K3: Advancing Electron-Counting Cryo-EM

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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
More degrees of freedom means more particles.
Higher resolution demands more particles.
Better motion correction and dose weighting mean more **frames**.
Throughput
K3 Camera Framerate – 1500 fps

→ 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)
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


Cryo-EM methods leverage Electron-Counting DQE

Better DQE

Better drift correction

Better CTF measurement

Operation at lower defocus

Better specimen images

Better processing

Higher resolution

Smaller molecules
Coincidence Loss Causes Lowering of DQE

Count Rate vs Dose Rate

SNR(0) reduction vs Dose Rate

SNR(s) vs Dose Rate

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

(Based on fit to curve at left)

Chiu, et al, JSB 2015

SNR(0) reduction vs Dose Rate

Based on fit to curve at left
K2 200kV DQE is higher at low spatial frequency
High resolution being achieved at 200 kV

2.6 Å at 200 kV without image filtering or phase plate

Herzik, Wu and Lander, Nature Methods 2017

Image courtesy of Gabriel Lander
What is the Best Magnification and Binning?

K2-XP DQE at 2 e/pix/s

- 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

Constant temporal sampling
Framerate based on specimen speed and resolution content

FFT
iFFT
And resolving conformational states demands better $DQE$
Realtime DQE
Coincidence Loss – Exposure Time Tradeoff

Count Rate vs Dose Rate (K2 300kV)

DQE derating vs Dose Rate

Li et al, Nature Methods (2013) Figure 1b.
The spatial side of counting speed.

200 counters/mm²
Improving SNR with Correlated Double Sampling

(same-contrast images of 200 keV electrons from K3 camera prototype)
Lower read noise allows lower counting threshold

Counts per pixel per frame

Threshold value (multiples of non-CDS rms noise)

- nonCDS dark
- CDS dark
- non-CDS 1e/p/s
- CDS 1e/p/s
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.
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

Summit Processor

Computer

K3 Camera

Computer
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.
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.