K3:
Advancing Electron-Counting Cryo-EM

Paul Mooney
Gatan, Inc.

October 31, 2017

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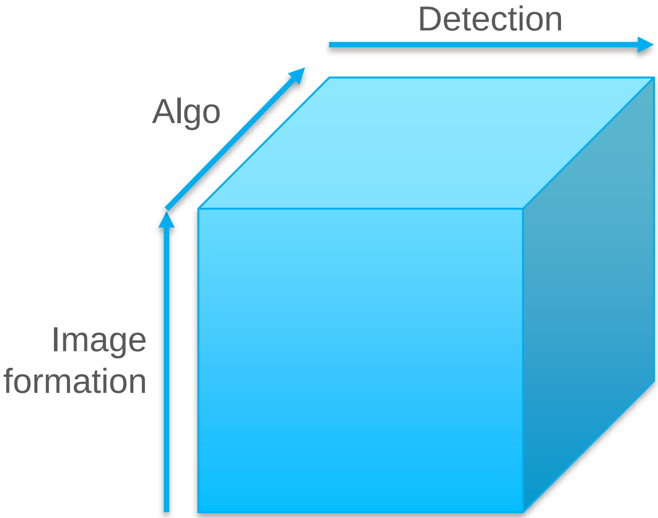
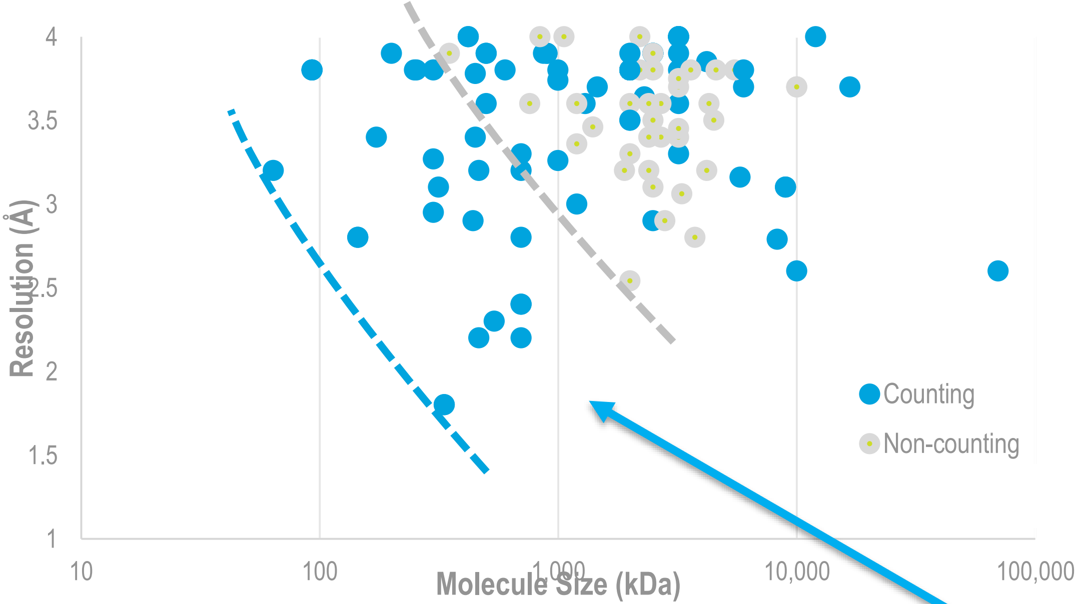
Leverage



"Electron-counting cryo-electron microscopy*"



*Hong Zhou in:

Science, 6/30/2017

and

J. General Virology, Oct. 2017

New biological territory

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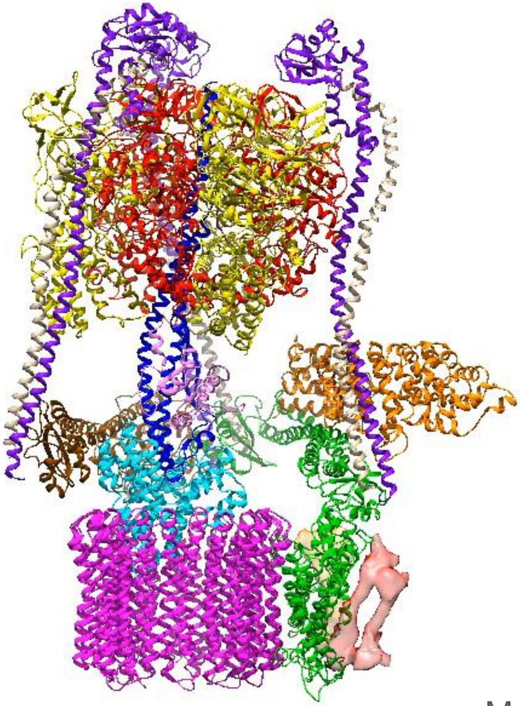
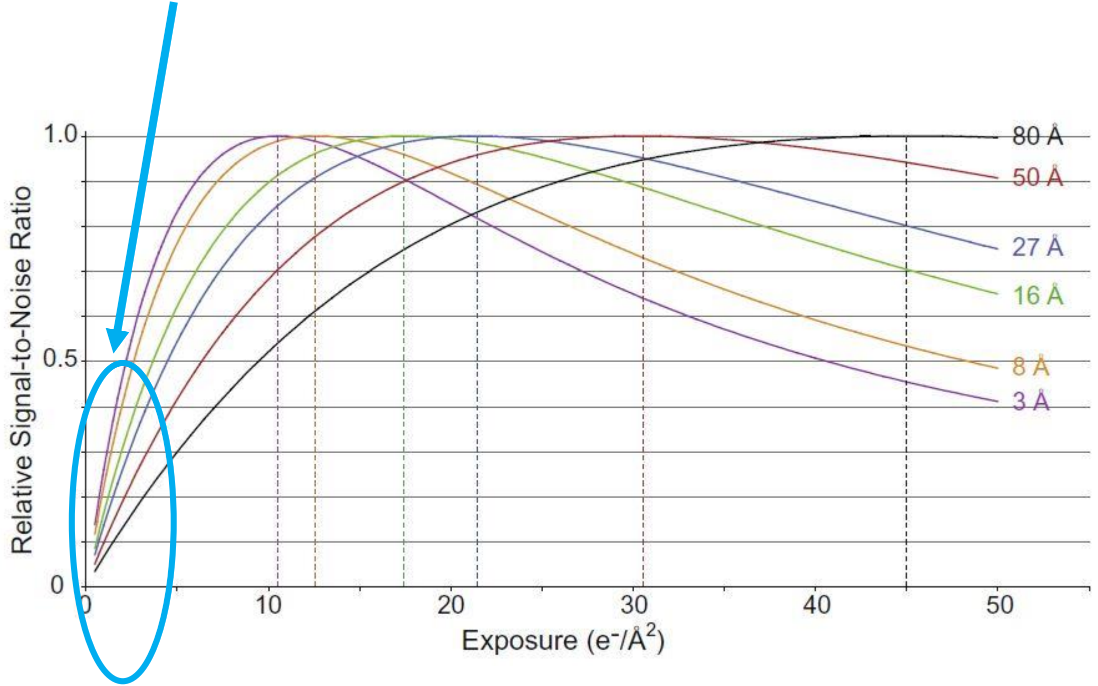
More degrees of freedom means more particles.



Movie courtesy of John Rubinstein

Higher resolution demands more particles.



L.A. Baker et al. / Journal of Structural Biology 169 (2010) 431–437

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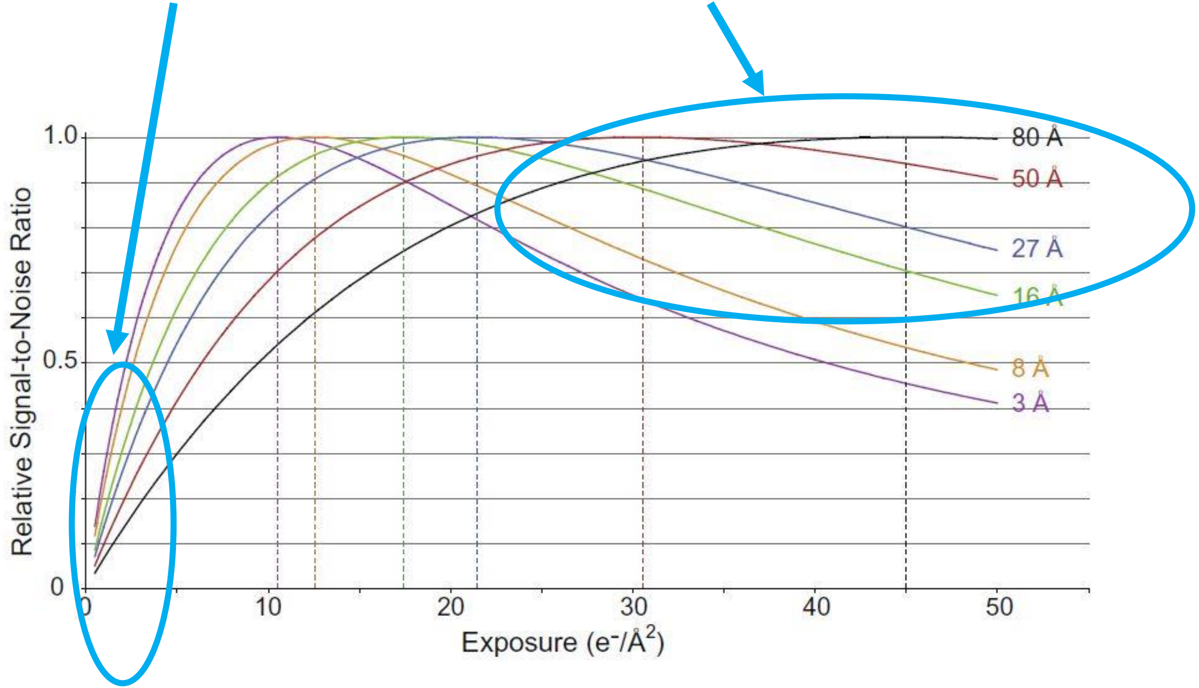
Better motion correction and dose weighting mean more *frames*.



L.A. Baker et al./Journal of Structural Biology 169 (2010) 431–437

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Throughput

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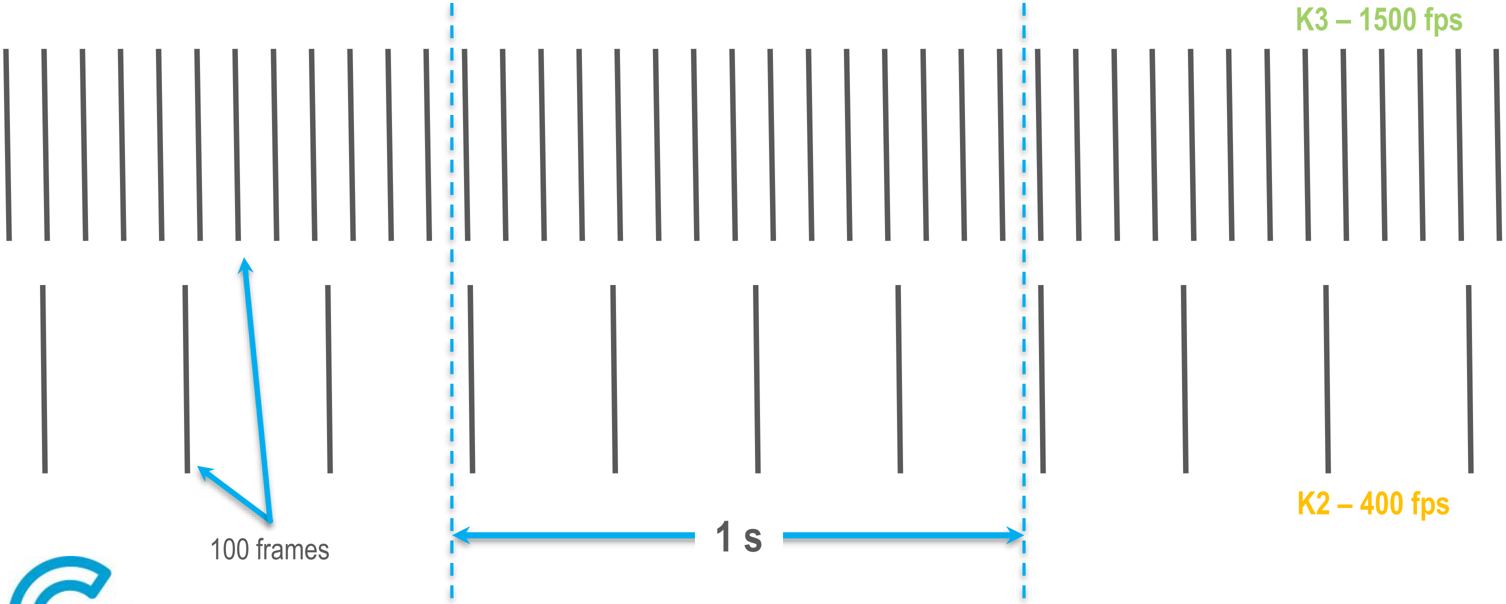
K3 Camera Framerate – 1500 fps



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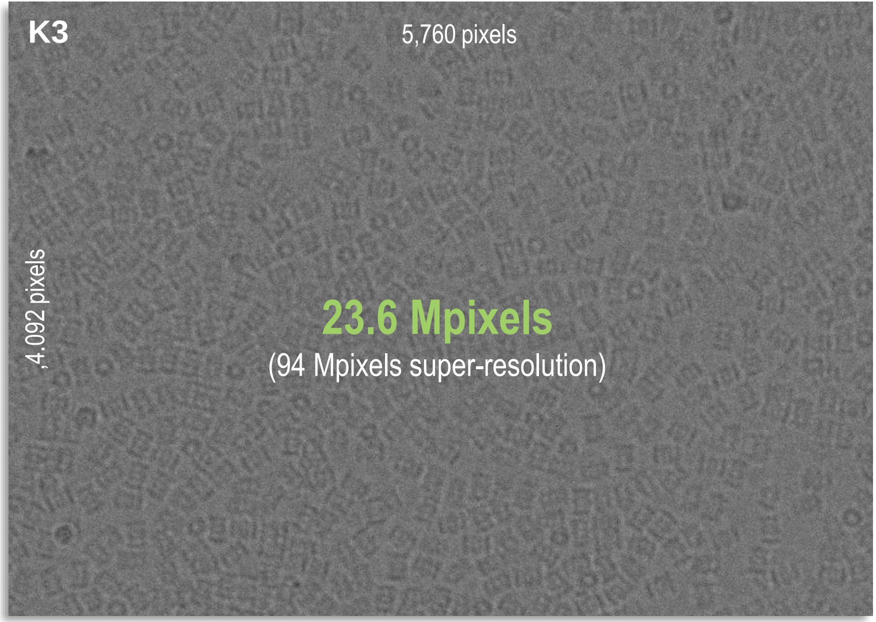
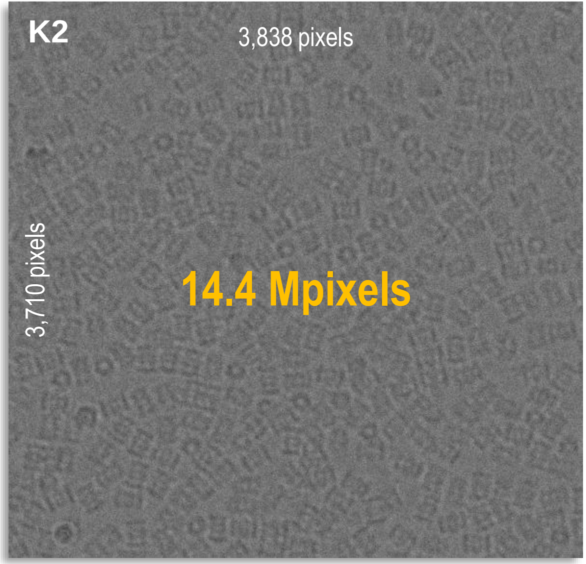
→ **3.75 times** the throughput of the K2® camera (frames per second)

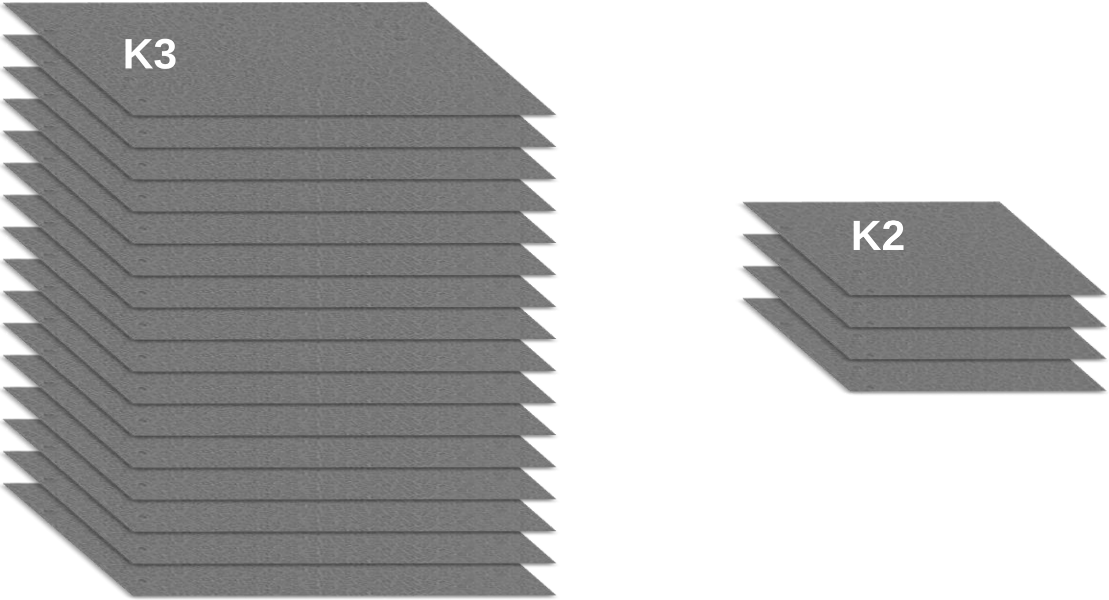
K3 Sensor – 23.6 Mpixels





→ **1.65 times** the throughput of the K2 camera (pixels/frame)

K3 Sensor – Throughput



→ **6.2 times** the raw sensor throughput of the K2 camera (pixels/s)

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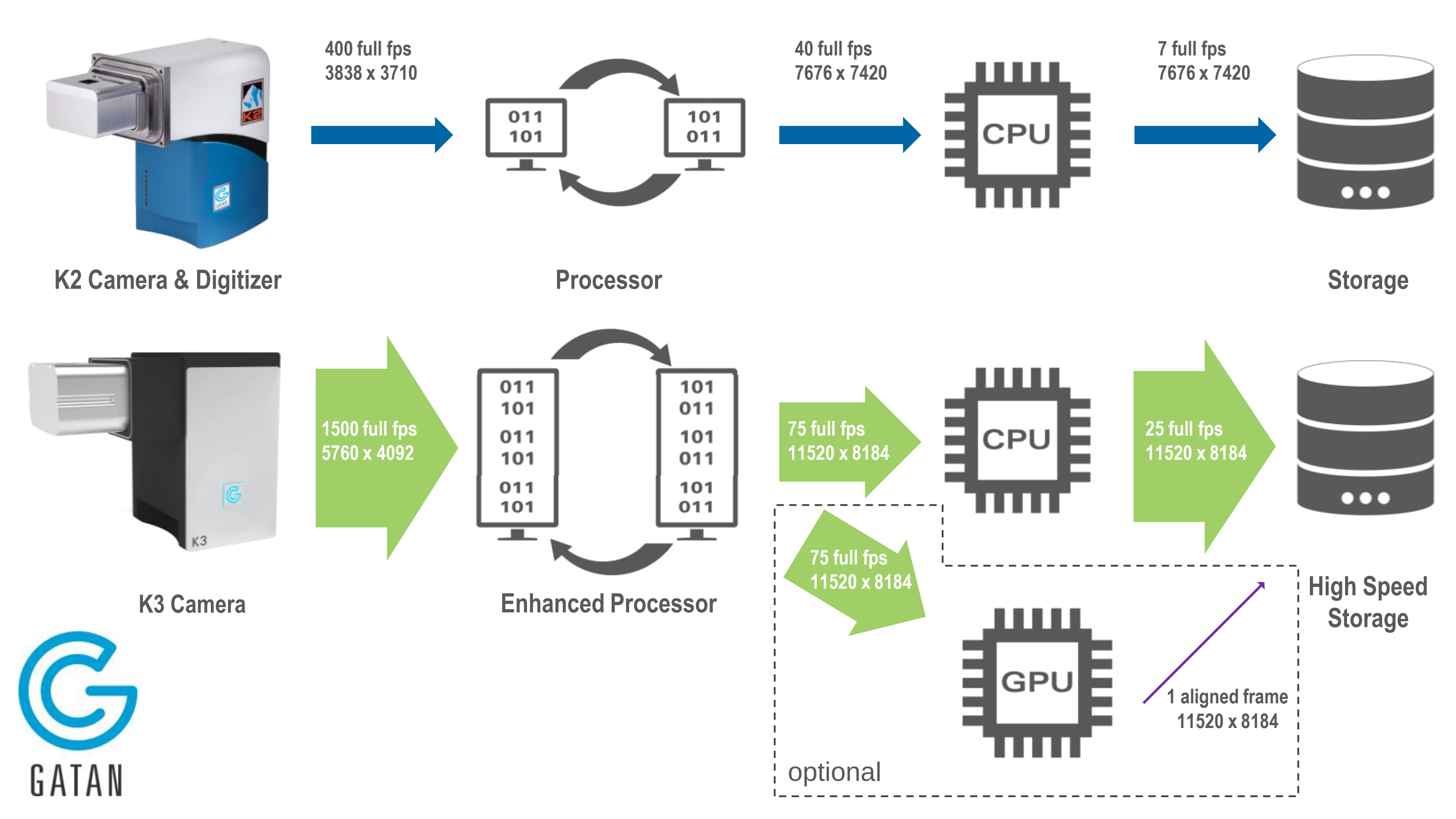
400 full fps
3838 x 3710
40 full fps
7676 x 7420
7 full fps
7676 x 7420
011
101
101
011
CPU
K2 Camera & Digitizer
Processor
Storage
1500 full fps
5760 x 4092
75 full fps
11520 x 8184
25 full fps
11520 x 8184
75 full fps
11520 x 8184
CPU
GPU
1 aligned frame
11520 x 8184
K3 Camera
Enhanced Processor
High Speed
Storage
optional
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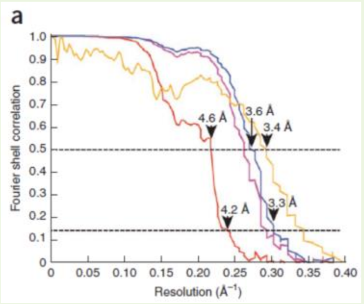
Counting vs. Motion Correction

20S Proteasome structure resolution



Film by cryo-EM	Electron-counting cryo-EM		
5.6 Å	4.2 Å	3.5 Å	3.3 Å
	No motion correction	Motion correction	Distortion and motion correction

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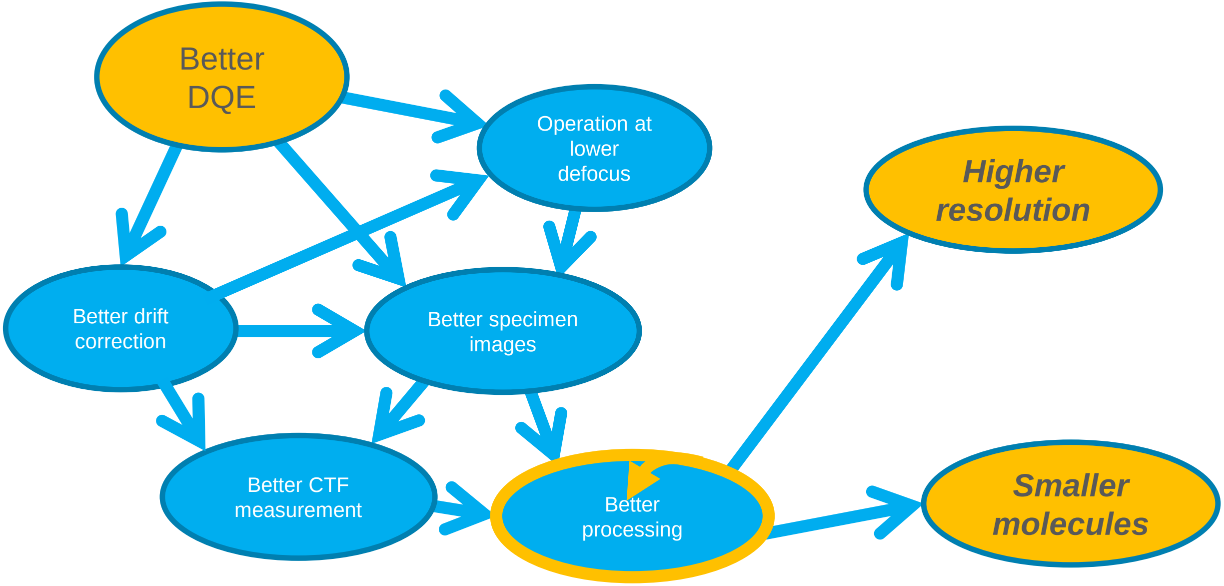
Incl. Cheng, Y.

Rabl, J. et al. Mechanism of gate opening in the 20S proteasome by the proteasomal ATPases. Mol. Cell 30, 360–368 (2008).

Li, X., Mooney, P., Zheng, Q., Booth, C.R., Braunfeld, M.B., Gubbens, S., Agard, D.A., Cheng, Y., 2013. Electron counting and beam-induced motion correction enable near-atomic-resolution single-particle cryo-EM. Nature Methods. http://dx.doi.org/10.1038/nmeth.2472.

Cryo-EM methods leverage Electron-Counting DQE



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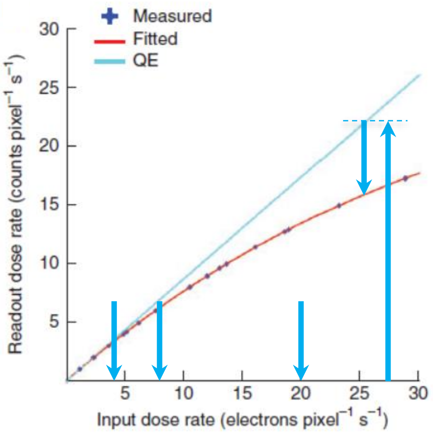
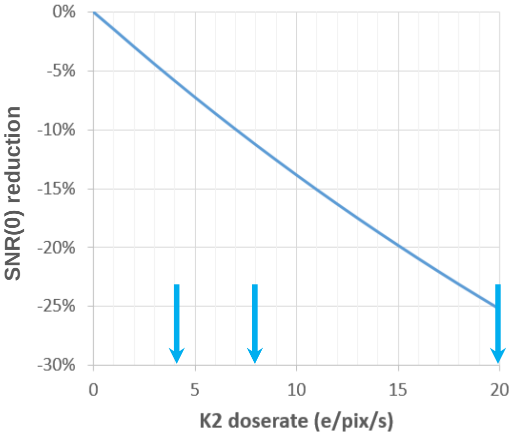
Coincidence Loss Causes Lowering of DQE

Count Rate vs Dose Rate



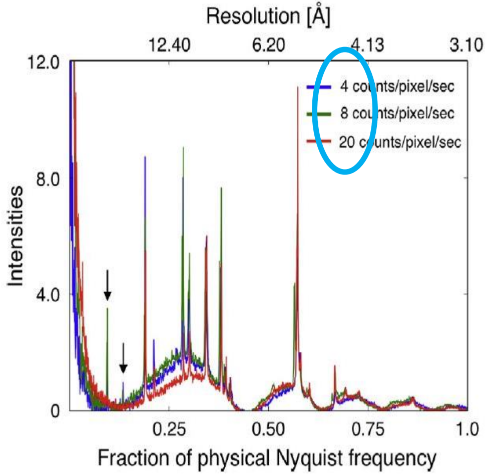
Li et al, Nature Methods (2013) Figure 1b.

SNR(0) reduction vs Dose Rate



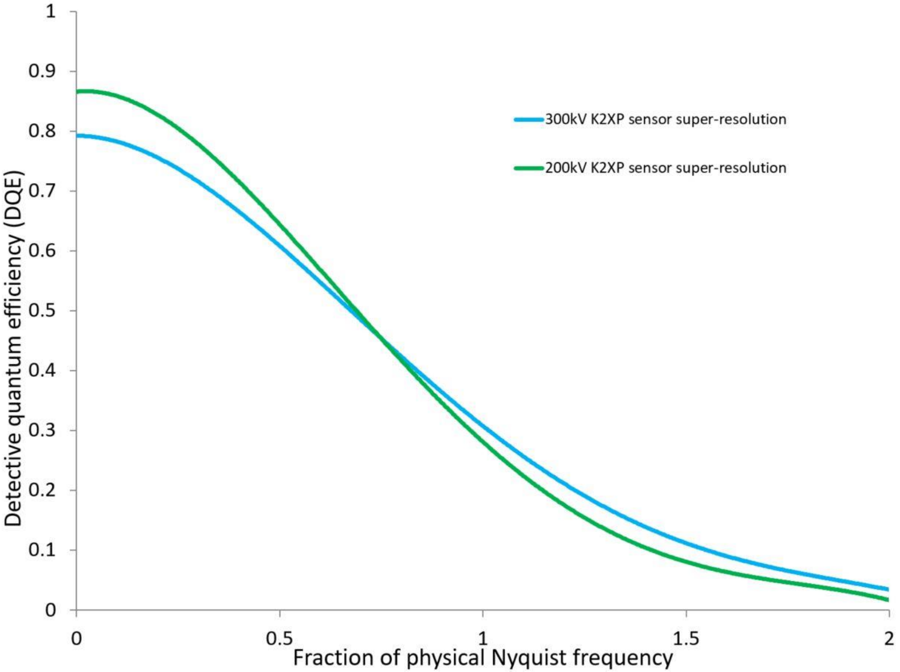
(Based on fit to curve at left)

SNR(s) vs Dose Rate



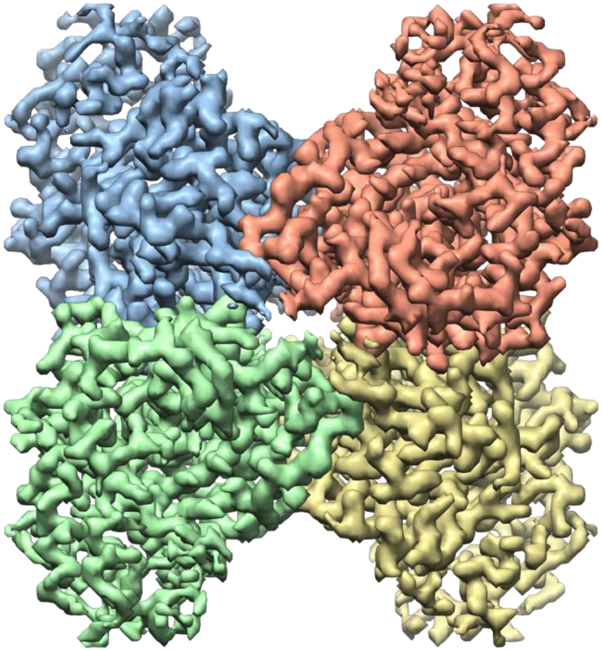
Chiu, et al, JSB 2015

K2 200kV DQE is higher at low spatial frequency



High resolution being achieved at 200kV

2.6 Å at 200 kV without image filtering or phase plate



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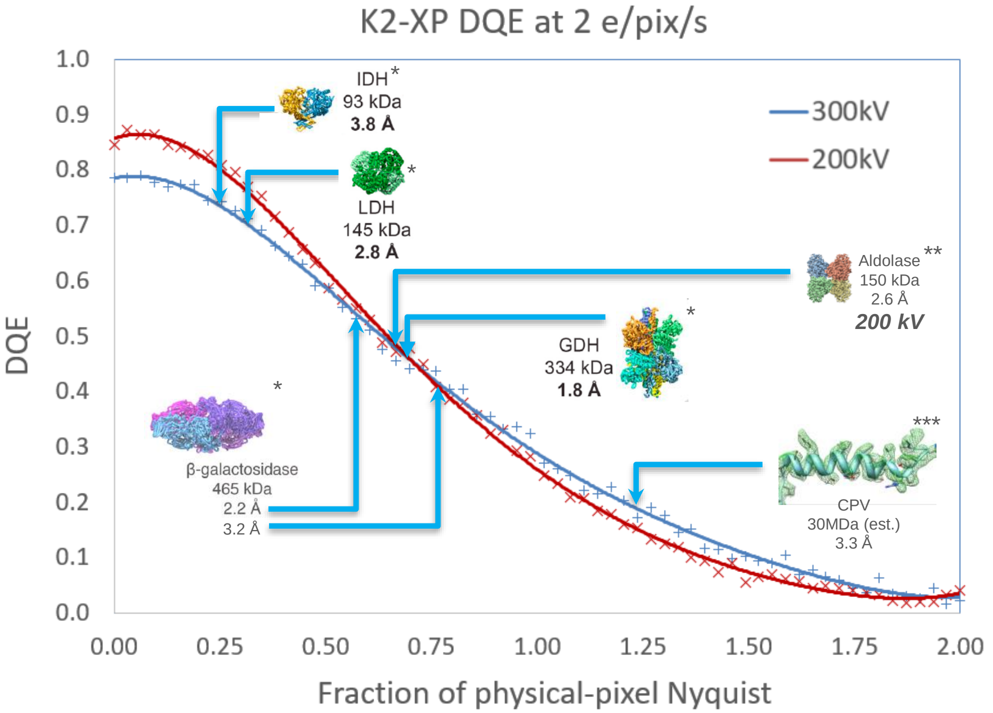
Herzik, Wu and Lander, Nature Methods 2017

Image courtesy of Gabriel Lander

What is the Best Magnification and Binning?



* 300 kV energy-filtered Krios structures from Merk et al, Cell, 2016

** 200 kV Talos Arctica density map from Herzik et al, Nat. Meth., 2017

*** 300 kV energy-filtered Krios structure, Hong Zhou (private communication)

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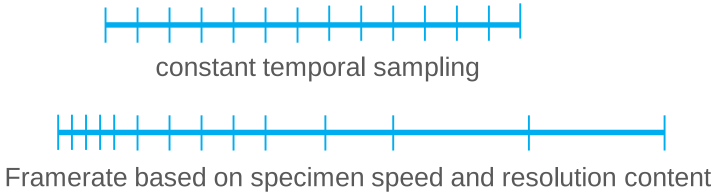
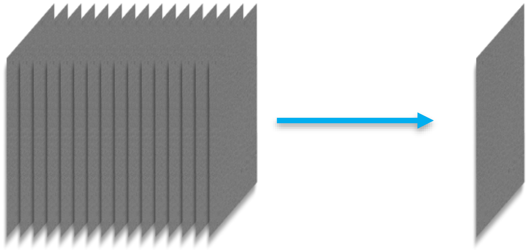
Data Size Reduction

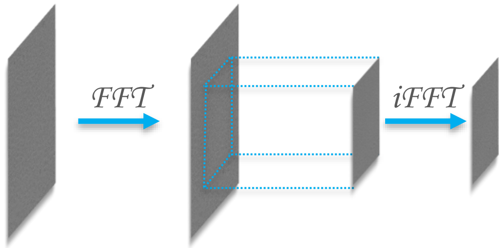
- Variable sub-frame exposure time.



- Motion correction



- Anti-aliased binning



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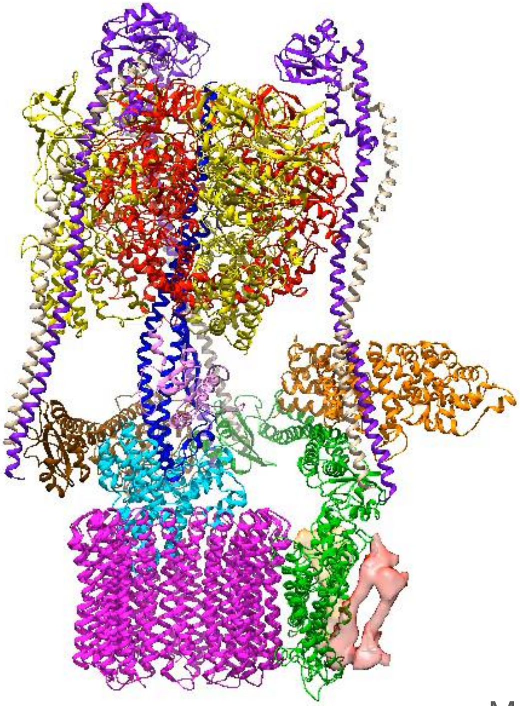
And *resolving* conformational states demands better *DQE*



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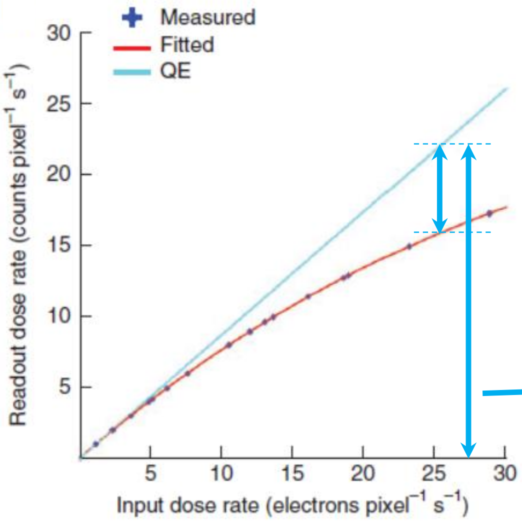
Movie courtesy of John Rubinstein

Realtime DQE

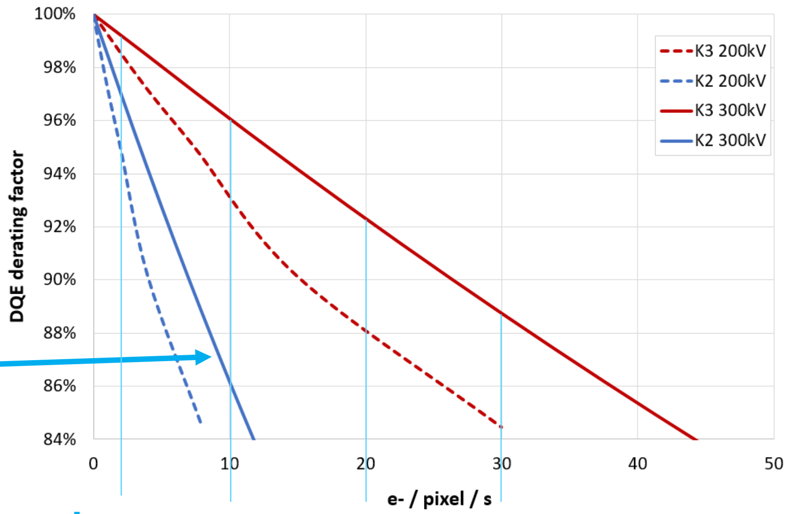
Coincidence Loss – Exposure Time Tradeoff

Count Rate vs Dose Rate (K2 300kV)



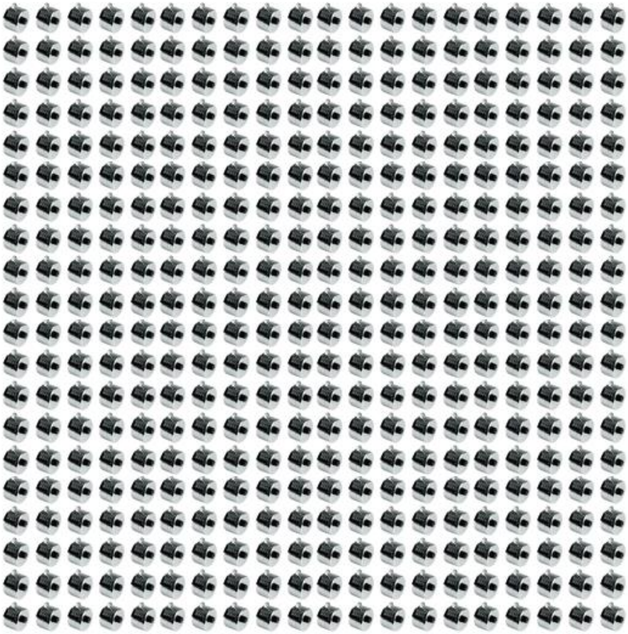
Li et al, Nature Methods (2013) Figure 1b.

DQE derating vs Dose Rate



15 e-	7.5s	1.5s	.75s	.5s
50 e-	25 s	5 s	2.5 s	1.4 s

The spatial side of counting speed.



200 counters/mm^2

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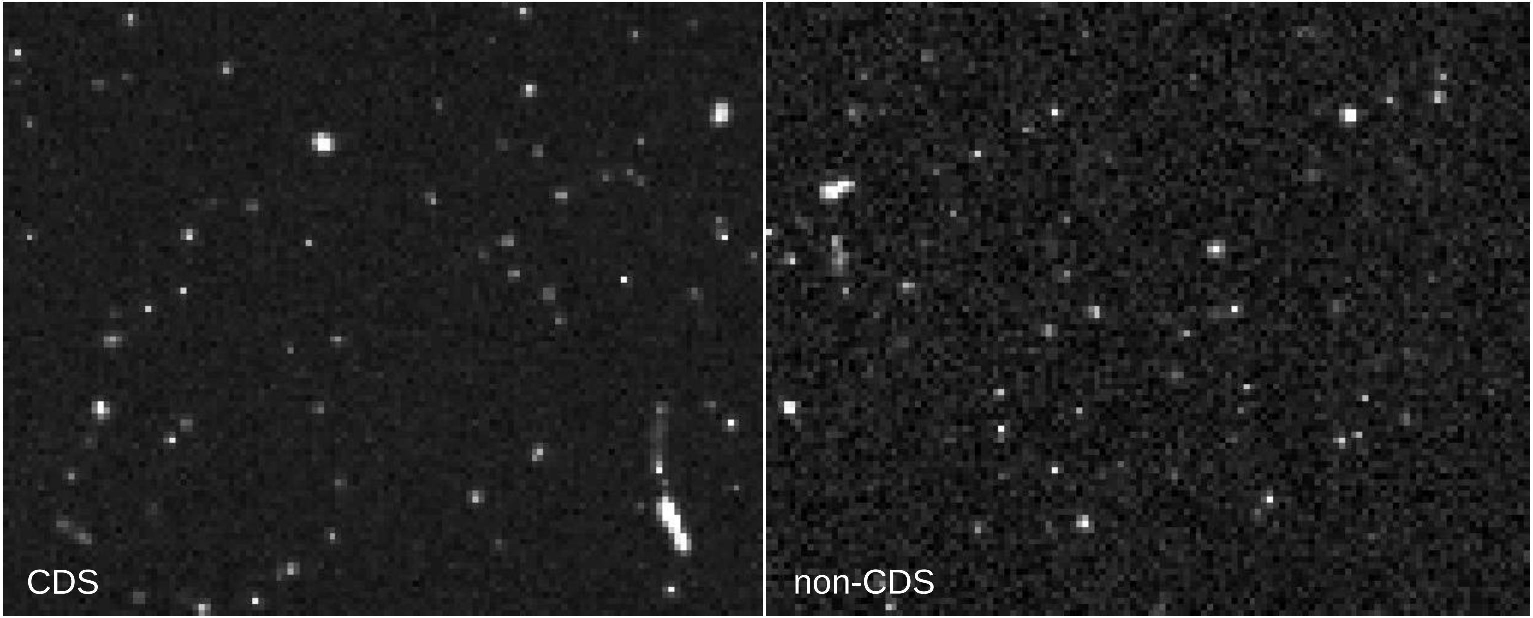
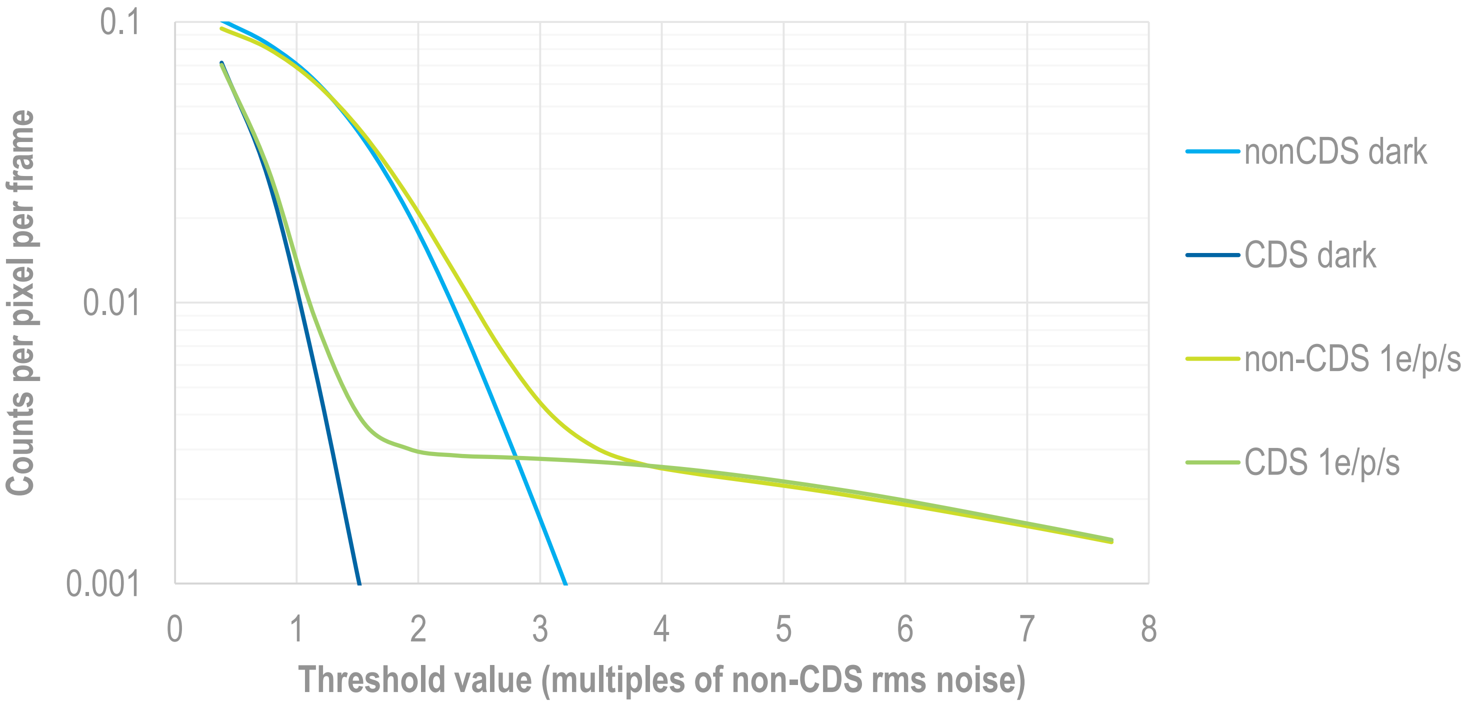
Improving SNR with Correlated Double Sampling



(same-contrast images of 200 keV electrons from K3 camera prototype)

Lower read noise allows lower counting threshold



Summary of Tradeoffs Between DQE and Throughput

Correlated double sampling

Coincidence loss vs. detection SNR

Framerate

Coincidence loss vs. Exposure time

Magnification

DQE vs particles/frame

K3's larger area and higher read rate can be spent on all of these flexibly according to the needs of the project.

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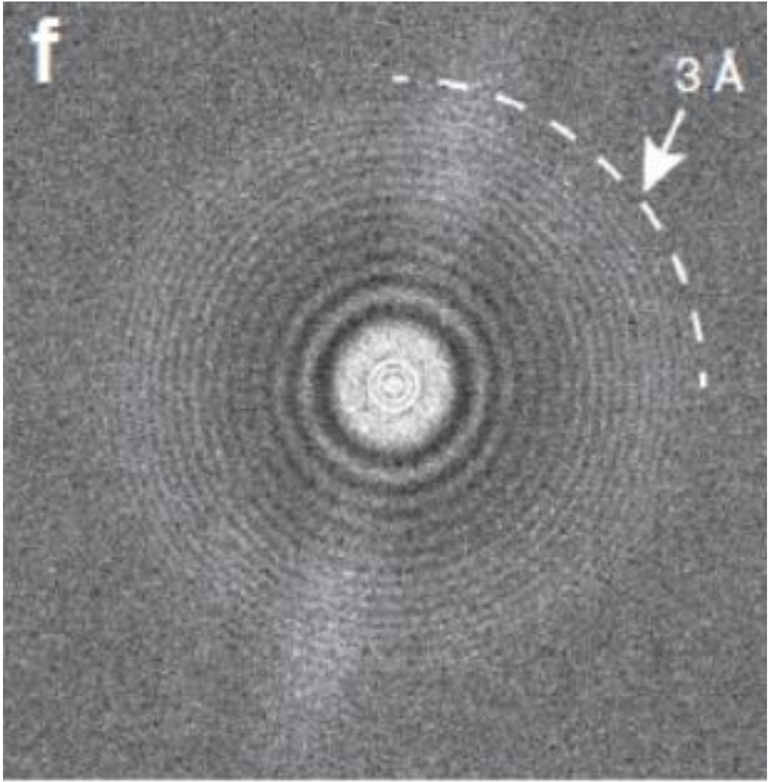
Correlated Noise

- Motion correction algorithms deal with it as this figure illustrates.
- Improvements to correction software in 2012 (in response to this result) eliminated the problem shown here.
- Further improvements coming through reduction of time from reference to sample.



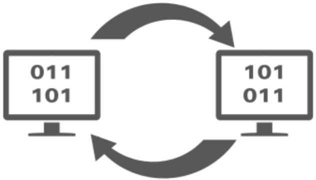
Li et al. 2013 Nature Methods, figure 2.

Looking forward: Platform integration

K2 Camera

Digitizer

K3 Camera



Summit Processor

Computer

Computer

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In Summary, K3 will provide:

- Electron counting cryo-EM for a wider base of users through accelerated workflow and 200kV performance.

- Reduced read noise and fixed pattern noise.

- Flexibility to further optimize use of speed and size for the DQE needed for a given experiment.

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Thank you for listening!

And thanks to the teams that worked to put the K3 together, especially Peter Denes and his group at LBNL,

… and to our collaborator and advisor, David Agard.

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