Direct Electron Detectors in Electron Microscopy

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Renato Turchetta RAL-STFC
Nicola Guerinni RAL-STFC

FEI team

New LMB nearing completion 2012

..... and now officially open!

Courtesy MRC-LMB archives

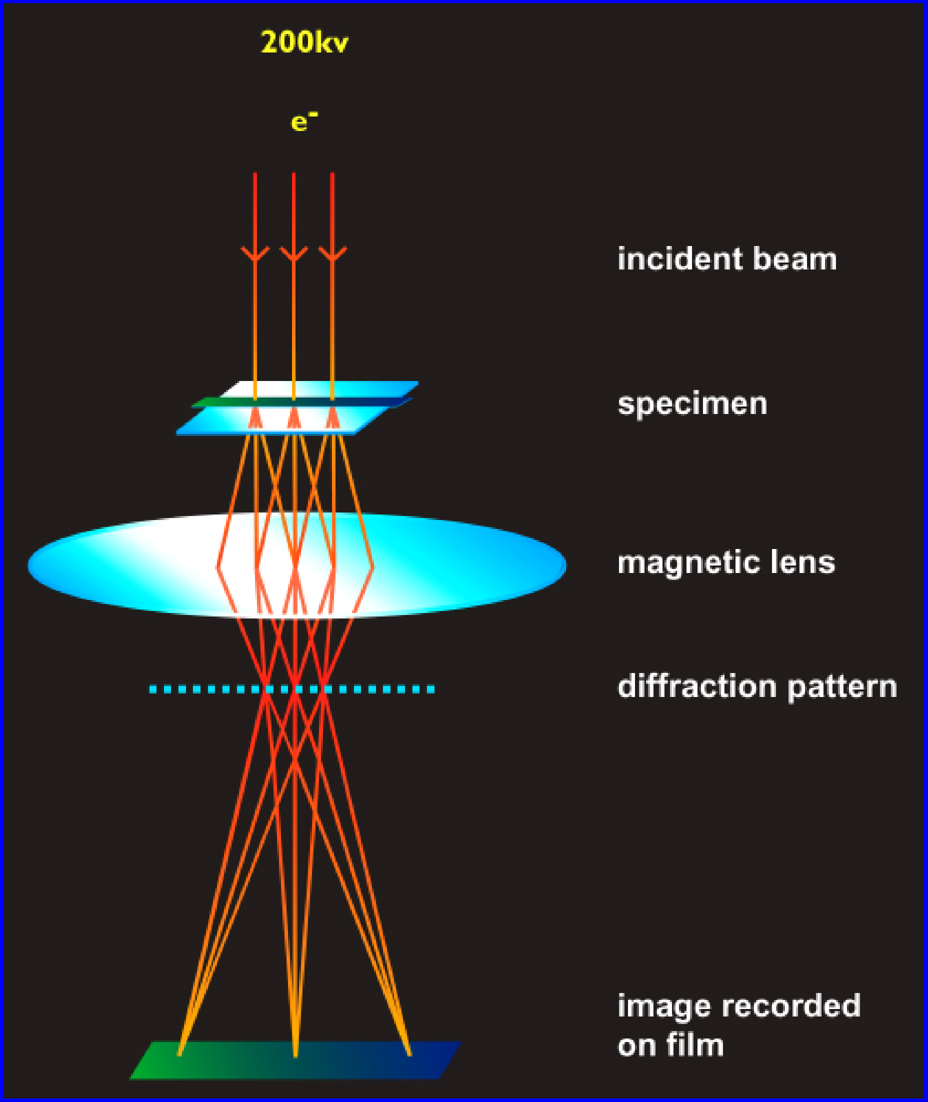
Electron Microscopy at different energies

LEEM/PEEM Low Energy Electron/Photon Microscopy	~20 keV
Electron Diffraction	~100 keV
Electron Cryo-Microscopy	100 – 300 keV

Single Particle Imaging (single molecules)

Tomography (larger structures – cells)

Schematic of a Transmission Microscope



Brief background to cryo-microscopy

Applications: single particle analysis, diffraction, electron tomography

Examples: Ribosome, beta-galactosidase,

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 sophisicated 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/Å^2 at the specimen

Detectors: Quality Factors

Sensitivity: Detective Quantum Efficiency (DQE), $(S/N)^2_{output} / (S/N)^2_{input}$ (=1 for perfect detector)

DQE(0) zero spatial frequency

DQE(spatial frequency) includes MTF, NTF

Resolution: Modulation Transfer Function (MTF)

Framing Speeds… inverse of readout time; 20-1000 Frames/sec

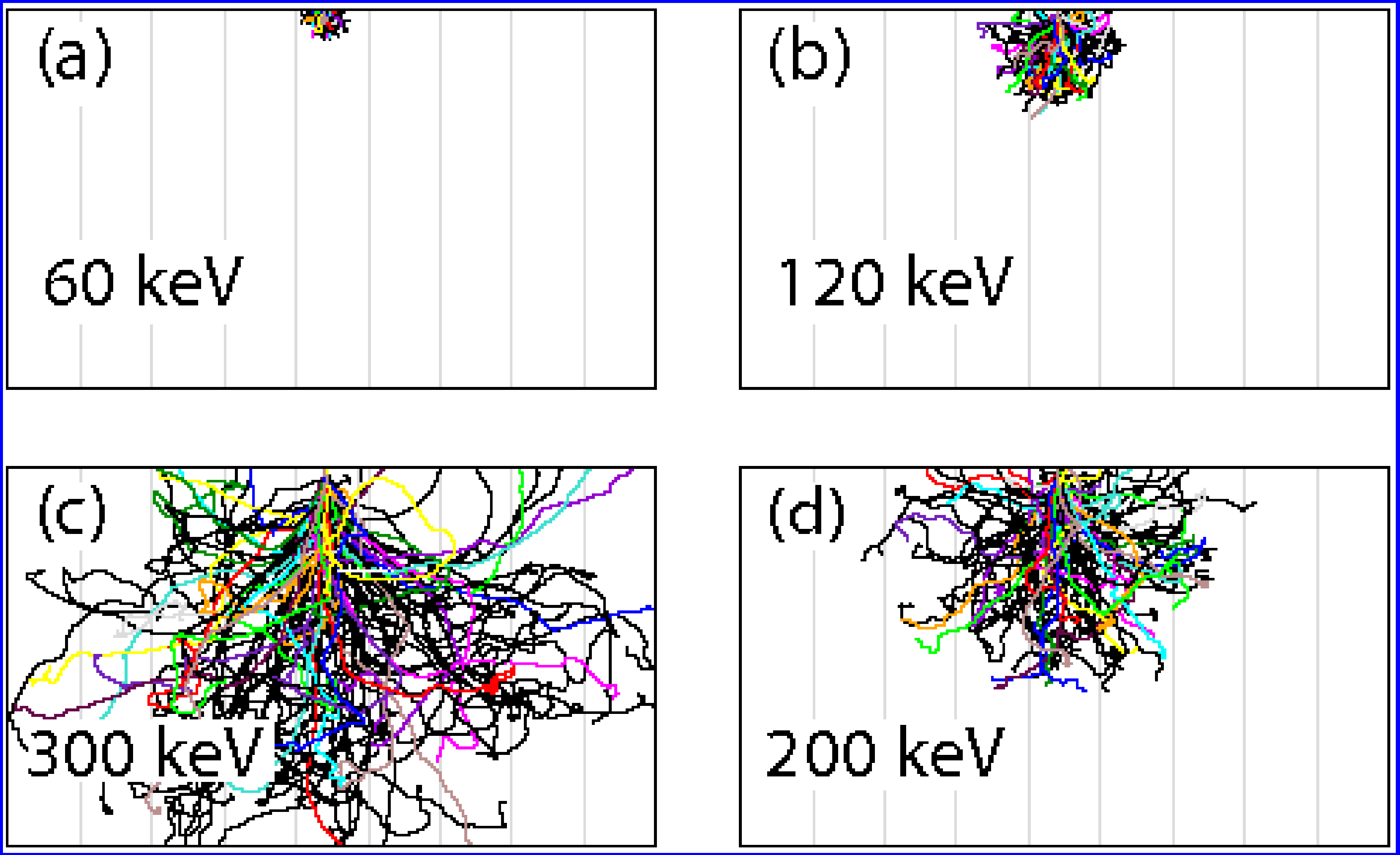
Radiation Hardness … useful lifetime > 1 year

Dynamic Range … ability to record very weak and very strong parts of an image simultaneously (diffraction only)

Defects …… Faults in fabrication, etc

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

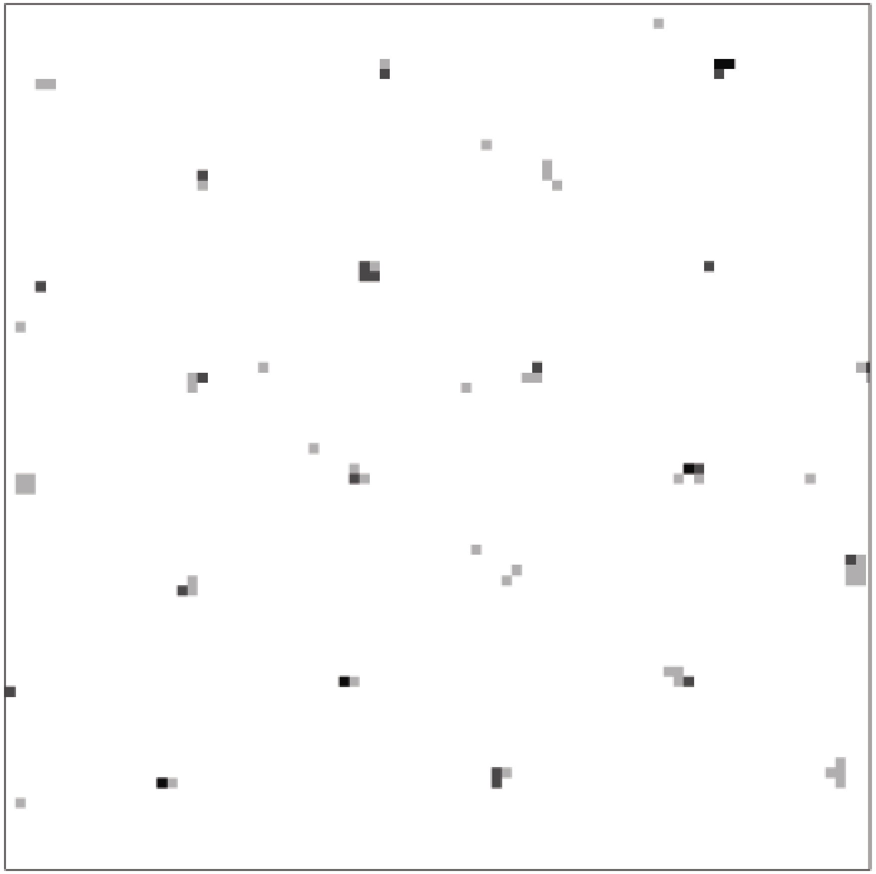
Extension of simulations to include energy deposition



Noiseless direct detection of electrons in Medipix2 for electron microscopy

A.R.Faruqi, R.Henderson and L.Tlustos

NIM A (546), 2005, Pages 160–163



120 keV electrons ‘spotscan’ in Medipix2

Mean:4.7, σ : 1.8

Comparison of Medipix2 with film at 120 keV

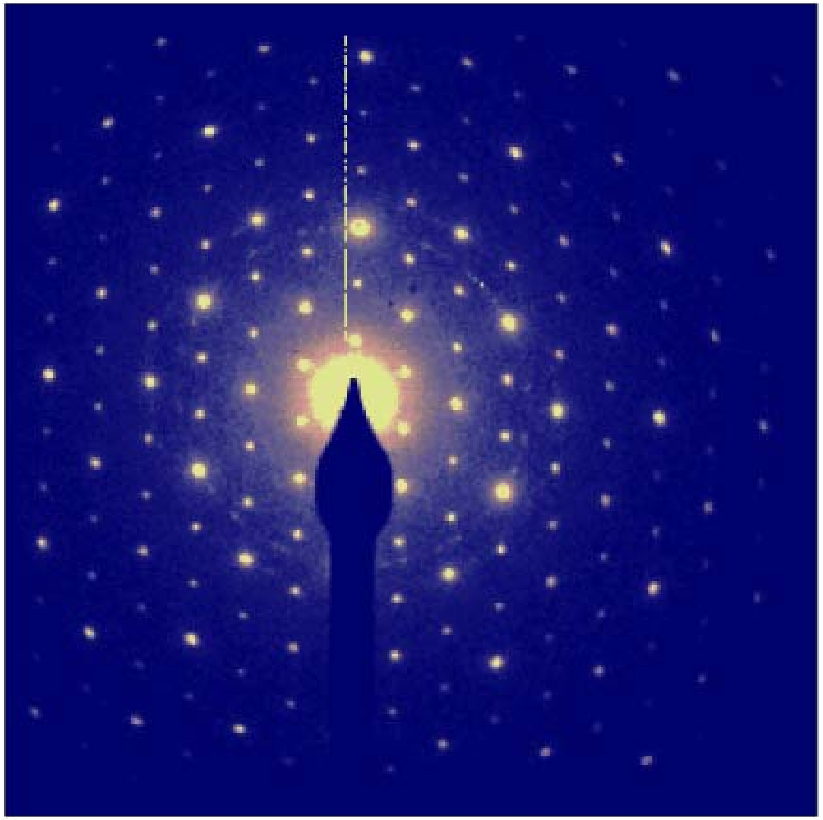
Spotscan 1

	mean	σ
Medipix2	111	11
Film	116	24

Spotscan 7

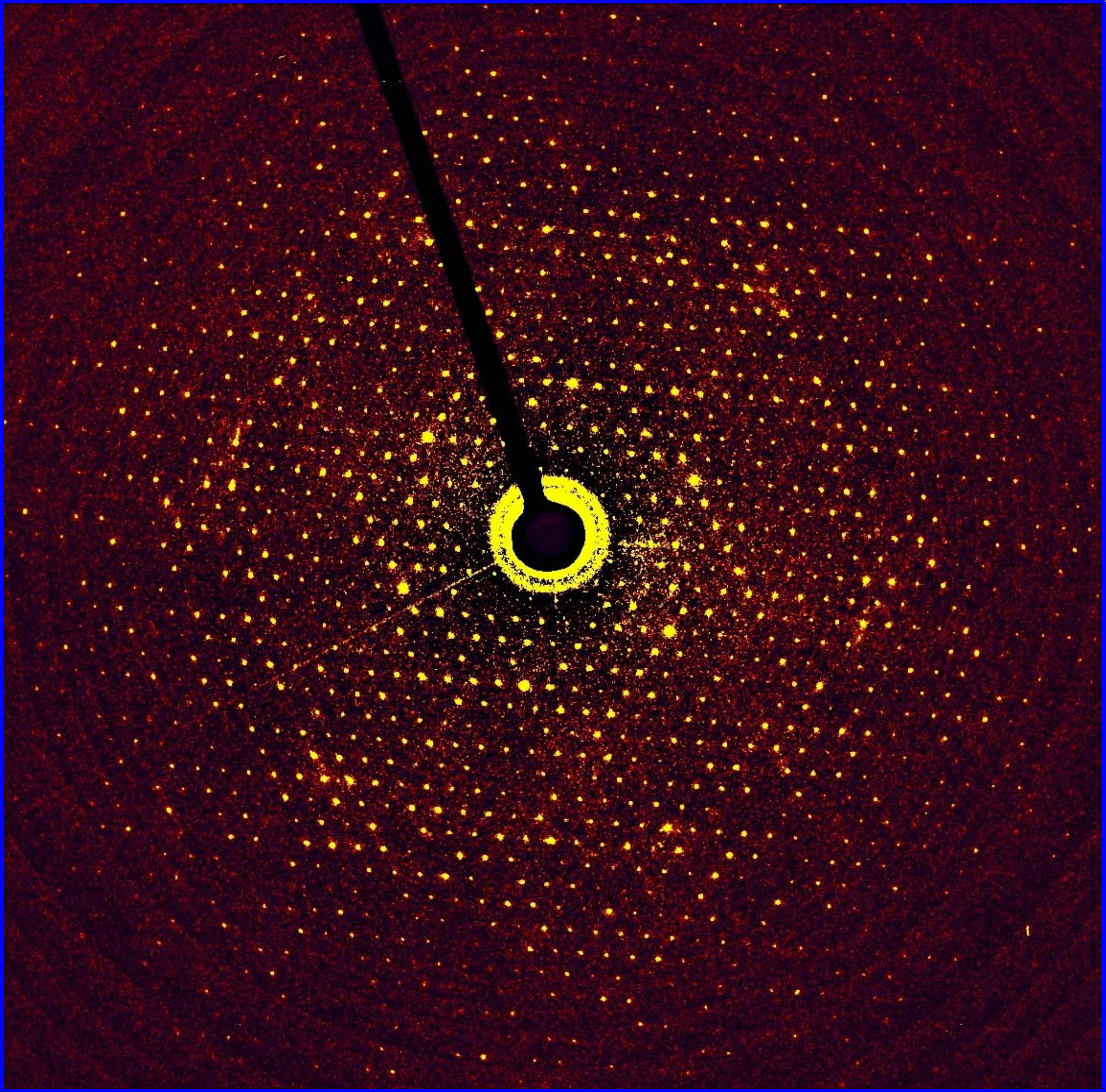
Medipix2	4.7	1.8
Film	spots invisible	

Electron diffraction pattern from Vermiculite on Medipix2 (120keV)

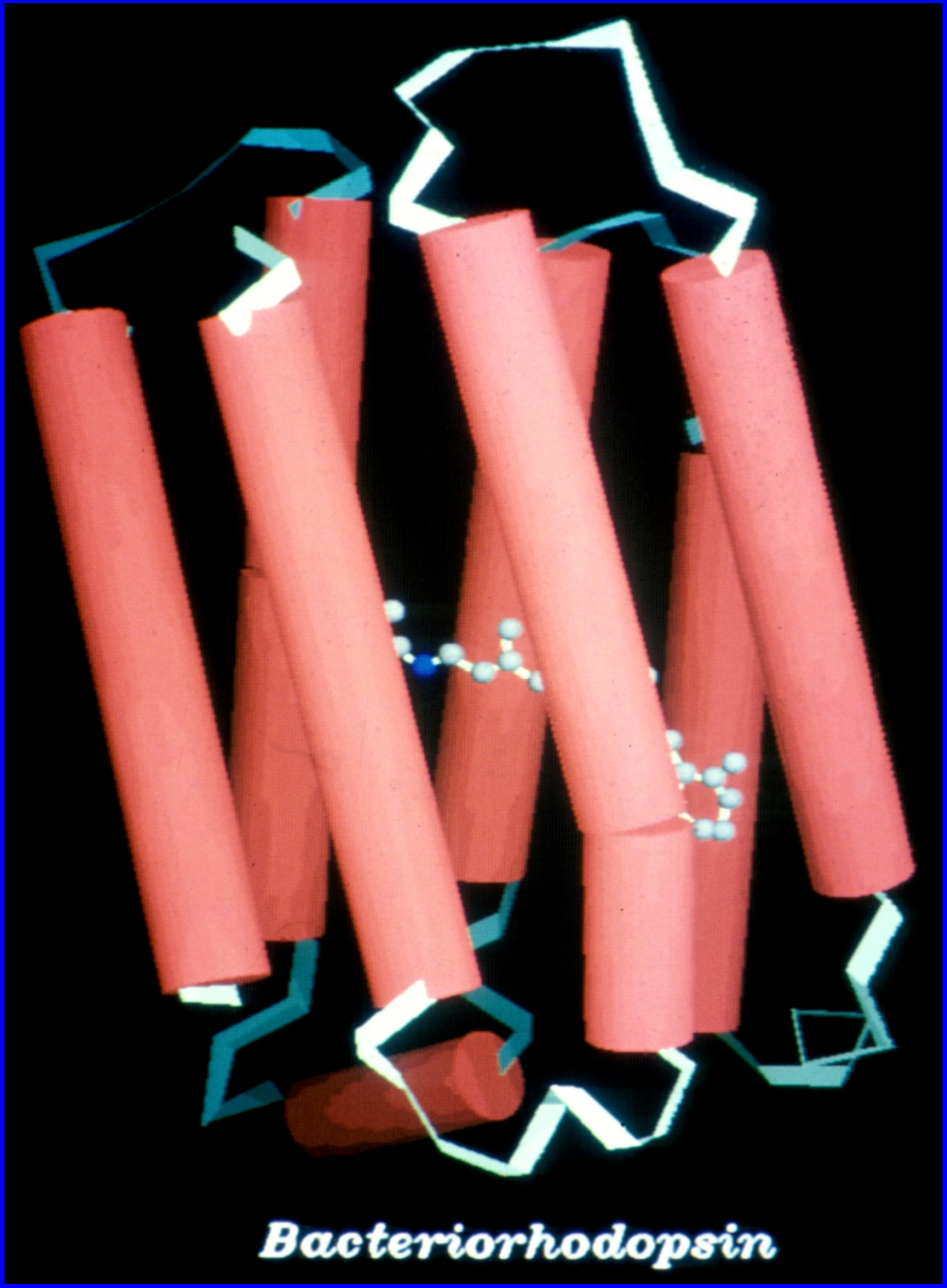


Electron Diffraction Studies on Bacteriorhodopsin

(with R.Henderson and S. Subramaniam)



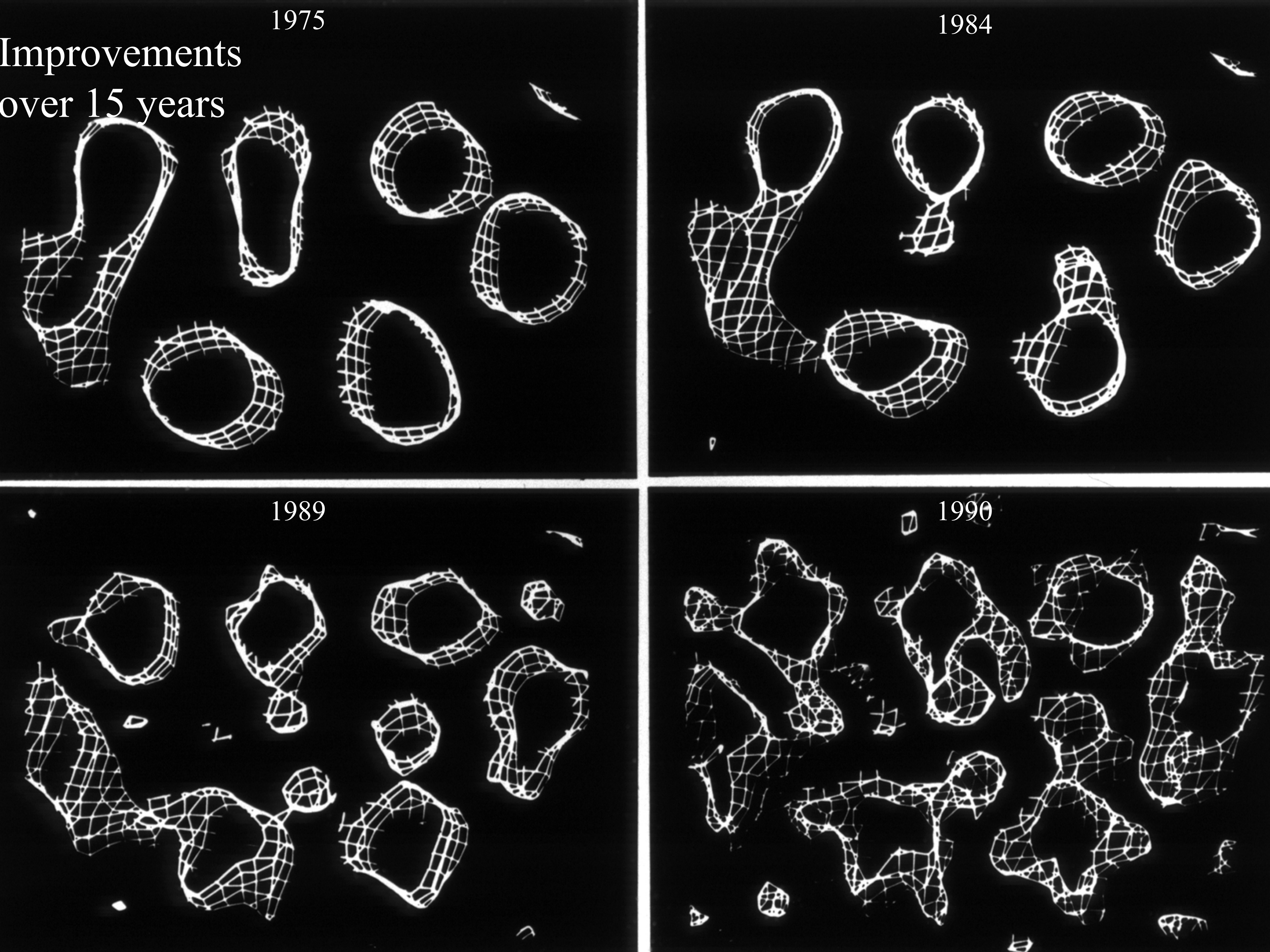
A 7-second diffraction pattern from bacteriorhodopsin with spots visible to 2Å^{-1}



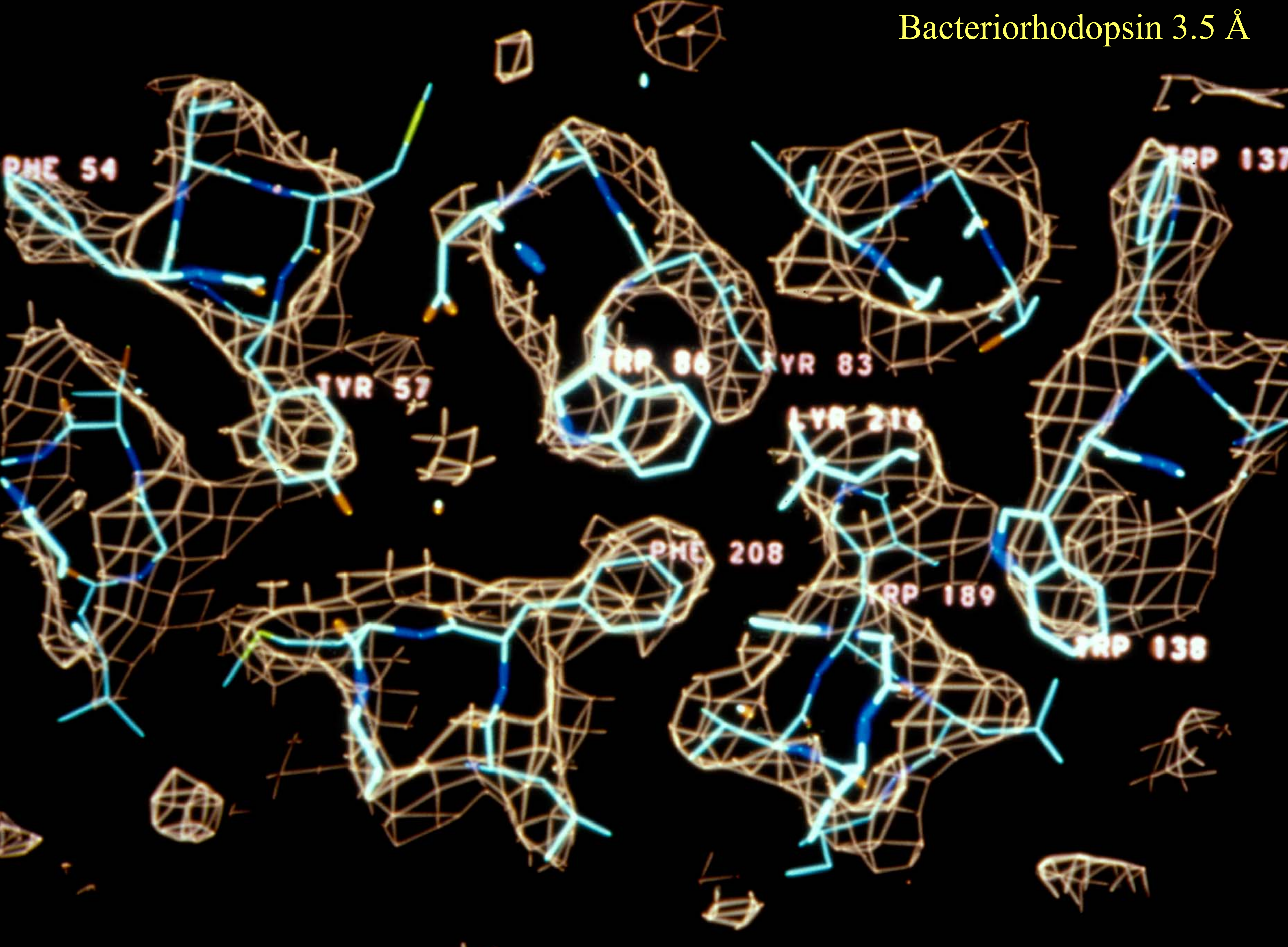
Bacteriorhodopsin

Light-driven proton pump.
Consists of seven trans-membrane helices, shown here in low resolution.
Following slides show how obtaining higher resolution maps can allow atomic resolution models to be built.

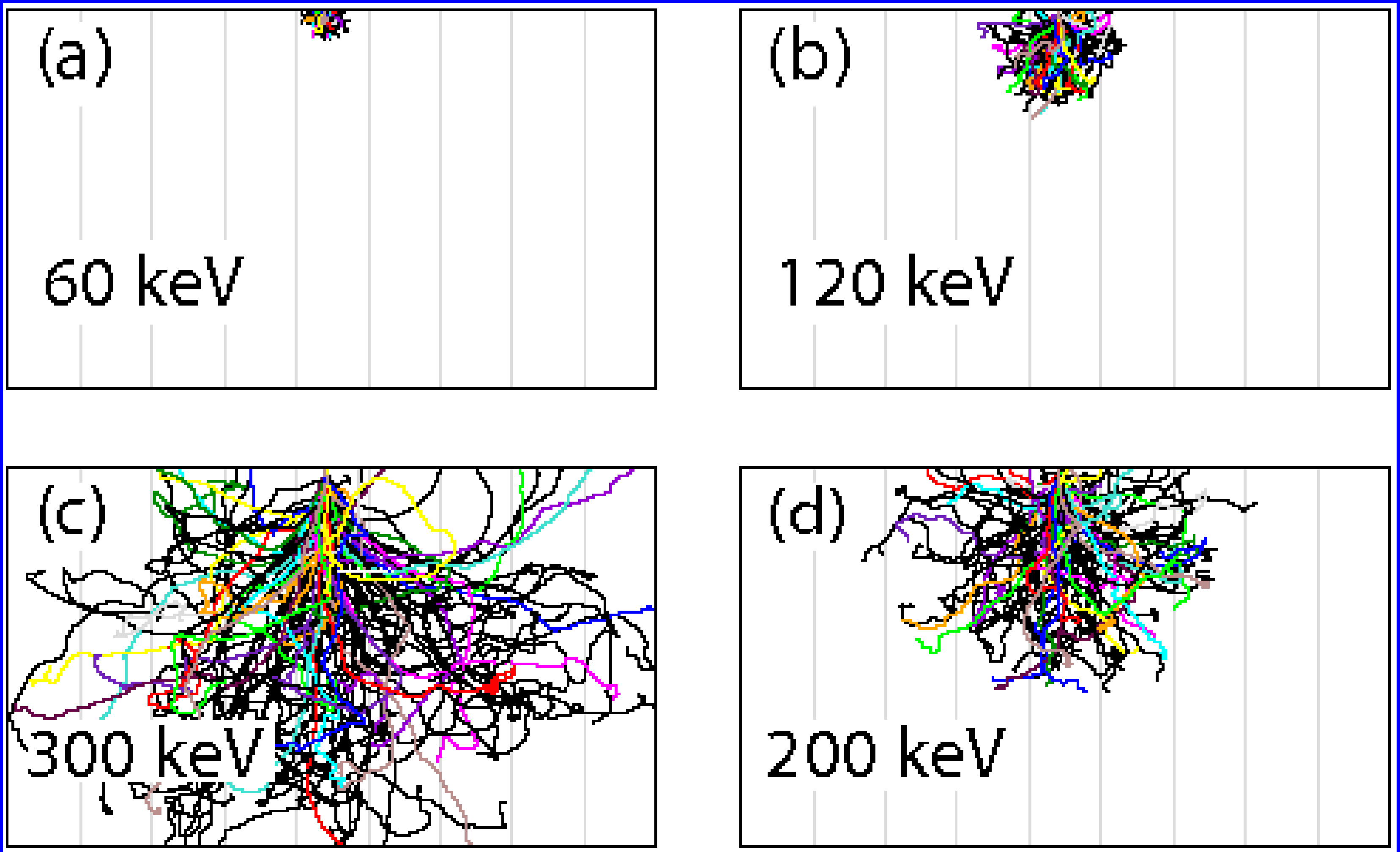
Henderson, et al JMB, **213**, 899-929 (1990)


Improvements
over 15 years
1975
1984
1989
1990

Bacteriorhodopsin 3.5 Å

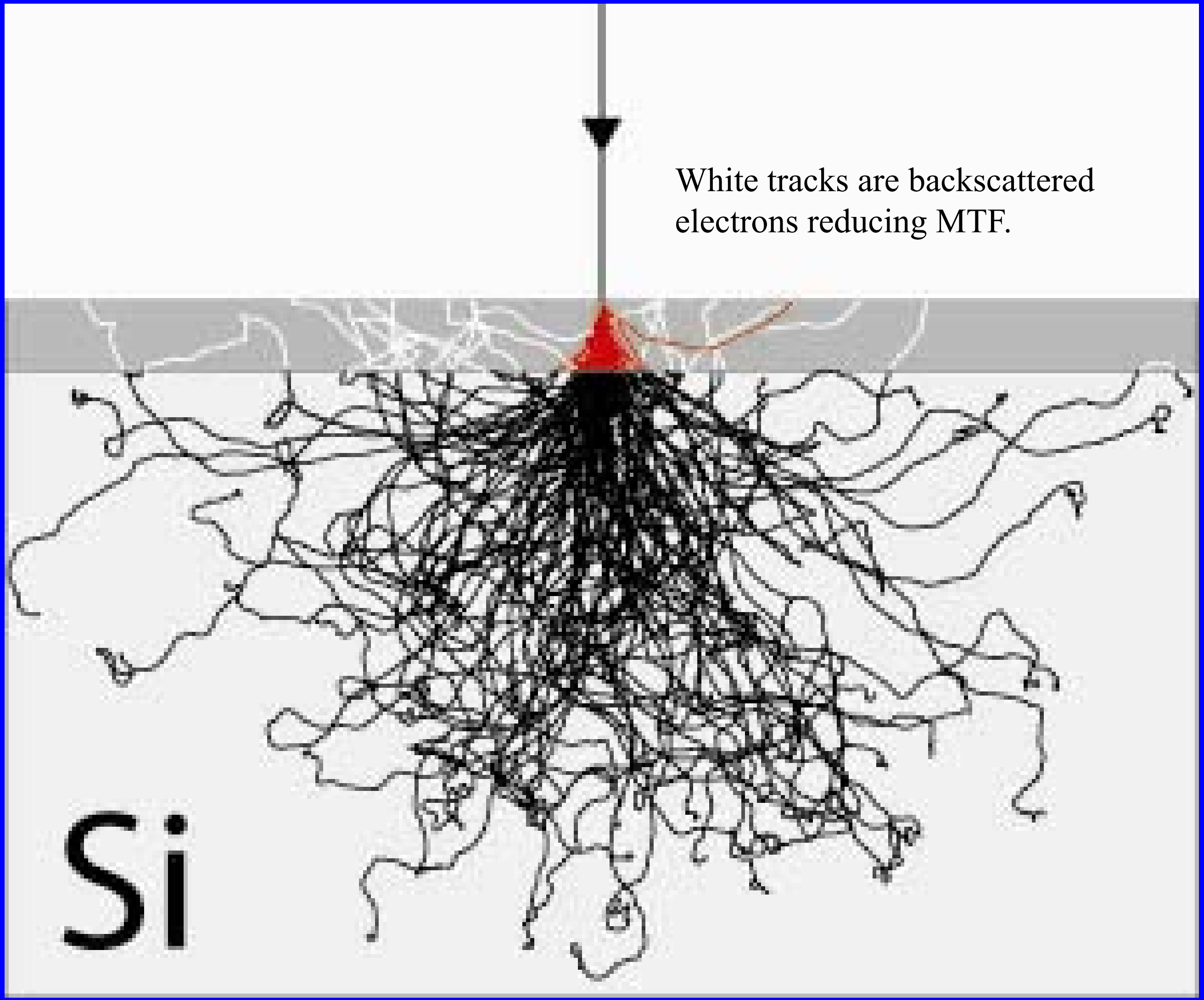


Direct detection of electrons in silicon : Monte Carlo simulation of electron trajectories in silicon. Detector thickness = 300 microns, pixel=55 microns



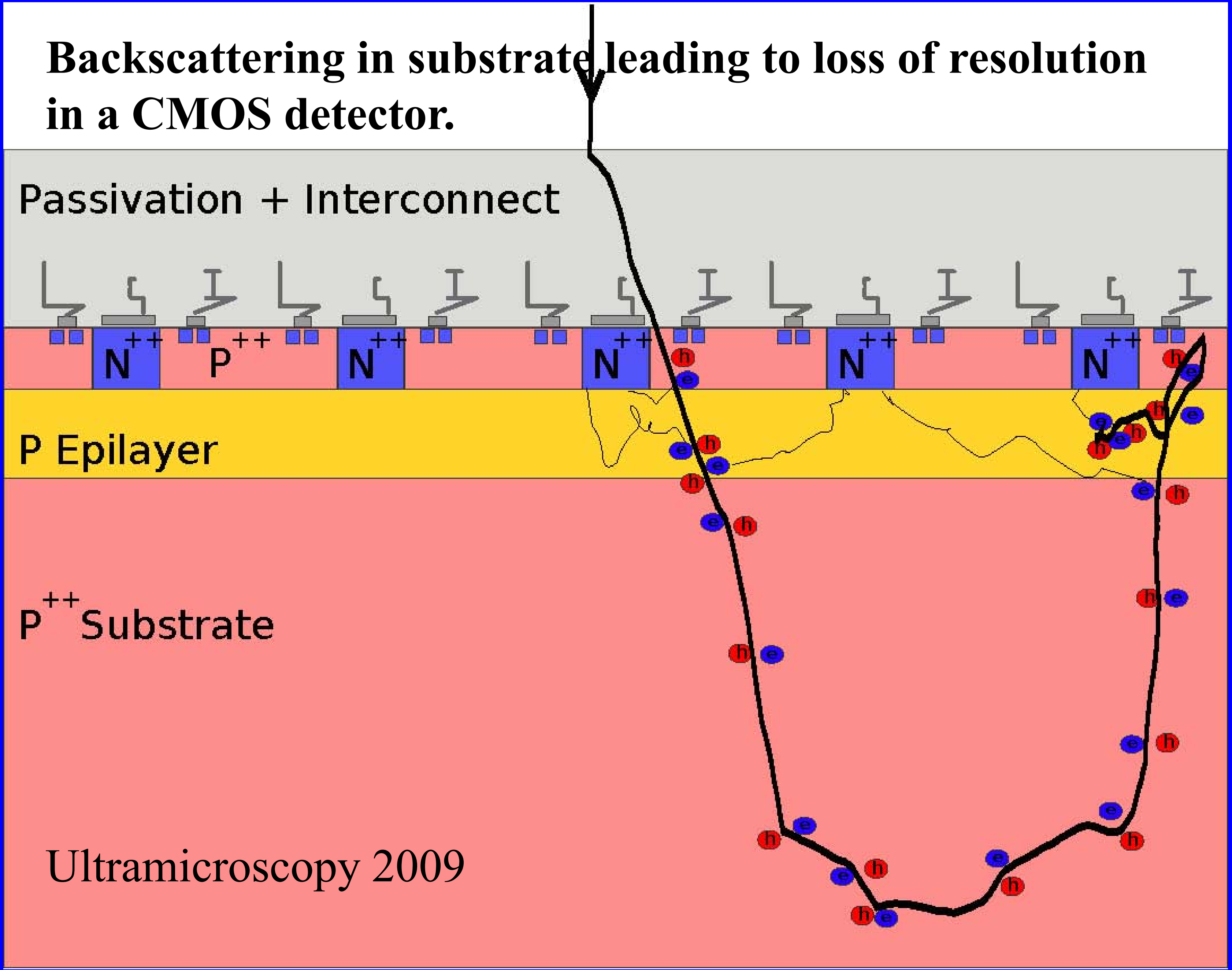
Simulation of 300 keV electron trajectories in silicon.

Total thickness 350 μm, grey thickness 35 μm

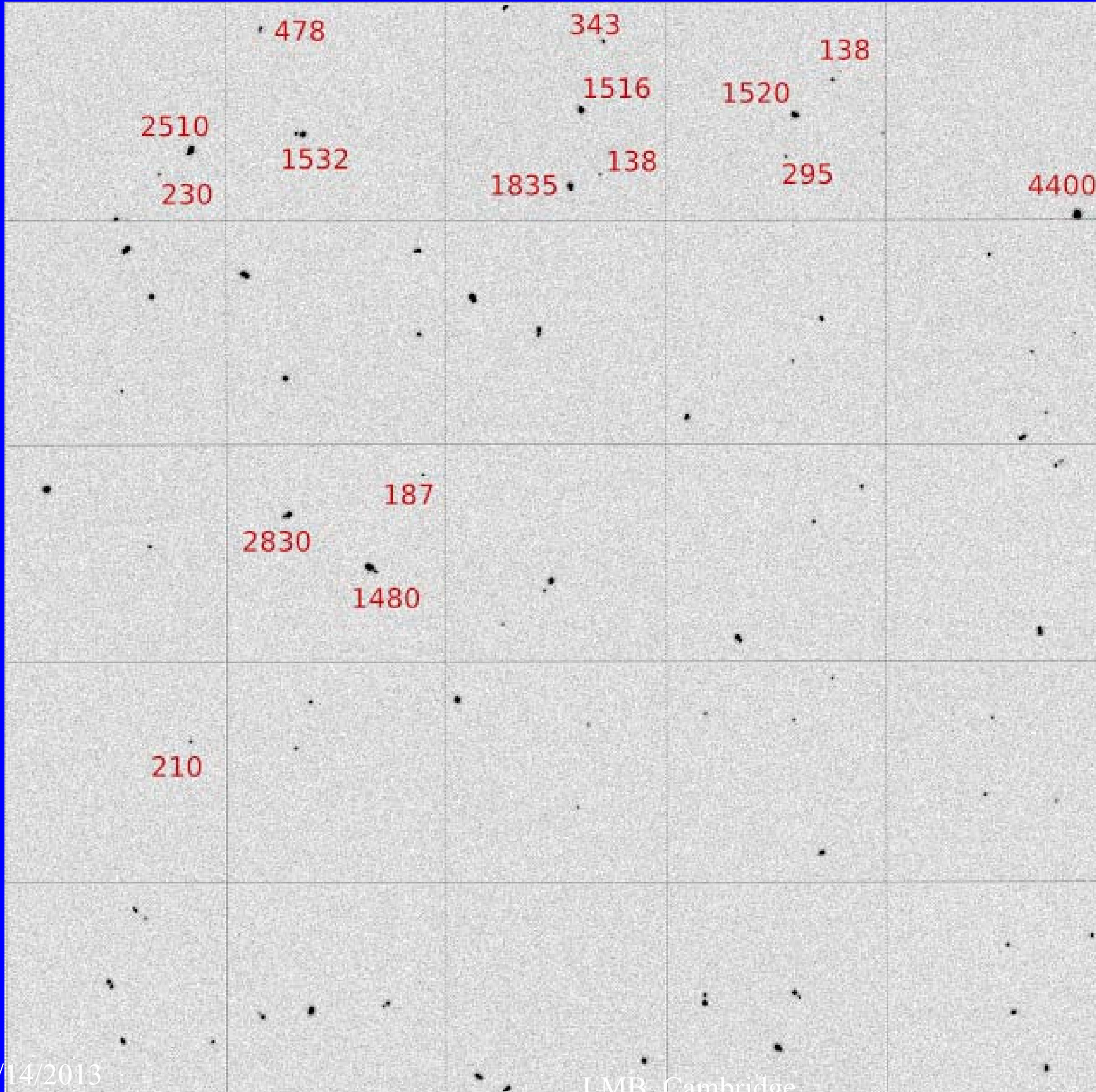


Ultramicroscopy 2009

Backscattering in substrate leading to loss of resolution in a CMOS detector.

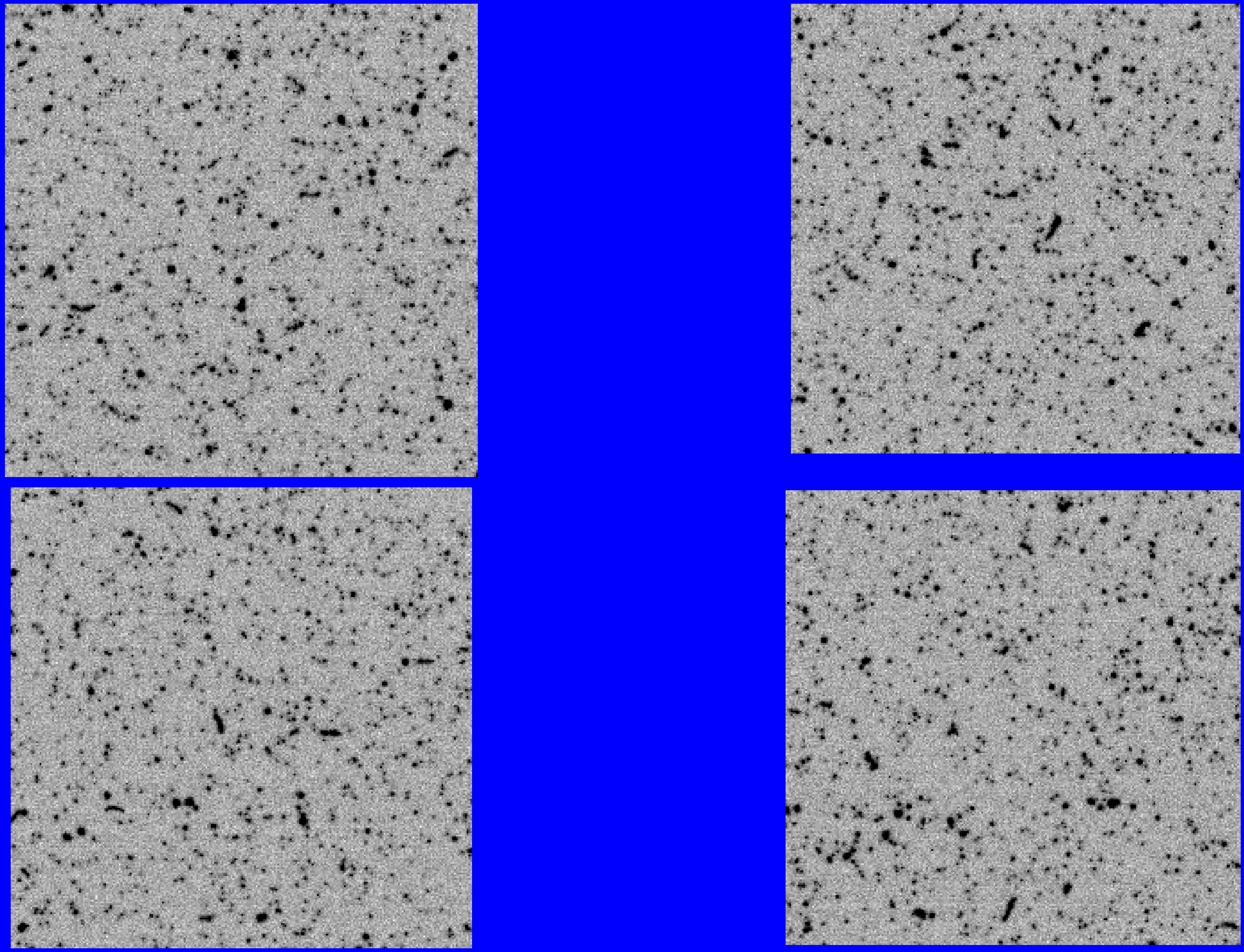


Ultramicroscopy 2009



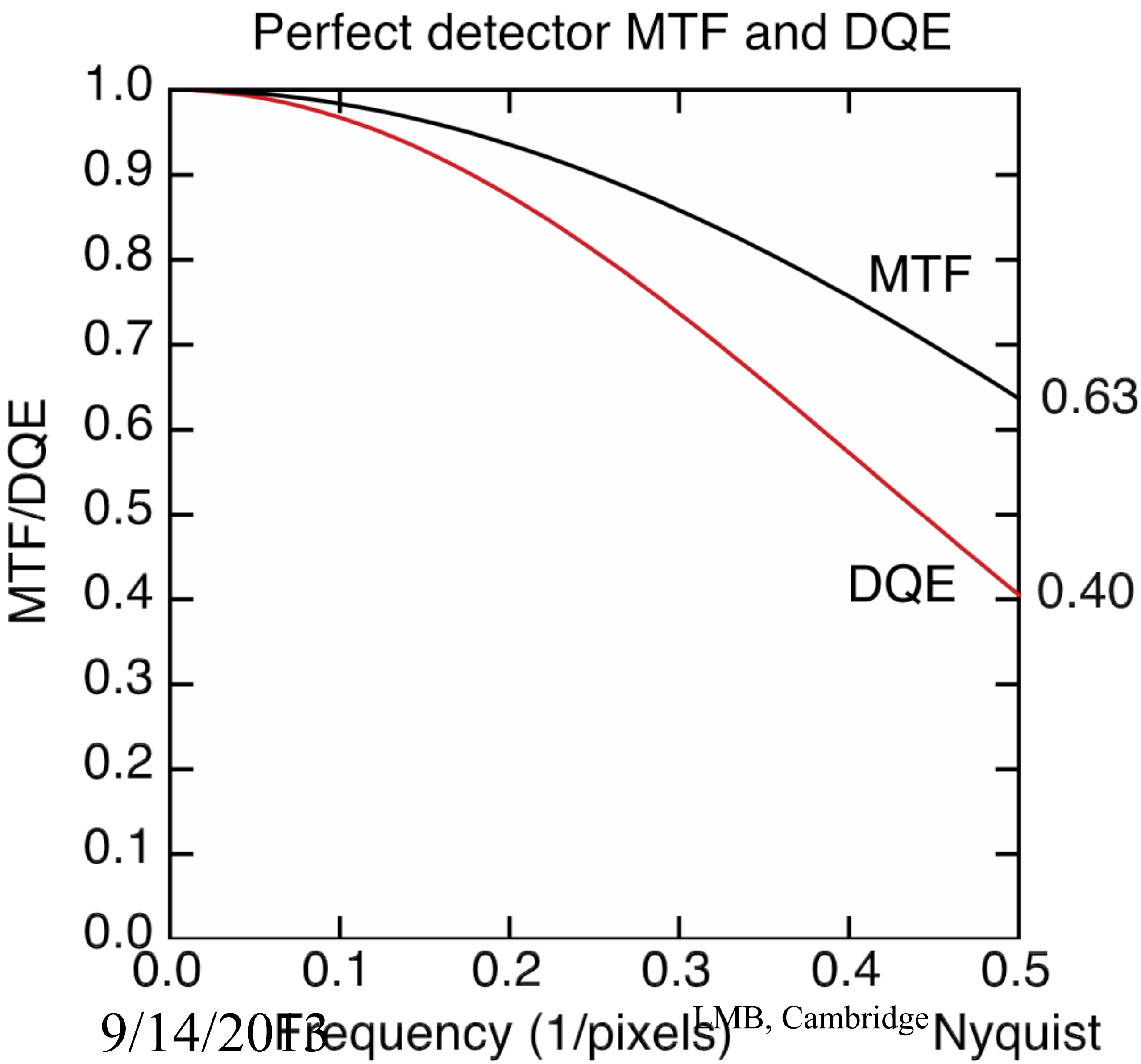
Single electron events

300 keV electron tracks in (50 μm thick) backthinned Falcon (Greg McMullan LMB-MRC)

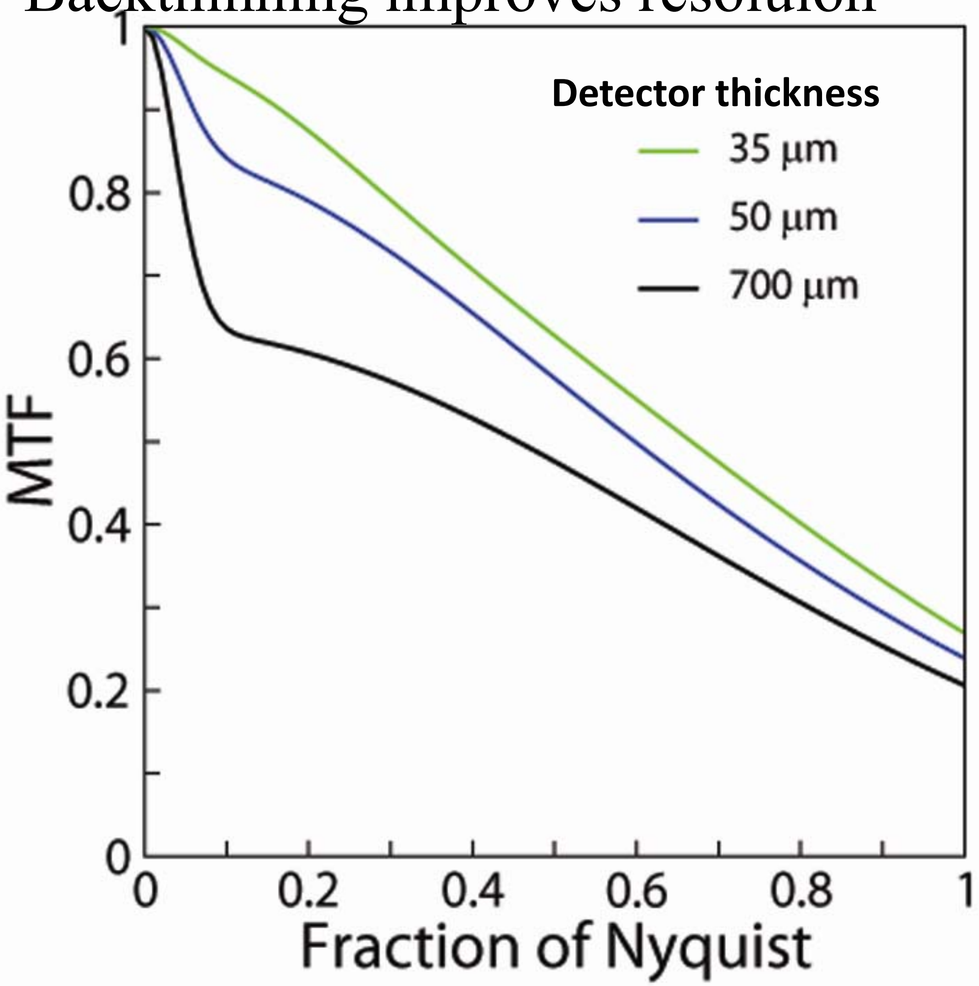


$$DQE(\omega) = DQE(0)*MTF^2/NTF^2$$

Meyer & Kirkland (2000)
De Ruijter (1995)

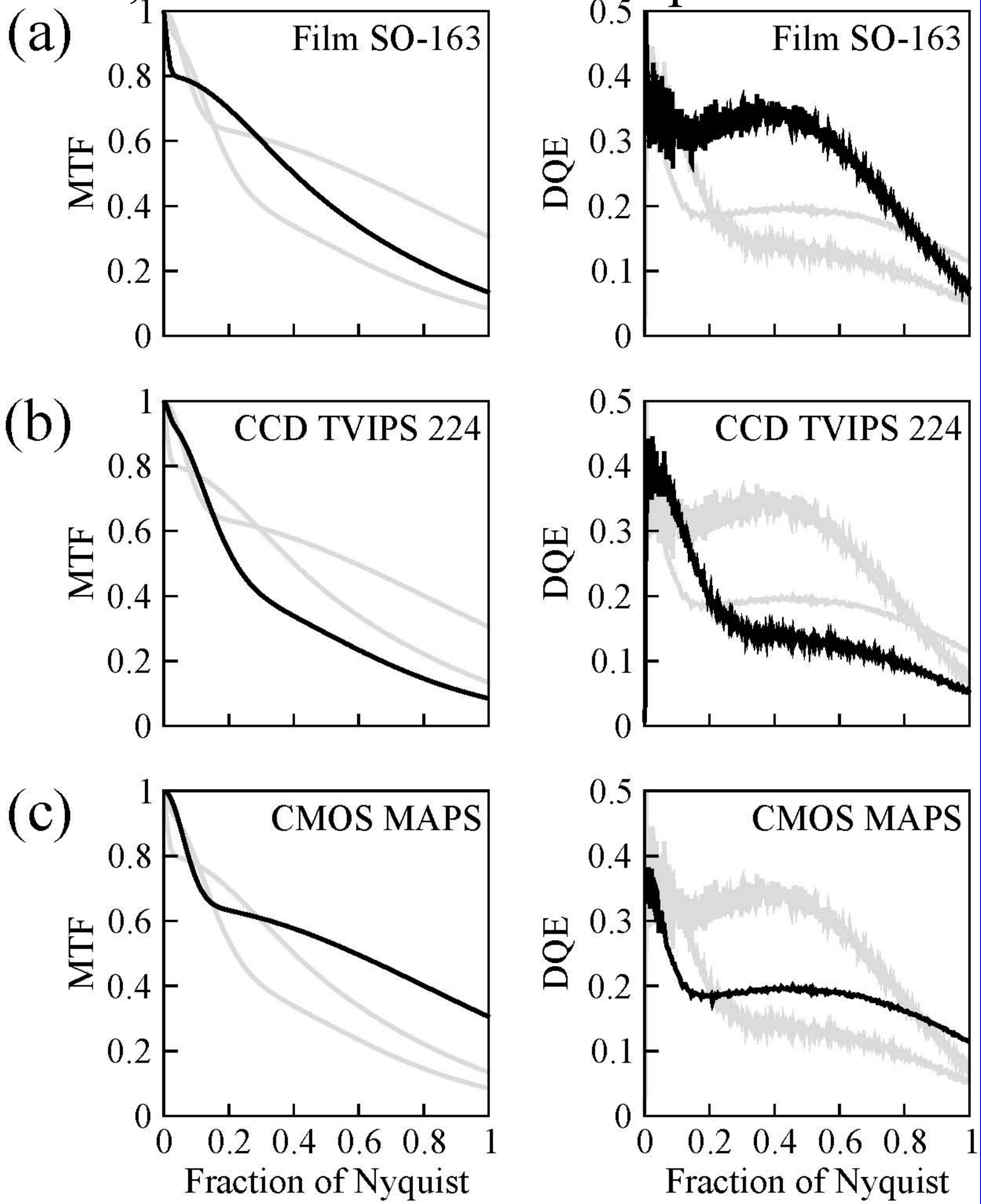


Backthinning improves resoluion



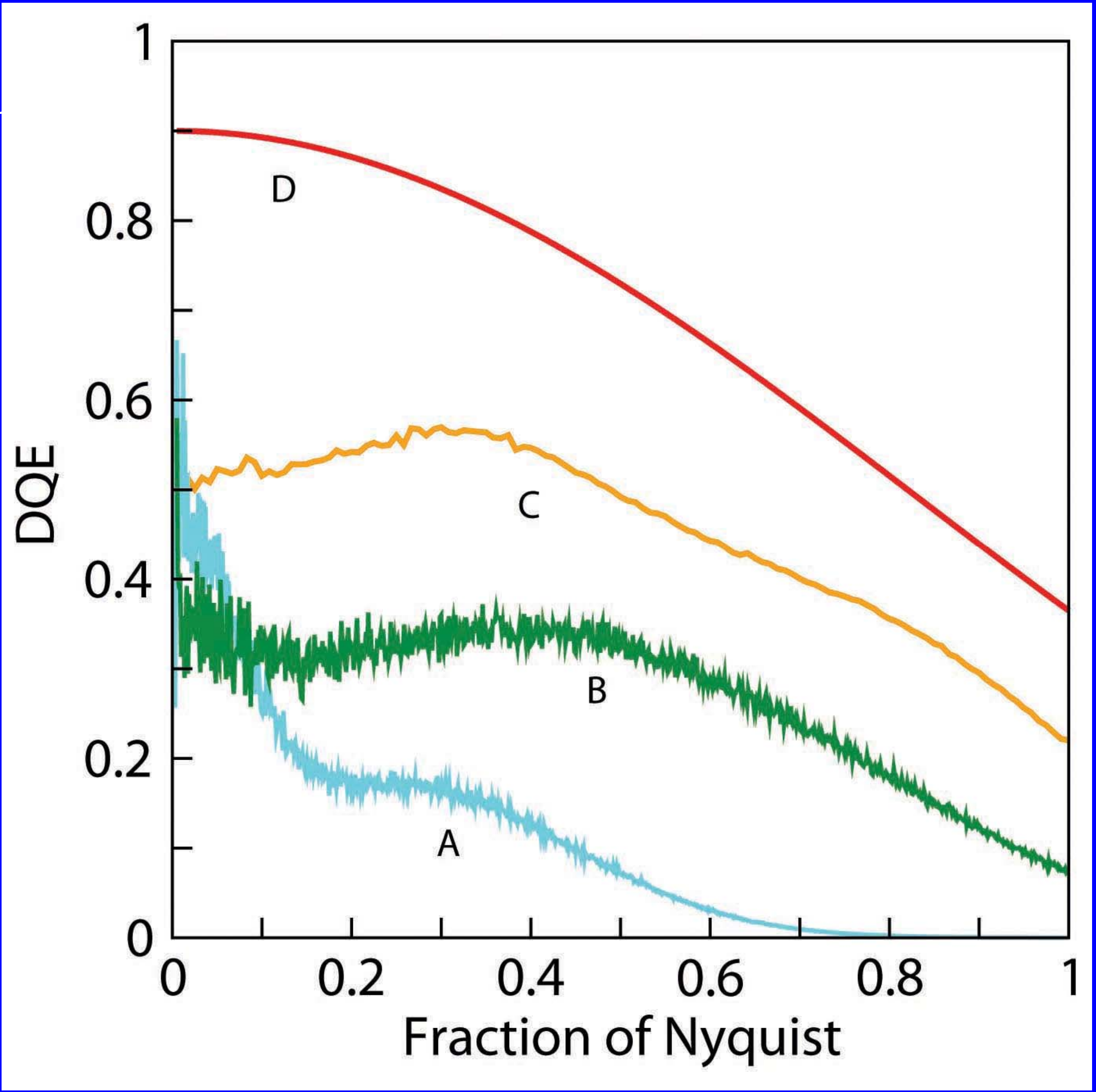
McMullan, et al Ultramicroscopy 2009

300 keV
Film, CCD and CMOS comparison



300keV

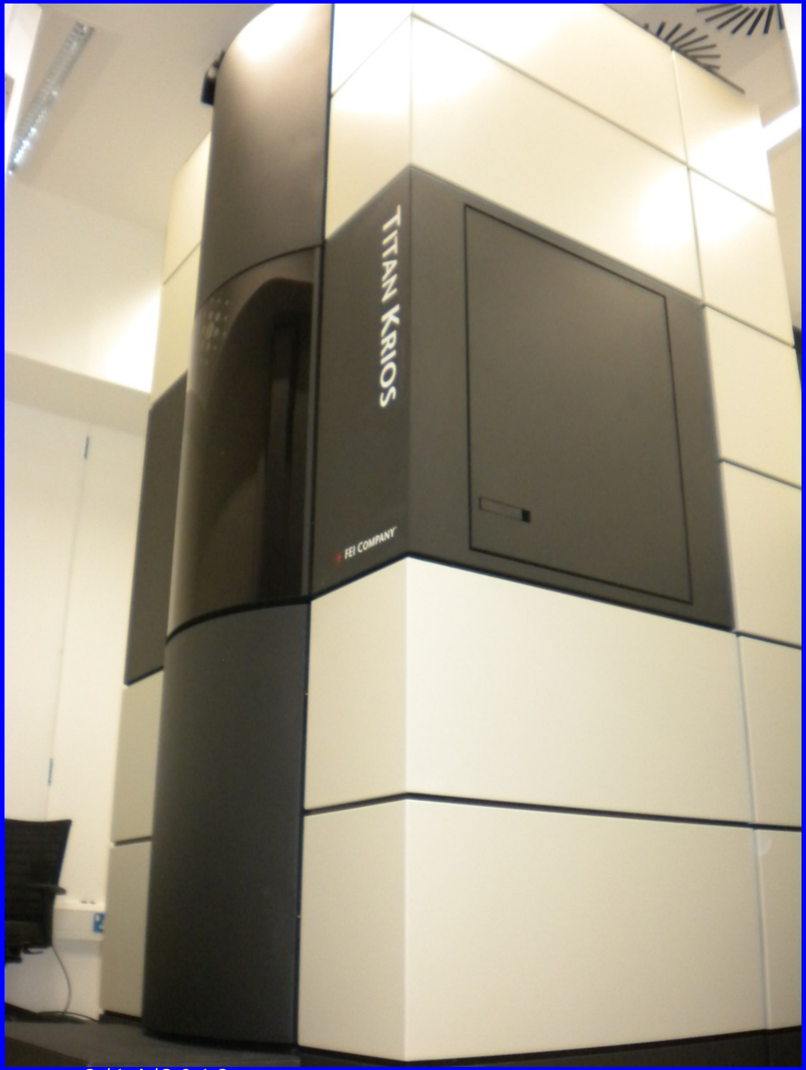
A Ultrascan 4000 15μm
B SO-163 film 7μm
C Backthinned CMOS
D Electron counting

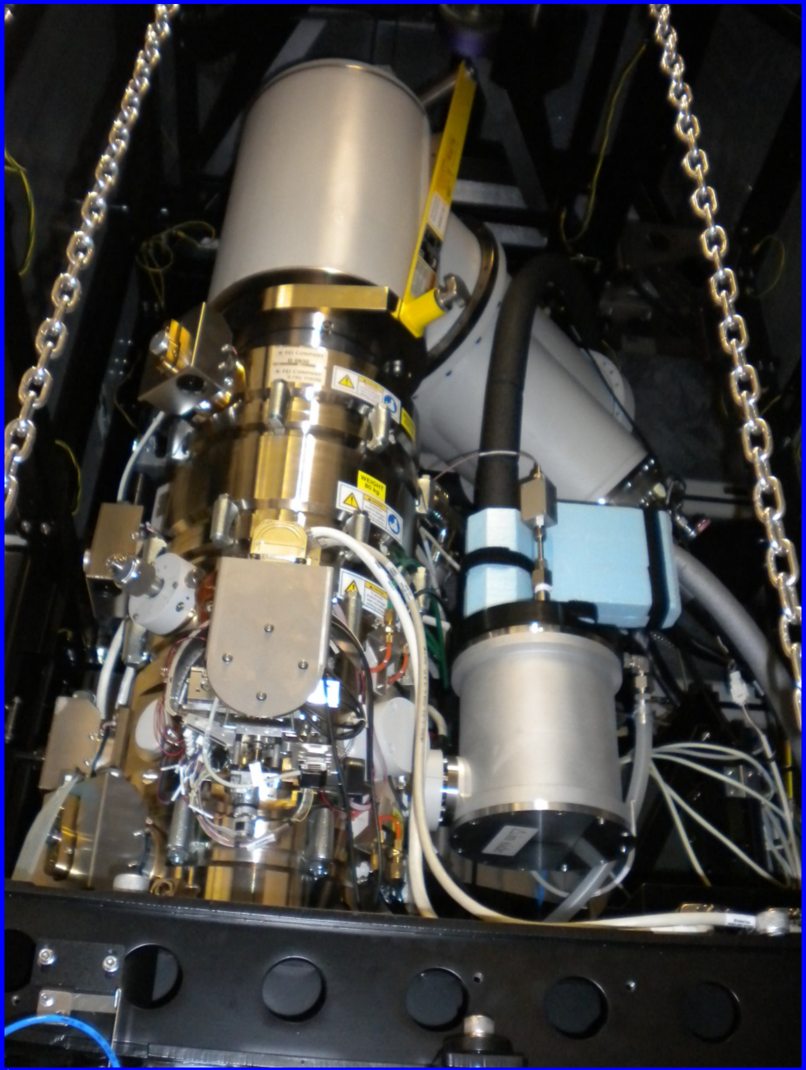


D represents the performance of the proposed new electron counting detector

Falcon mounting for EM

FEI KRIOS 300 kV Electron Microscope

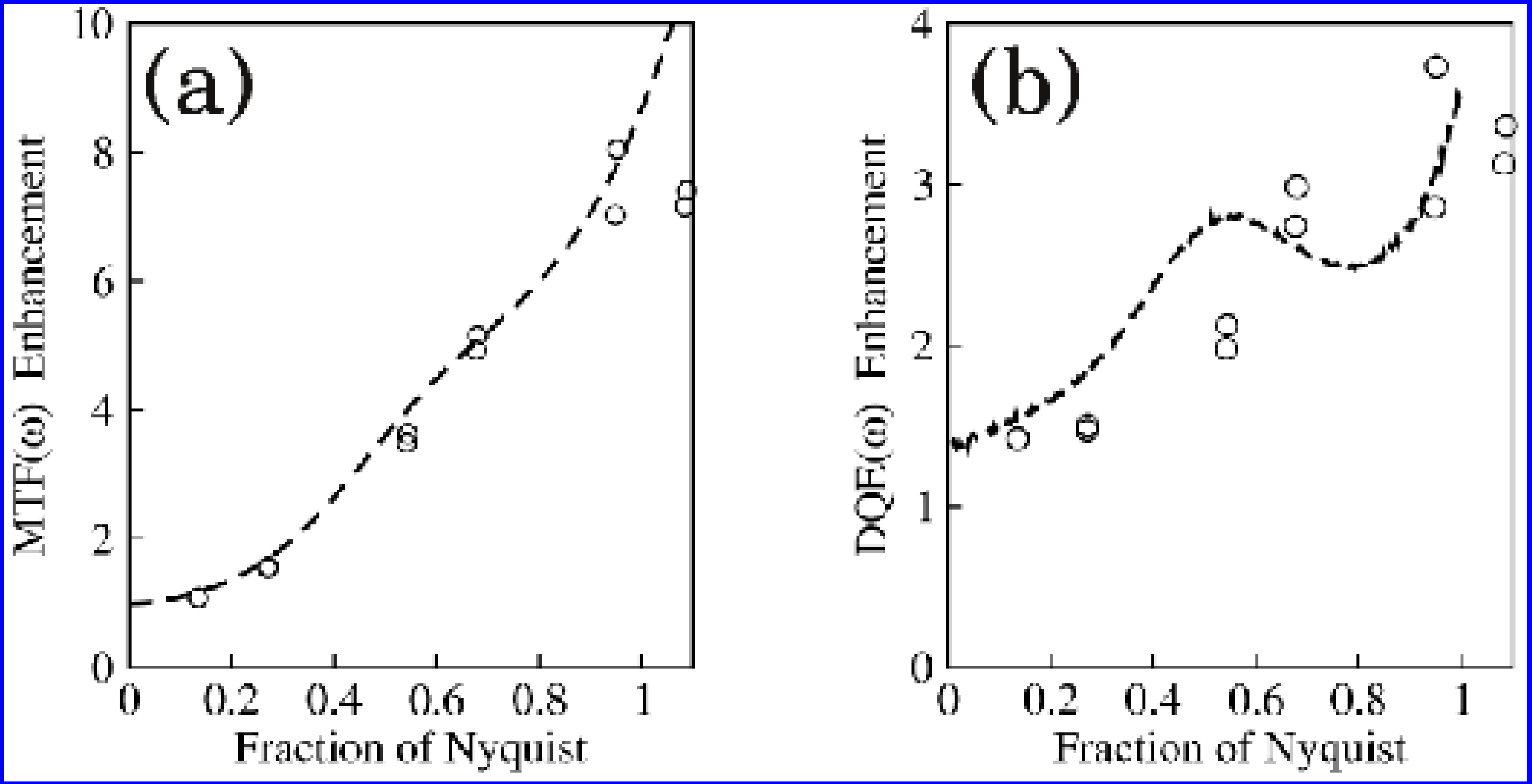




Can we make further improvements to the detector?

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)

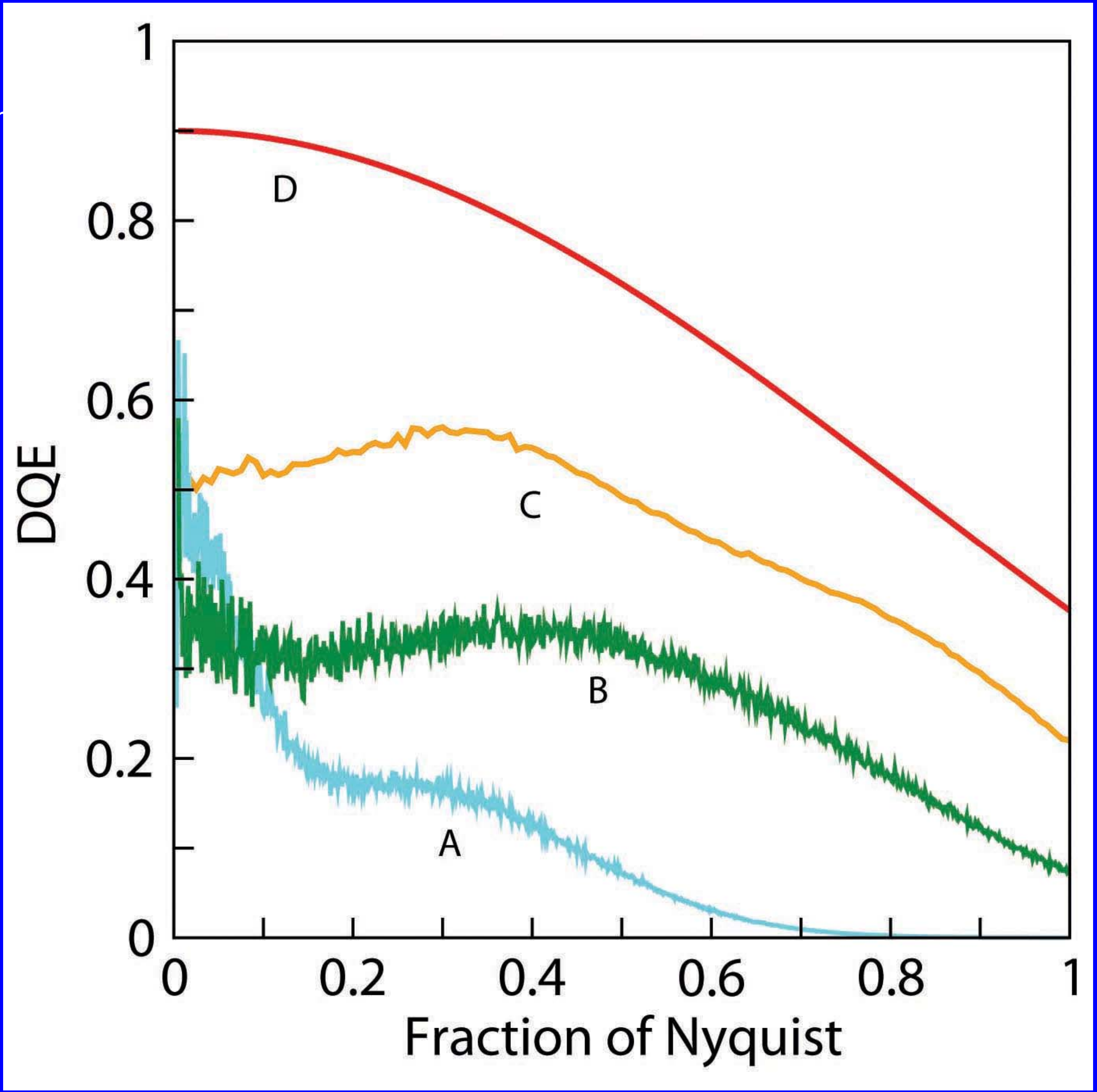
Enhancement of MTF(ω) and DQE(ω) for 'electron counting' compared to analog readout



Ultramicroscopy, (2009), 109, 1411-1416

300keV

A Ultrascan 4000 15µm
B SO-163 film 7µm
C Backthinned CMOS
D Electron counting



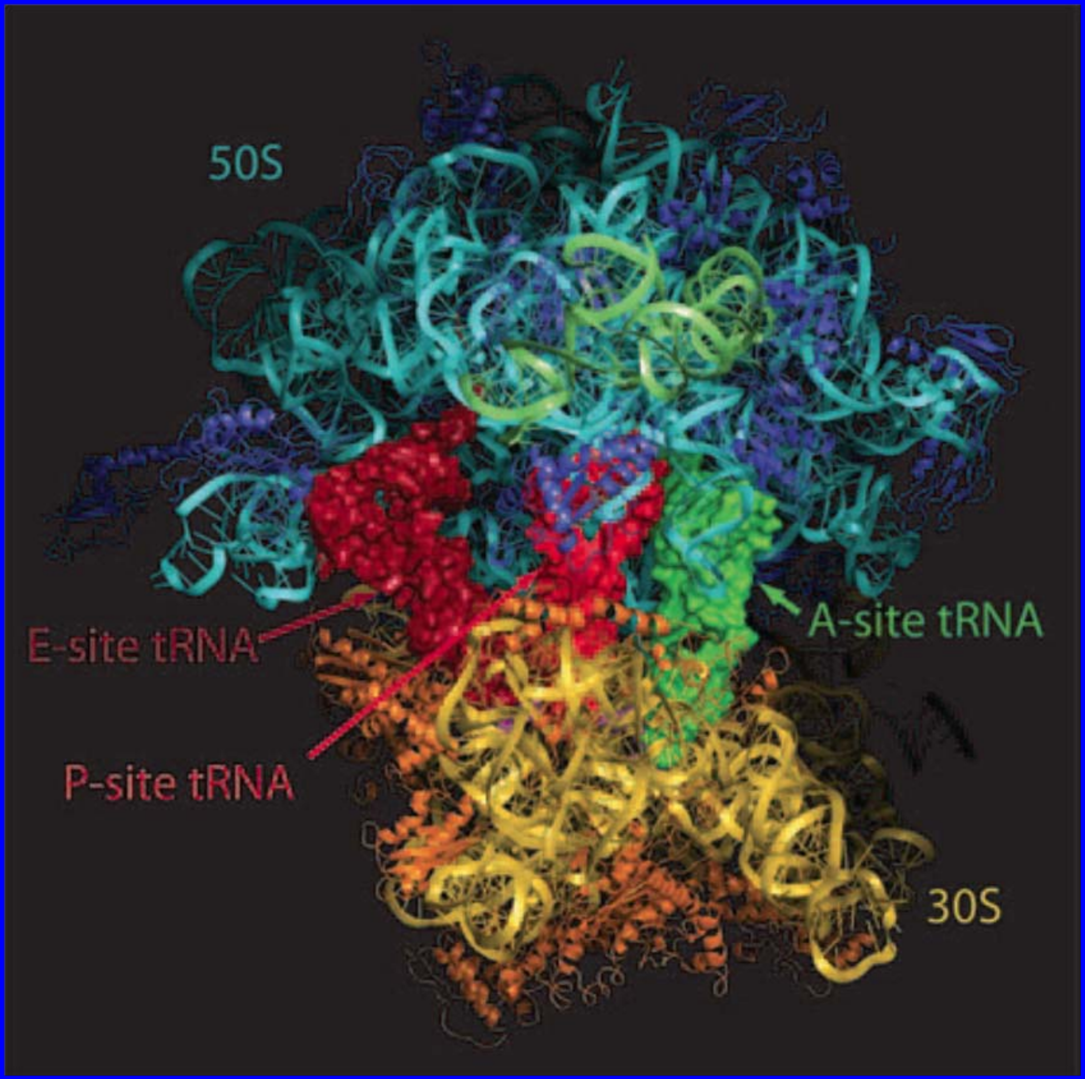
D represents the performance of the proposed new electron counting detector

X.-C. Bai, I.S. Fernandez, G. McMullan & <u>S.H.W. Scheres</u> (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

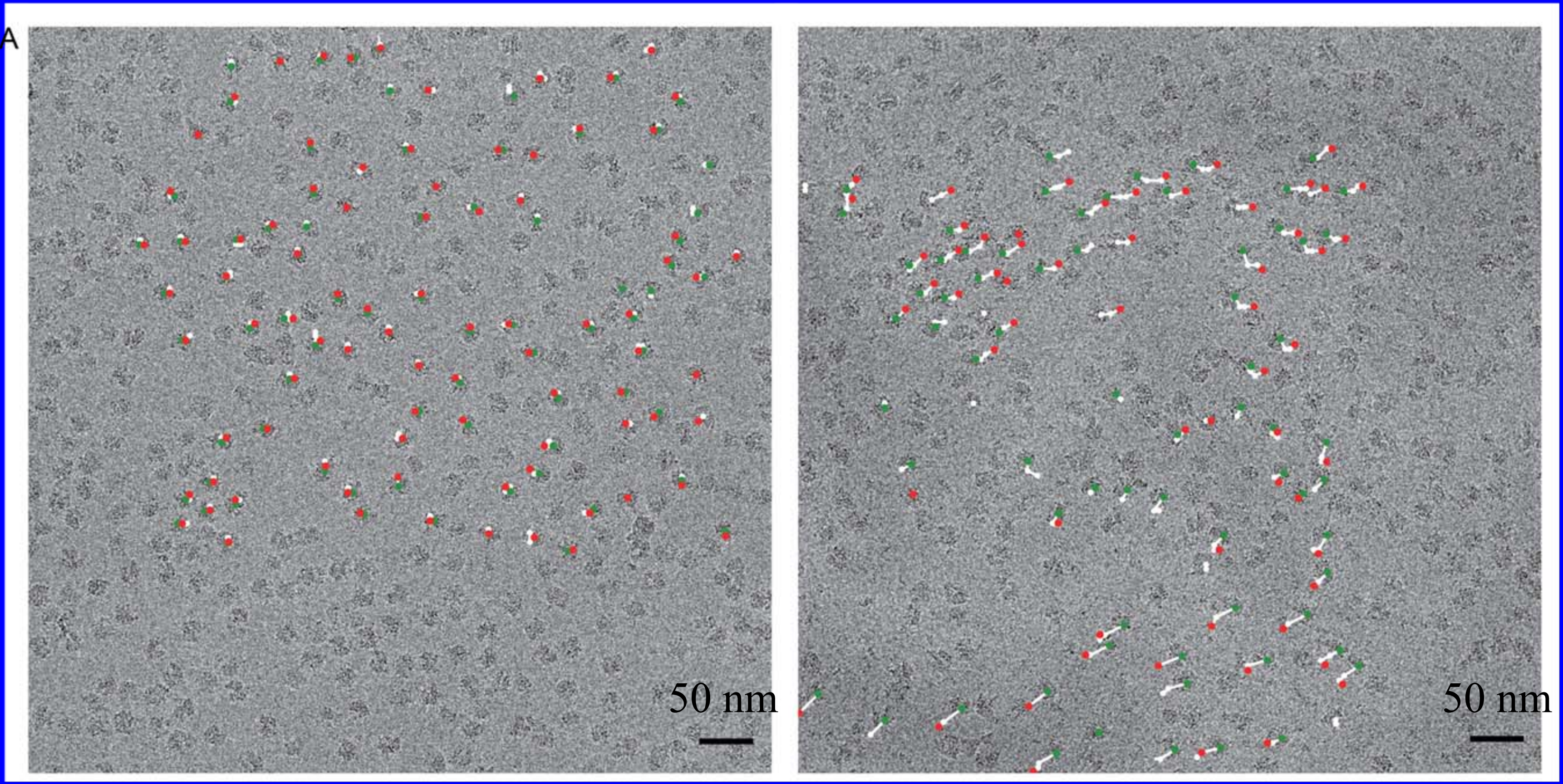
DOI:10.1002/anie.2001001436

Can resolution be improved by reducing the effects of beam-induced specimen movements?

Bai, et al have used the fast readout in a backthinned Falcon to divide an image (1 second) of ribosome into 16 frames. Next slide shows the specimen movement during exposure to electrons.

Beam-induced movements in 70S Ribosome (movie mode)

Average of 16 frames shown for two areas. Green circles are average positions from 4 frames at start of exposure and red circles are positions from last 4 frames. White lines show movements, which are exaggerated by 25 times for clarity (Bai, et al eLife).



Ribosome -Summary

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 improves SNR

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

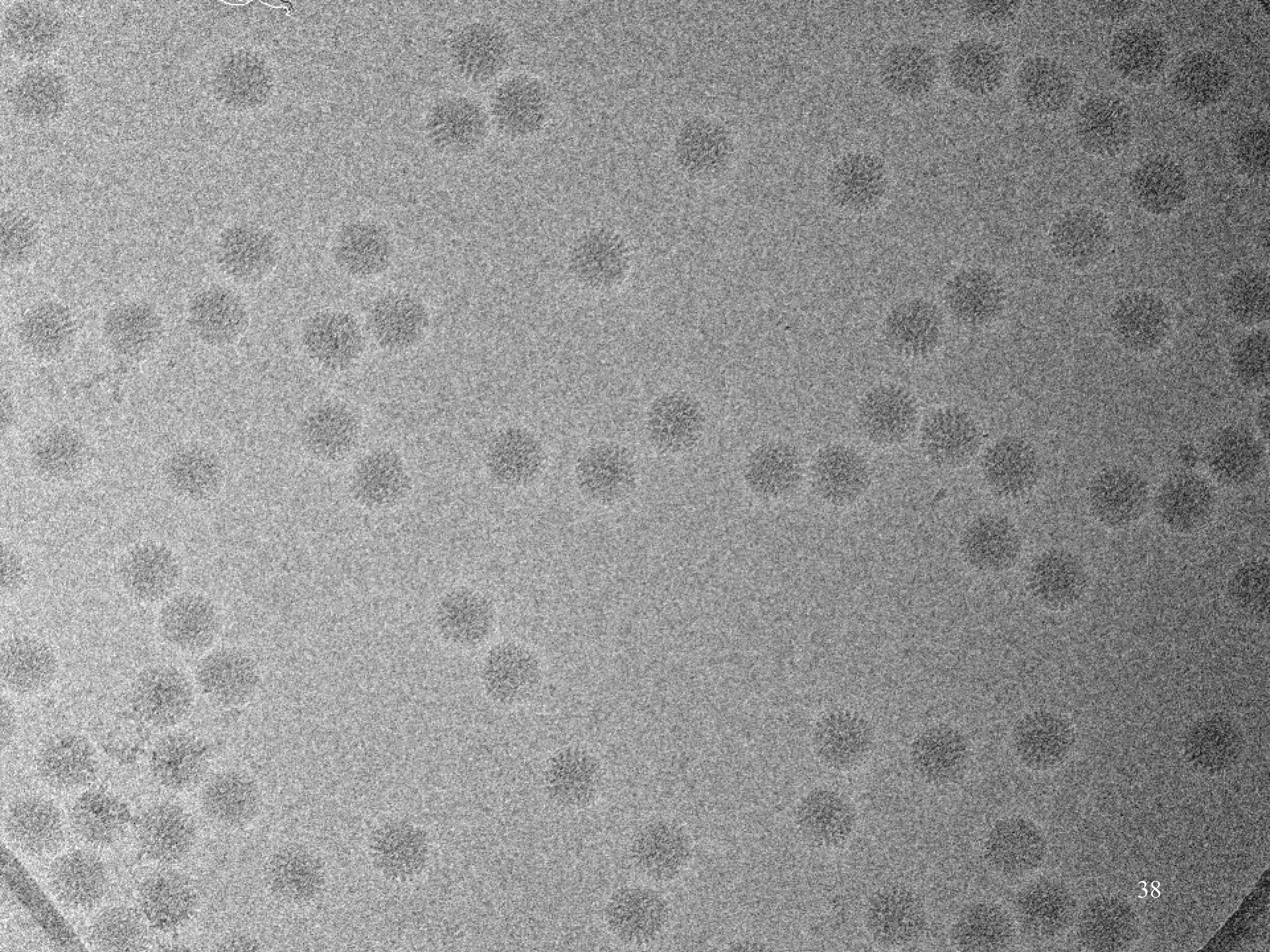
this is close to what has been achieved with x-ray crystallography

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/Å^2.
Total dose: 20 electron/Å^2. 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, Jue 2013
http://dx.doi.org/10.1016/j.ultramic.2013.06.004

Beta- galactosidase

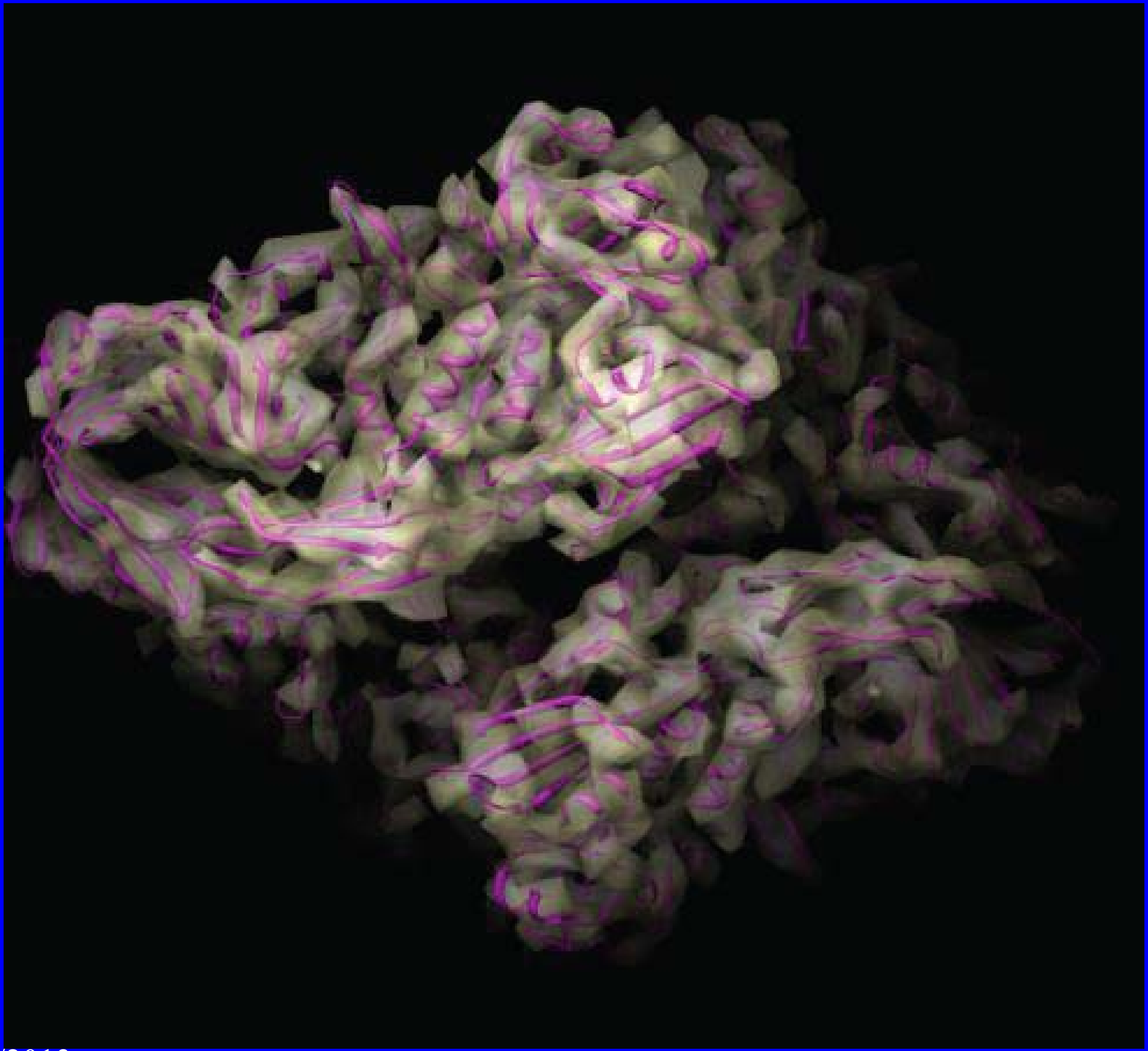
Three dimensional structure of beta-galoctosidase along with comparison with atomic model.
Higher DQE of backthinned Falcon II allows higher resolution data with fewer single particles.

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 2013 Ultramicroscopy

3D map of beta-galactosidase with atomic model superimposed



Chen, et al 2013

UNIVERSITY OF TWENTE.

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.

High DQE and the ability to conveniently collect large amounts of data should lead to routinely attaining atomic resolution, so far only possible using crystals.

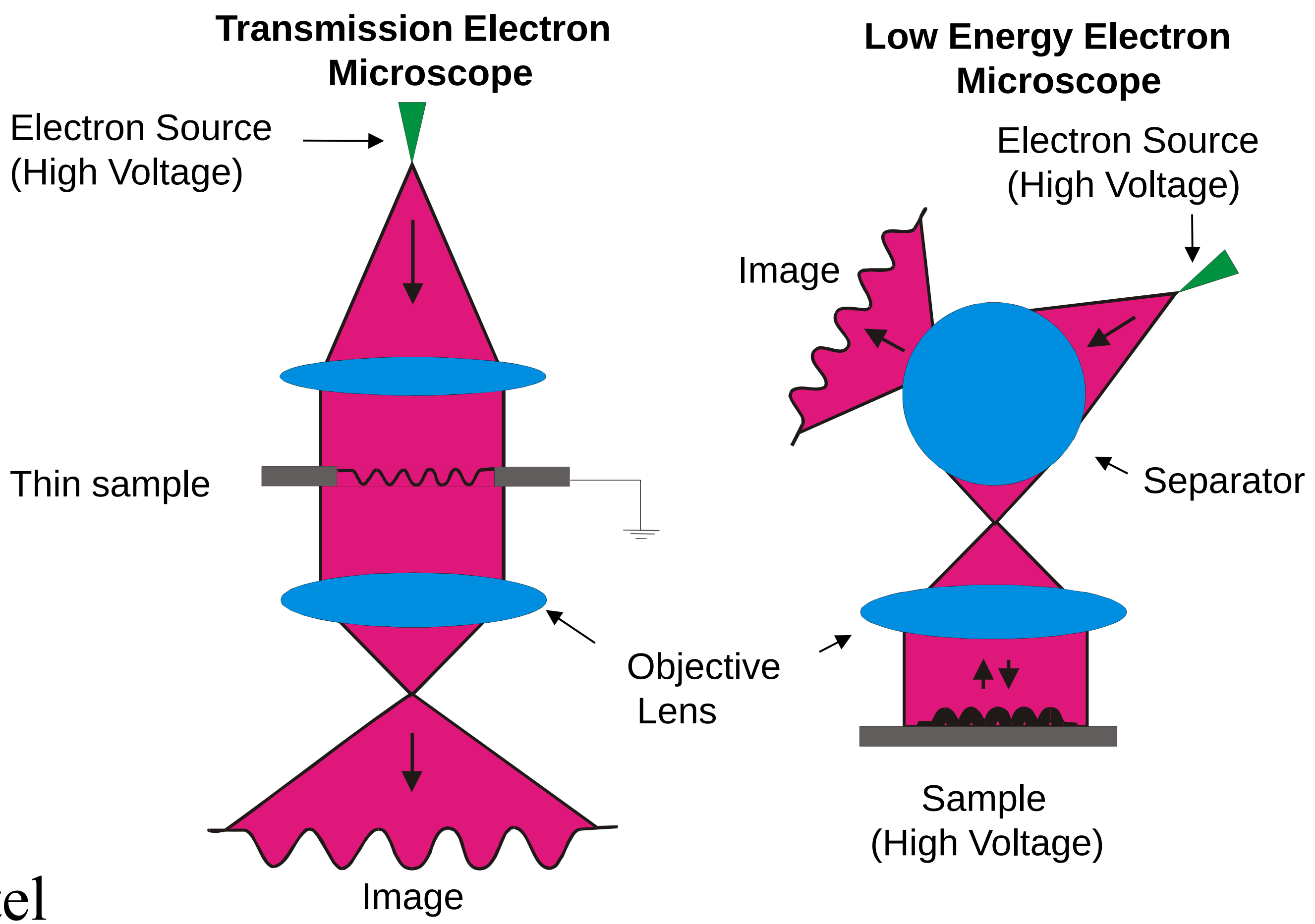
Even better performance than for backthinned sensors should be possible with electron counting –using fitted centroids to obtain sub-pixel resolution.

Low energy electron microscopy imaging using Medipix2 detector

Sikharulidze, R.van Gastel et al
Nucl. Instr. & Meth. A633, (2011), S239-S242

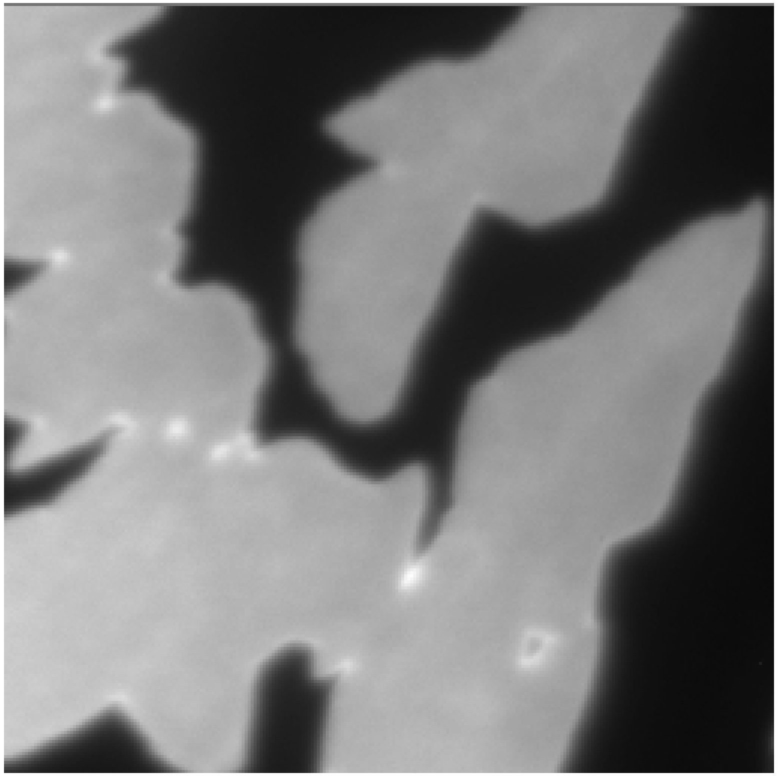
Surface imaging using low-energy backscattered electrons
Electrons decelerated to 0-100 eV near surface
Scattered electrons accelerated to ~20 keV and guided to detector
Much improved S/N in counting detectors cpd to CCD

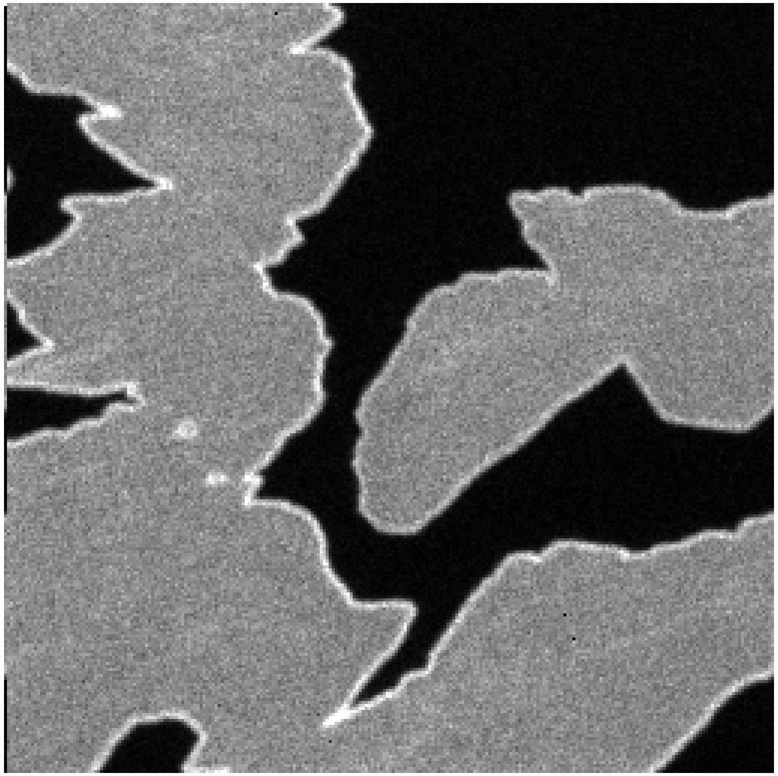
Comparison of LEEM to TEM



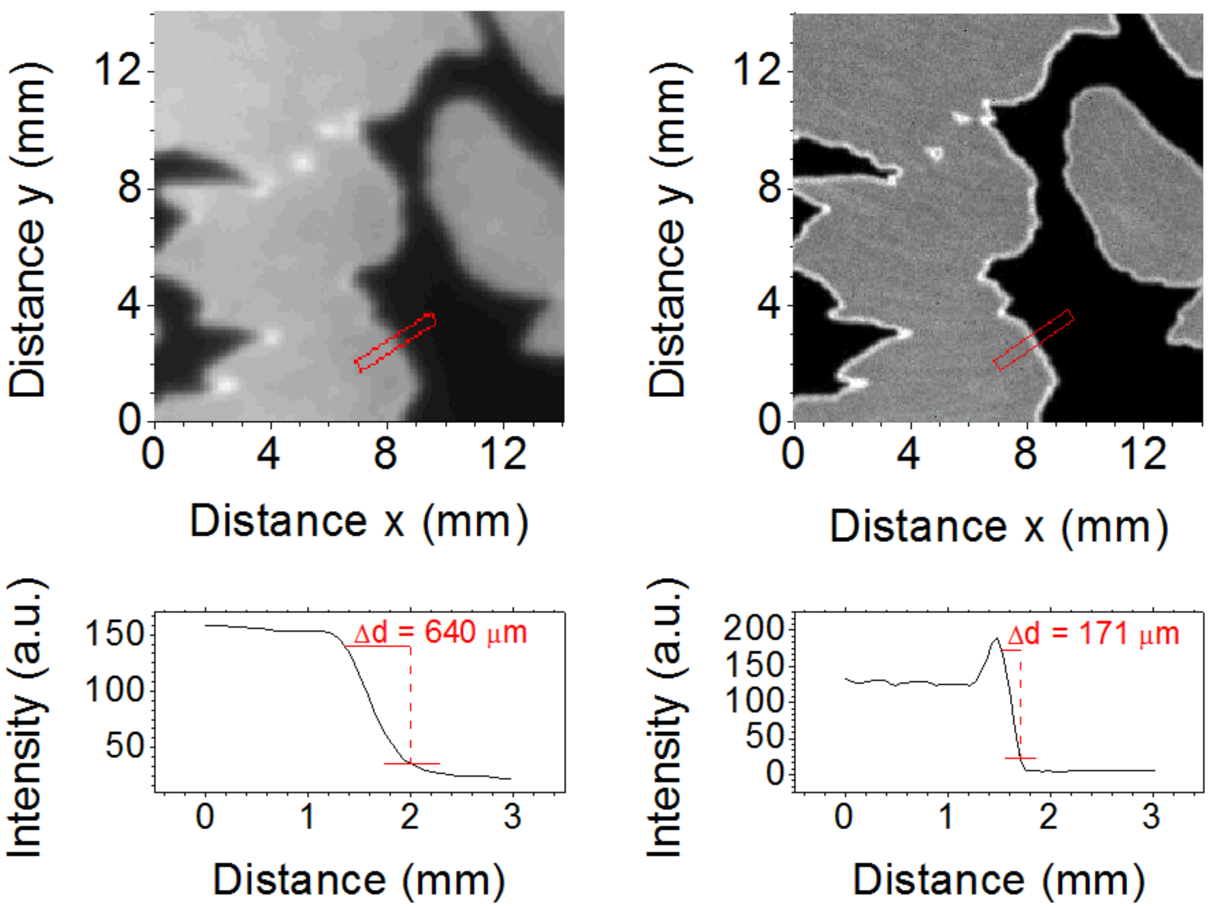
van Gastel

Improvement in LEEM resolution with Medipix2 compared to Microchannel Plate based detectors

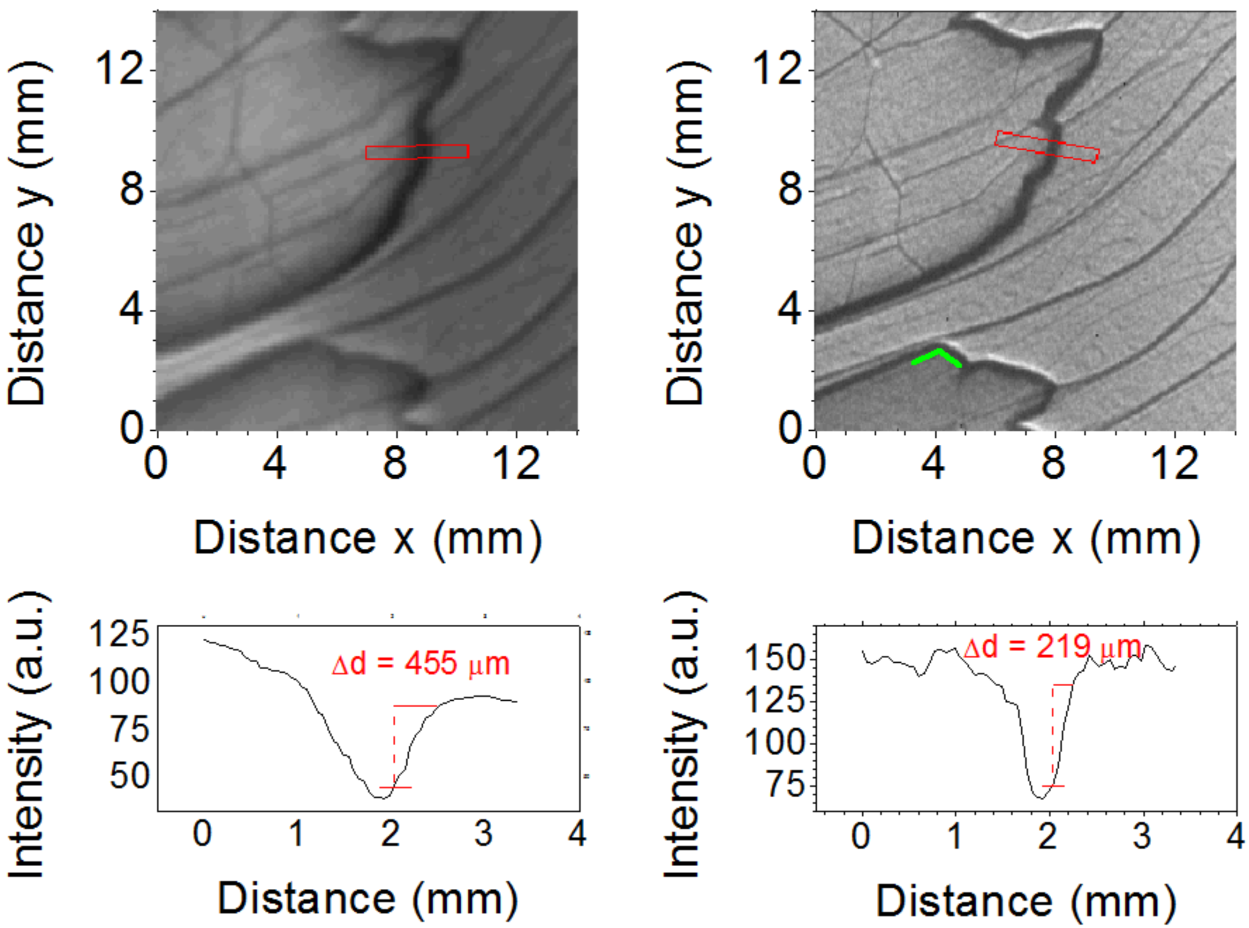




Enhanced edge resolution in PEEM using Medipix2 compared with Microchannel Plate based detectors



Enhanced edge resolution in LEEM



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