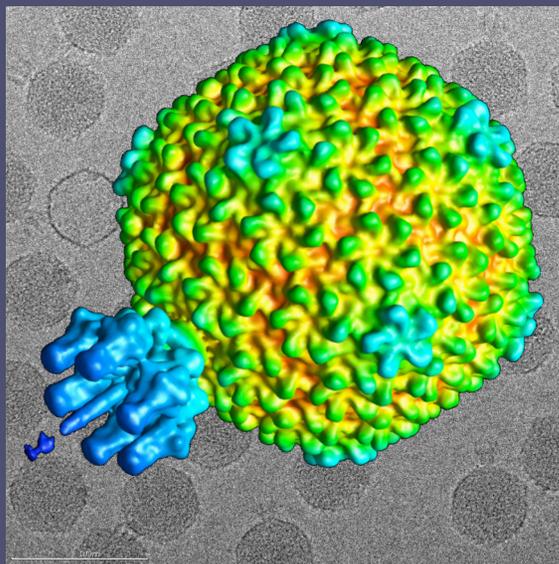


Automated Molecular Microscopy

Bridget Carragher

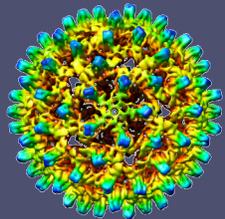
November 2007



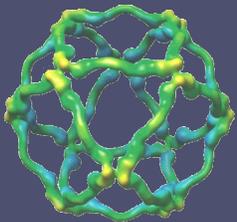
NRAMMM

National Resource for Automated Molecular Microscopy

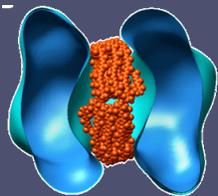
The overall mission of NRAMMM is to develop, test and apply technology aimed towards automating and streamlining cryo-electron microscopy (cryoEM) for structural biology.



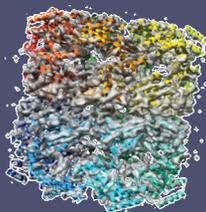
HBV



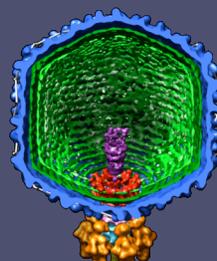
COPII



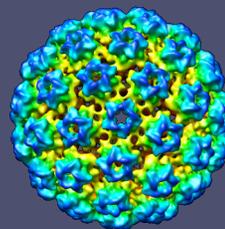
LCP



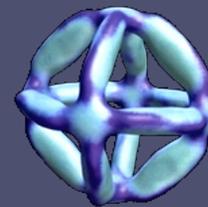
GroEL



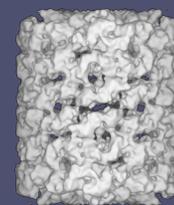
P22



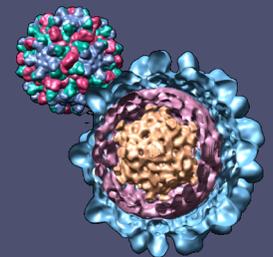
HPV



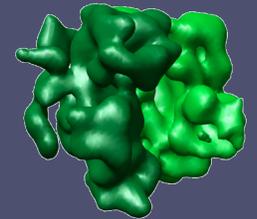
DNA



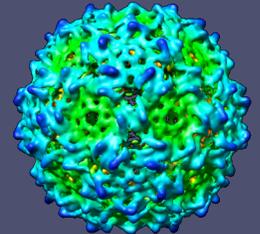
KLH



CNV



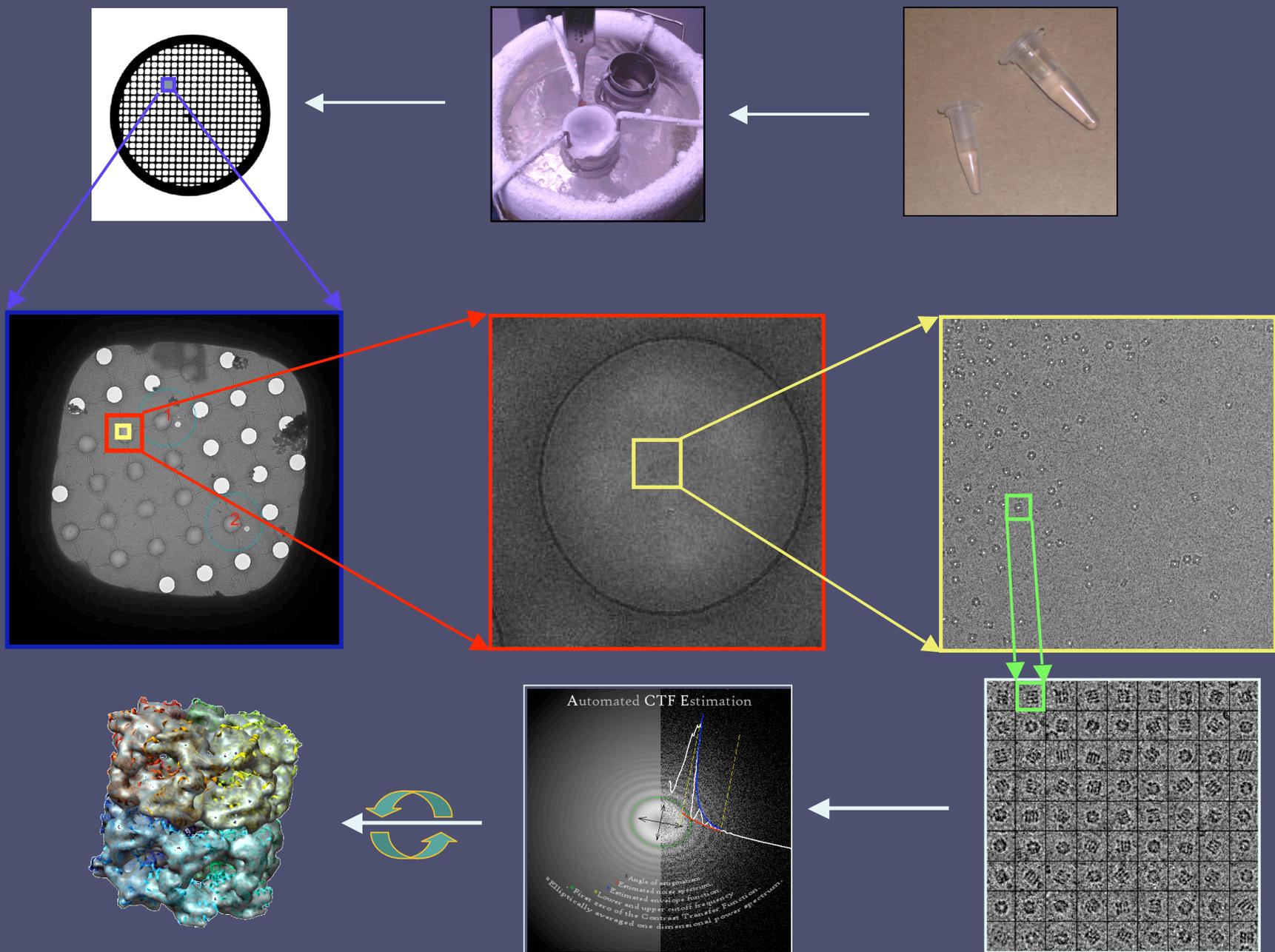
Ribosome



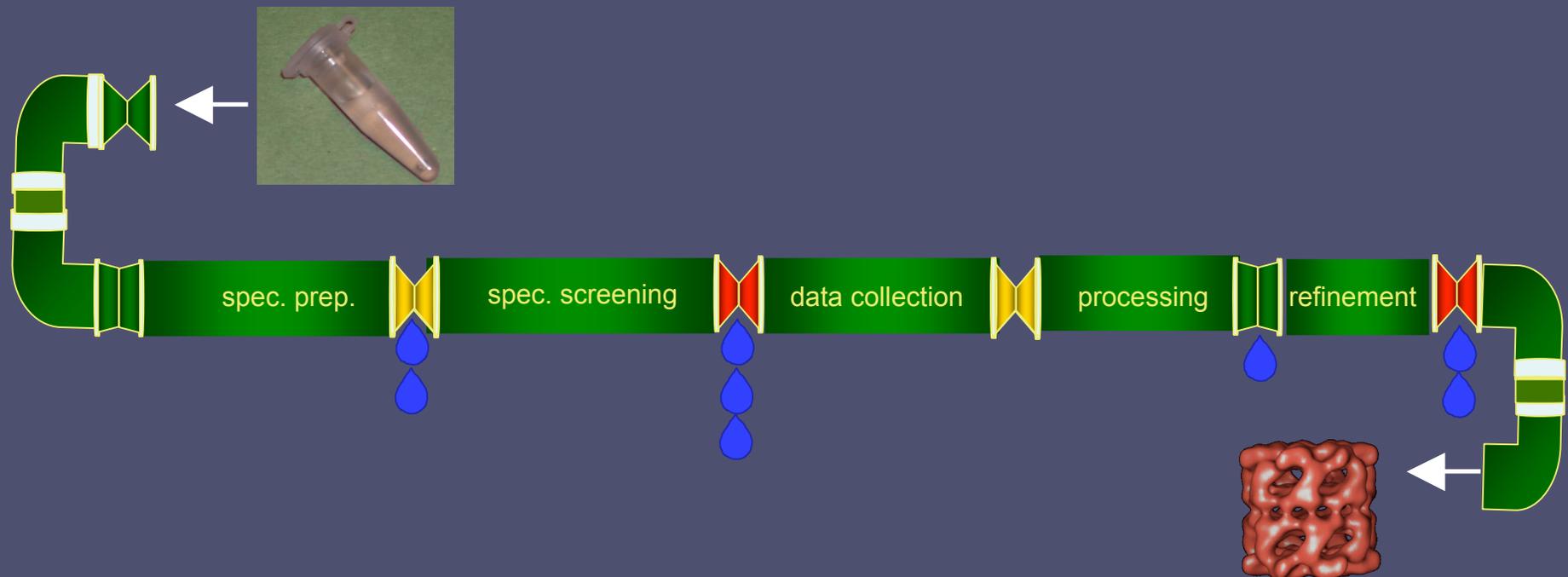
Qbeta

Automation goals
Facilitate the process
Increase throughput
Optimize resolution
Expand the possibilities
Open the technology to wider audience

Cryo-electron microscopy: structure of macromolecular machines



Automated Pipeline for Molecular Microscopy

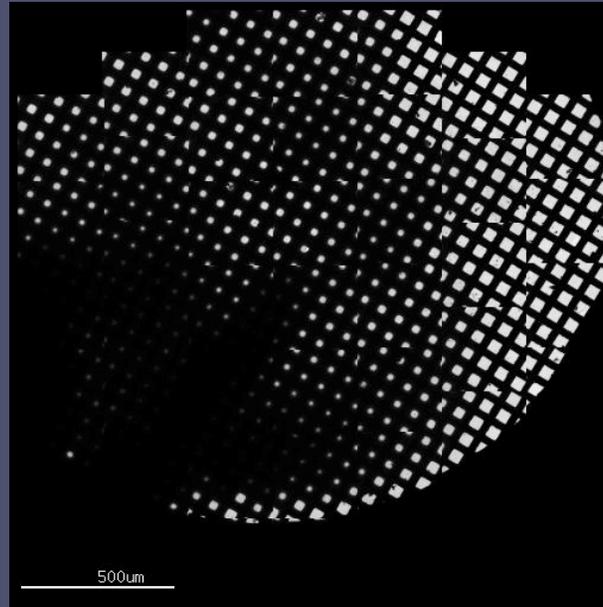


Adapted from a slide courtesy of: Peter Kuhn, Scripps-PARC Institute for Advanced Biomedical Sciences, TSRI

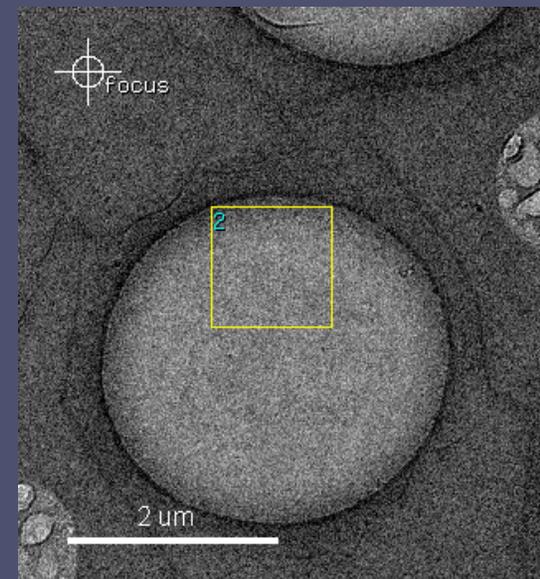
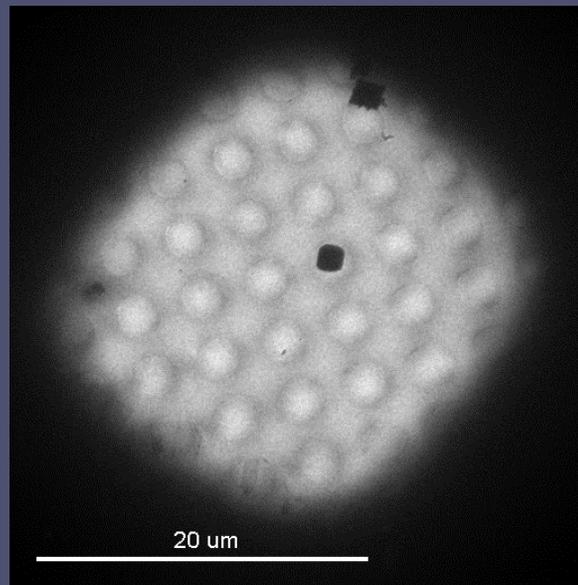
Step 1: Freezing EM specimens



Gatan Solarus Plasma Cleaner



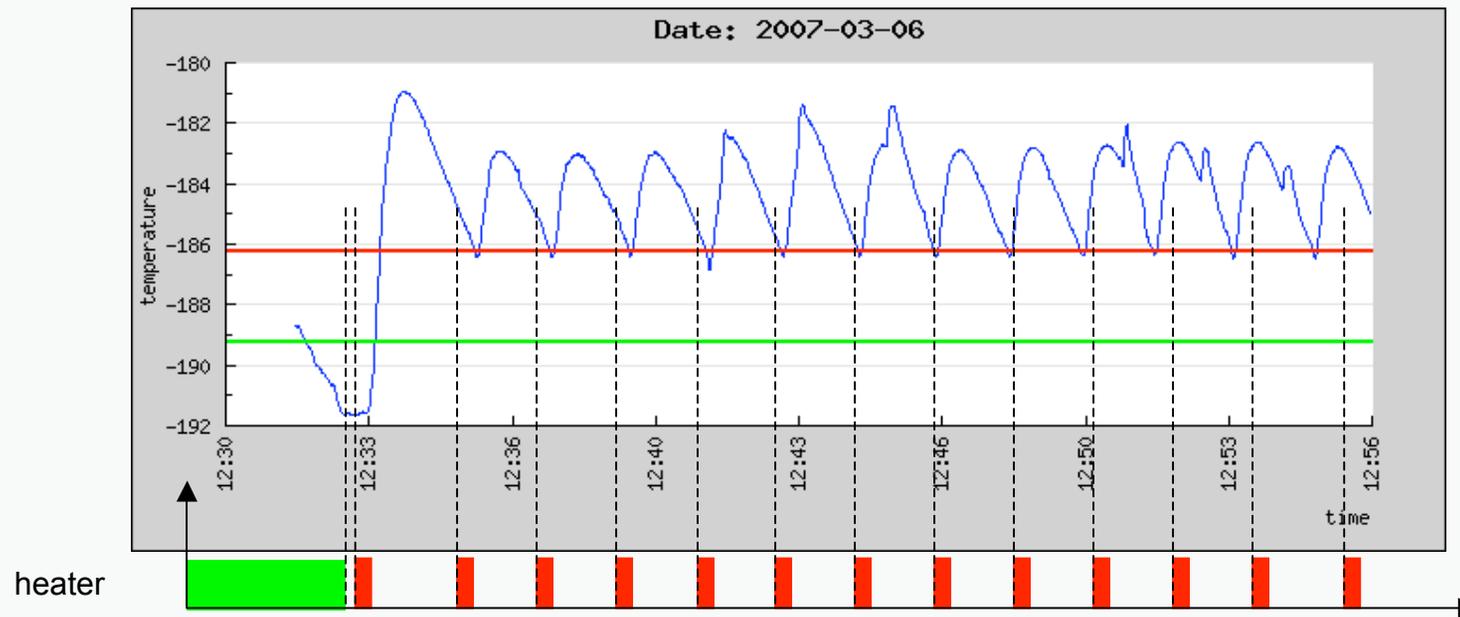
FEI Vitrobot

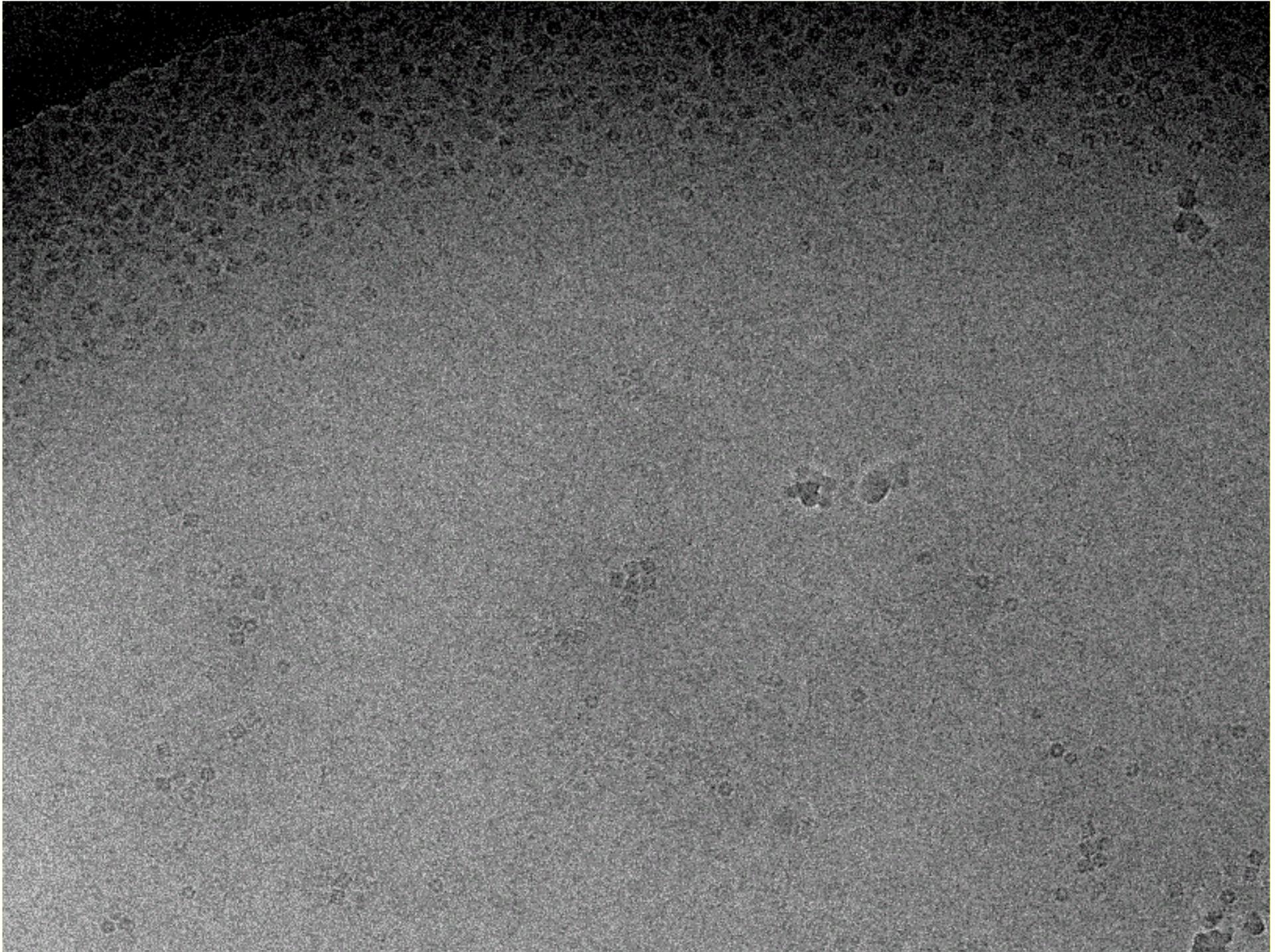


Automated temperature control of liquid ethane



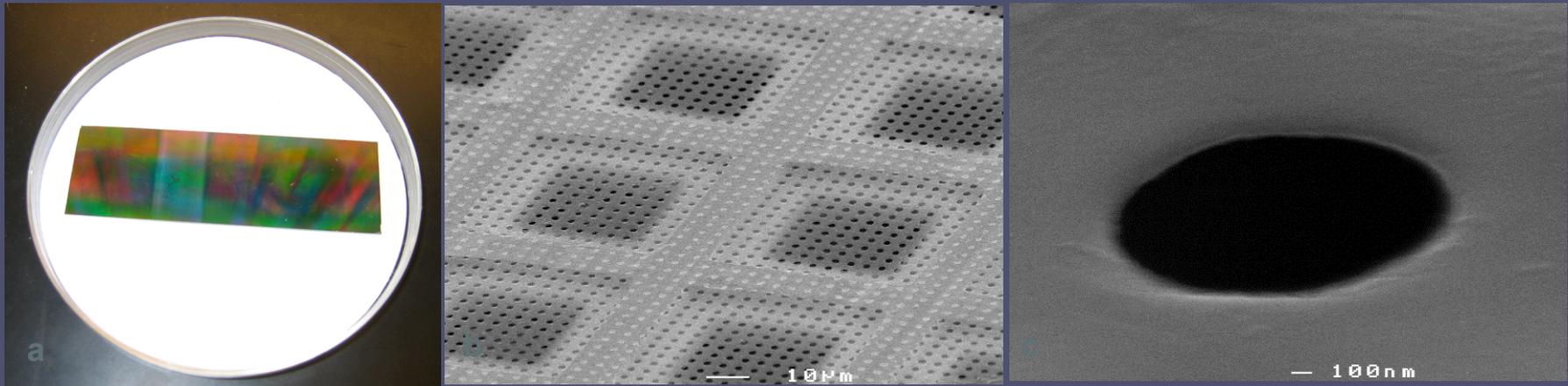
Automated temperature control of liquid ethane





Alternative technique for making a holey carbon film (C-Flats).

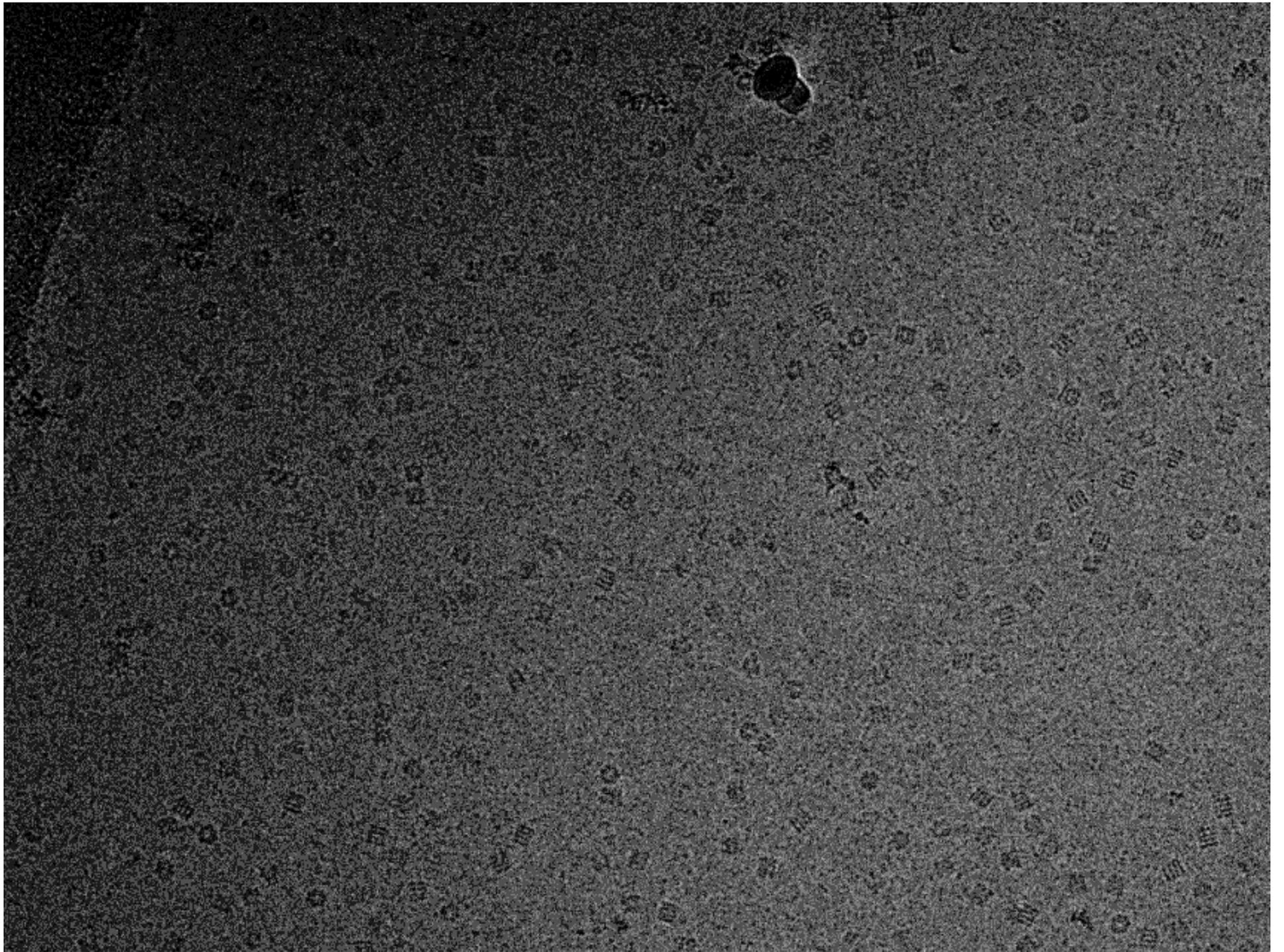
- Use a silicon-nitride template with regularly spaced wells. Developed by the Protochips Co. We are currently working with a template similar to the R2/4 from Quantifoil, but many different geometries are possible.
- Make a carbon replica of the template using Victawet as a releasing agent.



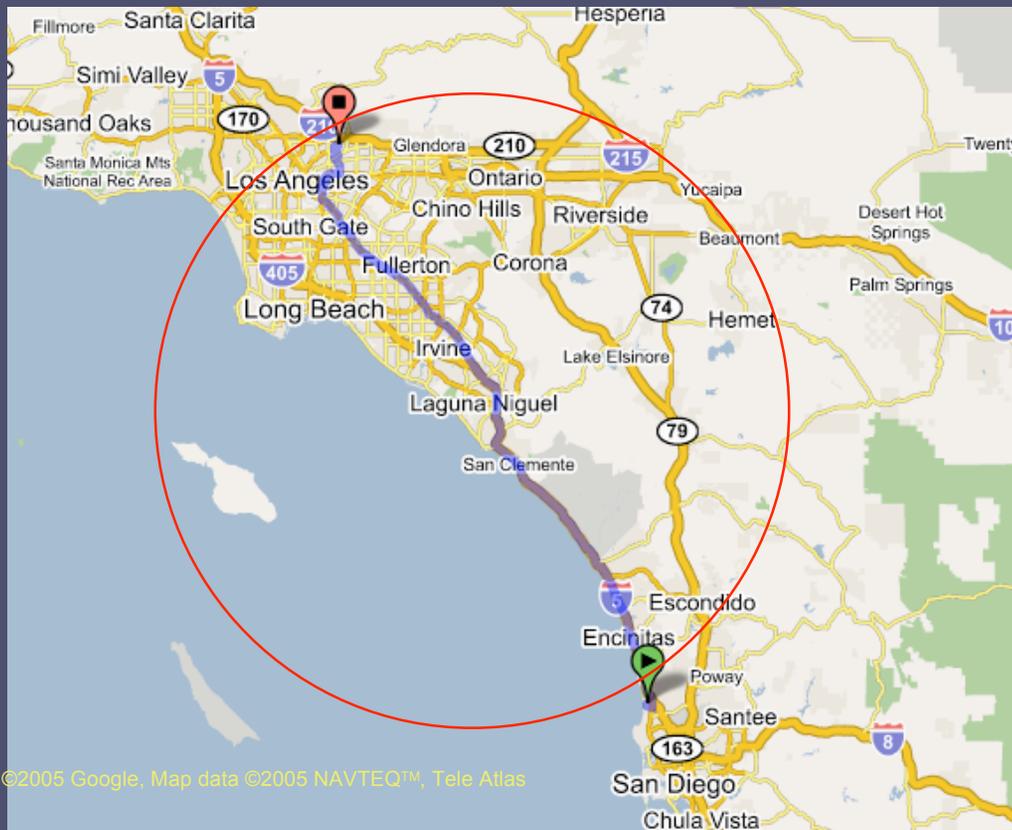
Quispe, J., et al. (2006). A New Holey Carbon Film for Cryo Electron Microscopy. Microscopy and Microanalysis..

<http://www.protochips.com/>

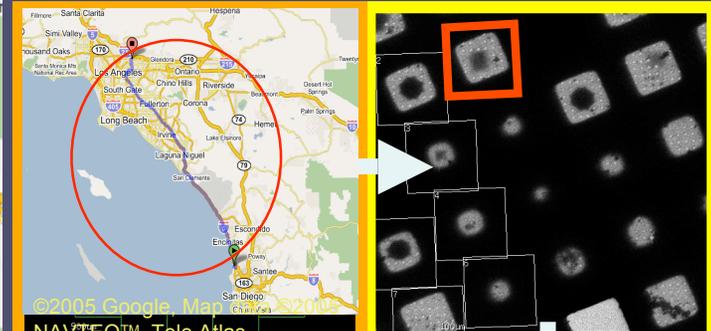




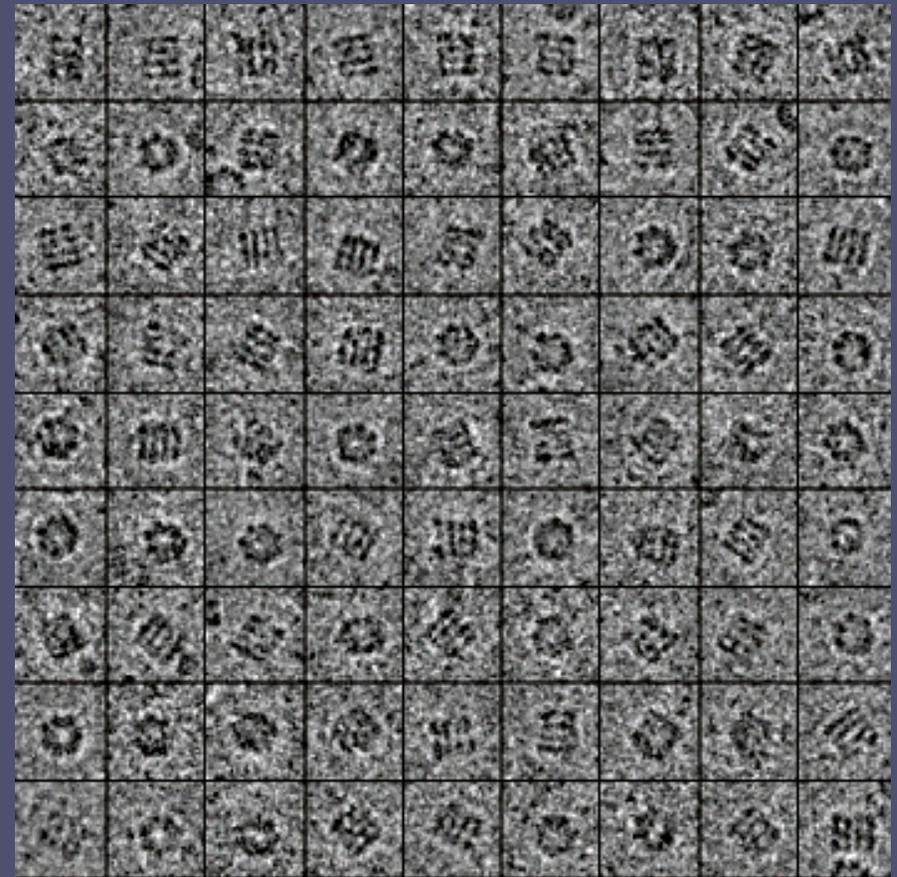
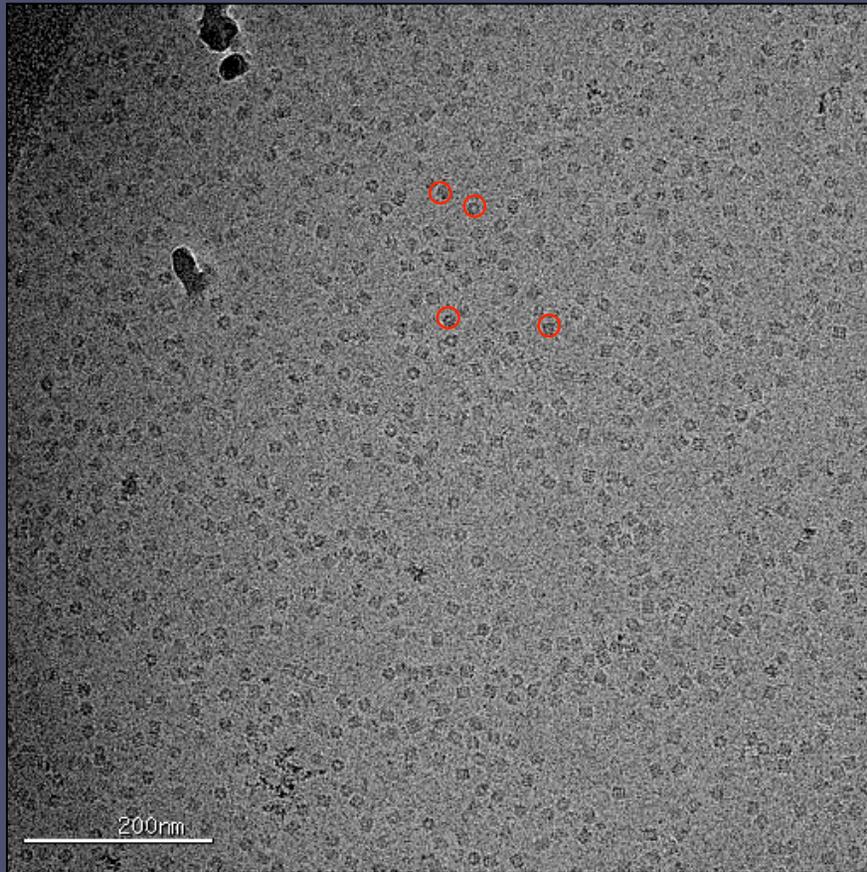
Step 2: Automated data acquisition using Legimon



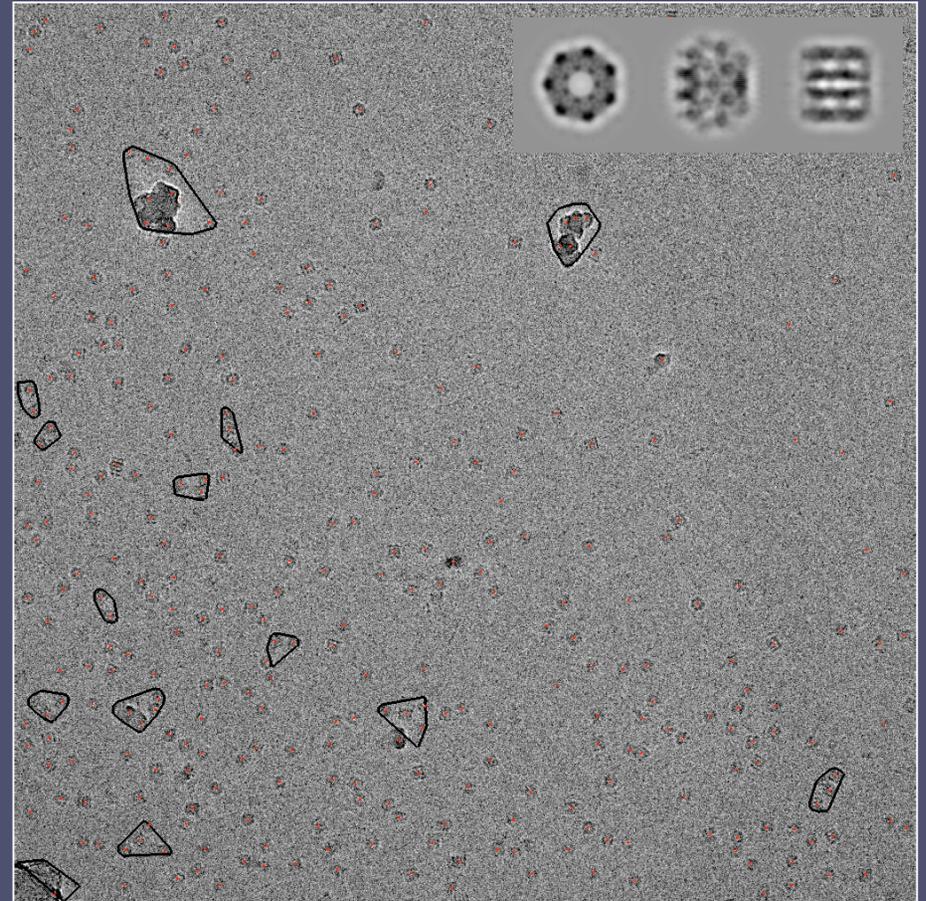
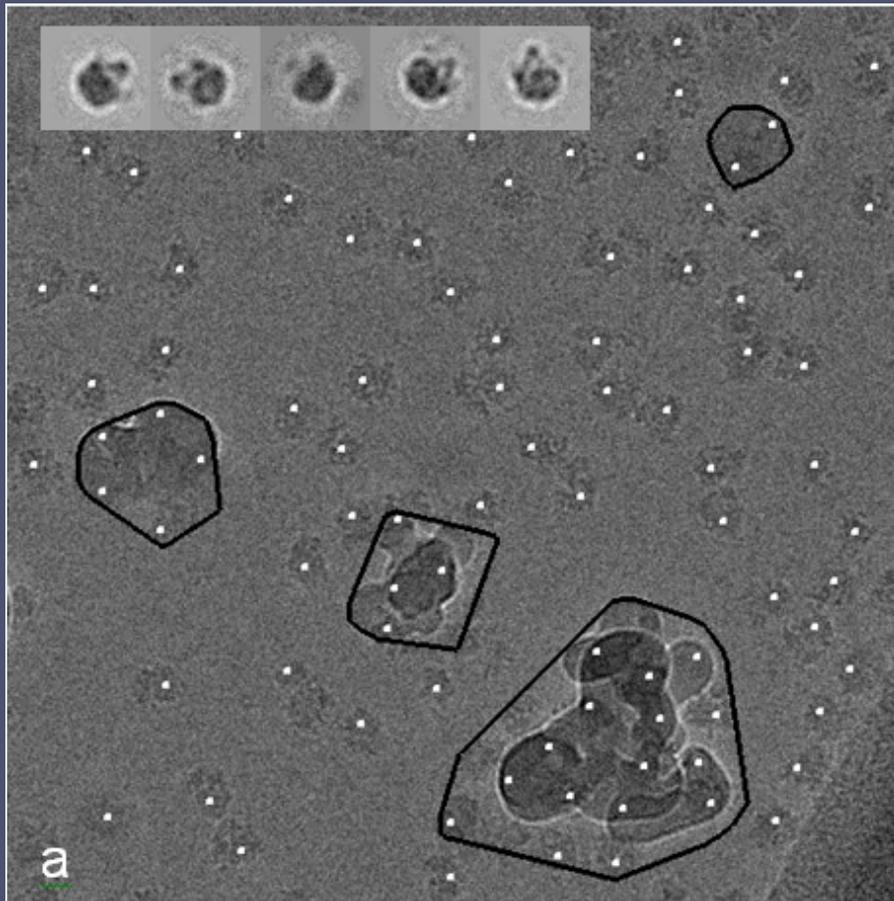
Multiscale Imaging



Step 3: Particle selection



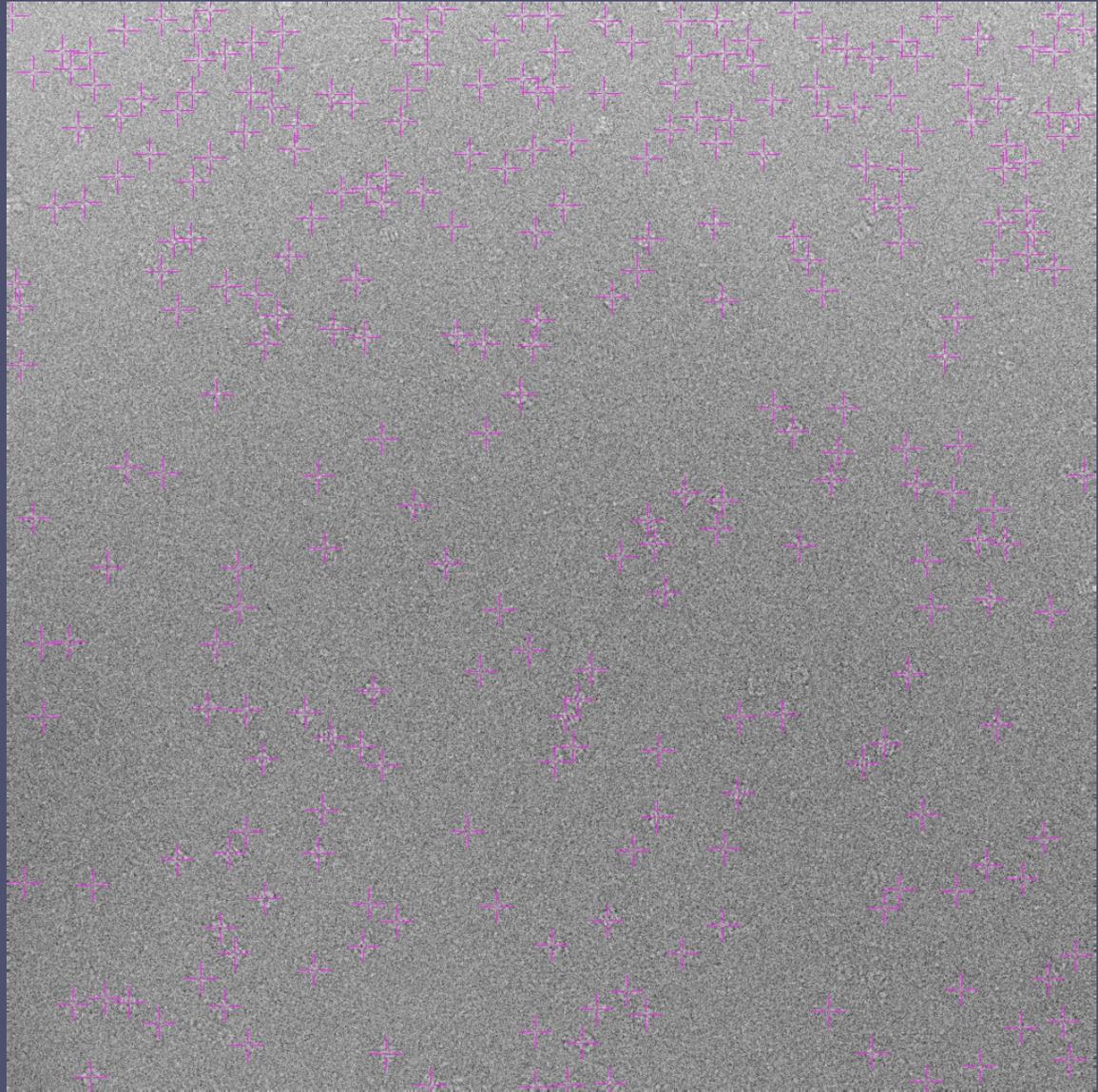
Automated particle selection with Selexon



- ~95% accurate for GroEL
- runs concurrently with data collection
- integrated with database

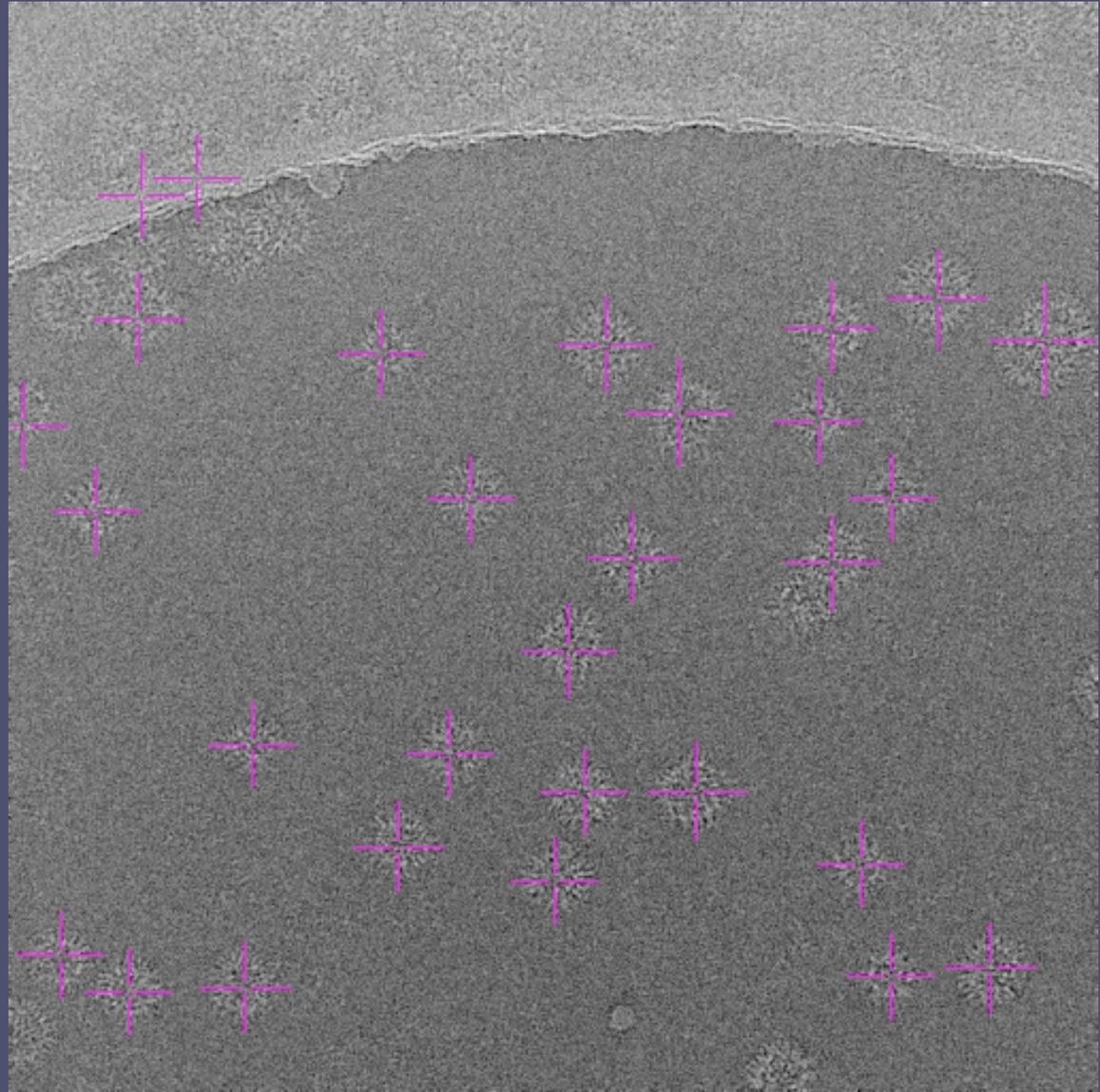
Roseman (2004) JSB, 145, 91
Zhu *et al.* (2004) IEEE, 22, 1053

Automated particle selection with DogPicker



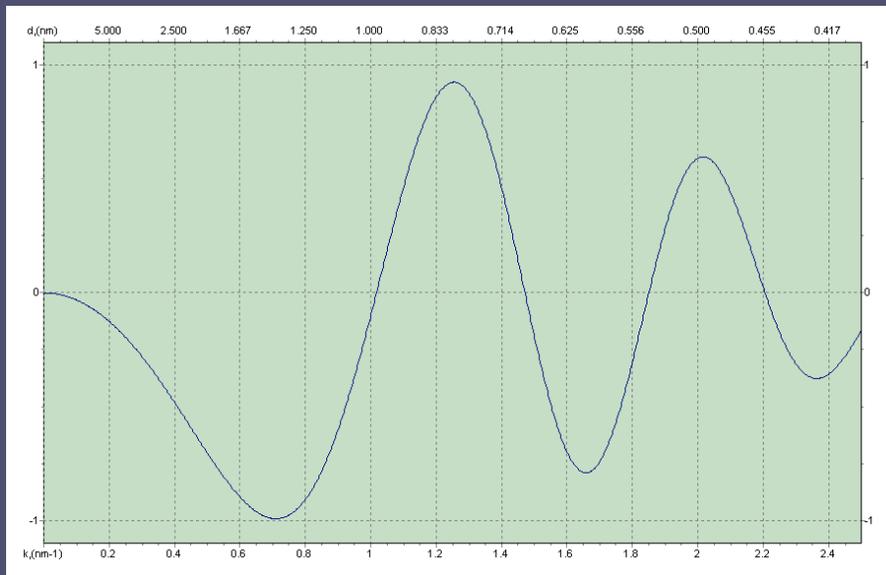
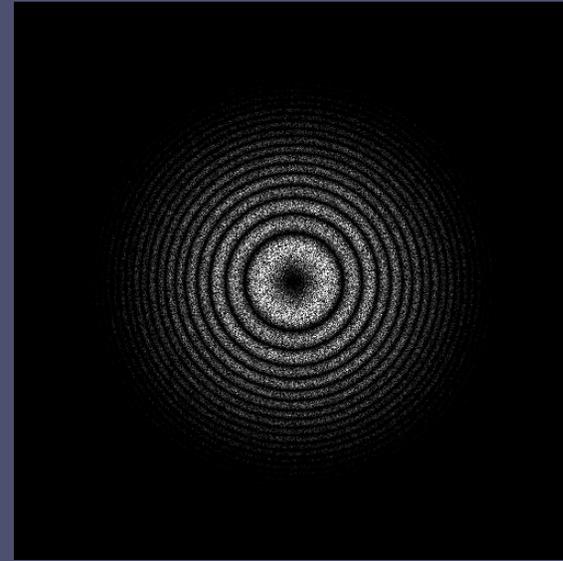
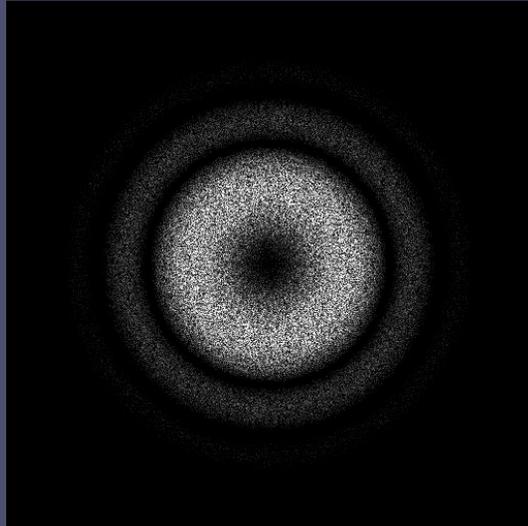
GroEL

Automated particle selection with DogPicker

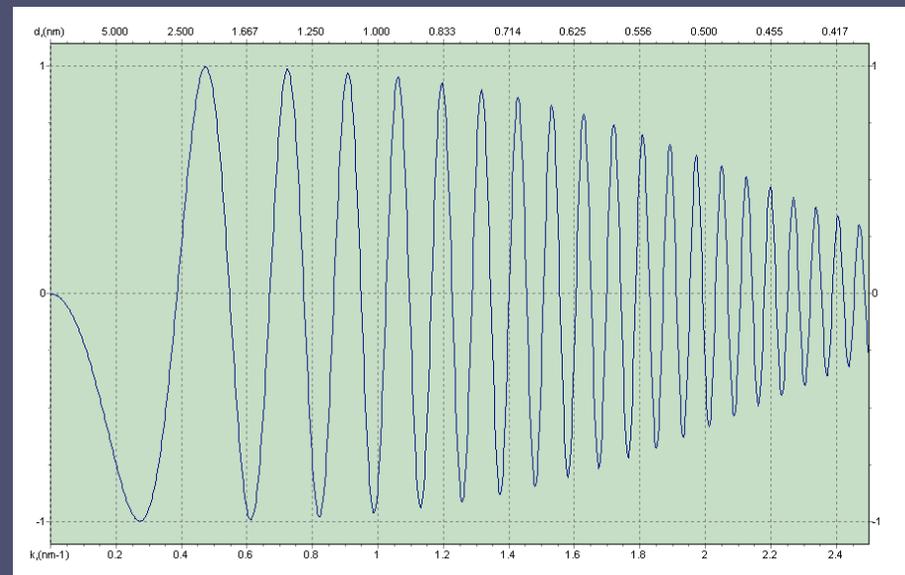


Virus like particles

Step 4: CTF correction

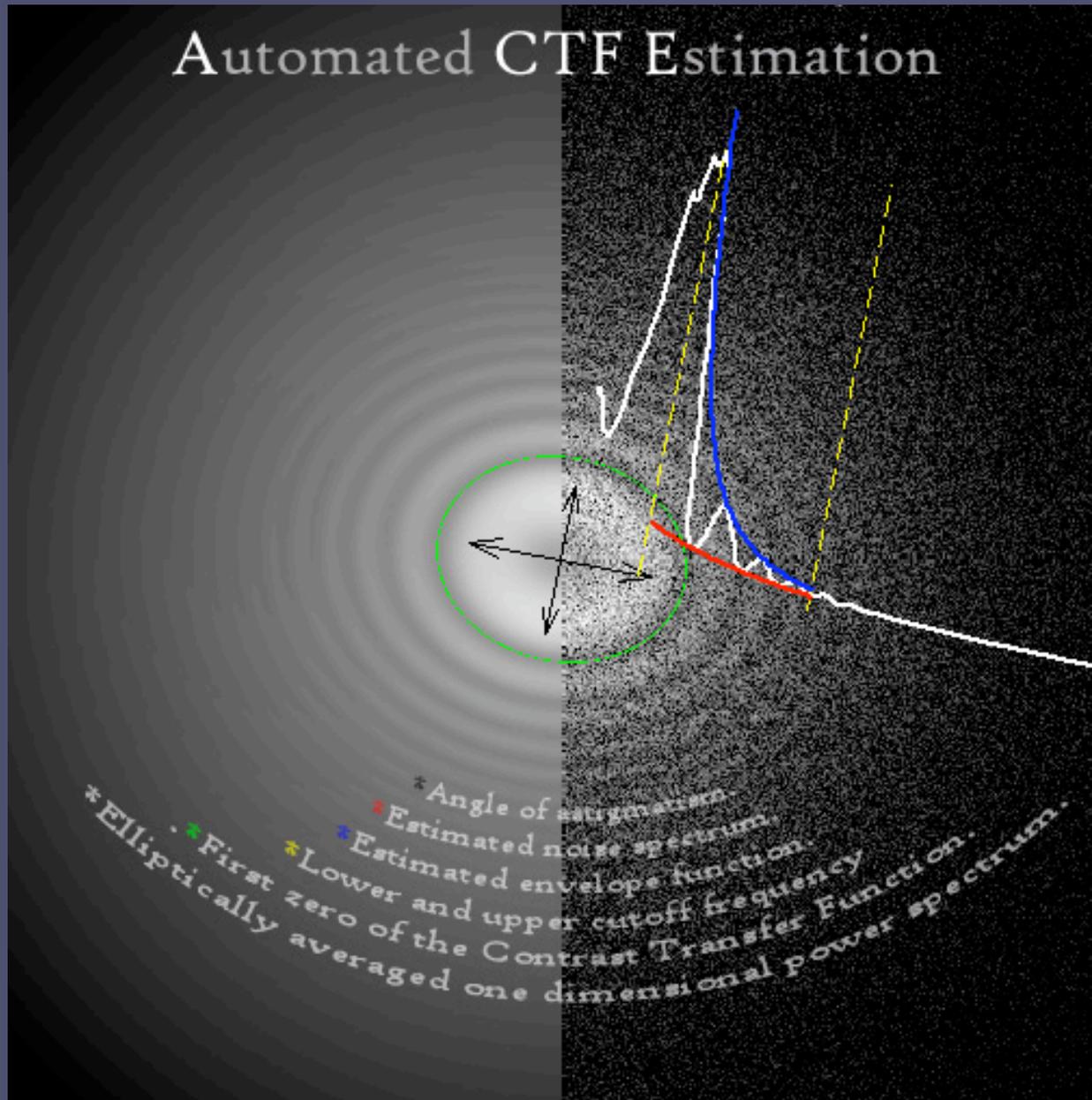


Defocus -300nm



Defocus -2000 nm

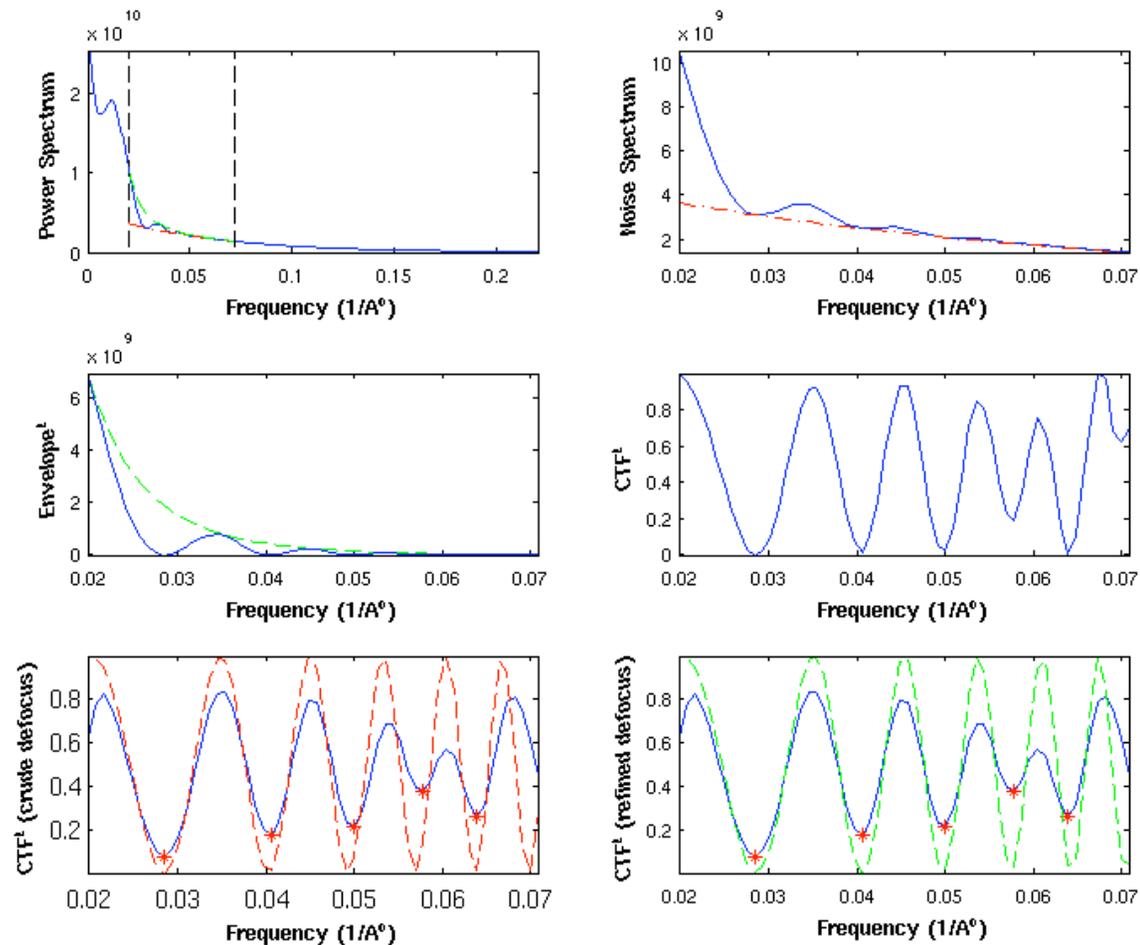
Automated CTF Estimation



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



Automated CTF estimation

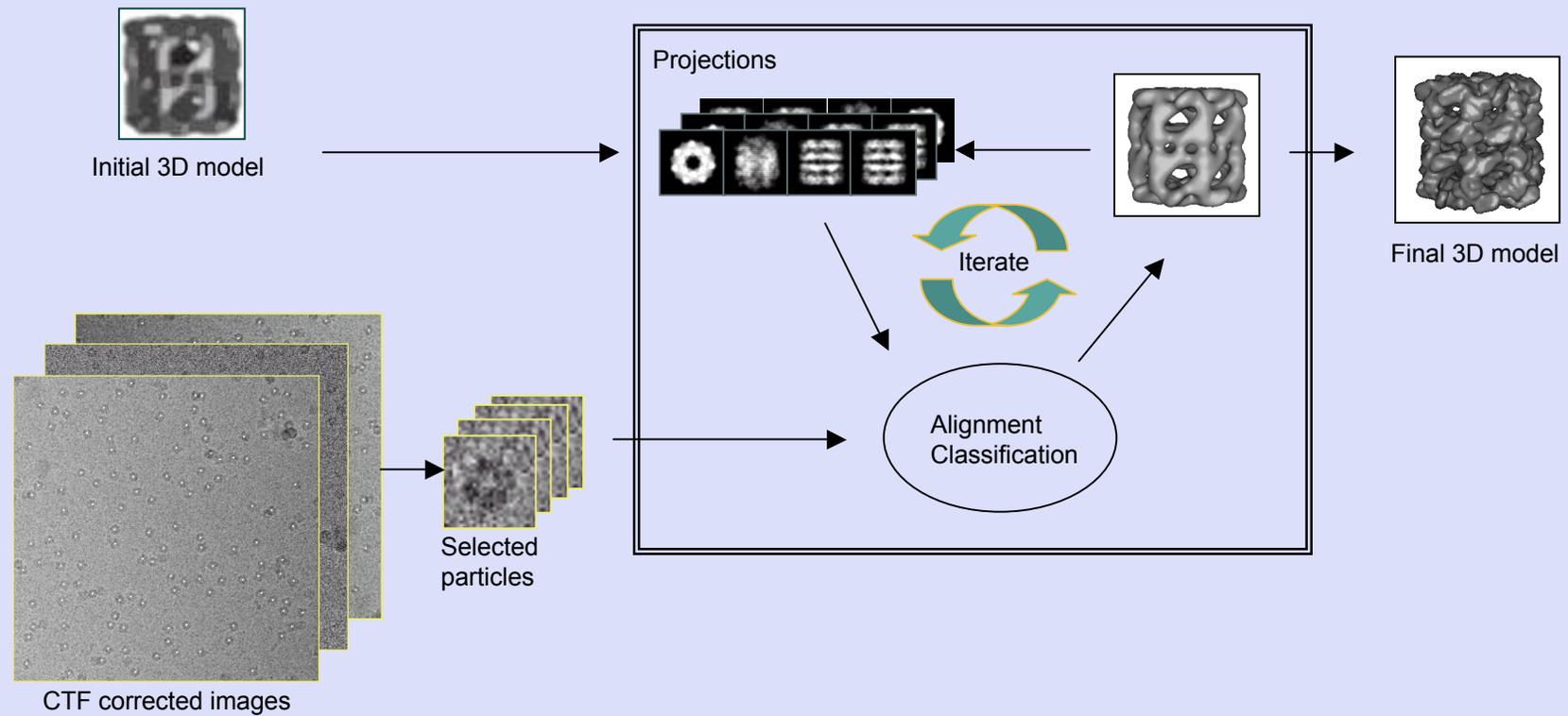


- fitness factor >0.8 ~100% accurate for GroEL
- runs concurrently with data collection
- integrated with database

Mallick *et al.* (2005) Ultramicroscopy, 104

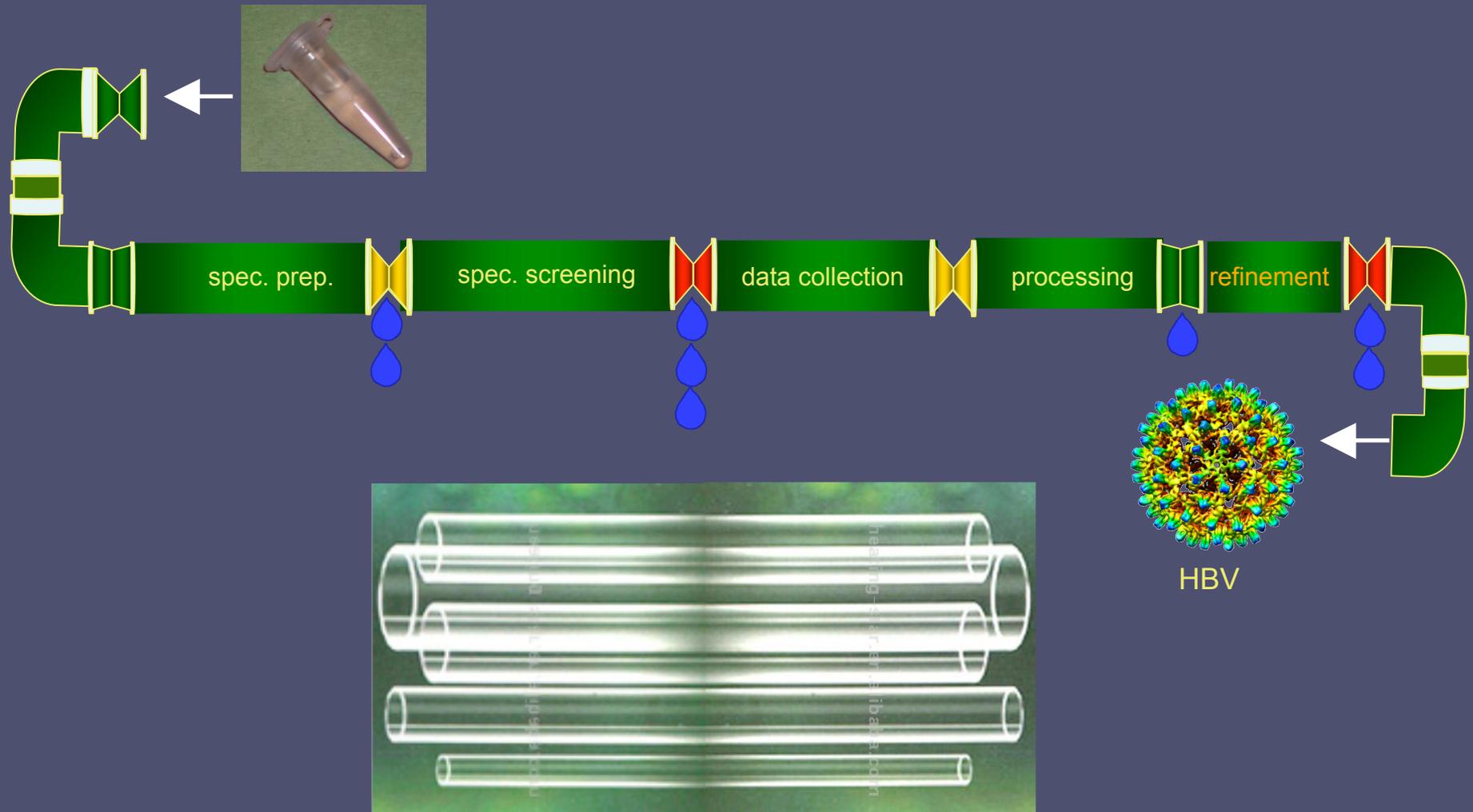


Step 5: Refinement and reconstruction



EMAN, Spider, Imagic, Frealign, etc.

Automated Pipeline for Molecular Microscopy



Appion Data Processing

Username: Password:

Project: Lander - P22

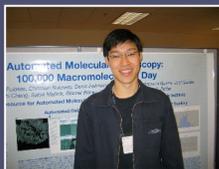
Session:

Image Path: /ami/data00/leginon/07oct18c/rawdata

Action	Results	New run
 Particle Selection	1 completed	Template Picking DoG Picking Manually Edit Picking
 CTF Estimation	1 completed	ACE Estimation
 Micrograph Assessment	All 657 completed	Re-Assess Images
 Region Mask Creation	none	Crud Finding Manual Masking
 Stacks	3 completed	Stack creation
 Reference-free Classification	1 completed	Ref-free Classification
 Reference-based Alignment	none	Ref-based Alignment
 Reconstructions	1 uploaded	EMAN Reconstruction Upload Reconstruction

Pipeline tools:

 Templates	2 available	Upload template
 Initial Models	3 available	Upload model



Create an Image Stack

logged in as **bcarr** [Log Out]

Project: [NRAMM - GroEL 100K](#)

Session: [GroEL 5X, Box 3, Slot 3](#)

Stack File Name:

Stack Run Name:

Stack Description:

Output Directory:

Particles:

No Mask Assessed for this Session

Density:

- Invert image density
- Normalize Stack Particles
- Phaseflip Images
- Use Inspected Images
- Commit to Database

File Format:

Box Size (Unbinned, in pixels)

Filter Values:

Low Pass

High Pass

Binning

ACE Confidence Cutoff

Use Values Above: (between 0.0 - 1.0)

Particle Correlation Cutoff

(between 0.0 - 1.0)

Use Values Above:

Use Values Below:

Defocal pairs:

Calculate shifts for defocal pairs

Defocus Limits

Minimum

Maximum

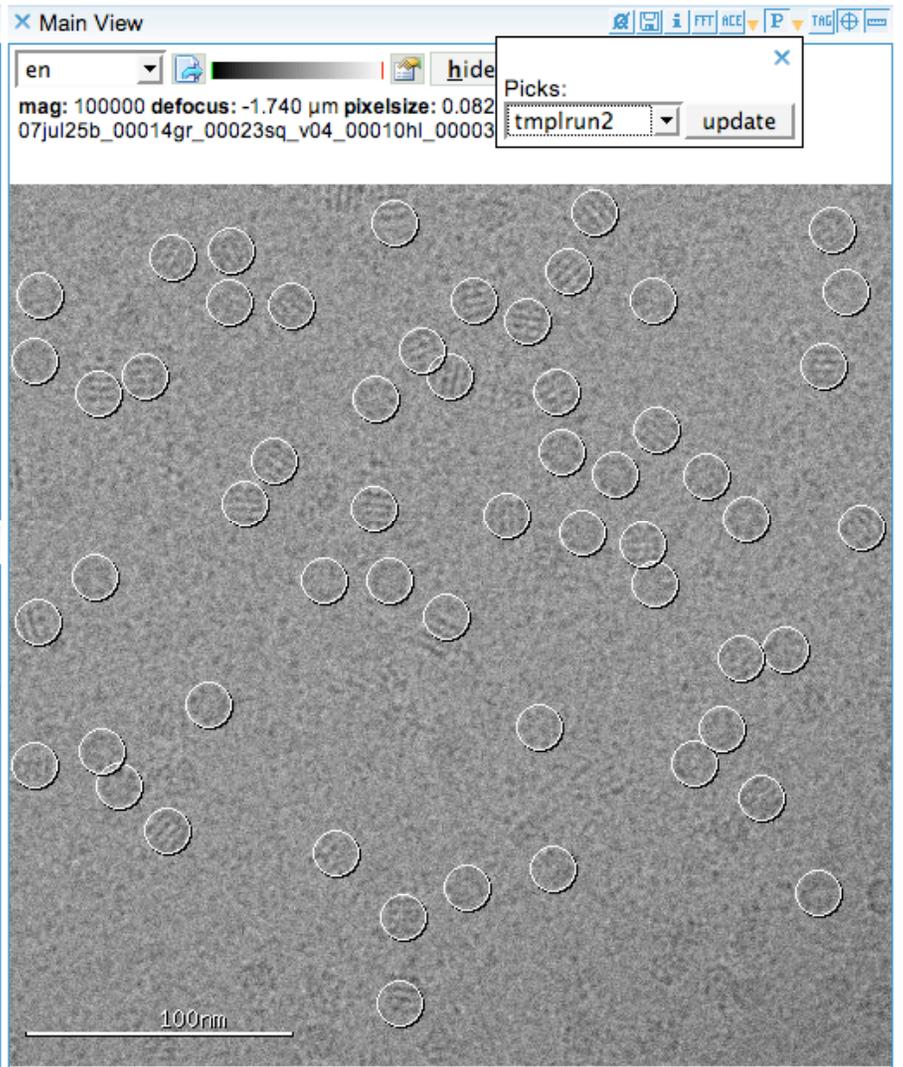
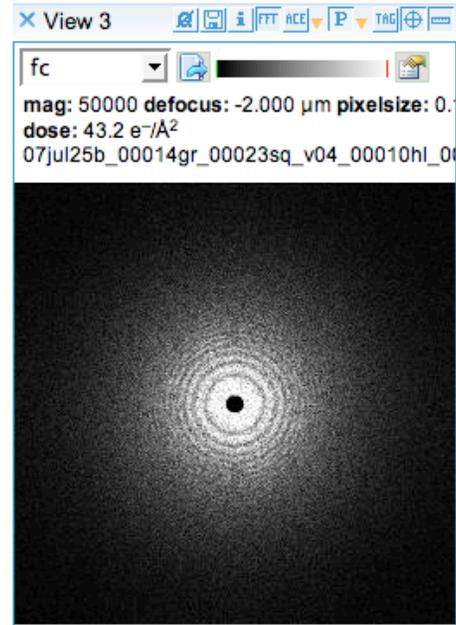
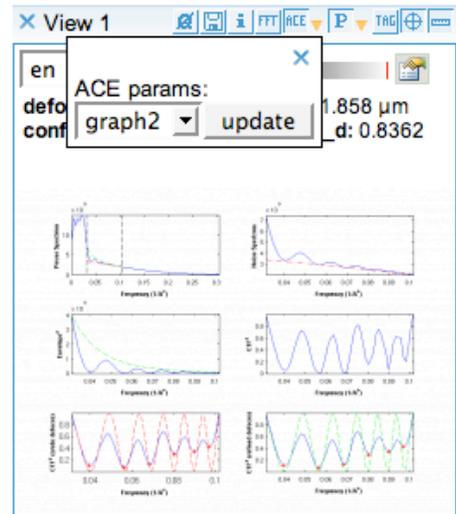
Limit # of particles to:

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

all 07jul25b - GroEL 5X, Box 3, Slot 3

Select **NRAMM - GroEL 100K project**

- 00014gr_23sq_v04_10hl_3en.mrc
- 00014gr_23sq_v04_10hl_2en.mrc
- 00014gr_23sq_v04_9hl_3en.mrc
- 00014gr_23sq_v04_9hl_2en.mrc
- 00014gr_23sq_v04_8hl_3en.mrc
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- 00014gr_23sq_v04_6hl_3en.mrc
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- 00014gr_23sq_v03_4hl_v13_3en.mrc
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- 00010gr_22sq_v02_14hl_2en.mrc



Reconstruction Report Page

logged in as *bcarr* [Log Out]

Run Description:

Stack: [/ami/data15/appion/07jul25b/stacks/stack1/start.hed](#)

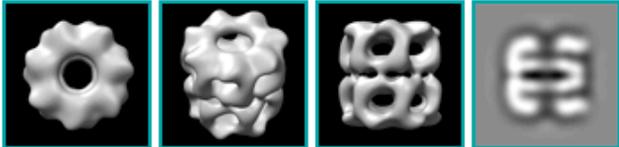
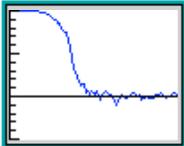
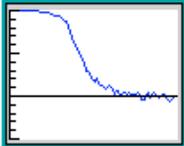
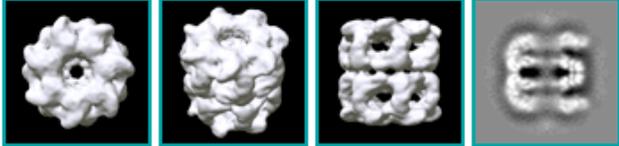
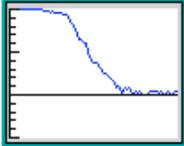
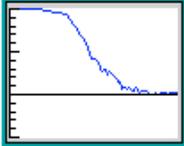
Reconstruction path: [/ami/data15/appion/07jul25b/refine/logsplit/run43720/](#)

Particles: 43720

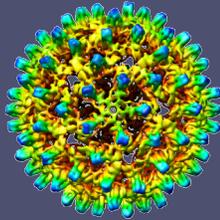
Initial Model: [/ami/data13/appion/06jul12a/refine/logsplit2/run100/threed.0a.mrc](#)

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

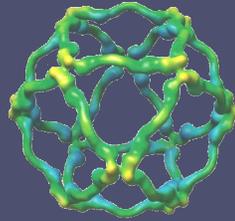
download: compare

iteration	ang incr	resolution	fsc	classes	distr	# particles	density	snapshot
0								
1	5	8.62		classes.1.img	61	[35778 good] [7942 bad]	threed.1a.mrc	
2	5	7.75		classes.2.img	61	[36037 good] [7683 bad]	threed.2a.mrc	
3	5	6.89		classes.3.img	61	[35597 good] [8123 bad]	threed.3a.mrc	
4	5	6.78		classes.4.img	61	[35600 good] [8120 bad]	threed.4a.mrc	

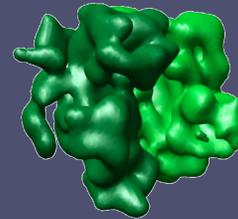
3D maps



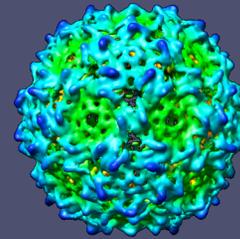
HBV



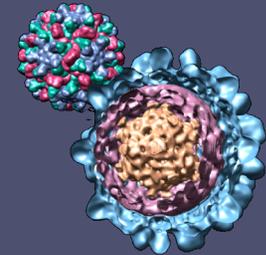
COPII



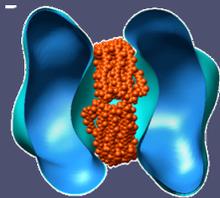
Ribosome



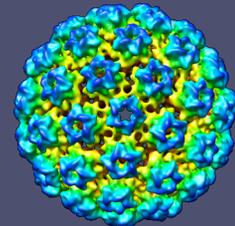
Qbeta



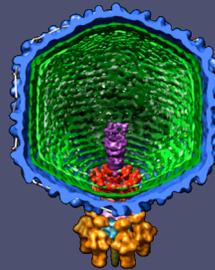
CNV



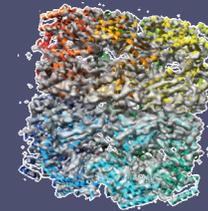
LCP



HPV



P22



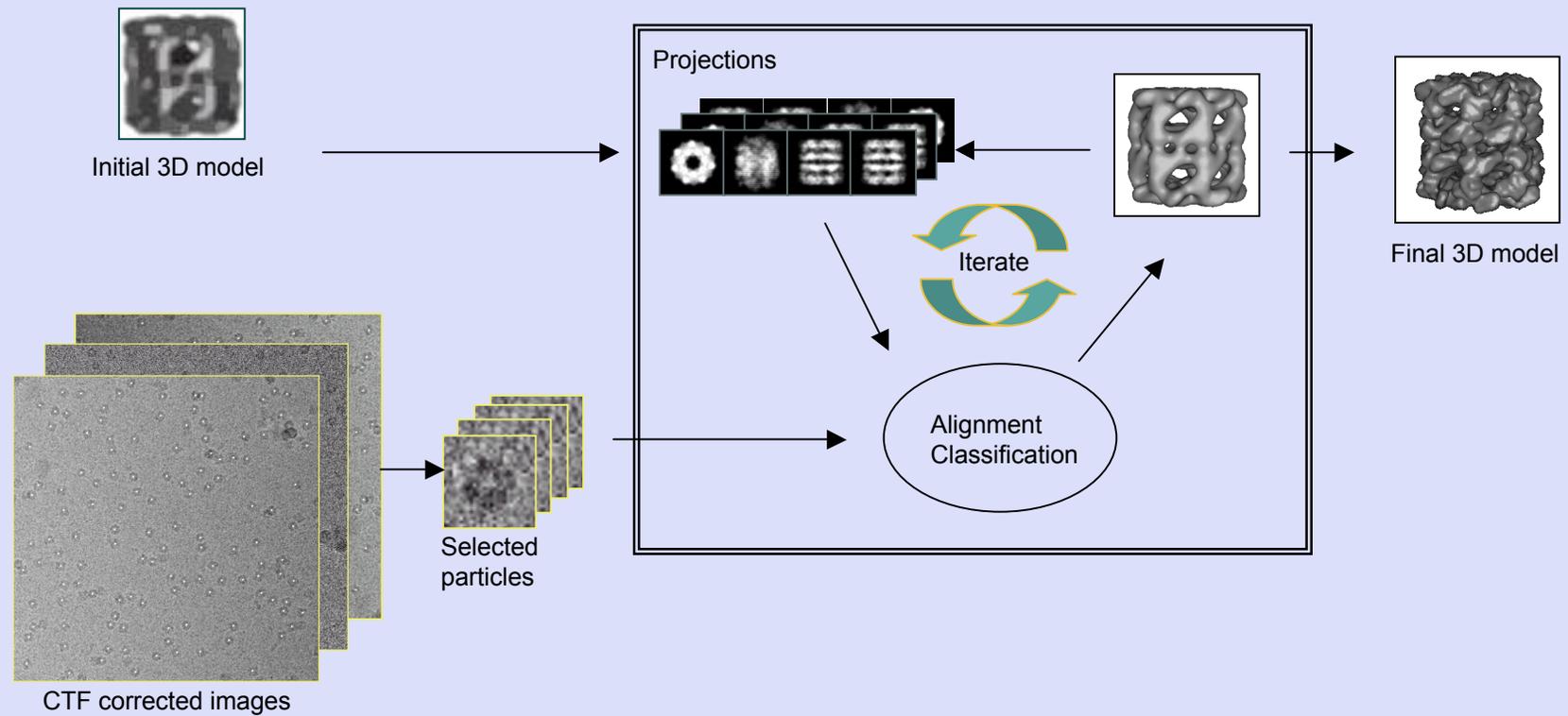
GroEL



DNA

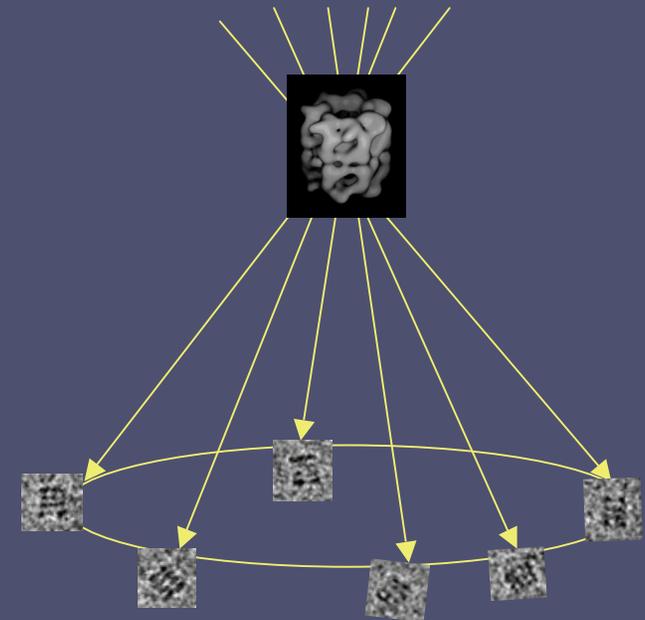
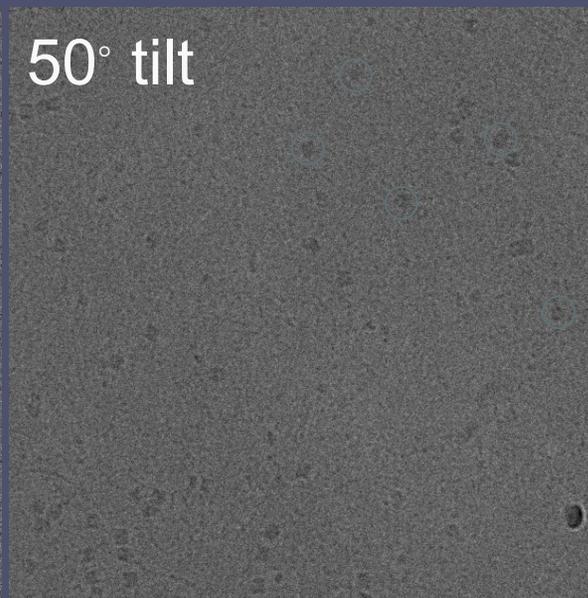
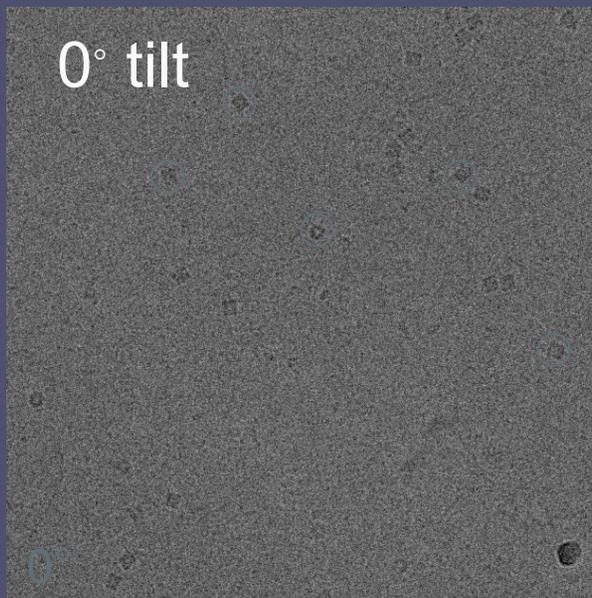
But what about the initial model?

Step 5: Refinement and reconstruction



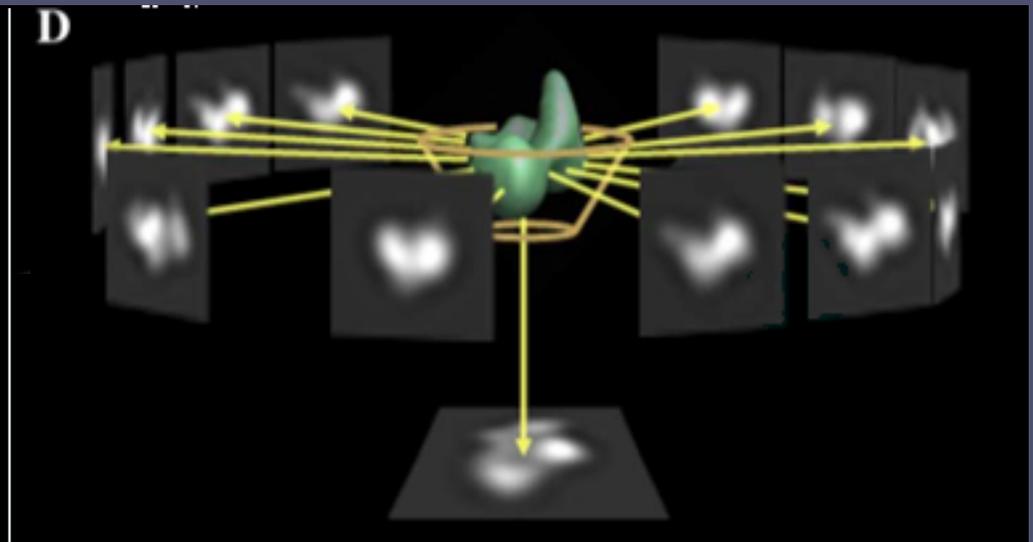
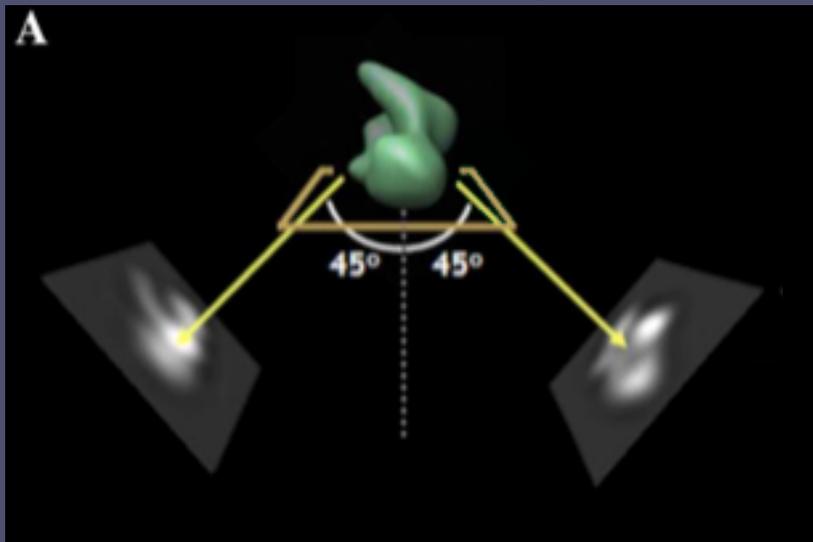
EMAN, Spider, Imagic, Frealign, etc.

Random Conical Reconstruction (RCT)

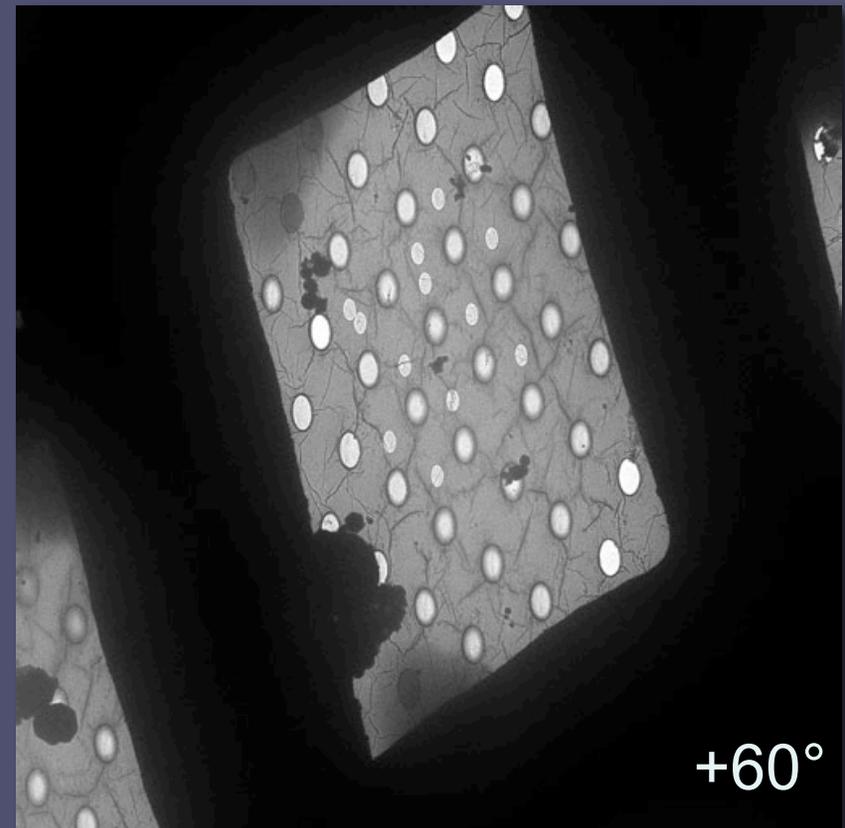
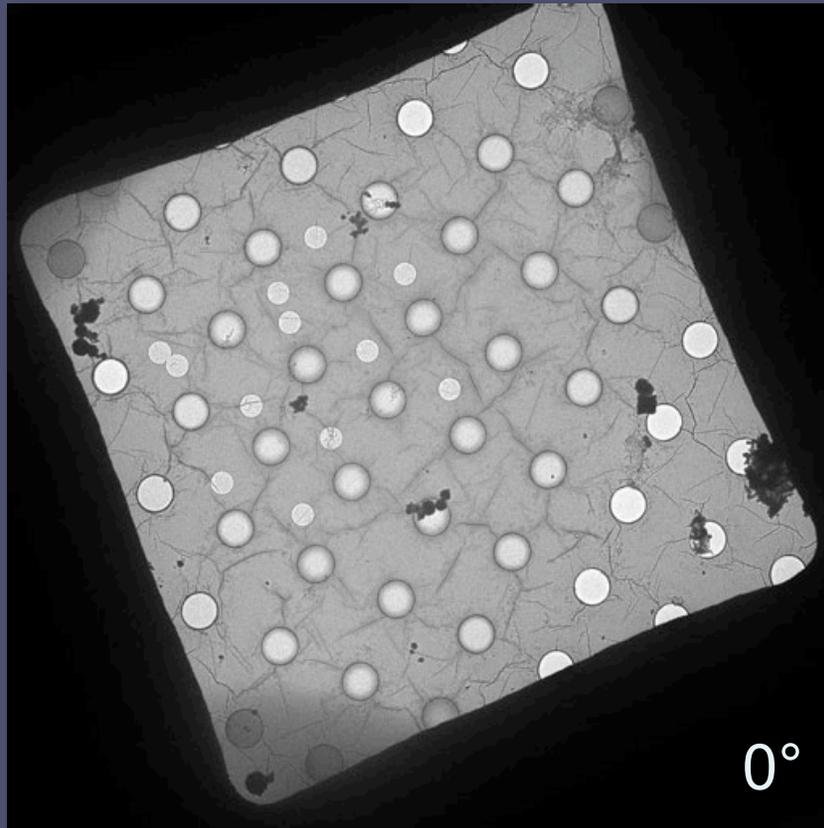


Or better yet: Orthogonal Tilt Reconstruction (OTR)

Leschziner and Nogales. *J Struct Biol.* 2006 Mar;153(3):284-99.



Automated Random Conical Tilt Data Collection

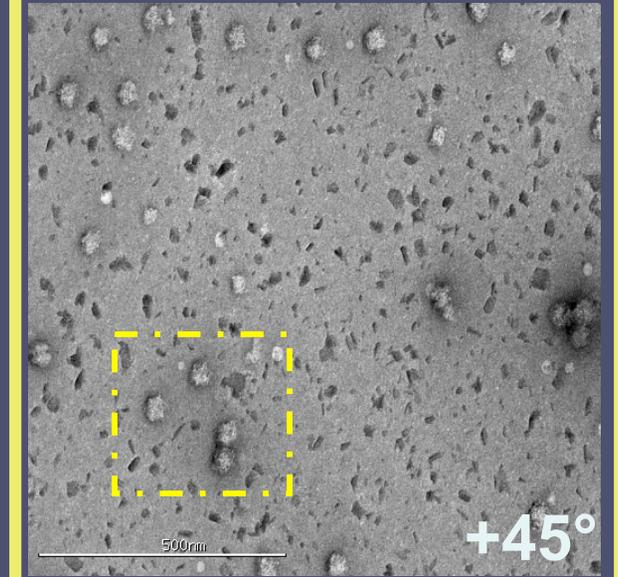
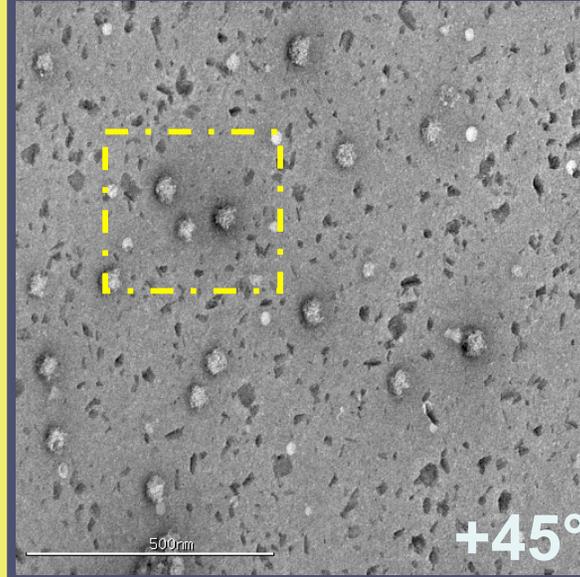
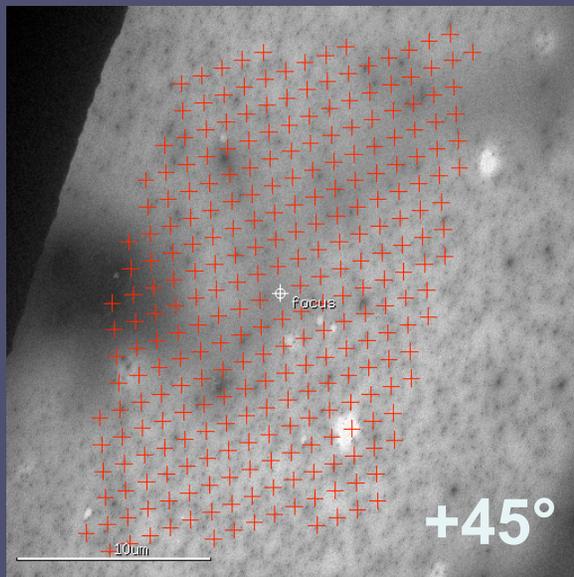
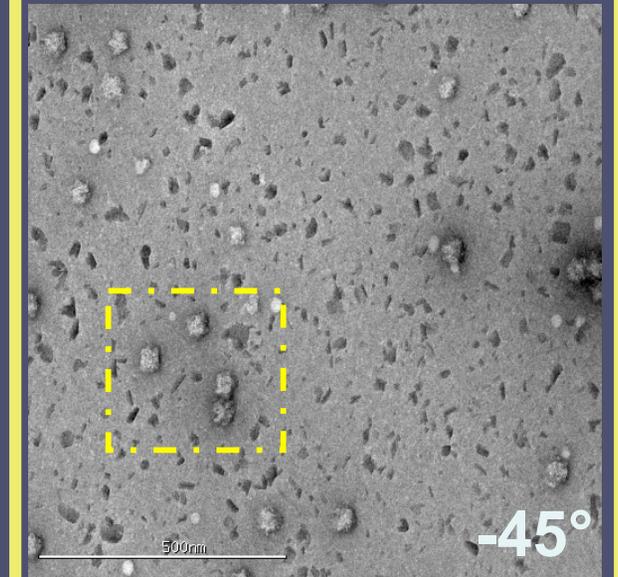
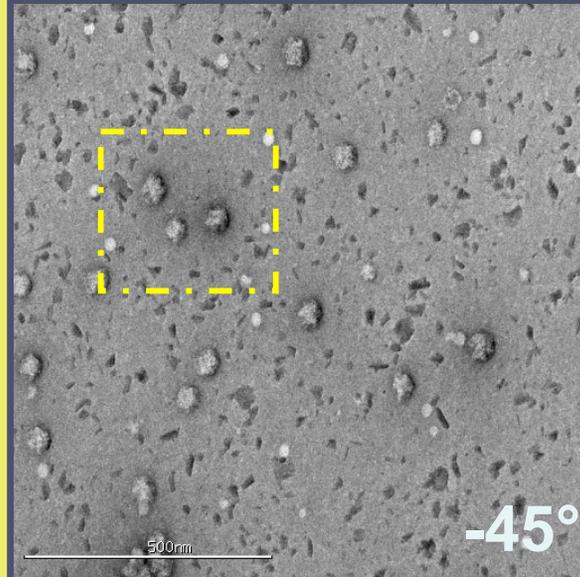
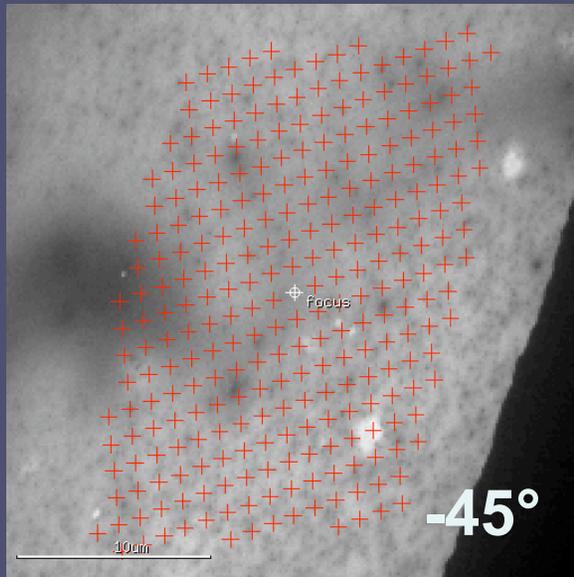


Large image variations

- Non-planar foreground / background variations
- Mechanical stage instabilities

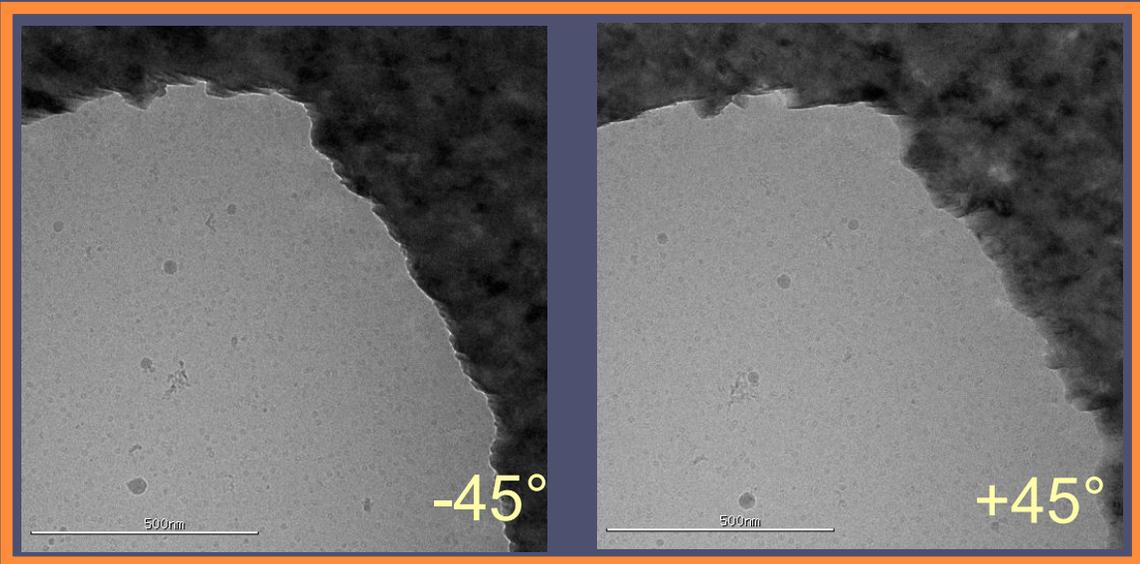
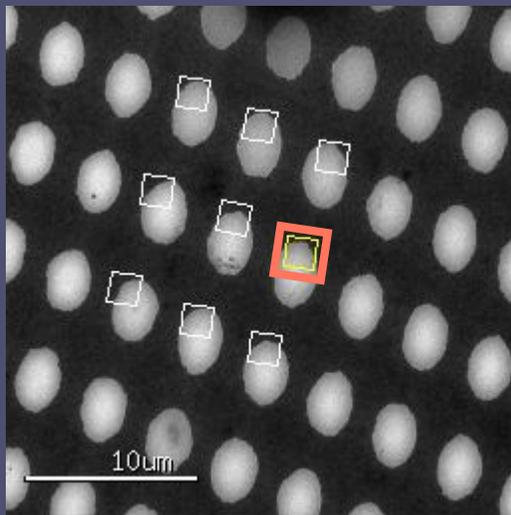
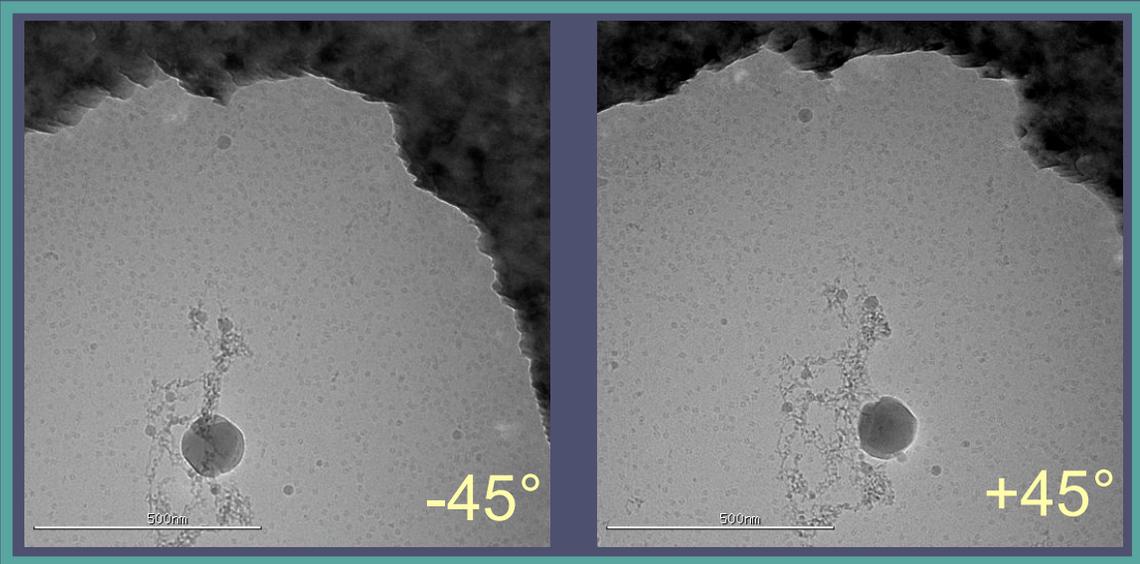
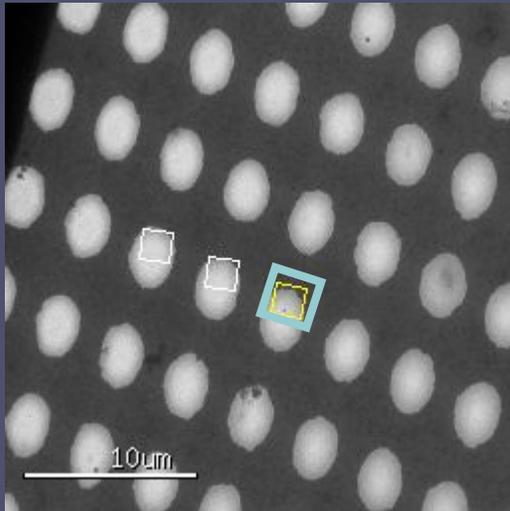


Leginon: Automated OTR for stained grids



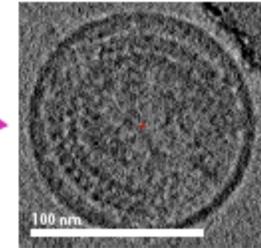
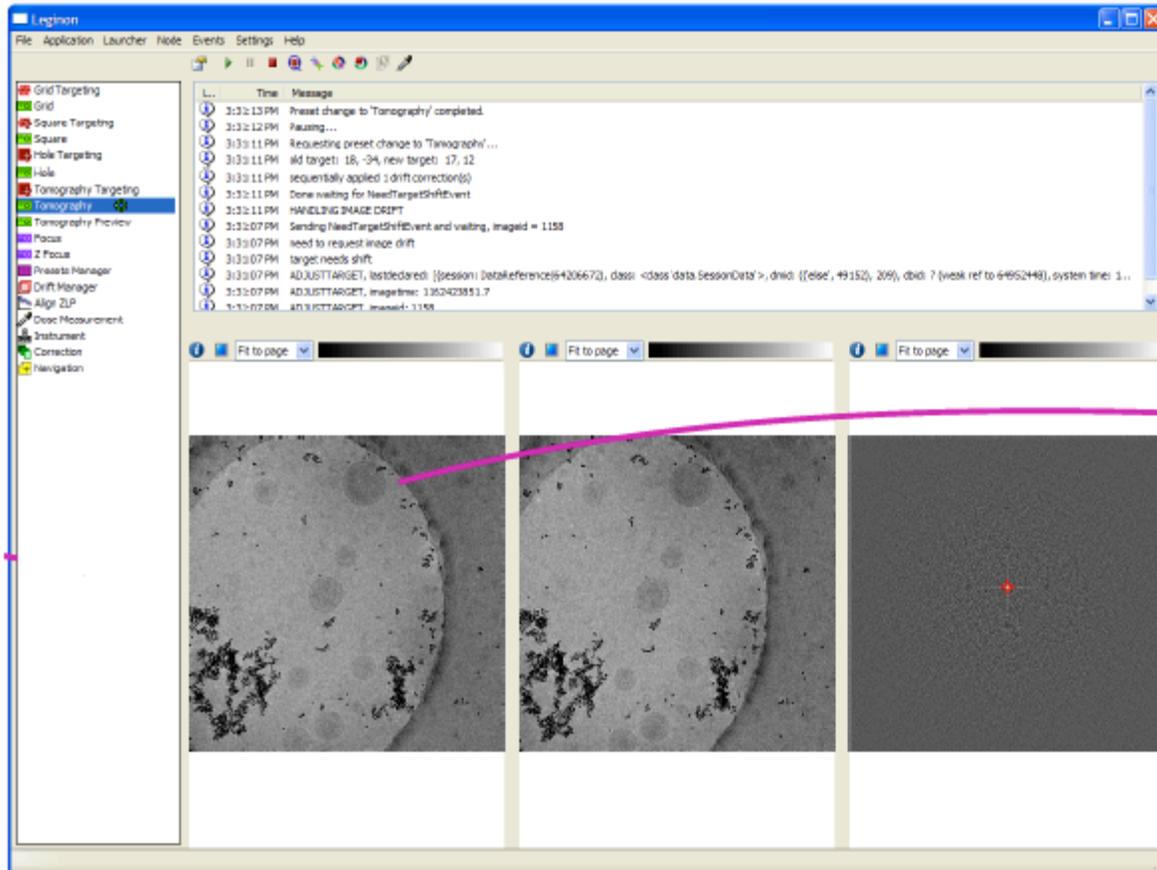
370 tilt pairs in 8 hours (1 pair every 1.8 minutes)

Leginon: Automated OTR for vitreous ice grids (GroEL)



57 tilt pairs in 2 hours (1 pair every 2.2 minutes)

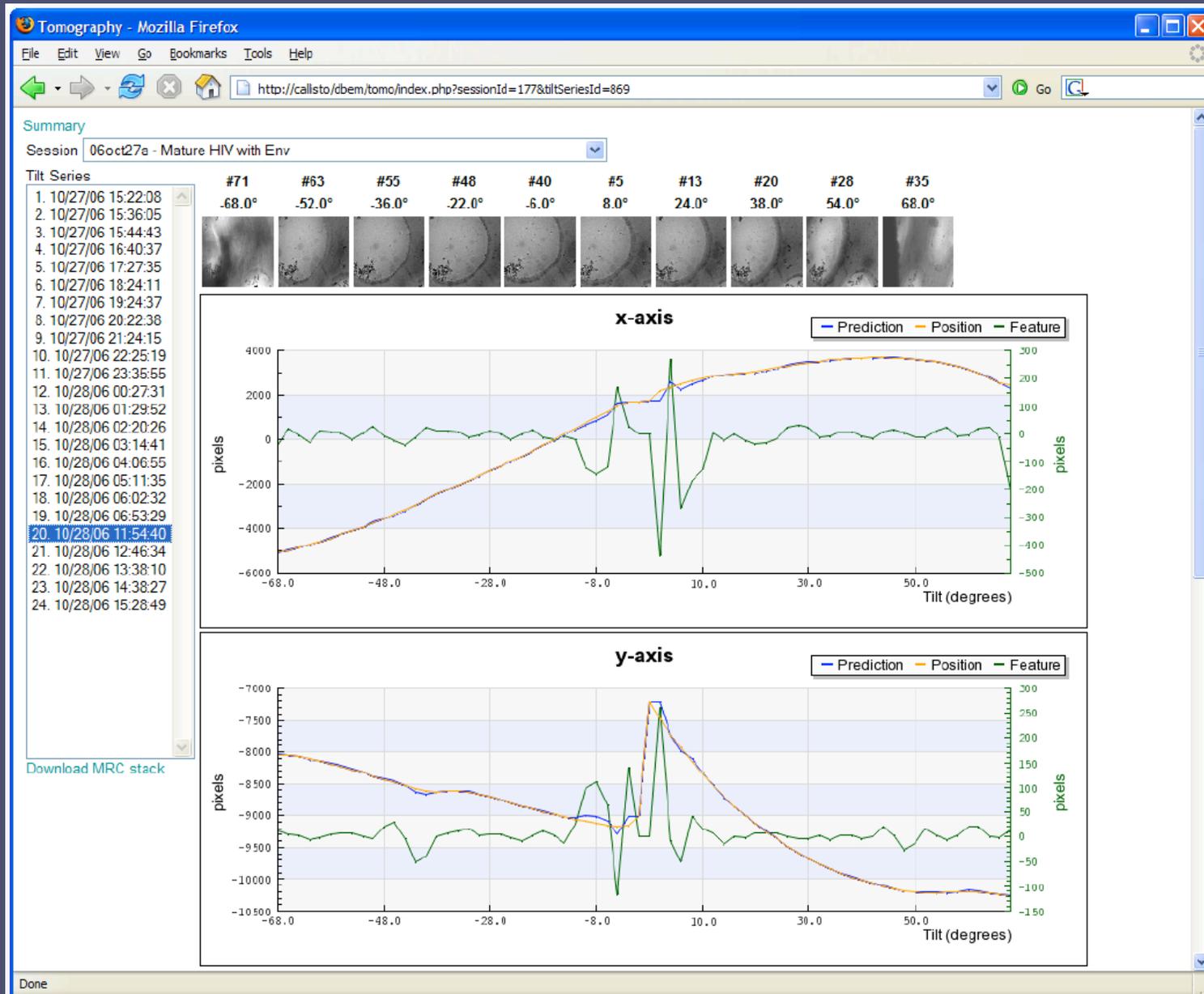
Leginon Tomography Application:



An immature HIV from an IMOD reconstruction (32 central slices averaged).

Leginon will record tilt series for each target, adjusting for specimen drift, beam intensity changes, and energy filter drift.

Christian Suloway, Jensen Lab, Caltech;
Shawn Zheng, Agard Lab, UCSF



Tomography node released in Legion 1.3 (November 2006)

Automated Pipeline for Molecular Microscopy Results

Automation goals

Facilitate the process

Increase throughput

Optimize resolution

Expand the possibilities

Open the technology to wider audience



Automated pipeline case study #1

Virus particles as platforms for display



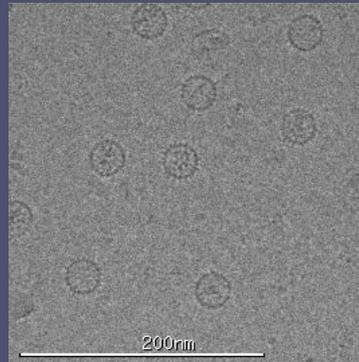
Sample arrives
(1 week to several years)



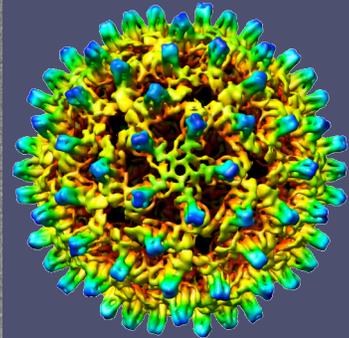
Grid prep using Vitrobot
(1 hour)



Microscope setup, grid
loading, Legimon setup
(1-2 hours)

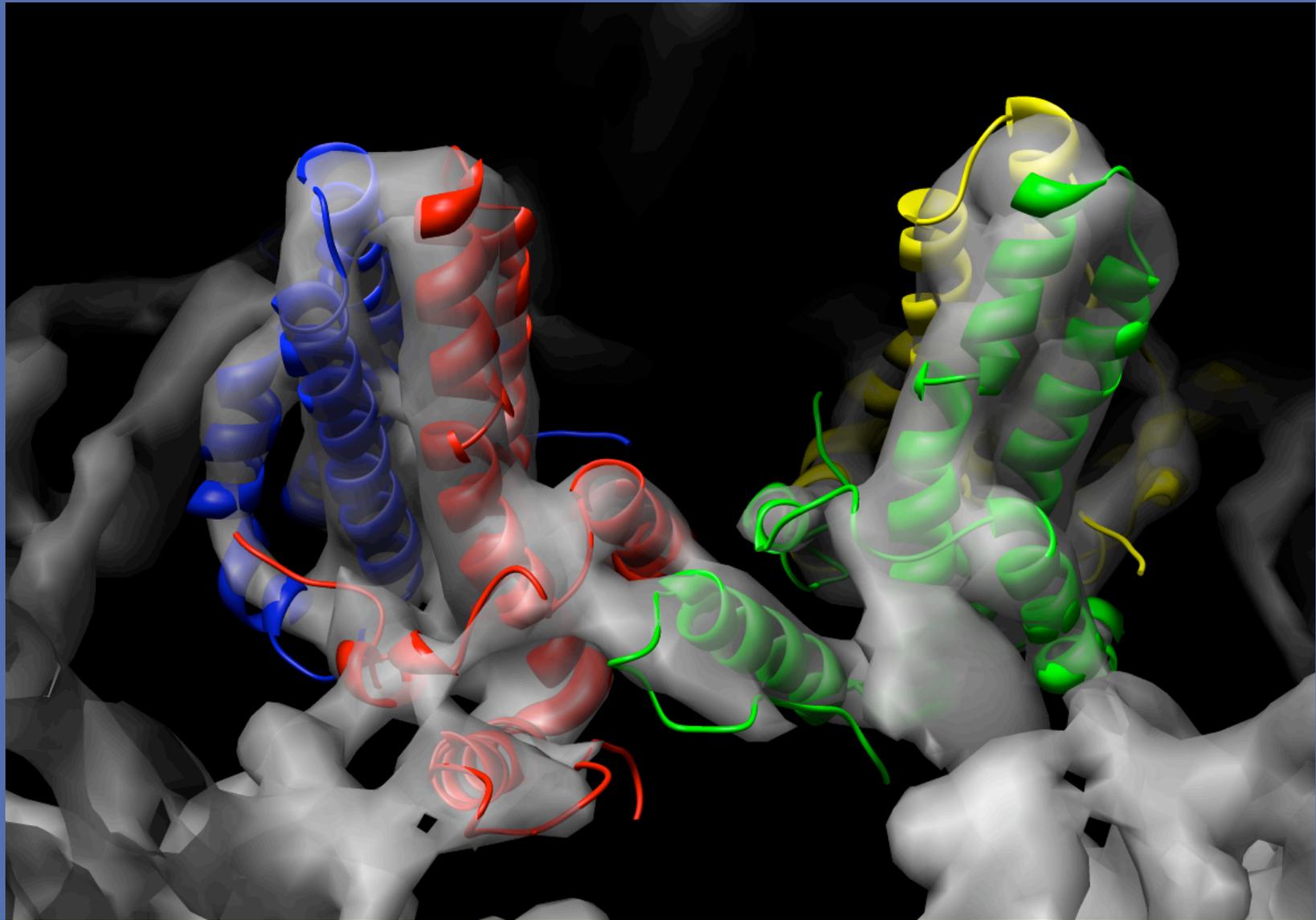


Data collection & pre-
processing
(12 hours)



Reconstruction (resolution
<1nm)
(5 hours)

Interpretation
(1 week to several years)



Hepatitis B virus

Gabe Lander, Erica Strable, MG Finn

Case Study #2: The Structure of Intact Bacteriophage

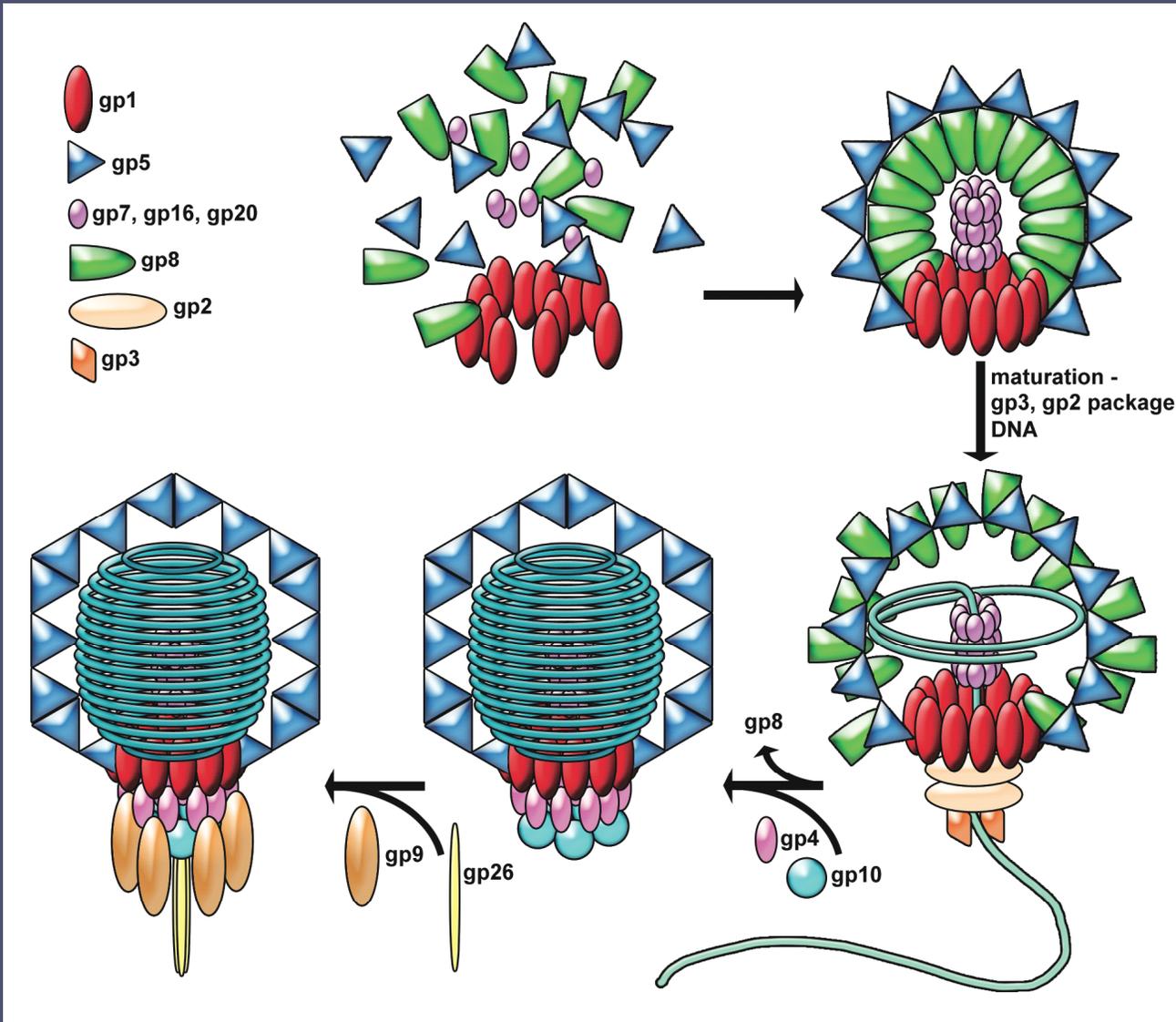
Infests Salmonella

Capsid T=7 icosahedral lattice

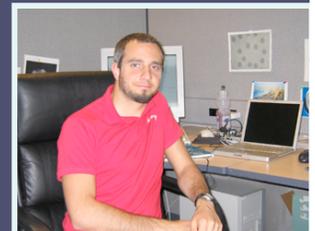
~55-65 nm diameter

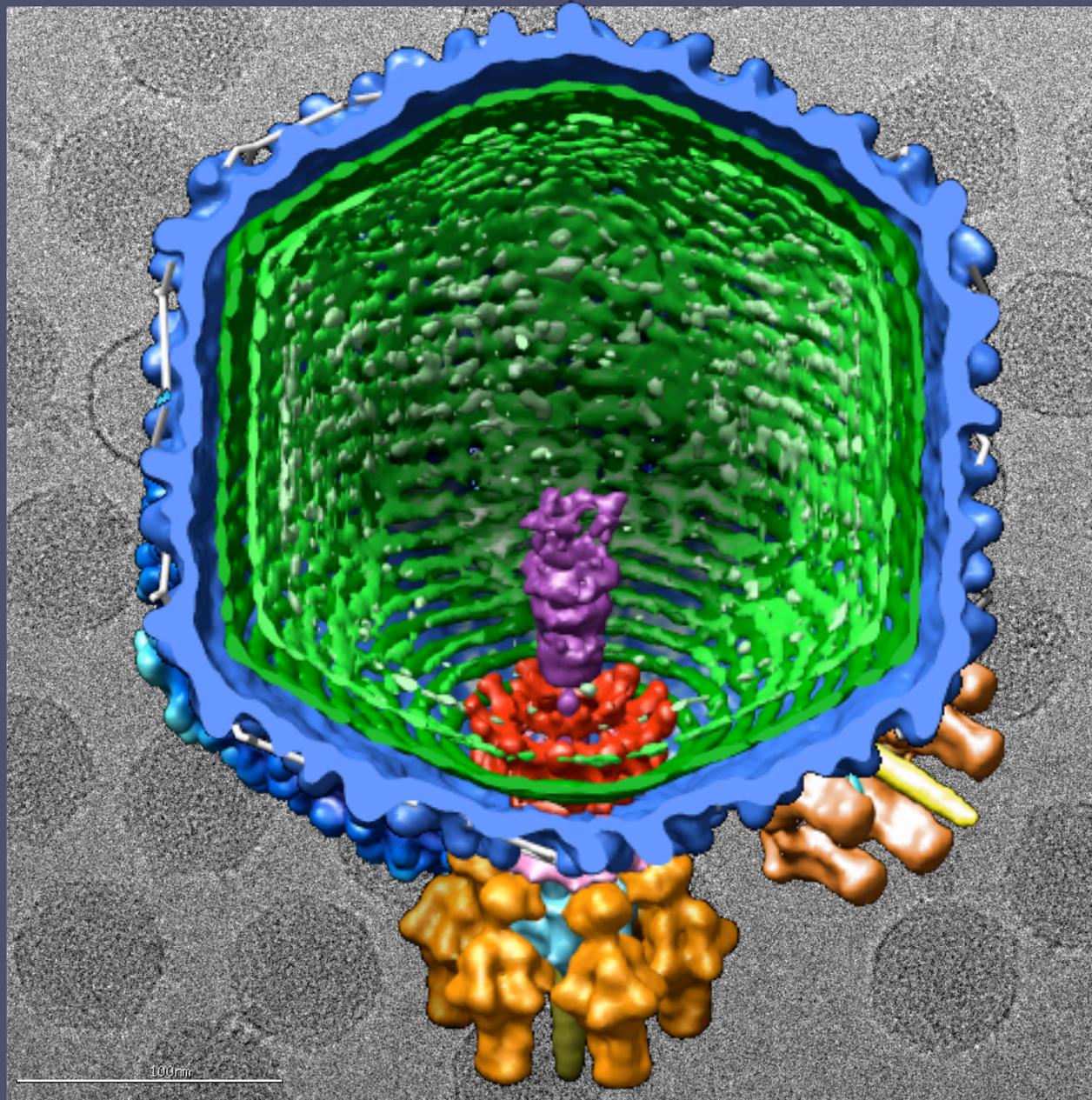
Multi-stage maturation from procapsid to mature capsid -

Packages a ~43.5 kb dsDNA genome, but its wild type nucleotide sequence is only 41.7 kb. Termination of packaging is not triggered by sequence.



Gabe Lander, Jack Johnson, TSRI;
Sherwood Casjens, University of Utah; Peter Prevelige, University of Alabama, Birmingham.

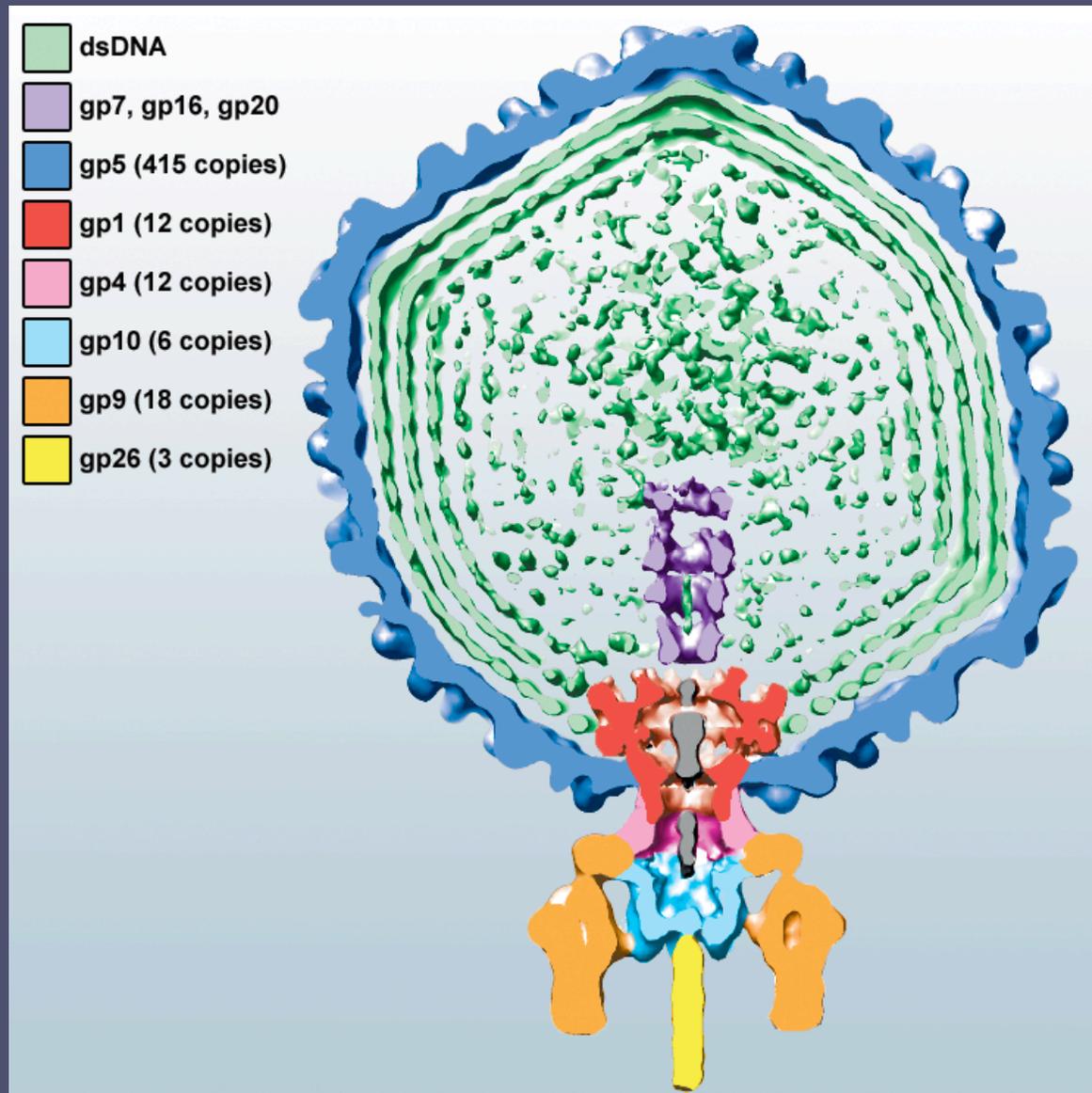




Lander, G. C., Tang, L., Gilcrease, E. B., Privelige, P., Poliakov, A., Potter, C. S., Carragher, B., and Johnson, J. E. (2006). A protein sensor for head-to-tail DNA packaging is activated by spooled dsDNA. *Science*. 312, 1791.

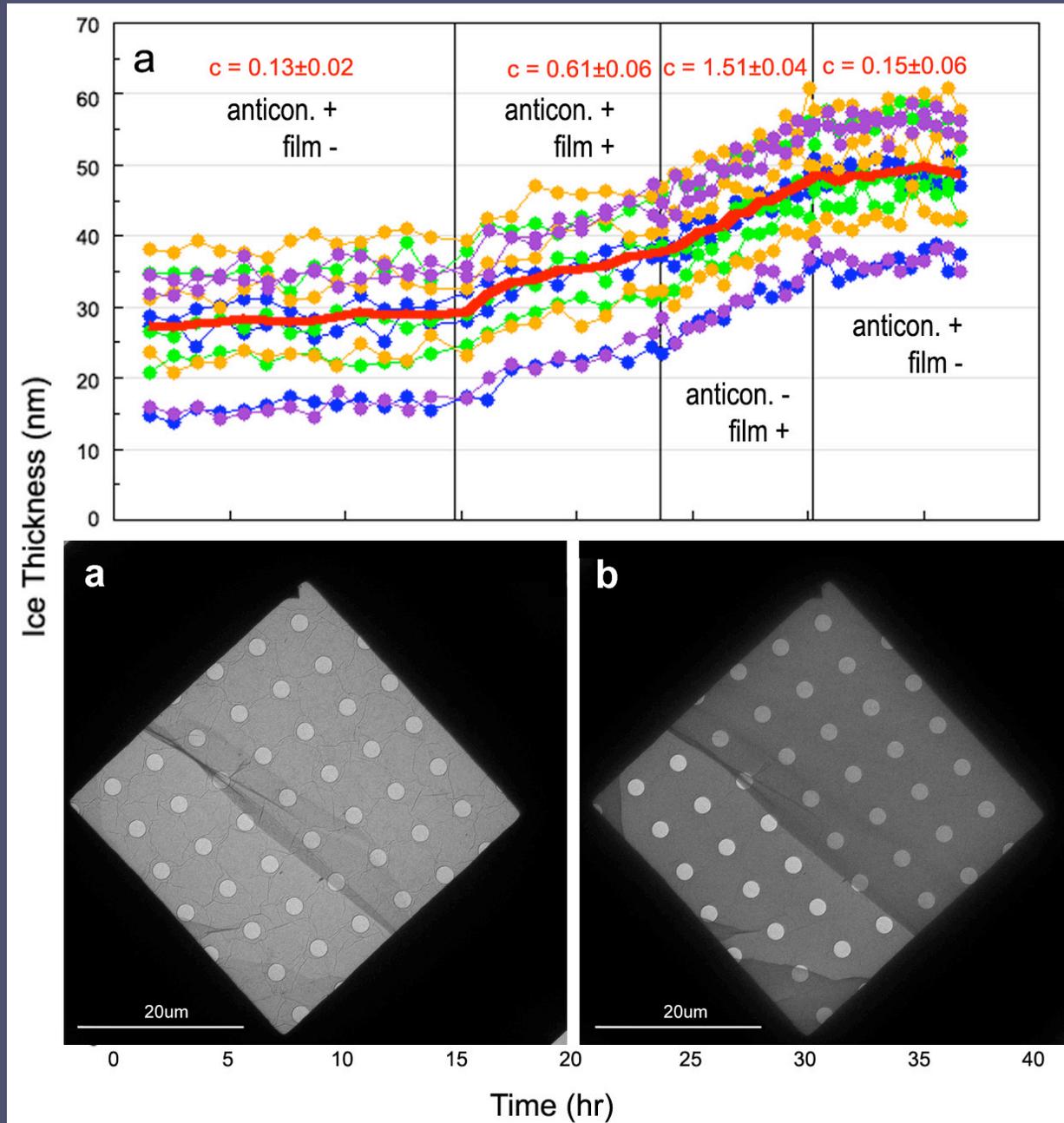
4,800 images, 25,703 particles

A challenge for higher throughputs: multiple states



Lander, G. C., Tang, L., Gilcrease, E. B., Privelige, P., Poliakov, A., Potter, C. S., Carragher, B., and Johnson, J. E. (2006). A protein sensor for head full viral chromosome packaging is activated by spooled dsDNA. *Science*. In Press.

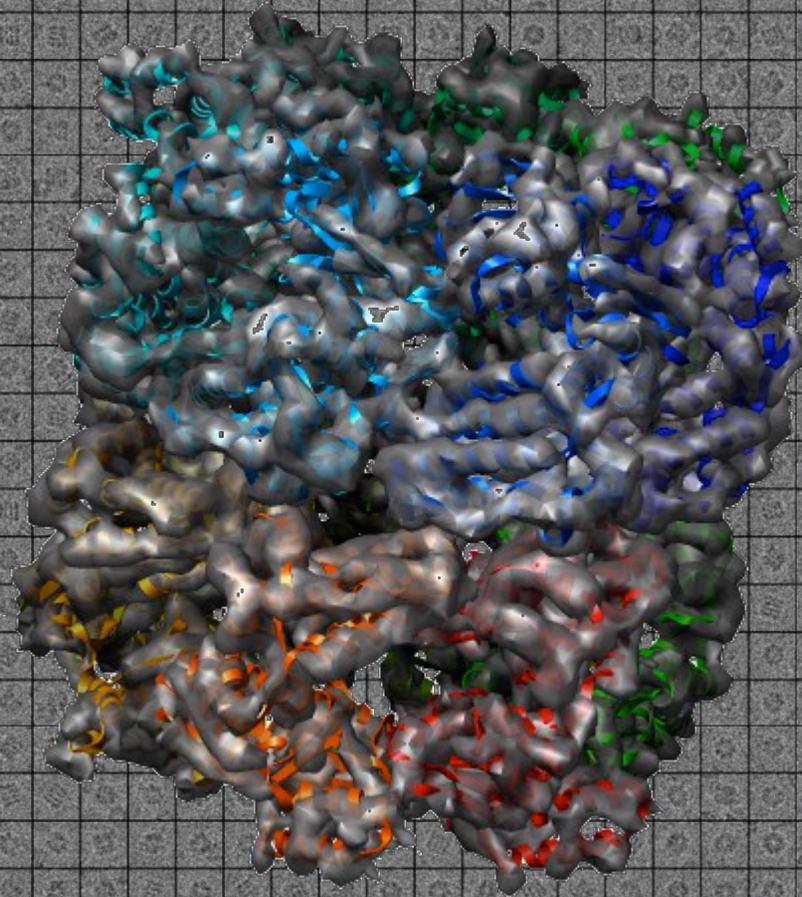
What about contamination during long data acquisitions?



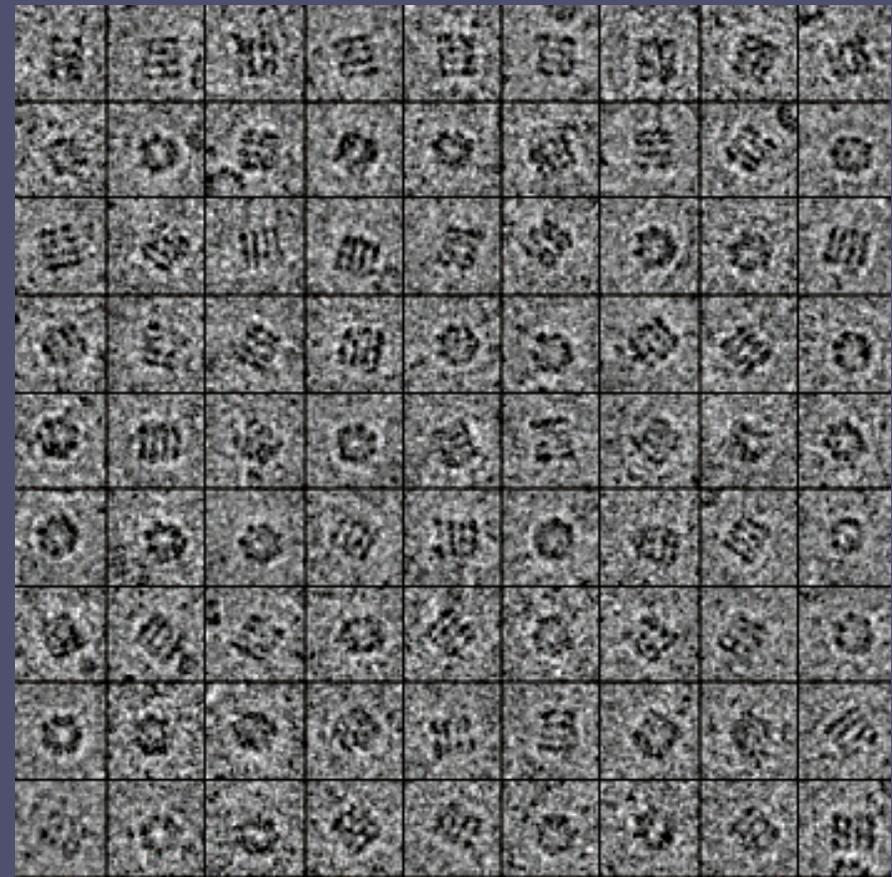
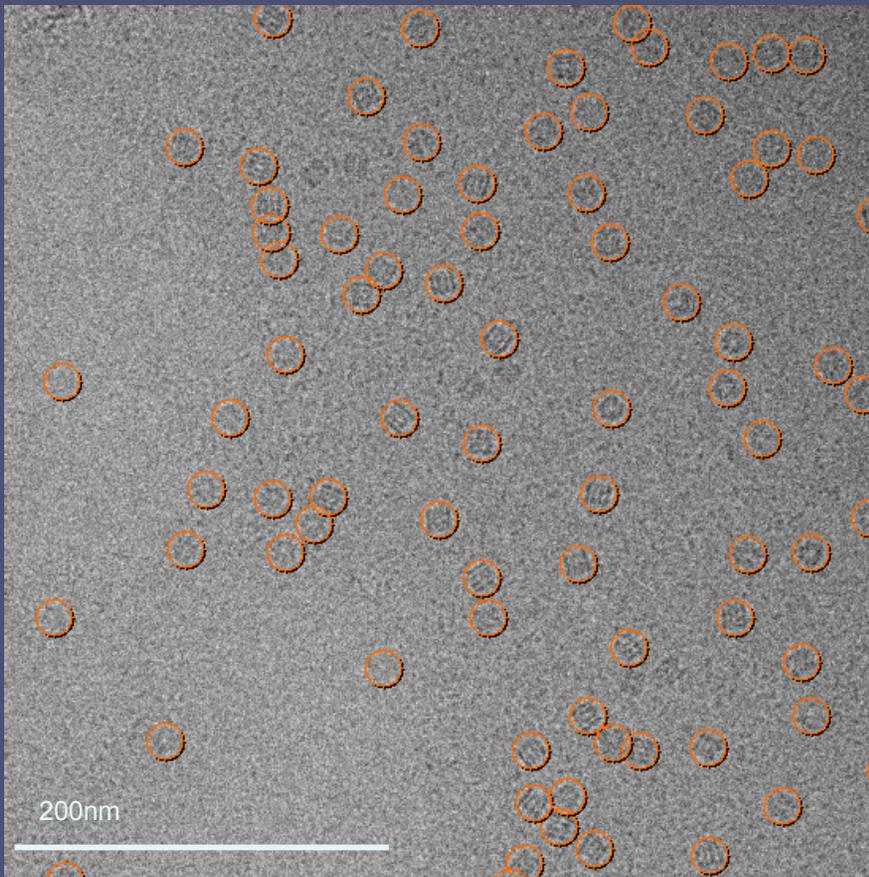
Cheng, A., et al.
(2006) JSB, 154,
303-311.

Case Study #3

GroEL as a testbed

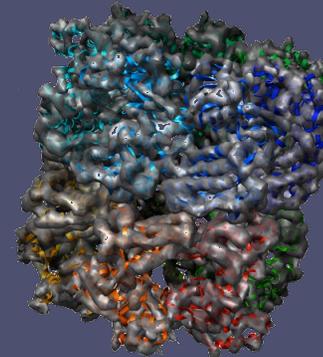


Automated throughput for single particles (GroEL)



Throughput:

# grids:	1
# squares:	32
# holes:	318
# defocus pairs:	552
Duration:	26 hrs
# particles found:	~280,000



Leginon Database: Images and Acquisition Parameters

04mar16a - PBCV-1, 25mg/ml, blot 2.5sec., new batch from J. Gurgon in Nebraska

View 1

grid **adjust**

mag: 120 **defocus:** 0.0000 μm **pixelsize:** 93.0800 nm

04mar16a_0036grid.mrc

Main View

efar **adjust**

mag: 62000 **defocus:** -2.0000 μm **pixelsize:** 0.1791 nm **dose:** 10.6336 $\text{e}^{-}/\text{\AA}^2$

04mar16a_0036grid_0007sq_0003hole_0002efar.mrc

View 3

hole **adjust**

mag: 5000 **defocus:** -150.0000 μm **pixelsize:** 2.2328 nm

04mar16a_0036grid_0007sq_0003hole.mrc

Image Report: 04apr07a_00046grid_00019sq_00005hole_00003efar.mrc - Microsoft Internet Explorer

Created with HyperSnap-DX 4
To avoid this stamp, buy a license at <http://www.hyperionics.com>

Address: <http://cronus3.scripps.edu/dbem/imgreport.php?id=33287&preset=efar>

General	
Filename:	04apr07a_00046grid_00019sq_00005hole_00003efar.mrc
Size:	513 kB
Acquired:	2004-06-04 17:44:45
Path:	/ami/data04/leginon/04apr07a/
Session:	04apr07a - PBCV-1, 25 mg/ml, 200kV, qfoil R2/4, blot 3.5 secs., good grid
Instrument:	Tecnai 1 - Tecnai F20 and Gatan 4k

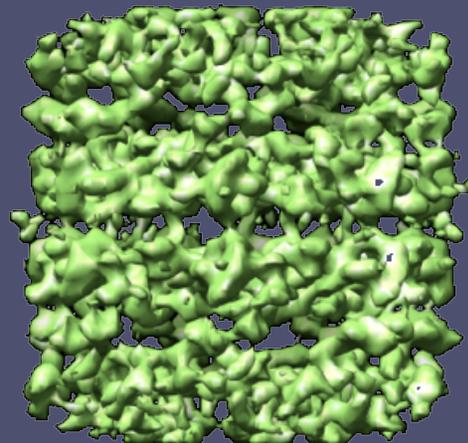
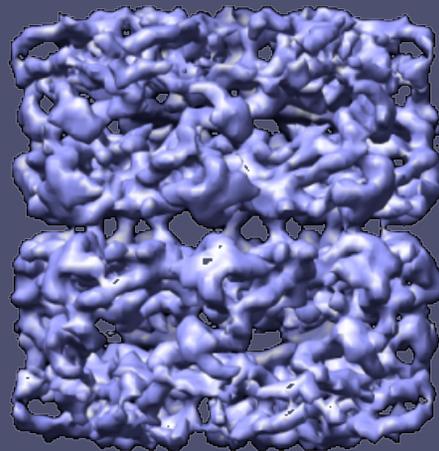
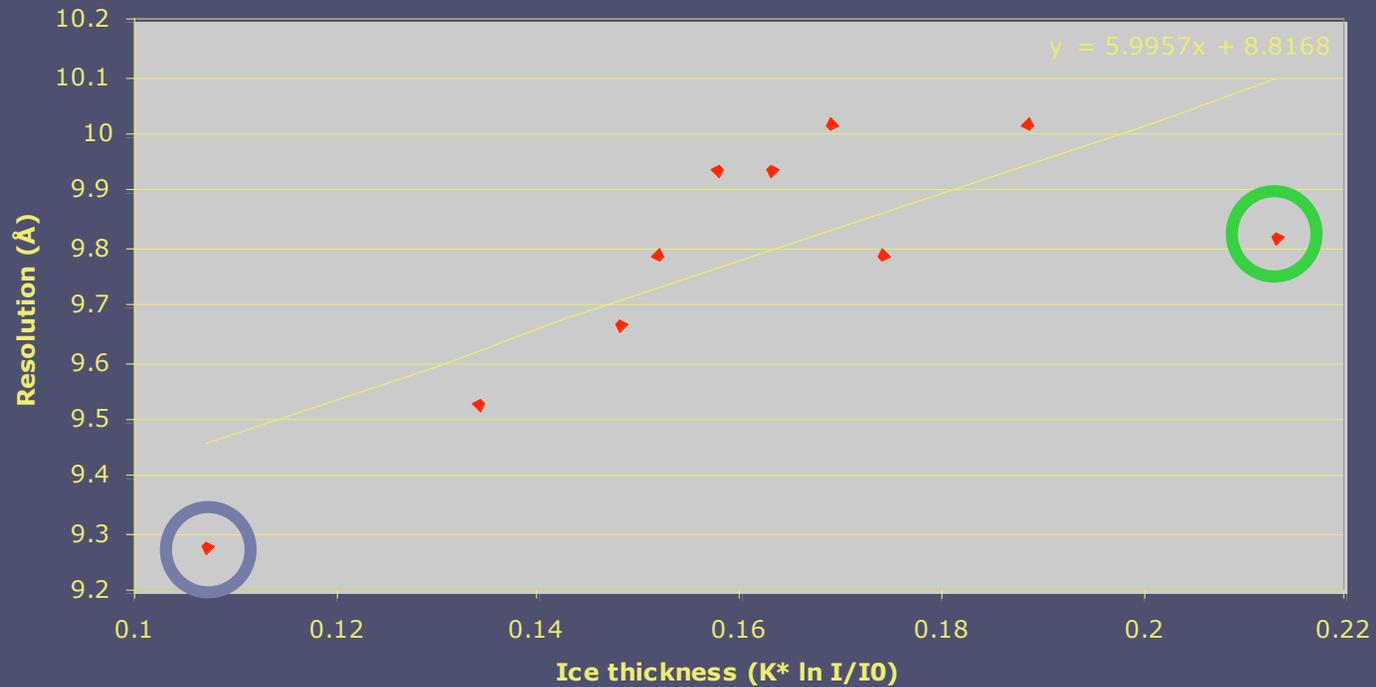
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mag: 62000	amin: 26886
defocus: -1.0000 μm	amax: 45680
pixelsize: 0.1791 nm	amean: 0.162334442139
	xorigin: 2.34128947419E-41
	yorigin: 35943.1914062

Parent Image Information	Image Relations
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parentpreset: hole	hole: 04apr07a_00046grid_00019sq_00005hole.mrc
parenttype: acquisition	enr: 04apr07a_00046grid_00019sq_00005hole_00003enr.mrc
parentnumber: 3	foc: 04apr07a_00046grid_00019sq_00005hole_00001foc.mrc
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targety: 165	
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targetdiam: 58.094076824089	

Thumbnail

Data Tree view >

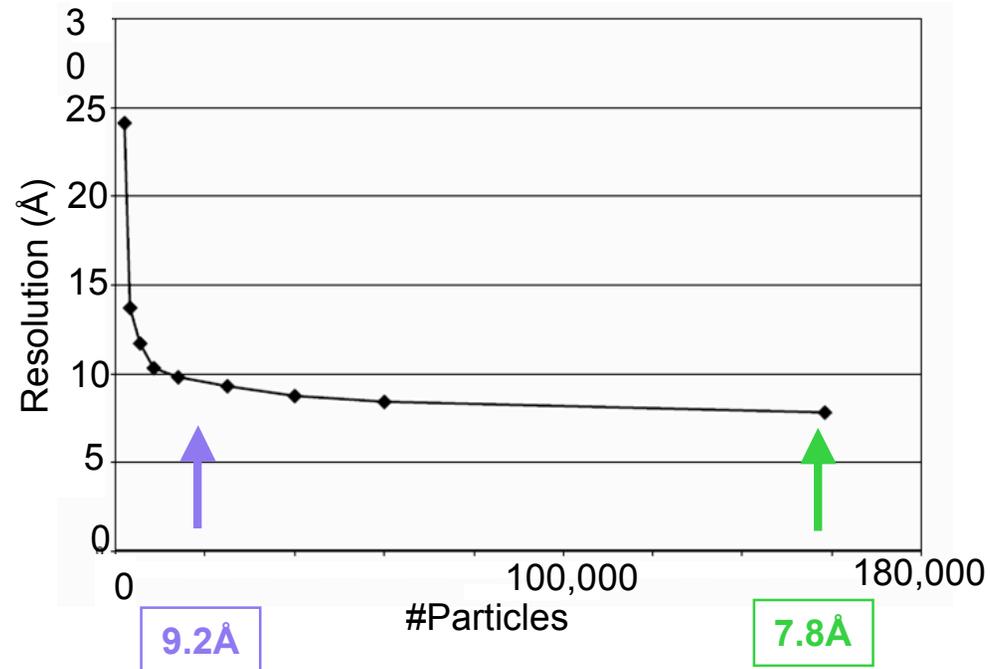
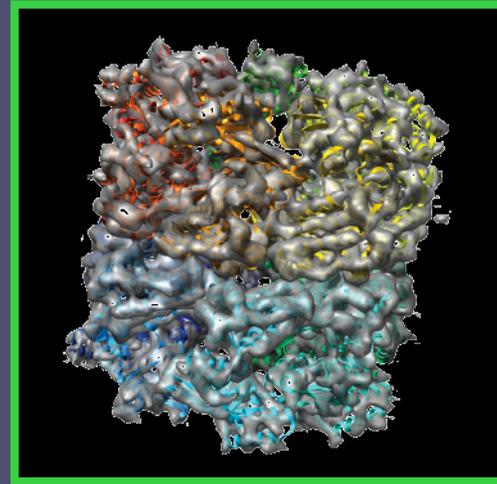
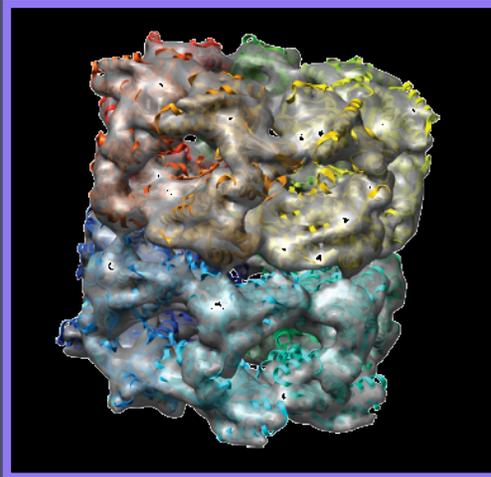
Resolution vs. Ice thickness

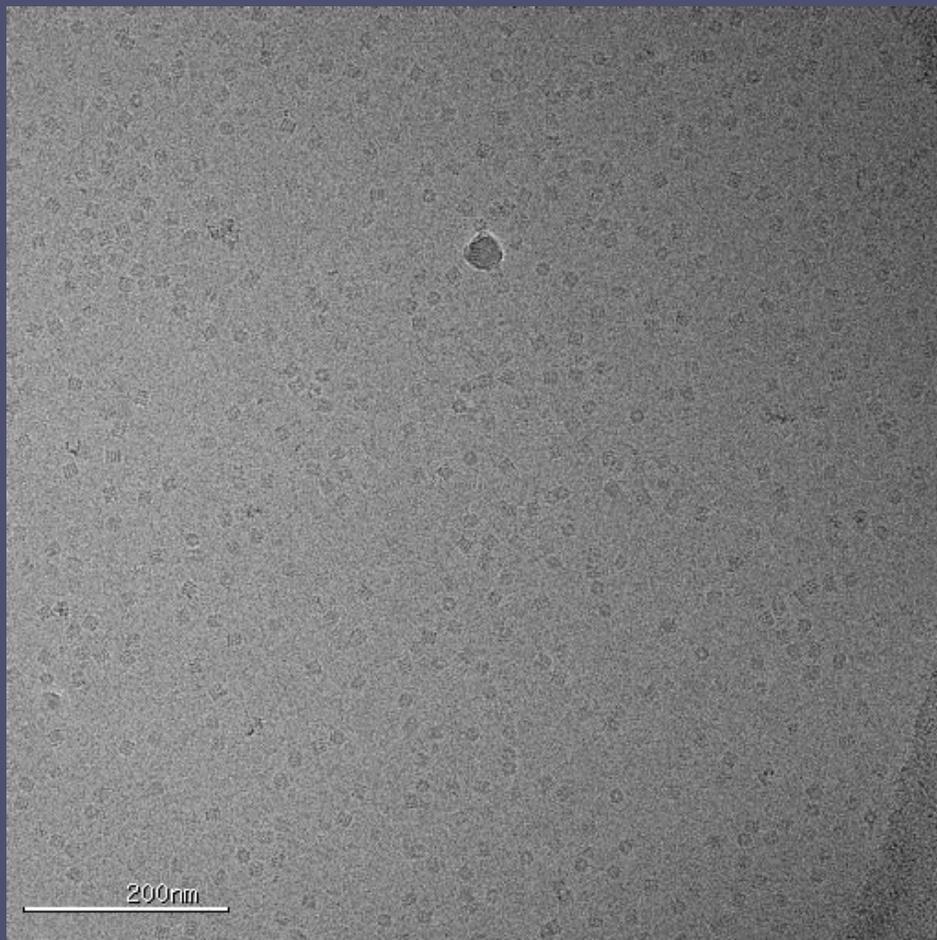


Stagg, et al. "Automated CryoEM Data Acquisition and Analysis of 284,742 Particles of GroEL"
J. Struct. Biol. Journal of Structural Biology 155, 470-481.



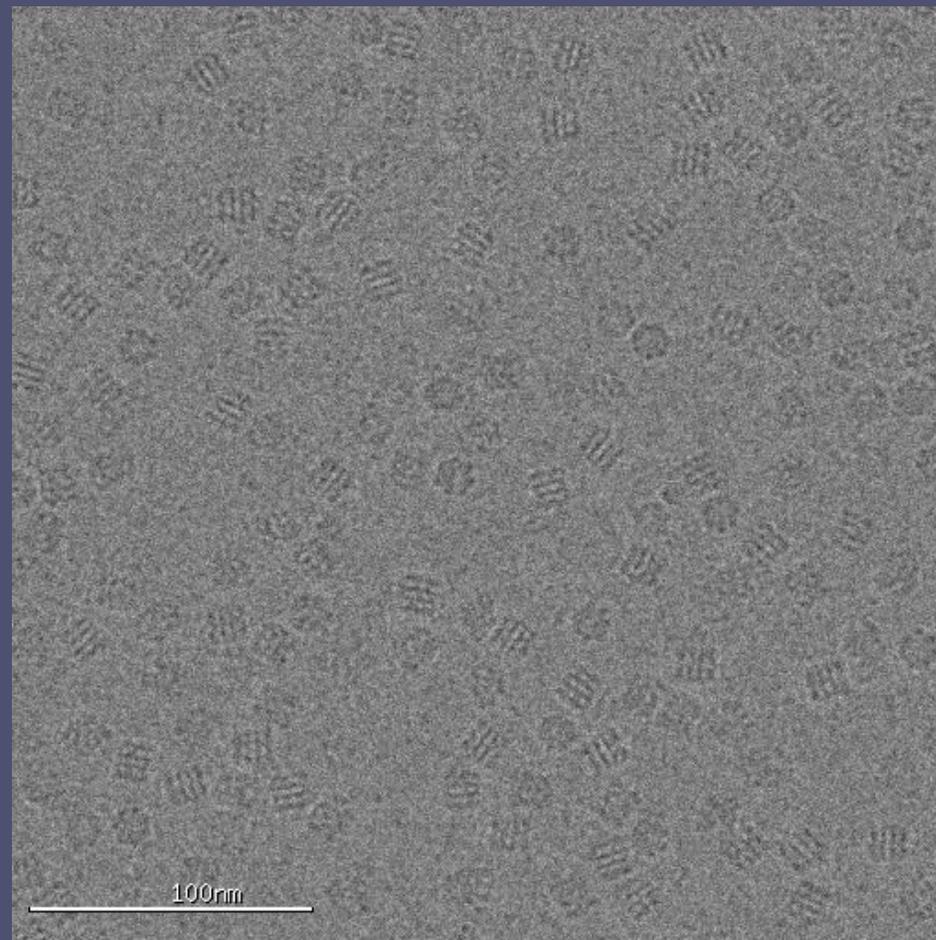
Resolution vs. number of particles





0.2nm/pix

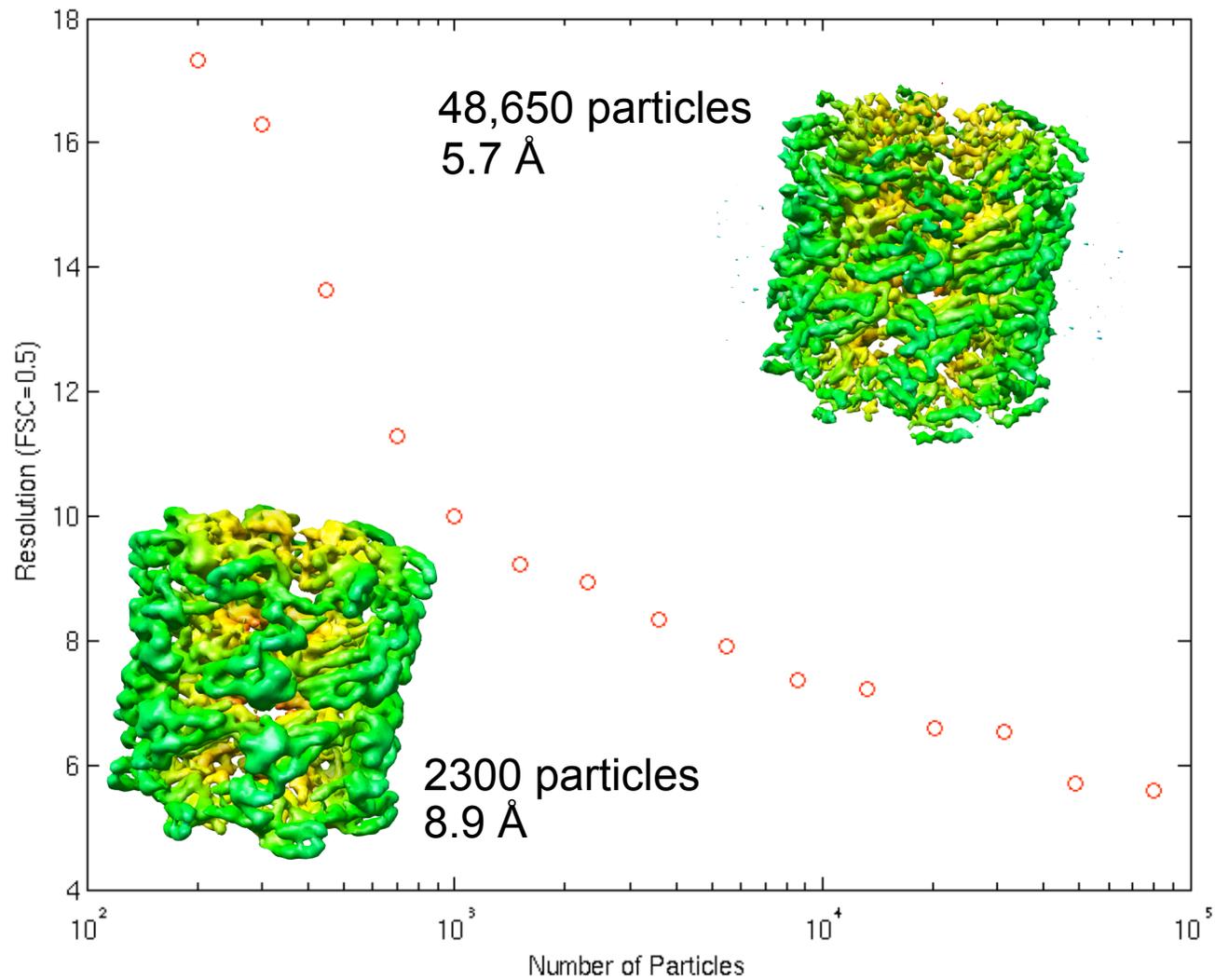
~500 particles/image



0.08 nm/pix

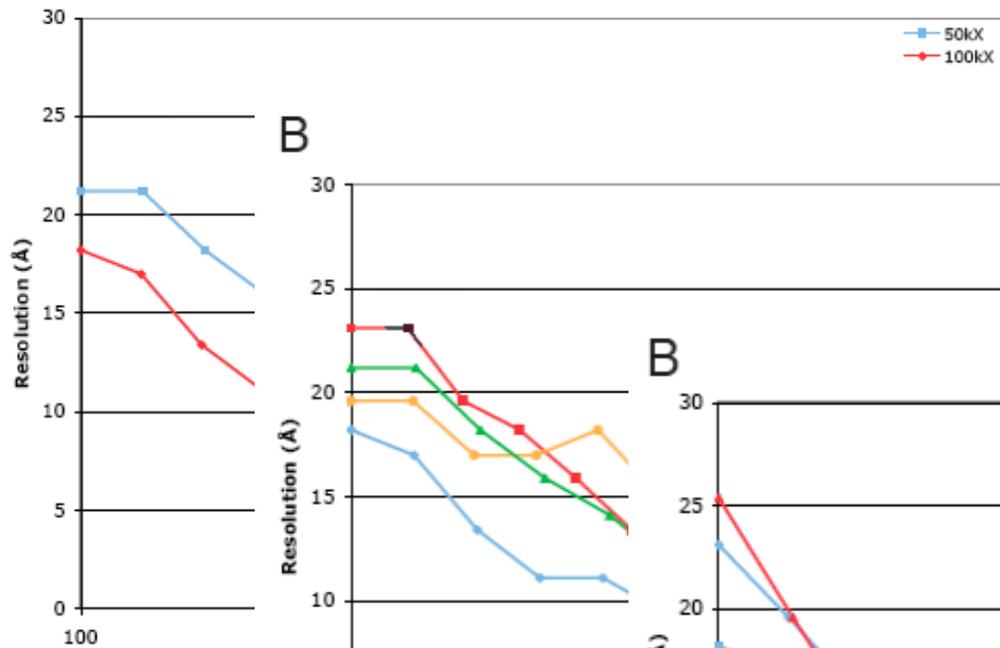
~80 particles/image

Resolution as a function of number of particles

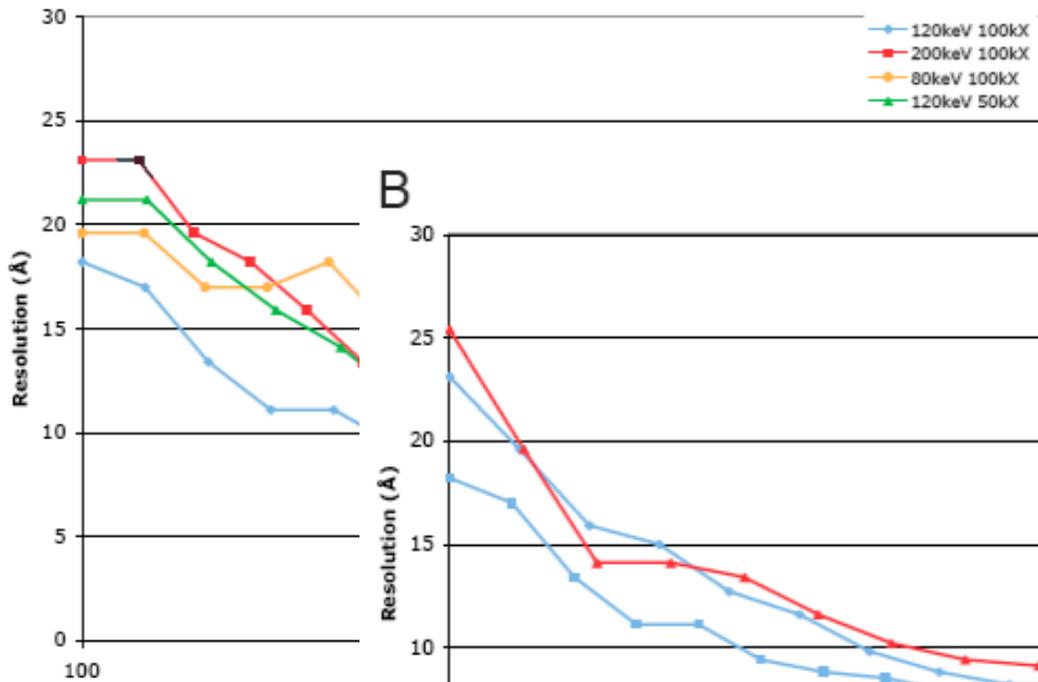


Resolution as a function of mag, KeV, dose...

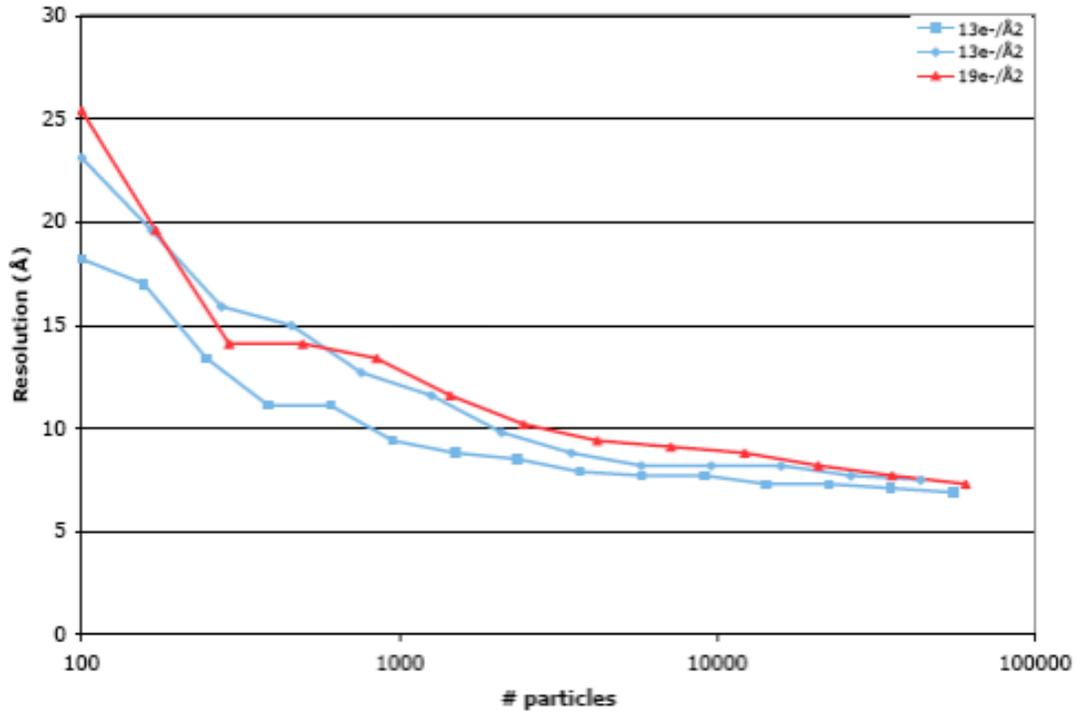
B



B



B



Imaging Technology Group at TSRI:



Jim Pulokas



Denis Fellmann



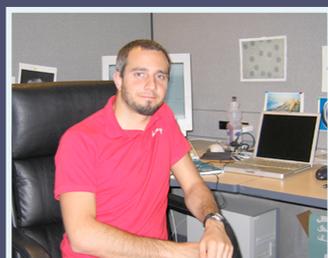
Joel Quispé



Anchi Cheng



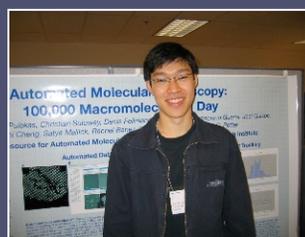
Teddy Ajero



Gabriel Lander



Craig Yoshioka



Pick-Wei Lau



Anke Mulder



Sunita Nayak



Neil Voss



Christopher Irving



Lorraine Lathrop



Clint Potter



Bridget Carragher



Scott Stagg



National Resource for Automated Molecular Microscopy
<http://nramm.scripps.edu>

