

# Automated Molecular Microscopy



THE SCRIPPS RESEARCH INSTITUTE

Bridget Carragher  
CryoCourse, 2005

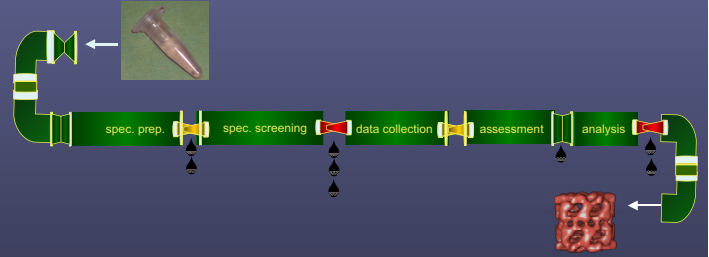
National Resource for Automated Molecular Microscopy  
namm.scripps.edu



Center for Integrative Molecular Biosciences

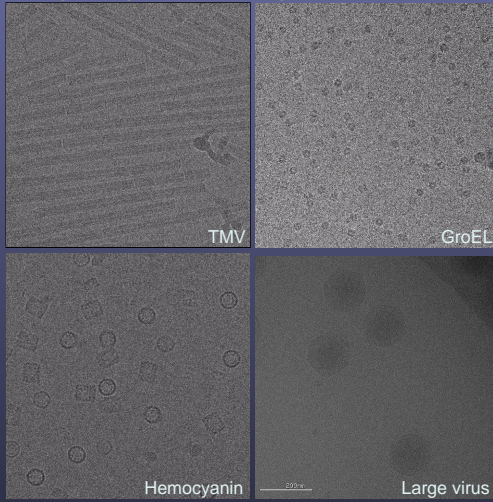


## Pipeline for Molecular Microscopy



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

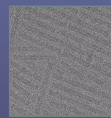
## 5. High magnification images (~60,000x)



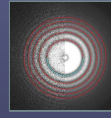
Acquired as defocus pairs or sequences

Total dose: ~ 10 e/A<sup>2</sup>

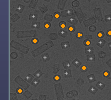
## Automated image analysis and reconstruction



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\_00030gr\_data/  
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

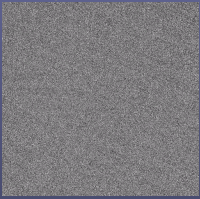
parentId: 124660  
parentImage: 05jun28a\_00030gr\_00002sq\_00012hl.mrc  
parentPreset: hl  
parentType: acquisition  
parentNumber: 2  
target: 250  
targety: 243  
targetDim: 20.864  
targetDim: 29.506151765352

### 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

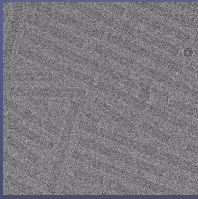
## Assessing the images: TMV

Exposure 1



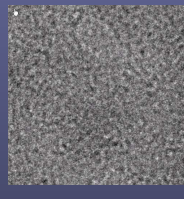
dose  $\sim 12e^-/\text{\AA}^2$   
 $\Delta f = -30\text{nm}$

Exposure 2

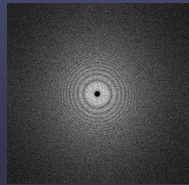
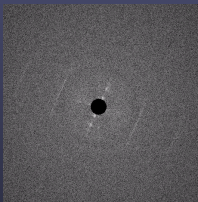


dose  $\sim 24e^-/\text{\AA}^2$   
 $\Delta f = -200\text{nm}$

Focus image

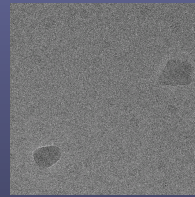


$\Delta f = -200\text{nm}$



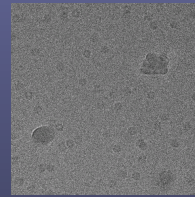
## Assessing the images: GroEL

Exposure 1



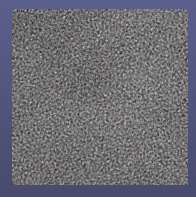
$\Delta f = -60\text{nm}$

Exposure 2

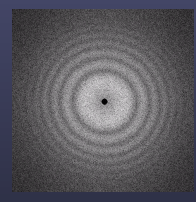


$\Delta f = -200\text{nm}$

Focus image



$\Delta f = -60\text{nm}$



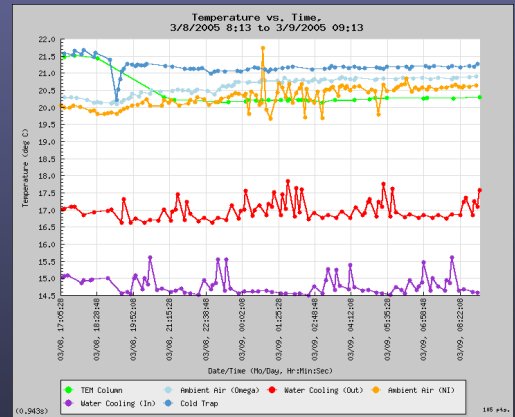
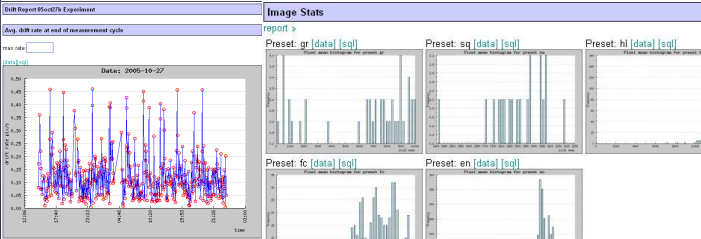
## Summary of 05oct27b Experiment

### Experiment Information

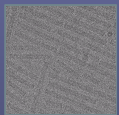
SessionId: 2553  
Name: 05oct27b  
Purpose: p22 mature virion 2nd collection- qfoil 5 sec blot slot #2  
Begin Time: 2005-27-10 11:02:30  
End Time: 2005-28-10 23:43:31  
Total Duration: 36:41:01  
Image path: /ami/data06/legion/05oct27b/rawdata  
Instrument: Tecnai - Tietz SCX  
InstrumentId: 20  
User: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

### Images Acquired

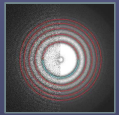
Preset label # images  
en: 1628  
fc: 417  
gr: 43  
hl: 431  
sq: 20  
Total images: 2539



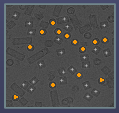
## Automated image processing and reconstruction



Assess the images



Determine the CTF

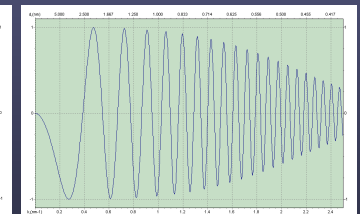
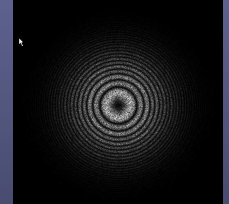
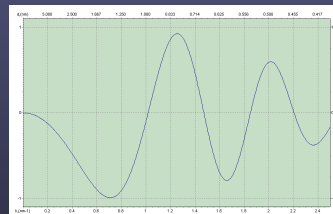
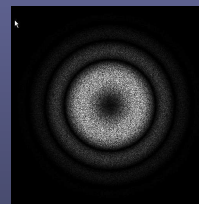


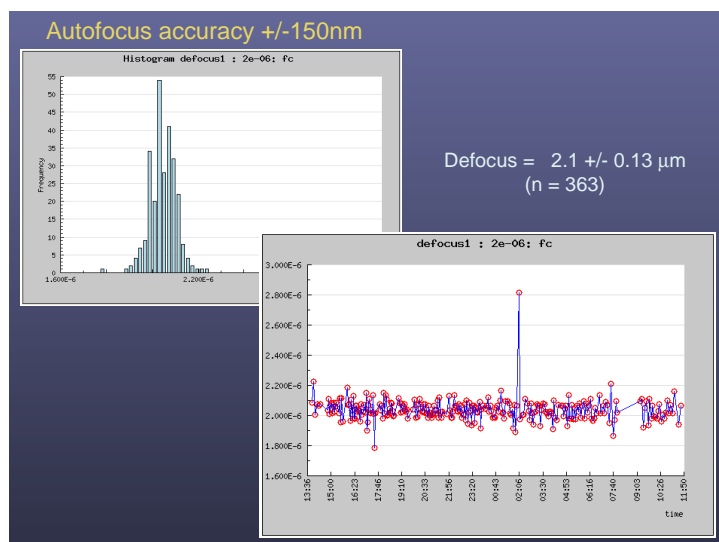
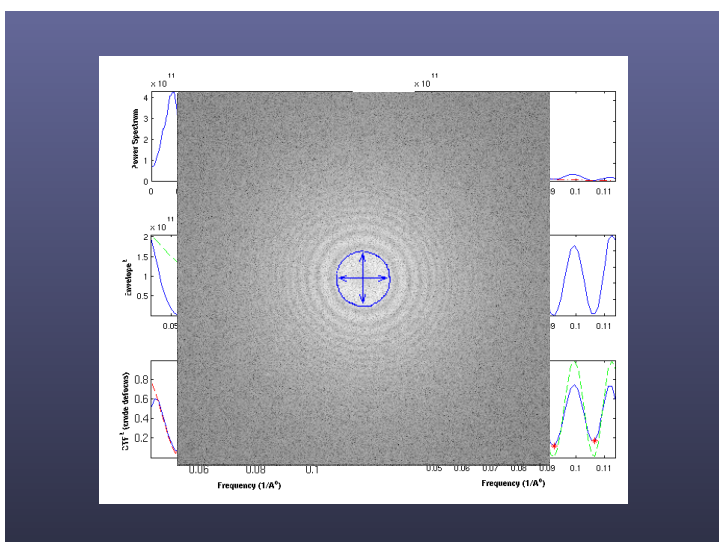
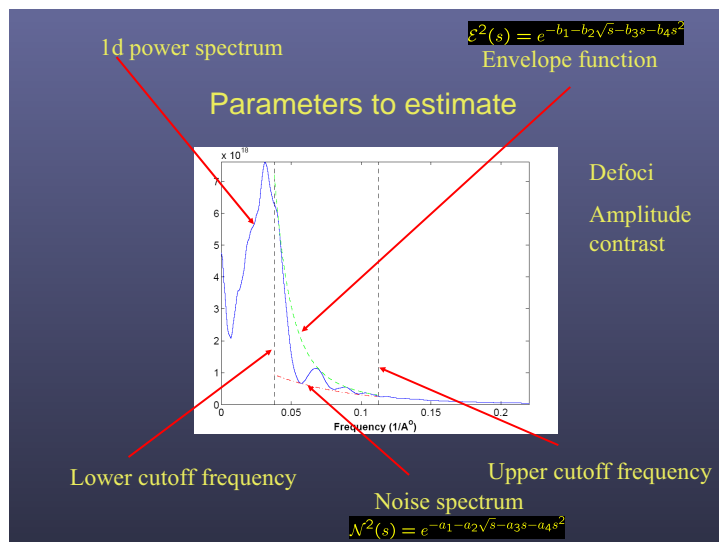
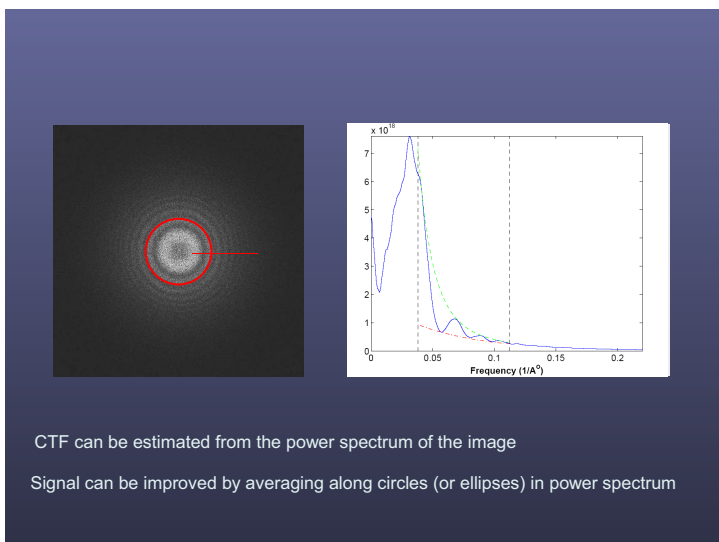
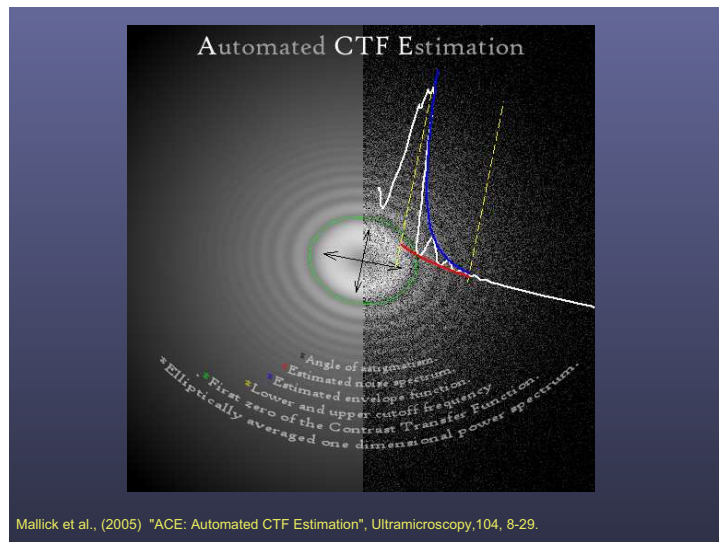
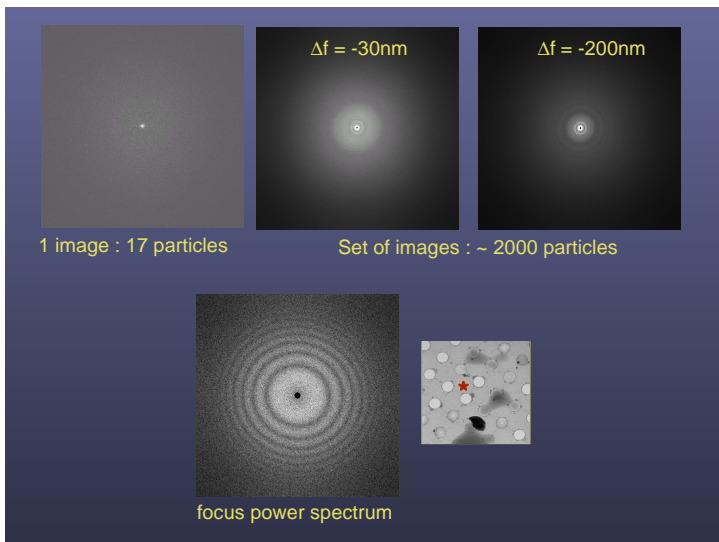
Select and segment particles



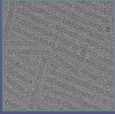
Reconstruct 3D map

## Defocus determination

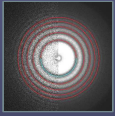




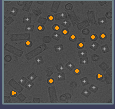
### Automated image processing and reconstruction



Assess the images



Determine the CTF

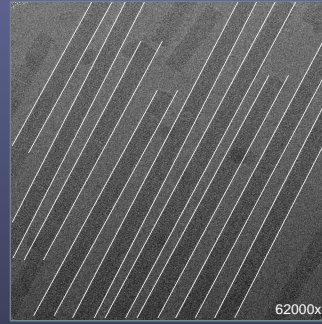


Select and segment particles



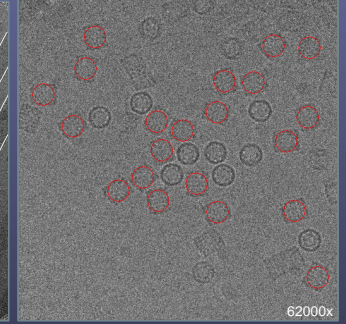
Reconstruct 3D map

### Automated specimen selection and segmentation



Filaments

411 image pairs  
686 filaments automatically selected

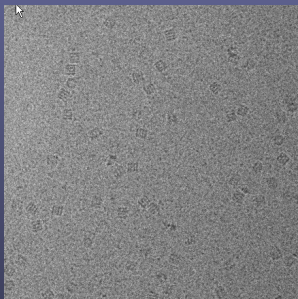


Single particles

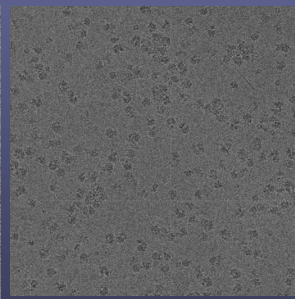
816 image pairs  
23,000 particles automatically selected

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

### Automated specimen selection and segmentation



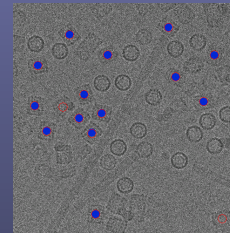
GroEL



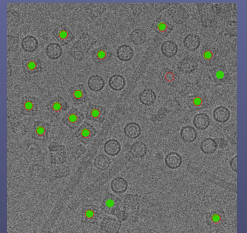
Ribosomes

### Automated particle selection "bakeoff"

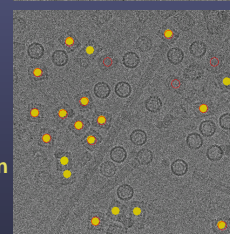
human vs. human



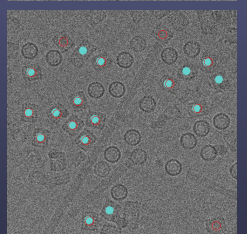
human vs. template matching



human vs. feature recognition

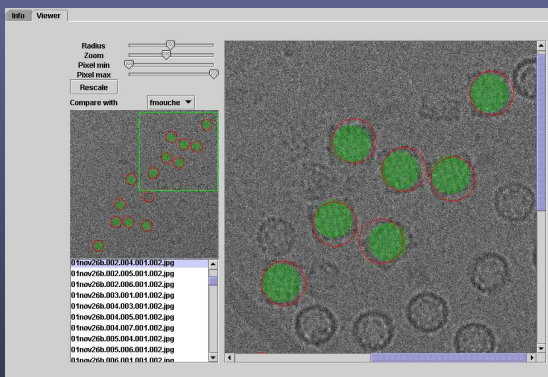


human vs. machine learning



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

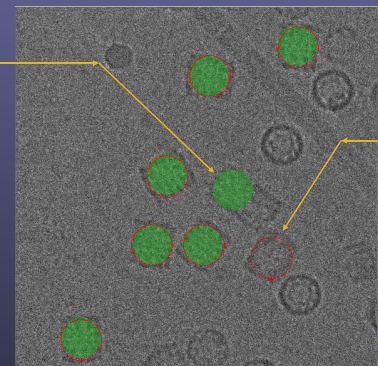
### Automated particle selection Bakeoff



[http://ami.scripps.edu/legion/particle\\_viewer/](http://ami.scripps.edu/legion/particle_viewer/)

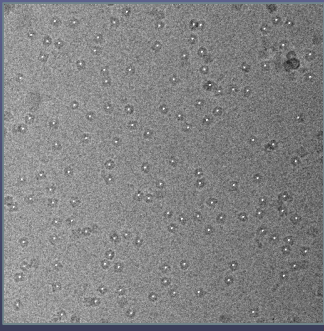
### Bakeoff Results

False Positive

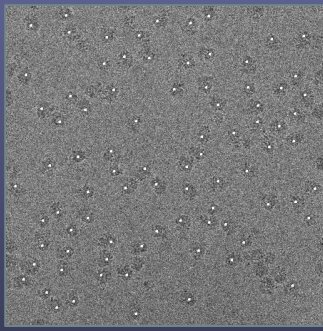


False Negative

### Some examples using "Roseman" + "Selexon"

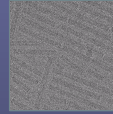


GroEL  
# images: 550  
# particles: 270,000  
Time: 24 hours

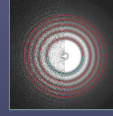


Ribosomes  
# images: 551  
# particles: 26,000  
Time: 36 hours

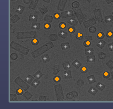
### Automated image processing and reconstruction



Assess the images



Determine the CTF



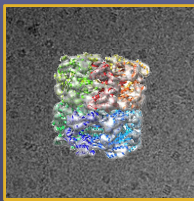
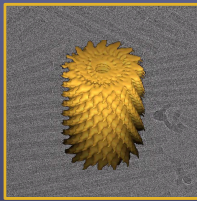
Select and segment particles



Reconstruct 3D map

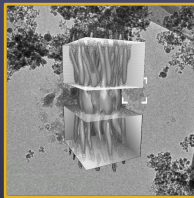
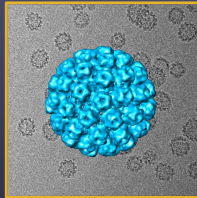
### Reconstruction:

helices



single particles

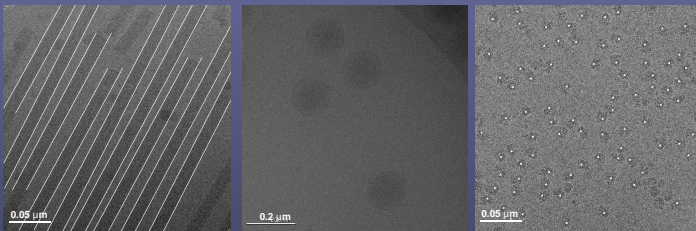
viruses



2D crystals

## Throughput and Resolution

### How many images do we need?



Resolution?	# "particles"*	# images			
		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

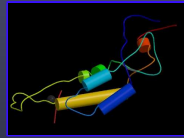
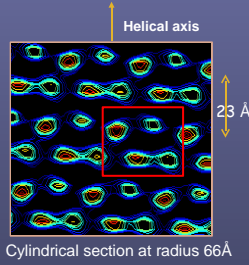
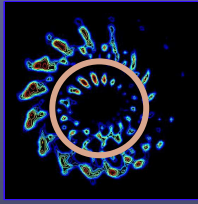
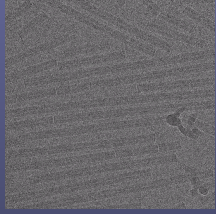
\*Except for:  
Yonekura, K., Maki-Yonekura, S. and K. Namba (2003) Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. *Nature* 424:643-50.

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

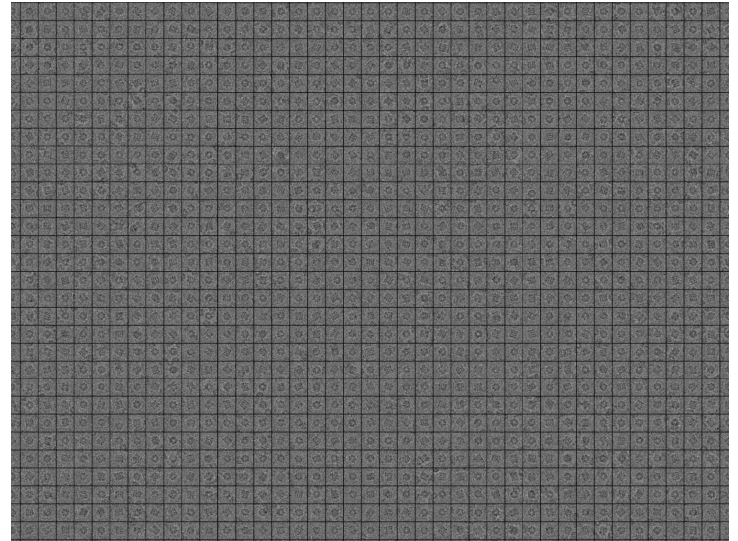


TMV

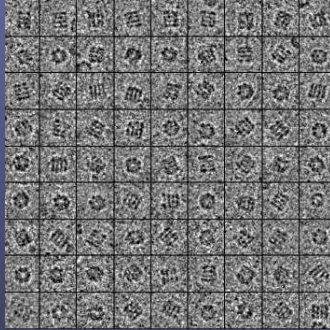
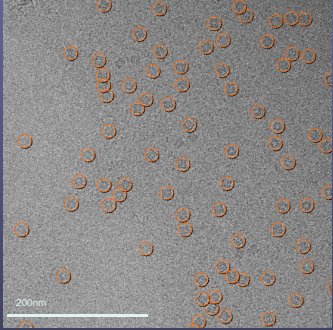
## What is the throughput/resolution?



# 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: ~7.6 Å  
 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

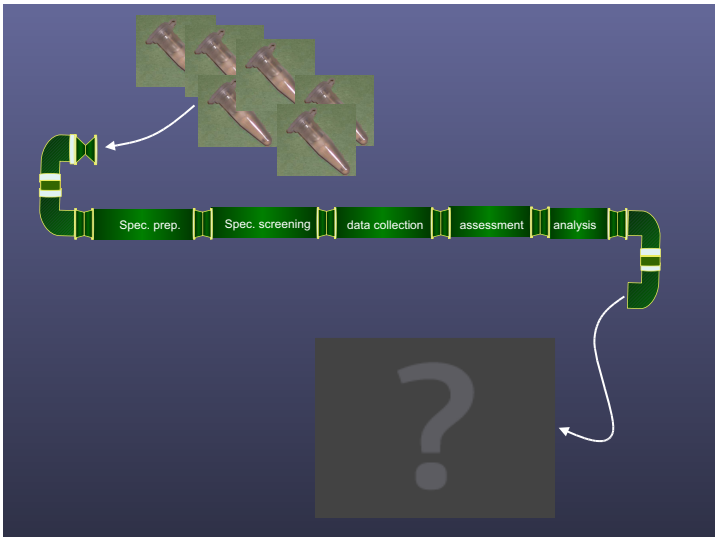
### C. Service Projects

### D. Training- workshops + courses.

### E. Dissemination



<http://nramm.scripps.edu>



## 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:



(RR 17573)

National Institutes of Health (GM61939)  
 National Science Foundation (DBI-9730056, DBI-0296063)