Issues – image formation & acquisition

1. Amplitude and phase contrast in cryoEM
2. Recording the image, detectors, MTF and DQE

We don’t detect all the electrons (half or less than half)

Those electrons which lose energy (inelastically scattered) are often badly focussed and contribute only noise

We don’t extract the optimum phase contrast signal.

- **Better detectors with higher DQE and MTF**
- **C_c correctors to focus electrons with a wider ΔE**
- **Quarter-wave plates to optimize phase contrast**

Ultramicroscopy (1988) 25, 279-292

CONTRAST TRANSFER FOR FROZEN-HYDRATED SPECIMENS: DETERMINATION FROM PAIRS OF DEFOCUSED IMAGES

Chikashi TOYOSHIMA *

Department of Cell Biology, Stanford University School of Medicine, Stanford, California 94305, USA

and

Nigel UNWIN

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Received 14 January 1988; received in revised form 30 March 1988

Electron imaging of frozen-hydrated biological molecules allows density maps to be obtained directly, without the need for fixatives or stains. The appearance of such maps may, however, be strongly influenced by the contrast transfer properties, which have not previously been evaluated by quantitative experiments. Here we determine the contribution due to amplitude contrast in a typical (~300 Å thick) frozen specimen, consisting of arrays of acetylcholine receptor, by comparing pairs of images recorded with different defocuses. We find that this specimen is imaged as a "weak-phase–weak-amplitude" object and that the contribution due to amplitude contrast is 7%. **(at 120kV)**

See also Toyoshima et al, Ultramicroscopy (1993) 48, 165-176

120kV – 5.8%

200kV – 4.8%

Toyoshima & Unwin, Ultramicroscopy 1988

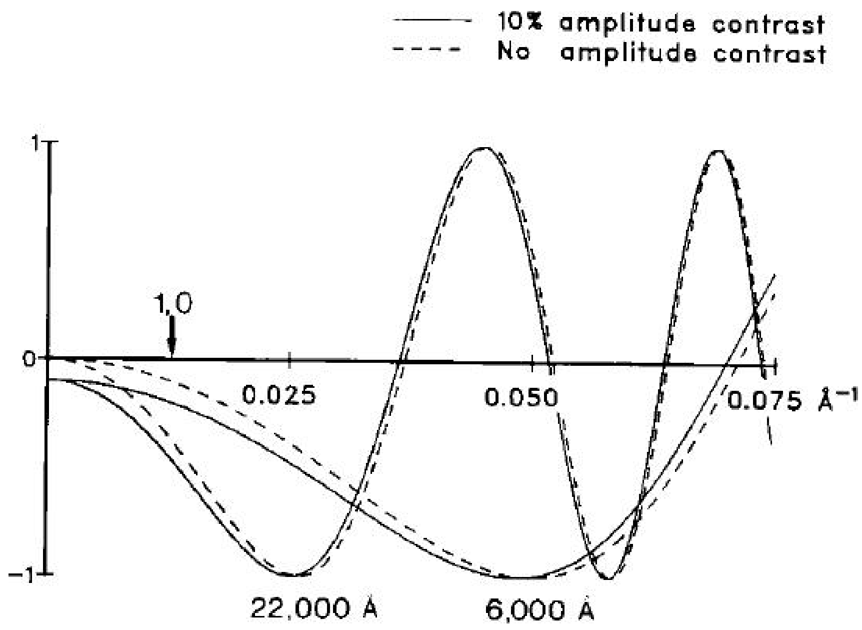


Fig. 1. Theoretical CTFs, $C(\nu)$, for 6000 Å and 22000 Å underfocus, assuming pure phase contrast (broken lines) or 10% amplitude contrast (solid lines). By comparing the ratio of the CTFs at a given spatial frequency, it is possible to estimate the proportion of amplitude contrast. For example, at a spatial

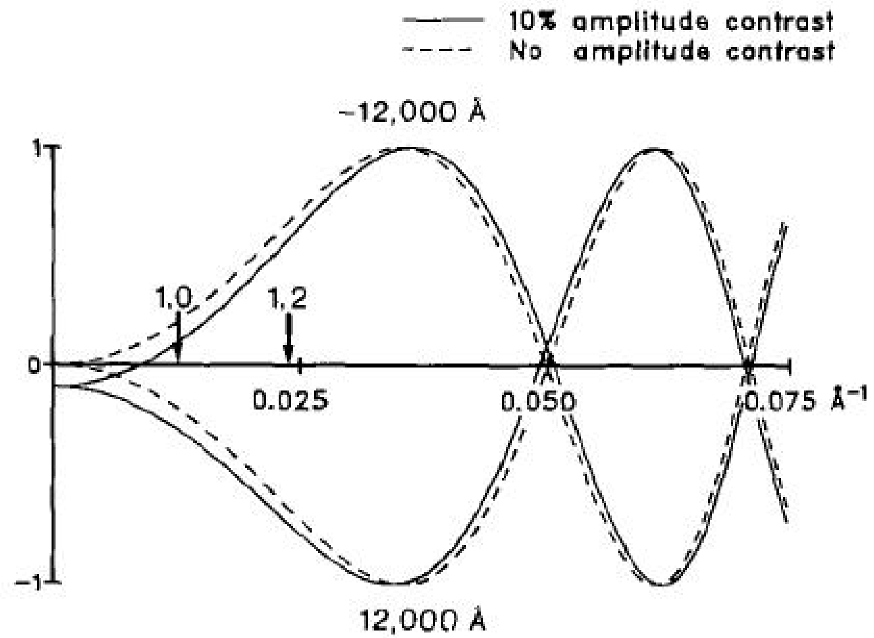


Fig. 6. Theoretical CTFs for the defocus conditions realized in fig. 7 (±12000 Å defocus). Solid and broken lines correspond to 10% and 0% amplitude contrast, respectively. The spatial frequencies corresponding to the (1,0) and (1,2) reflections are indicated; at these low spatial frequencies the phase and amplitude contrast contributions are of opposite sign and therefore tend to cancel when the image is overfocused, but are of the same sign and reinforce one another when the image is underfocused. Thus at low spatial frequencies comparison of underfocused–overfocused pairs of images provides a sensitive means of measuring the amplitude contrast contribution.

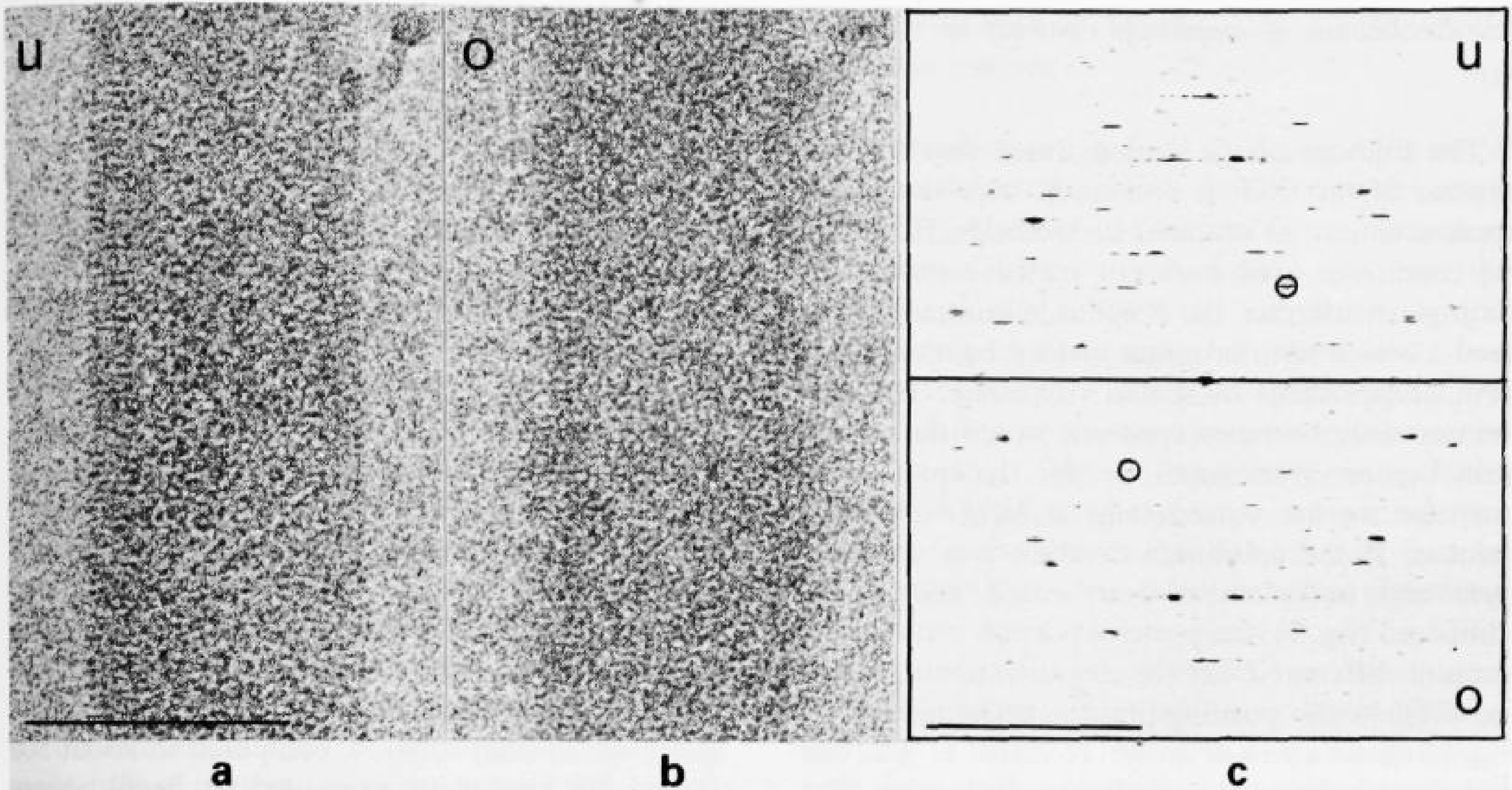


Fig. 7. Images of an ice-embedded tube recorded at 12000 Å underfocus (a) and 12000 Å overfocus (b), and a composite of their diffraction patterns (c). The (1,0) reflections from one side of the tube are marked. U and O denote under- and overfocus, respectively. Note that lower resolution reflections appear much weaker in the diffraction pattern from the overfocused image (lower half, (c)) than in the underfocused one (upper half, (c)), due to a partial cancellation of phase and amplitude contrast. The overfocused image was recorded after the underfocused image so that the effect cannot be due to radiation damage. Bars correspond to 0.1 μm (a) and 1/50 $Å^{-1}$ (b).

Understanding images of unstained biological molecules

100 keV electron scattering factors (IAM)

	|f| per atom	η
H	0.63	0.008
C	2.90	0.037
N	2.57	0.045
O	2.34	0.052
S	6.08	0.08
U	22	0.22

Some problems in understanding images of unstained biological molecules

100 keV electron scattering factors (IAM)

	$\lvert f \rvert$ per atom	η
H	0.63	0.008
C	2.90	0.037
N	2.57	0.045
O	2.34	0.052
S	6.08	0.08
U	22	0.22

Embedding in ice

Ratio of amplitude to phase contrast

$$\eta_{(p-w)} = (f_p \eta_p - f_w \eta_w)/(f_p - f_w)$$

$$= \eta_p + (f_w/(f_p - f_w)).(\eta_p - \eta_w)$$

M.W.		|f|/mol or /res	moles /Å^3	|f|/Å3	η	estimated “fudge” from bonding		density used (g/cc)
18	**H_2O (ice)**	3.6	0.033	0.12	0.036	0.13	0.03	1.0
108.5	**protein (lo dose)**	25.7	0.008	0.198	0.035	0.18	0.04	1.4
	protein (hi dose)	~13	~0.008	~0.10	~0.04	~0.10	~0.04	0.7
376	**UA (lo dose)**	48.7	0.0048	0.23	0.12	0.22	0.14	3.0
~300	**UA (hi dose)**	~35	~0.006	~0.21	~0.17	~0.21	~0.17	3.0
	UA - prot (lo dose)	-	-	0.032	0.64	0.05	0.48	-
	UA - prot (hi dose)	-	-	~0.11	~0.27	~0.11	~0.27	-
	prot - ice	-	-	0.08	0.035	0.05	0.066	

Note: for high dose images of negatively stained specimens, a 50% mass loss of organic matter has been assumed compared with low does images.

Detectors for Electron Microscopy

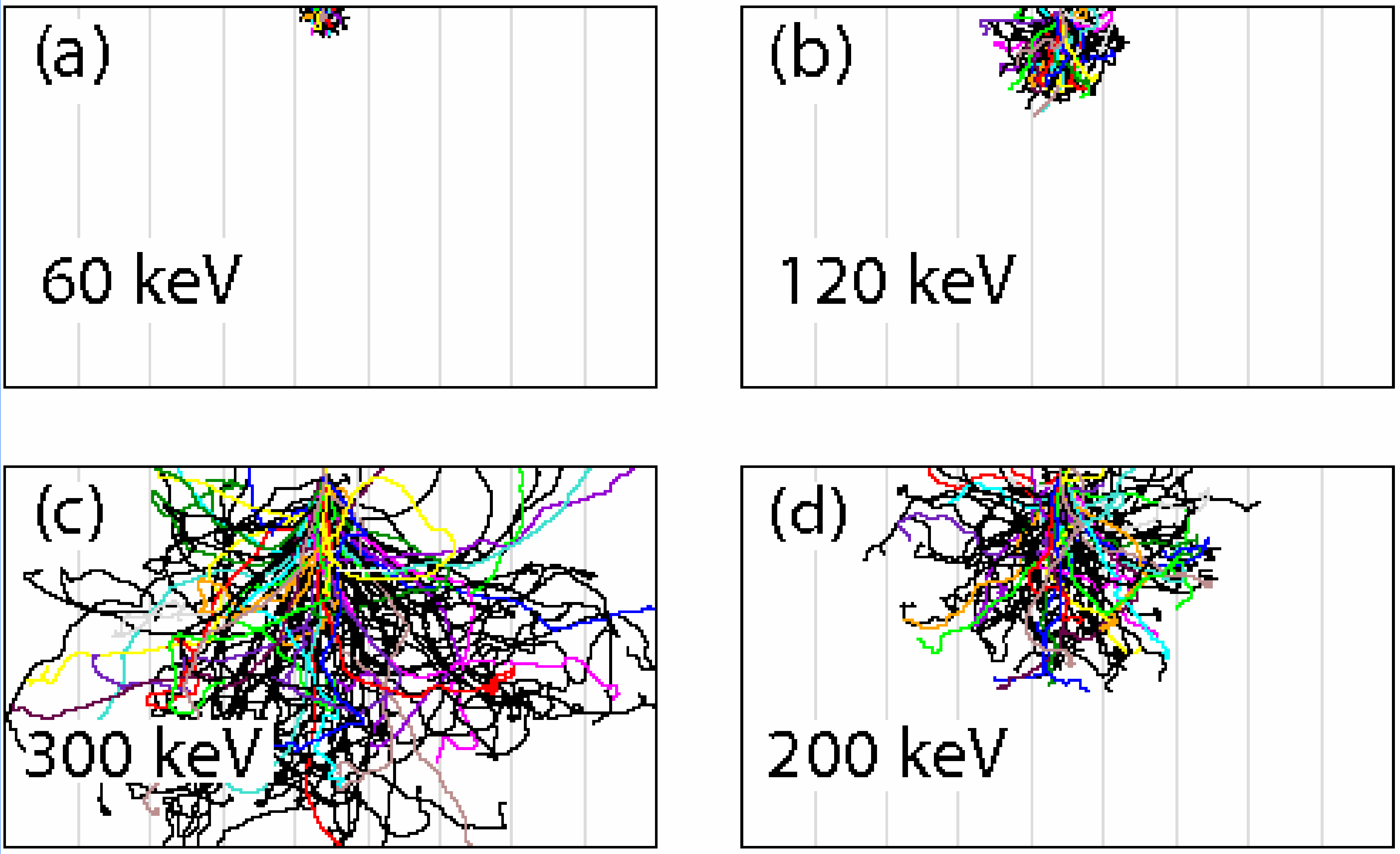
Film (SO-163)
Phosphor/Fibre Optics/cooled CCD
Phosphor/Lens/cooled CCD

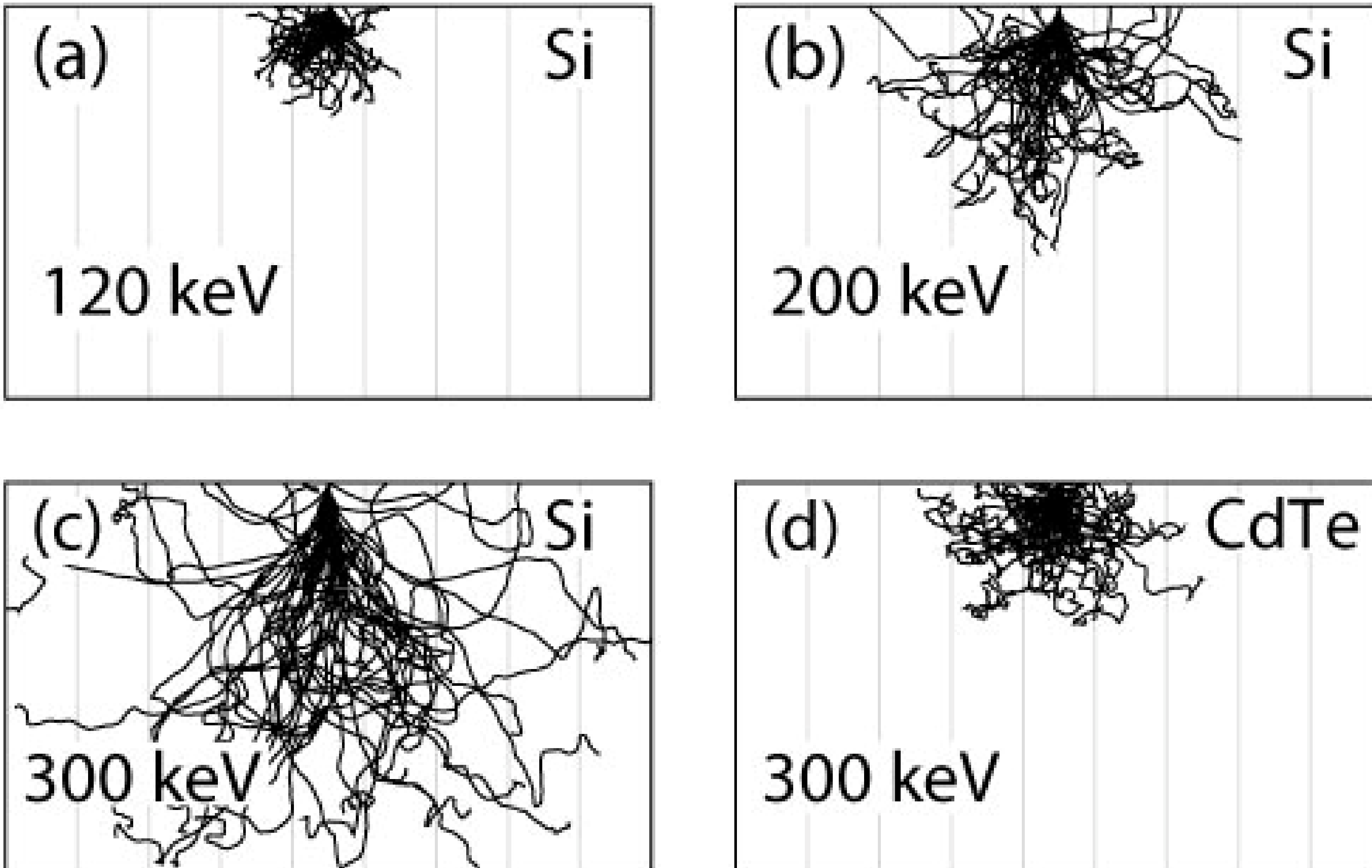
Hybrid Pixel Detectors (Medipix)

Monolithic Active Pixel Sensors (MAPS/CMOS)

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

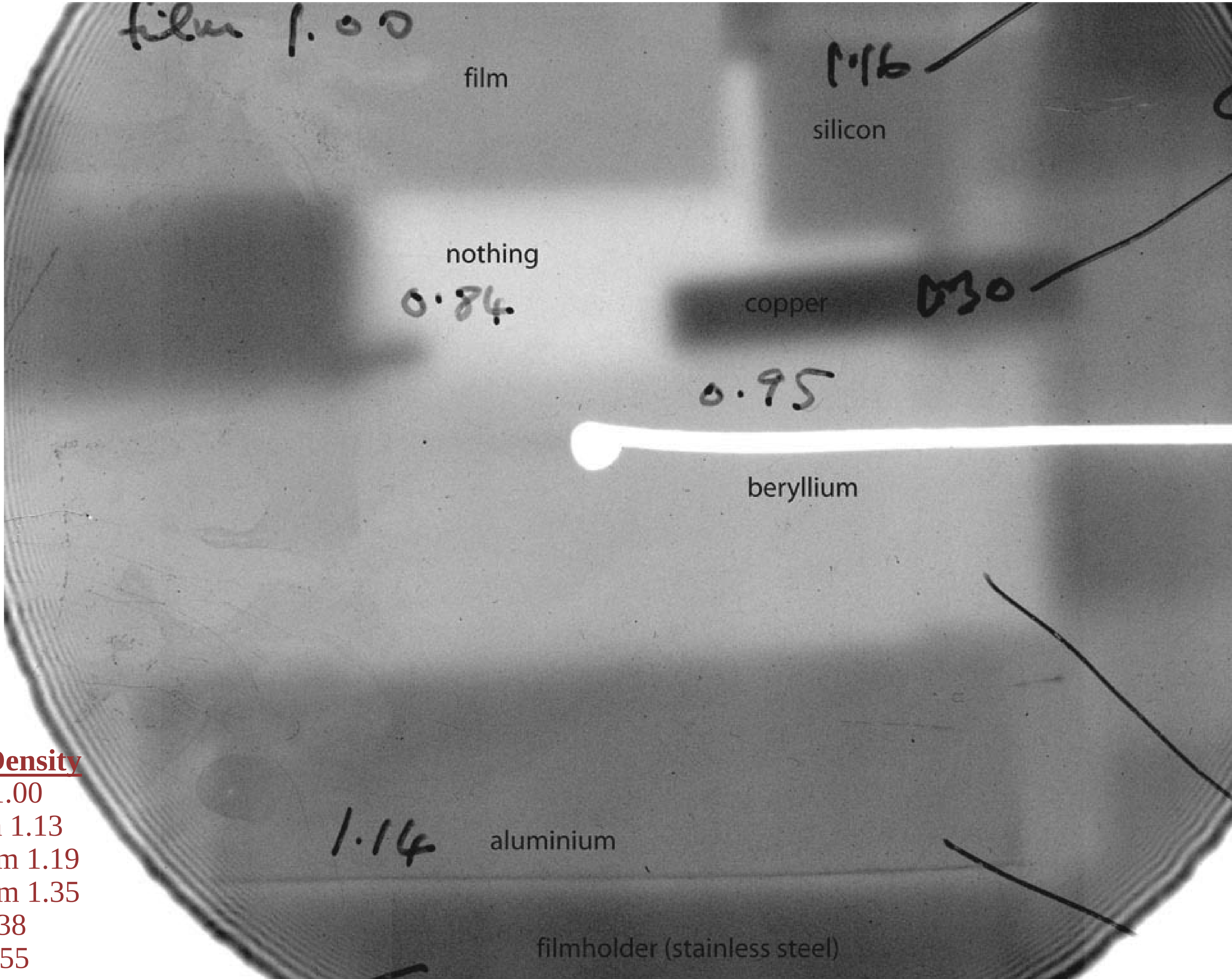
Extension of simulations to include energy deposition (GM)





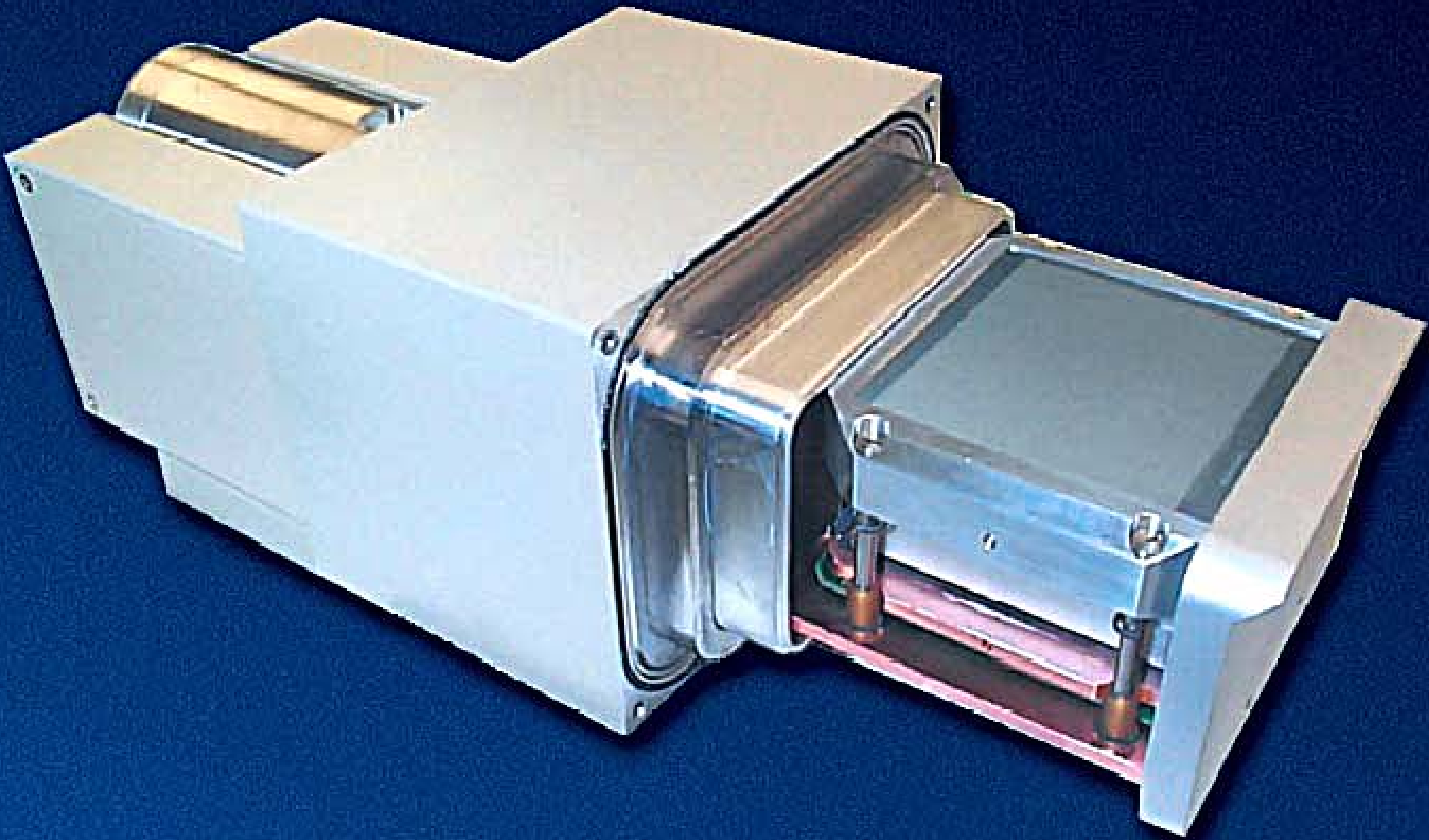
(a)
Si
120 keV
(b)
Si
200 keV
(c)
Si
300 keV
(d)
CdTe
300 keV

Backscattering test – film loaded upside down above test materials

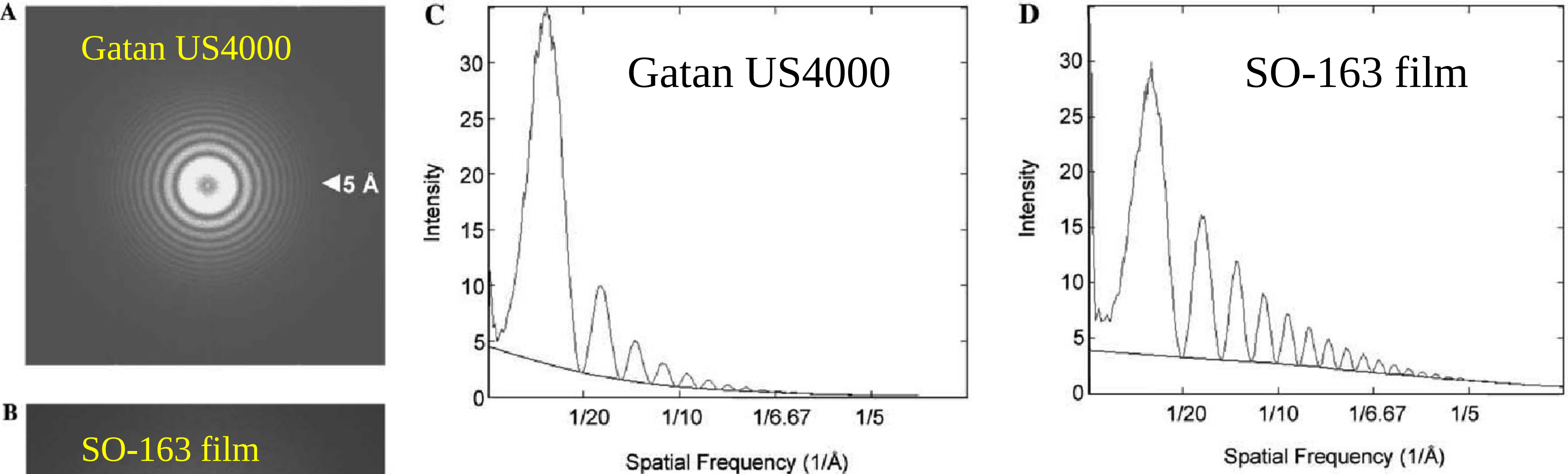


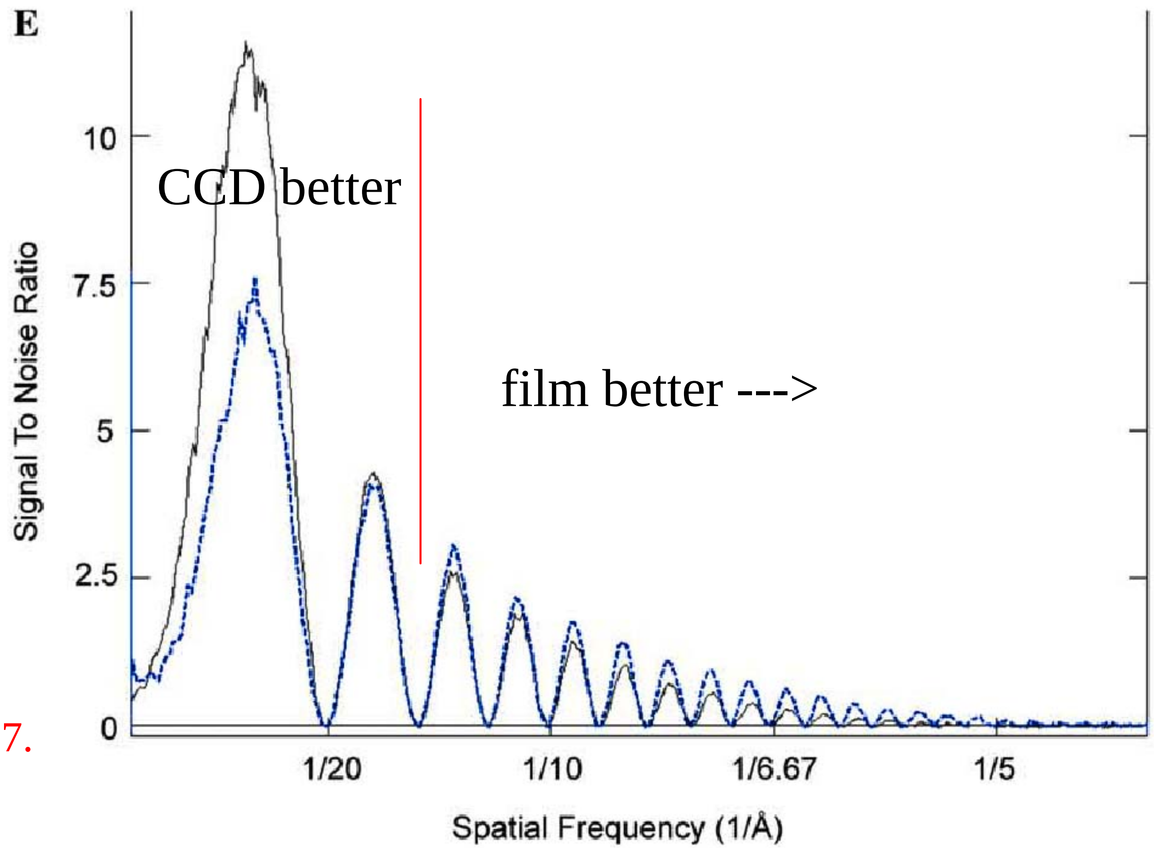
Optical Density

Vacuum 1.00
Beryllium 1.13
Plastic film 1.19
Aluminium 1.35
Silicon 1.38
Copper 1.55



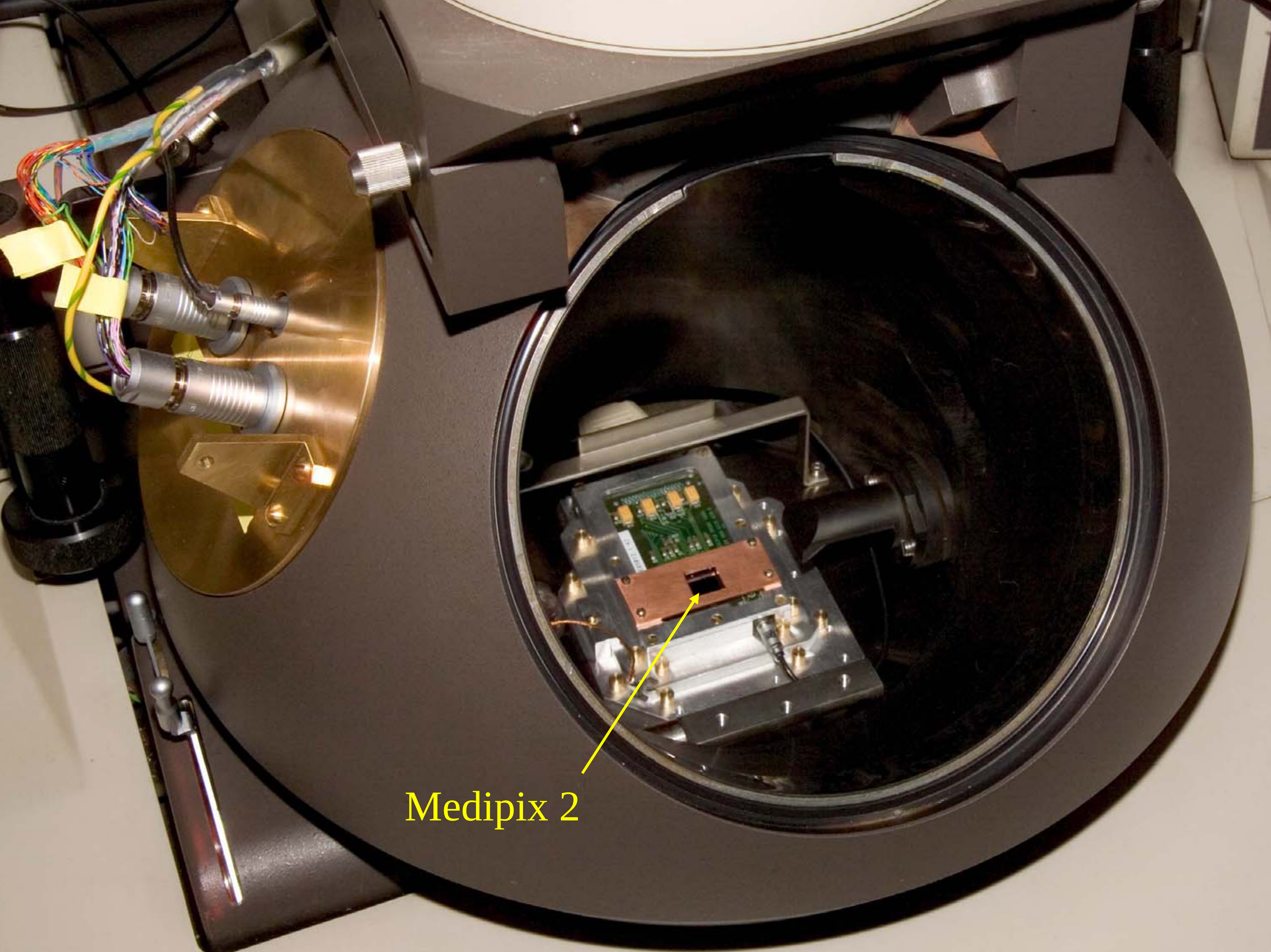
Gatan Ultrascan 4000

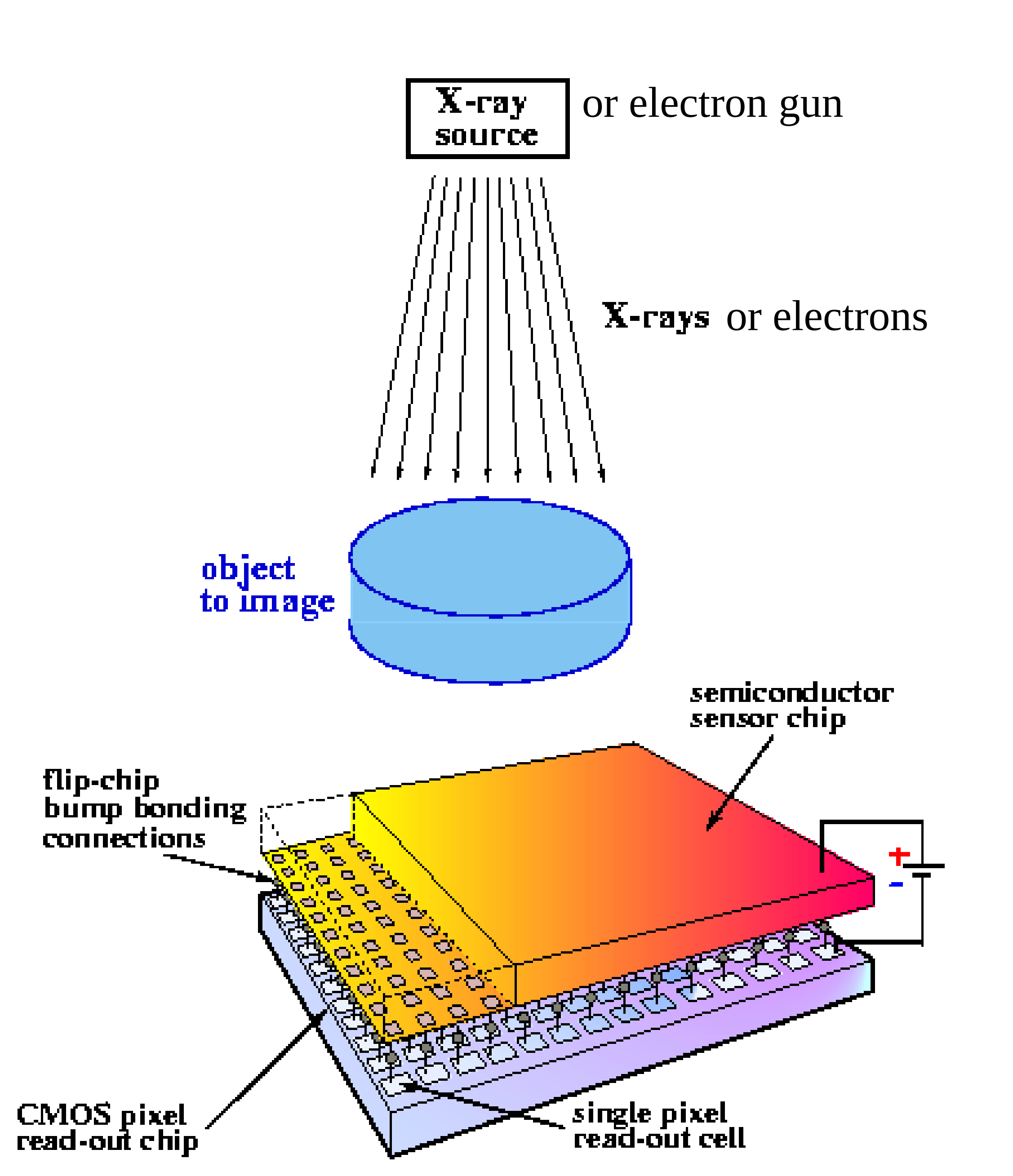




Gatan US4000 CCD camera
versus Kodak SO-163 film
for 200keV electrons

from
Booth et al (2004) JSB, **147**, 116-127.


Medipix 2

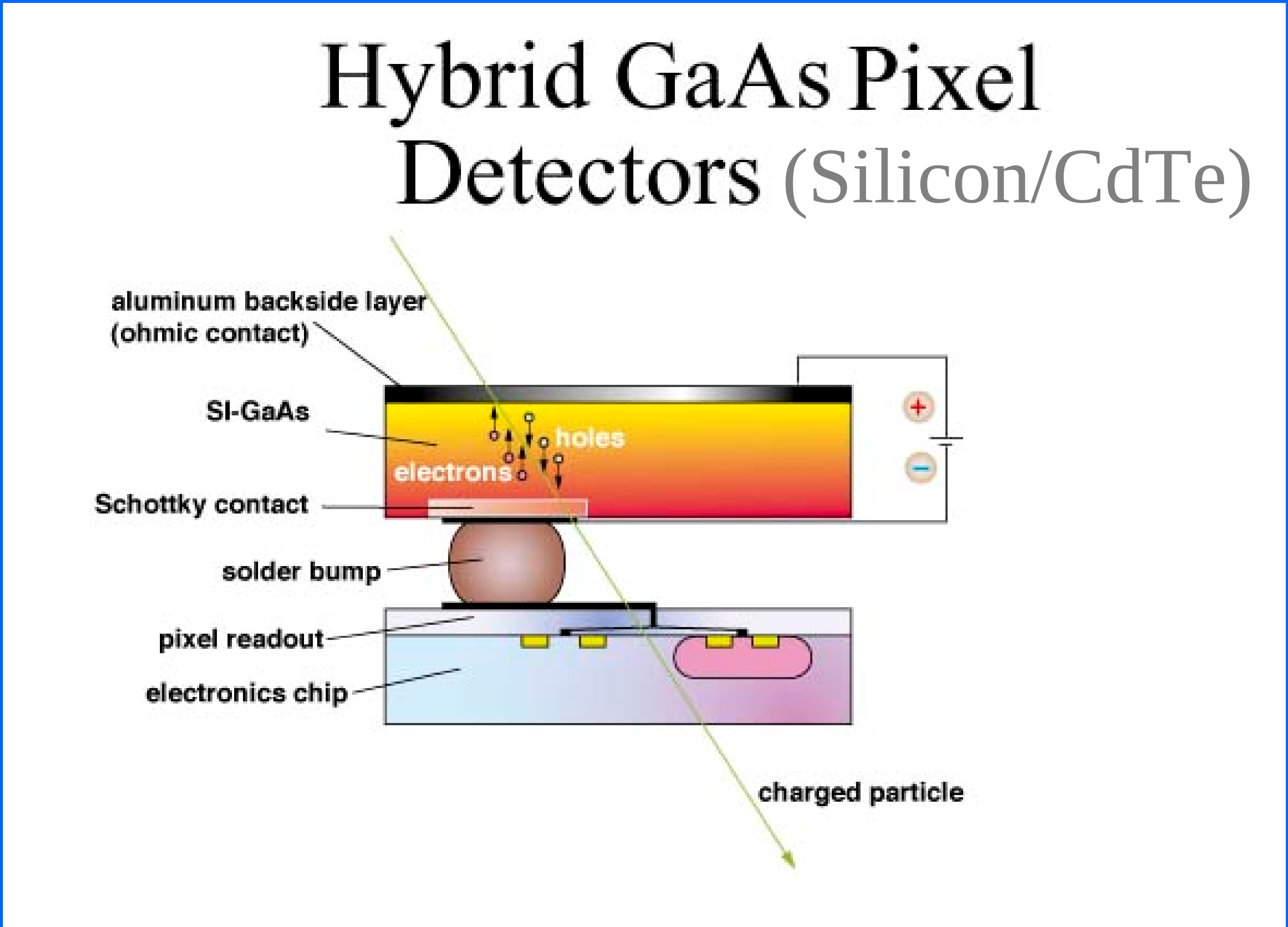


MediPix 2

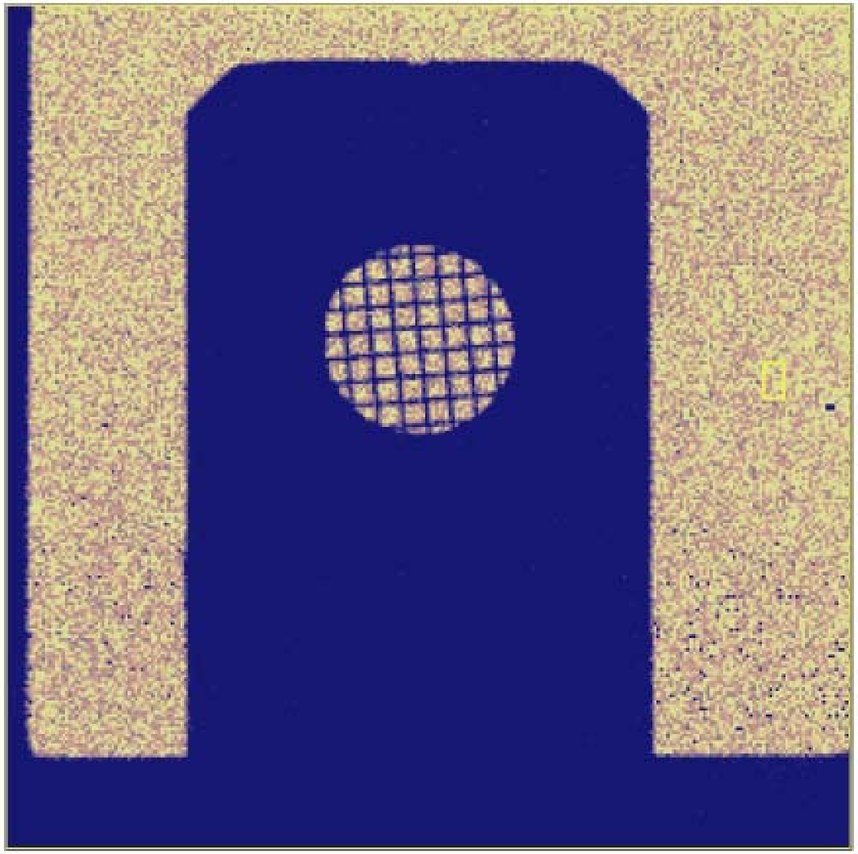
hybrid pixel sensor

Campbell et al at CERN

Single pixel schematic



Medipix 2 - grid shadow



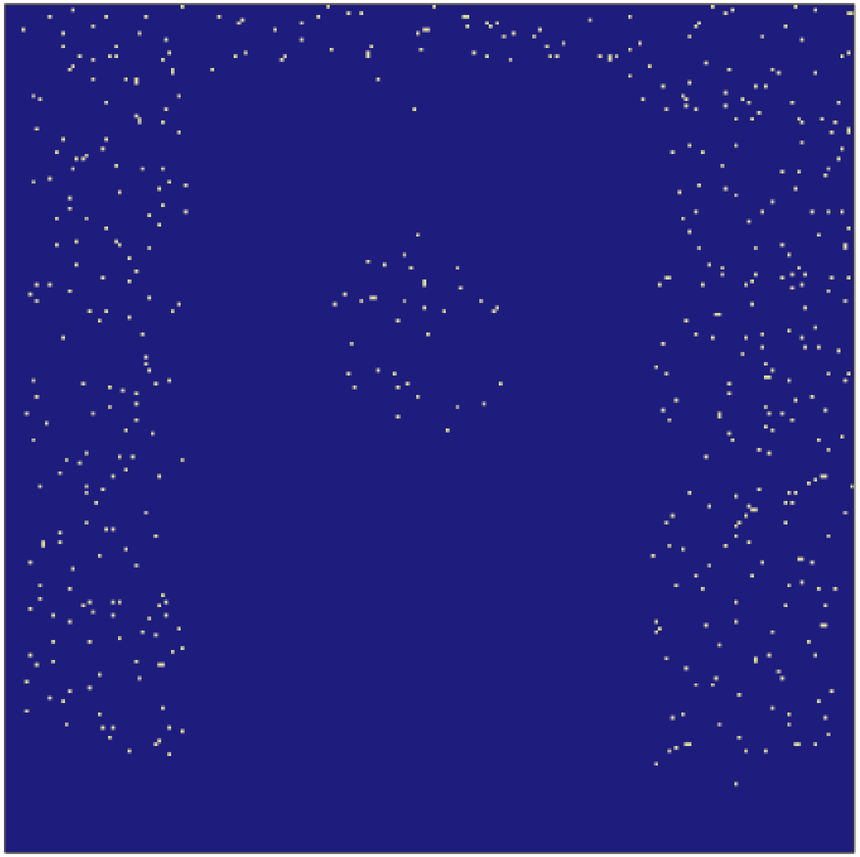


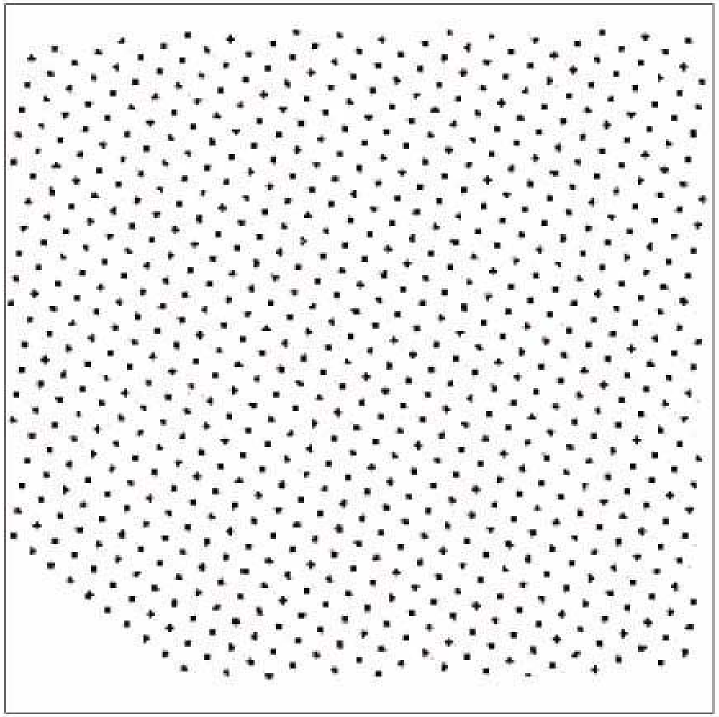
Image of grid with **18 electrons/pixel**

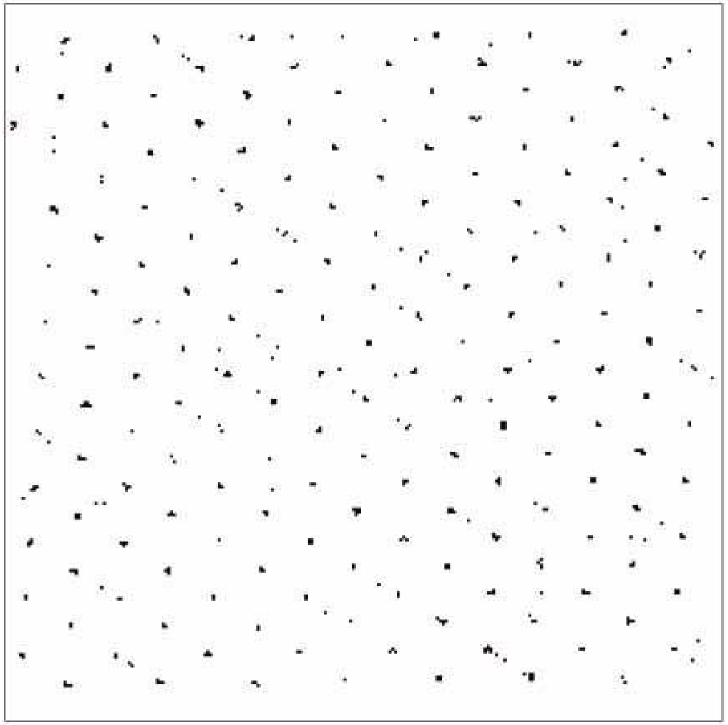
No counts are recorded in areas not exposed to incident electrons

Image with **< 0.01 electron/pixel**

Electrons are almost all recorded in one pixel

Medipix 2 - raster of spots





Mean Intensity	111
Std Deviation	11

Mean Intensity	4.7
Std Deviation	1.8

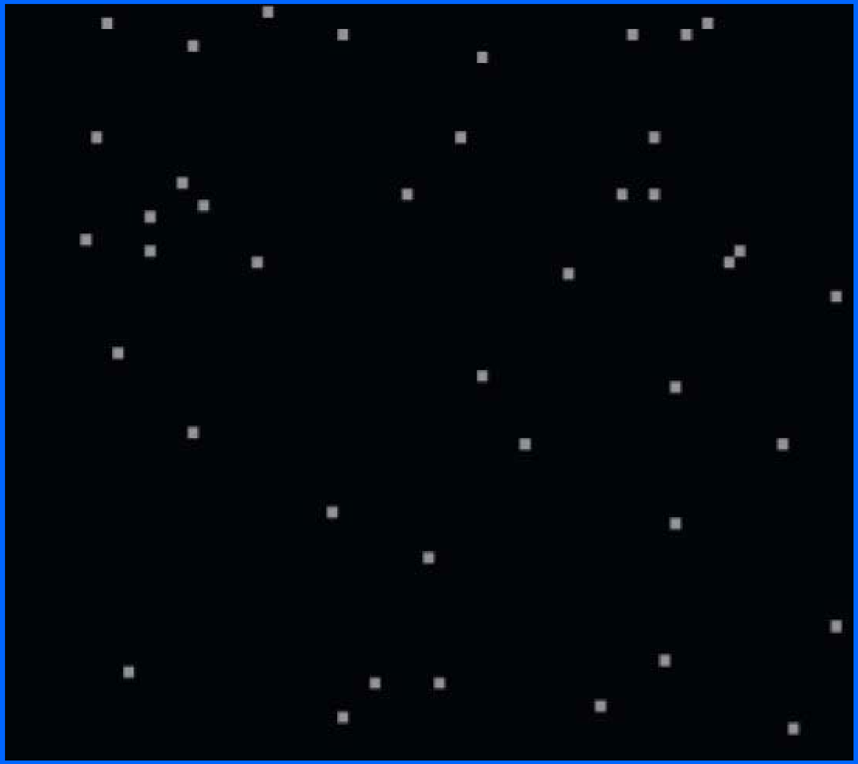
Film exposure equivalent to above

Mean Intensity 116

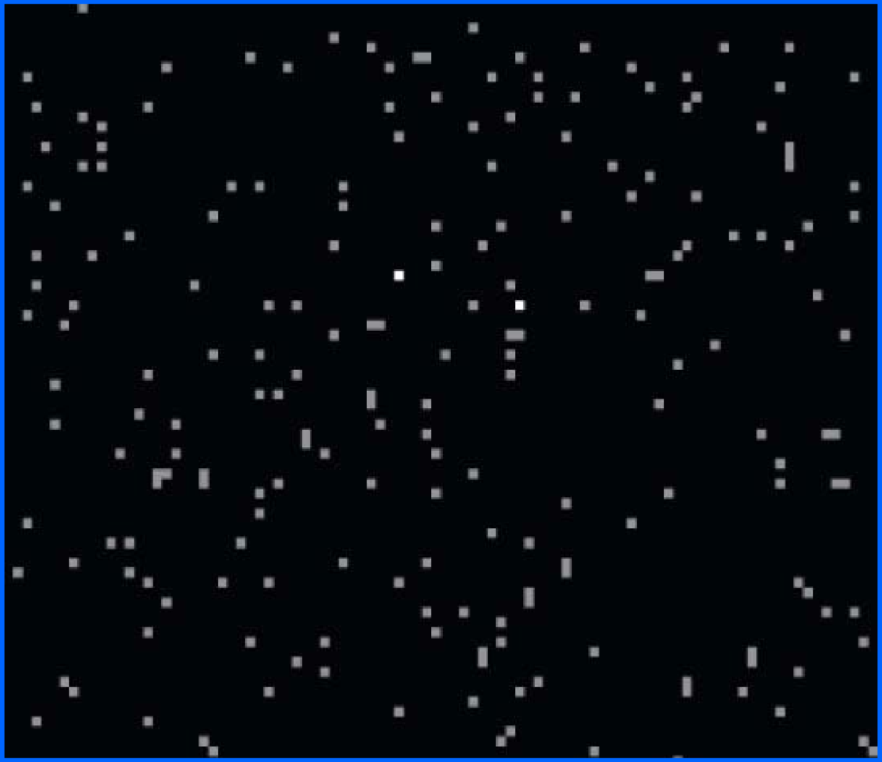
Std Deviation 24

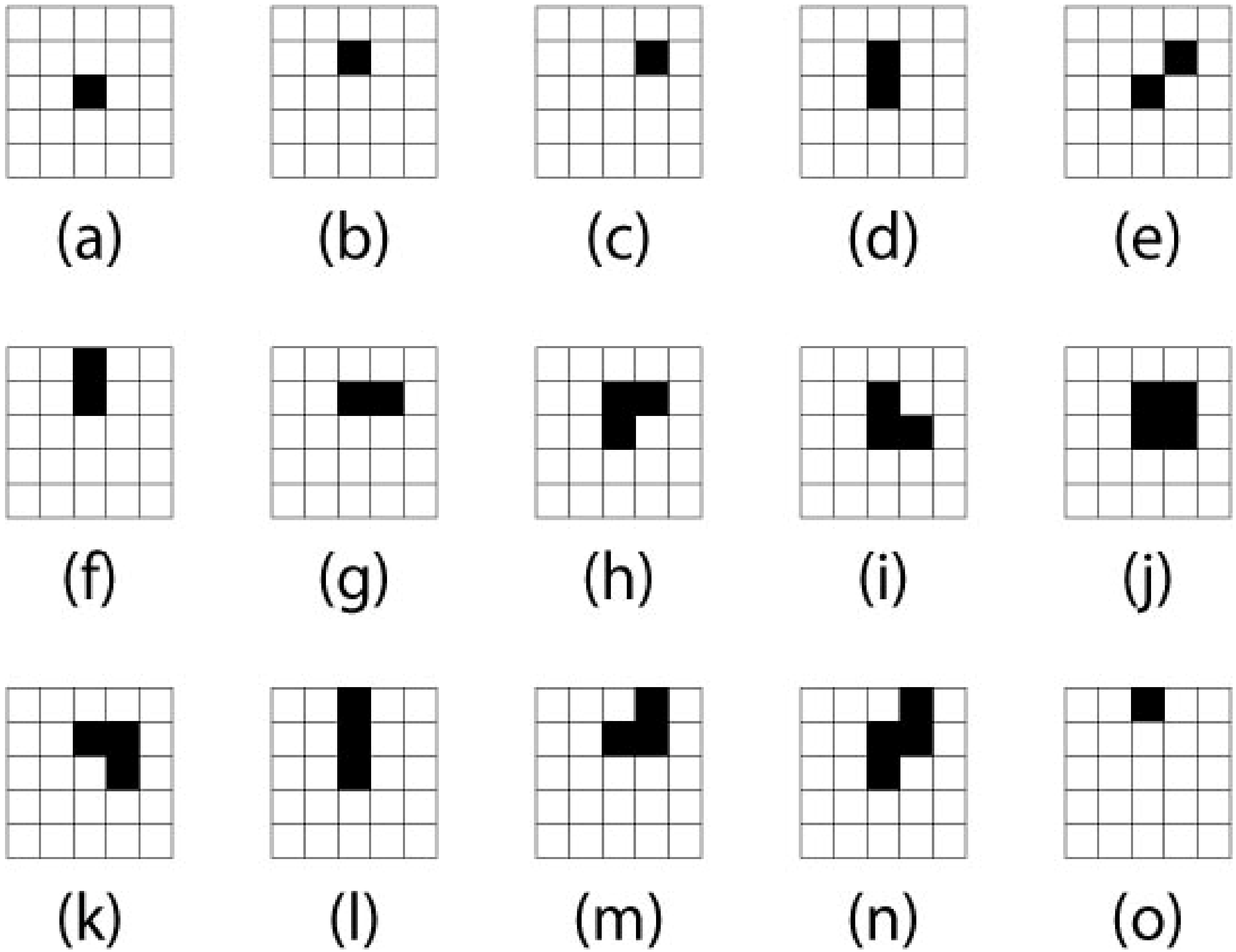
spots not visible, unmeasurable

120keV electrons/45keV threshold



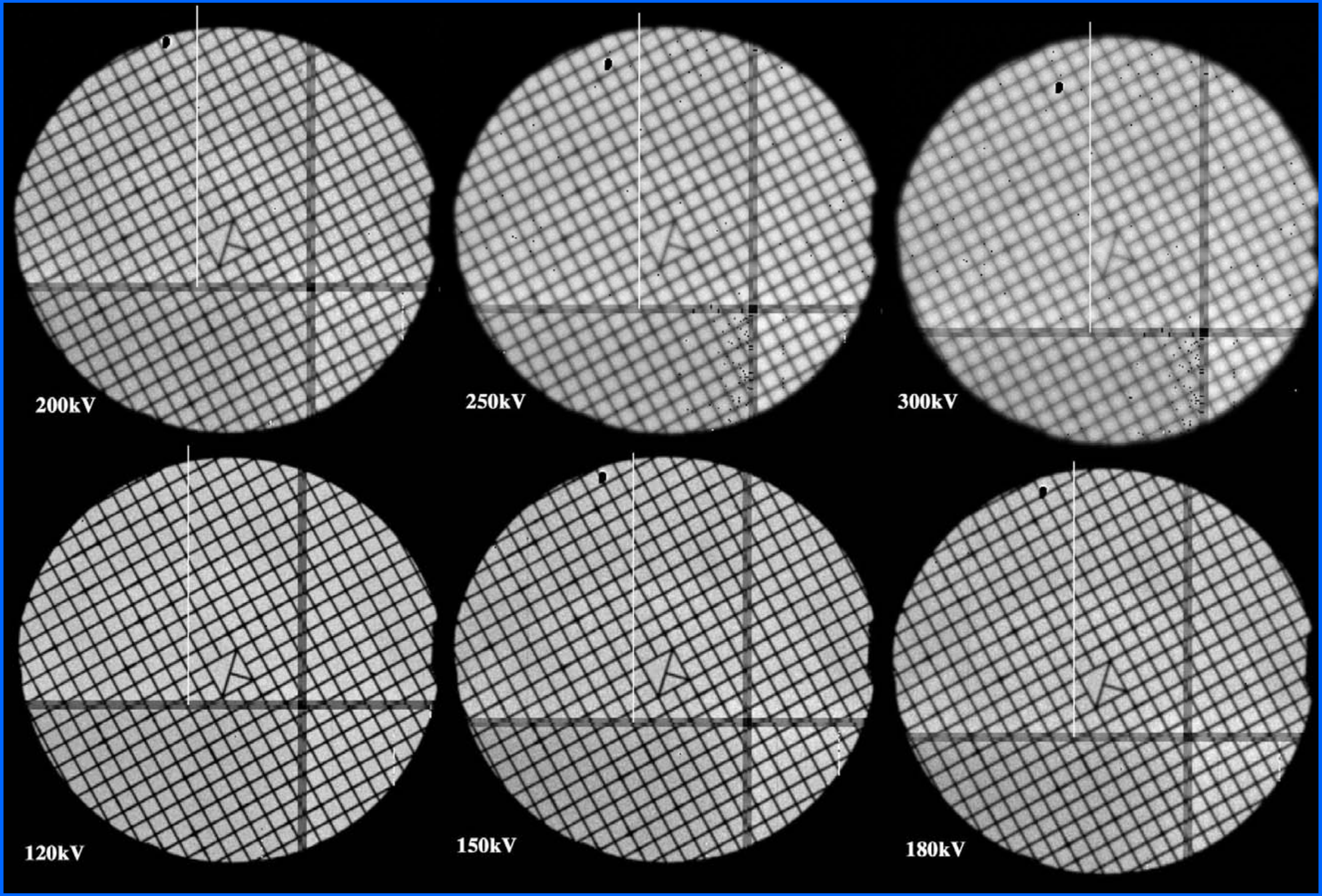
300keV electrons/80keV threshold



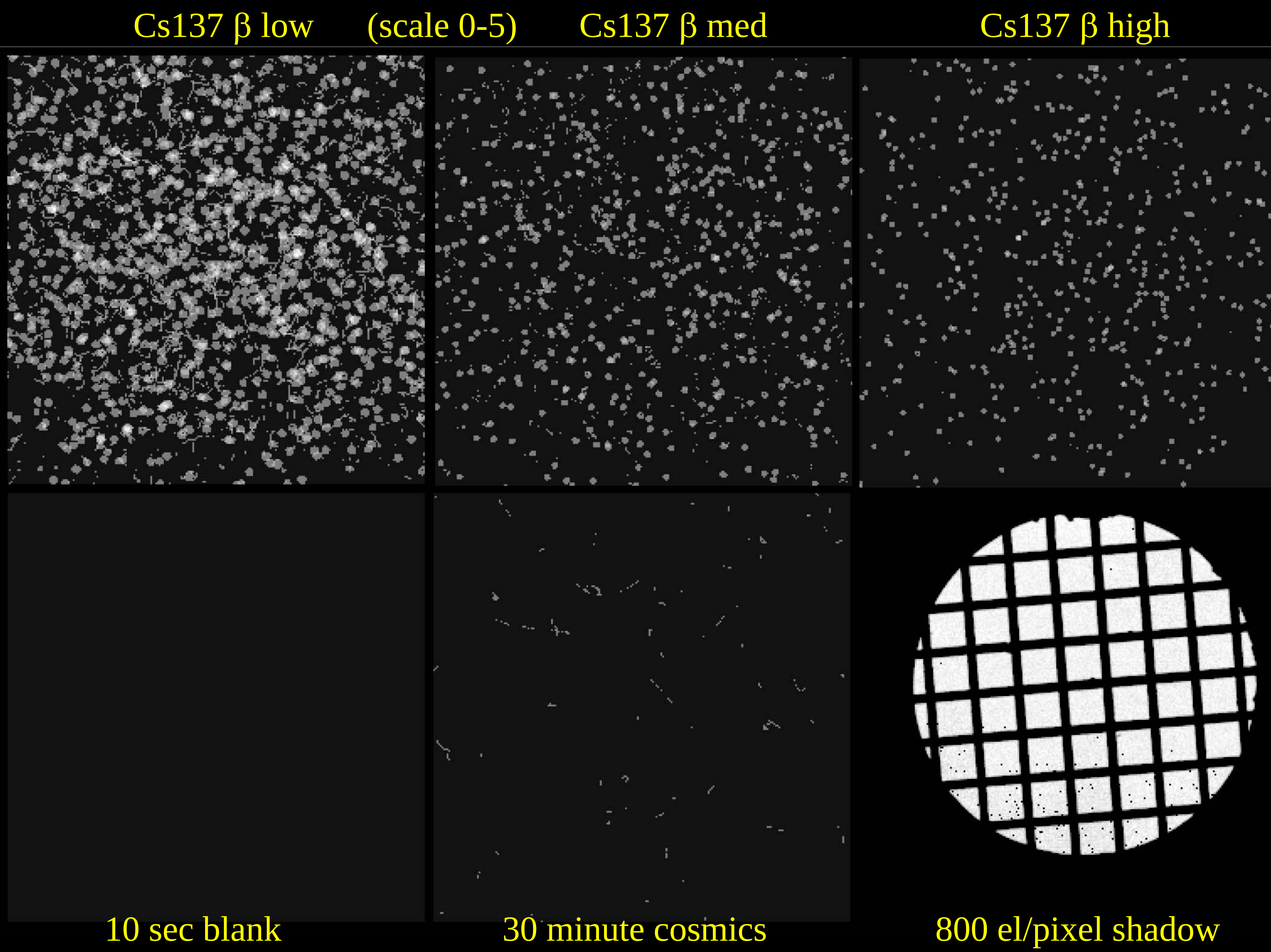


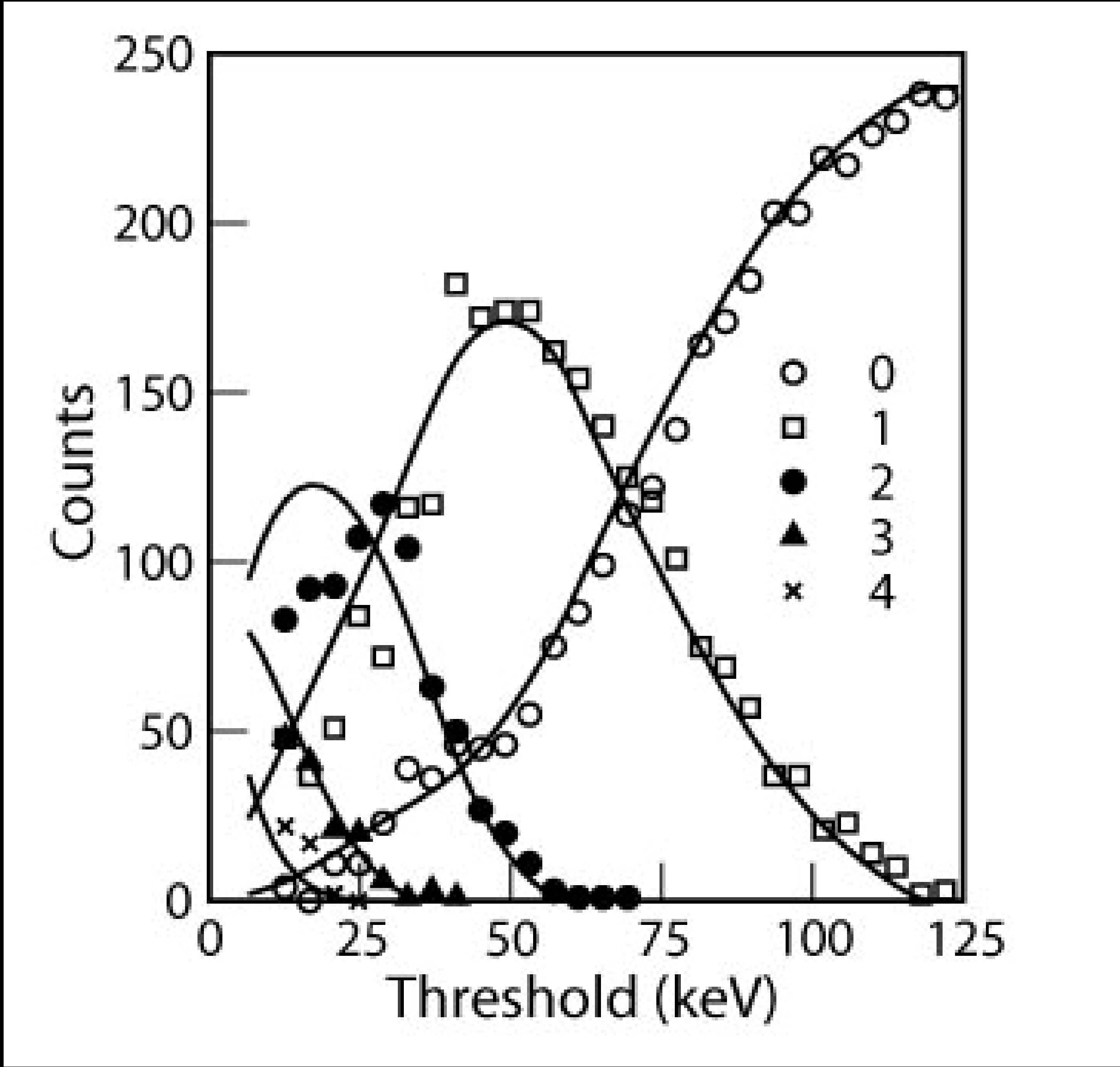
120keV electrons; a-c ΔE=80keV, a-g 40keV, a-j 20keV, a-n >0.5%, o - rare

Resolution of Quad_Medipix2 from 120 – 300 keV



300 mesh grid, spacing ~650 microns in image


Cs137 β low
(scale 0-5)
Cs137 β med
Cs137 β high
10 sec blank
30 minute cosmics
800 el/pixel shadow


Counts
250
200
150
100
50
0
0
25
50
75
100
125
Threshold (keV)
0
1
2
3
4

McMullan et al, Ultramicroscopy (2007) 107, 401-413)

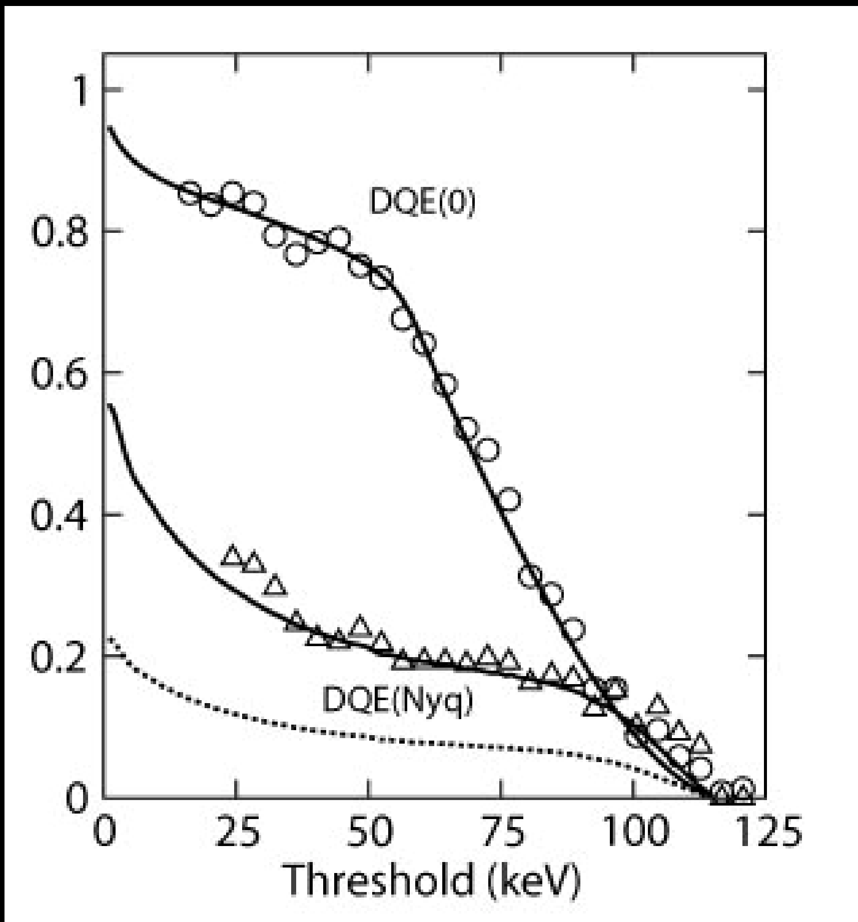
Some DQE formulae

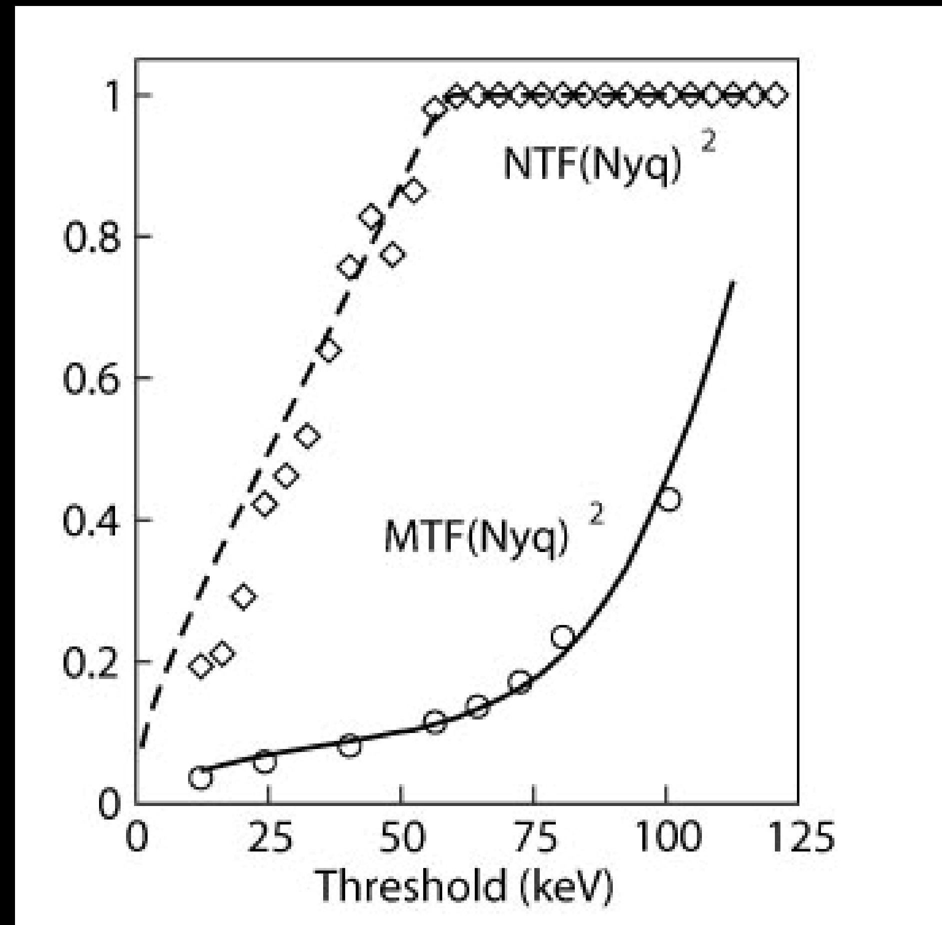
$DQE(0) = (S/N_{out})^2 / (S/N_{in})^2$ - any detector

$DQE(0) = (\Sigma i p_i)^2 / (\Sigma i^2 p_i)$ - digital detector

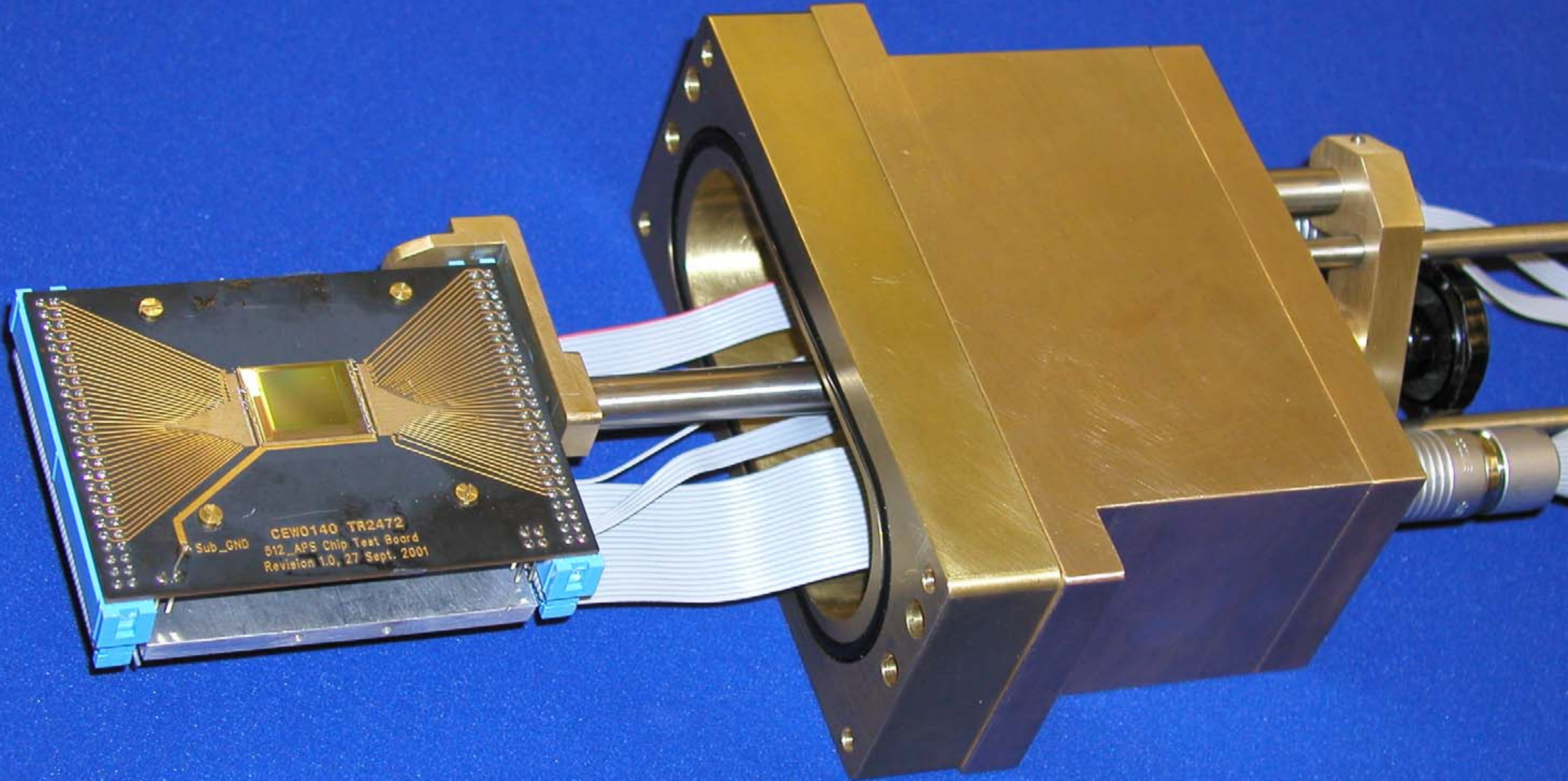
(where pi is probablility of recording a single incident electron in i pixels)

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

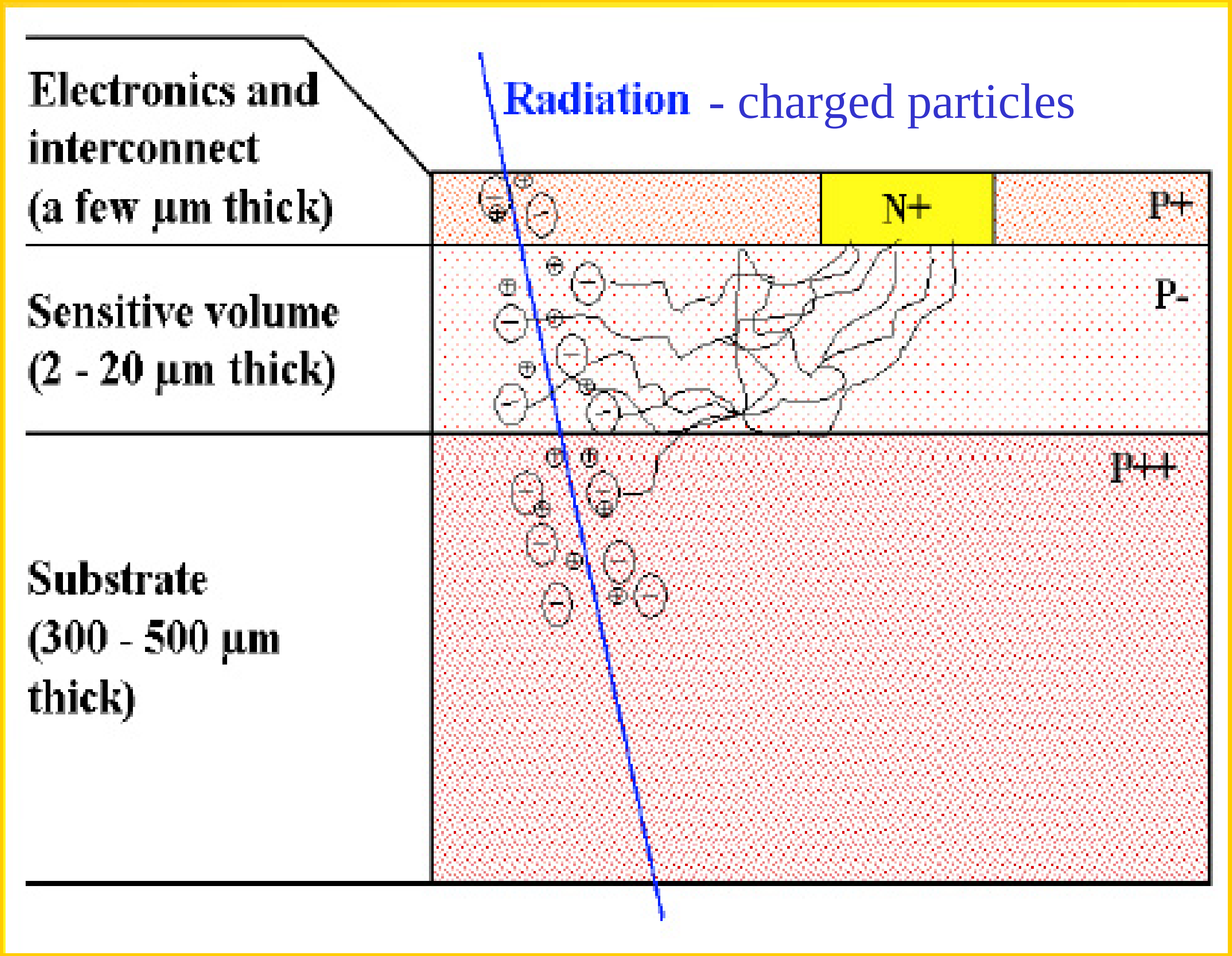

DQE(0)
DQE(Nyq)
0
0.2
0.4
0.6
0.8
1
0
25
50
75
100
125
Threshold (keV)


NTF(Nyq) 2
MTF(Nyq) 2
0
0.2
0.4
0.6
0.8
1
0
25
50
75
100
125
Threshold (keV)

MAPS/CMOS in 35mm port on CM12

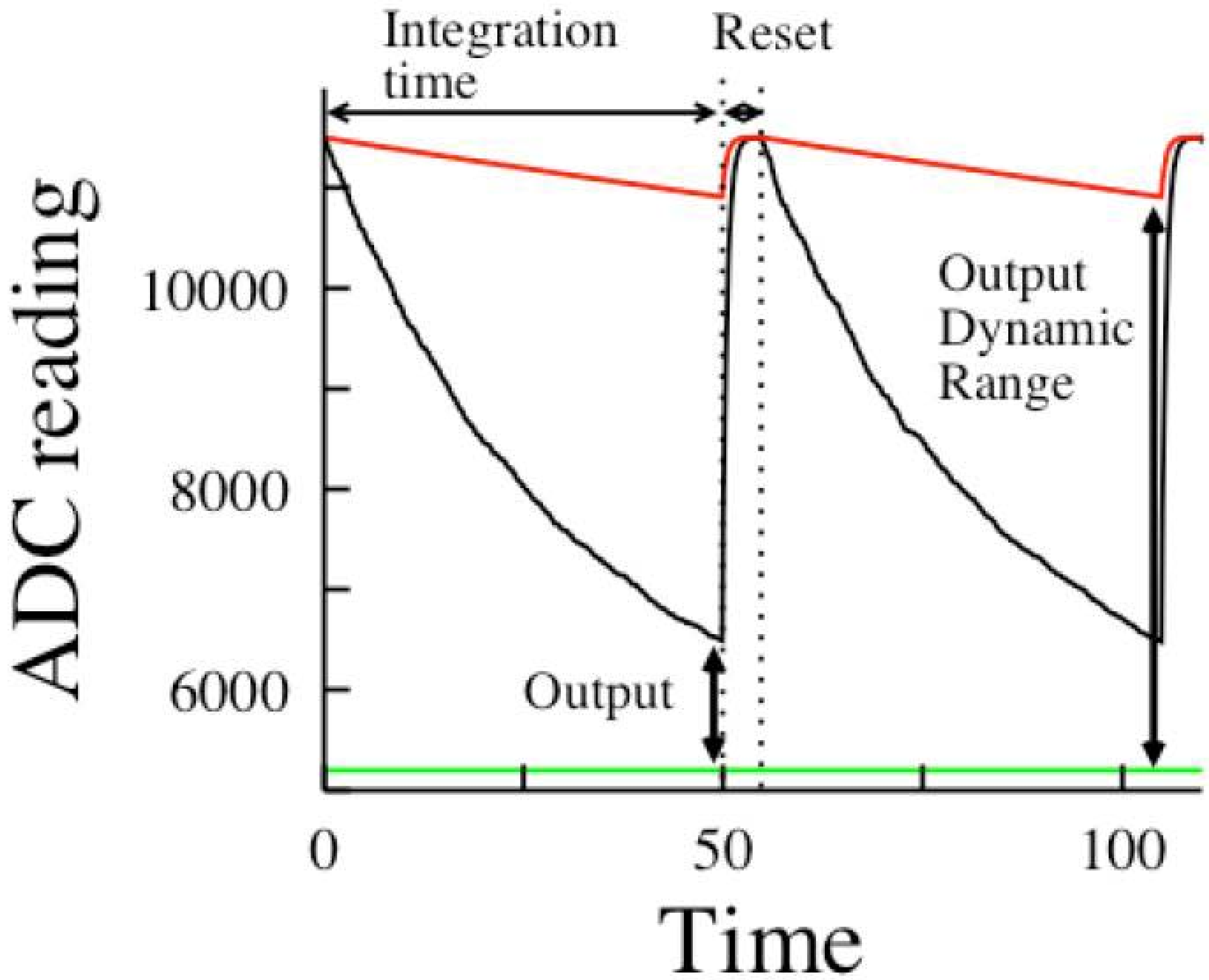


MAPS CMOS Detector



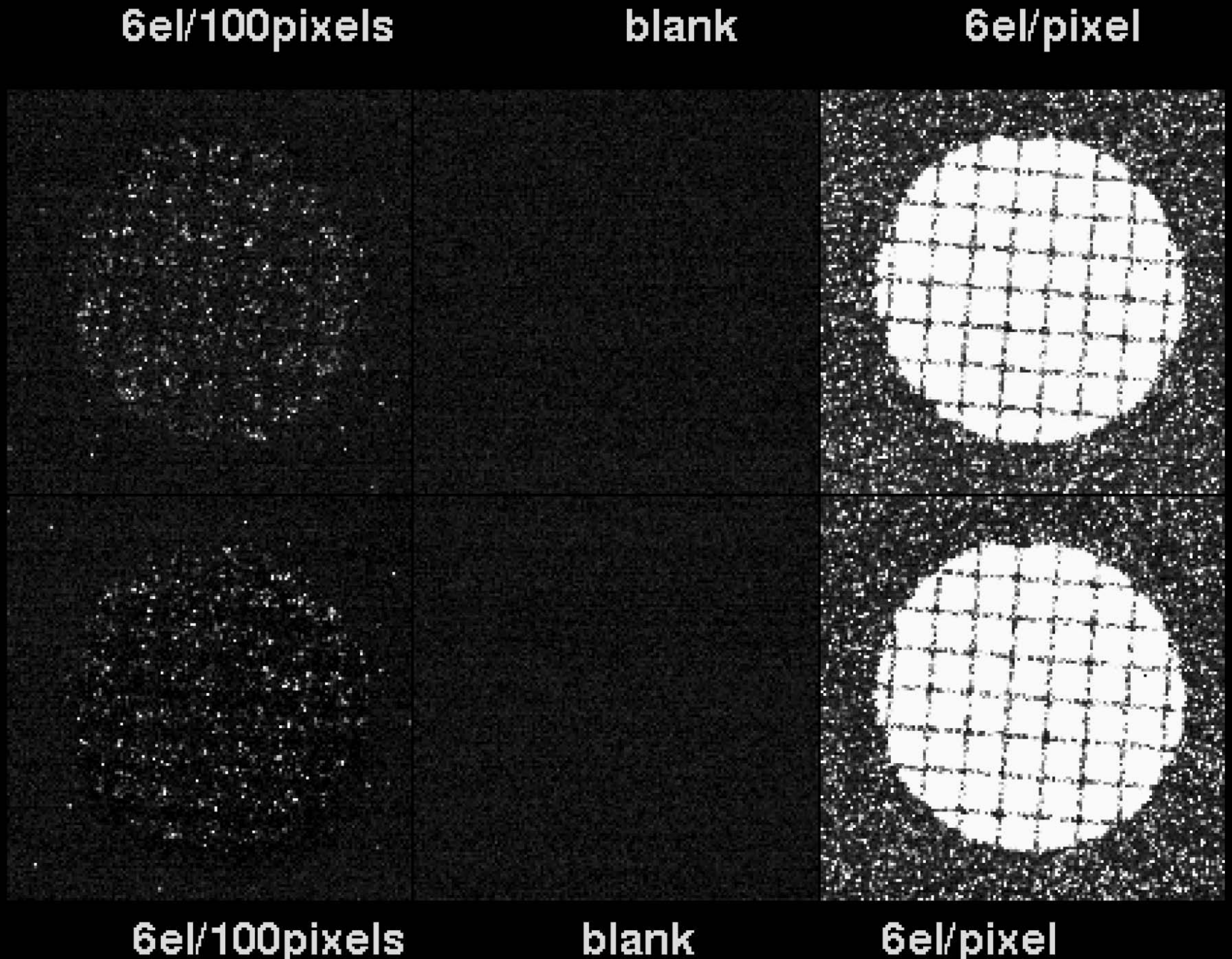
- no bias voltages
- charge diffusion
- 100% fill factor

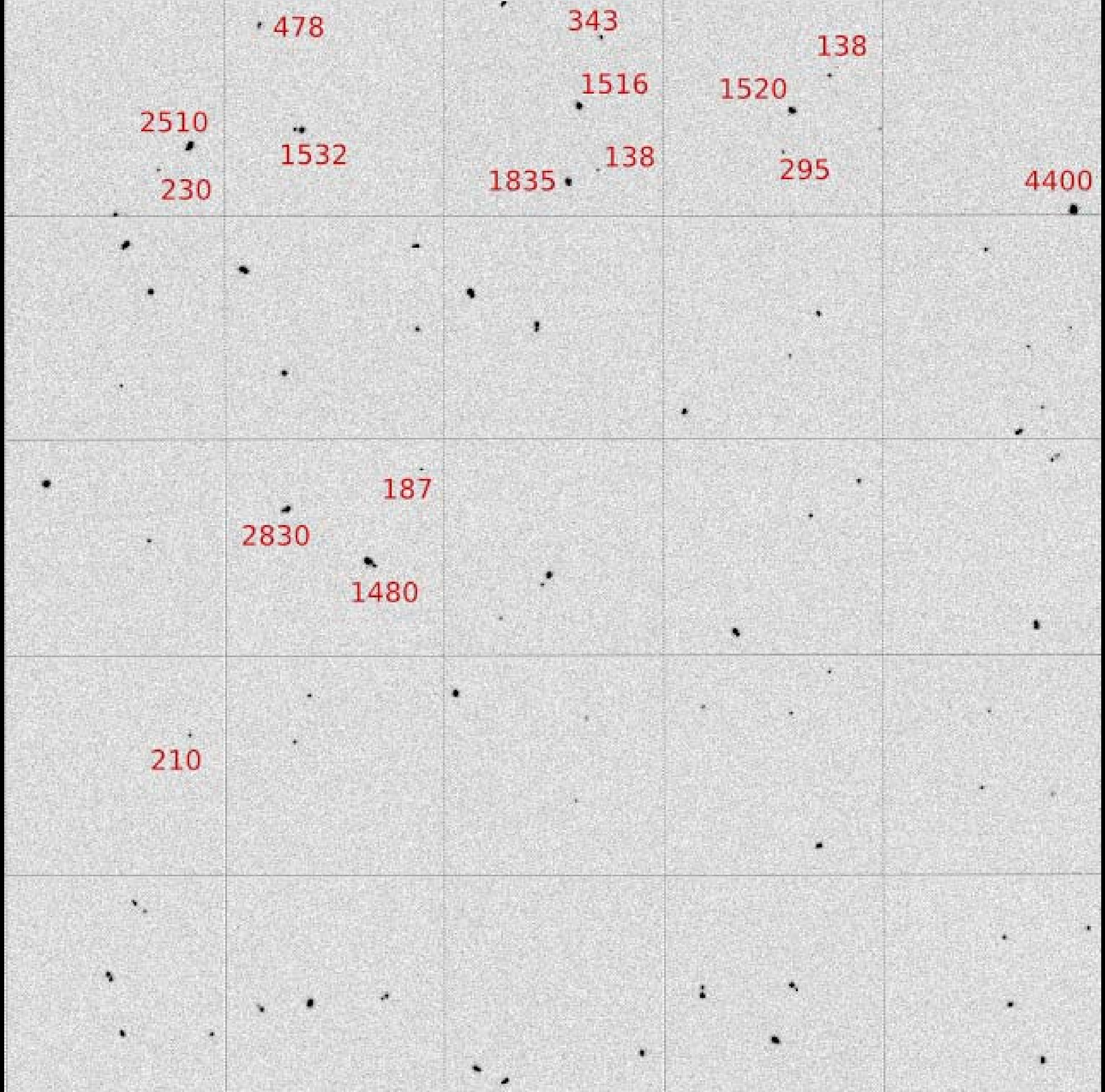
Turchetta et al
NIM A458 (2001) 677-689



Integration time
Reset
ADC reading
10000
8000
6000
Output
Output Dynamic Range
0
50
100
Time

MAPS CMOS detector




478
343
138
1516
1520
2510
1532
138
1835
295
4400
230
187
2830
1480
210

MTF at Nyquist limit (% of maximum = $2/\pi$)

Detector	Energy	Noise	MTF	pixel size	# pixels on edge
SO-163 film	120keV	~ 4	50%	7µm	12000
Gatan US4000	200keV	0.8	<10%	15µm	4000
Tietz 224	200keV	0.3	25%	24µm	2000
	300keV	0.5	24%	24µm	2000
Tietz 224 HD	120keV	0.06	25%	24µm	2000
Tietz 224 HD*	300keV	0.1	24%	24µm	2000
MAPS/CMOS	120keV	0.05	52%	25µm	525
HEPAPS-2	300keV	0.08	-	15µm	525
SIRA Star250	300keV	0.2	-	25µm	512
Medipix2	120keV	0.000001	50%	55µm	256

* = extrapolated

Detectors – Medipix & CMOS

Wasi Faruqi
Greg McMullan
David Cattermole