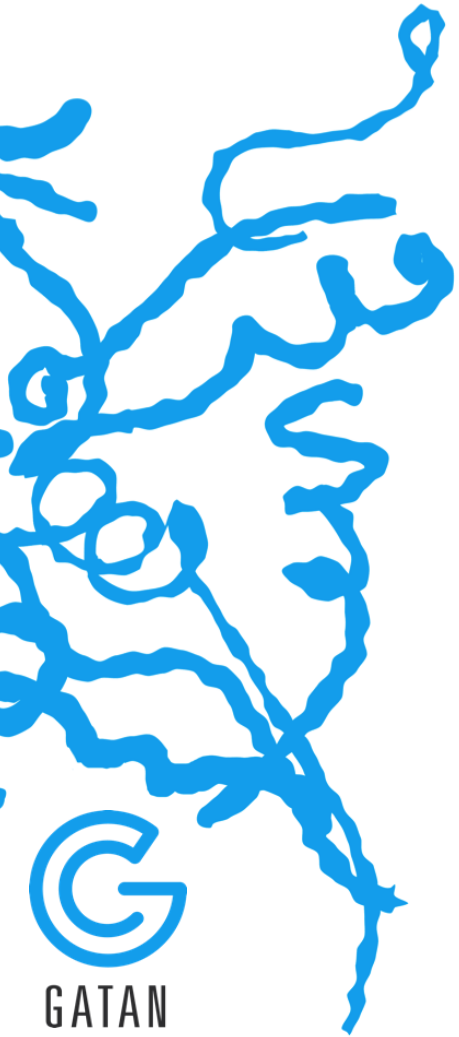


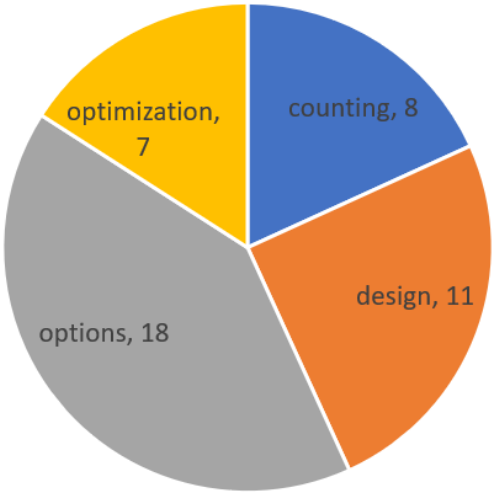
Paul Mooney

EMBO Cryo School, Birkbeck College, 9.9.2019

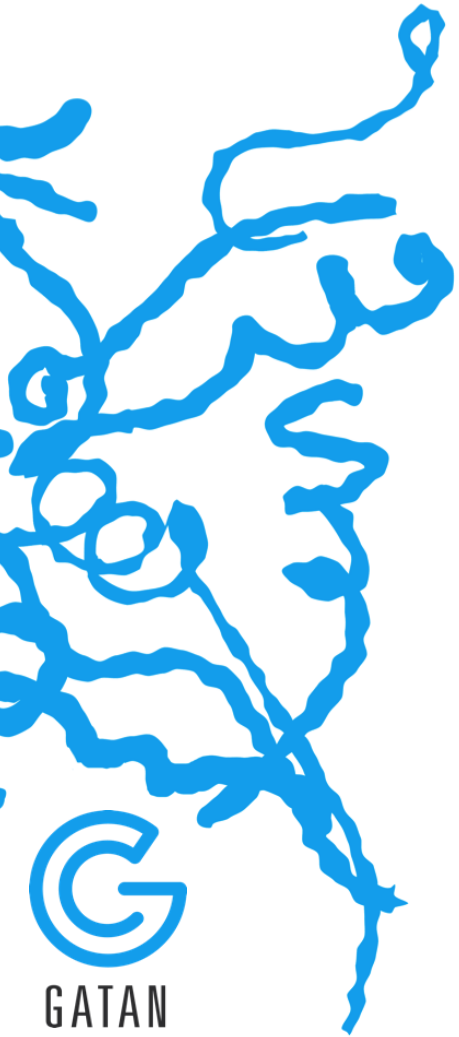
Getting the most out of a counting direct detector

Talk Plan

- What is counting? — Slides 4-11
- Getting the most with design. — Slides 13-22
- Getting the most in operation: what are the choices? — Slides 24-41
- Getting the most: how do we decide? — Slides 42-48



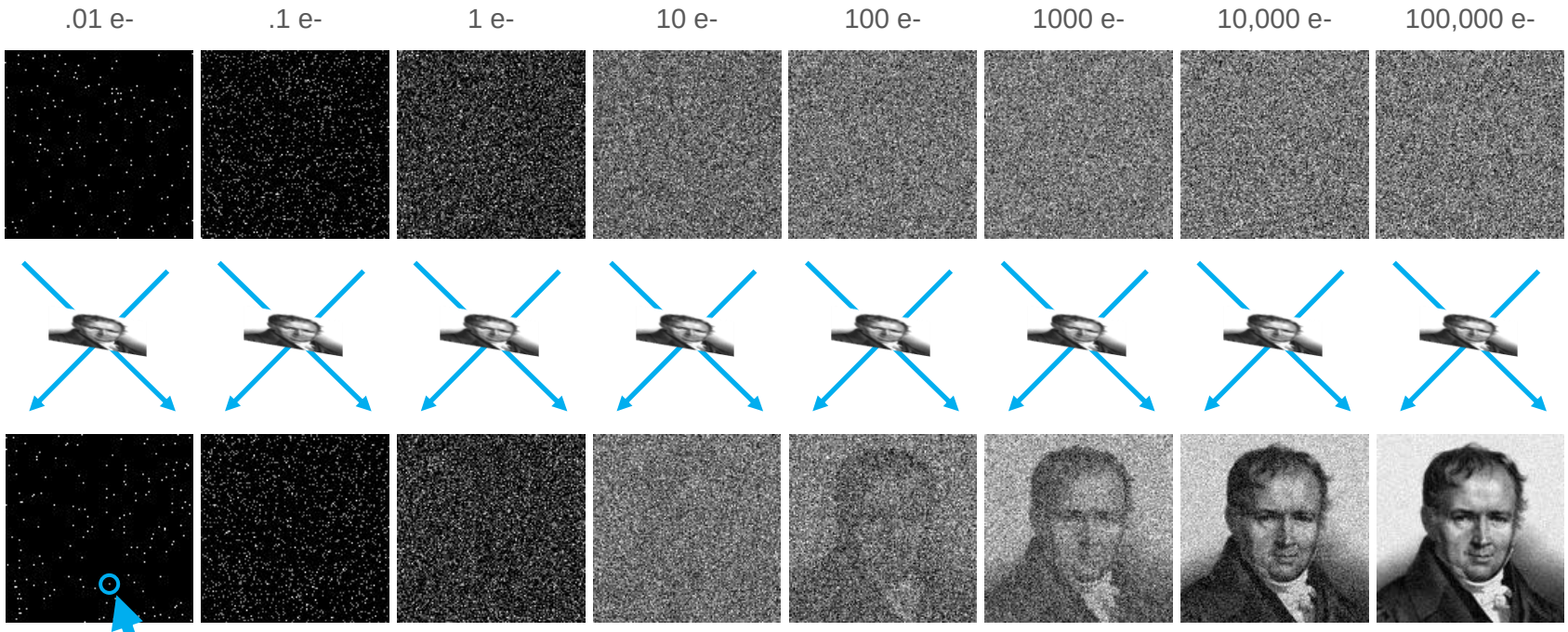
GATAN



Section 1

What is counting and why do we do it?

All Imaging uses quanta

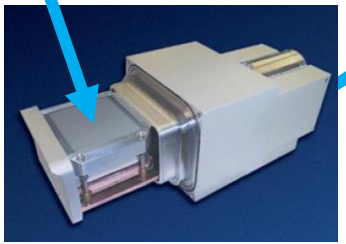


Siméon Denis Poisson

Before image capture with a camera or an eyeball.

So there is something to count if one choses to.

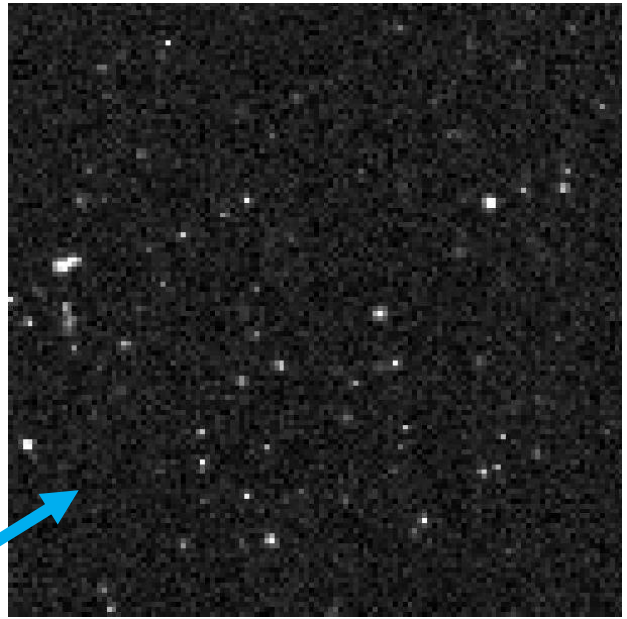
Analog capture of quanta at high dose



Analog capture of quanta at low dose

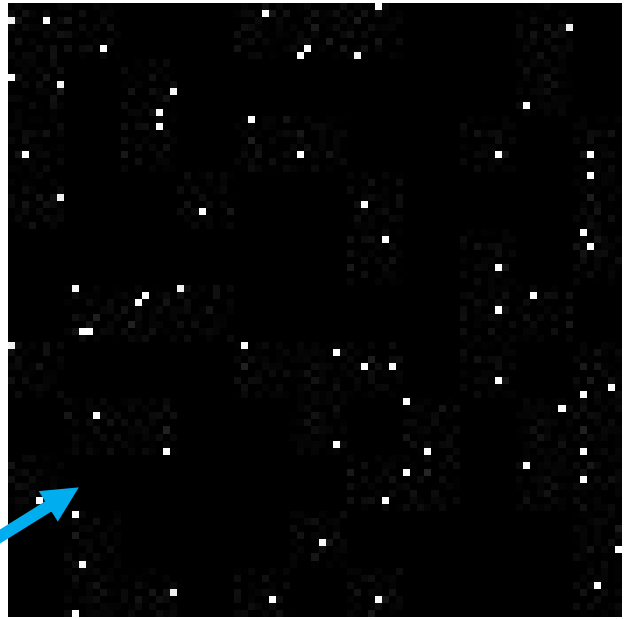
Gatan K3 in linear mode

Electrons as they appear before counting.



Counted capture of quanta at low dose

Electrons counted where they arrived.



Gatan K3 in counting mode

Counted capture of quanta at high dose

Gatan K3 in counting mode

Counted image after summing.

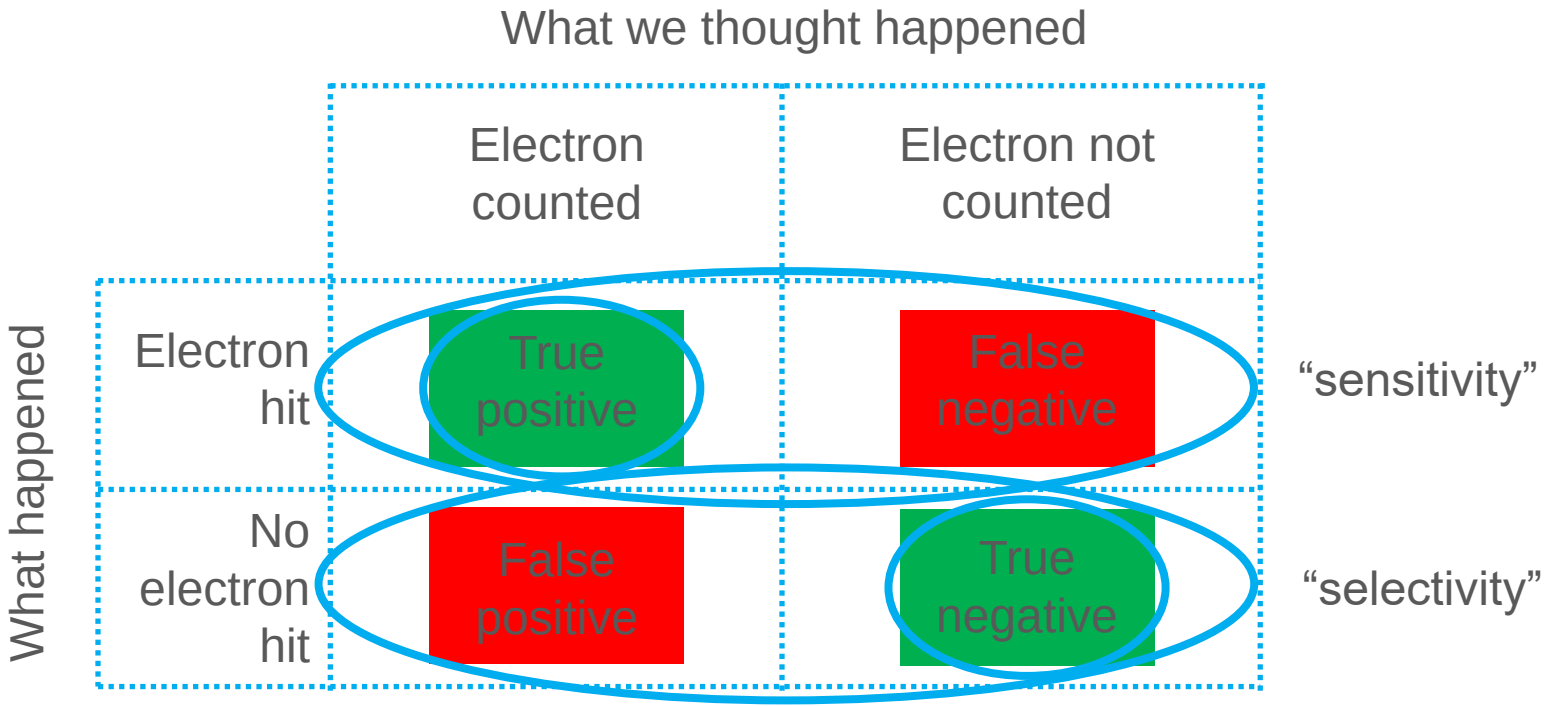
Counting reduces image acquisition noise

Analog accumulation

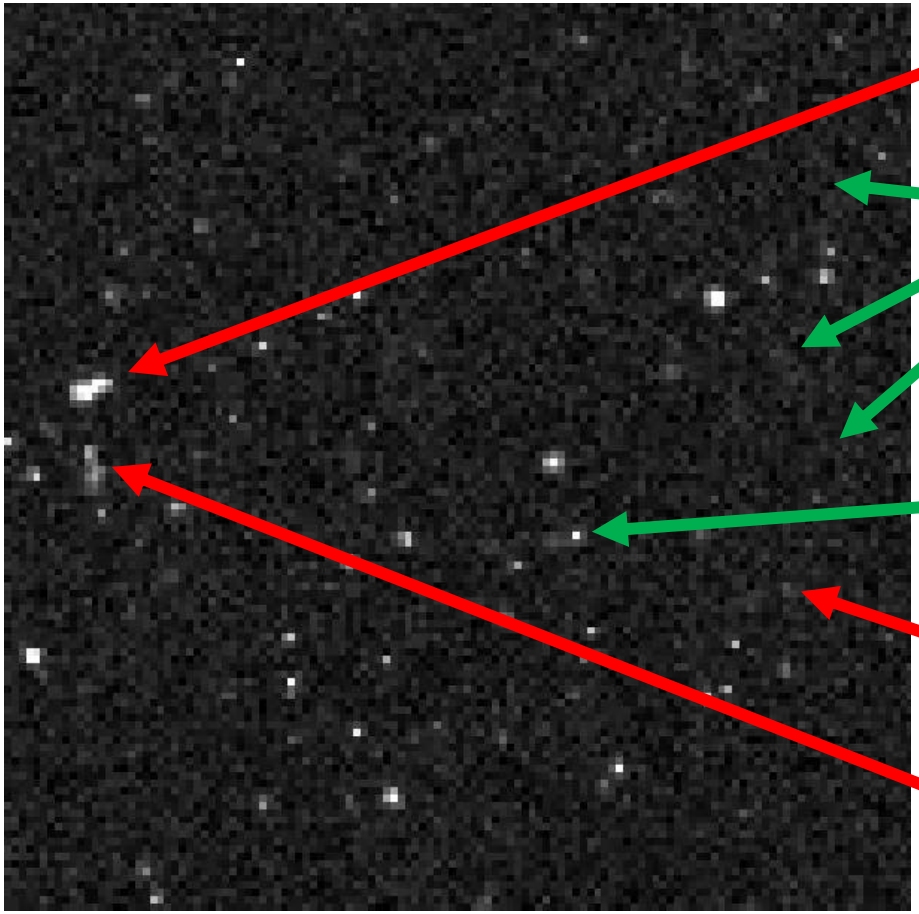
Counted accumulation

GATAN

“Counting” is done by discrimination



And discrimination is non-trivial



One electron or two?

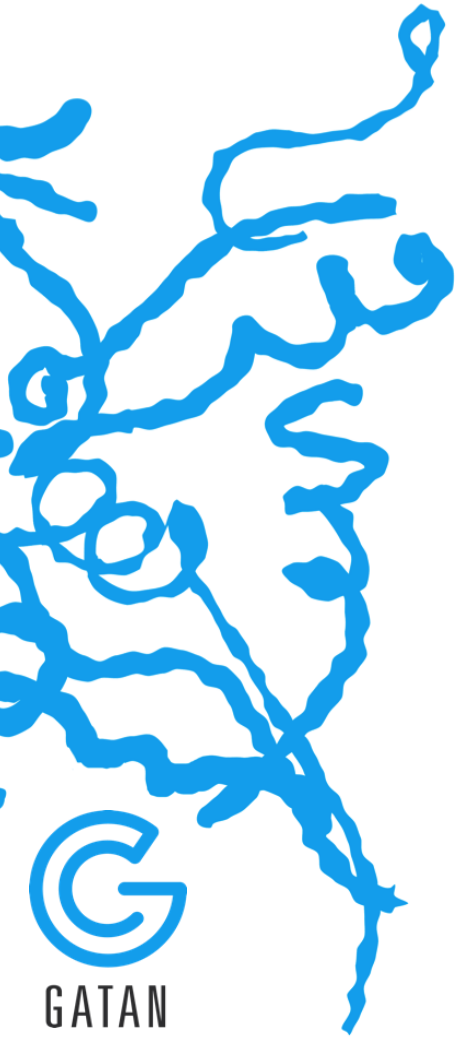
Probably no electron –but have to get this right many many times

Probably one electron

Maybe an electron

Possibly a scattered electron

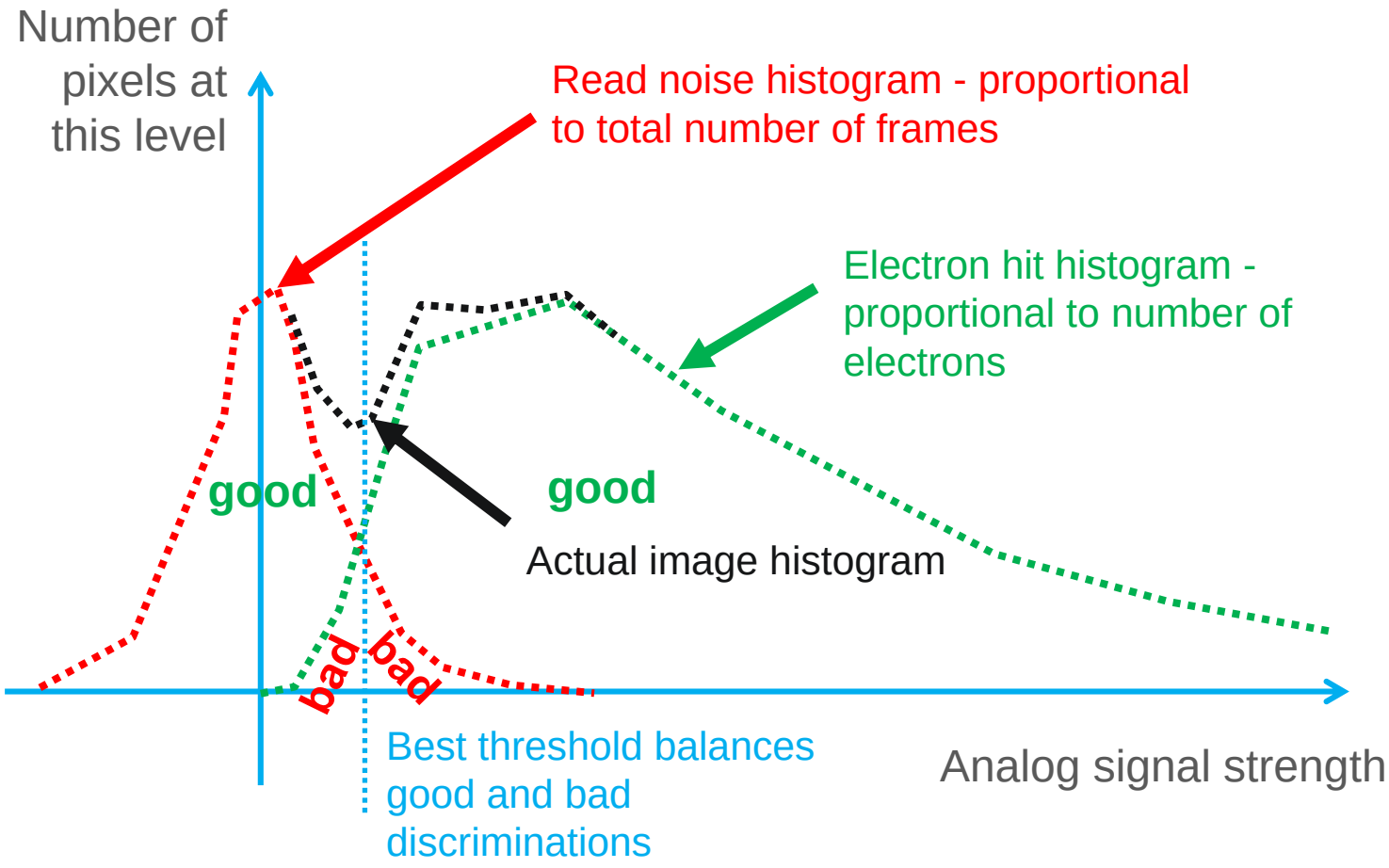
GATAN



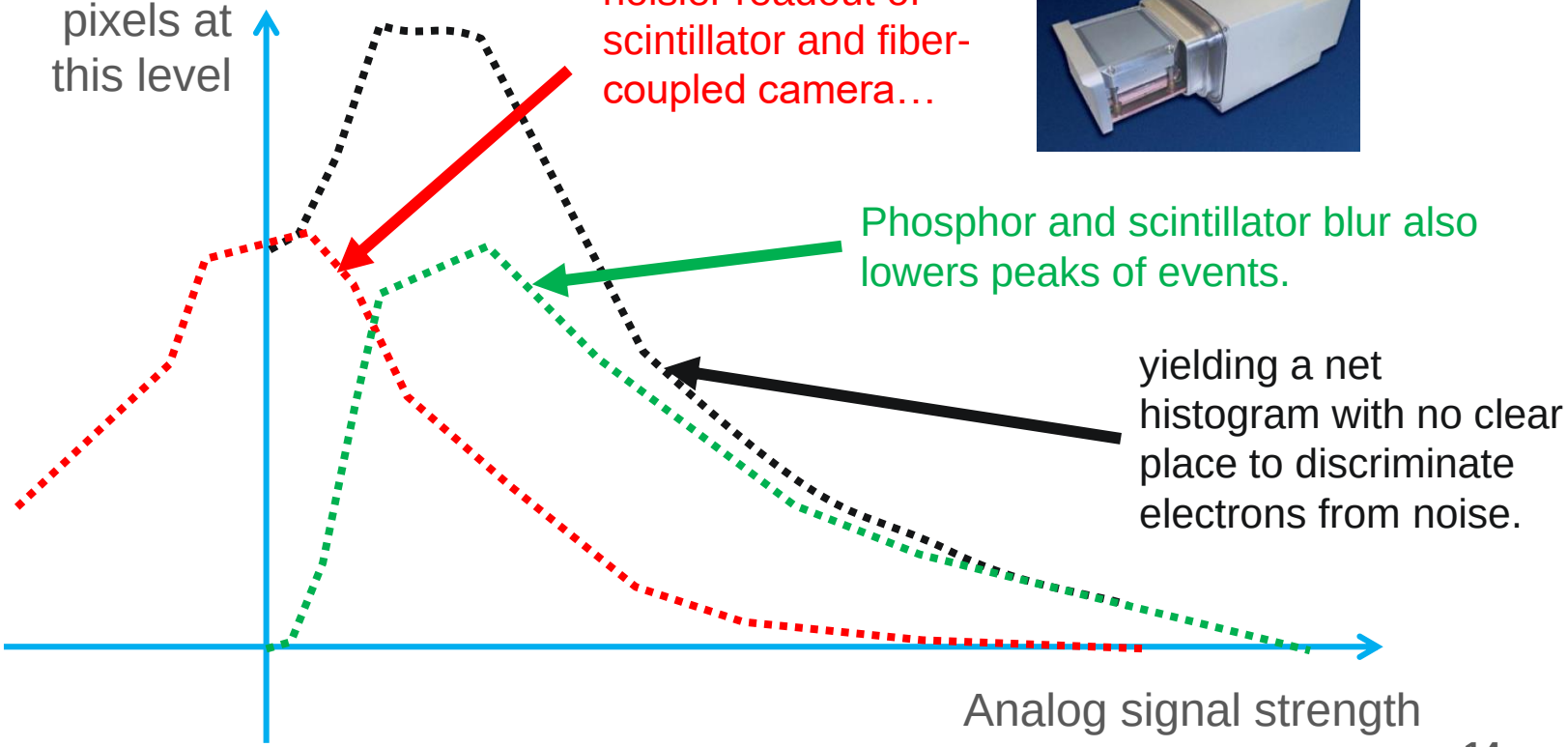
Section 2:

Getting the most out of counting with design

Discriminating electrons from background

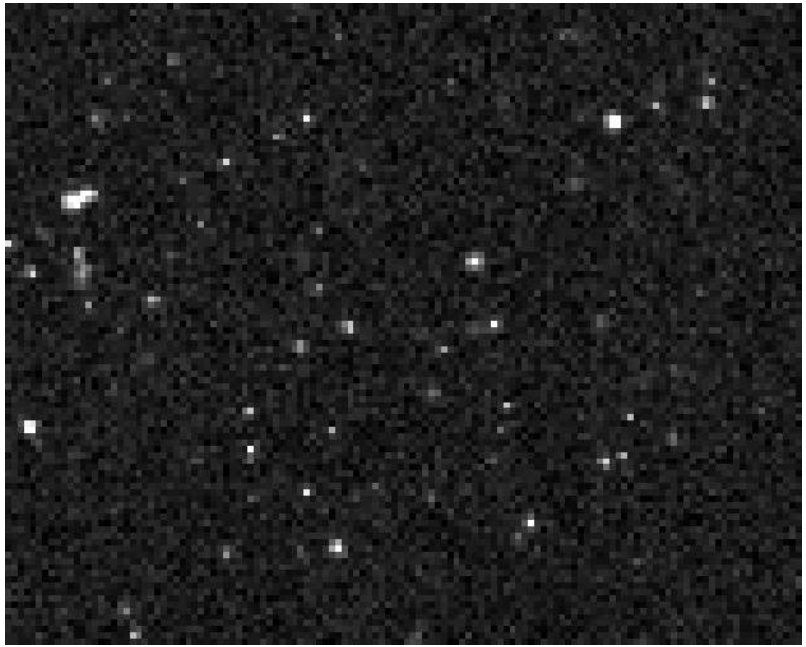


Indirect detectors won't work for counting



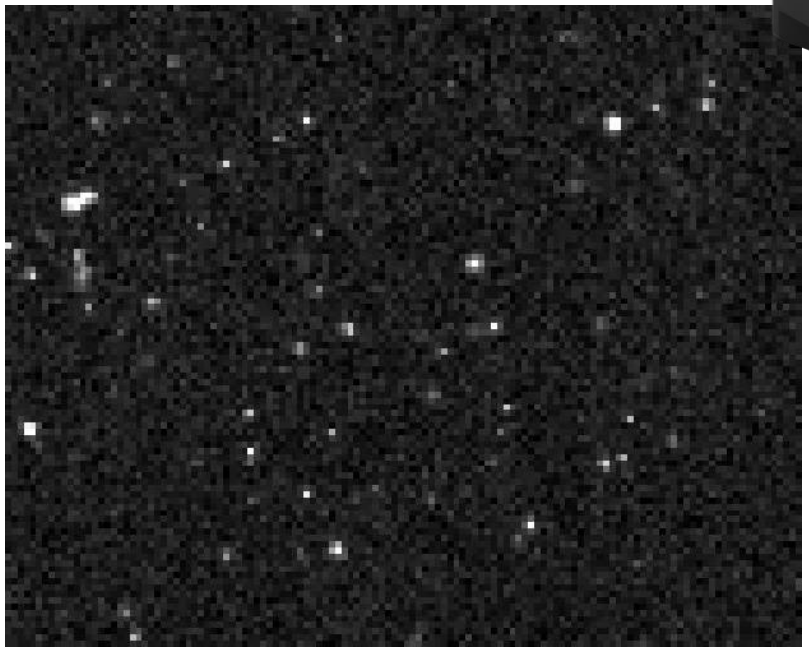
GATAN

A silicon direct-detection sensor (as in the Gatan K3) makes electrons and background noise more easily distinguishable

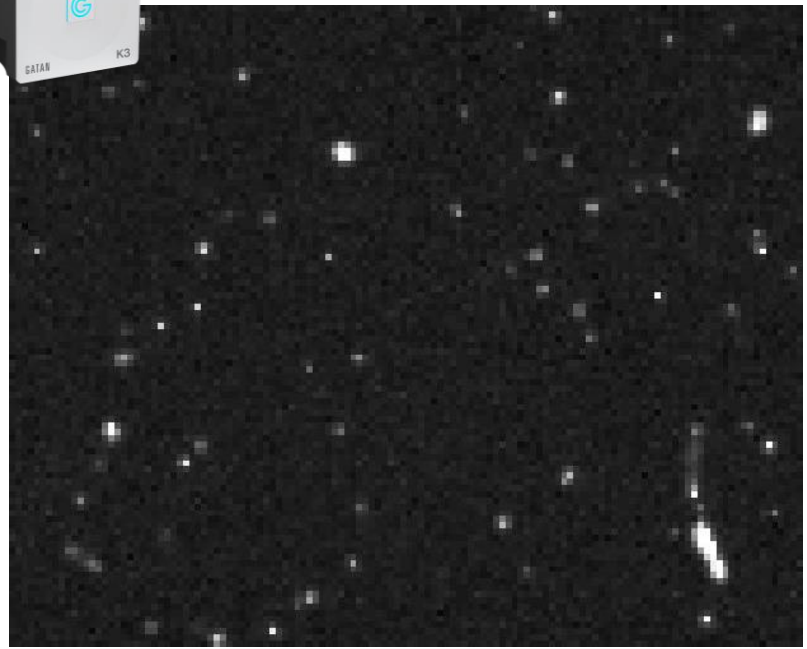


GATAN

Read noise can be lowered even more with Correlated Double Sampling

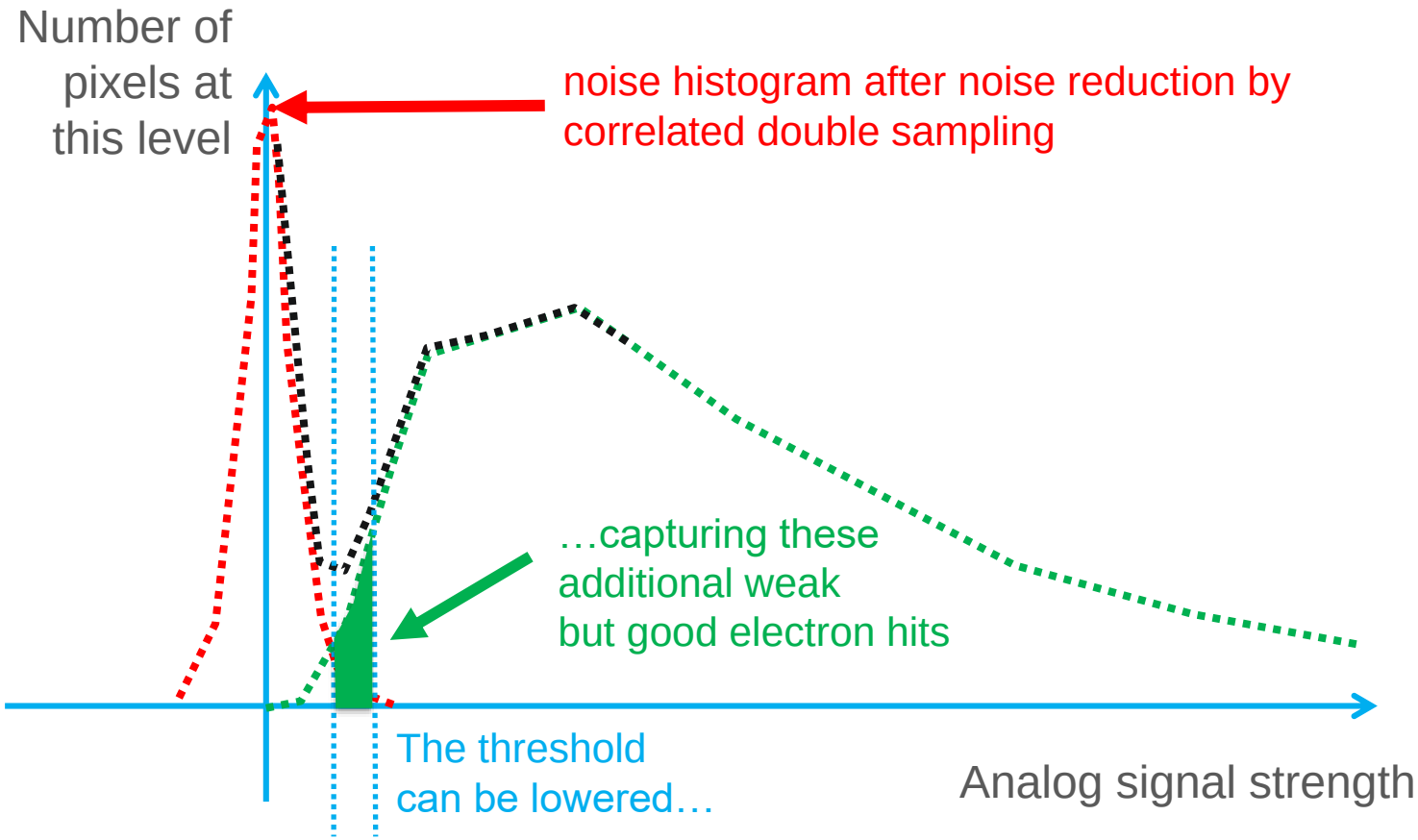


non-CDS

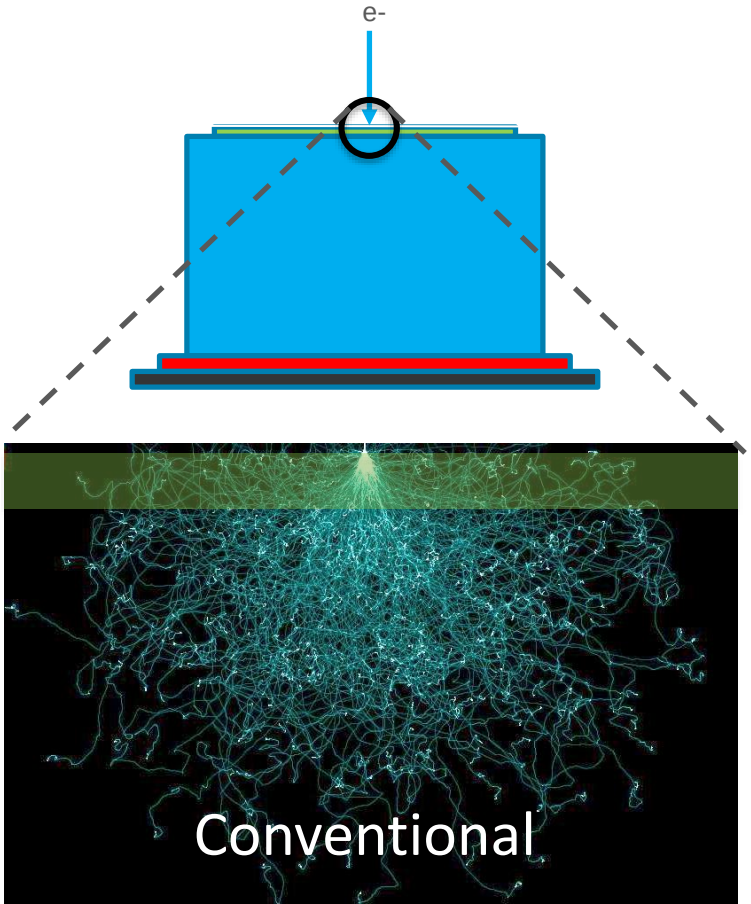


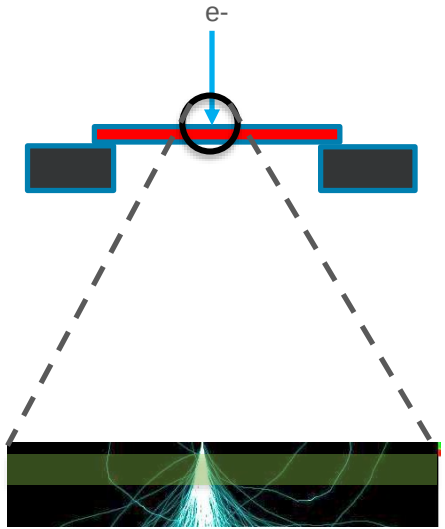
CDS

CDS allows the discrimination threshold to be lowered



Thinning minimizes false positives from scatter

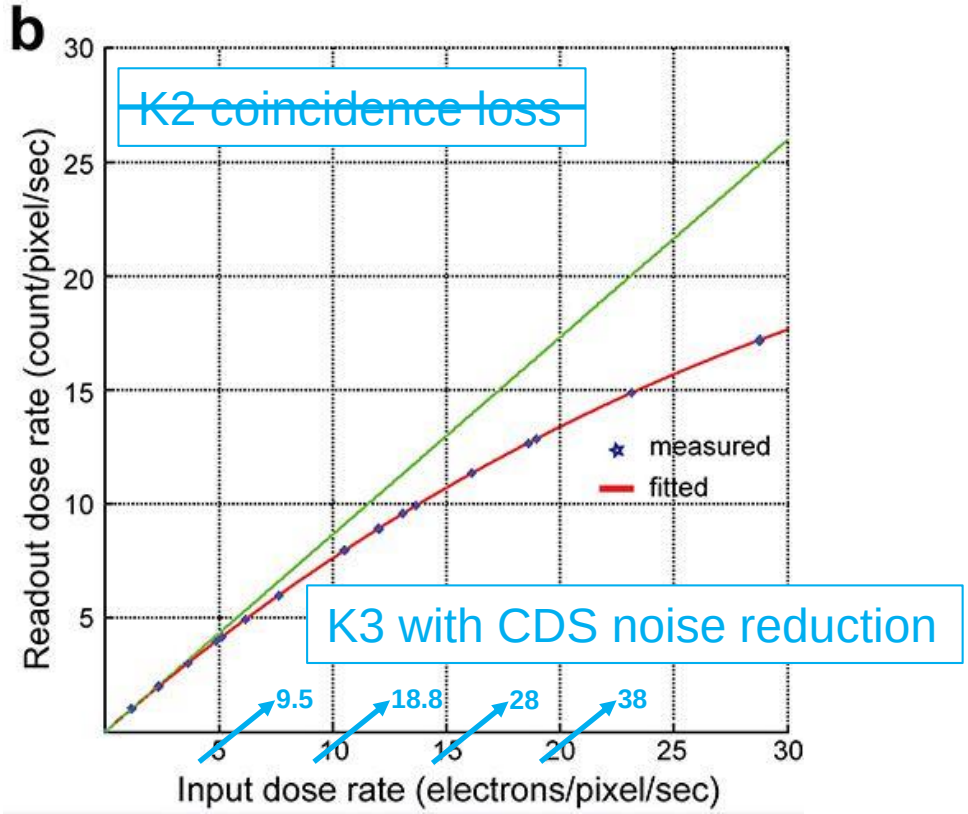




Direct Detection

GATAN

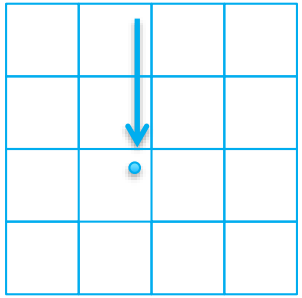
High speed minimizes false negatives from coincidence Loss



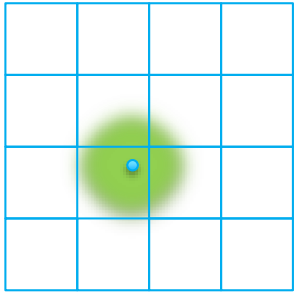
Li et al, Nature Methods 10, 584–590 (2013).

Super-resolution by centroiding extracts more location information from each event

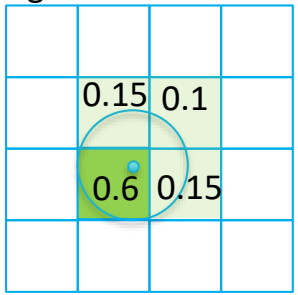
1. Electron enters detector



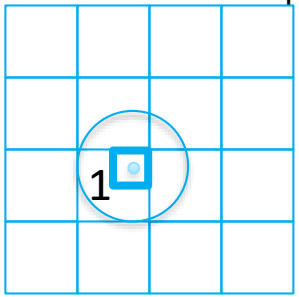
2. Signal is scattered



3. Charge collects in each pixel

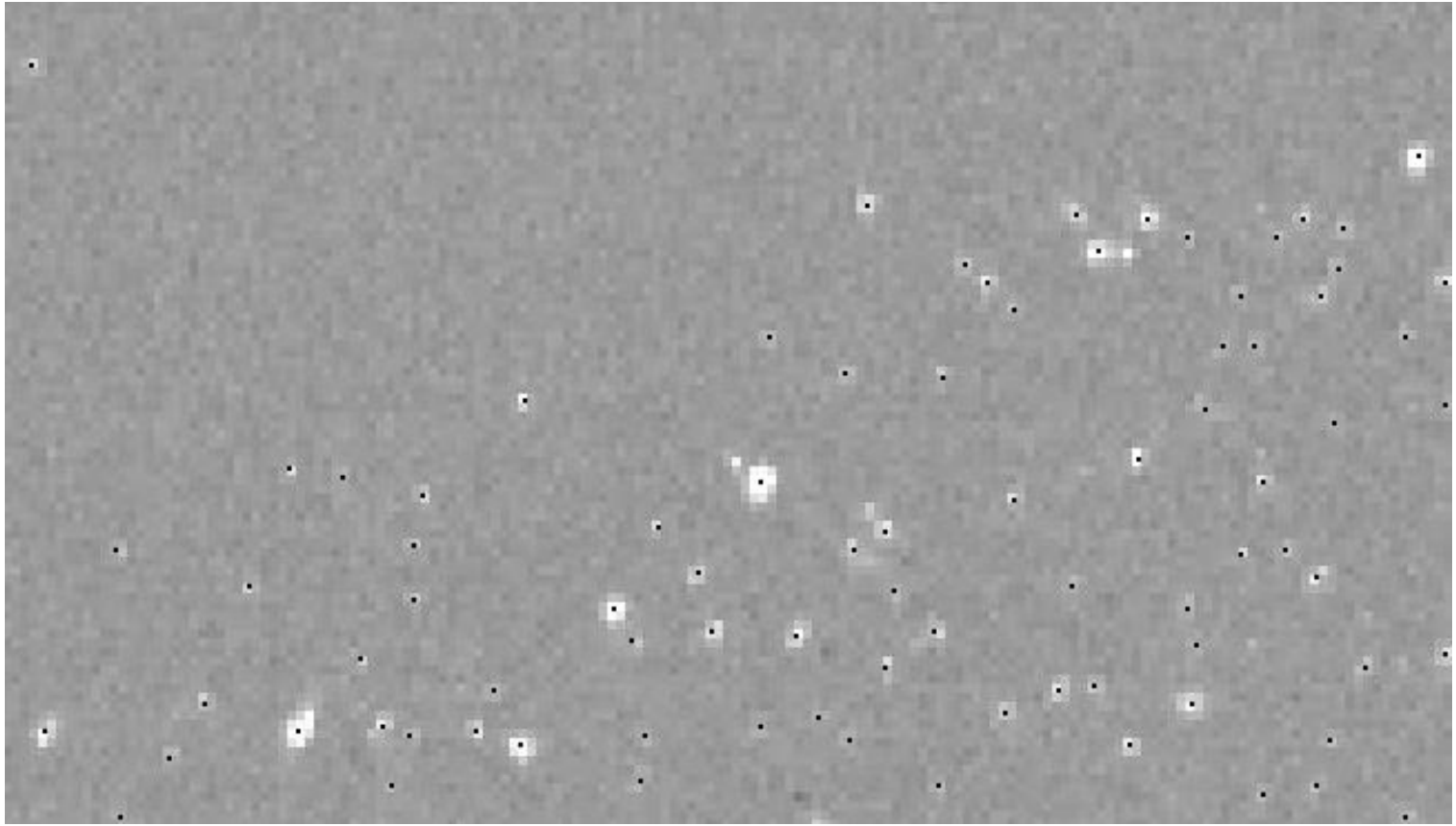


4. Events are localized with sub-pixel accuracy



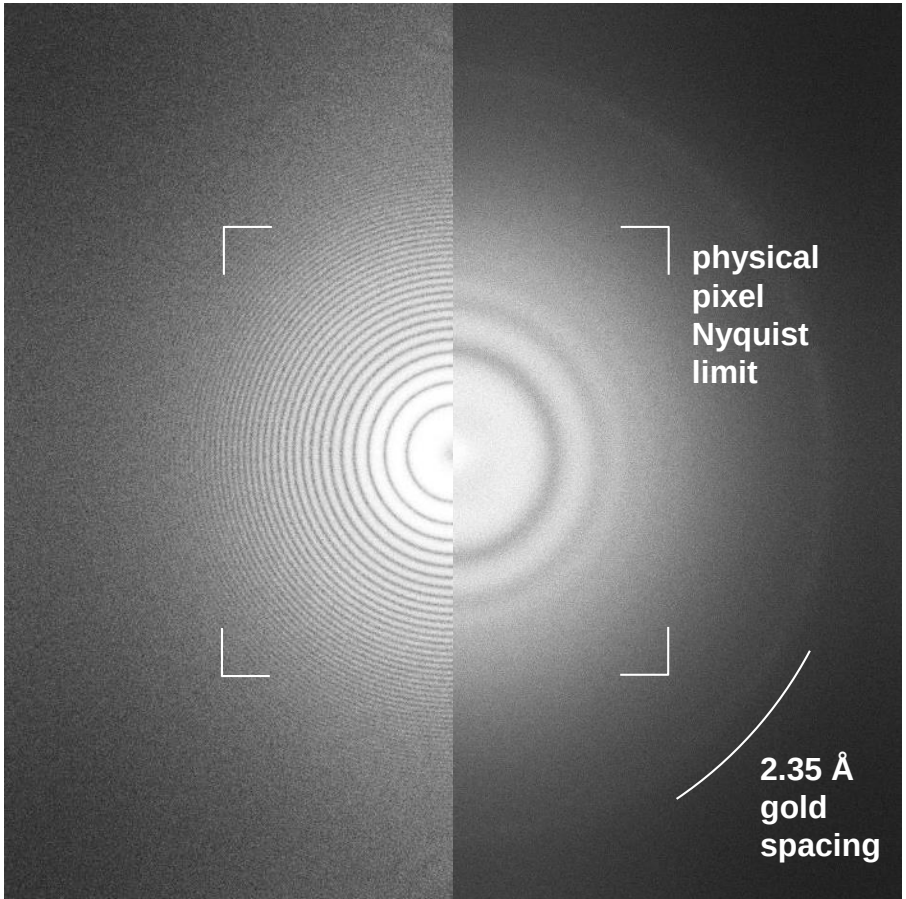
GATAN

This example shows that centroid locations are plausible



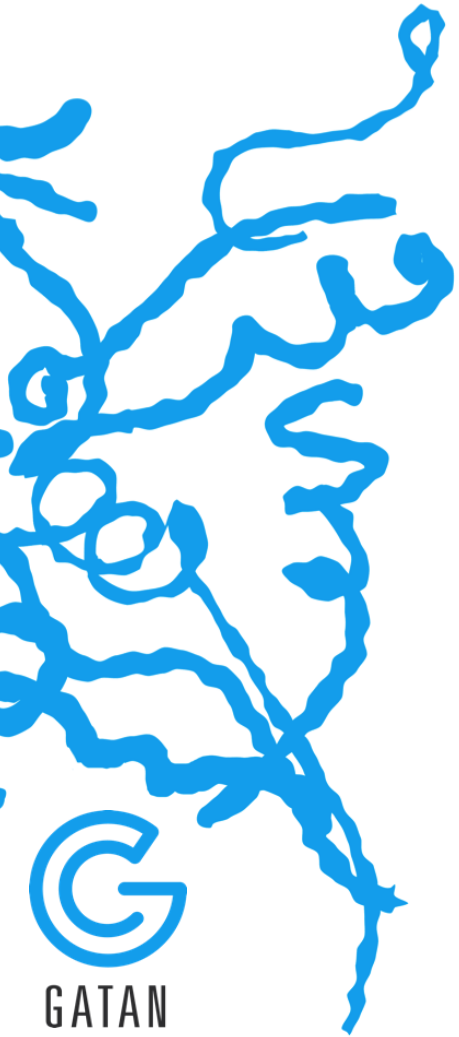
GATAN

And these examples show that super-resolution is real and free of artifacts



Pt/Ir
55 e/Å2

K2 Summit in super-resolution mode, 1Å super-pixel, 300kV, end of GIF.



Section 3:

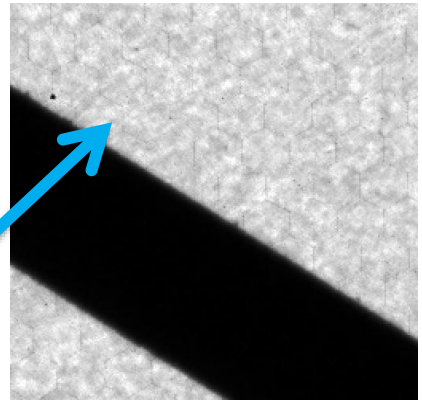
Getting the most out of a counting detector in operation: the options

Choices to make when setting up to image

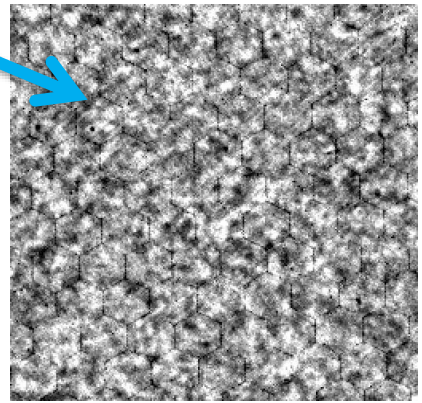
- Gain correction – how long?
- kV
- Dose rate – how fast?
- Magnification – how fine, how big?

GATAN

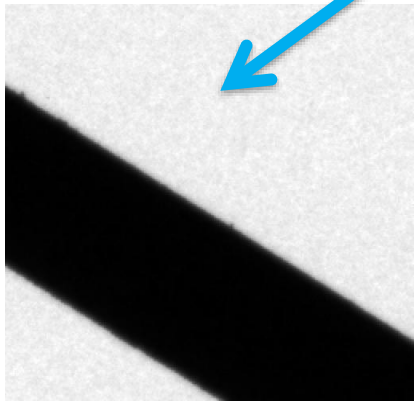
Gain Normalization corrects fixed pattern noise



Same fiber-optic pattern in image and reference

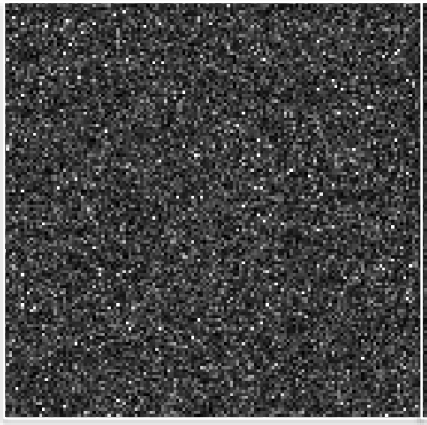
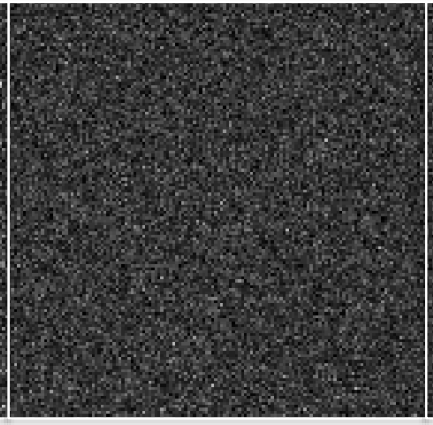
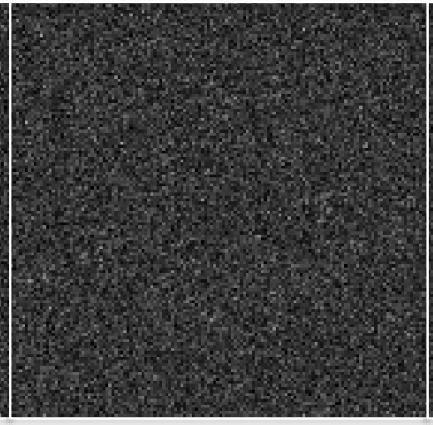
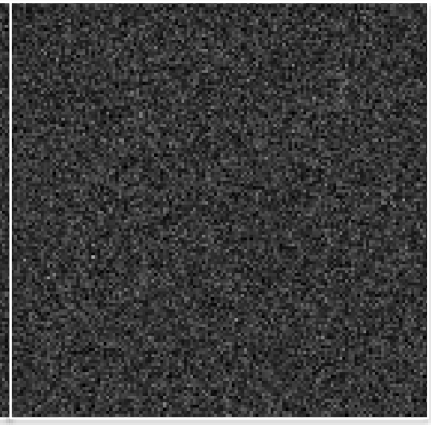


=



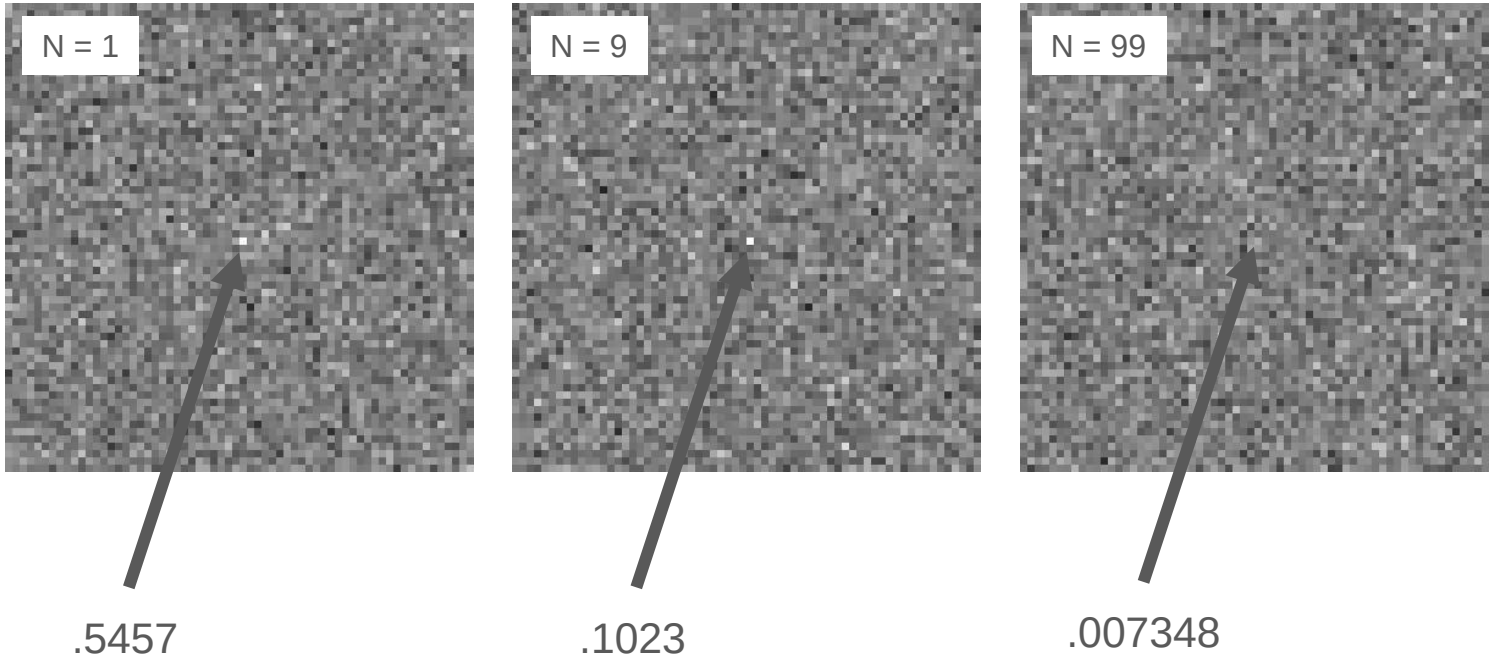
Cancelled in ratio image

Beware: gain normalization by a noisy gain reference can *create* fixed pattern noise while trying to correct it.

10 e- image	10 e- image	10 e- image	10 e- image
			
10 e- gain reference	30 e- gain reference	100 e- gain reference	300 e- gain reference

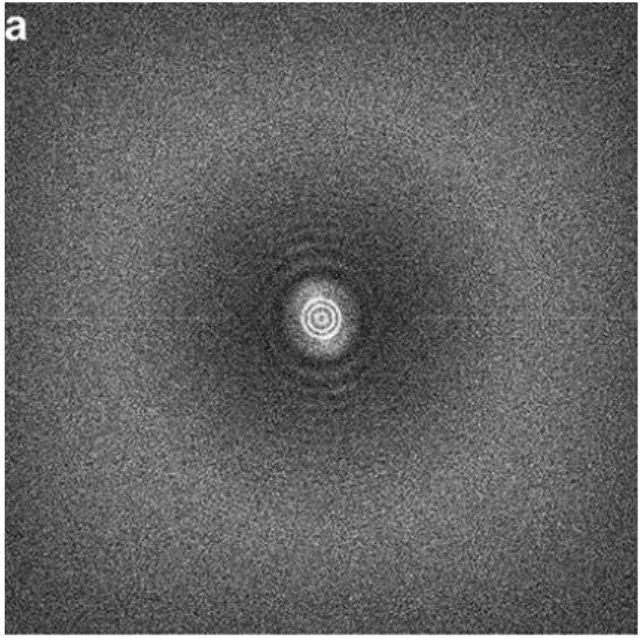
Uncorrected fixed pattern noise causes self-correlation

Cross-correlated 20e- images with N times that dose in the gain-reference (simulated)

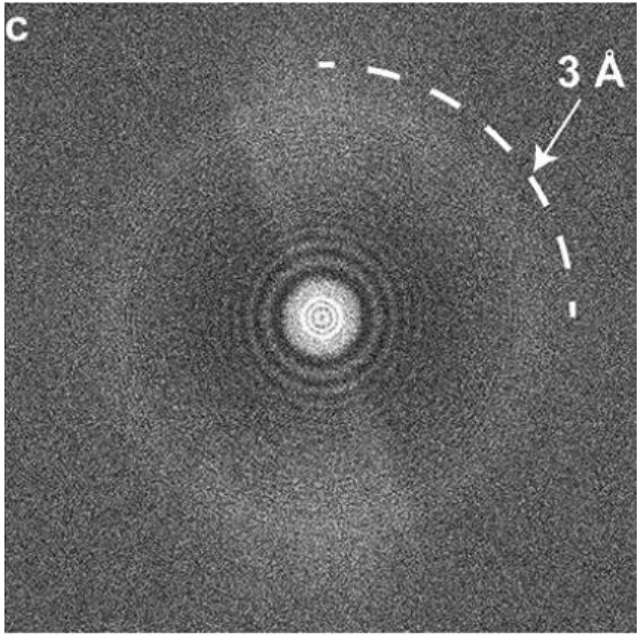


Fixed pattern noise also causes… uh… noise

Images from Li et al, Nature Methods, 2013



Without motion correction, high-frequency specimen contrast is destroyed.



With motion correction high-frequency fixed pattern contrast is destroyed because now it is the detector that is drifting.

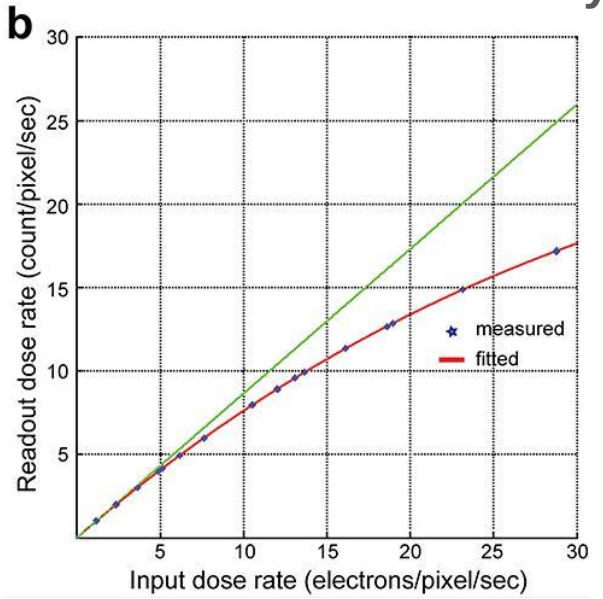
The stripe of fixed pattern contrast that remains shows that fixed pattern noise IS noise and reduces image quality, even when it isn't obvious.

GAIN

GATAN

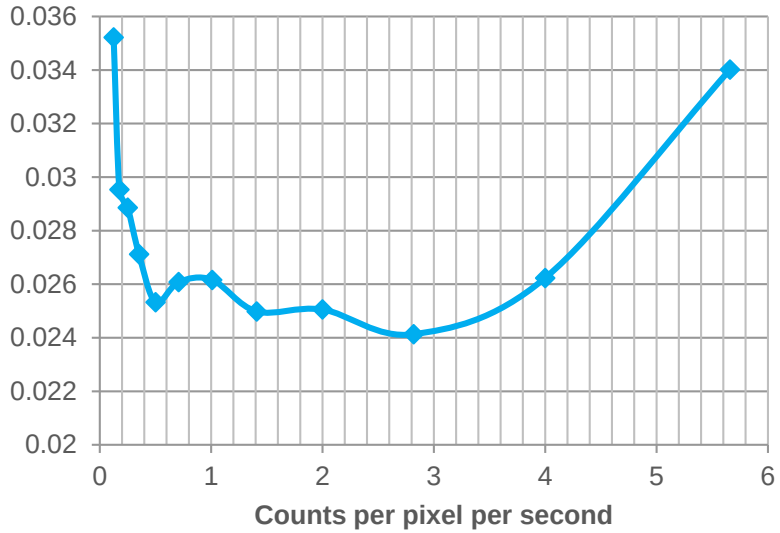
Coincidence loss creates mild non-linearity – but the gain reference can still be used over a large range of dose rates

K2 conversion efficiency

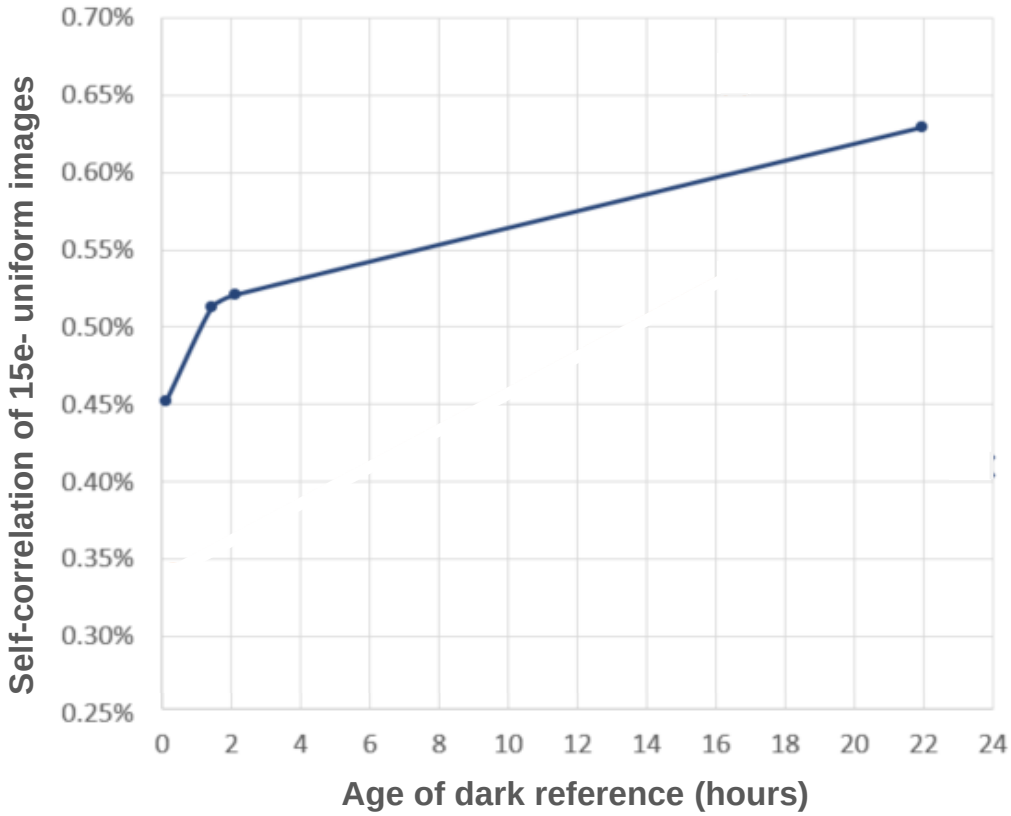


(from Li et al, 2013)

K2 fixed pattern noise fraction after normalization



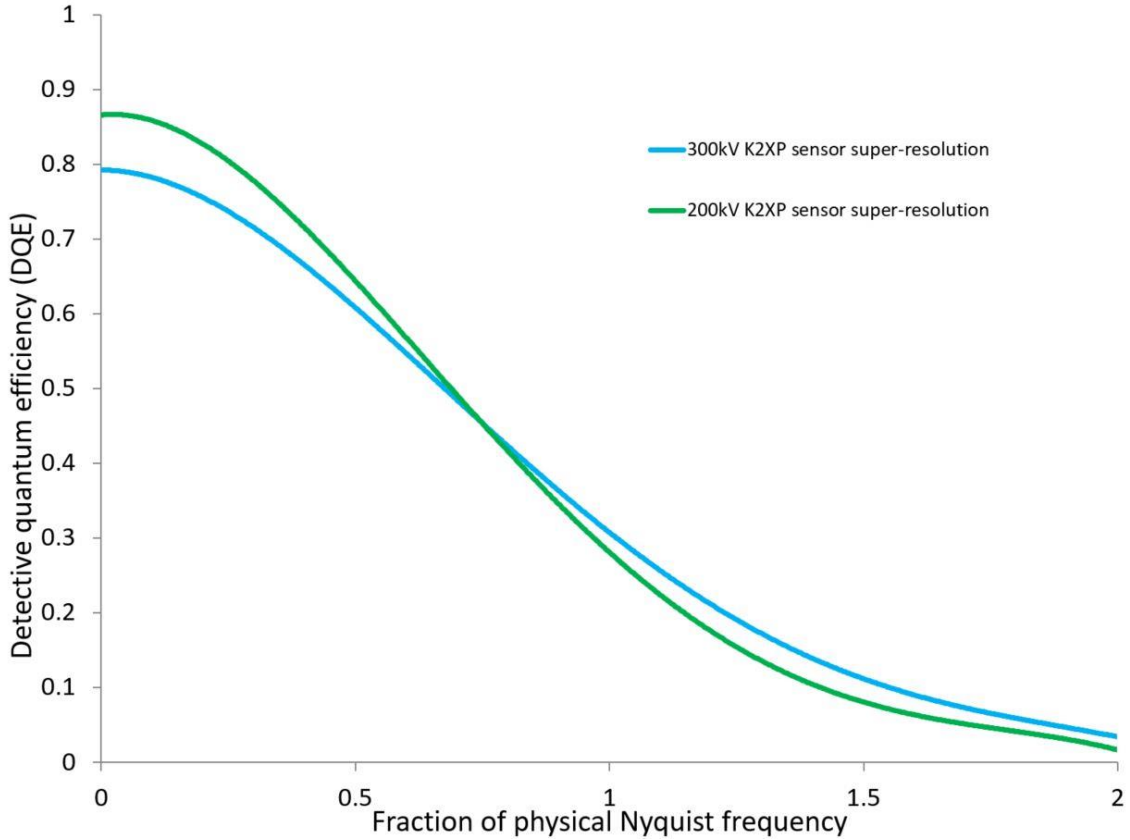
A 6000 electron gain reference provides < 1% self-correlation for > 1 day



So take a long gain reference at the target dose rate

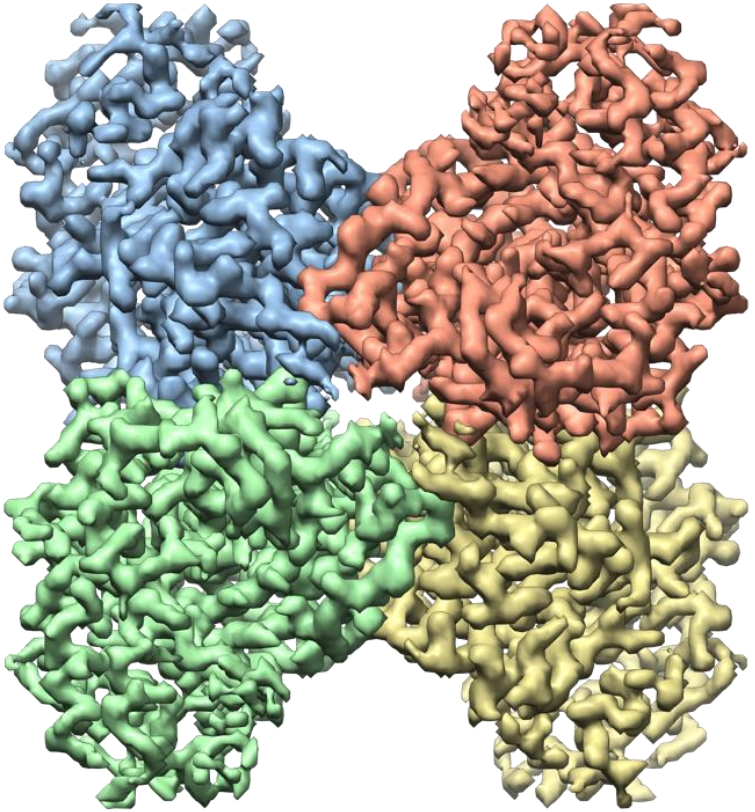
- If your exposures are to have total dose of 20 electrons, a 2000 electron gain reference will increase noise power by only 1% and yield self-correlations between 20 electron exposures of only 1%
- A 6000 electron gain reference will give extra margin, so that as the gain reference ages over a day or two, the effects will still remain under 1%.
- Dark reference update restores the validity of the gain reference – so the time invested in making the gain reference lasts for much longer than a few days.
- Note that motion correction *does* reduce high-frequency fixed pattern noise, so the gain reference dose requirement may be somewhat lower in practice.

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

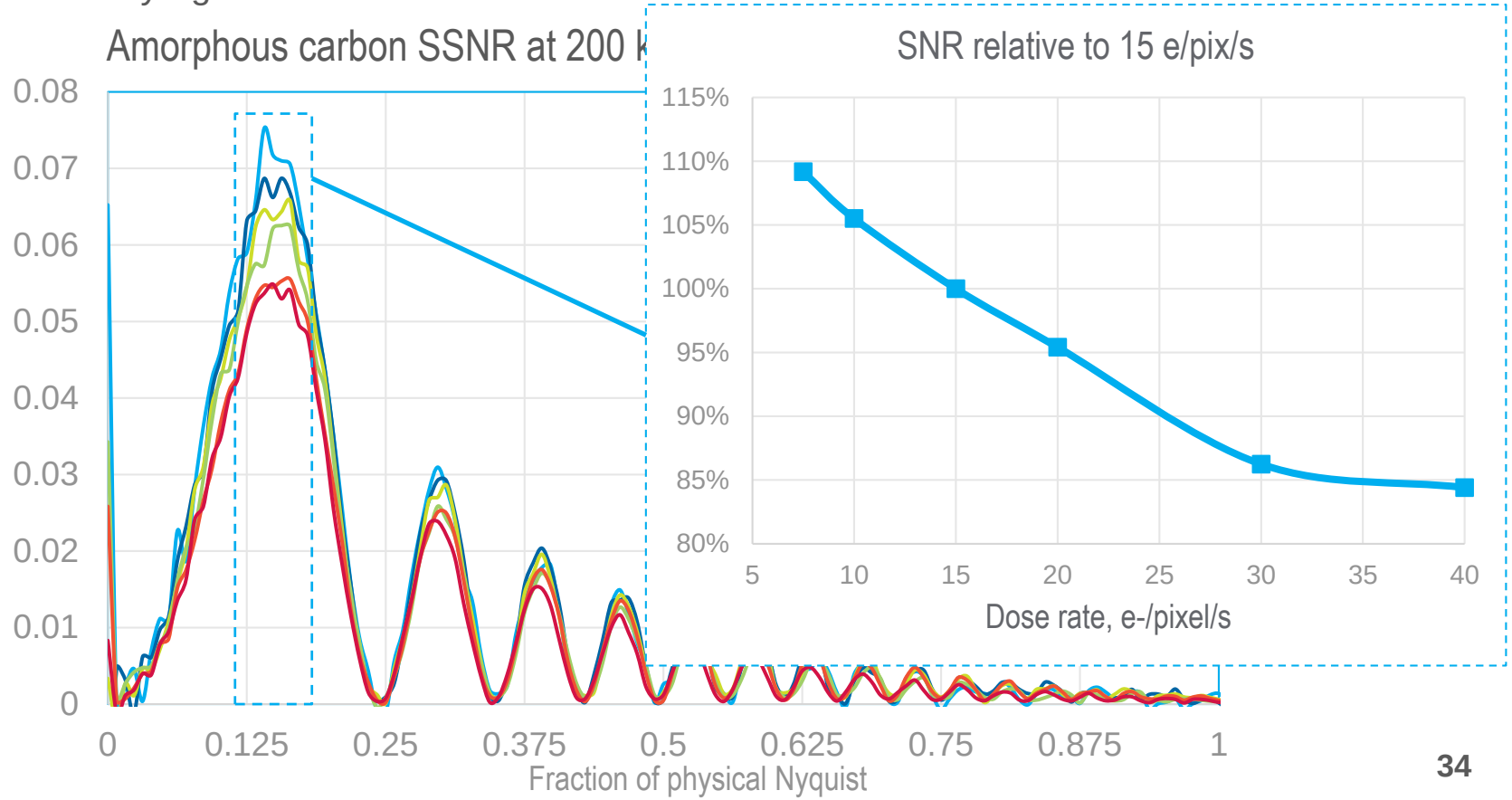


Herzik, Wu and Lander, Nature Methods 2017

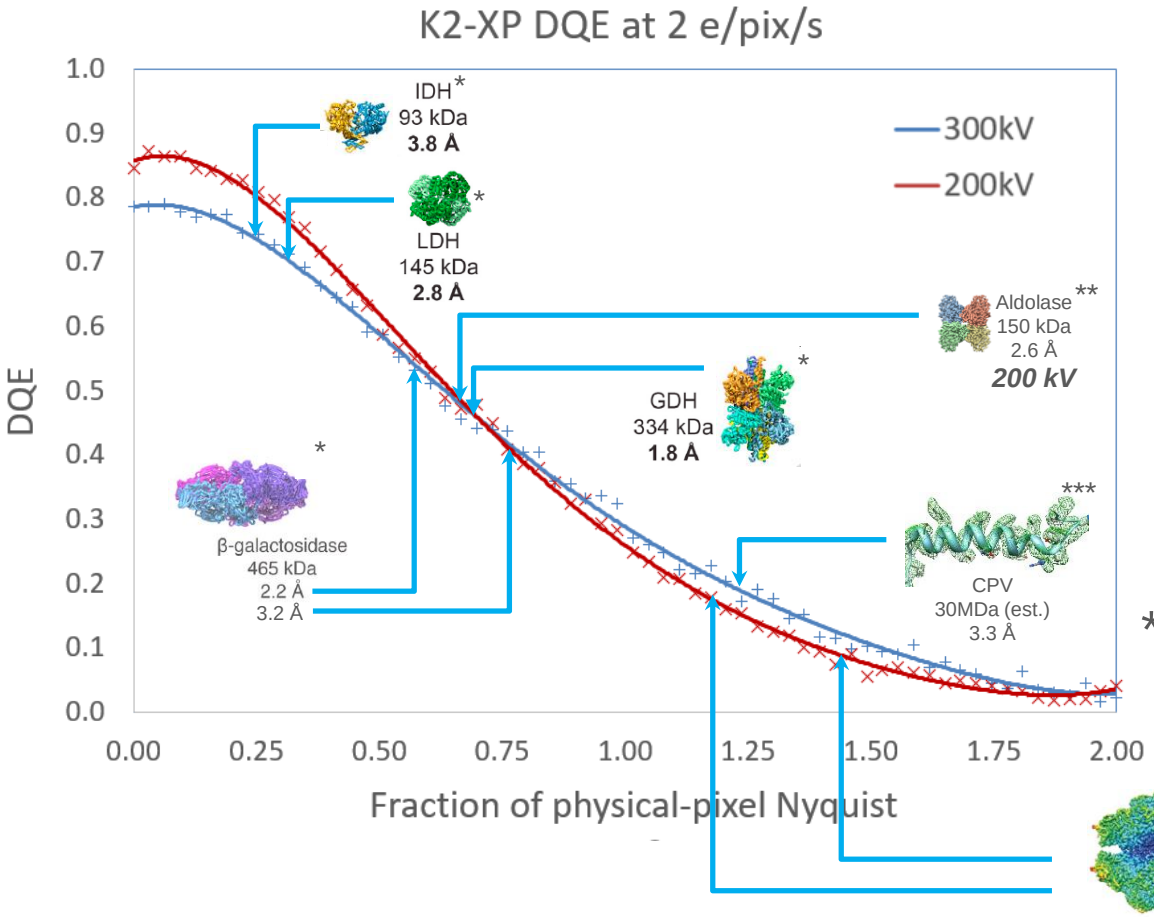
Image courtesy of Gabriel Lander

Small-signal AmC SNR implies higher DQE at lower dose rate

Work by Agustin Avila-Sakar



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)

**** 200kV, Feathers, Spoth, Fromme, BioArxiv 2019

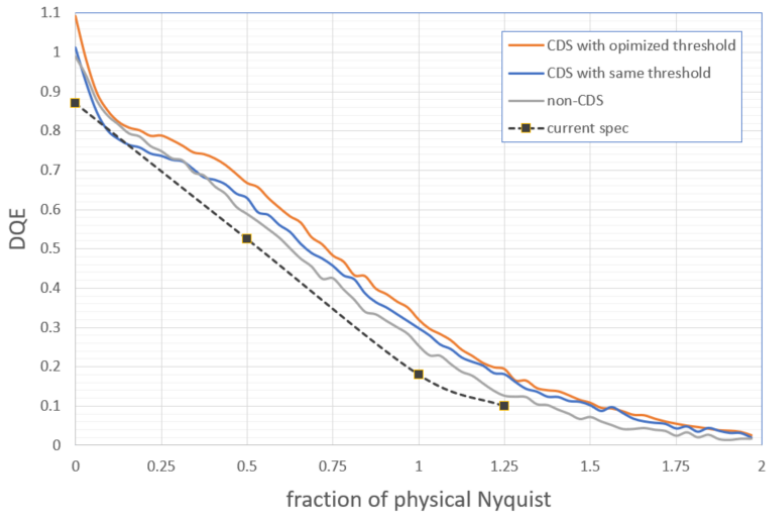
How do we decide the best parameters?

- DQE – a measure of the camera's efficiency at generating the above kind of SNR from limited dose.

$$DQE(N, \boldsymbol{s}) = \frac{SNR_{out}(N, \boldsymbol{s})}{SNR_{in}(N, \boldsymbol{s})}$$

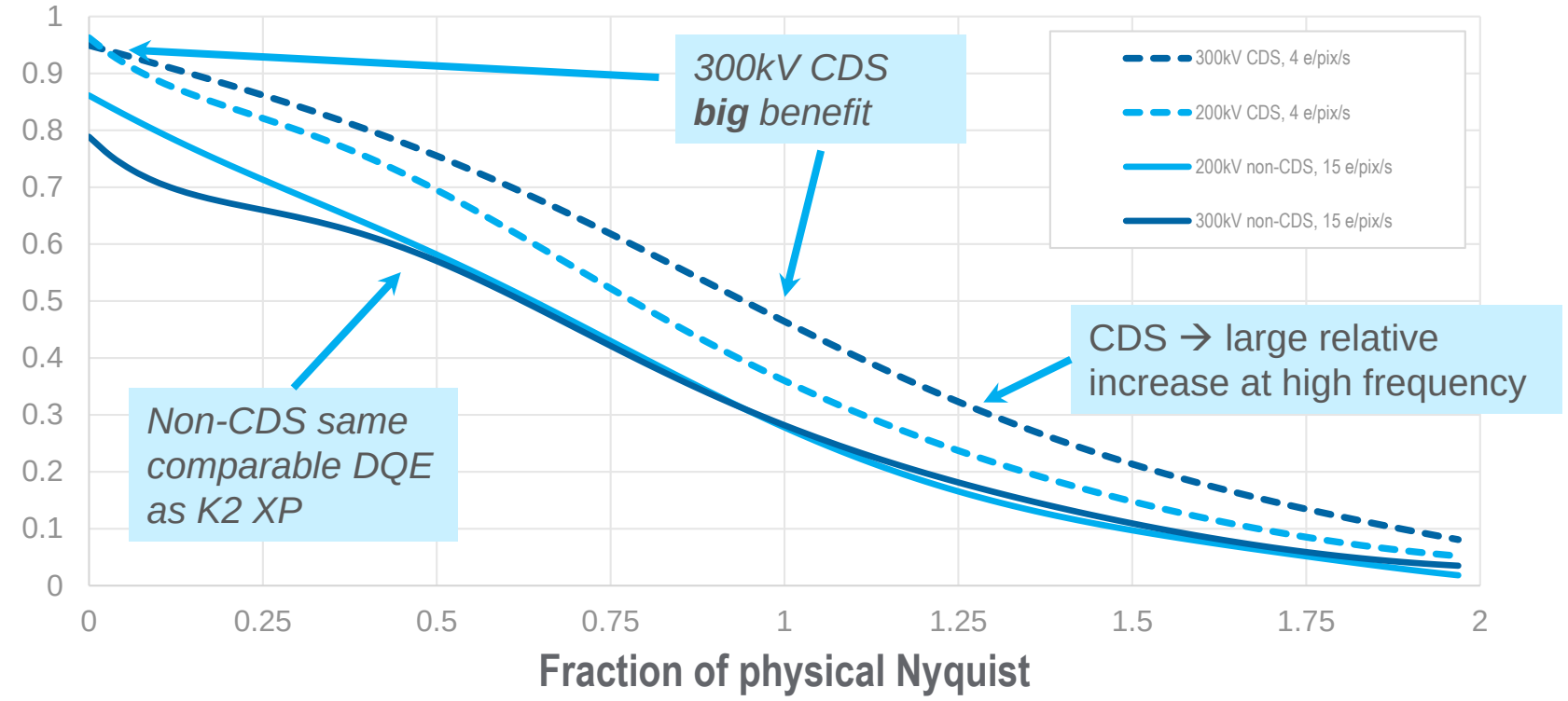
N = incoming average dose in primary electrons / pixel
s = spatial frequency

DQE at same (15 e/pix/s) dose rate for non-CDS and CDS



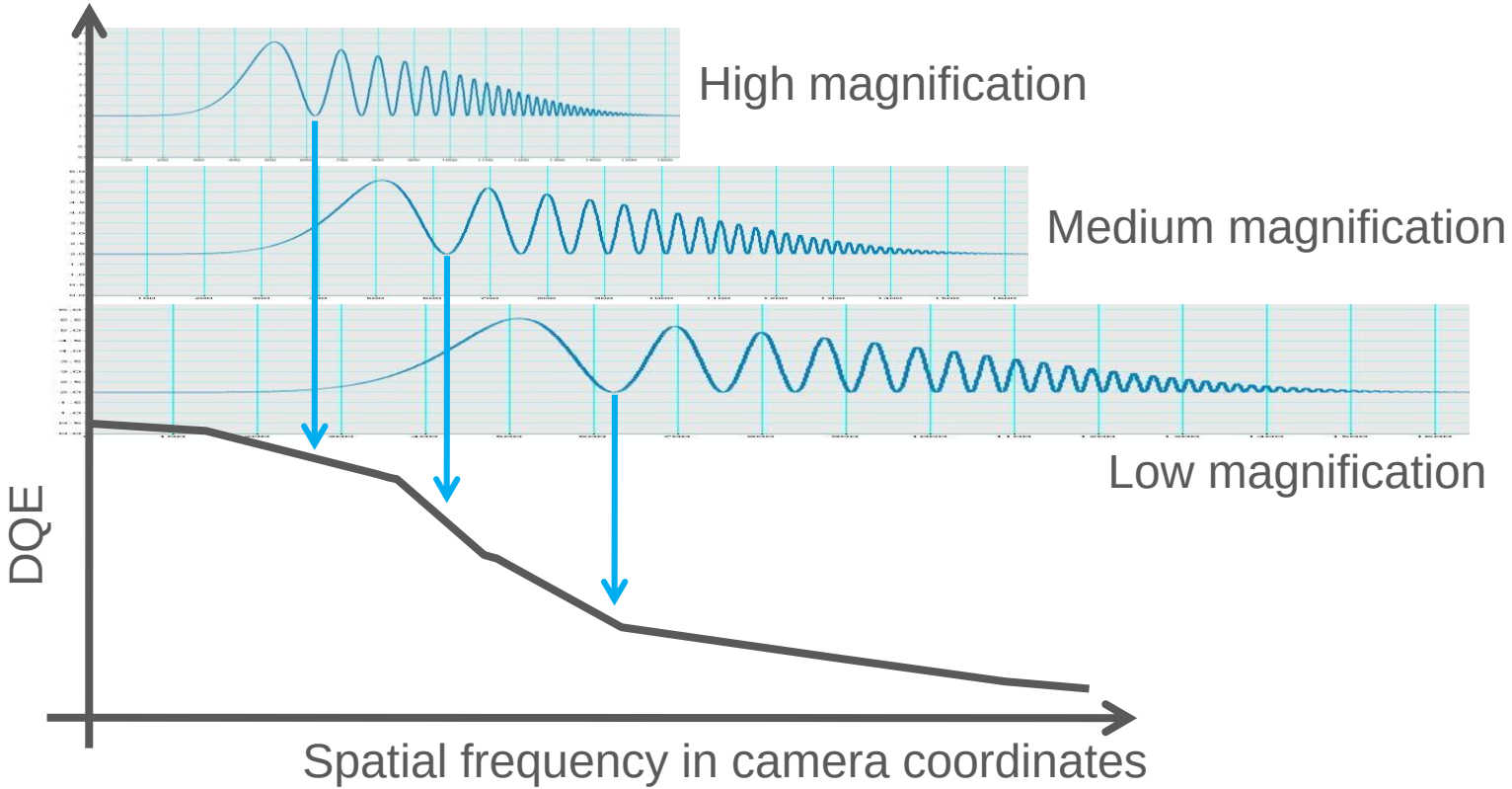
Nyquist fraction	absolute increase	relative increase
0-.3	0-5%	0-6%
1	7%	28%
1.25	5%	38%

DQE allows specimen- and microscope- independent camera characterization as for the K3 shown here



GATAN

Higher magnification maps specimen contrast to lower spatial frequencies on the camera – where the DQE is higher



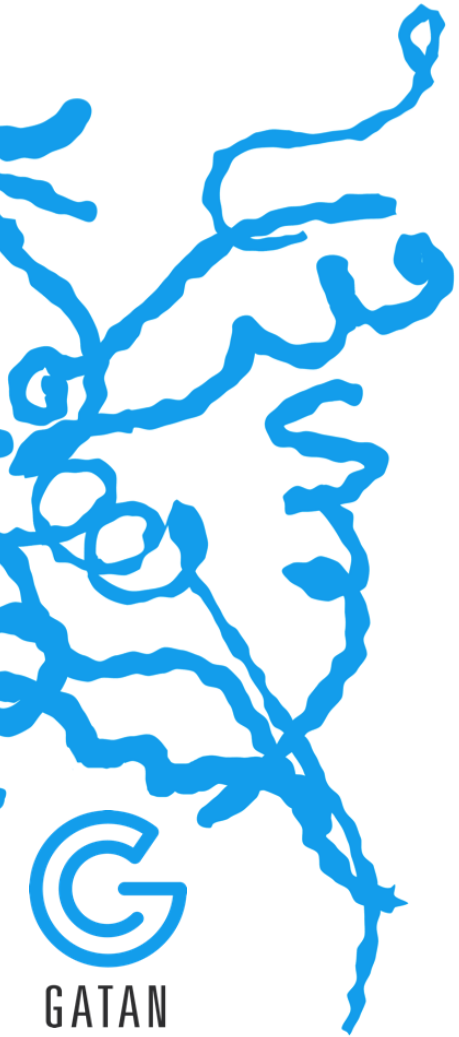
High magnification reduces field of view

But this may not be an issue for small tightly-packing molecules

Aldolase image courtesy of Gabriel Lander and Mark Herzik

Tradeoff Summary: Image Quality vs Throughput

- Lower kV improves beam-specimen interaction but affects spatial frequencies at the camera differently, raising low frequency DQE and lowering high frequency DQE
- Higher dose rate reduces exposure time but also reduces image quality as measured by DQE.
- Higher magnification increases the DQE with which specimen contrast is detected but at the expense of field of view.



Section 4:

Getting the most out of a counting detector in operation: how to decide

“throughput”

~~molecules / day~~

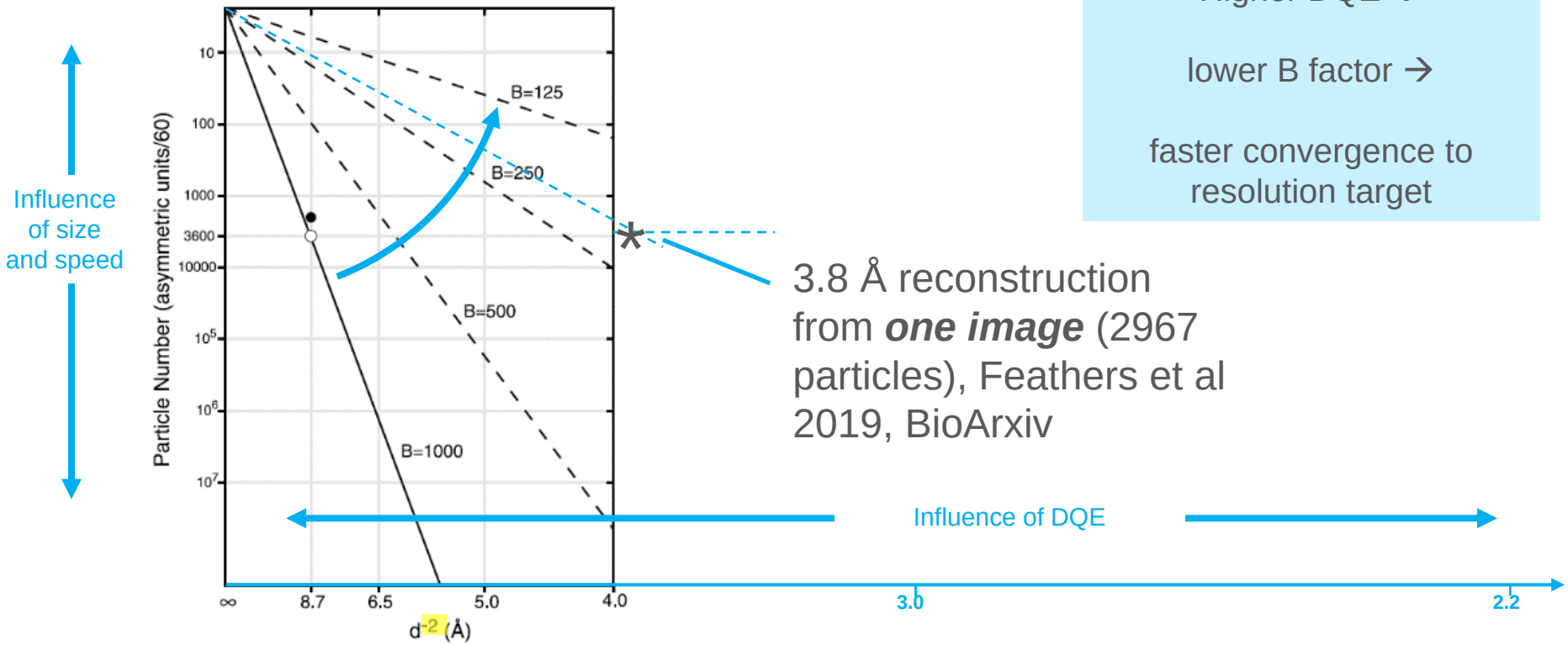
structures / day

Image *quality* is also “throughput”

Higher DQE →

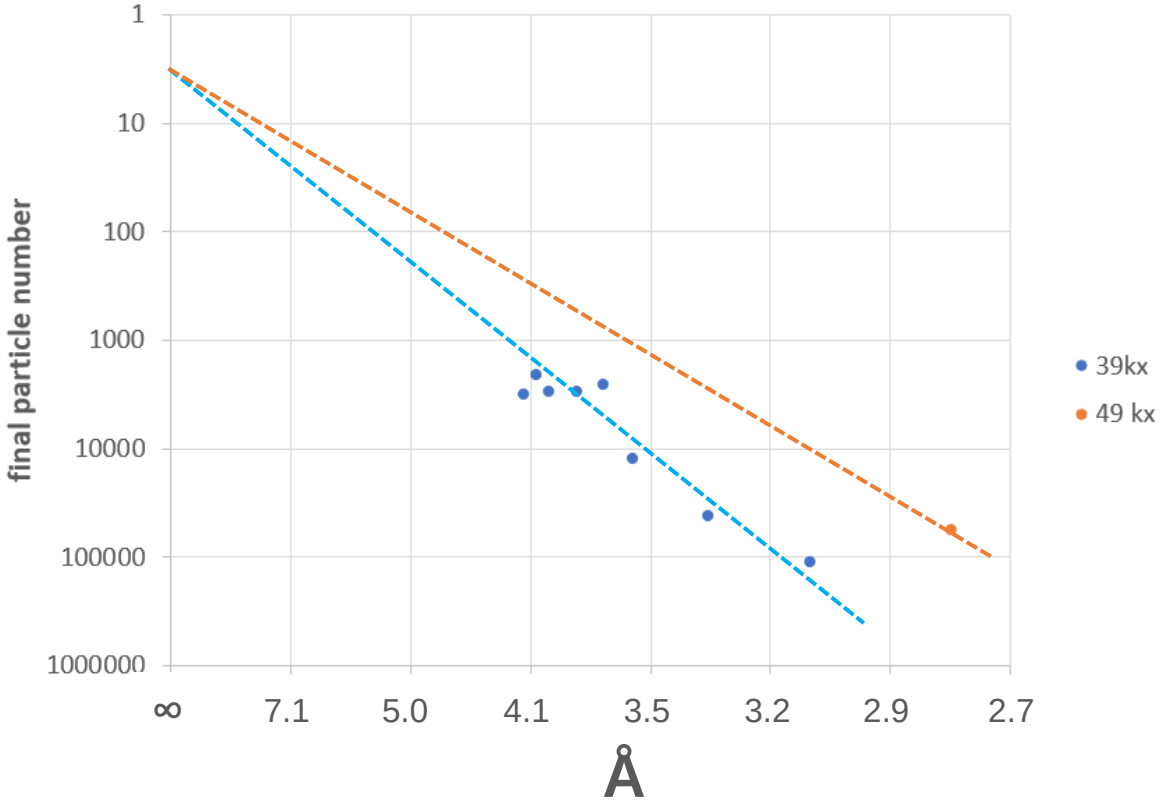
lower B factor →

faster convergence to resolution target



Rosenthal and Henderson, JMB, 2003, figure 11.

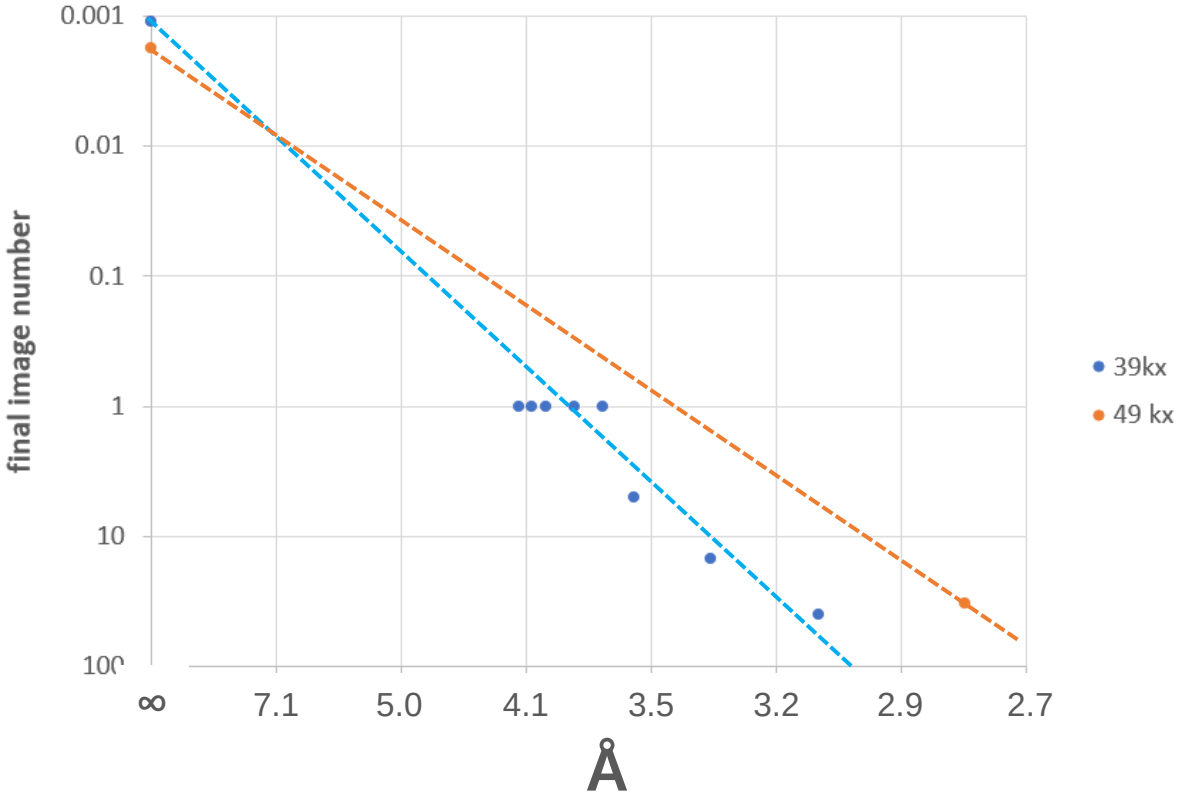
Rosenthal-Henderson plot of Feather et al table 2



Higher mag has lower B-factor – but is benefit outweighed by slower particle accumulation?

Derived from Feathers et al, BioArxiv, 6/19/19, table 2

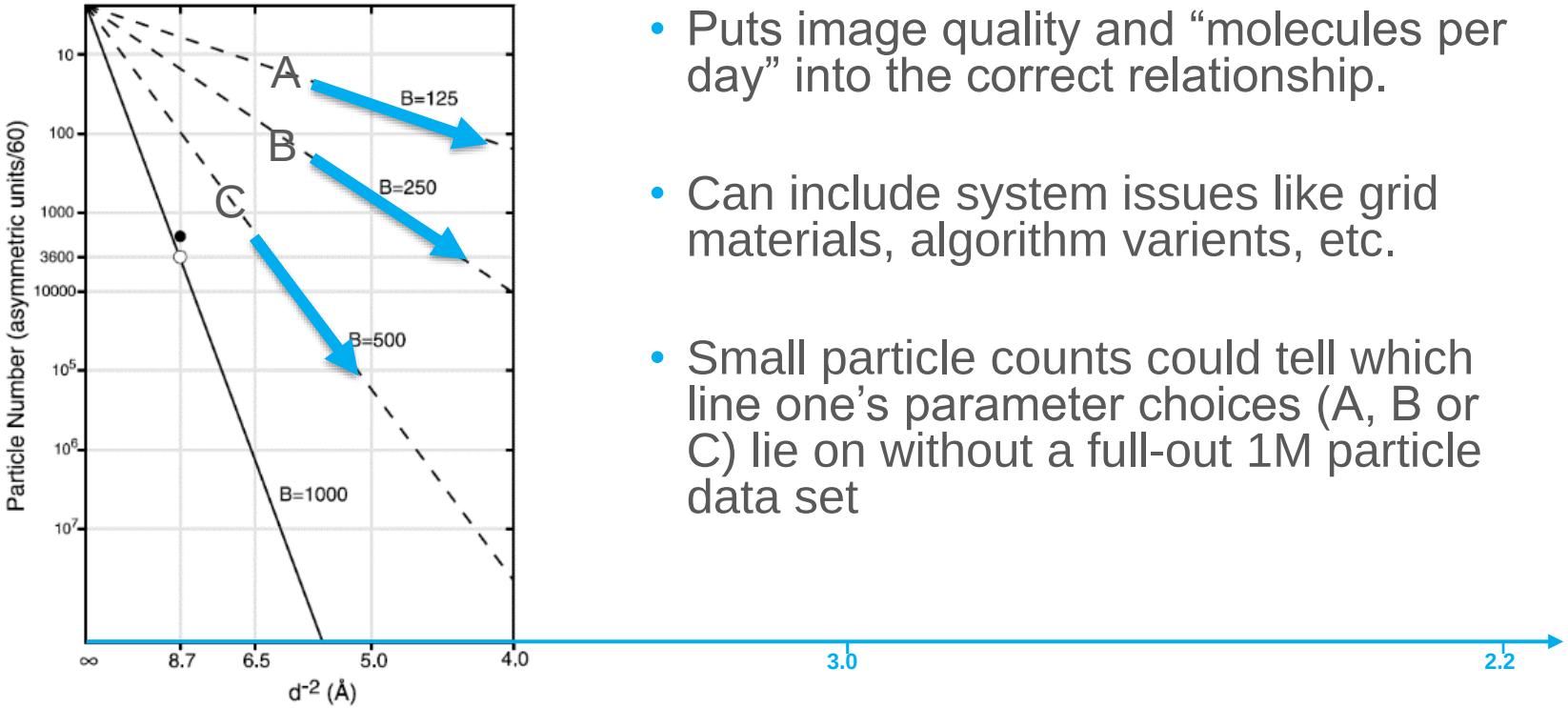
Rosenthal-Henderson plot redrawn vs *image* count



Higher mag converges faster even with fewer particles per frame.

Derived from Feathers et al, BioArxiv, 6/19/19, table 2 using table 1 average particles per image

→ Use B-factor for optimization of real throughput



- Puts image quality and “molecules per day” into the correct relationship.
- Can include system issues like grid materials, algorithm varients, etc.
- Small particle counts could tell which line one’s parameter choices (A, B or C) lie on without a full-out 1M particle data set

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Reading List

- Chiu, et al, Evaluation of super-resolution performance of the K2 electron-counting camera using 2D crystals of aquaporin-0, JSB 2015.
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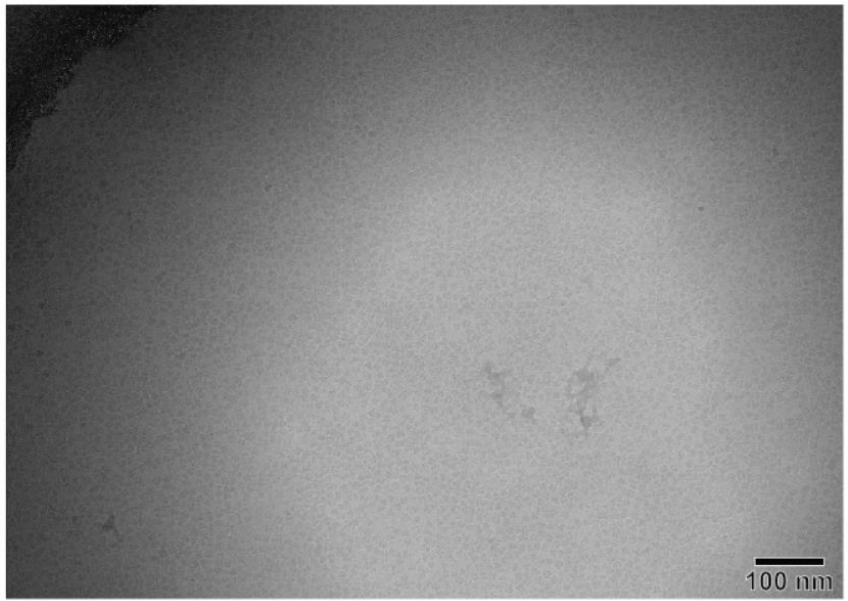
Backup

3.8Å reconstruction from one K3 image

Figure 3

A



Surpassing the physical Nyquist limit to produce super-resolution cryo-EM reconstructions

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Figure 4