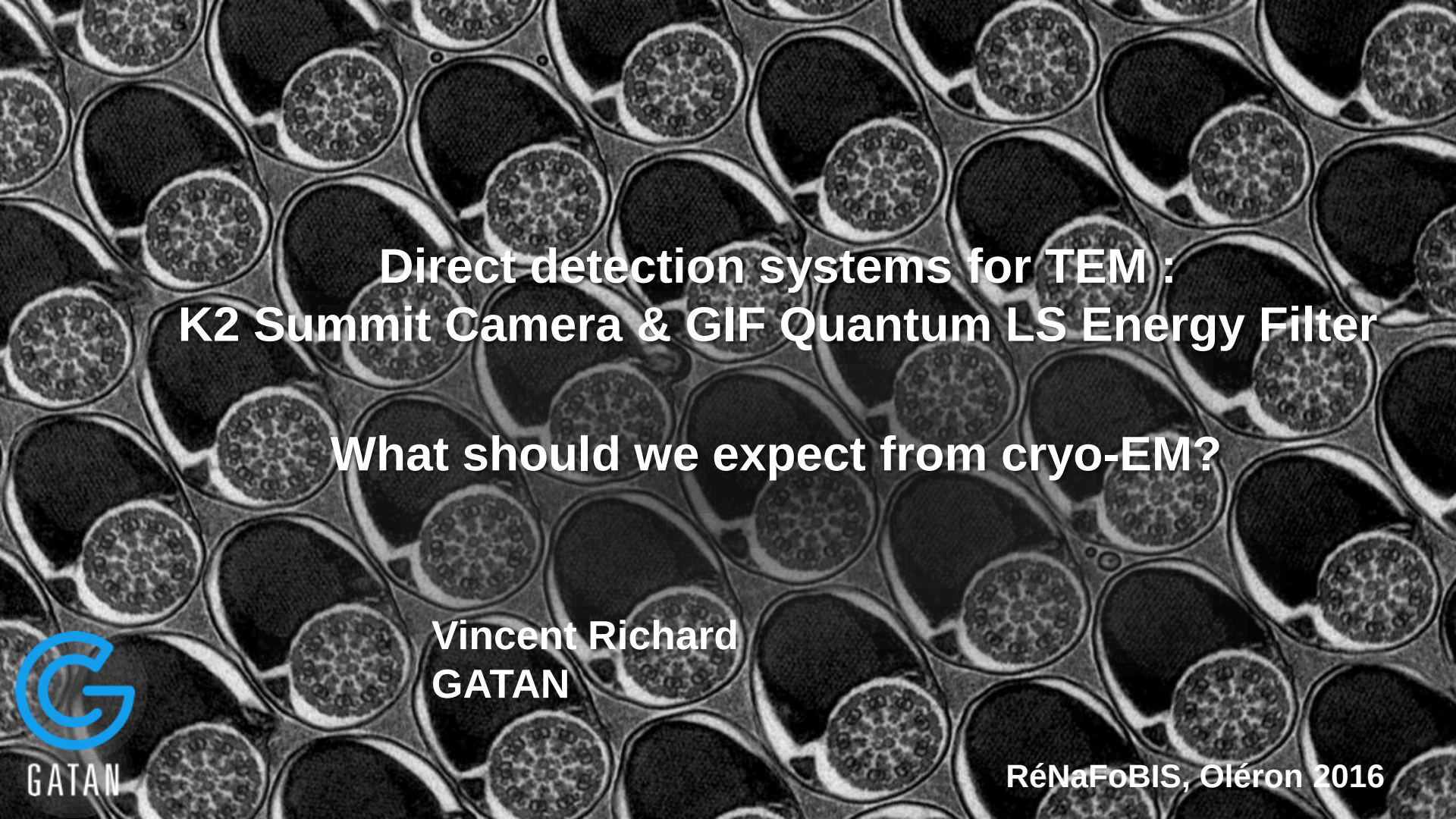


Direct detection systems for TEM :
K2 Summit Camera & GIF Quantum LS Energy Filter

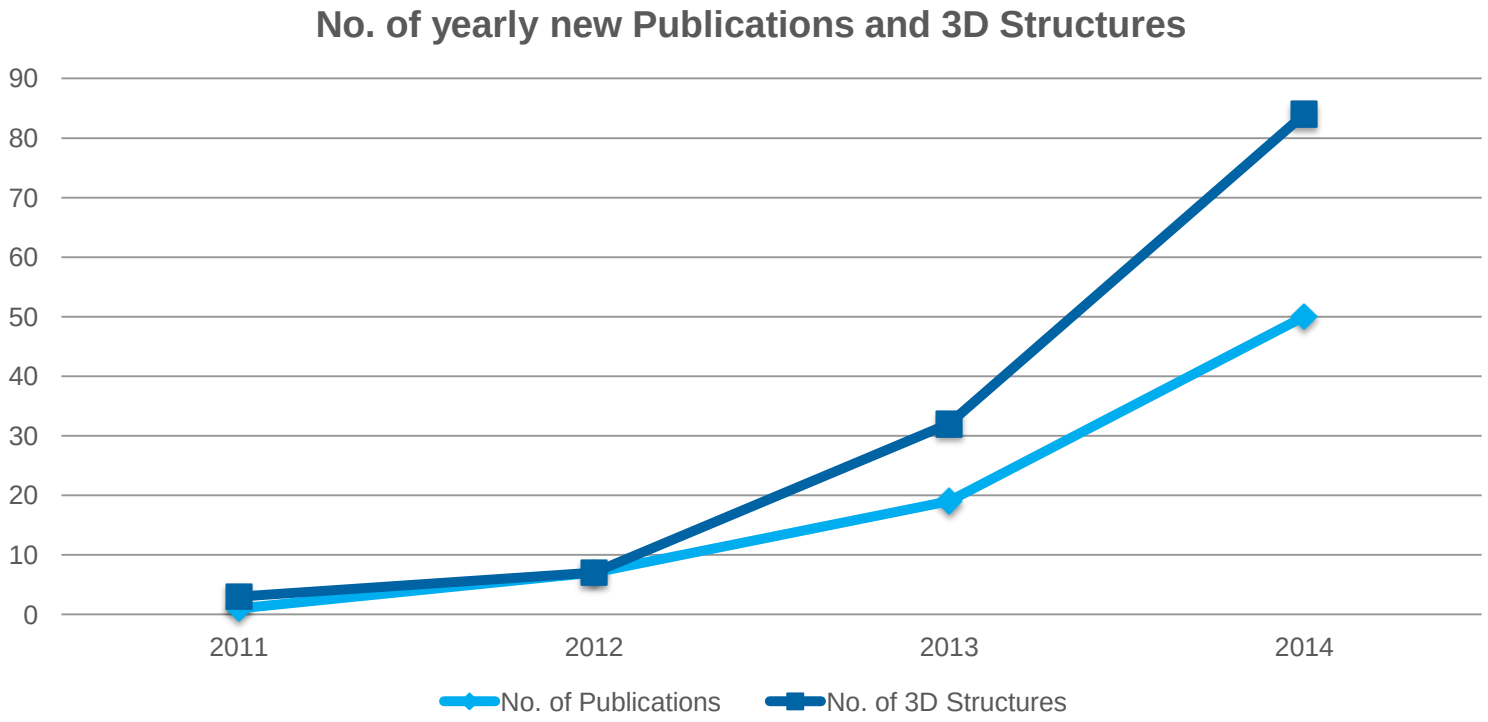
What should we expect from cryo-EM?

Vincent Richard
GATAN

GATAN

RéNaFoBIS, Oléron 2016

Influence of detectors on published data (EMDB, 20. January 2015)



GATAN

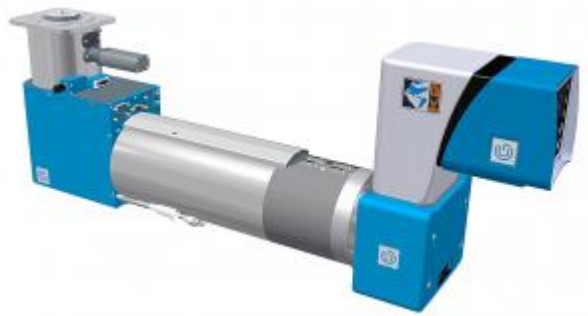
Detectors Designed for Structural Biology

K2 Summit®

- Electron counting camera
- K2 direct detection sensor
- Unmatched performance
- Highest contrast for thin specimens
- Gatan Latitude support
- SerialEM/Leginion support

GIF Quantum® LS

- Electron counting energy filter
- K2 direct detection sensor
- Unmatched performance
- Highest contrast for thick and thin specimens
- Gatan Latitude support
- SerialEM/Leginon support
- FEI embedding supported



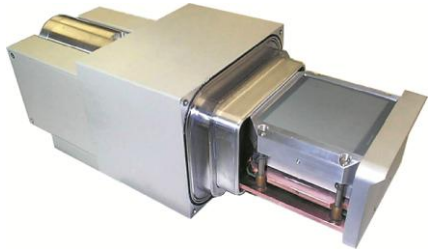
GATAN

History of detection devices

Fluorescence Screen

Film

TV rate cameras



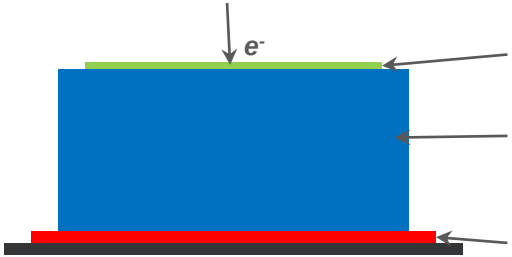
Later CCD and CMOS

Direct Electron Detector

GATAN

What is direct detection?

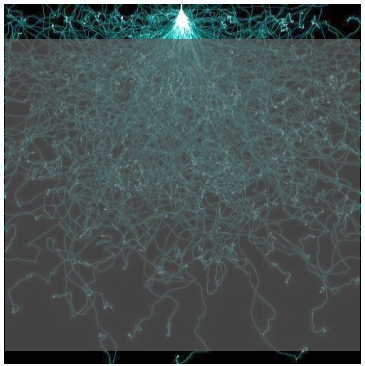
Traditional fiber-coupled camera (CCD or CMOS)



Scintillator
electron to light conversion

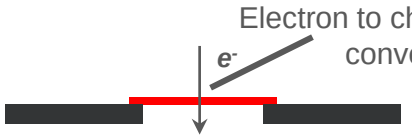
Fiber optic
light image transfer

CCD or CMOS sensor
light to charge conversion



Direct detection camera

Electron to charge conversion

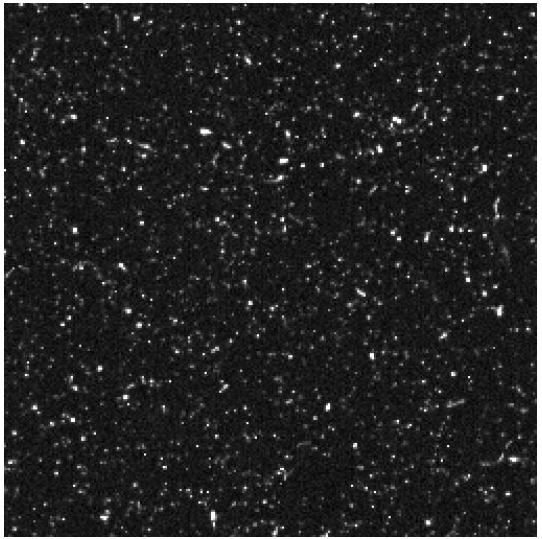


DQE limiting factors: low-Z Si sensor

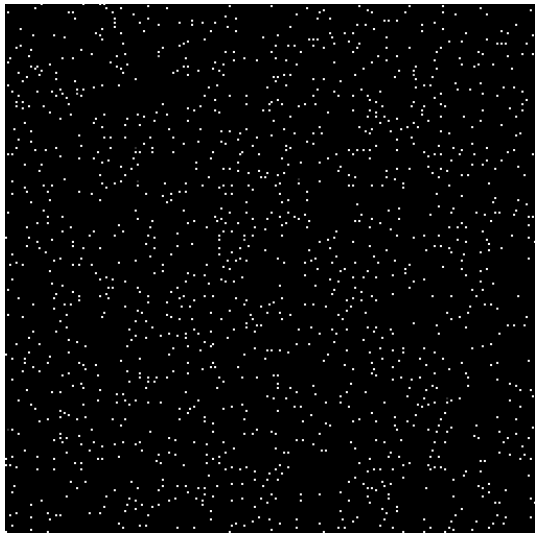
- Electron scattering in ~~high-Z scintillator~~
- ~~Electron back-scattering from fiber optic~~
- ~~Scattering of light in fiber optic~~
- ~~Distortions from fiber optic~~
- Electronic read noise

GATAN

What is electron counting?



Single 2.5 ms frame using conventional charge read-out



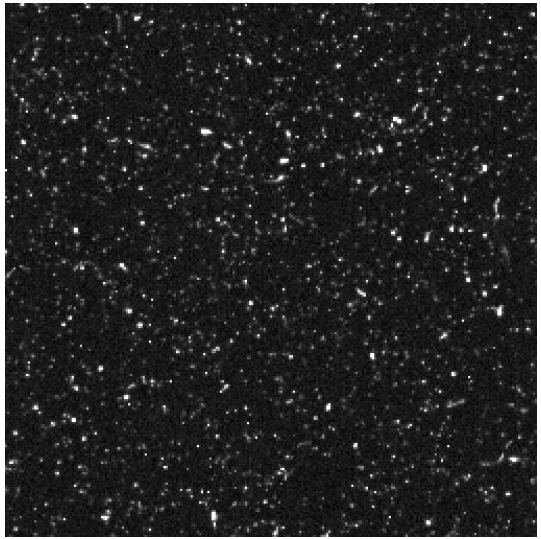
Same frame after counting

Counting removes the variability from scattering, rejects the electronic read-noise, and restores the DQE

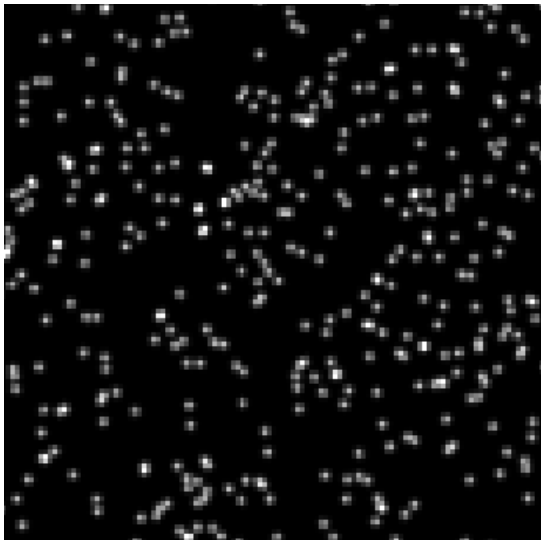
GATAN

So why doesn't every camera allow counting?

Typical dose rate of 10 e^-/pix/s



40 fps: events overlap and cannot be resolved



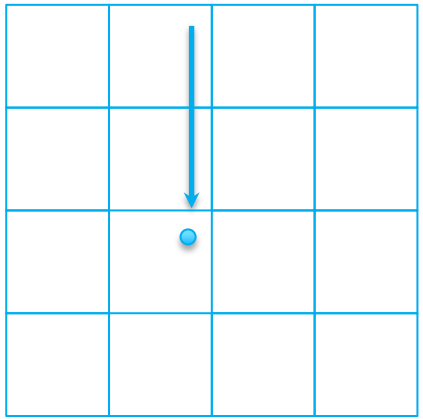
400 fps: events are resolved

It takes 400 fps to resolve electrons at a dose rate of 10 e^-/pix/s

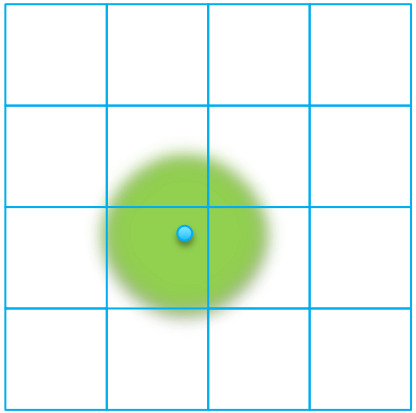
GATAN

Improved DQE at high frequency

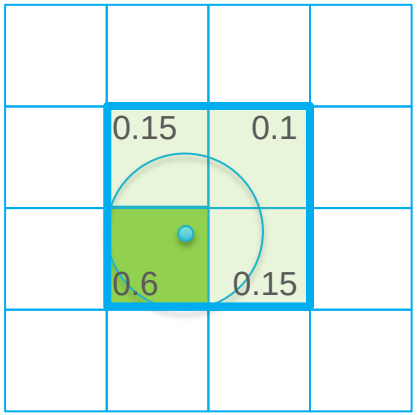
K2 Base: charge integration
Improved DQE at high frequency



1. Electron enters detector



2. Signal is scattered

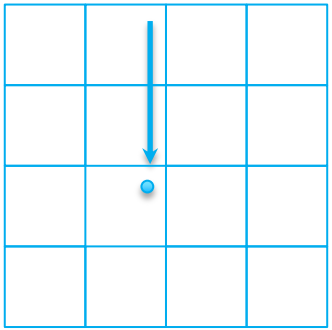


3. Charge collects in each pixel

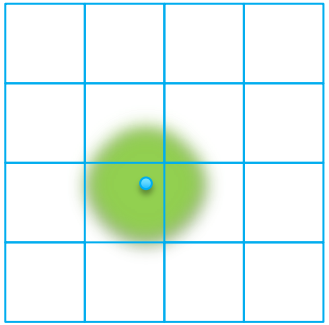
GATAN

Improved DQE at high and low frequency

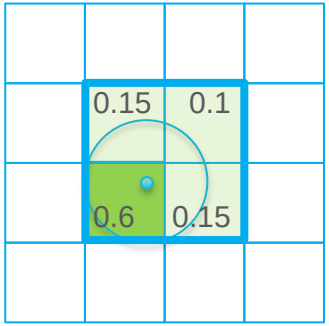
K2 Summit: counting
Improved DQE at low and high frequency



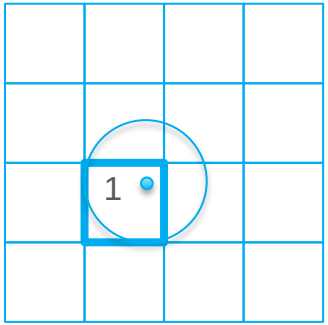
1. Electron enters detector



2. Signal is scattered



3. Charge collects in each pixel

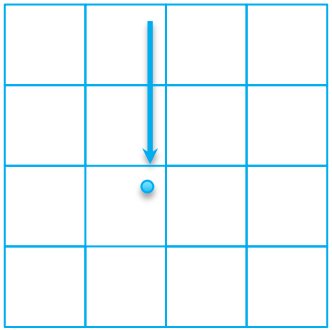


4. Events are reduced to the highest charge pixels

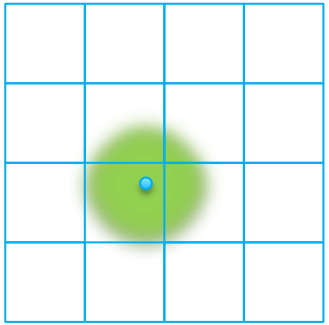
GATAN

Improved DQE at high and low frequency

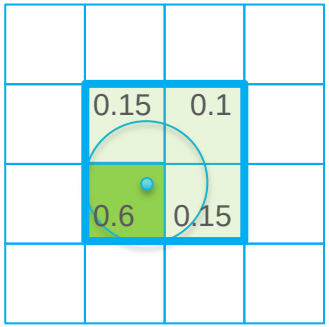
K2 Summit: super-resolution
Improved DQE at low and high frequency 7680 x 7424 pix



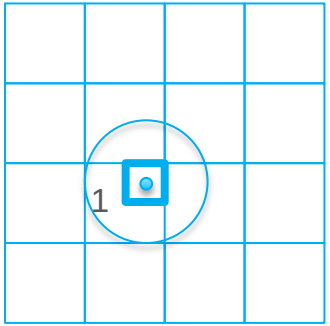
1. Electron enters detector



2. Signal is scattered



3. Charge collects in each pixel



4. Events are localized with sub-pixel accuracy

GATAN

The impact of DQE: why is it important?

Detective quantum efficiency

Input image: Low contrast picture of Siméon Denis Poisson

Detector
DQE of 0.33

Output Image: Image after recording with a camera with uniform 33% DQE

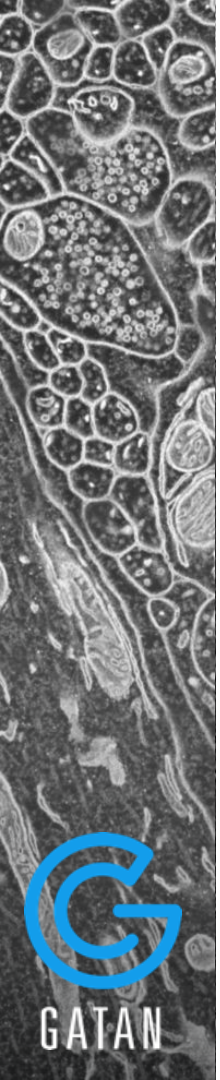
Image signal detail is lost in the noise added by the camera

$$DQE(s) = \frac{SNR^2{}_{out}(s)}{SNR^2{}_{in}(s)} = \frac{MTF(s)}{NTF(s)}$$

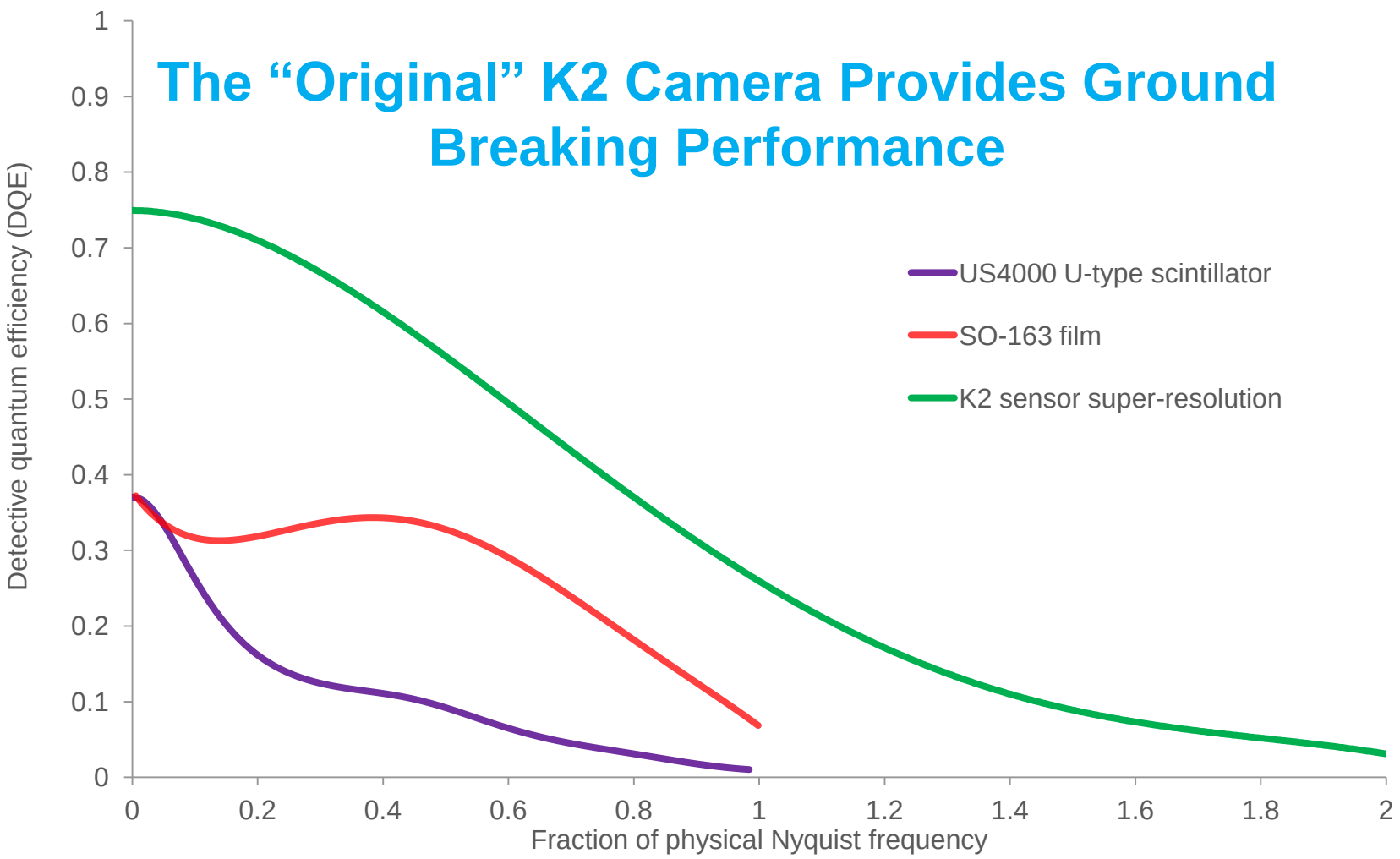
Cryo **:** low contrast samples require highest possible SNR output

Camera is most critical element

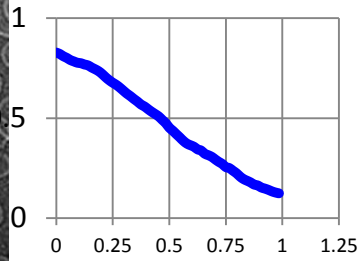
GATAN



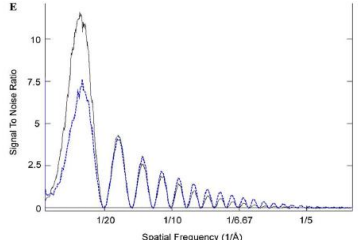
The “Original” K2 Camera Provides Ground Breaking Performance



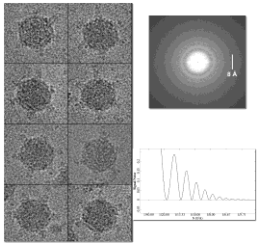
What Does DQE Mean for Cryo-EM?



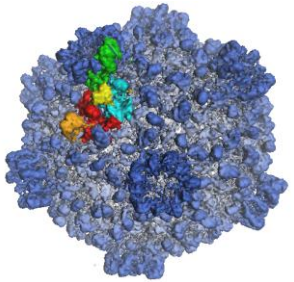
Improved DQE



Improved carbon Thon rings



Improved particle resolution



Better/easier reconstructions

Improvement in DQE → **Better Thon rings from amorphous C** → **Better Thon rings from real samples** → **Higher quality cryo-EM images**

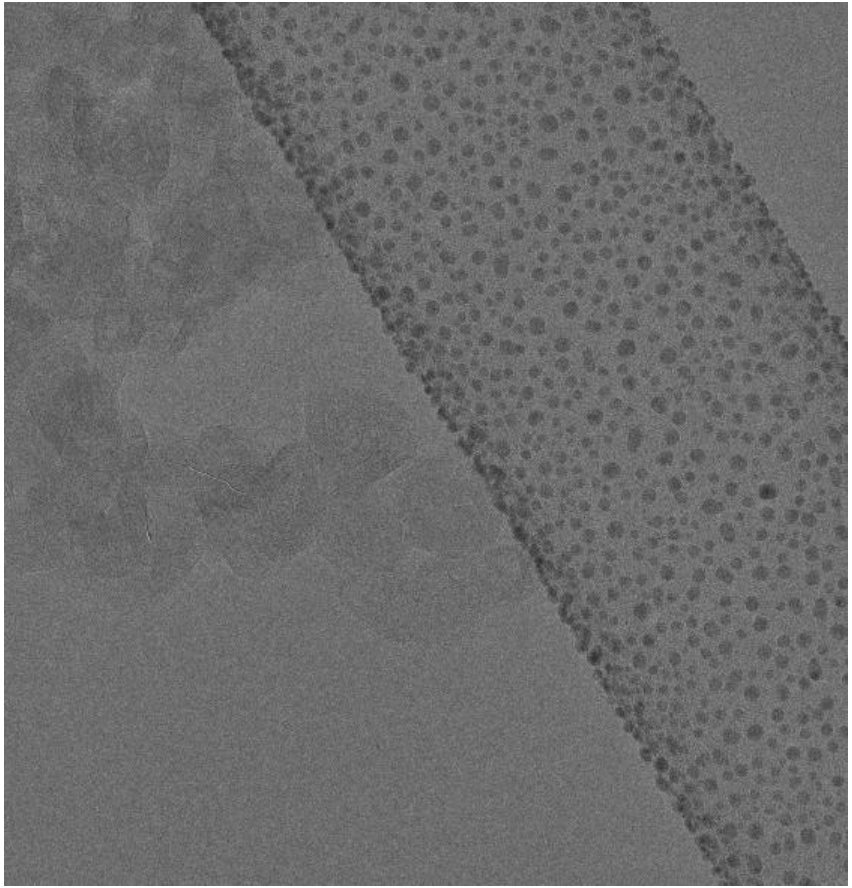
Higher resolution reconstructions with fewer images required.

GATAN

What is Dose Fractionation ?

- Dose fractionation is the distribution of a total electron dose over a series of sub-frames
- 21 x 0.33 sec = 7 sec

7 sec exposure time without drift correction

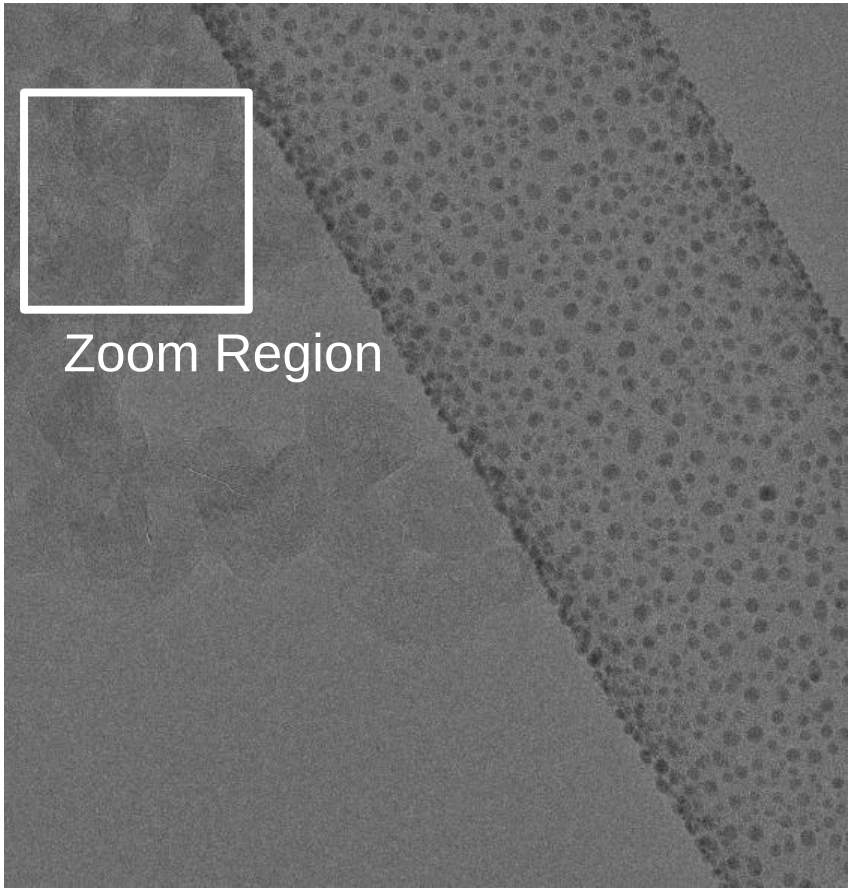


GATAN

Dose Fractionation

- Dose fractionation is the distribution of a total electron dose over a series of sub-frames
- 21 x 0.33 sec = 7 sec

7 sec exposure time with drift correction

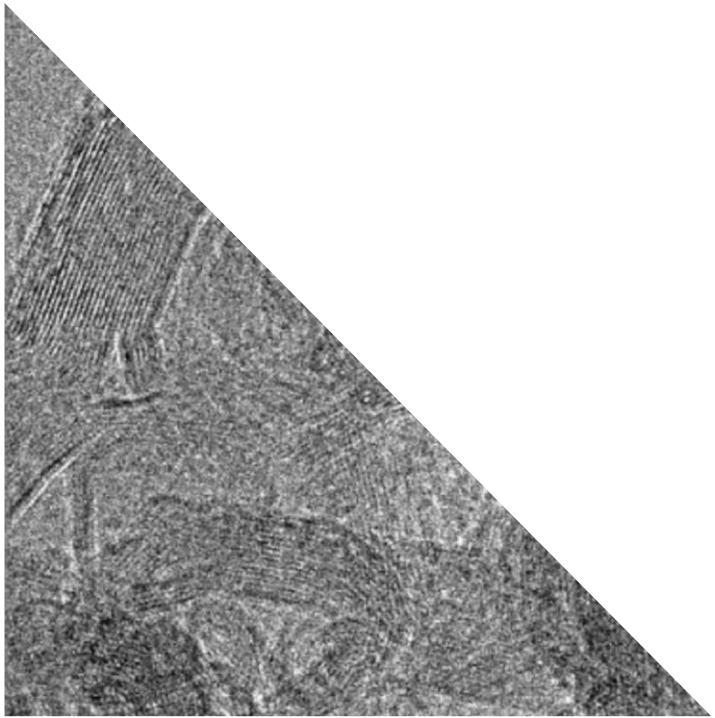


GATAN

Dose Fractionation

- Dose fractionation is the distribution of a total electron dose over a series of sub-frames

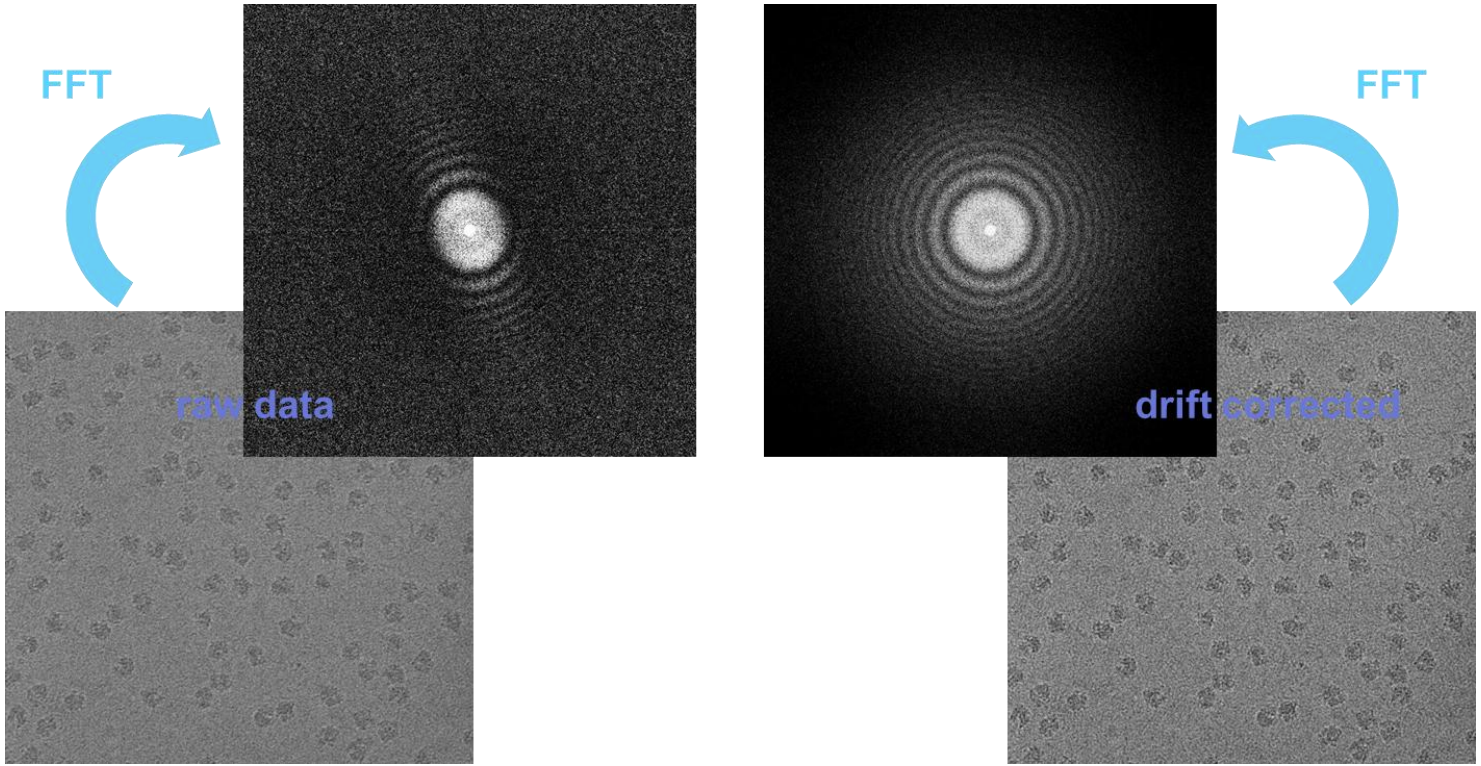
without sub-frame drift correction



with sub-frame drift correction

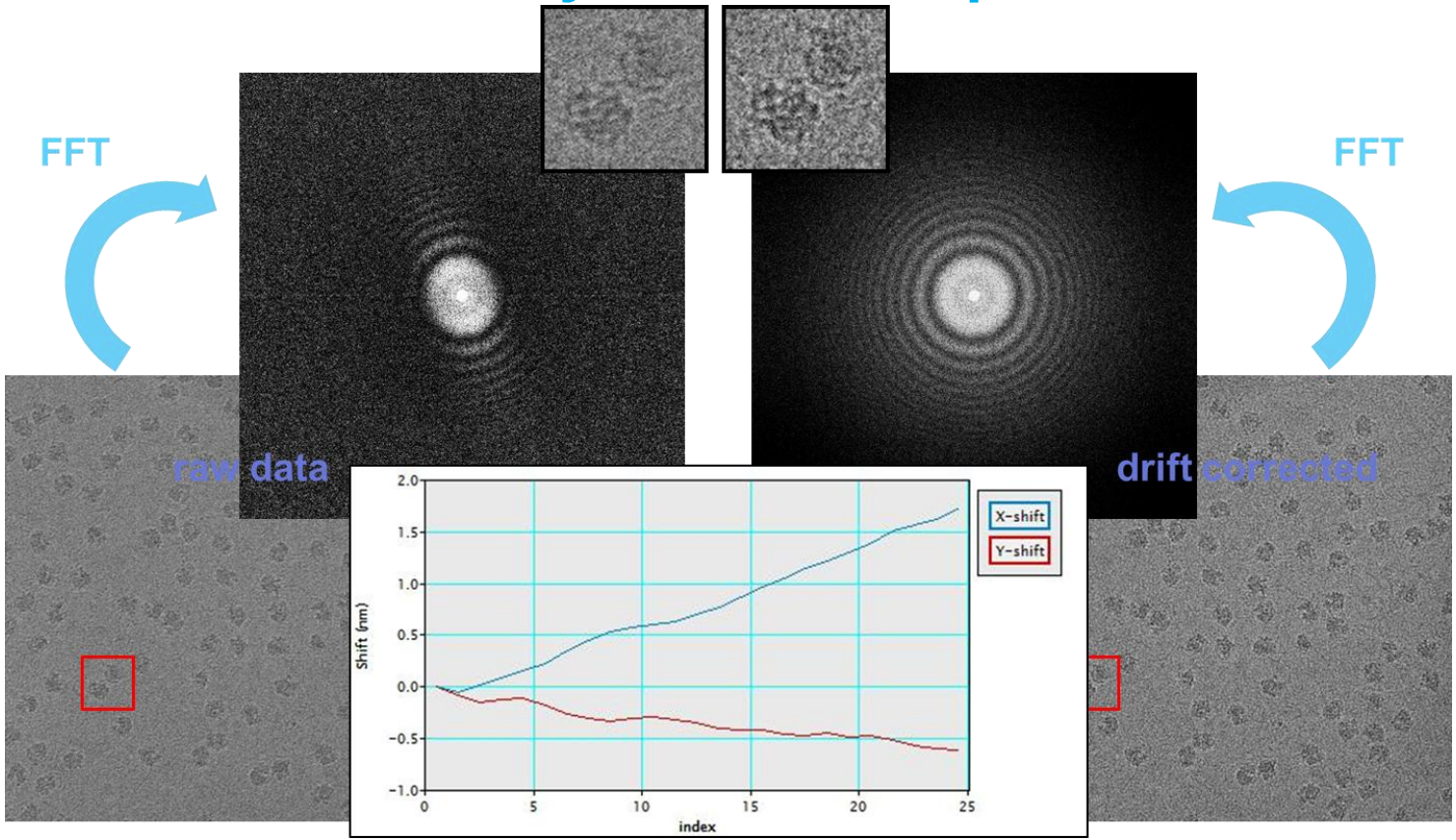
GATAN

Drift correction: Cryo-TEM example of Ribosome



GATAN

Drift correction: Cryo-TEM example of Ribosome



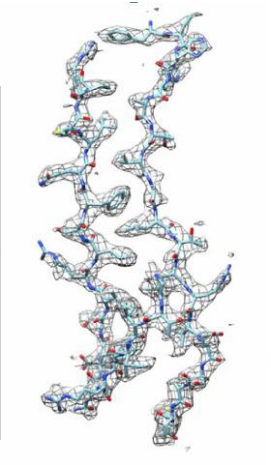
GATAN

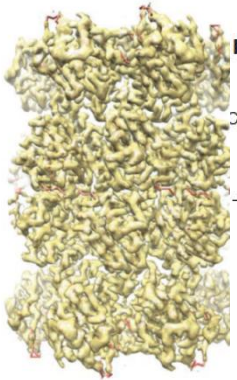
Benefits coming from K2 :

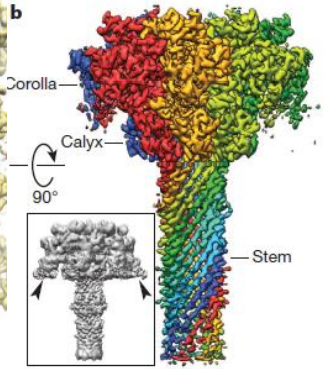
- Direct detection ☺
- Counting ☺
- SuperResolution ☺
- Dose fractionation ☺
- Drift correction ☺

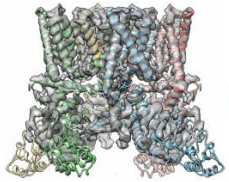
GATAN

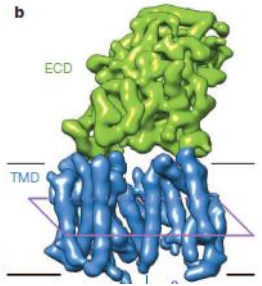
K2 Summit/K2 Quantum: Powerful Tools for High Impact Science







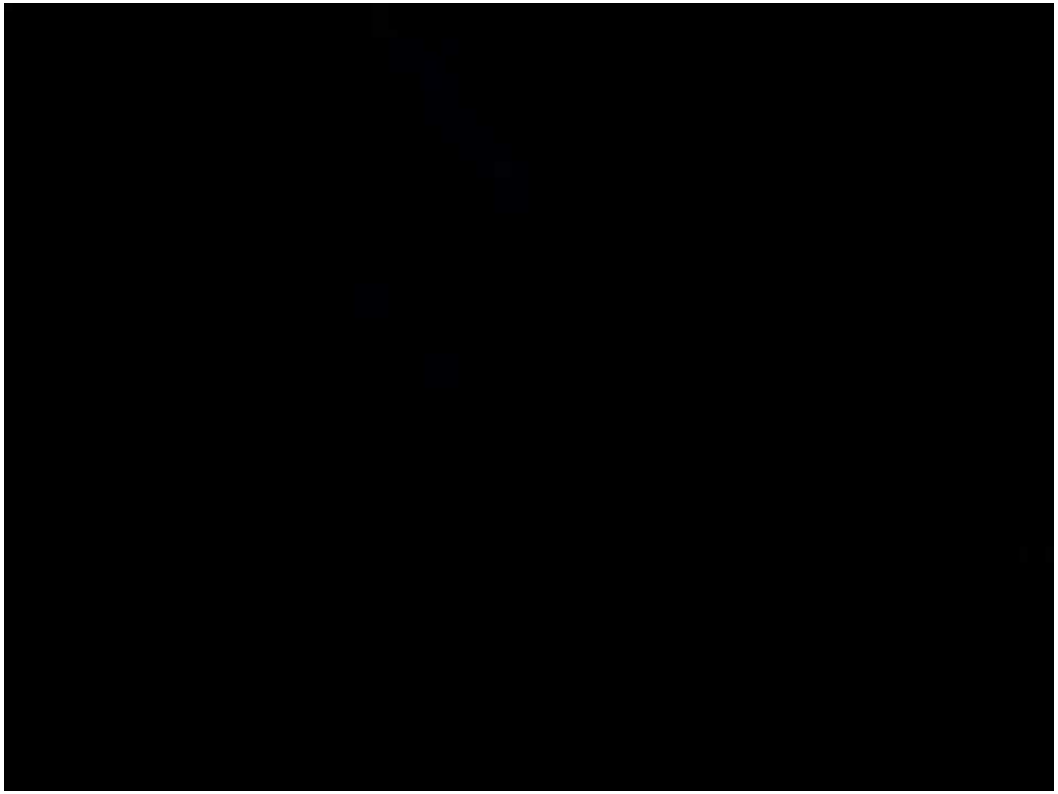




2.2 Å **β-galactosidase**	**2.6 Å** **Rotavirus**	**2.8 Å** **Proteasome**	**2.9 Å** **Anthrax Pore**	**3.4 Å** **TRPV1**	**4.5 Å** **γ-secretase**
465 kDa Bartesaghi et al., Science 2015	126 MDa Grant/Grigorieff (EMDB-6272)	700 kDa Campbell et al. eLife 2015	425 kDa Jiang et al., Nature 2015	380 kDa Liao et al., Nature 2013	170 kDa Lu et. al., Nature 2014
NIH	Janelia Farms	NRAMM	Scripps Research Inst.	UCSF	MRC-LMB/Tsinghua
K2 Quantum	K2 Summit	K2 Summit	K2 Summit	K2 Summit	K2 Quantum
Frealign	Frealign	Relion	Frealign	Relion	Relion
Manual Imaging	Leginon	Leginon	Leginon	Manual/UCSF Image	Manual Imaging

GATAN

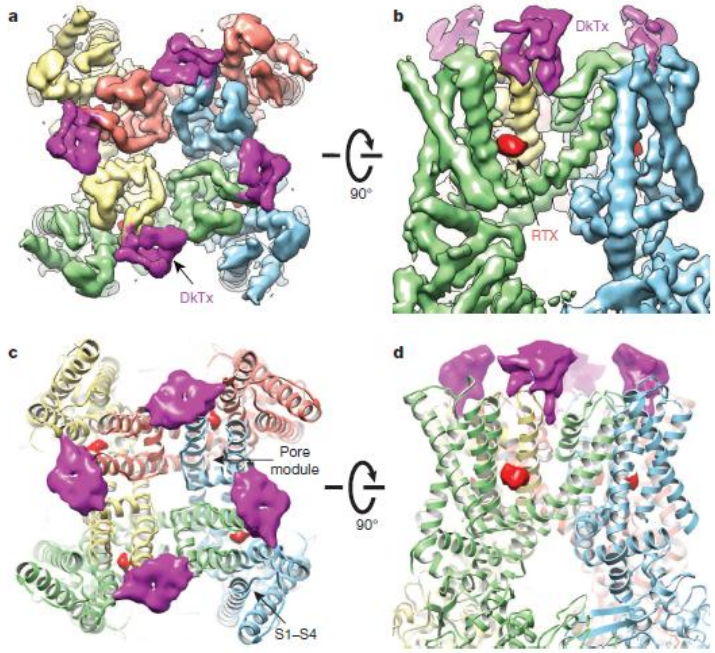
High Resolution Helps with Drug Development



GATAN

Cryo Electron Microscopy (Cryo-EM) Shows Impact of Drug Binding on Protein

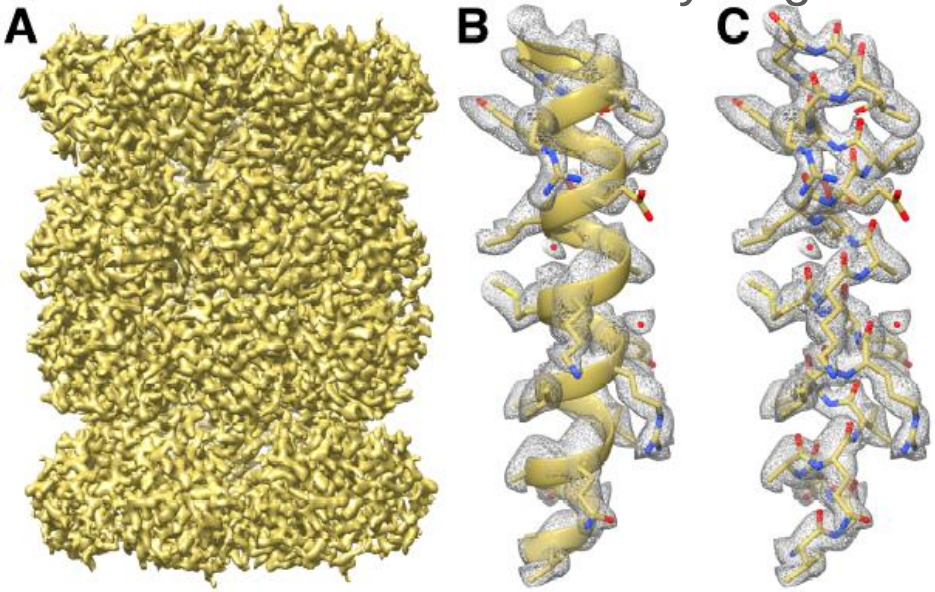
- TRPV1 is an important drug target: chronic pain
- AstraZeneca, Bayer, Eli Lilly, Janssen, Johnson&Johnson, Novartis all have drugs targeting TRPV in clinical trials

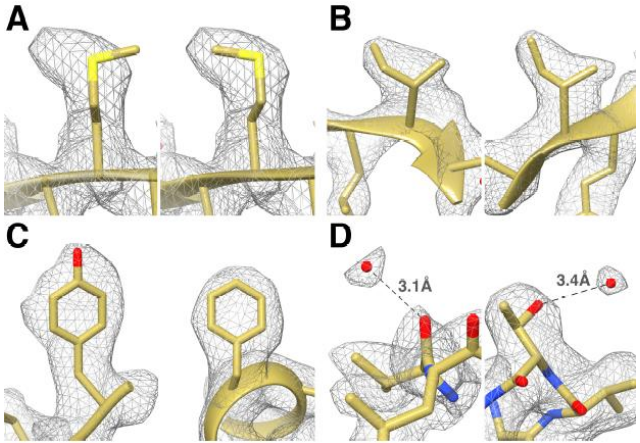


Data courtesy Yifan Cheng, UCSF

2.8 Å Resolution Reconstruction of the *Thermoplasma acidophilum* 20 S Proteasome using Cryo-electron Microscopy

- 2.8 Å resolution
- Side chain conformations
- Water molecules and hydrogen bonds





eLife 2015;10.7554/eLife.06380

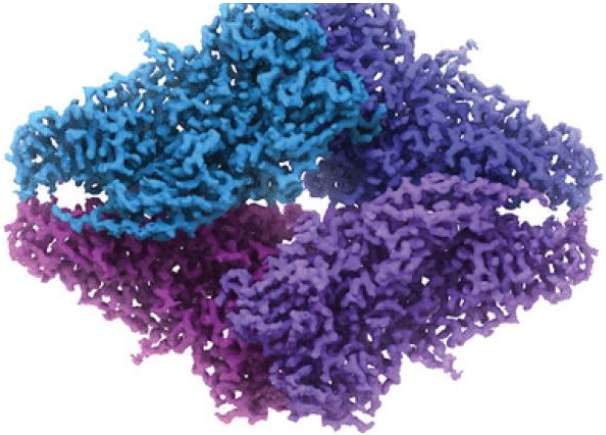
GATAN

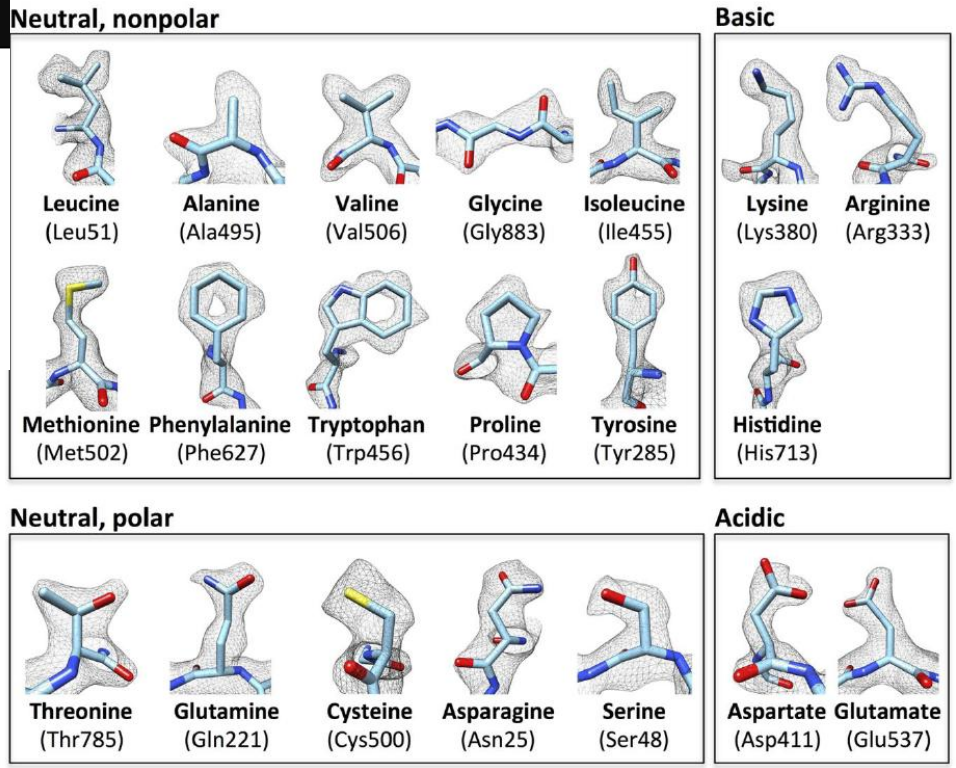
Highest Resolution Structure – GIF Quantum LS

Sciencexpress

2.2 Å resolution cryo-EM structure of β-galactosidase in complex with a cell-permeant inhibitor

Alberto Bartesaghi,[1]* Alan Merk,[1]* Soojay Banerjee,[1] Doreen Matthies,[1] Xiongwu Wu,[2] Jacqueline L. S. Milne,[1] Sriram Subramaniam[1]†





GATAN

Merci pour votre attention !!

GATAN