

Direct Detectors used in Electron Cryo- microscopy – Status and Challenges

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With thanks to:

Richard Henderson MRC-LMB

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Renato Turchetta RAL-STFC

Nicola Guerinni RAL-STFC

FEI team

New LMB nearing completion 2012



Courtesy MRC-LMB archives

Background to cryo-microscopy

Applications: single particle analysis, electron tomography, materials science,...

Examples: Ribosome, beta-galactosidase, rotavirus...

Detectors: Hybrid pixel detectors (i.e. Medipix2), MAPS based on CMOS

Recent developments and future improvements

Conclusions

Electron Cryo-Microscopy

Image individual molecules in native aqueous environment in vitreous ice (i.e no de-hydration). No crystals required. Need homogenous sample.

• **Images of biological molecules have very low contrast : need sophisticated software for orienting and averaging a large number of individual molecules (>10,000) to produce near-atomic resolution. High DQE detectors can get to same resolution with fewer particles**

• **Radiation damage to specimen a severe limitation. Limits dose to ~20 electrons/Å² at the specimen**

High Resolution Imaging Detector Requirements for Cryo-EM

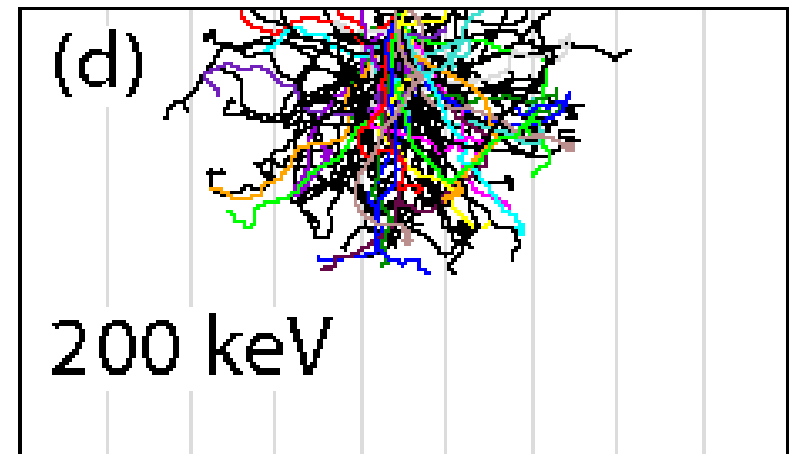
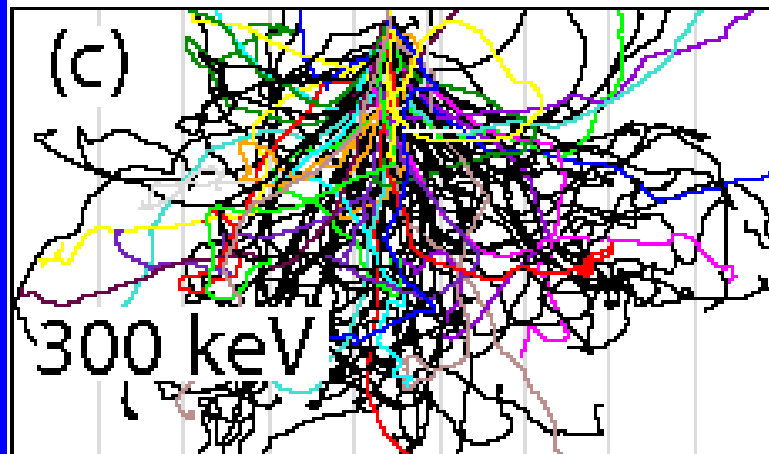
- 1. Electronic detector needed (rather than film)**
- 2. Number of *independent* pixels : > 4000 by 4000**
- 3. Pixel Size 5 –20 μm**
- 4. High sensitivity with low noise – ability to add multiple frames (high Detective Quantum Efficiency)**
- 5. Radiation damage: should be able to withstand at least 1 Mrad, preferably 10 Mrad – use for ~years**
- 6. Readout time short – 1000 frames/second; movie mode**

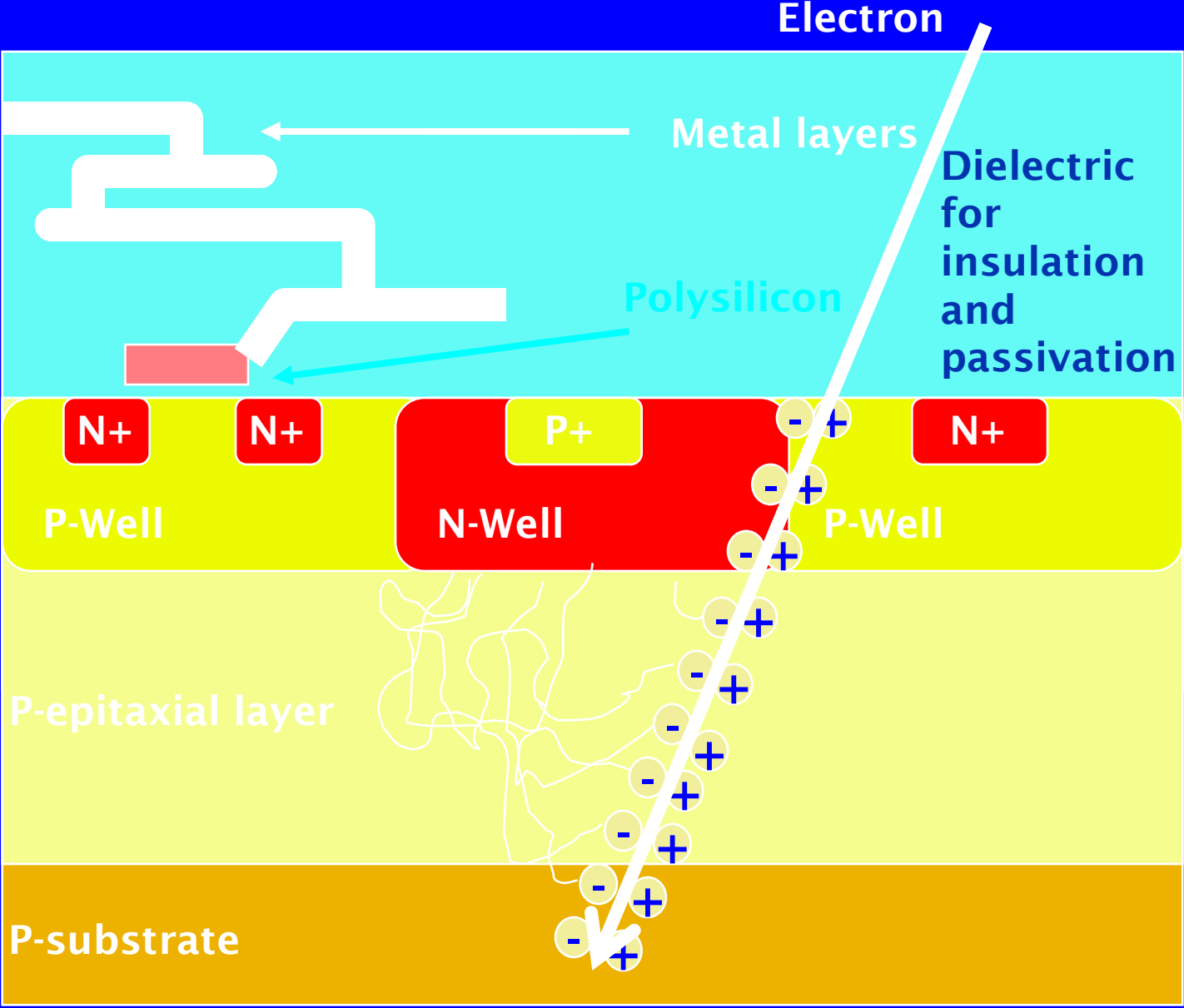
Direct Detection in Silicon Pixel Detectors

- **Hybrid Pixel Detectors**
- **Sensor bonded to readout ASIC**
e.g. Medipix2
- **CMOS Detectors based on Monolithic Active Pixel Sensors (MAPS)**
Pixellated silicon, readout built into each pixel.

Monte Carlo simulation of electron trajectories in silicon. Detector thickness = 300 microns, pixel=55 microns

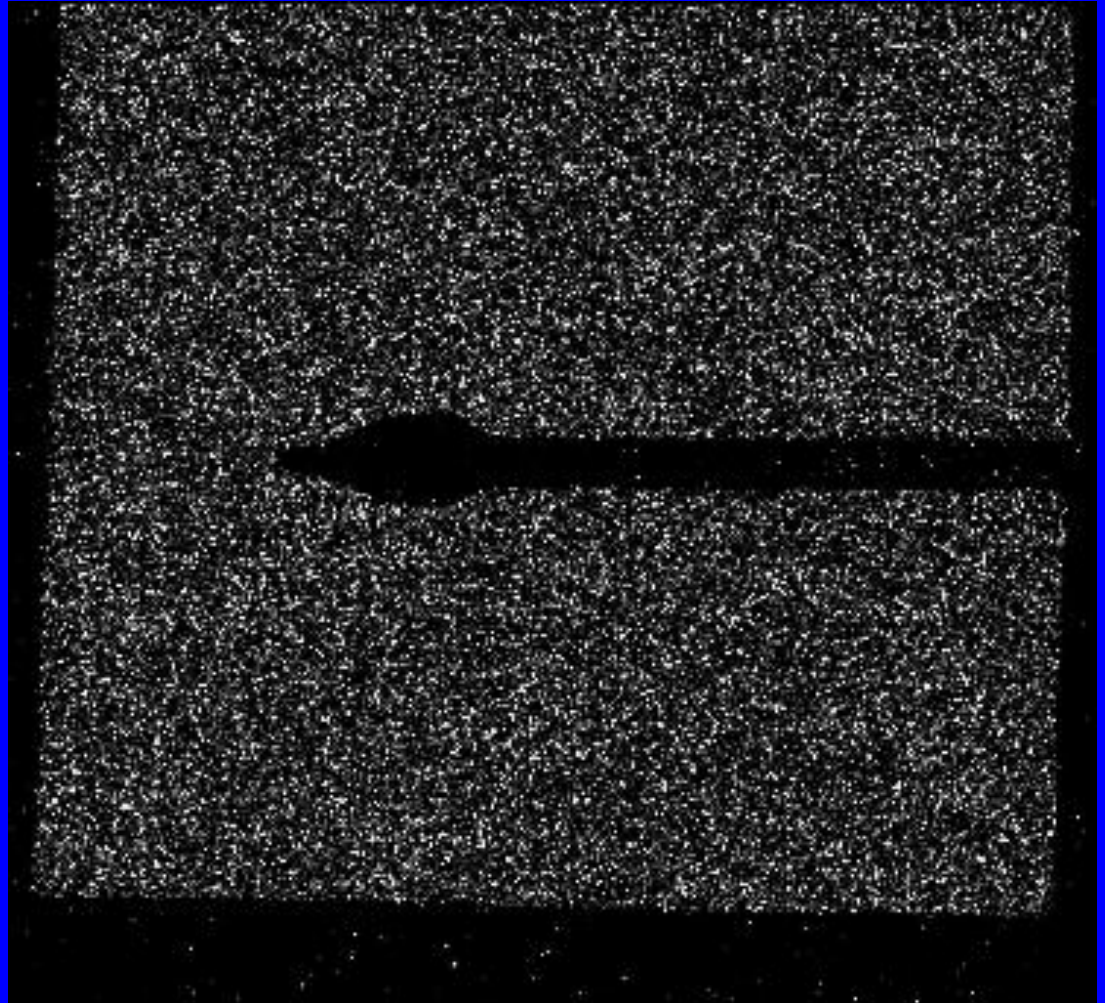
Extension of simulations to include energy deposition





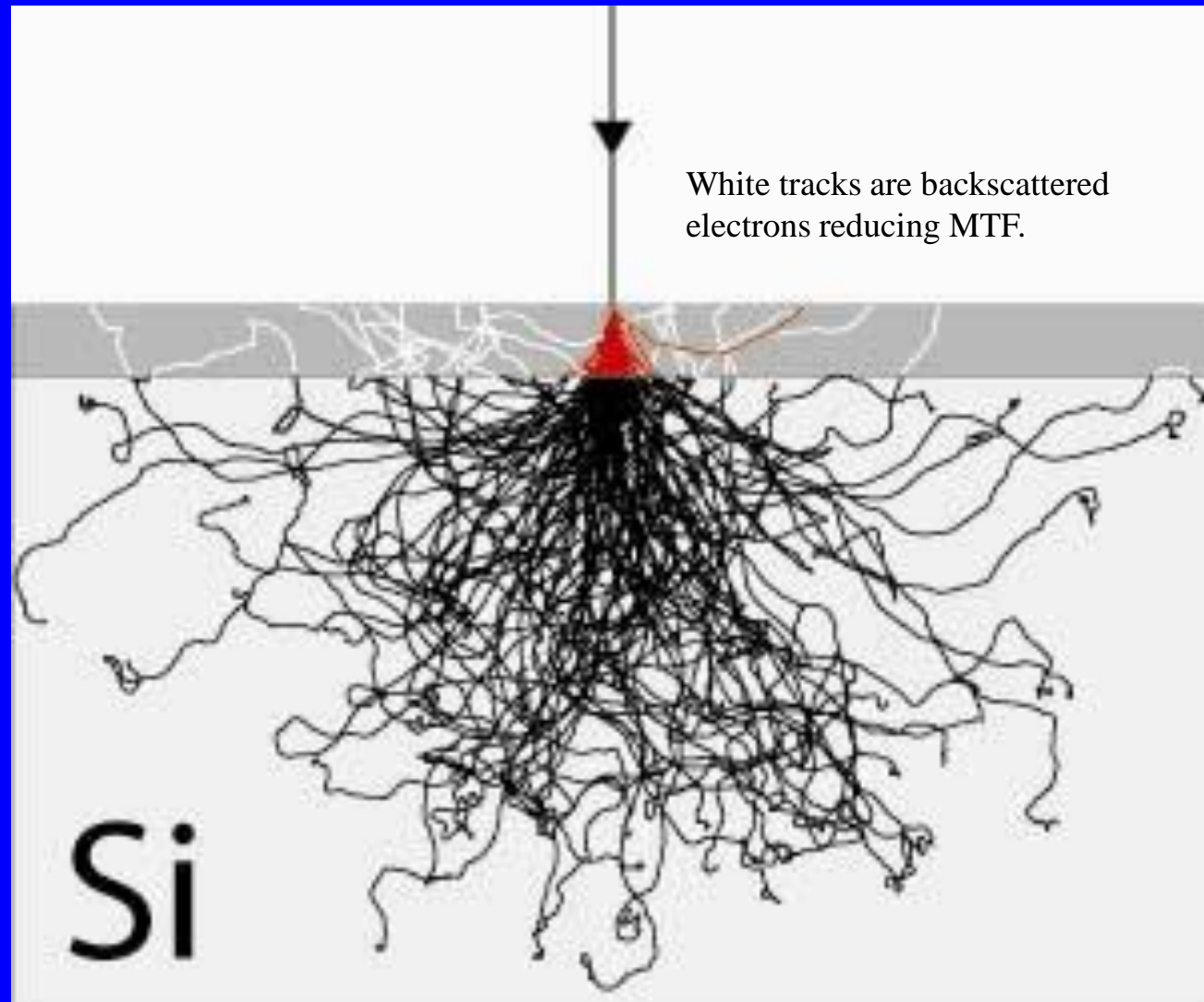
First movie made with Startracker in Feb/March 2003 with Turchetta (STFC). Good signal-to noise, individual electrons clearly visible behind the backstop.

512x512 pixels
120 keV
10 frames/second



Simulation of 300 keV electron trajectories in silicon.

Total thickness 350 μm , grey thickness 35 μm

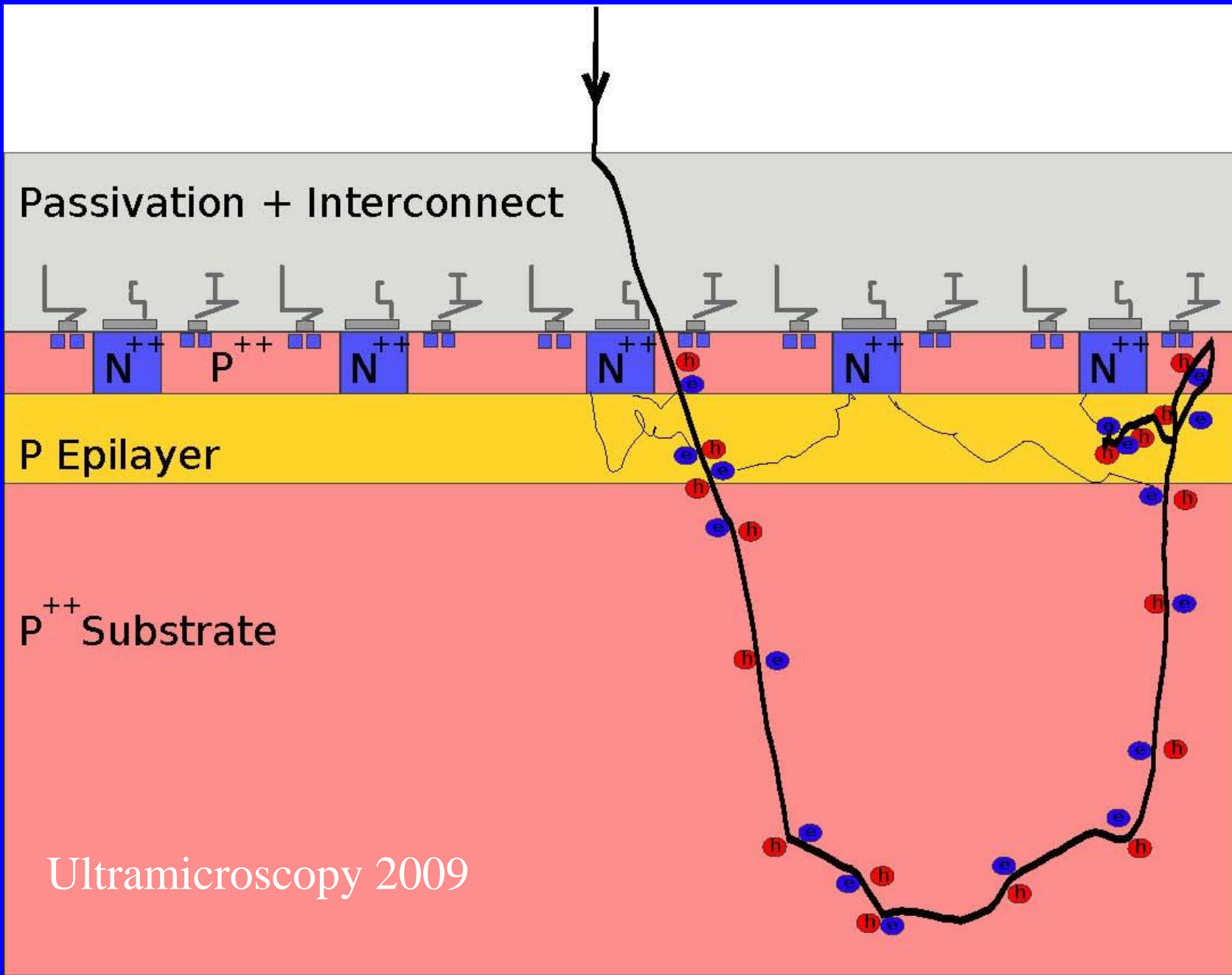


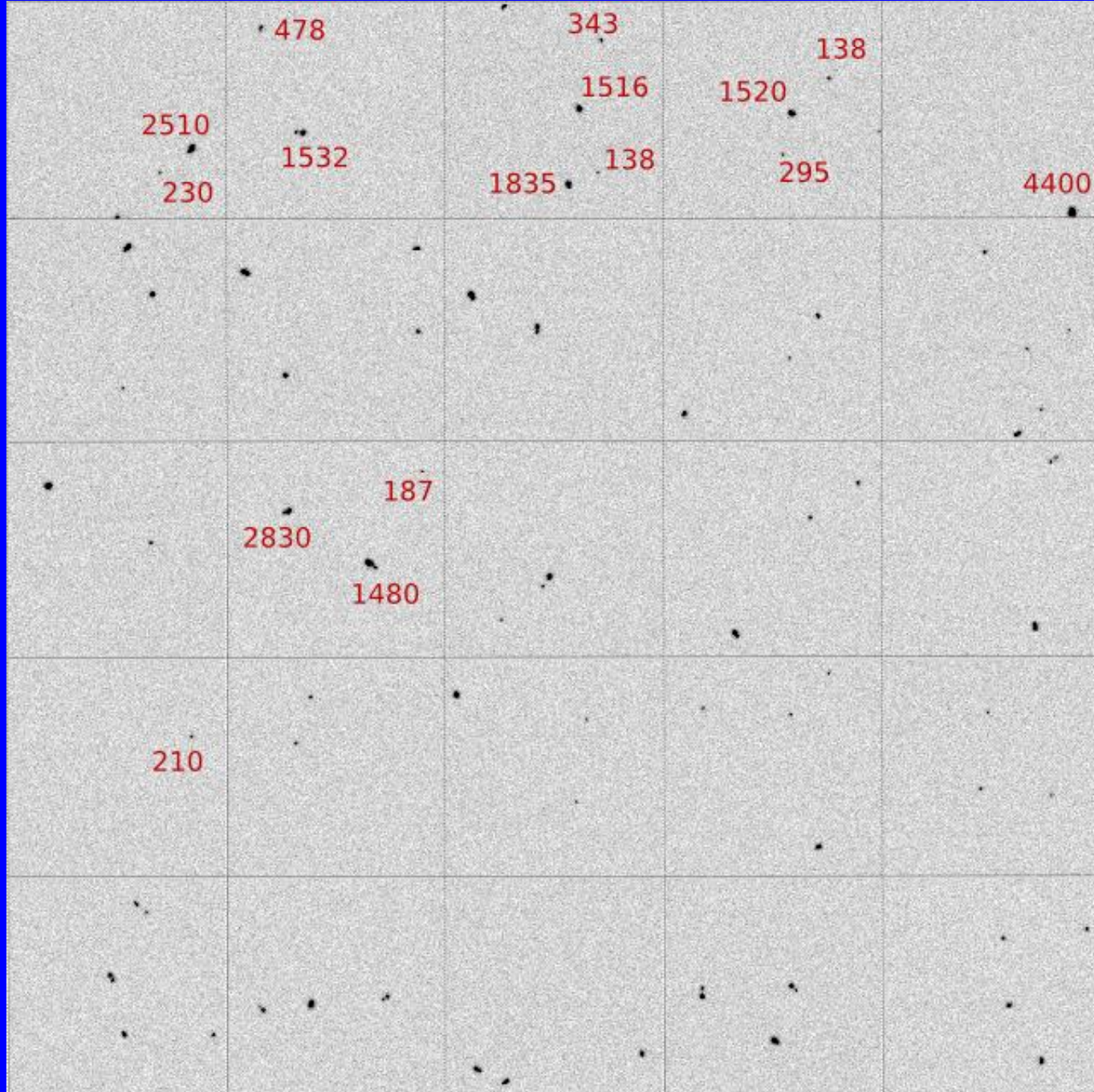
Passivation + Interconnect

P Epilayer

P⁺⁺ Substrate

Ultramicroscopy 2009

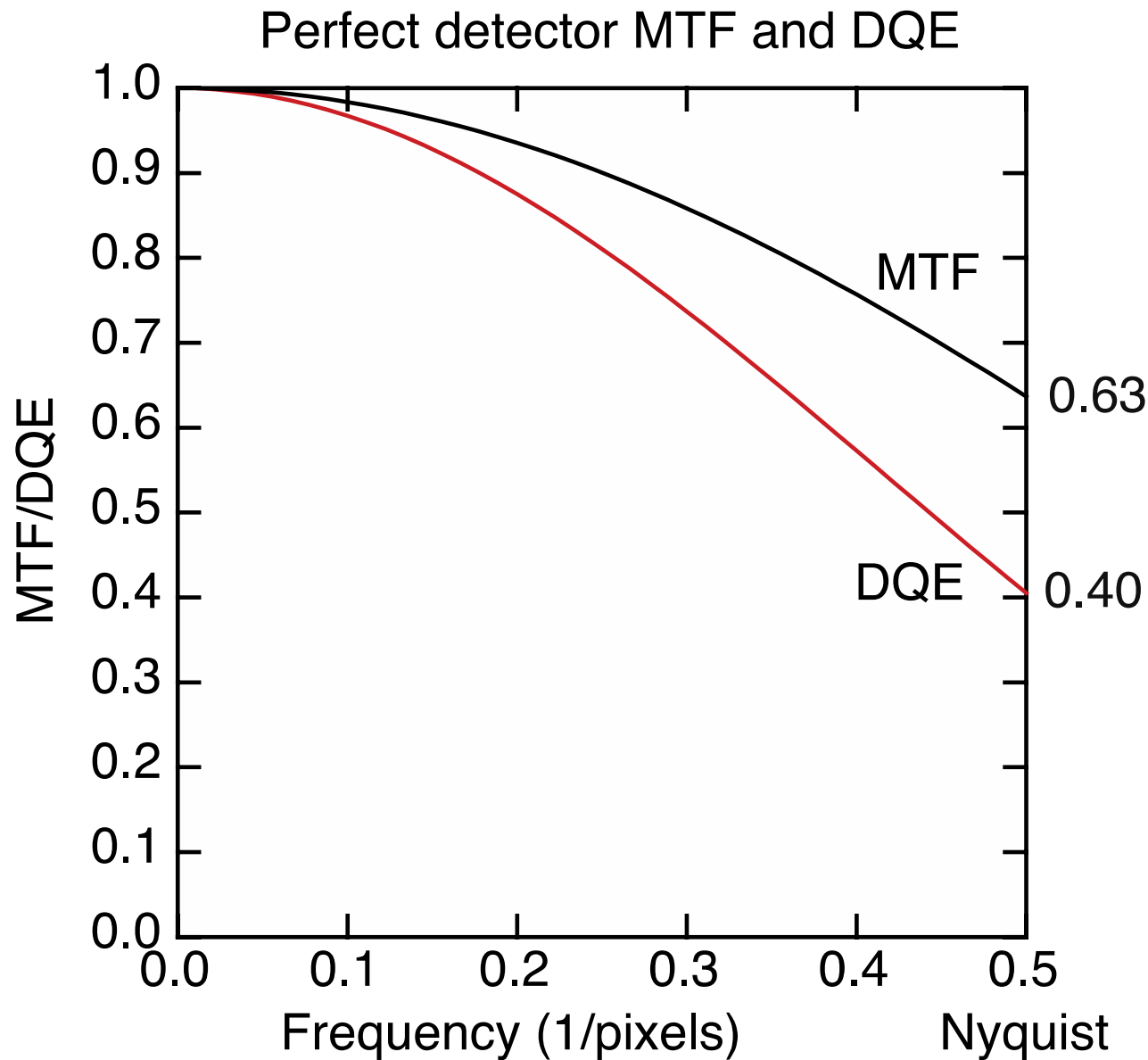


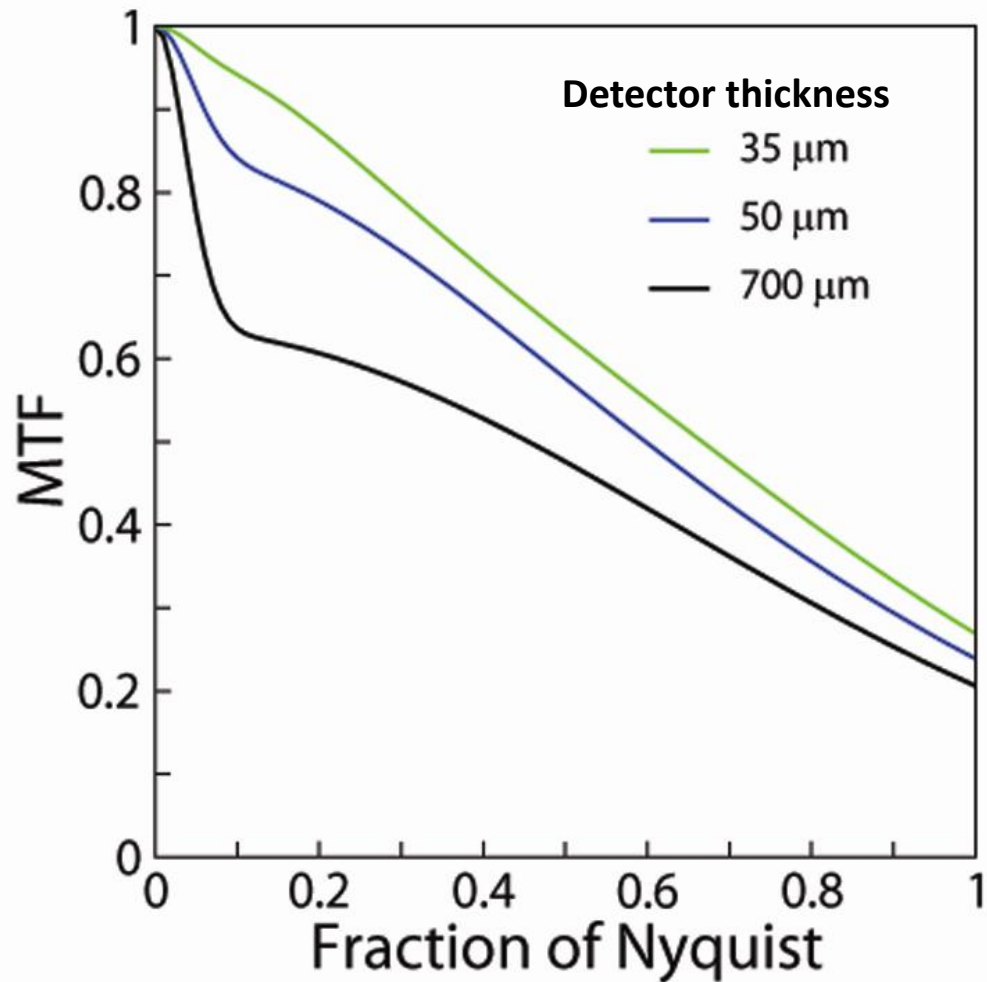


Single electron events

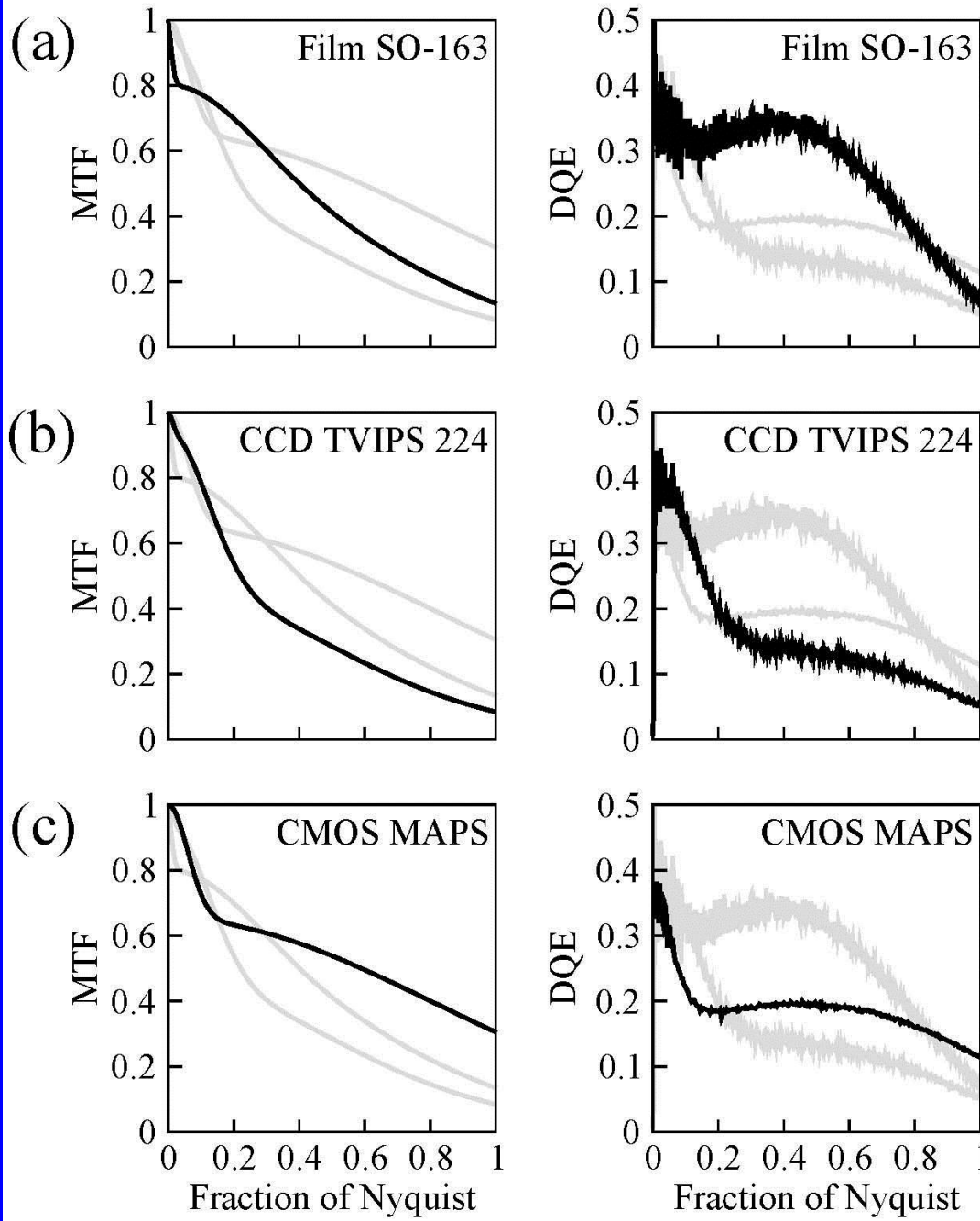
$$DQE(\omega) = DQE(0) * MTF^2 / NTF^2$$

Meyer & Kirkland (2000)
De Ruijter (1995)



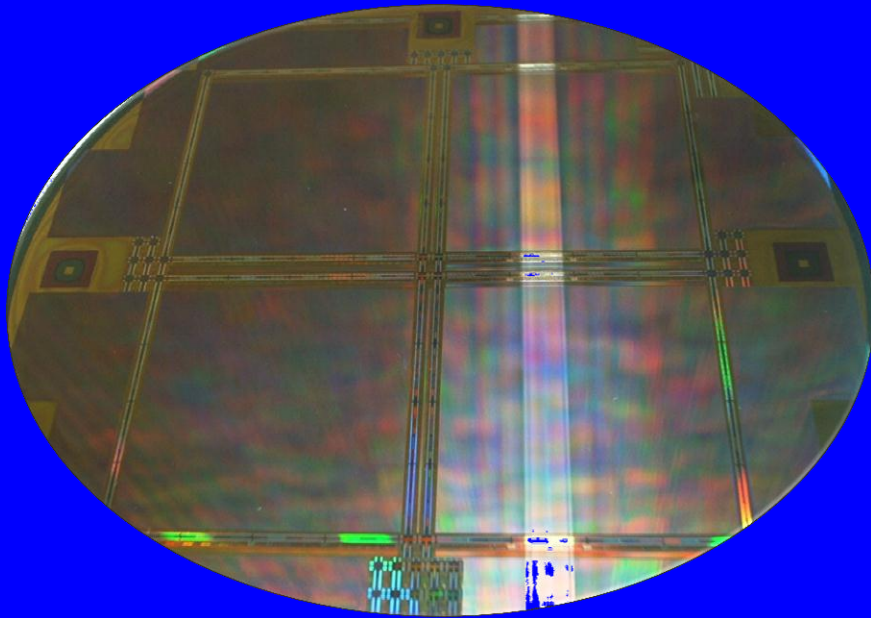


300 keV



Ultramicroscopy
2009

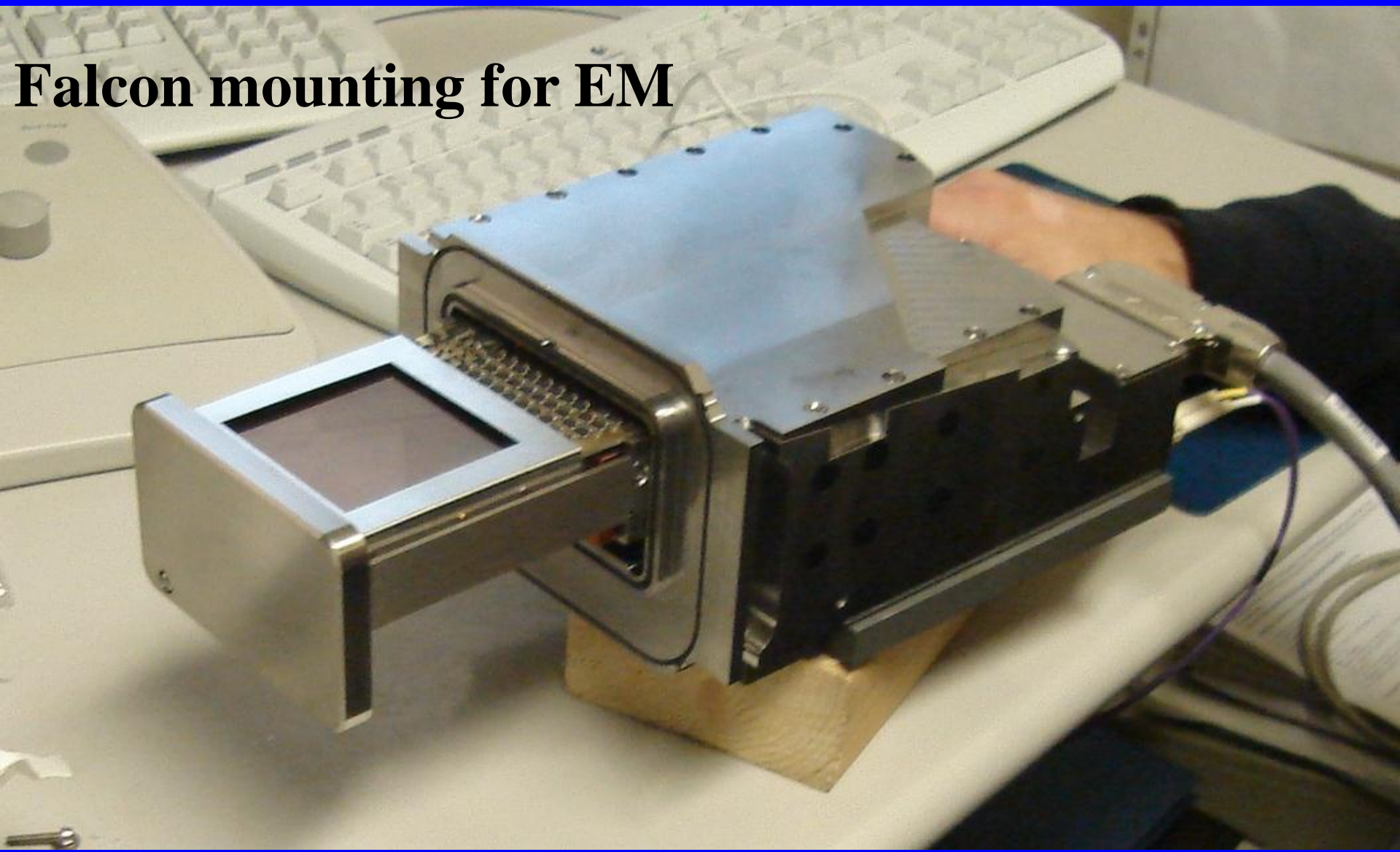
Sensor for EM applications



- 61x63 mm² silicon area
- 16 million pixels
- 4Kx4K array
- Analogue outputs
- Frame rate in excess of video rate.
- Radiation hard characteristics.
- Pixel binning.
- Region Of Interest readout
- 0.35µm CMOS process, including stitching

Commercial detector system
produced by FEI, Eindhoven, NL

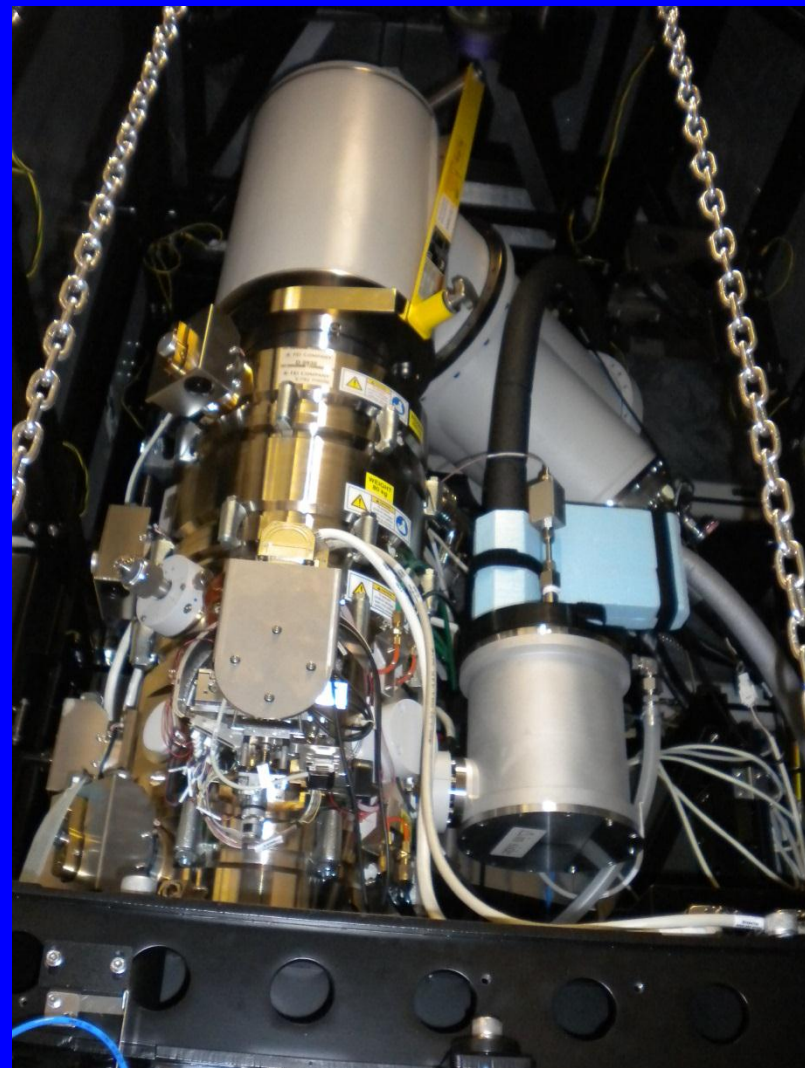
Falcon mounting for EM



FEI KRIOS 300 kV Electron Microscope



4/21/2013



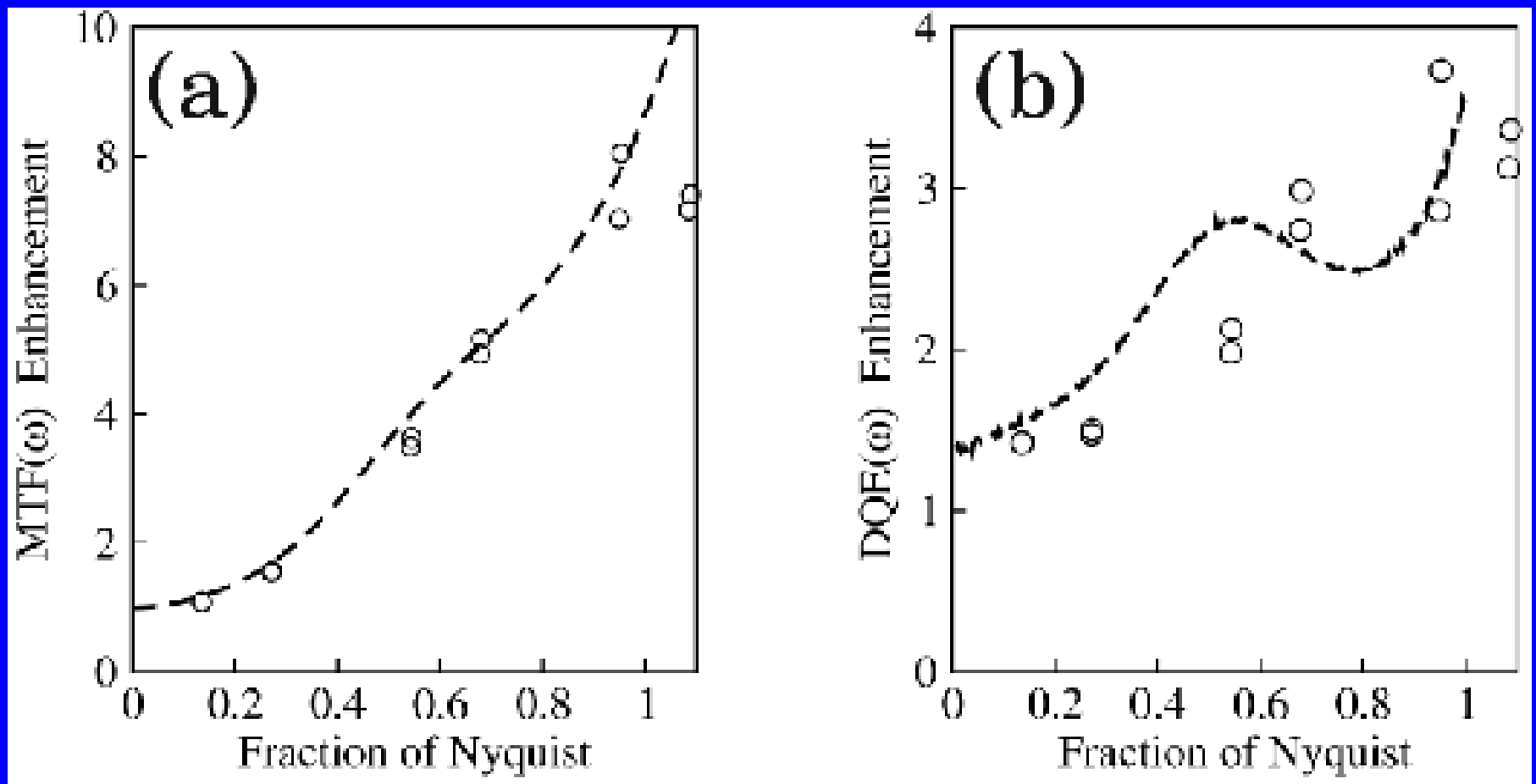
LMB, Cambridge

Can we make further improvements to the detector?

Recording individual electrons with sub-pixel resolution, considerable improvements are possible.

But, this requires reducing the number of electrons to eliminate overlapping events (which can not be distinguished)

Enhancement of $MTF(\omega)$ and $DQE(\omega)$ for 'electron counting' compared to analog readout

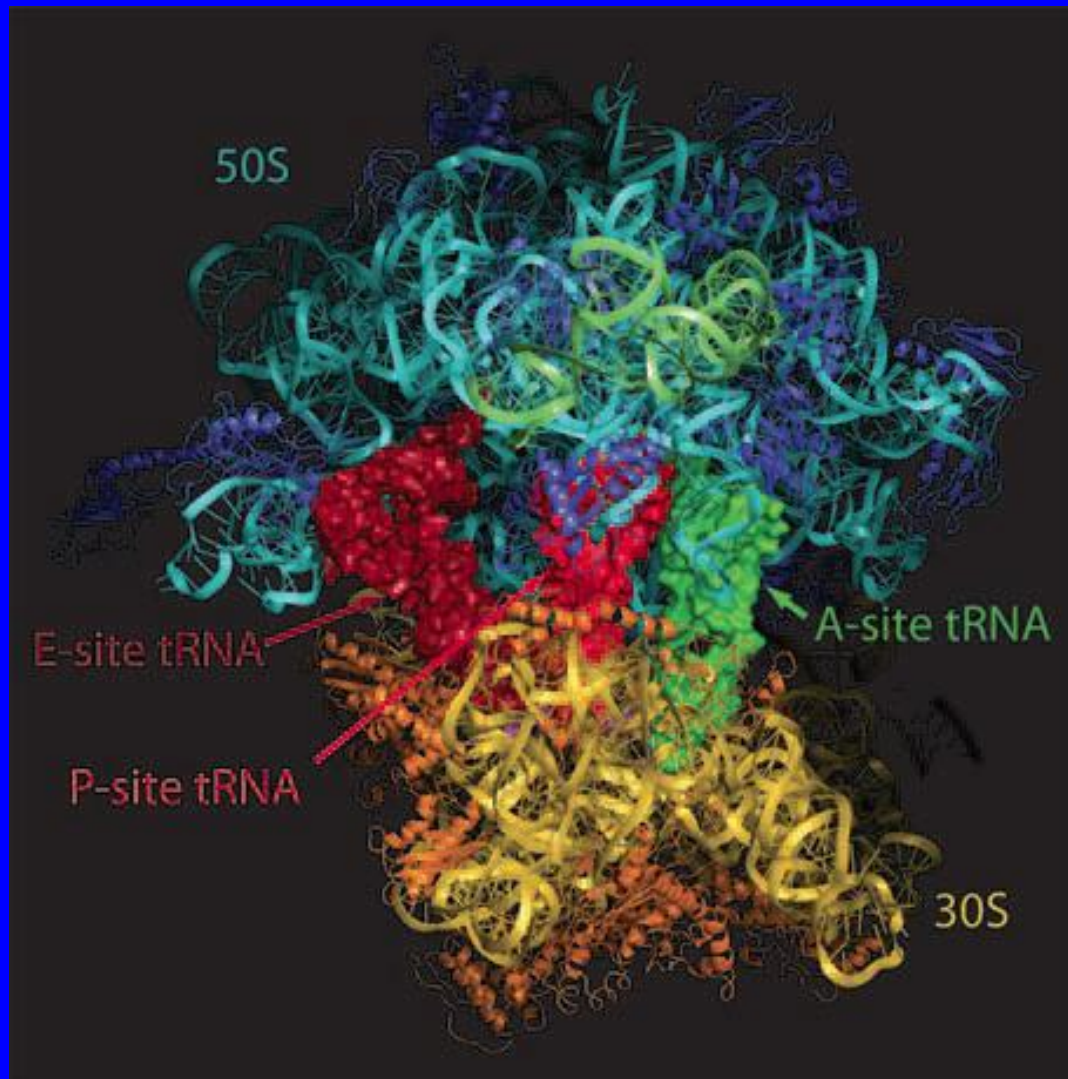


**X.-C. Bai, I.S. Fernandez, G. McMullan &
S.H.W. Scheres (2013)**

**'Ribosome structures to near-atomic
resolution from thirty thousand cryo-EM
particles'**

***eLife*, 2:e00461**

Data collected using backthinned Falcon at MRC-LMB



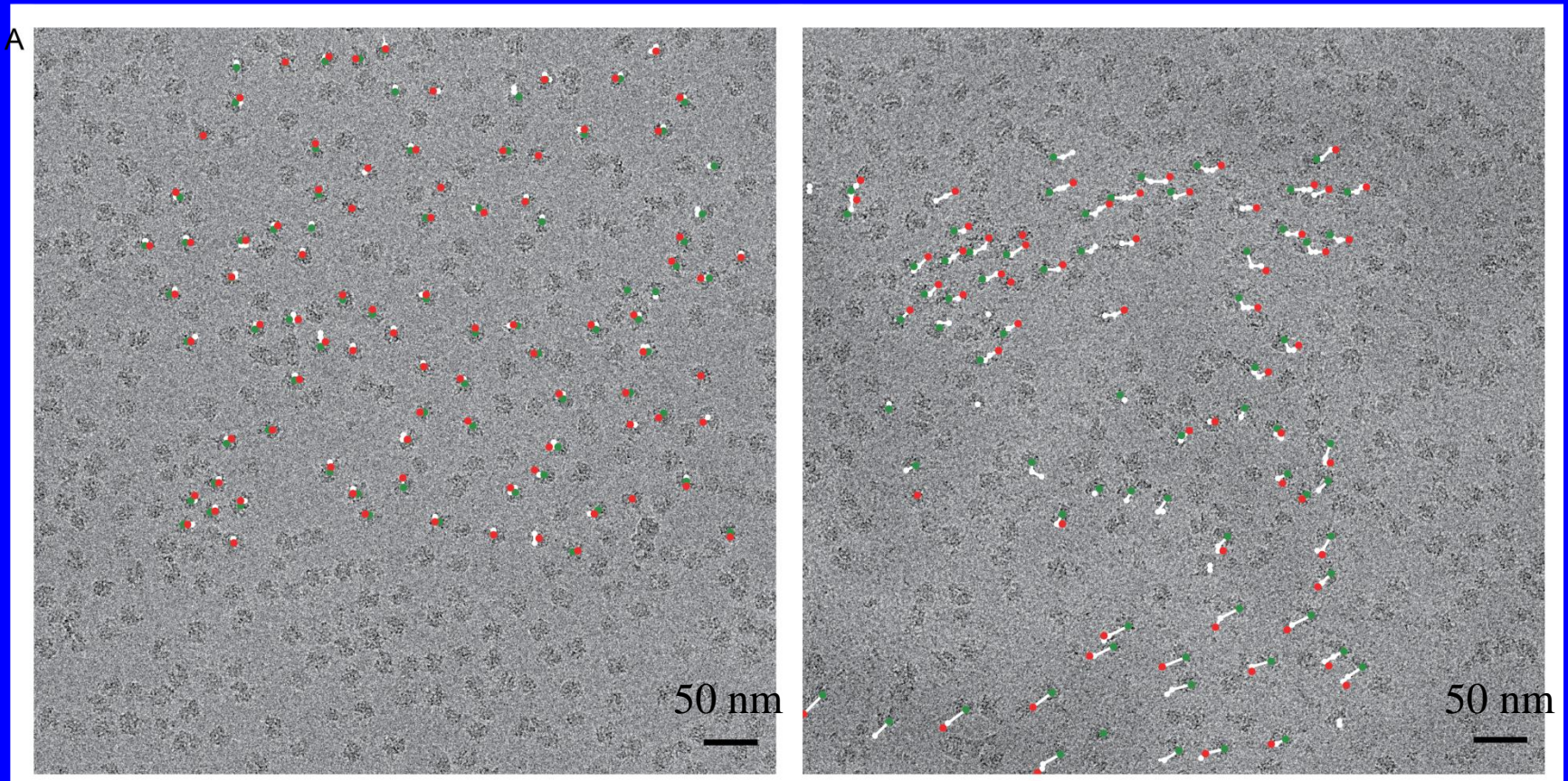
70S ribosome drawn
from a structure at 2.8 Å

V. Ramakrishnan
(2010)

Nobel Lecture

DOI:10.1002/anie.2001001436

Beam-induced movements in 70S Ribosome, average of 16 frames shown for two areas. Movement shown as white lines; green circles are 4 frame averages at start of exposure and red circles last 4 frames (Bai, et al eLife)



Ribosome

Number of ribosome particles required for structure determination

Images collected in 'movie' mode with Falcon

Need fewer particles for higher resolution as:

1. Movie mode reduces blurring
2. Higher DQE(Nyquist frequency) for Falcon

Film	1 million particles	5.5 Å	Armache, et al (2010)
Falcon	10,000 particles	~4 Å	Bai, et al eLife (2013)

Beam-induced motion of vitrified specimen on holey carbon film

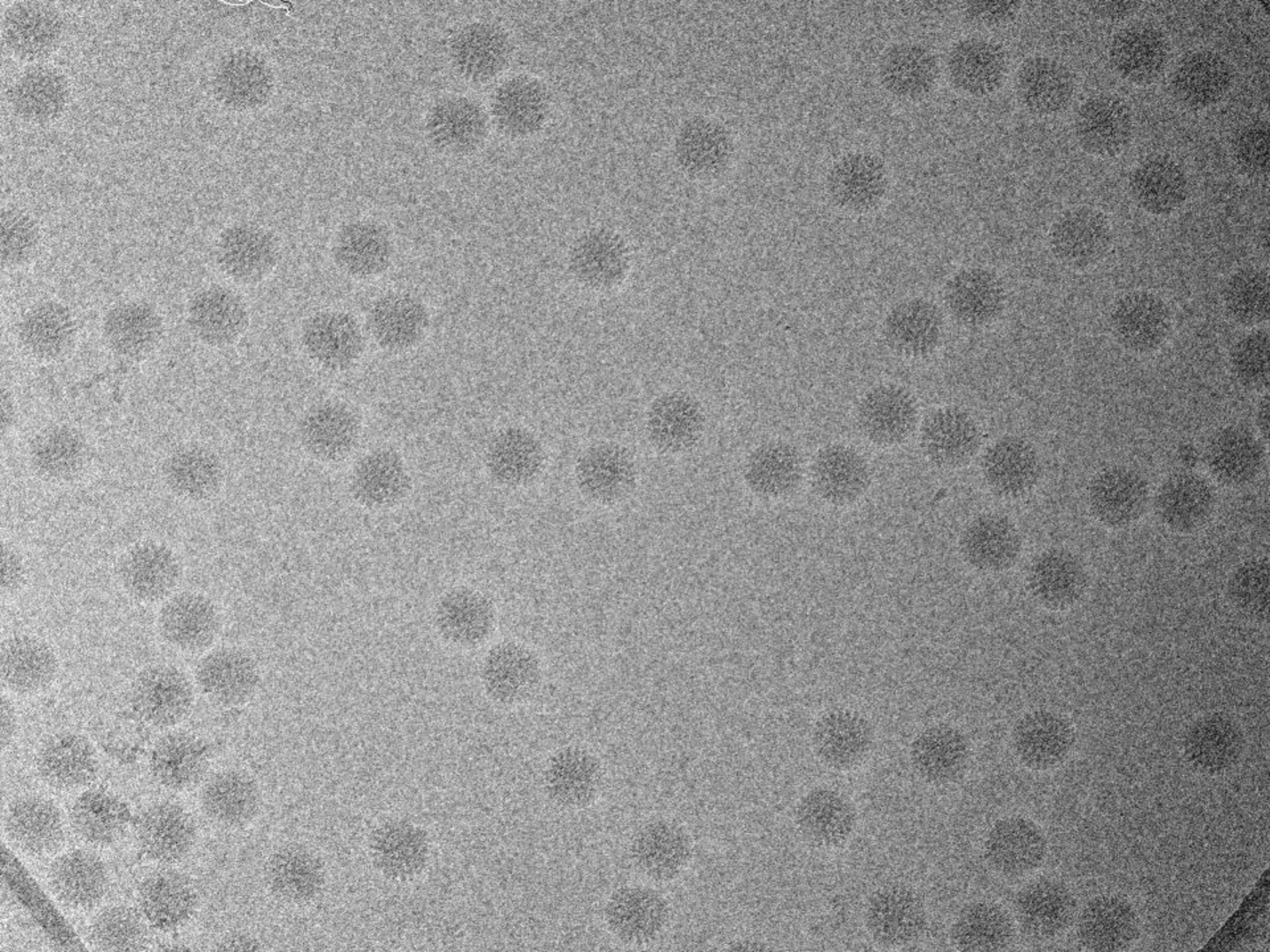
Axel F. Brilot , James Z. Chen, Anchi Cheng, Junhua Pan,
Stephen C. Harrison , Clinton S. Potter ,
Bridget Carragher , Richard Henderson , Nikolaus Grigorieff

Journal of Structural Biology 177 (2012) 630–637

Rotavirus, 700 Å used as test specimen, previously structure determined to 3.8 Å resolution.

Data collected at 40 frames/second, exposure 0.5 electron/Å².

Total dose: 20 electron/Å². To improve contrast sum in groups of 10 frames. Measure vector displacement and angular rotation.



High-resolution noise substitution to measure overfitting and validate resolution in 3D structure determination by single particle electron cryomicroscopy

Shaoxia Chen; Greg McMullan; Abdul R Faruqi; Garib N Murshudov; Judith M Short; Sjors H Scheres; Richard Henderson

Ultramicroscopy, Accepted April (2013)

Beta- galactosidase

Three dimensional structure of beta-galactosidase along with comparison with atomic model.

Higher DQE of backthinned Falcon II allows higher resolution data with fewer single particles.

How does Falcon II compare with film?

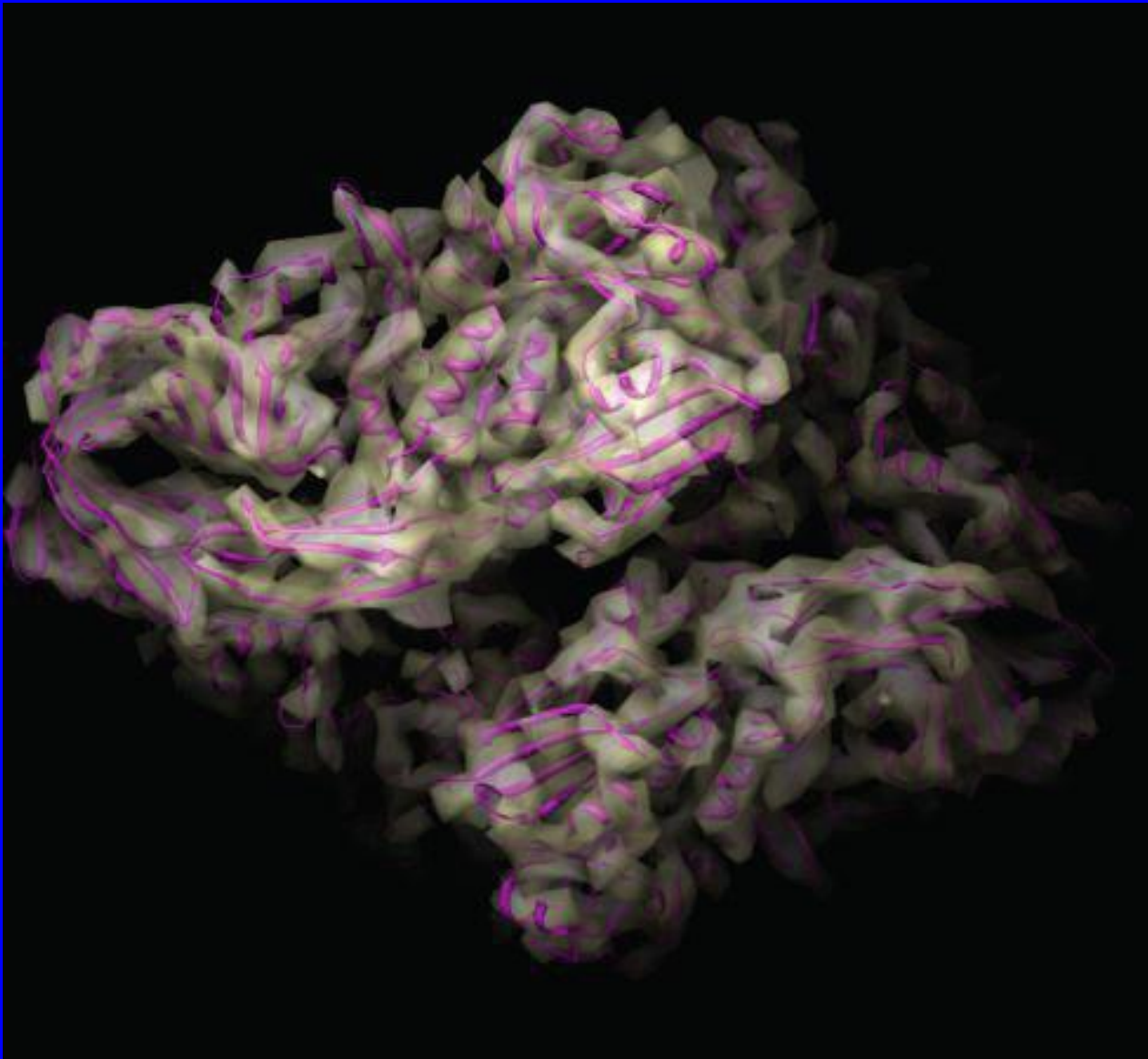
Film: 49000 particles – 11 Å resolution 52 films

Falcon II 43000 particles – 6 Å resolution 89 images

Chen, et al Ultramicroscopy accepted April, (2013)

3D map of beta-galactosidase with atomic model superimposed

Chen, et al 2013



4/21/2013

LMB, Cambridge

Summary

A 4k x 4k rad-hard backthinned sensor for Electron Microscopy described; commercially produced and routinely installed in high-end FEI Electron Microscopes

Falcon II performance exceeds that of film at 300 keV

Examples from two structures, Ribosome and Beta-galactosidase demonstrate the excellent performance of the detector.

Even better performance than for backthinned sensors should be possible with electron counting –using fitted centroids to obtain sub-pixel resolution. With the improved resolution in electron counting mode the DQE(Nyquist frequency) could be increased by up to ~ 3 fold (G. McMullan, A.T.Clark, R.Turchetta, A.R.Faruqi Ultramicroscopy 109 (2009) 1411–1416)

Future Technical Improvements

Finer linewidth layout used for chip design – 130 nm/ 65 nm?

Larger sensors : 8k x 8k pixels

Faster readout, more frames/sec, more frames/exposure (more parallel readout)

Improved image processing software

Sensors with greater radiation hardness

Higher energy 1 MeV sensors