's next?

Continue testing...

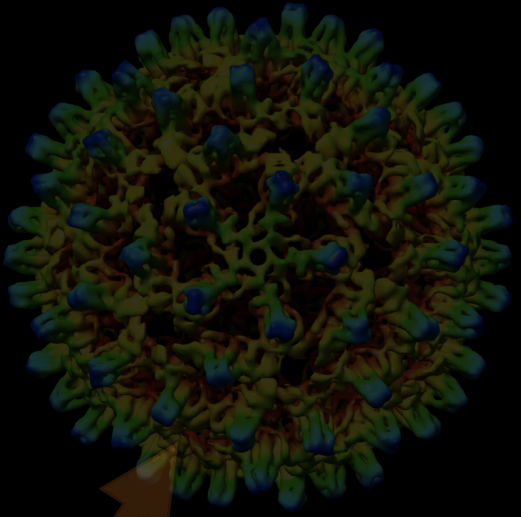


CryoEM Pipeline at NRAMM

Sample

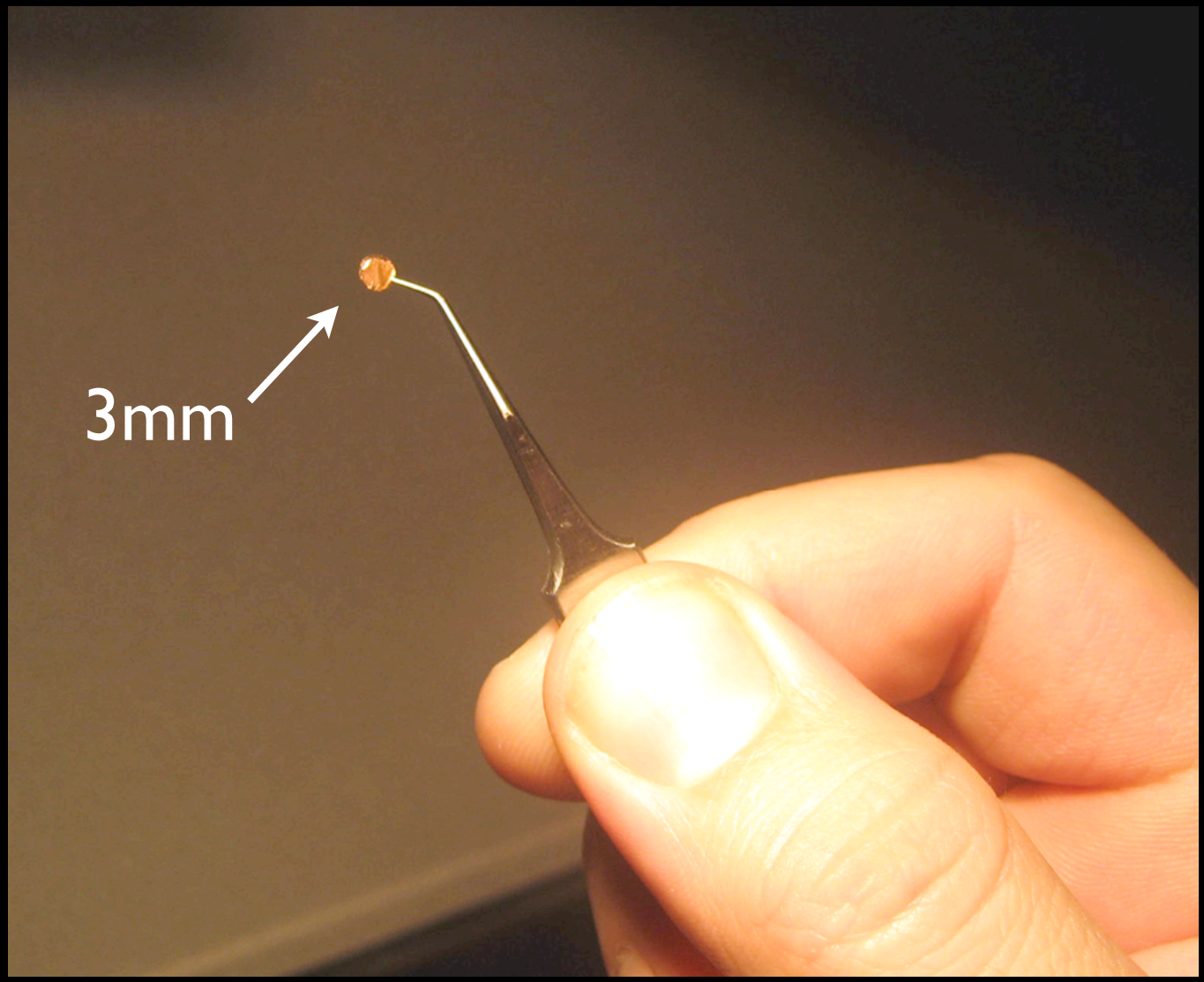
Automated
Data Collection
(Leginon)

3D EM Density

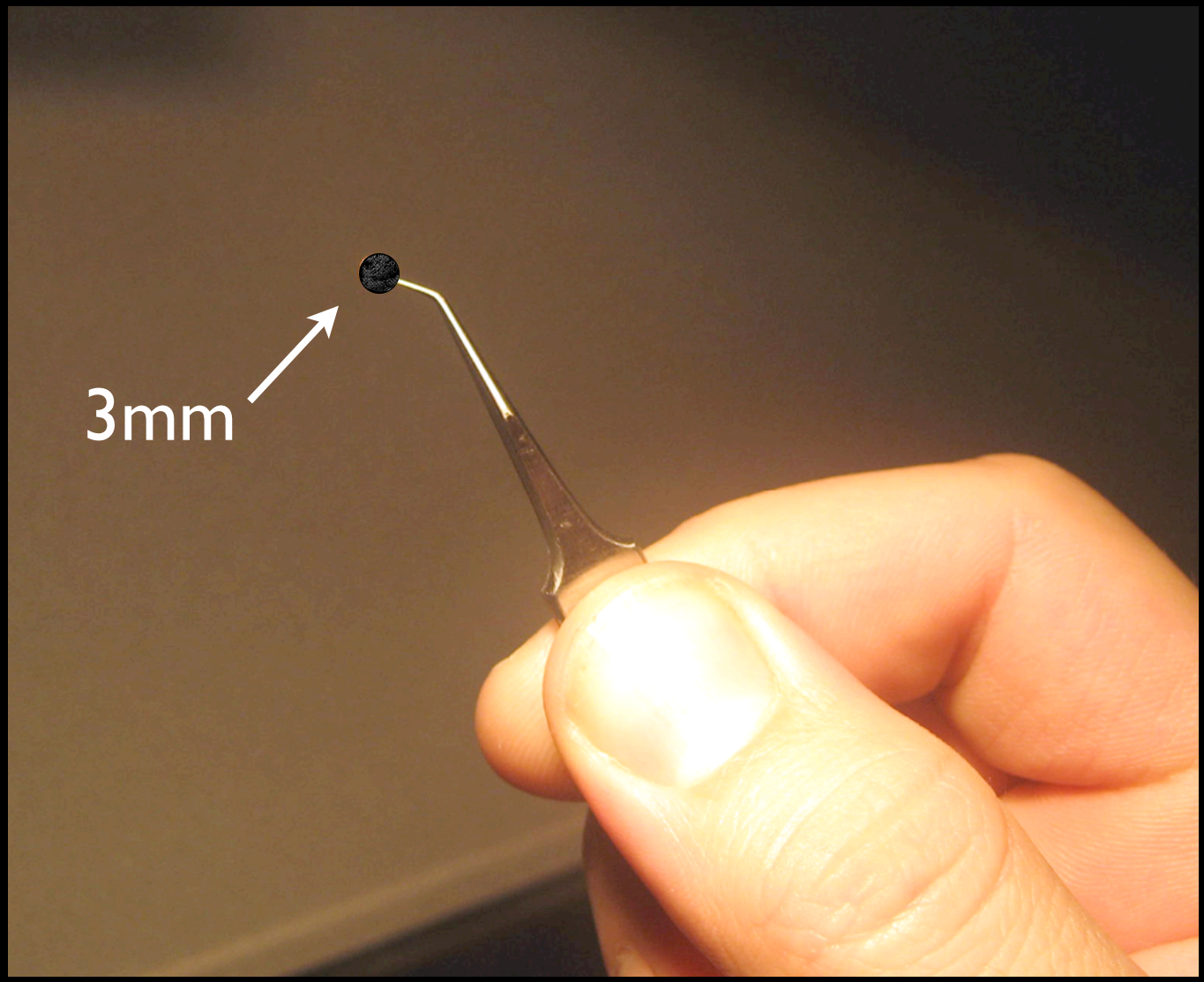


Specimen Vitrification

Streamlined
Processing
(Appion)



Courtesy Gabriel Lander (TSRI)



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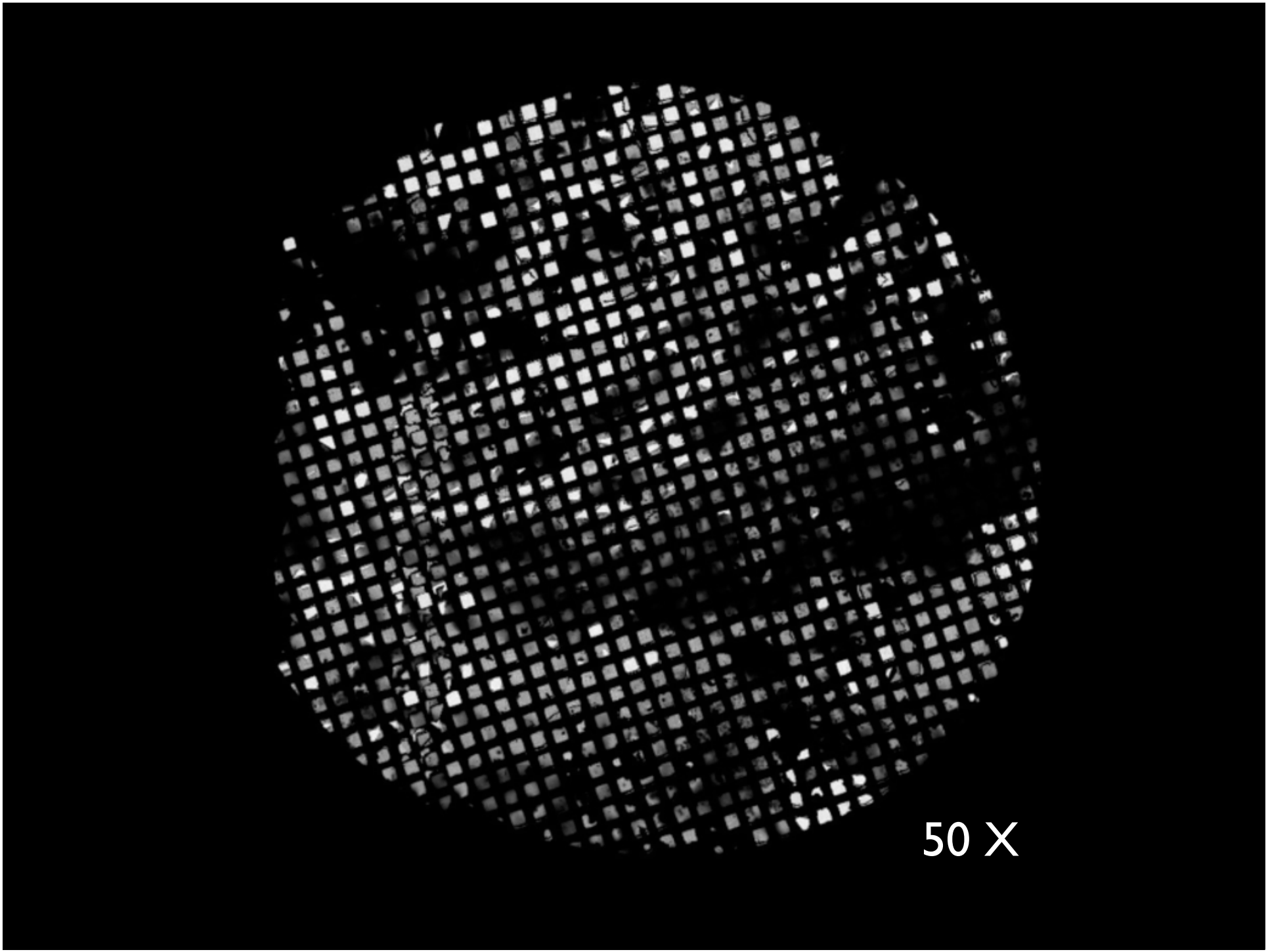
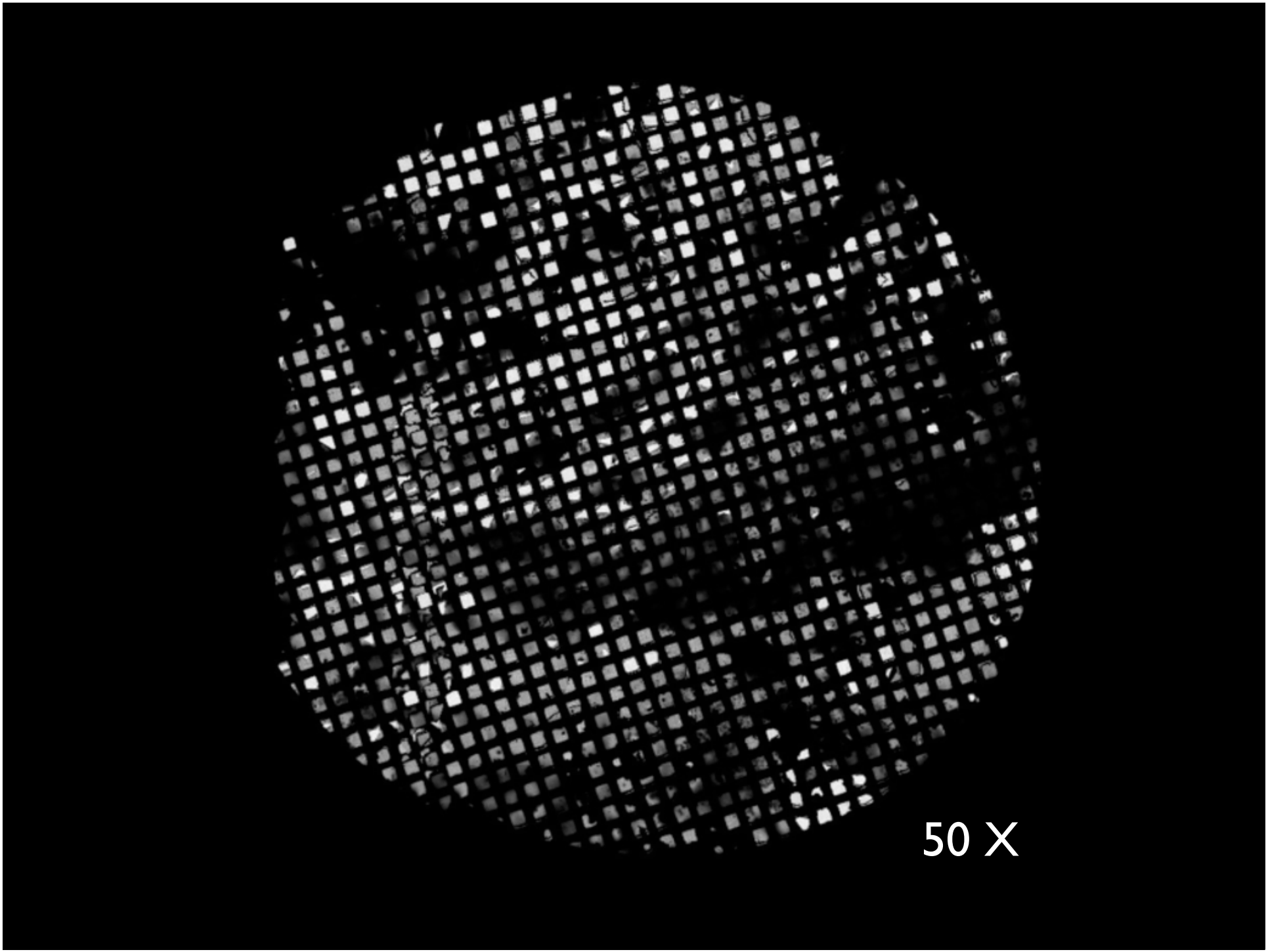

50 X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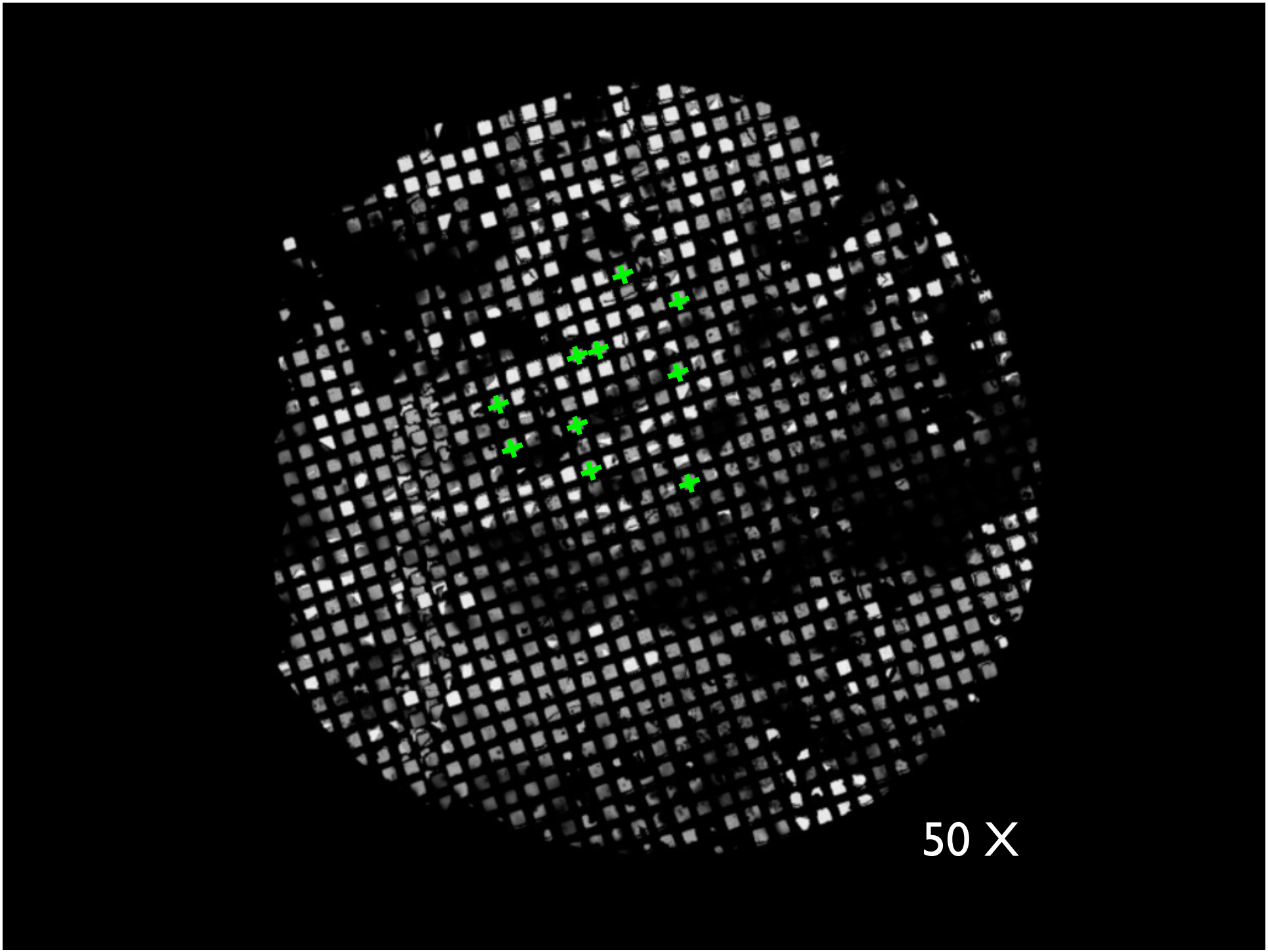
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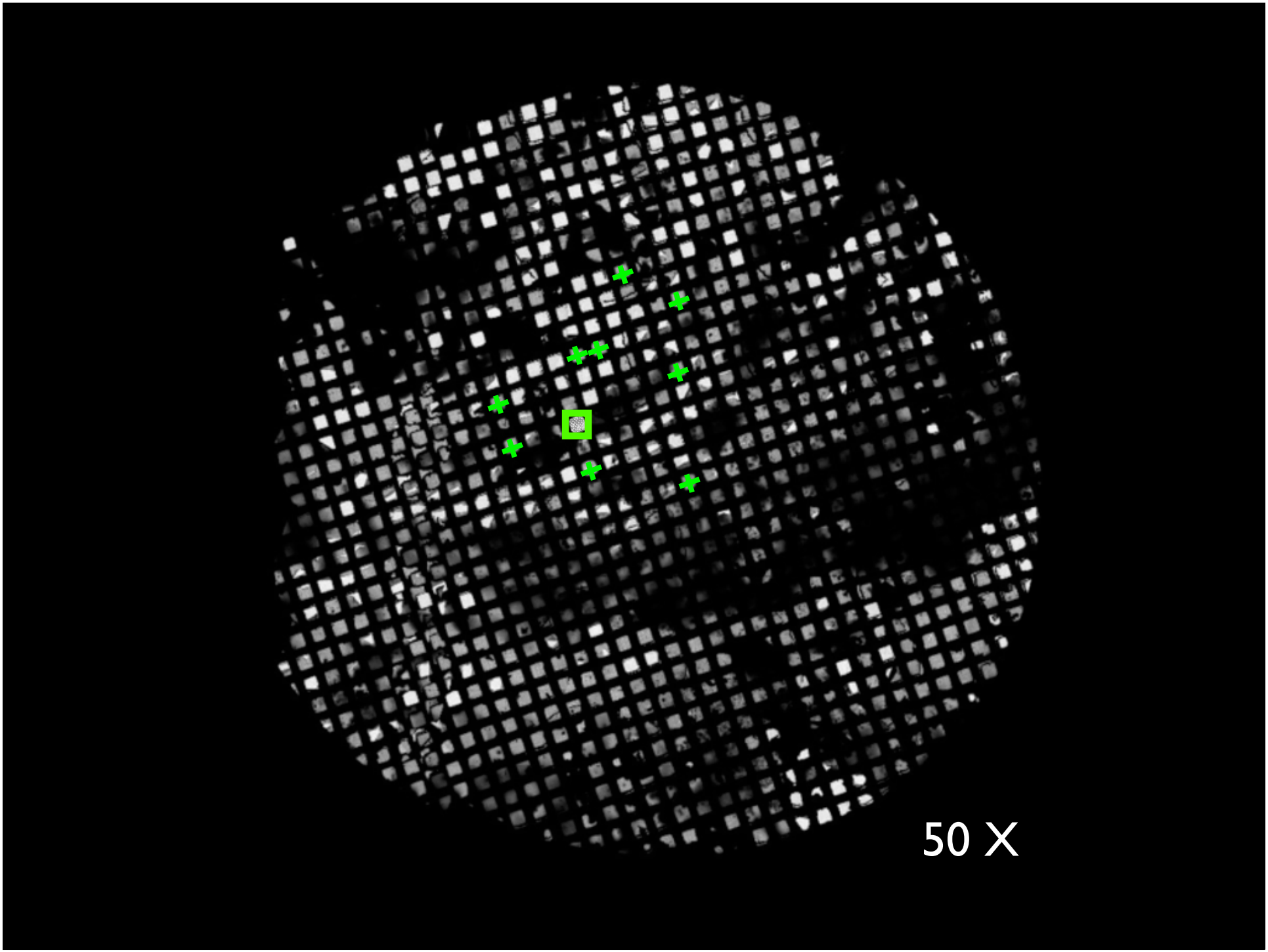
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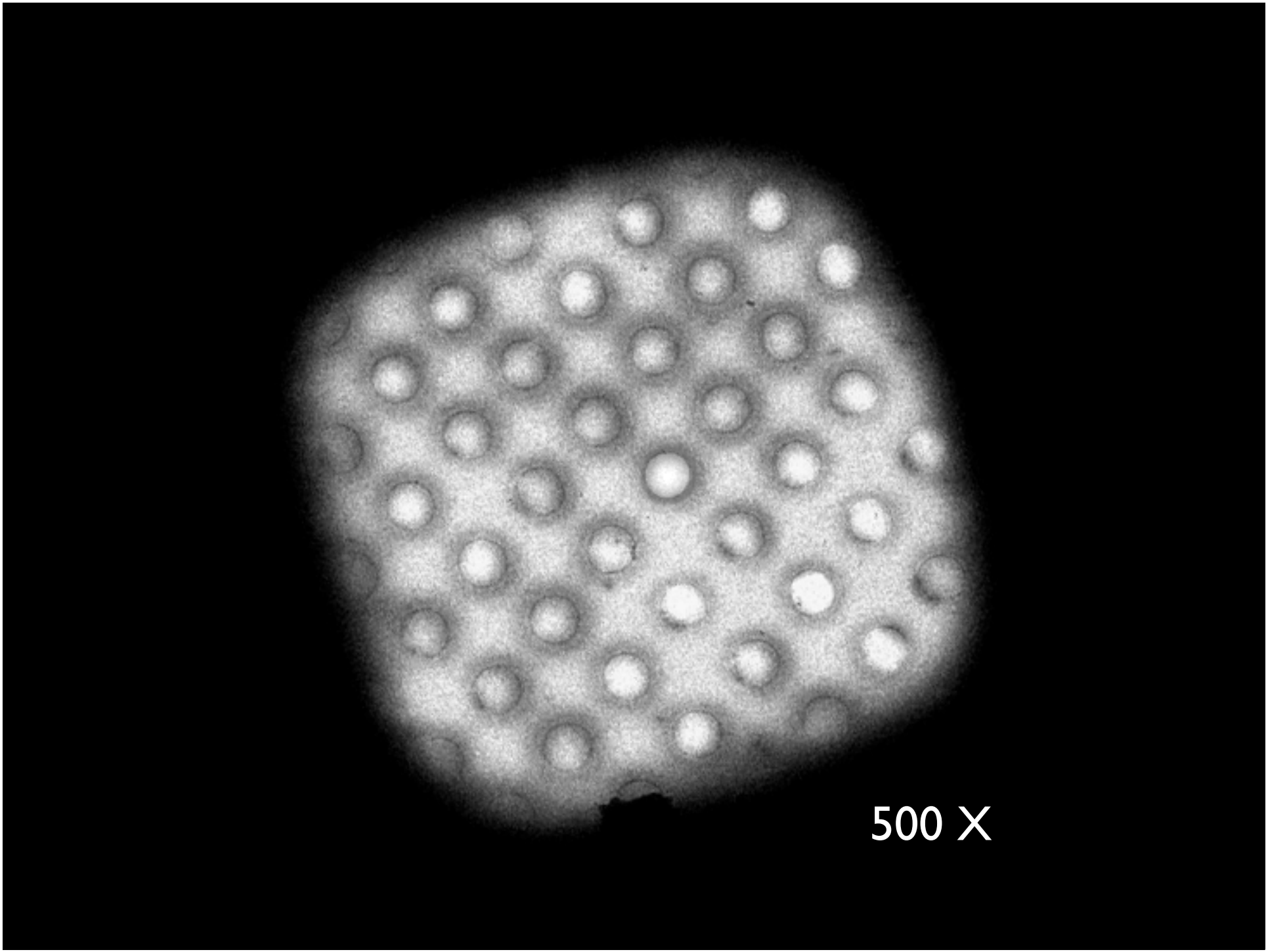
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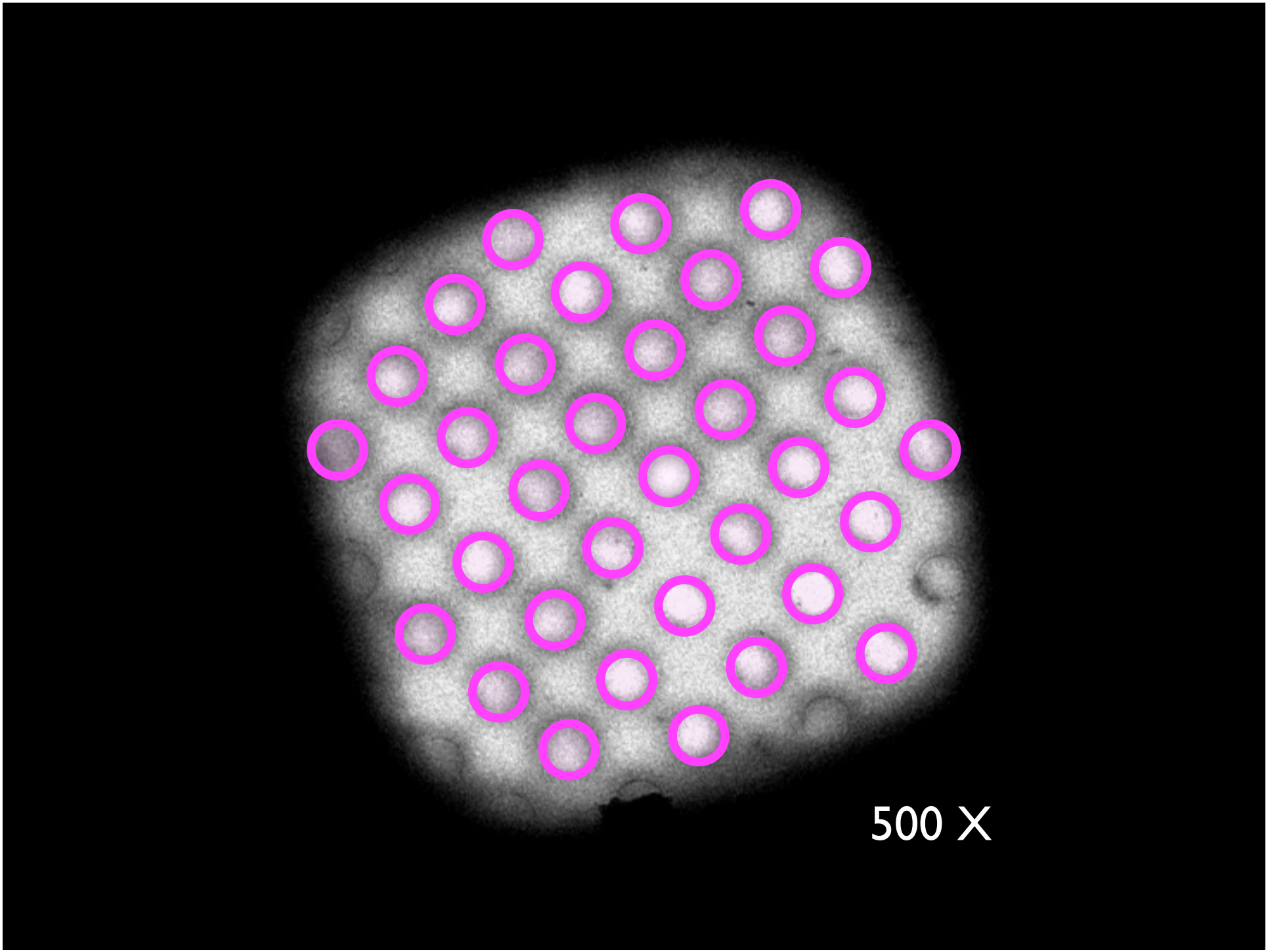
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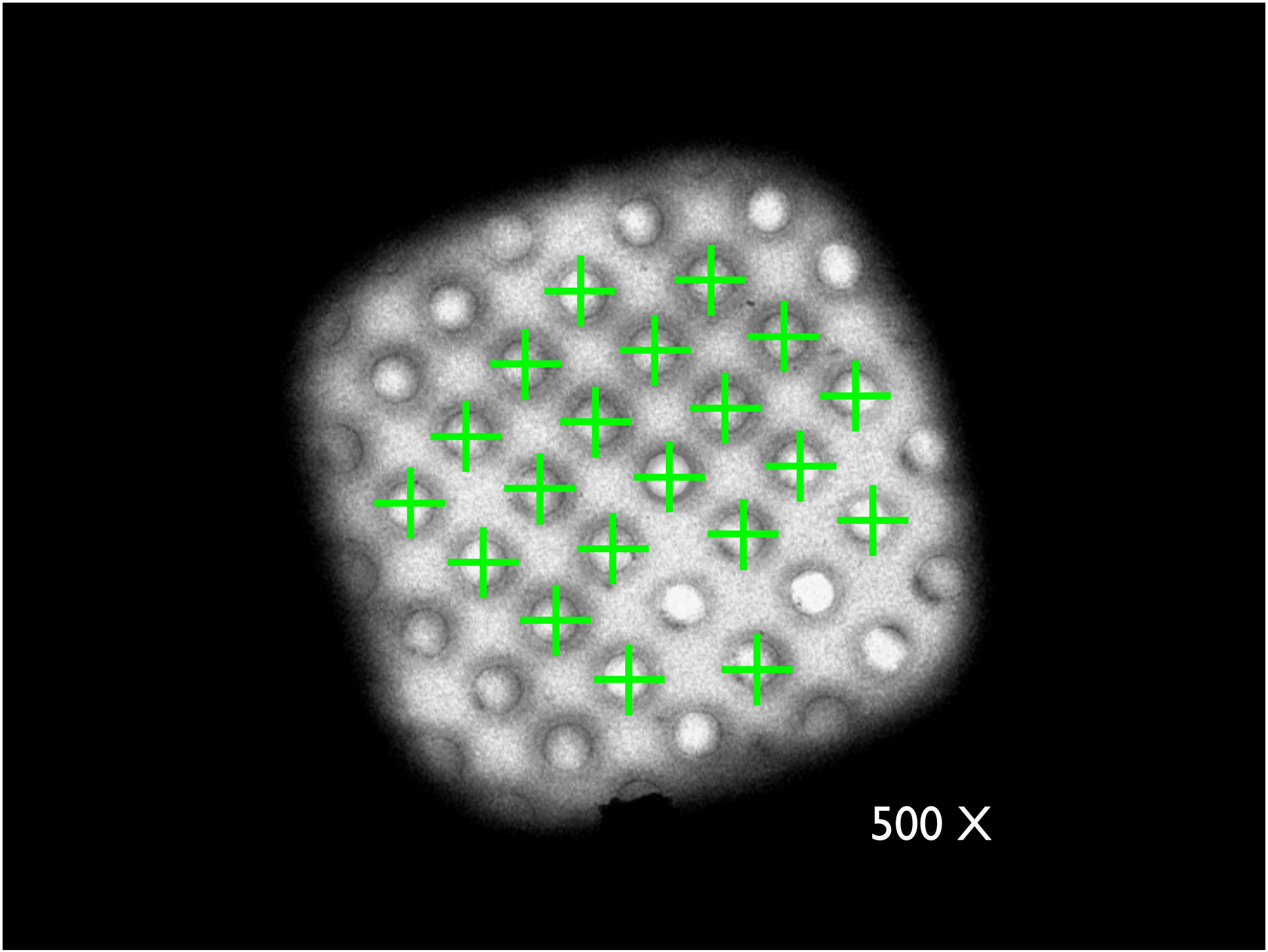
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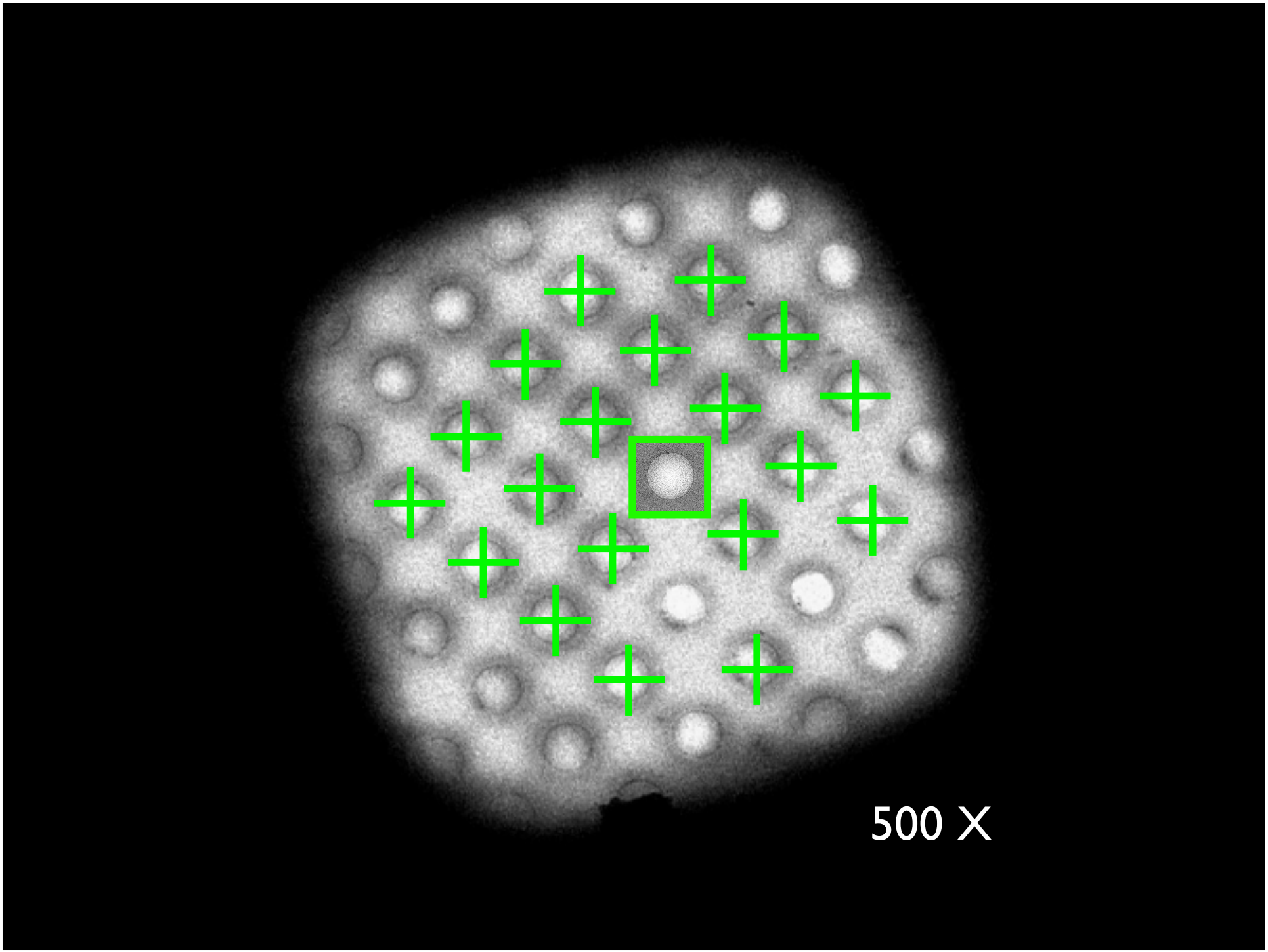
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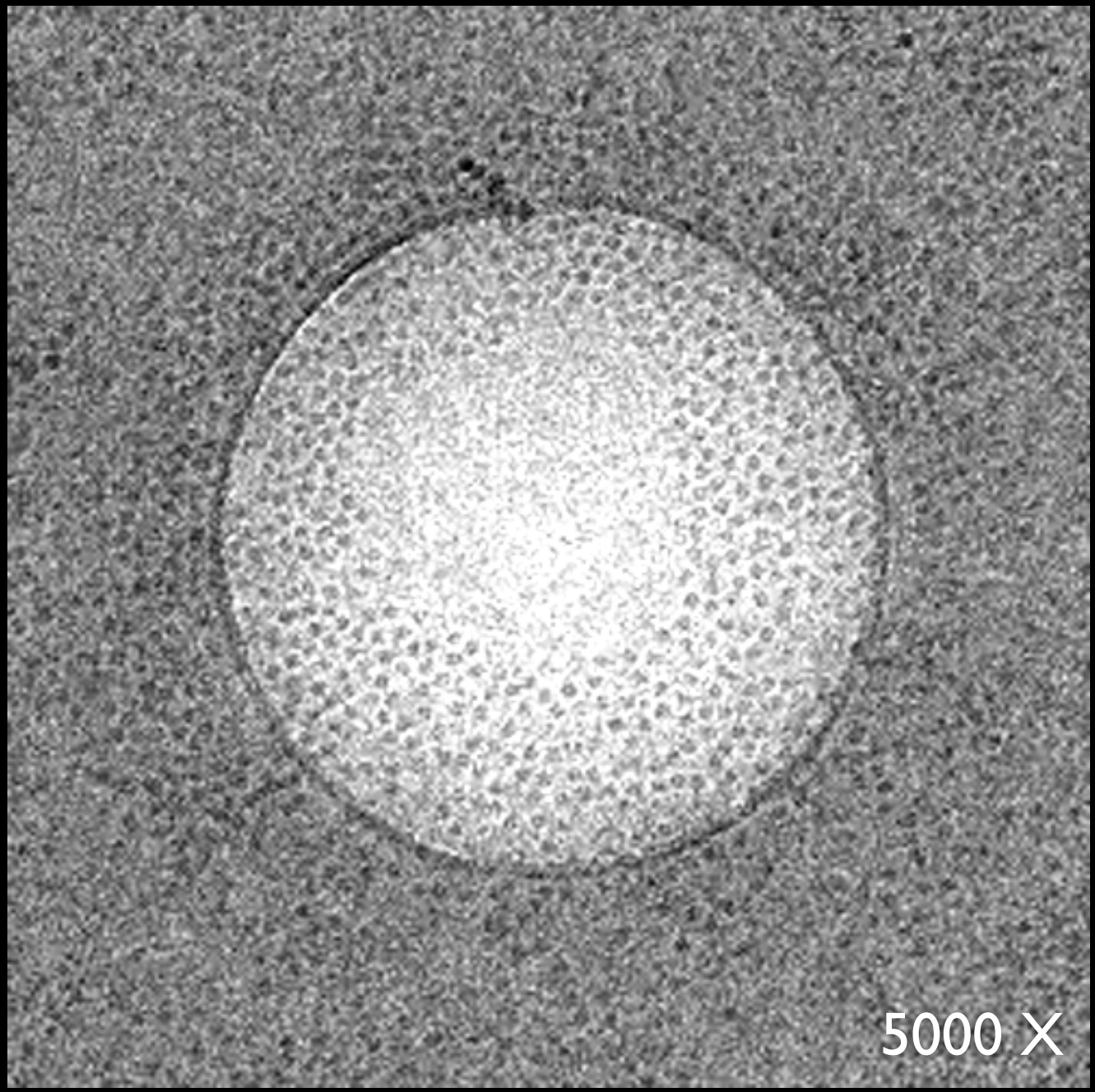
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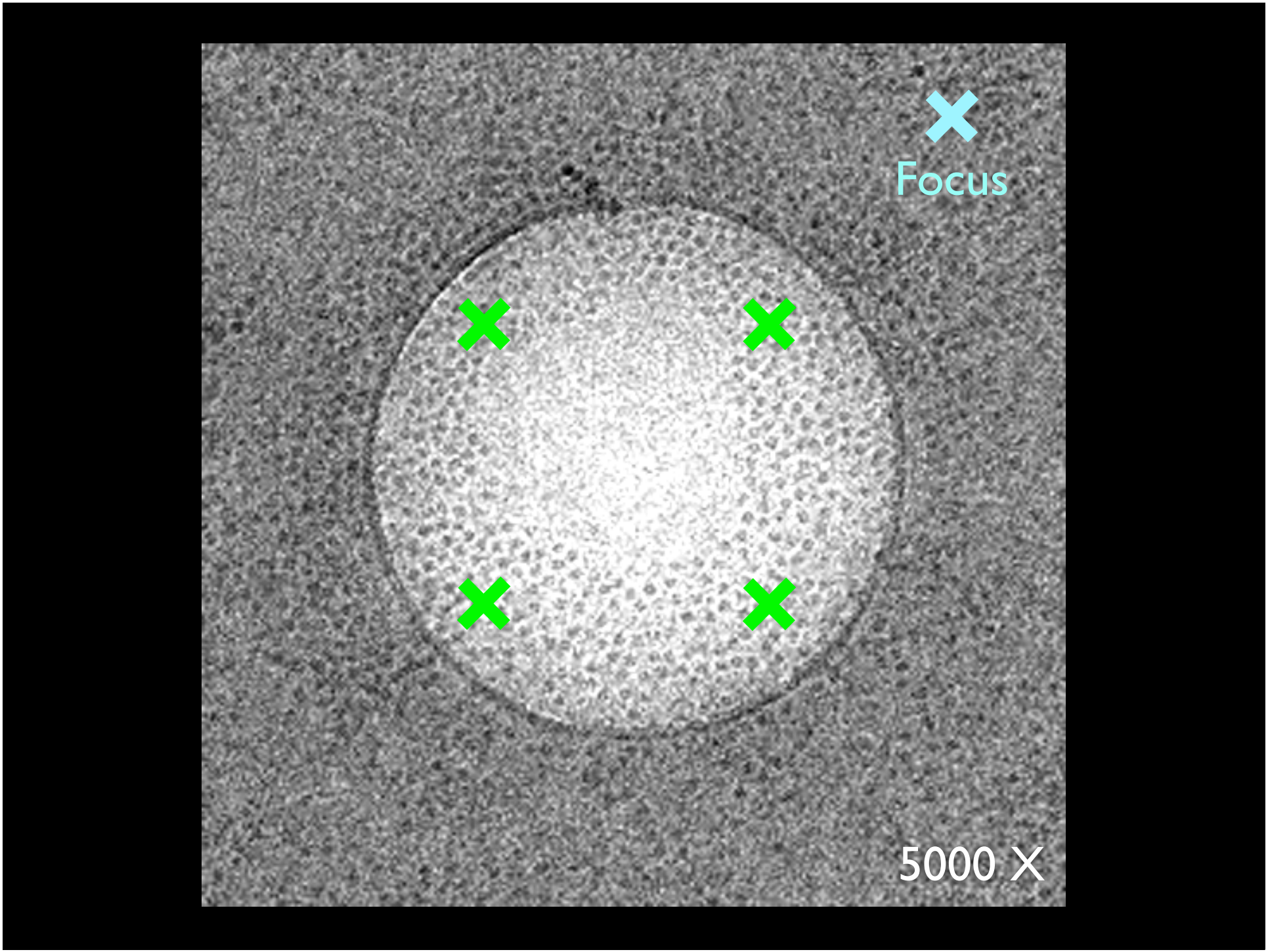
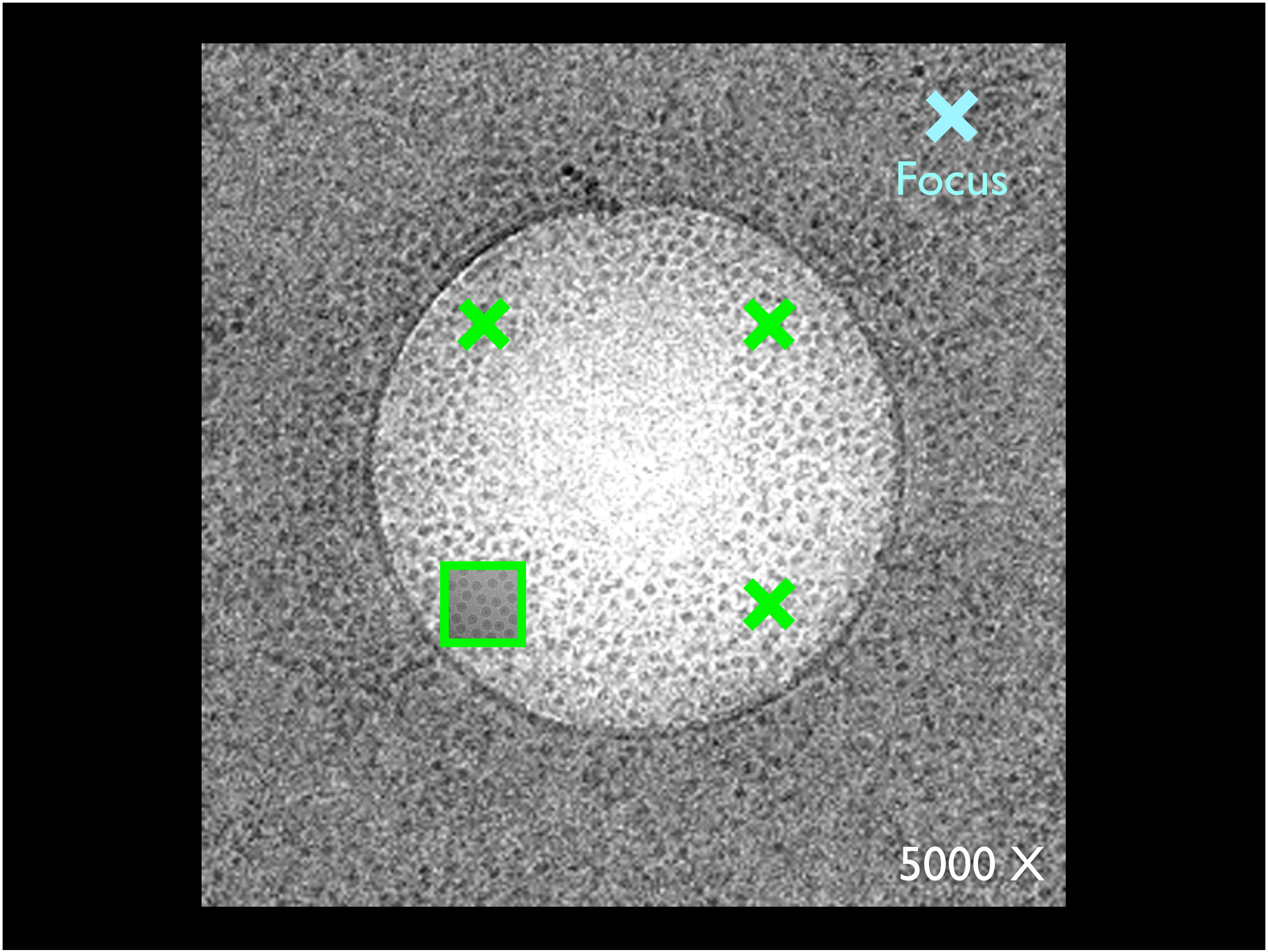

Focus
5000 X


Focus
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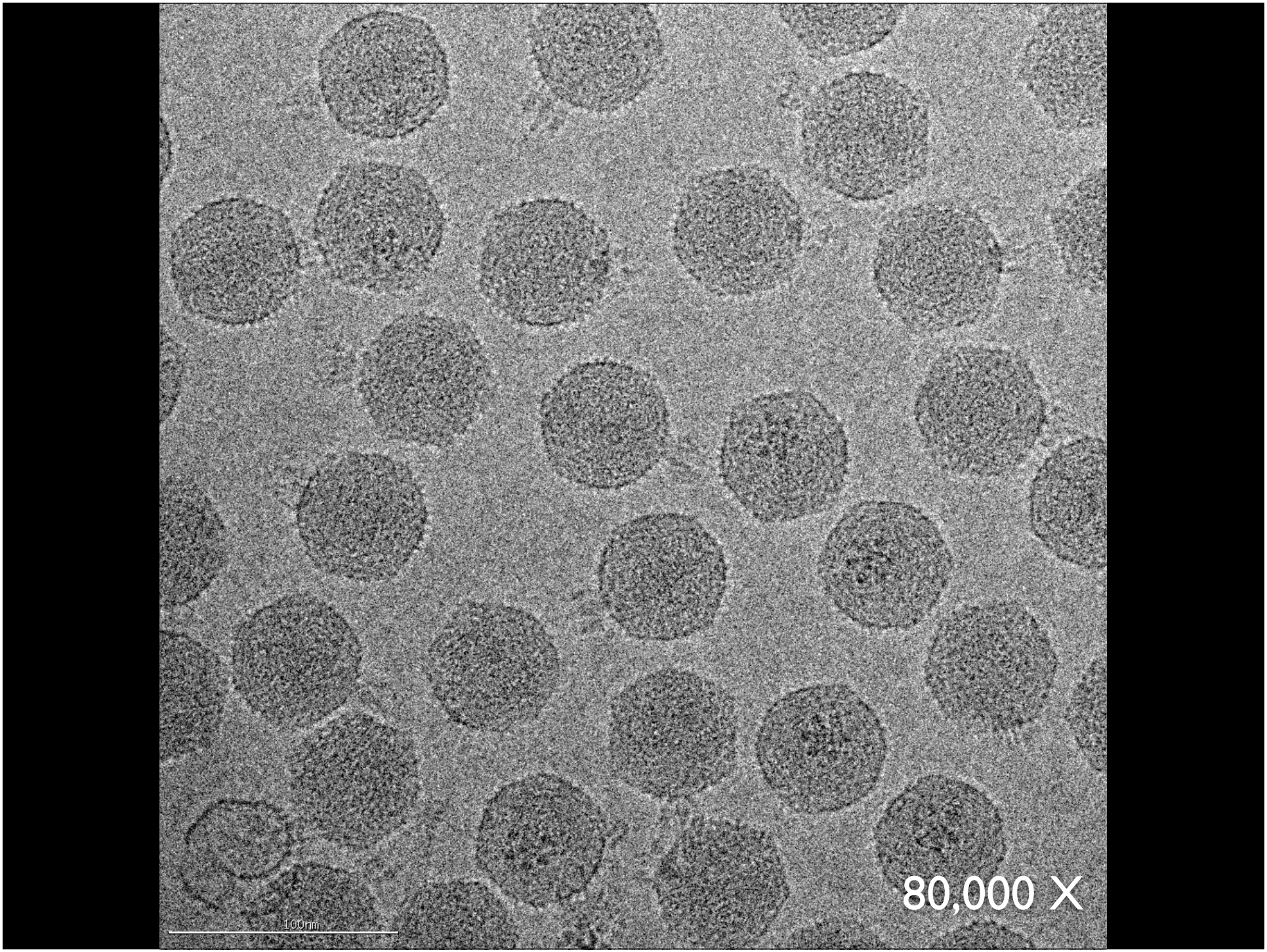
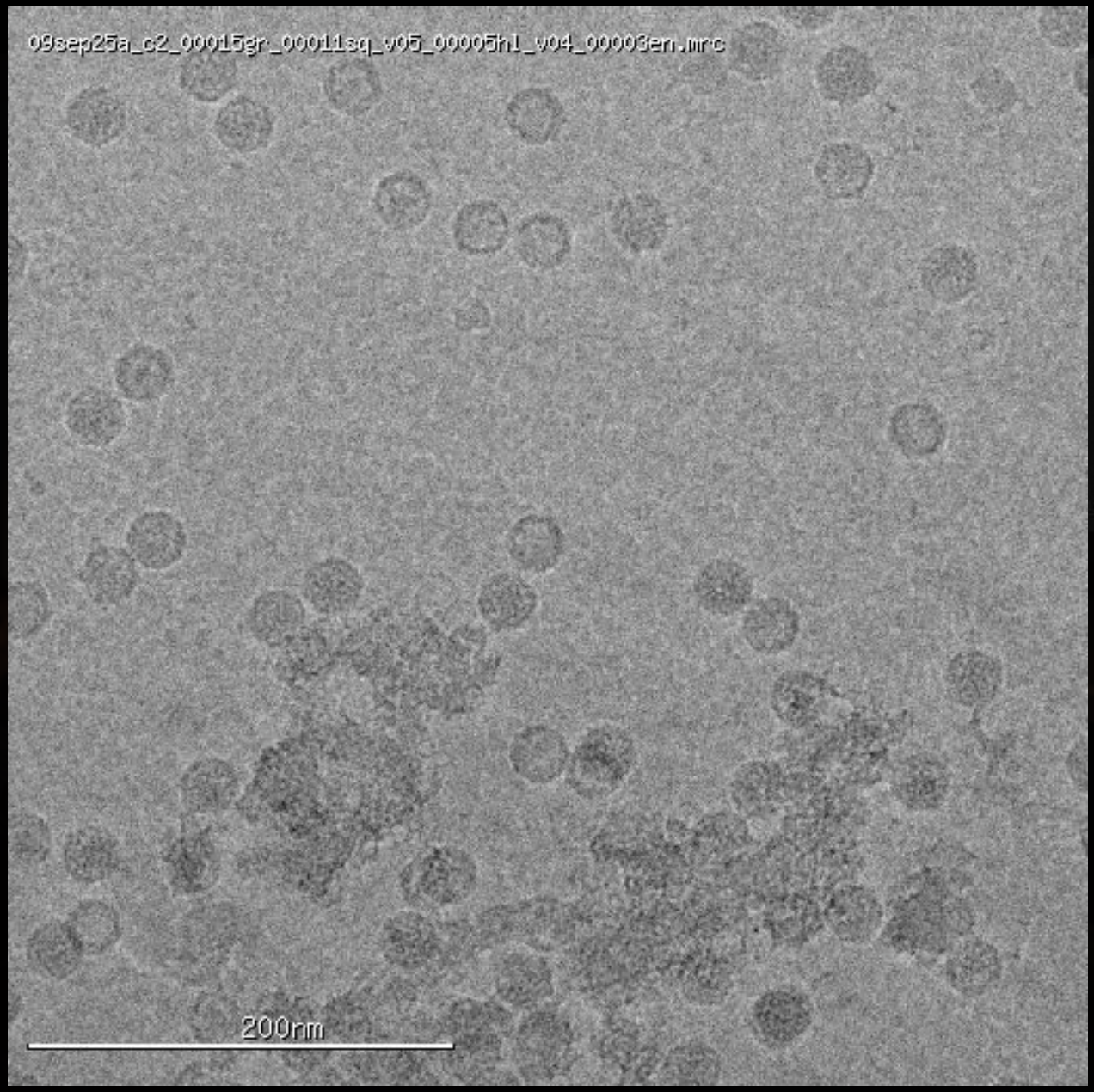

80,000 X
100nm



Recent experiment: Characterize intermediates in cell entry pathway of poliovirus (Hogle group): 15,000 images; 430,000 particles

What would improve throughput:

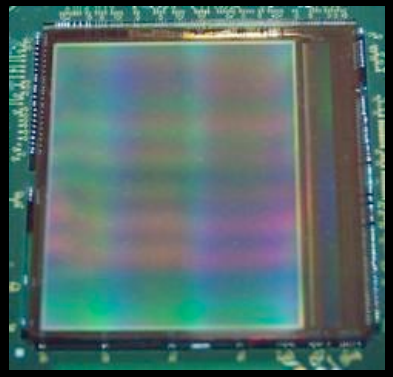
What would improve throughput:

- A Titan Krios

What would improve throughput:

- A Titan Krios

- A CMOS camera



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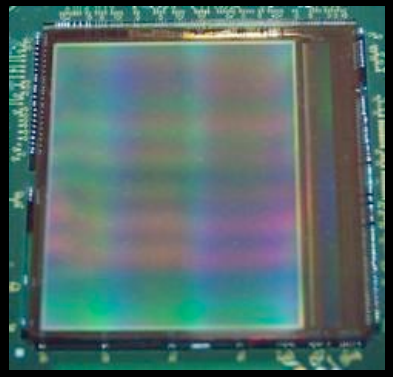
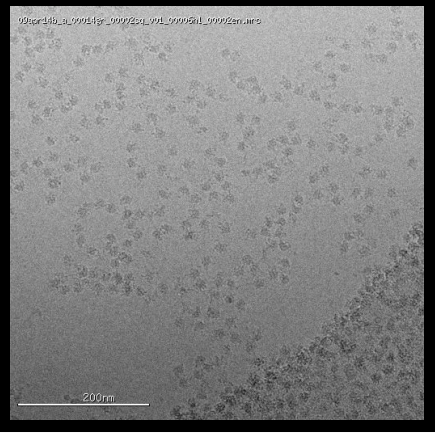
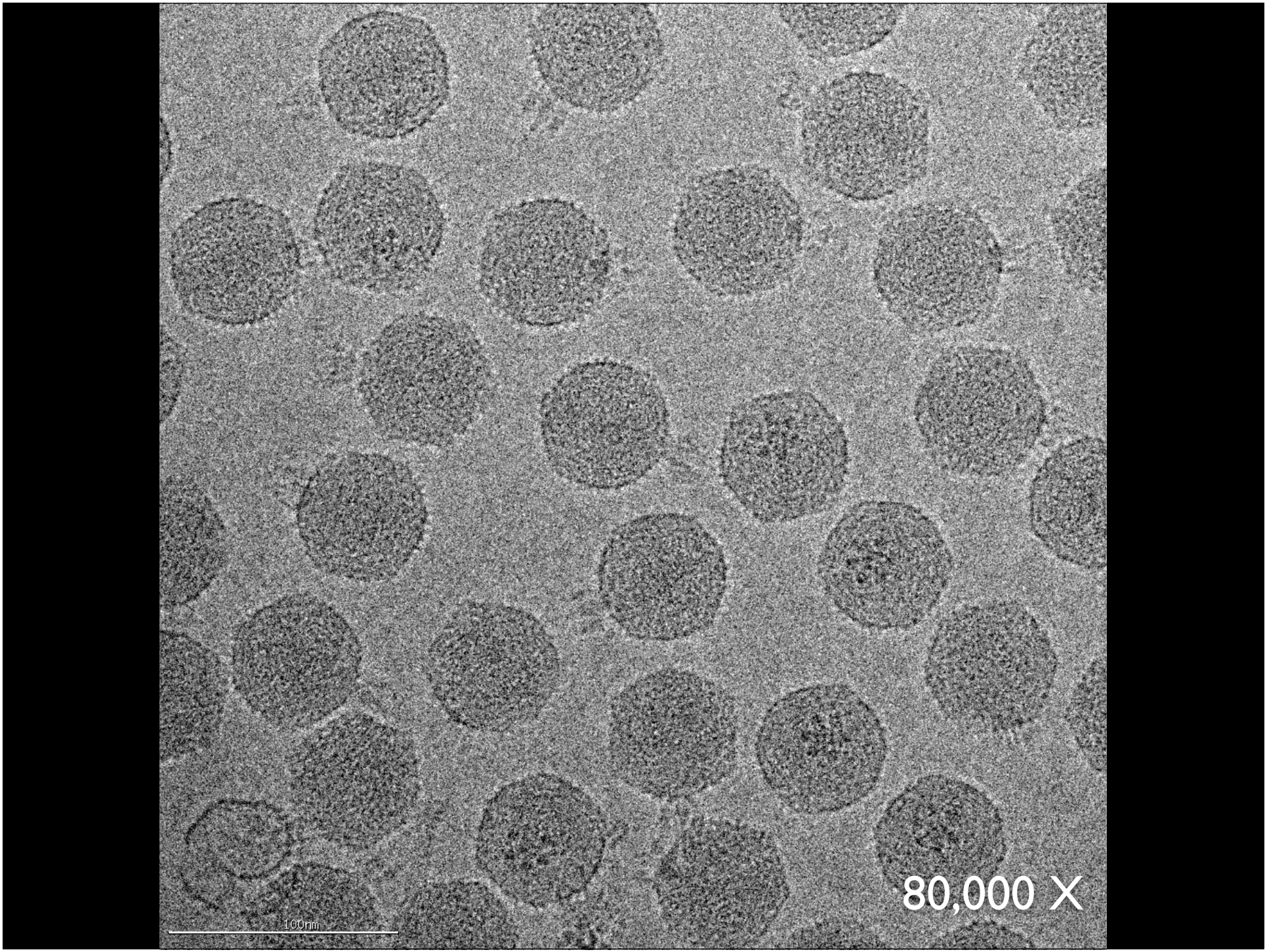
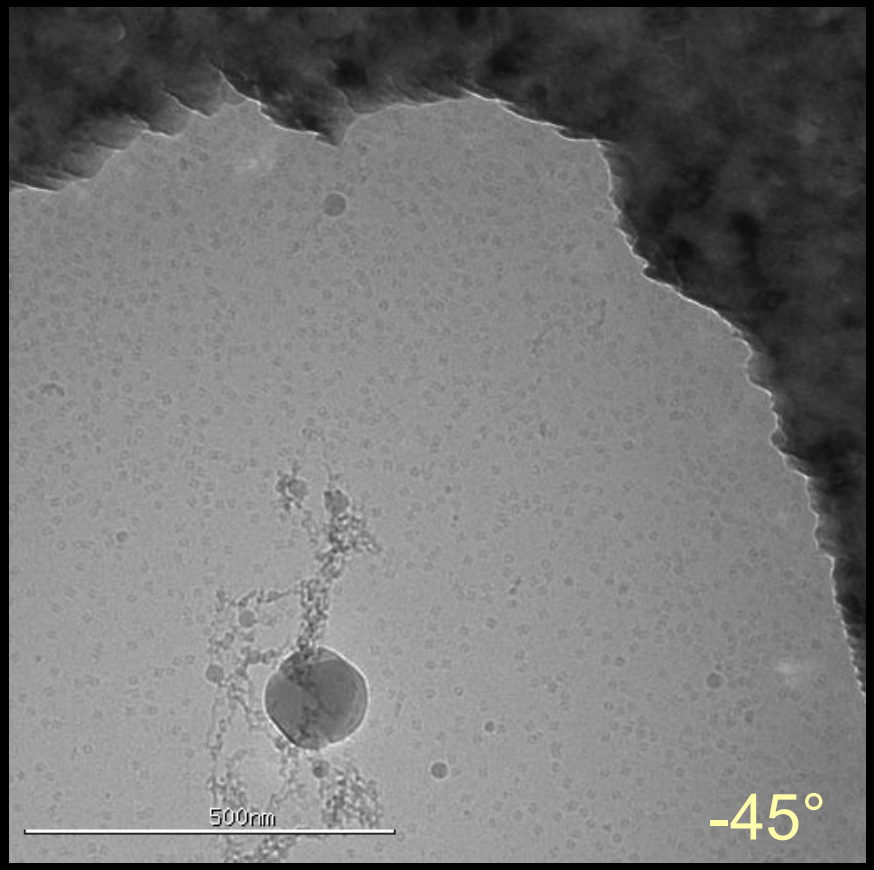


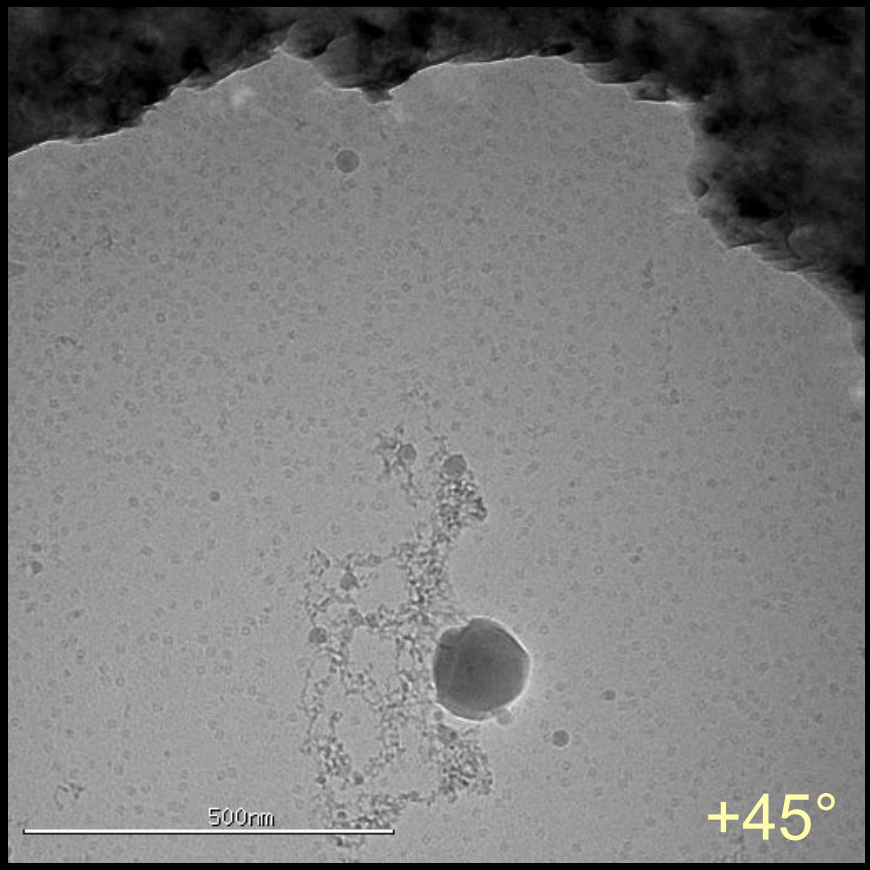
- A ribosome




80,000 X
100nm

Automated Multi-Scale Image Acquisition using Leginon ORT (or RCT or SAT) acquisition





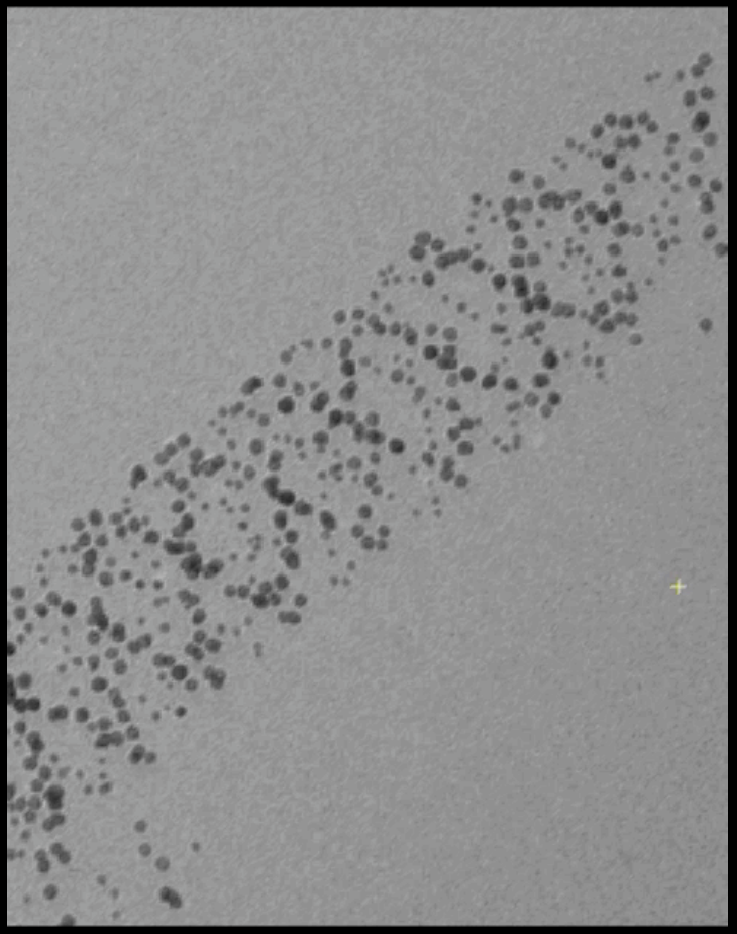
Automated Multi-Scale Image Acquisition using Leginon Tomography acquisition

Courtesy of Jason Lanman (TSRI)

Courtesy of Hao Yan (U. Arizona)
and Anchi Cheng (TSRI)

Automated Multi-Scale Image Acquisition using Leginon Tomography acquisition

Courtesy of Jason Lanman (TSRI)



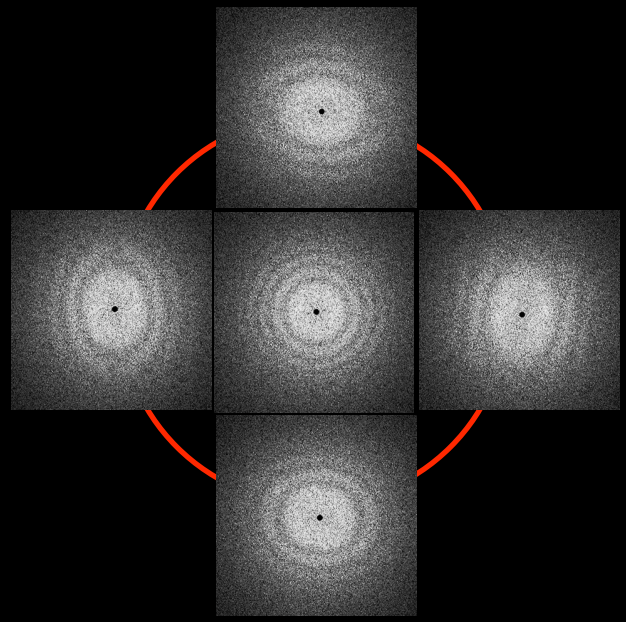
Courtesy of Hao Yan (U. Arizona)
and Anchi Cheng (TSRI)

Automated Multi-Scale Image Acquisition using Leginon Tomography acquisition

Courtesy of Jason Lanman (TSRI)

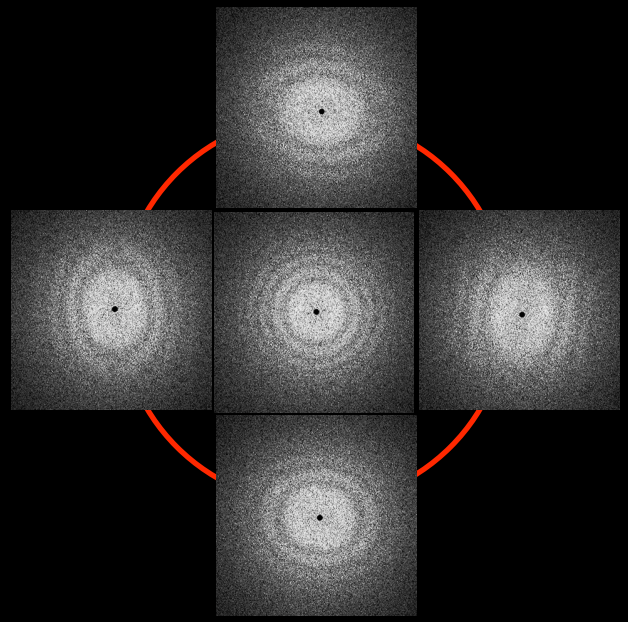
Courtesy of Hao Yan (U. Arizona)
and Anchi Cheng (TSRI)

What's next:



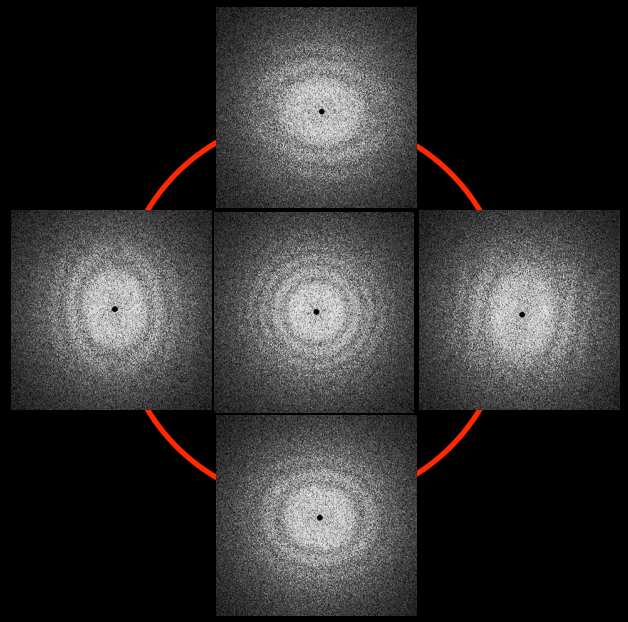
What's next:

- Automated alignments



What's next:

- Automated alignments



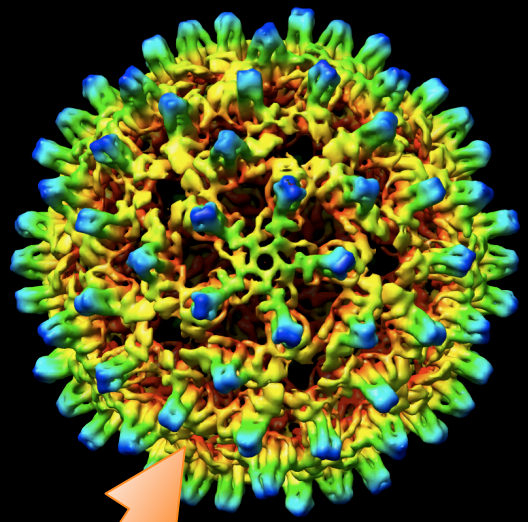
- Add more intelligence into the system

CryoEM Pipeline at NRAMM

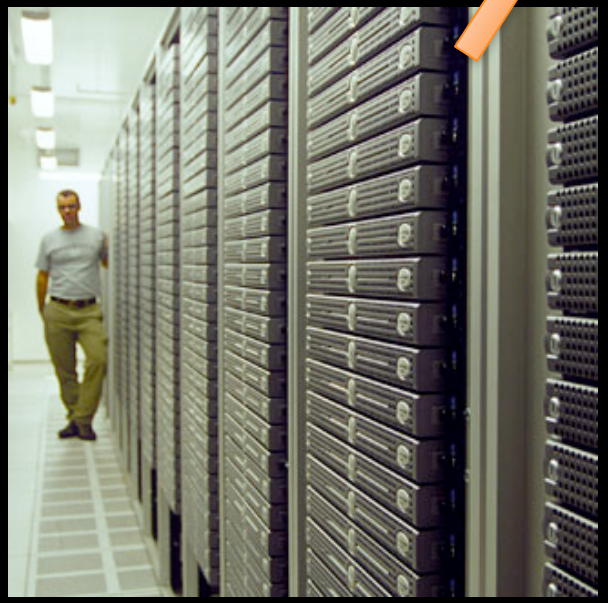
Sample

Automated
Data Collection
(Leginon)

3D EM Density

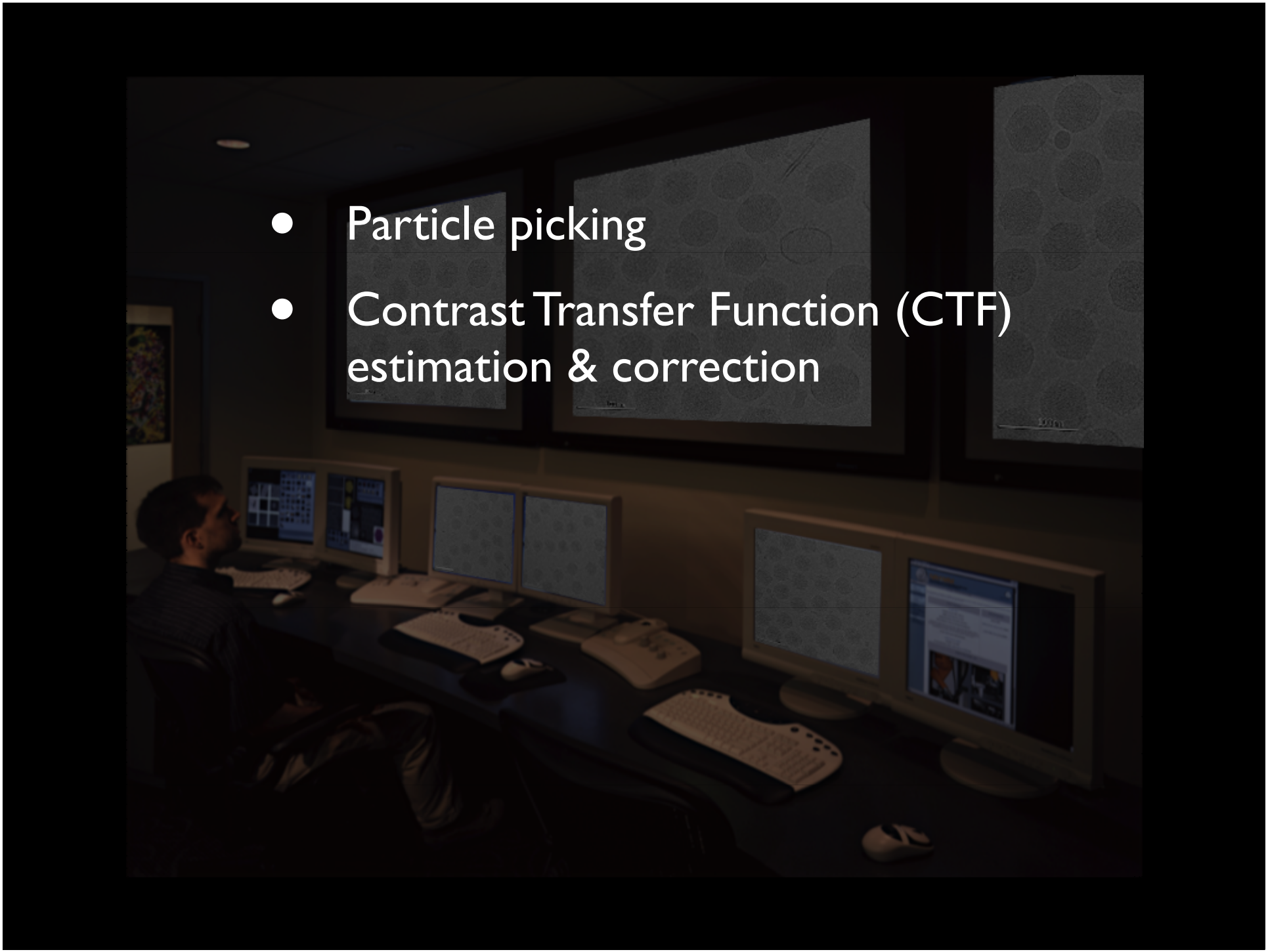


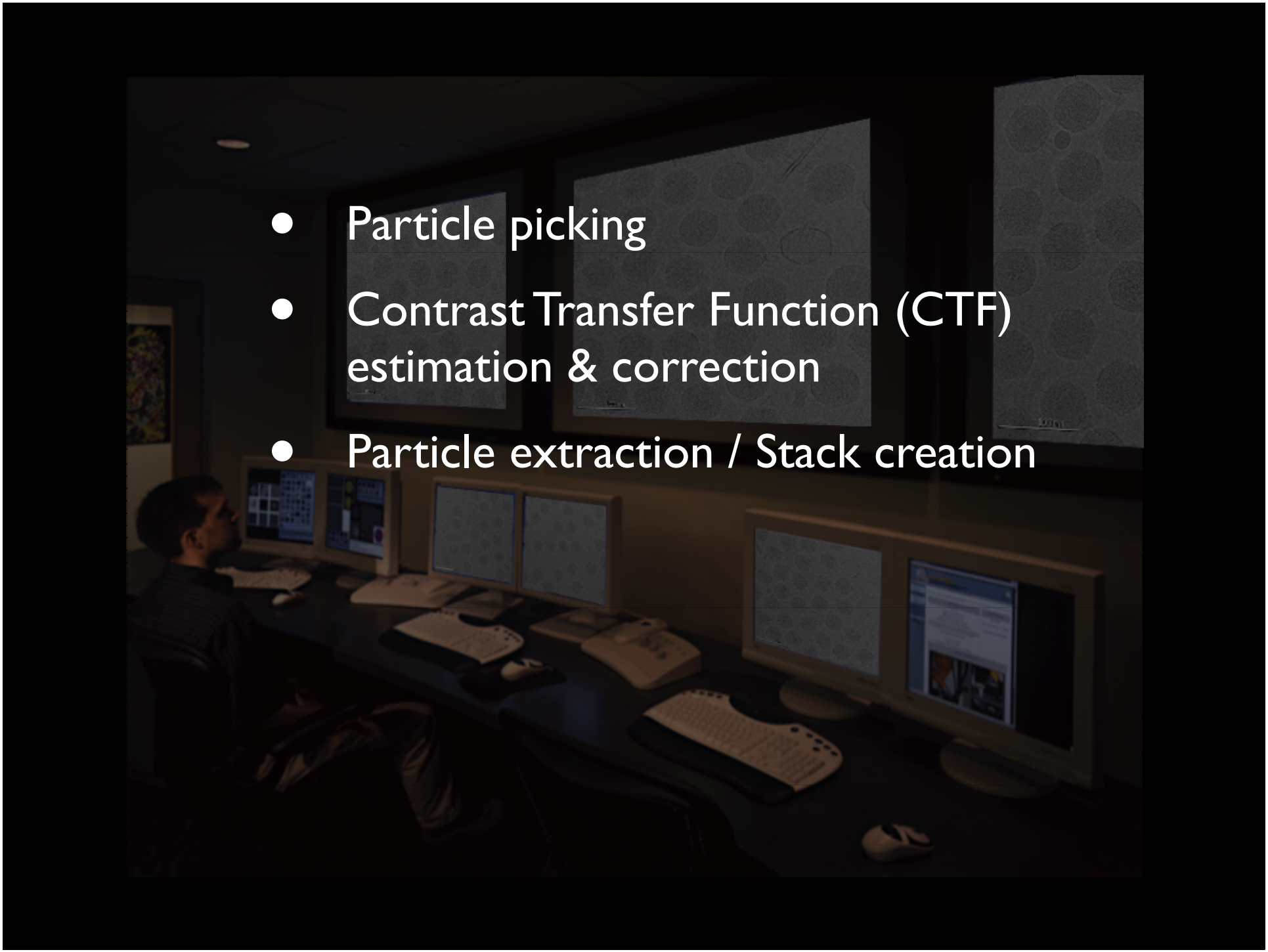
Specimen Vitrification

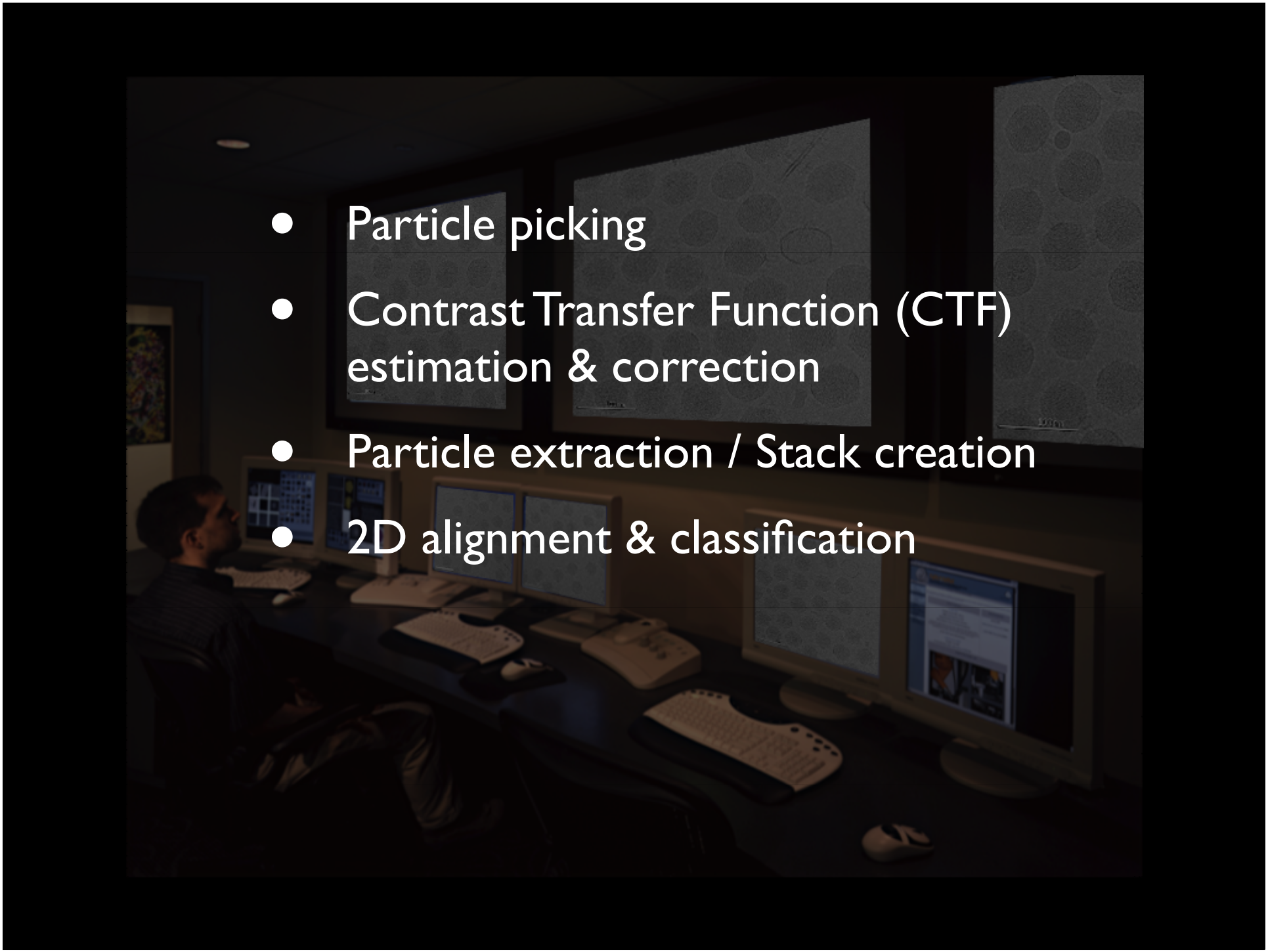


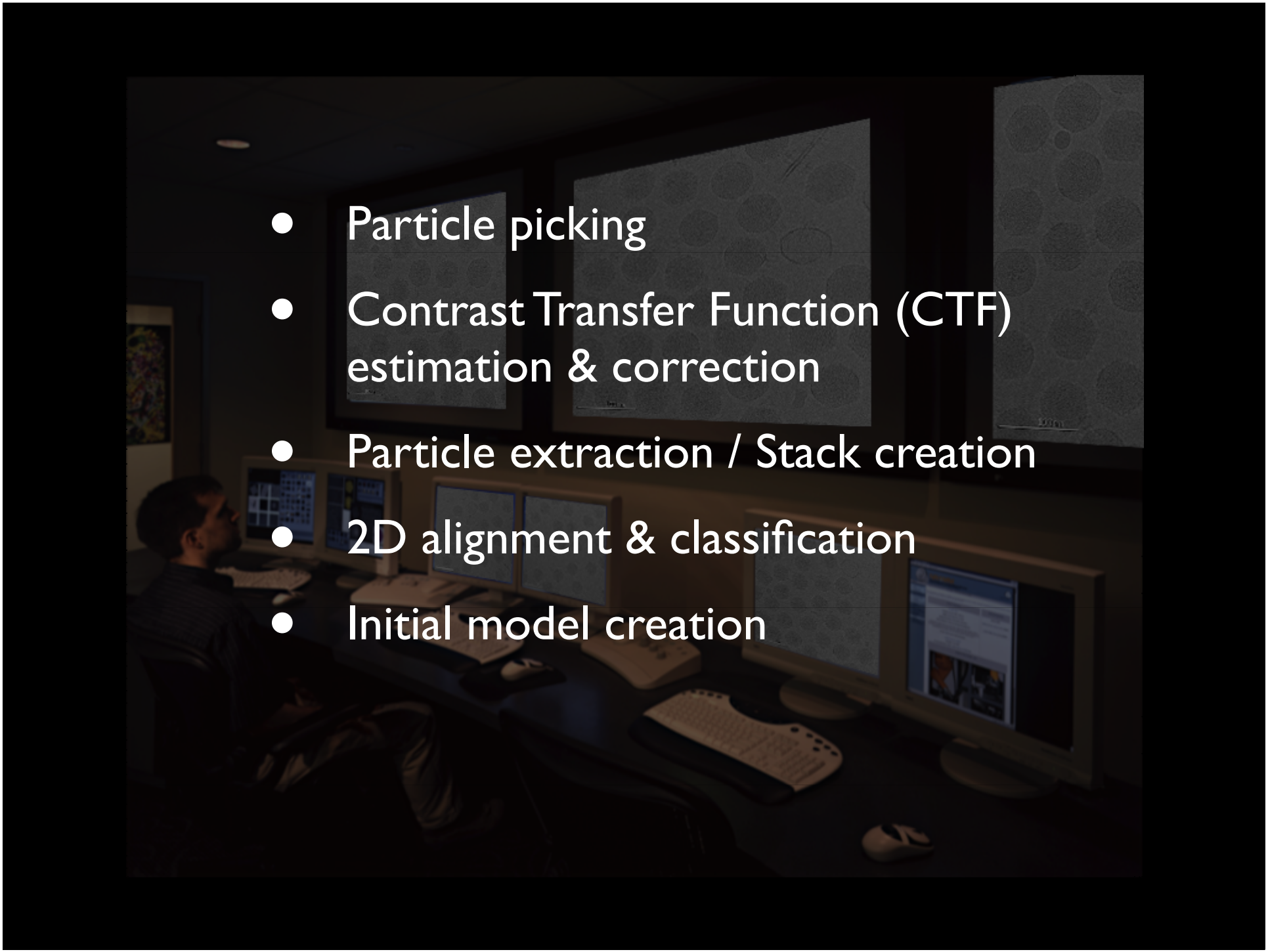
Streamlined
Processing
(Appion)

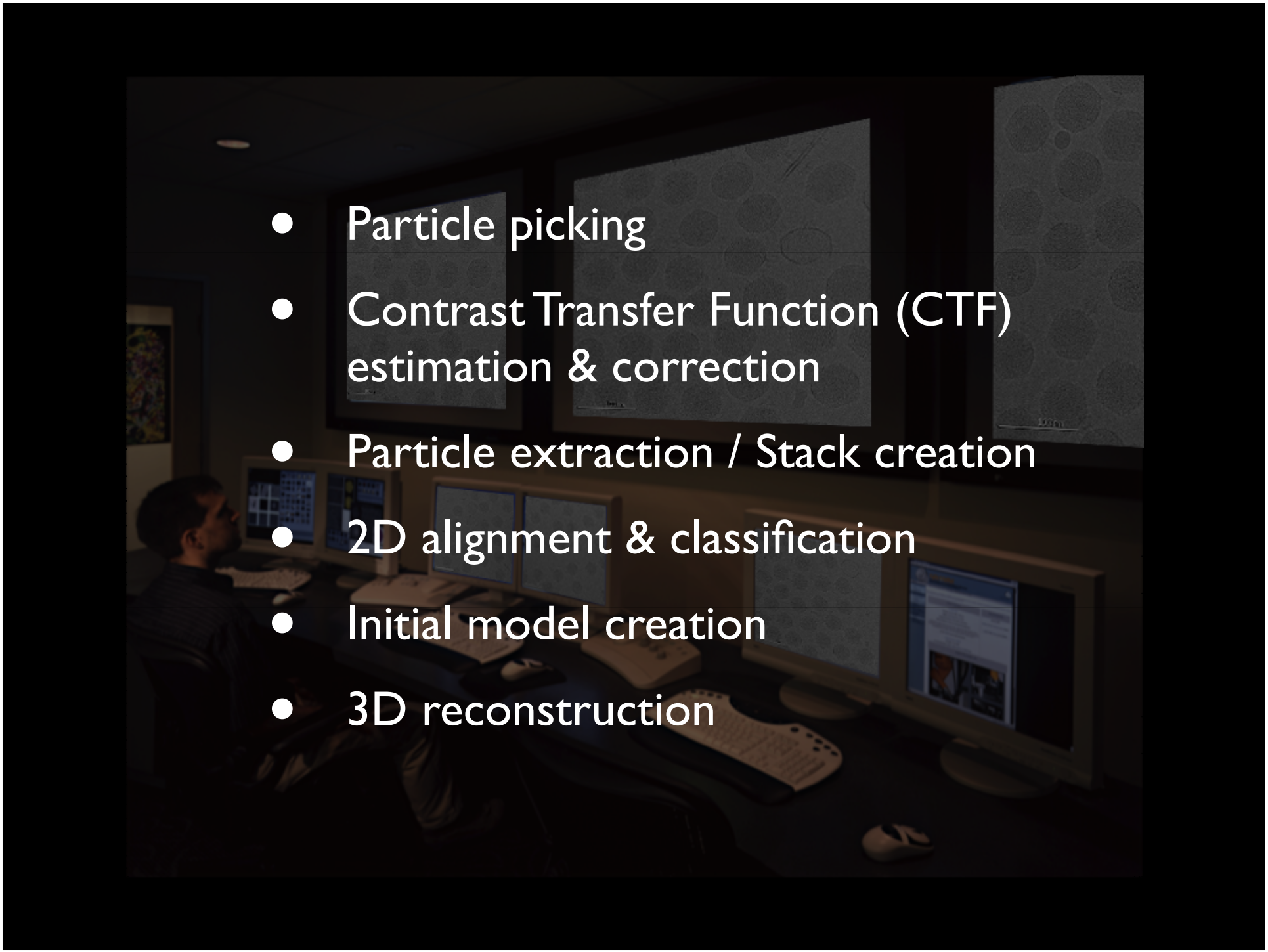

• Particle picking


• Particle picking
• Contrast Transfer Function (CTF) estimation & correction

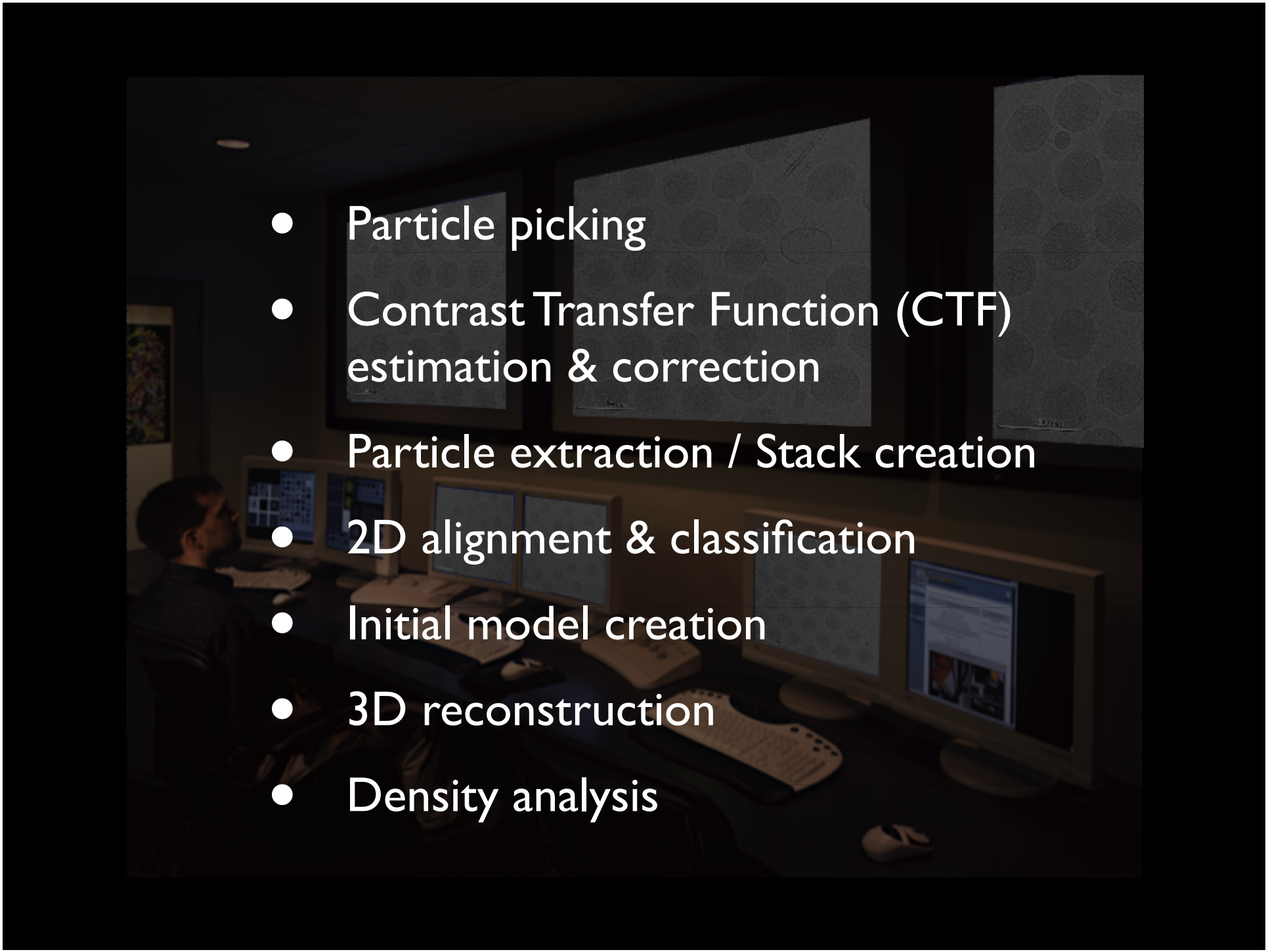

• Particle picking
• Contrast Transfer Function (CTF) estimation & correction
• Particle extraction / Stack creation


• Particle picking
• Contrast Transfer Function (CTF) estimation & correction
• Particle extraction / Stack creation
• 2D alignment & classification


• Particle picking
• Contrast Transfer Function (CTF) estimation & correction
• Particle extraction / Stack creation
• 2D alignment & classification
• Initial model creation

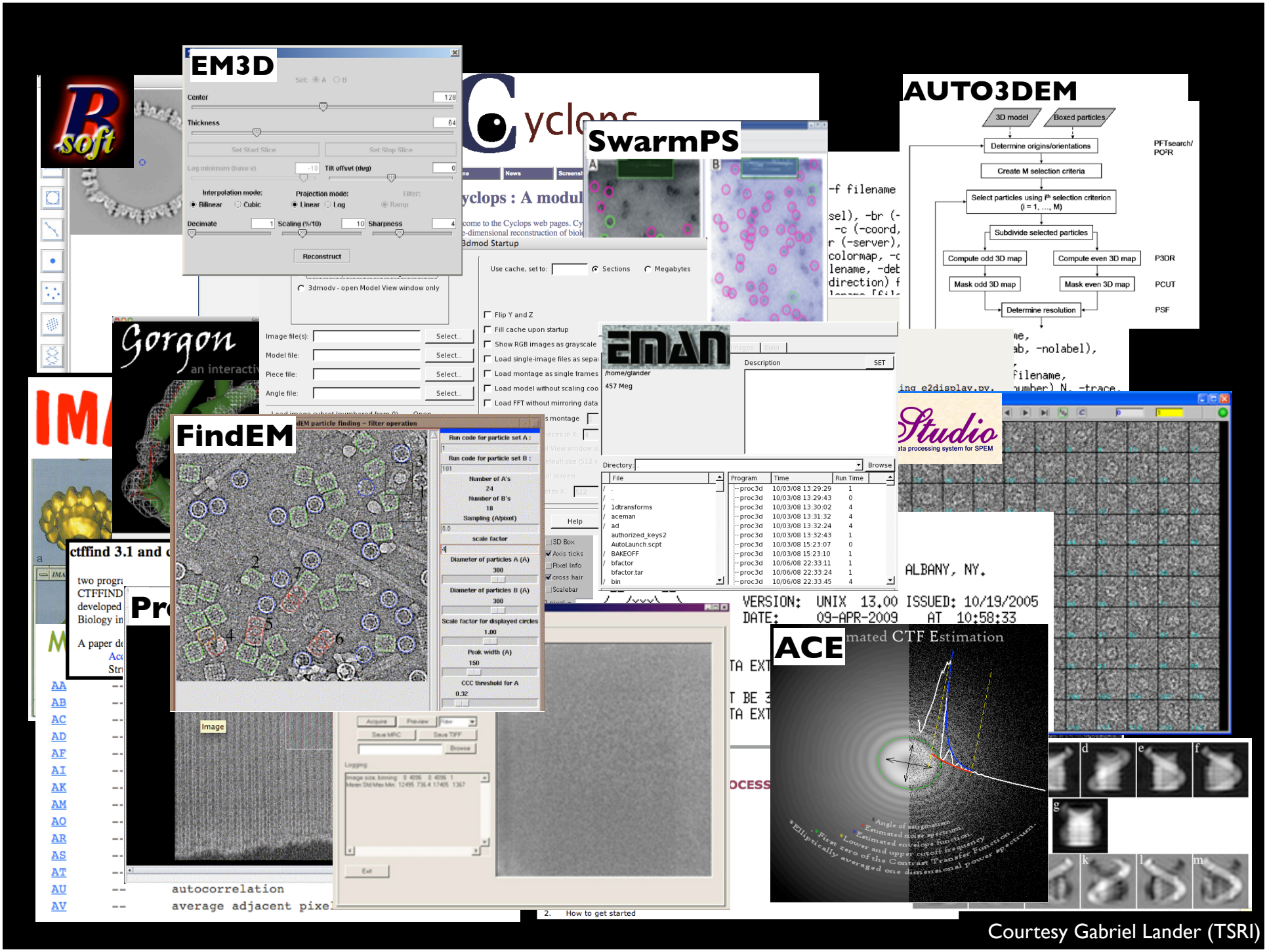


- Particle picking
- Contrast Transfer Function (CTF) estimation & correction
- Particle extraction / Stack creation
- 2D alignment & classification
- Initial model creation
- 3D reconstruction

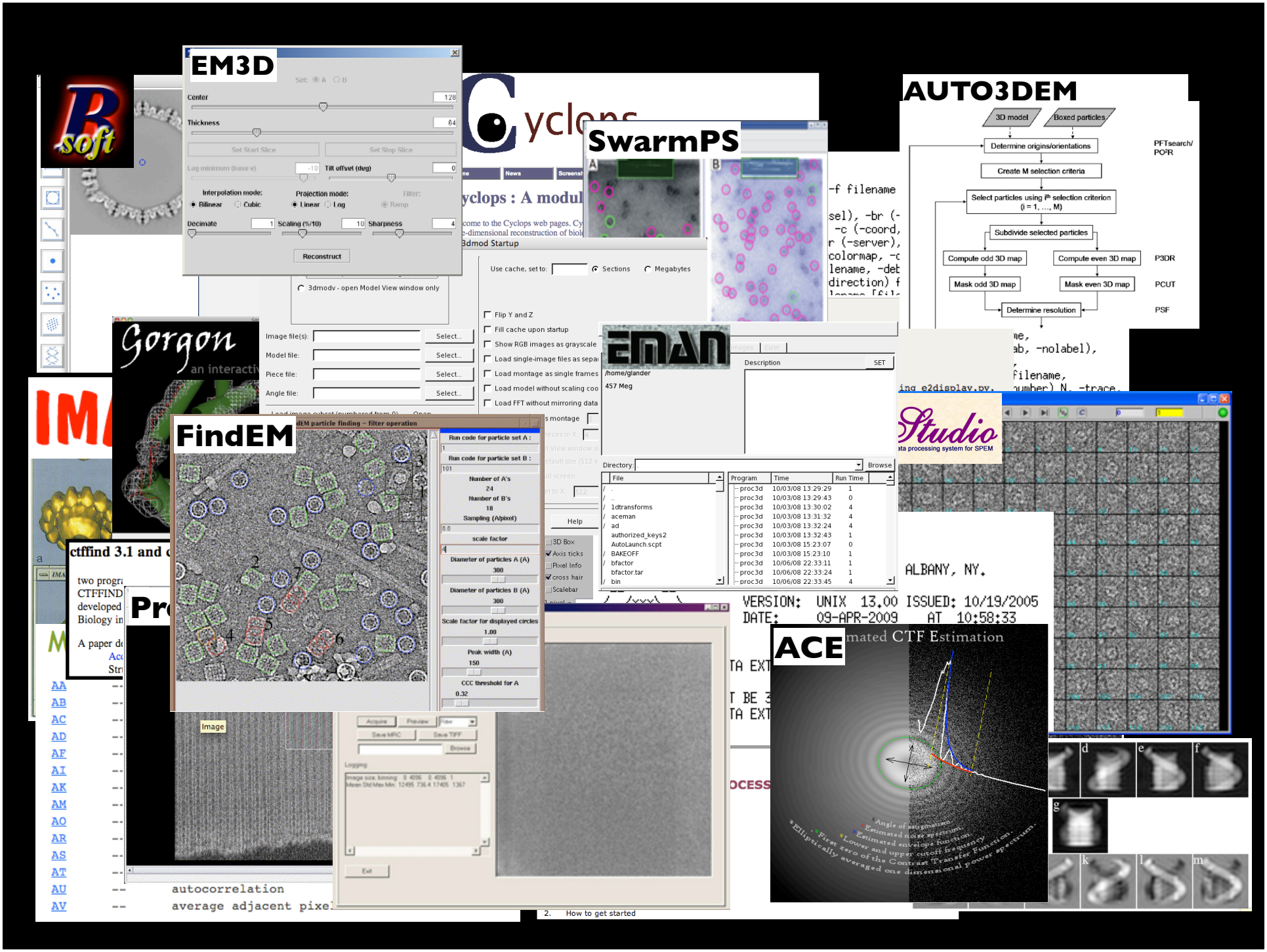

• Particle picking
• Contrast Transfer Function (CTF) estimation & correction
• Particle extraction / Stack creation
• 2D alignment & classification
• Initial model creation
• 3D reconstruction
• Density analysis

EM Processing

Courtesy Gabriel Lander (TSRI)



Courtesy Gabriel Lander (TSRI)


EM3D
Center
Thickness
Reconstruct
Cyclops
SwarmPS
AUTO3DEM
3D model
Boxed particles
Determine origins/orientations
Create M selection criteria
Select particles using ith selection criterion (i = 1, ..., M)
Subdivide selected particles
Compute odd 3D map
Compute even 3D map
Mask odd 3D map
Mask even 3D map
Determine resolution
PFTsearch/ POR
P3DR
PCUT
PSF
Gorgon
FindEM
EMAN
Studio
ACE
ctffind 3.1 and
ALBANY, NY.
VERSION: UNIX 13.00 ISSUED: 10/19/2005
DATE: 09-APR-2009 AT 10:58:33
autocorrelation
average adjacent pixel
How to get started

Enter filename: ?

Enter filename: ?

raw data:
/ami/data00/leginon/08mar29b/rawdata/

Particle picking:
/ami/data10/leginon/08mar29b/picking/findem1/
/ami/data09/leginon/08mar29b/TestPicks/findem2/
/home/glander/particlePickers/march29/runningPicks/

CTF estimation & correction
/home/glander/processing/march29b/ctfrun/
/ami/data15/temp/processing/march29b/ctfrun2/

Particle extraction / Stack creation
/ami/data00/leginon/08mar29b/stacks/stack1/
/ami/data00/leginon/08mar29b/stacks/stack2/
/home/glander/08march29/stacks/stackTest/
/home/glander/08march29/stacks/allParticles/

3D reconstruction
/home/glander/march29b/reconstruction/recon1/
/home/glander/march29b/reconstruction/recon2/
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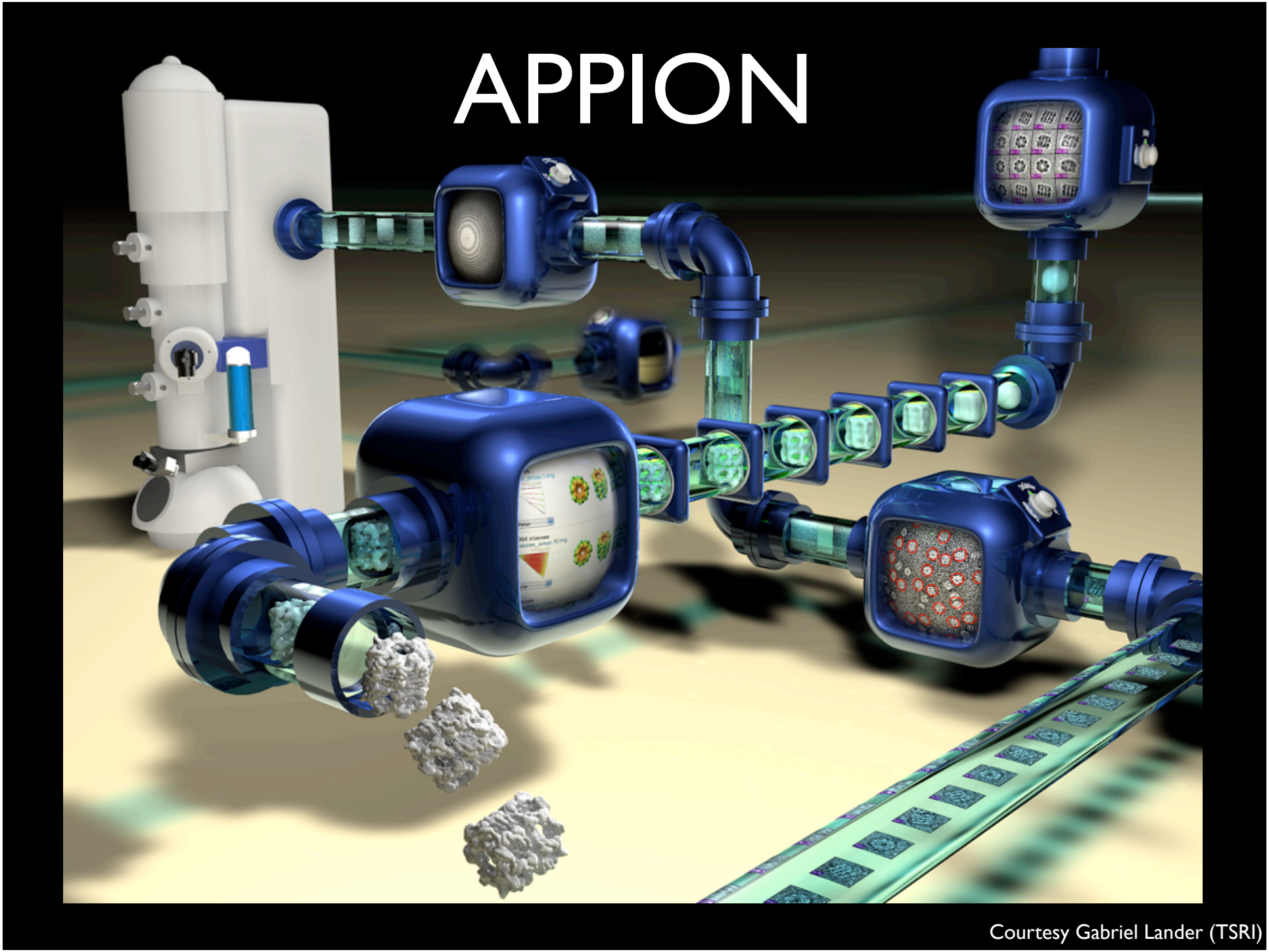
/ami/data00/leginon/08mar29b/refine/refinement/
/ami/data00/leginon/08mar29b/recon/EMAN/
newRecon/
/ami/data00/leginon/08mar29b/recon/SPIDER/recon3/

Density analysis
/home/glander/processing/work1/segmentation/
/home/glander/SPIDER/processing/amplitudes/
/ami/data00/leginon/08mar29b/postProc/bFactors/
/ami/data00/leginon/08mar29b/recon/EMAN/
newRecon/



Courtesy Gabriel Lander (TSRI)

[Login][register][summary] [processing] [make jpgs]

all | 06jul12a - GroEL 100K at 100K at 120KeV

Select NRAMM - GroEL 100K project

00027gr_65sq_9hl_5en.mrc
00027gr_65sq_9hl_4en.mrc
00027gr_65sq_9hl_3en.mrc
00027gr_65sq_9hl_2en.mrc
00027gr_65sq_9hl_1fc.mrc
00027gr_65sq_9hl.mrc
00027gr_65sq_8hl_5en.mrc
00027gr_65sq_8hl_4en.mrc
00027gr_65sq_8hl_3en.mrc
00027gr_65sq_8hl_2en.mrc
00027gr_65sq_8hl_1fc.mrc
00027gr_65sq_8hl.mrc
00027gr_65sq_7hl_5en.mrc
00027gr_65sq_7hl_4en.mrc
00027gr_65sq_7hl_3en.mrc
00027gr_65sq_7hl_2en.mrc
00027gr_65sq_7hl_1fc.mrc
00027gr_65sq_7hl.mrc
00027gr_65sq_6hl.mrc
00027gr_65sq_5hl_5en.mrc
00027gr_65sq_5hl_4en.mrc
00027gr_65sq_5hl_3en.mrc
00027gr_65sq_5hl_2en.mrc
00027gr_65sq_5hl_1fc.mrc
00027gr_65sq_5hl.mrc
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00027gr_65sq_4hl_4en.mrc
00027gr_65sq_4hl_3en.mrc
00027gr_65sq_4hl_2en.mrc
00027gr_65sq_4hl_1fc.mrc
00027gr_65sq_4hl.mrc
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00027gr_65sq_2hl_5en.mrc
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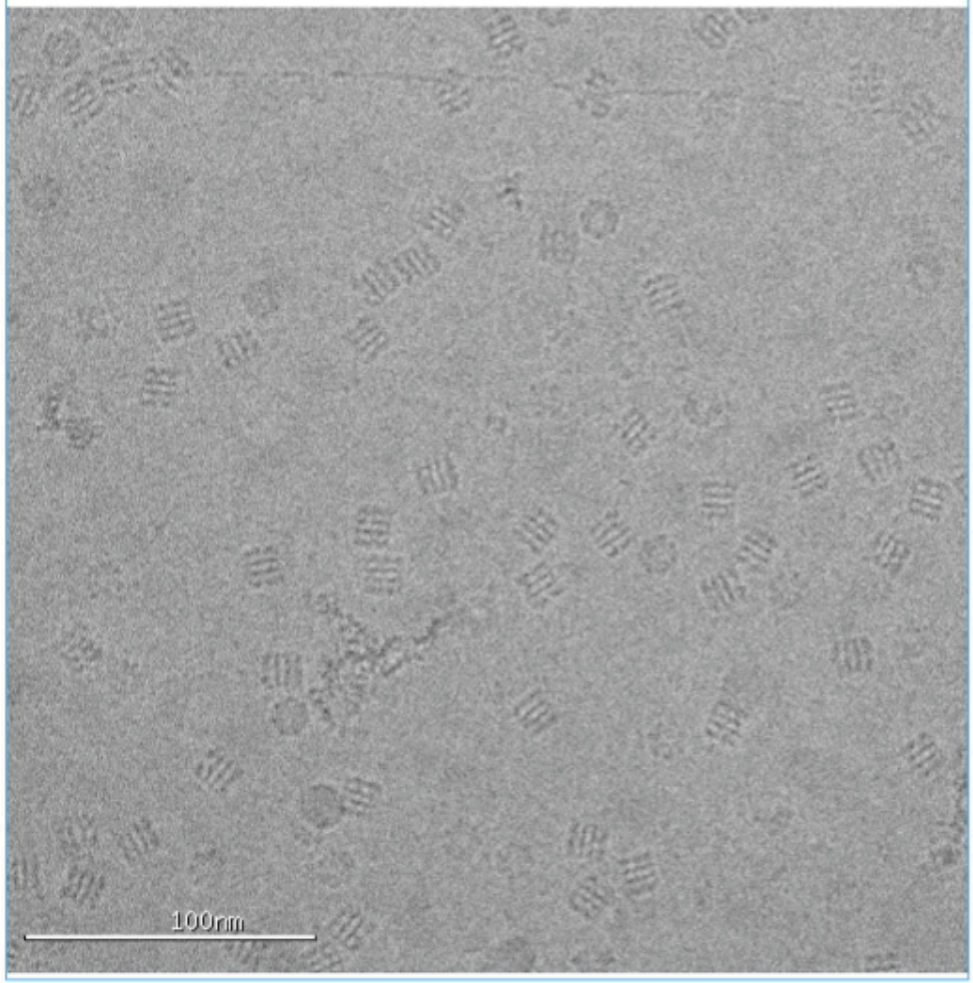
00027gr_65sq_2hl_2en.mrc
00027gr_65sq_2hl_1fc.mrc
00027gr_65sq_2hl.mrc
00027gr_65sq.mrc
00024gr_64sq_17hl.mrc

Main View

all | hide | next | exemplar

mag: 100000 **defocus:** -0.839 µm **pixelsize:** 0.082 nm **dose:** 13.4 $e^{-}/Å^{2}$
06jul12a_00027gr_00065sq_00009hl_00005en.mrc



[Login][register][summary] [processing] [make jpgs]

all

Select NRAMM - GroEL 100K project

00027gr_65sq_9hl_5en.mrc
00027gr_65sq_9hl_4en.mrc
00027gr_65sq_9hl_3en.mrc
00027gr_65sq_9hl_2en.mrc
00027gr_65sq_9hl_1fc.mrc
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00027gr_65sq_8hl_1fc.mrc
00027gr_65sq_8hl.mrc
00027gr_65sq_7hl_5en.mrc
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00027gr_65sq_5hl_5en.mrc
00027gr_65sq_5hl_4en.mrc
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00027gr_65sq_5hl_2en.mrc
00027gr_65sq_5hl_1fc.mrc
00027gr_65sq_5hl.mrc
00027gr_65sq_4hl_5en.mrc
00027gr_65sq_4hl_4en.mrc
00027gr_65sq_4hl_3en.mrc
00027gr_65sq_4hl_2en.mrc
00027gr_65sq_4hl_1fc.mrc
00027gr_65sq_4hl.mrc
00027gr_65sq_3hl.mrc
00027gr_65sq_2hl_5en.mrc
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00027gr_65sq_2hl_3en.mrc
00027gr_65sq_2hl_2en.mrc
00027gr_65sq_2hl_1fc.mrc
00027gr_65sq_2hl.mrc
00027gr_65sq.mrc
00024gr_64sq_17hl.mrc

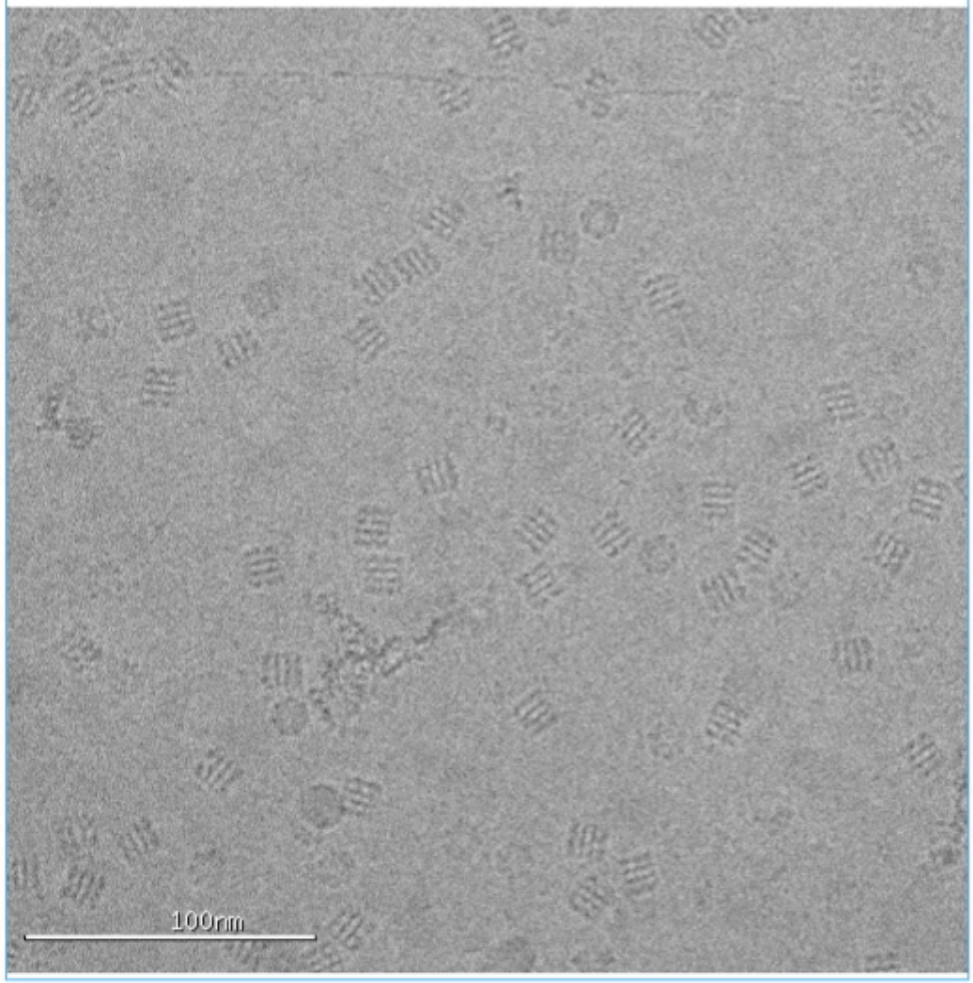
06jul12a – GroEL 100K at 100K at 120Kev

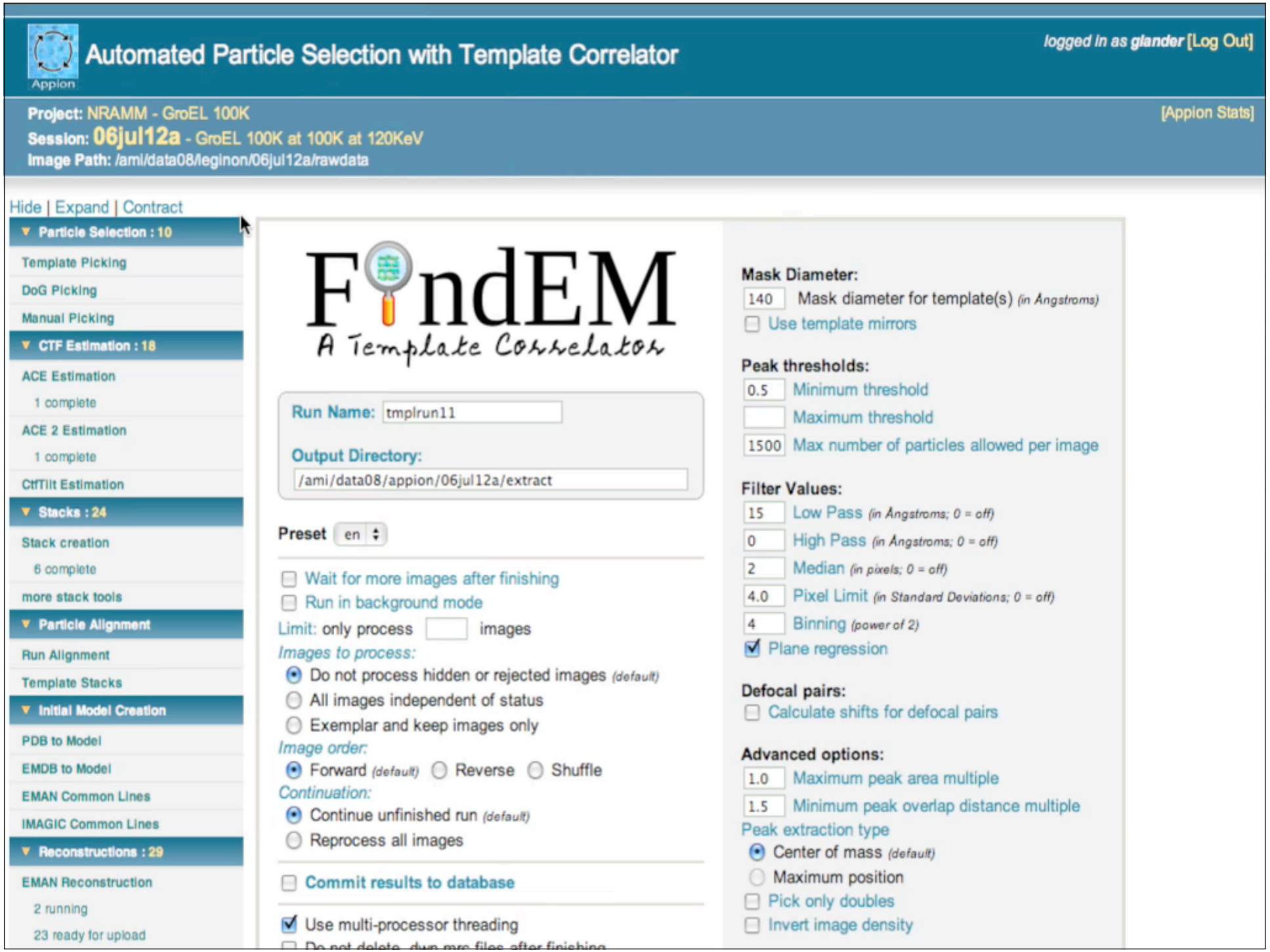
Main View

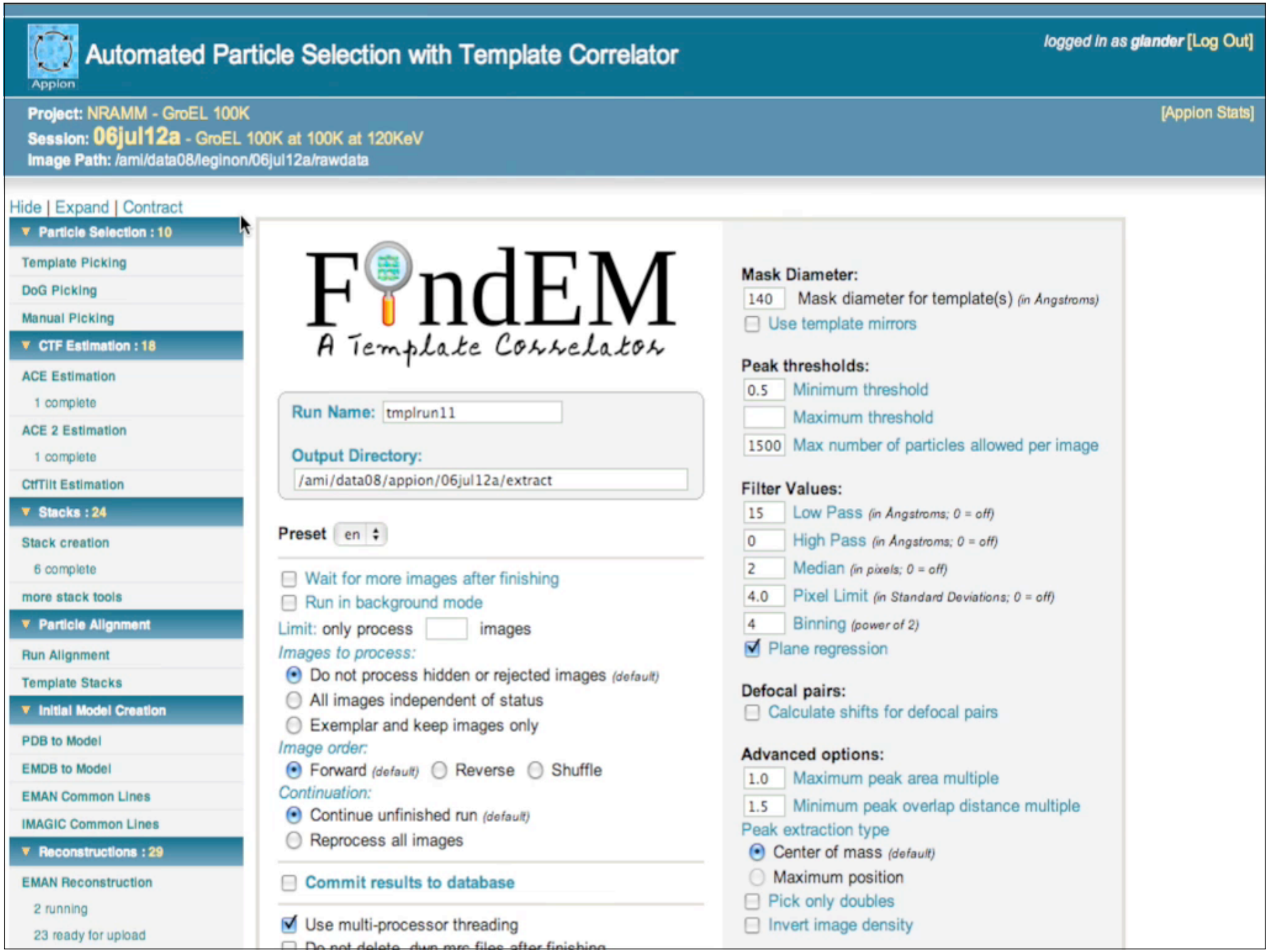
all

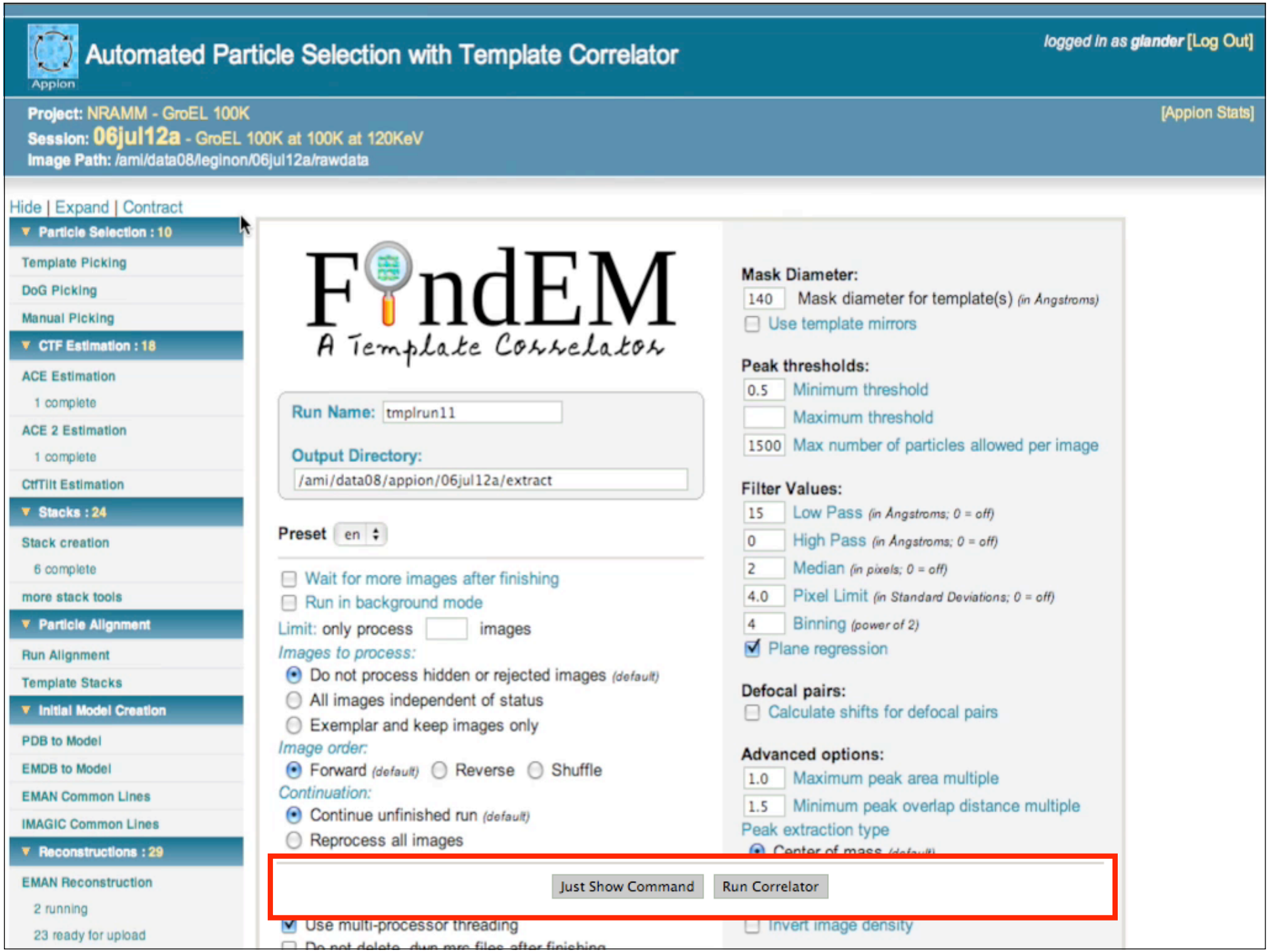
hide next exemplar

mag: 100000 **defocus:** -0.839 µm **pixelsize:** 0.082 nm **dose:** 13.4 $e^{-}/Å^{2}$
06jul12a_00027gr_00065sq_00009hl_00005en.mrc

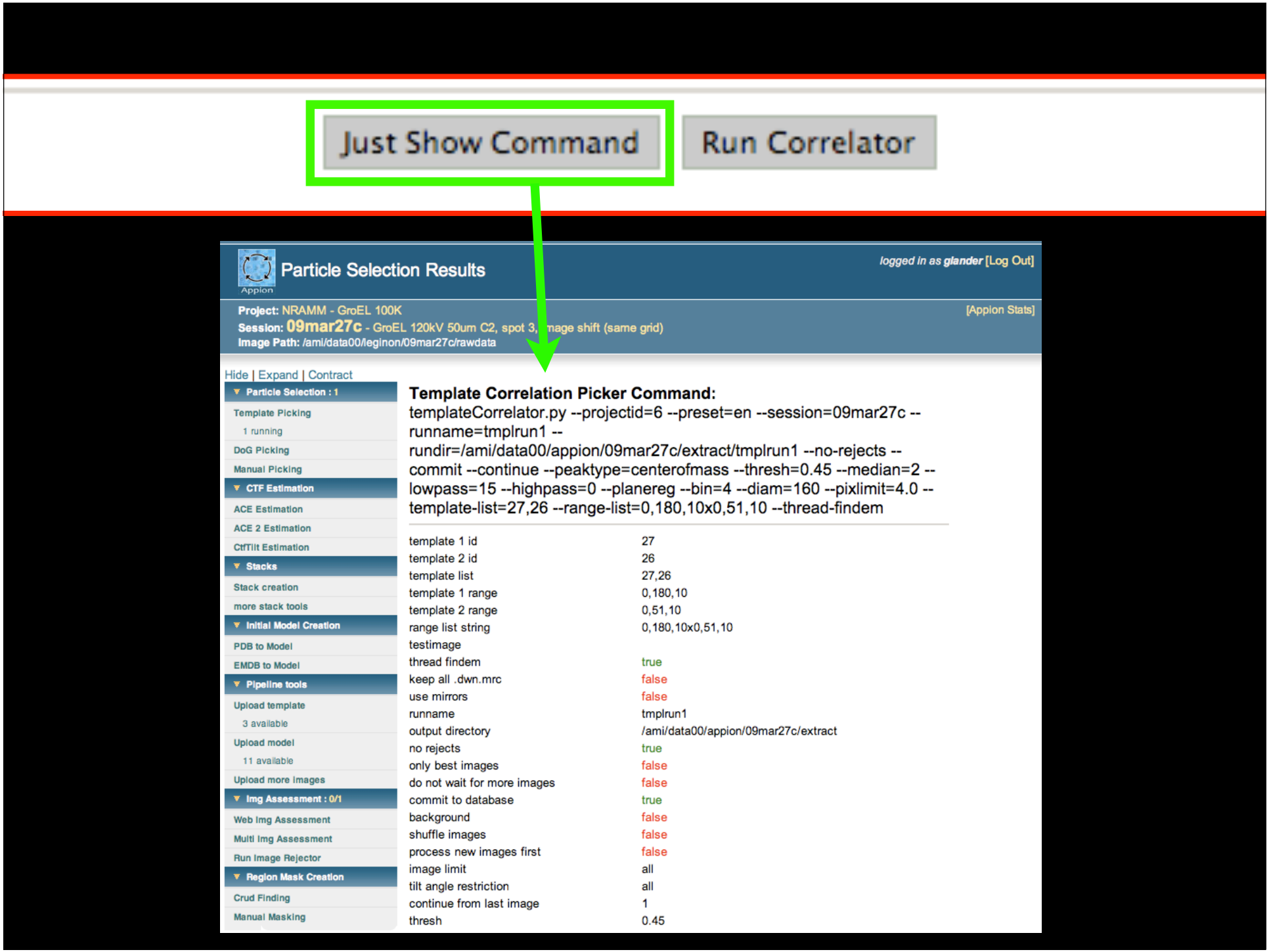



Appion
Automated Particle Selection with Template Correlator
logged in as glander [Log Out]
Project: NRAMM - GroEL 100K
Session: 06jul12a - GroEL 100K at 100K at 120KeV
Image Path: /ami/data08/leginon/06jul12a/rawdata
[Appion Stats]
Hide | Expand | Contract
Particle Selection : 10
Template Picking
DoG Picking
Manual Picking
CTF Estimation : 18
ACE Estimation
1 complete
ACE 2 Estimation
1 complete
CtfTilt Estimation
Stacks : 24
Stack creation
6 complete
more stack tools
Particle Alignment
Run Alignment
Template Stacks
Initial Model Creation
PDB to Model
EMDB to Model
EMAN Common Lines
IMAGIC Common Lines
Reconstructions : 29
EMAN Reconstruction
2 running
23 ready for upload
FindEM
A Template Correlator
Run Name: tmplrun11
Output Directory:
/ami/data08/appion/06jul12a/extract
Preset en
Wait for more images after finishing
Run in background mode
Limit: only process images
Images to process:
Do not process hidden or rejected images (default)
All images independent of status
Exemplar and keep images only
Image order:
Forward (default) Reverse Shuffle
Continuation:
Continue unfinished run (default)
Reprocess all images
Commit results to database
Use multi-processor threading
Mask Diameter:
140 Mask diameter for template(s) (in Angstroms)
Use template mirrors
Peak thresholds:
0.5 Minimum threshold
Maximum threshold
1500 Max number of particles allowed per image
Filter Values:
15 Low Pass (in Angstroms; 0 = off)
0 High Pass (in Angstroms; 0 = off)
2 Median (in pixels; 0 = off)
4.0 Pixel Limit (in Standard Deviations; 0 = off)
4 Binning (power of 2)
Plane regression
Defocal pairs:
Calculate shifts for defocal pairs
Advanced options:
1.0 Maximum peak area multiple
1.5 Minimum peak overlap distance multiple
Peak extraction type
Center of mass (default)
Maximum position
Pick only doubles
Invert image density


Appion
Automated Particle Selection with Template Correlator
logged in as glander [Log Out]
Project: NRAMM - GroEL 100K
Session: 06jul12a - GroEL 100K at 100K at 120KeV
Image Path: /ami/data08/leginon/06jul12a/rawdata
[Appion Stats]
Hide | Expand | Contract
Particle Selection : 10
Template Picking
DoG Picking
Manual Picking
CTF Estimation : 18
ACE Estimation
1 complete
ACE 2 Estimation
1 complete
CtfTilt Estimation
Stacks : 24
Stack creation
6 complete
more stack tools
Particle Alignment
Run Alignment
Template Stacks
Initial Model Creation
PDB to Model
EMDB to Model
EMAN Common Lines
IMAGIC Common Lines
Reconstructions : 29
EMAN Reconstruction
2 running
23 ready for upload
FindEM
A Template Correlator
Run Name: tmplrun11
Output Directory:
/ami/data08/appion/06jul12a/extract
Preset en
Wait for more images after finishing
Run in background mode
Limit: only process images
Images to process:
Do not process hidden or rejected images (default)
All images independent of status
Exemplar and keep images only
Image order:
Forward (default) Reverse Shuffle
Continuation:
Continue unfinished run (default)
Reprocess all images
Commit results to database
Use multi-processor threading
Mask Diameter:
140 Mask diameter for template(s) (in Angstroms)
Use template mirrors
Peak thresholds:
0.5 Minimum threshold
Maximum threshold
1500 Max number of particles allowed per image
Filter Values:
15 Low Pass (in Angstroms; 0 = off)
0 High Pass (in Angstroms; 0 = off)
2 Median (in pixels; 0 = off)
4.0 Pixel Limit (in Standard Deviations; 0 = off)
4 Binning (power of 2)
Plane regression
Defocal pairs:
Calculate shifts for defocal pairs
Advanced options:
1.0 Maximum peak area multiple
1.5 Minimum peak overlap distance multiple
Peak extraction type
Center of mass (default)
Maximum position
Pick only doubles
Invert image density

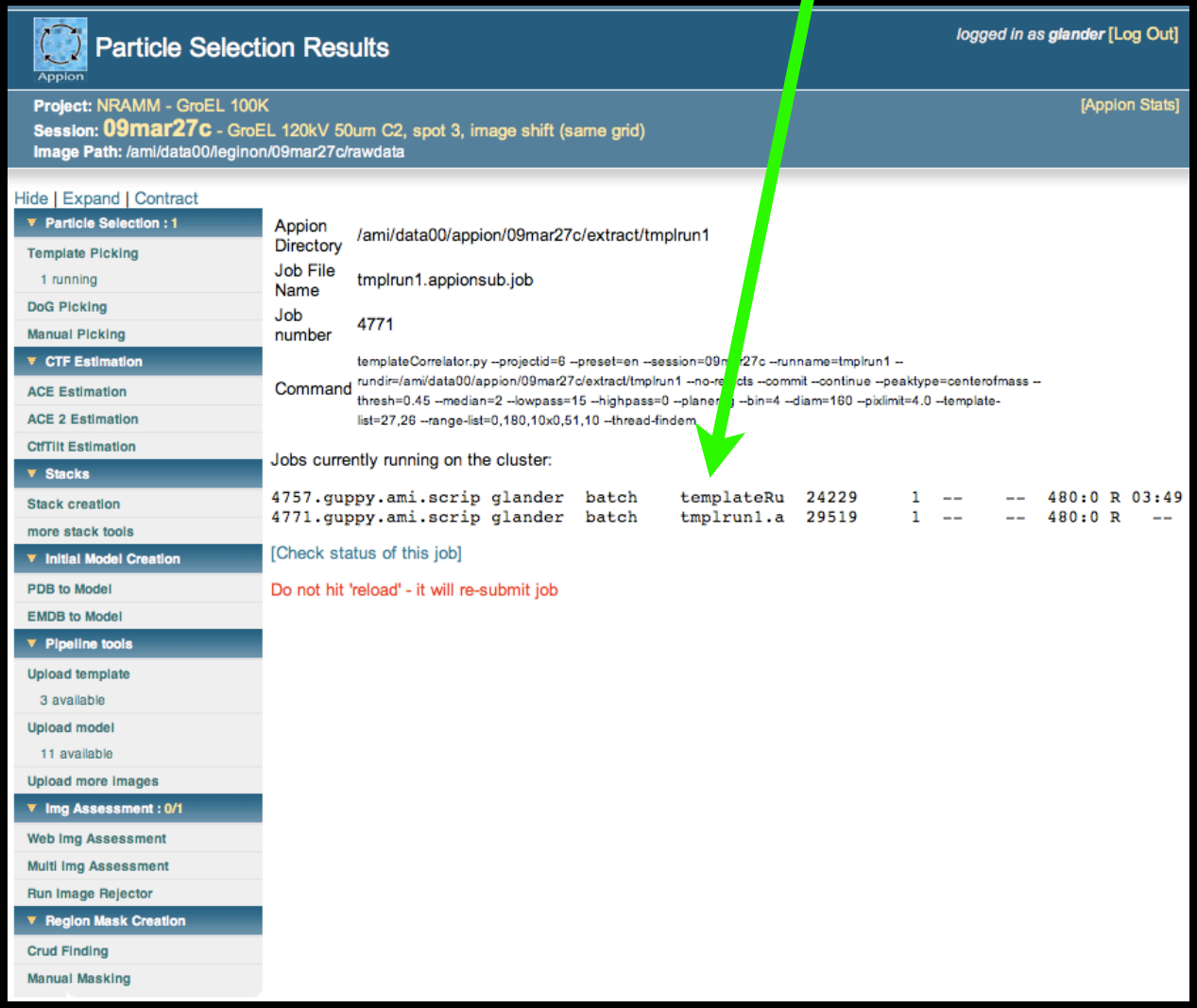

Appion
Automated Particle Selection with Template Correlator
logged in as glander [Log Out]
Project: NRAMM - GroEL 100K
Session: 06jul12a - GroEL 100K at 100K at 120KeV
Image Path: /ami/data08/leginon/06jul12a/rawdata
[Appion Stats]
Hide | Expand | Contract
Particle Selection : 10
Template Picking
DoG Picking
Manual Picking
CTF Estimation : 18
ACE Estimation
1 complete
ACE 2 Estimation
1 complete
CtfTilt Estimation
Stacks : 24
Stack creation
6 complete
more stack tools
Particle Alignment
Run Alignment
Template Stacks
Initial Model Creation
PDB to Model
EMDB to Model
EMAN Common Lines
IMAGIC Common Lines
Reconstructions : 29
EMAN Reconstruction
2 running
23 ready for upload
FindEM
A Template Correlator
Run Name: tmplrun11
Output Directory:
/ami/data08/appion/06jul12a/extract
Preset en
Wait for more images after finishing
Run in background mode
Limit: only process images
Images to process:
Do not process hidden or rejected images (default)
All images independent of status
Exemplar and keep images only
Image order:
Forward (default) Reverse Shuffle
Continuation:
Continue unfinished run (default)
Reprocess all images
Mask Diameter:
140 Mask diameter for template(s) (in Angstroms)
Use template mirrors
Peak thresholds:
0.5 Minimum threshold
Maximum threshold
1500 Max number of particles allowed per image
Filter Values:
15 Low Pass (in Angstroms; 0 = off)
0 High Pass (in Angstroms; 0 = off)
2 Median (in pixels; 0 = off)
4.0 Pixel Limit (in Standard Deviations; 0 = off)
4 Binning (power of 2)
Plane regression
Defocal pairs:
Calculate shifts for defocal pairs
Advanced options:
1.0 Maximum peak area multiple
1.5 Minimum peak overlap distance multiple
Peak extraction type
Center of mass
Just Show Command
Run Correlator
Use multi-processor threading
Invert image density


Just Show Command
Run Correlator


Just Show Command
Run Correlator
Particle Selection Results
logged in as glander [Log Out]
Project: NRAMM - GroEL 100K
[Appion Stats]
Session: 09mar27c - GroEL 120kV 50um C2, spot 3, image shift (same grid)
Image Path: /ami/data00/leginon/09mar27c/rawdata
Hide | Expand | Contract
Particle Selection : 1
Template Picking
1 running
DoG Picking
Manual Picking
CTF Estimation
ACE Estimation
ACE 2 Estimation
CtfTilt Estimation
Stacks
Stack creation
more stack tools
Initial Model Creation
PDB to Model
EMDB to Model
Pipeline tools
Upload template
3 available
Upload model
11 available
Upload more images
Img Assessment : 0/1
Web Img Assessment
Multi Img Assessment
Run Image Rejector
Region Mask Creation
Crud Finding
Manual Masking
Template Correlation Picker Command:
templateCorrelator.py --projectid=6 --preset=en --session=09mar27c --runname=tmplrun1 --rundir=/ami/data00/appion/09mar27c/extract/tmplrun1 --no-rejects --commit --continue --peaktype=centerofmass --thresh=0.45 --median=2 --lowpass=15 --highpass=0 --planereg --bin=4 --diam=160 --pixlimit=4.0 --template-list=27,26 --range-list=0,180,10x0,51,10 --thread-findem
template 1 id 27
template 2 id 26
template list 27,26
template 1 range 0,180,10
template 2 range 0,51,10
range list string 0,180,10x0,51,10
testimage
thread findem true
keep all .dwn.mrc false
use mirrors false
runname tmplrun1
output directory /ami/data00/appion/09mar27c/extract
no rejects true
only best images false
do not wait for more images false
commit to database true
background false
shuffle images false
process new images first false
image limit all
tilt angle restriction all
continue from last image 1
thresh 0.45

Just Show Command

Run Correlator



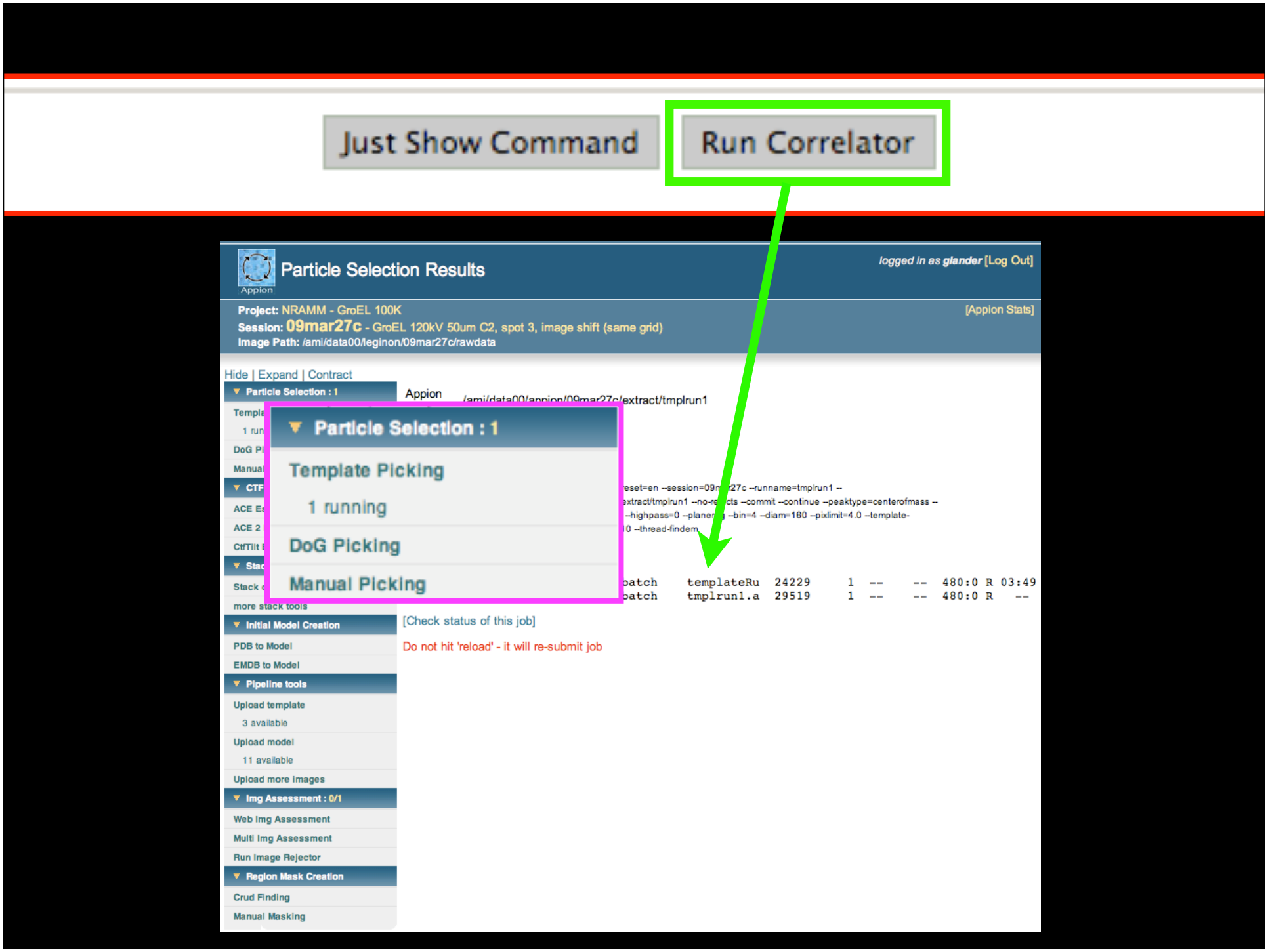

Just Show Command
Run Correlator
Particle Selection Results
logged in as glander [Log Out]
Project: NRAMM - GroEL 100K
Session: 09mar27c - GroEL 120kV 50um C2, spot 3, image shift (same grid)
Image Path: /ami/data00/leginon/09mar27c/rawdata
[Appion Stats]
Hide | Expand | Contract
Particle Selection : 1
Template Picking
1 running
DoG Picking
Manual Picking
Appion
/ami/data00/appion/09mar27c/extract/tmplrun1
templateRu 24229 1 -- -- 480:0 R 03:49
tmplrun1.a 29519 1 -- -- 480:0 R --
[Check status of this job]
Do not hit 'reload' - it will re-submit job
Initial Model Creation
PDB to Model
EMDB to Model
Pipeline tools
Upload template
3 available
Upload model
11 available
Upload more images
Img Assessment : 0/1
Web Img Assessment
Multi Img Assessment
Run Image Rejector
Region Mask Creation
Crud Finding
Manual Masking

image viewer

http://cronus3.scripps.edu/glander/dbem/imageviewer.php

Google

Mininova Viper weather PubMed NRAMM phpMyAdmin Leginon Image Viewer SPIDER myscripps dlsrs brew CIMBio - Scheduler

[Logout glander][glander Prefs][summary] [processing] [make jpgs]

all

08feb14j - tail machines 3rd data set

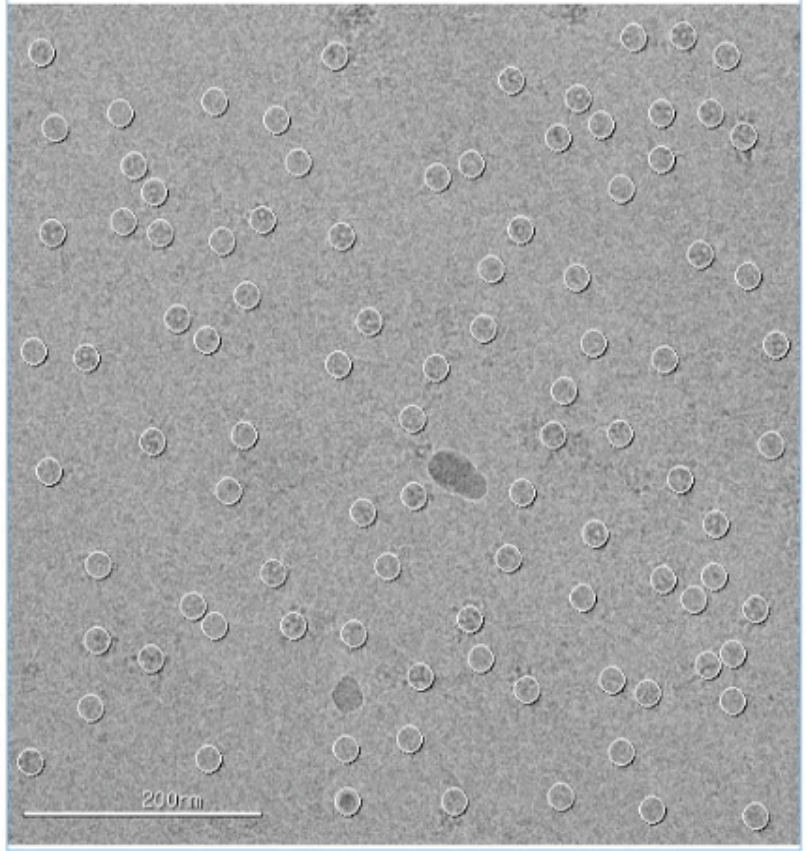
Select Lander - P22 project

00032gr_76sq_v02_15hl_2en.mrc
00032gr_76sq_v02_15hl_1fc.mrc
00032gr_76sq_v02_15hl.mrc
00032gr_76sq_v02_14hl_2en.mrc
00032gr_76sq_v02_14hl_1fc.mrc
00032gr_76sq_v02_14hl.mrc
00032gr_76sq_v02_13hl_2en.mrc
00032gr_76sq_v02_13hl_1fc.mrc
00032gr_76sq_v02_13hl.mrc
00032gr_76sq_v02_12hl_2en.mrc
00032gr_76sq_v02_12hl_1fc.mrc
00032gr_76sq_v02_12hl.mrc
00032gr_76sq_v02_11hl_2en.mrc
00032gr_76sq_v02_11hl_1fc.mrc
00032gr_76sq_v02_11hl.mrc
00032gr_76sq_v02_10hl_2en.mrc
00032gr_76sq_v02_10hl_1fc.mrc
00032gr_76sq_v02_10hl.mrc
00032gr_76sq_v02_9hl_1fc.mrc
00032gr_76sq_v02_9hl.mrc
00032gr_76sq_v02_8hl_2en.mrc
00032gr_76sq_v02_8hl_1fc.mrc
00032gr_76sq_v02_8hl.mrc
00032gr_76sq_v02_7hl_2en.mrc
00032gr_76sq_v02_7hl_1fc.mrc
00032gr_76sq_v02_7hl.mrc
00032gr_76sq_v02_6hl_2en.mrc
00032gr_76sq_v02_6hl_1fc.mrc
00032gr_76sq_v02_6hl.mrc
00032gr_76sq_v02_5hl_2en.mrc
00032gr_76sq_v02_5hl_1fc.mrc
00032gr_76sq_v02_5hl.mrc
00032gr_76sq_v02_4hl_1en.mrc
00032gr_76sq_v02_4hl.mrc
00032gr_76sq_v02_3hl_1en.mrc
00032gr_76sq_v02_3hl.mrc
00032gr_76sq_v02_2hl_1en.mrc
00032gr_76sq_v02_2hl.mrc
00032gr_76sq_v02.mrc
00032gr_76sq_v01.mrc

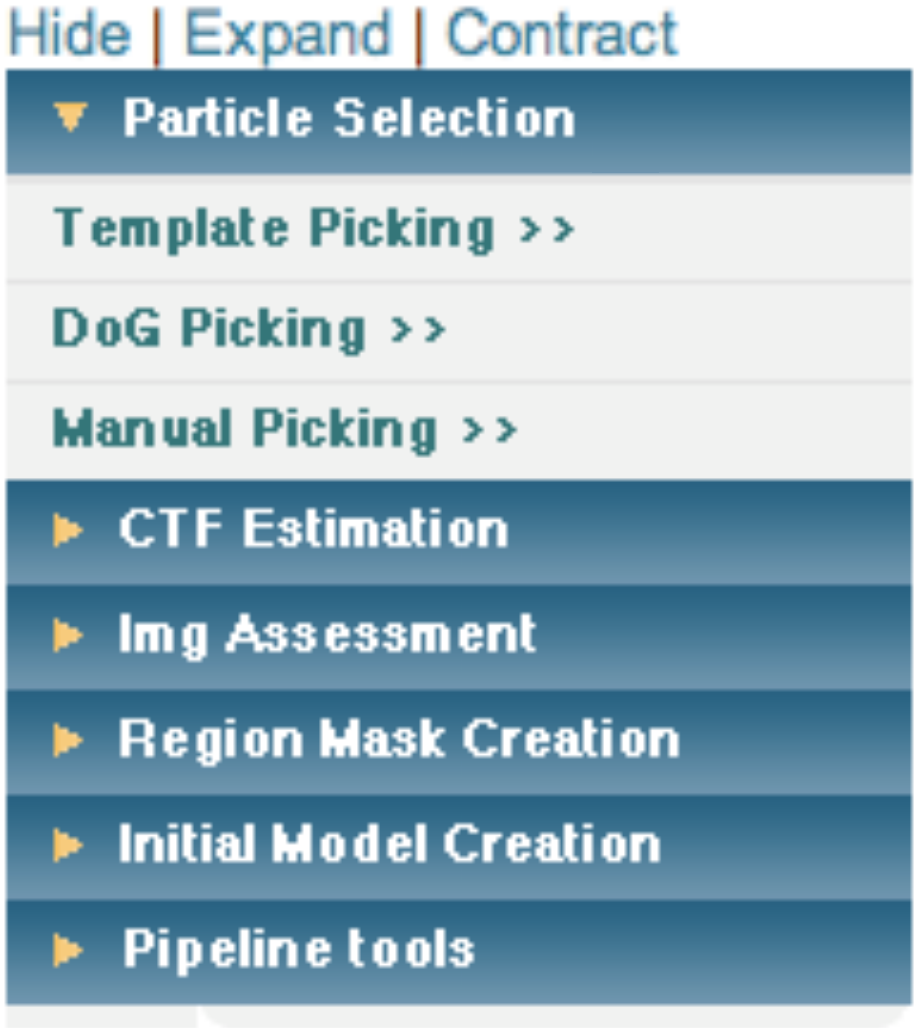
Main View

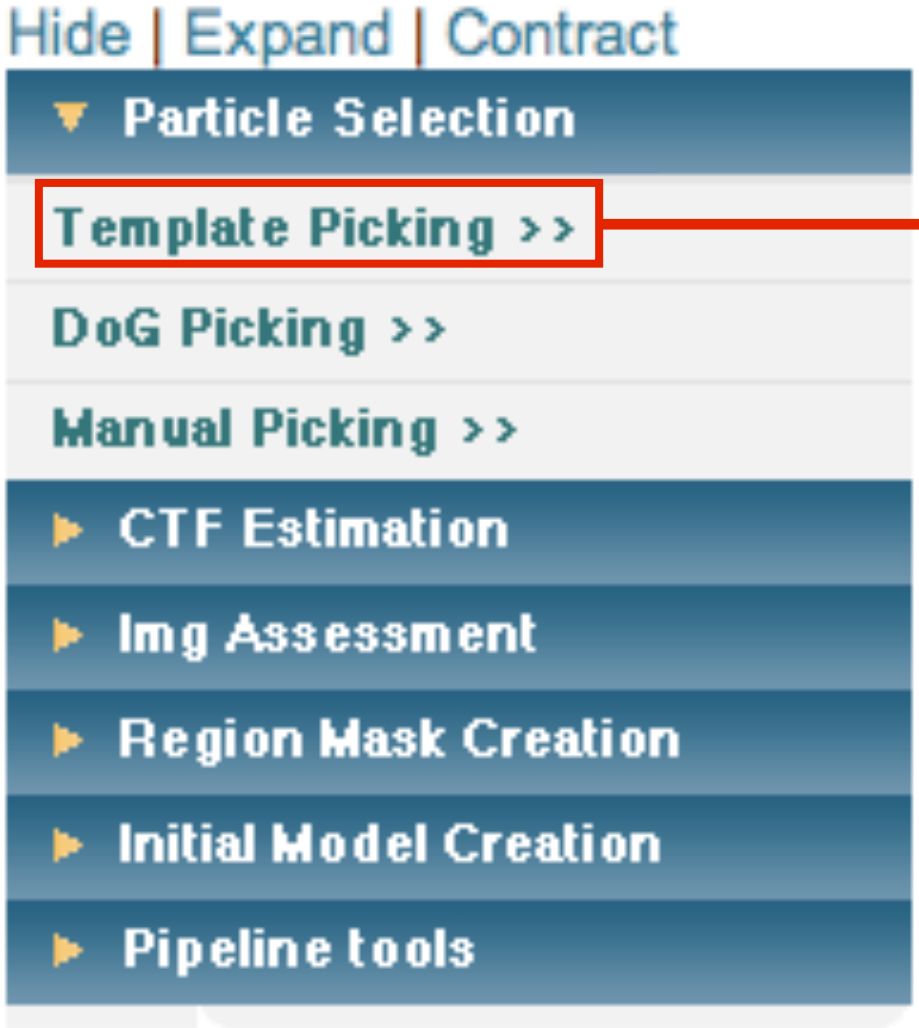
all hide next exemplar

mag: 50000 **defocus:** -2.026 µm **pixelsize:** 0.163 nm **dose:** 18.4 $e^{-}/Å^{2}$
08feb14j_00032gr_00076sq_v02_00015hl_00002en.mrc



Display a menu for "javascript:v1btnptclaction(v1nptcl_bt_st)"


Hide | Expand | Contract
Particle Selection
Template Picking >>
DoG Picking >>
Manual Picking >>
CTF Estimation
Img Assessment
Region Mask Creation
Initial Model Creation
Pipeline tools



Cross-correlation based template matching wrapper for "FindEM" by Alan Roseman

"Feature finding" method based on difference of gaussians algorithm by Craig Yoshioka

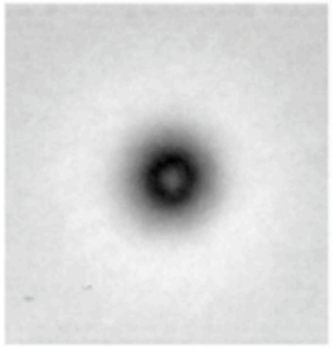
Manually select particles or edit automatically picked particles

Manual or automatic alignment of tilted pairs if RCT/OTR data is collected

Initial models within Appion

- **Particle Alignment**
  - Run Alignment
  - Run Feature Analysis
  - Run Particle Clustering
- **Initial Model Creation**
  - PDB to Model
  - EMDB to Model
  - RCT Volume
  - EMAN Common Lines
  - IMAGIC Common Lines
  - 3d Density Volumes
- **Tomography**
  - Create Full Tomogram
  - Upload Tomogram
  - Create Tomogram Subvolume
  - Average subvolumes
- **Pipeline tools**
  - Upload Template
  - Upload Model

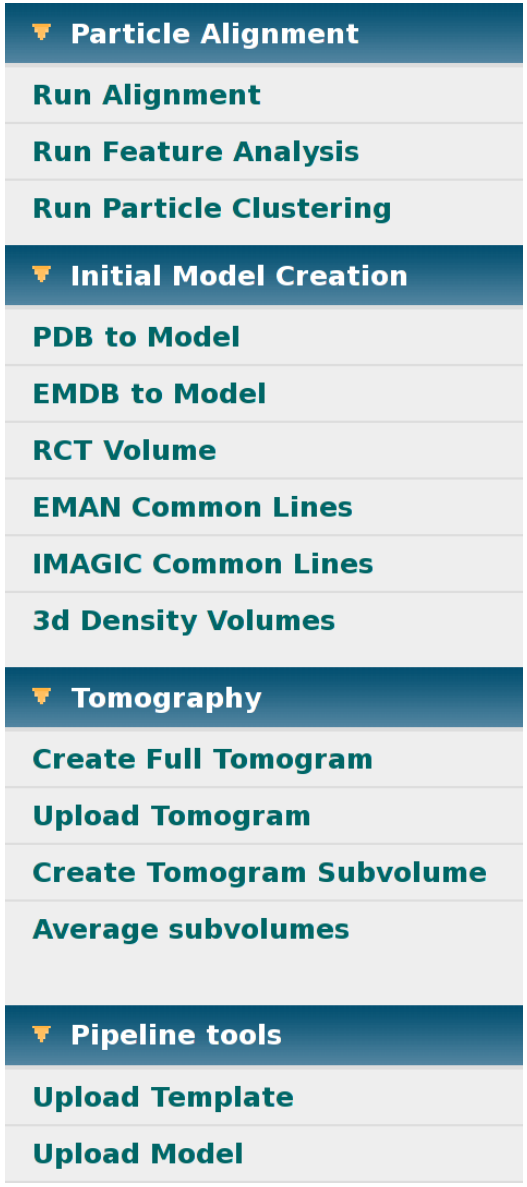
Stack: dogpicked1 (ID: 878) hide



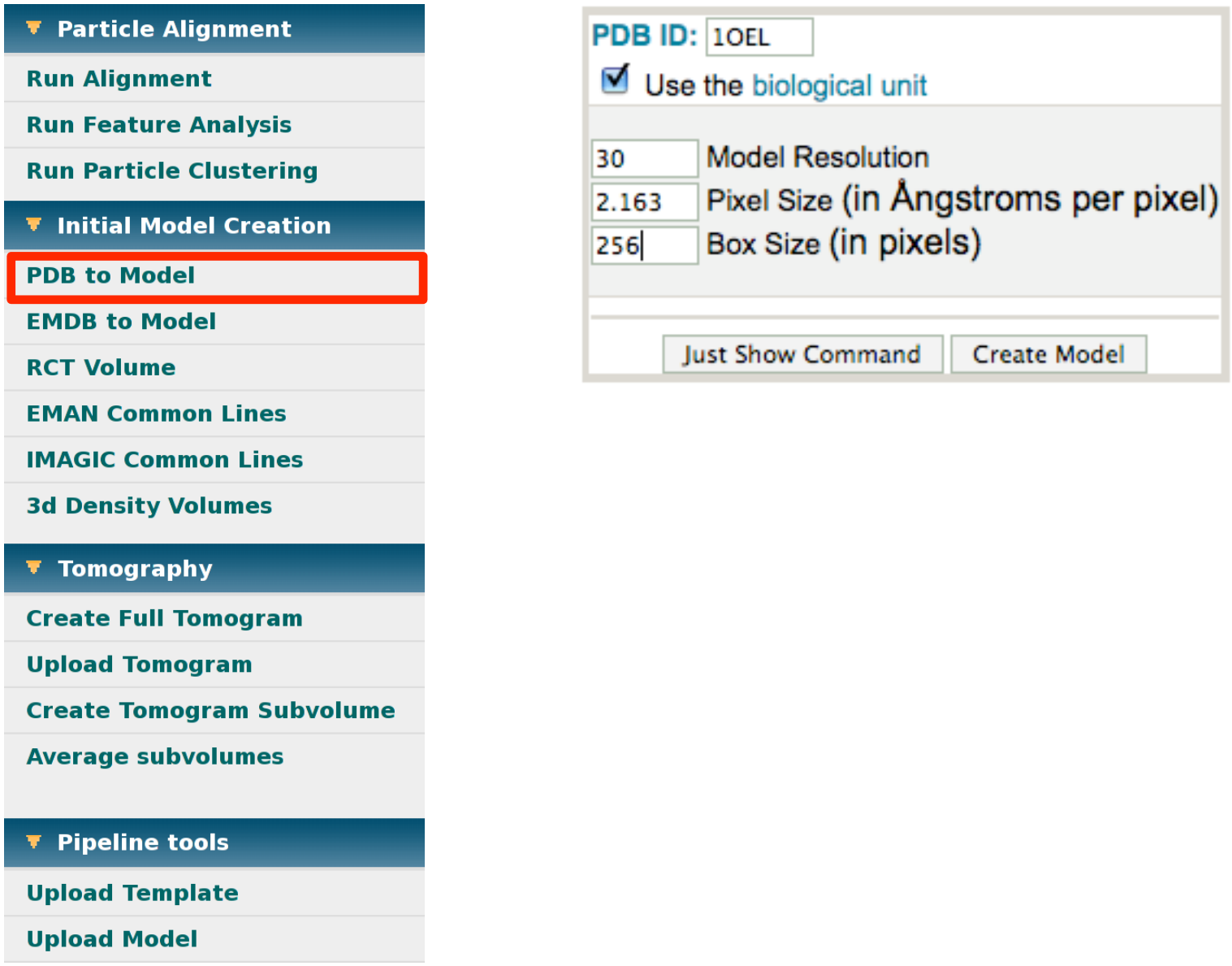
averaged stack image

description:	stack for ref-free classification edit
# prtls:	11,046
path:	/ami/data15/appion/07dec13a/stacks/dogpicked1
name:	start.hed
box size:	192 pixels
pixel size:	3.26 Å/pixel
phase flipped:	Yes
density:	dark on light background
normalization:	On
file type:	imagic

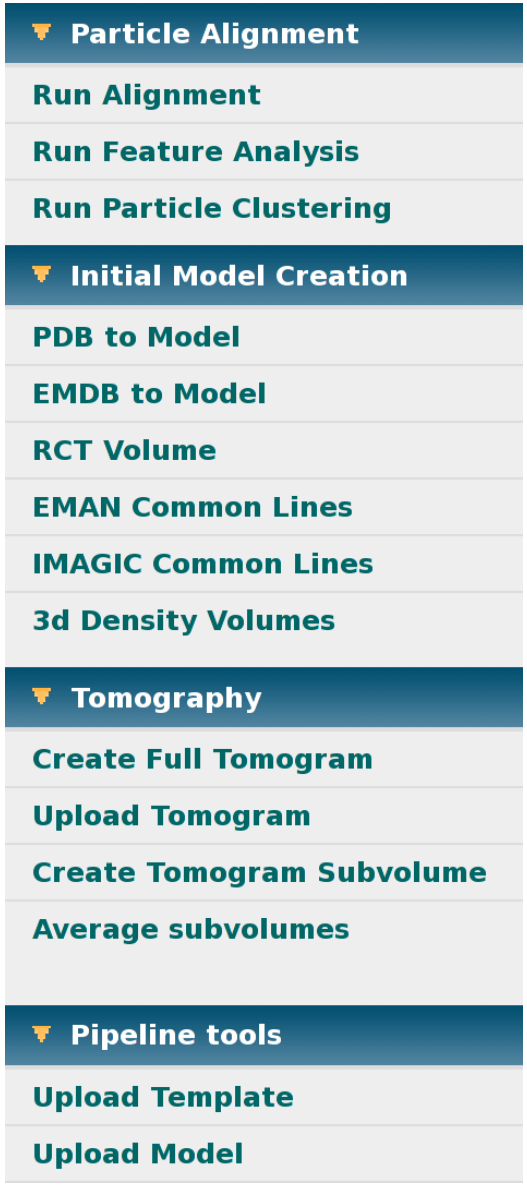
Initial models within Appion



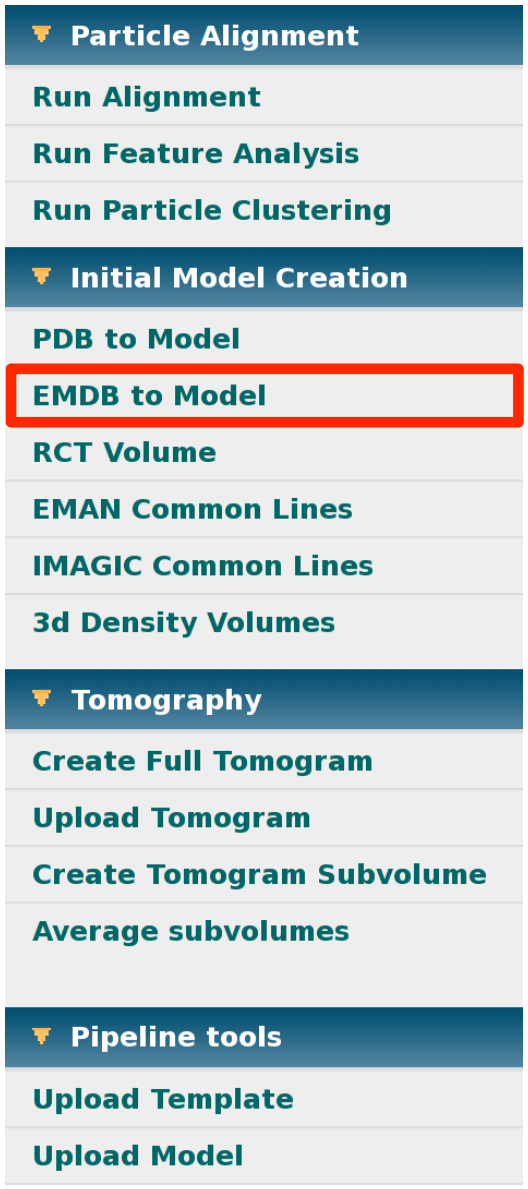
Initial models within Appion

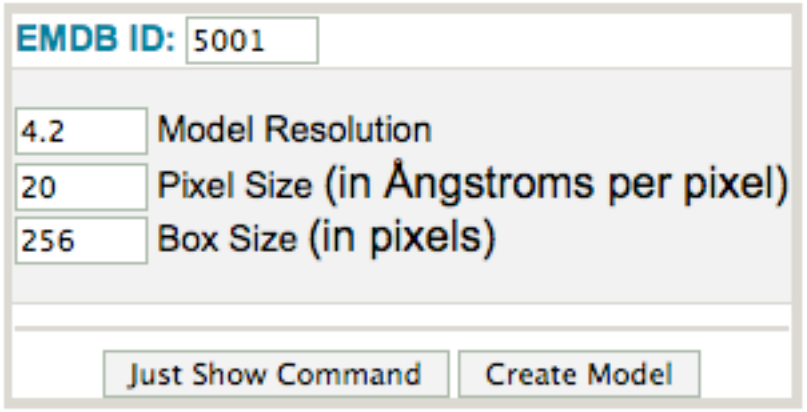


Initial models within Appion



Initial models within Appion

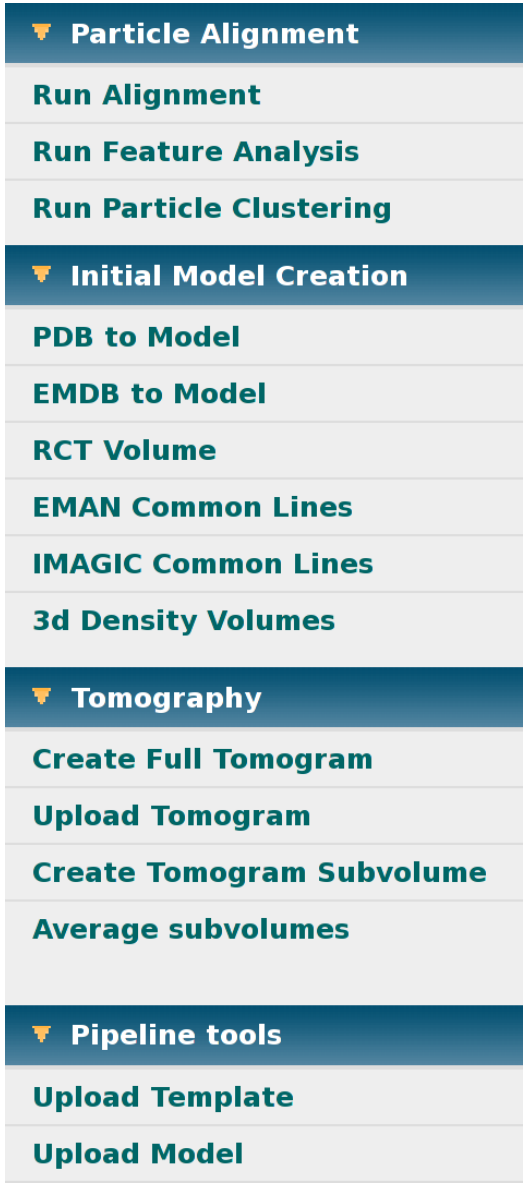




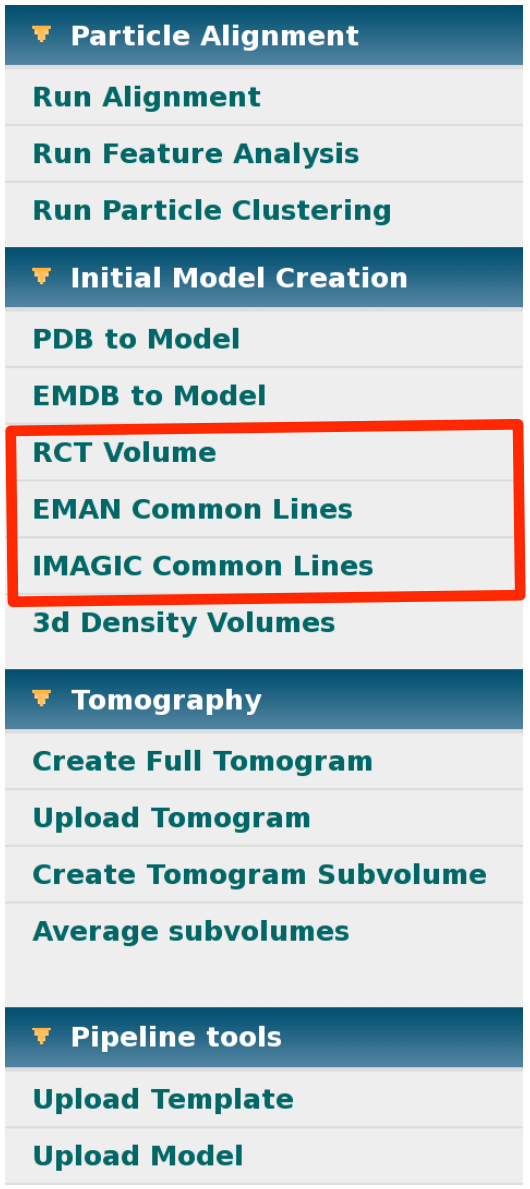
DownloadModel Command:
modelFromEMDB.py --projectid=159 --runname=1OEL_09feb10p03 -e 1OEL -s 08nov02a -a 2.163.0 -r 30.0 -b 256

EM Density file to be created:
/ami/data15/appion/08nov02a/models/emdb/1OEL_09feb10p03/1OEL-2.163.0-30.0-256.mrc

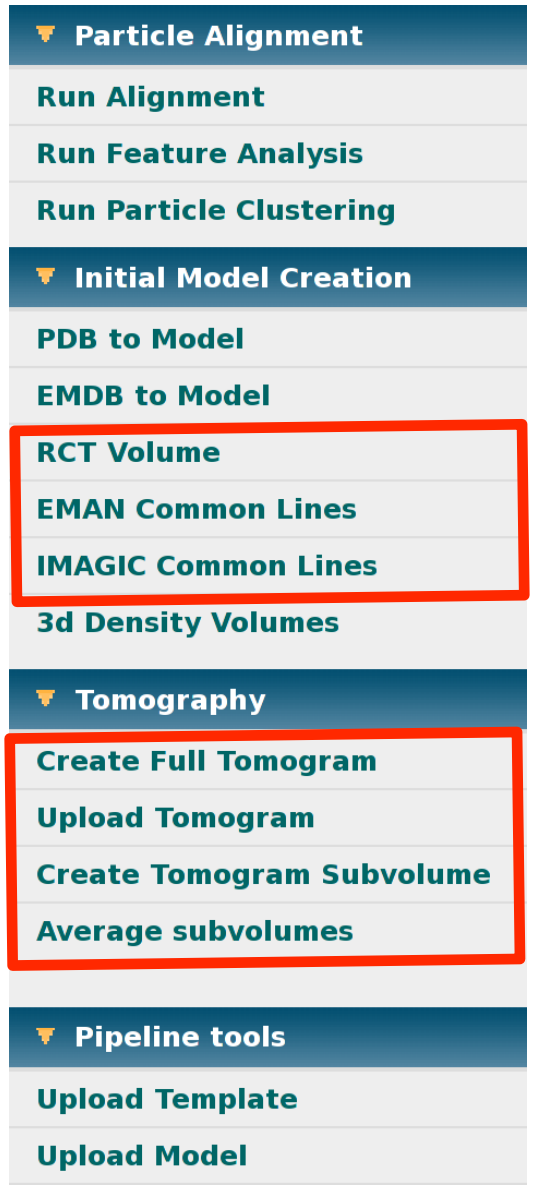
Initial models within Appion



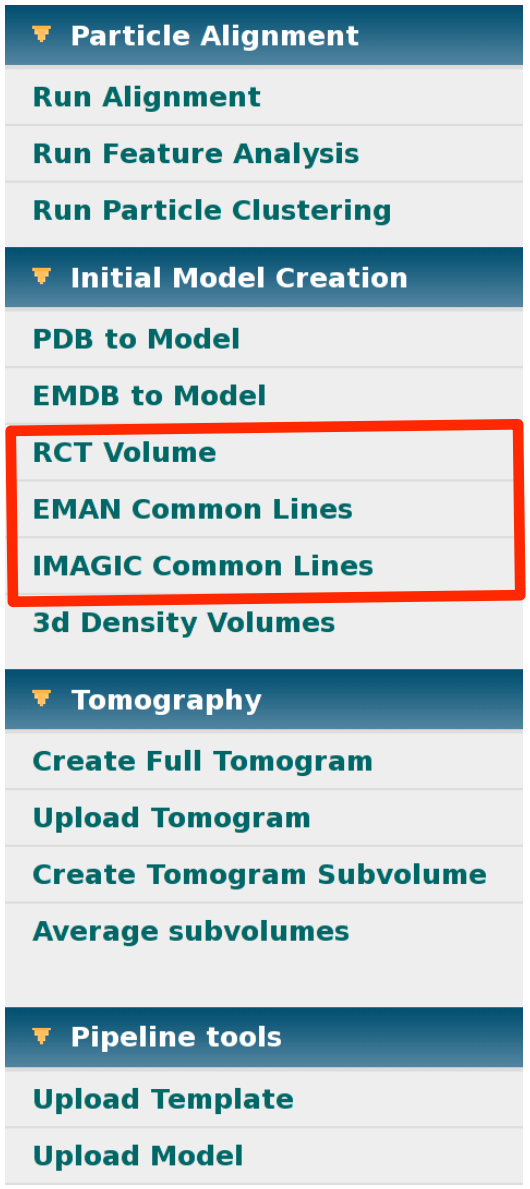
Initial models within Appion



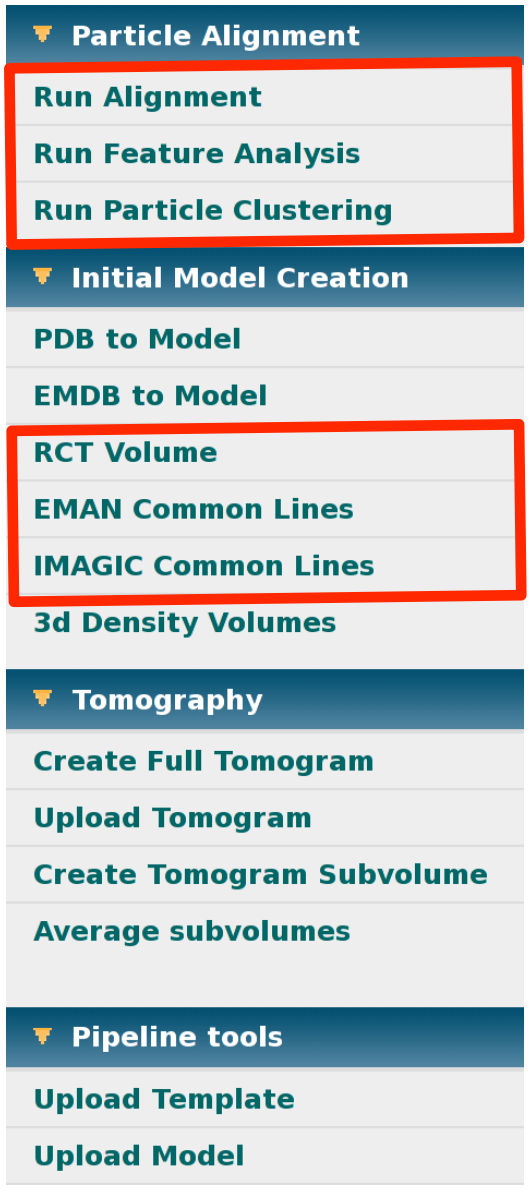
Initial models within Appion



Initial models within Appion



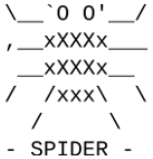
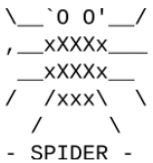
Initial models within Appion



Alignment and Classification Pipeline

- Particle Selection : 5
- CTF Estimation : 2
- Stacks : 7
- Particle Alignment
  - **Run Alignment**
    2 complete
  - **Run Feature Analysis**
    4 complete
  - **Run Particle Clustering**
    18 complete
  - **Template Stacks**
    6 complete
- Initial Model Creation
- Reconstructions
- Pipeline tools
- Img Assessment : 1/189
- Region Mask Creation
- Synthetic Data

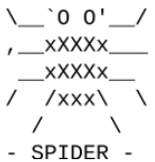
Particle Alignment Procedures

	Xmipp Maximum Likelihood Alignment
	Spider Reference-based Alignment
	IMAGIC Multi Reference Alignment (MRA)
	Ed's Iteration Alignment
	Spider Reference-free Alignment

Alignment and Classification Pipeline

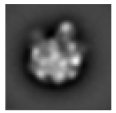
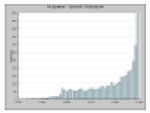
- Particle Selection : 5
- CTF Estimation : 2
- Stacks : 7
- Particle Alignment
  - **Run Alignment**
    2 complete
  - **Run Feature Analysis**
    4 complete
  - **Run Particle Clustering**
    18 complete
  - **Template Stacks**
    6 complete
- Initial Model Creation
- Reconstructions
- Pipeline tools
- Img Assessment : 1/189
- Region Mask Creation
- Synthetic Data

Feature Analysis Procedures

	Spider Coran Classification
	Xmipp Kerden Self-Organizing Map
IMAGIC	IMAGIC Multivariate Statistical Analysis (MSA)

Alignment and Classification Pipeline

Align Stack Info: maxlike7 (ID: 110) hide

avg image spread distribution

date time:	2009-06-22 17:30:22
description:	quick alignment for poster edit
size:	9,026 particles (178.5 MB)
original stack:	1444
reference stack:	part09jun22r12_average.hed
pixel / box size:	4.64 Å/pixel and 72 pixels
stack file:	/ami/data16/appion/09feb12b/align/

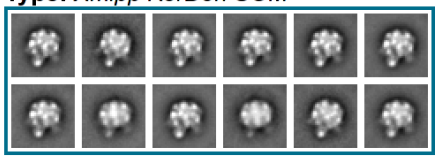
Clustering Info: coran5 (ID: 79) with 3 clusters

Type: *SPIDER Coran*
Method: *hierarch*
Factor list: *4,8,9,10,11,13,16*

- 64 Class Averages [variance] (ID 194)
- 16 Class Averages [variance] (ID 193)
- 4 Class Averages [variance] (ID 192)

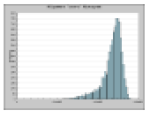
Clustering Info: kerden5 (ID: 78) with 1 clusters

Type: *Xmipp KerDen SOM*



View Montage View as Stack

Align Stack Info: refbased3 (ID: 59) hide

avg image score distribution

date time:	2009-02-19 11:30:08
description:	more accurate alignment edit
size:	9,026 particles (178.5 MB)
original stack:	1444
reference stack:	templatestack03.hed
pixel / box size:	4.64 Å/pixel and 72 pixels
stack file:	/ami/data16/appion/09feb12b/align/re

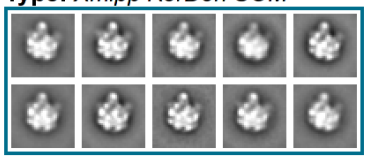
Clustering Info: coran4 (ID: 57) with 3 clusters

Type: *SPIDER Coran*
Method: *hierarch*
Factor list: *1,2,3*

- 64 Class Averages [variance] (ID 139)
- 16 Class Averages [variance] (ID 138)
- 4 Class Averages [variance] (ID 137)

Clustering Info: kerden2 (ID: 45) with 1 clusters

Type: *Xmipp KerDen SOM*



View Montage View as Stack

Xmipp Maximum Likelihood Alignment
SPIDER Correspondence Analysis
Xmipp KerDen Self-Organizing Map

SPIDER Reference-based Alignment
SPIDER Correspondence Analysis
Xmipp KerDen Self-Organizing Map

Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

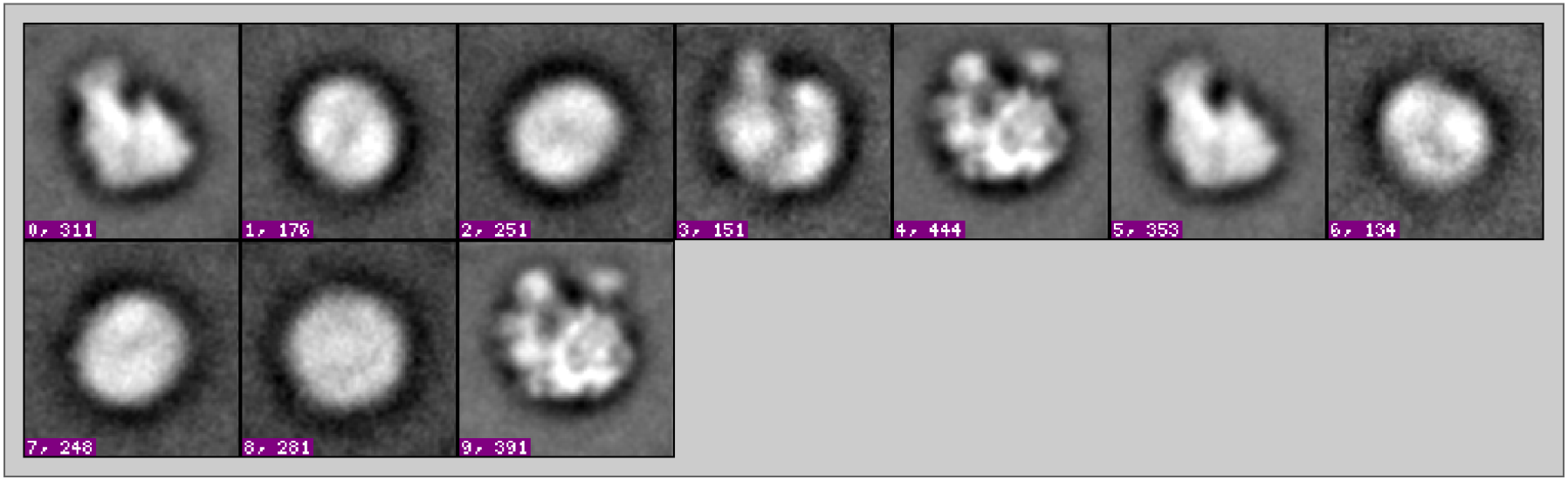
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

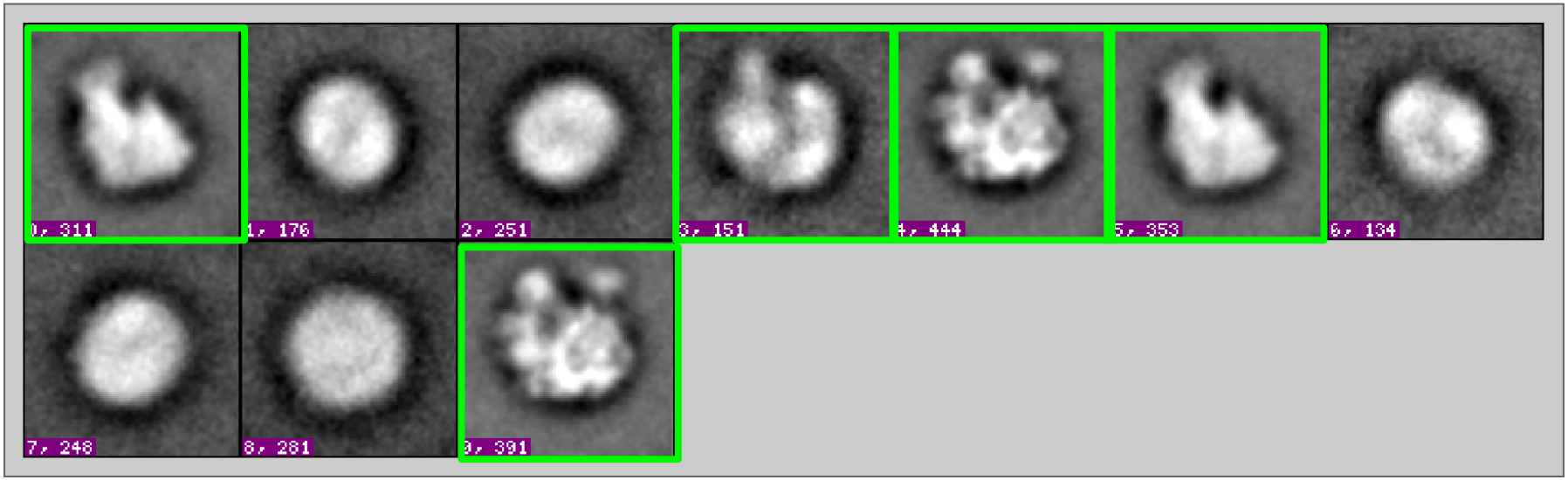
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

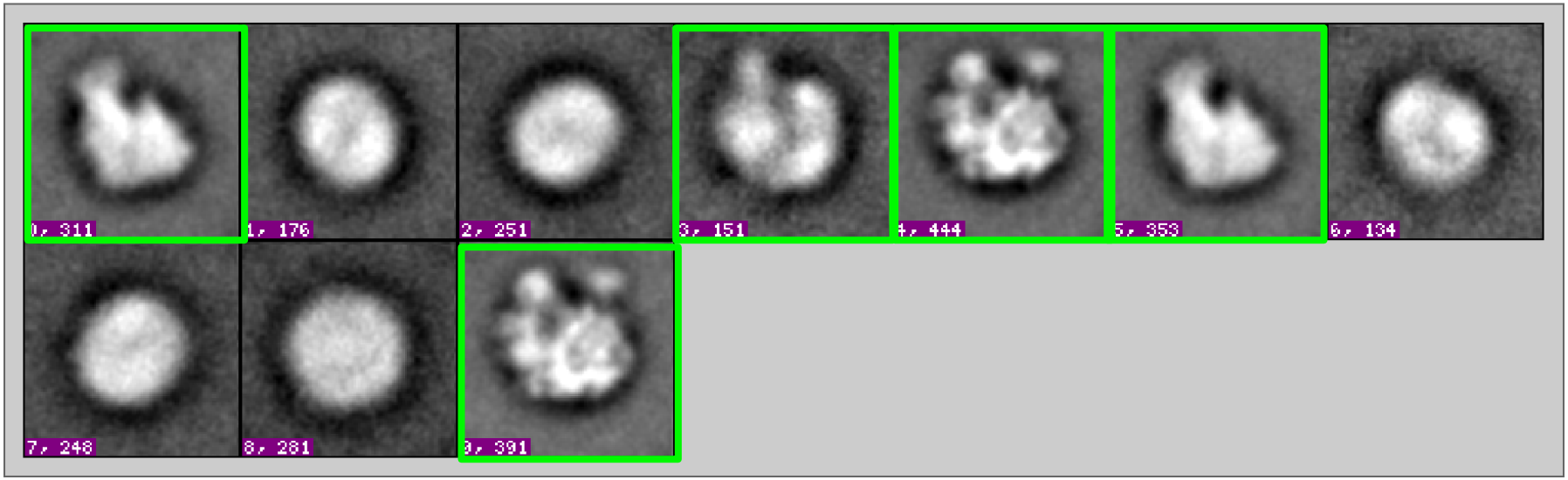
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

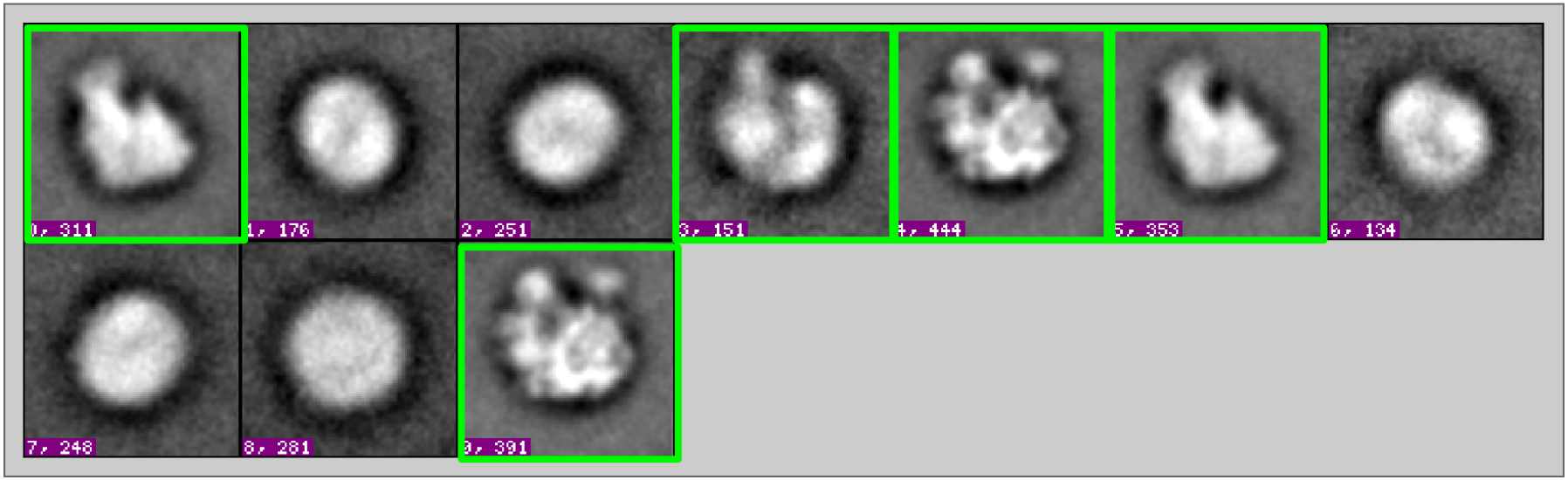
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

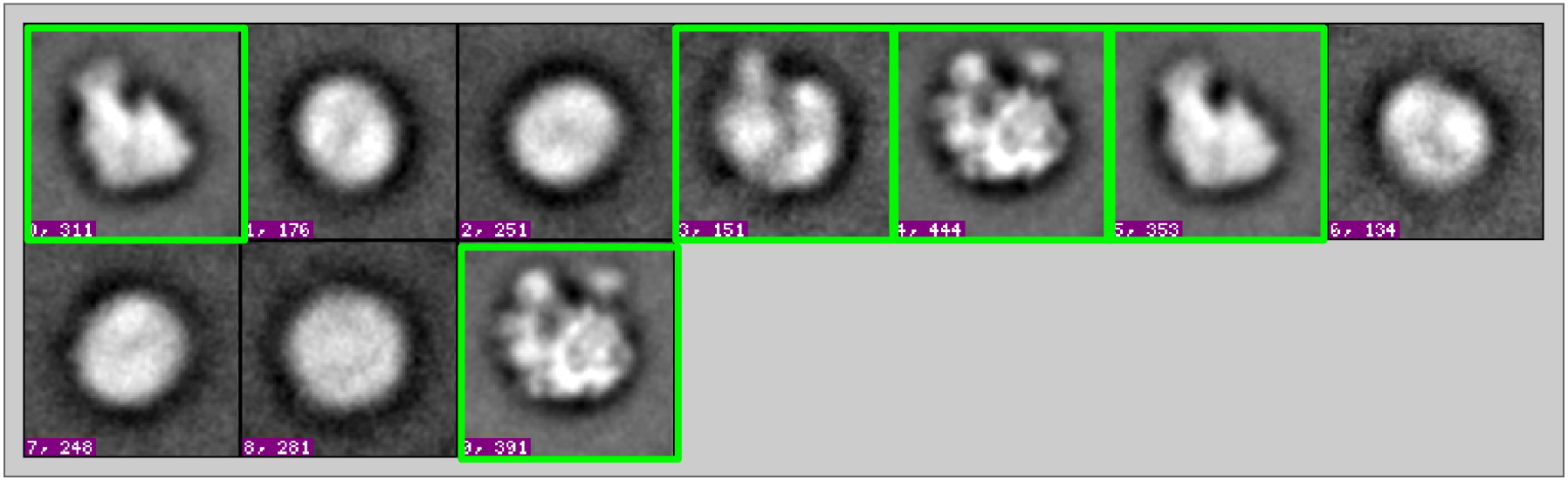
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

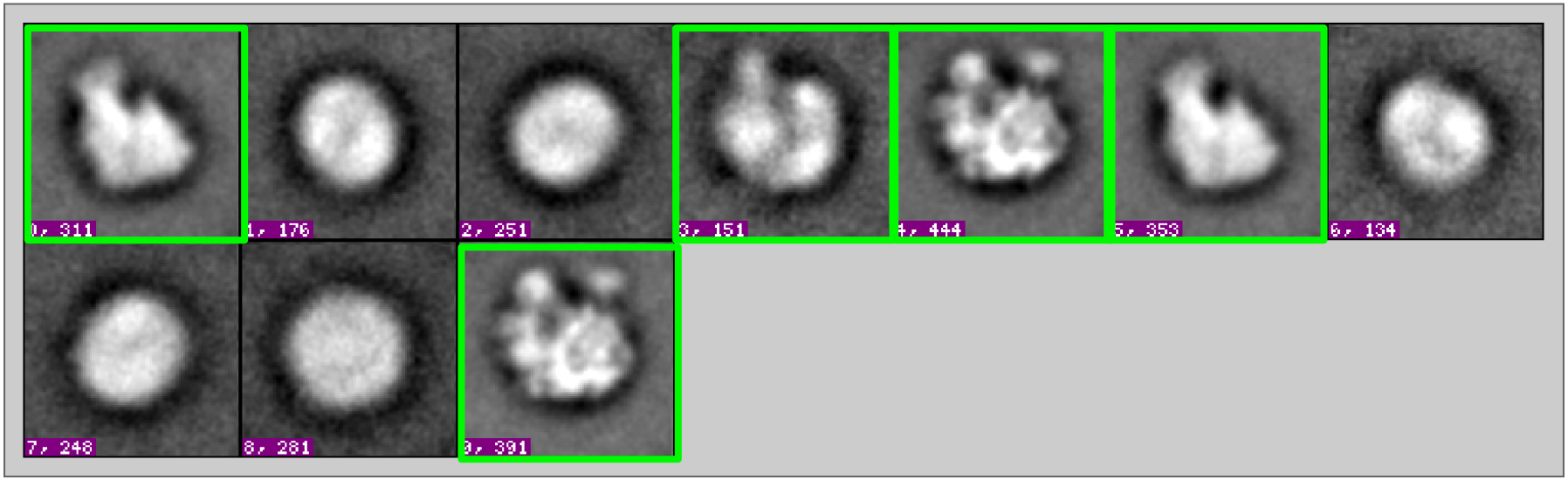
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

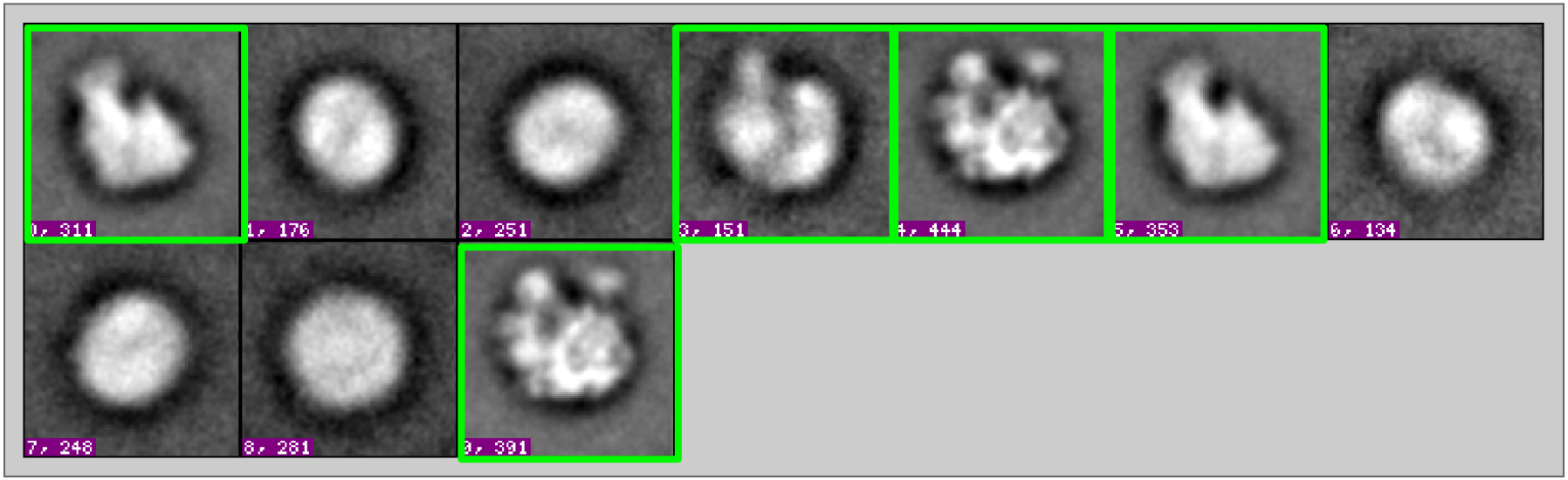
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

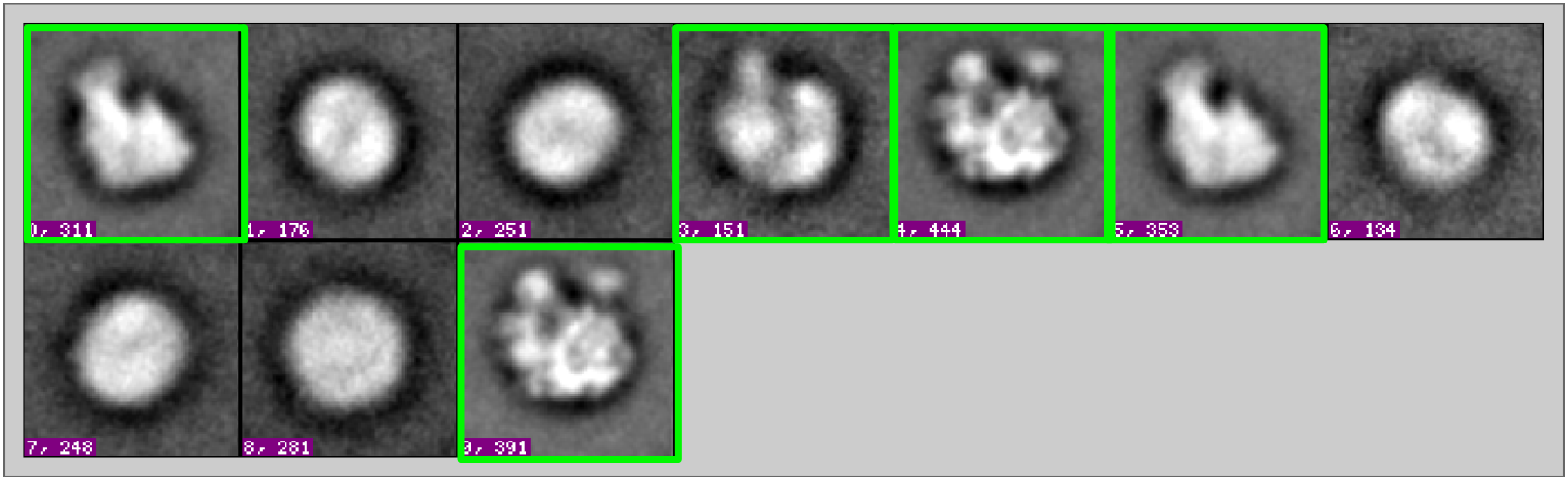
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Use classification for more tasks

stack: /ami/data00/appion/09jun11a/align/kerden2/kerdenstack09jun18r29.hed
#images: 10
from: 0 to: 9 binning: 1 quality: png Load

Selection mode: select

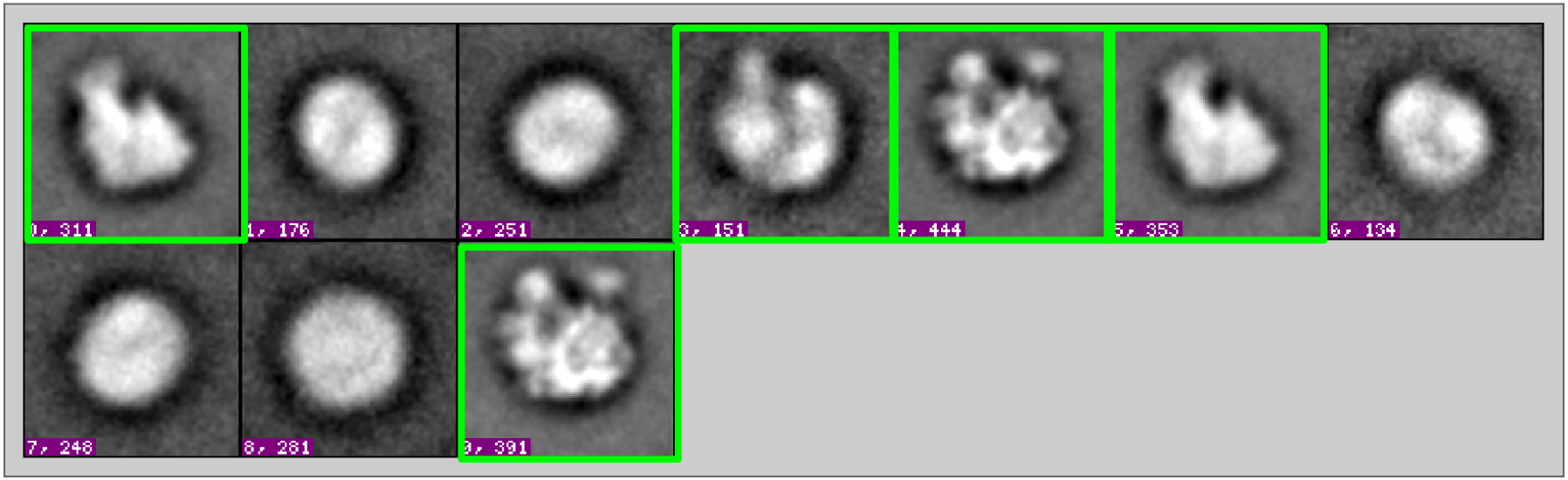
Excluded images: 1,2,6,7,8 Create SubStack

Create Template Stack | Run Common Lines

Selected images: 0,3,4,5,9 Create SubStack | Create Template Stack

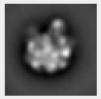
Create RCT Volume | Create OTR Volume | Create Templates | Run Imagic 3d0

Run Common Lines | View Raw Particles



Random Conical Tilt (RCT)

Align Stack Info: maxlike7 (ID: 110) hide


avg image


spread distribution

date time:	2009-06-22 17:30:22
description:	quick alignment for poster edit
size:	9,026 particles (178.5 MB)
original stack:	1444
reference stack:	part09jun22r12_average.hed
pixel / box size:	4.64 Å/pixel and 72 pixels
stack file:	/ami/data16/appion/09feb12b/align/maxlike7/alignstack.hed

Class Numbers to generate volume:
Selected class numbers '**0**'

Tilted Stack Id:
1475: faketiltstack7 (216 boxsize, 9,163 parts) tilted stack for RCT...

Mask Radius:
(in pixels)

Volume Median Filter:
3 (in pixels)

Volume Low Pass Filter:
10 (in Ångstroms)

Particle High Pass Filter:
800 (in Ångstroms)

Min Score/Spread cutoff:
(see graph)

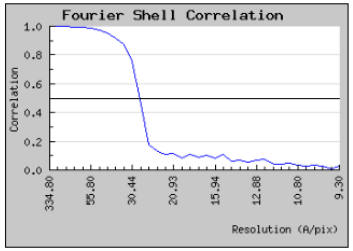
Number of Particle centering iterations:
3

Chimera snapshot settings:
Contour: 3.0 *Zoom:* 0.9 *Mass:* kDa

Just Show Command | Rct Volume

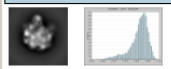
RCT Run: rct9align93class0 (ID: 12)

Number of Particles:	8,039 of 9,163
Pixel size:	1.55 Å
Box size:	216 pixels
Mask radius:	100 pixels
Volume Lowpass filter:	10 Å
Particle Highpass filter:	1500 Å
Median filter:	3
Class numbers:	('0', '0')
Path name:	/ami/data16/appion/09feb12b/rctvolume/rct9align93class0
Description:	improved alignment volume edit
FSC Resolution:	28.19 Å
Rmeasure Resolution:	4.62 Å



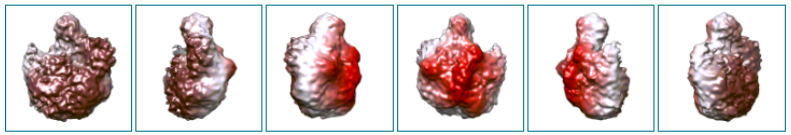
Alignment information

Align Stack Info: refbased4 (ID: 93) hide

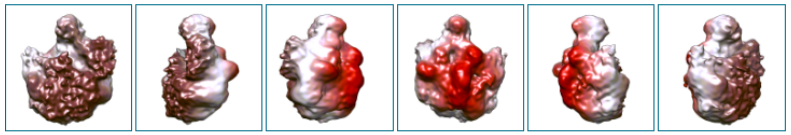

avg image score distribution

date time:	2009-05-11 10:37:11
description:	alignment with fixed iteration updates edit
size:	9,026 particles (1.57 GB)
original stack:	1444
reference stack:	templatestack03.hed
pixel / box size:	1.55 Å/pixel and 216 pixels
stack file:	/ami/data16/appion/09feb12b/align/refbased4/aligned.hed

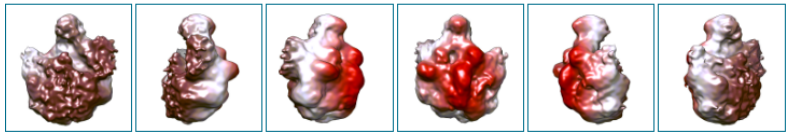
RCT Run, *iteration 0*



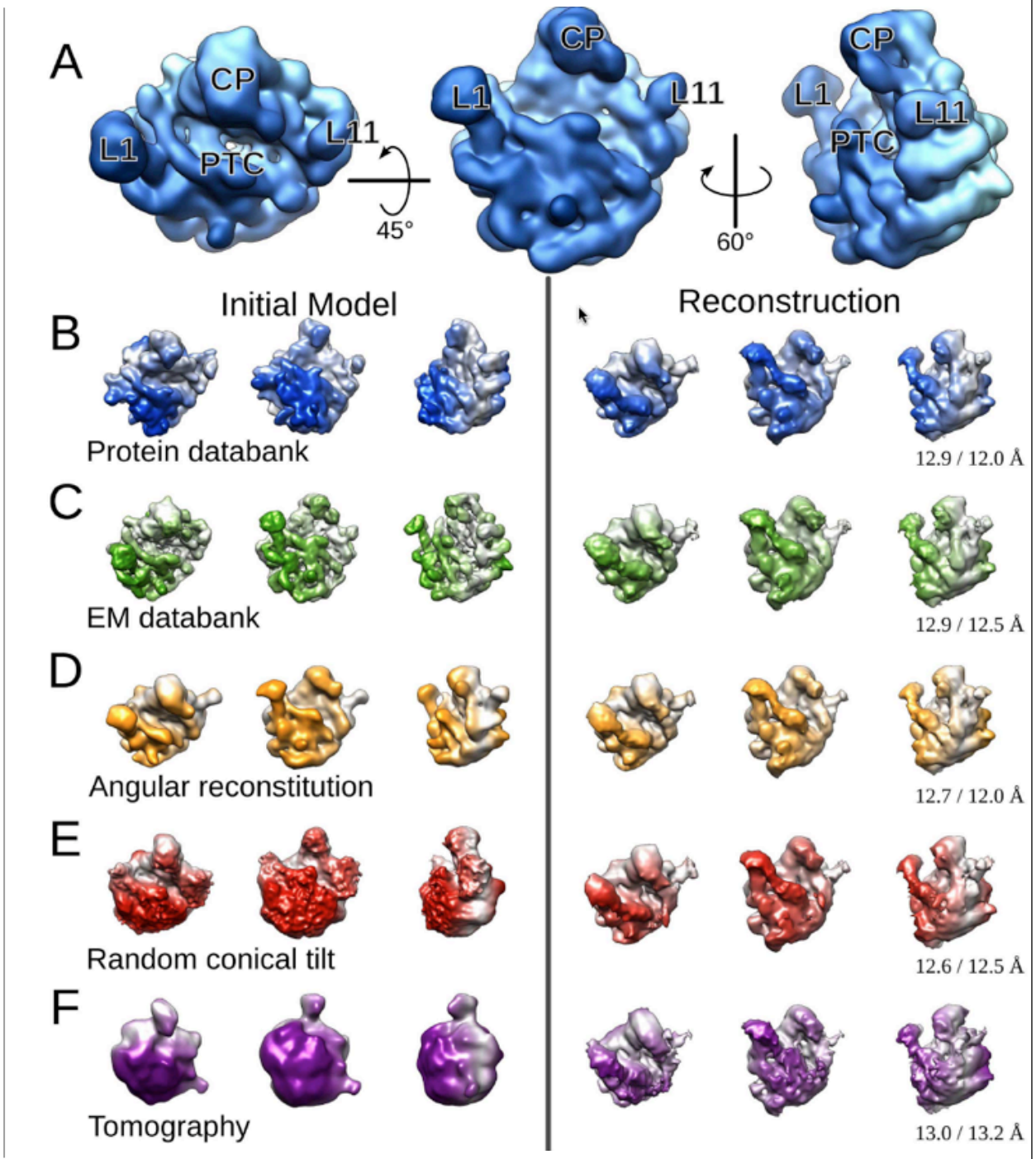
RCT Run, *iteration 1*



RCT Run, *iteration 2*



- Initial models can be calculated using a variety of independent methods
- The toolbox facilitates and largely automates all procedures
- This is helpful for determining the reliability of the results



- Initial models can be calculated using a variety of independent methods
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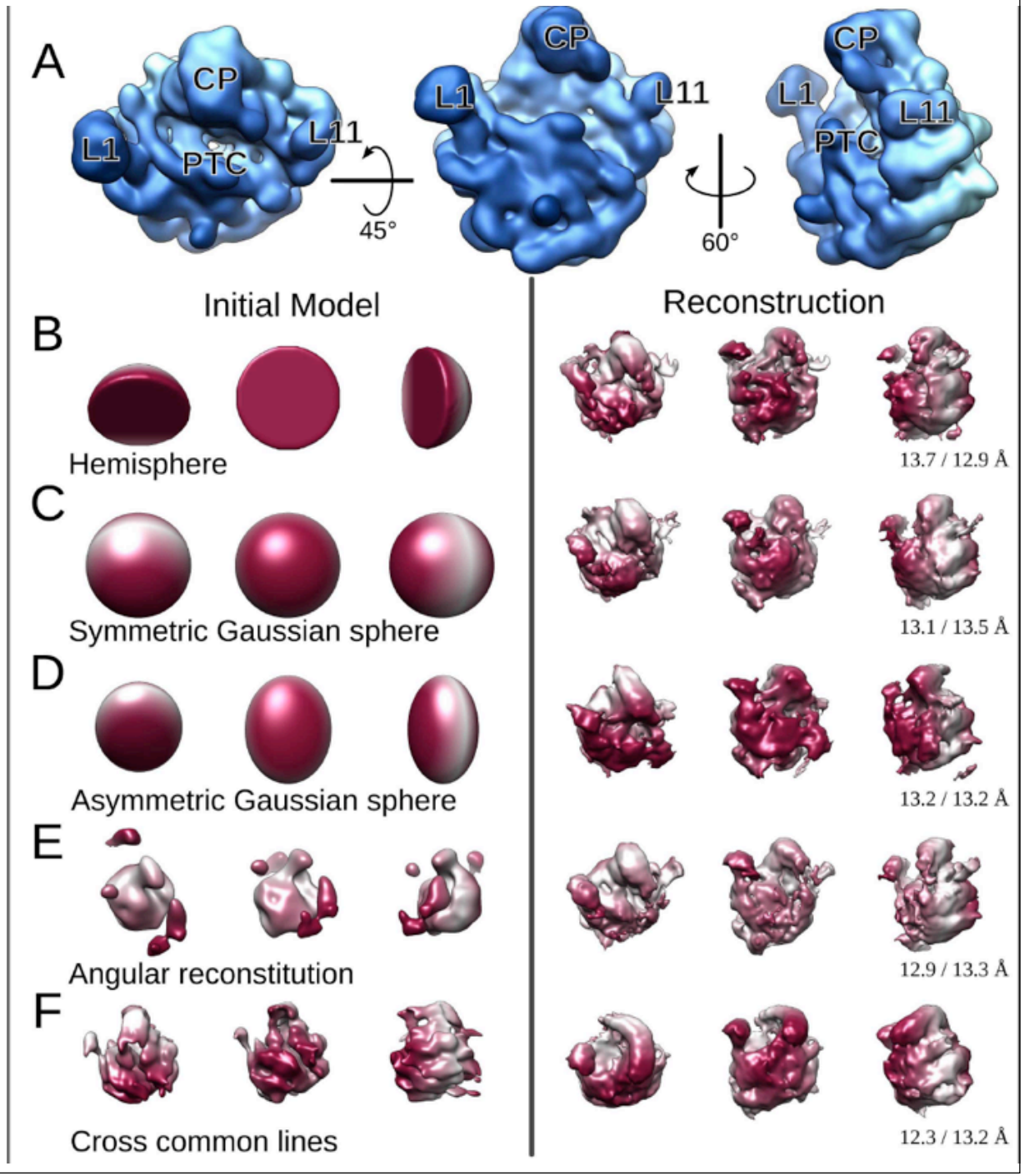


Cluster Job Status

Project: Lander - P22
Session: 08feb14j - tail machines 3rd data set
Image Path: /ami/data15/leginon/08feb14j/rawdata

Hide | Expand | Contract

- ▸ Particle Selection : 2
- ▸ CTF Estimation : 1
- ▸ Img Assessment : 592/592
- ▸ Region Mask Creation
- ▸ Stacks : 2
- ▸ Particle Alignment : 1
- ▾ Reconstructions
  - EMAN Reconstruction
    - 1 running
  - Upload Reconstruction
- ▾ Pipeline tools
  - Upload template >>
    - 3 available
  - Upload model >>
    - 1 available

Check Jobs in Queue

Job: tailrecon.job (ID: 466)

abort job

name:	tailrecon.job
appion path:	/ami/data15/appion/08feb14j/recon/tailrecon
dmf path:	/home/glander/appion/08feb14j/recon/tailrecon
cluster path:	~glander/appion/08feb14j/recon/tailrecon
cluster:	garibaldi
status:	Running

Iteration 1 finished in 12 hrs 51 min, resolution: 14.81 Å
Iteration 2 finished in 12 hrs 21 min, resolution: 13.42 Å
Iteration 3 finished in 11 hrs 39 min, resolution: 12.74 Å

Processing iteration 4 of 9

reconstruction step	started	duration	status
creating projections	Jun 11, 2008 22:58:49	28 sec	Done
classifying particles	Jun 11, 2008 22:59:17	7 hrs 33 min	Done
iterative class averaging	Jun 12, 2008 06:32:25	1 hr 52 min	Done
creating 3d model	Jun 12, 2008 08:24:52	8 hrs 13 min	running

Reconstruction Report Page

logged in as glander [Log Out]

Project: NRAMM - GroEL 100K [Appion Stats]
Session: 06jul12a - GroEL 100K at 100K at 120KeV
Image Path: /ami/data08/leginon/06jul12a/rawdata

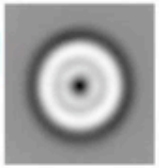
Hide | Expand | Contract

- Particle Selection : 10
  - Template Picking
  - DoG Picking
  - Manual Picking
- CTF Estimation : 18
  - ACE Estimation
    - 1 complete
  - ACE 2 Estimation
    - 1 complete
  - CtfTilt Estimation
- Stacks : 24
  - Stack creation
    - 6 complete
  - more stack tools
- Particle Alignment
  - Run Alignment
  - Template Stacks
- Initial Model Creation
  - PDB to Model
  - EMDB to Model
  - EMAN Common Lines
  - IMAGIC Common Lines
- Reconstructions : 29
  - EMAN Reconstruction
    - 2 running
    - 23 ready for upload

recon info

id	388
name	recon49
description	GroEL, 77465 particles, w/ D7 symmetry, 'classiter=0' for 2 additional rounds
path	/ami/data15/appion/06jul12a/recon/recon49
refine package	EMAN
best resolution	5.31 / 7.27 Å (10)
median euler jump	5.56 ± 12.0

Stack Info: stack **(ID: 207)**



average

date time: 2007-06-23 09:01:18
description: 120kv 100kx combined stack from session 06mar09a and 06jul12a
size: 77,465 parts / 7.02 GB
path: /ami/data13/appion/06mar09a_06jul12a/stack
stack file: start.hed
pixel / box size: 156 pixels / 1.63 Å/pixel

initial model info

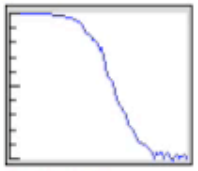
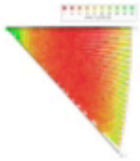
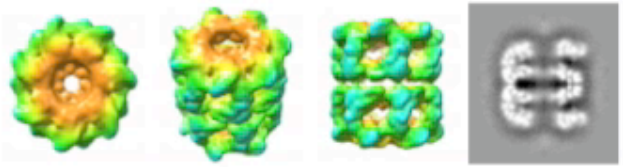
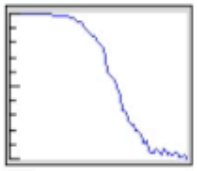
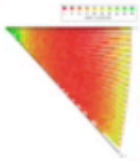
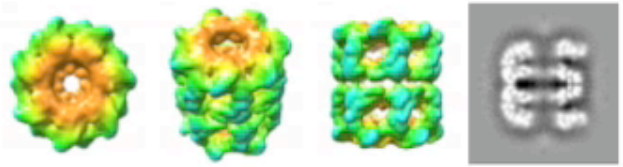
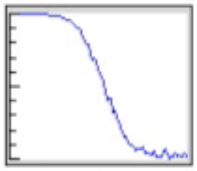
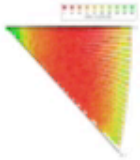
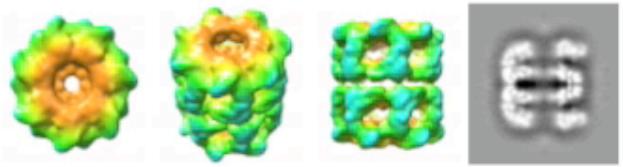
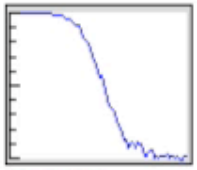
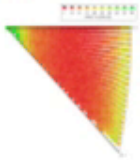
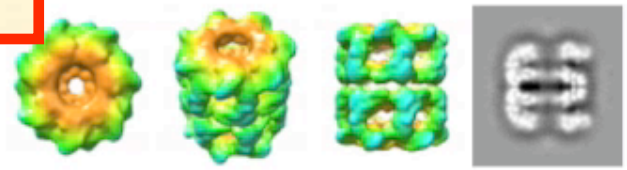
id	4
description	groel low pass filtered to 30 angstroms resolution
path	/ami/data13/appion/06jul12a/refine/logsplit2/run100/threed.0a.mrc
symmetry	D7 (z), 7-fold symmetry along the z axis, 2-fold rotational axis 90 degrees from z
pixel/box size	1.63 Å/pixel; 156 pixels

Compare Iterations: Eulers Iteration 1: 1 Iteration 2: 1

download: compare

Show Common Particles Between Iterations: Bad by EMAN refine From Iteration: 1 To: 1 Show Common Particles

		FSC 0.5: 6.12 *Rmeas:* 7.06	Polar	[12690-bad]	exemplar	
9	1°	*FSC 0.5:* 5.48 *Rmeas:* 7.27	1304 classes classes_eman.9.img Polar	EMAN [62277-good] [15188-bad]	threed.9a.mrc [post processing] [new averages] exemplar	
10	1°	*FSC 0.5:* 5.31 *Rmeas:* 7.27	1304 classes classes_eman.10.img Polar	EMAN [62185-good] [15280-bad]	threed.10a.mrc [post processing] [new averages] exemplar	
11	1°	*FSC 0.5:* 6.01 *Rmeas:* 7.27	1304 classes classes_eman.11.img Polar	EMAN [64667-good] [12798-bad]	threed.11a.mrc [post processing] [new averages] exemplar	
12	1°	*FSC 0.5:* 6.01 *Rmeas:* 7.27	1304 classes classes_eman.12.img Polar	EMAN [64695-good] [12770-bad]	threed.12a.mrc [post processing] [new averages] exemplar	

		FSC 0.5: 6.12 *Rmeas:* 7.06	Polar	[12690-bad]	exemplar	
9	1°	 *FSC 0.5:* 5.48 *Rmeas:* 7.27	1304 classes classes_eman.9.img  Polar	EMAN [62277-good] [15188-bad]	threed.9a.mrc [post processing] [new averages] exemplar	
10	1°	 *FSC 0.5:* 5.31 *Rmeas:* 7.27	1304 classes classes_eman.10.img  Polar	EMAN [62185-good] [15280-bad]	threed.10a.mrc [post processing] [new averages] exemplar	
11	1°	 *FSC 0.5:* 6.01 *Rmeas:* 7.27	1304 classes classes_eman.11.img  Polar	EMAN [64667-good] [12798-bad]	threed.11a.mrc [post processing] [new averages] exemplar	
12	1°	 *FSC 0.5:* 6.01 *Rmeas:* 7.27	1304 classes classes_eman.12.img  Polar	EMAN [64695-good] [12770-bad]	[post processing] [new averages] exemplar	

Appion Data Processing

logged in as glander [Log Out]

Project: NRAMM - GroEL 100K [Appion Stats]
Session: 06jul12a - GroEL 100K at 100K at 120KeV
Image Path: /ami/data08/leginon/06jul12a/rawdata

Hide | Expand | Contract

- Particle Selection : 10
  - Template Picking
  - DoG Picking
  - Manual Picking
- CTF Estimation : 18
  - ACE Estimation
    - 1 complete
  - ACE 2 Estimation
    - 1 complete
  - CtfTilt Estimation
- Stacks : 24
  - Stack creation
    - 6 complete
  - more stack tools
- Particle Alignment
  - Run Alignment
  - Template Stacks
- Initial Model Creation
  - PDB to Model
  - EMDB to Model
  - EMAN Common Lines
  - IMAGIC Common Lines
- Reconstructions : 29
  - EMAN Reconstruction
    - 2 running
    - 23 ready for upload

Exemplar Iteration ID: 4877

CTF info

report »

	name	nb	min	max	avg	stddev
nominal	en	1756	-5.330 µm	-0.801 µm	-1.392 µm	0.367 µm
defocus1	en	1756	0.412 µm	5.594 µm	0.813 µm	1.672 µm
	fc	390	0.908 µm	10.000 µm	2.081 µm	0.422 µm
confidence	en	1756	6.385×10^{-3}	0.986	0.794	0.226
	fc	390	6.871×10^{-3}	0.889	0.761	9.495×10^{-2}
confidence_d	en	1756	1.639×10^{-2}	0.986	0.822	0.196
	fc	390	5.181×10^{-2}	0.883	0.764	9.234×10^{-2}
difference	en	1756	2.106×10^{-9}	4.443×10^{-6}	-5.737×10^{-7}	1.719×10^{-6}
	fc	390	8.233×10^{-11}	8.000×10^{-6}	8.131×10^{-8}	4.224×10^{-7}

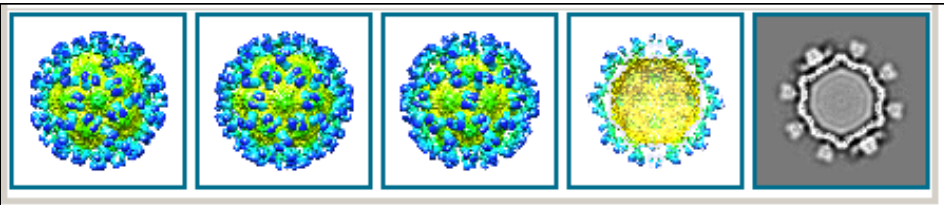
Particle Selection info

Inpected images: 605, [inspected data]

name	dbid	totparticles	numimgs	min	max	avg	stddev
test1	249	34082	561 (60.8 prtl/img)	0.3500	0.5473	0.4023	3.6425×10^{-2}

Inpected images: 605, [inspected data]

name	dbid	totparticles	numimgs	min	max	avg	stddev
run4	279	79890	1703 (46.9 prtl/img)	0.5000	0.7309	0.5736	4.7857×10^{-2}



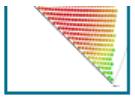
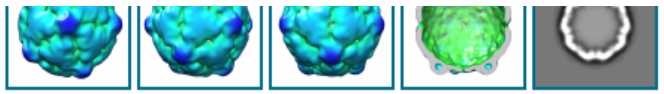
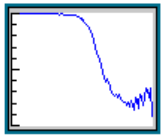
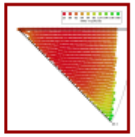
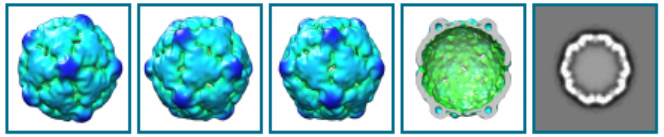
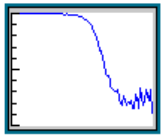
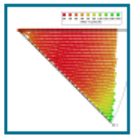
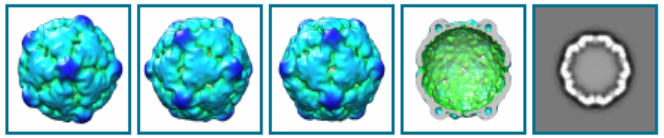
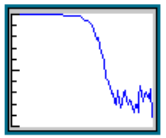
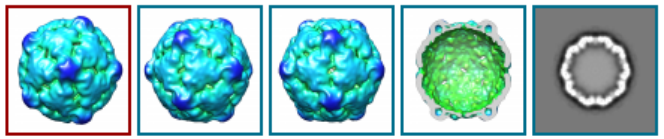
Data Collection

Data were acquired using a Tecnai F20 Twin transmission electron microscope operating at 120 kV, using a dose of ~15 e-/Å² and a nominal underfocus ranging from 1.0 to 2.0 µm. 912 images were automatically collected at a nominal magnification of 50,000X at a pixel size of 0.1547 nm at the specimen level. All images were recorded with a Tietz F415 4k x 4k pixel CCD camera (15 µm pixel) utilizing the Leginon data collection software (Suloway *et al.*, 2005). Experimental data were processed by the Appion software package (Lander *et al.*, 2009), which interfaces with the Leginon database infrastructure. The contrast transfer function (CTF) for each micrograph was estimated using the ACE package (Mallick*et al.*, 2005). 22,333 particles were automatically selected from the micrographs using a template-based particle picker (Roseman, 2003) and extracted at a box size of 384 pixels. Stacked particles were binned by a factor of 1 for the final reconstruction. The final stack contained 21,921 particles. The 3D reconstruction was carried out using the EMAN reconstruction package (Ludtke *et al.*, 1999). Resolution was assessed by calculating the Fourier Shell Correlation (FSC) at a cutoff of 0.5, which provided a value of 7.74 Å resolution. Calculation of the resolution by Rmeasure (Sousa & Gridgorieff, 2007) at a 0.5 cutoff yielded a resolution of 8.14 Å.

- Frank, Radermacher, Penczek, Zhu, Li, Ladjadj, and Leith. (1996). *"SPIDER and WEB: processing and visualization of images in 3D electron microscopy and related fields."* J Struct Biol v116(1): pp. 190-9.
- Lander, Stagg, Voss, Cheng, *et al.*, Potter, and Carragher (2009). *"Appion: an integrated, database-driven pipeline to facilitate EM image processing."* J Struct Biol v166(1): pp. 95-102.
- Ludtke, Baldwin, and Chiu (1999). *"EMAN: semiautomated software for high-resolution single-particle reconstructions."* J Struct Biol v128(1): pp. 82-97.
- Mallick, Carragher, Potter, and Kriegman (2005). *"ACE: automated CTF estimation."* Ultramicroscopy v104(1): pp. 8-29.
- Roseman (2003). *"Particle finding in electron micrographs using a fast local correlation algorithm."* Ultramicroscopy v94(3-4): pp. 225-36.
- Sousa and Grigorieff (2007). *"Ab initio resolution measurement for single particle structures."* J Struct Biol v157(1): pp. 201-10.
- Suloway, Pulokas, Fellmann, Cheng, Guerra, Quispe, Stagg, Potter, and Carragher (2005). *"Automated molecular microscopy: the new Leginon system."* J Struct Biol v151(1): pp. 41-60.
- Voss, Yoshioka, Radermacher, Potter, and Carragher (2009). *"DoG Picker and TiltPicker: tools to facilitate particle selection in single particle electron microscopy."* J Struct Biol v166(2): pp. 205-13.

What's next:

- Continue adding new software

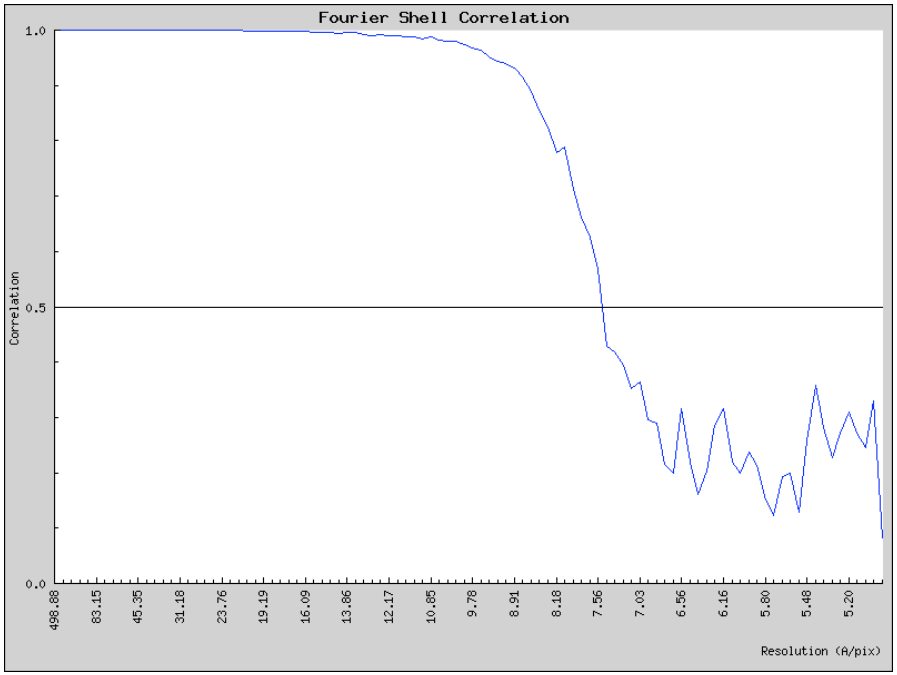
11	1°	 *FSC 0.5:* 8.62 *Rmeas:* 6.10	 Polar	[211058-good] [97419-bad]	Post Processing Remove Jumpers Make Exemplar	
12	0.7°	 *FSC 0.5:* 7.72 *Rmeas:* 142.54	classes_eman.12.img 620 classes  Polar	EMAN [210906-good] [97571-bad]	threed.12a.mrc Post Processing Remove Jumpers Make Exemplar	
13	0.7°	 *FSC 0.5:* 7.50 *Rmeas:* 124.72	classes_eman.13.img 620 classes  Polar	EMAN [210899-good] [97578-bad]	threed.13a.mrc Post Processing Remove Jumpers Make Exemplar	
14	0.7°	 *FSC 0.5:* 7.50 *Rmeas:* 124.72	classes_eman.14.img 620 classes  Polar	EMAN [210965-good] [97512-bad]	threed.14a.mrc Post Processing Remove Jumpers Make Exemplar	

Run Name:
refine29_09nov12m55

Density File: /ami/data17/appion/09sep25a/recon/toomanyrecon2/threed.14a.mrc
Pixel Size: 2.49 Å/pixel

Reported Resolution:

FSC½: 7.50 Å
Rmeasure: 124.72 Å



B-factor sharpening:

☐ Apply sharpening using automated b-factor determination from FSC curve (description)

Apply amplitude correction

○ None

Resolution limit: 4.6
Source: GroEL SAXS data
○ Use this amplitude curve

Resolution limit: 4.808
Source: Wild type CCMV Virus SAXS data collected by Kelly Lee (closed form, RNA-filled)
○ Use this amplitude curve

Resolution limit: 7.854
Source: Experimental X-ray curve, smoothed by Dmitri Svergun
○ Use this amplitude curve

Resolution limit: 11.519
Source: Experimental X-ray curve, smoothed by Dmitri Svergun
○ Use this amplitude curve

UCSF Chimera Snapshot Options:

7.5	Resolution
3.0	Contour Level
	Mass (in kDa)
1.0	Zoom

Low-pass filter:	3.75	Ångstroms
Median filter:	3	Pixels
Radius of outer mask:		Ångstroms
Radius of inner mask:		Ångstroms

☐ Flip handedness of density
☐ Invert the magnitude of the density
☐ Rotate density from EMAN to Viper orientation
☑ Normalize the resulting density

Software

EM-BFACTOR

A package for sharpening high resolution information in single particle electron cryomicroscopy.

J.J. Fernandez [1,2], D. Luque [1], J.R. Caston [1], J.L. Carrascosa [1]
P.B. Rosenthal [3], R. Henderson [3]

[1] Centro Nacional de Biotecnologia - CSIC, Madrid 28049, Spain.

[2] Dept. Computer Architecture. University of Almeria. Almeria 04120. Spain.

[3] MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, UK

Contact: jjfdez_at_ual.es

Description

This package allows sharpening of high resolution information in 3D maps obtained by electron cryomicroscopy according to the approach by Rosenthal and Henderson (JMB 333:721-745, 2003). The program allows automatic determination of the Bfactor for maps at a resolution higher than 10 Angstroms. The program also allows application of the Bfactor found, or a Bfactor value provided by the user, to sharpen the map and compensate the decay of the amplitudes. Before sharpening, the map is weighted in Fourier space by C_{ref}, a function computed from the FSC that represent the correlation between the map and a perfect reference, in order to avoid noise amplification. Furthermore, the program also places the structure factor amplitudes of the map on an absolute scale. The program accepts 3D maps in any format common in EM (e.g. MRC, Spider, Xmipp, PIF, EM, etc) and also accepts FSC curves in the format of most common packages for single particles cryoEM (Frealign, Spider, EMAN, Xmipp, Bsoft). As output, the program produces the sharpened map and a Guinier plot that allows assessment of the sharpening.

A detailed description of the procedures implemented in the package are described in the following articles:

- Optimal Determination of Particle Orientation, Absolute Hand, and Contrast Loss in Single-particle Electron Cryomicroscopy.
  P.B. Rosenthal and R. Henderson
  Journal of Molecular Biology, 333:721-745, 2003.

- Sharpening high resolution information in single particle electron cryomicroscopy.
  J.J. Fernandez, D. Luque, J.R. Caston, J.L. Carrascosa
  Journal of Structural Biology, 164:170-175, 2008.
  [PDF]

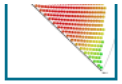
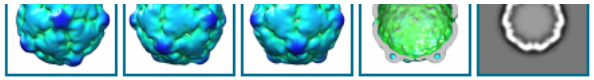
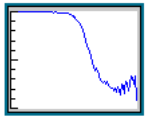
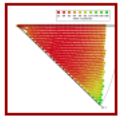
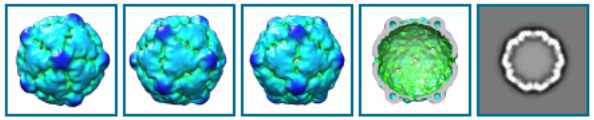
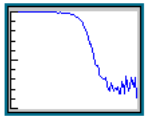
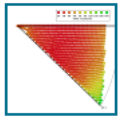
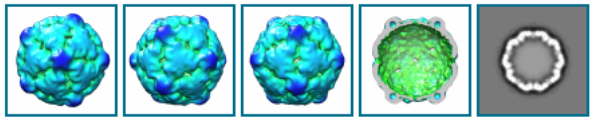
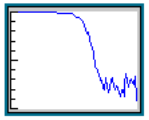
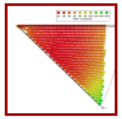
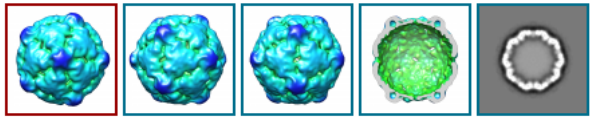
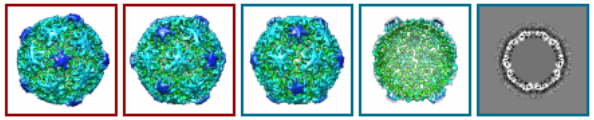
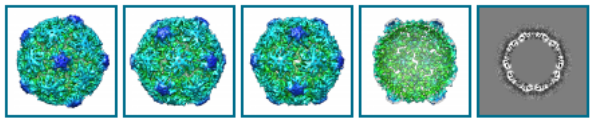
Please, cite these articles if you use EM-BFACTOR in your works.

Download

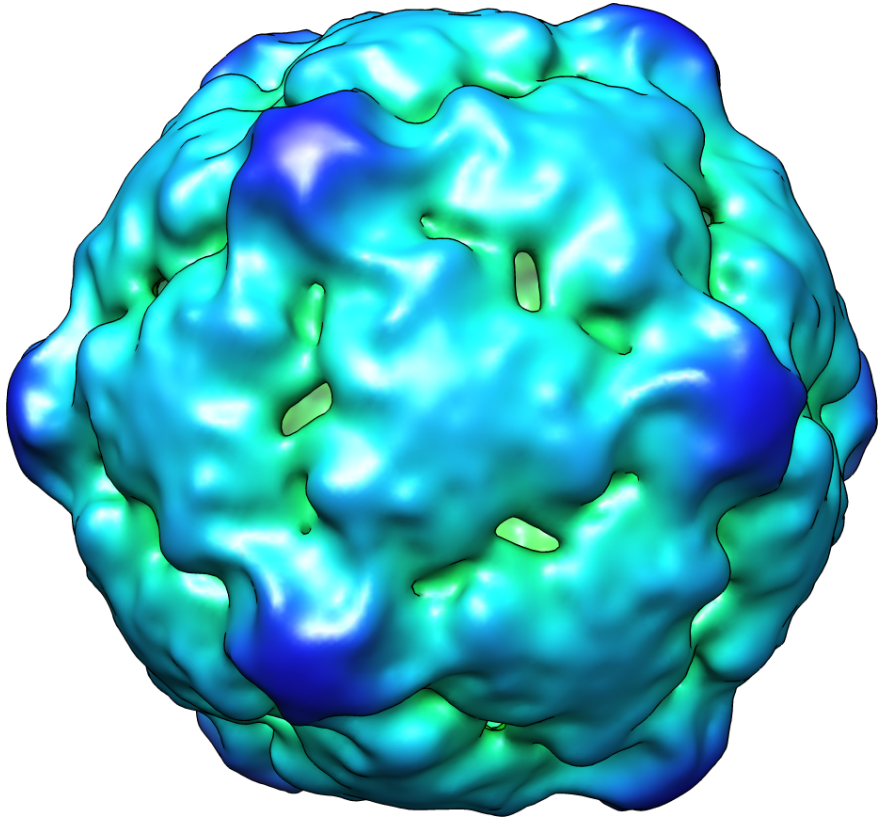
Current version: 18-Apr-2008

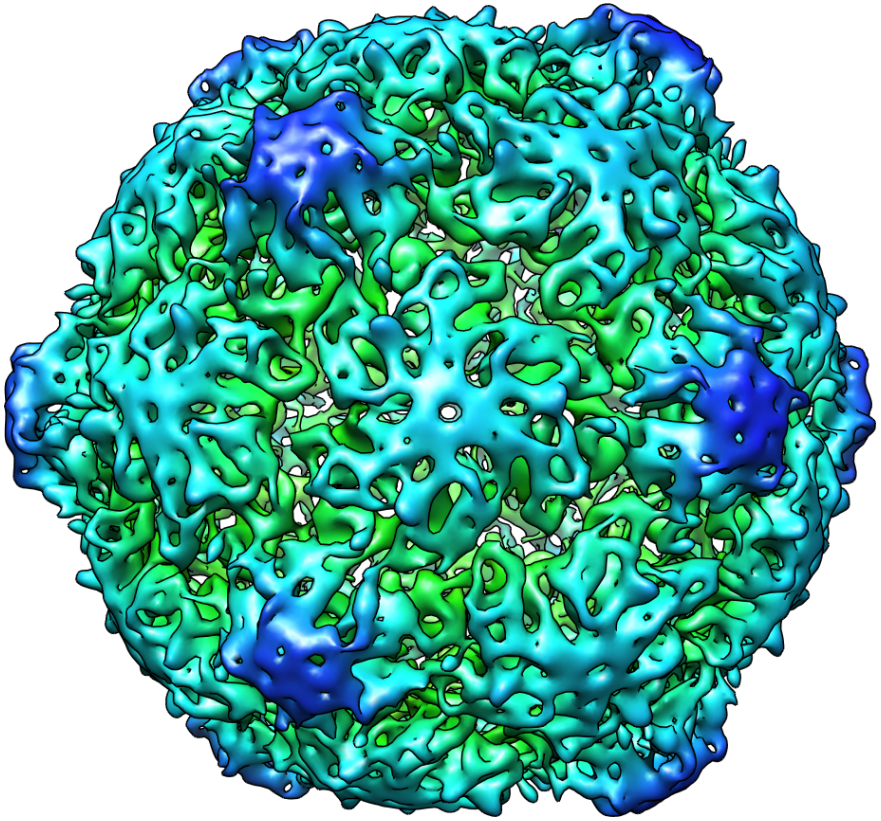
Register: Please, send us an email (jjfdez_at_ual.es) with your name, affiliation and email address. We would like to know who are using the package so that we can have them updated about future versions.

- **embfactor.pdf** *(updated: 10 october 2008)*

11	1°	 *FSC 0.5:* 8.62 *Rmeas:* 6.10	 Polar	[211058-good] [97419-bad]	Post Processing Remove Jumpers Make Exemplar	
12	0.7°	 *FSC 0.5:* 7.72 *Rmeas:* 142.54	classes_eman.12.img 620 classes  Polar	EMAN [210906-good] [97571-bad]	threed.12a.mrc Post Processing Remove Jumpers Make Exemplar	
13	0.7°	 *FSC 0.5:* 7.50 *Rmeas:* 124.72	classes_eman.13.img 620 classes  Polar	EMAN [210899-good] [97578-bad]	threed.13a.mrc Post Processing Remove Jumpers Make Exemplar	
14	0.7°	 *FSC 0.5:* 7.50 *Rmeas:*	classes_eman.14.img 620 classes  Polar	EMAN [210965-good] [97512-bad]	threed.14a.mrc Post Processing Remove Jumpers Make Exemplar	
B-factor sharpening of iteration #14 **path:** /ami/data17/appion/09sep25a/recon/toomanyrecon2/postproc/refine29_09nov12m13 **name:** threed.14a-09nov12m21.lp3.m180.im105.norm.mrc **low pass filter:** 3 angstroms **mask:** 179.595792 Angstroms (72 pixels) **inner mask:** 104.8 Angstroms (42 pixels) **FSC 0.5:** 7.50					
Amplitude correction of iteration #14 **path:** /ami/data17/appion/09sep25a/recon/toomanyrecon2/postproc/refine29_09nov12m42 **name:** threed.14a-09nov12m50.amp.amp.lp3.m180.im105.norm.mrc **ampcor file:** /ami/sw/packages/pyappion/lib/ampcor5.spi **low pass filter:** 3 angstroms **mask:** 179.595792 Angstroms (72 pixels) **inner mask:** 104.8 Angstroms (42 pixels) **FSC 0.5:** 7.50					

Poliovirus





unsharpened

308,477 particles
at 2.5 A/pixels
7.5/5.8 Angstroms

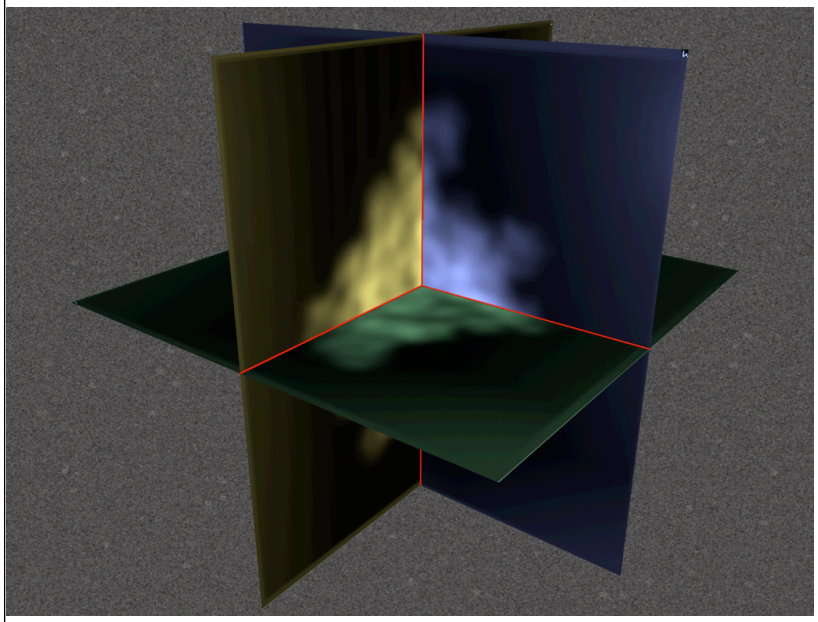
sharpened

What's next:

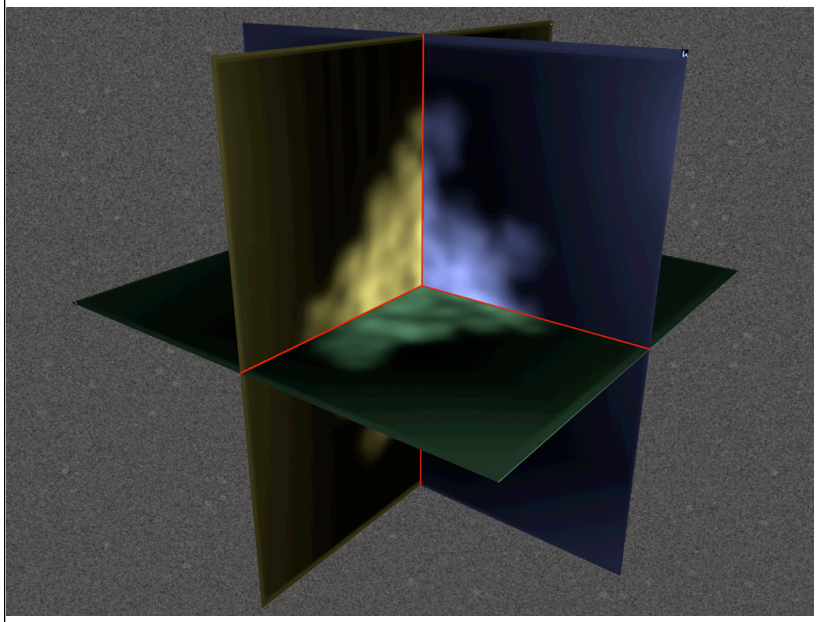
What's next:

- Continue developing new methods

Angular Reconstitution

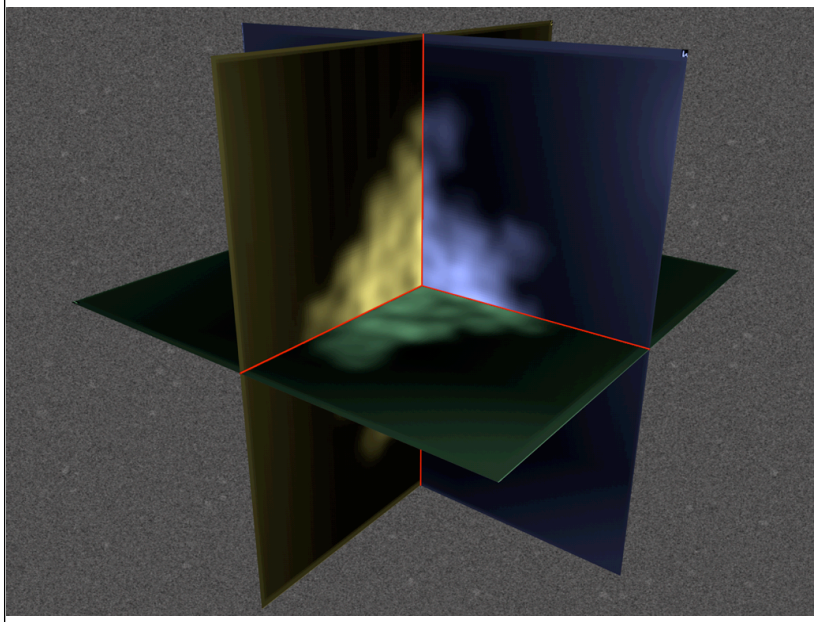


Angular Reconstitution



Create N models (specified by user) using automated angular reconstitution

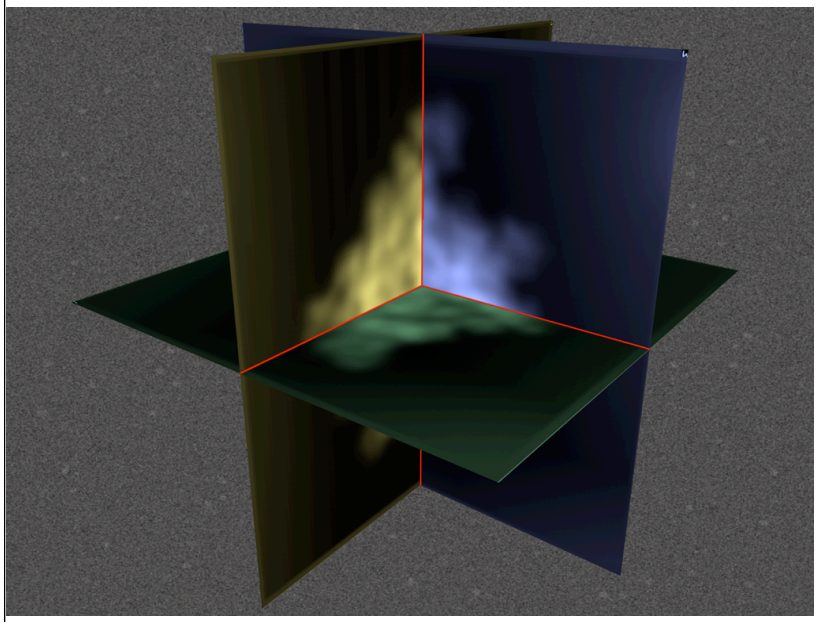
Angular Reconstitution



Create N models (specified by user) using automated angular reconstitution

maximum-likelihood 3-D alignment of resulting models

Angular Reconstitution

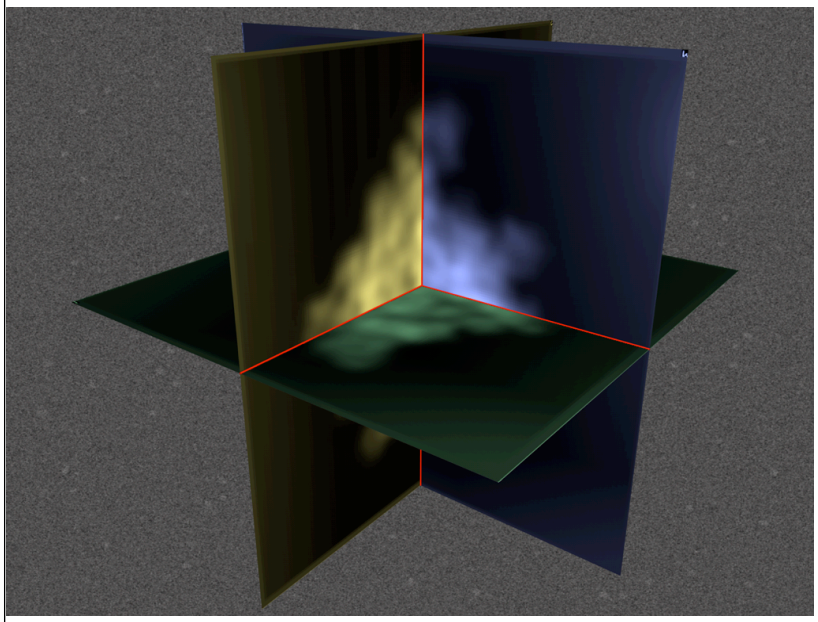


Create N models (specified by user) using automated angular reconstitution

maximum-likelihood 3-D alignment of resulting models

3-D clustering using affinity propagation

Angular Reconstitution

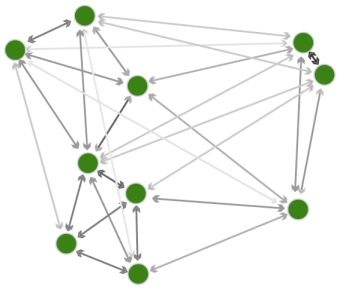


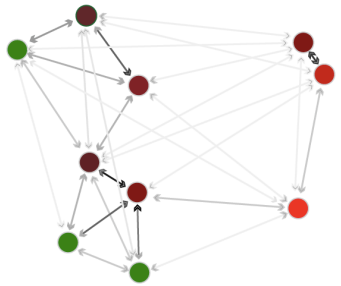
Create N models (specified by user) using automated angular reconstitution

maximum-likelihood 3-D alignment of resulting models

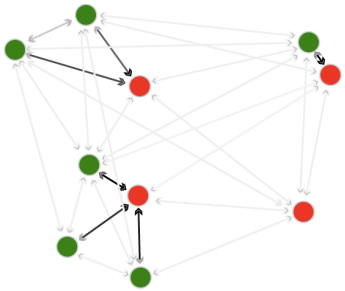
3-D clustering using affinity propagation

Initialization

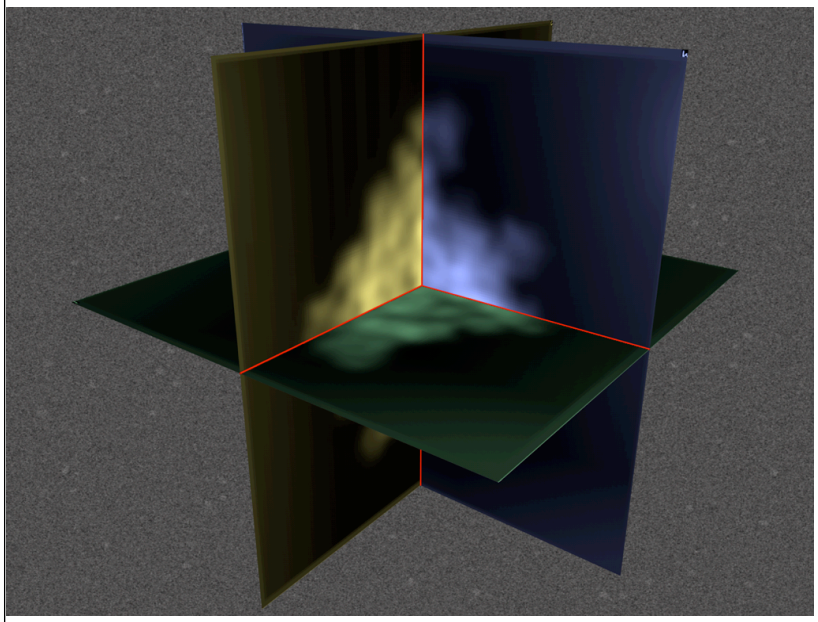




Convergence



Angular Reconstitution

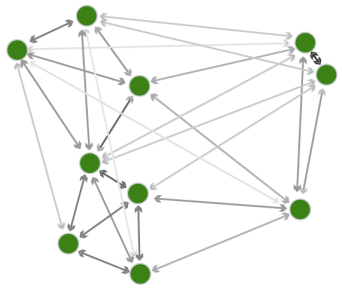


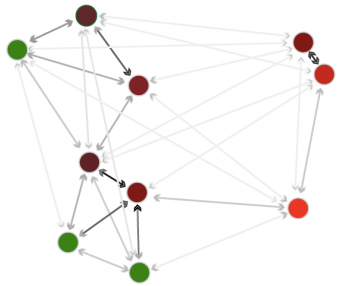
Create N models (specified by user) using automated angular reconstitution

maximum-likelihood 3-D alignment of resulting models

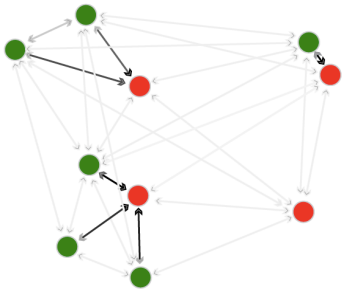
3-D clustering using affinity propagation

Initialization





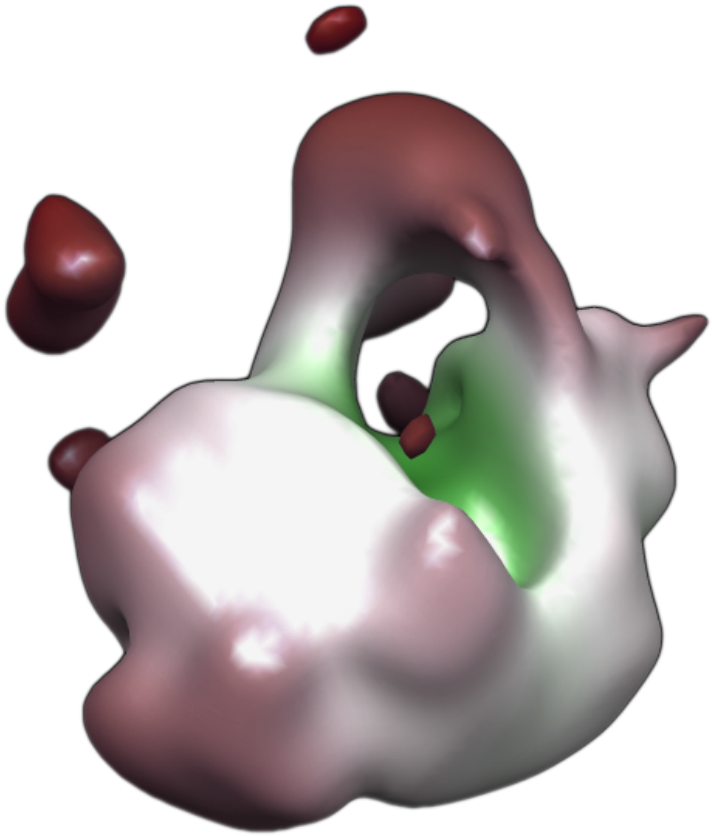
Convergence



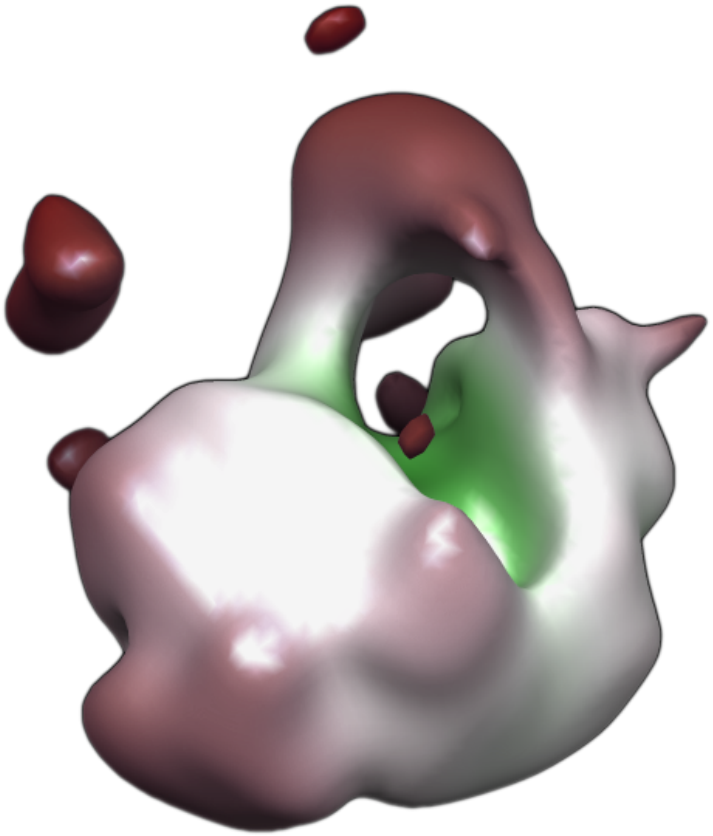
Analysis of 3-D averages

Best Model

Best 50S model from Automated Angular Reconstitution

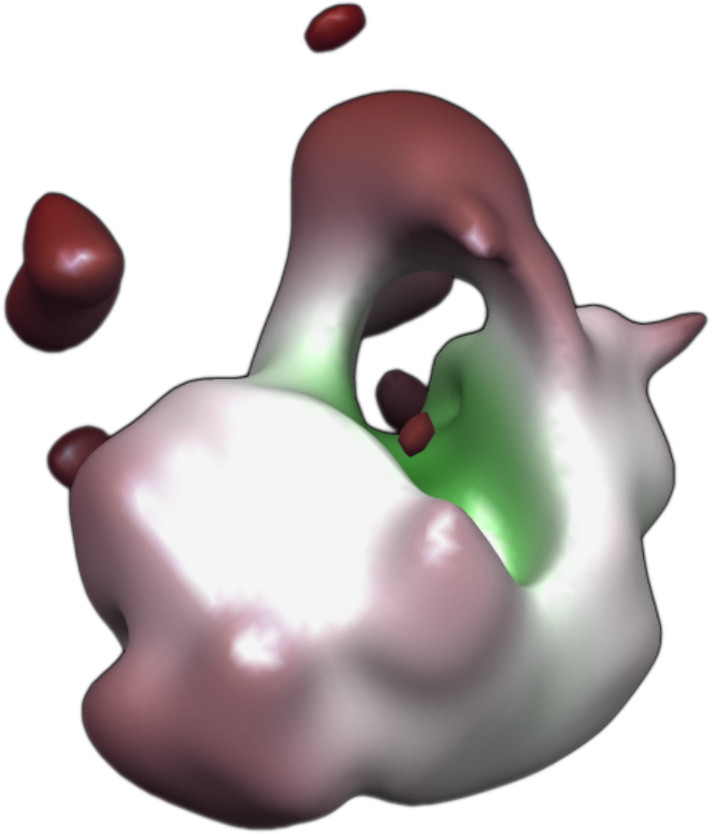


Best 50S model from Automated Angular Reconstitution



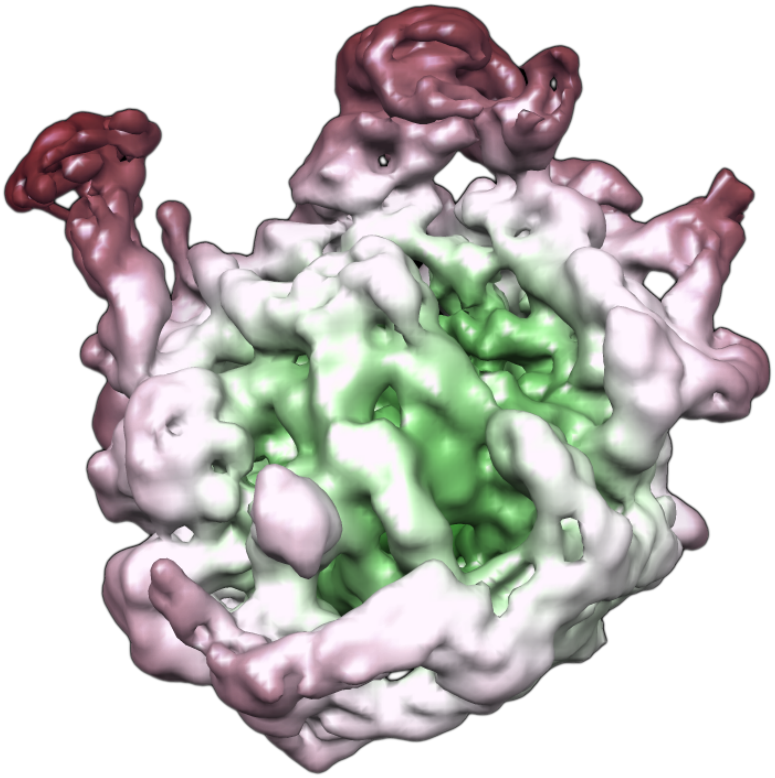
3-D Batch Refinement within Appion

Best 50S model from Automated Angular Reconstitution

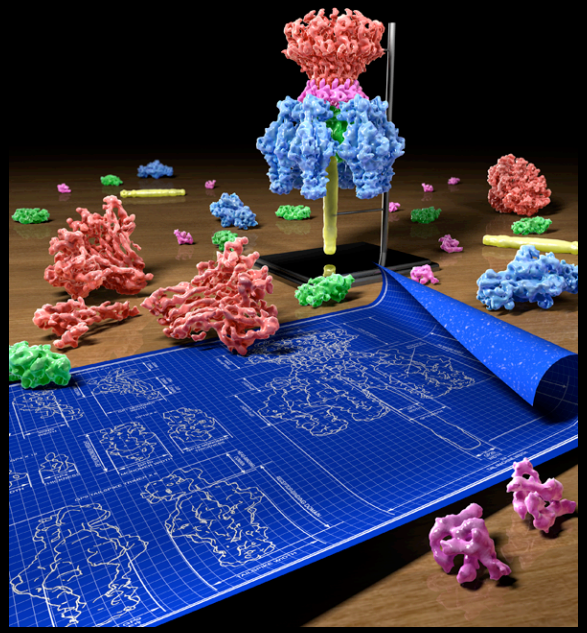


3-D Batch Refinement within Appion

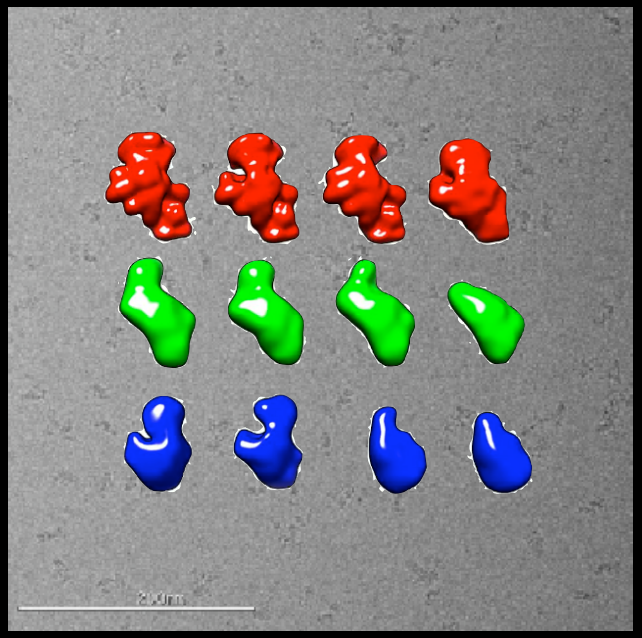
Refined 50S Subunit



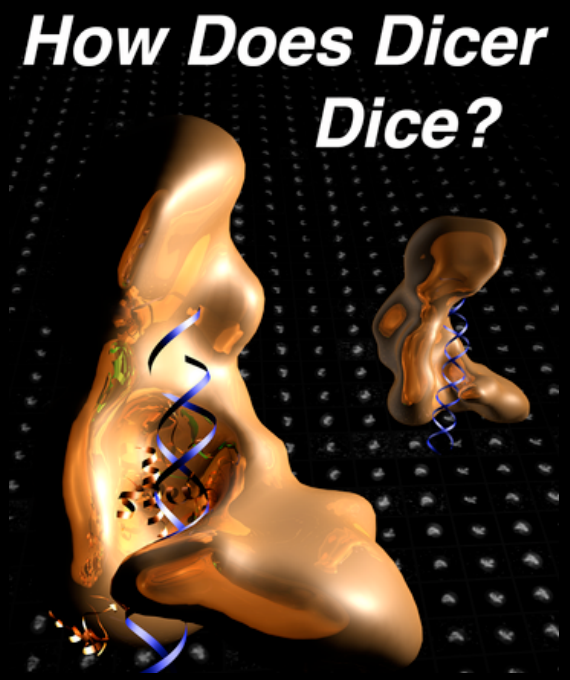
Collaborative Projects at NRAMM



Gabe Lander
Jack Johnson



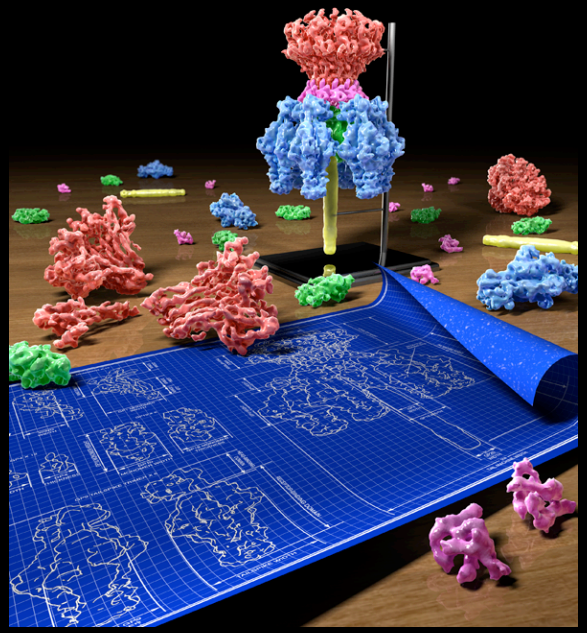
Anke Mulder
Jamie Williamson



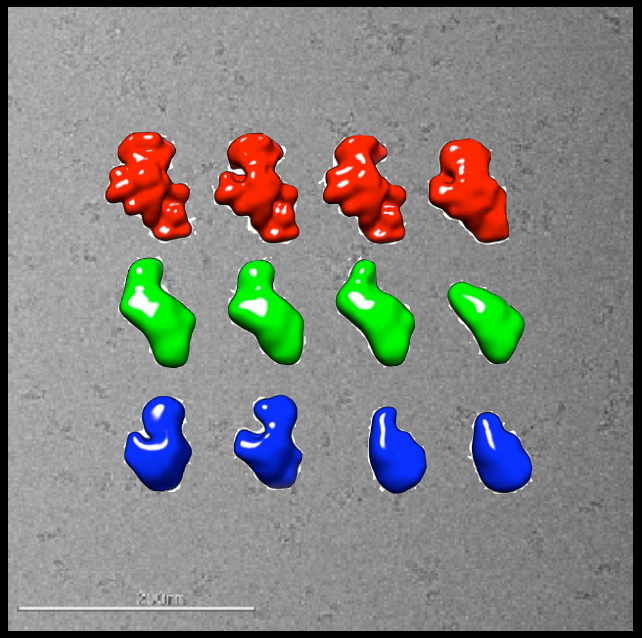
Pick-Wei Lau
Ian MacRae

Arne Moeller
Ray Stevenson

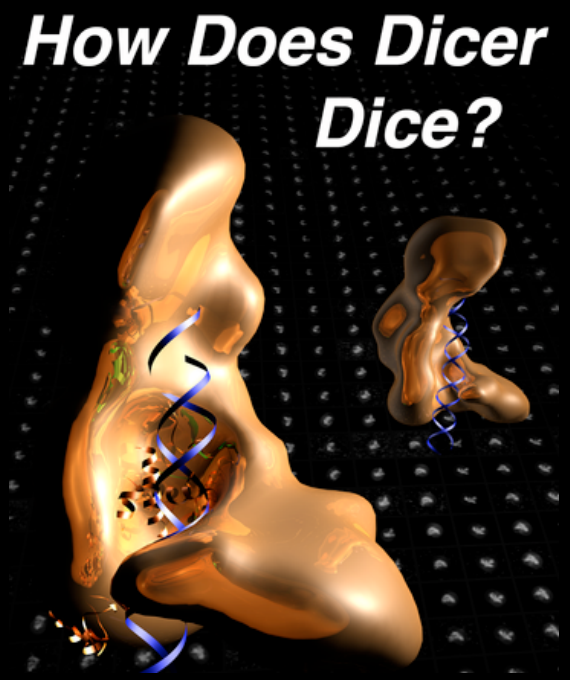
Collaborative Projects at NRAMM



Gabe Lander
Jack Johnson



Anke Mulder
Jamie Williamson

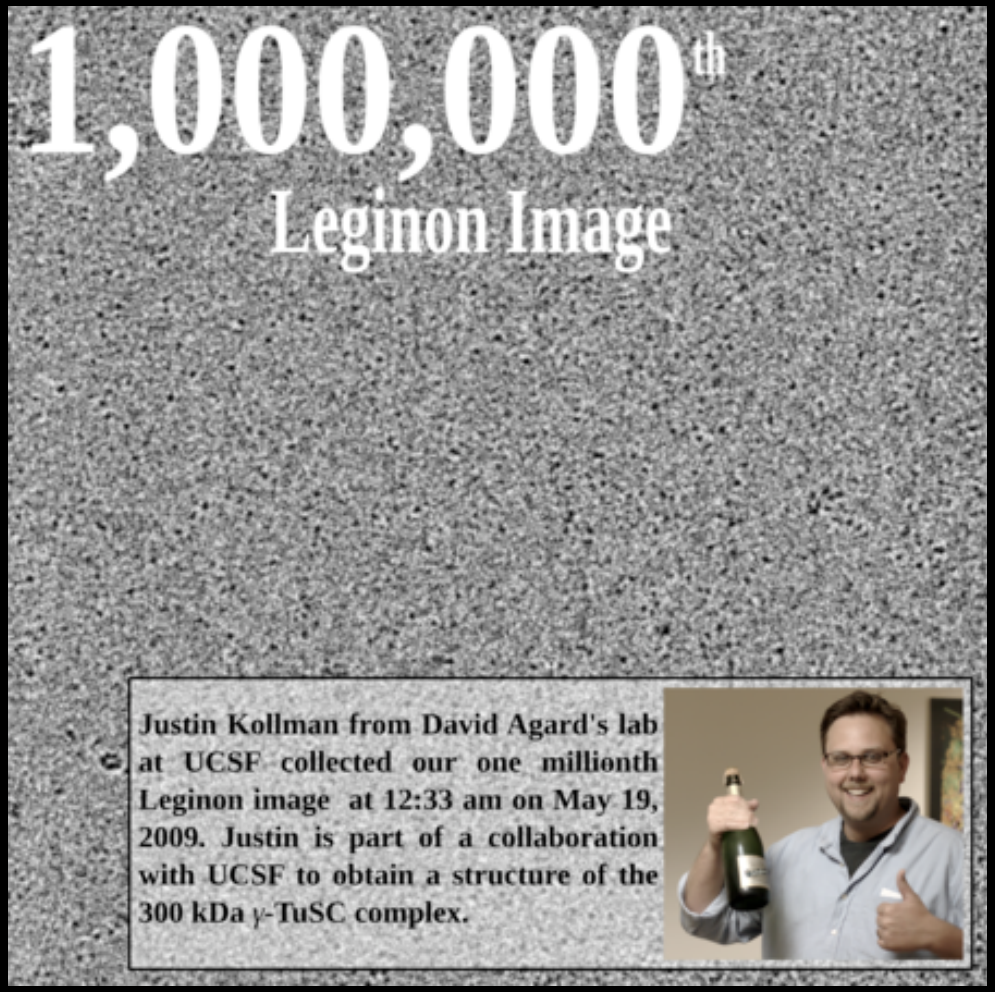


Pick-Wei Lau
Ian MacRae

Membrane Proteins

Arne Moeller
Ray Stevenson

Service Projects at NRAMM



THE SCRIPPS INSTITUTE

Workshop on Advanced Topics in EM Structure Determination: Challenging Molecules.
November 8-13, 2009

National Resource for Automated Molecular Microscopy
The Scripps Research Institute, La Jolla, CA

Participants

David Agard, Thorsten Althoff, Ernesto Arias-Palomo, Rocio Arranz, Chris Arthur, Francisco Asturias, Benjamin Bammes, Andrew Barthel, Tanmay Bharat, Robert Bilhorn, Daniel Bose, Edward Brignole, Stan Burgess, Sarah Butcher, Gang Cai, Tony Carpenter, Bridget Carragher, Anchi Cheng, Johnathan Chittuluru, Wah Chiu, Rebeca Choy, Michael Cianfrocco, Claudio Ciferri, Aguang Dai, Sacha De Carlo, David DeRosier, Amedee des Georges, Frank Dickerson, Mark Dods, Radoslav Enchev, MaryBeth Evans, Vince Fernando, Lauren Fisher, Sibylle Franckenberg, Achilleas Frangakis, Joachim Frank, Alexander Freiberg, Xiaofeng Fu, Brian Gibbons, Bob Glaeser, Robert Grassucci, Niko Grigorieff, Megan Guelker, Ricardo Guerrero, Richard Gursky, Florian Hauer, Richard Henderson, Amber Herold, Thomas Heuser, Andy Hoenger, Eric Hou, Thomas Hrabe, Christopher Irving, Erica Jacovetty, Jerry Jasso, Grant Jensen, Lei Jin, Matthew Johnson, Jack Johnson, Ahmad Jomaa, Hiroo Katayama, Farida Khan, Zdravko Kochovski, Rebecca Kohler, Abhay Kotecha, Andre Krueger, Gabriel Lander, Jason Lanman, Keren Lasker, Lorraine Lathrop, Pick-Wei Lau, Catherine Lawson, Jinwoo Lee, Jeffrey Lengyel, Yael Levi-Kalisman, Justus Loerke, Shee-Mei Lok, Steven Ludtke, Dmitry Lyumkis, Jaime Martin-Benito, Linda Melanson, Zoltan Metlage, Thorsten Mielke, Anna-Clare Milazzo, Ron Milligan, Arne Moeller, Paul Mooney, David Morgan, Anke Mulder, Daniel Nemecek, Eva Nogales, Joaquin Ortega, Jesper Pallesen, Radosav Pantelic, Lori Passmore, Pawel Penczek, Steve Pfieffer, Clint Potter, Jim Pulokas, Joel Quispe, Bilal Qureshi, Maria Angeles Recuero Checa, Fabiana Renzi, Andrey Reshetnyak, William Rice, Alan Roseman, John Rubinstein, Christopher Russell, Eduardo Sanz Garcia, Anne Schreiber, Tanvir Shaikh, Gyanesh Sharma, Fred Sigworth, Daniel Southworth, Holger Stark, Alasdair Steven, Raymond Stevens, David Stokes, Hemant Tagare, Rebecca Taurog, Derek Taylor, David Taylor, Gokhan Tolun, Judi Townsend, Vinzenz Unger, Nigel Unwin, Neil Voss, Tom Walz, Andrew Ward, Elizabeth Wilson-Kubalek, John Wineman, Elena Yakubovskaya, Xiao Yang, Mark Yeager, Craig Yoshioka, Yadong Yu, Hong Zhou, Benoit Zuber.

Dissemination:

Leginon and Appion dissemination

Dissemination:

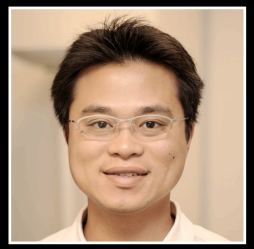
Leginon and Appion dissemination

- Make it easier to use
- Make it easier to add new features
- Develop a “portal”

The National Resource for Automated Molecular Microscopy


Jim Pulokas

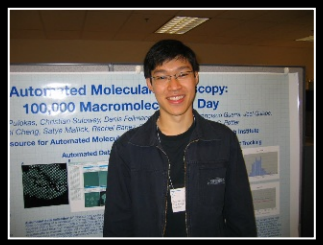

Amber Herold

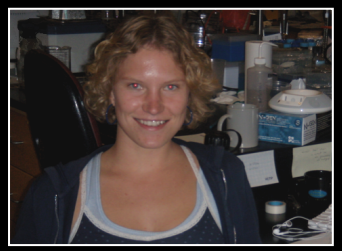

Eric Hou

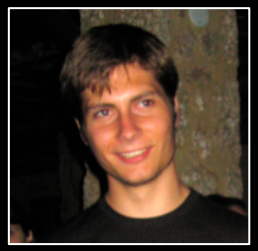

Joel Quispe


Anchi Cheng

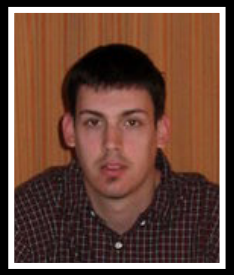

Erica Jacovetty


Pick-Wei Lau


Anke Mulder


Dmitry Lyumkis


Arne Moeller


Neil Voss


Gabe Lander


Craig Yoshioka


Christopher Irving


Lorraine Lathrop


Ron Milligan


Clint Potter


Bridget Carragher

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