

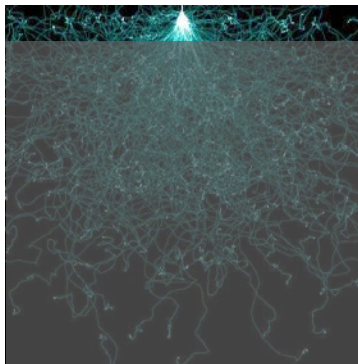
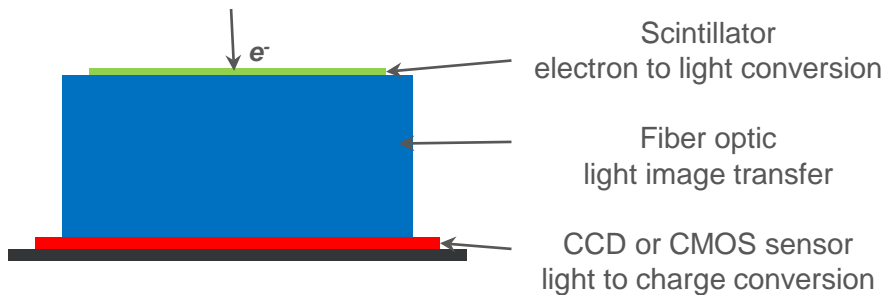


# Applications of electron counting direct detection cameras in high resolution cryo-EM

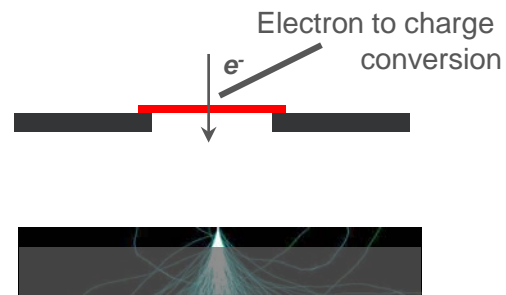
Christopher Booth  
2016 July 11  
CEM3DIP Workshop

## 2 Types of Detectors

### Fiber-coupled camera (OneView, Orius)



### Direct detection camera (K2 Summit, K2-IS)



# OneView™ Camera

No compromises



High-Speed + Full Resolution  
...delivered *at the same time, all the time.*



# Speed vs. Resolution

## Always fast

- 25 frames per second – at full 4k x 4k pixel resolution
- Search and navigate in high resolution at video frame rates
- Perform all your alignments and corrections at full speed

## No longer need to bin to go fast

- ...but *in-situ* experiments can benefit from higher frame rates

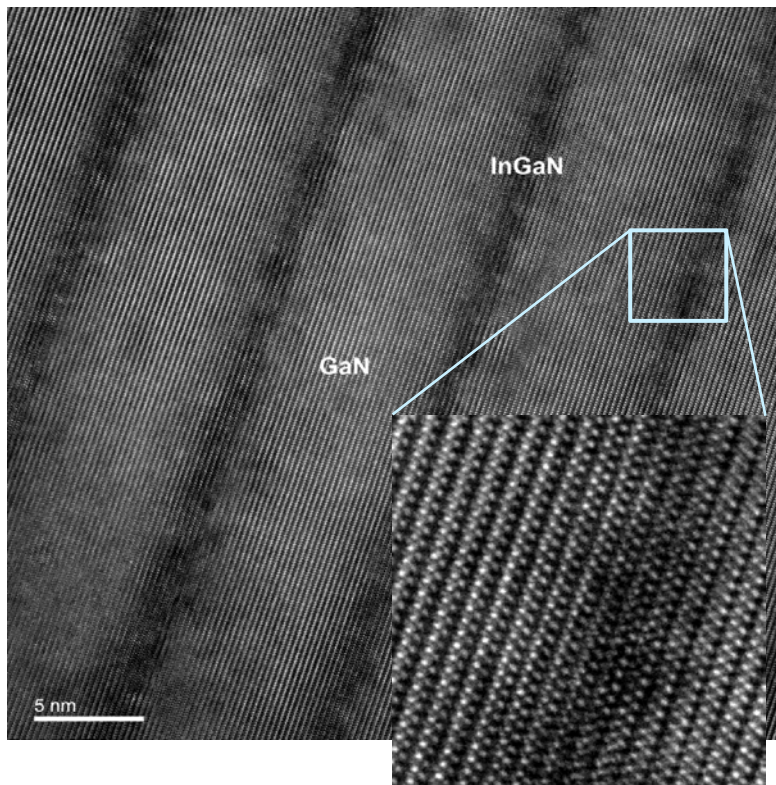
## Easy to use

- Auto-exposure mode
- Redesigned GMS (Gatan Microscopy Suite®) 3.0 user interface
  - Better integration and control of the TEM
  - Based on Techniques workflow
- New “TruAlign” feature

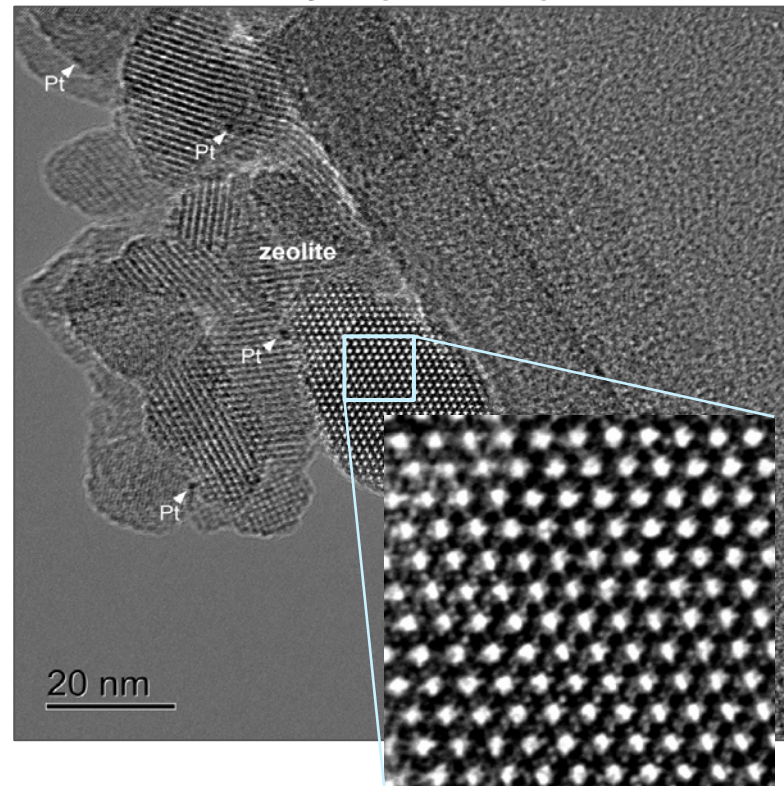


# Material Science Applications

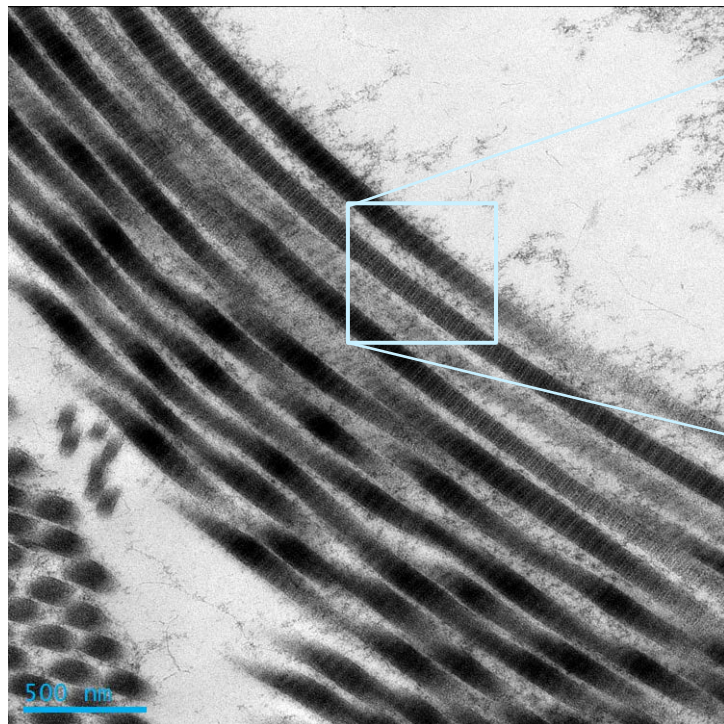
## Semiconductors



## Catalyst (Zeolites)



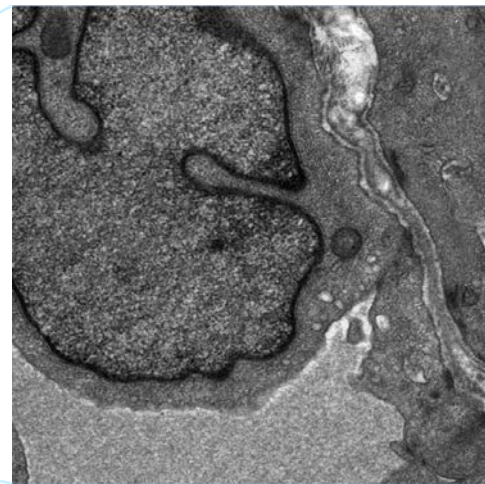
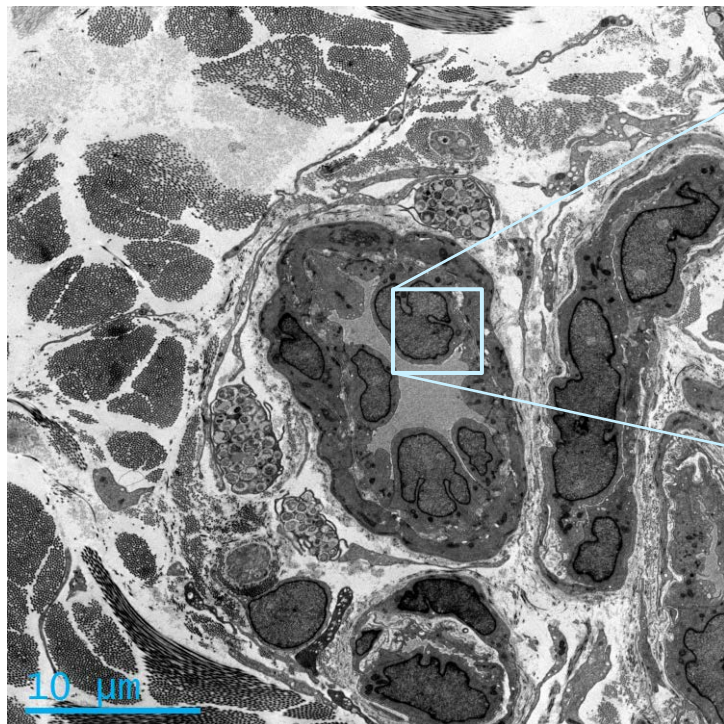
# Life Science Applications



Collagen fibrils are clearly resolved in this 4k x 4k image. OneView delivers high resolution and large FOV.



# Life Science Applications



Crisp detail revealed in high contrast imaging.

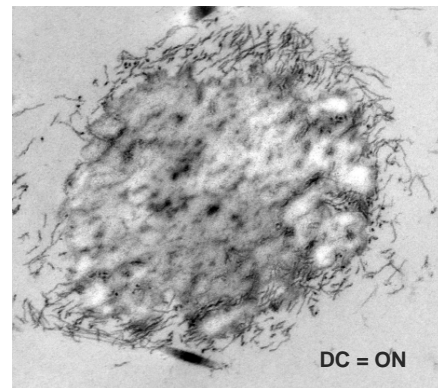
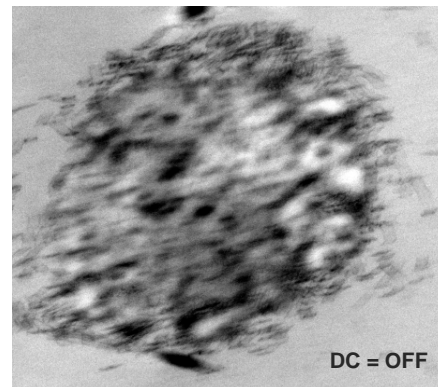
# Live Drift Correction

## Every TEM drifts

- Multiple sources affect images
  - Environmental, thermal, beam-induced, stage, etc.
  - Even subtle drift causes a loss of resolution and clarity

## Live drift correction

- Real-time correction provides immediate results
- One-click set up





# Zeolite Catalyst Example

Some samples are too sensitive to image easily

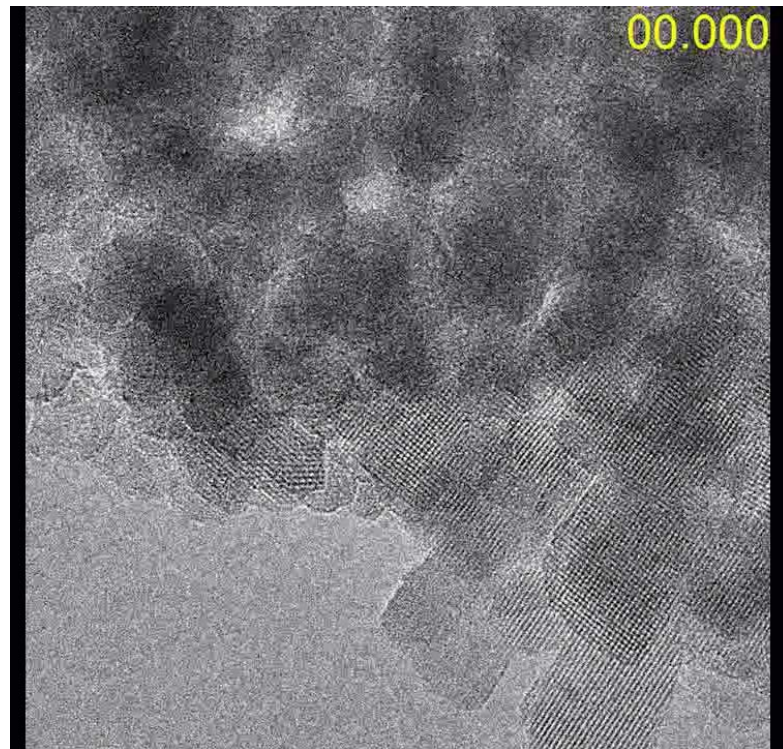
**Unstable under e-beam**

**Radiation damage**

- Sample drift
- Structure modification

**Stage drift**

- Moving to a fresh area introduces mechanical stage drift



Chevron SSZ-57 zeolite (4k x 4k)

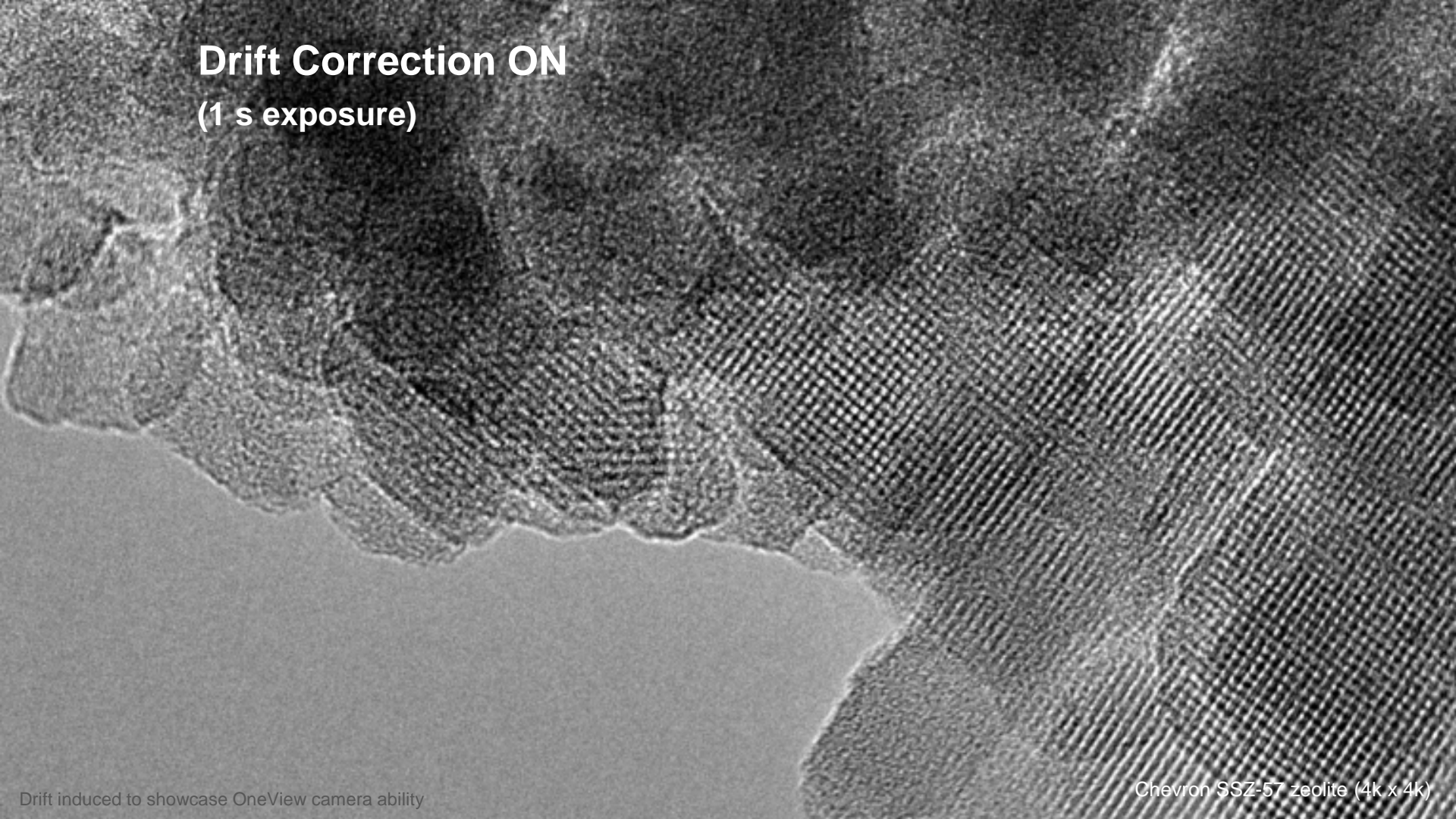
**Drift Correction OFF**

**(1 s exposure)**



Chevron SSZ-57 zeolite (4k x 4k)

**Drift Correction ON**  
**(1 s exposure)**

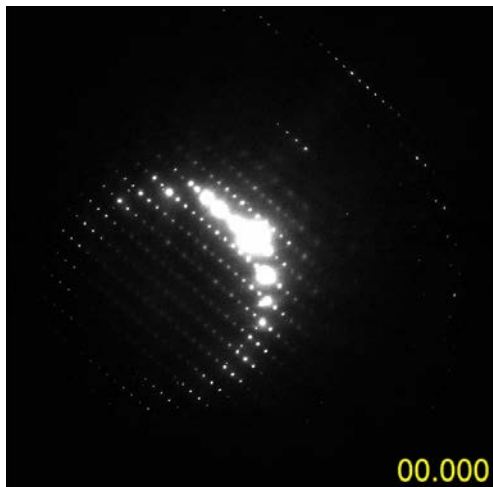


Chevron SSZ-57 zeolite (4k x 4k)

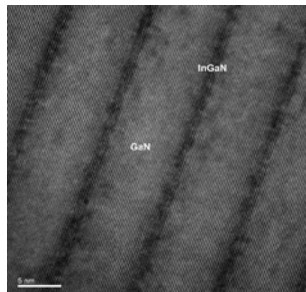


# CBED, Nanobeam Diffraction Studies

Parallel beam diffraction of GaN/InGaN



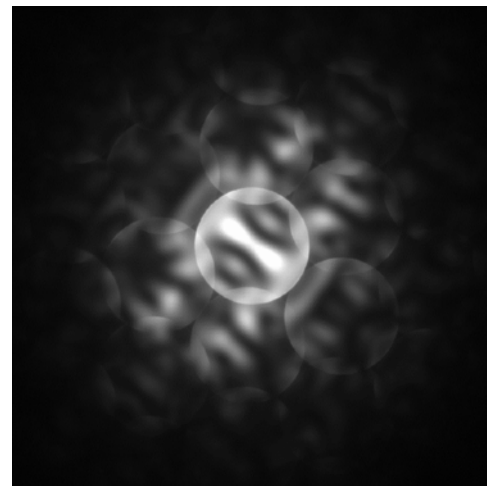
Record diffraction movies with optional *in-situ* mode



Bright field  
semiconductor image



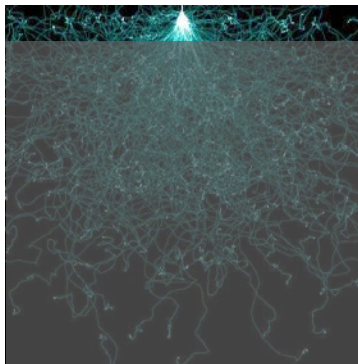
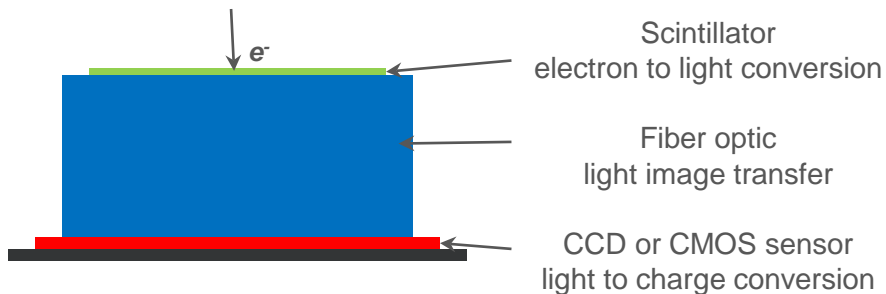
[110] CBED of GaN/InGaN



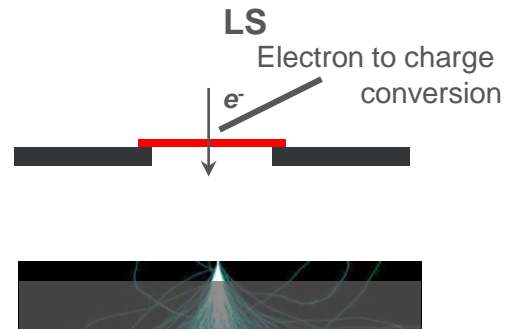
Synchronize frames for 4D STEM applications

## 2 Types of Detectors

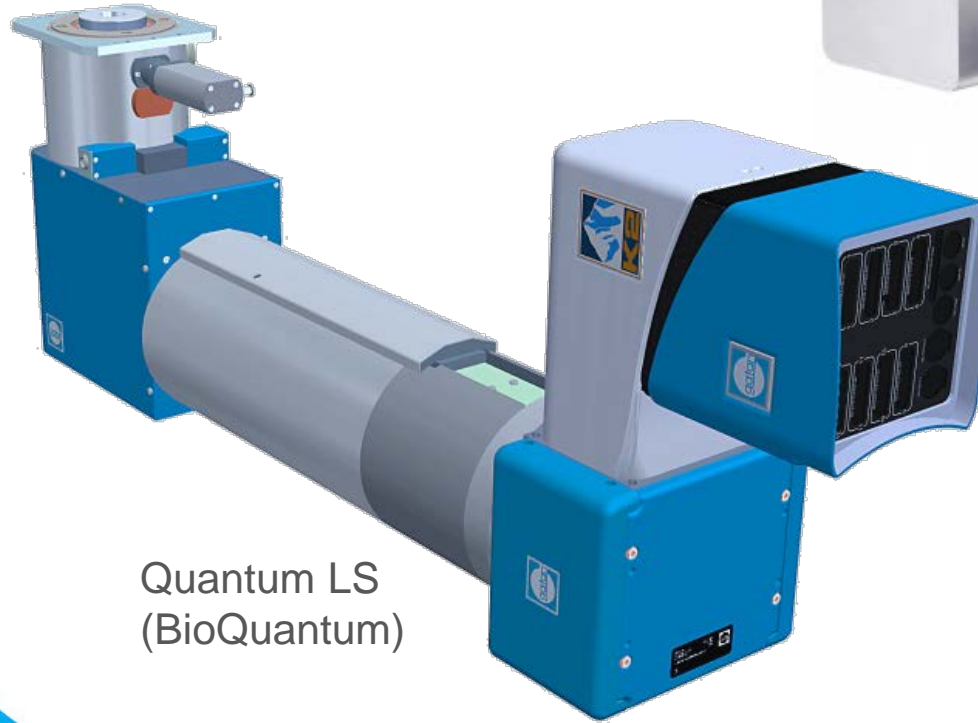
### Fiber-coupled camera (OneView, Orius)



### Direct detection camera (K2 Summit, K2-IS, Quantum)



# K2 Summit and Quantum LS



Quantum LS  
(BioQuantum)



K2 Summit

# Detectors designed for structural biology

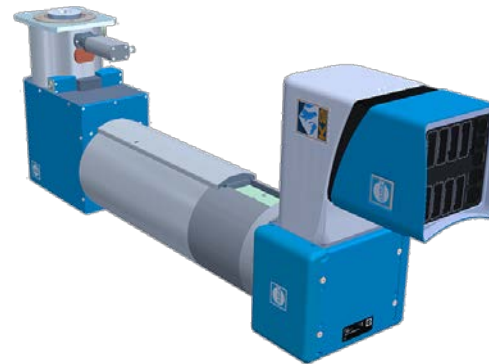
## K2 Summit

- **Electron counting** camera
- 400 full frames per second
- K2 direct detection sensor
- Unmatched performance
- Highest contrast for thin specimens



## GIF Quantum LS (BioQuantum)

- **Electron counting** energy filter
- 400 full frames per second
- K2 direct detection sensor
- Unmatched performance
- Highest contrast for thick and thin specimens

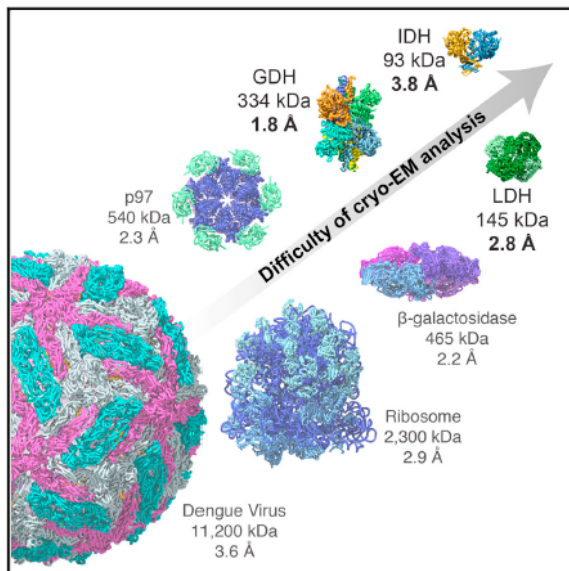


# K2 Summit/Quantum LS. A record of firsts!

## Cell

### Breaking Cryo-EM Resolution Barriers to Facilitate Drug Discovery

#### Graphical Abstract



#### Article

Higher DQE values ... **can only be obtained by operating in counting mode**... High frame rates are required for counting, to avoid double hits on individual pixels or very long exposures times, **at present only the Gatan K2, when operated in counting mode, can produce a DQE(0) as high as 80% in conjunction with reasonably small exposure times.** The K2 detector frame rate is about 10 times higher than that available with the two other detector brands

#### Authors

Alan Merk, Alberto Bartesaghi, Soojay Banerjee, ..., Lesley A. Earl, Jacqueline L.S. Milne, Sriram Subramaniam

#### Correspondence

ss1@nih.gov

#### In Brief

By using cryo-EM methods, the structure of small metabolic enzymes as well as the localization of small-molecule inhibitors that bind to them can be determined at near-atomic resolution.

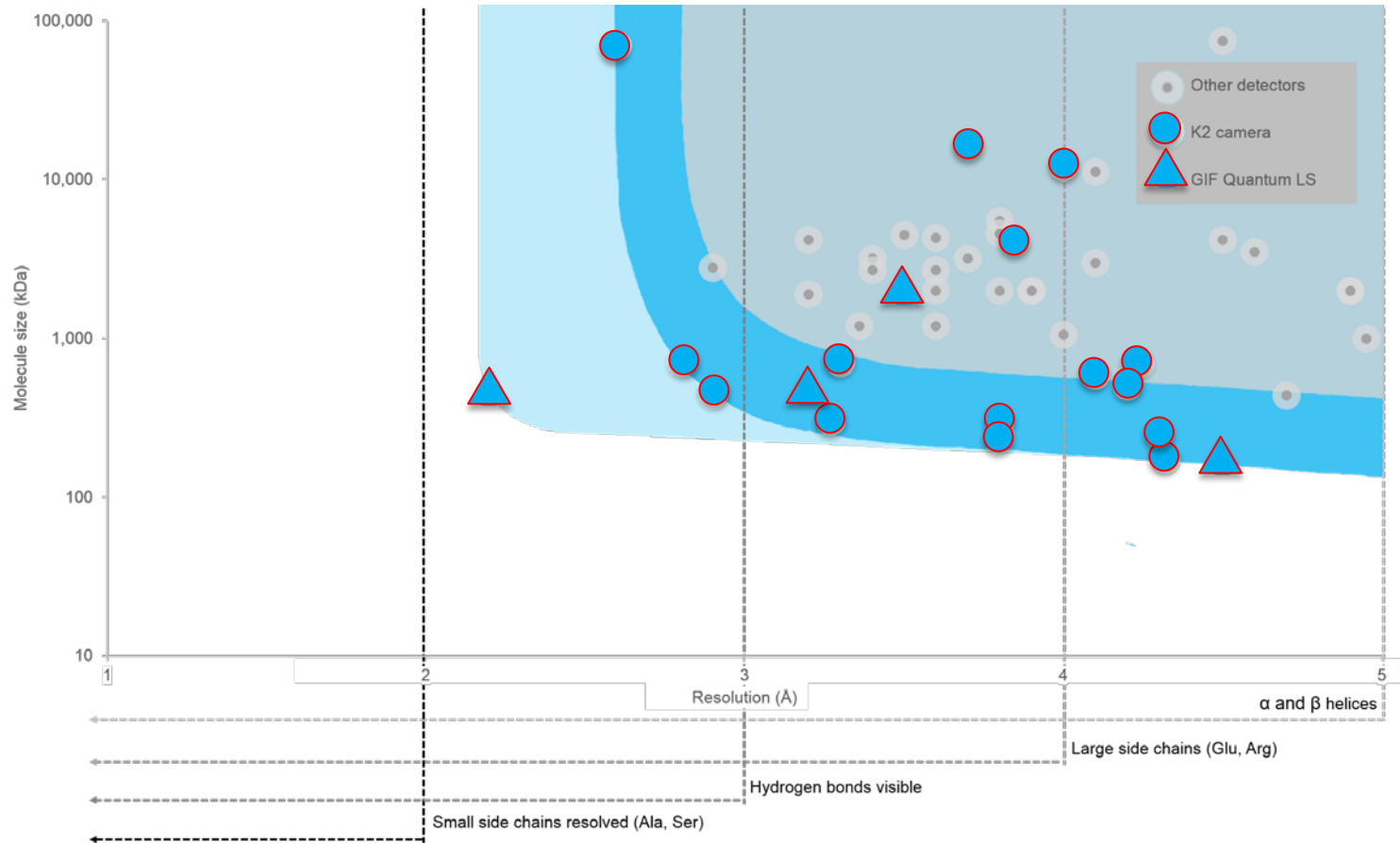
CryoEM at **IUCr**: a new era

Sriram Subramaniam,<sup>a</sup> Werner Kühlbrandt<sup>b</sup> and Richard Henderson<sup>c\*</sup>

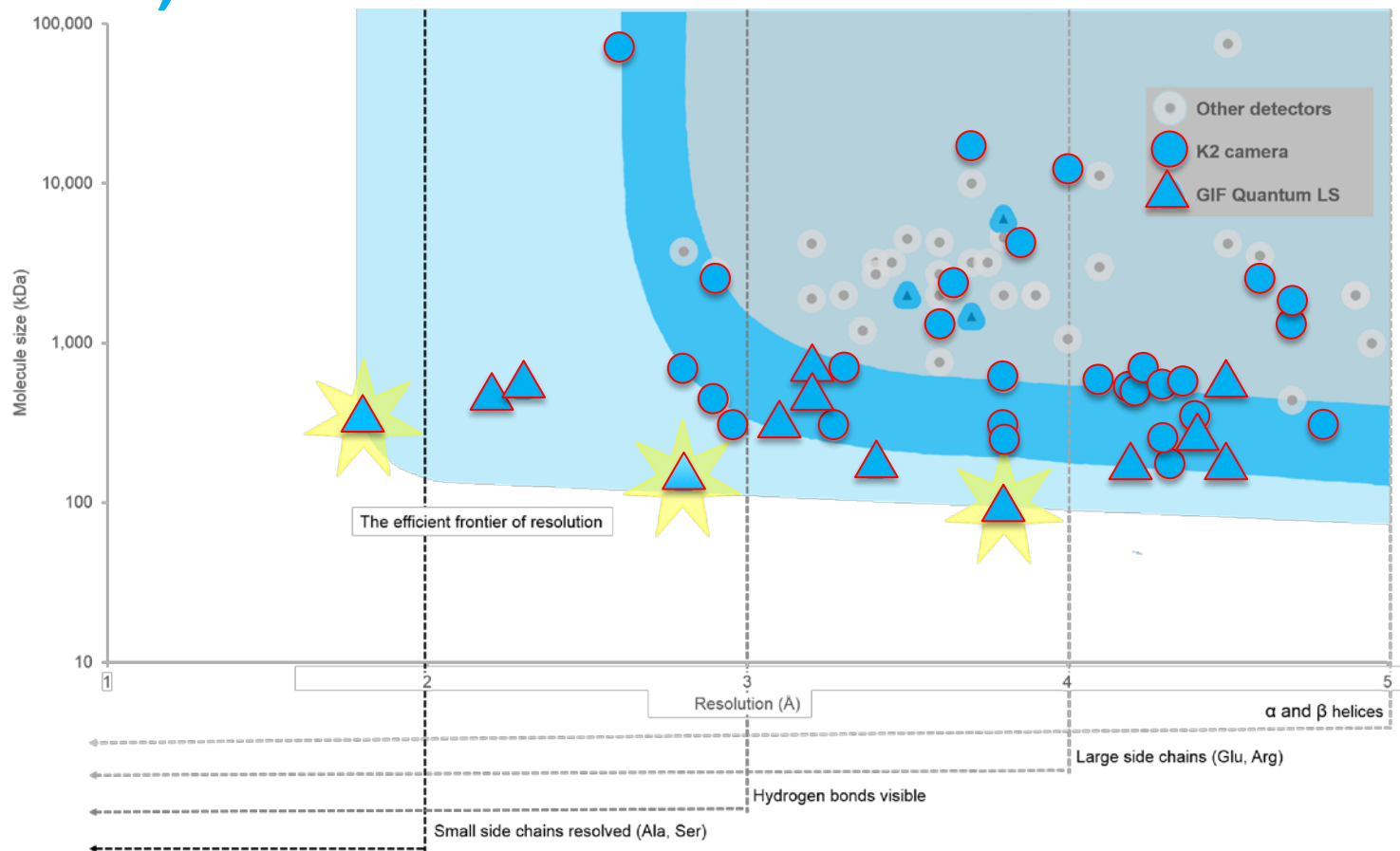
<sup>a</sup>Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA, <sup>b</sup>Department of Structural Biology, Max Planck Institute of Biophysics, Frankfurt, 60538, Germany, and <sup>c</sup>MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, UK.  
\*Correspondence e-mail: rh15@mrc-lmb.cam.ac.uk



# Breaking the 3 Å barrier... (May 2015)



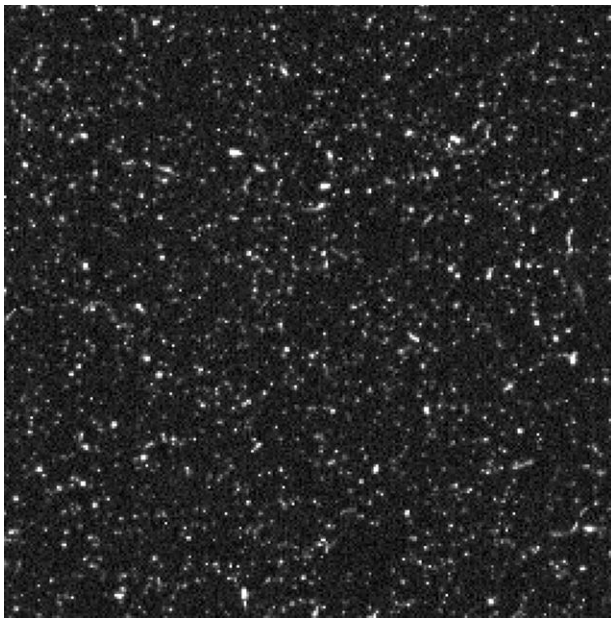
# Breaking the 2 Å and 100 kDa barrier (May 2016)



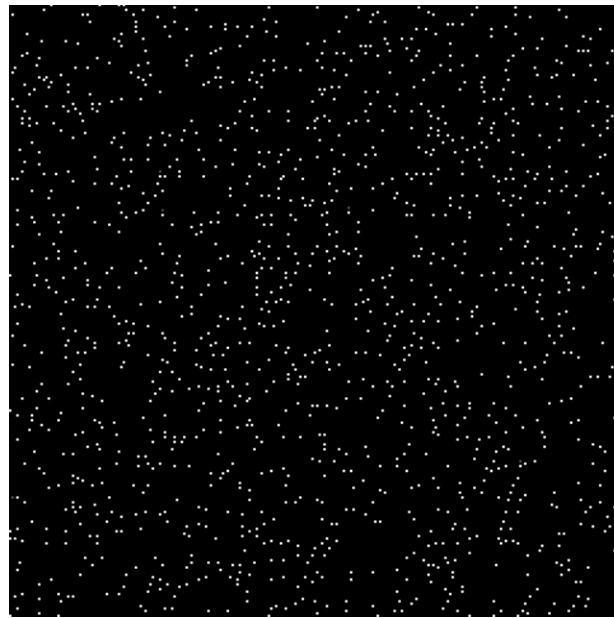


# Electron Counting – Remove the remaining noise

Typical dose rate of  $10 \text{ e}^-/\text{pix}/\text{s}$



Single 2.5 ms frame using conventional  
CCD-style charge read-out

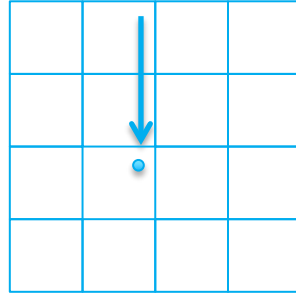


Same frame after counting

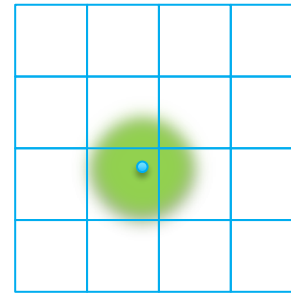
Counting removes the variability from scattering,  
rejects the electronic read-noise, and restores the DQE.

# “Integration” or “linear mode”

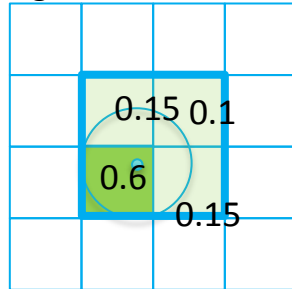
1. Electron enters detector



2. Signal is scattered



3. Charge collects in each pixel



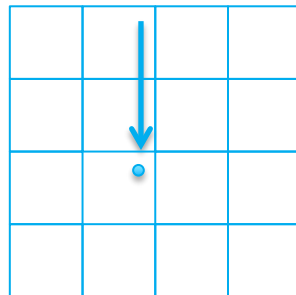
Stop at step 3:

**Charge Integration**

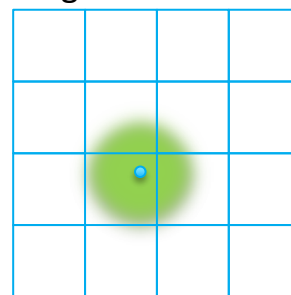
Improved DQE at high Frequency

# Counting mode

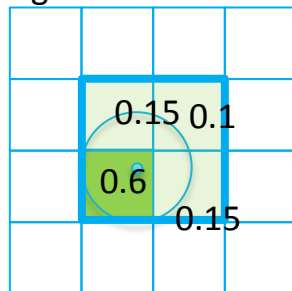
1. Electron enters detector



2. Signal is scattered



3. Charge collects in each pixel

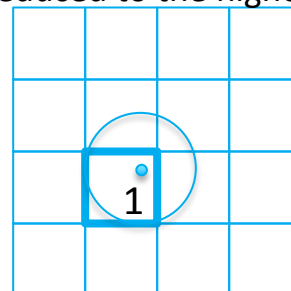


Stop at step 3:

**Charge Integration**

Improved DQE at high Frequency

4. Events are reduced to the highest charge pixels



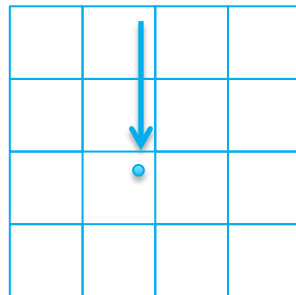
Continue to step 4:

**Counting**

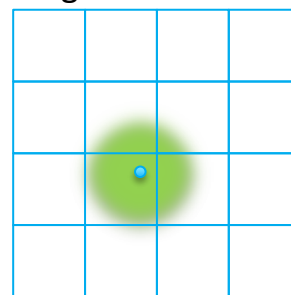
Improved DQE at low AND high Frequency

# Super-resolution mode

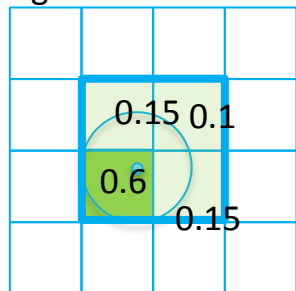
1. Electron enters detector



2. Signal is scattered



3. Charge collects in each pixel

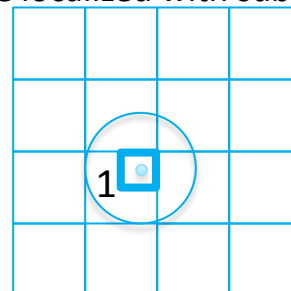


Stop at step 3:

**Charge Integration**

Improved DQE at high Frequency

4b. Events are localized with sub-pixel accuracy



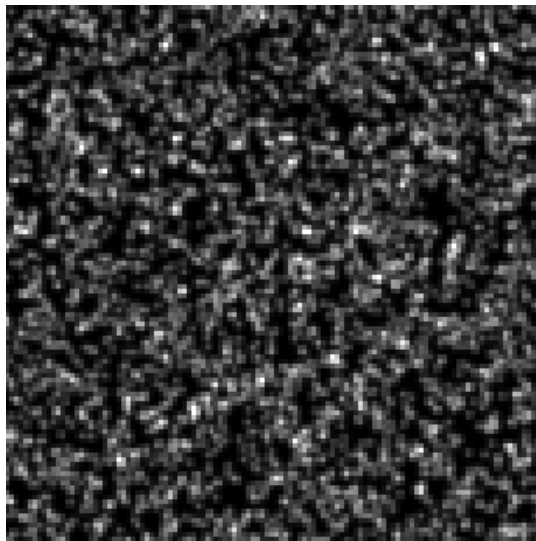
Continue to step 4:

**Super-Resolution**

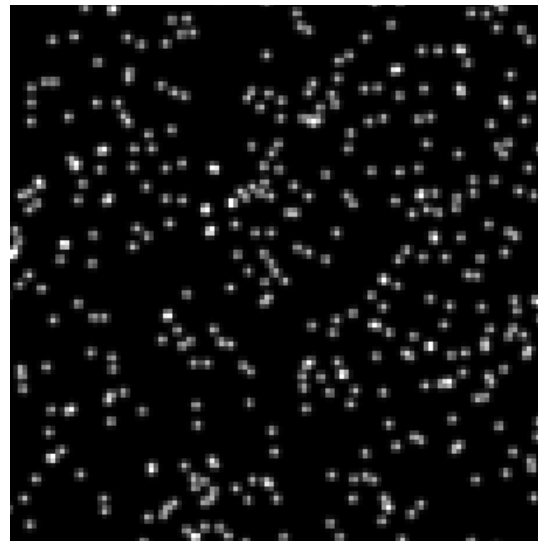
Improved DQE at low AND high Frequency  
and 7680 x 7424 pixels

# Electron counting requires high speed image readout

Typical dose rate of 10 e<sup>-</sup>/pix/s



40 fps: events overlap and  
cannot be resolved



400 fps: events are resolved

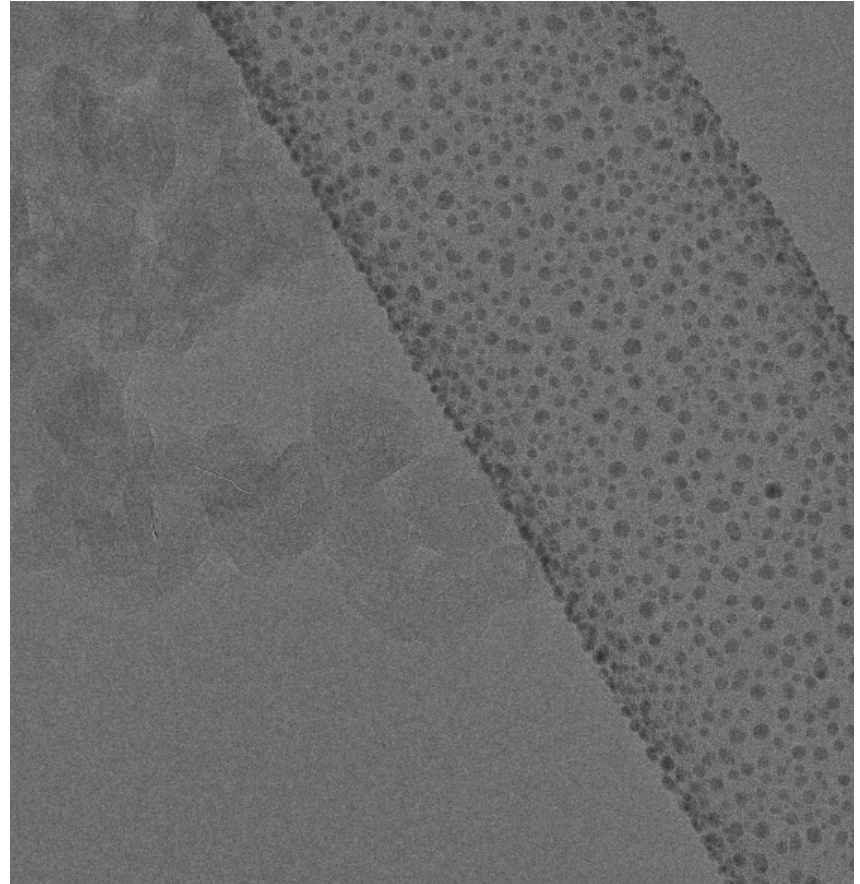
**It takes 400 fps to resolve electrons at a dose rate of 10 e<sup>-</sup>/pix/s**



# Dose Fractionation

7 sec exposure time without drift correction

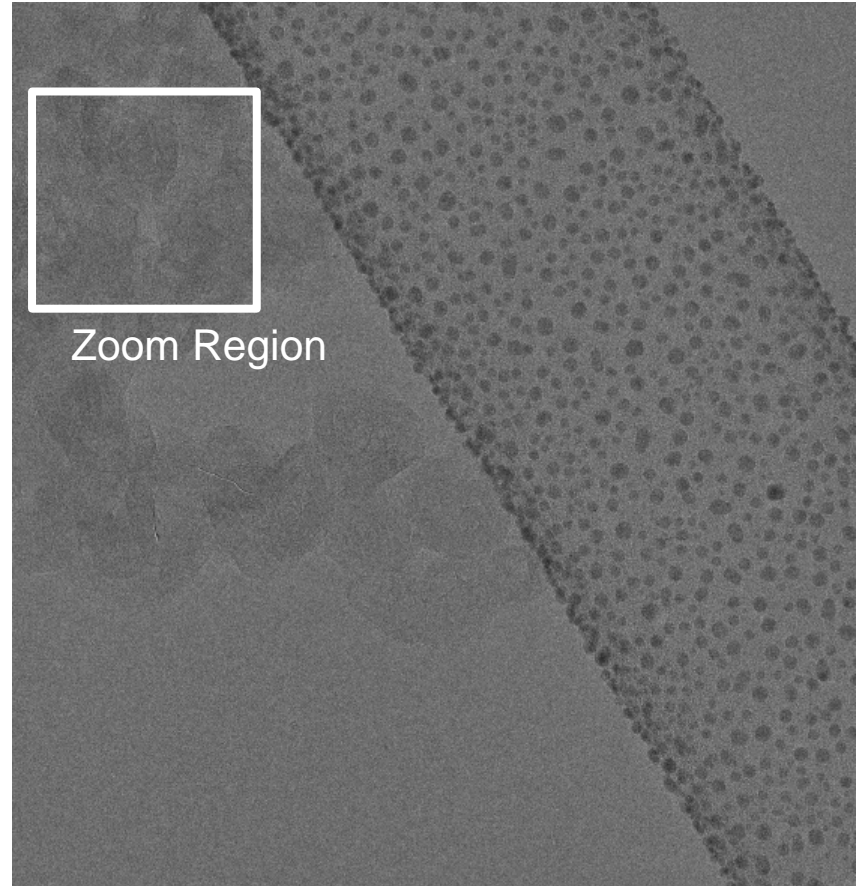
- Dose fractionation is the distribution of a total electron dose over a series of sub-frames
- $21 \times 0.33 \text{ sec} = 7 \text{ sec}$



# Dose Fractionation

- Dose fractionation is the distribution of a total electron dose over a series of sub-frames
- $21 \times 0.33 \text{ sec} = 7 \text{ sec}$

7 sec exposure time with drift correction

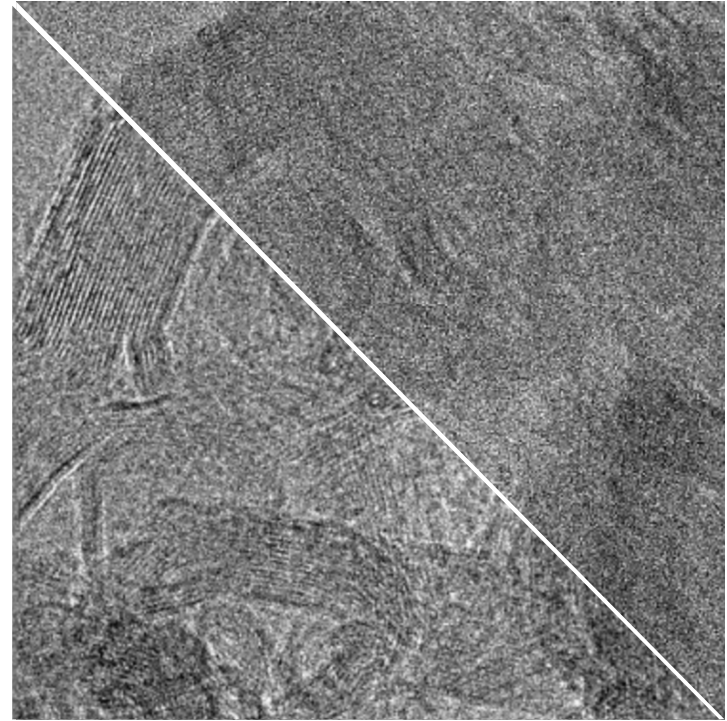




# Dose Fractionation

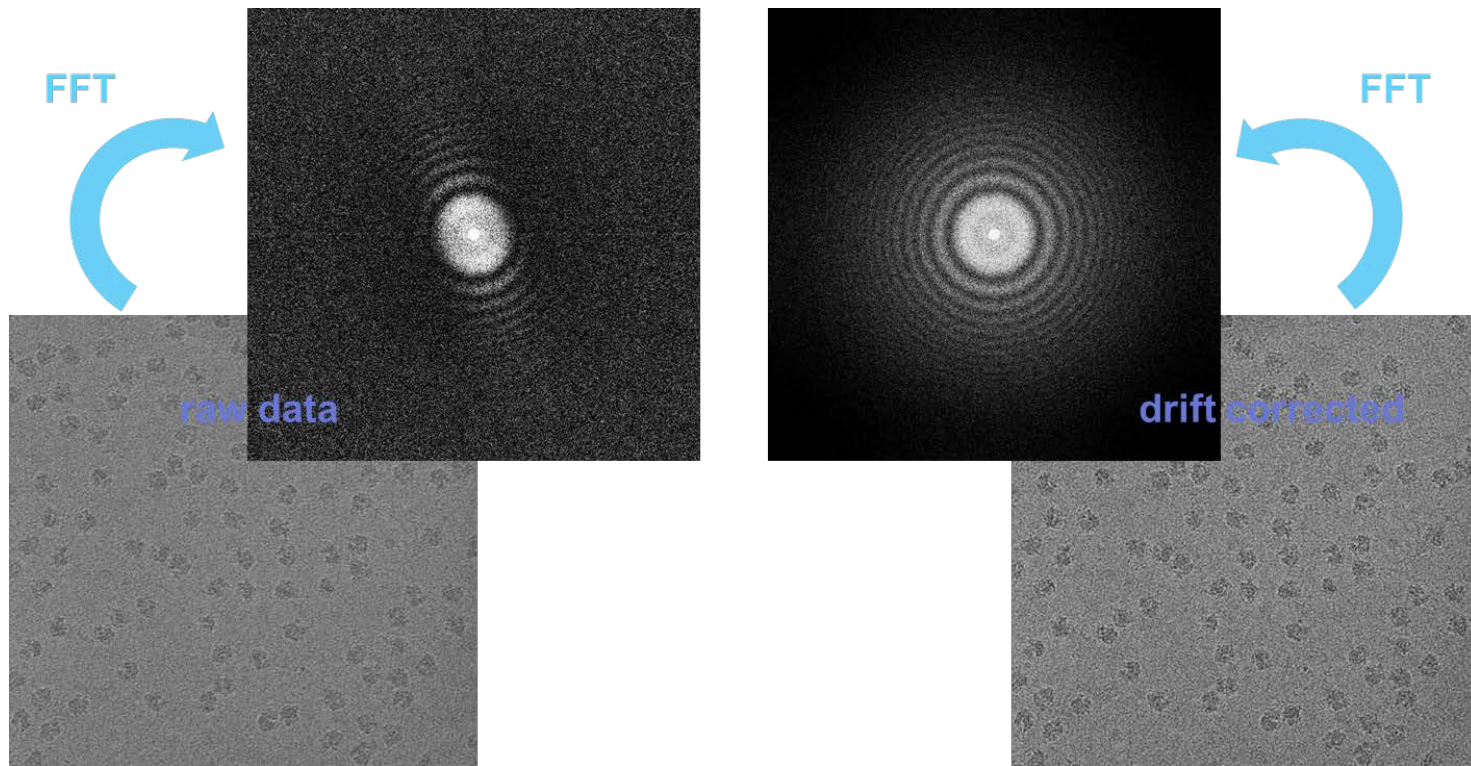
- Dose fractionation is the distribution of a total electron dose over a series of sub-frames

**without sub-frame drift correction**



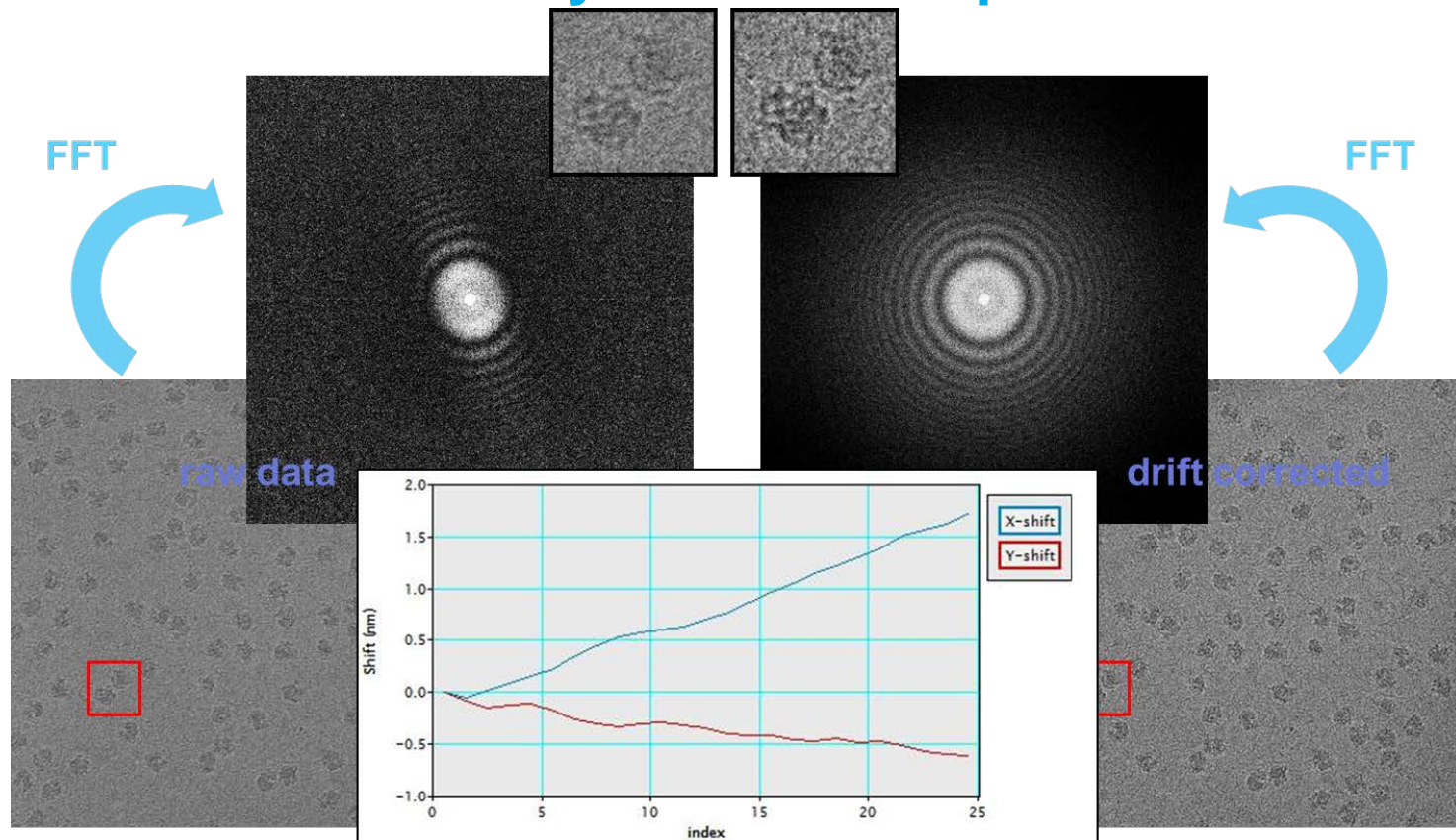
**with sub-frame drift correction**

# Drift correction: Cryo-TEM example of Ribosome

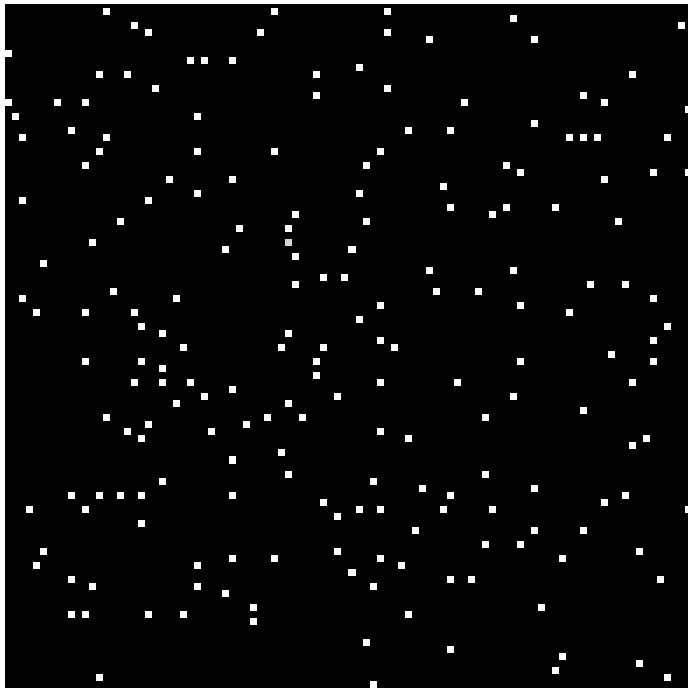




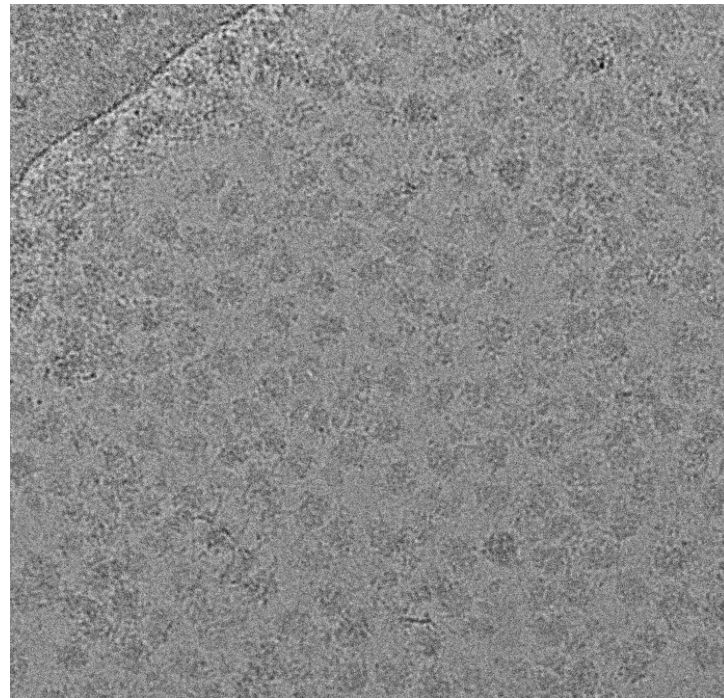
# Drift correction: Cryo-TEM example of Ribosome



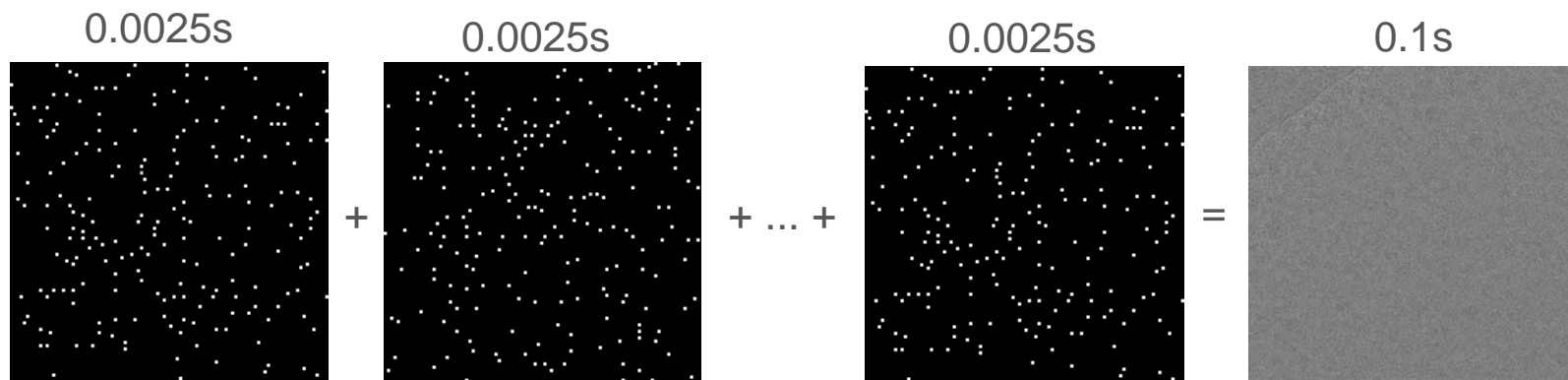
## How frame alignment works.



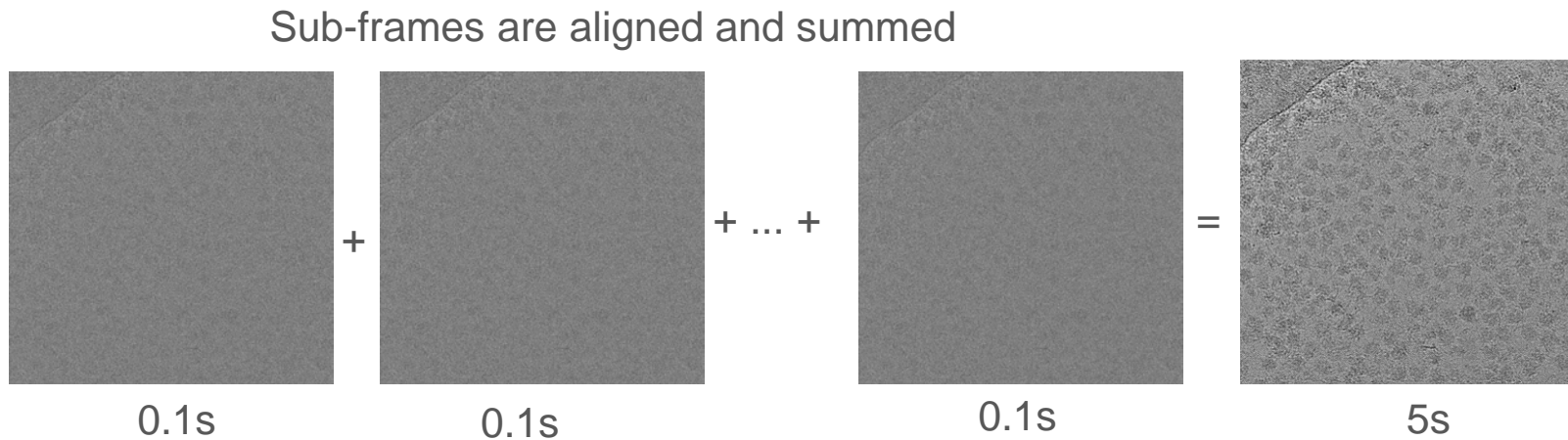
Raw counted frame



Final aligned image



Raw counted frames are summed





# Slower frame rates mean you cannot correct fast sample movement

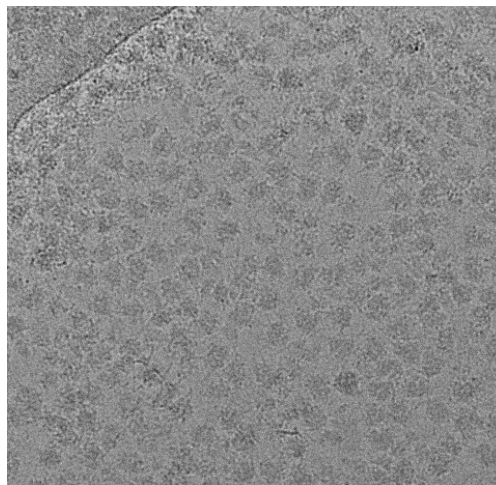
Counting frame rate	400	100	30
Dose rate (e/pix/sec)	10	2.5	1
Total exposure time (sec)	3	12	40
Sub-frame exposure time (sec)	0.1	0.4	1



Very slow drift correction



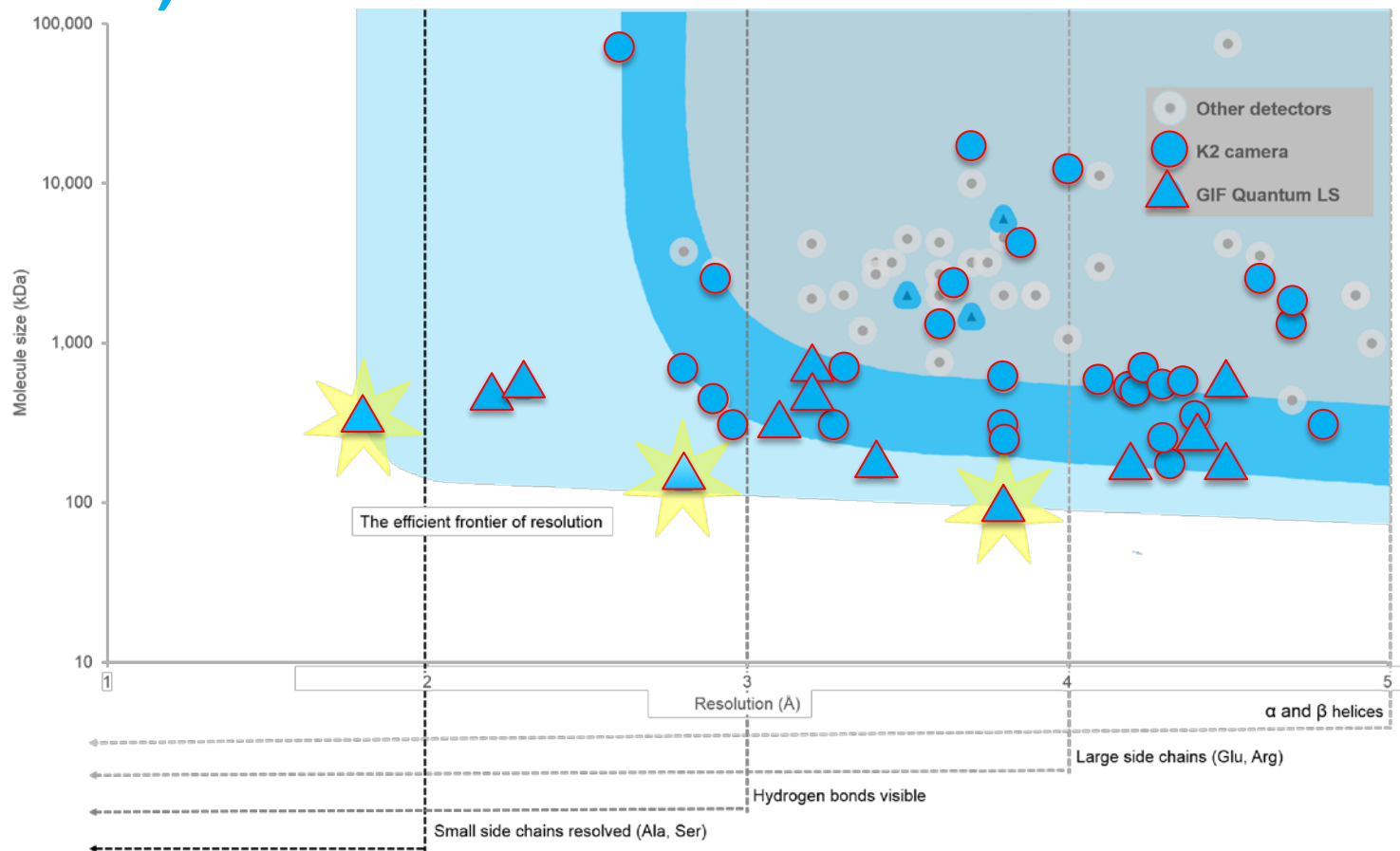
Raw frame



Final aligned frame

Raw counted frames contain only event information they must be accumulated to be aligned

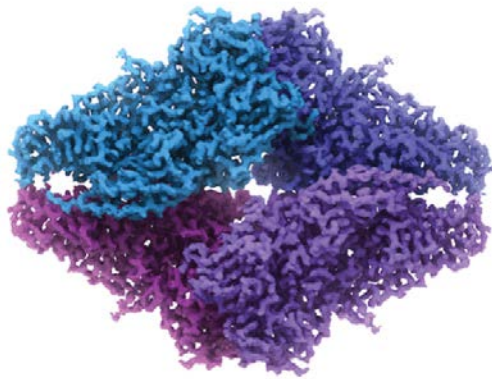
# Breaking the 2 Å and 100 kDa barrier (May 2016)





# High contrast imaging is essential to high resolution

- “improved image contrast and maximization of amplitudes at low resolution [allowed] us to go closer to focus and still correctly pick particles”



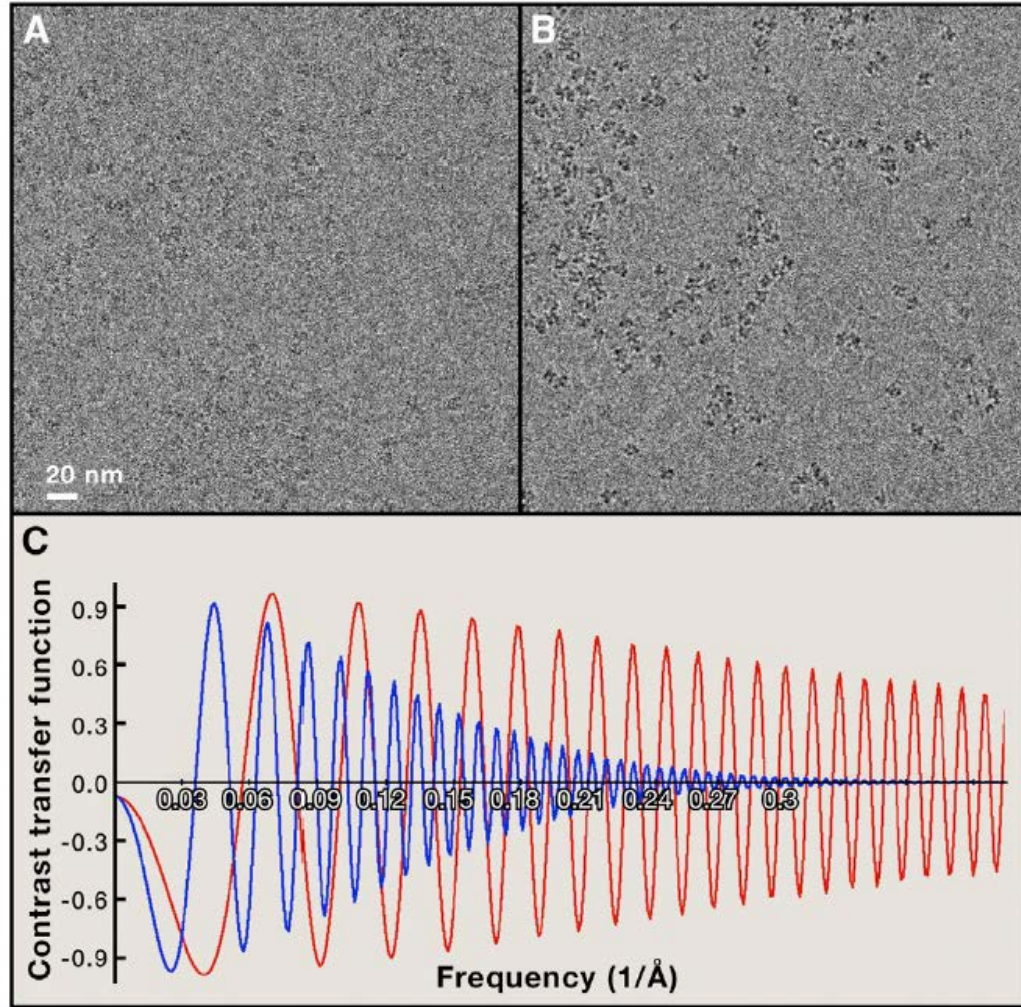
## 2.2 Å resolution cryo-EM structure of $\beta$ -galactosidase in complex with a cell-permeant inhibitor

**Alberto Bartesaghi,<sup>1\*</sup> Alan Merk,<sup>1\*</sup> Soojay Banerjee,<sup>1</sup> Doreen Matthies,<sup>1</sup> Xiongwu Wu,<sup>2</sup> Jacqueline L. S. Milne,<sup>1</sup> Sriram Subramaniam<sup>1†</sup>**

<sup>1</sup>Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA. <sup>2</sup>Laboratory of Computational Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA.

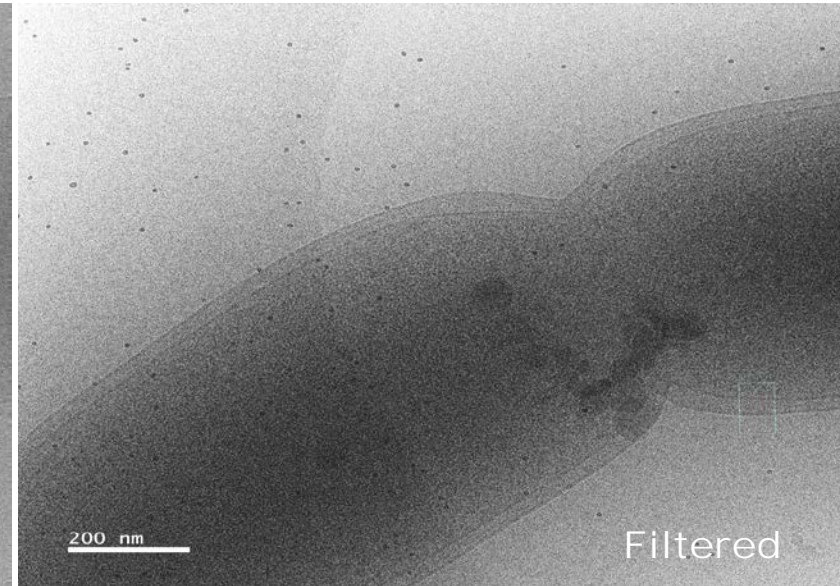
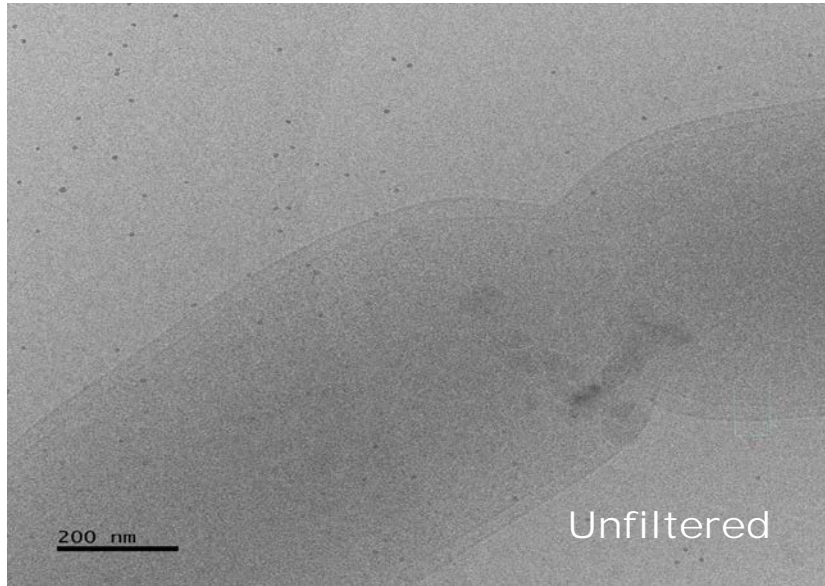
## Contrast and focus

In cryo-EM (phase contrast TEM) an image in focus means that you can't see the sample!





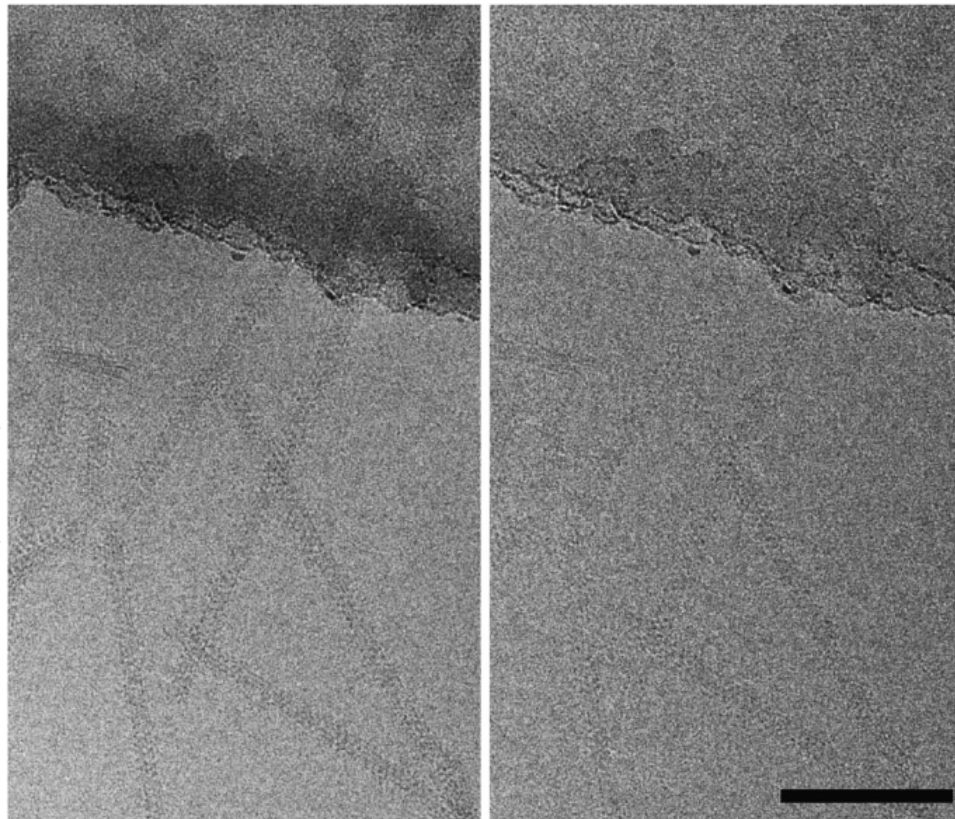
# Energy filters improves contrast for imaging low dose specimens



It is widely accepted that if you are doing cryo-tomography, you must have an energy filter

# Energy filtering improves image contrast even for (small) particles

- Filtered Image (left)
- Unfiltered Image (right)



Journal of Structural Biology

Volume 156, Issue 3, December 2006, Pages 524–536



Electron energy filtering significantly improves amplitude contrast of frozen-hydrated protein at 300 kV

Koji Yonekura<sup>a</sup>,  , Michael B. Braunfeld<sup>b</sup>, Saori Maki-Yonekura<sup>a, c</sup>, David A. Agard<sup>b, d</sup>

# Energy Filters Improve Contrast

## Small Particles

Single-particle cryo-EM benefits from an energy filter.



Journal of Structural Biology

Volume 156, Issue 3, December 2006, Pages 524–536



Electron energy filtering significantly improves amplitude contrast of frozen-hydrated protein at 300 kV

Koji Yonekura<sup>a,\*</sup>, Michael B. Braunfeld<sup>b</sup>, Saori Maki-Yonekura<sup>a,c</sup>, David A. Agard<sup>b,d</sup>

- Red = Filtered Images
- Blue = Unfiltered Images
- Few % improvement in contrast even with TMV

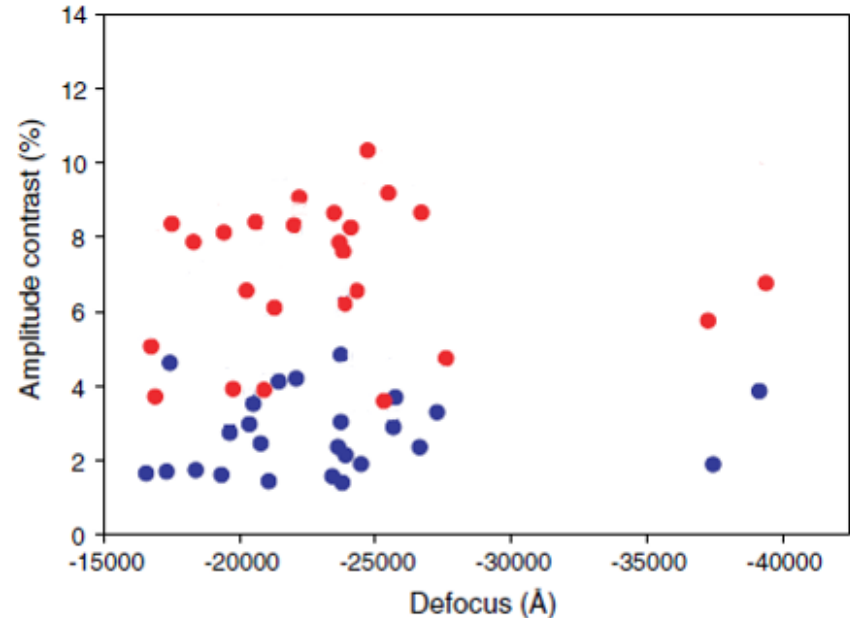
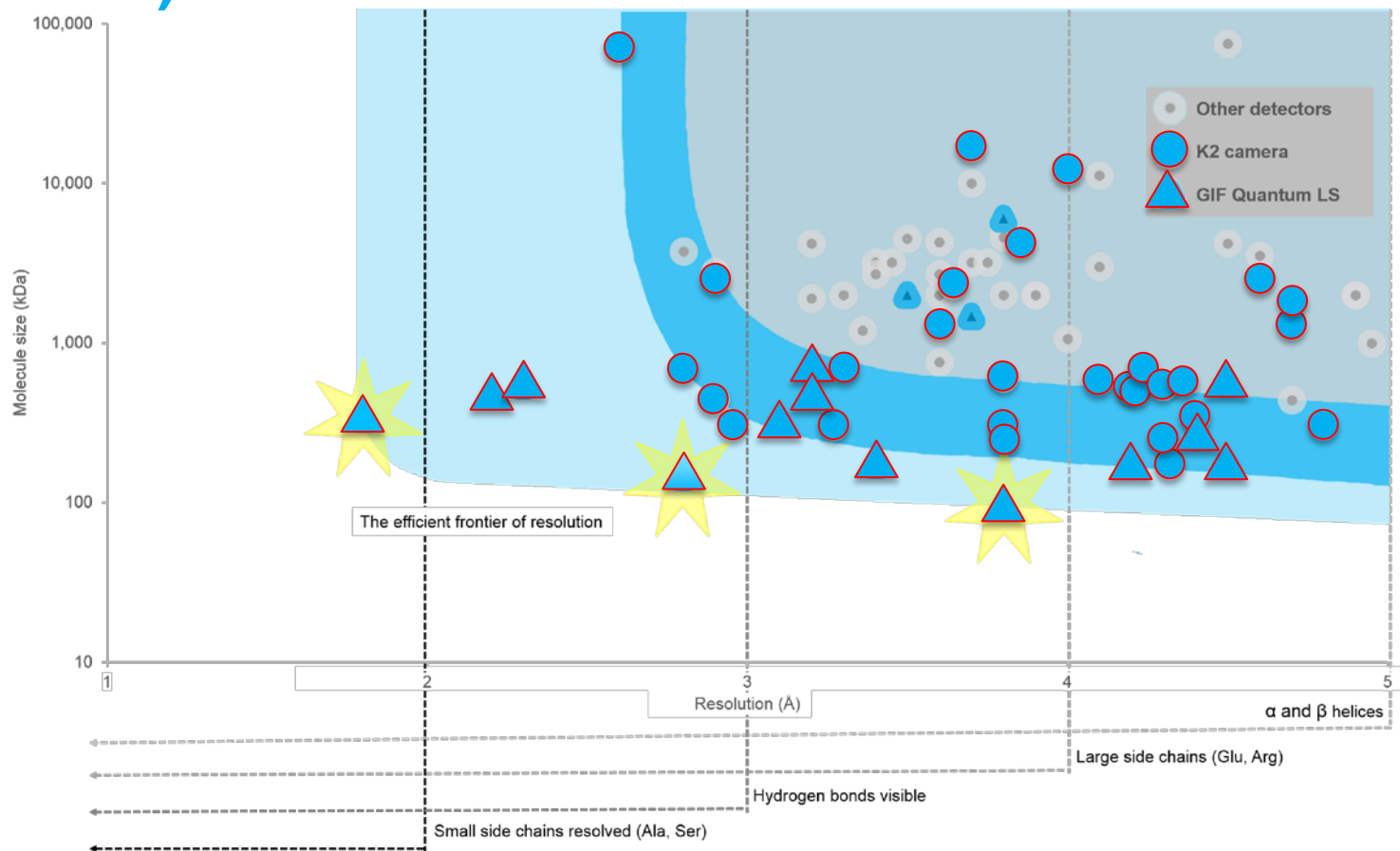


Fig. 7. Distribution of the amplitude contrast from flagellar filaments or the carbon film plotted against defocus. Red and blue symbols represent filtered and unfiltered data, respectively, recorded in either the 1st or 2nd exposure, and filled and open symbols indicate the filaments or the carbon film, respectively.



# Breaking the 2 Å and 100 kDa barrier (May 2016)



# The best camera now, and in the future...

- Make your K2 camera “future proof” between now and the end of 2016



K3?

## Latitude-S: Next step in automation software

- Improved ease of use
  - Better integration of microscope and camera
- Flexibility
  - Avoiding the pitfalls of “guided automation”
  - Scripting is supported
- Improved system stability for a wide range of microscopes
  - (Not only supported on Titan Krios)
- Improved stability for longer continuous runs
- Comprehensive status, tracking and logging of progress

# Latitude-S: Improving ease of use

**Guided, but not rigid workflow**

**Straightforward menu sequence, yet flexible choice of setup order, checked by built in conditionals.**

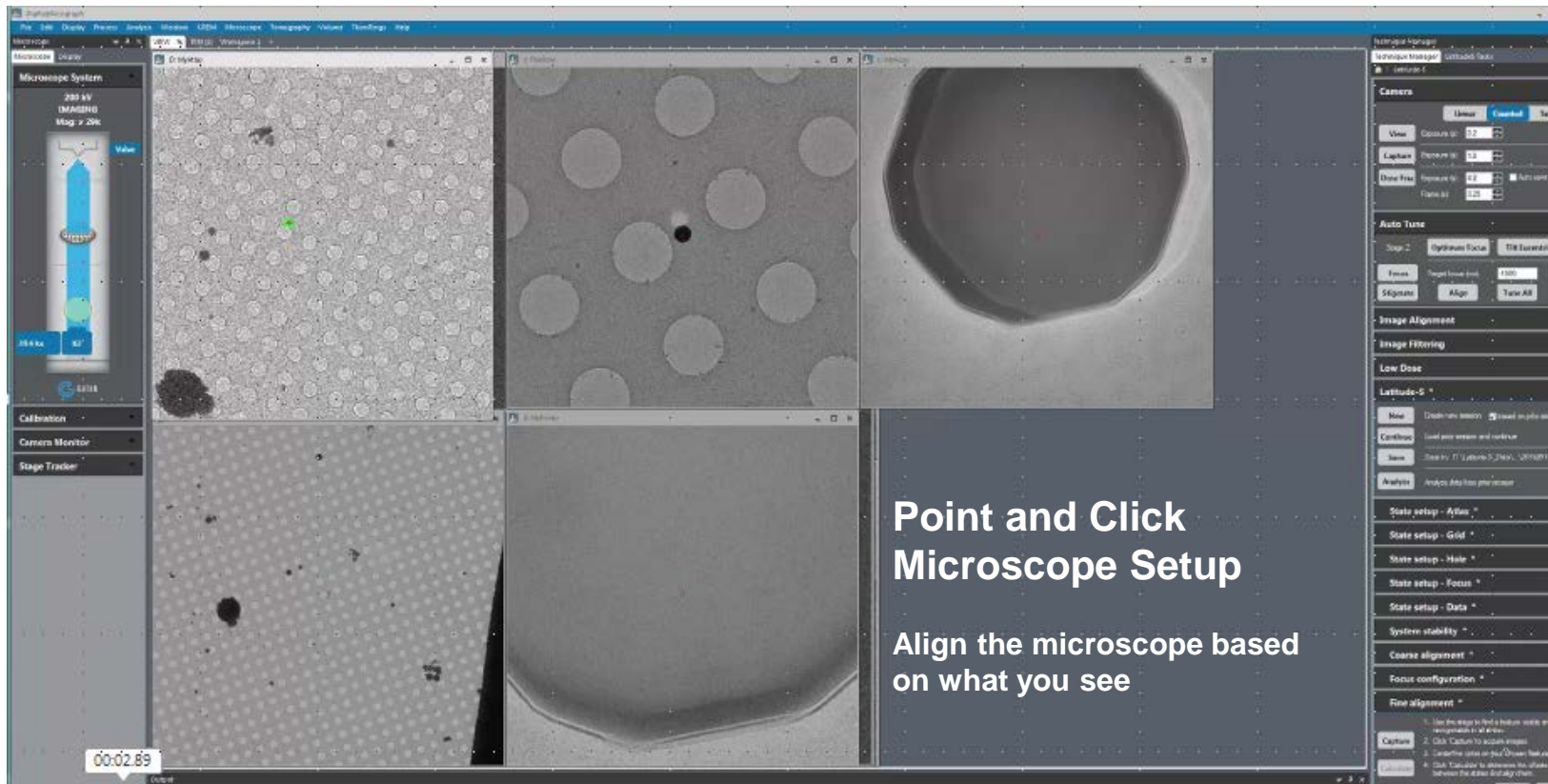
**Output**

```
07/06/2016, 14:28:17 Start Tuning Stage Z with optimum focus
Changing stage Z by: 0.0 µm
07/06/2016, 14:28:40 End Tuning Stage Z with optimum focus
- Z-change to reach optimum focus height: 0.02796 µm, optimum focus height at: 0.
07/06/2016, 14:29:06 Start Tuning Stage Z with optimum focus
Changing stage Z by: 0.0 µm
07/06/2016, 14:29:29 End Tuning Stage Z with optimum focus
- Z-change to reach optimum focus height: 0.02796 µm, optimum focus height at: 0.
```





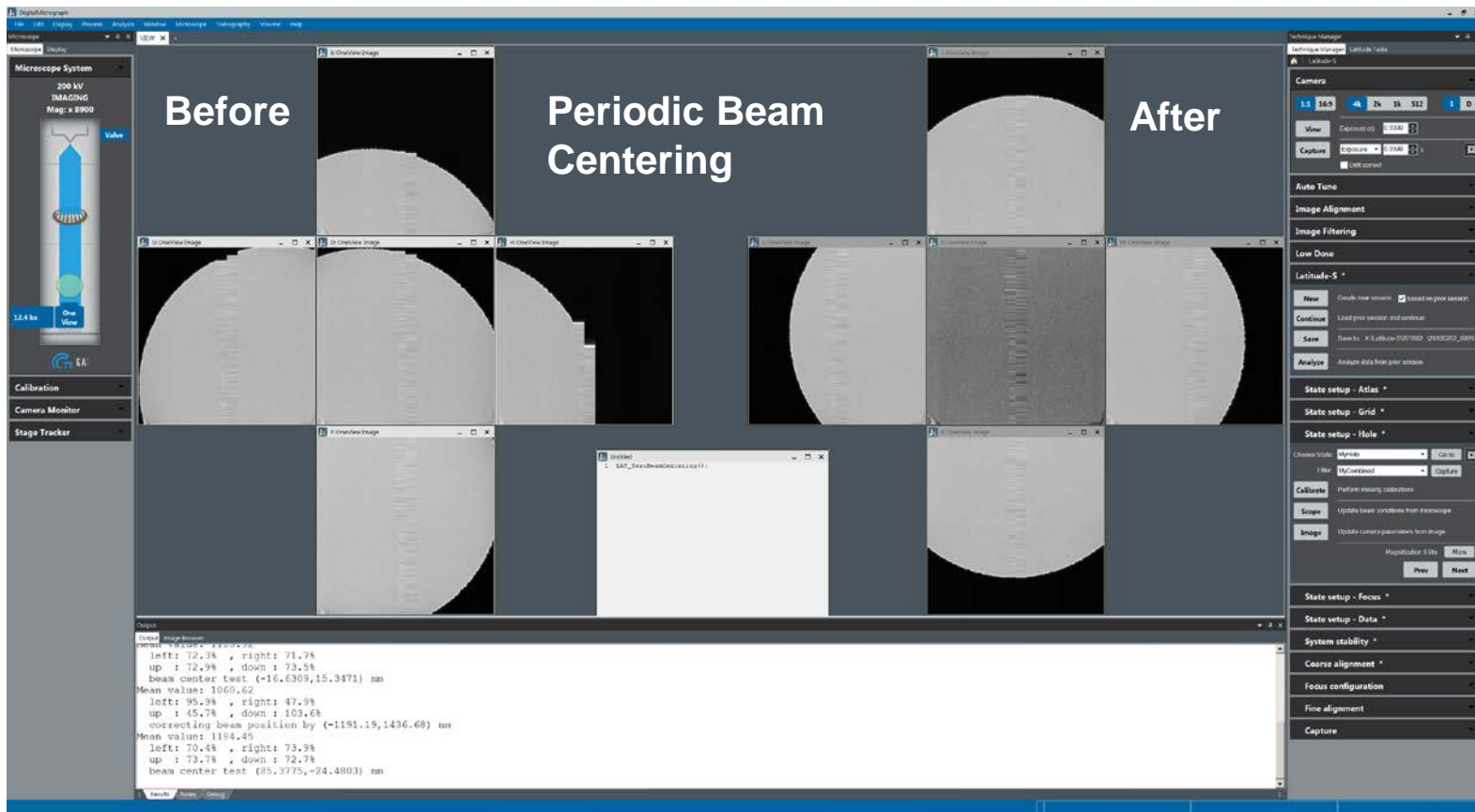
# Latitude-S: Improving ease of use



**Point and Click  
Microscope Setup**

Align the microscope based  
on what you see

# Latitude-S: Improving System Stability To support more microscopes



# Latitude-S: Comprehensive control of the automated acquisition

The screenshot displays the Latitude-S software interface, which is used for controlling automated acquisition. The interface is divided into several panels:

- Left Panel:** Contains 'Image Status' (showing image 4, 1024 x 1024), 'Display Control' (a histogram), 'Control' (buttons for 'Free', 'Next', 'Act', 'Stop'), and 'Image Info'.
- Top Center:** A large panel showing a grid of acquisition points (green dots) on a dark background. A scale bar indicates 2  $\mu\text{m}$ .
- Top Right:** A 'Summary' panel showing the 'Detailed status of Current Task'. It includes a 'Summary' table with columns for 'Name', 'Grid', 'Hole', 'Focus', and 'Data'. Below this is a 'Current Task' section with a 'Task' table.
- Bottom Center:** A 'Task schedule' panel showing a sequence of images (AFI, K2, OI, etc.) and a 'Current Task' section.
- Bottom Right:** A 'Current Task' panel showing a detailed task schedule table with columns for 'Name', 'Grid', 'Hole', 'Focus', 'Data', and 'Status'.

The 'Summary' table in the top right panel is as follows:

Name	Grid	Hole	Focus	Data
Unscheduled				
Scheduled	4	5	141	129

The 'Current Task' table in the bottom right panel is as follows:

Name	Grid	Hole	Focus	Data	Status
0000	0000	0000	0000	0000	Finished
0001	0001	0001	0001	0001	Finished
0002	0002	0002	0002	0002	Finished
0003	0003	0003	0003	0003	Finished
0004	0004	0004	0004	0004	Finished
0005	0005	0005	0005	0005	Finished
0006	0006	0006	0006	0006	Finished
0007	0007	0007	0007	0007	Finished
0008	0008	0008	0008	0008	Finished
0009	0009	0009	0009	0009	Finished
0010	0010	0010	0010	0010	Finished
0011	0011	0011	0011	0011	Finished
0012	0012	0012	0012	0012	Finished
0013	0013	0013	0013	0013	Finished
0014	0014	0014	0014	0014	Finished
0015	0015	0015	0015	0015	Finished
0016	0016	0016	0016	0016	Finished
0017	0017	0017	0017	0017	Finished
0018	0018	0018	0018	0018	Finished
0019	0019	0019	0019	0019	Finished
0020	0020	0020	0020	0020	Finished
0021	0021	0021	0021	0021	Finished
0022	0022	0022	0022	0022	Finished
0023	0023	0023	0023	0023	Finished
0024	0024	0024	0024	0024	Finished
0025	0025	0025	0025	0025	Finished
0026	0026	0026	0026	0026	Finished
0027	0027	0027	0027	0027	Finished
0028	0028	0028	0028	0028	Finished
0029	0029	0029	0029	0029	Finished
0030	0030	0030	0030	0030	Finished
0031	0031	0031	0031	0031	Finished
0032	0032	0032	0032	0032	Finished
0033	0033	0033	0033	0033	Finished
0034	0034	0034	0034	0034	Finished
0035	0035	0035	0035	0035	Finished
0036	0036	0036	0036	0036	Finished
0037	0037	0037	0037	0037	Finished
0038	0038	0038	0038	0038	Finished
0039	0039	0039	0039	0039	Finished
0040	0040	0040	0040	0040	Finished
0041	0041	0041	0041	0041	Finished
0042	0042	0042	0042	0042	Finished
0043	0043	0043	0043	0043	Finished
0044	0044	0044	0044	0044	Finished
0045	0045	0045	0045	0045	Finished
0046	0046	0046	0046	0046	Finished
0047	0047	0047	0047	0047	Finished
0048	0048	0048	0048	0048	Finished
0049	0049	0049	0049	0049	Finished
0050	0050	0050	0050	0050	Finished
0051	0051	0051	0051	0051	Finished
0052	0052	0052	0052	0052	Finished
0053	0053	0053	0053	0053	Finished
0054	0054	0054	0054	0054	Finished
0055	0055	0055	0055	0055	Finished
0056	0056	0056	0056	0056	Finished
0057	0057	0057	0057	0057	Finished
0058	0058	0058	0058	0058	Finished
0059	0059	0059	0059	0059	Finished
0060	0060	0060	0060	0060	Finished
0061	0061	0061	0061	0061	Finished
0062	0062	0062	0062	0062	Finished
0063	0063	0063	0063	0063	Finished
0064	0064	0064	0064	0064	Finished
0065	0065	0065	0065	0065	Finished
0066	0066	0066	0066	0066	Finished
0067	0067	0067	0067	0067	Finished
0068	0068	0068	0068	0068	Finished
0069	0069	0069	0069	0069	Finished
0070	0070	0070	0070	0070	Finished
0071	0071	0071	0071	0071	Finished
0072	0072	0072	0072	0072	Finished
0073	0073	0073	0073	0073	Finished
0074	0074	0074	0074	0074	Finished
0075	0075	0075	0075	0075	Finished
0076	0076	0076	0076	0076	Finished
0077	0077	0077	0077	0077	Finished
0078	0078	0078	0078	0078	Finished
0079	0079	0079	0079	0079	Finished
0080	0080	0080	0080	0080	Finished
0081	0081	0081	0081	0081	Finished
0082	0082	0082	0082	0082	Finished
0083	0083	0083	0083	0083	Finished
0084	0084	0084	0084	0084	Finished
0085	0085	0085	0085	0085	Finished
0086	0086	0086	0086	0086	Finished
0087	0087	0087	0087	0087	Finished
0088	0088	0088	0088	0088	Finished
0089	0089	0089	0089	0089	Finished
0090	0090	0090	0090	0090	Finished
0091	0091	0091	0091	0091	Finished
0092	0092	0092	0092	0092	Finished
0093	0093	0093	0093	0093	Finished
0094	0094	0094	0094	0094	Finished
0095	0095	0095	0095	0095	Finished
0096	0096	0096	0096	0096	Finished
0097	0097	0097	0097	0097	Finished
0098	0098	0098	0098	0098	Finished
0099	0099	0099	0099	0099	Finished
0100	0100	0100	0100	0100	Finished



120 kV  
IMAGING  
Mag: x 11k

Valve

14.5 kV US4000

GATAN

Output

Beam tilt Calibration, rad/gad:  
Calibrated magnification: 85618  
Image rotation wrt beam tilt X  
Beam tilt, rad: 0.01  
Measured displacement, pix: 1.6  
Measured detector plane displa  
Measured object plane displac  
Measured defocus: -49.6 nm  
Measured defocus accuracy: 35.4  
03/22/2016, 14:07:59 End Meas

Latitude-S Single Particle Acquisition

Atlas 0000: MyAtlas

Select Add Position Remove Position

Dose: 879.6counts/pixel

Schedule

Prev Next

Grid 0001: MyGrid

Add Position Add Template Remove Position

Dose: 1071.3counts/nixel

Schedule

Prev Next

Hole 0837: TheHole

Select Add Position Remove Position

Dose: 2163.1counts/pixel

Schedule

Prev Next

Save as Template

Informative color coding:

- Selected
- Scheduled
- Finished
- Failed

### Summary

	Atlas	Grid	Hole	Focus	Data
Unscheduled			129	129	129
Scheduled			110	110	111
Acquired	9	2	42	47	41

Time for unscheduled positions: 4 hours, 48 minutes  
Time remaining: 4 hours, 7 minutes

Schedule

### Current Task

Atlas 0000, Grid 0001, Hole:0837, Data:0001

Status	Details
Finished	move to (-160.1,-182.5) μm
Active	position refinement (stage)
Scheduled	position refinement 2 (stage)
Scheduled	acquisition

### Tasks

☒ Auto navigate to current task and acquired image

Show: ☒ Finished Tasks ☒ Scheduled Tasks

Atlas	Grid	Hole	Data	Status	Duration (s)
0000	0001	0829	0001	Finished	63.7
0000	0001	0830		Finished	26.3
0000	0001	0830	Focus	Finished	38.1
0000	0001	0830	0001	Finished	63.6
0000	0001	0831		Finished	26.7
0000	0001	0831	Focus	Finished	38.3
0000	0001	0831	0001	Finished	64.0
0000	0001	0832		Finished	26.2
0000	0001	0832	Focus	Finished	40.3
0000	0001	0832	0001	Finished	63.6
0000	0001	0833		Finished	26.0
0000	0001	0833	Focus	Finished	37.9
0000	0001	0833	0001	Finished	64.1
0000	0001	0834		Finished	26.2
0000	0001	0834	Focus	Finished	38.4
0000	0001	0834	0001	Finished	63.9
0000	0001	0835		Finished	26.2
0000	0001	0835	Focus	Finished	38.4
0000	0001	0835	0001	Finished	63.7
0000	0001	0836		Finished	26.1
0000	0001	0836	Focus	Finished	65.4
0000	0001	0836	0001	Finished	64.1
0000	0001	0837		Finished	26.3
0000	0001	0837	Focus	Finished	38.5
0000	0001	0837	0001	Active	



Thank You!

Questions?

