

Molecular Biology Consortium

Protein Crystallographic Detectors at Synchrotron Radiation Sources

E. M. Westbrook¹, J. Morse¹, A.C. Thompson¹, C. Kenney¹

Sherwood Parker²

E. Mandelli³, G. Medeler³, H. von der Lippe³

C. Da Via⁴, J. Hasi⁴, A. Kok⁴

J. Morse⁵

¹Molecular Biology Consortium Inc.

²University of Hawaii

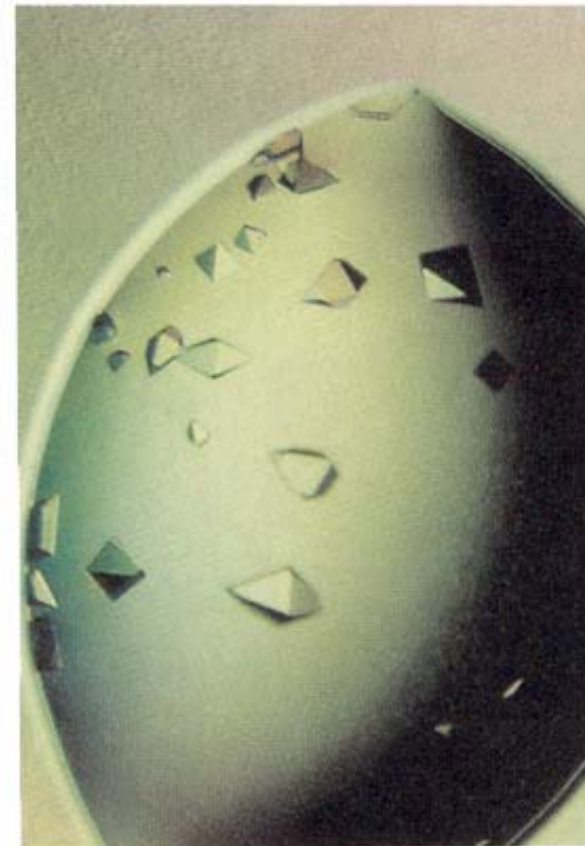
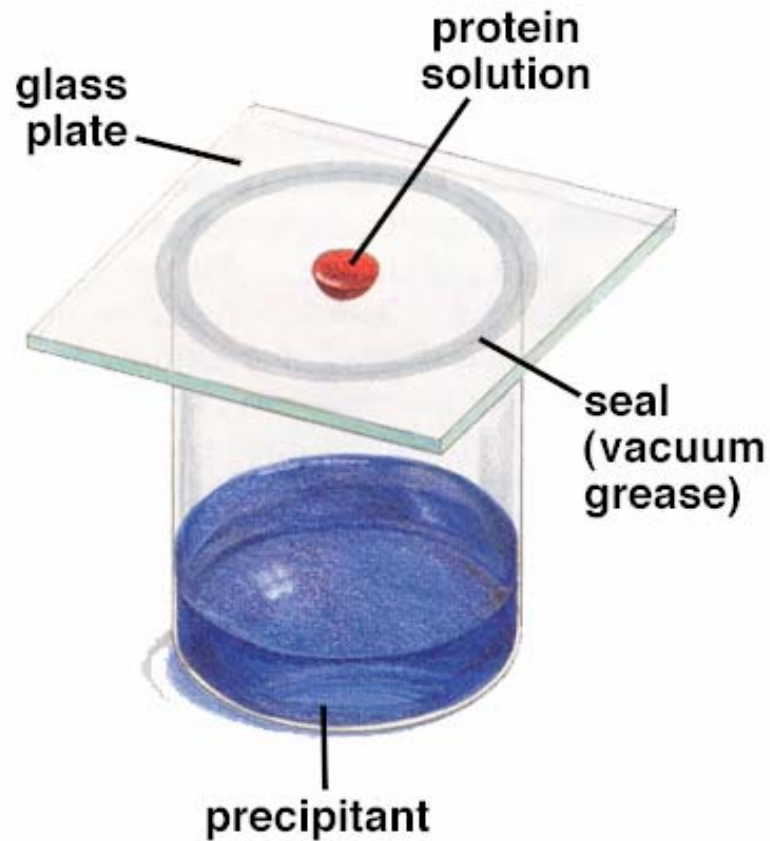
³Lawrence Berkeley Laboratory

⁴Brunel University, London

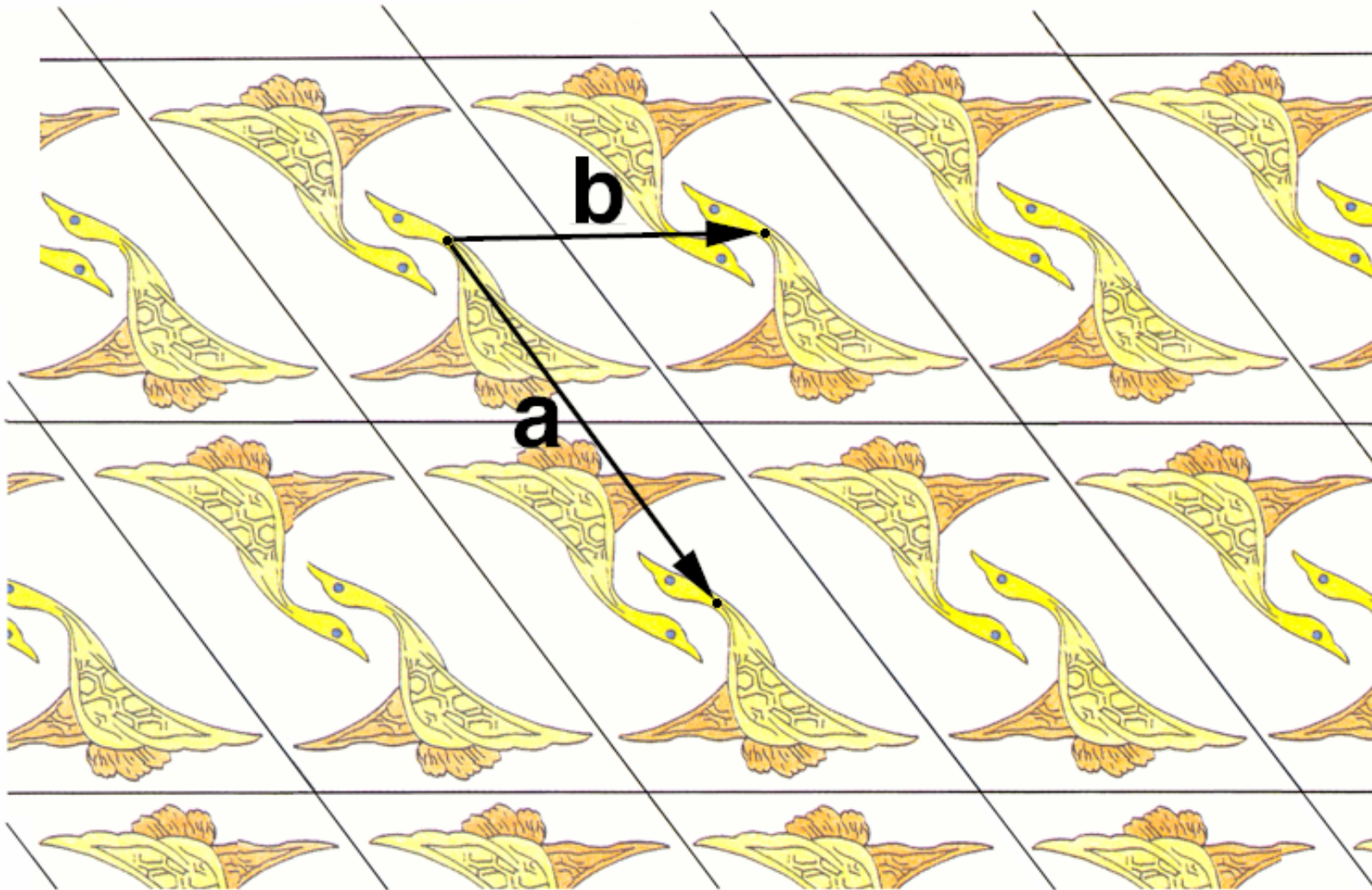
⁵European Synchrotron Radiation Facility, Grenoble

This work is funded by NIH grant 1 R01 RR16230

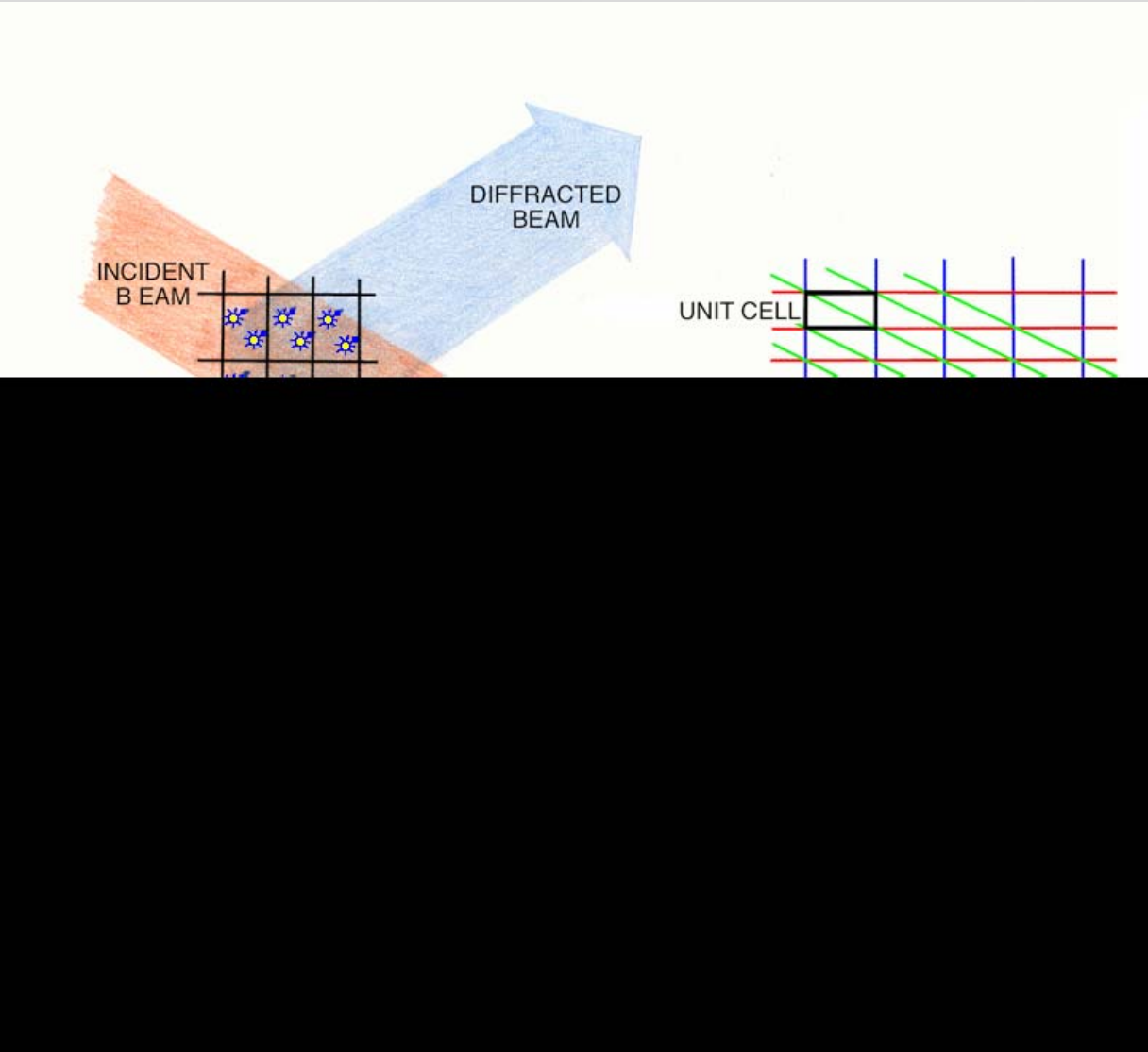
Protein Crystals are (almost) always grown from vapor diffusion droplets.



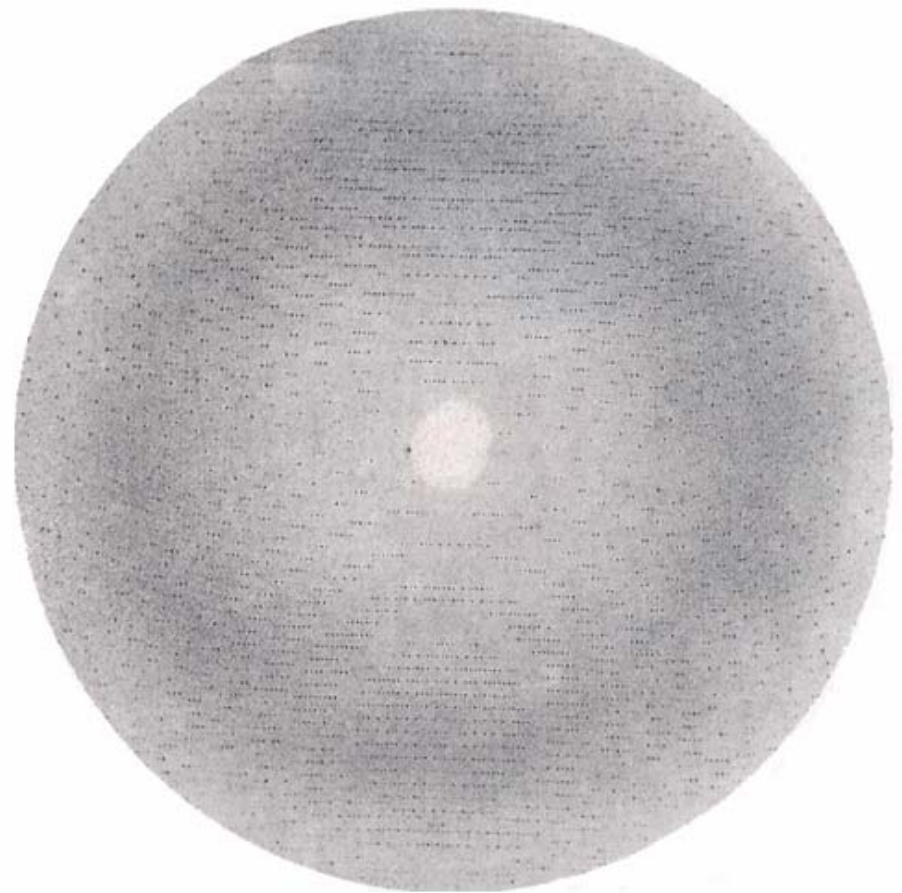
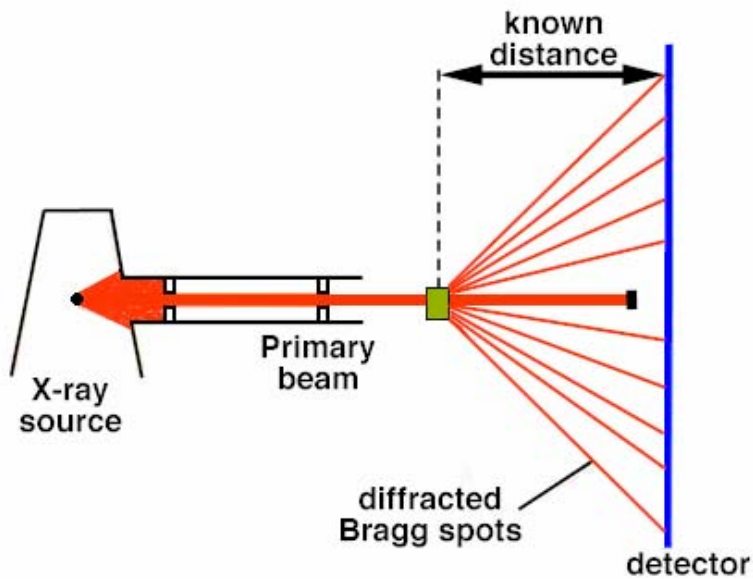
**Crystals are symmetric w.r.t.
translation operations:
 $(x',y',z') = (x,y,z) + (na,mb,lc)$
a,b,c vectors, n,m,l integers**



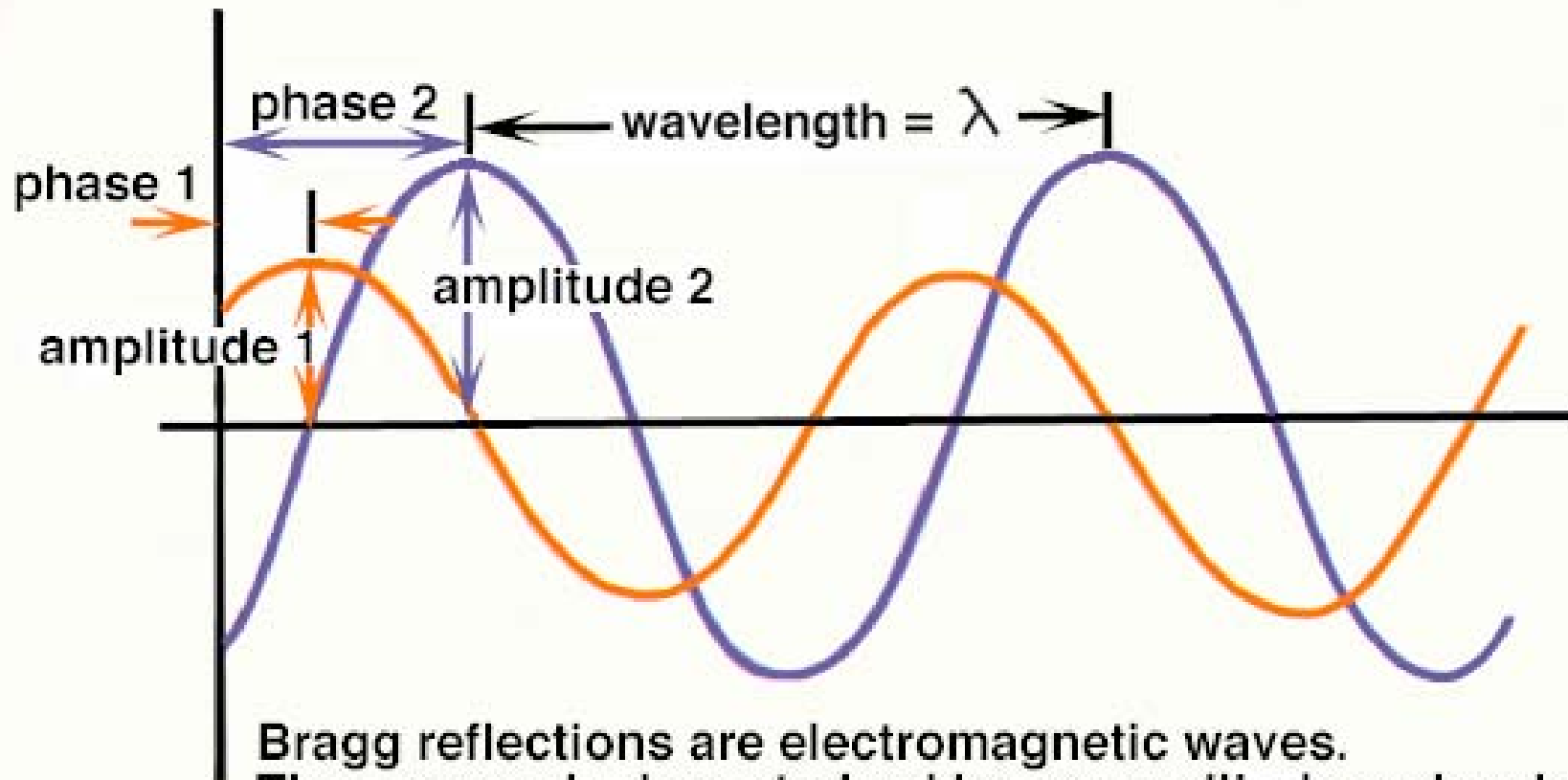
Crystal Diffraction Occurs only at discrete momentum transfer directions: the Bragg Reflections.



The Diffraction Pattern of Discrete Bragg Spots is Captured by the Detector



You can measure amplitude, but phases must be derived indirectly.



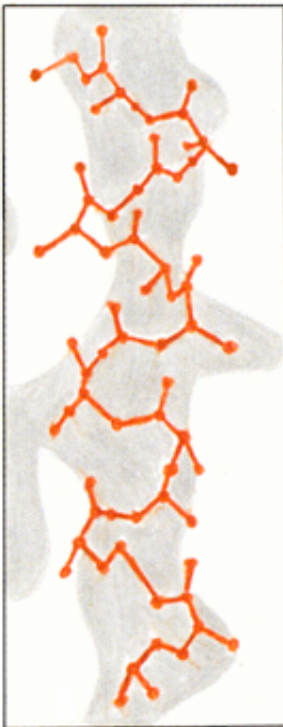
Bragg reflections are electromagnetic waves.
They are each characterized by an amplitude and a phase.

$$F(h,k,l) = A(h,k,l) e^{i\theta(h,k,l)}$$

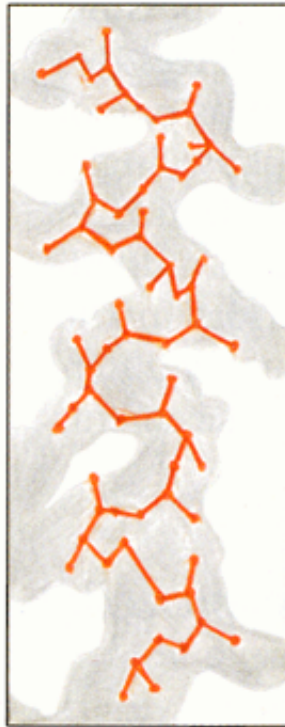
Once phases are known, the electron density map can be calculated by a Fourier transform.

$$\rho(x, y, z) = \sum_h \sum_k \sum_l |F(h, k, l)| e^{i\phi(h, k, l)} e^{-2\pi i(hx + ky + lz)}$$

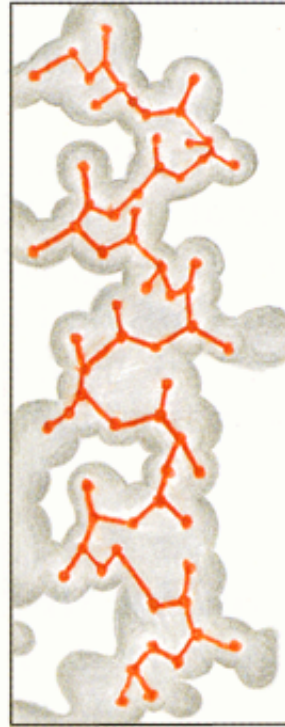
(a) 5.0 Å



(b) 3.0 Å



(c) 1.5 Å



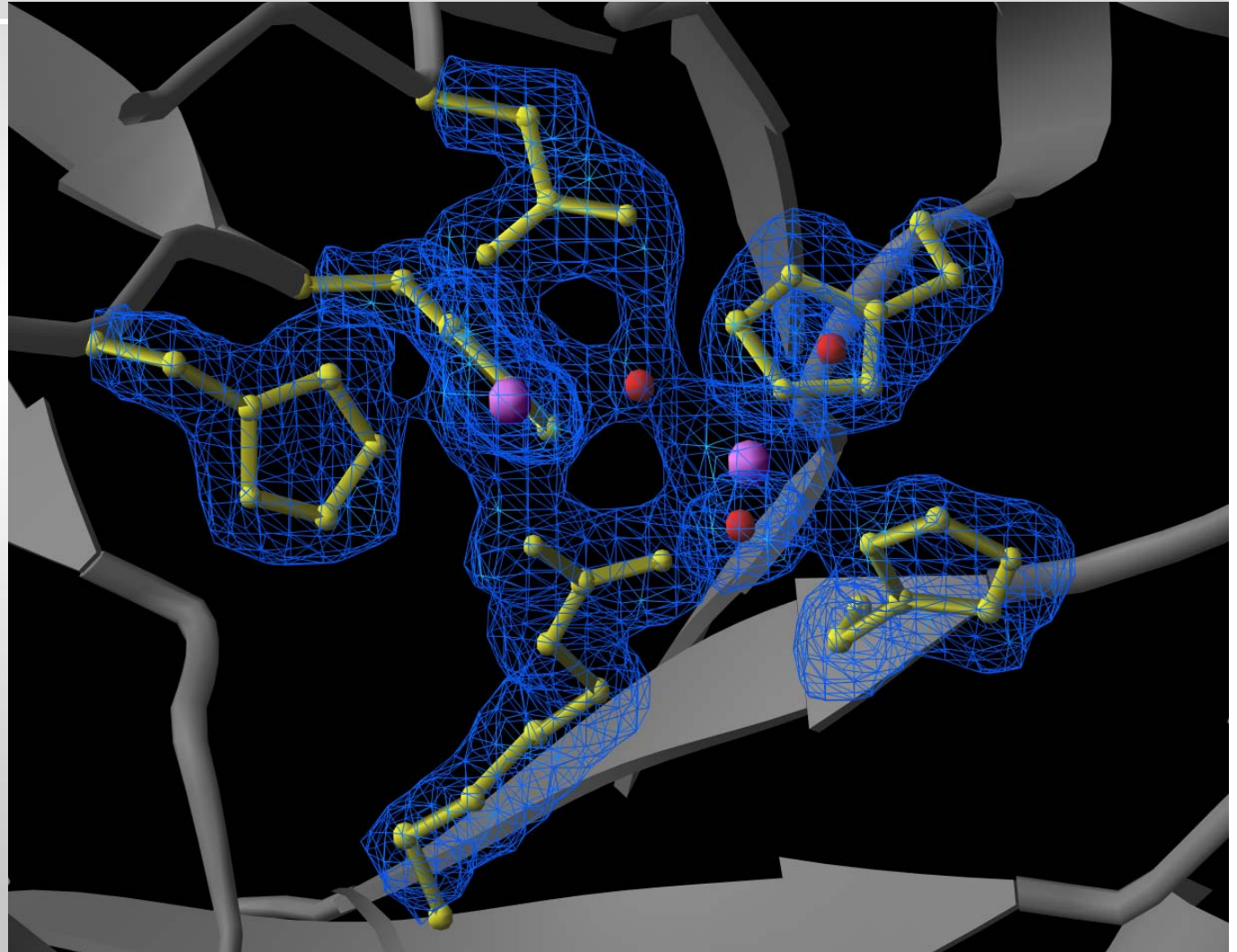
The molecular structure must be inferred from the map by someone competent in physical biochemistry.

The precision of the map interpretation depends on the resolution of the data to which you collected your data.

Example: Structure of an Enzyme Active Site



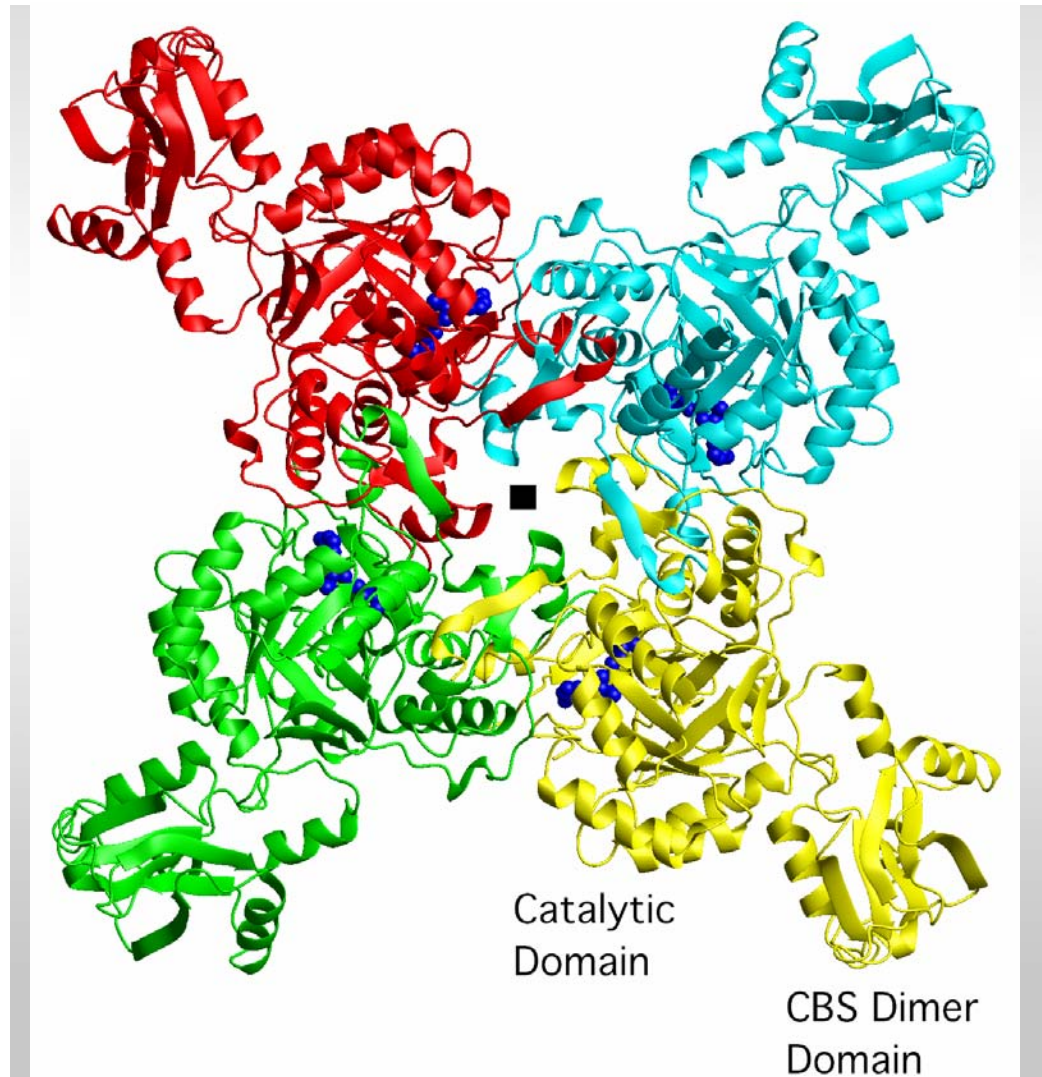
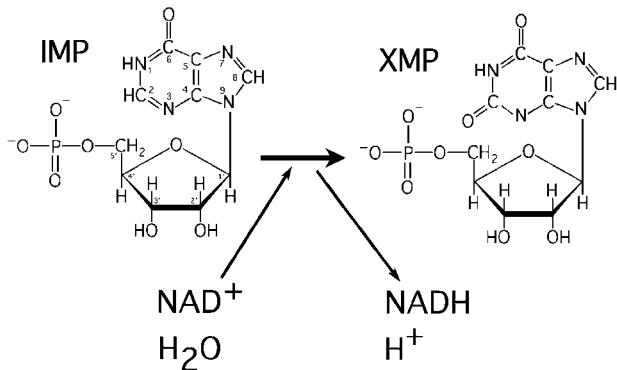
- *Pseudomonas diminuta*
phosphotriesterase:
This enzyme catalyzes the hydrolysis of organophosphorus pesticides and nerve agents. Its crystal structure is being studied by Hazel Holden's research group at the University of Wisconsin, Madison (see PDB file 1DPM).
- Purple atoms: zinc
- Red: bound water
- Yellow: side chains
- 1.8 Å resolution map,
- 21% R-factor



IMPDH Story: Compare Bacterial (Staphylococcus) Enzyme with Human: drug design

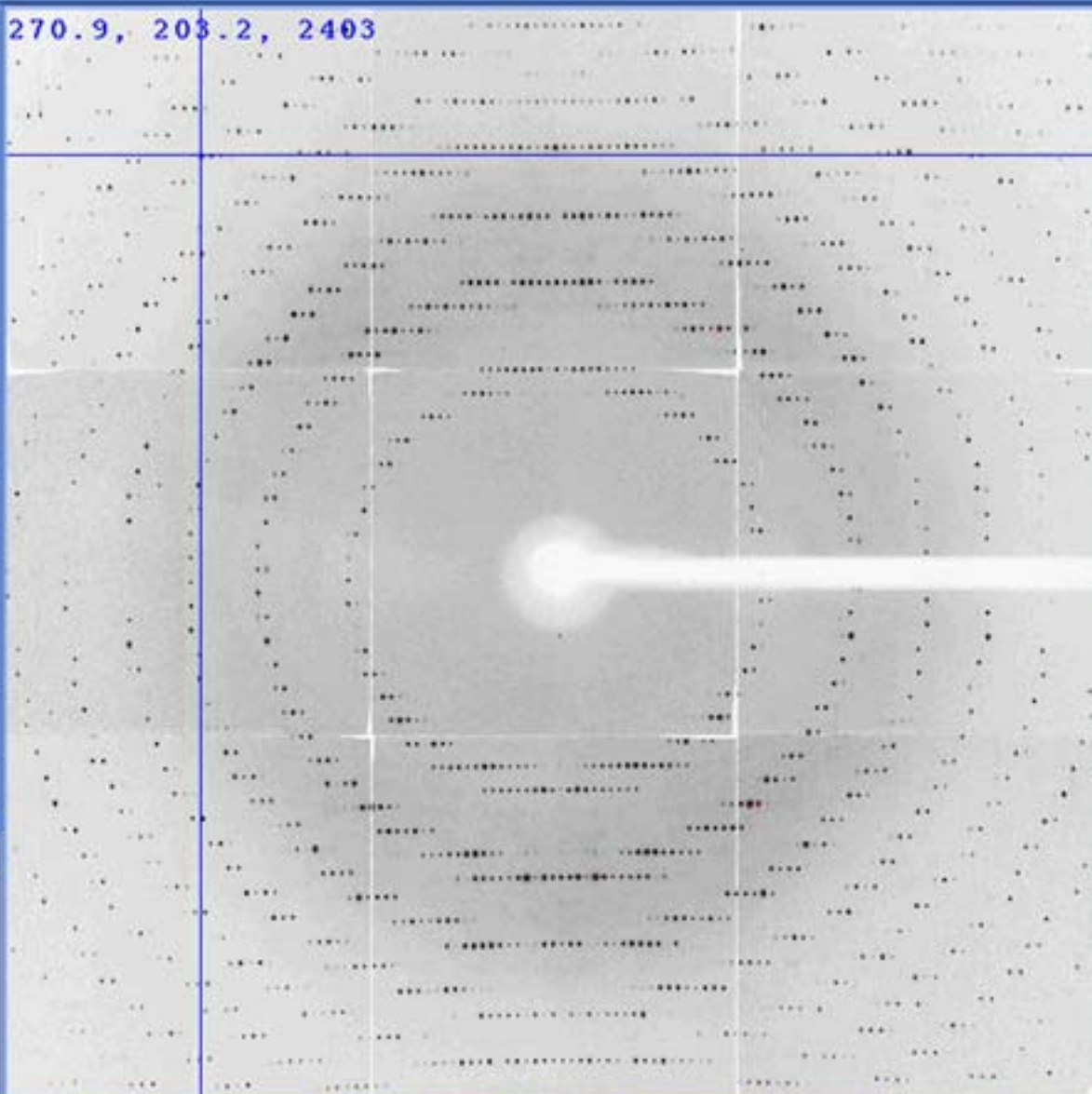


Structure and Function of Inosine Monophosphate Dehydrogenase



Value: 2403
Mm pos: 74.87, 86.19
Intensity: 129126 (405)
Int/Sigl: 319.0
Size: 3 by 5
Backgrnd: 145 (156)
Resol: 2.21 A, 2T: 30

270.9, 203.2, 2403



Prev

Next

No: ?

Det dist: 200.00

Det swing: -0.29

Rot start: 0.000

Rot end: 0.200

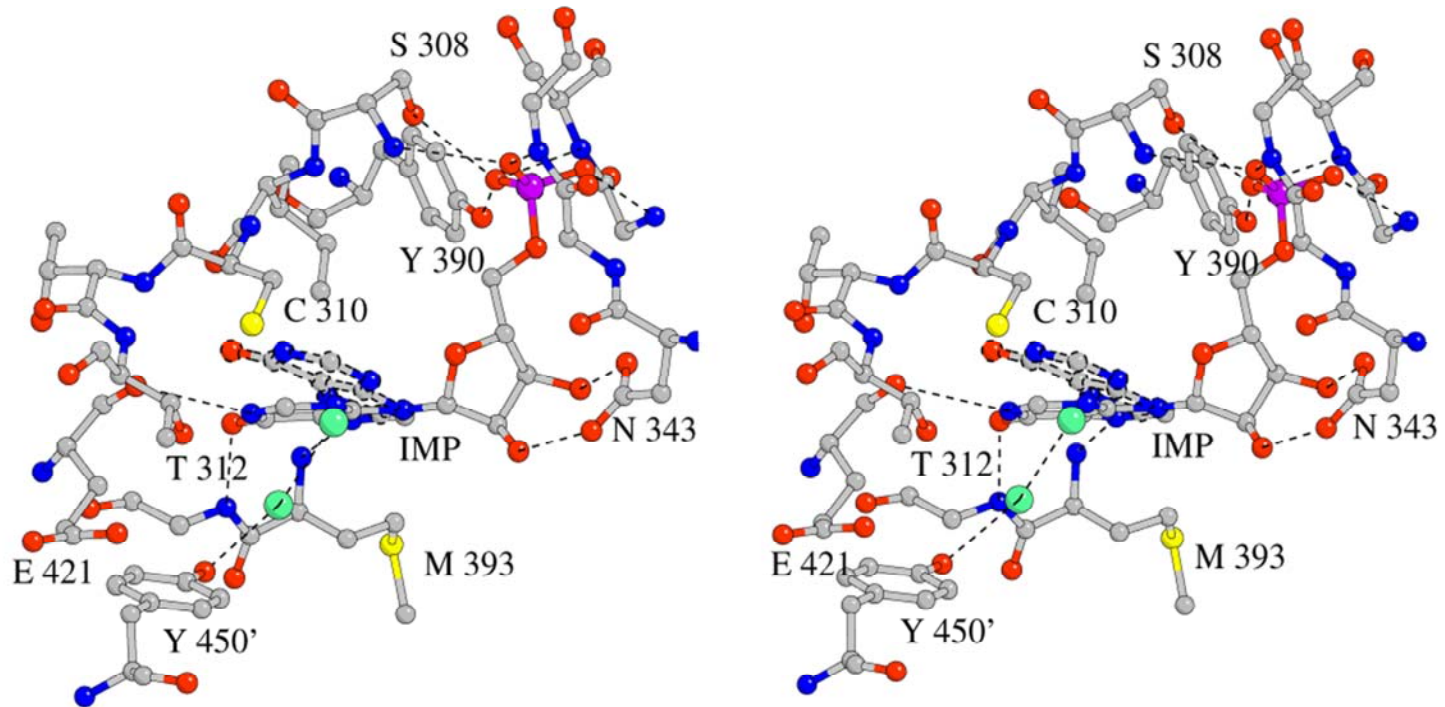
Wavelength: 1.12710

258.0, 191.0, 81

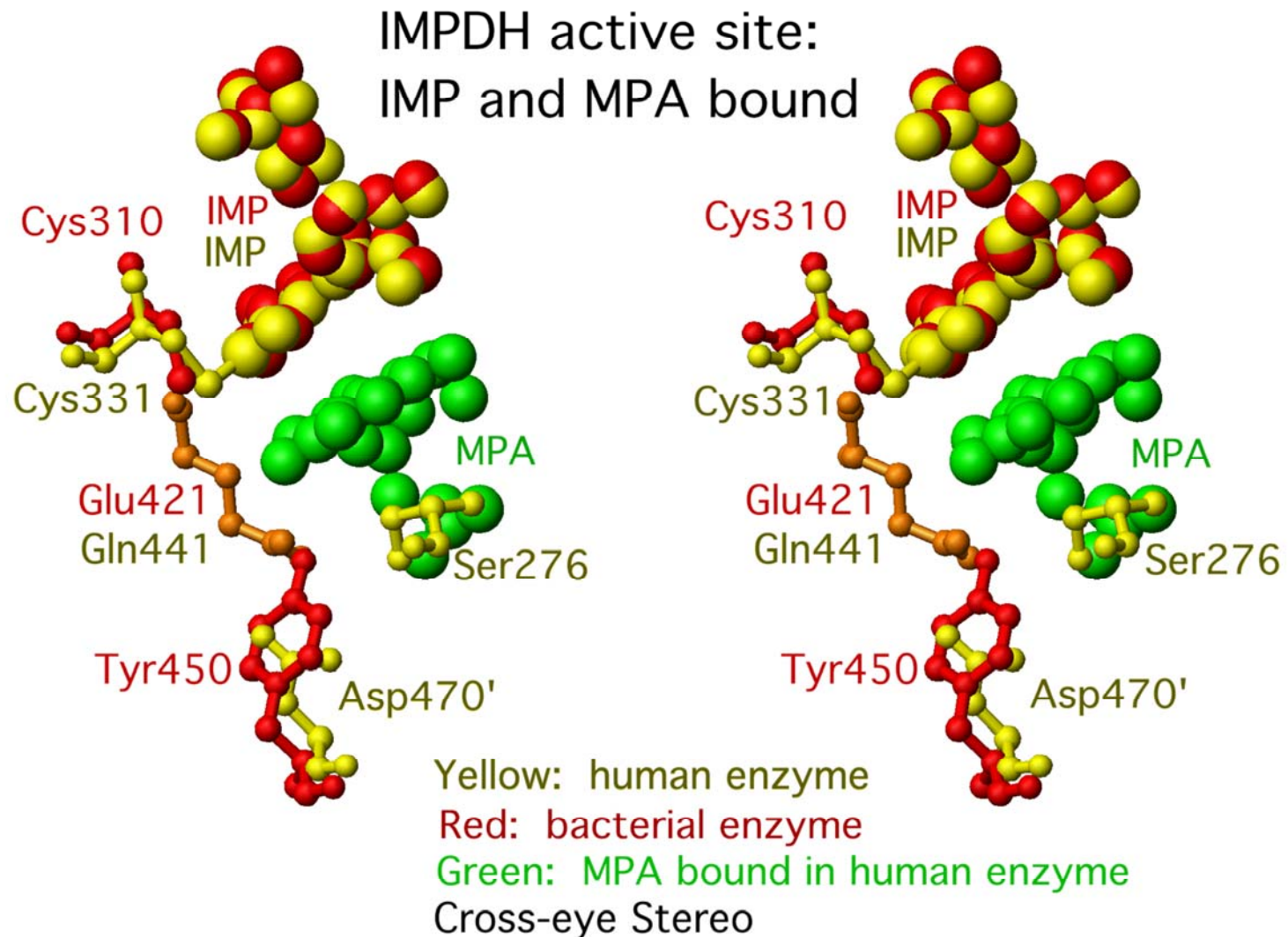


0 893

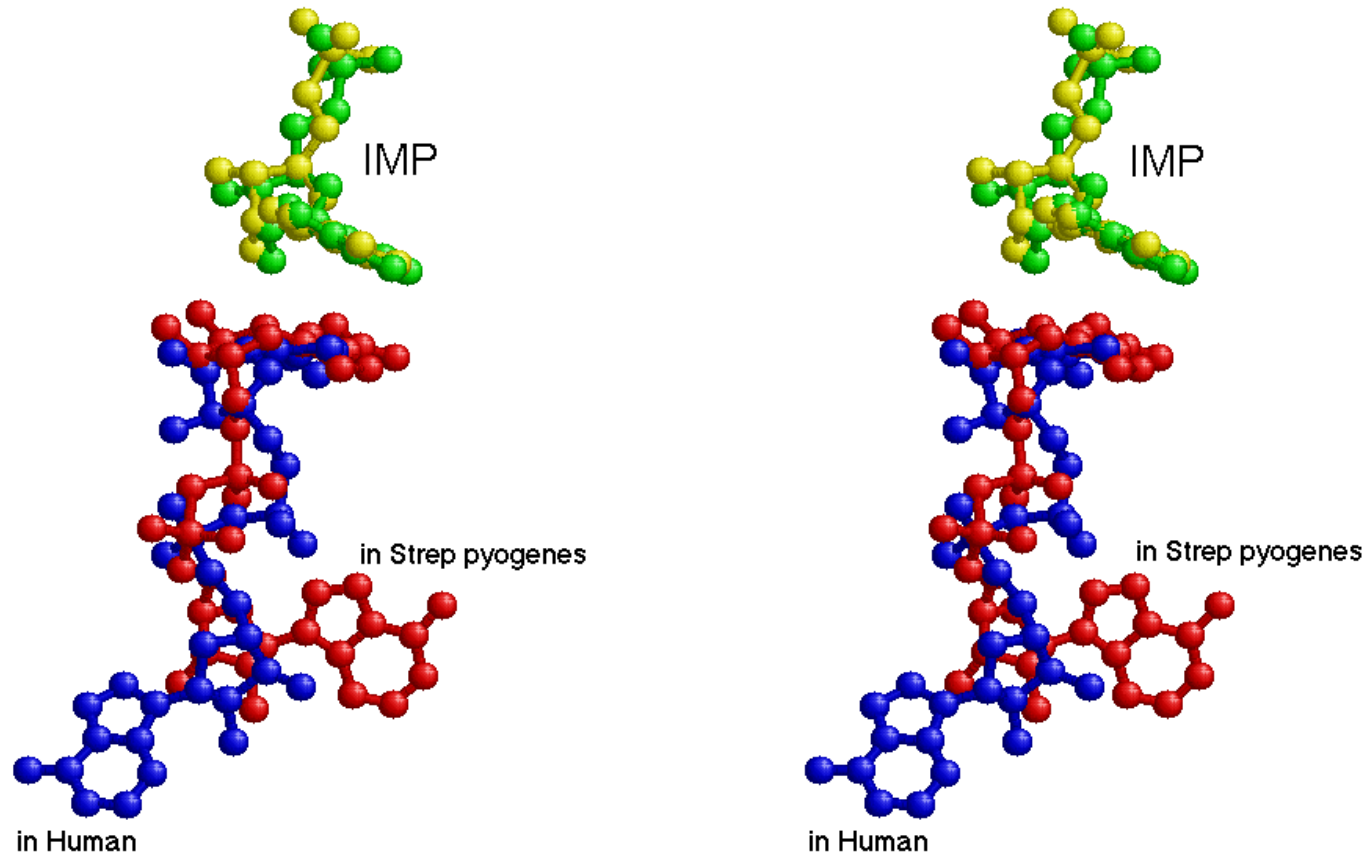
Molecular Model of Bacterial IMPDH Enzyme Active Site



Correspondence between Bacterial and Human IMPDH Active Sites: IMP/MPA binding

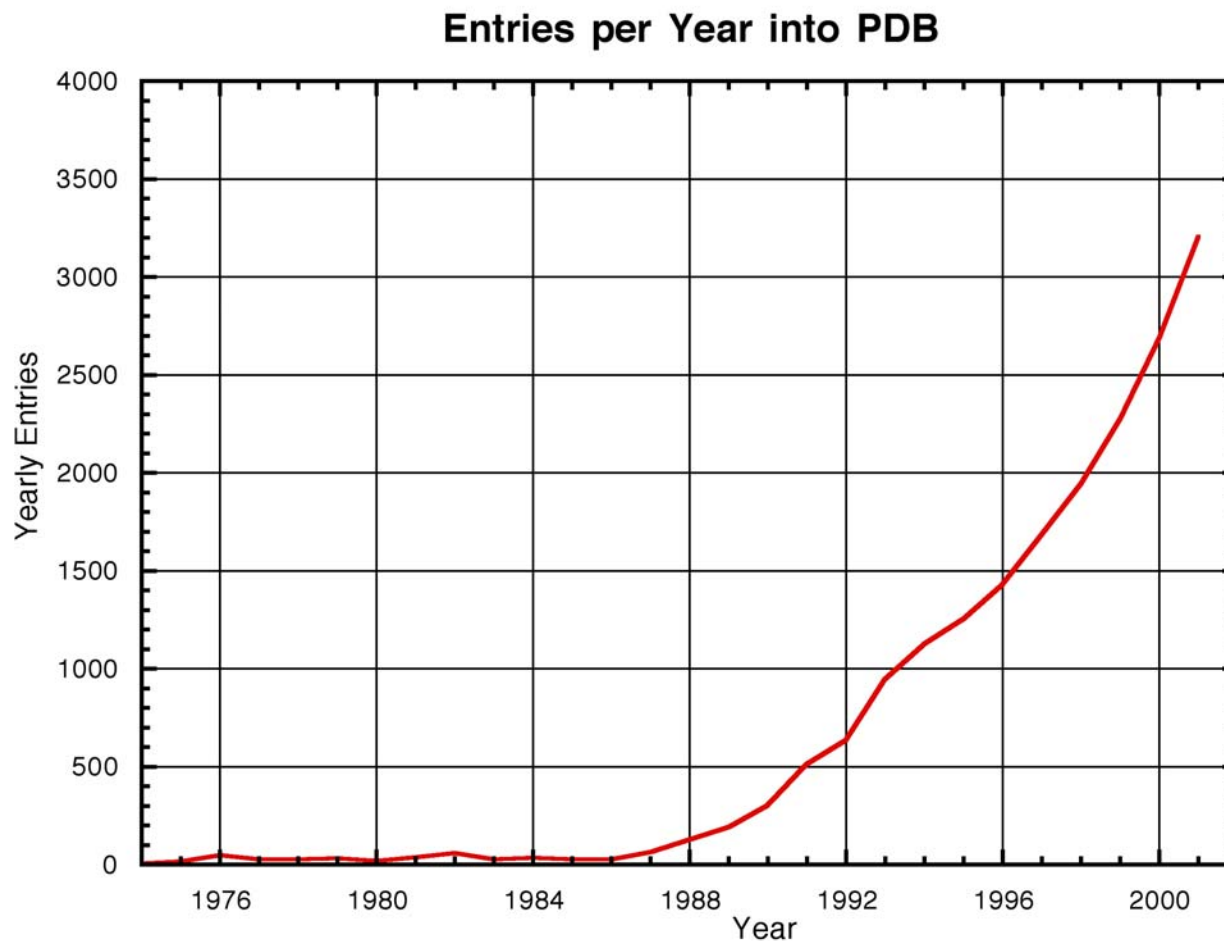


A Striking Difference Between Human and Bacterial Binding of NAD Analog TAD



Alternate sites of Adenine in 2 crystal structures

Entries into the Protein Data Bank are increasing exponentially



There are now more than 20,000 entries in the PDB.

Quality Parameters For Protein Crystallographic X-ray Detectors



(A) Dynamic Range
Noise
Efficiency
Sensitivity

(B) Speed: driven by crystal sample decay

(C) Resolving Power =
(# Bragg orders across face)
Physical Size

Fiberoptically-coupled CCD Detectors: Today's "standard"



- Integrating detector; exhibits dark image and read noise.
- Spatial resolution is good, but we want it better than this.
- Very dense pixel raster; can be made modular.
- Fast readout, but not fast enough.
- Detective Quantum Efficiency (DQE) > 50%: good, but we want more.
- Dynamic range limited to ~ 14 bits; not perfect linear response.
- Image is degraded by the fibers.
- Expensive to make very large.

Pixel Detector Benefits:



Direct absorption in silicon:

Single photon sensitivity

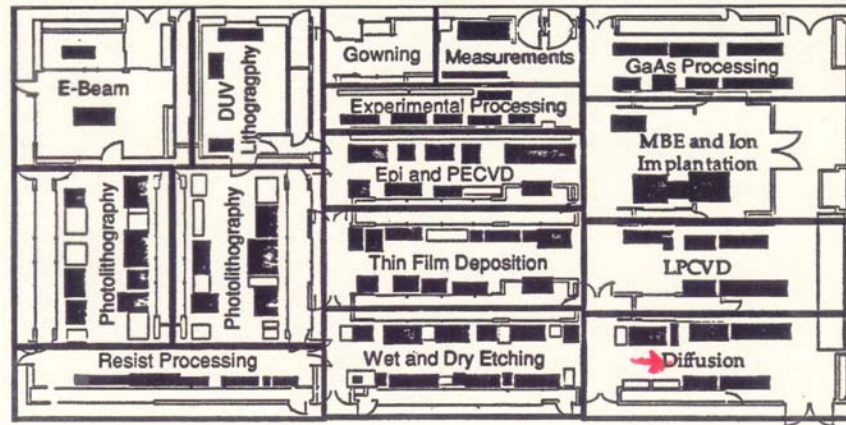
(Pseudo) counting detector: dynamic range??

Quasi-continuous readout with no deadtime

'Single pixel response' (150 μ m typical) point spread function

*Use 3D silicon with active edges => large area can then be covered with small sensors (yield!) with **no insensitive border areas***

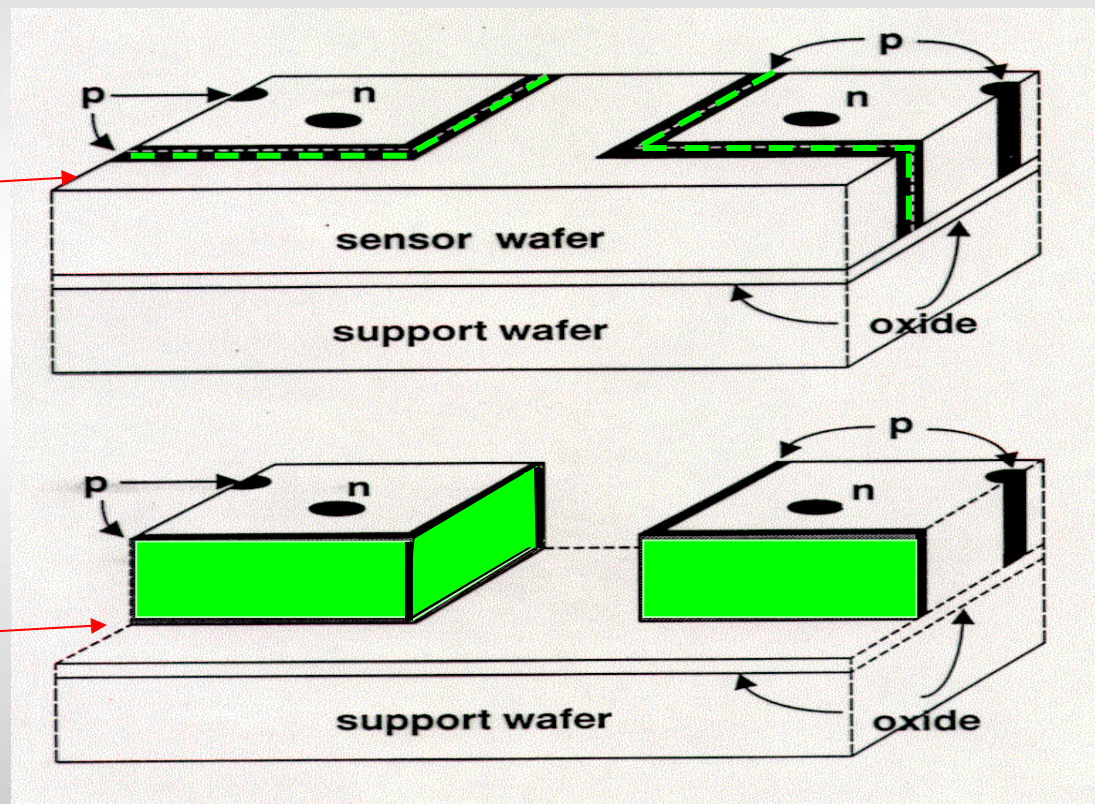
The Stanford Nanofabrication Facility



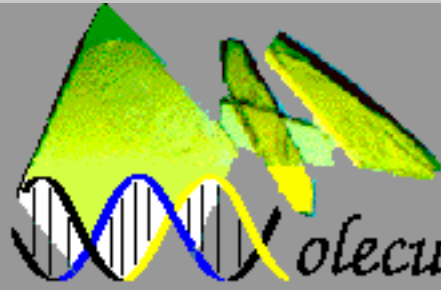
ACTIVE EDGE PROCESSING



A TRENCH IS ETCHED,
DOPED TO TERMINATE
THE E-FIELD LINES,
OXIDIZED (FOR CAPPING
AS WELL AS FOR AN
ETCH STOP), AND FILLED
WITH POLY.



AFTER THE FULL PROCESS
IS COMPLETED THE
MATERIAL SURROUNDING
THE DETECTOR IS ETCHED
AWAY TO THE OXIDE ETCH
STOP LAYER, AND THE
SUPPORT
WAFER REMOVED : NO
SAWING NEEDED!!!
(NO CHIPS, NO CRACKS)



Molecular Biology Consortium

3D Advantages

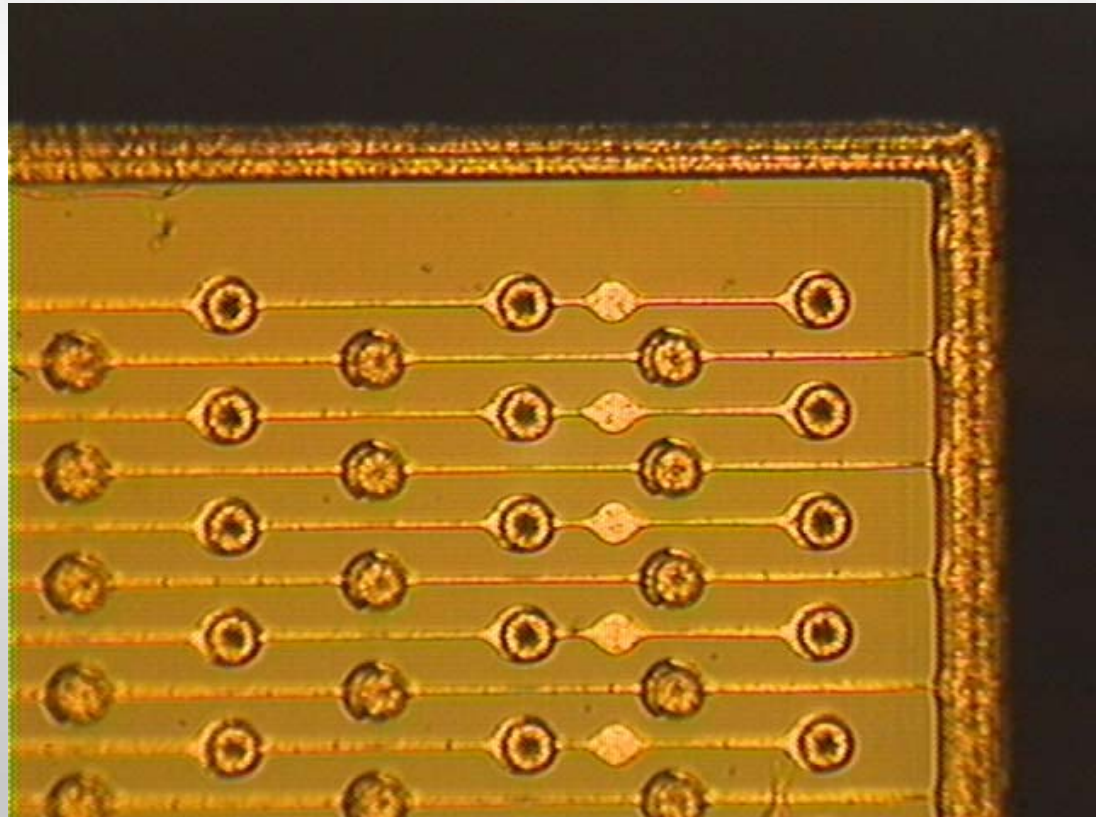
■ **Radiation Hardness**

■ **Speed**

■ **No Guard Rings**

■ **Active Edges:**

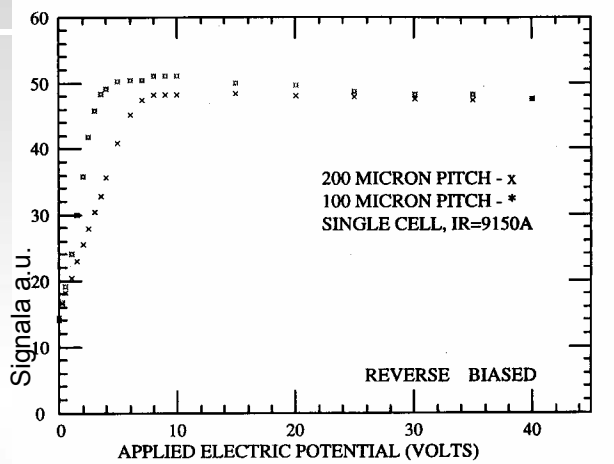
- no dead areas
- seamless tiling of large areas



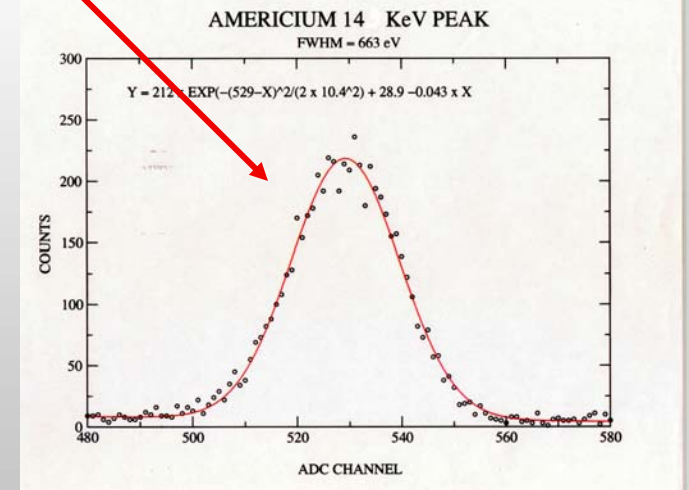
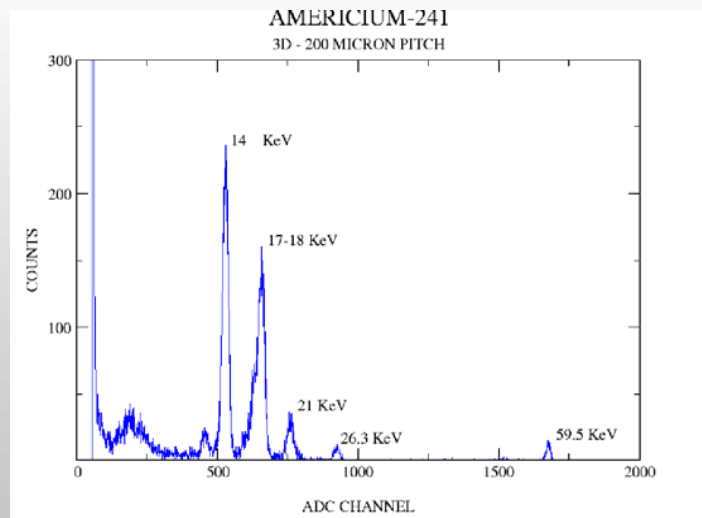
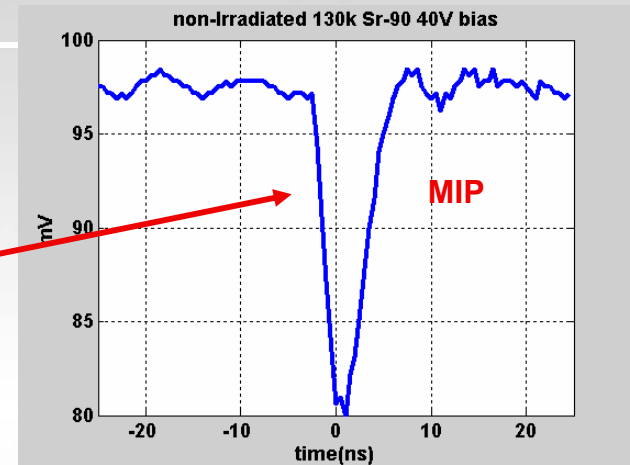
RESULTS



$I_{leak} = 0.45 \text{ nA}$ (average) 200 μm
 $I_{leak} = 0.26 \text{ nA}$ (average) 100 μm
 $C = 0.2 \text{ pF}$ per 120 μm electrode

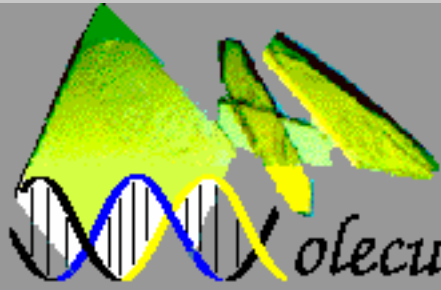


- ❖ Small V_{FD} 5-8 V
- ❖ Speed 1.5, 3.5 ns
- ❖ Energy resolution
- ❖ Gaussian response



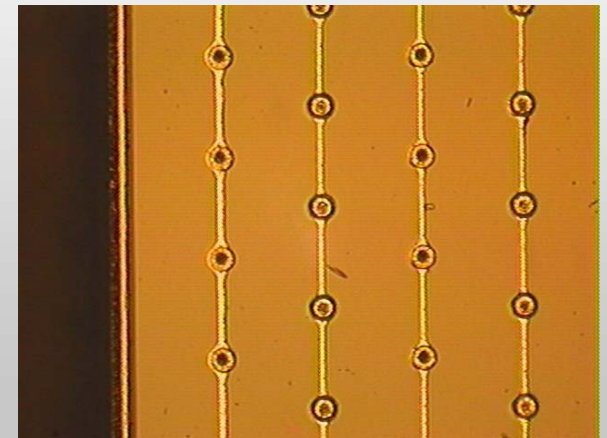
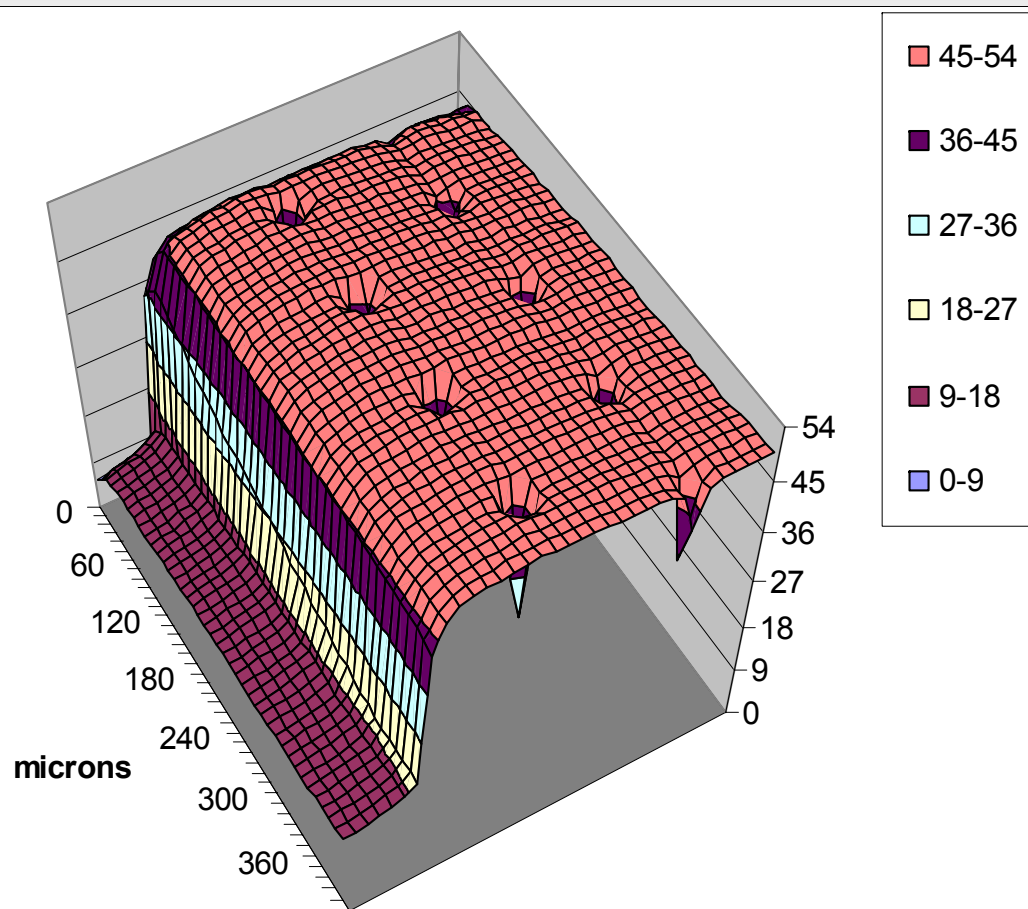
*Fast Electronics CERN MIC : P. Jarron et al. NIM A 377 (1996) 435

*2 μs electronics made at LBL

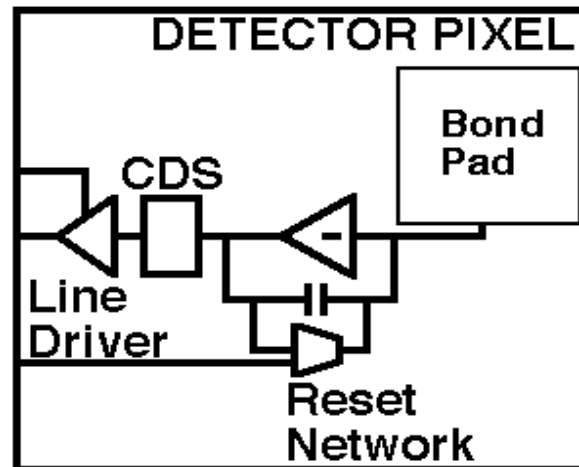
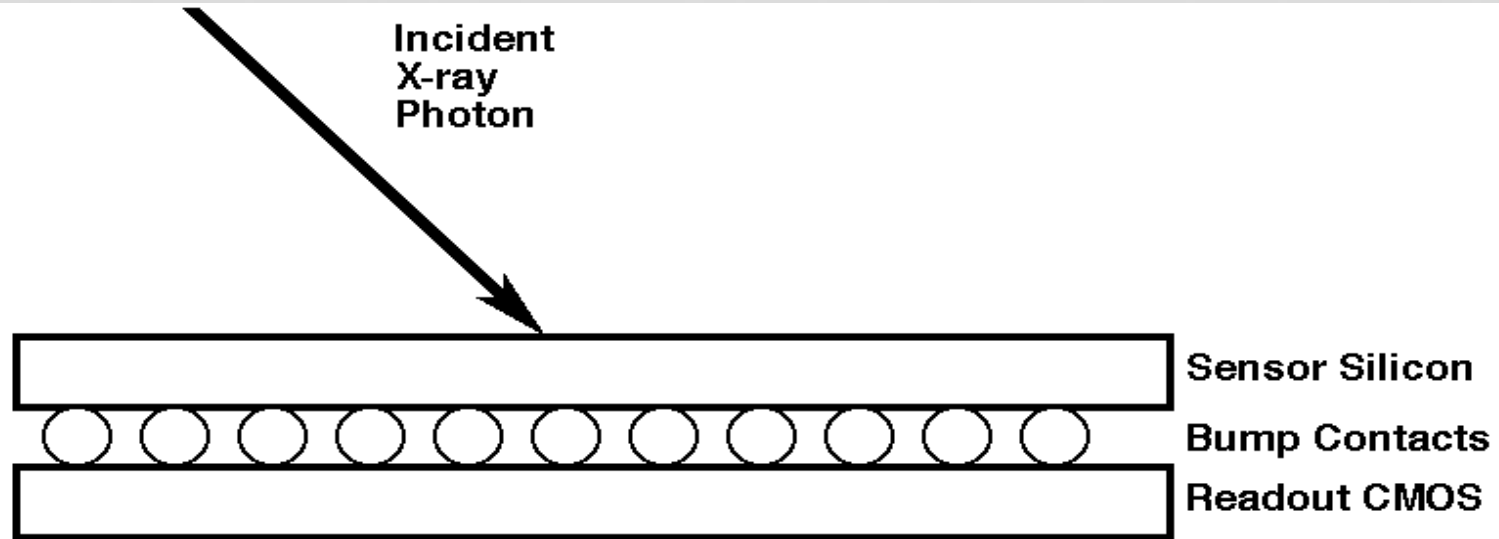


Molecular Biology Consortium

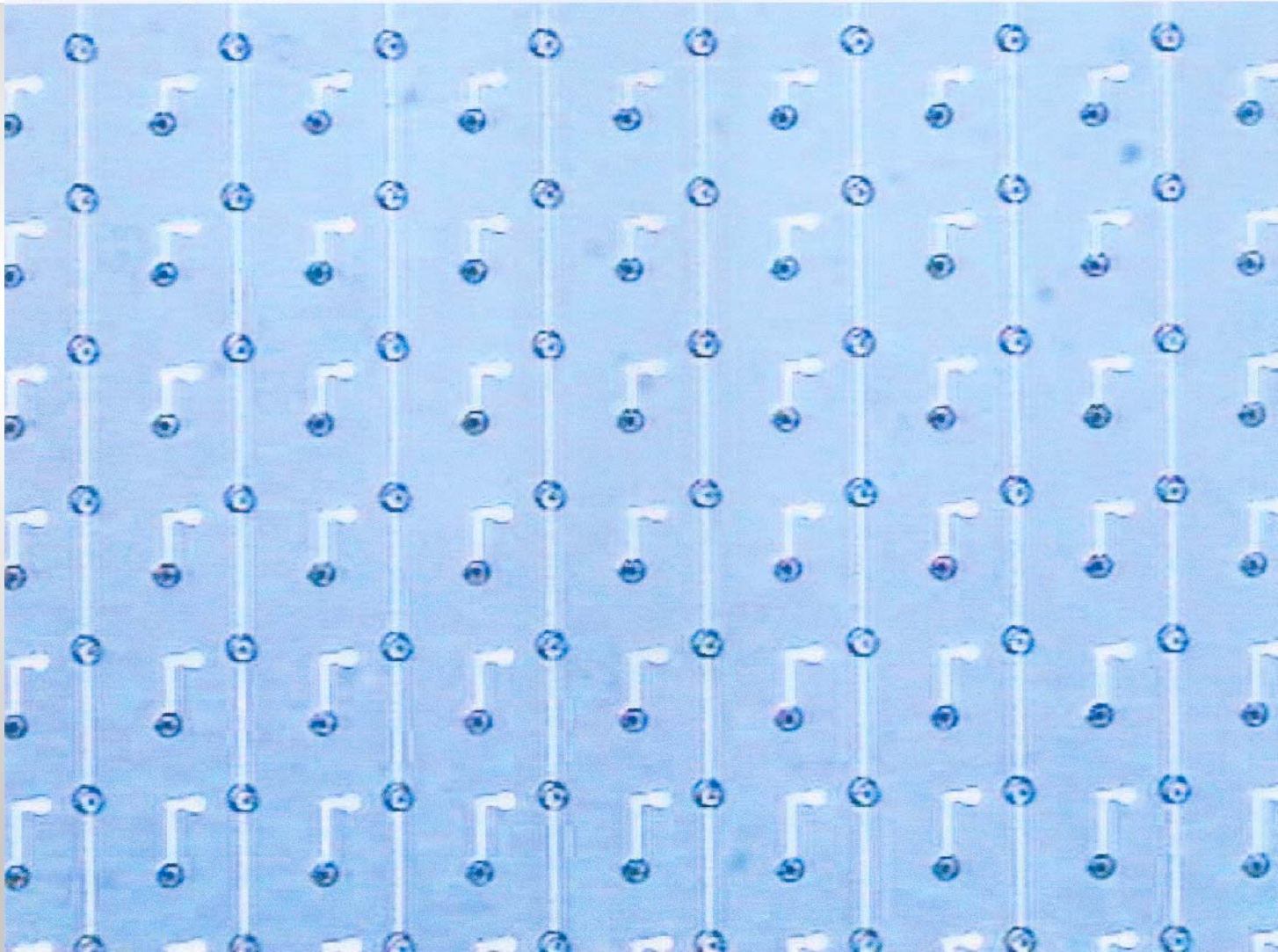
■ 12 KeV X Rays at ALS beamline 10.3



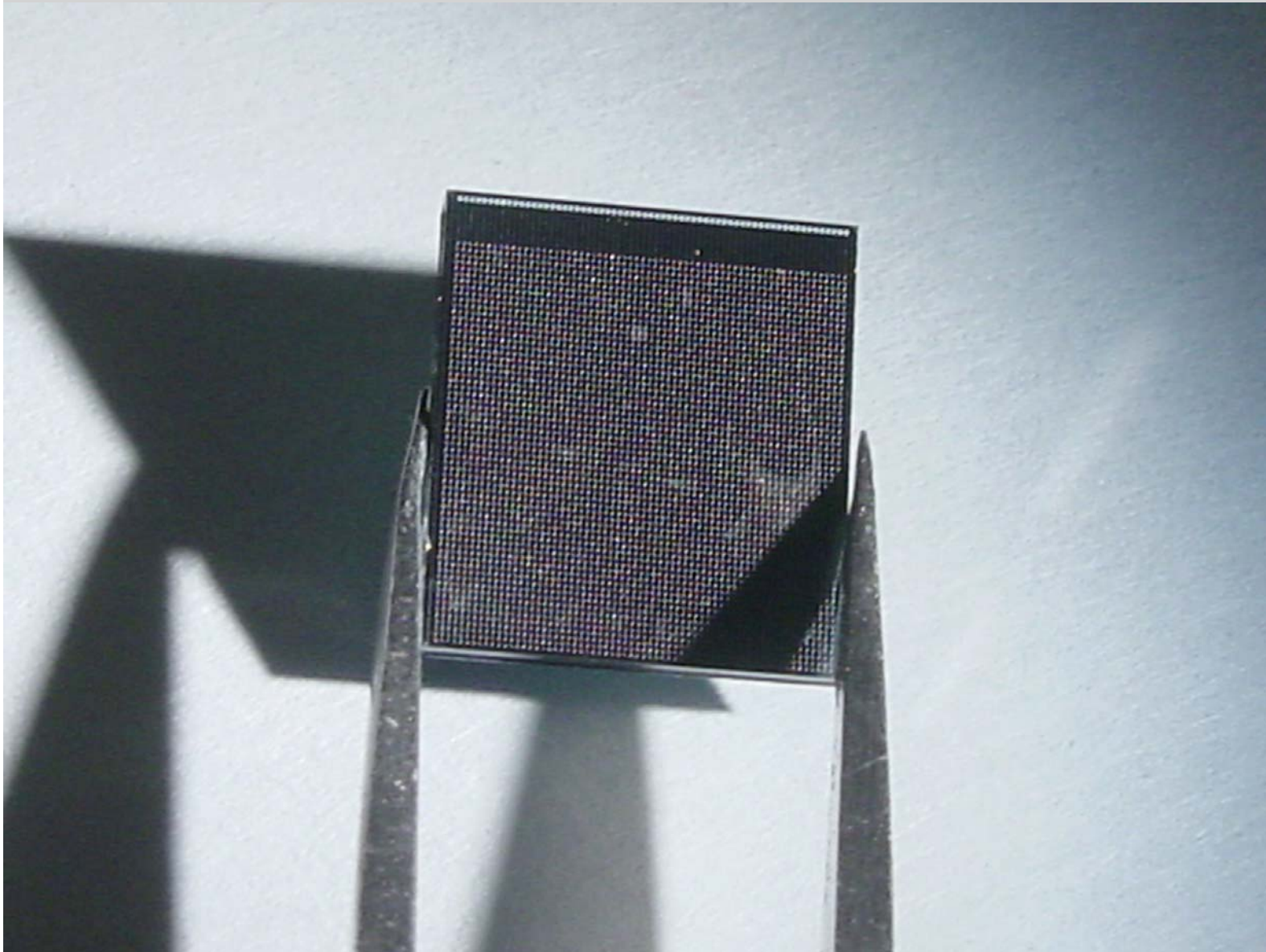
Each sensor pixel must be hybridized (bump-bonded) to an ASIC pixel:



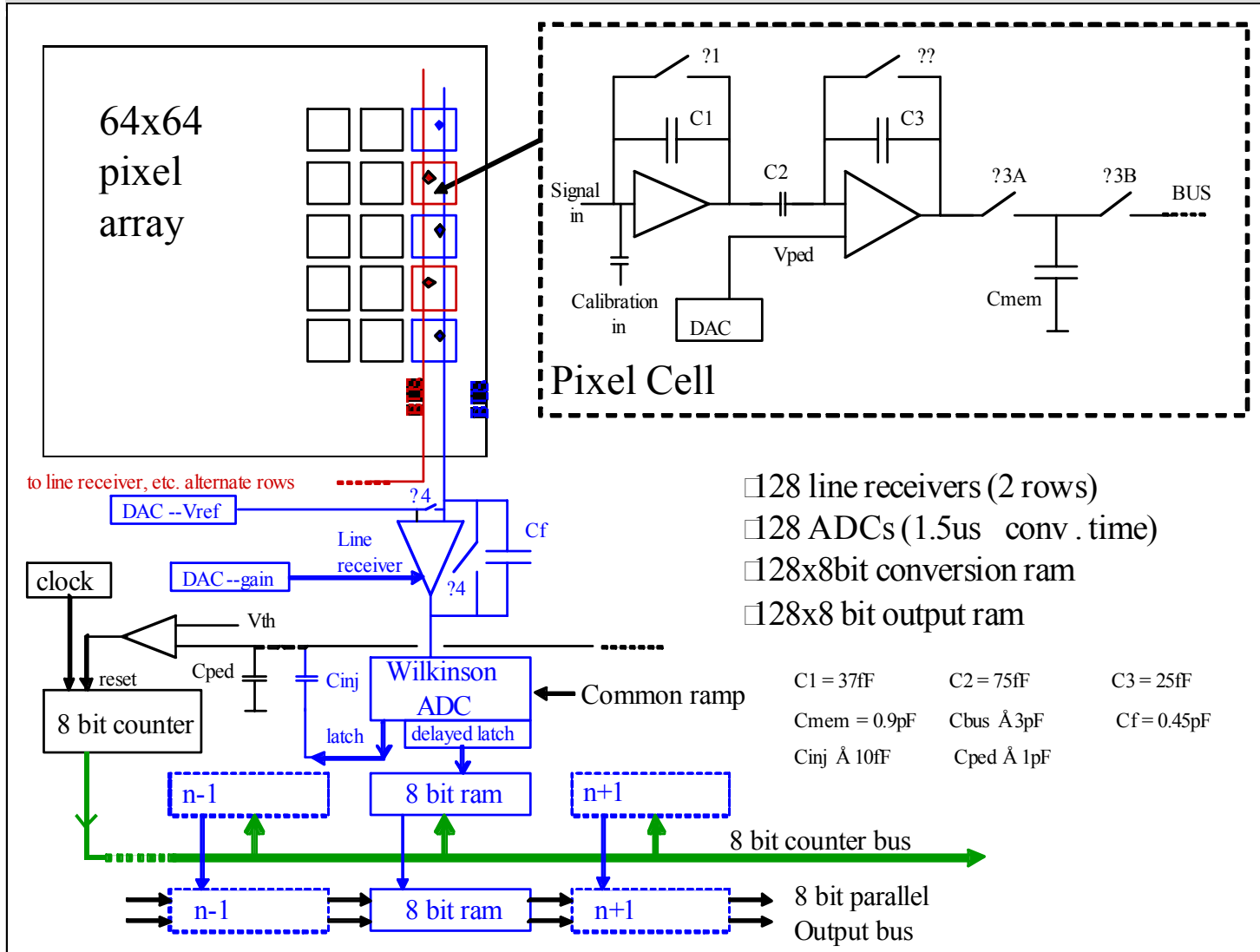
Electron micrograph of actual sensor chip, with electrodes and bump-bonding pads



One complete sensor, with indium bumps



ASIC Schematic: derived from ATLAS SVX-4: 128-fold parallel architecture



In each pixel:
Charge preamp,
gain trim, CDS

6 - 8 bit ADC
encoding

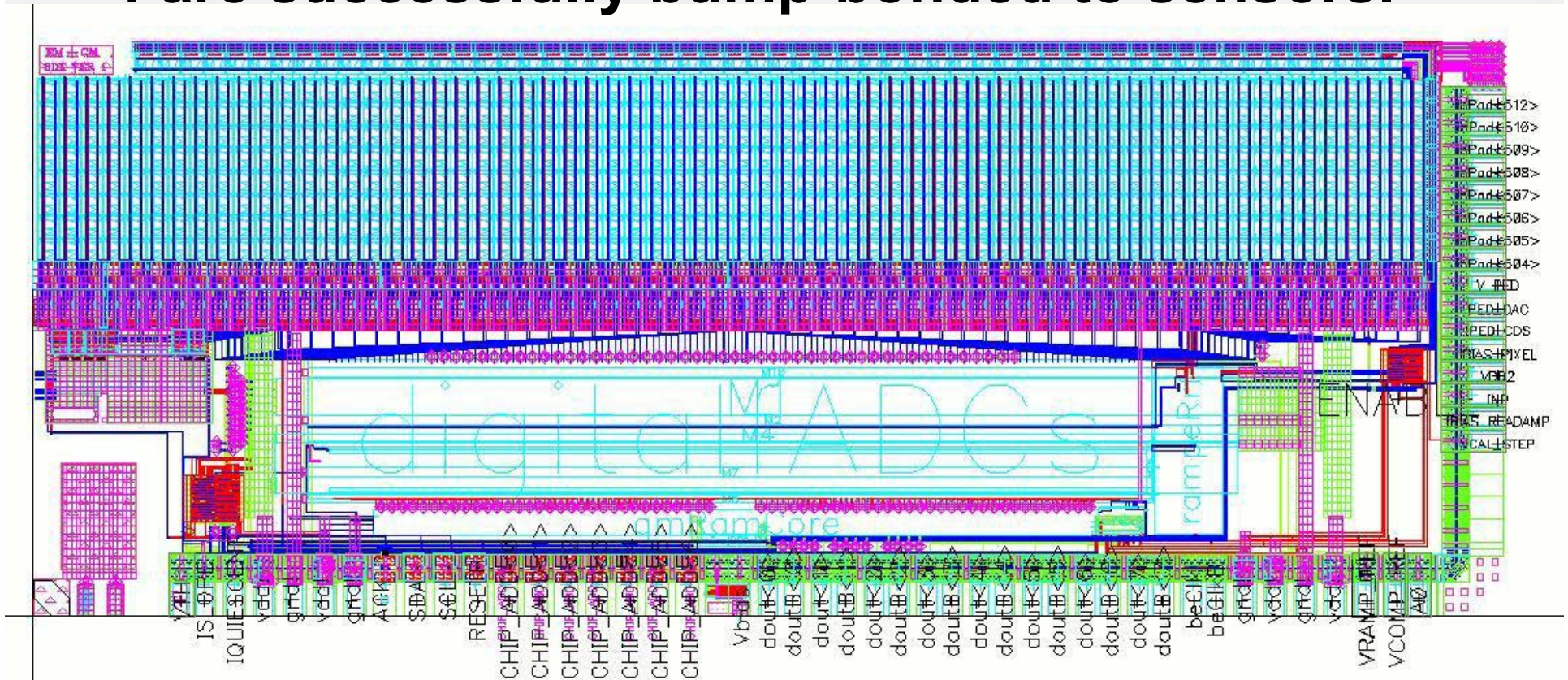
Continuous
readout cycling,
64 μsec for entire
chip

- 128 line receivers (2 rows)
- 128 ADCs (1.5us conv. time)
- 128x8bit conversion ram
- 128x8 bit output ram

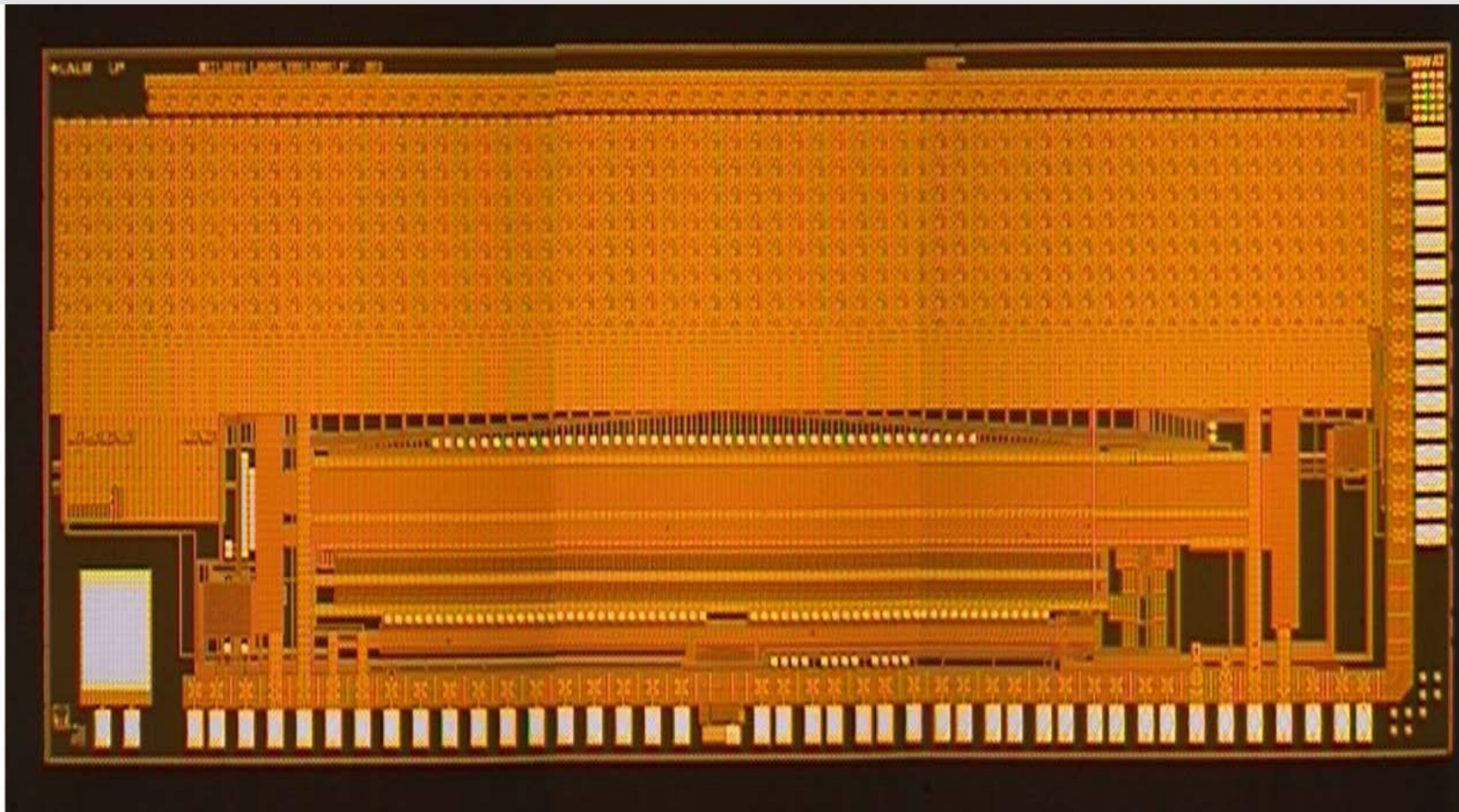
The ASIC was designed at LBL, based on ATLAS SVX-4. It took 2 MOSIS runs to get it right.



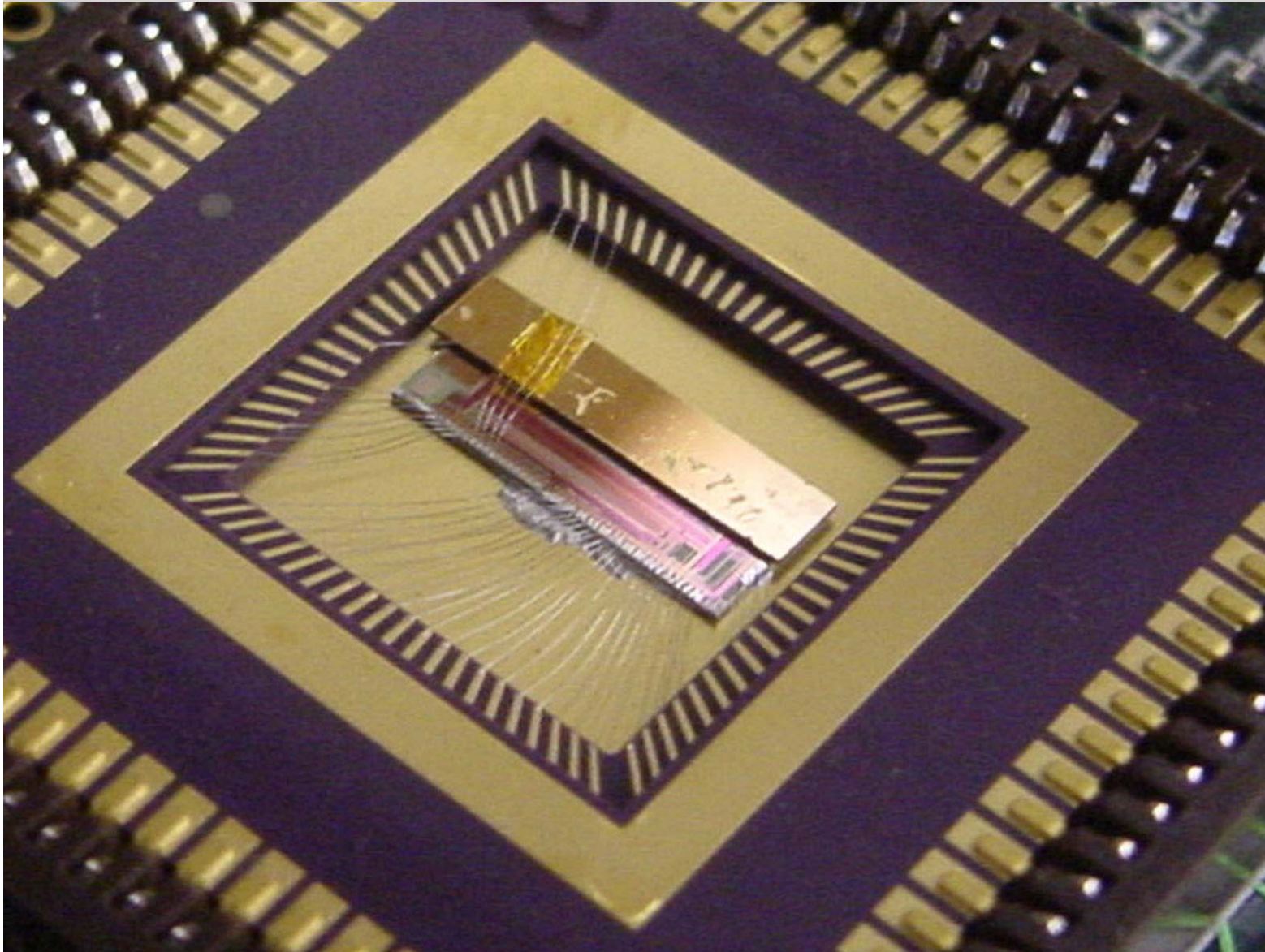
As of March 2003, we have 40 working ASICS. 4 are successfully bump-bonded to sensors.



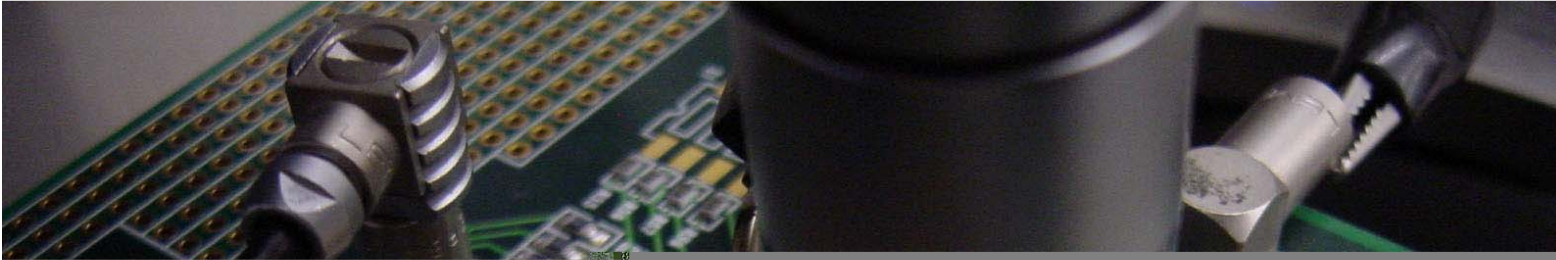
**For testing purposes,
the ASIC has an 8 x 64 pixel
array (to save cost)**

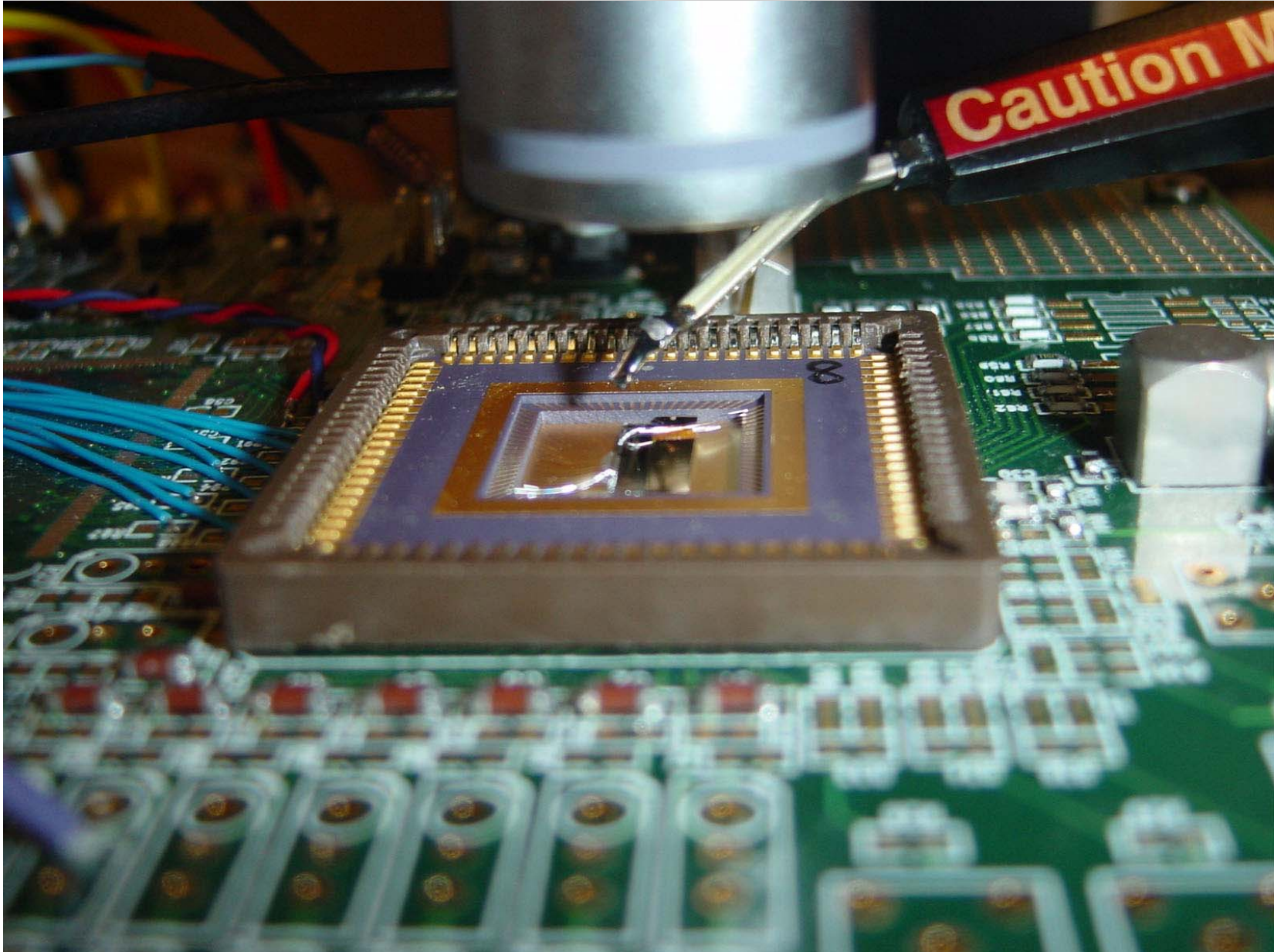


Sensor, bonded to ASIC, in a testing carrier

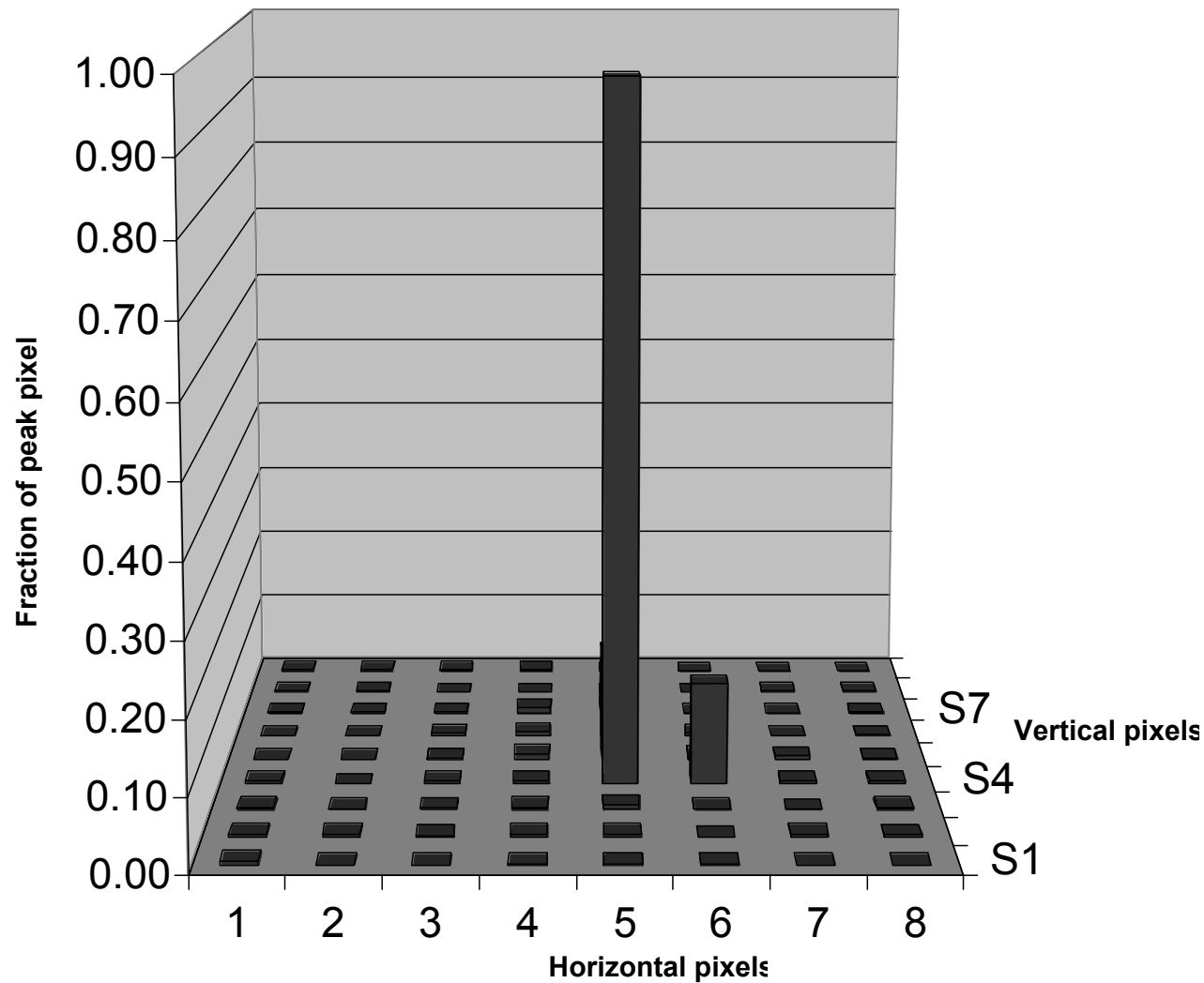


Assembly on a PC board, being electronically evaluated





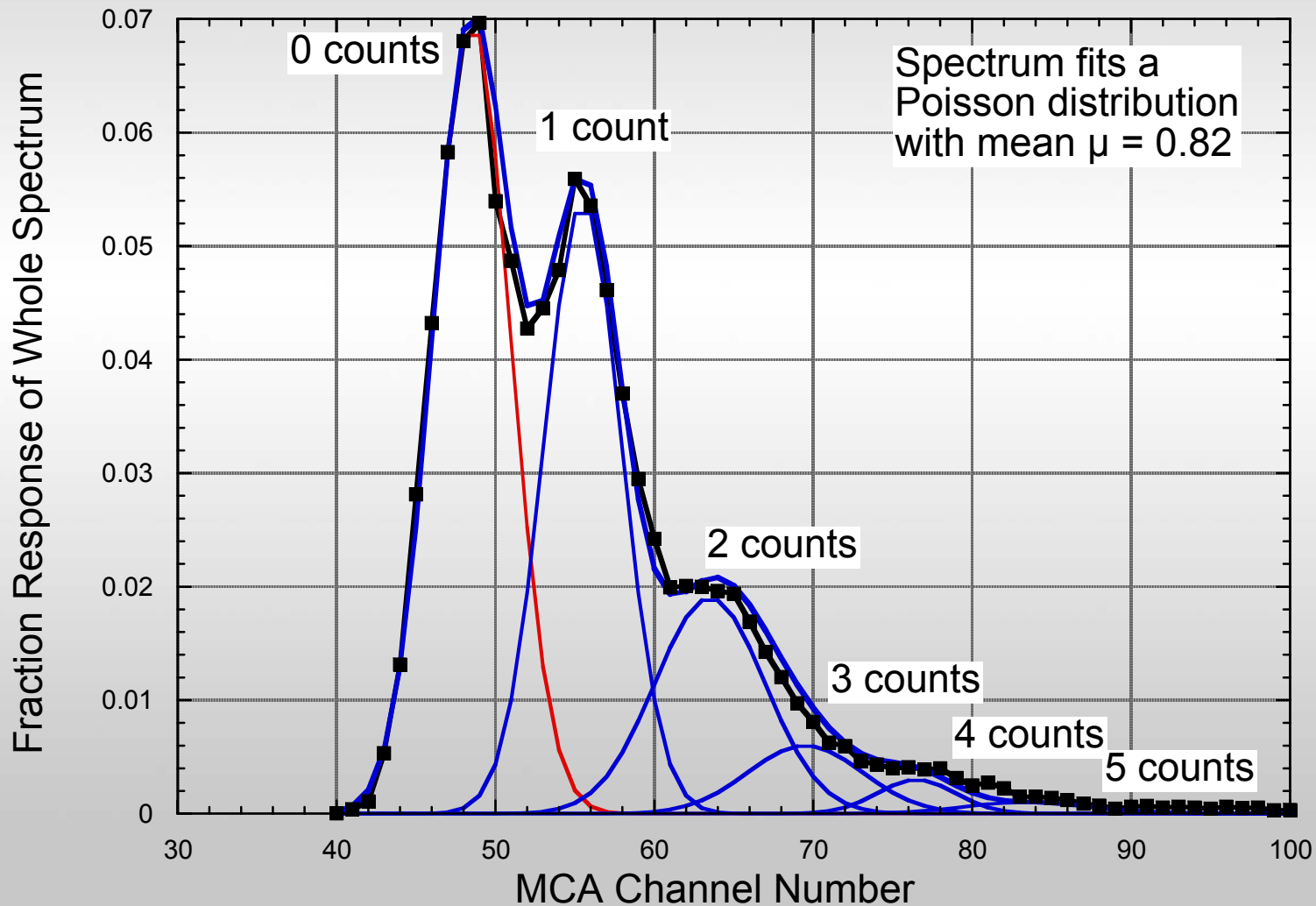
In the X-ray beam: Geometric Signal Distribution: Single-Pixel Response

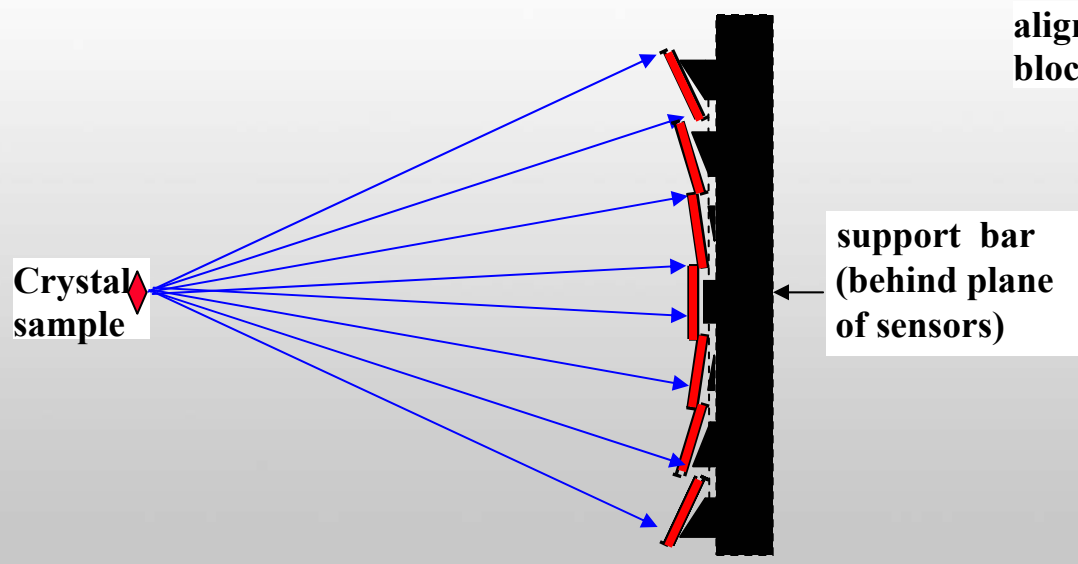
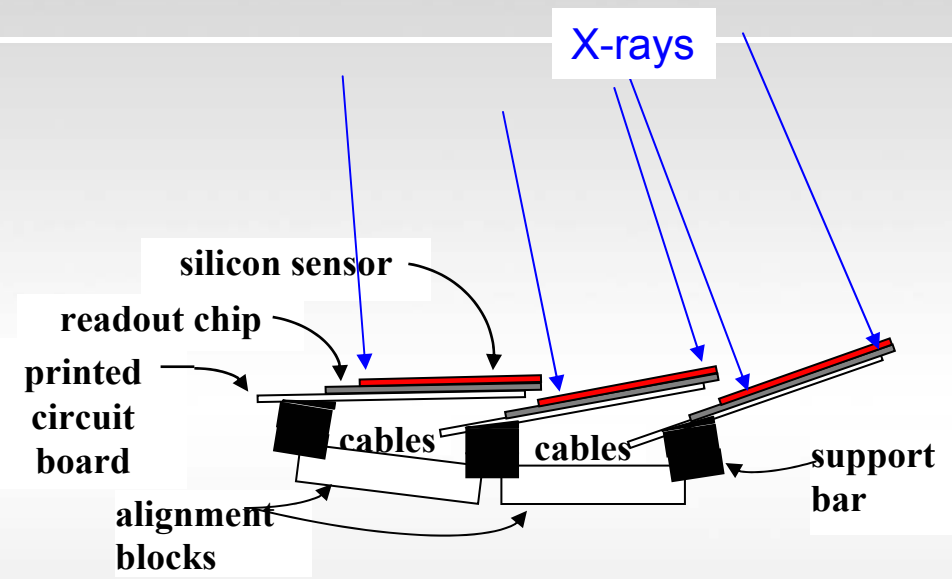
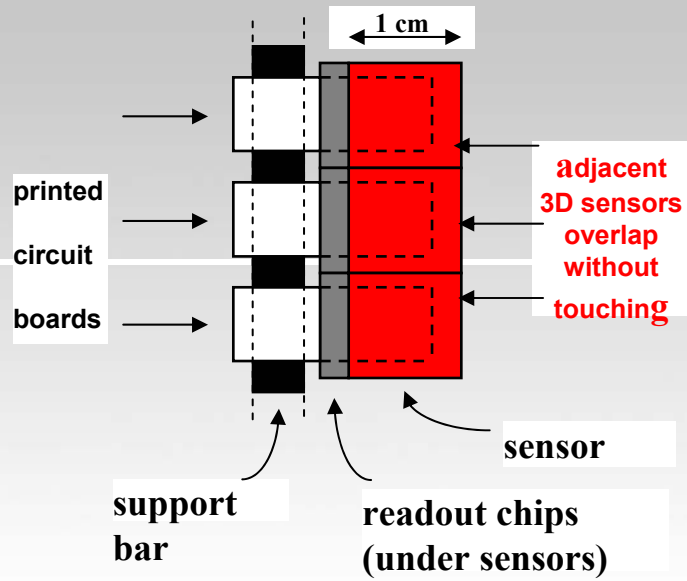


Direct X-ray beam response: Poisson distribution of counts in each pixel



Spectrum in a Multichannel Analyzer





Schematic design for full scale protein crystallography detector (~300 x 300mm²)