Dec 2020

Electron microscopy

Detectors

Francisco Javier Chichón

Recording Images In Electron Microscopy

A little bit of history

- Oldest recording medium: photographic film
- 1970: Charge coupled device (CCD) was invented
- 1976: CCD camera was used for astronomy
- 1982: 100 x 100 CCD was *directly* exposed to 100 kV electrons…radiation damage
- 1988: 576 x 382 CCD used with scintillator and optical coupler
- 1990: **Gatan** made the world's first commercial CCD camera
- 2002: 128 x 128 direct detection camera developed
- 2008 – 2009: commercial complementary metal-oxide semiconductor (CMOS) cameras and radiation hard CMOS cameras were introduced

Image & pixels

Thus, the so-called Nyquist limit is defined as the maximum frequency of a signal that can be reproduced at a certain sampling rate (->), i.e. what we can reproduce at best at that sampling rate. This theoretical maximum possible is one pair of lines for every 2 pixels.

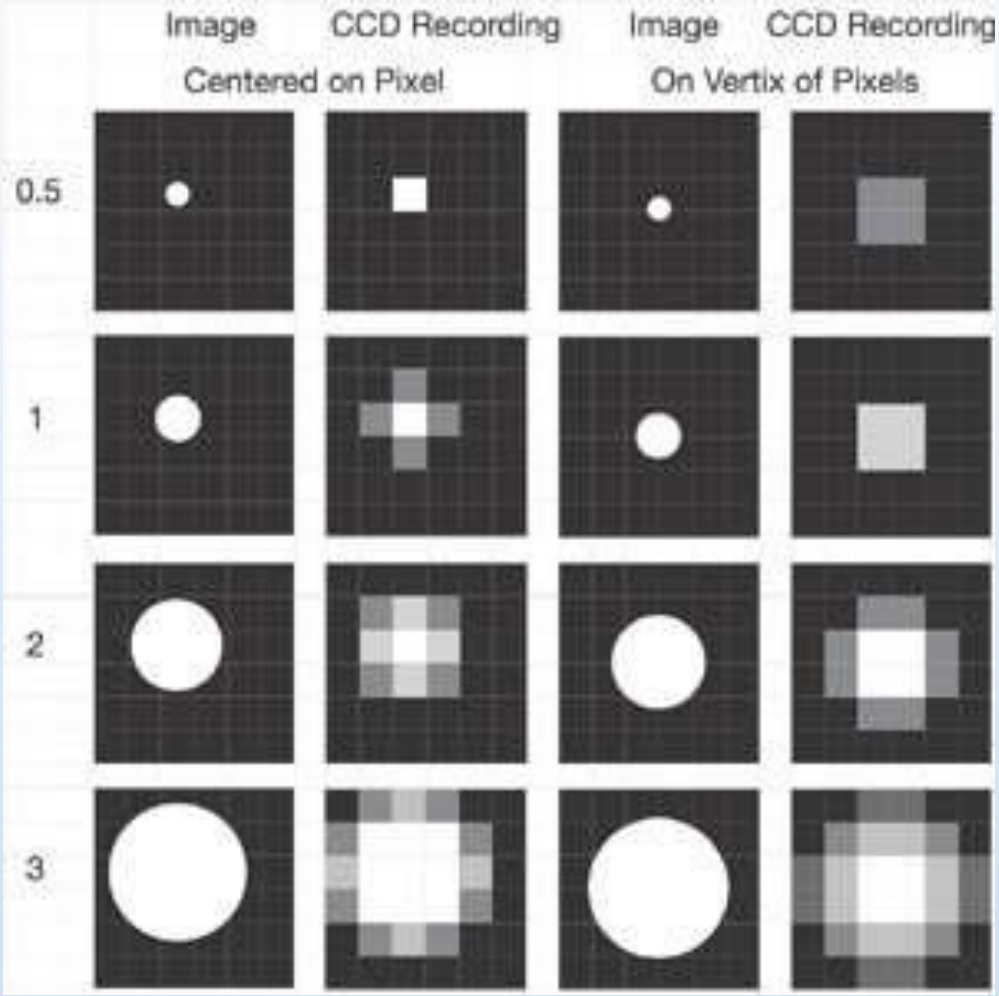
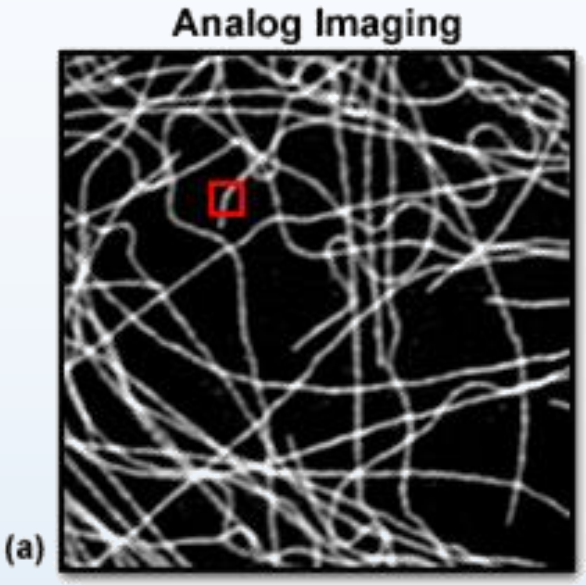
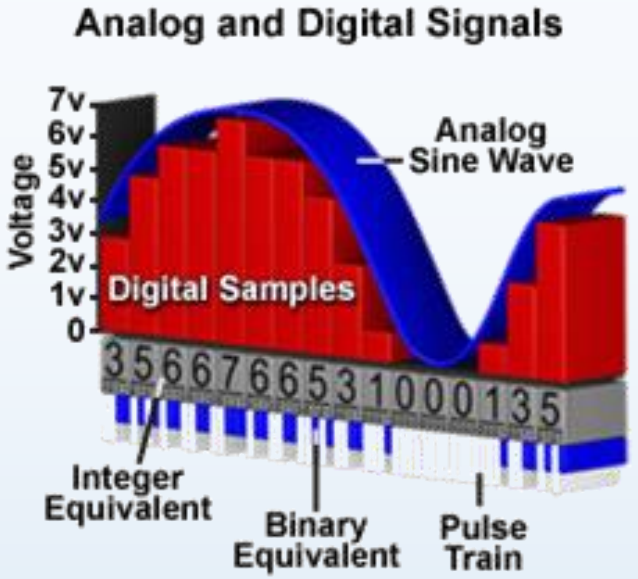
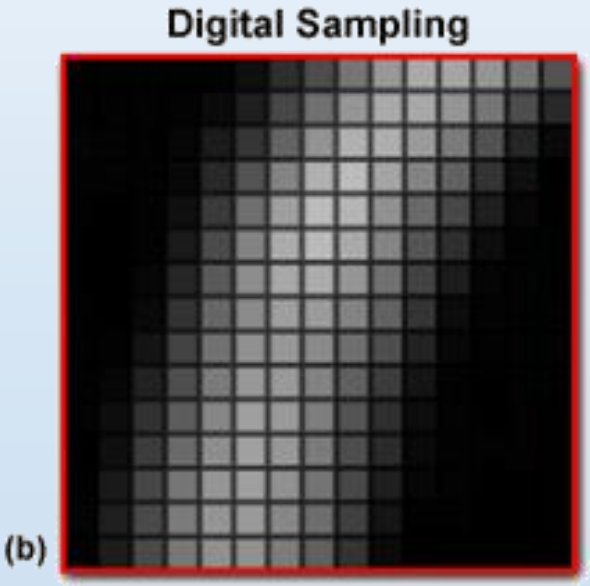


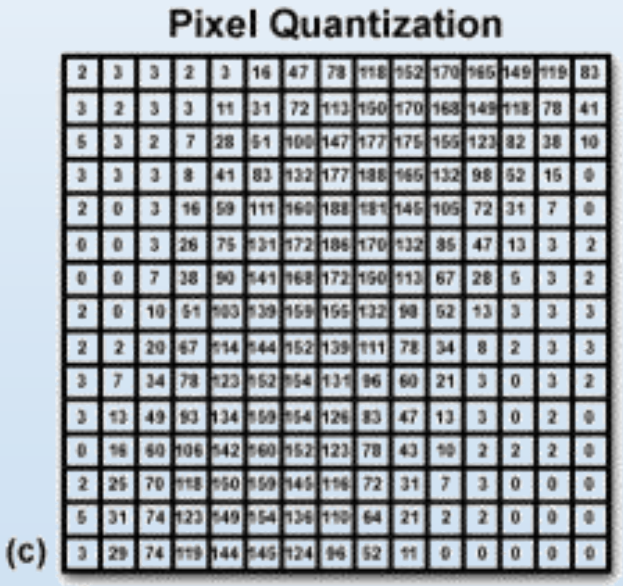
Image & pixels

- It is possible to produce an image from a discrete signal.
- A digital image is not more than a matrix.

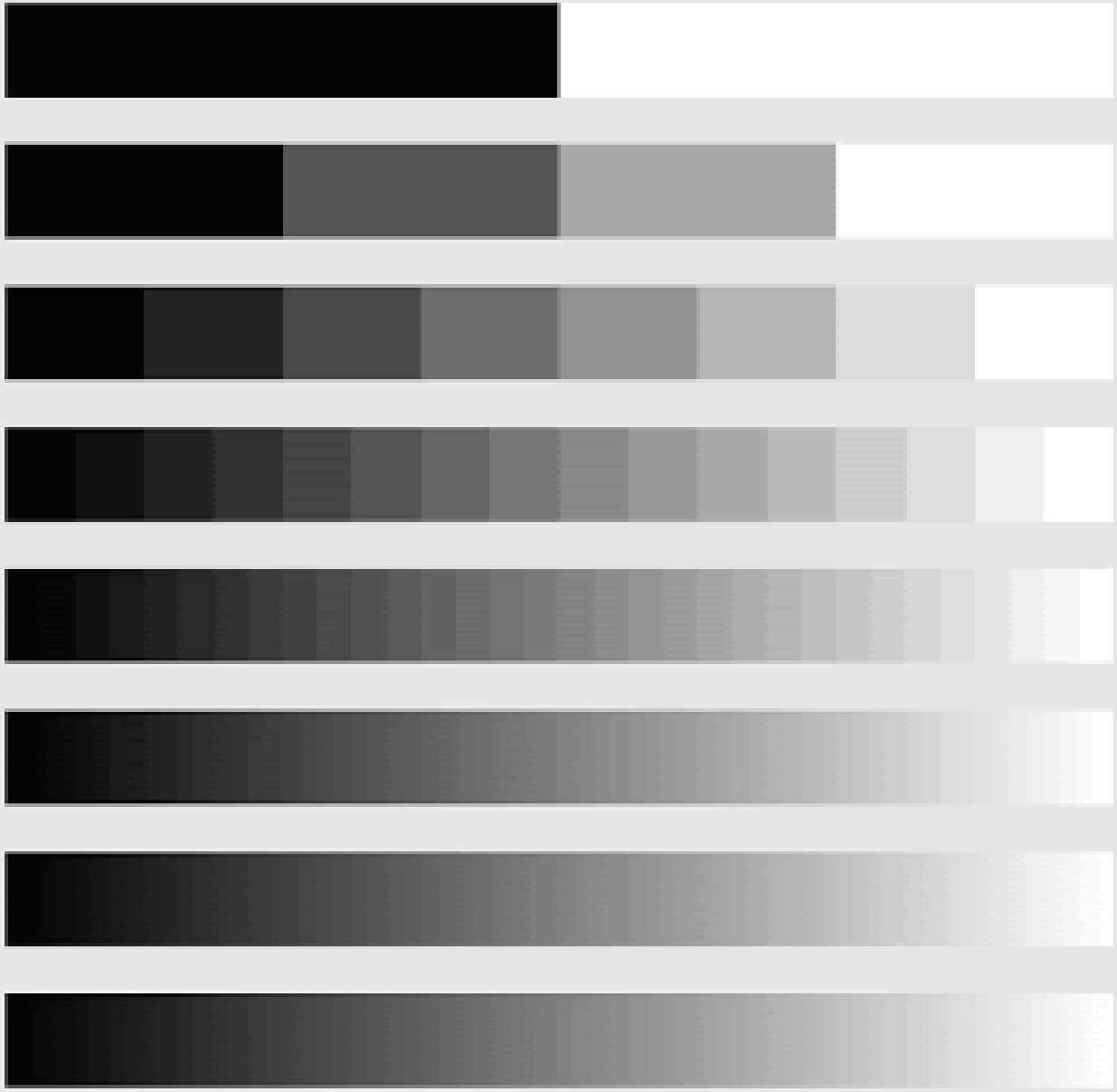








Bit Depth



bit x pixel	colores
1	2
2	4
3	8
4	16
5	32
6	64
7	128
8	256

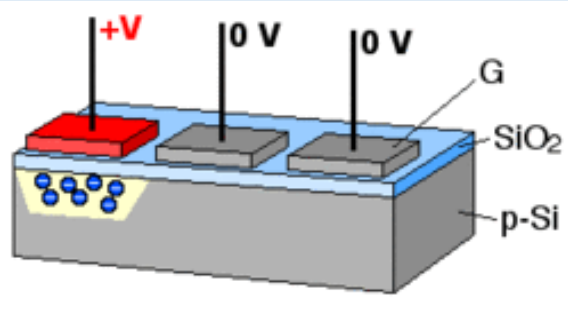
16 bits = 65.536

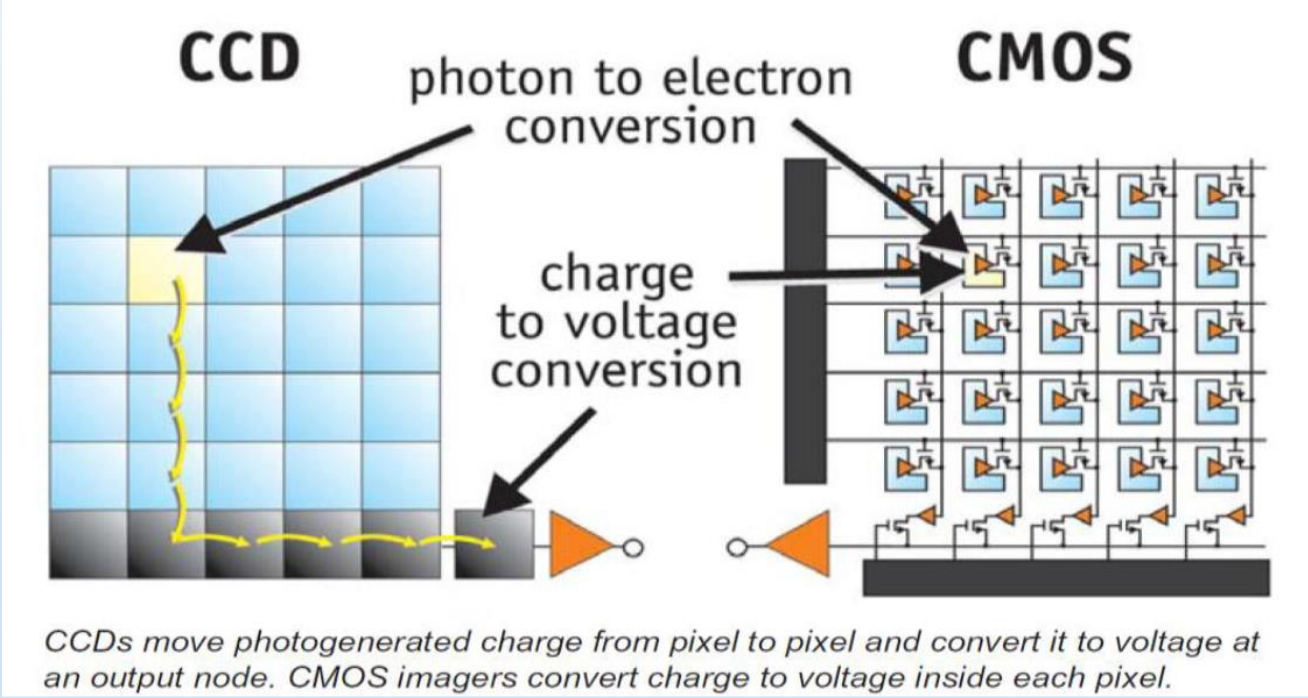
32 bits =4.294.967.296

CCD vs. CMOS

Both CCD and CMOS use photo diodes to convert photons to electrons, the difference is how they store charge and transfer it.

- **CCD**: Charge is transferred between neighboring cells, and read-out
- **CMOS** : Charge immediately converted to voltage (read out with digital output)

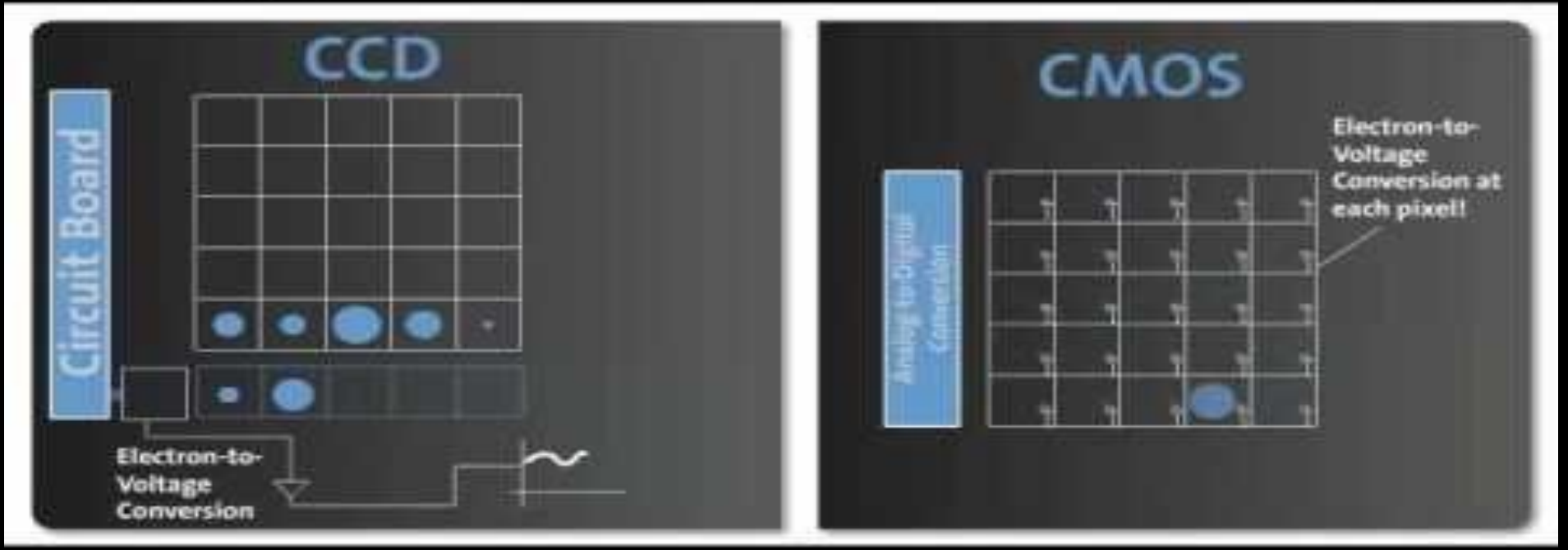




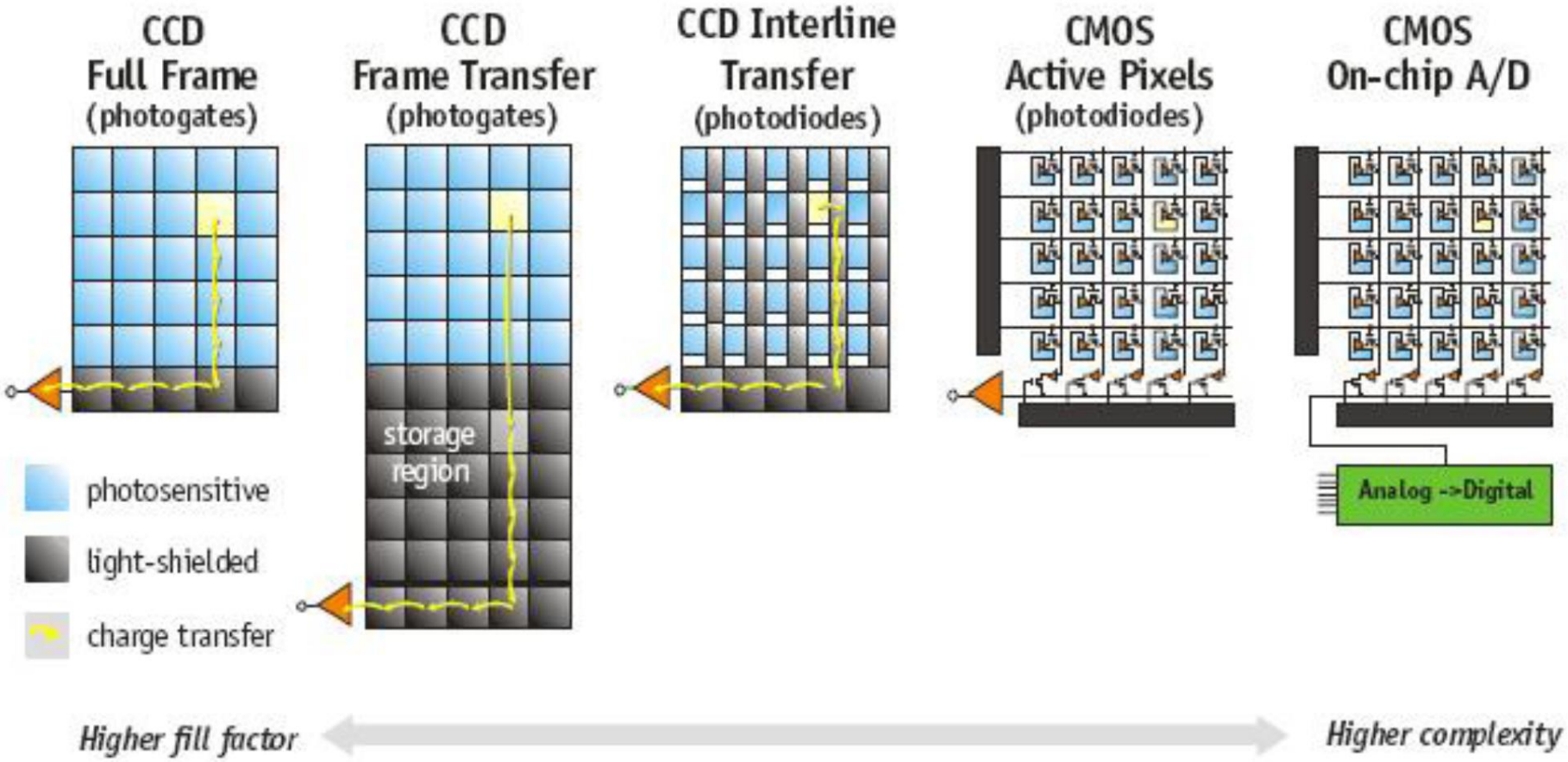
CCDs move photogenerated charge from pixel to pixel and convert it to voltage at an output node. CMOS imagers convert charge to voltage inside each pixel.

CCD vs. CMOS

Dallmeier



CCD and CMOS types



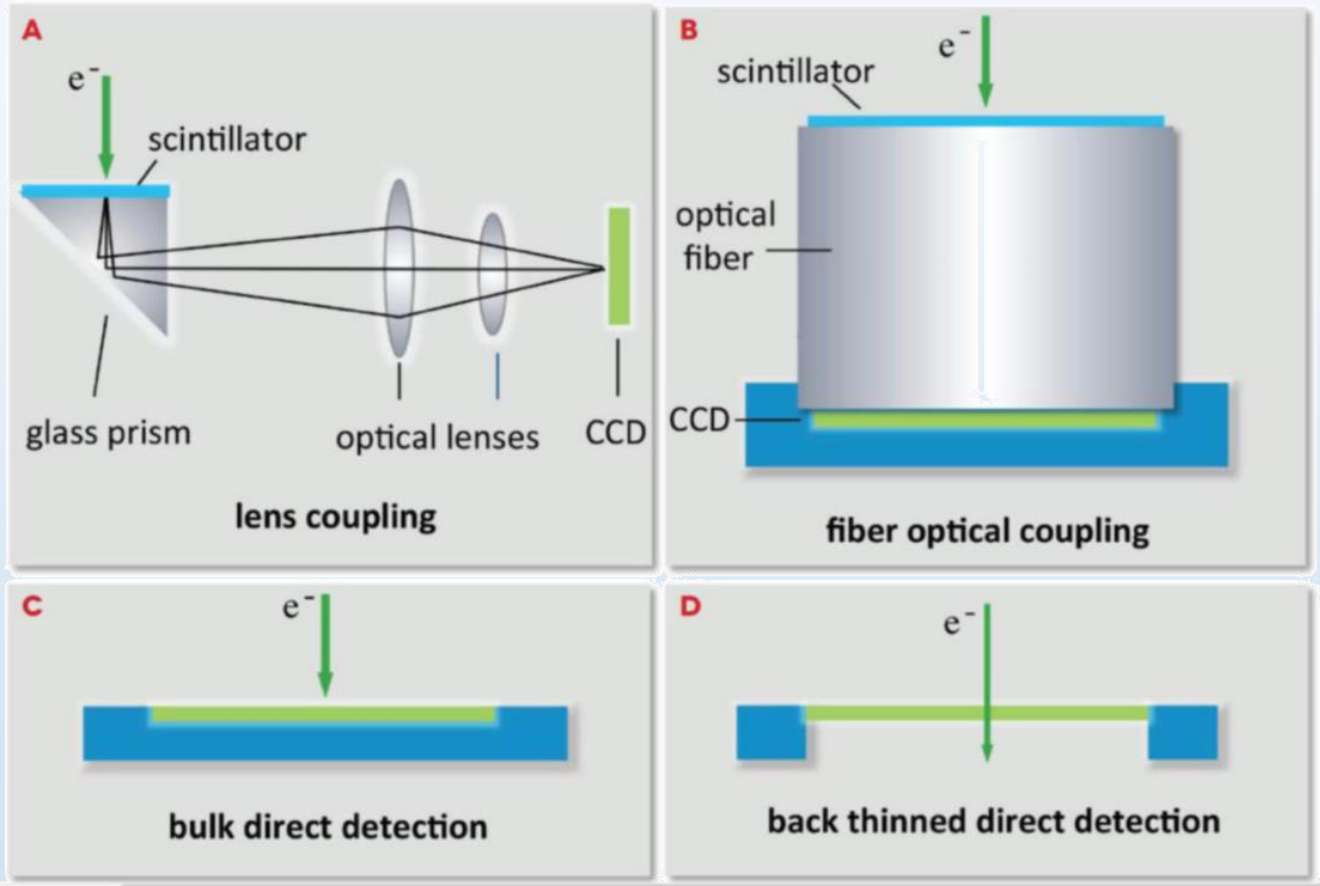
Detectors in Electron Microscopy

A. Optically coupled

B. Fiber-optic coupling

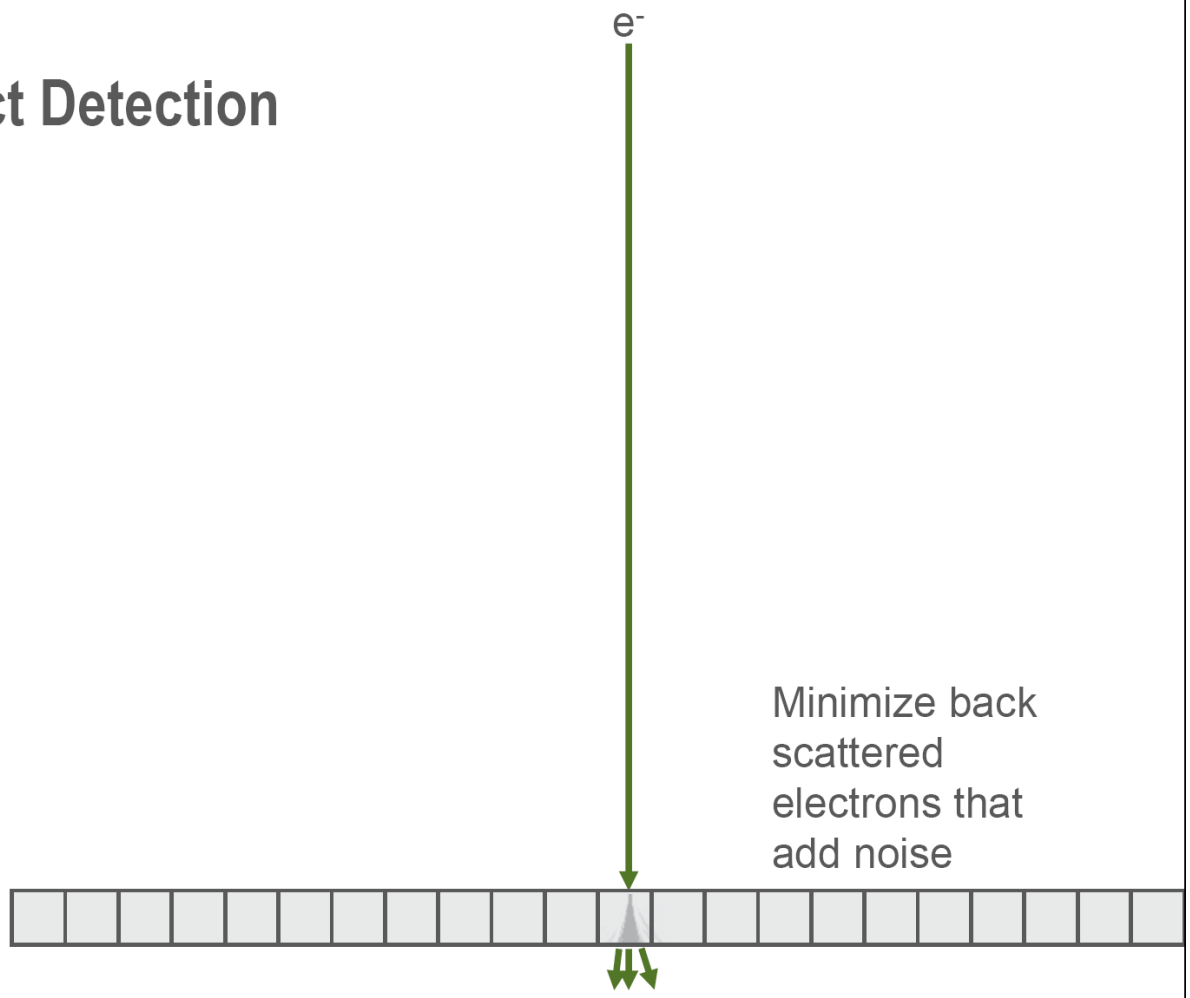
C. Direct detection

D. Transmission direct detection



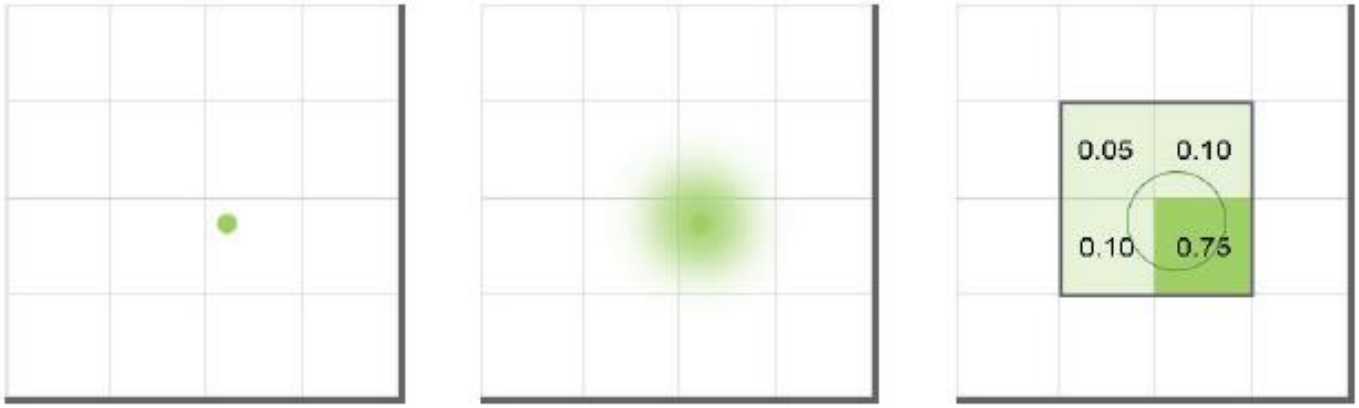
Transmission Direct Detection

1. ~~Convert electrons to light~~
2. ~~Transfer light to detector~~
3. Detect electron and convert to signal



GATAN

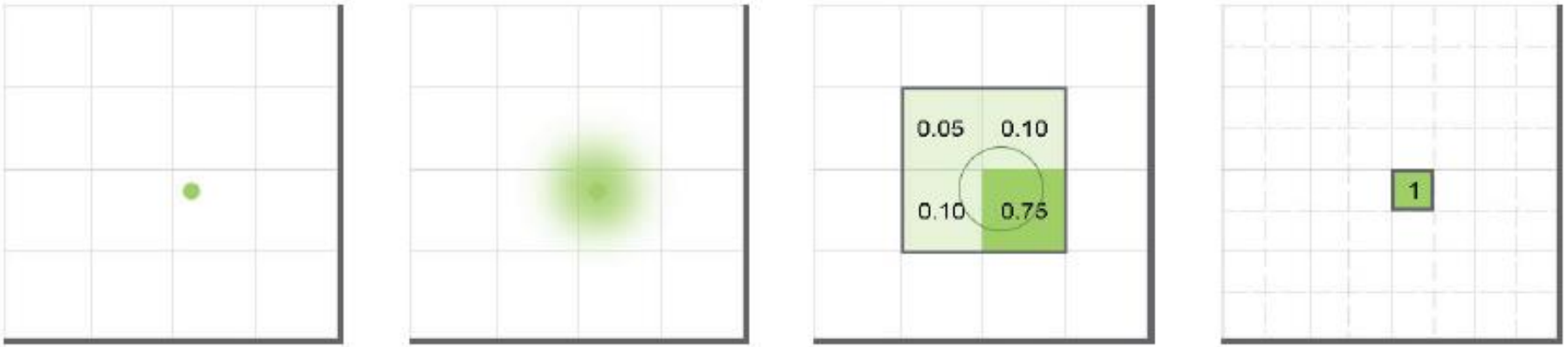
SISTEMA LINEAL O INTEGRATIVO



Electron enters Detector. | Electron signal is scattered. | Charge collects in each pixel.

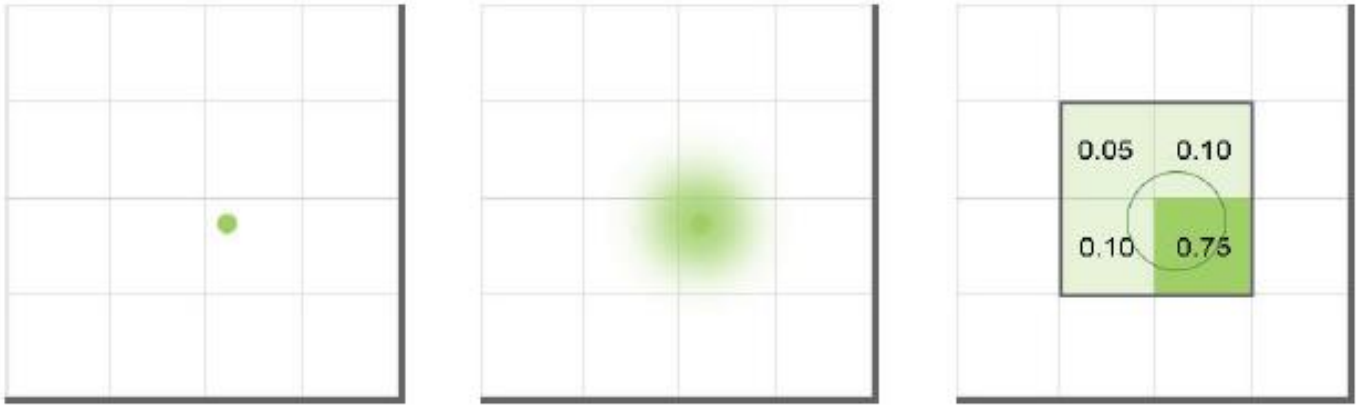
Super-RESOLUCION

CONTEO



Electron enters Detector. | Electron signal is scattered. | Charge collects in each pixel. | Event localized to sub-pixel accuracy.

SISTEMA LINEAL O INTEGRATIVO



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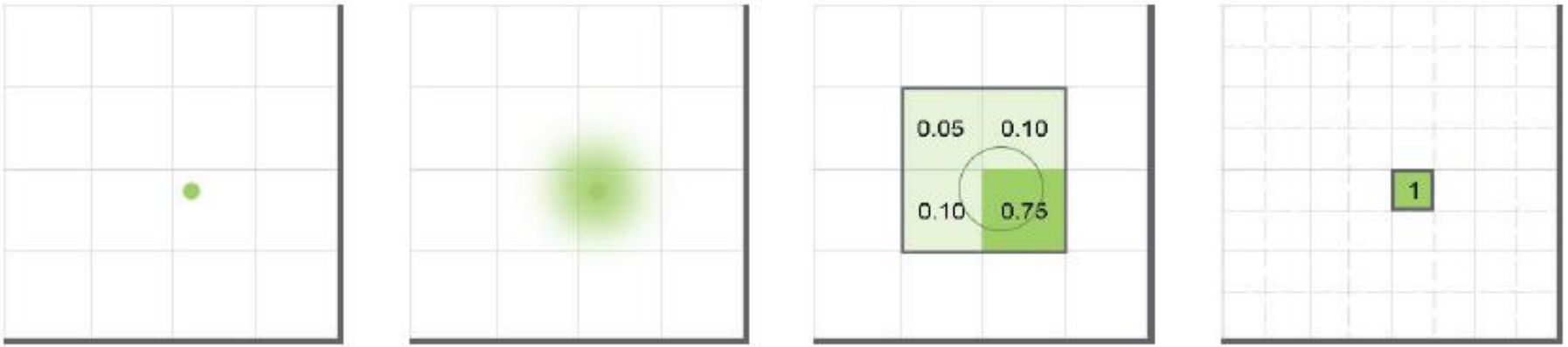
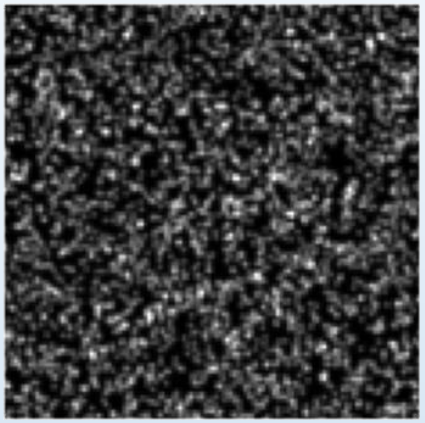


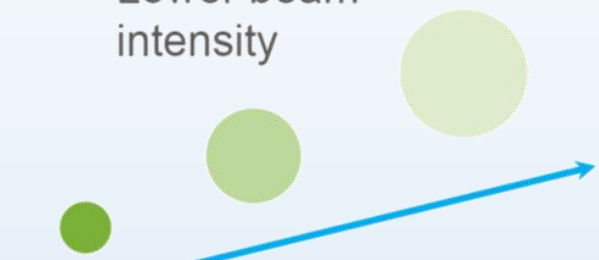
Electron enters Detector. | Electron signal is scattered. | Charge collects in each pixel. | Event localized to sub-pixel accuracy.


K3
GATAN

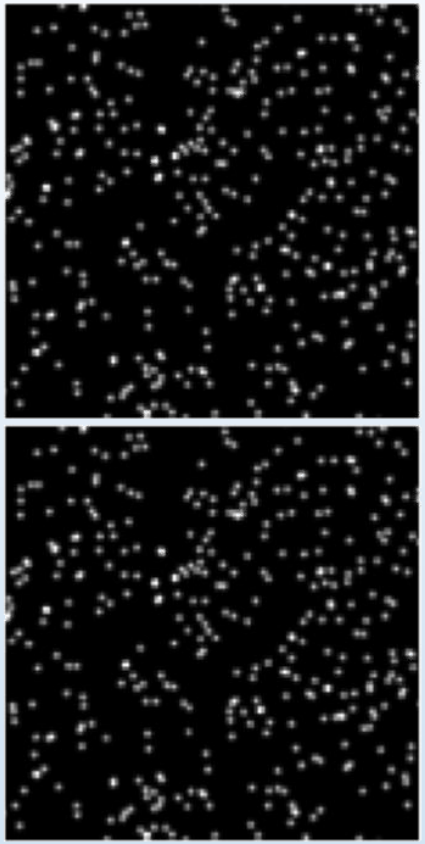
Electron Counting Requires that Electrons Don't Overlap on the Sensor



Lower beam intensity

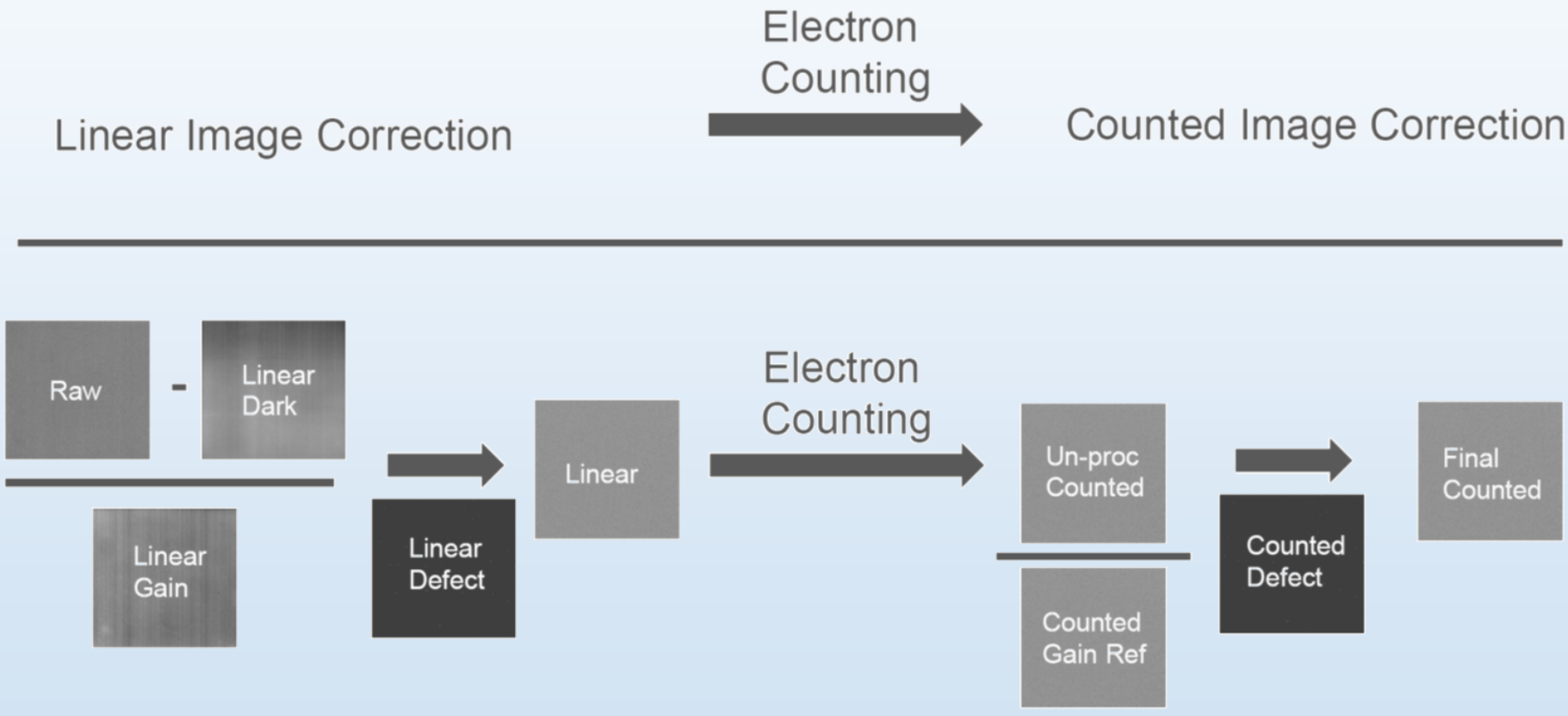


Faster frame rate

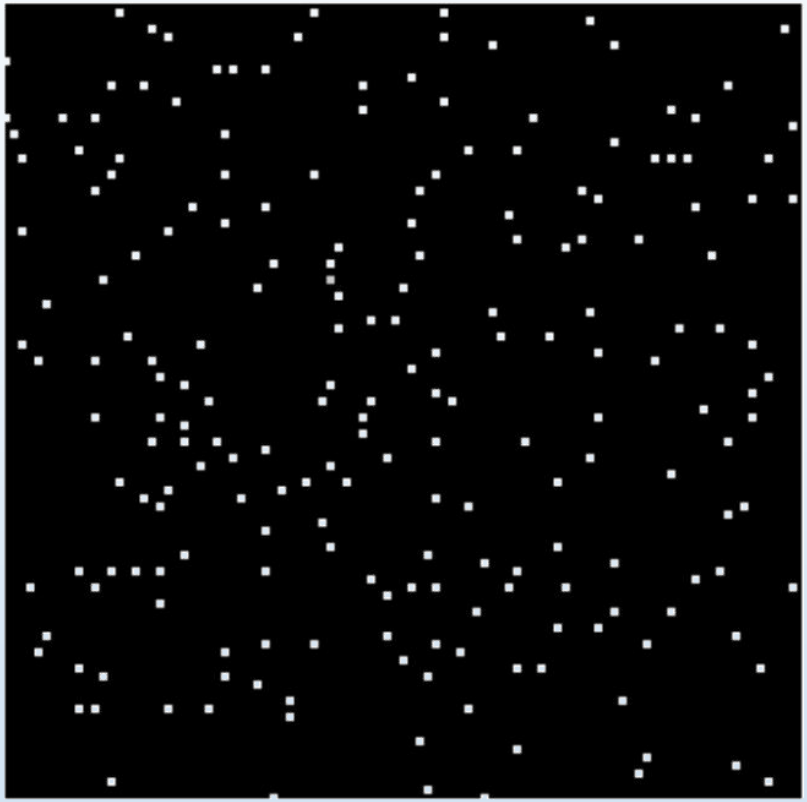


Both methods allow counting, but the effect is not equivalent!

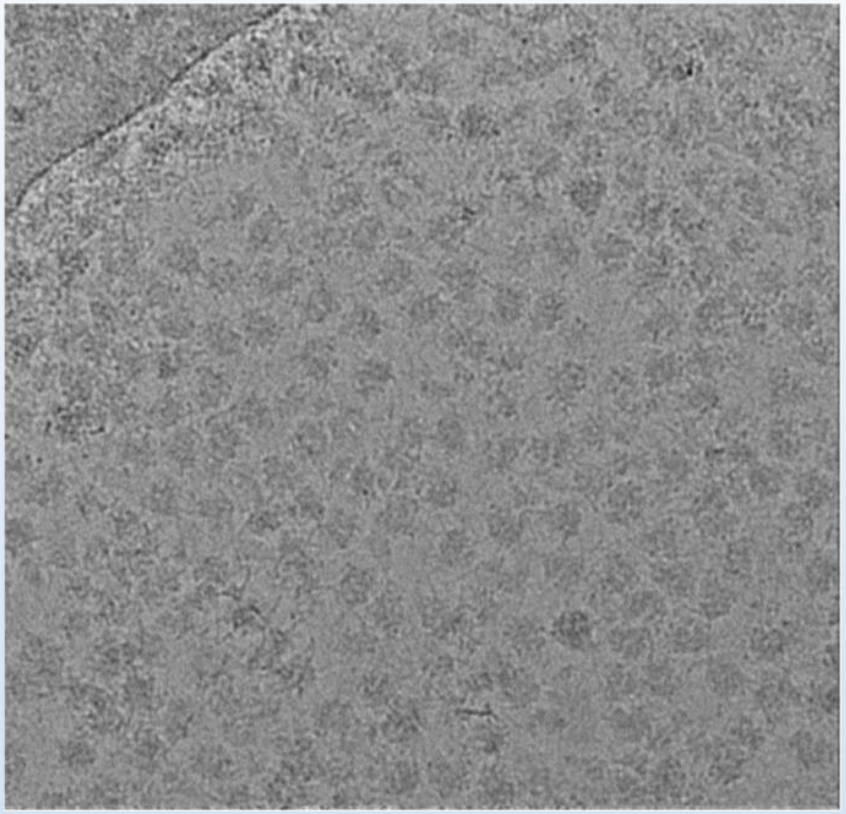
Counted Gain Correction Scheme



How Frame Alignment Works



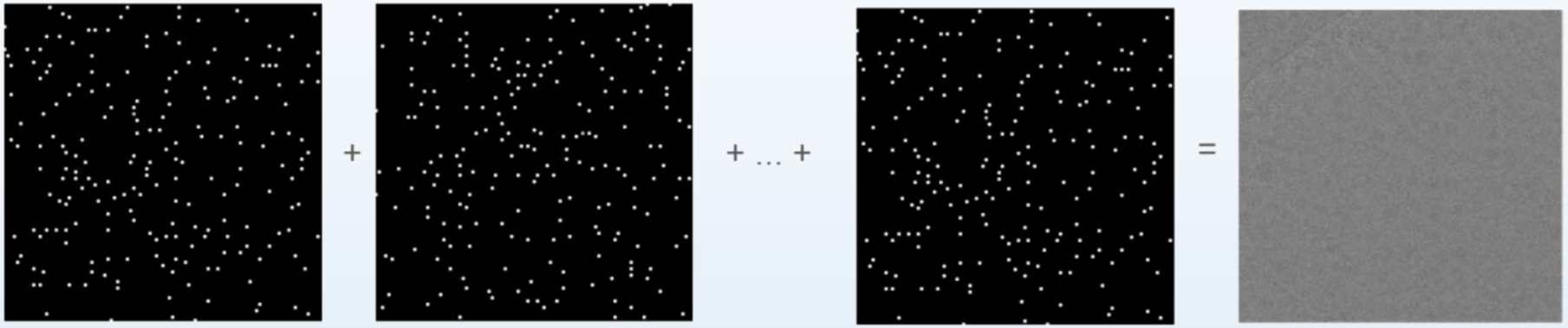
Raw counted frame

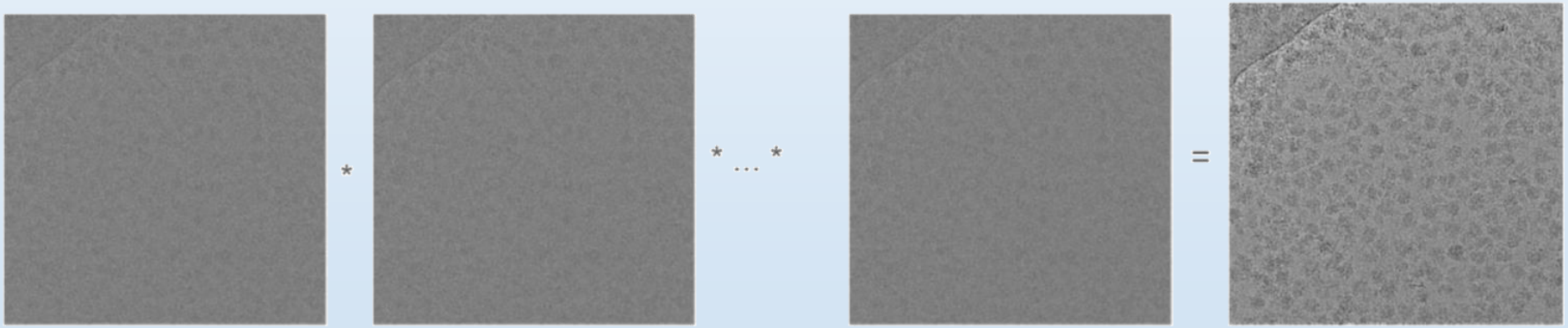


Final aligned image

Raw counted frames are summed

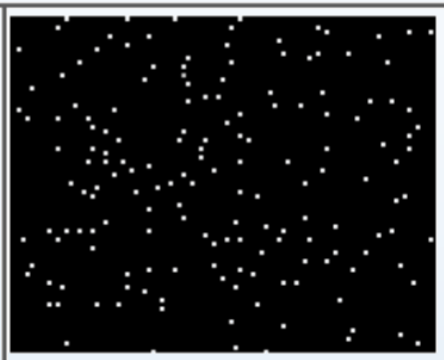
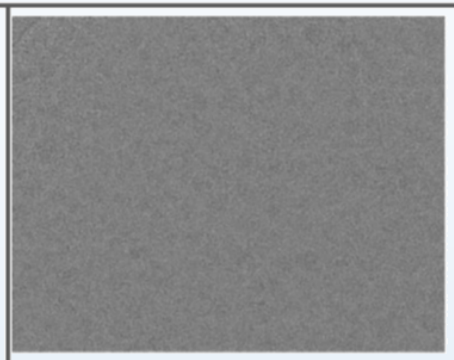
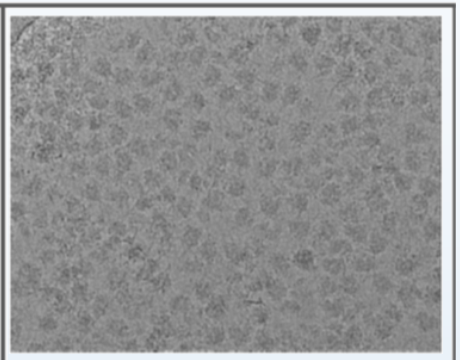
1 sub-frame





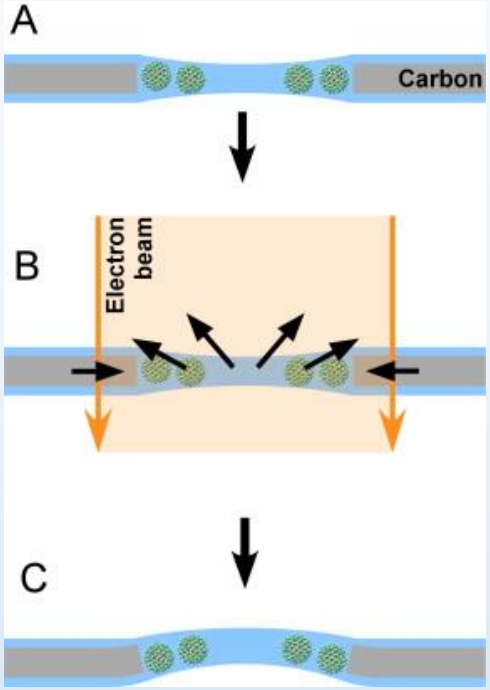
Sub-frames are aligned and summed

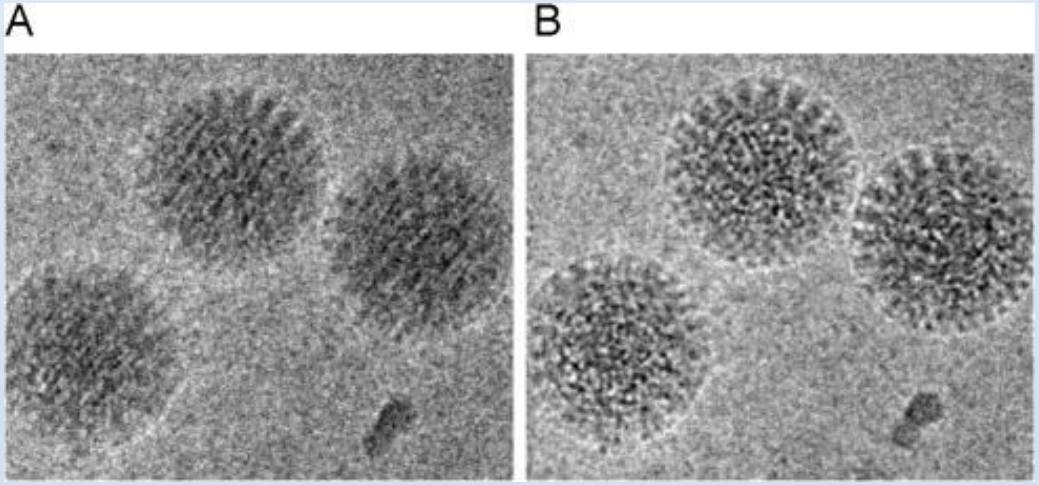
1 final image

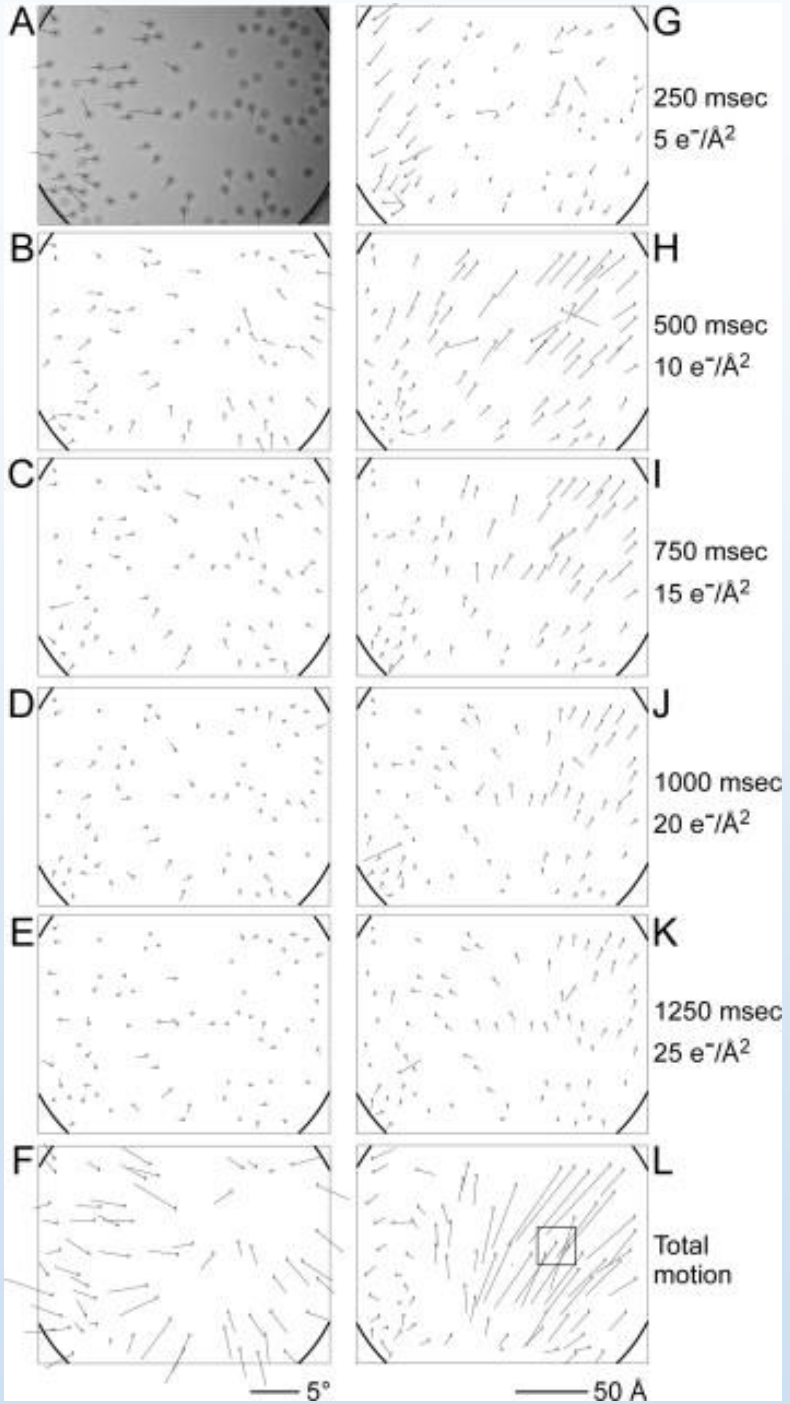
	Sensor Frame Rate	Dose Rate	 Counted Frame	 Sub-frame	 Summed/Aligned Frame
Other	40	0.8 e/pix/s	0.025 s	1 s (1 fps)	100 s
K2	400	8 e/pix/s	0.0025 s	0.1 s (10 fps)	10 s
K3	1500	30 e/pix/s	0.00066 s	0.027 s (37 fps)	2.7 s

Frame alignment.

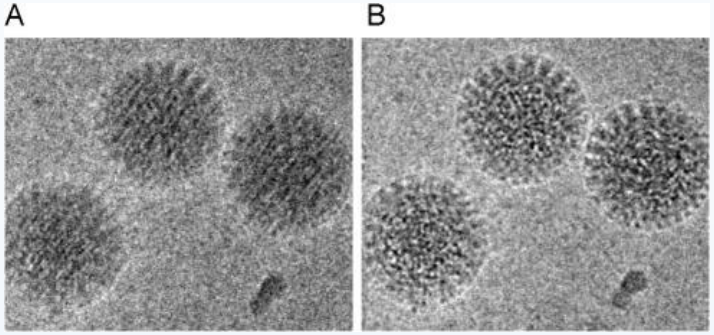
The way to correct Beam Induced Movement.
This type of process are always present and almost random.

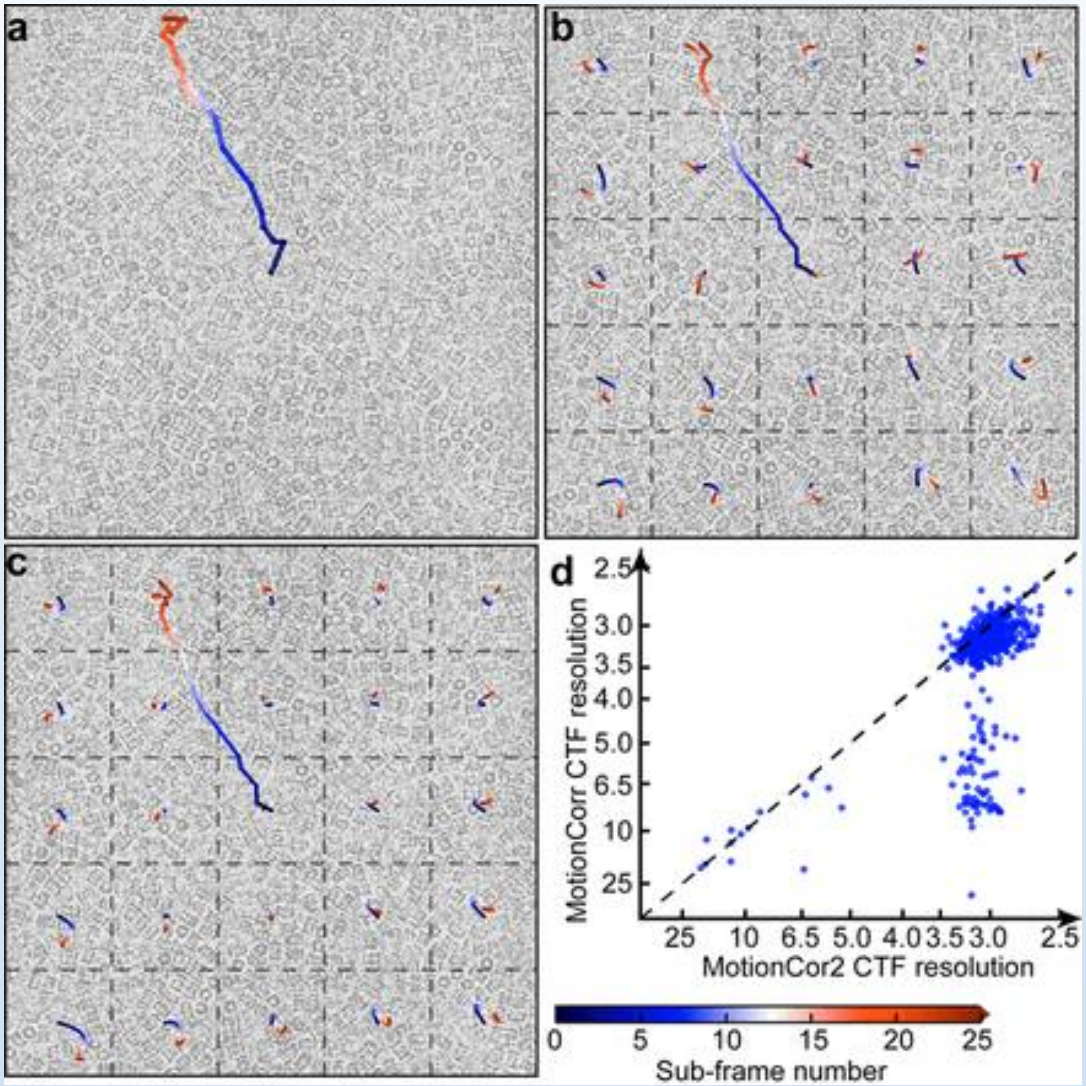






Frame Aligment





Motion correction of cryo-EM images by MotionCor2.(a) Image of frozen hydrated archaeal 20S proteasome overlaid with the traces of motions determined. The long trace originated from the center of image is the global motion based upon full-frame alignment. The whole frame is divided into 5 × 5 patches, and traces of each patch are predicted from the polynomial function. (b) The same image overlaid with the motion traces of each patch as determined from MotionCor2. (c) The trace of globe motion determined from the same image using the original MotionCorr. (d) Resolutions of micrographs estimated using CTFFIND4 [22] from the correction by both MotionCorr (vertical axis) and MotionCor2 (horizontal axis). Circles on the lower right side of the dashed line represent micrographs which MotionCor2 produced better correction.