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

Christopher Booth

2016 July 11

CEM3DIP Workshop

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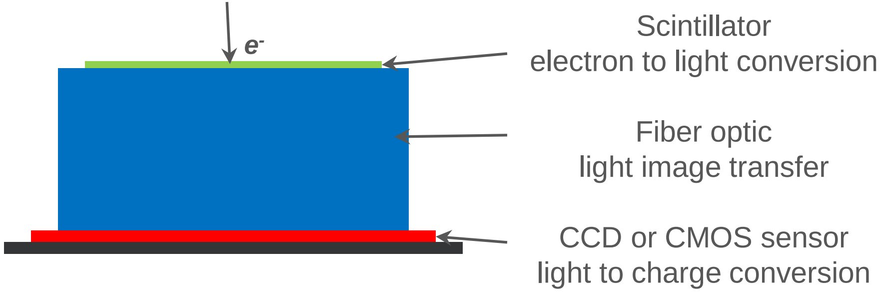
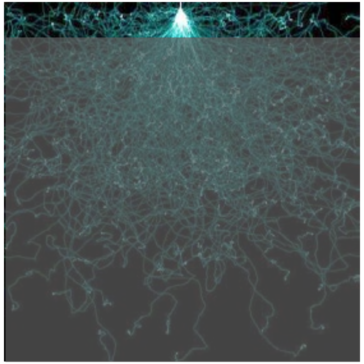
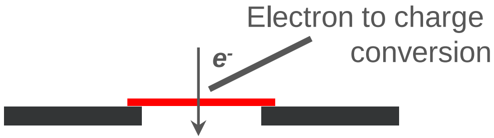
2 Types of Detectors

Fiber-coupled camera (OneView, Orius)





Direct detection camera (K2 Summit, K2-IS)



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OneView™ Camera
No compromises

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

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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

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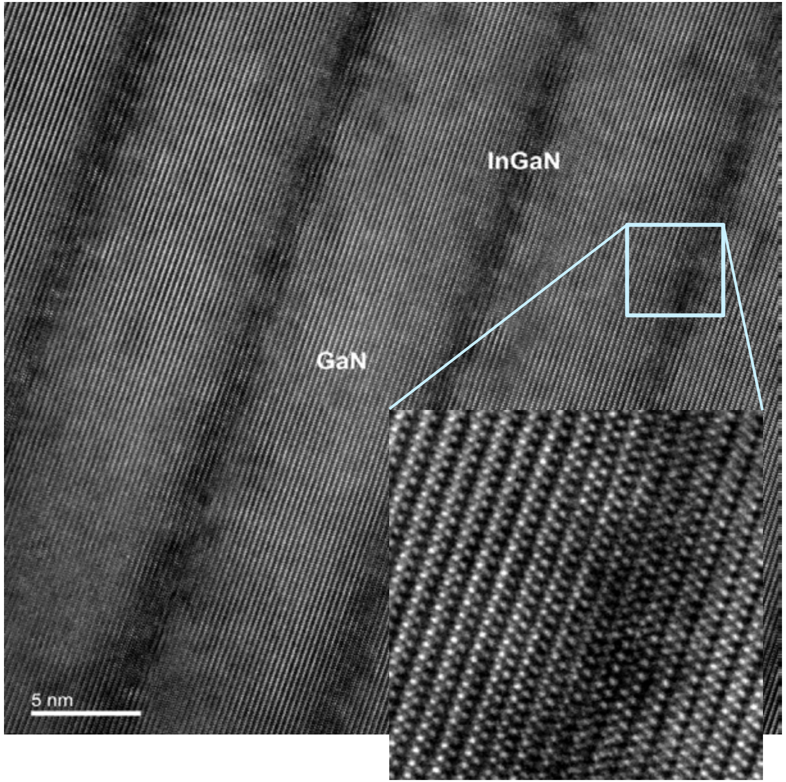
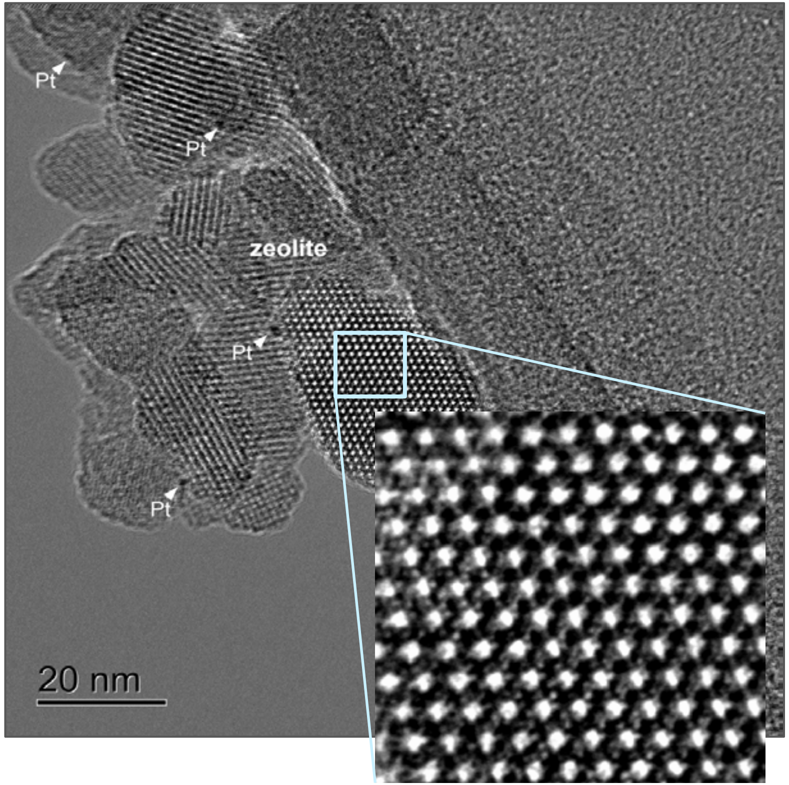
Material Science Applications

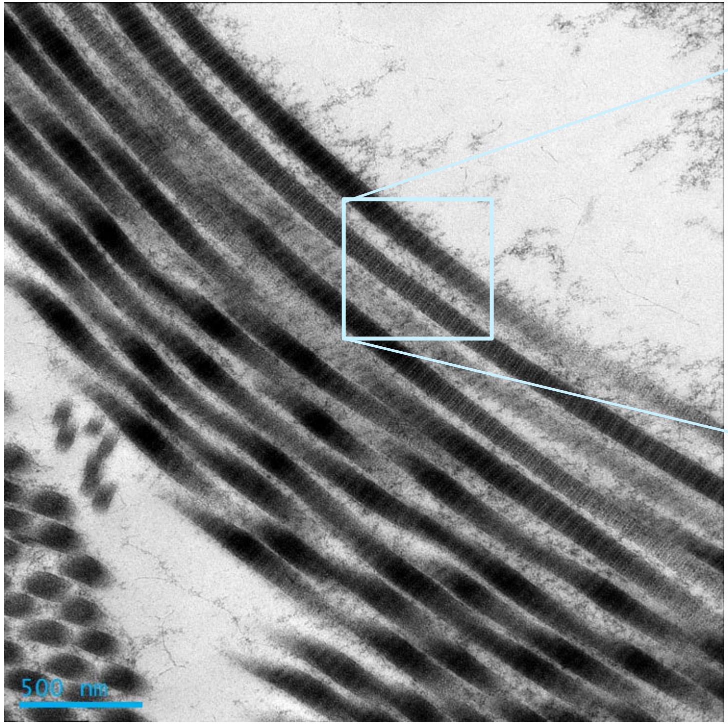
Semiconductors

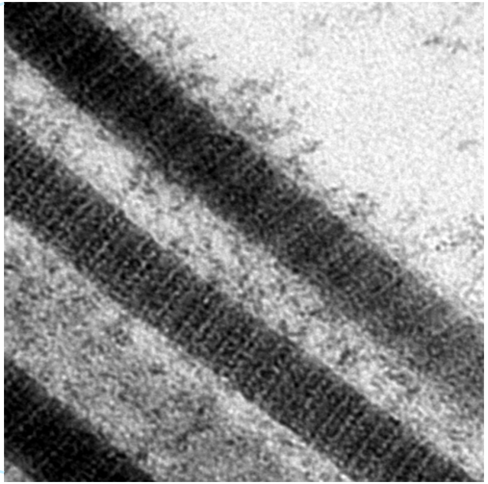


Catalyst (Zeolites)



Life Science Applications



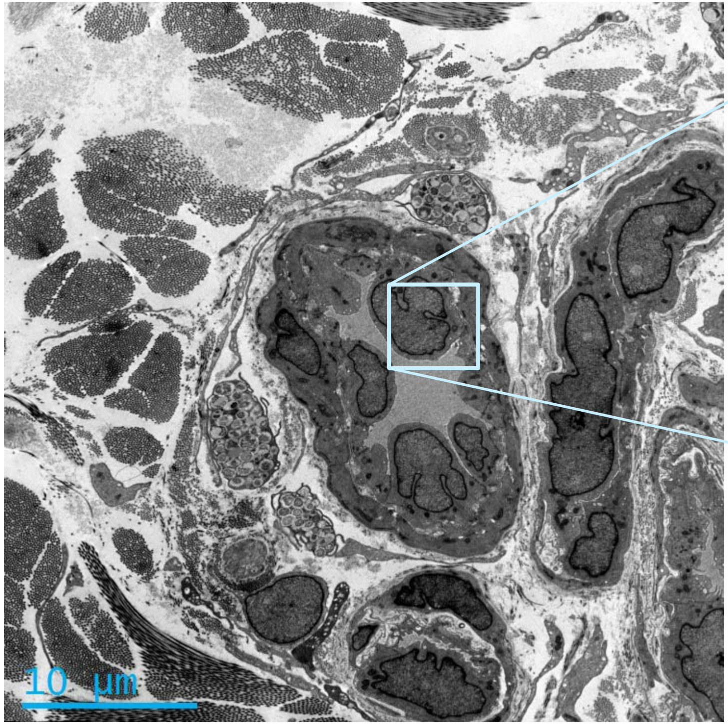


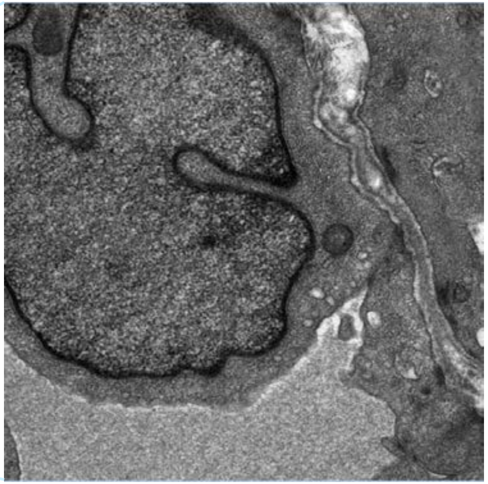
Collagen fibrils are clearly resolved in this 4k x 4k image.

OneView delivers high resolution and large FOV.

Image information: sample: collagen; beam energy: 120 kV; image size: 4k x 4k; exposure: 1 s; number of frames: 25.

Life Science Applications





Crisp detail revealed in high contrast imaging.

Image information: sample: skin; beam energy: 120 kV; image size: 4k x 4k; exposure: 1 s; number of frames: 25.

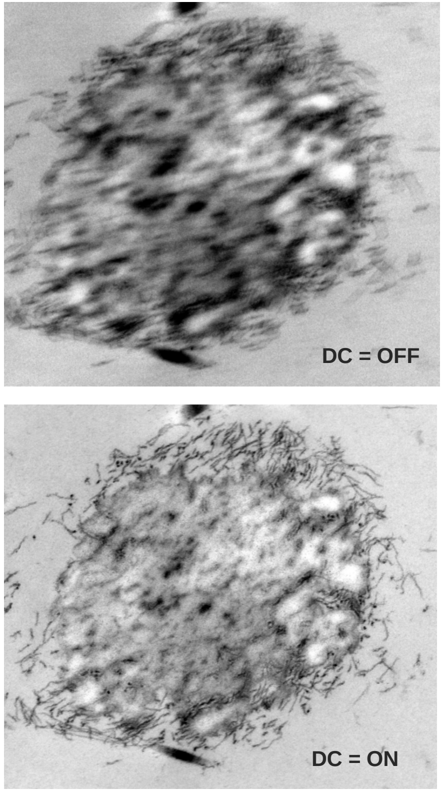
Live Drift Correction

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



Drift induced to showcase OneView camera ability

1 s exposure @ 25 fps (full 4k x 4k resolution)

Zeolite Catalyst Example

Some samples are too sensitive to image easily

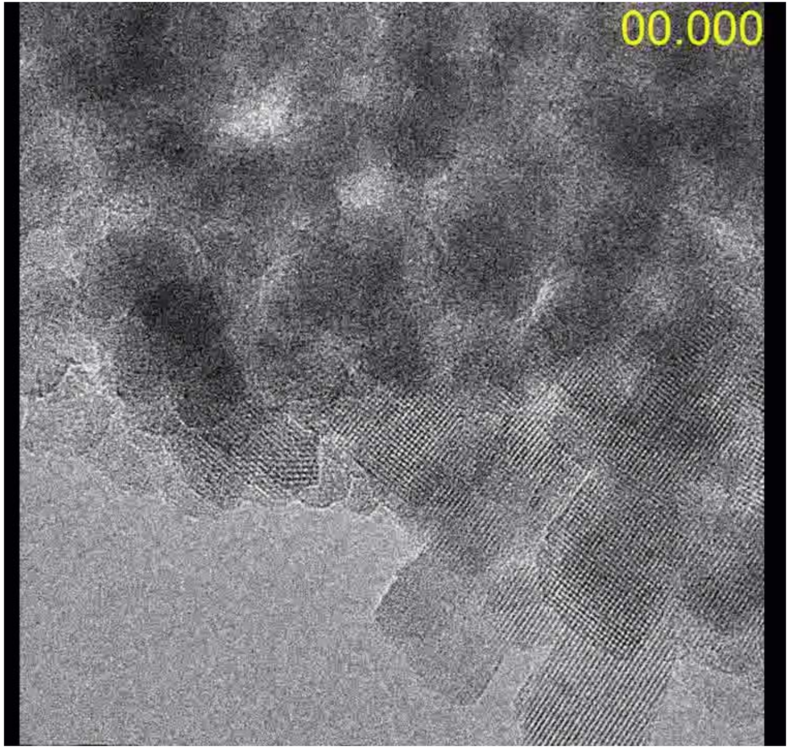
Unstable under e-beam

Radiation damage

- Sample drift
- Structure modification

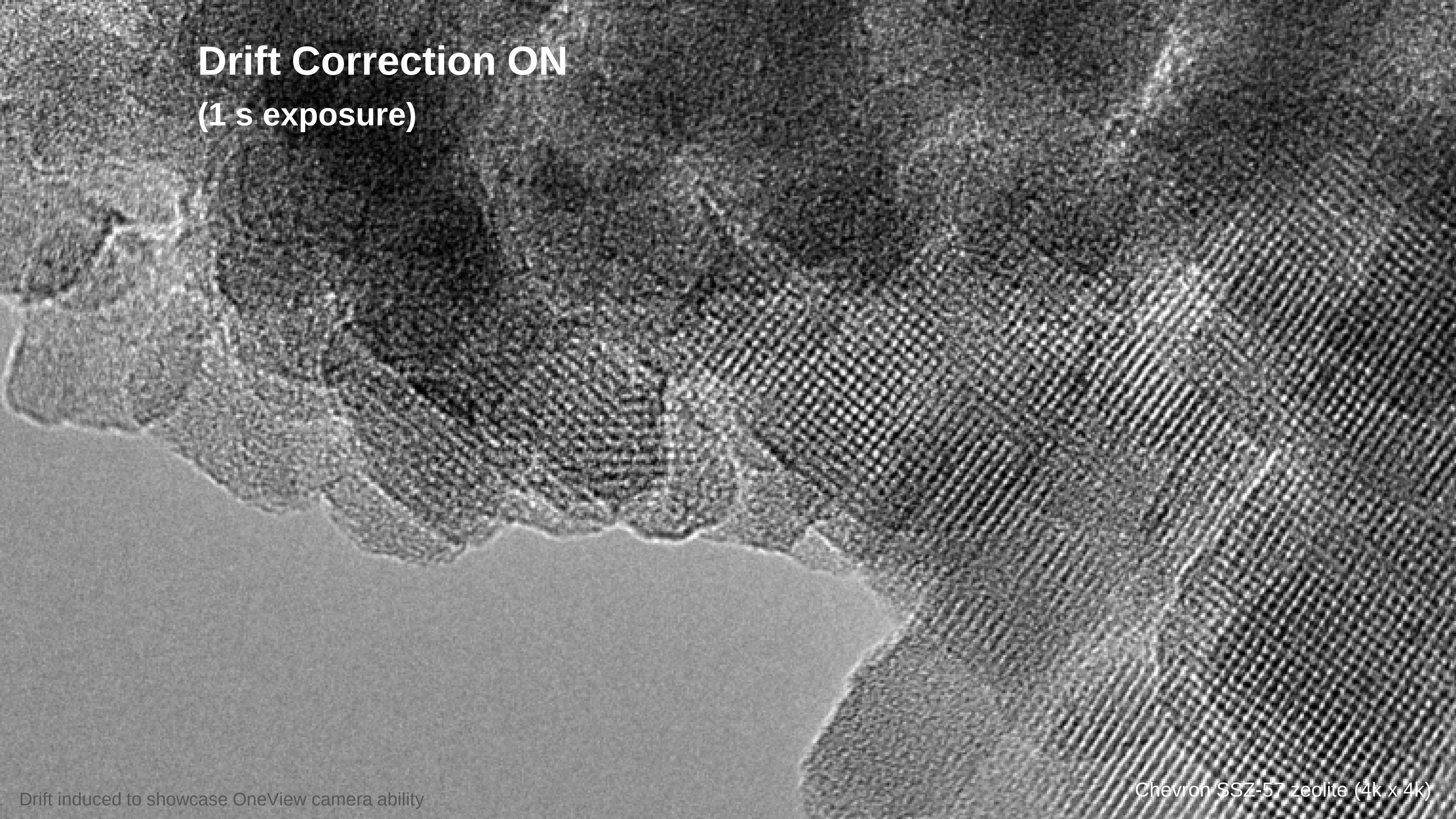
Stage drift

- Moving to a fresh area introduces mechanical stage drift



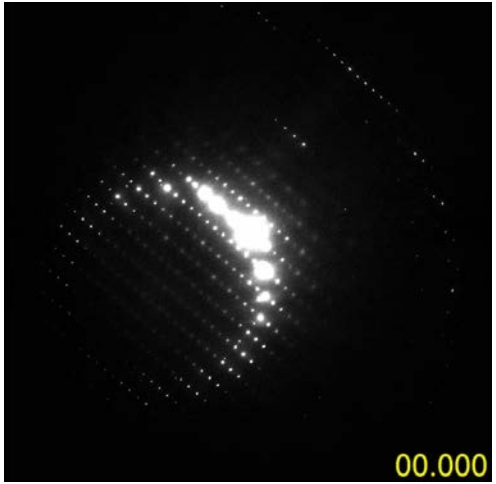
Chevron SSZ-57 zeolite (4k x 4k)


Drift Correction OFF
(1 s exposure)
Drift induced to showcase OneView camera ability
Chevron SSZ-57 zeolite (4k x 4k)

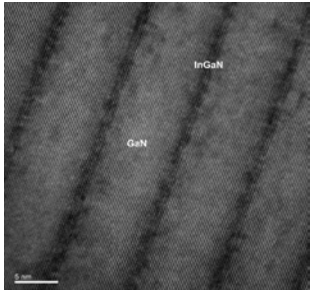

Drift Correction ON
(1 s exposure)
Drift induced to showcase OneView camera ability
Chevron SSZ-57 zeolite (4k x 4k)

CBED, Nanobeam Diffraction Studies

Parallel beam diffraction of GaN/InGaN

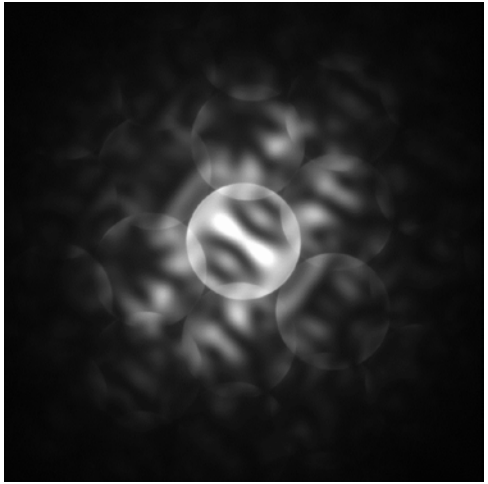


Record diffraction movies with optional *in-situ* mode



Bright field semiconductor image

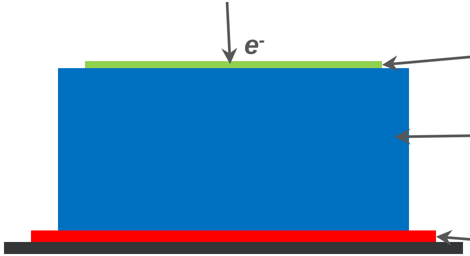
[110] CBED of GaN/InGaN



Synchronize frames for 4D STEM applications

2 Types of Detectors

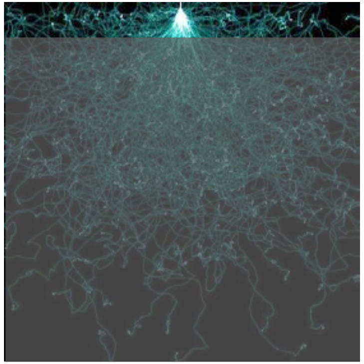
Fiber-coupled camera (OneView, Orius)



Scintillator
electron to light conversion

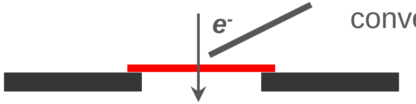
Fiber optic
light image transfer

CCD or CMOS sensor
light to charge conversion



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

Electron to charge conversion



K2 Summit and Quantum LS

Detectors Designed for Structural Biology

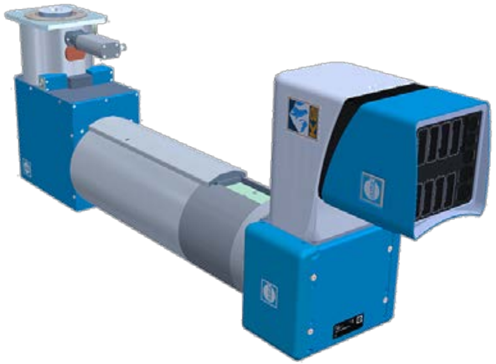
Detectors designed for structural biology

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



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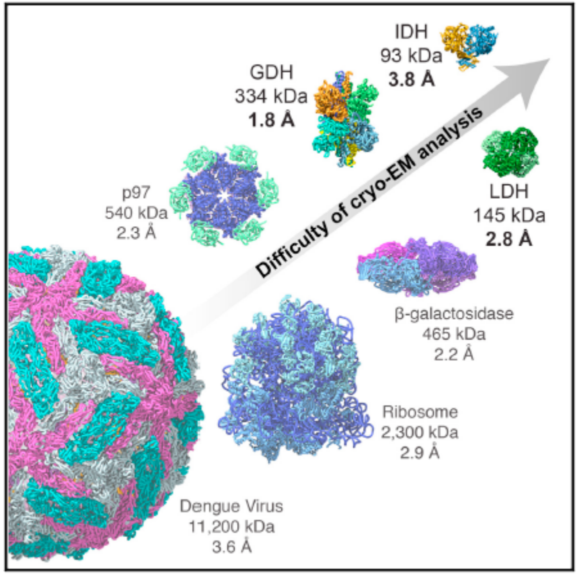
K2 Summit/Quantum LS. A record of firsts!

Article

Cell

Breaking Cryo-EM Resolution Barriers to Facilitate Drug Discovery

Graphical Abstract



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Correspondence

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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.

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

CryoEM at IUCrJ: a new era

Sriram Subramaniam,[a] Werner Kühlbrandt[b] and Richard Henderson[c]*

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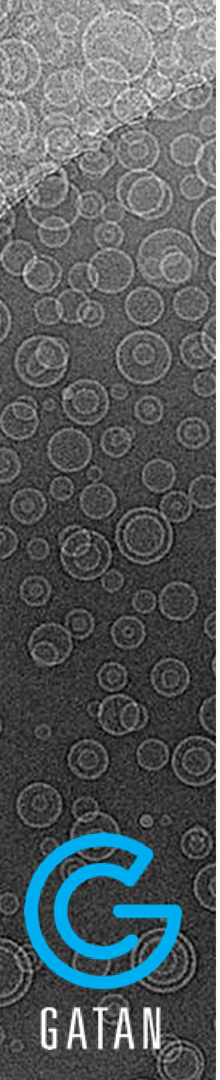
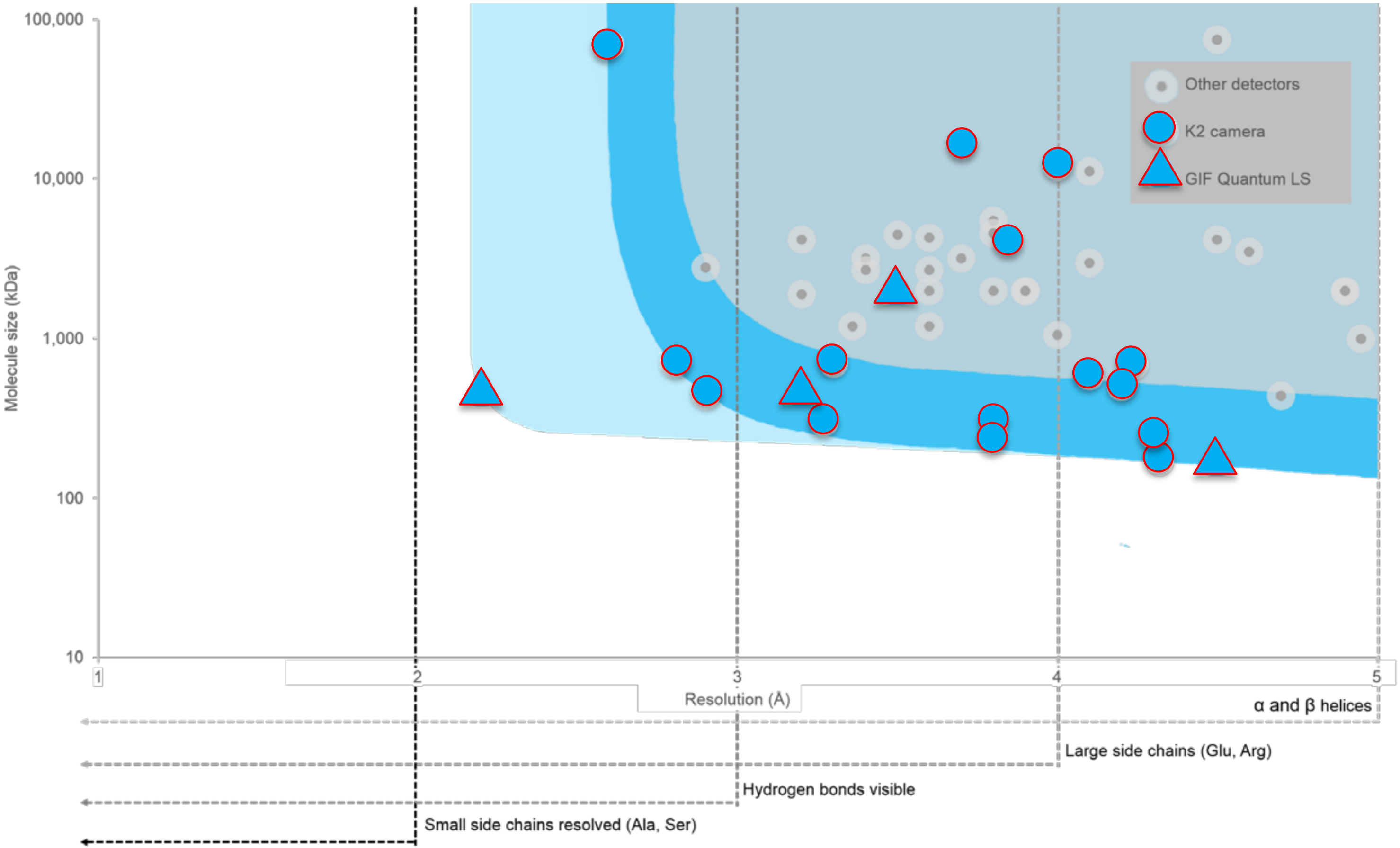
*Correspondence e-mail: rh15@mrc-lmb.cam.ac.uk



Breaking the 3 Å barrier… (May 2015)



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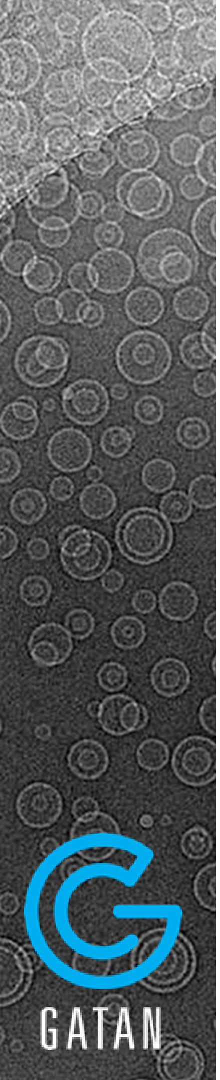
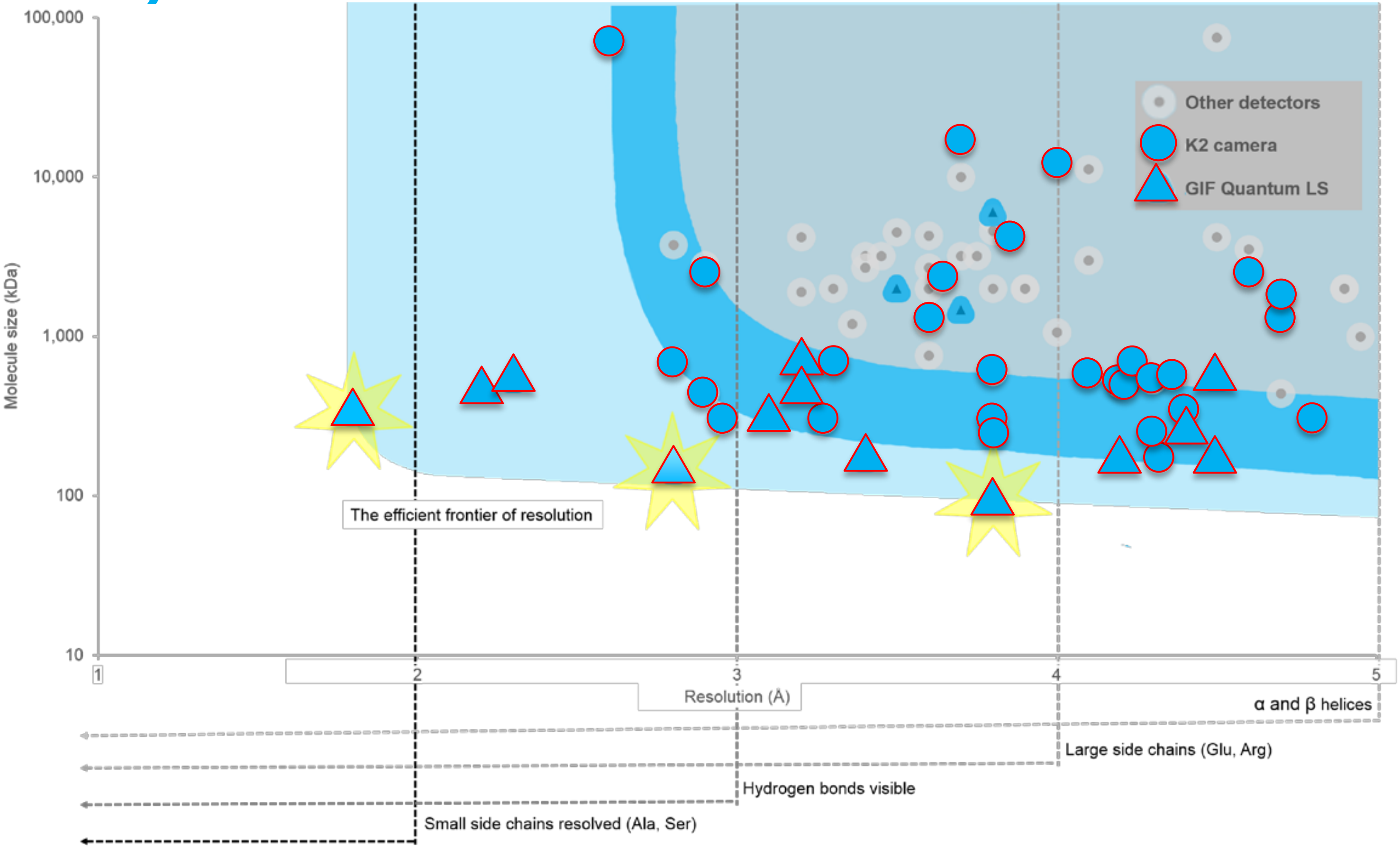
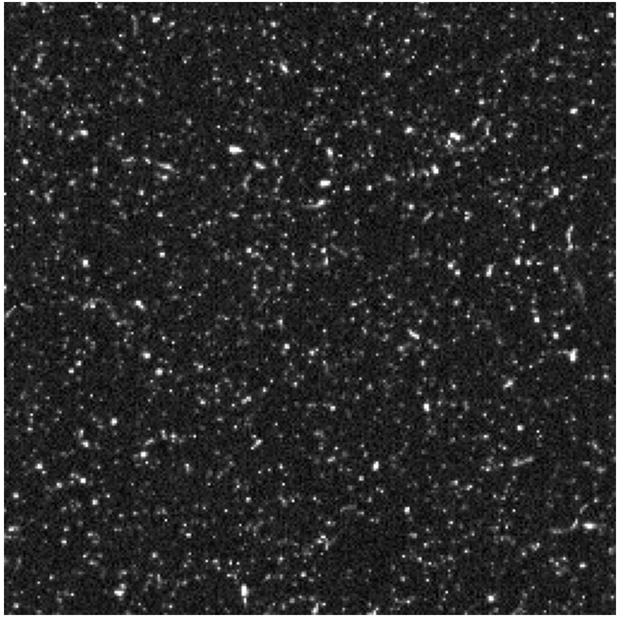
Breaking the 2 Å and 100 kDa barrier (May 2016)



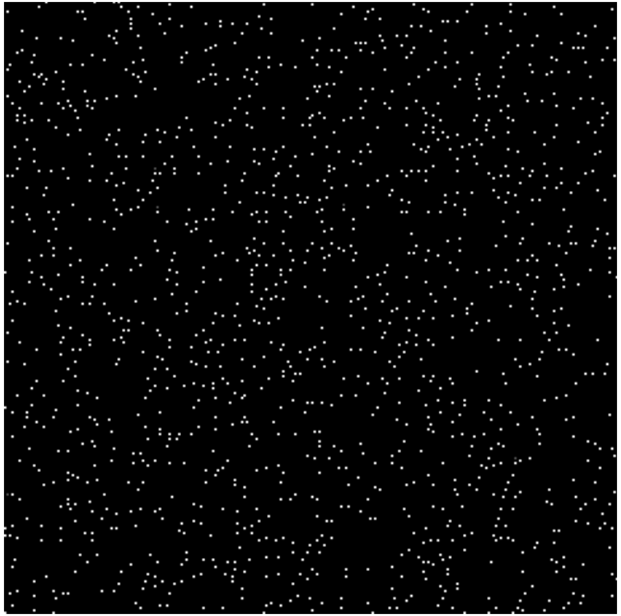


Electron Counting – Remove the remaining noise

Typical dose rate of 10 e^-/pix/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.

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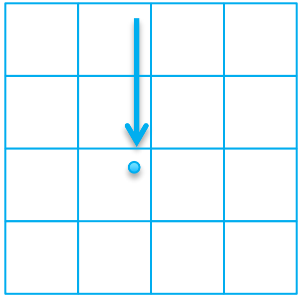
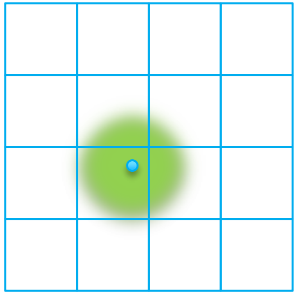
“Integration” or “linear mode”

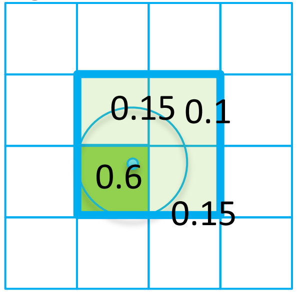
1. Electron enters detector



2. Signal is scattered



3. Charge collects in each pixel



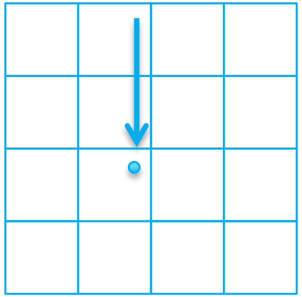
Stop at step 3:

Charge Integration

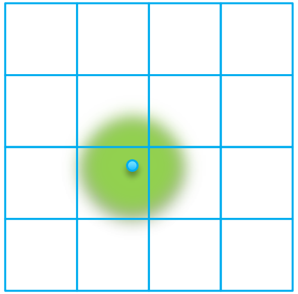
Improved DQE at high Frequency

Counting mode

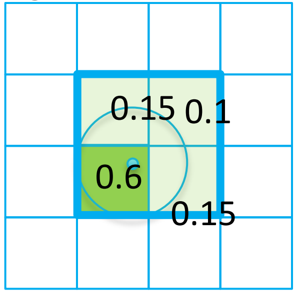
1. Electron enters detector



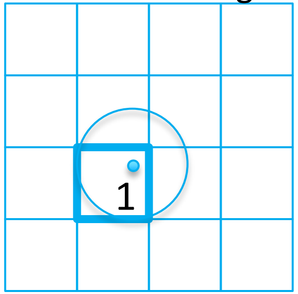
2. Signal is scattered



3. Charge collects in each pixel



4. Events are reduced to the highest charge pixels



Stop at step 3:
Charge Integration
Improved DQE at high Frequency

Continue to step 4:
Counting
Improved DQE at low AND high Frequency

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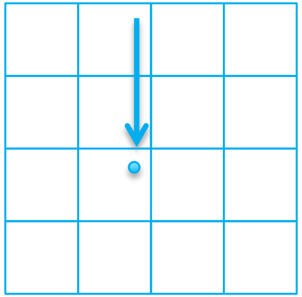
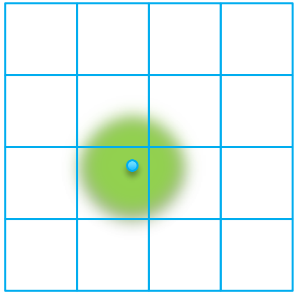
Super-resolution mode

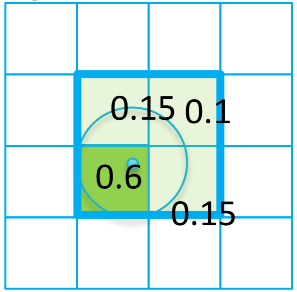
1. Electron enters detector



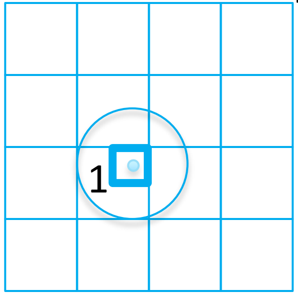
2. Signal is scattered



3. Charge collects in each pixel



4b. Events are localized with sub-pixel accuracy



Stop at step 3:
Charge Integration
Improved DQE at high Frequency

Continue to step 4:
Super-Resolution
Improved DQE at low AND high Frequency
and 7680 x 7424 pixels

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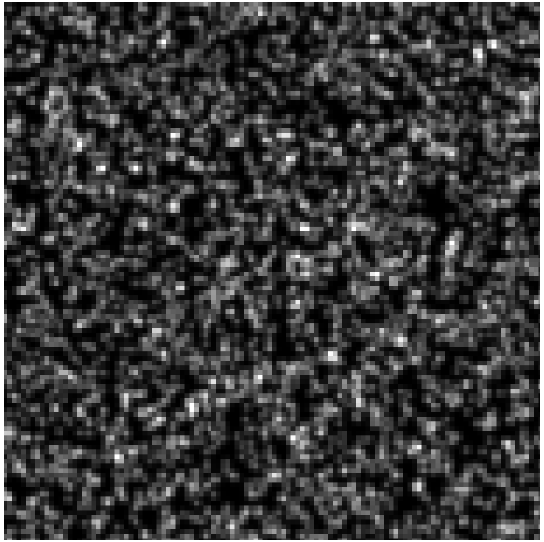
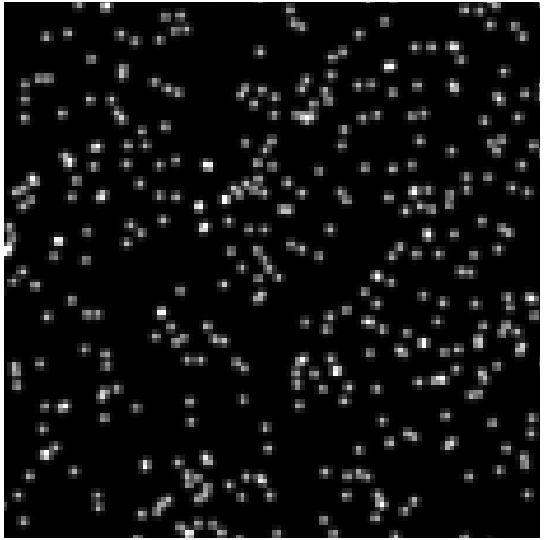
Electron counting requires high speed image readout

Typical dose rate of 10 e^-/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^-/pix/s

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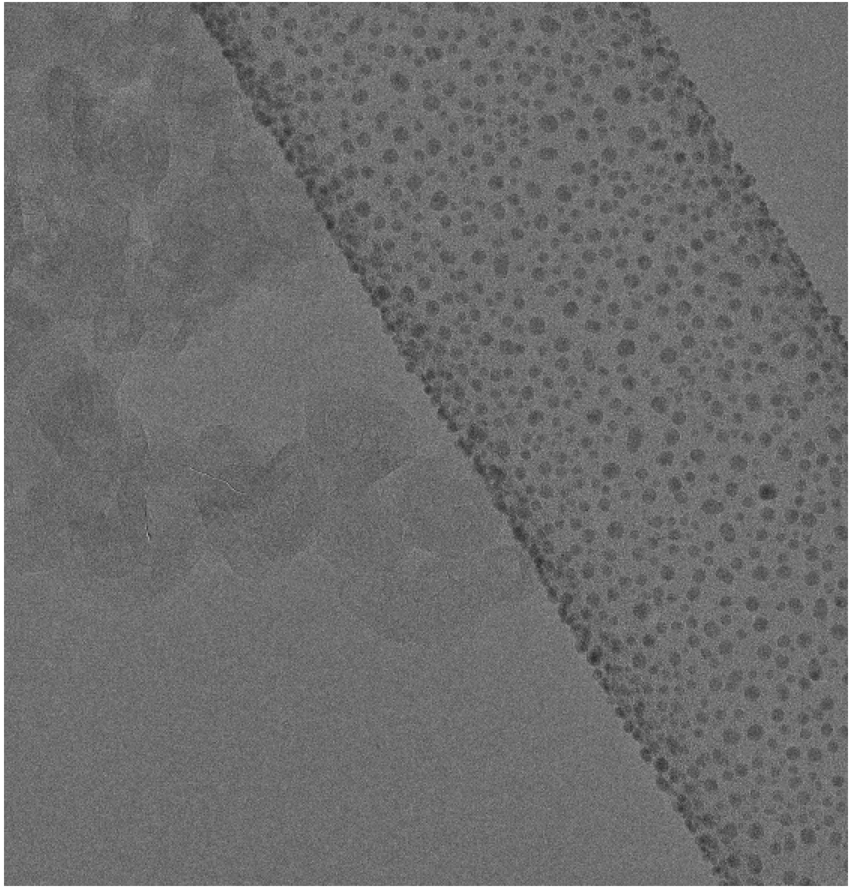
Dose Fractionation

7 sec exposure time without drift correction

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

- 21 x 0.33 sec = 7 sec



Dose Fractionation

- Dose fractionation is the distribution of a total electron dose over a series of sub-frames
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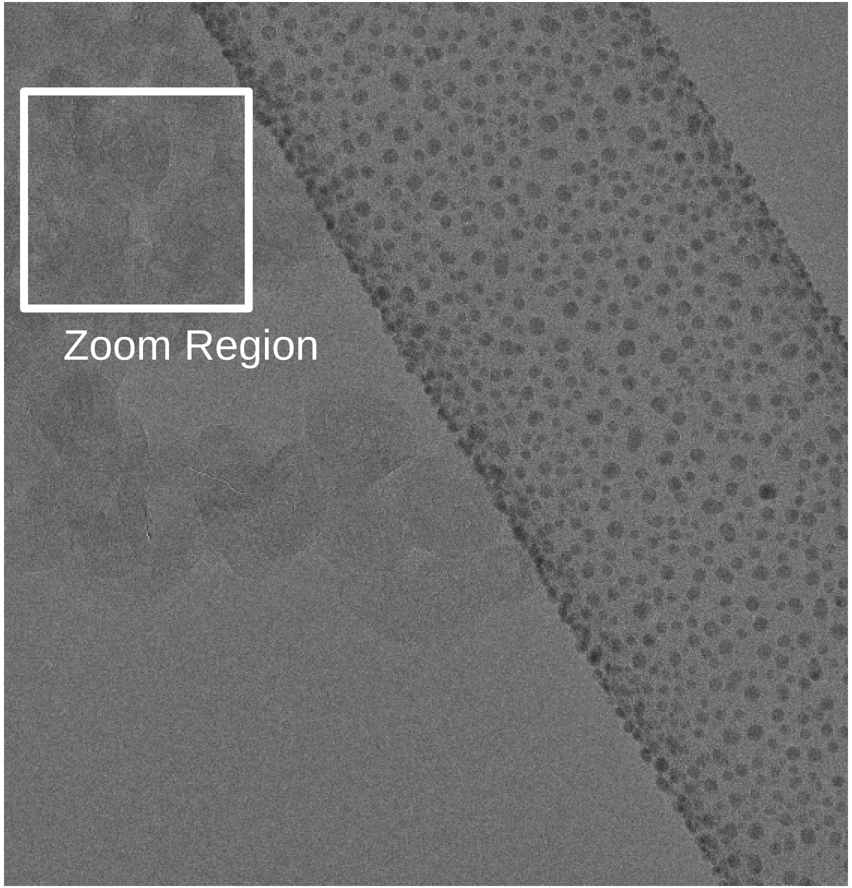
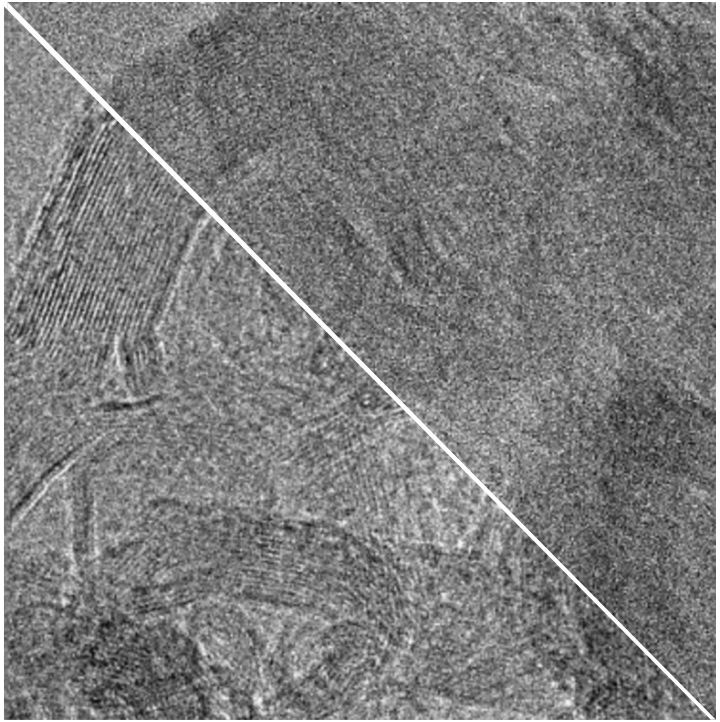
7 sec exposure time with drift correction



Dose Fractionation

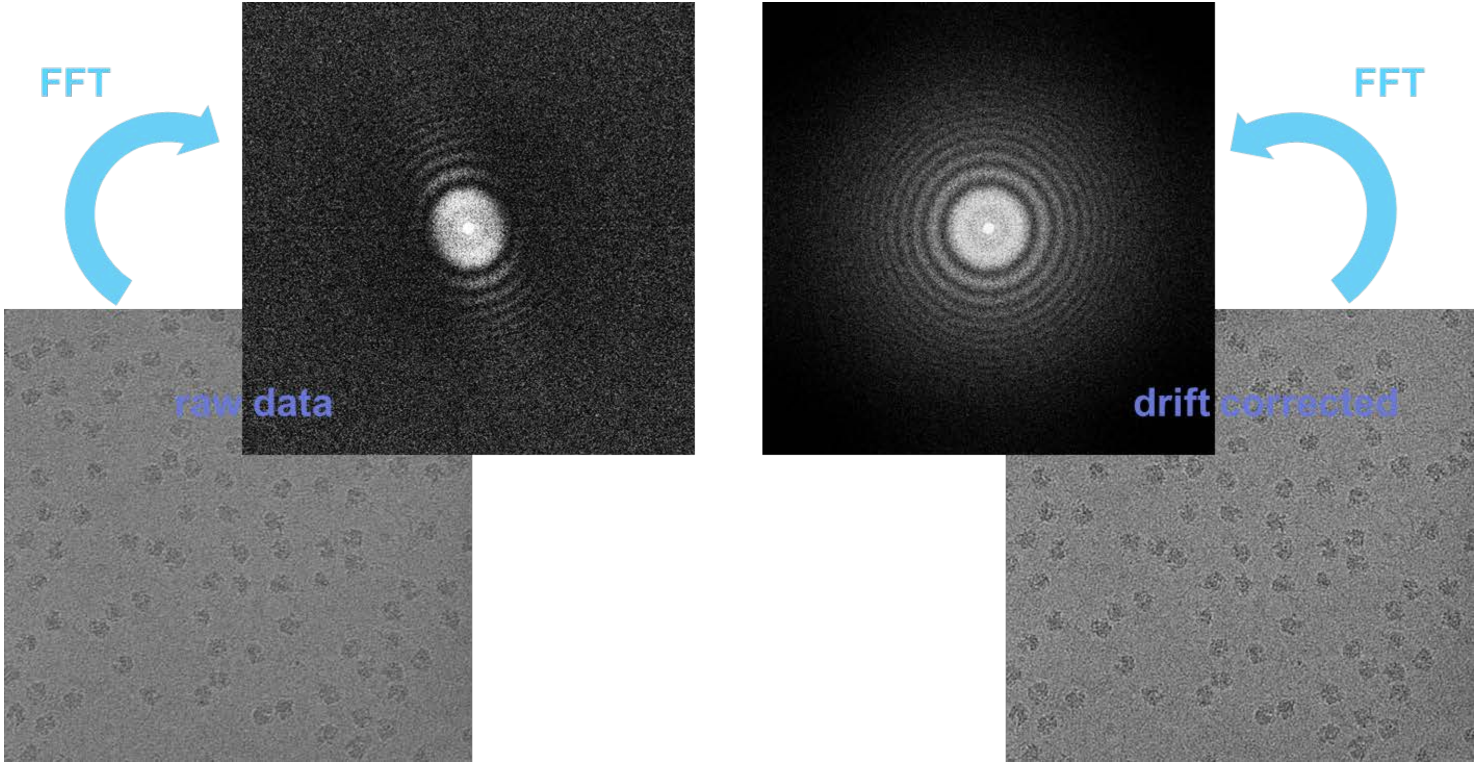
- Dose fractionation is the distribution of a total electron dose over a series of sub-frames

without sub-frame drift correction

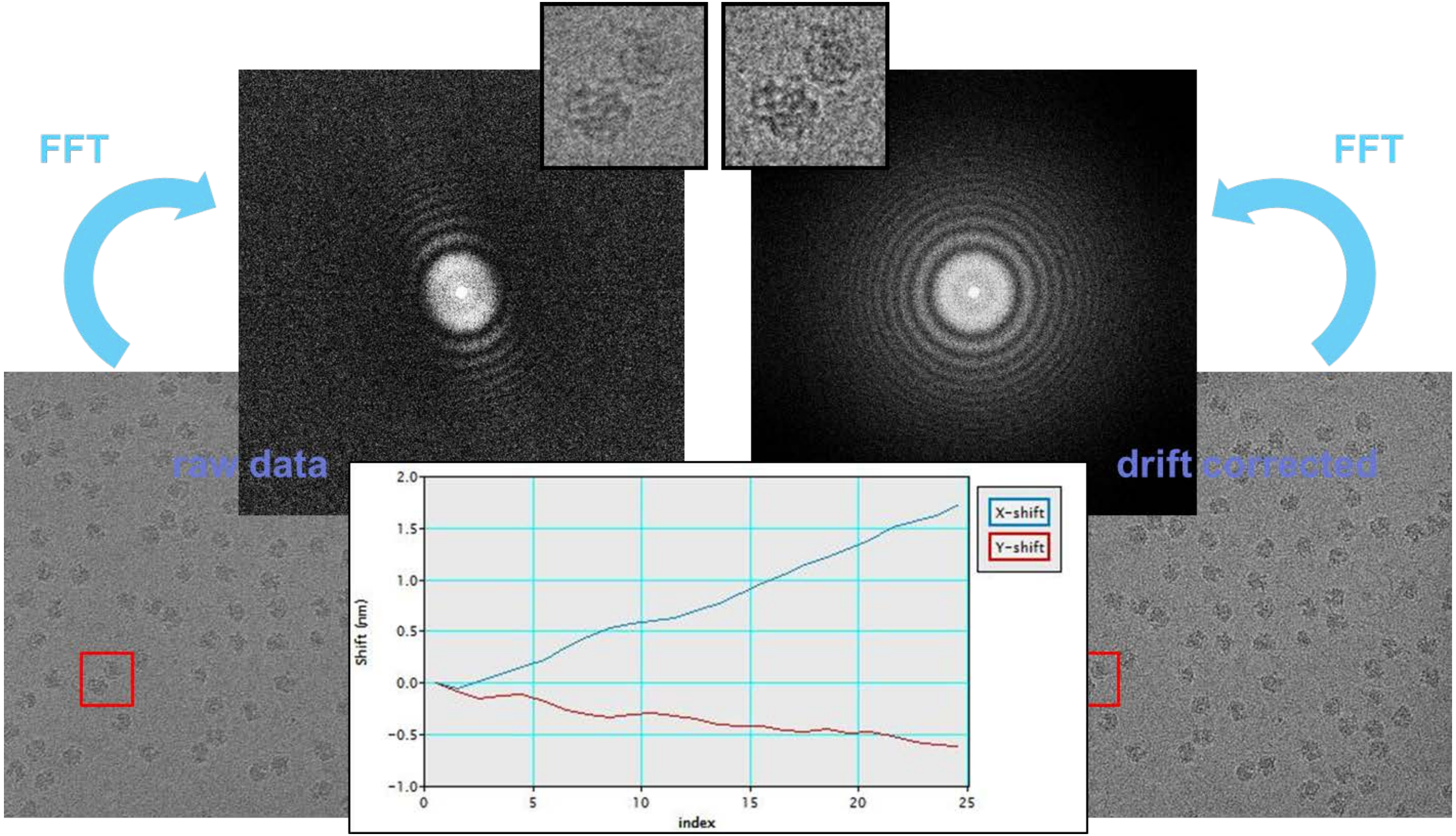


with sub-frame drift correction

Drift correction: Cryo-TEM example of Ribosome



Drift correction: Cryo-TEM example of Ribosome



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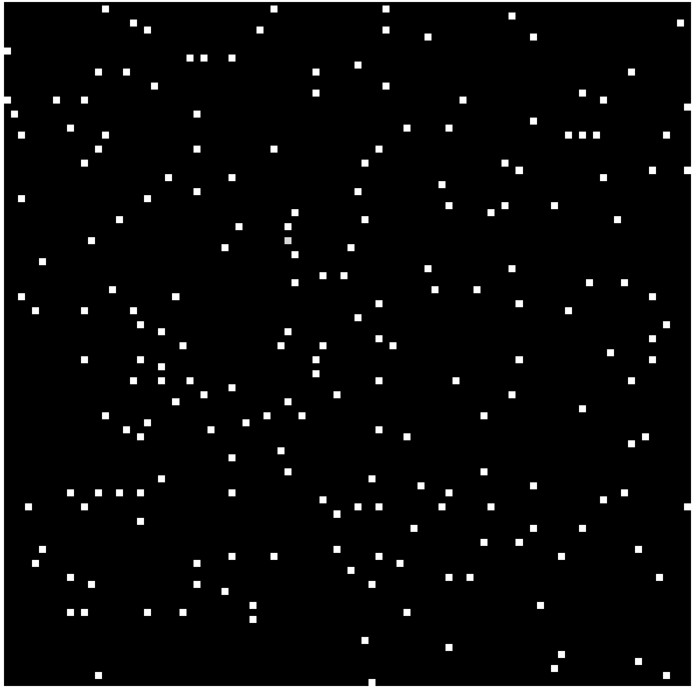
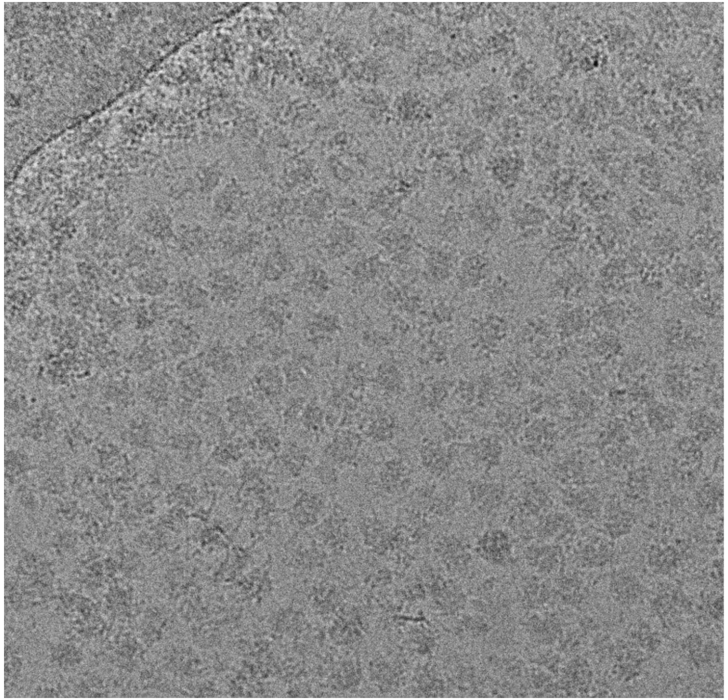
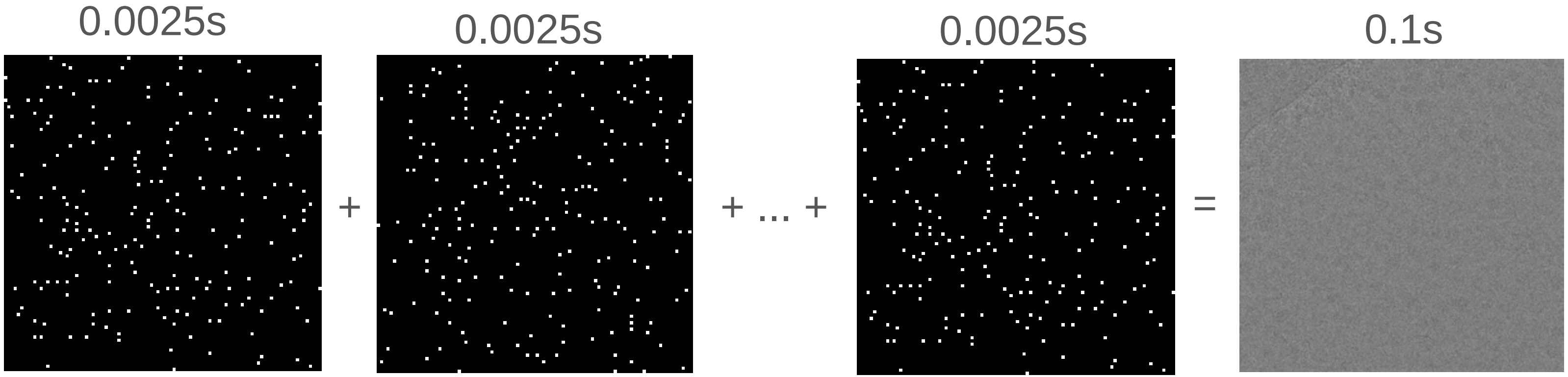
How frame alignment works.



Raw counted frame

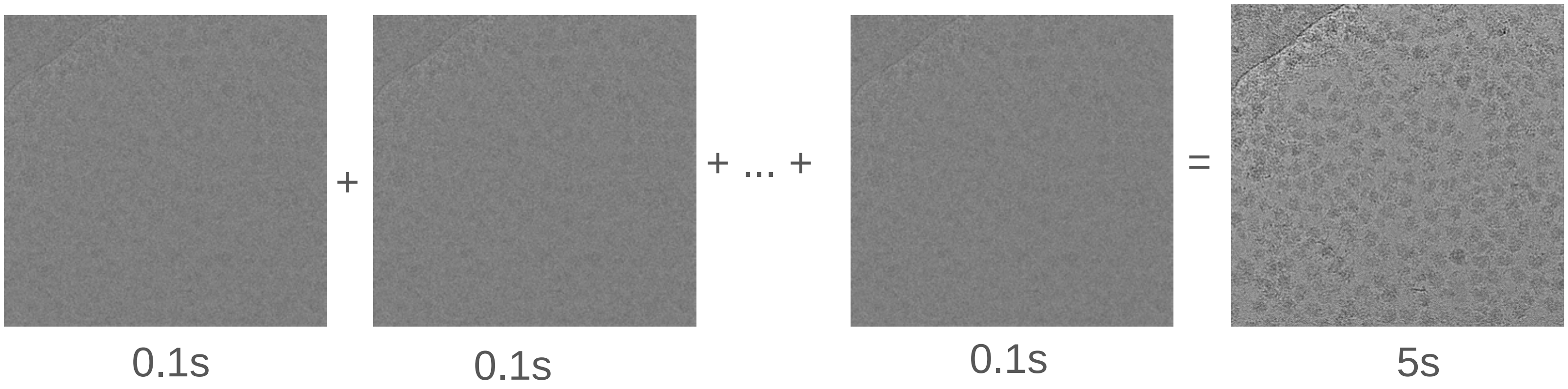


Final aligned image



Raw counted frames are summed

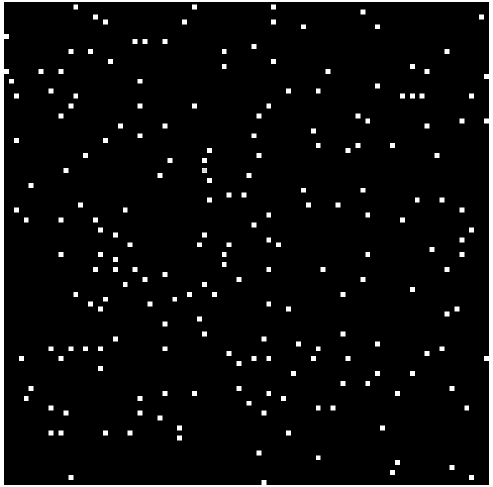
Sub-frames are aligned and summed



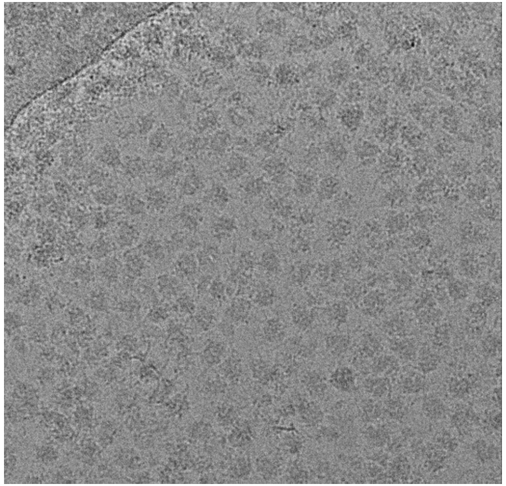
Slower frame rates mean you cannot correct fast sample movement

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



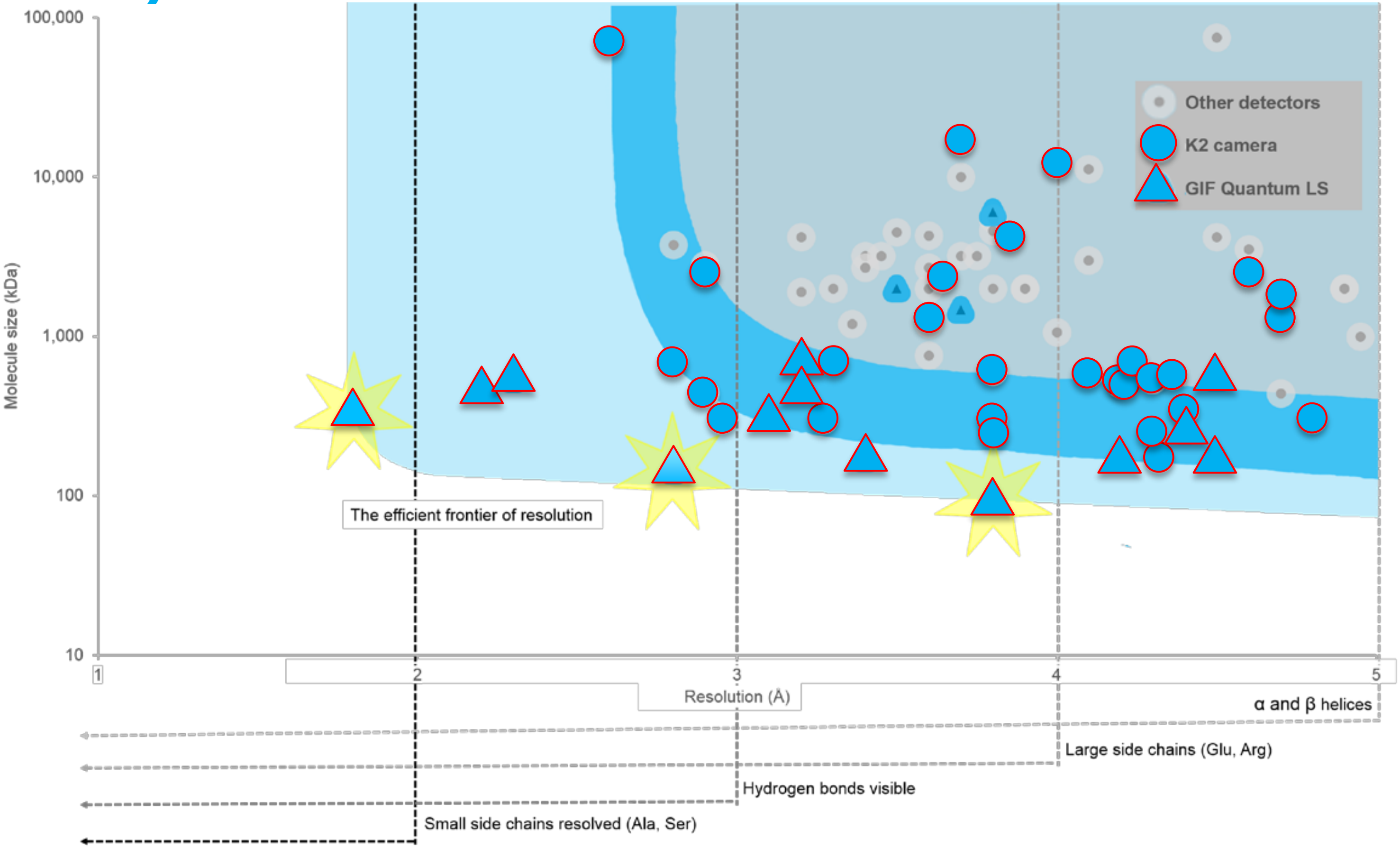
Raw frame



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)



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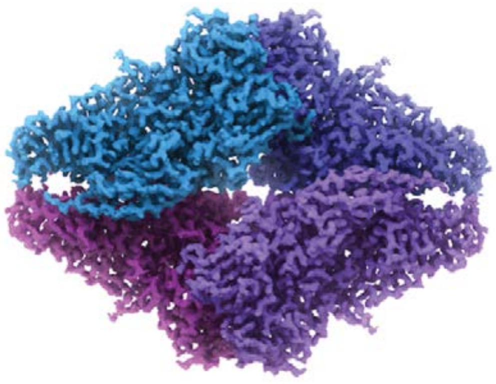
High contrast imaging is essential to high resolution

- “**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 β-galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,[1*] Alan Merk,[1*] Soojay Banerjee,[1] Doreen Matthies,[1] Xiongwu Wu,[2] Jacqueline L. S. Milne,[1] Sriram Subramaniam[1†]

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

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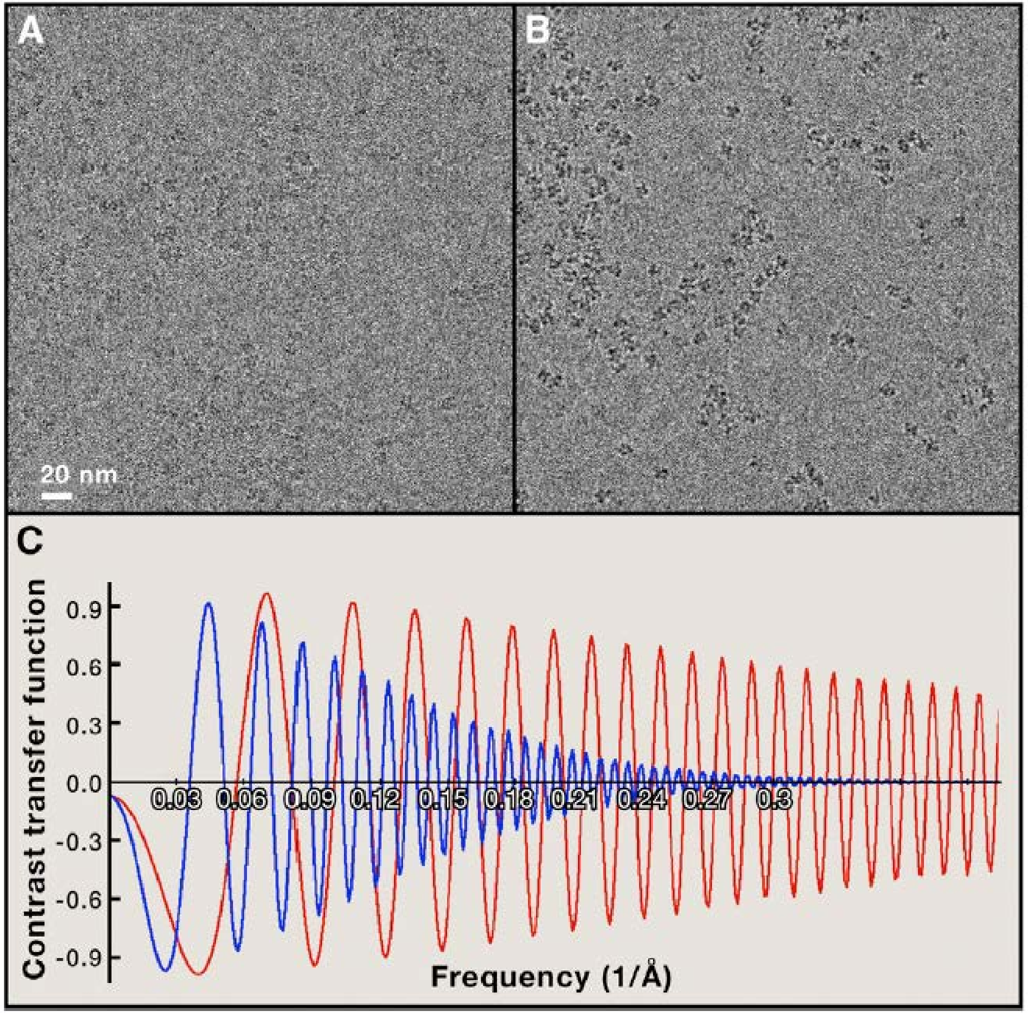
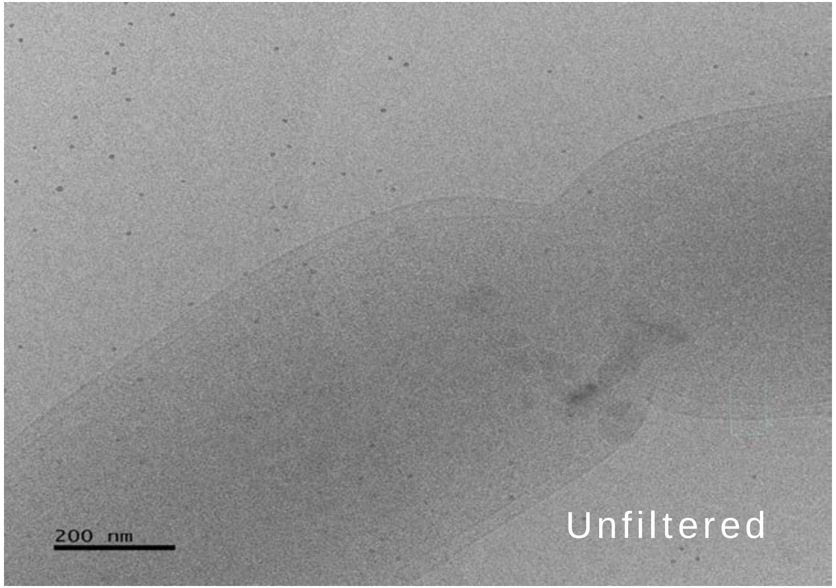
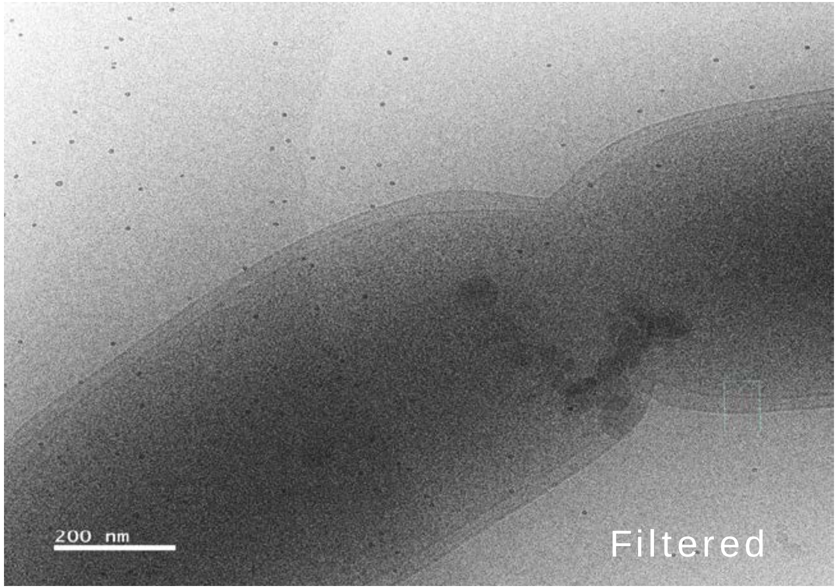
Contrast and focus

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

Data courtesy of Jan Löwe and Colin Palmer of LMB Cambridge @MPI Martinsried

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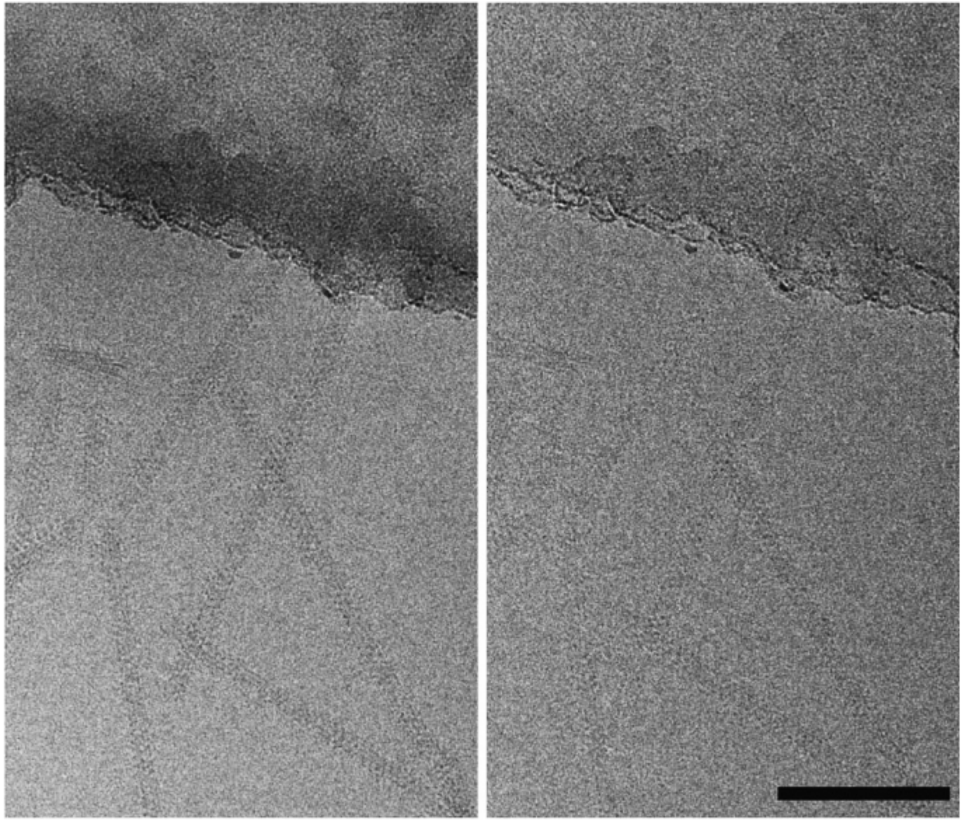
Energy filtering improves image contrast even for (small) particles

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



ELSEVIER

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[a], Michael B. Braunfeld[b], Saori Maki-Yonekura[a, c], David A. Agard[b, d]

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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

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- Red = Filtered Images
- Blue = Unfiltered Images

- Few % improvement in contrast even with TMV

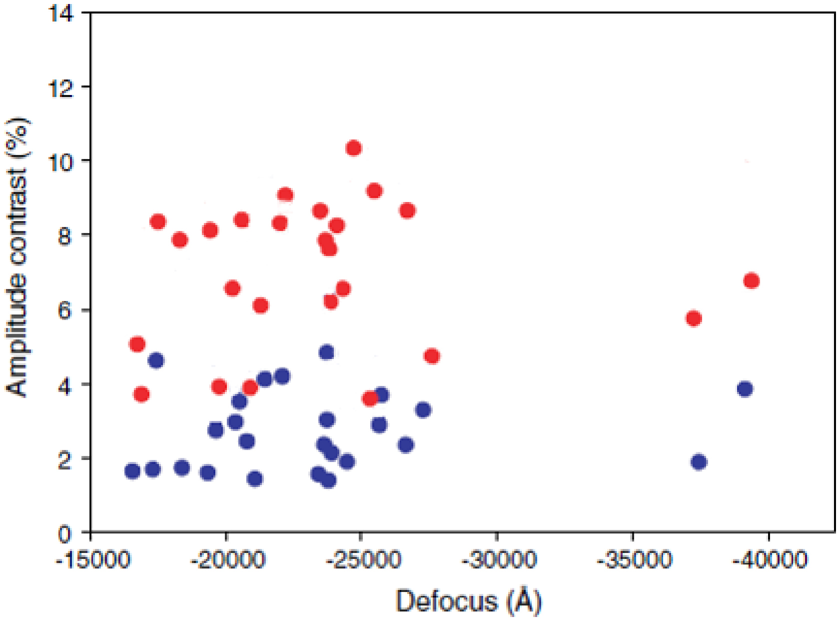
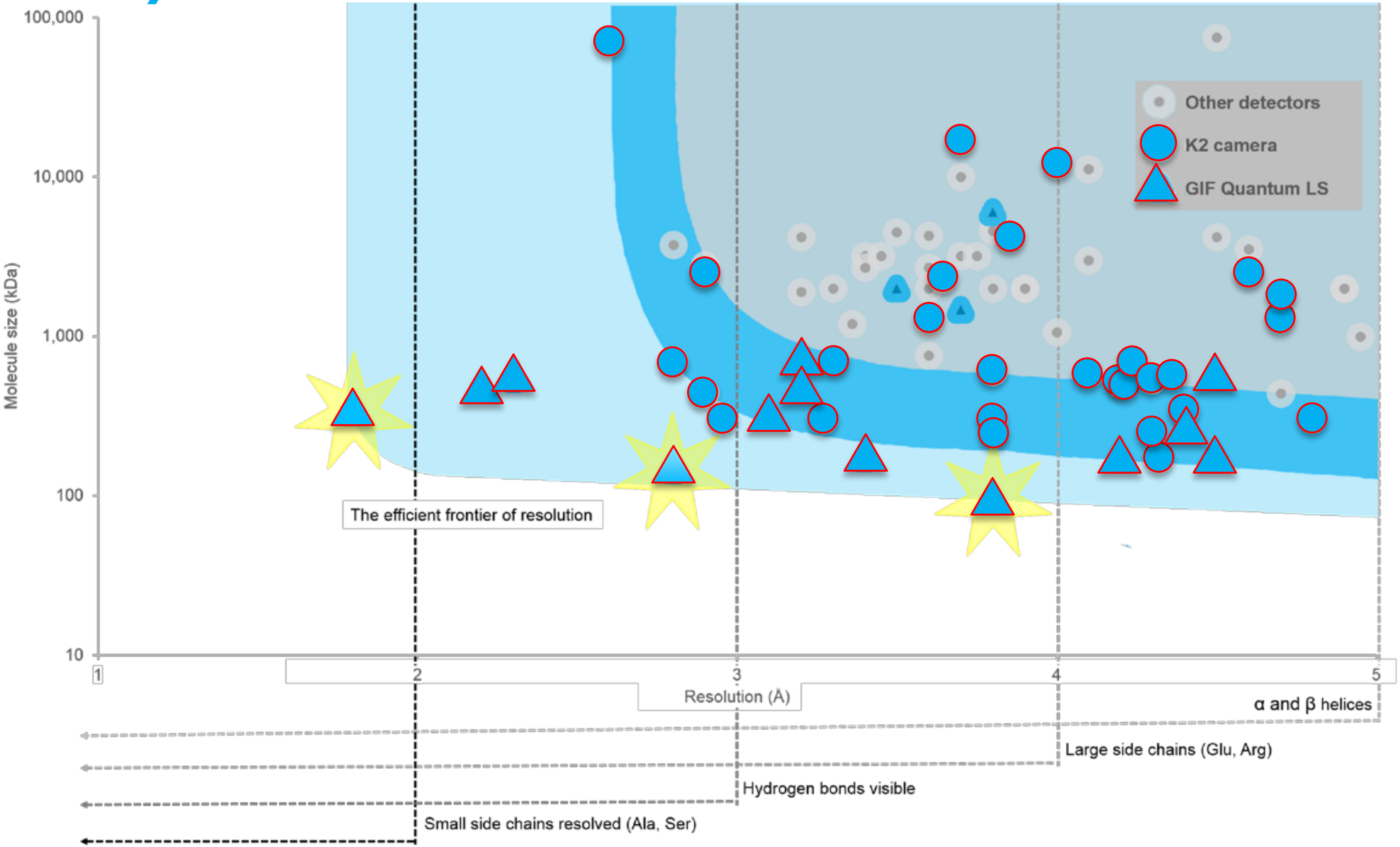


Fig. 7. Distribution of the amplitude contrast from flagellar filaments or the carbon film plotted against defocus. Red and blue symbols represent filtered and unfiltered data, respectively, recorded in either the 1st or 2nd exposure, and filled and open symbols indicate the filaments or the carbon film,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

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



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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

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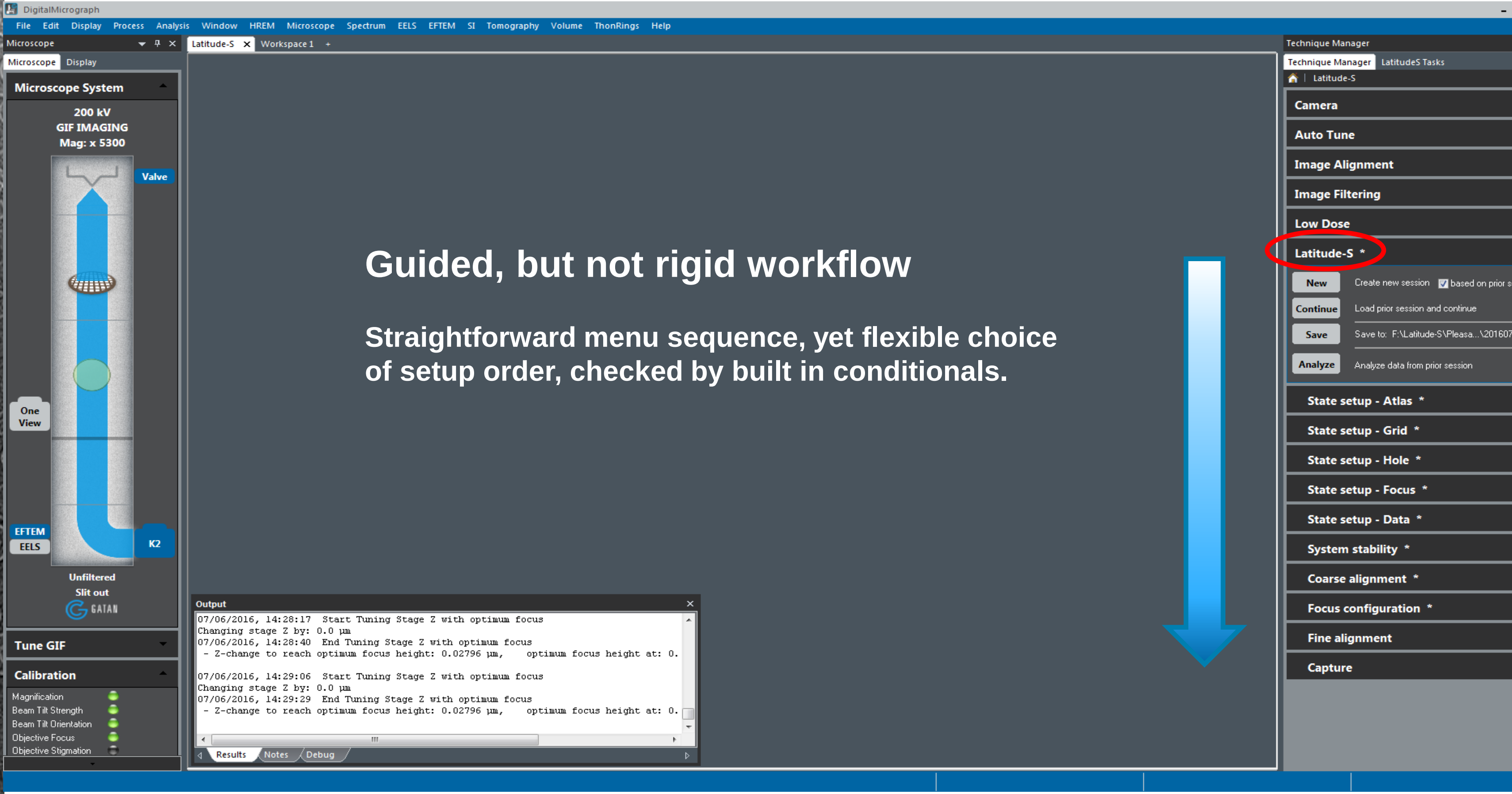
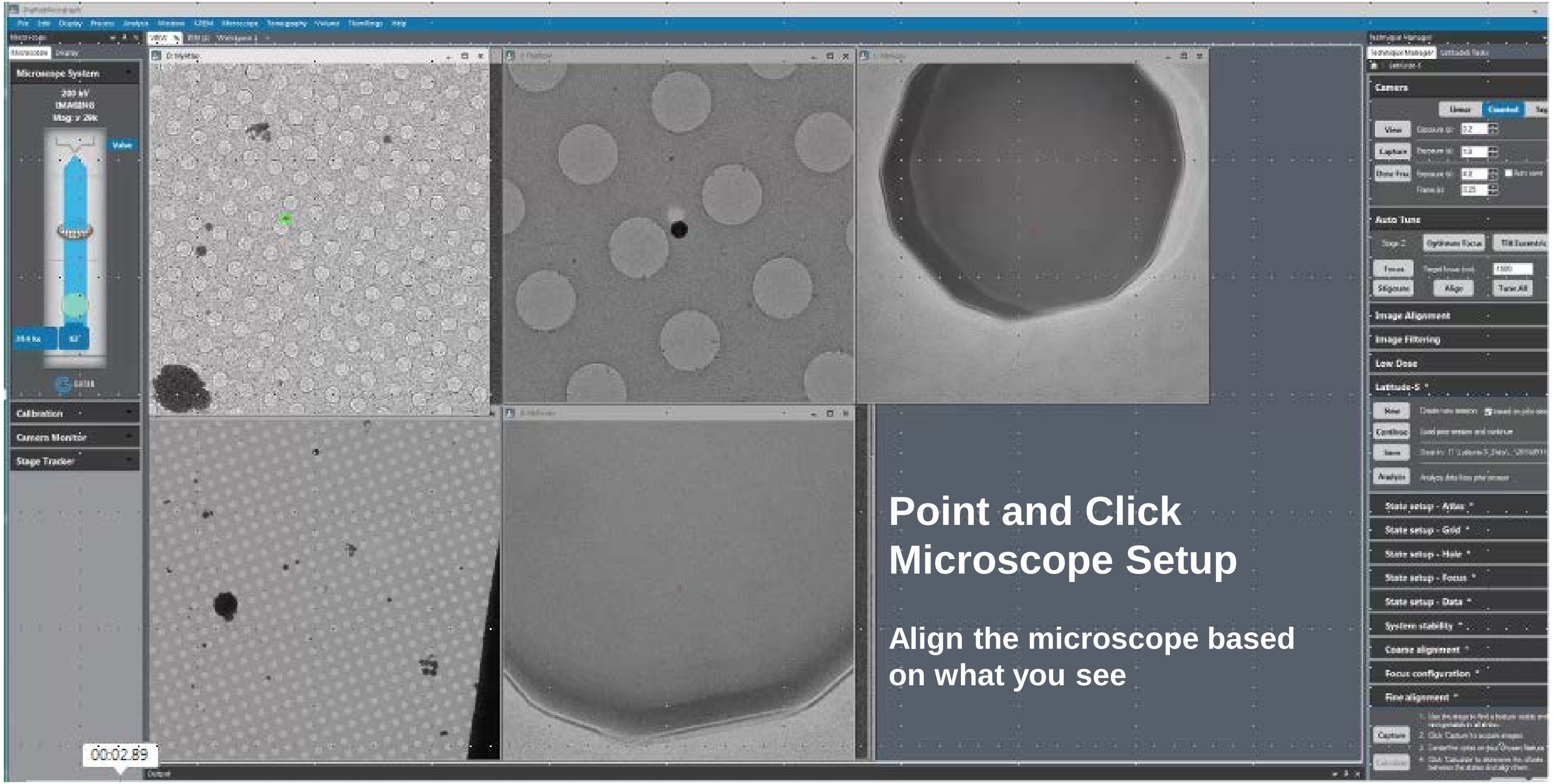
Latitude-S: Improving ease of use



Guided, but not rigid workflow

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

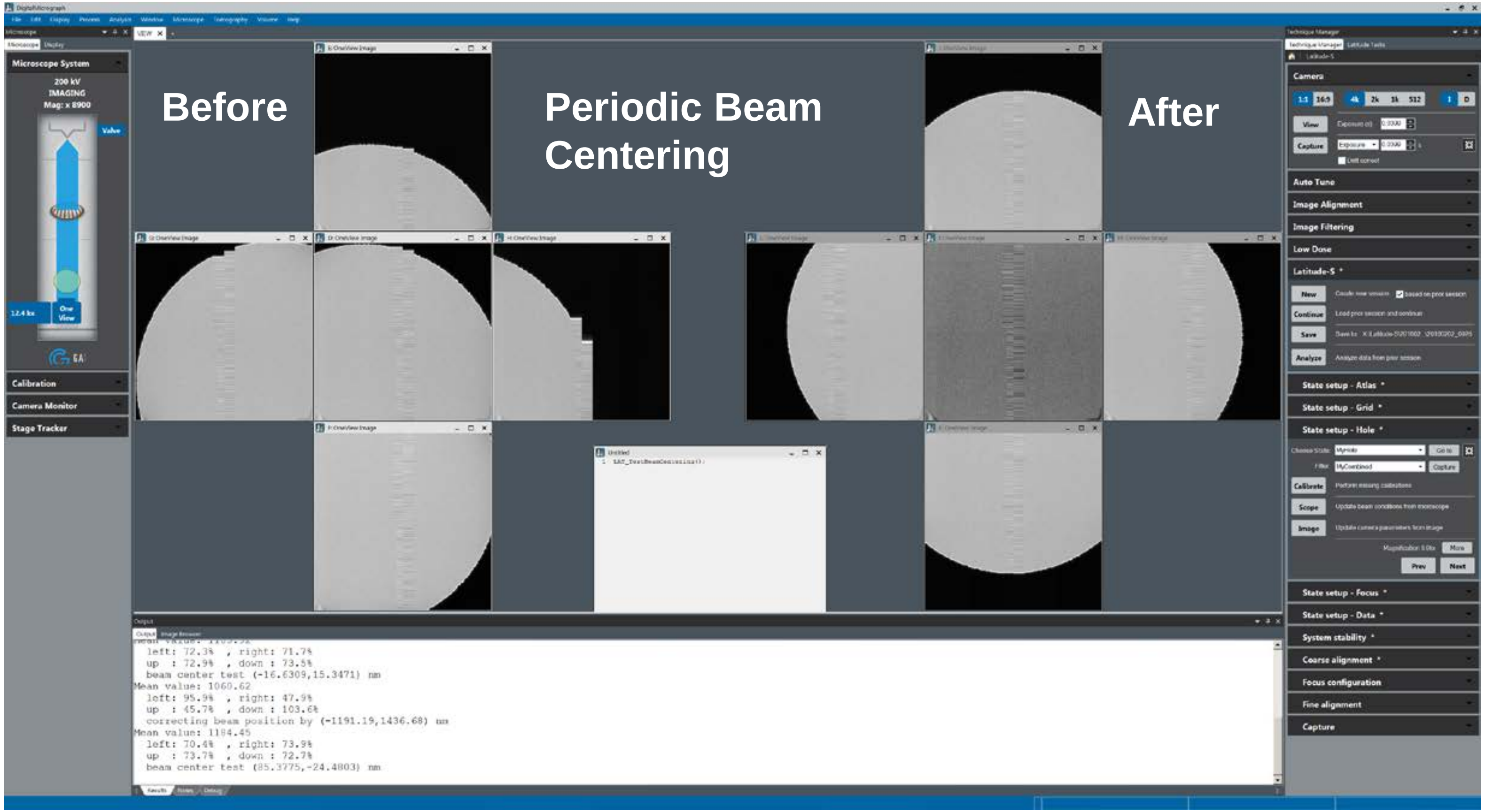
Latitude-S: Improving ease of use



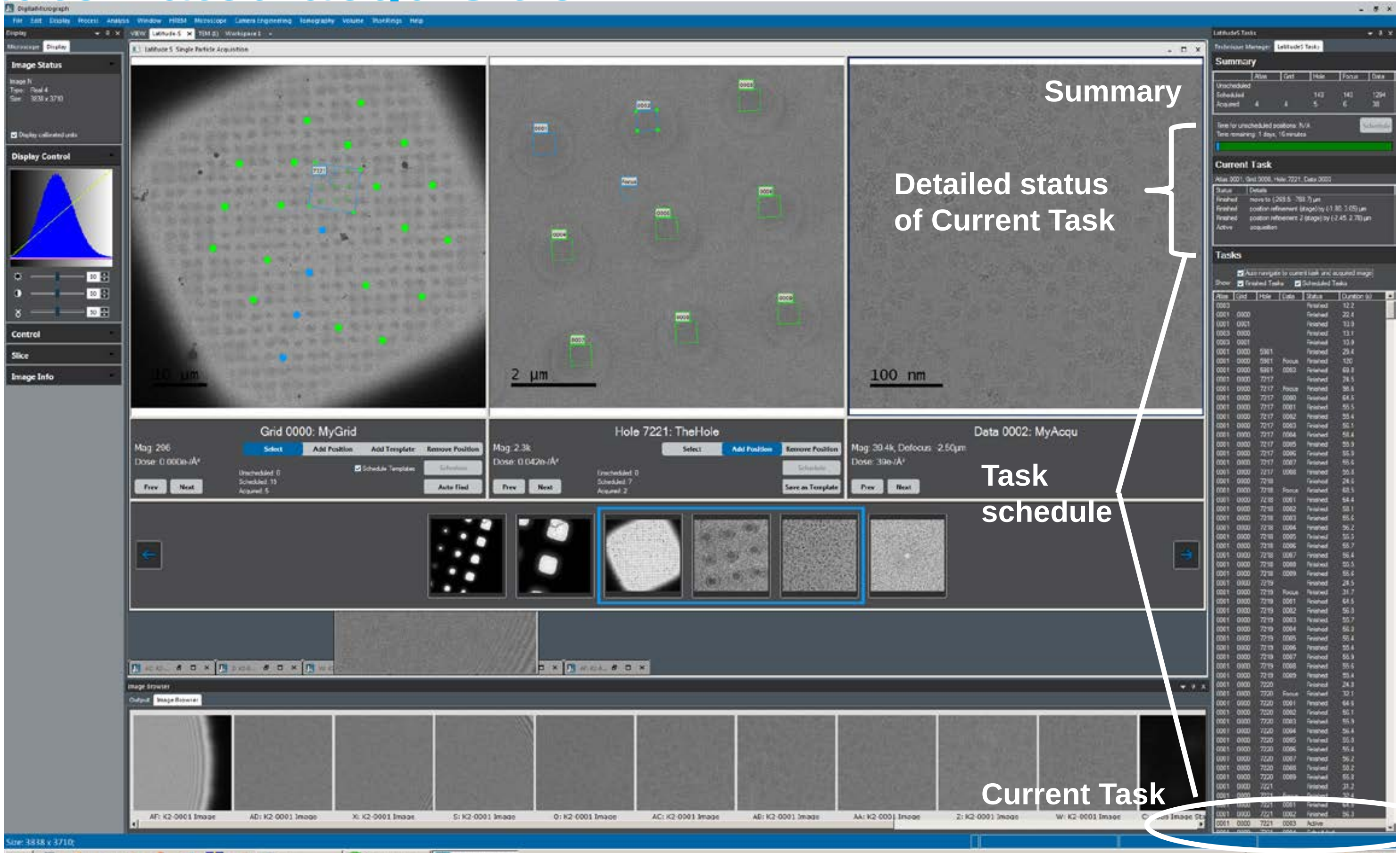
Point and Click Microscope Setup

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



Images from a test run, kindly provided by Adam's Frost group at UCSF.

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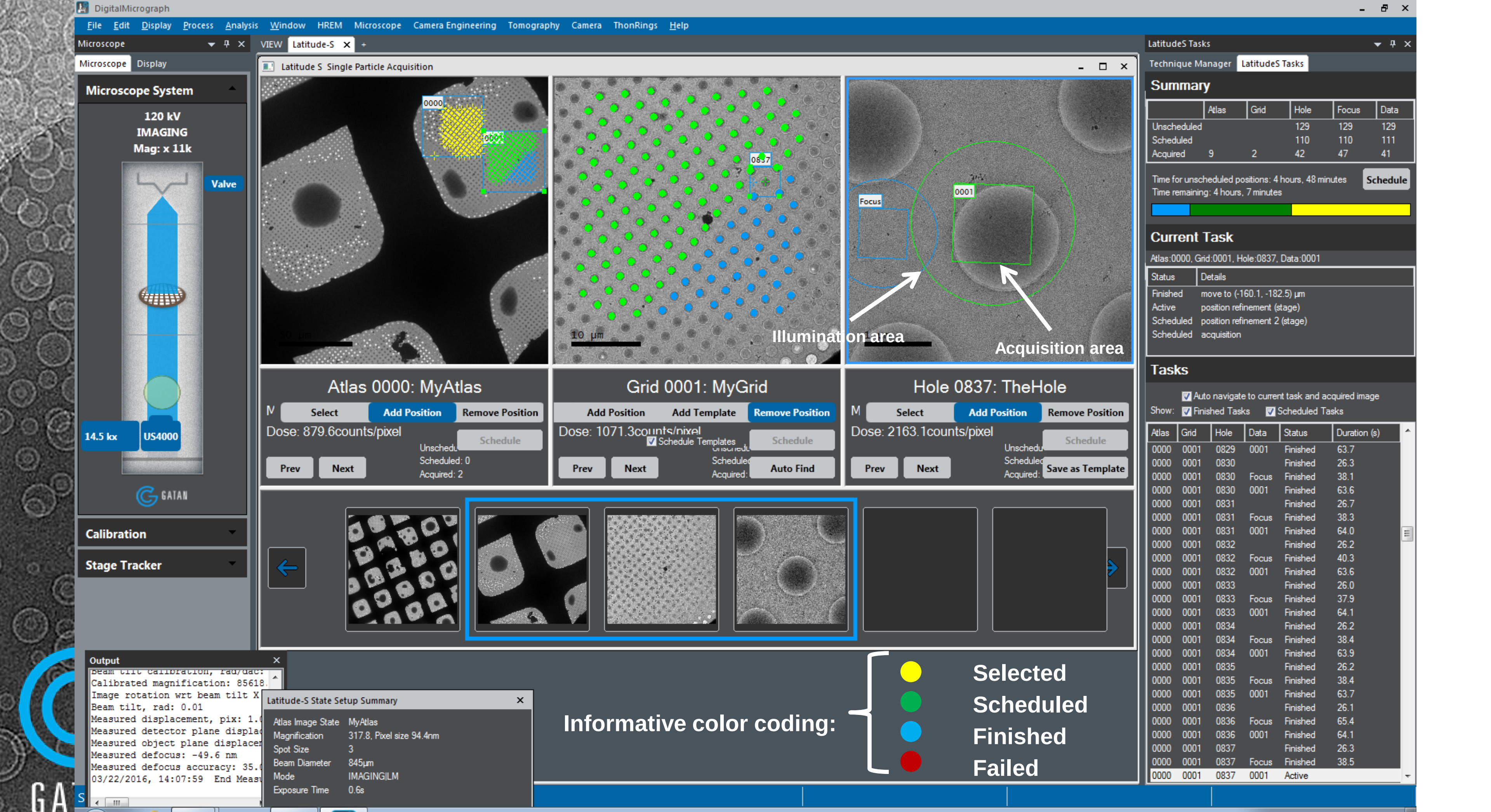

Images from a test run, kindly provided by Adam's Frost group at UCSF.

Thank You!

Questions?

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