Direct electron detectors in electron cyro-microscopy (cryoEM)

IWORID 2023

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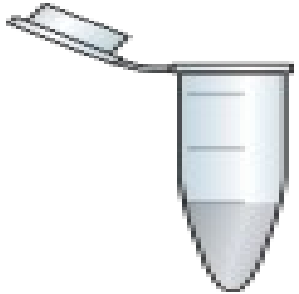
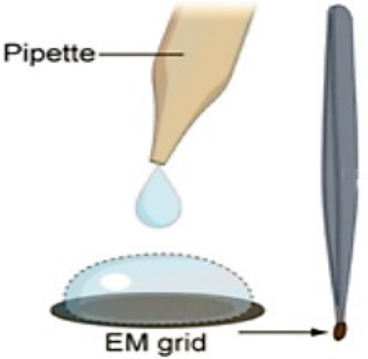
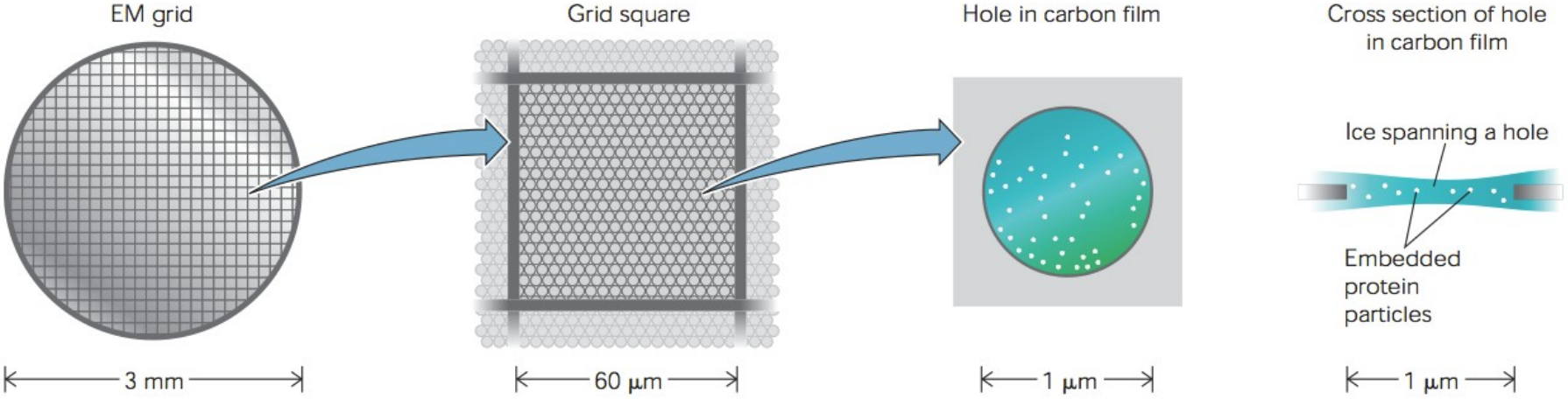
Overview:

- What is cryoEM
- Why detectors are so important to CryoEM
- Differences between imaging:
  - 100 keV electrons
  - 300 keV electrons
- Data handling problems
- Future detectors

CryoEM




Pipette
EM grid


EM grid
Grid square
Hole in carbon film
Cross section of hole in carbon film
Ice spanning a hole
Embedded protein particles
3 mm
60 µm
1 µm
1 µm

JEOL
(cryoARM)

ThermoScientific
(Krios G4)

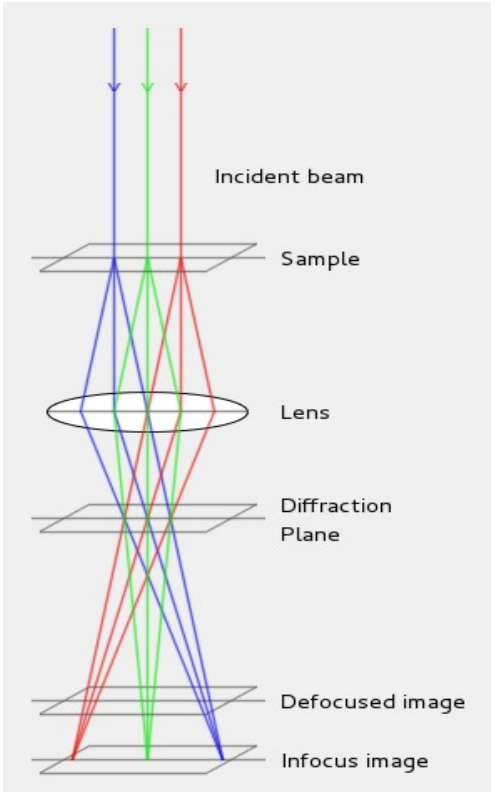
- 300 keV
- 100 000 000 kr
- Service costs
- “White boxes”
- Better than needed?

Electron Microscope

Cheaper
7 000 000 kr

Electron Microscope

- Based on JEOL's cheapest 120 keV electron microscope
- Need FEG (supplied by YPS – no SF6)
- Need excellent imaging detector (Dectris Singla)
- Really want 2k x 2k detector
- Current “lash-up” is good enough 2.6 Å resolution



Bright field phase contrast imaging

Field emission Source

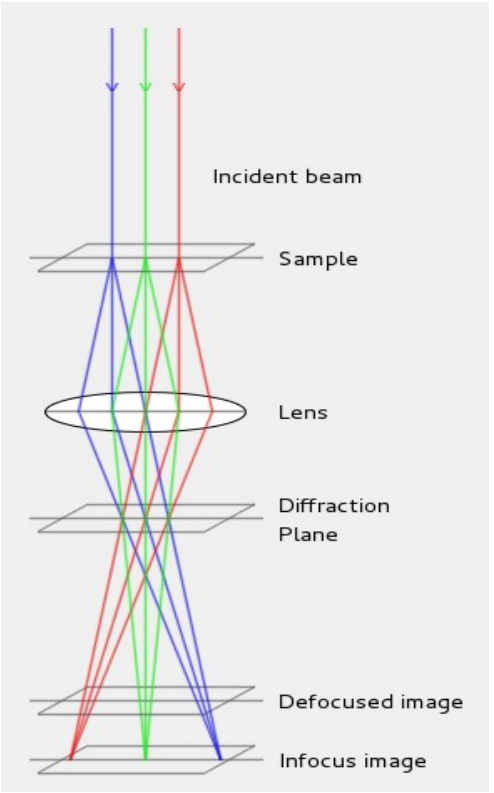
High DQE detector

Electron Microscope

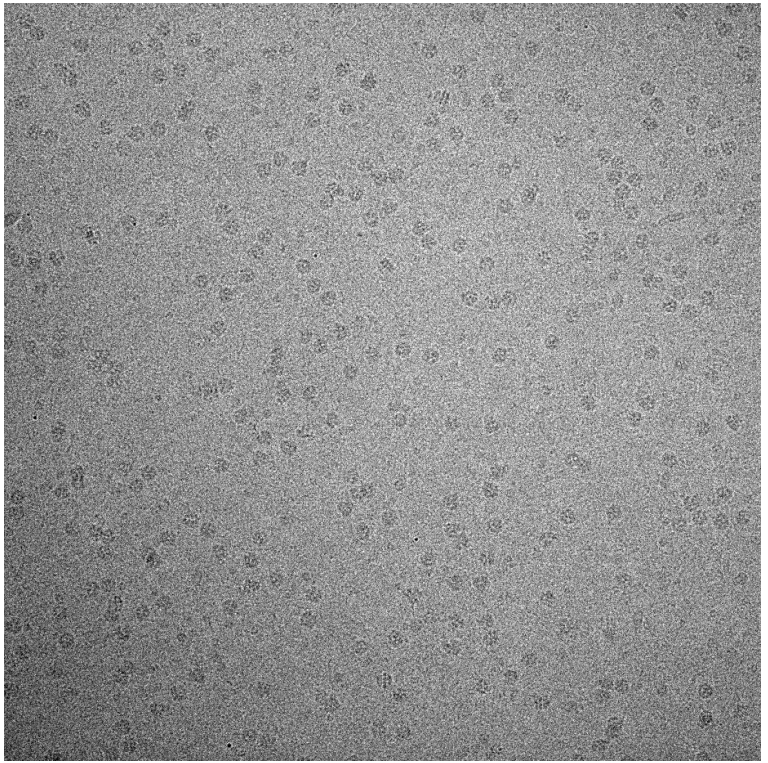
Only one electron in microscope at a time!

- **Limited to ~20 electrons per square Å**
- 2 Second exposure
- Image area ~ 20 x 10^6 pixels
- 1/(2Å) resolution (1Å pixel)
- Have 200 x 10^6 electrons per second
- Electrons moving at 200 x 10^6 m/s
- **1 m between electrons**

Electron Microscope

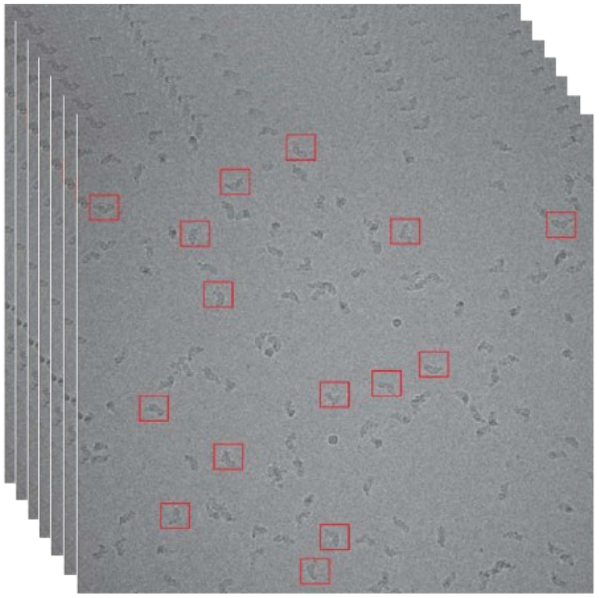


Bright field phase contrast imaging

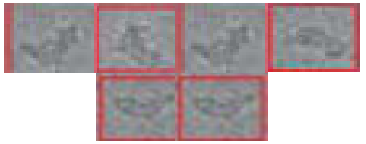


Low contrast noisy images

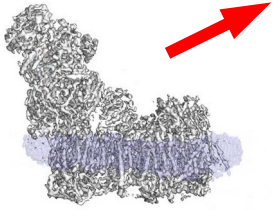
CryoEM data processing



Images



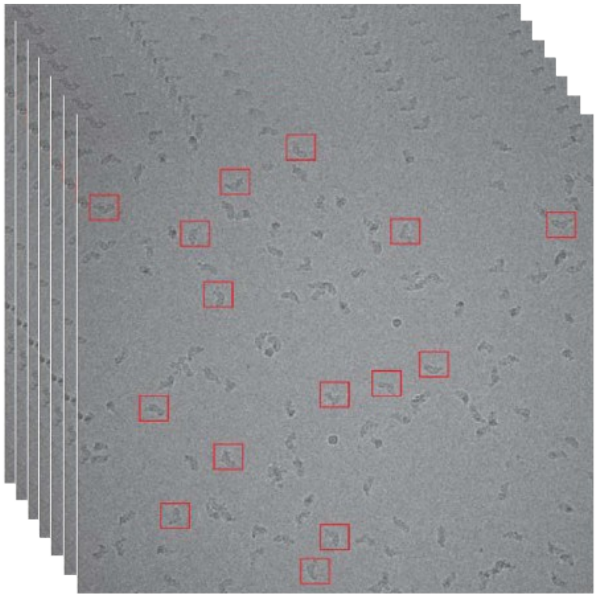
Select particles
(1000 to 10^6)



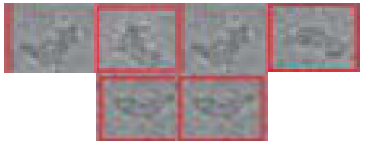
Generate model

Match
projections

CryoEM data processing



Images



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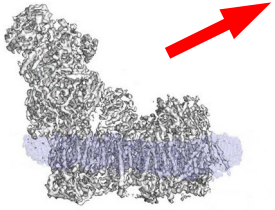

Generate model

Match
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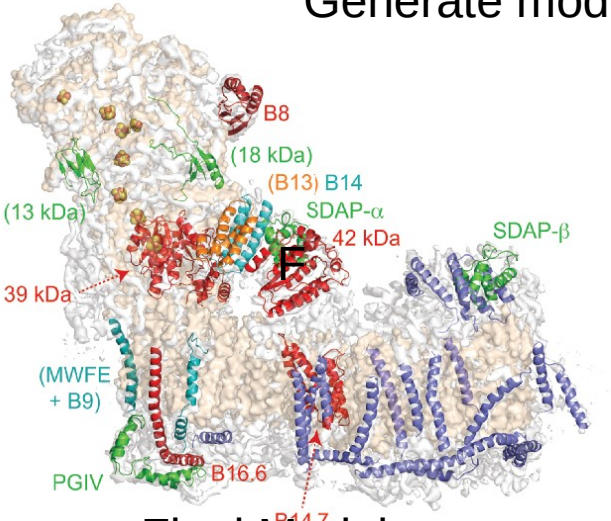
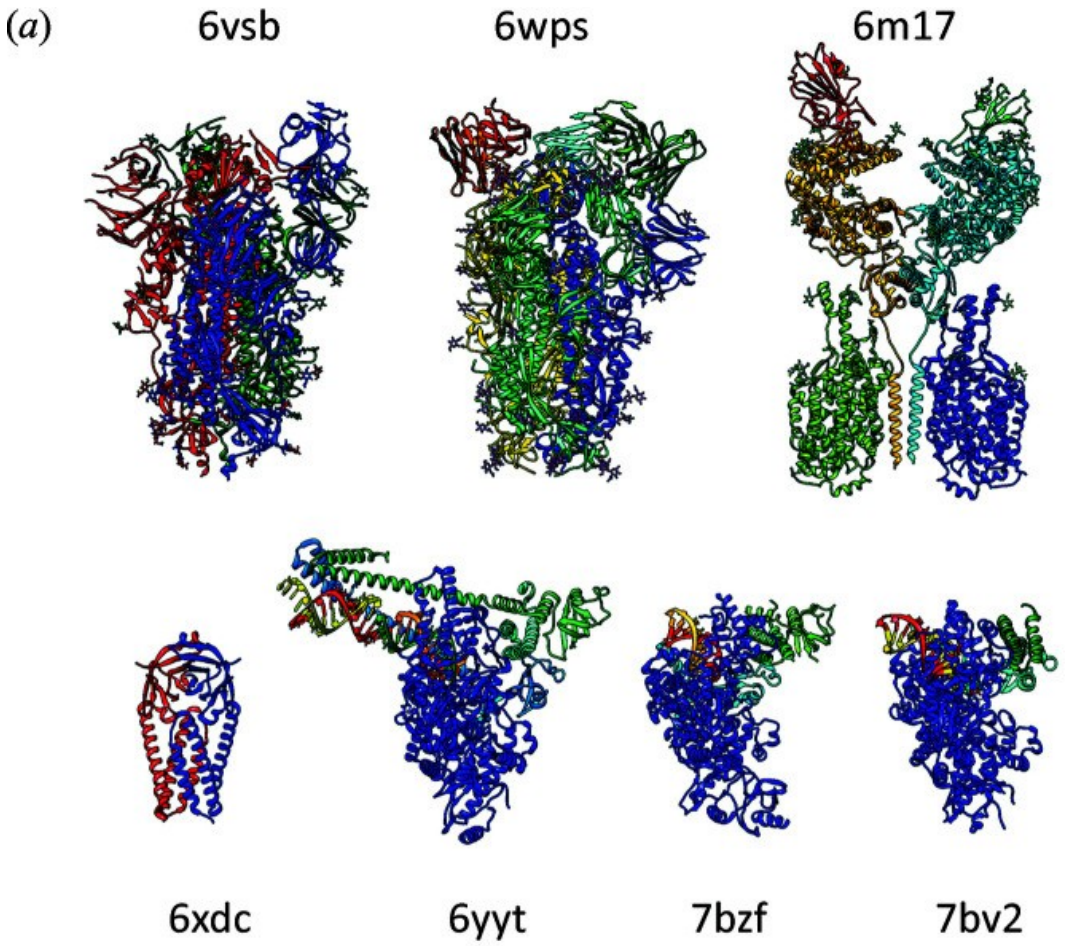


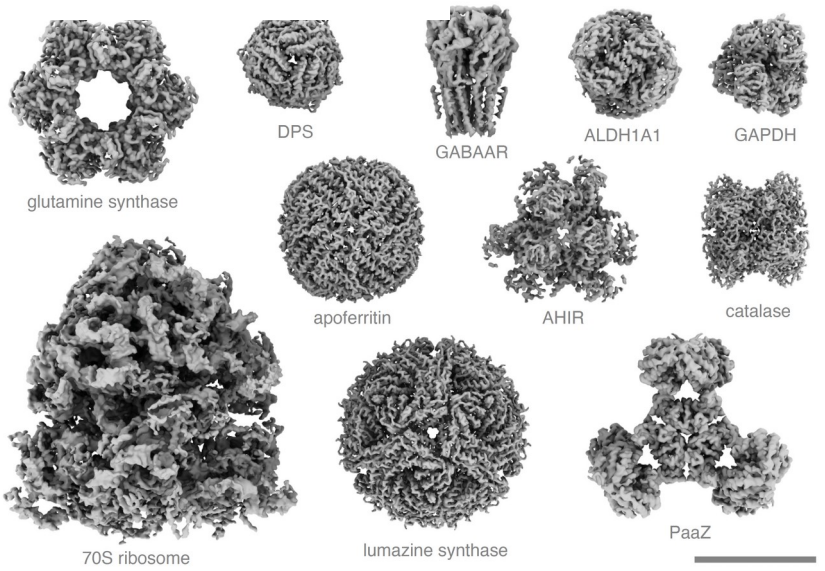
Final Model

Covid19 results



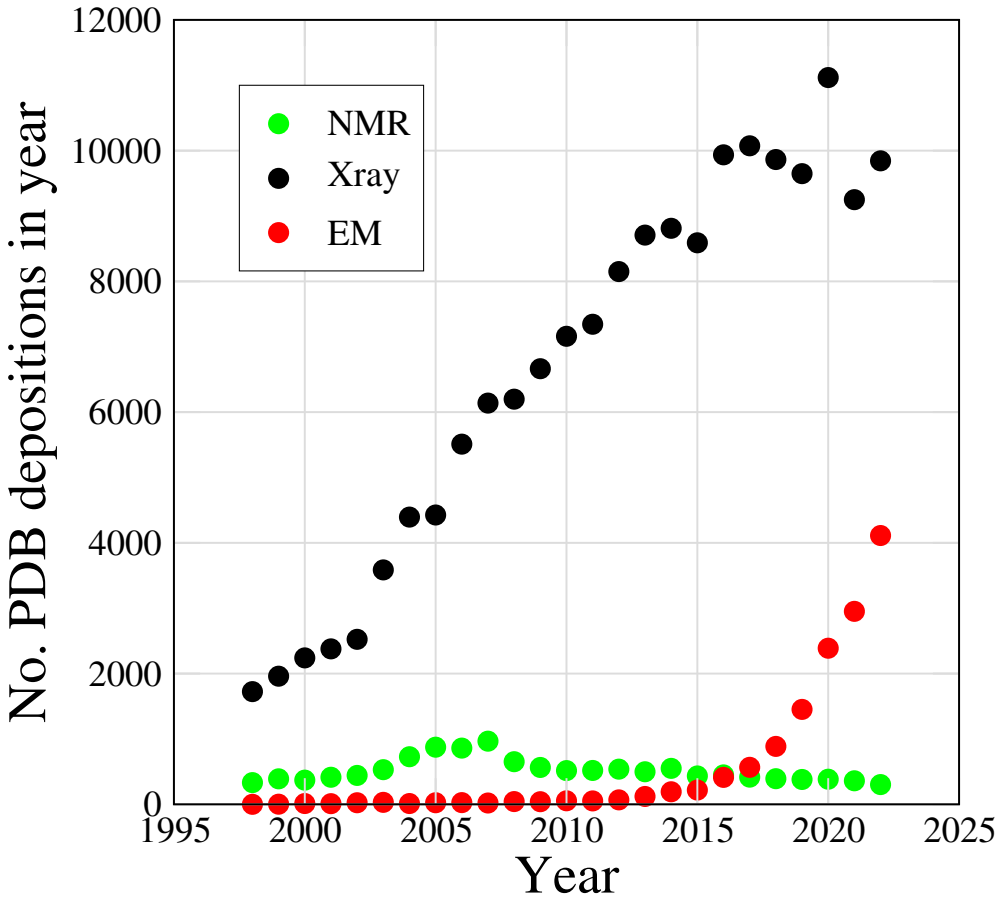
Subramaniam IJCrJ 2020 Jul 2 5750576

7SVR PDB ENTRY

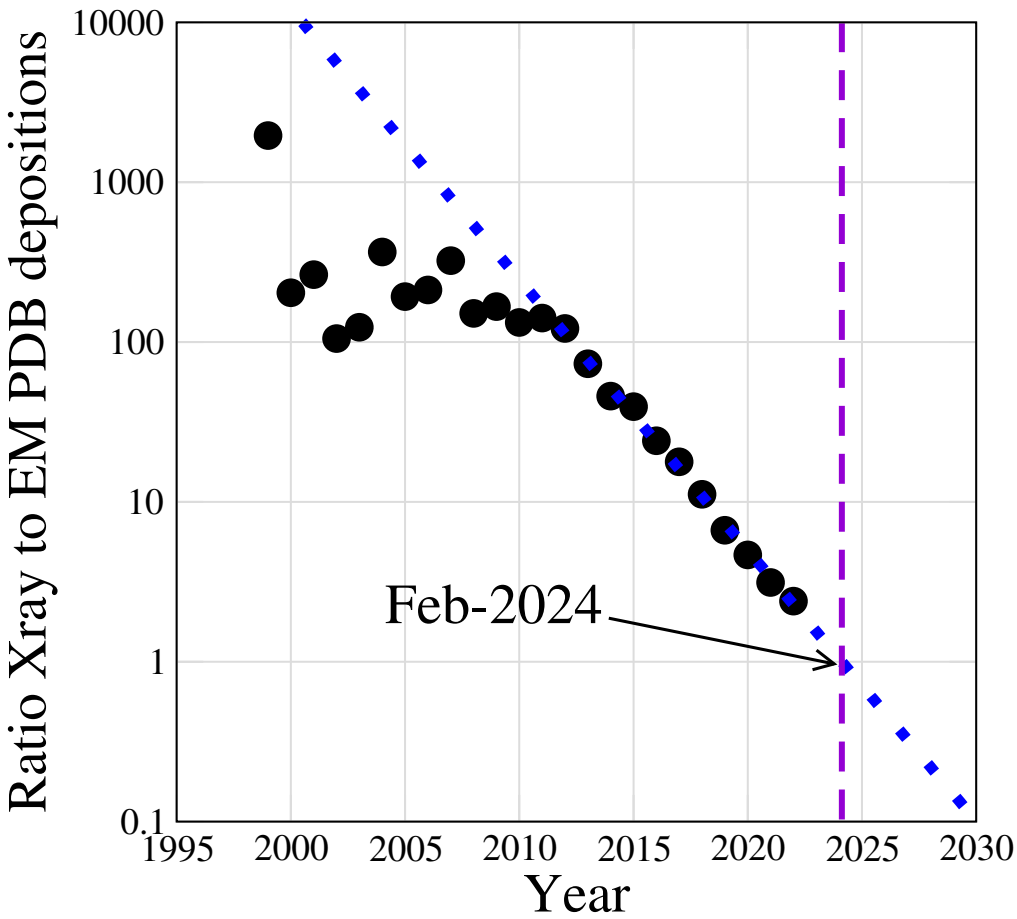


100 keV results

PDB depositions per annum by method



Xray versus EM depositions



Statistics from Worldwide Protein Data Bank (wwPDB.org)

Total coordinates deposited 204,826 (May 2023)

Why direct electron detectors have made such a difference

- **Higher DQE**
- Movies allowing motion correction
- Movies allowing radiation damage weighting
- Many more images

- Better reconstruction programs
- Microscope automation
- More people in the field
- Higher expectations
- Success attracts Money

CryoEM:

- Two tribes:
  - ➔ Tomography
  - ➔ Single particle:
    - 300 keV
    - 100 keV

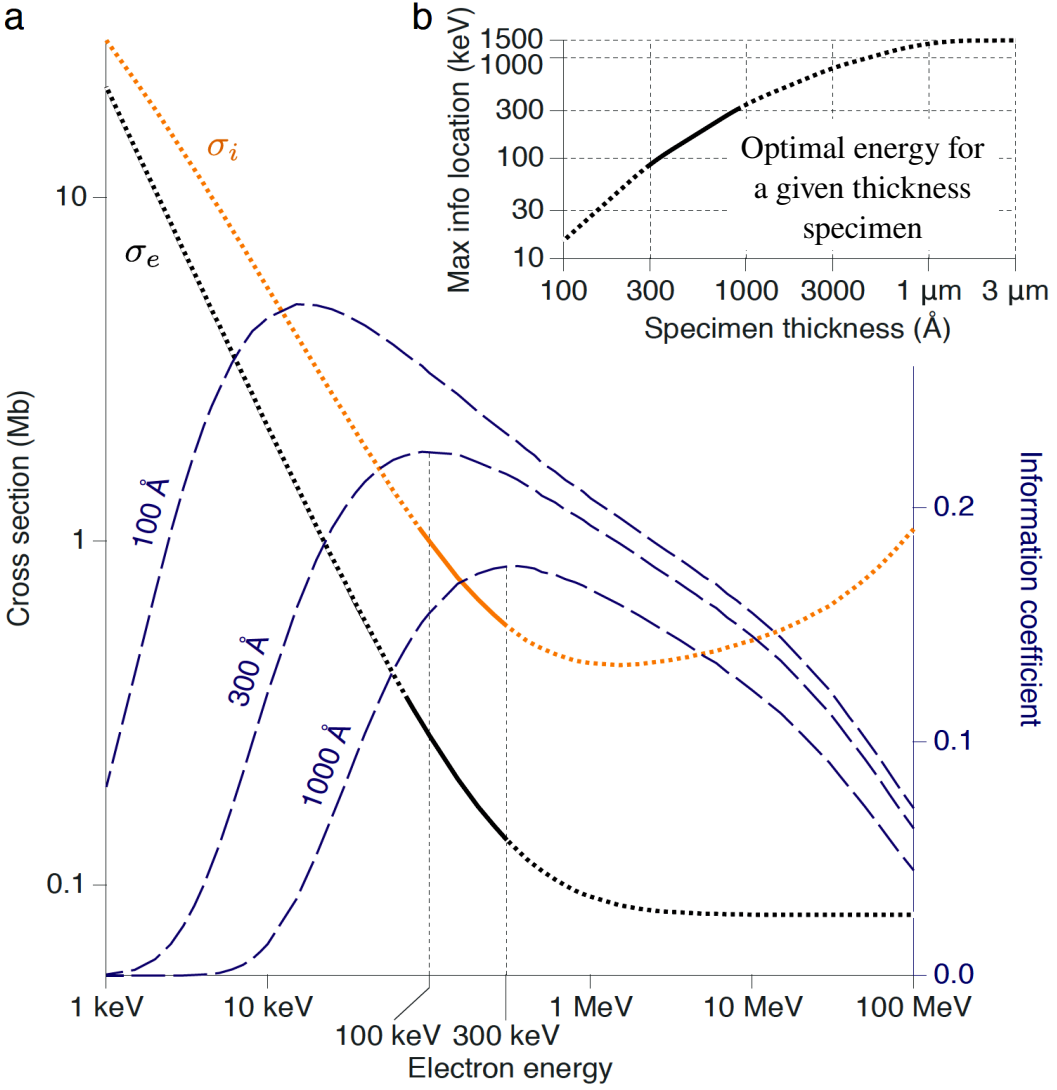
Peet et al (2019)
Ultramicroscopy
203, 125–131

Historical Theory by Bloch/Bethe confirmed in new measurements

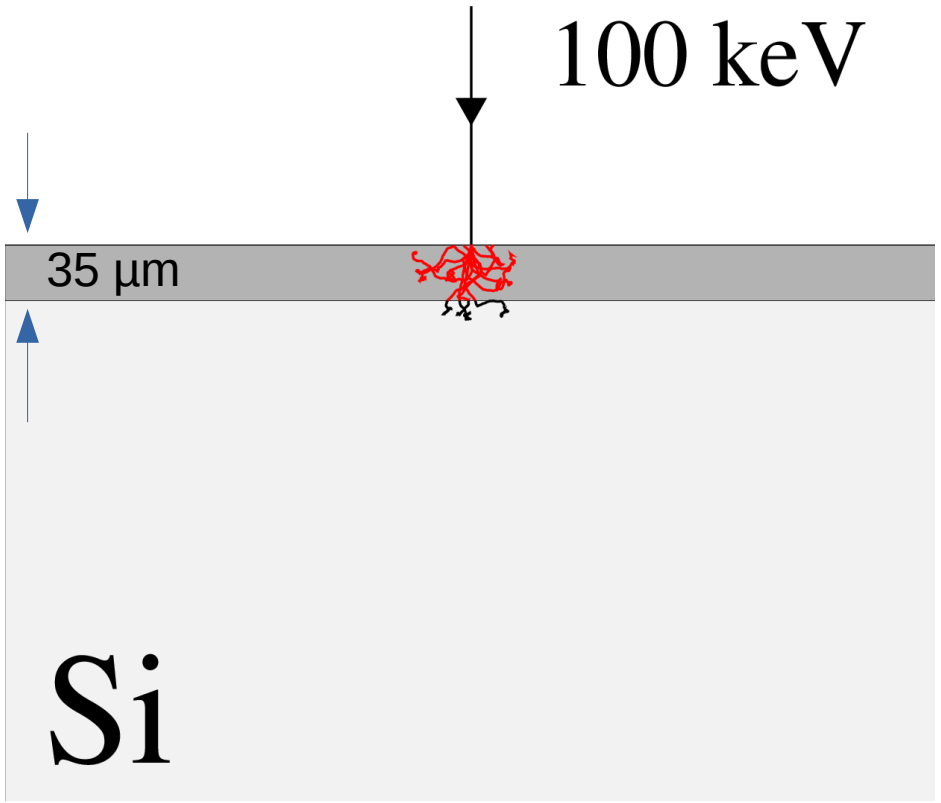
Structural information in images for same amou of radiation damage, as a function of electron energy.

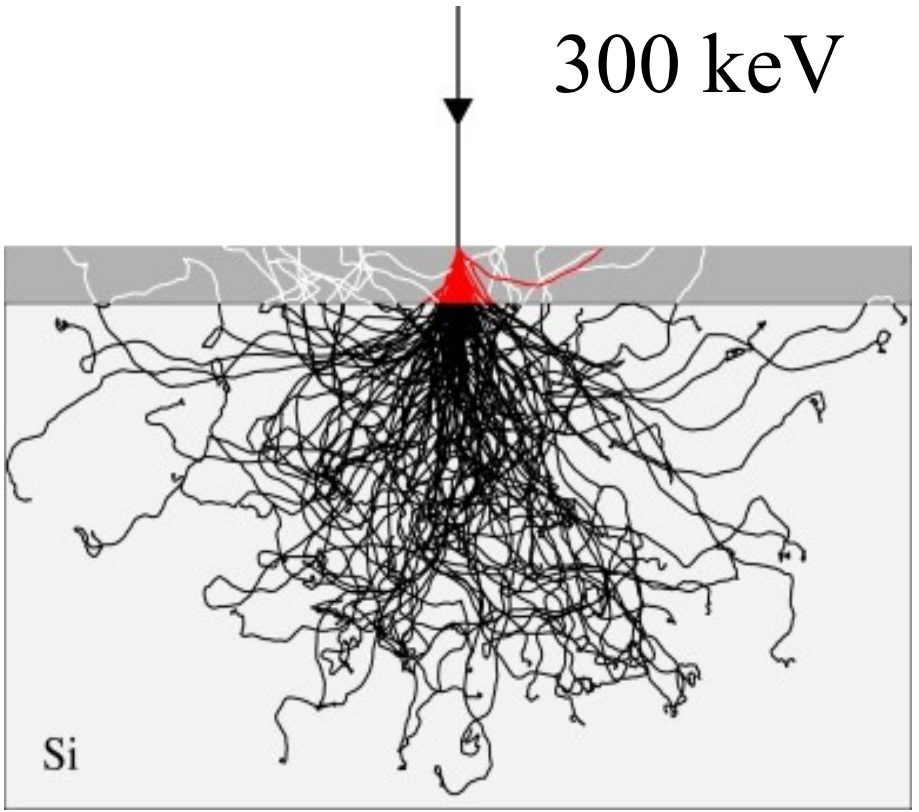
Cross section (megabarns)

Transmission = $e^{-t/\lambda}$
Information ≈ [Transmission * σe / σi]

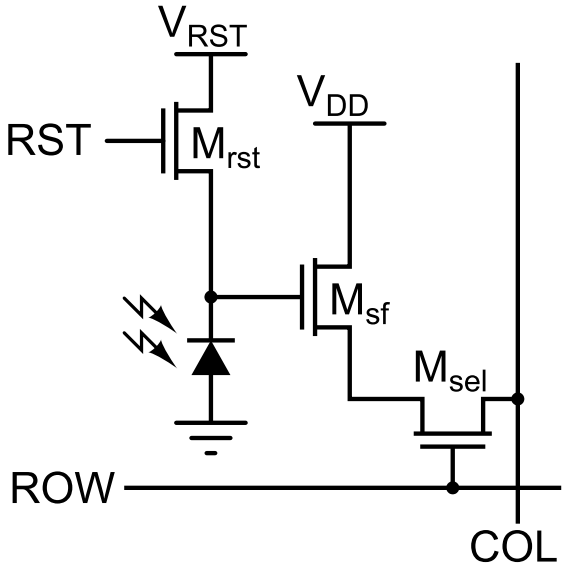


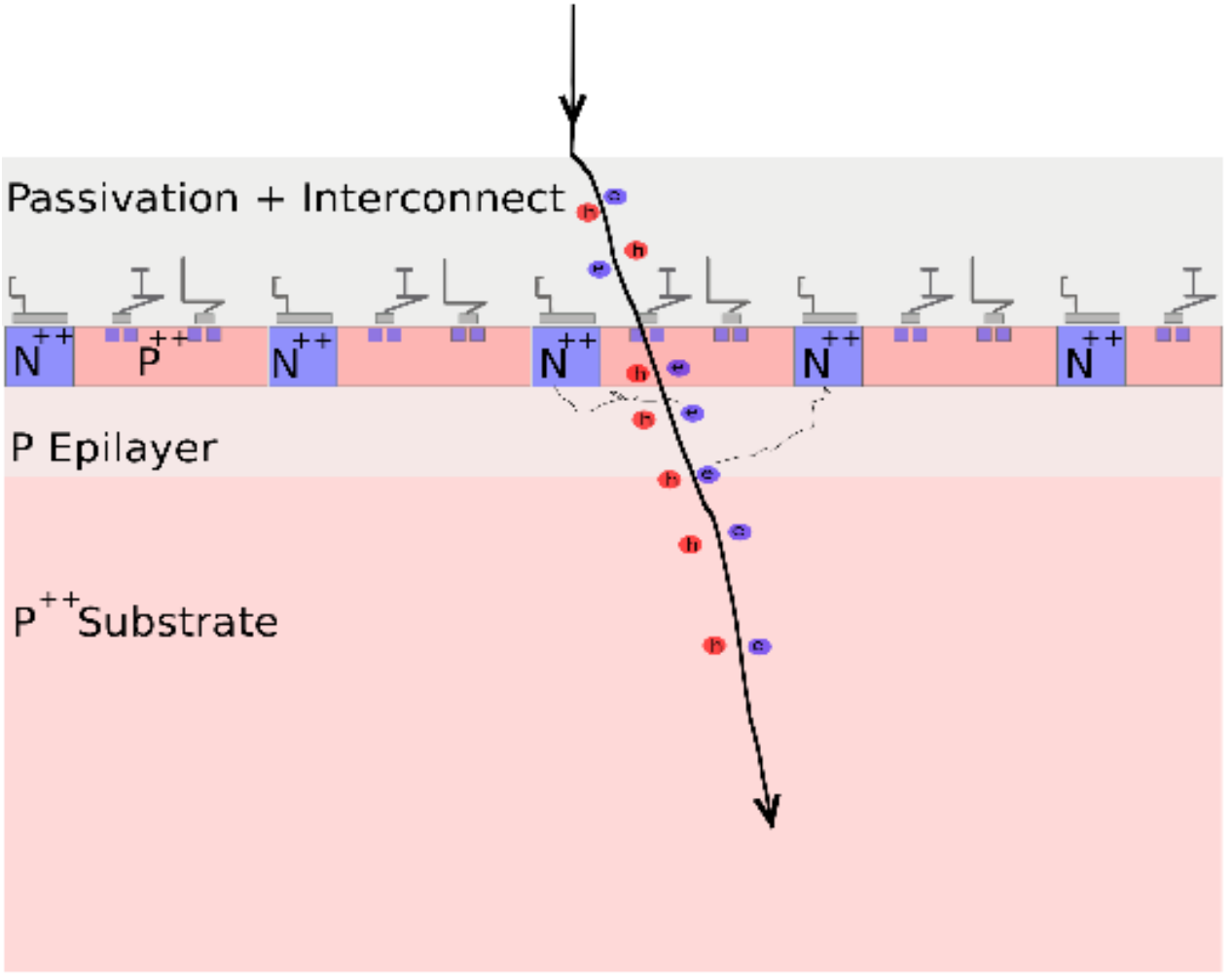
Difference in scattering of 100 and 300 keV electrons in Silicon



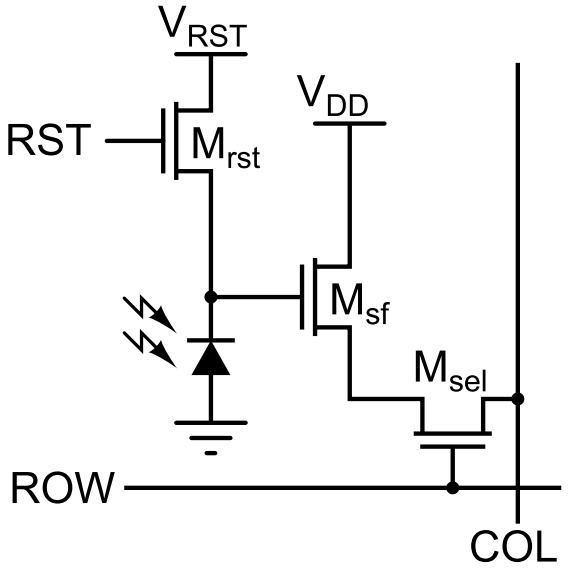


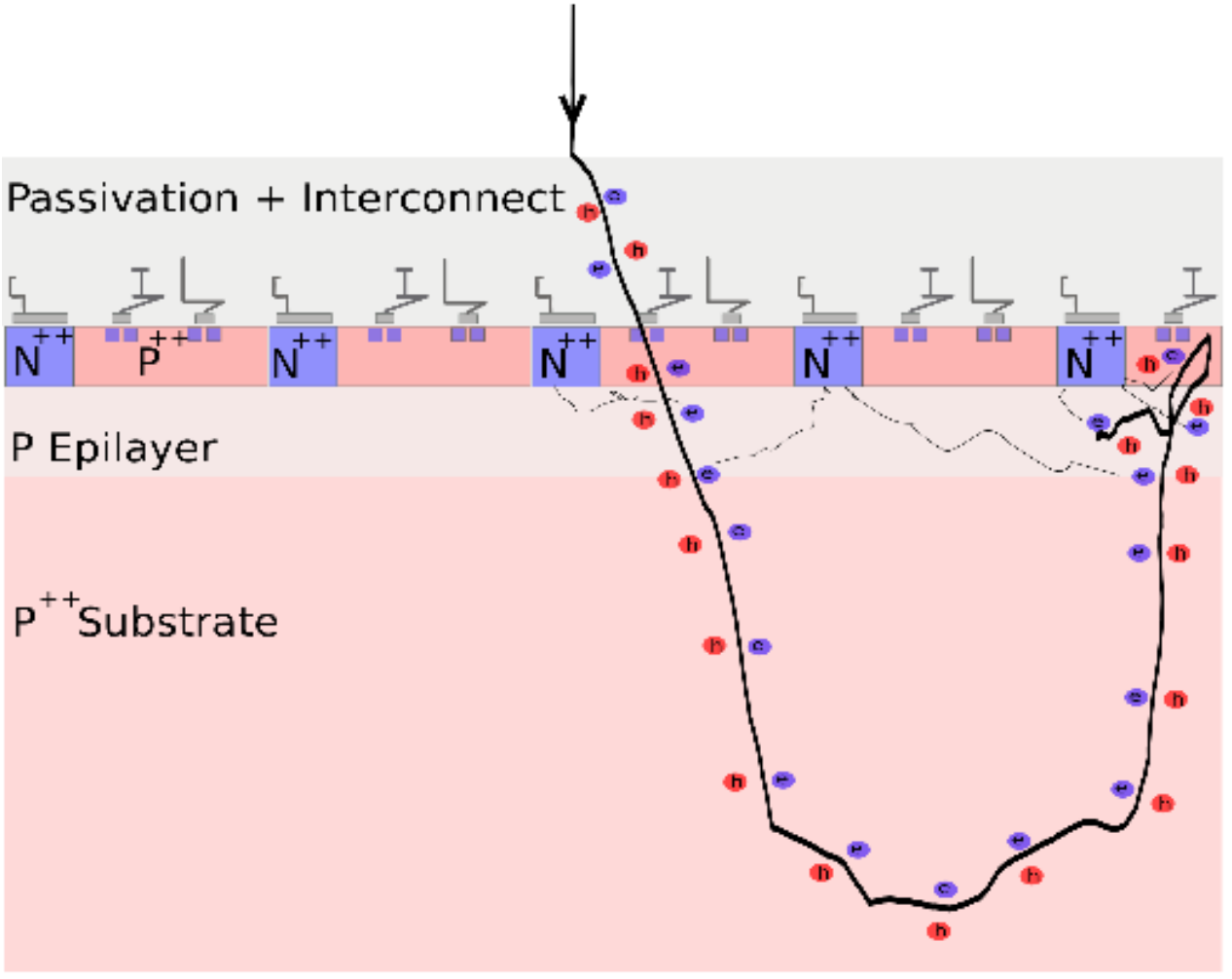
MAPS Detector





MAPS Detector





300 keV detectors

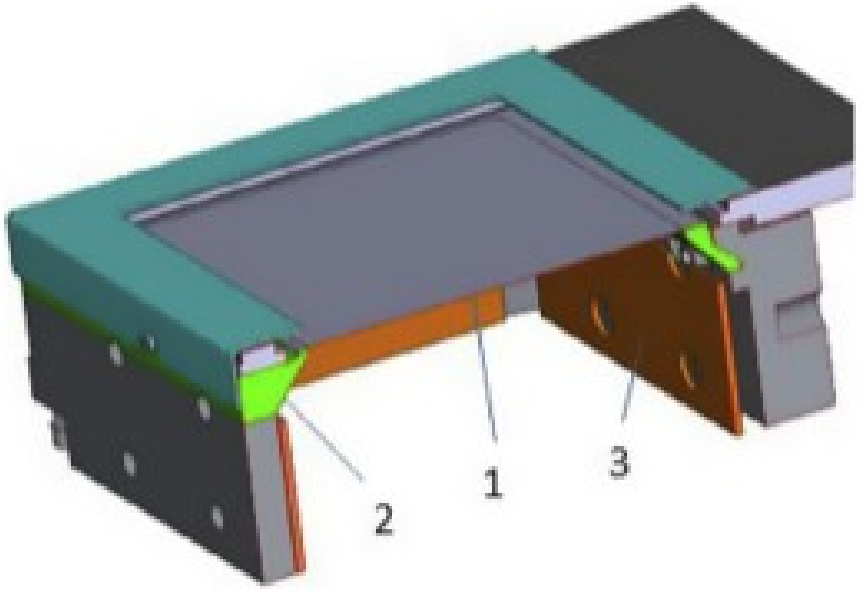


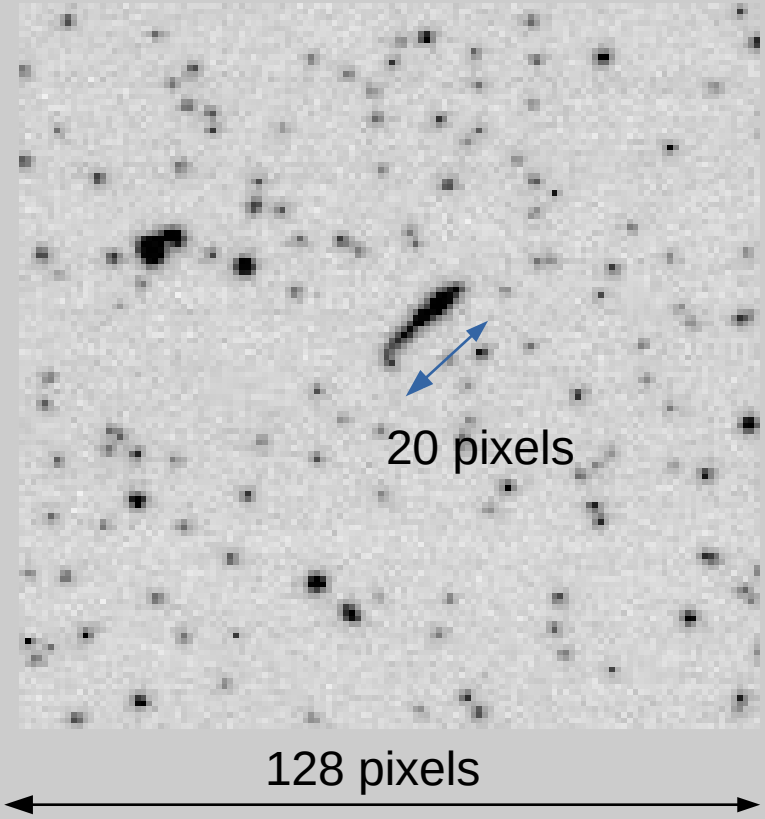
Fig. 4. Falcon 2 design with integrated low atomic mass back scatter reduction plates (3) and back thinned sensor membrane (1) mounted on the low-CTE carrier (2).

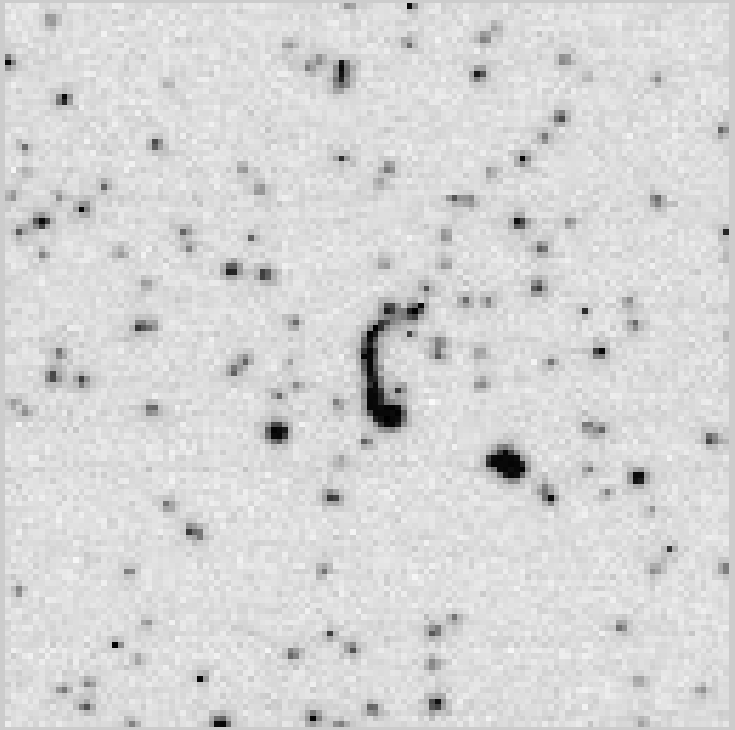
Detector must be backthinned!

Detector must be mounted carefully

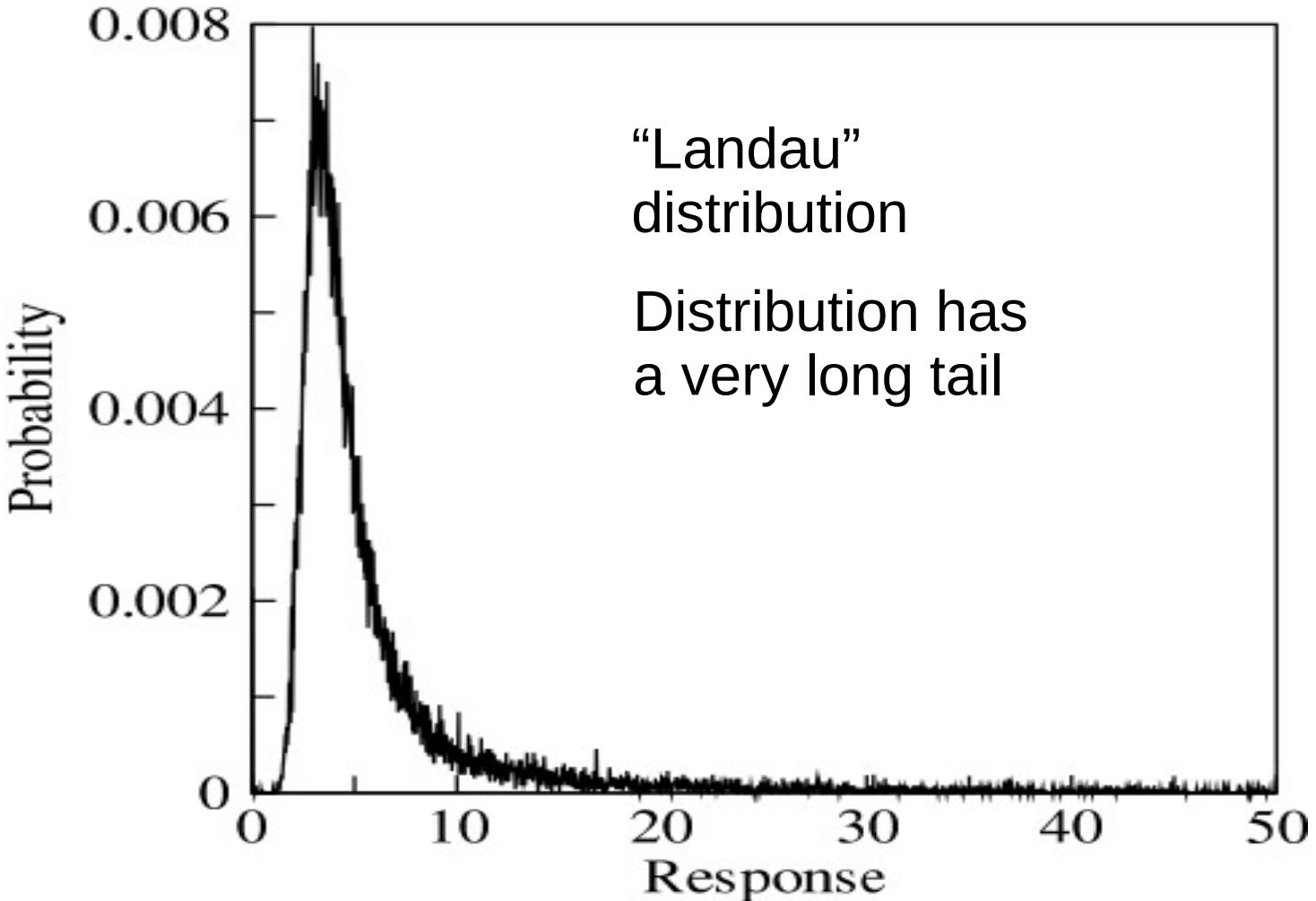
Kuijper etal, **JSB**, 192 (2015) 179-187

MAPS Electron Event Images
(14 μm pixel – backthinned ~30μm)





Variable energy deposition:

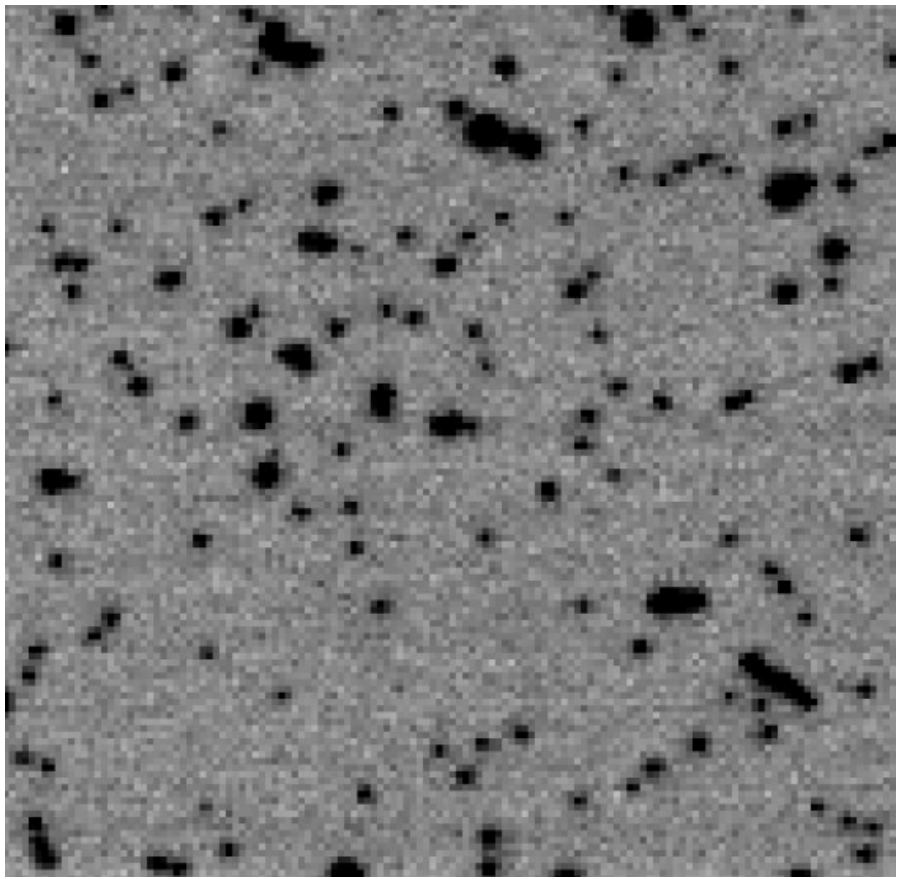


"Landau" distribution

Distribution has a very long tail

Variable singal lowers DQE (~0.6)

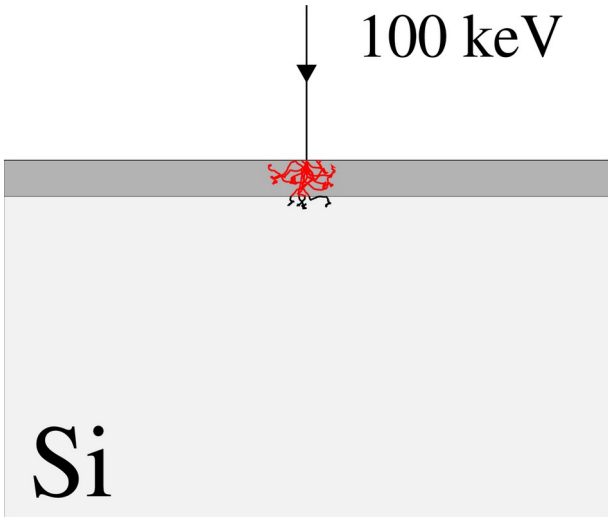
Solution: use counting mode for high DQE



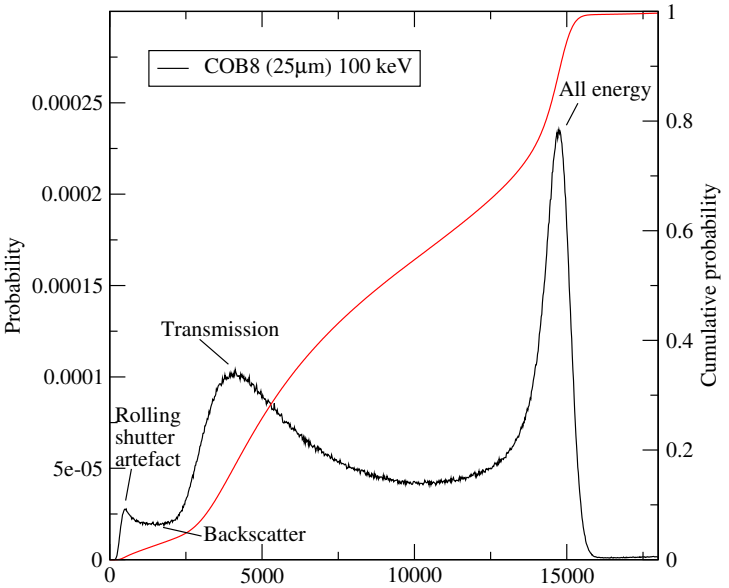
Commercial 300 keV Detectors

- Gatan (K2/K3)
- Direct Electron (DE20)
- ThermoScientific (Falcon)

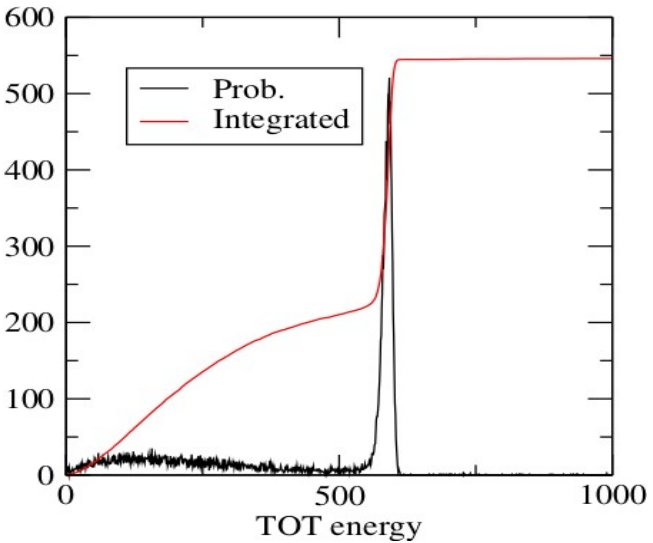
What about 100 keV?



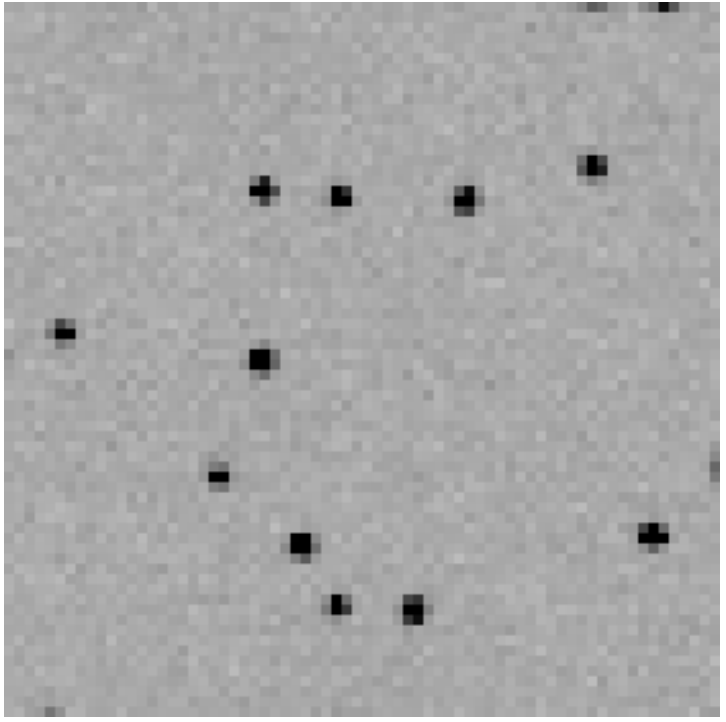
Big pixel MAPS

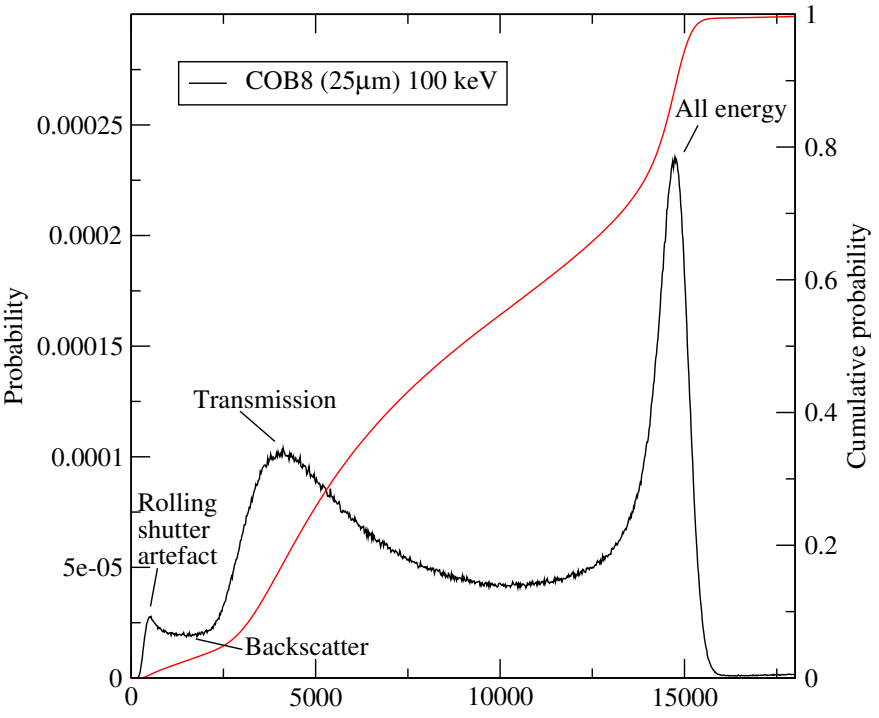


Hybrid pixel detector

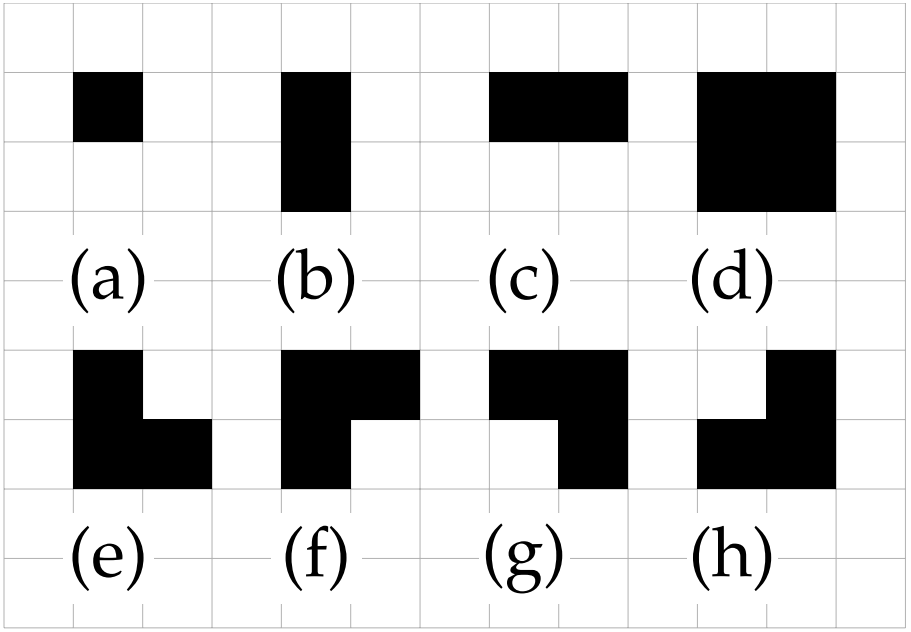


100 keV MAPS detector





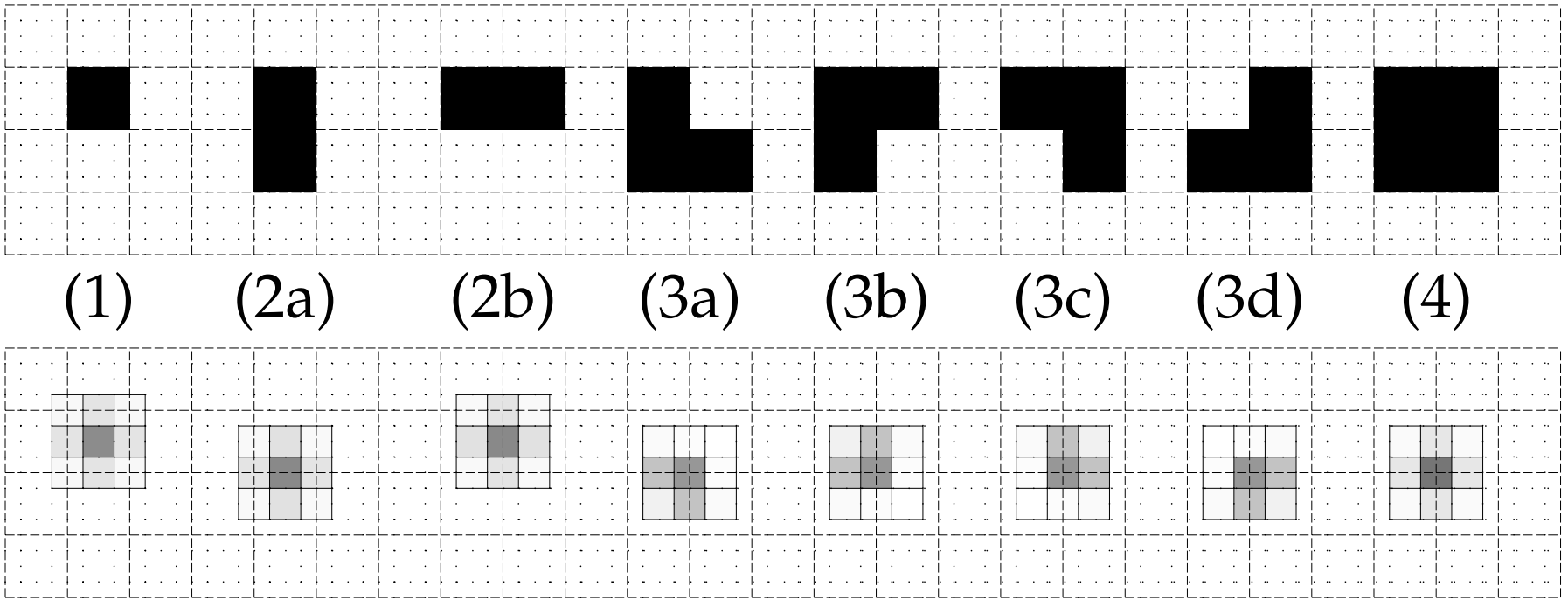
Hybrid Pixel Detector Approach



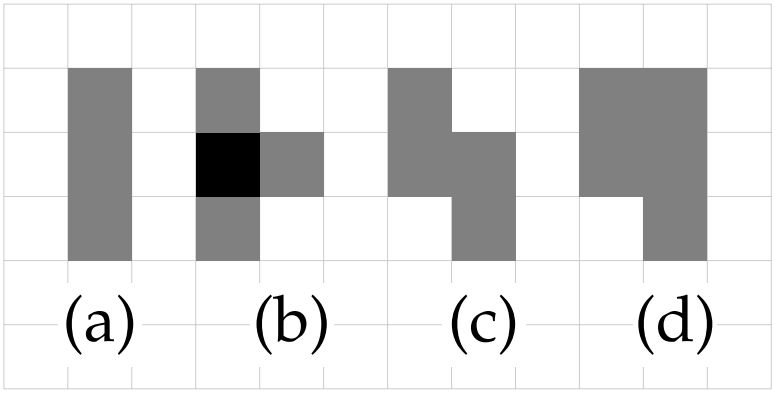
All possible responses to 100 keV electron with 75 μm pixel

- Threshold as low as possible to catch every electron
- Average response ~2/e-
- DQE(0) reduced to ~0.8

Improved Hybrid Pixel Detector



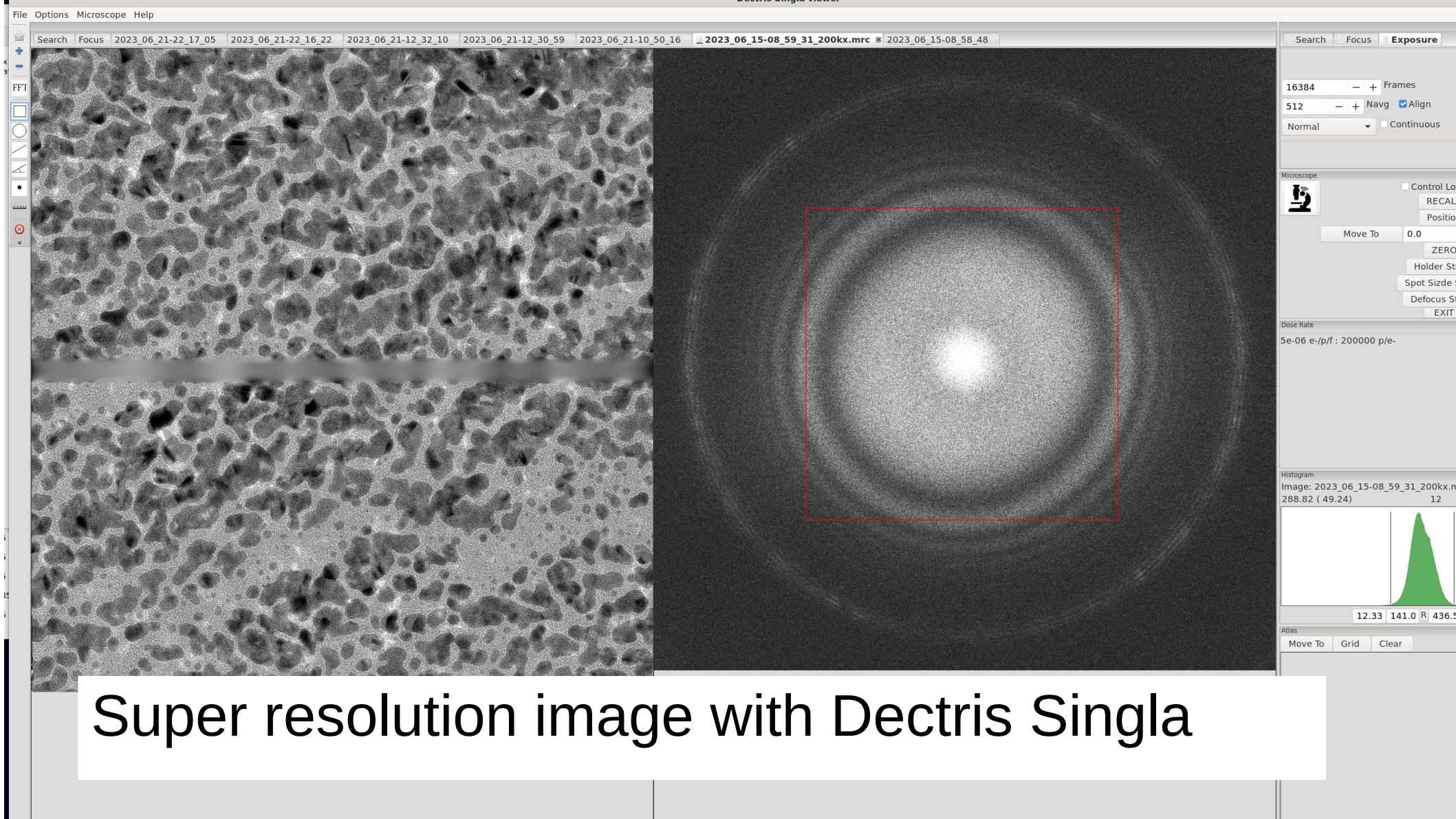
Improved Hybrid Pixel Detector (Smart pixel)



Find events “on pixel” to avoid multiple events. (Not CSM of Medipix)

Or

Read out very fast using 2 bits


File Options Microscope Help
Search Focus 2023_06_21-22_17_05 2023_06_21-22_16_22 2023_06_21-12_32_10 2023_06_21-12_30_59 2023_06_21-10_50_16 2023_06_15-08_59_31_200kx.mrc 2023_06_15-08_58_48
Search Focus Exposure
16384 Frames
512 Navg Align
Normal Continuous
Microscope
Move To 0.0
Dose Rate
5e-06 e-/p/f : 200000 p/e-
Histogram
288.82 (49.24) 12
12.33 141.0 R 436.5
Atlas
Move To Grid Clear
Super resolution image with Dectris Singla

What about the data

- 4 TB a day per microscope despite compression
- 365 Days per year
- MRC-LMB has 4 Krios + Glacios + others
- Storage is a major problem
- Data processing is a major problem

Future detector improvements:

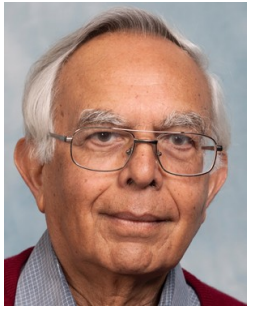
- 300 keV: (money runs out)
  6k x 6k
  > 2000 fps

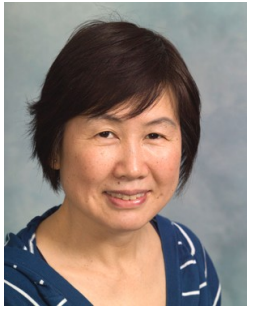
- 100 keV: (space runs out)
  3k x 3k
  5000 fps

Summary

- Introduction of direct electron detectors has made a huge difference to cryoEM
- 300 keV microscopes work very well but are expensive (380 + 200 currently in world)
- 100 keV microscopes can work well but only TFS Tundra commercially available
- **All depends on large area high DQE imaging detectors (DQE(0) > 0.9 DQE(Nyquist) > 0.4)**

Thanks




And many more ...

MRC Laboratory of Molecular Biology