Novel Pixel Detectors for Structural Biology

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Scientific Problems

Structural Biology (and Condensed Matter Physics)

Protein Crystallography X-rays for 3D crystals

Electrons for 2D crystals

Small Angle Diffraction/Scattering

Direct imaging with electrons

Single Molecule Imaging(& tomography)

Larger complexes (NMR also used)

General Classification of Detectors

Integrating Detectors

1. Film
2. Image Plate
3. **CCD (based)**
4. Amorphous Silicon (Flat Plate)
5. MAPS/CMOS (with scintillator for photons)

Photon Counting Detectors

1. Multiwire (incl. Gas Microstrip, Gas Microdot, etc)
2. Silicon (or GaAs/Cd(Zn)Te) Hybrid Pixel, derivatives

Electron Detectors & X-ray Detectors

Electron Detection: What are the main differences from X-ray Detectors?

Electrons very easily scattered and stopped,

Need to:

(a) work in vacuum, and

(b) no entrance window

i.e. cannot use gas detectors

Scientific Background to Cryo-Electron Microscopy

Three Main types of Analysis:

1. Single Particle Analysis 6 -10 Å
2. Electron Crystallography of ordered specimen, i.e. 2-D crystals ~3Å
3. Electron Tomography 20 - 100 Å

Electron Cryo-Microscopy

Image molecules in native aqueous environment in vitreous ice

Trap important conformations by rapid freezing : equivalent to time-resolved measurements

Low contrast : need clever software and lots of averaging

Radiation damage to specimen a severe limitation

Single Particle Analysis

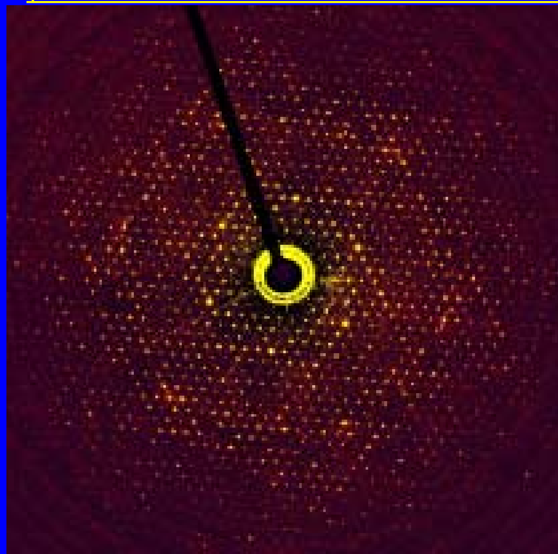
- No crystals required .. Makes it possible to analyse structure of membrane proteins, etc
- Can be applied to large macromolecular complexes.. Powerful technique when used in conjunction with atomic structures obtained with x-ray crystallography. Hep-B example
- Applications: Virus particles, ribosomes, etc
- 'Best' Resolution with this technique:Hepatitis B 7.4 Å
- Need many views for averaging

Electron Crystallography

- 2-D crystals required
- Averaging done in crystal… many identical scattering particles
- Main application: membrane proteins (but not exclusively)
- Near-atomic resolution (~2.5 Å) achieved.

Electron Diffraction Studies on Bacteriorhodopsin

(with R.Henderson and S. Subramaniam)



9/19/2005 LMB, Cambridge Faruqi,et al Ultramicroscopy, **75**,235-250 (1999)

Single crystal, Vermiculite

vermiculite single crystal

Medipix2, 120 keV

9/19/2005

Recent structures obtained using Cryo-EM on next slide

a. 70S E.coli ribosome complexed with mRNA and fMet-tRNA (11.5 Å)

b. Hepatitis B virus (7.4 Å)

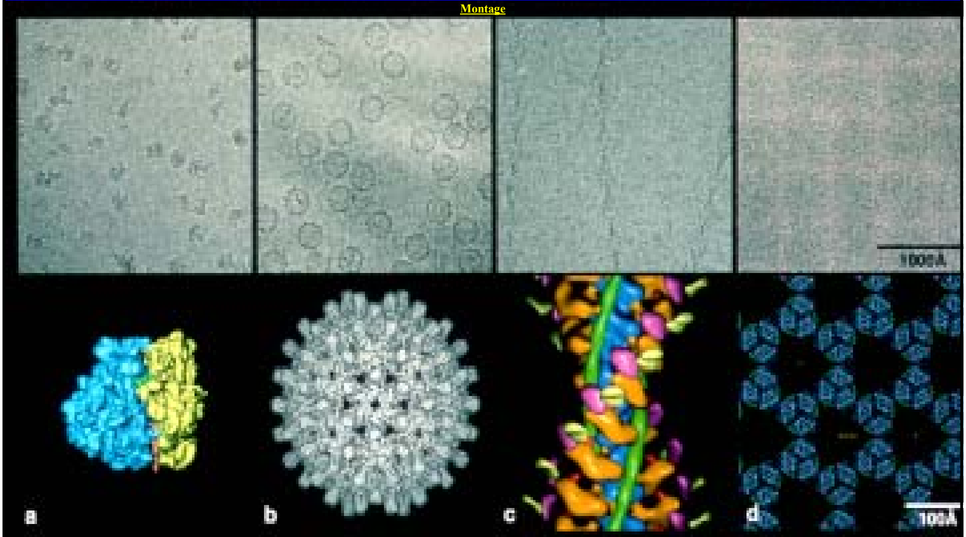
c. Actin filaments decorated with myosin heads (30-35 Å)

d. 2D crystal Light Harvesting Complex II (3.4 Å)

Baker & Henderson 'Electron cryomicroscopy' International Tables for Crystallography, 451-479,(2002)

Molecular structures obtained by Electron Cryo-Microscopy Magnification Micrographs:170K, Models: 1.2 Million

Montage



70S Ribosome | Hepatitis B Virus | Actin-Myosin (Muscle) | Light Harvesting Comple

High Resolution Imaging Detector Requirements for Cryo-EM

1. **Electronic detector with computer control.. eliminate film!**
2. **Number of *independent* pixels:4000 by 4000**
3. **Pixel Size 10 – 50 µm (has to fit in commercial microscopes)**
4. **High sensitivity with no noise – ability to add multiple frames**
5. **Radiation hardness important at least 1 MRad**
6. **Readout time preferably short**

Semiconductor Detectors (pixel detectors)

General Considerations

Good spatial resolution; no intermediate light conversion step in the phosphor or fibre optics

Photon (electron) Counting –low or no noise

Fast Readout – multiple frames per image

Compact size: limited space in microscopes

Tiling essential : for adequate area coverage

Cooling not required

Direct Detection in Silicon Pixel Detectors

- **Hybrid Pixel Detectors**

  Pixellated silicon detector, bump-bonded to readout chip with same size pixels

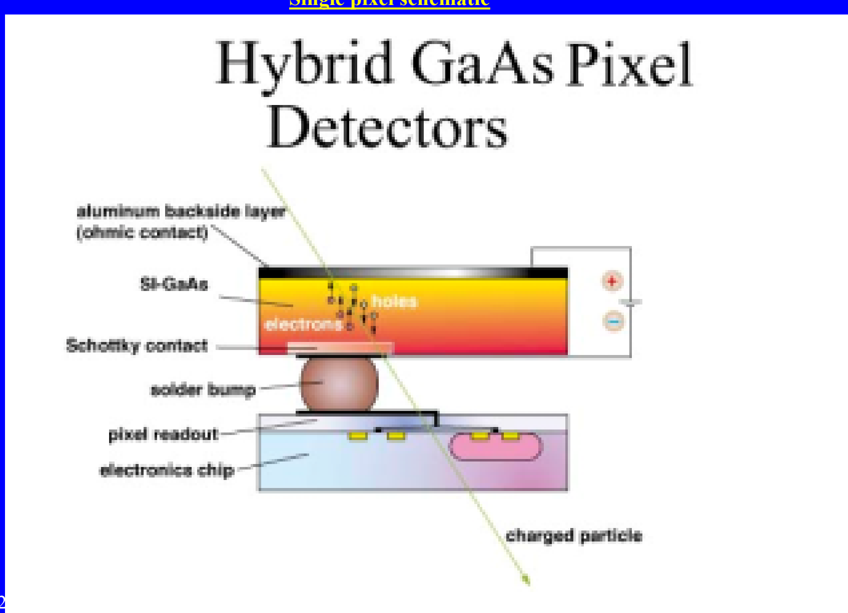
http://medipix.web.cern.ch/MEDIPIX

- **MAPS/CMOS Detectors**

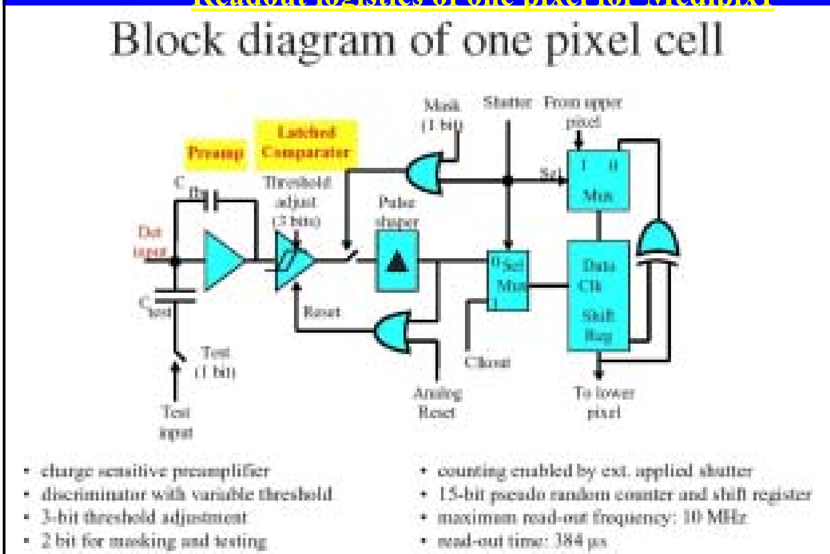
  Pixellated silicon, readout built into each pixel

http://www.rl.ac.uk/

Single pixel schematic



Readout logistics of one pixel for Medipix1

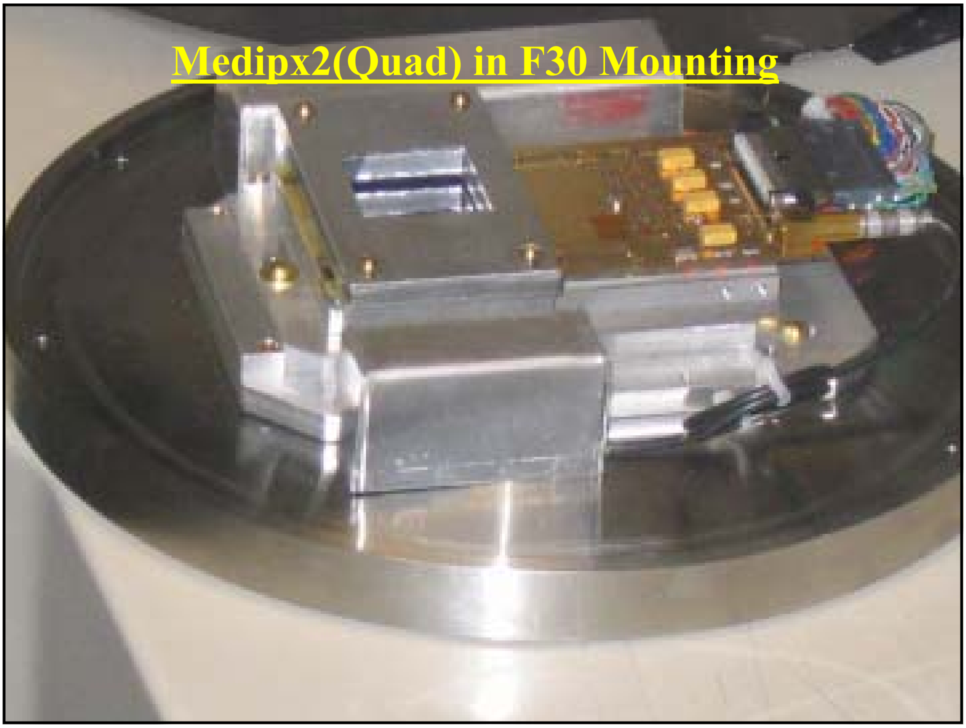


Medipix2 (Quad Chip) Main Parameters

• Pixel size	55 µm
• Number of Pixels	512 x 512 pixels
• Amplifier input	+ve or –ve input
• Current compensation	Pixel-wise current compensation
• Threshold	Two discriminators
• Max counting rate	1 MHz/pixel
• Tiling?	3-side buttable
• Layout	0.25 µm technology
• I/O	Serial/Parallel I/O
• No. Transistors?	142 M transistors/Quad chip

Llopart, etal IEEE Trans Nucl. Sci (2005)

Medipx2(Quad) in F30 Mounting



300 kV EM with detector installed

Detectors: Quality Factors

Sensitivity: Detective Quantum Efficiency (DQE), defined as:

$(S/N)^2_{output} / (S/N)^2_{input}$

DQE(0) zero spatial frequency

DQE(Nyquist)

Resolution: Modulation Transfer Function (MTF)

Framing Speeds

Radiation Hardness

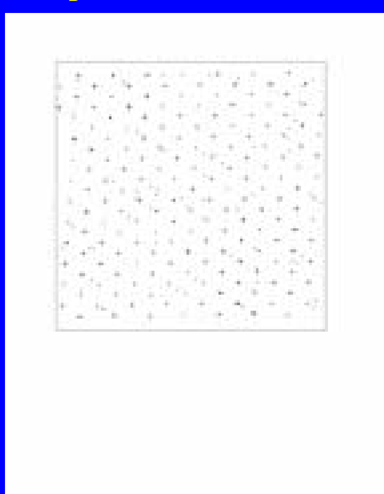
Dynamic Range

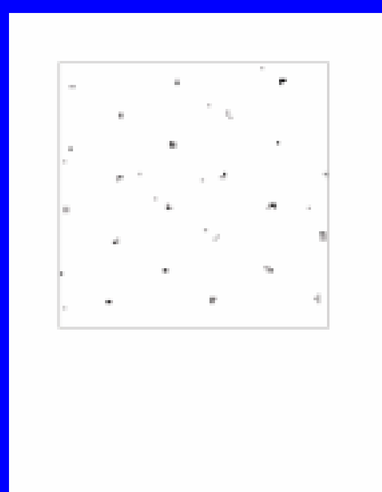
Defects

Raster of electrons at 120 keV

Spotscan **Magnified**

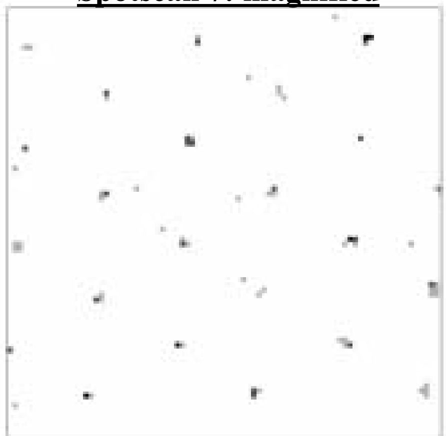
spots in a line – some electrons 'leak' in adjacent areas





Mean:4.7, σ : 1.8

Spotscan 7: magnified



Mean:4.7, σ : 1.8

Spots in a raster with some ‘leakage’ along the line of movement

Comparison with film

Spotscan1

	mean	σ
Medipix2	111	11
Film	116	24

Spotscan7

Medipix2	4.7	1.8
Film	spots invisible	

Medipix2 behaves like an electron counter with no noise.

18 electrons/pixel — 0.02 electron/pixel

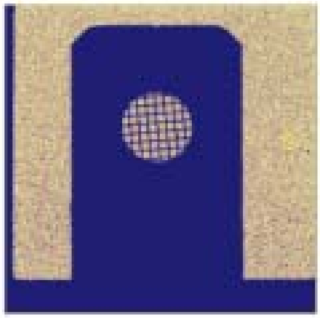


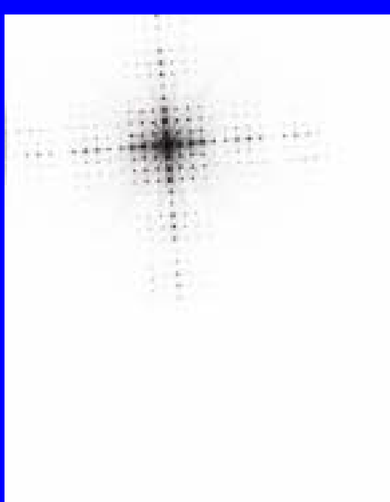
Image of grid used for computing MTF from FFT

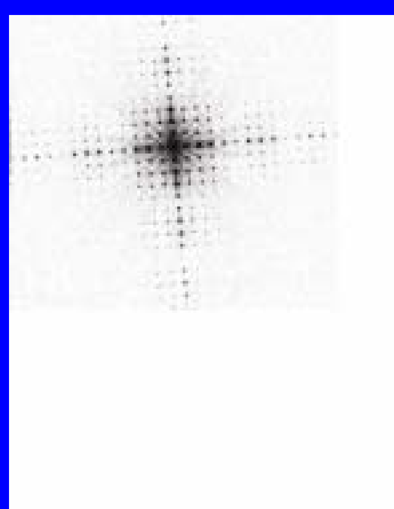
Single electrons recorded in single pixels

Wasi Faruqi, LMB

FFT of Grid image recorded on:

Film — **Medipix2 (MTF 90% of film at Nyquist)**





DQE Measurements

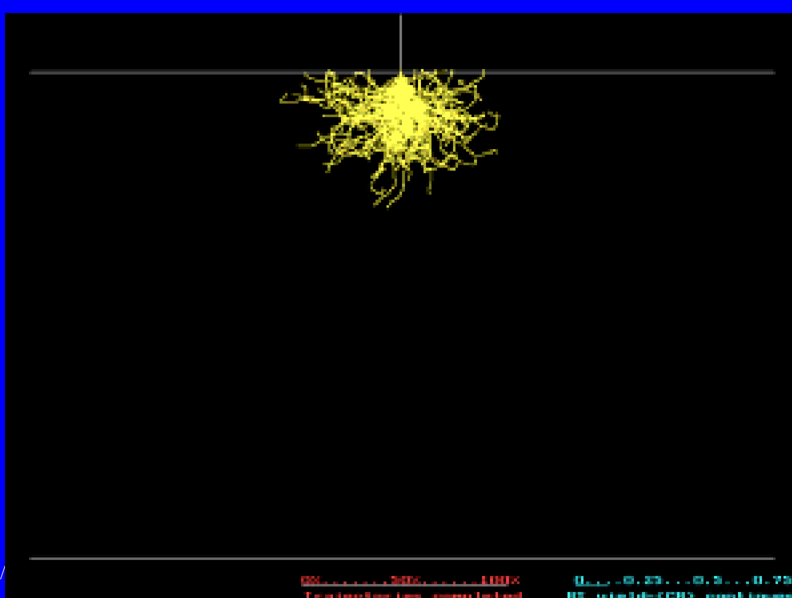
Measure **DQE** from Cluster Analysis of Single Electron events.

Charge sharing between adjacent pixels – unavoidable

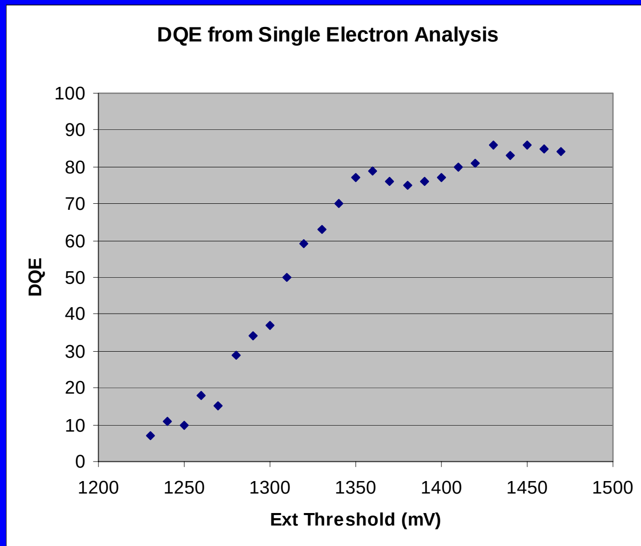
Counts recorded due to charge sharing can be controlled by changing threshold in pixel. **High threshold** cuts down number of counting pixels (**better MTF lower DQE**), **low threshold** increases number of counts from adjacent pixels **(lower MTF higher DQE).**

Counts/electron plotted as function of threshold

Monte Carlo simulation 120 keV, 300 µm silicon



DQE



low
threshold

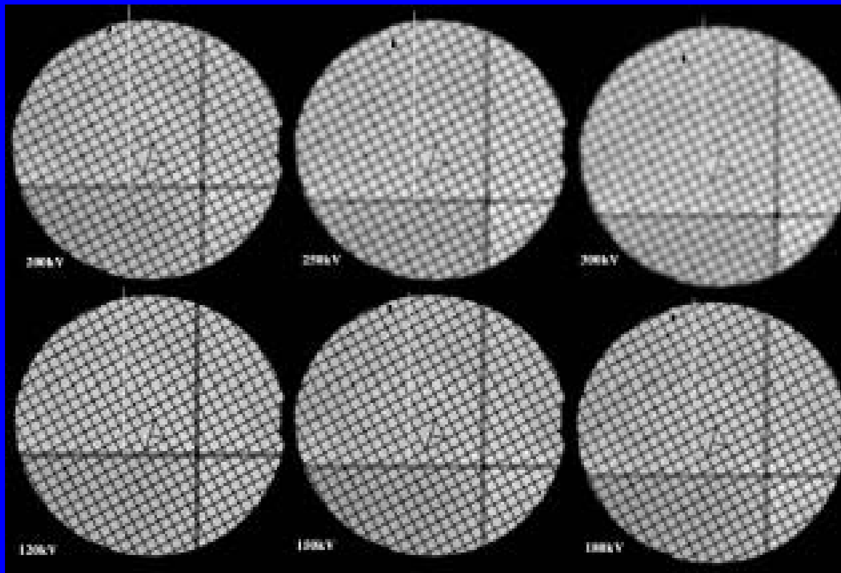
Medipix Summary

DQE, MTF at (120 keV):

Threshold	DQE(0)	DQE(Nyq.)	MTF(Nyq)
56(keV)	70%	63%	90%
16(keV)	85%	74%	45%

Radiation Damage: No detectable damage up to 200 keV (~1.5 MRads). Damage evident at 300 keV (~125 kRads).

Resolution of Quad_Medipix2 from 120 – 300 keV

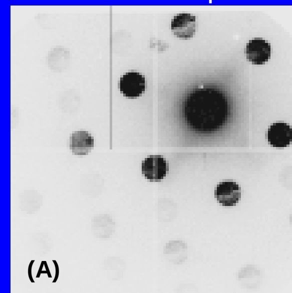


OBJECTIVE OF MEDIPIX EXPERIMENTS IN A 120 keV ELECTRON MICROSOCPE

CAN ELECTRON MICROSCOPY OF BIOLOGICAL MACROMOLECULES BE EXTENDED TO HIGHER RESOLUTION WITH THE USE OF A NOISE-FREE DETECTOR?

Robert Glaeser: MCB Dept., UC Berkeley and Phys. Bioscience Div., LBNL
Richard Henderson, et al: LMB, Cambridge

(A) One in a series of diffraction patterns used to study beam-Induced specimen tilting during Irradiation of the sample



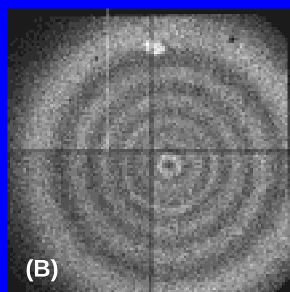
A CONFINED AREA OF ILLUMINATION AND EXTREMELY LOW EXPOSURES SHOW PROMISE FOR IMPROVING THE IMAGE QUALITY AT HIGH RESOLUTON

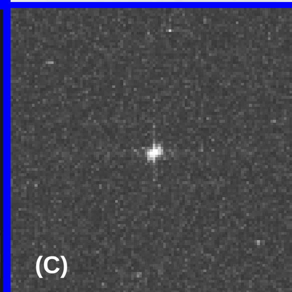
- Medipix has proven to be a superb detector for recording weak electron intensities:
  - **A long series of electron diffraction patterns extends well beyond 0.4 nm resolution, even though the exposure per frame is very small**
  - **images at 0.4 nm resolution have strong signal at the Nyquist limit (again recorded with very small exposures per frame, ~50 e/nm^2)**

(B) ~100 nm illumination spot shows Fresnel fringes due to high wave-coherence

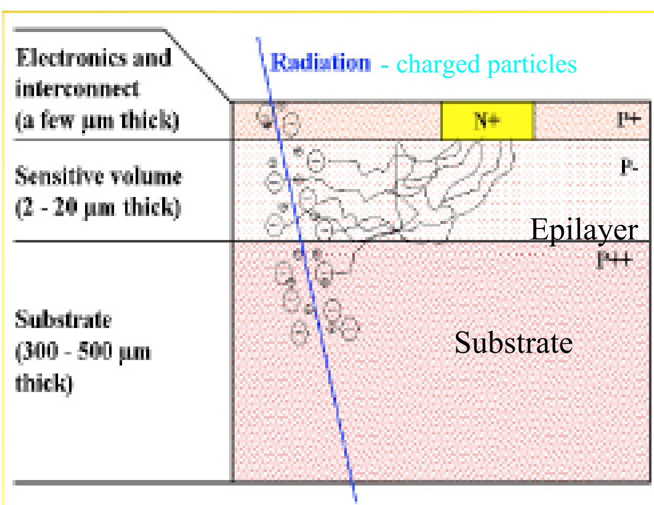
(C) An increased fraction of Images show 0.4 nm spots in their computed Fourier transforms, with stronger than usual signal

9/19/2005





MAPS CMOS Detector



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

Turchetta et al
NIM A458 (2001) 677-689

Monolithic Active Pixel Sensor (MAPS) General Background

Monte Carlo simulations of 120 keV electrons in silicon, 4 µm thick

lateral spreading of charge, ~1 µm,.

energy deposited: ~2.4 keV - >> Readout noise (~ 100 e^-), i.e. S/N expected to be high

Monolithic Active Pixel Sensor (MAPS) –designed at RAL

Size: 525 by 525, 25 µm pixels

Non-Radhard, standard 0.5 µm CMOS technology

Each pixel in MAPS contains four diodes in the sensitive epi-layer

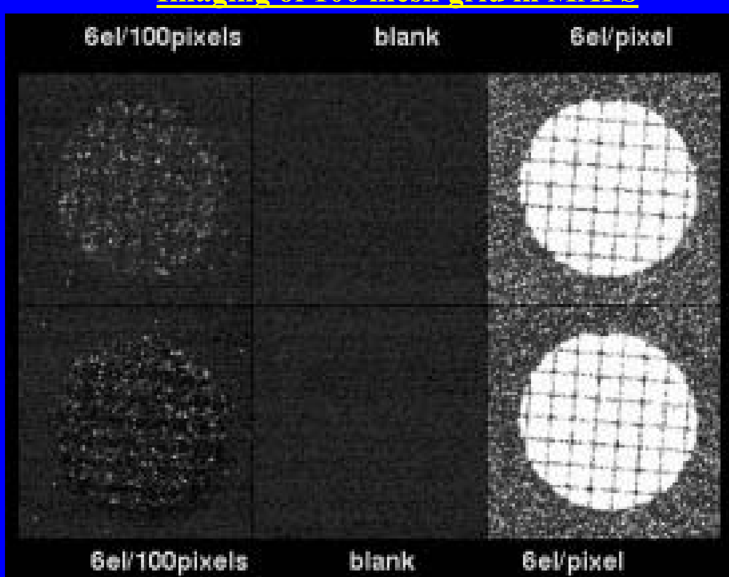
Electrons drift to one of the four diodes in pixel

Charge summed from all diodes and converted to a voltage

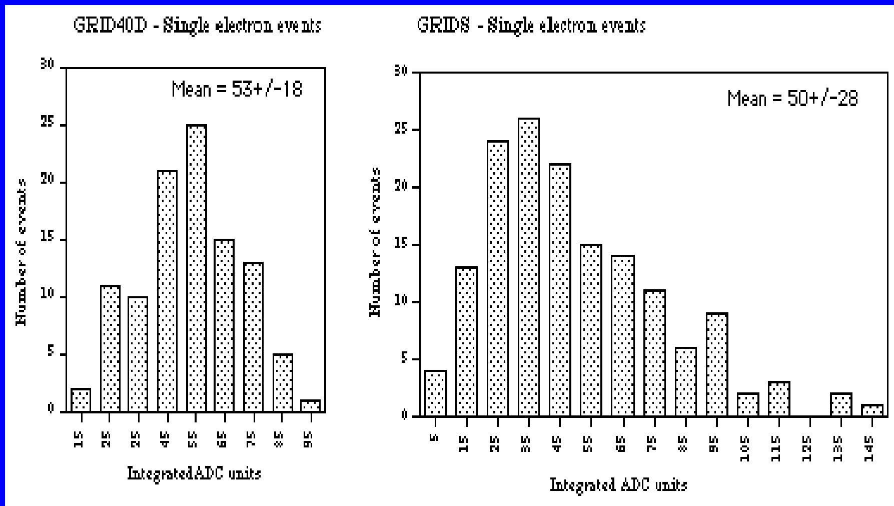
One ADC per column; all pixels in a row read out in parallel

MI-3 Project

Imaging of 100 mesh grid in MAPS



ADC Response for Single Electrons at 40 keV and 120 keV



Distribution of signal in ADC units from single electron hits at 120 keV

		0.2		
	0.8	5.2	1.4	
0.0	3.8	29.9	3.6	-0.2
	1.6	3.1	1.0	
		0.1		

Total = 50 ADC Units/electron from 152 events.
25% of signal in adjacent pixels (13% at 40 keV)

MAPS Summary at 120 keV

Sensitivity:	~50 ADC Units/electron
Noise:	~2 ADC Units
Signal/Noise:	20-25
Resolution:	52% of film at Nyquist Frequency
Radiation Hardness:	10-15 kRad . Needs improvement!
Active area	525 x 525 pixels need larger areas

Faruqi, Henderson, Turchetta et al Nucl. Instr.& Meth 546, 170-175, (2005)

Radiation Damage to Star250 (FillFactory) 300 keV

Radhard Technology

Pixels: 512 x 512, 25 μm pitch

Radiation Dose:

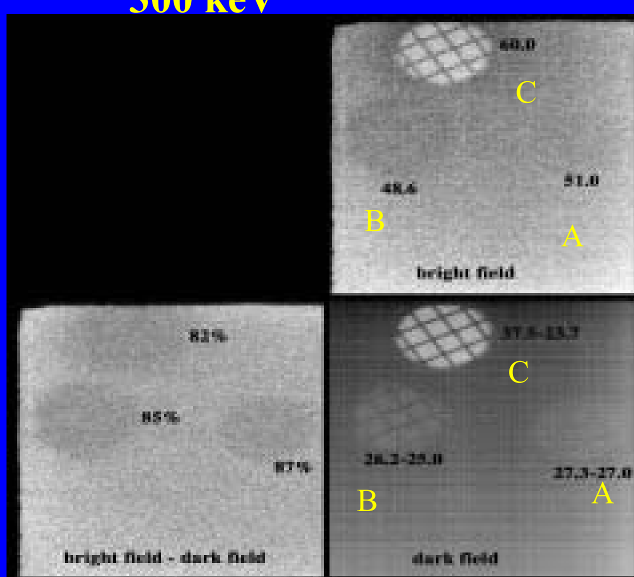
A:200kRad @300 keV (annealed for 4 weeks)

B:200 kRad @ 300 keV

C: 1000 kRad @300 keV

Faruqi,Henderson and Holmes

To be submitted to NIM(2005)



Detectors for Protein Crystallography : Main Requirements

- High Efficiency, low noise (high DQE)
- Large active area; can get better S/N by increasing distance
- Excellent spatial resolution; need to resolve ~500 orders (3K x3K)
- Very large dynamic range (strong & weak spots)
- High rate capability (no dead time, shutterless operation)
- No spatial distortions or non-uniformity of response; any corrections should be stable over long periods
- Ability to operate at a wide range of wavelengths for MAD, 0.6Å – 2.5Å
- Low cost, reliable (low maintenance)
- Example of Pixel Detector from PSI (C.Broenimann)

Hepatitis B Core Protein

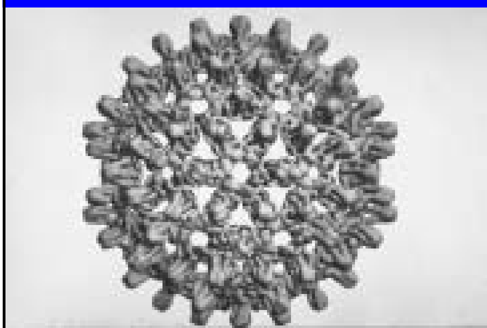
Crystal **Diffraction Pattern**

Andrew Leslie, LMB

Hepatitis B Structure

Cryo-EM

Resolution: 7.4 Å



X-ray Crystallography

Resolution: 3.3 Å

Andrew Leslie, Tony Crowther, et al LMB

PILATUS Detector with 3 Modules

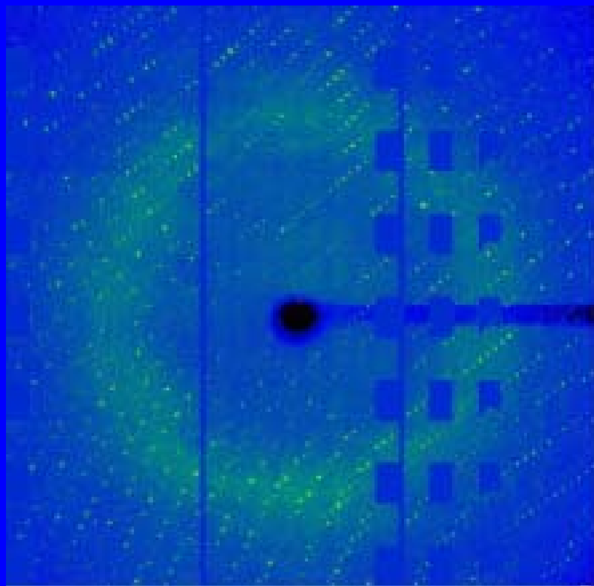
Bank Data

- Active Area: 238.7 x 35.3 mm^2
- 157 x 1098 = 172386 pixels
- 48 chips (radiation hard)
- 2.38 mm gap between modules
- Readout-time: 6 ms
- Energy Range: $E\gamma$ >4 keV
- XY-addressing of each pixel
- Threshold adjust of each pixel
- Analog signal of each pixel

Paul Scherrer Institut

Lysozyme

Diffraction pattern recorded with PILATUS Detector at Beamline 6s at the SLS



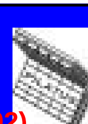
Data Taking

- Lysozyme crystal
- 1 deg. Rotation (of a 45 deg data set)
- 2s exposure, E=12 keV
- Data taken at 7 detector positions
- Flatfield correction for each detector position

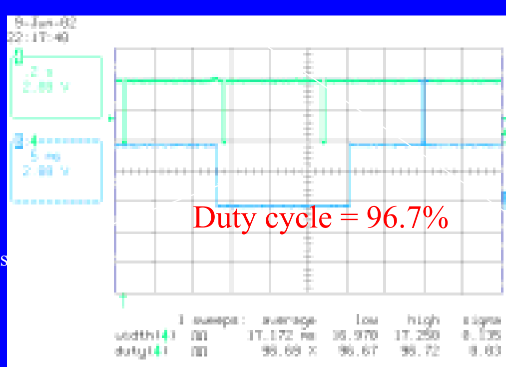
Ch. Brönnimann

PAUL SCHERRER INSTITUT

SLS Detector Group
Ch. Brönnimann



Continuous fine ϕ-slicing with the PILATUS-Detector (Jun02)



Exposure = 500 ms

Readout time= 17 ms
(Now 6 ms)

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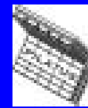
Ch. Brönnimann



Fine-ϕ-Slicing: Background and Rocking Curves

- Continuous rotation of the crystal
- Angular range: 10 deg
- Resolution of 0.02 deg/frame
- Exposure 500ms/frame (RO 17 ms)
- Total 500 frames, 250s exposure

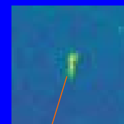
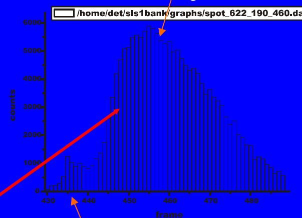
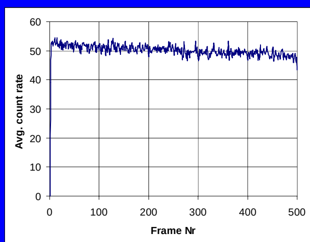


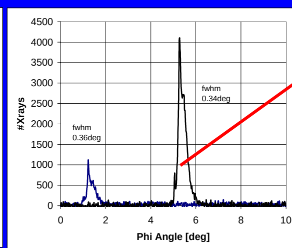
Image 455



Very stable background



Rocking curves background subtracted



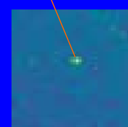


Image 435

Acknowledgements

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- **Richard Henderson**

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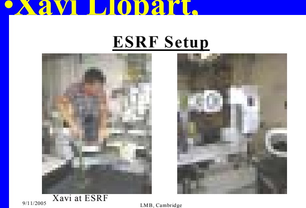
- **Lukas Tlustos, M.Campbell**
- **Xavi Llopart,**



CCLRC (RAL)

- **R.Turchetta, et al**
- **M. Prydderch**

Basic Technology Project: MI-3

Development of Active Pixel Sensors for Imaging

•PhilAllport	University of Liverpool
•RichardBates	University of Glasgow
•AndyCossins	University of Liverpool
•Mohamed El-Gomati	University of York
•WasiFaruqi	Medical Research Council (LMB)
•MarcusFrench	CCLRC(RAL)
•AndrewHolland	Brunel University
•ValO'Shea	University of Glasgow
•BobOtt	Institute of Cancer Research
•RobertSpeller	University College, London
•RenatoTurchetta	CCLRC (RAL)
•KevinWells	University of Surrey
•NigelAllinson	University of Sheffield (Program Leader)
•CatherineClayton	University of Sheffield (Program Manager)