not just the latest new toy: game changing

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- 1. how to select the best microscope parameters
- 2. how to pre-process frames: what does/doesn't work
- 3. how to assess the data quality
- 4. usability of cameras/interfaces
- 5. how do we handle data tsunami
- 6. what would we like in a next generation camera?

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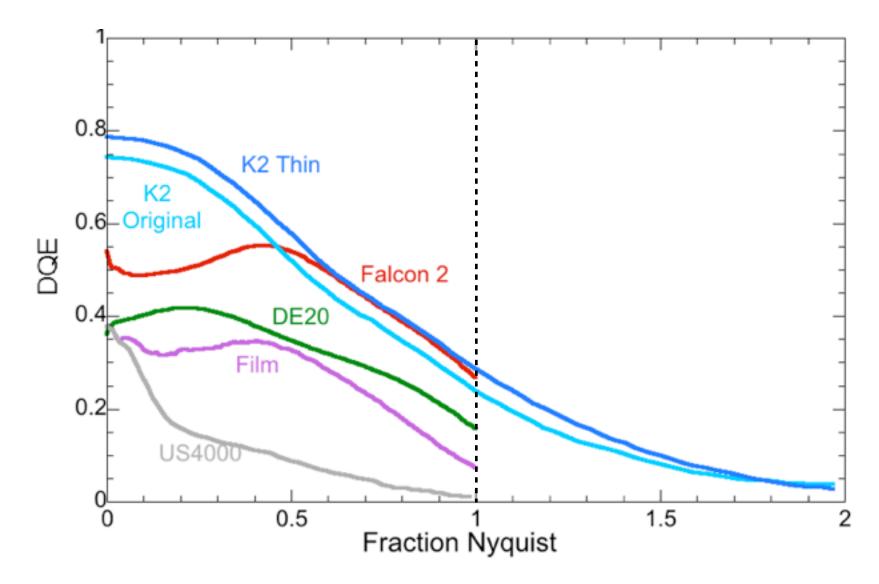
Yifan Cheng (UCSF) Gatan K2

Wah Chiu (Baylor) DE cameras

Carsten Sachse (EMBL) comparison of Falcon 2/K2

Sjors Scheres (MRC LMB) Falcon 2/K2

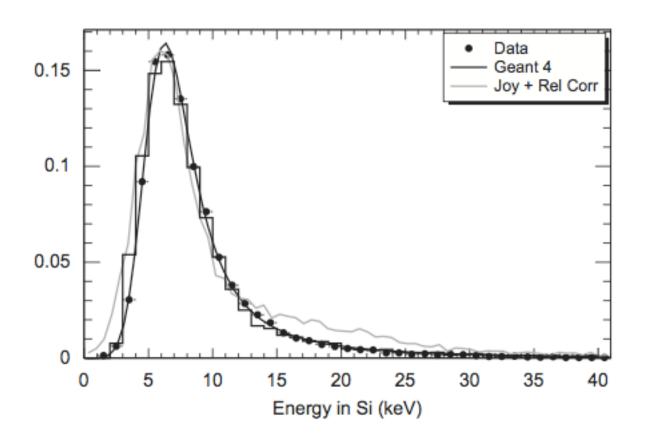
#### all Direct Detectors provide a huge leap forward



high sensitivity, minimal effective read out noise, high frame rates (10-40 fps)

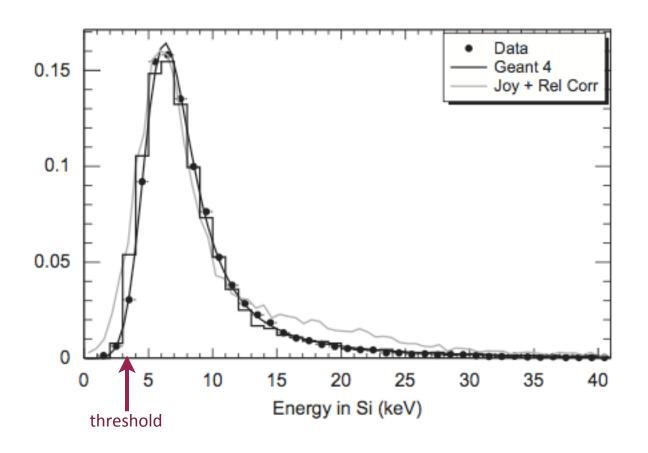
McMullan et al, 2014, Paul Mooney

# Landau noise: a problem for all integrating detectors

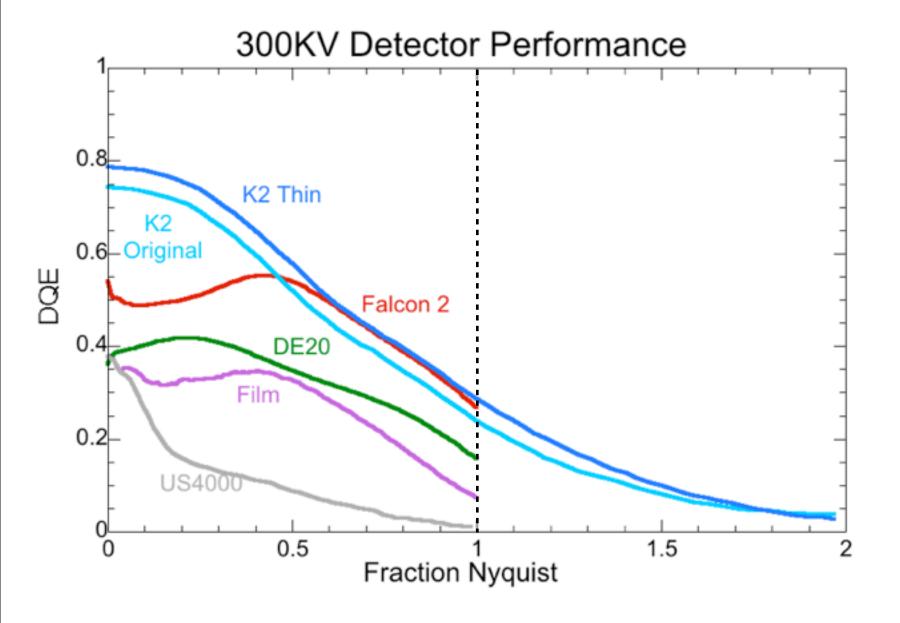


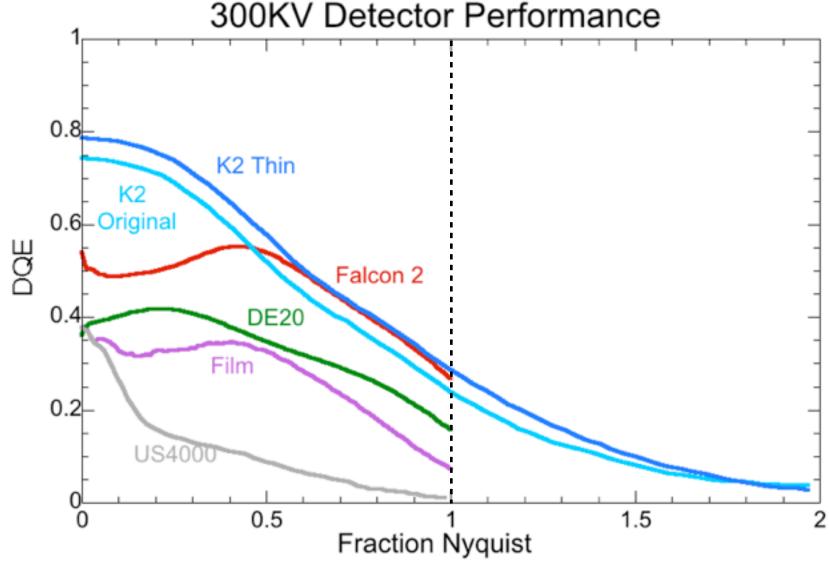
wide range of signal registered for each electron event reduces DQE significant contributor to image noise

# Landau noise: a problem for all integrating detectors



greatly improved DQE virtually eliminates noise centroid interpolation => super-resolution





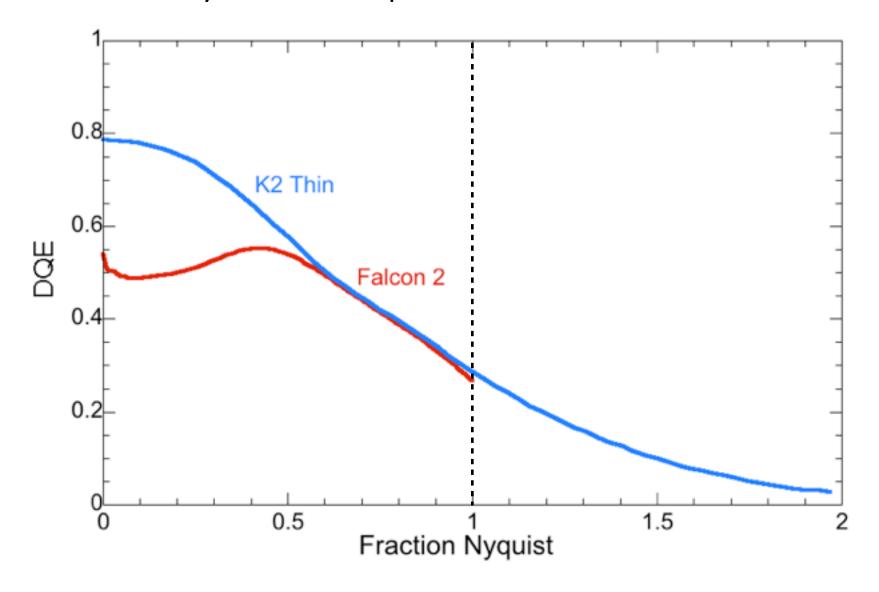
all have significant transfer at physical Nyquist => noise aliasing (JR point)

K2: super-resolution can provide some extra area (25% lower mag), aliasing suppression

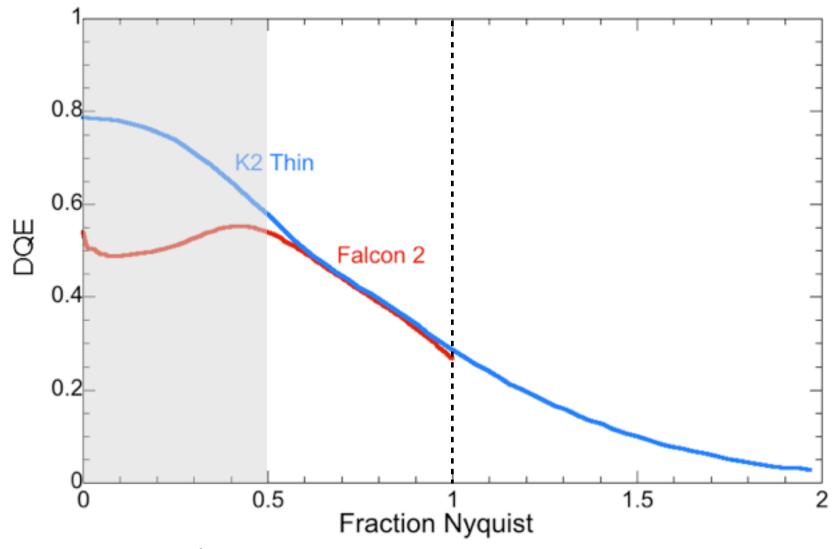
K2: coincidence losses reduce QE at dose rates above 8 e<sup>-</sup>/pix/sec

McMullan et al, 2014Paul Mooney

### "only need K2 for particles smaller than 400KDa"



#### "only need K2 for particles smaller than 400KDa"



information to 5-8Å used for all aspects of frame, particle alignment, classification, etc better information there, better for all samples higher DQE lower noise, better for dose fractionation => better classification, etc

#### Data Tsunami from movies

counted movies, only need to count to 4-6

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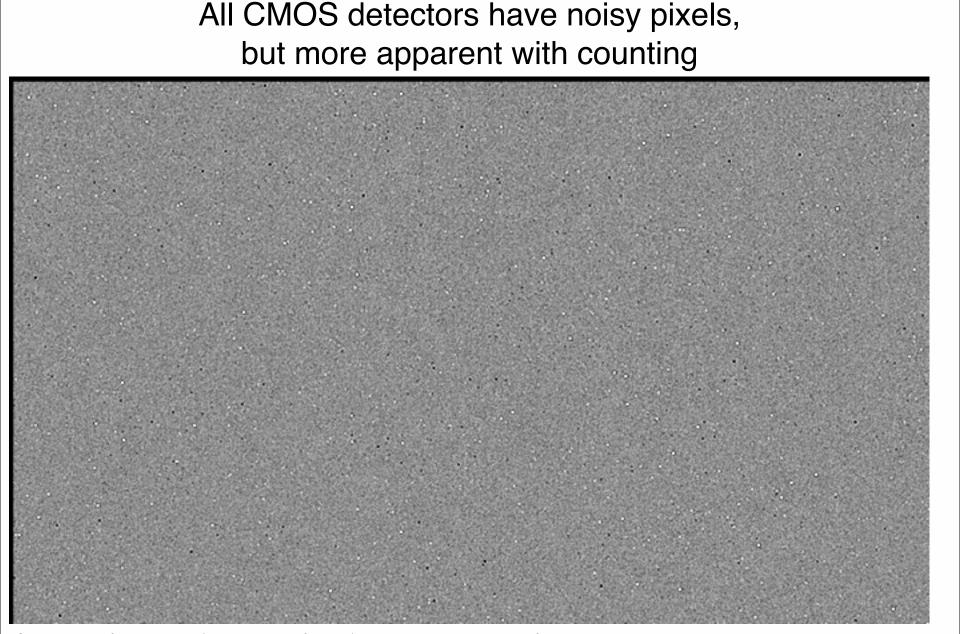
counted movies, only need to count to 4-6

Gatan offers 8 bit mode, we compress to 4 bit (8X reduction)

not gain corrected, or bad pixel fixed

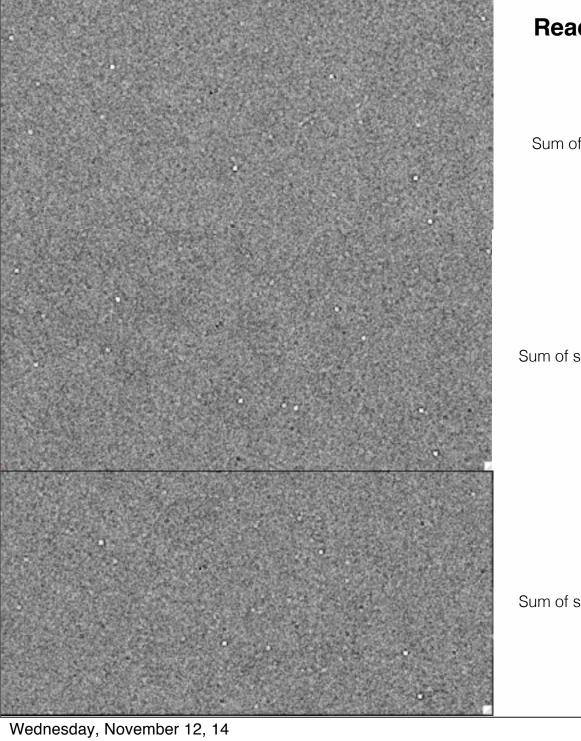
we store gain and bad pixel list in extended header

correct on fly, during frame alignment on GPUs, so fast



Summation of 150 stacks (4500 0.2 sec frames) yields a strong pattern of spots.

undetectable in individual frames or single stacks

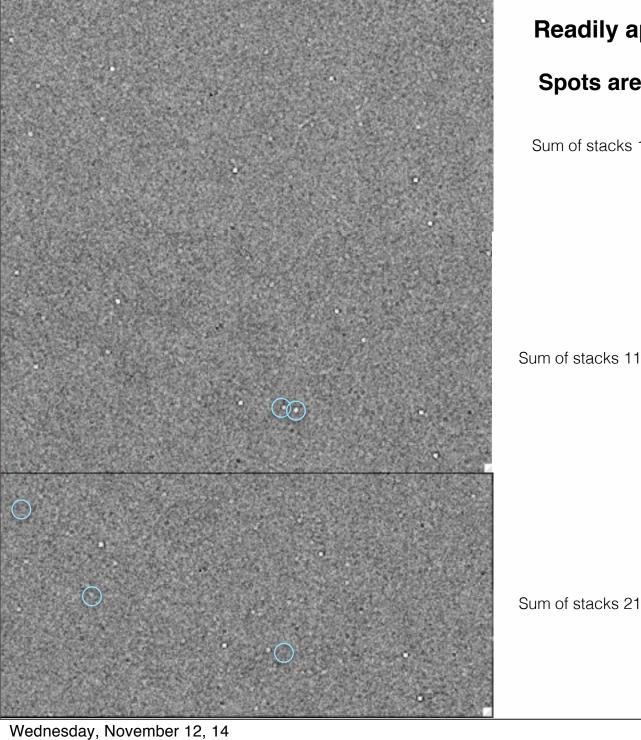


# Readily apparent in sum of 10 Stacks

Sum of stacks 1-10

Sum of stacks 11-20

Sum of stacks 21-30



Readily apparent in sum of 10 Stacks Spots are not consistent over time

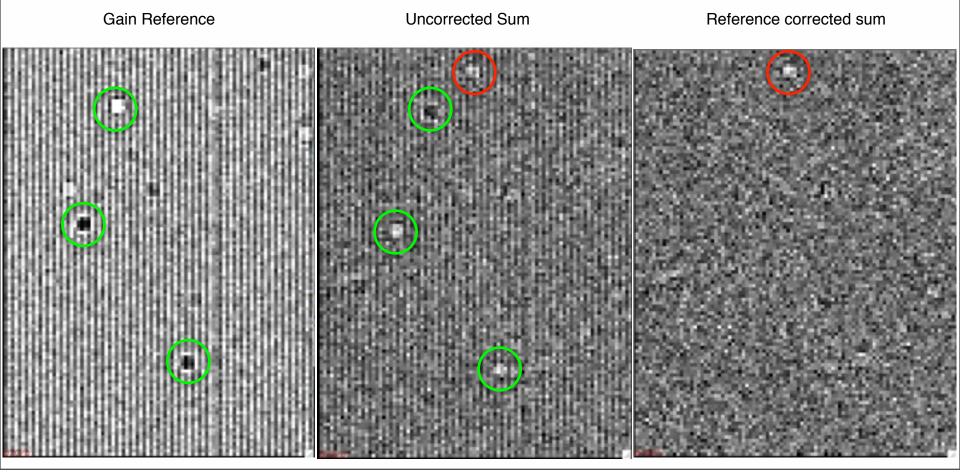
Sum of stacks 1-10

Sum of stacks 11-20

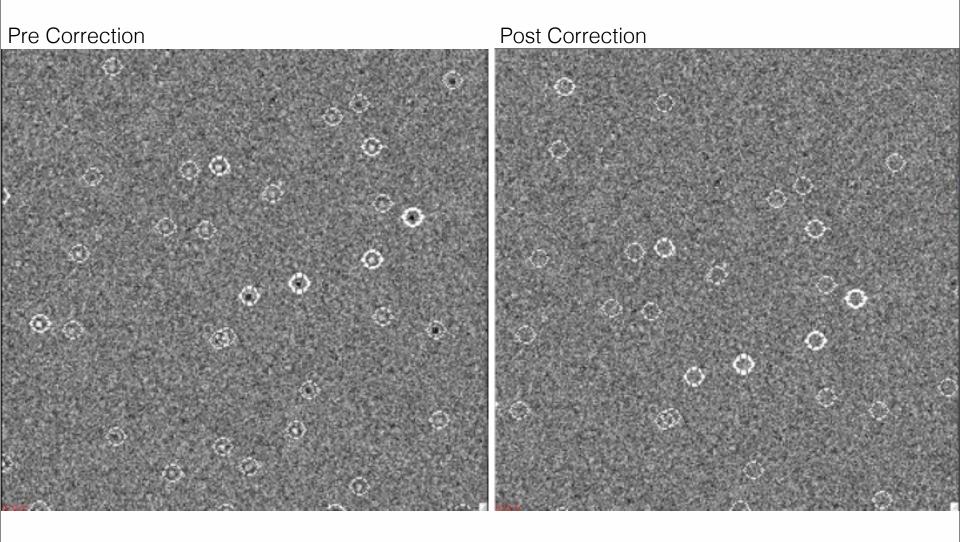
Sum of stacks 21-30

# Gain reference is not responsible for the defects but is actually fixing a lot of faulty pixels.

Green circles - spots which are on the camera chip, and which are corrected on the gain reference (hence contrast inversion) Red circles - spot which is on the gain reference corrected sum, and which is not on the gain reference itself.



#### Need to determine and fix from actual data



we have gpu code that does this efficiently can integrate with frame alignment

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