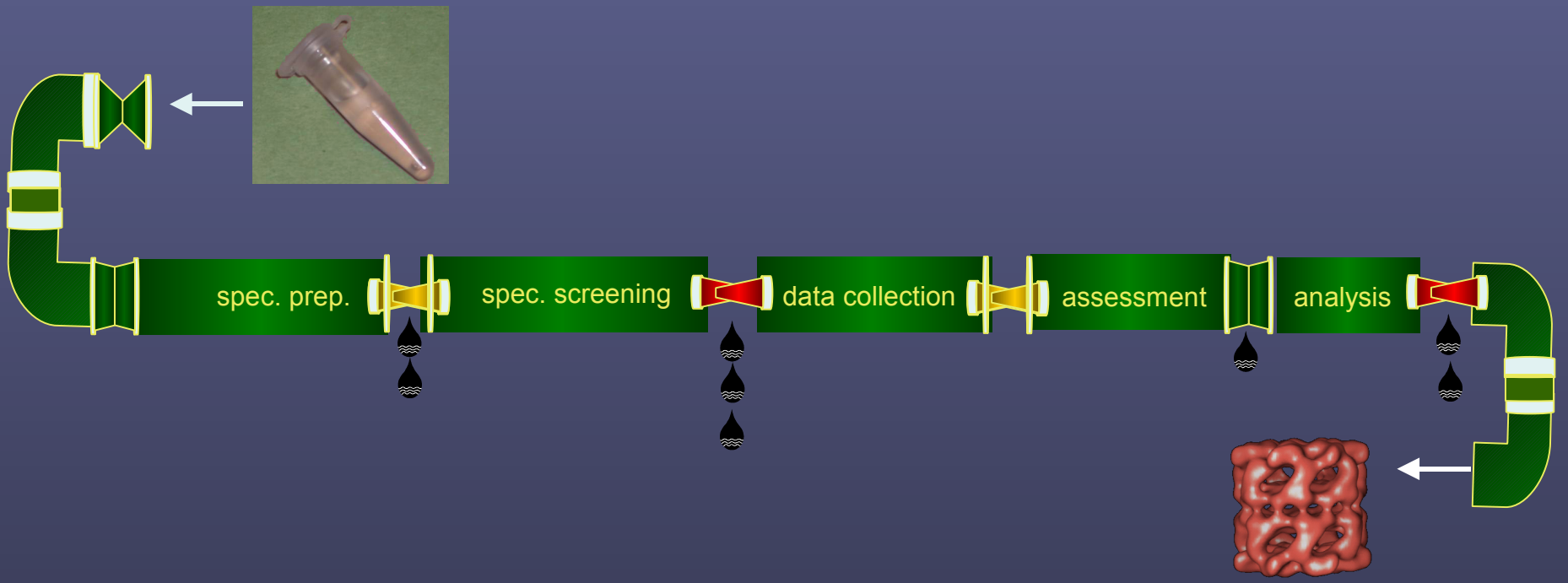
Automated Molecular Microscopy

Bridget Carragher
CryoCourse, 2005

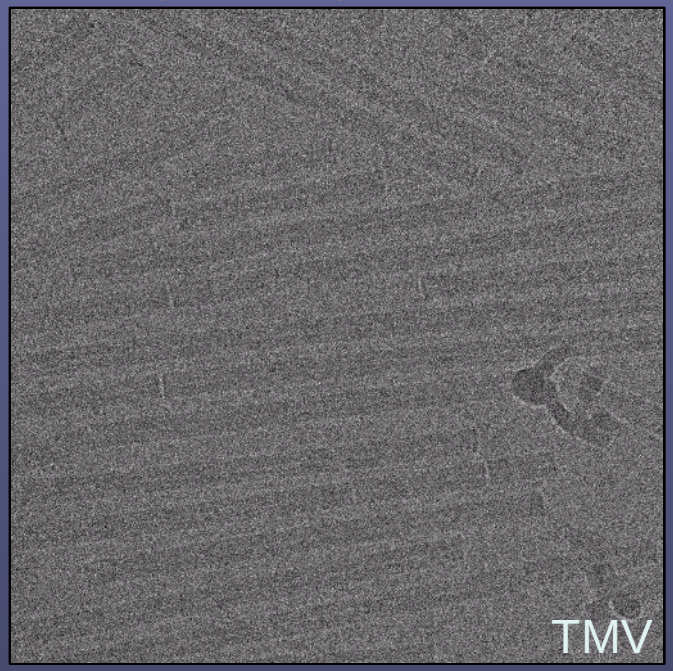
THE SCRIPPS RESEARCH INSTITUTE

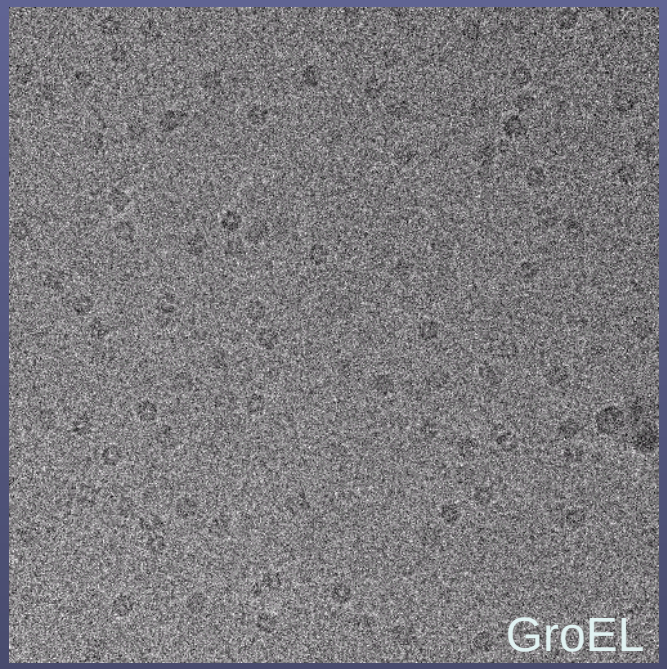
Pipeline for Molecular Microscopy

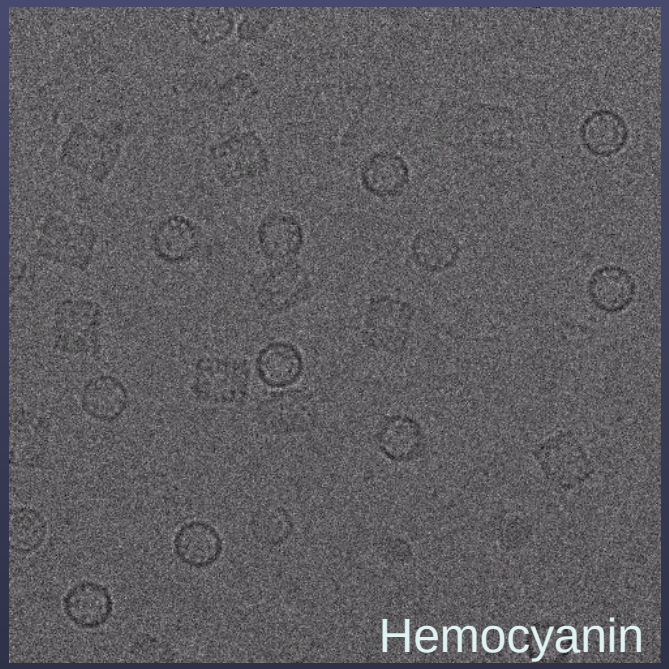


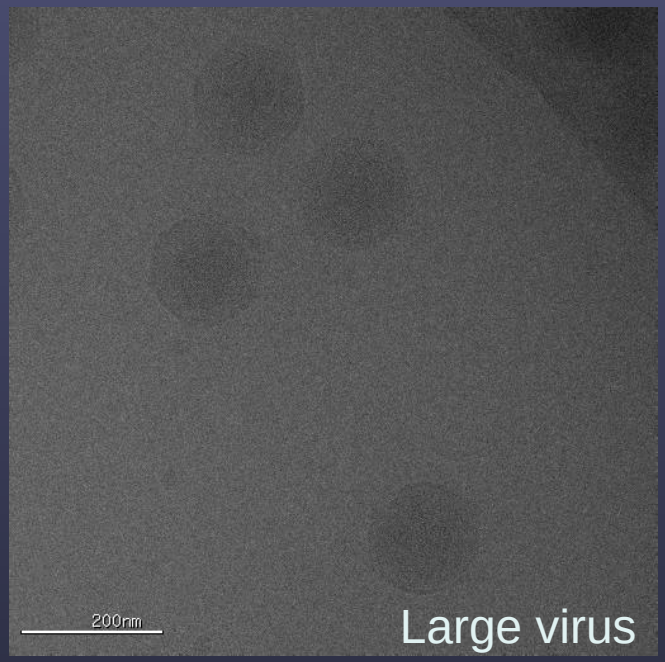
Adapted from a slide courtesy of: Peter Kuhn, Scripps-PARC Institute for Advanced Biomedical Sciences, TSRI

5. High magnification images (~60,000x)





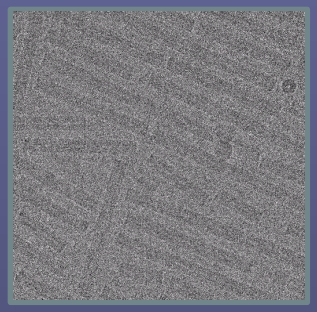




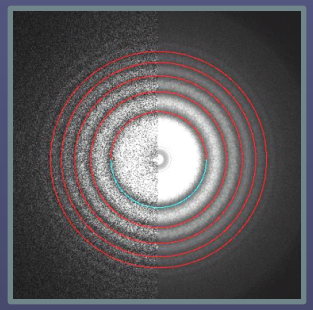
Acquired as defocus pairs or sequences

Total dose: ~ 10 e/A^2

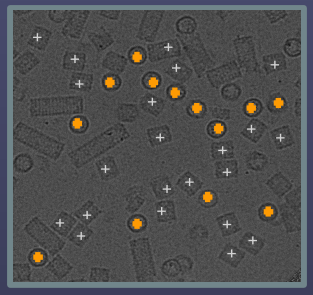
Automated image analysis and reconstruction



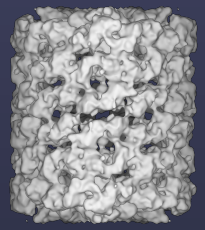
Assess the images



Determine the CTF

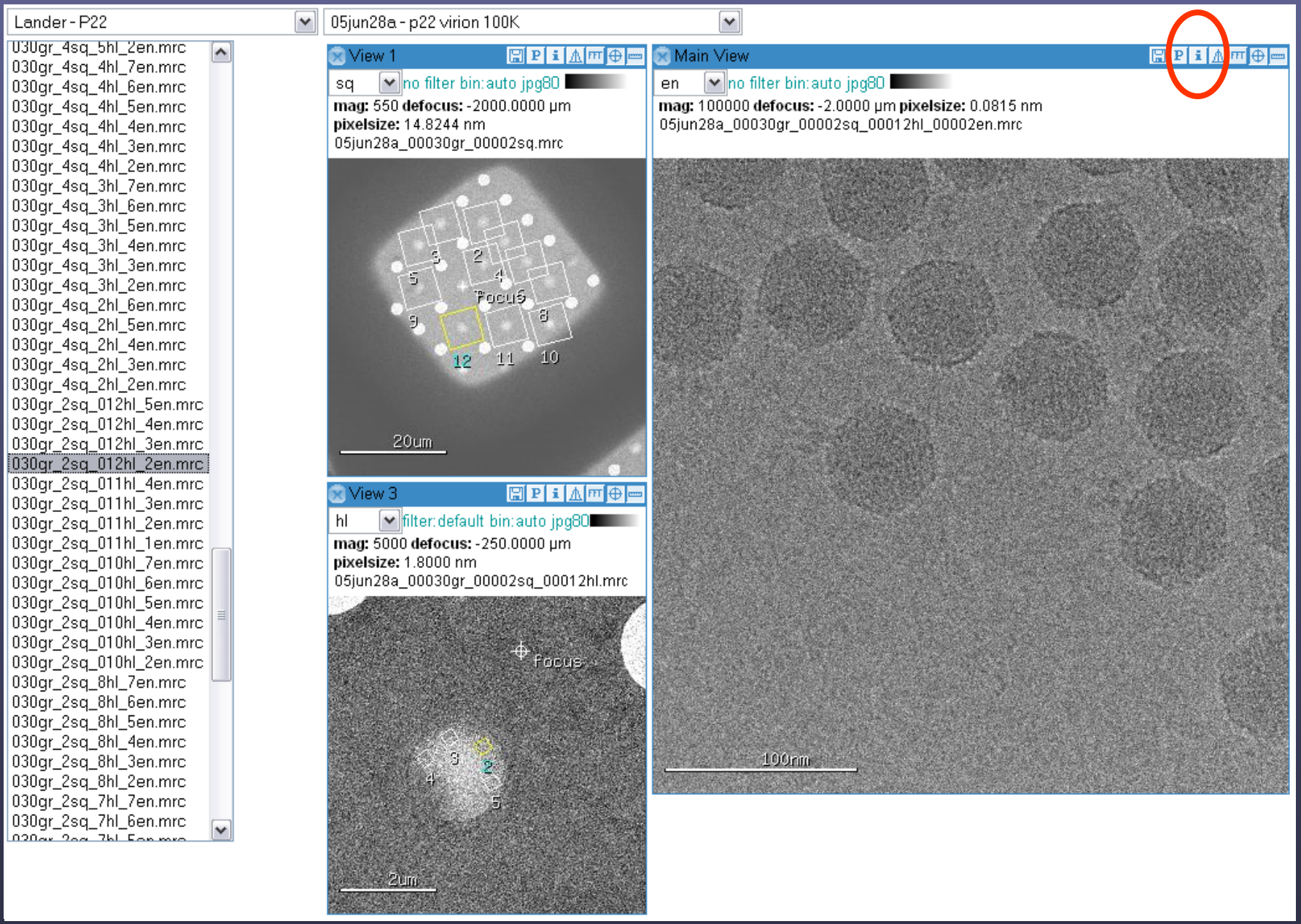


Select and segment particles



Reconstruct 3D map

Leginon Database: Images and Acquisition Parameters
- Multi-scale: Keeps track of relationships between scales.



General

Filename: 05jun28a_00030gr_00002sq_00012hl_00002en.mrc
Size: 64 MB
Acquired: 2005-28-06 17:29:07
Path: /ami/data06/leginon/05jun28a/rawdata/
Session: 05jun28a - p22 virion 100K
Instrument: Tecnai - Tietz SCX -

Image Information

imageId:	124720
preset:	en
dimx:	4096
dimy:	4096
binning:	1
high tension:	200000 V
exposure time:	260
mag:	100000
defocus:	-2.0000 µm
pixelsize:	0.0815 nm

Mrc Header Information

nx:	4096
ny:	4096
mode:	MRC_MODE_FLOAT
alpha:	90
beta:	90
gamma:	90
amin:	377.239227295
amax:	1133.19921875
amean:	670.582092285
xorigin:	2048
yorigin:	2048

Parent Image Information

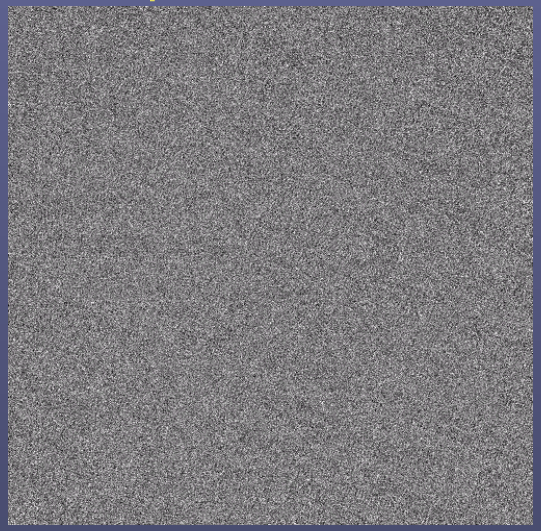
parentId:	124660
parentimage:	05jun28a_00030gr_00002sq_00012hl.mrc
parentpreset:	hl
parenttype:	acquisition
parentnumber:	2
targetx:	250
targety:	243
targetdim:	20.864
targetdiam:	29.506151765352

Image Relations

gr:	05jun28a_00030gr.mrc
sq:	05jun28a_00030gr_00002sq.mrc
hl:	05jun28a_00030gr_00002sq_00012hl.mrc
fc:	05jun28a_00030gr_00002sq_00012hl_00001fc.mrc
last:	« back

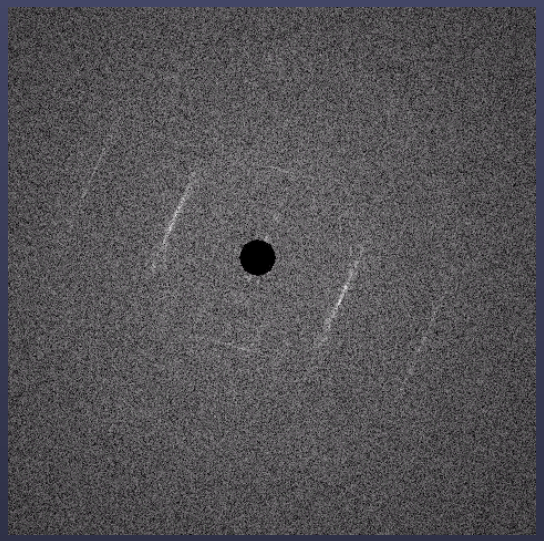
Assessing the images: TMV

Exposure 1

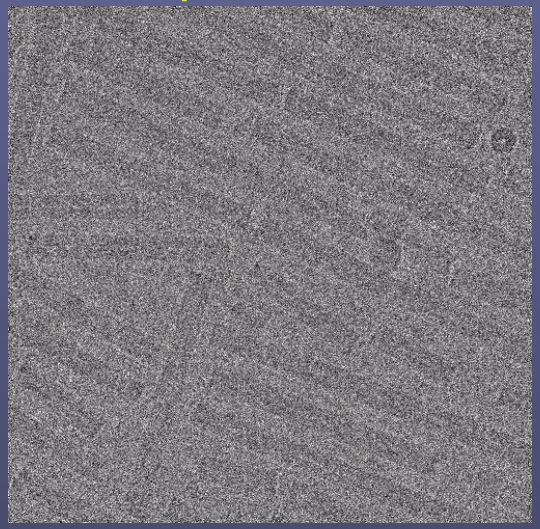


dose ~12e^{-}/Å^2

Δf = -30nm

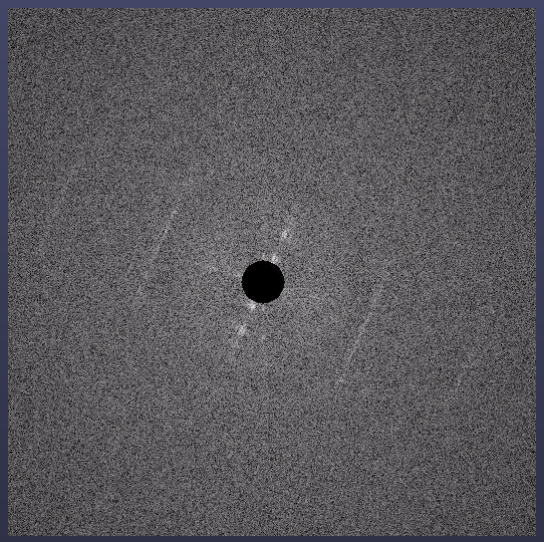


Exposure 2

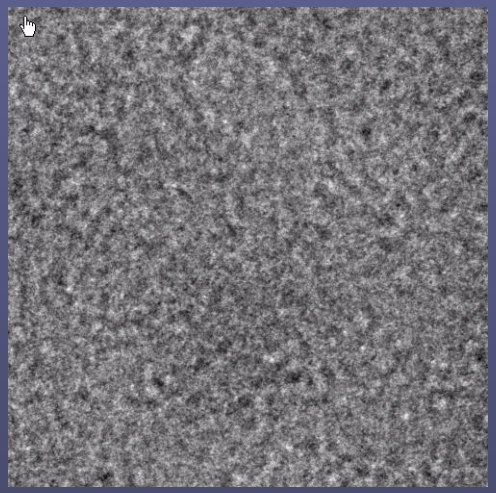


dose ~24e^{-}/Å^2

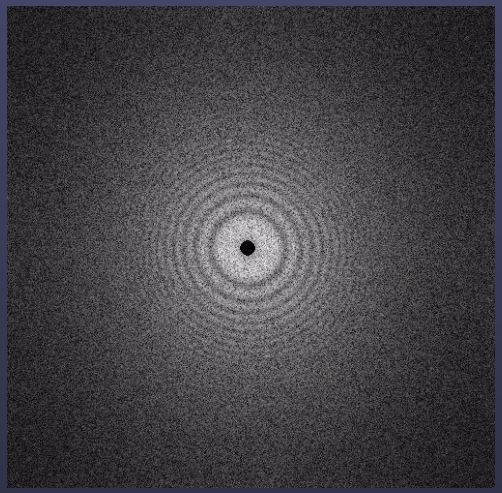
Δf = -200nm



Focus image

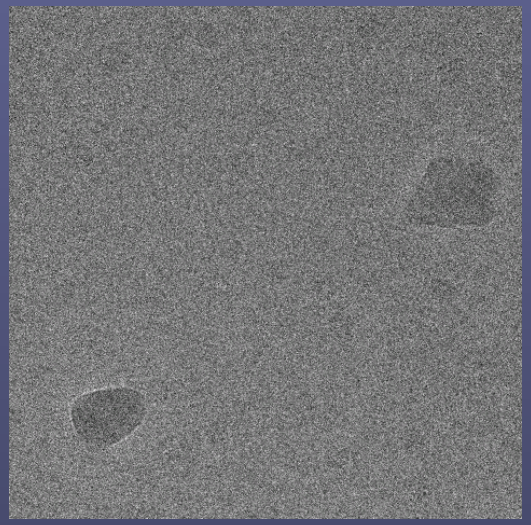


Δf = -200nm

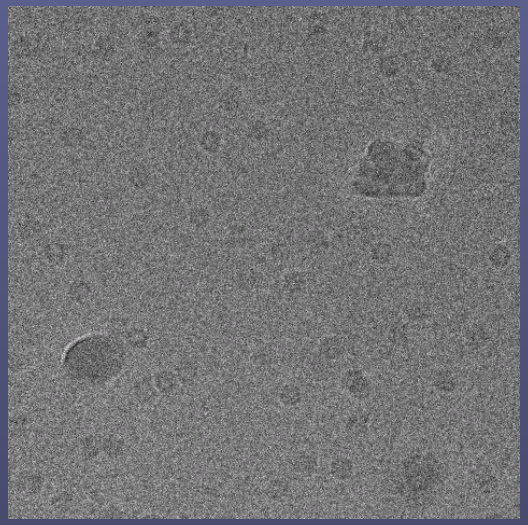


Assessing the images: GroEL

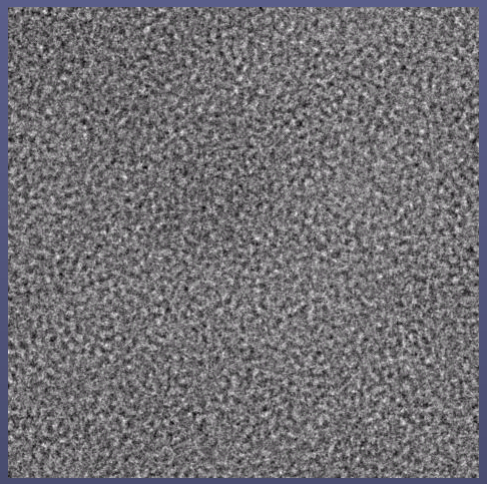
Exposure 1



Exposure 2

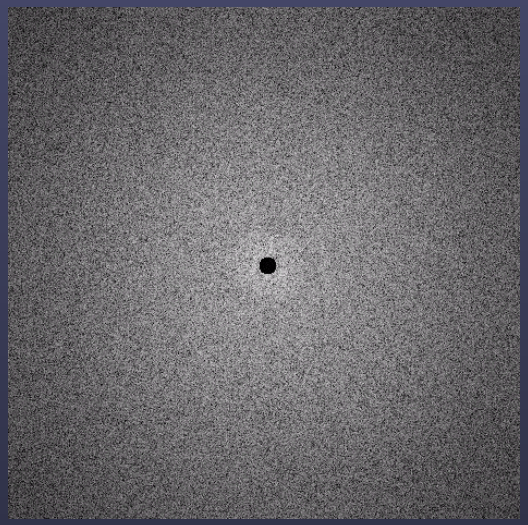


Focus image

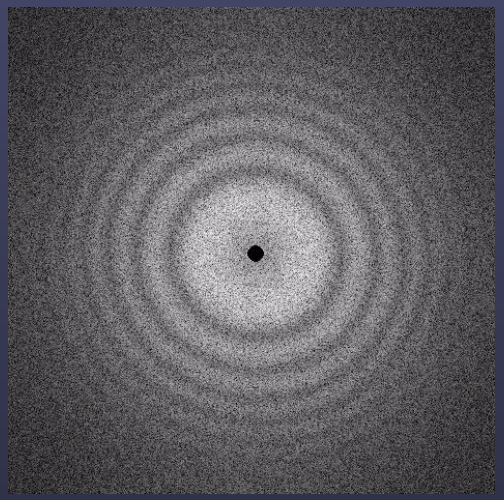


Δf = -60nm

Δf = -200nm



Δf = -60nm



Summary of 05oct27b Experiment

Experiment Information

SessionId:	2553
Name:	05oct27b
Purpose:	p22 mature virion 2nd collection- qfoil 5 sec blot slot #2
Begin Time:	2005-27-10 11:02:30
End Time:	2005-28-10 23:43:31
Total Duration:	36:41:01
Image path:	/ami/data06/leginon/05oct27b/rawdata
Instrument:	Tecnai - Tietz SCX
InstrumentId:	20
User:	Gabriel C Laner

Images Acquired

Preset label	# images
en:	1628
fc:	417
gr:	43
hl:	431
sq:	20

Total images:**2539**

Drift Report 05oct27b Experiment

Avg. drift rate at end of measurement cycle

max rate:

[data][sql]

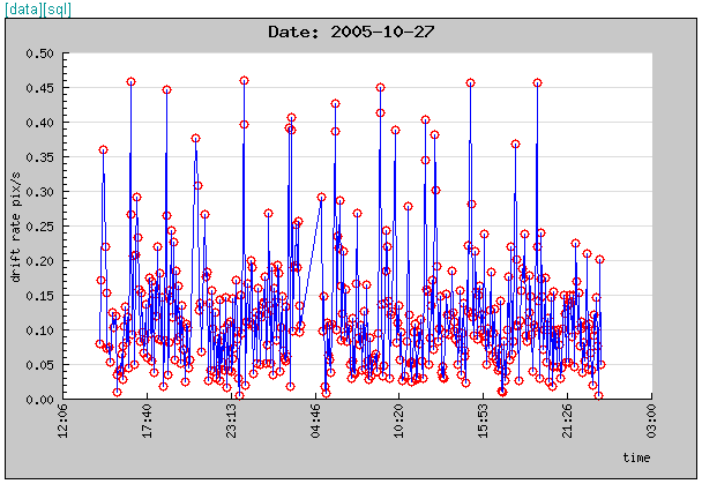
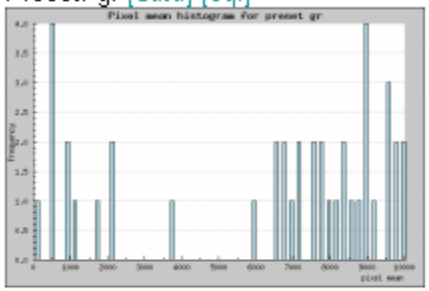


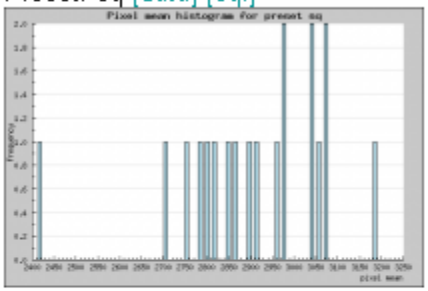
Image Stats

report »

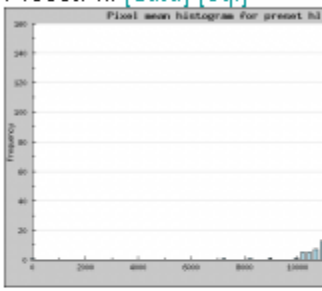
Preset: gr [data] [sql]



Preset: sq [data] [sql]

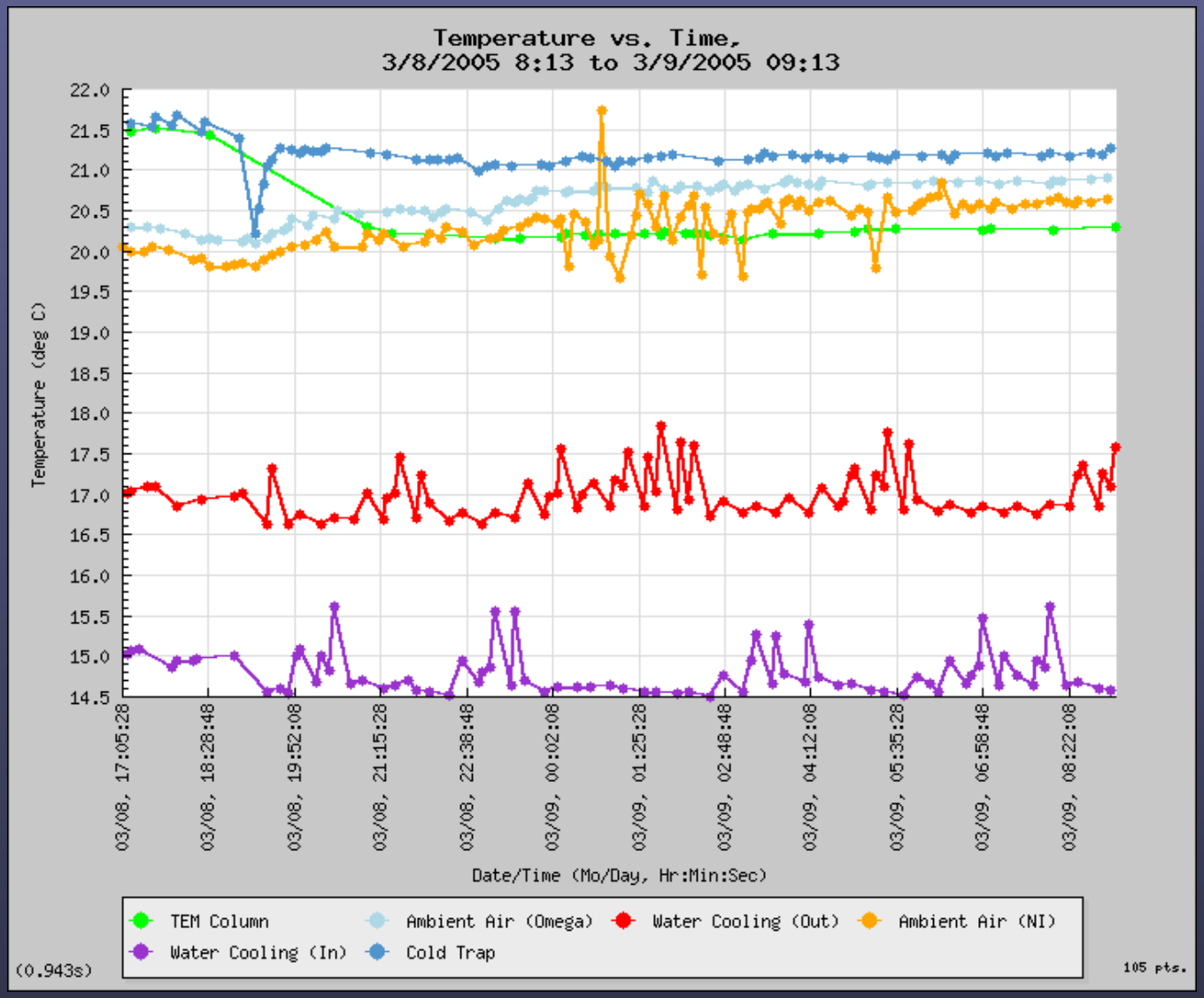


Preset: hl [data] [sql]

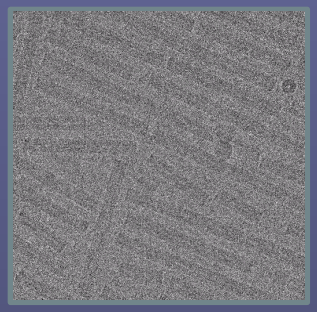


Preset: fc [data] [sql]

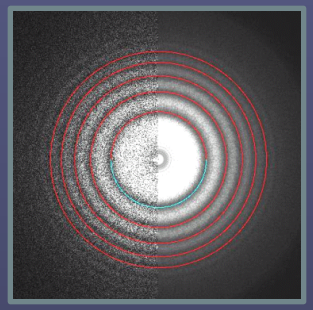
Preset: en [data] [sql]


Temperature vs. Time,
3/8/2005 8:13 to 3/9/2005 09:13
Temperature (deg C)
22.0
21.5
21.0
20.5
20.0
19.5
19.0
18.5
18.0
17.5
17.0
16.5
16.0
15.5
15.0
14.5
03/08, 17:05:28
03/08, 18:28:48
03/08, 19:52:08
03/08, 21:15:28
03/08, 22:38:48
03/09, 00:02:08
03/09, 01:25:28
03/09, 02:48:48
03/09, 04:12:08
03/09, 05:35:28
03/09, 06:58:48
03/09, 08:22:08
Date/Time (Mo/Day, Hr:Min:Sec)
TEM Column
Ambient Air (Omega)
Water Cooling (Out)
Ambient Air (NI)
Water Cooling (In)
Cold Trap
(0.943s)
105 pts.

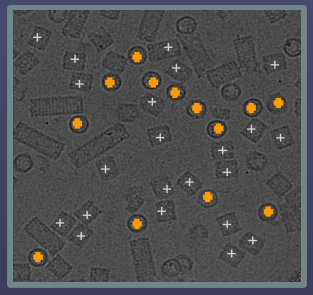
Automated image processing and reconstruction



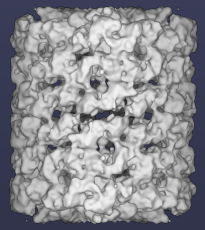
Assess the images



Determine the CTF

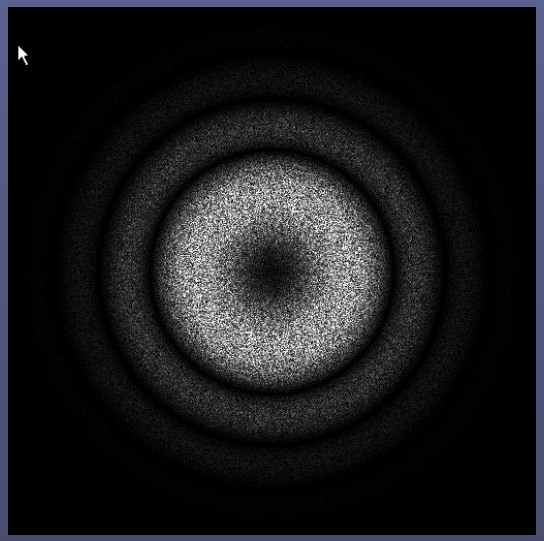


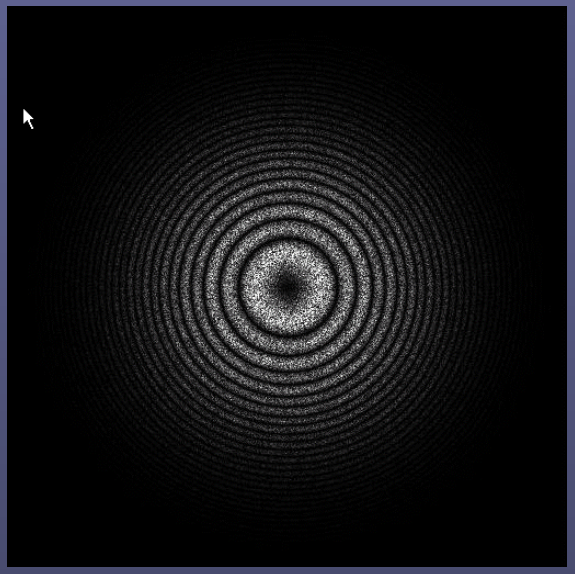
Select and segment particles

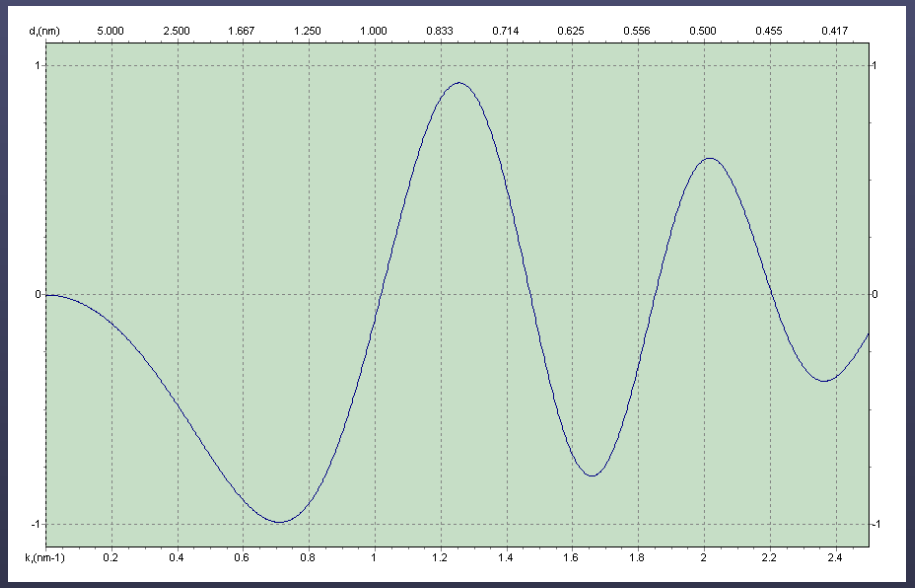


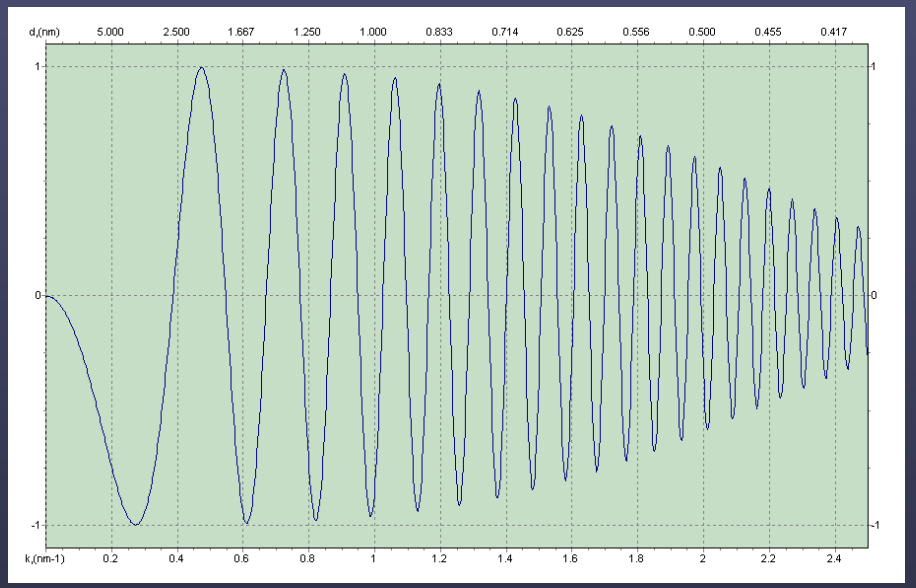
Reconstruct 3D map

Defocus determination





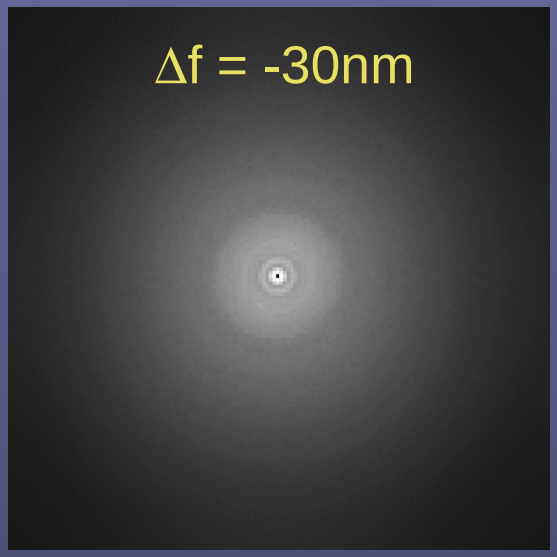


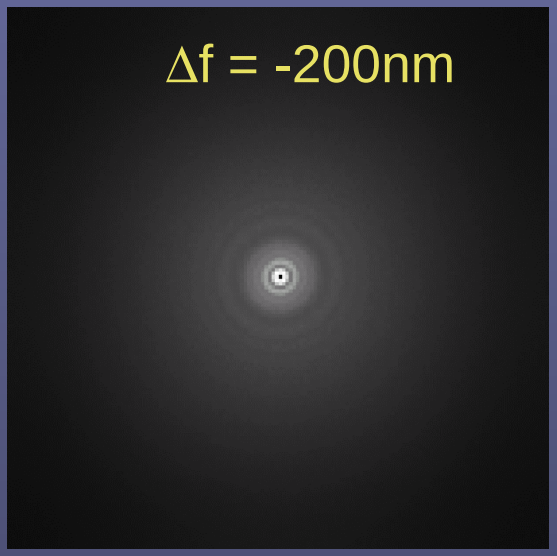


Defocus –300nm

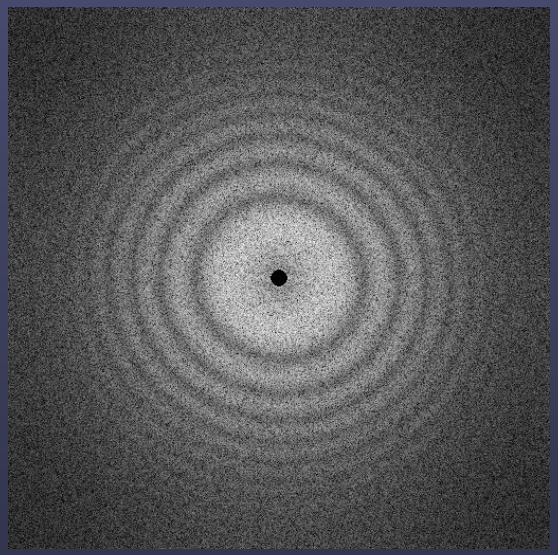
Defocus –2000 nm

1 image : 17 particles

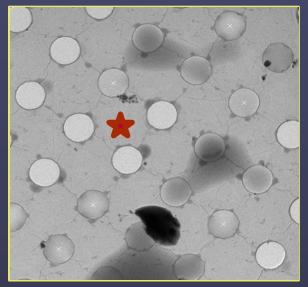


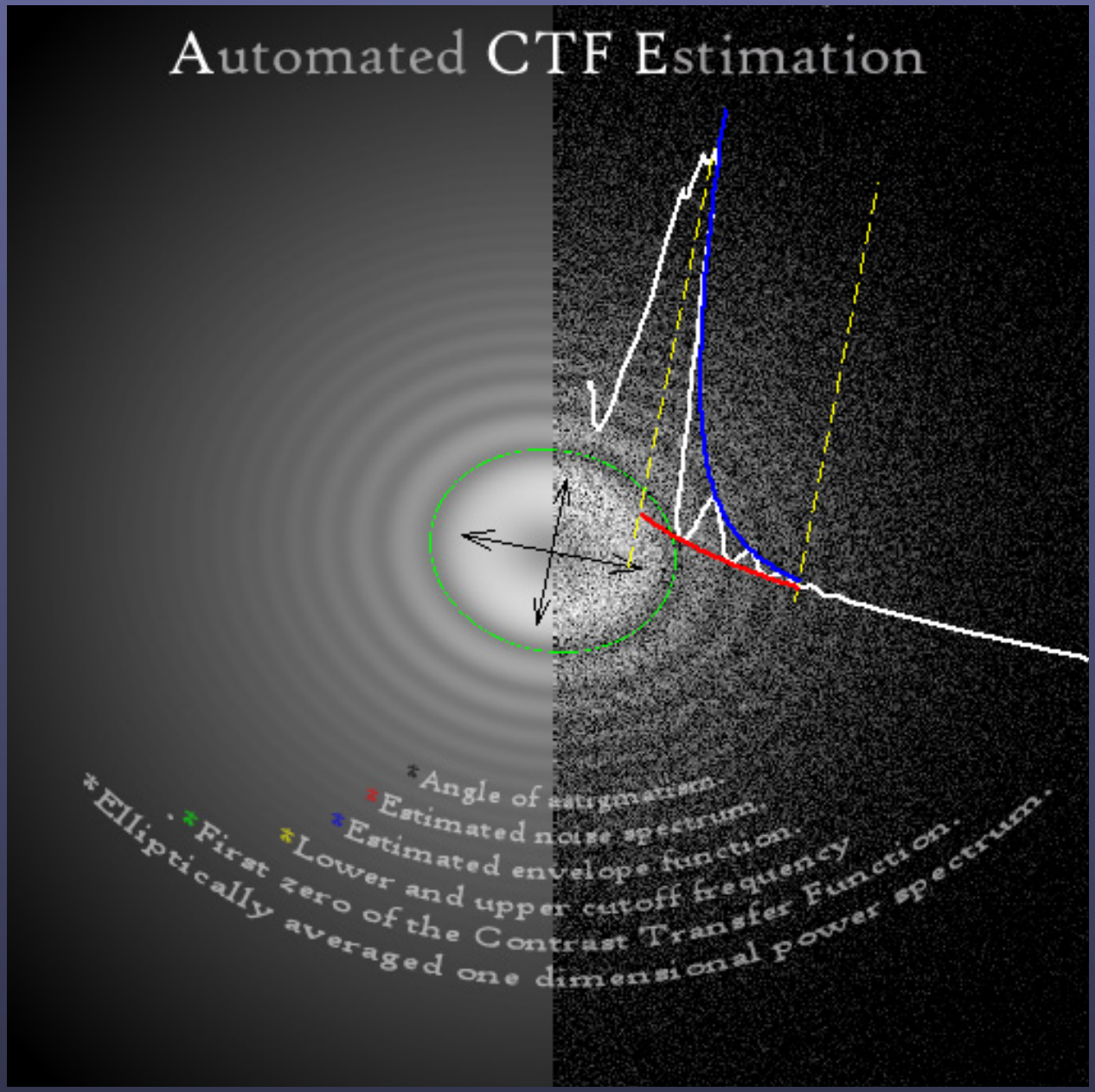


Set of images : ~ 2000 particles

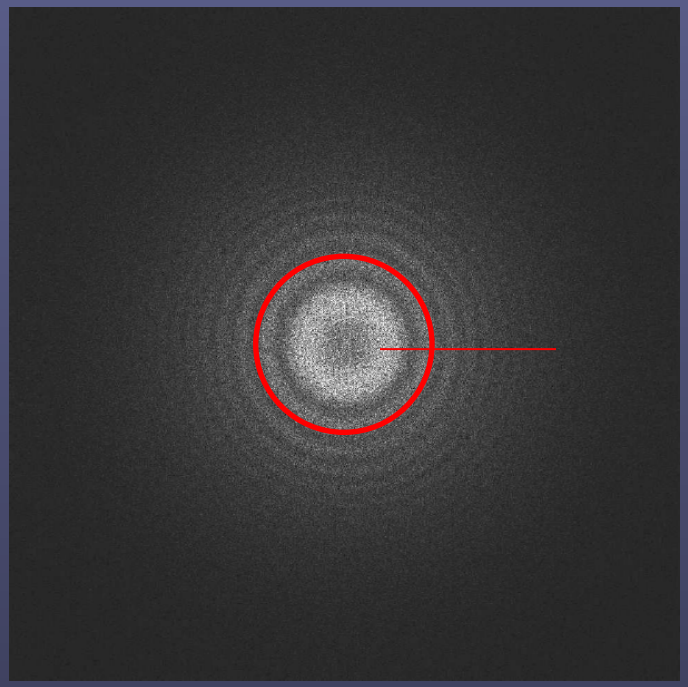


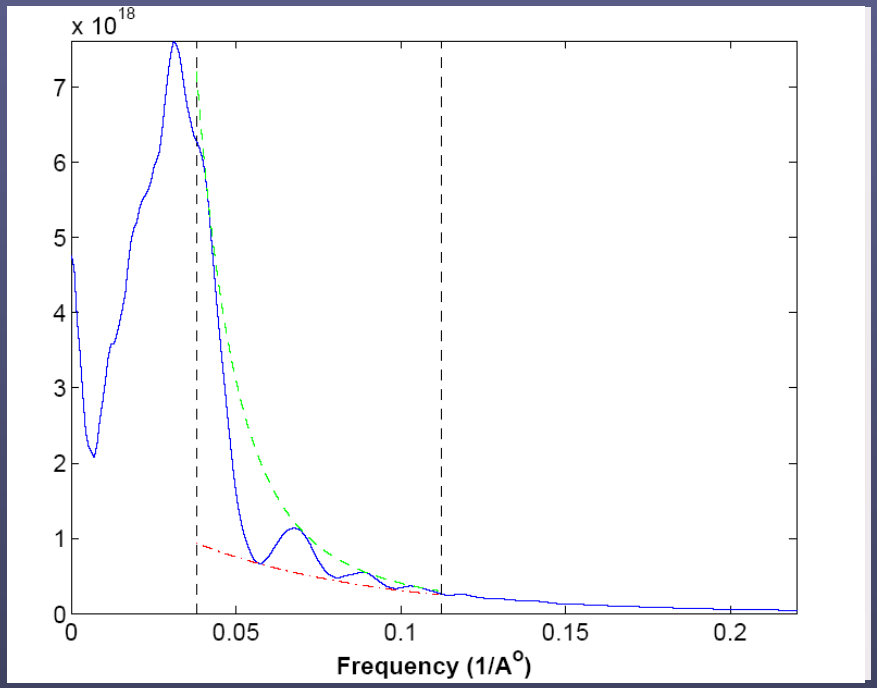
focus power spectrum





Mallick et al., (2005) "ACE: Automated CTF Estimation", Ultramicroscopy,104, 8-29.





CTF can be estimated from the power spectrum of the image

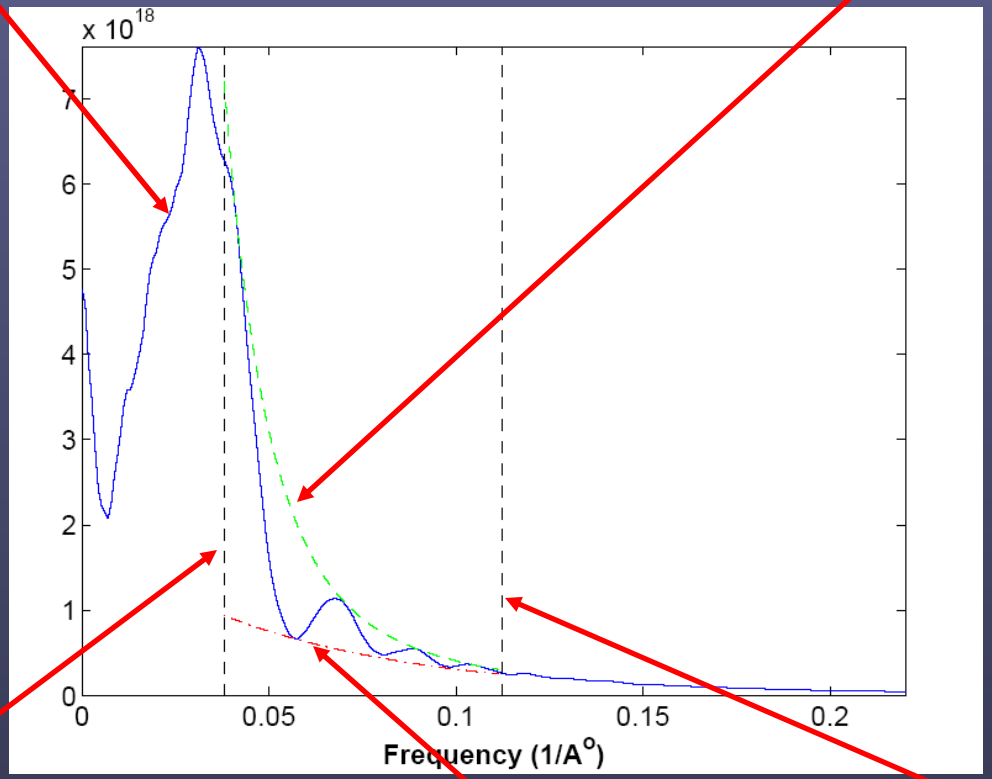
Signal can be improved by averaging along circles (or ellipses) in power spectrum

1d power spectrum

$\mathcal{E}^2(s) = e^{-b_1 - b_2\sqrt{s} - b_3 s - b_4 s^2}$

Envelope function

Parameters to estimate



Defoci

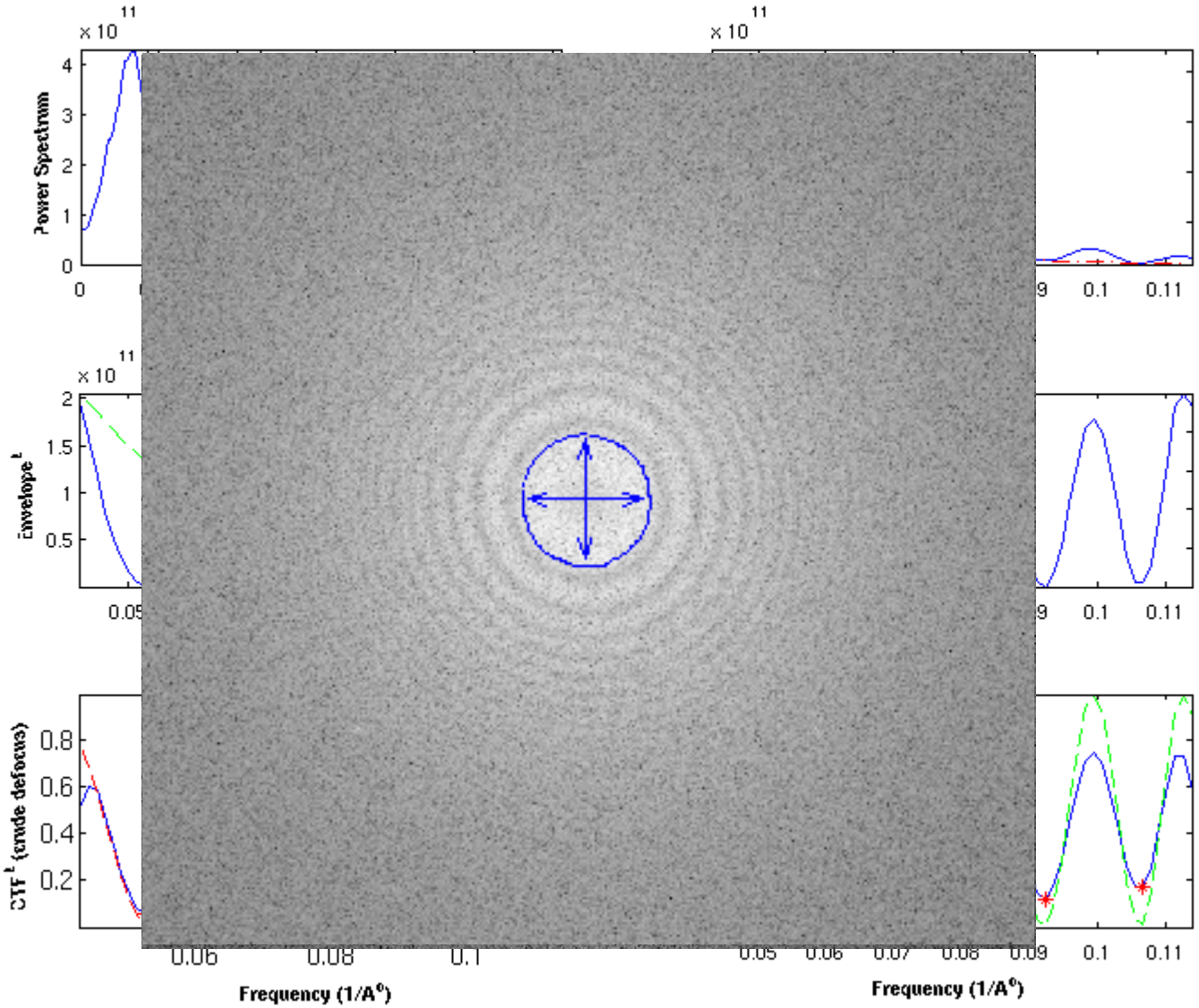
Amplitude contrast

Lower cutoff frequency

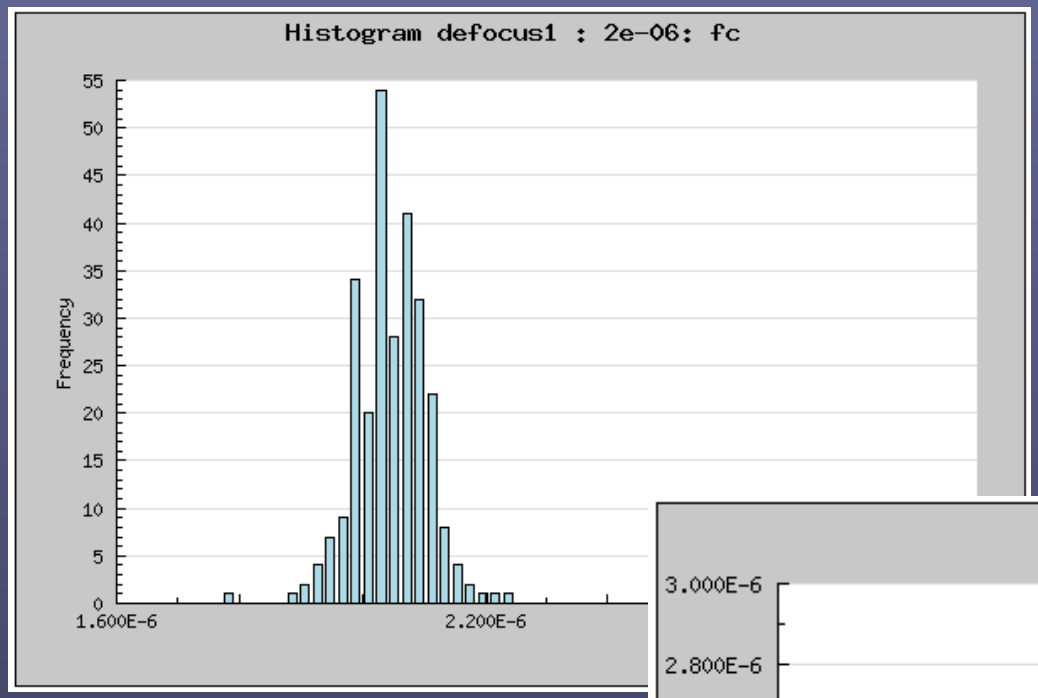
Upper cutoff frequency

Noise spectrum

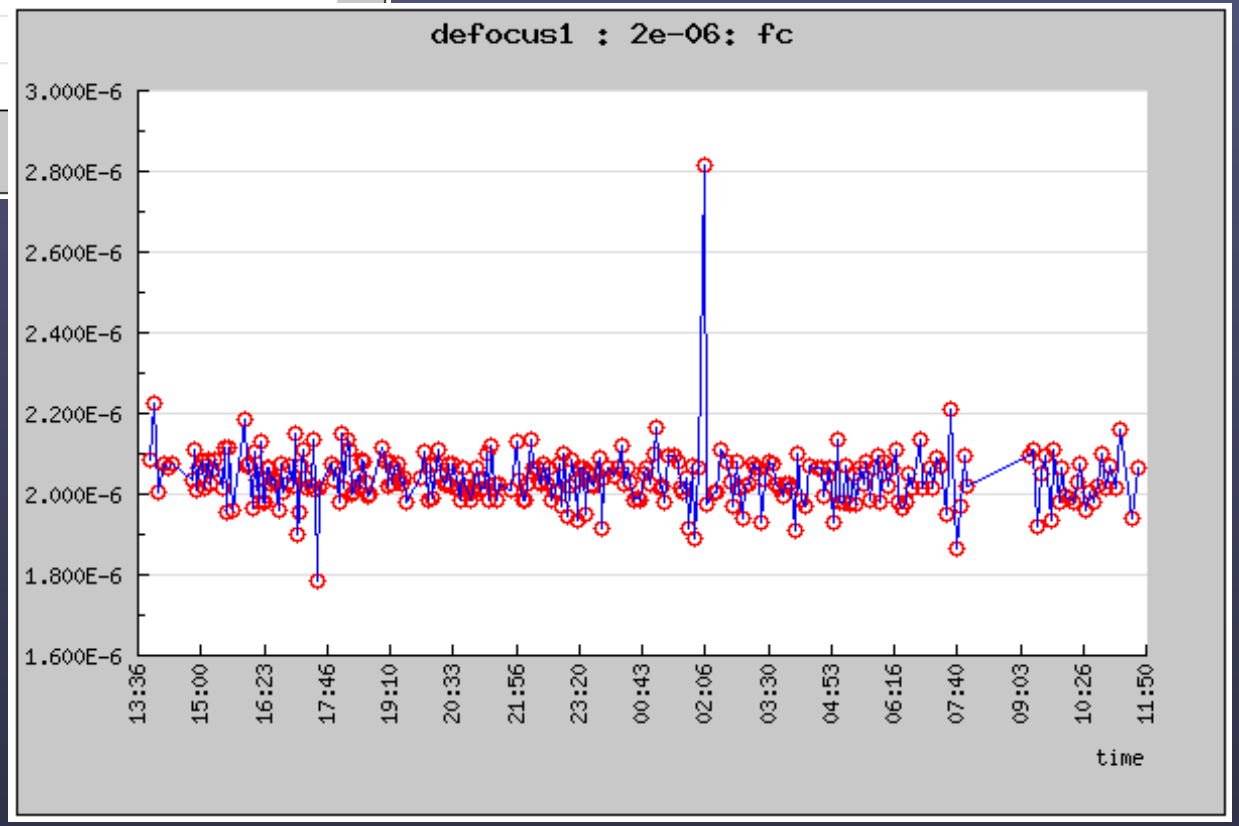
$\mathcal{N}^2(s) = e^{-a_1 - a_2\sqrt{s} - a_3 s - a_4 s^2}$


x 10^11
Power Spectrum
x 10^11
Envelope
CTF (crude defocus)
Frequency (1/A°)
Frequency (1/A°)

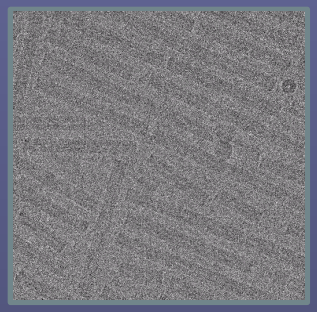
Autofocus accuracy +/-150nm



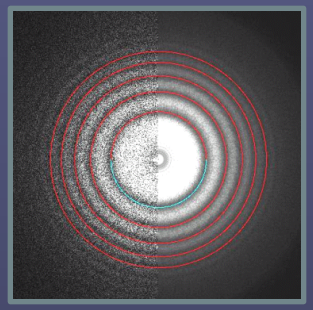
Defocus = 2.1 +/- 0.13 µm
(n = 363)



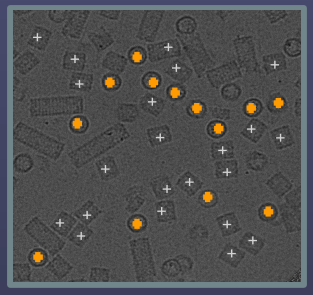
Automated image processing and reconstruction



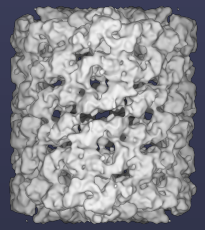
Assess the images



Determine the CTF

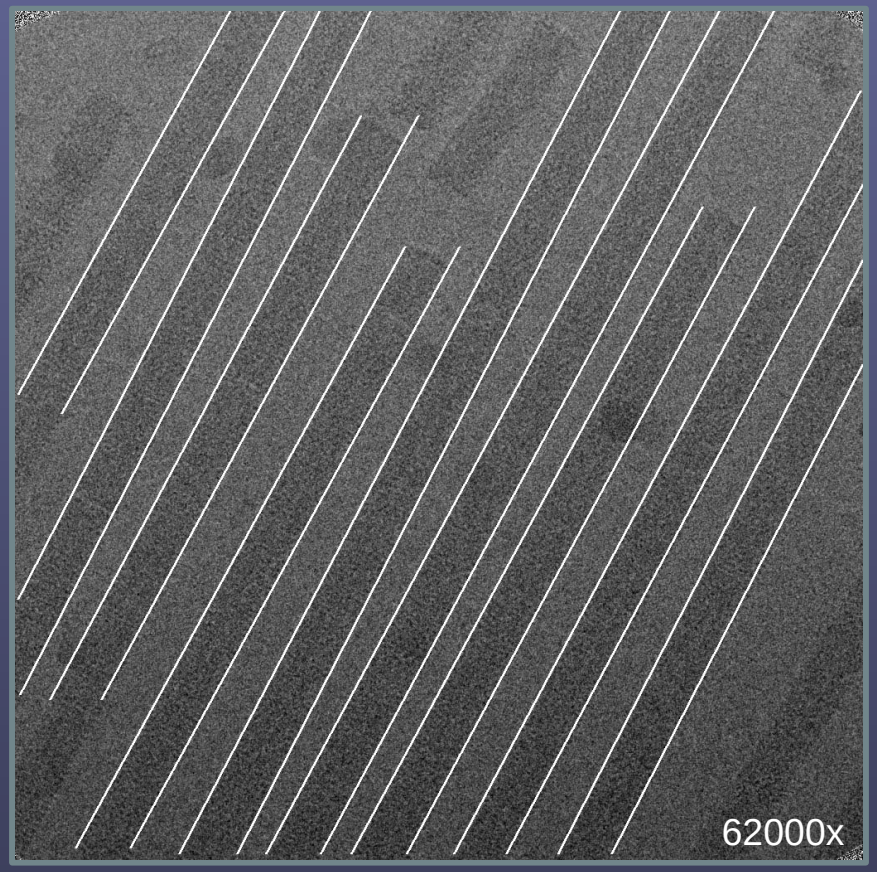


Select and segment particles



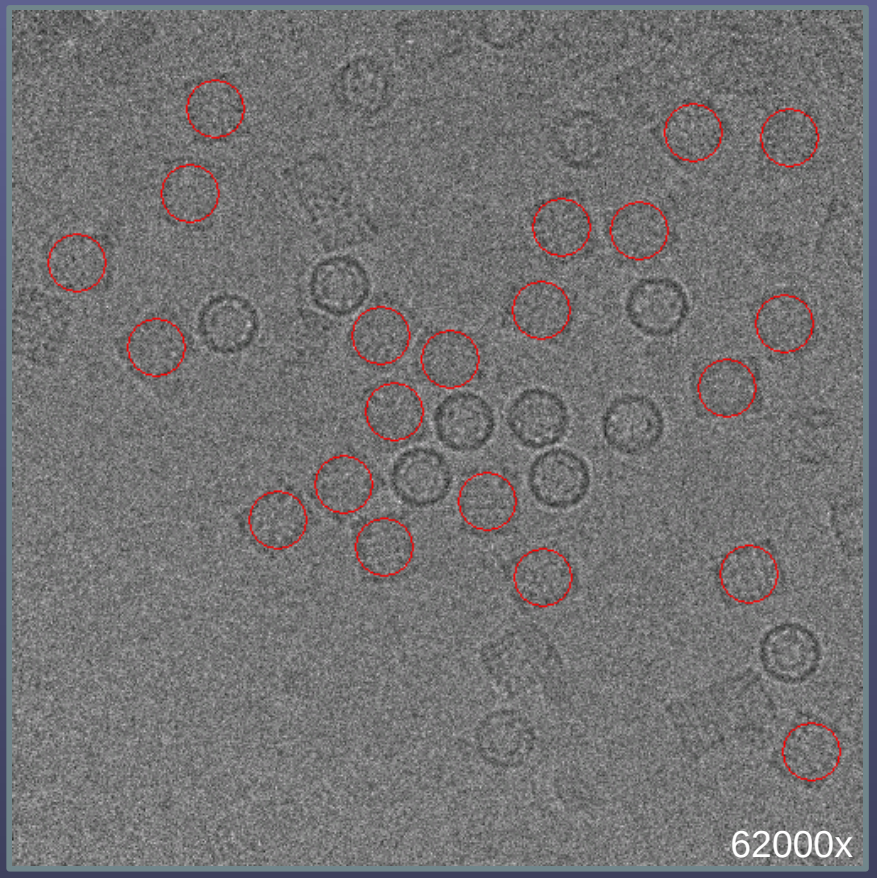
Reconstruct 3D map

Automated specimen selection and segmentation



Filaments

411 image pairs
686 filaments automatically selected

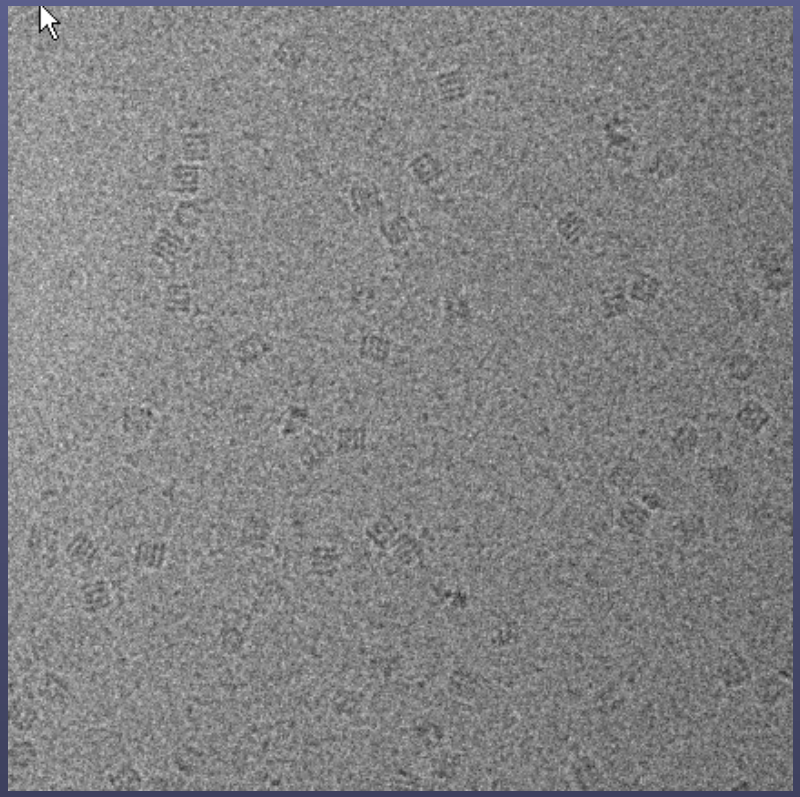


Single particles

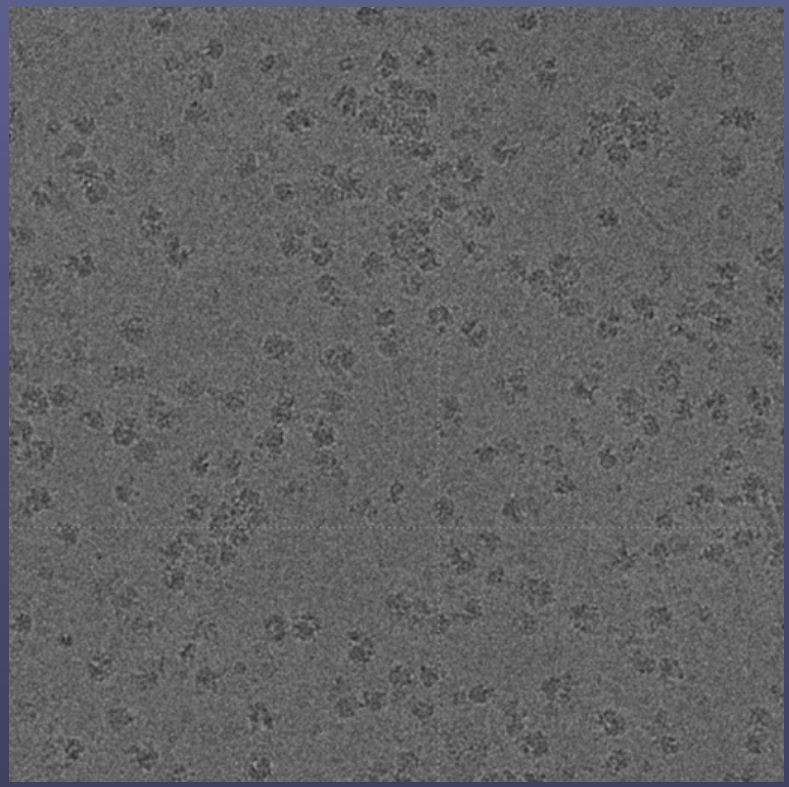
816 image pairs
23,000 particles automatically selected

Zhu, et al. IEEE Trans. on Med. Img., 22, 1053-1062 (2003).

Automated specimen selection and segmentation



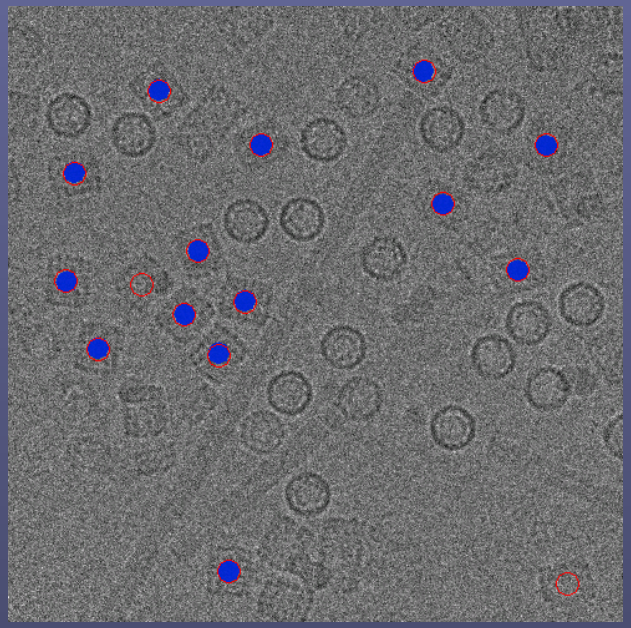
GroEL

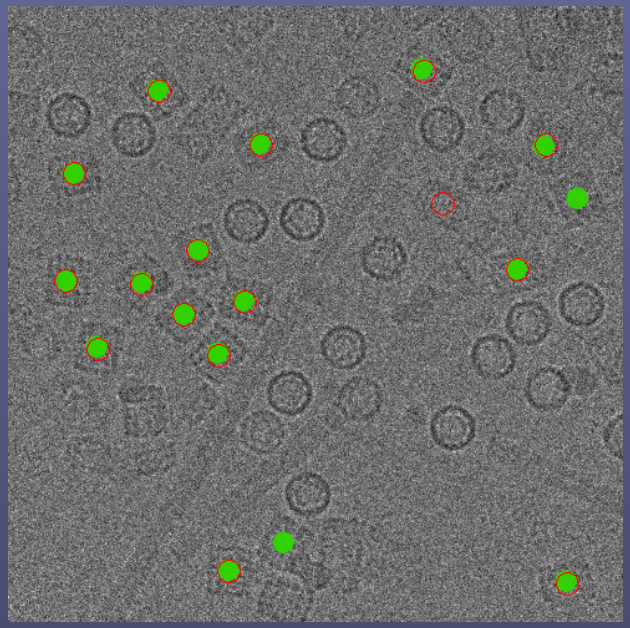


Ribosomes

Automated particle selection “bakeoff”

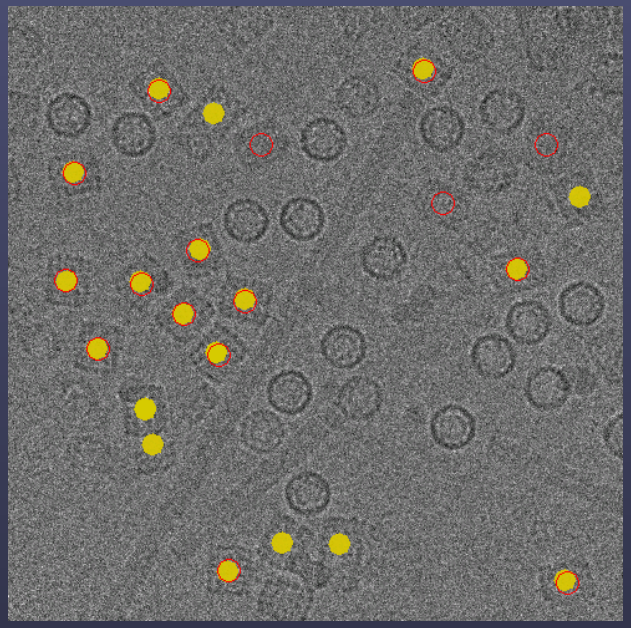
human vs. human

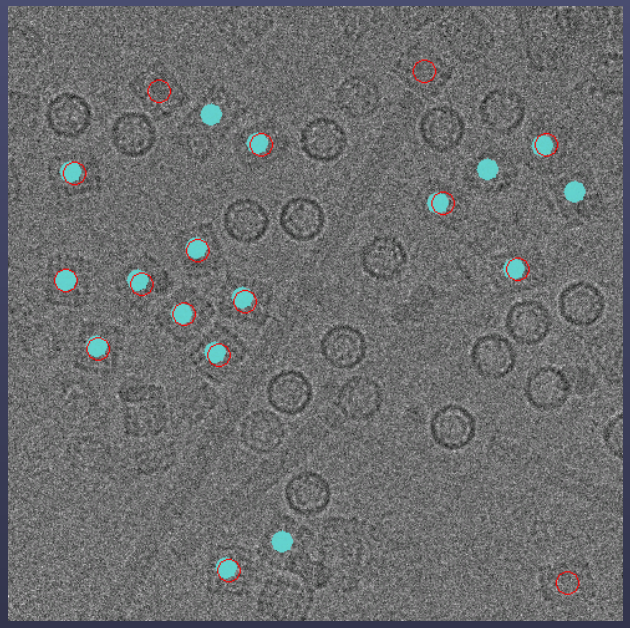




human vs. template matching

human vs. feature recognition

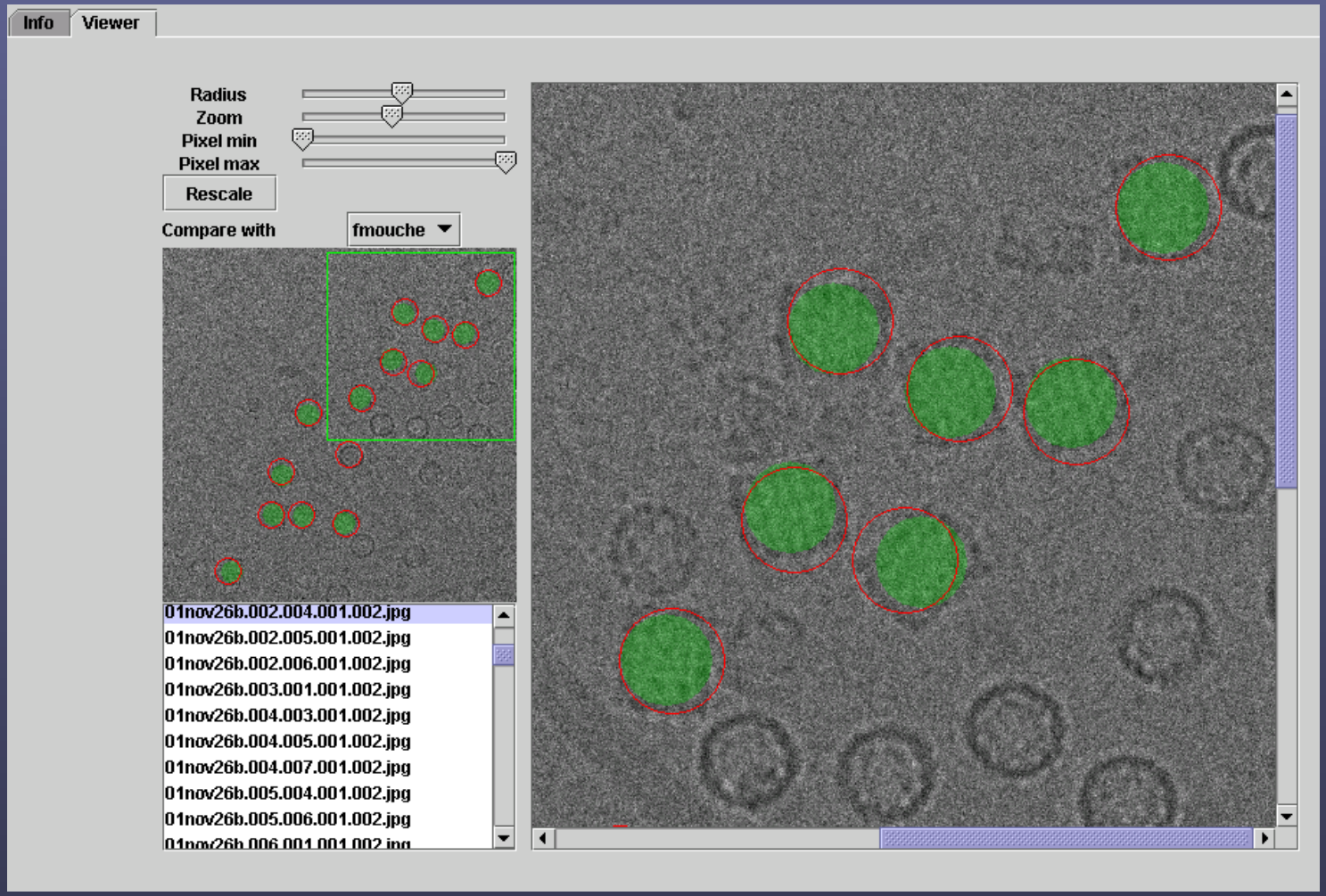




human vs. machine learning

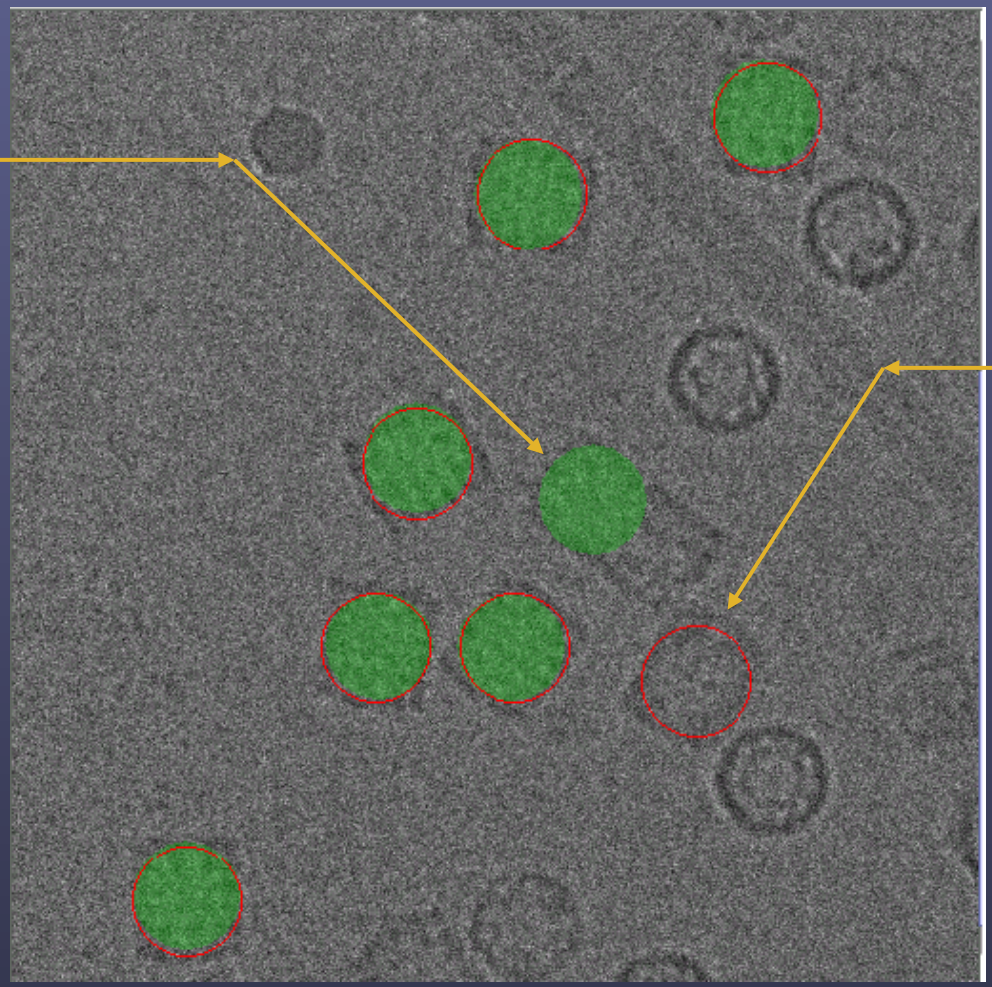
Zhu, et al., JSB, 145, 3-14 (2004).

Automated particle selection Bakeoff

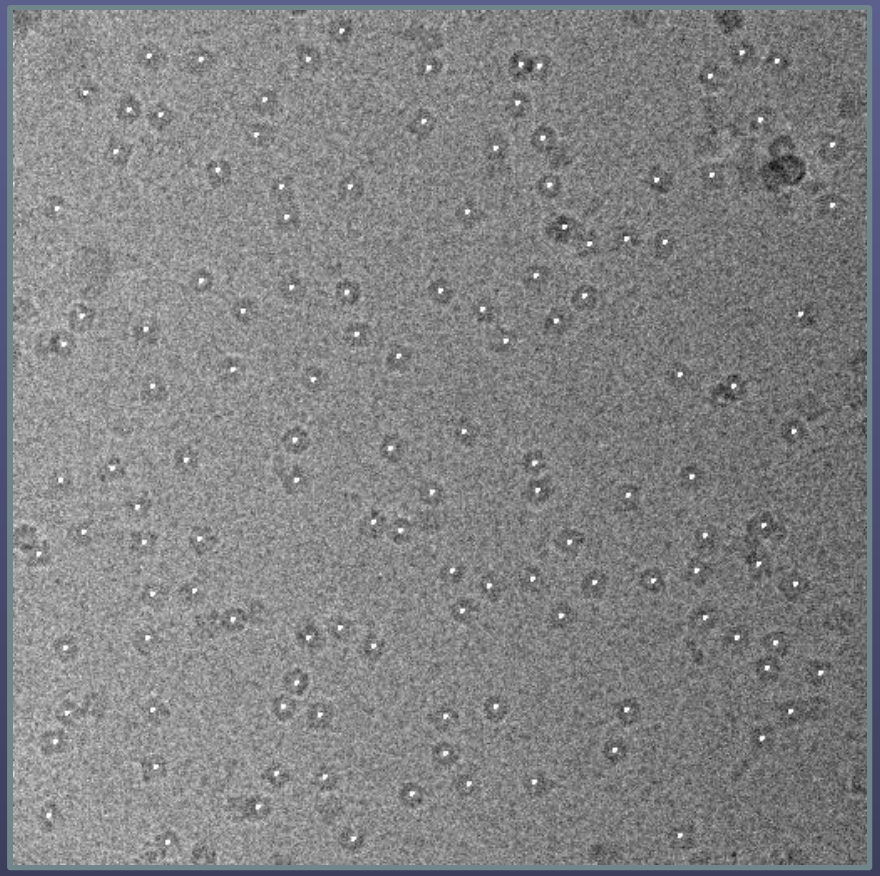


http://ami.scripps.edu/leginon/particle_viewer/

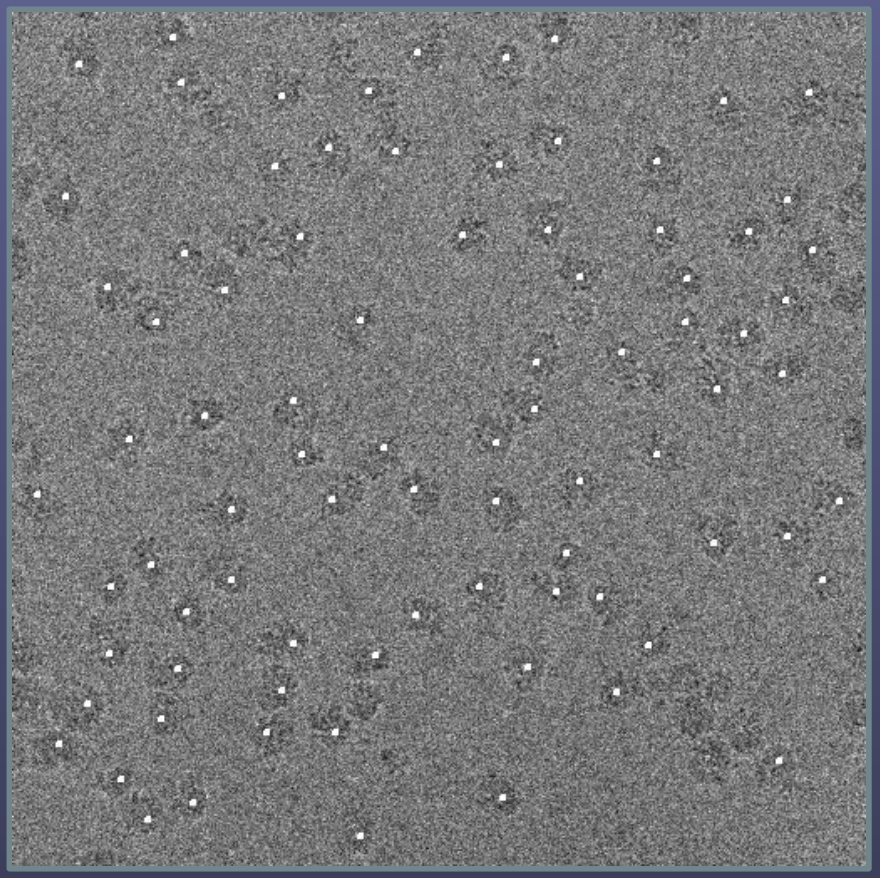
Bakeoff Results



Some examples using “Roseman” + “Selexon”

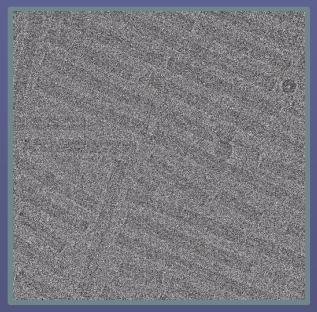


GroEL
images: 550
particles: 270,000
Time: 24 hours

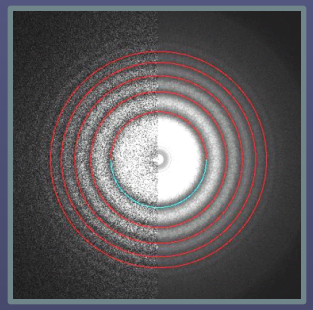


Ribosomes
images: 551
particles: 26,000
Time: 36 hours

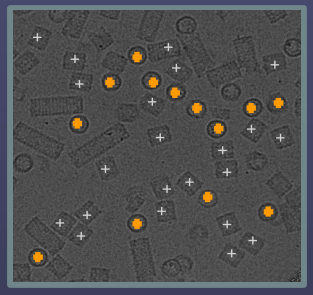
Automated image processing and reconstruction



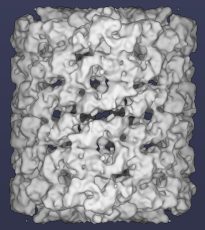
Assess the images



Determine the CTF



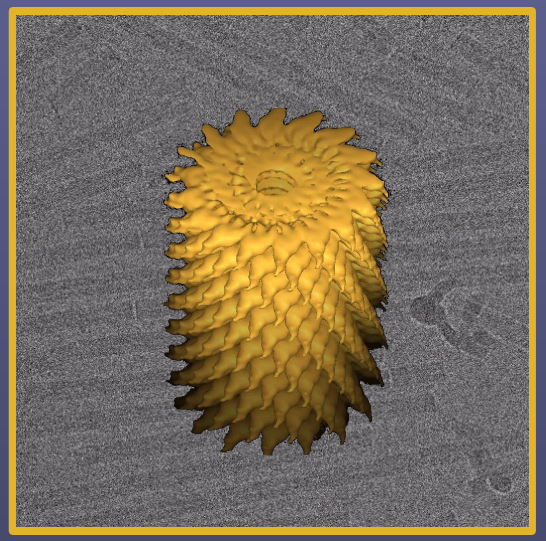
Select and segment particles

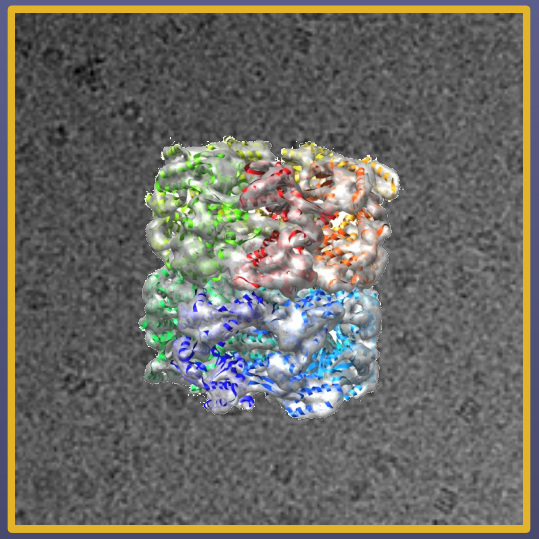


Reconstruct 3D map

Reconstruction:

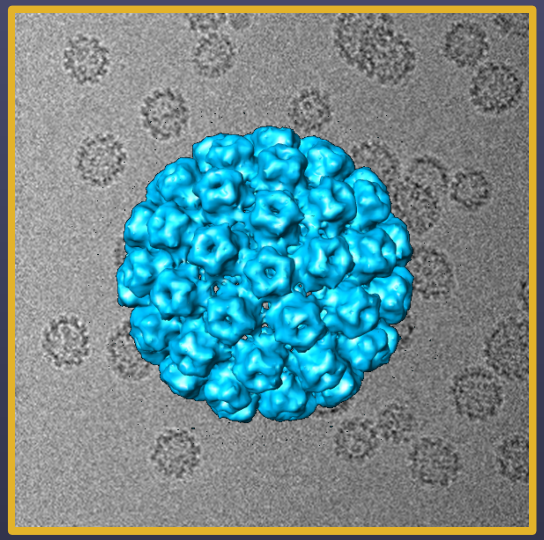
helices

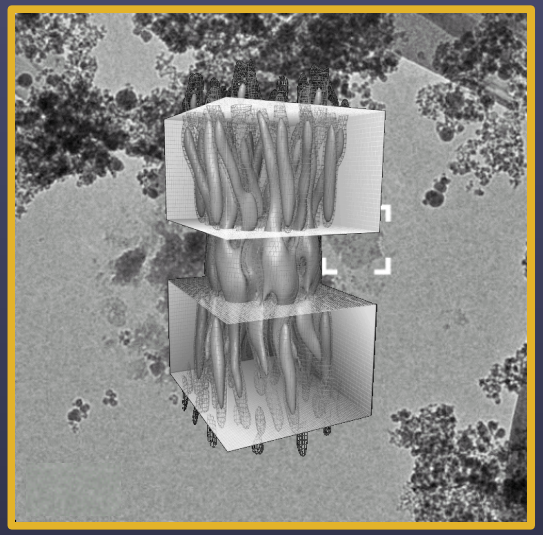




single particles

viruses

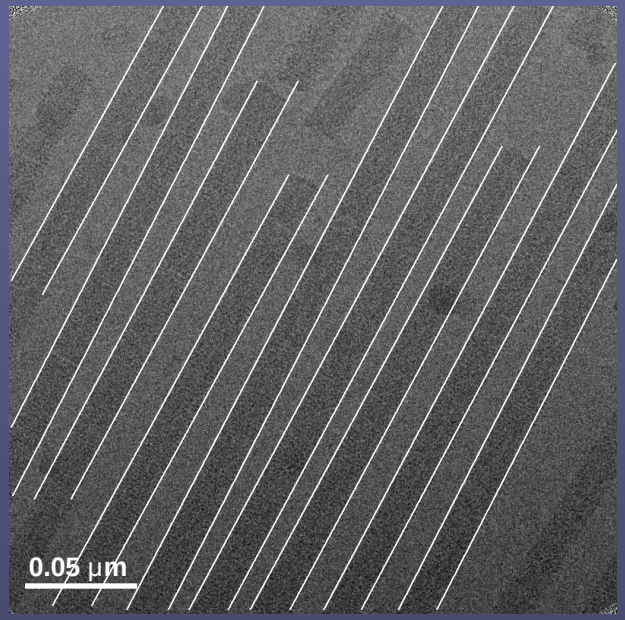


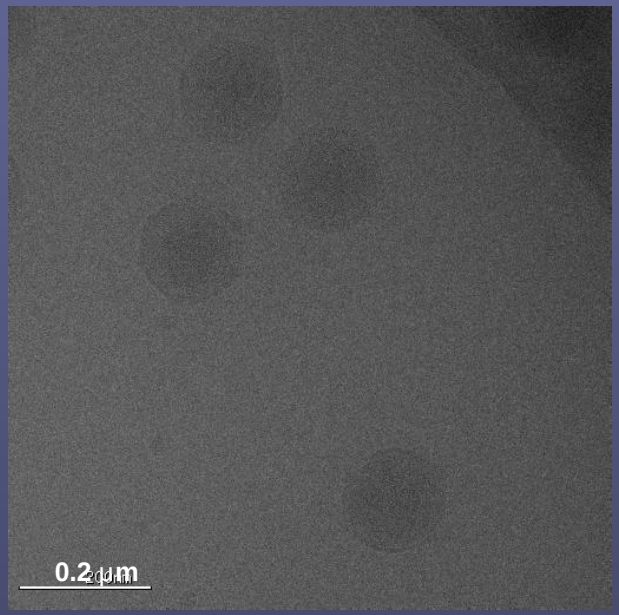


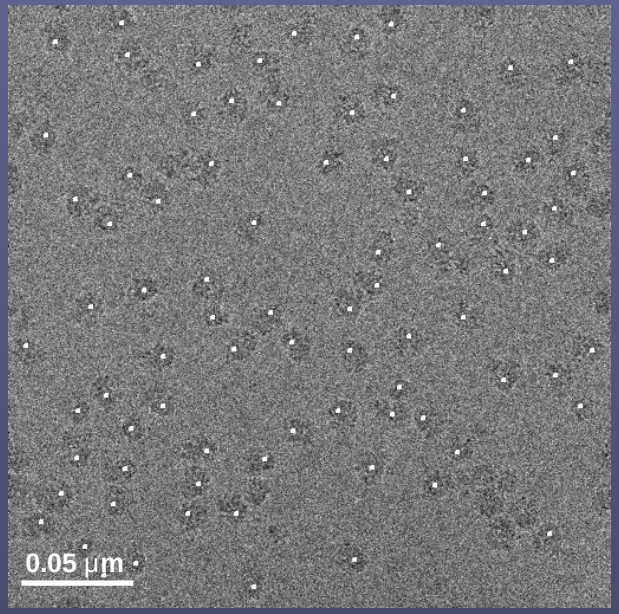
2D crystals

Throughput and Resolution

How many images do we need?







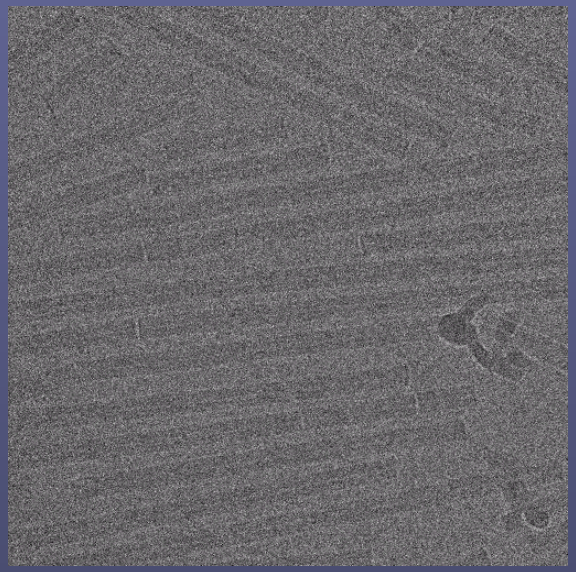
		# images			
Resolution?	**# “particles”***	**crystal**	**helix**	**big virus**	**SP’s**
4 Å	1,000,000	(100)	1000	5000	3000
8 Å	100,000	(10)	100	500	300
12 Å	10,000	(1)	10	50	30

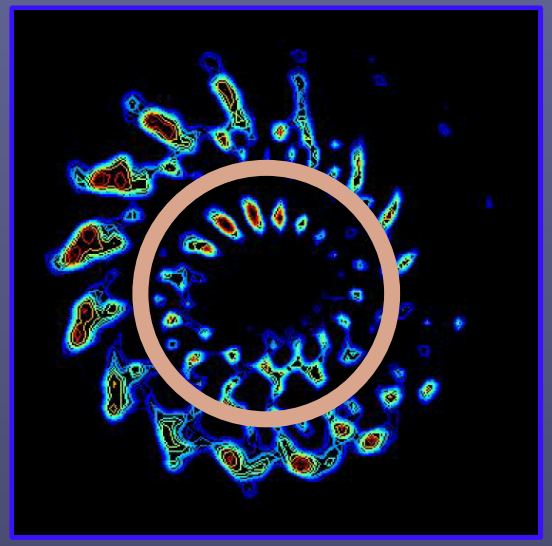
*Except for:
Yonekura, K., Maki-Yonekura, S. and K. Namba (2003) Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. *Nature* 424:643-50.

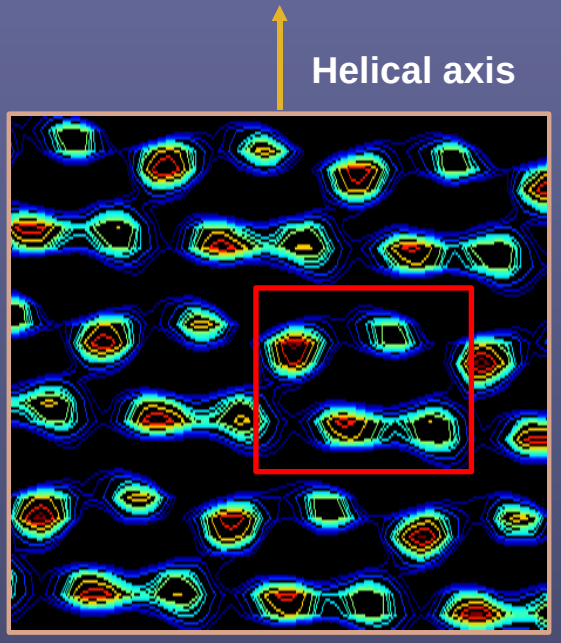
Reconstruction of TMV
Grid to map: ~7.5A within 24 hours.

TMV

What is the throughput/resolution?

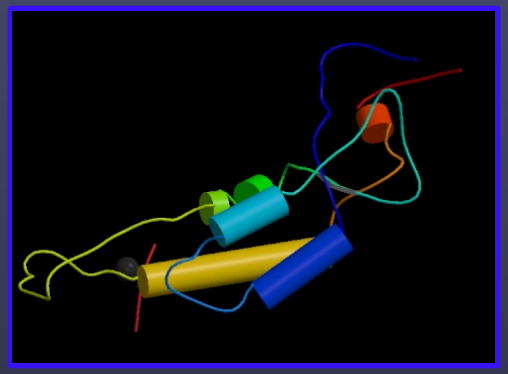




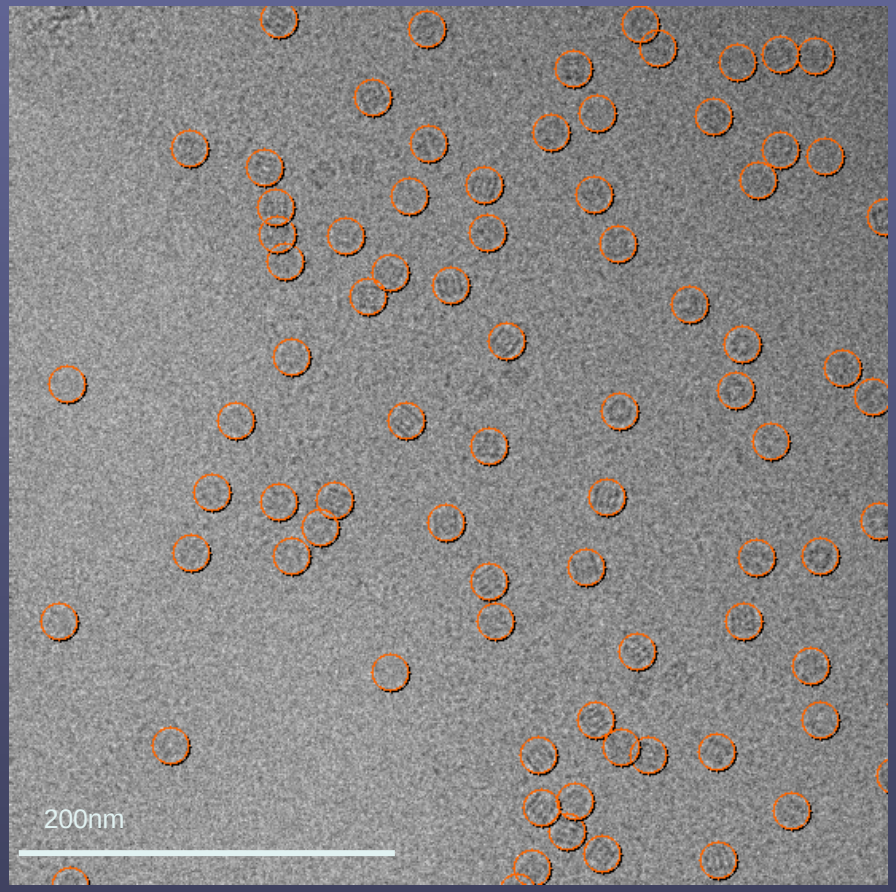


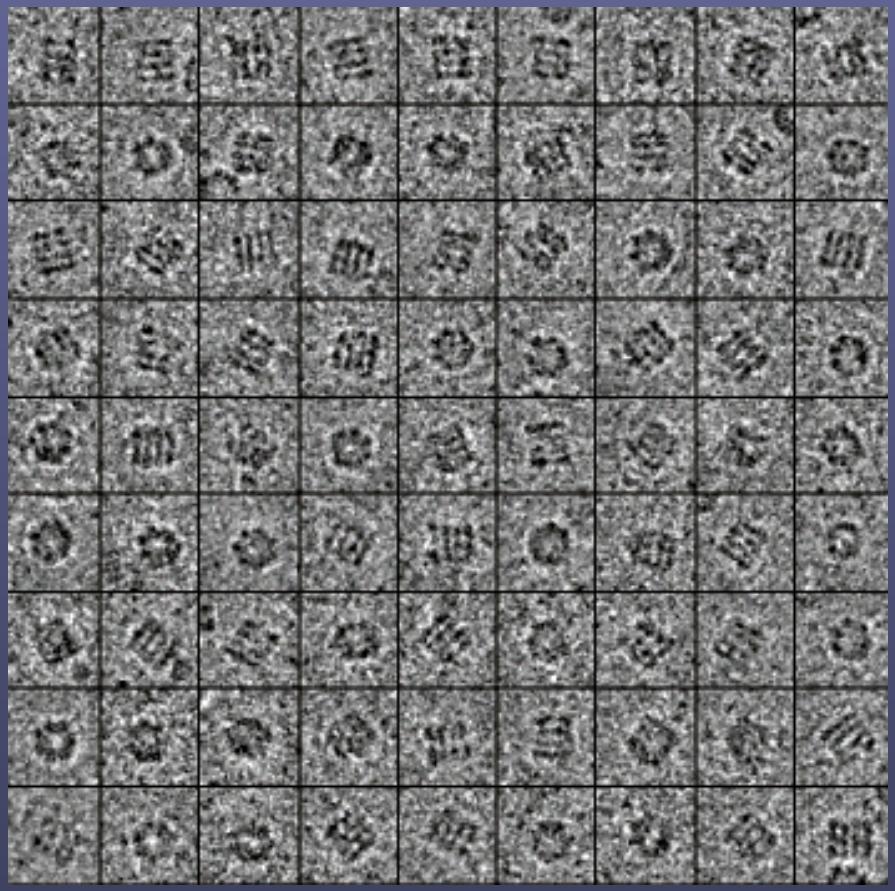
Cylindrical section at radius 66Å

# grids:	1
# squares:	19
# holes:	91
# defocus pairs:	131
# filaments found:	243
Duration:	12 hrs
Phoelix reconstruction	
# filaments used in map:	45
# molecules in map:	~70,000
Resolution of map:	~7.6Å
Yield:	~20%



Automated throughput for single particles (GroEL)





Throughput:

# grids:	1
# squares:	32
# holes:	318
# defocus pairs:	552
Duration:	26 hrs
# particles found:	~270,000

NRAMM

National Resource for Automated Molecular Microscopy

NIH NCRR Biomedical Technology Resource Center

A. Core TR&D Projects
   1. Specimen Handling
   2. Automated Acquisition
   3. Automated Processing
   4. Information Handling

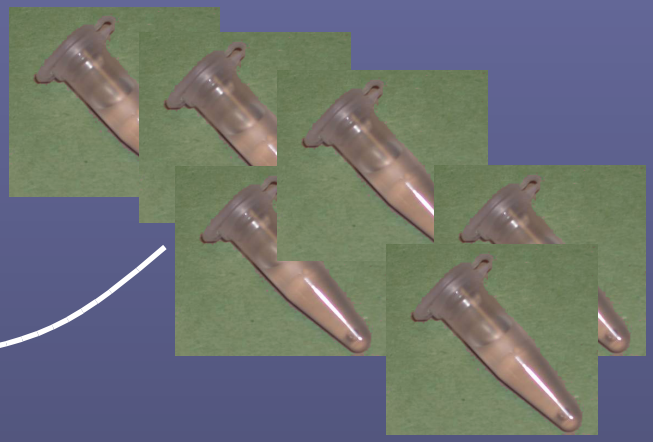
B. Collaborative Projects

C. Service Projects

D. Training- workshops + courses.

E. Dissemination

http://nramm.scripps.edu



Spec. prep. | Spec. screening | data collection | assessment | analysis

Automated Microscopy Imaging Group:

Jim Pulokas

Denis Fellmann

Joel Quispe

Anchi Cheng

Scott Stagg

Craig Yoshioka

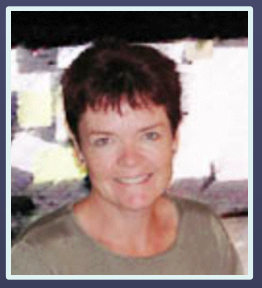
Gabriel Lander

Satya Mallick

Phil Mercurio

Jill Krawczyk

Clint Potter



Bridget Carragher

Support:

NCRR (RR 17573)

National Institutes of Health (GM61939)

National Science Foundation (DBI-9730056, DBI-0296063)