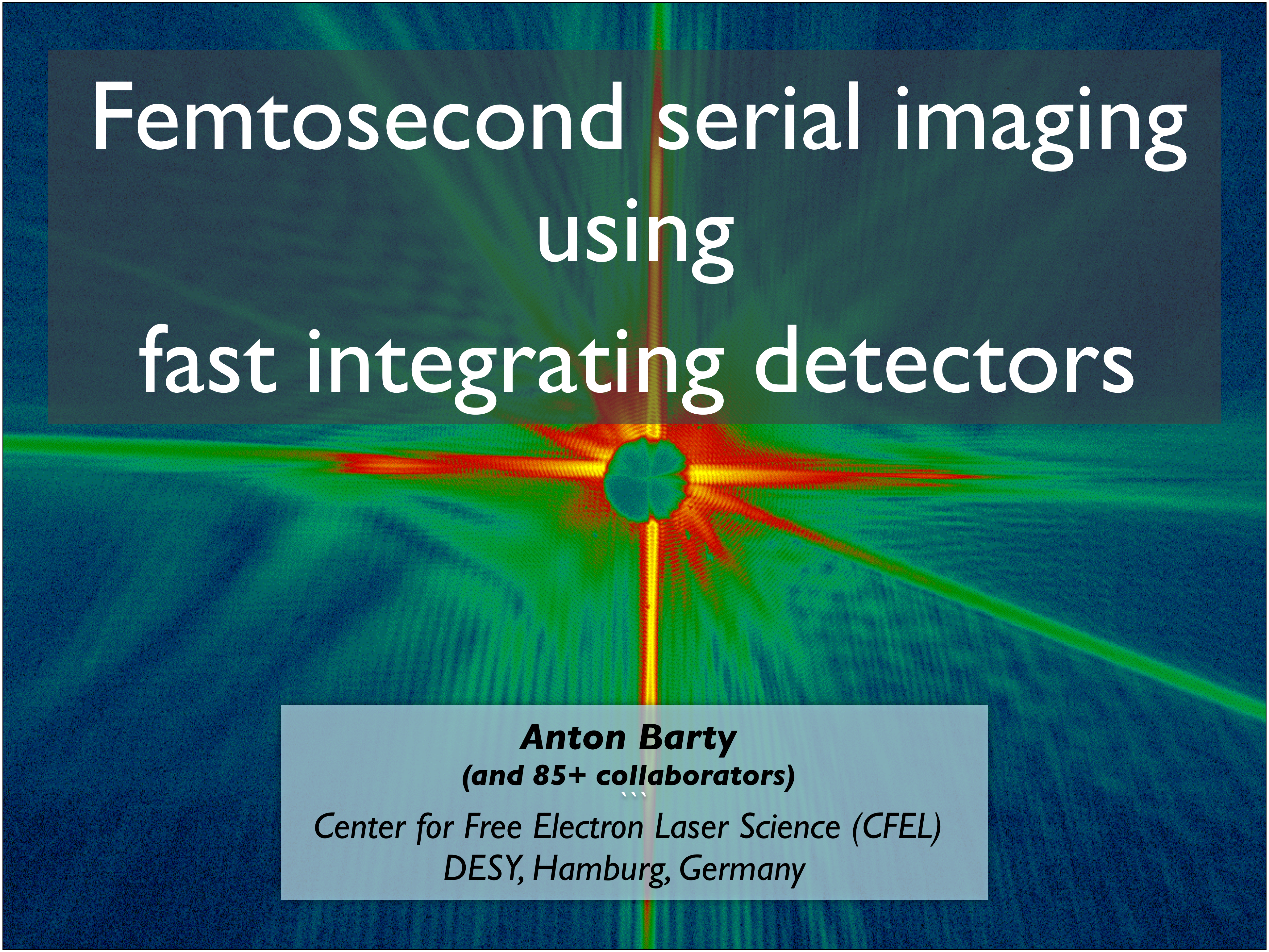
Femtosecond serial imaging using fast integrating detectors



Anton Barty
(and 85+ collaborators)
Center for Free Electron Laser Science (CFEL)
DESY, Hamburg, Germany

This work was the product of a large international team

Henry N. Chapman[1,2], Petra Fromme[3], Anton Barty[1], Thomas A. White[1], Richard A. Kirian[4], Andrew Aquila[1], Mark S. Hunter[3], Joachim Schulz[1], Daniel P. DePonte[1], Uwe Weierstall[4], R. Bruce Doak[4], Filipe R.N.C. Maia[5], Andrew Martin[1], Ilme Schlichting[6,7], Lukas Lomb[7], Nicola Coppola[1], Robert L. Shoeman[7], Sascha Epp[6,8], Robert Hartmann[9], Daniel Rolles[6,7], Artem Rudenko[6,8], Lutz Foucar[6,7], Nils Kimmel[10], Georg Weidenspointner[11,10], Peter Holl[9], Mengning Liang[1], Miriam Barthelmess[12], Carl Caleman[1], Sébastien Boutet[13], Michael J. Bogan[14], Jacek Krzywinski[13], Christoph Bostedt[13], Saša Bajt[12], Lars Gumprecht[1], Benedikt Rudek[6,8], Benjamin Erk[6,8], Carlo Schmidt[6,8], André Hömke[6,8], Christian Reich[9], Daniel Pietschner[10], Lothar Strüder[6,10], Günther Hauser[10], Hubert Gorke[15], Joachim Ullrich[6,8], Sven Herrmann[10], Gerhard Schaller[10], Florian Schopper[10], Heike Soltau[9], Kai-Uwe Kühnel[8], Marc Messerschmidt[13], John D. Bozek[13], Stefan P. Hau-Riege[16], Matthias Frank[16], Christina Y. Hampton[14], Raymond Sierra[14], Dmitri Starodub[14], Garth J. Williams[13], Janos Hajdu[5], Nicusor Timneanu[5], M. Marvin Seibert[5], Jakob Andreasson[5], Andrea Rocker[5], Olof Jönsson[5], Stephan Stern[1], Karol Nass[2], Robert Andritschke[10], Claus-Dieter Schröter[8], Faton Krasniqi[6,7], Mario Bott[7], Kevin E. Schmidt[4], Xiaoyu Wang[4], Ingo Grotjohann[3], James Holton[17], Stefano Marchesini[17], Sebastian Schorb[18], Daniela Rupp[18], Marcus Adolph[18], Tais Gorkhover[18], Martin Svenda[5], Helmut Hirsemann[12], Guillaume Potdevin[12], Heinz Graafsma[12], Björn Nilsson[12], and John C. H. Spence[4]

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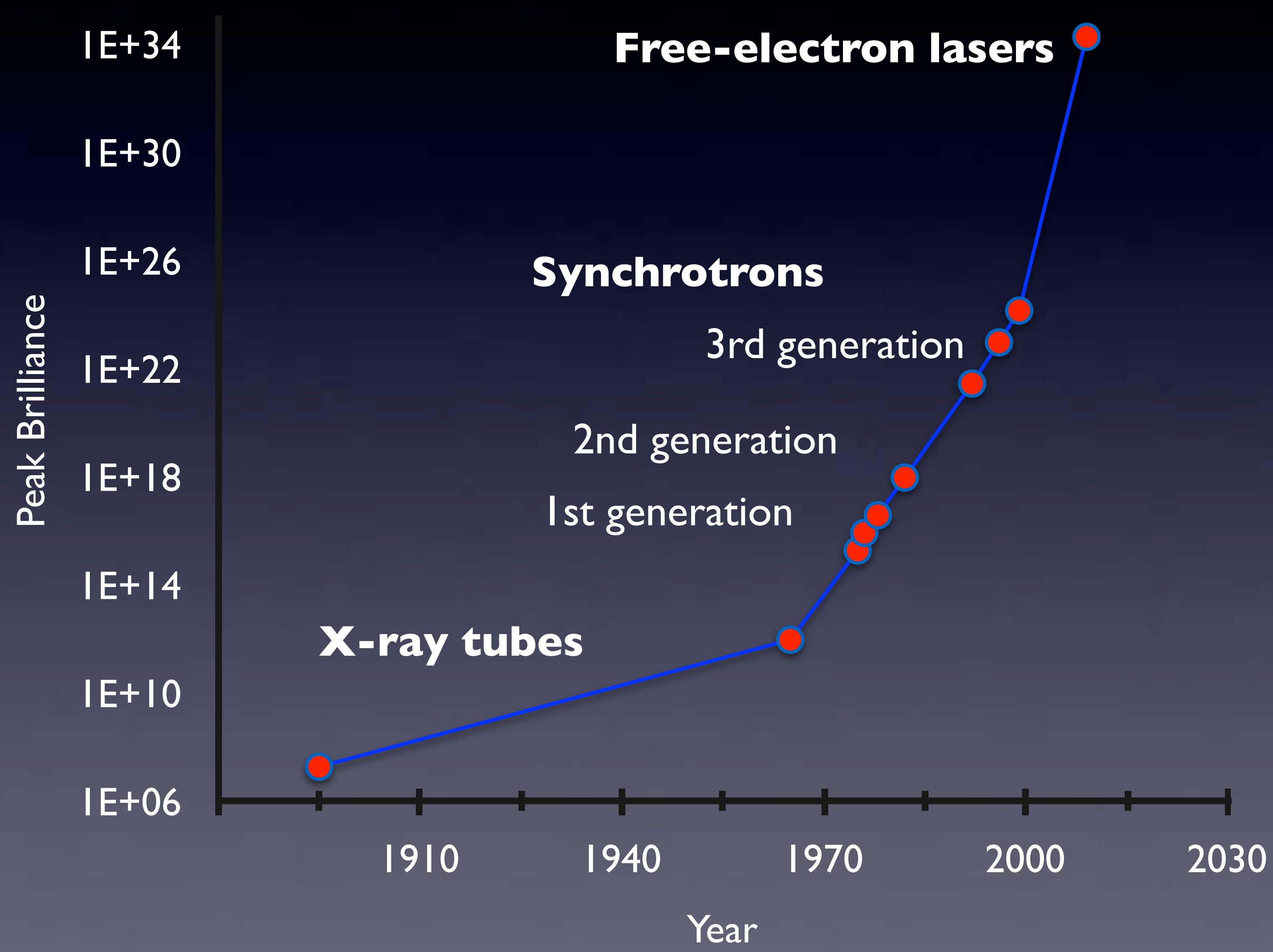
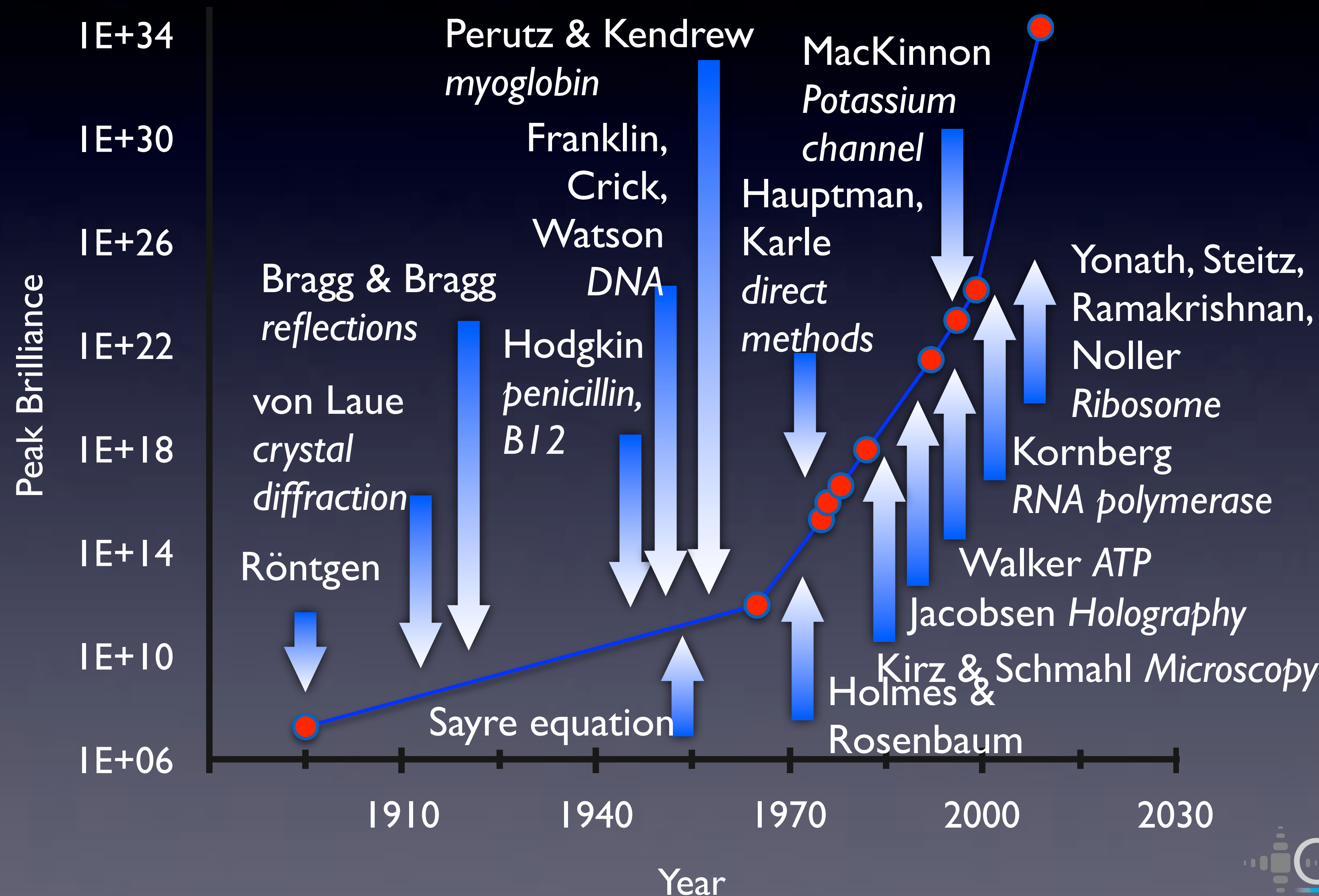
X-ray sources have developed at a staggering pace since their discovery in 1895



CFEL SCIENCE

Some great moments in X-ray science



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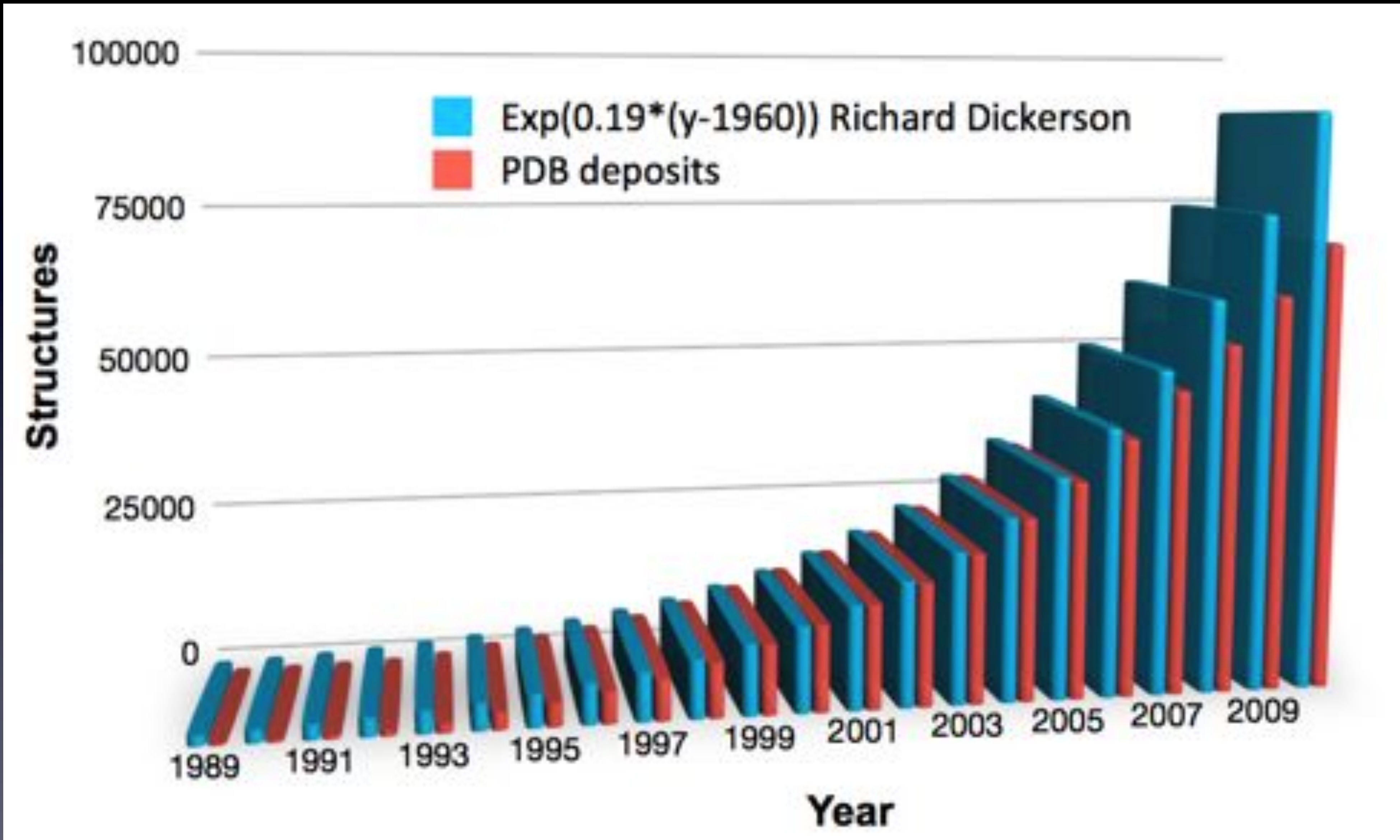
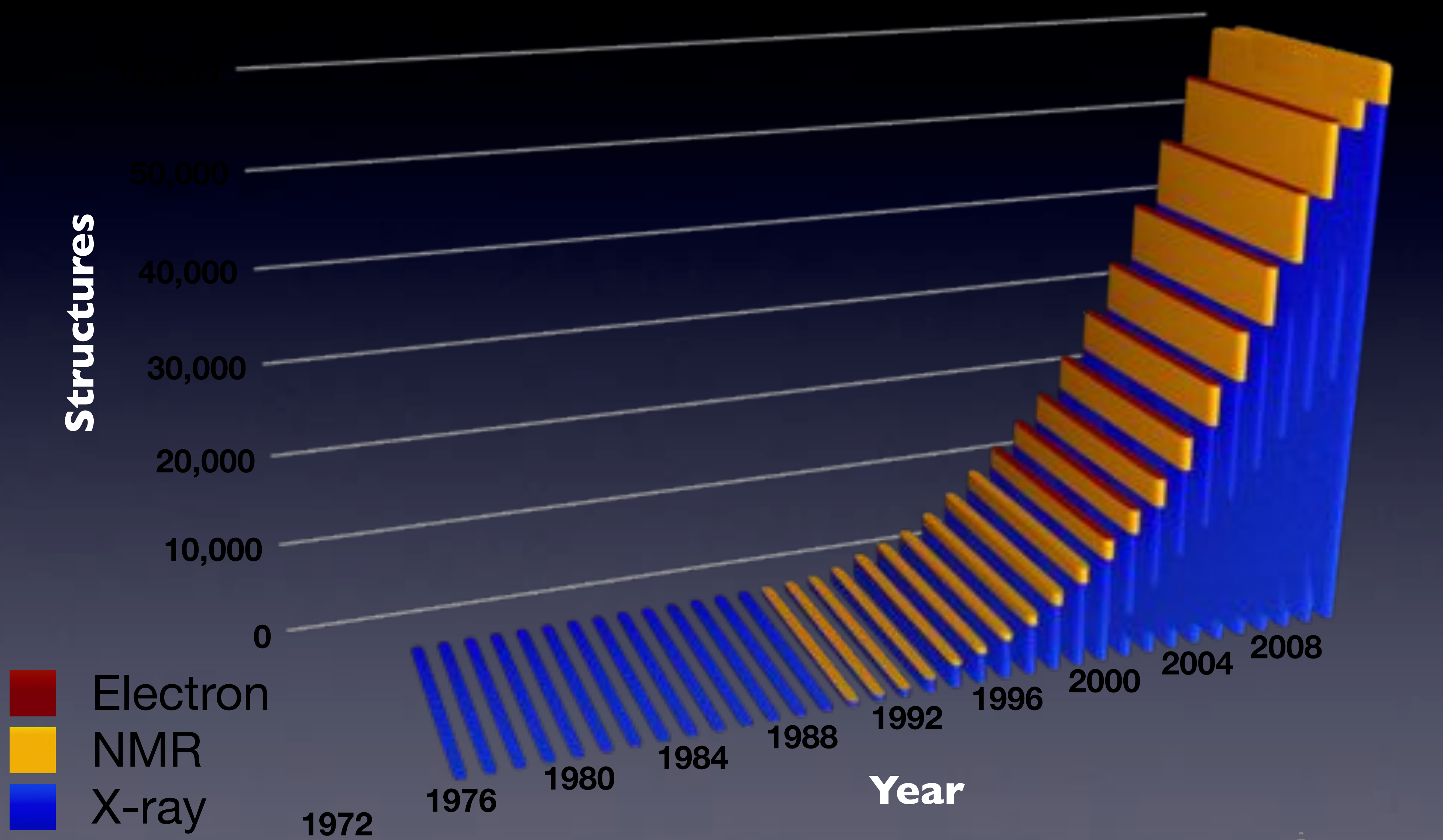
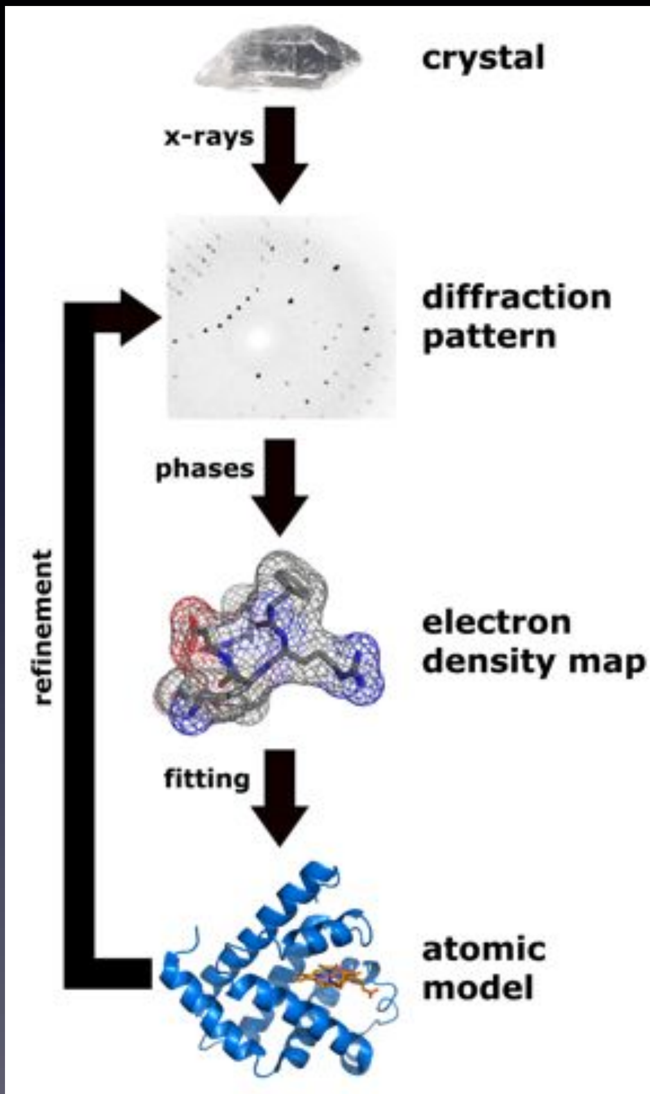
The number of solved protein structures is now increasing linearly with time



The bulk of protein structures have been solved using X-ray crystallography



X-ray crystallography requires large, well ordered crystals to overcome radiation damage



http://en.wikipedia.org/wiki/Image:X_ray_diffraction.png

- >52,684 PDB entries
- but only
  - ~10,000 *distinct* structures
  - 114 integral membrane proteins
- **The bottleneck is in growing good crystals**
- Membrane proteins are especially important (eg: for drug delivery)
- *Grand challenge:* Can we revolutionise molecular biology by imaging isolated molecules ?

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X-ray crystallography is powerful, but growing the crystals is often difficult

Consider the case of RNA polymerase II

1972: started to investigate structure

1983: 2D 'crystal' obtained

Nature 301, 125 (1983)

1991: 3D crystal growth observed

J.Mol.Biol. 221, 347 (1991); Nat.Struct.Biol 1, 195 (1994)

1999: Crystal oxidation problem solved

Cell, 98, 799 (1999)

2000: First detailed crystal structure

Cramer et.al, Science 228, 640 (2000)

Why crystals?

Signal-to-noise

Radiation damage

The bottleneck in structural biology is frequently growing good crystals

RNA Polymerase II
Extracted from Cramer et.al, Science 228, 640 (2000)

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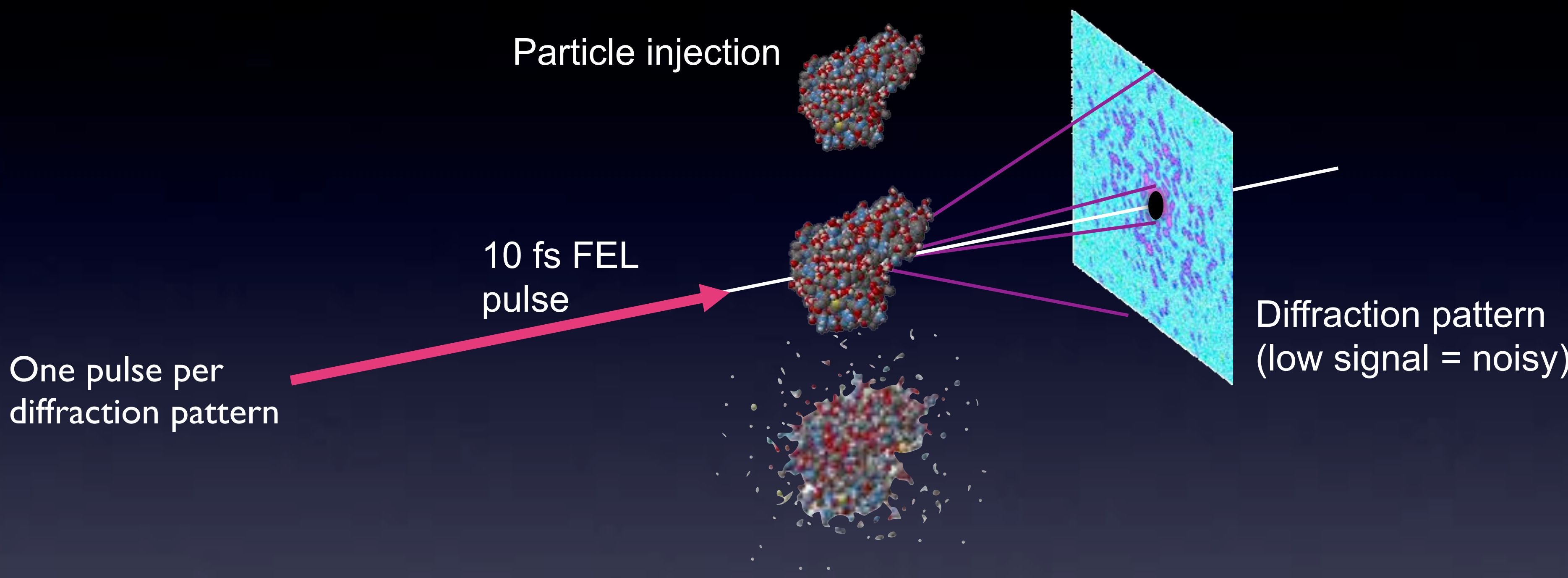
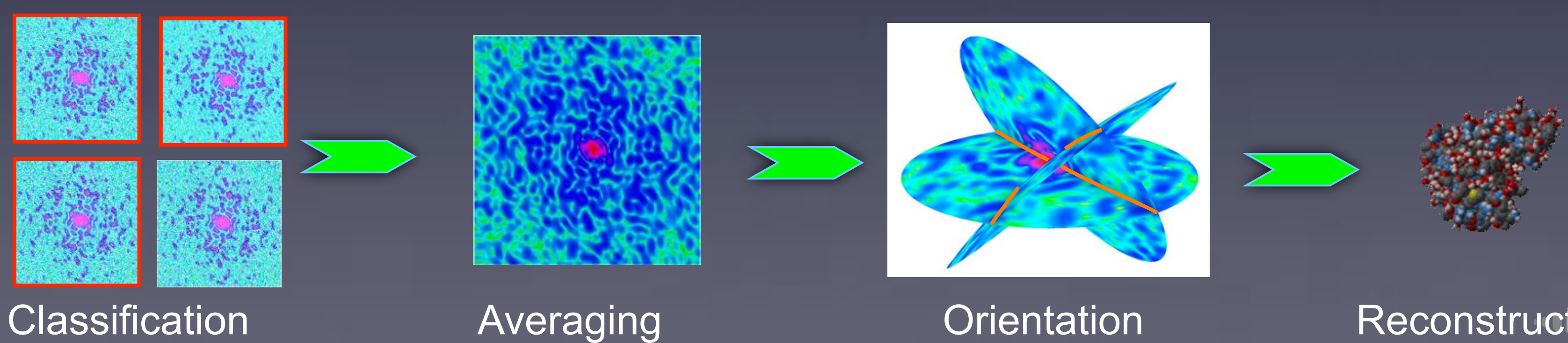
X-ray free-electron lasers may enable atomic-resolution imaging of macromolecules without the need to grow large crystals

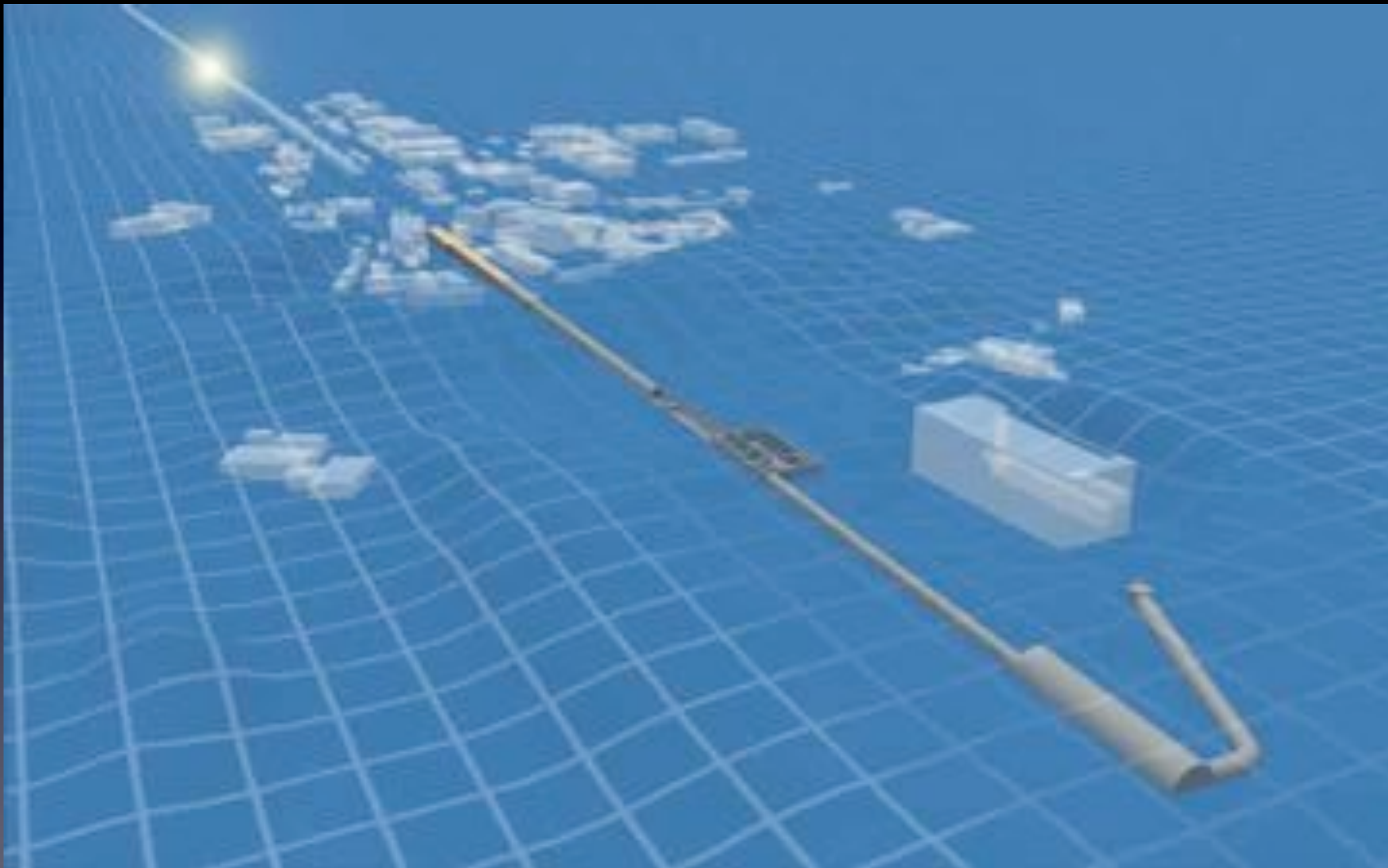


Combine 10^5-10^7 measurements



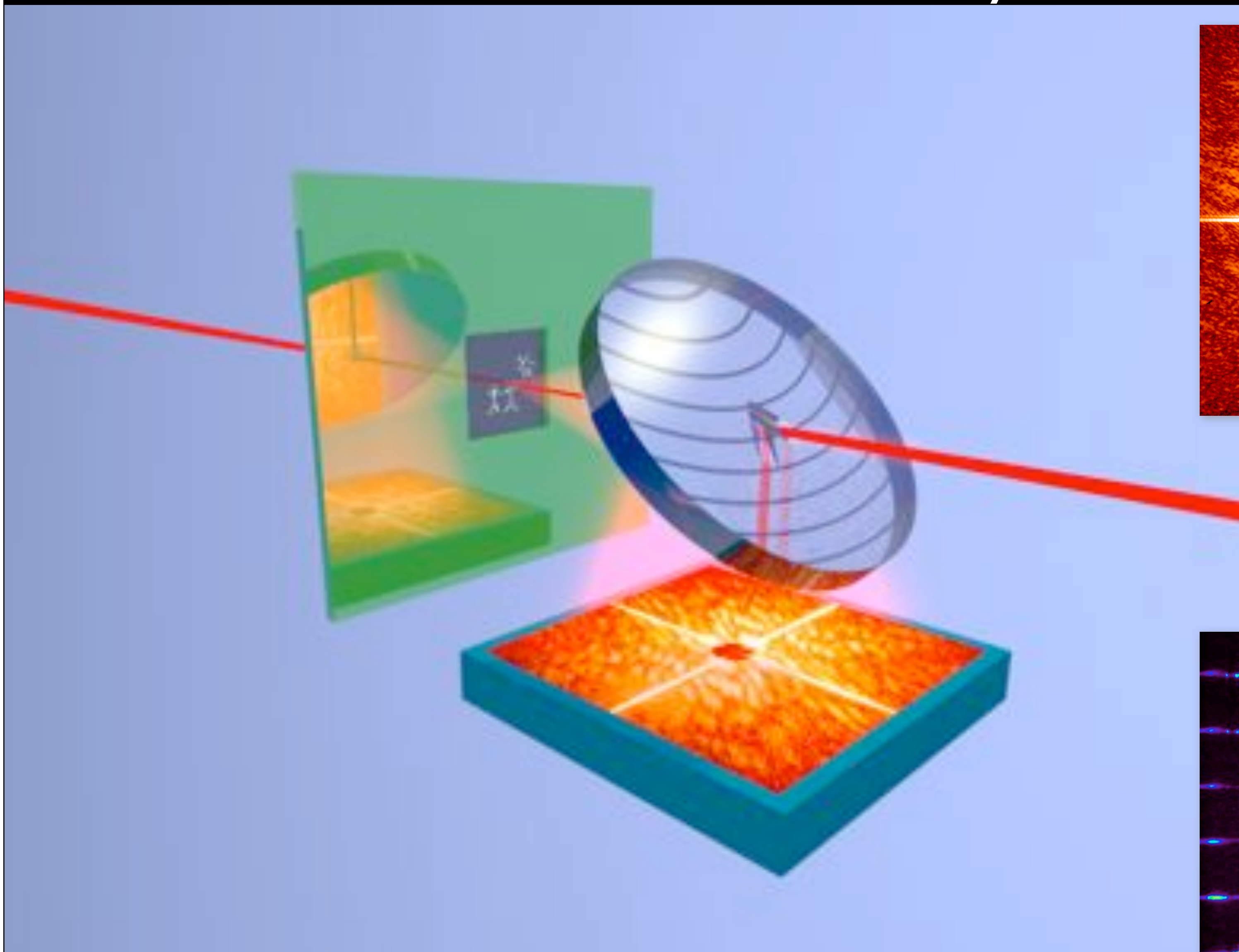
CFEL SCIENCE

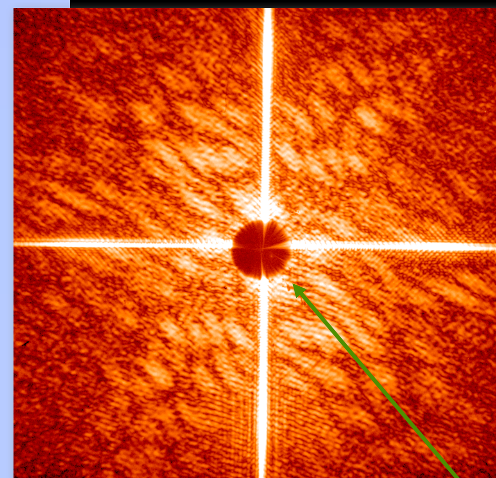
Single particle imaging at LCLS



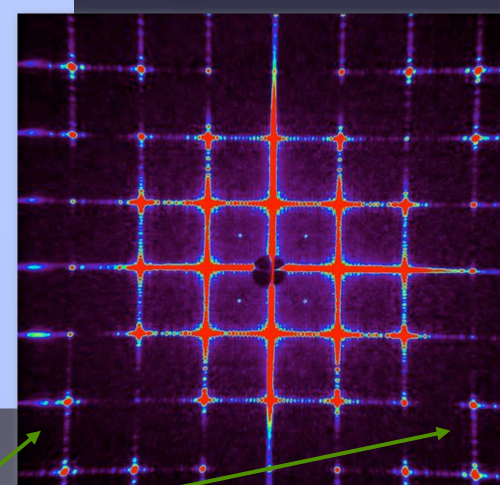
Animation courtesy of Sébastien Boutet,
CXI instrument scientist, SLAC

Our diffraction camera can measure forward scattering close to the direct soft-X-ray FEL beam





"Soft edge" prevents any scatter from the hole

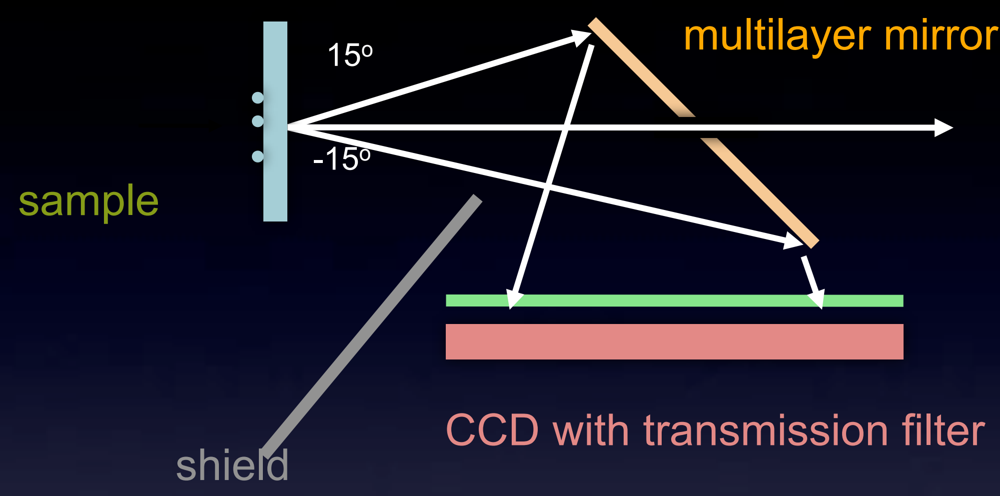


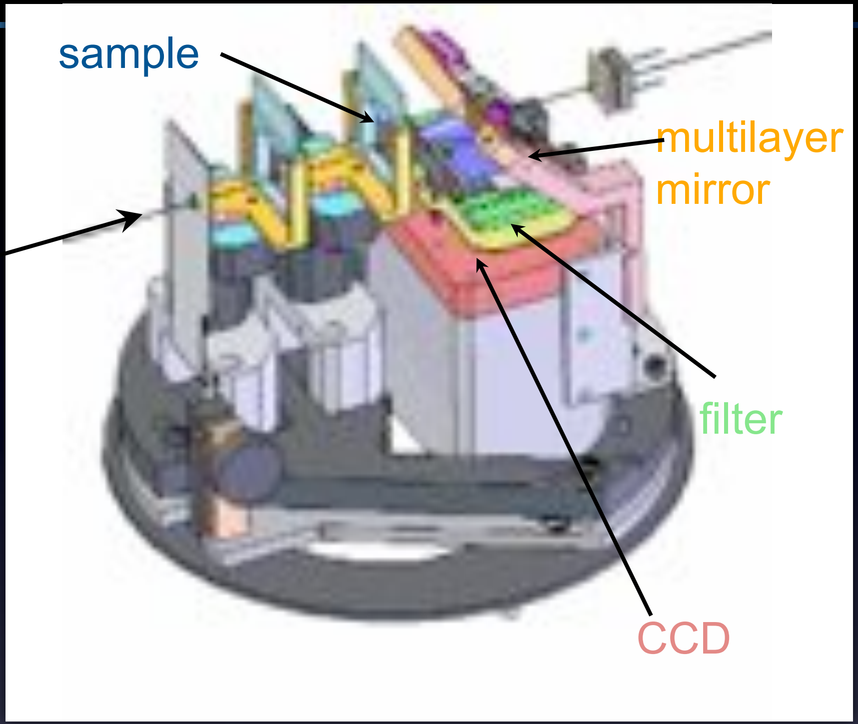
Multilayer reflectivity is uniform across the 30° to 60° gradient

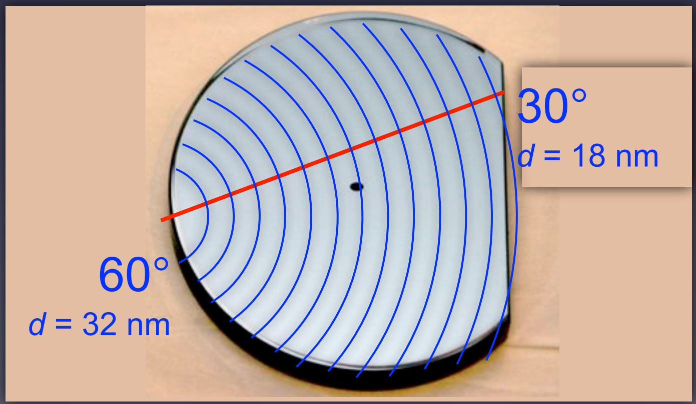
Bajt *et.al.* Appl.Opt. 47, 1673 (2008)

CFEL SCIENCE

The VUV-FEL diffraction experiment employs a unique camera to measure forward scattering with high SNR







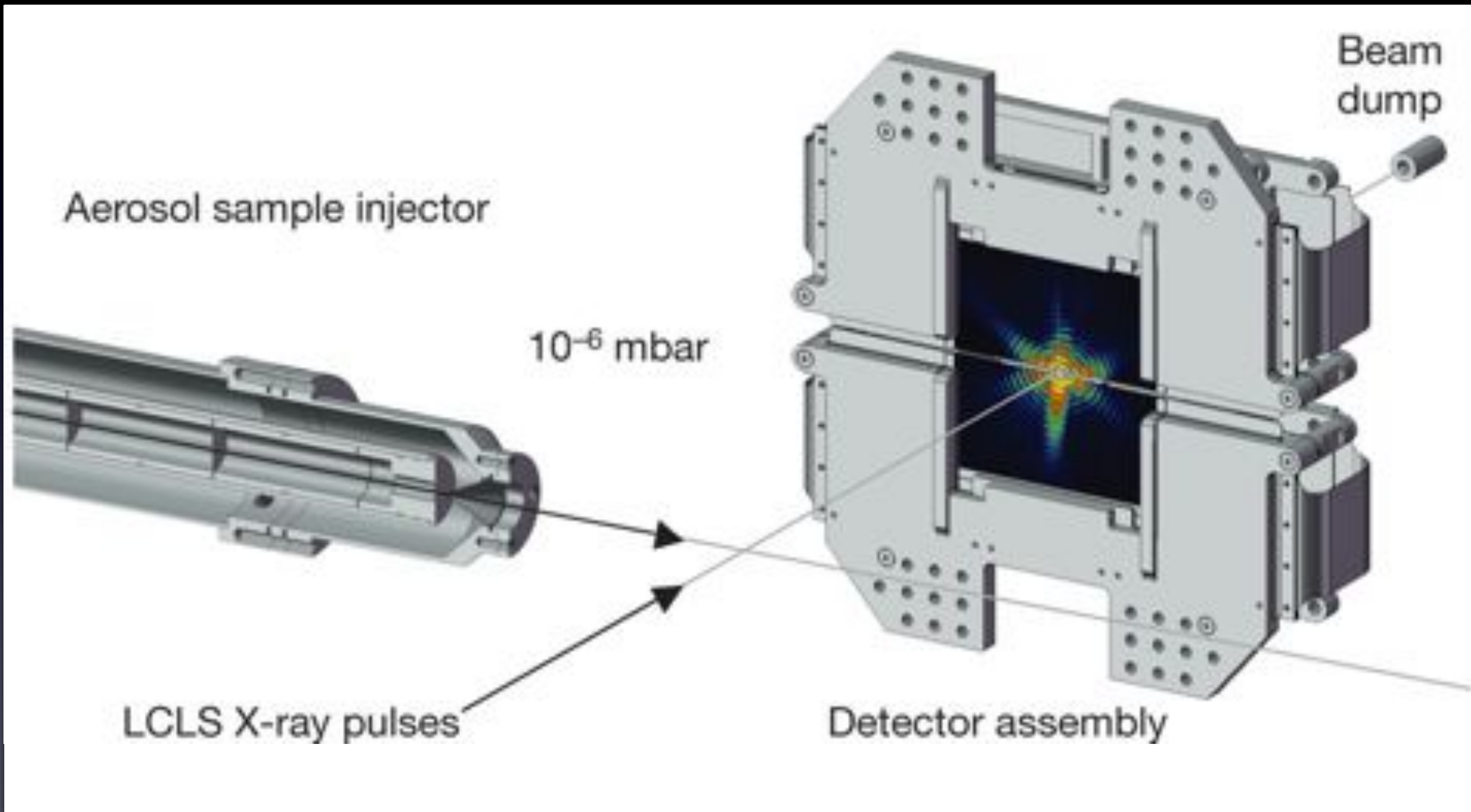
GRADED MULTILAYER MIRROR:
Si, Mo, and B_4C layers, period graded laterally. Variation matches angle of incidence (30° to 60°) to maintain Bragg condition for λ = 32 nm. Reflectivity: 45% for 32 nm pulses.

The mirror protects the CCD and works as a
(i) bandpass filter (bandwidth = 9 nm at 45°)
(ii) filter for stray light (1% off-axis reflectivity)
(iii) low-scatter beam-stop

Sasa Bajt, Eberhard Spiller, and Jennifer Alameda

CFEL SCIENCE

We performed single particle imaging of viruses in the CAMP instrument at LCLS



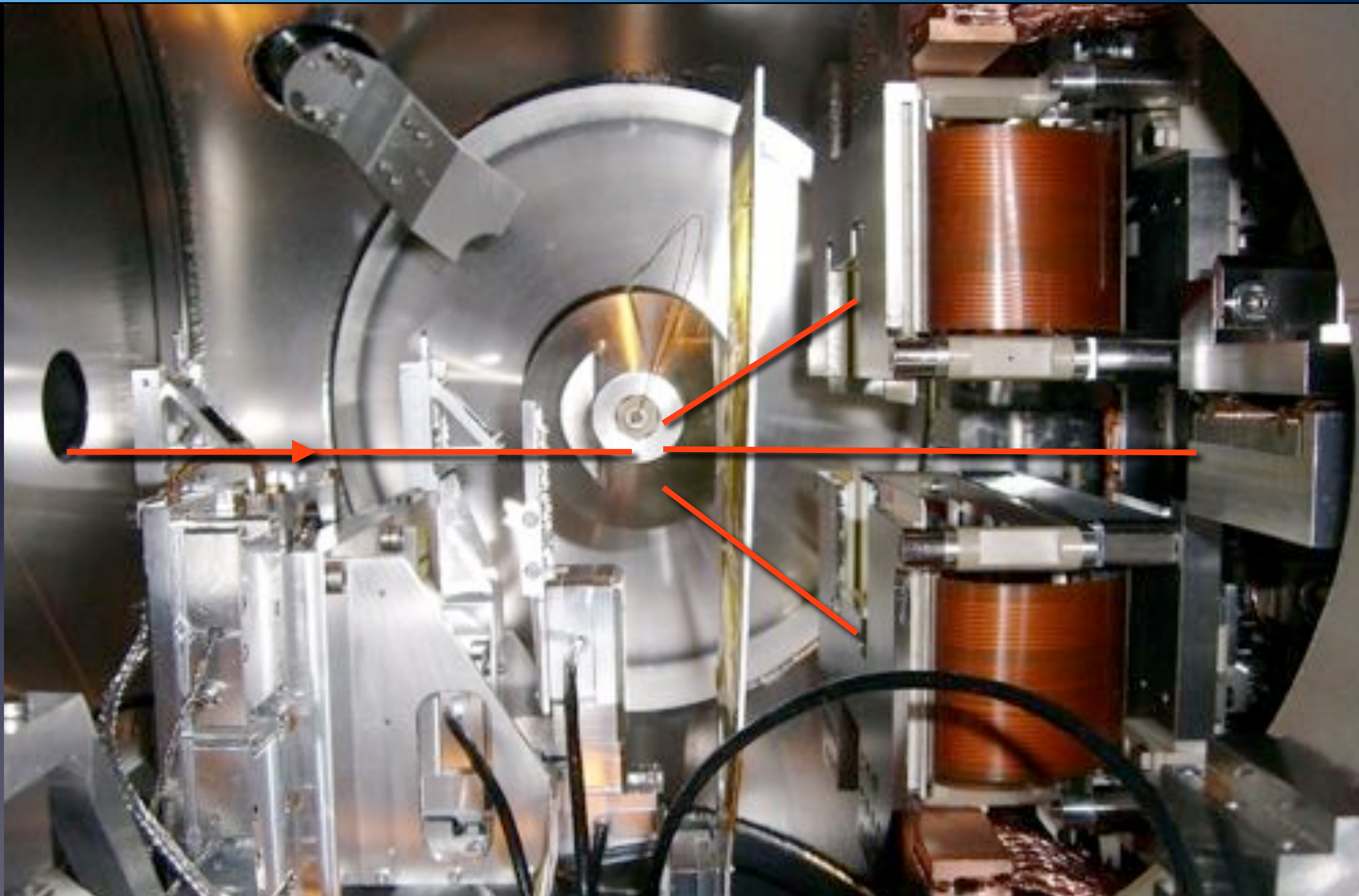
Seibert *et al.* Nature **470** 78–81 (2011)

CFEL SCIENCE

We have performed experiments in the CAMP instrument

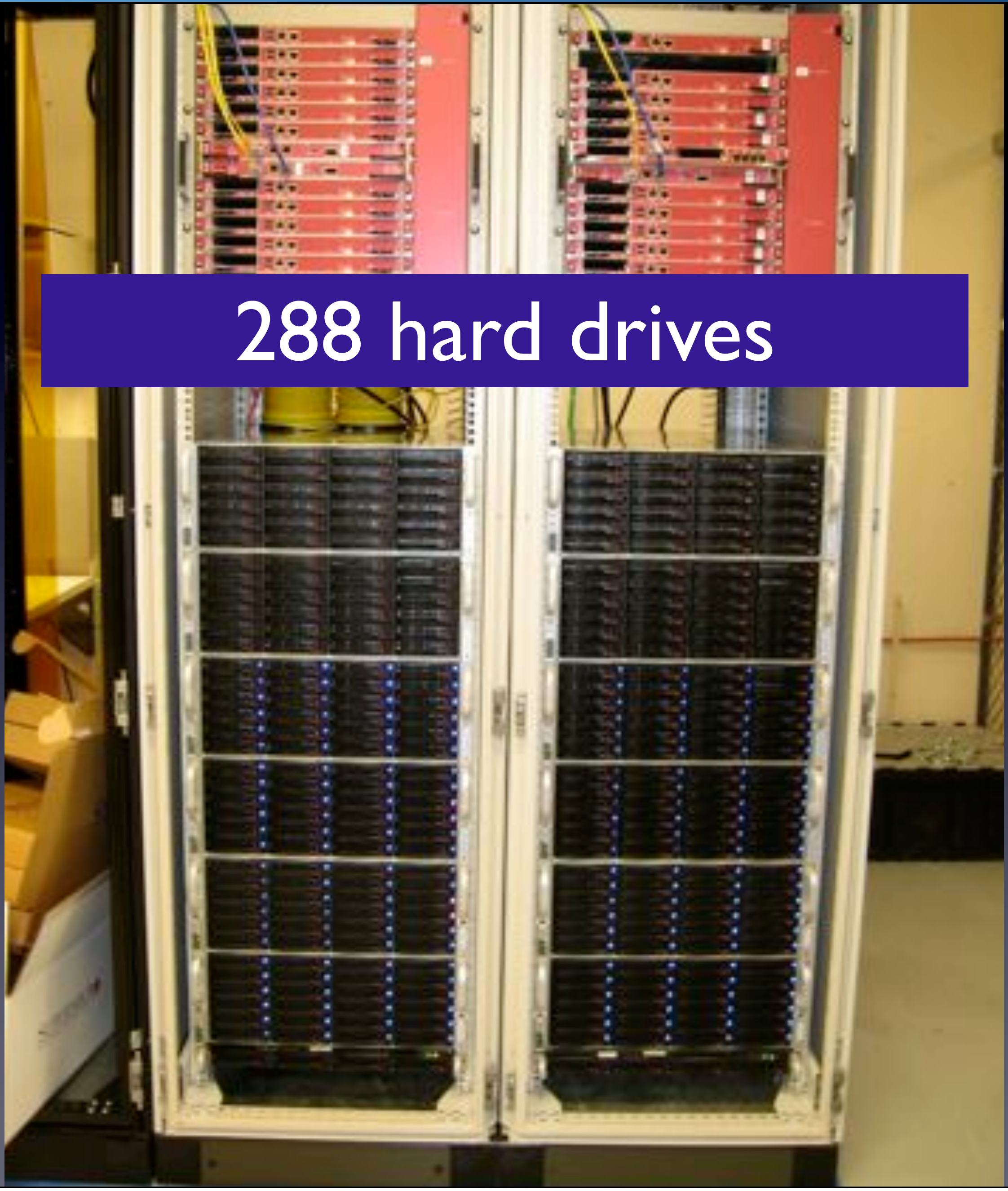
Sascha Epp[1], Robert Hartmann[1,2], Daniel Rolles[1], Artem Rudenko[1], Lutz Foucar[1], Benedikt Rudek[1], Benjamin Erk[1], Carlo Schmidt[1], André Hömke[1], Nils Kimmel[2], Christian Reich[2], Günther Hauser[2], Daniel Pietschner[2], Peter Holl[2], Hubert Gorke[3], Helmut Hirsemann[4], Guillaume Potdevin[4], Tim Erke[4], Jan-Henrik Mayer[4], Heinz Graafsma[4], Michael Matysek[5], Sebastian Schorb[6], Daniela Rupp[6], Marcus Adolph[6], Tais Gorkhover[6], Christoph Bostedt[7], John Bozek[7], Marc Messerschmidt[7], Joachim Schulz[4], Lars Gumprecht[4], Andrew Aquila[4], Nicola Coppola[4], Frank Filsinger[8], Kai-Uwe Kühnel[9], Christian Kaiser[9], Claus-Dieter Schröter[9], Robert Moshammer[9], Faton Krasniqi[1], Simone Techert[1,10], Georg Weidenspointer[2], Robert L. Shoeman[11], Ilme Schlichting[1,11], Lothar Strüder[1,2], Joachim Ullrich[1,9]

Experiments were performed in the CAMP chamber on the AMO beamline at LCLS



SCIENCE

Imaging experiments at LCLS generate large volumes of data



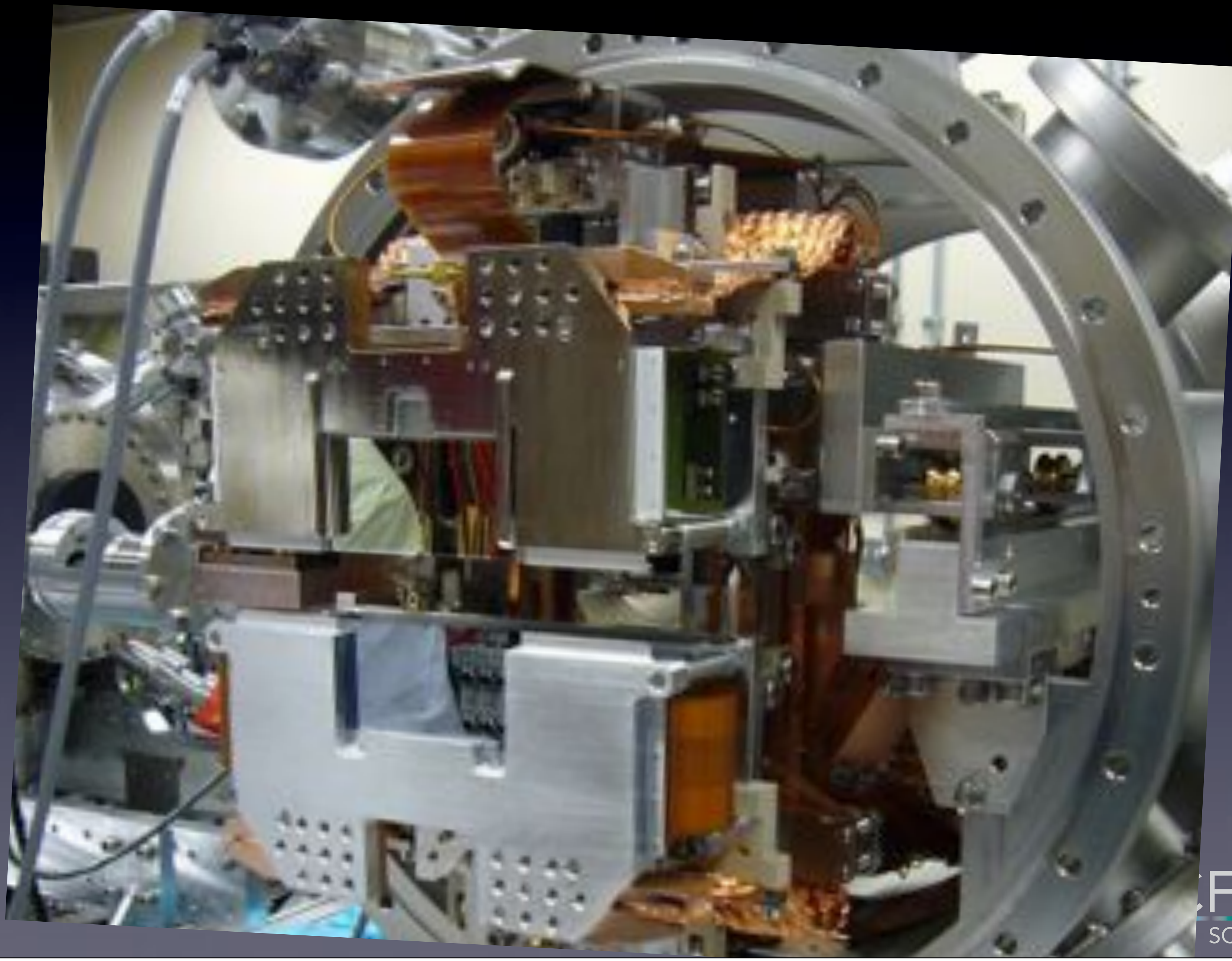
Data rate:
4 MB/image
430 GB/hr (30 Hz)
1.7 TB/hr (120 Hz)

Feb 2011:
120 Hz
200 TB data...
>20,000,000 images

CFEL SCIENCE

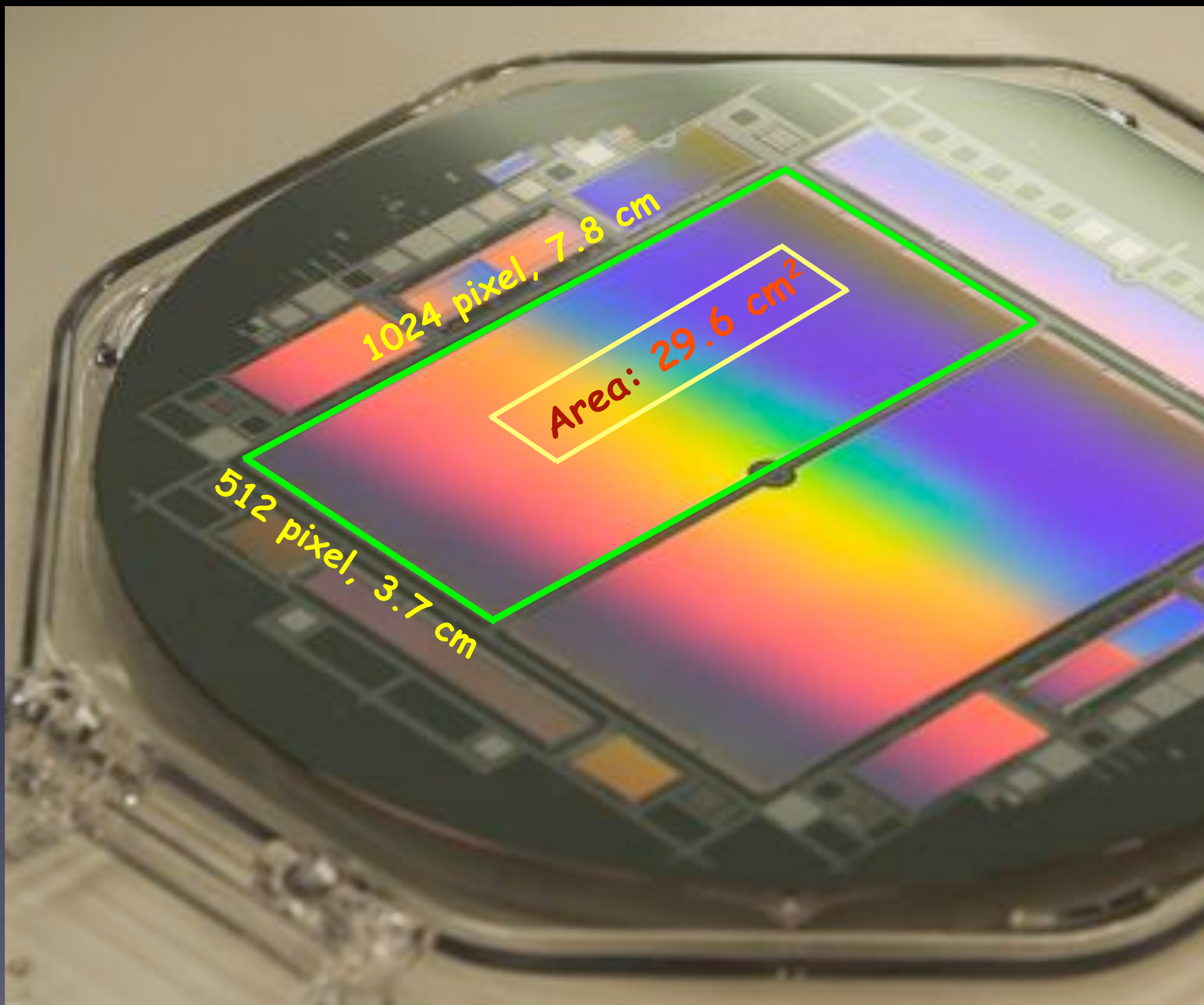
High-speed area detectors are essential for LCLS imaging experiments

Max-Planck-Institut
für Kernphysik

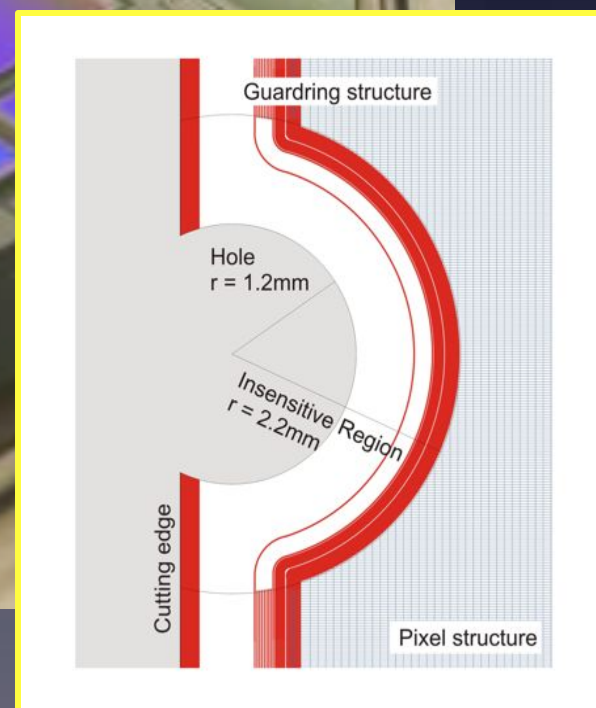


CFEL
SCIENCE

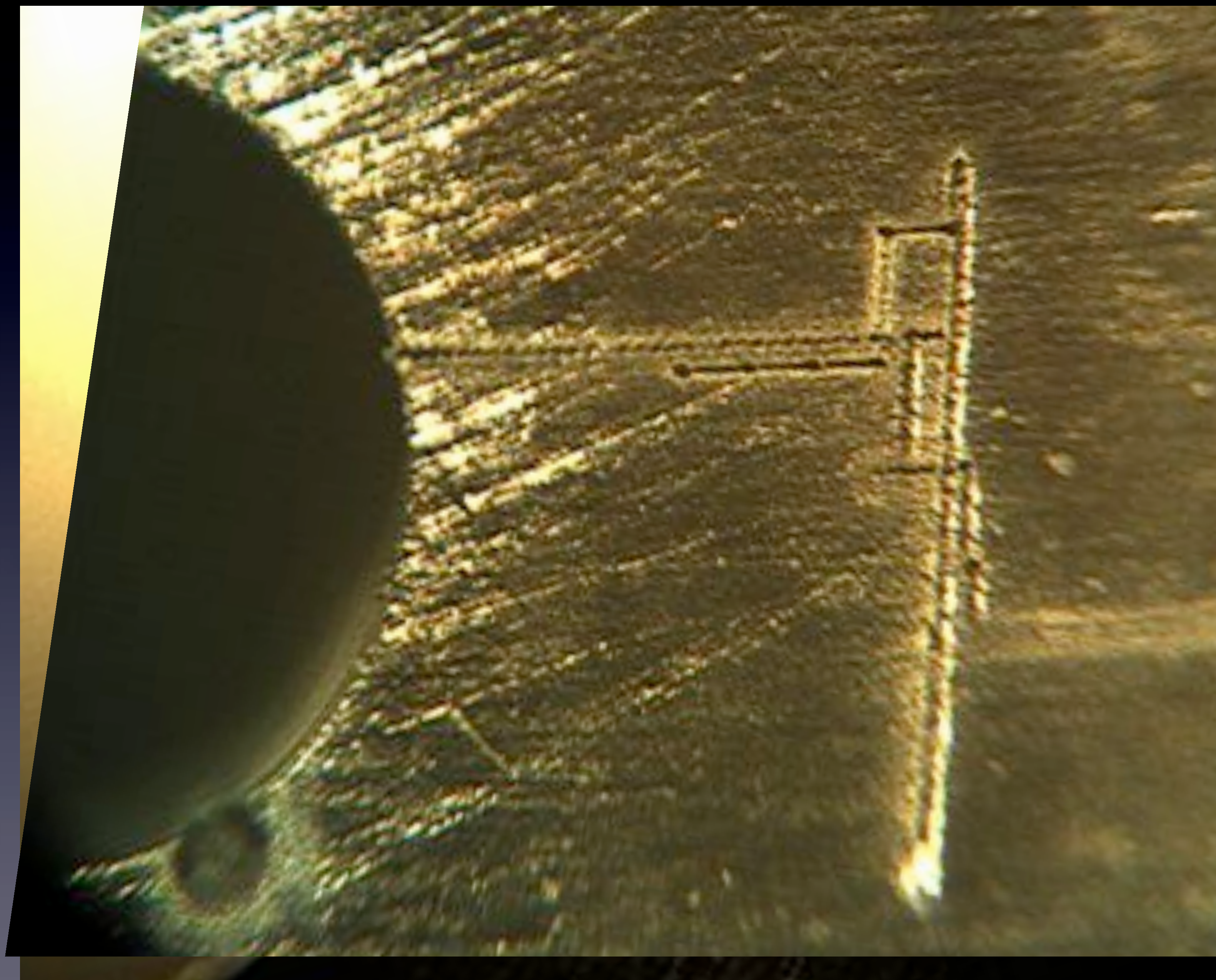
The pnCCD enables direct detection at X-ray wavelengths; central hole allows the direct beam to pass through



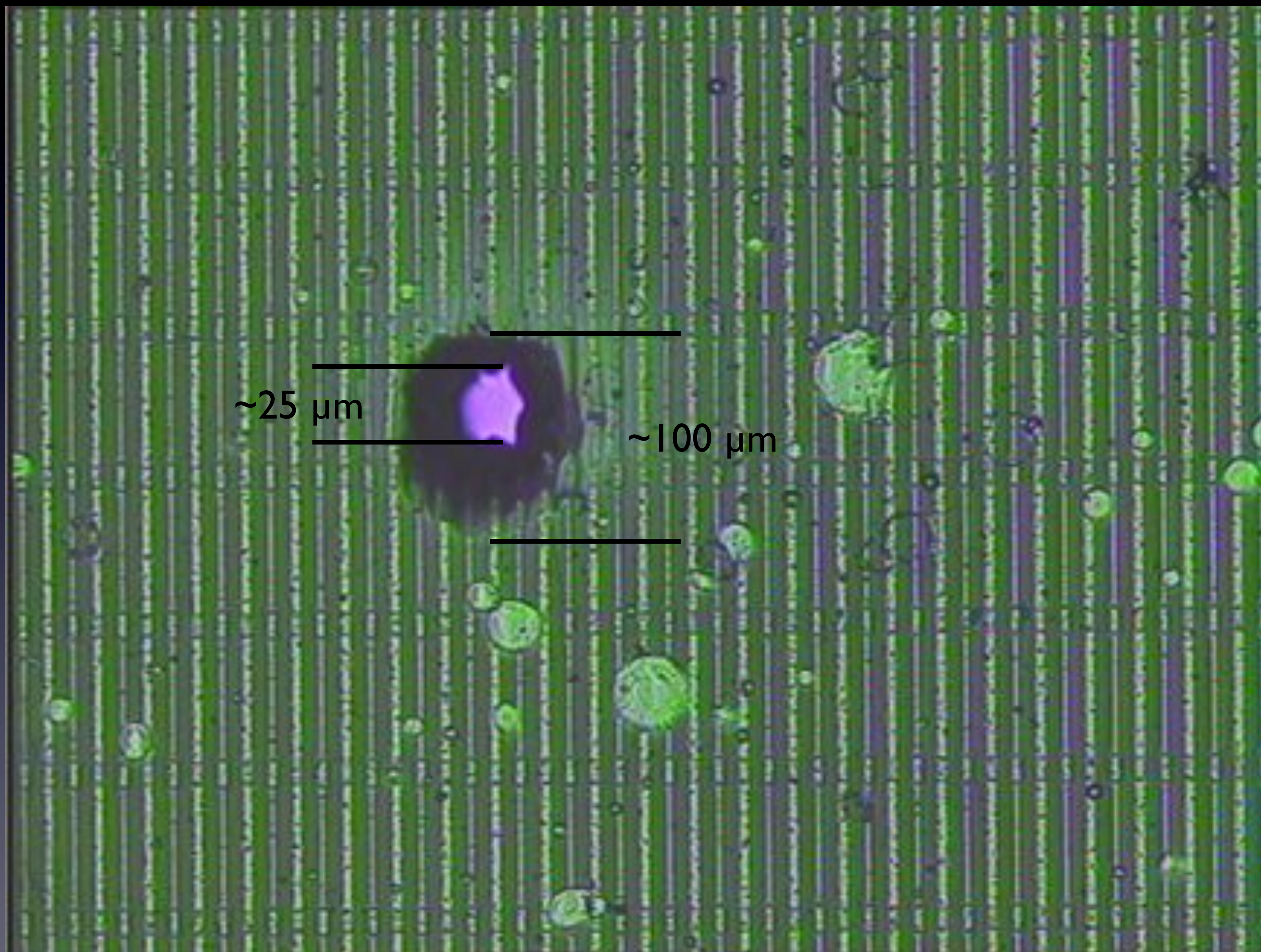
- 1024x1024 pixels
- pixel size: 75 x 75 μm²
- active area 60 cm²
- frame rate 125-900 Hz
- single-photon resolution
- ΔE=50/80eV @ 800/2000eV
- Q.E. ≥ 90 % from 0.4 to 10 keV
- operating range 0.1 < E < 24 keV



The LCLS beam easily cuts through stainless steel



An accidental direct hit on the pnCCD at full power drilled straight through the detector

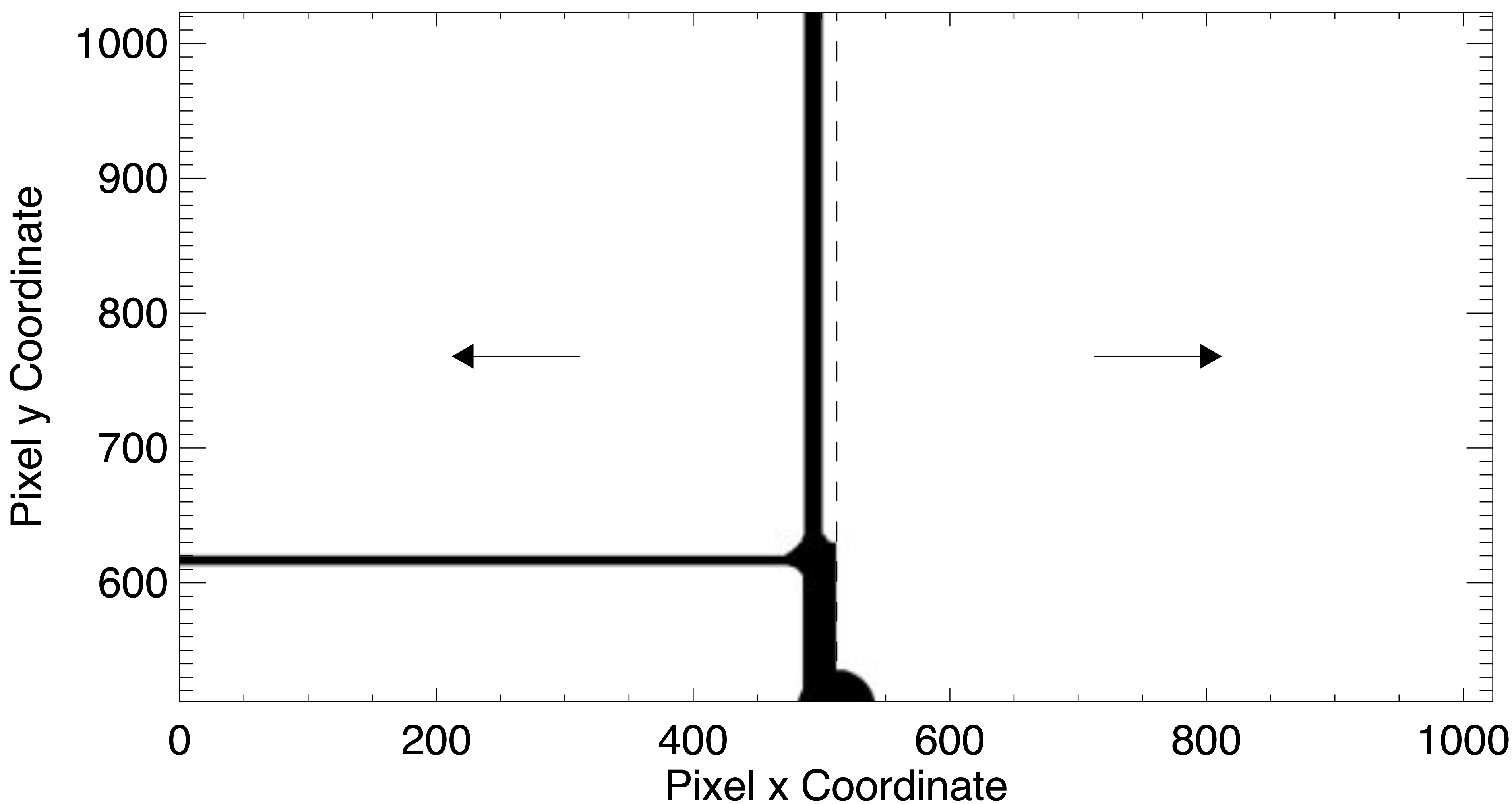


Georg Weidenspointer, HLL

Proc. SPIE 8070 (May 2011)

CFEL SCIENCE

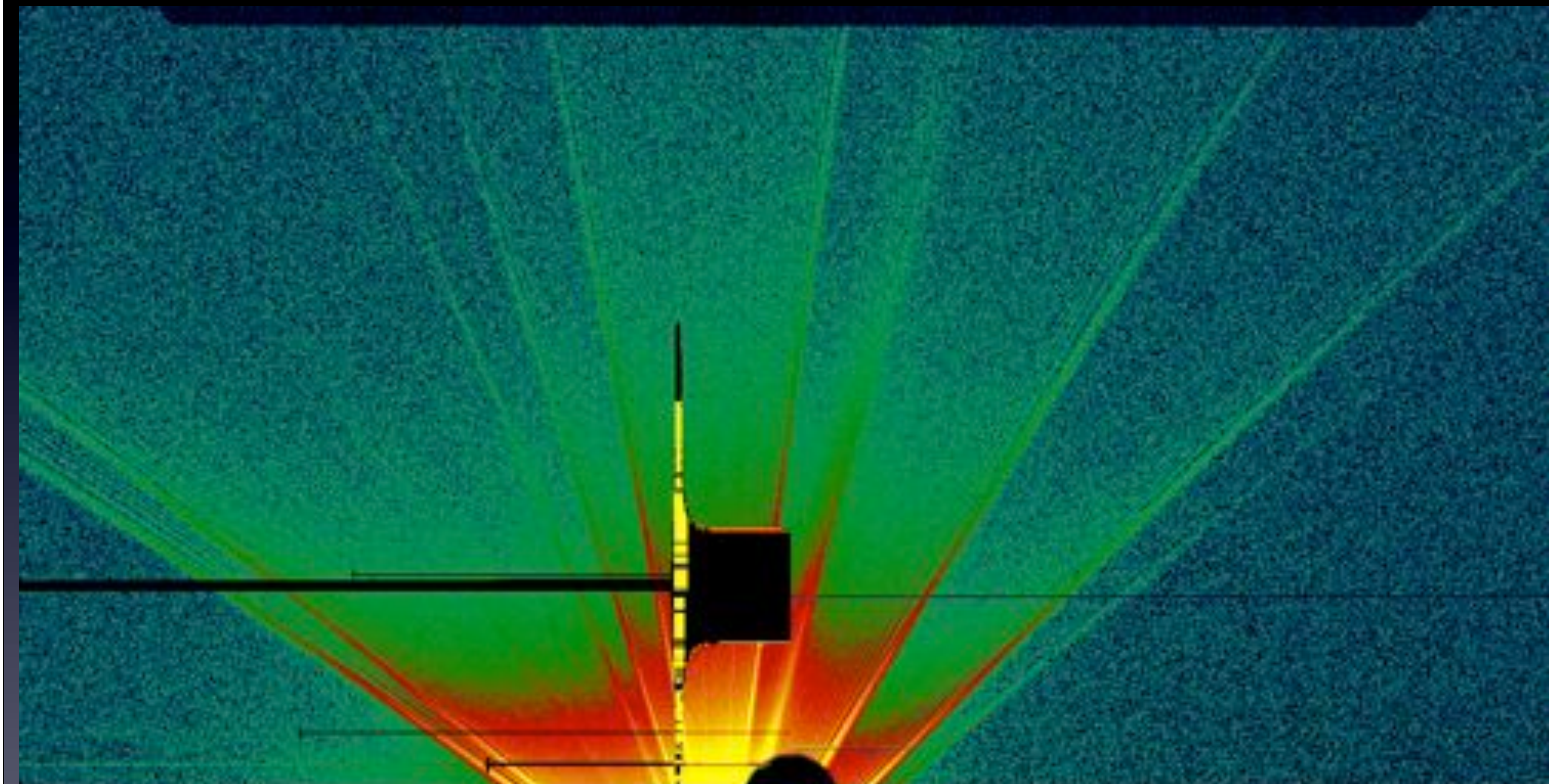
Amazingly, most of the detector remained useable despite the direct hit



Georg Weidenspointer, Robert Hartmann, MPG-HLL

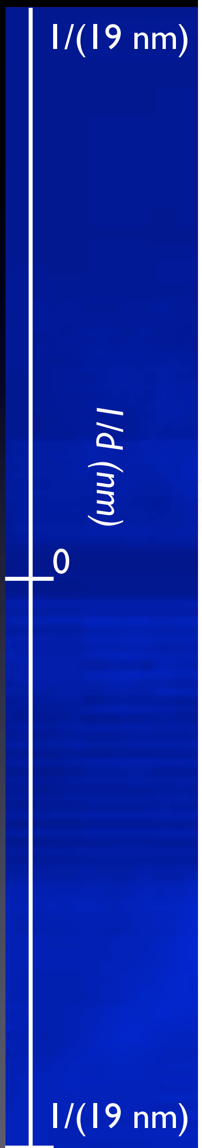
Proc SPIE 8070 (May 2011)

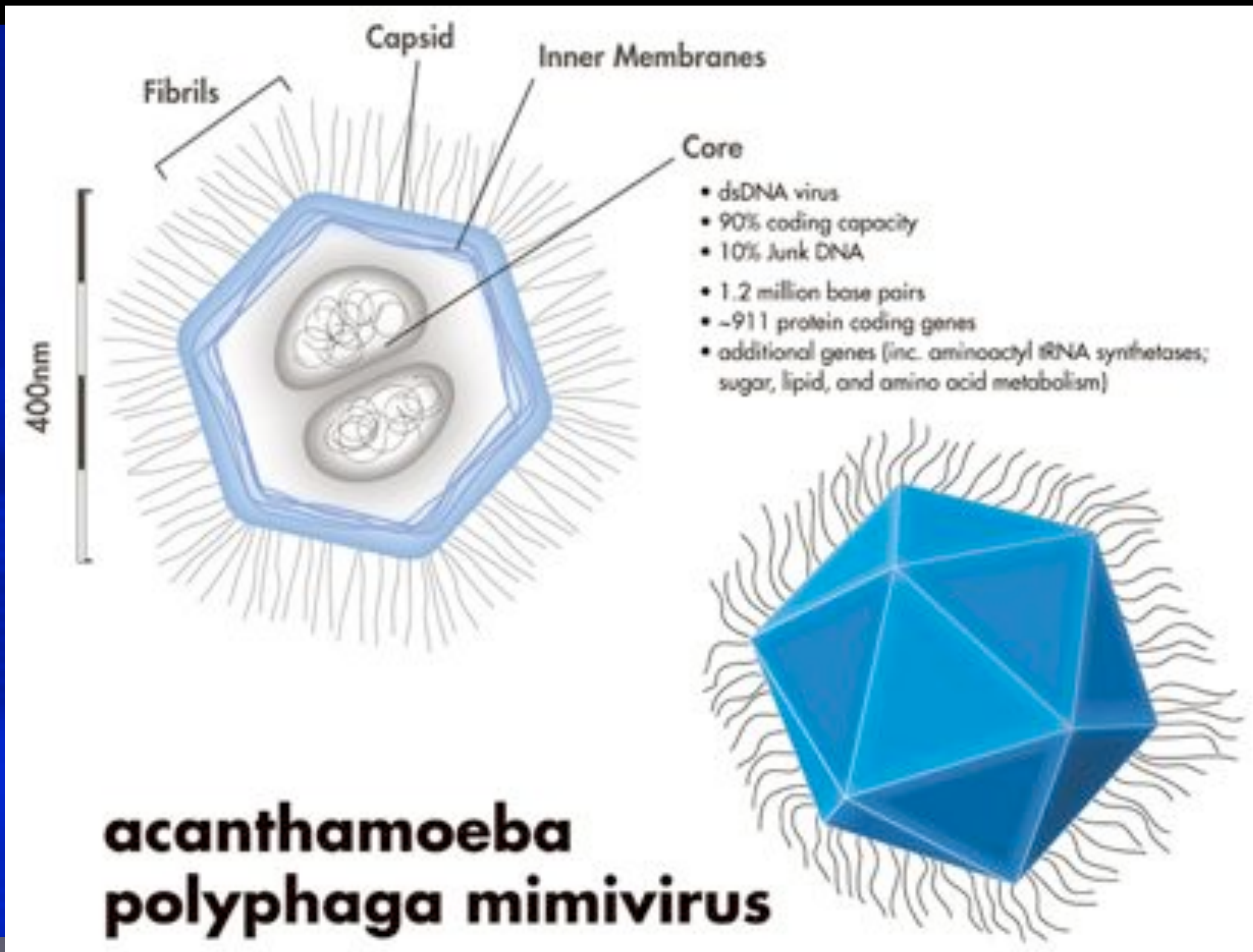
CFEL SCIENCE



CFEL SCIENCE

Clear diffraction is measured from individual mimivirus





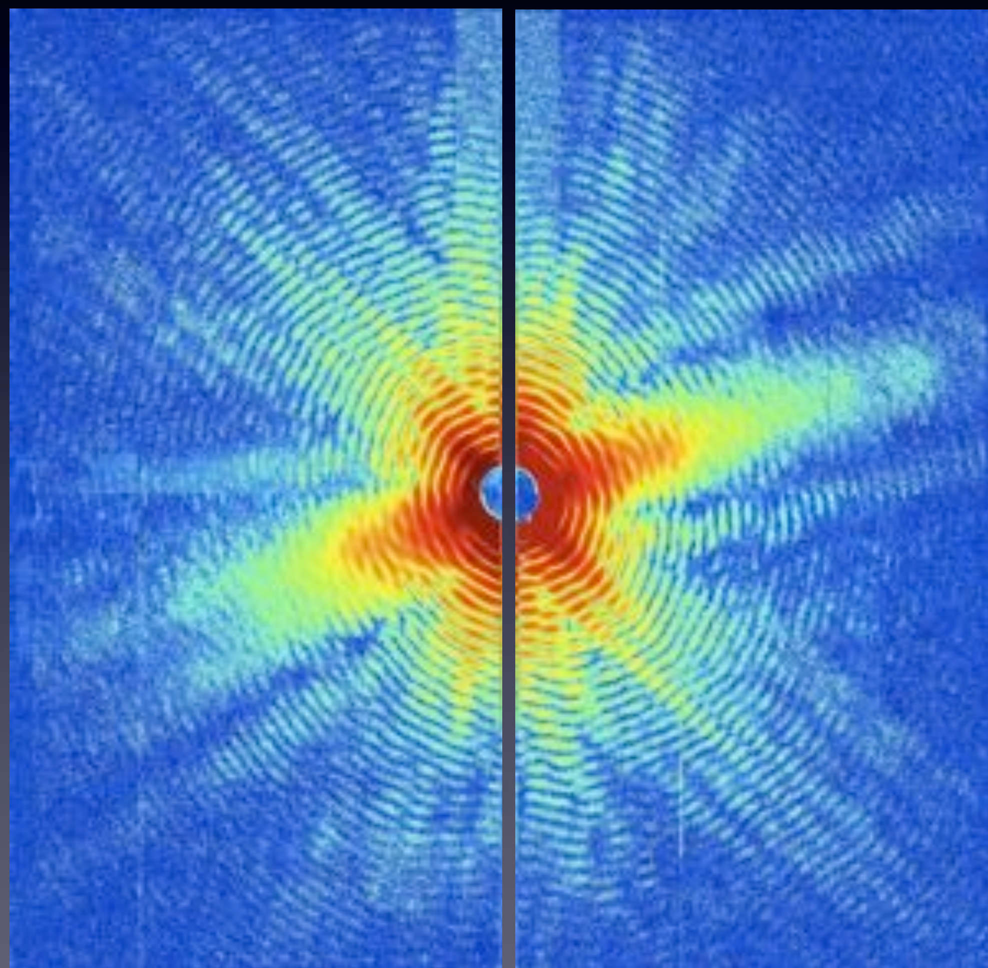
Single particles at 20 nm resolution

on of 2

Janos Hajdu, Uppsala University
CNRS Marseille

We achieved an unprecedented 43% hit rate using an aerosol injector

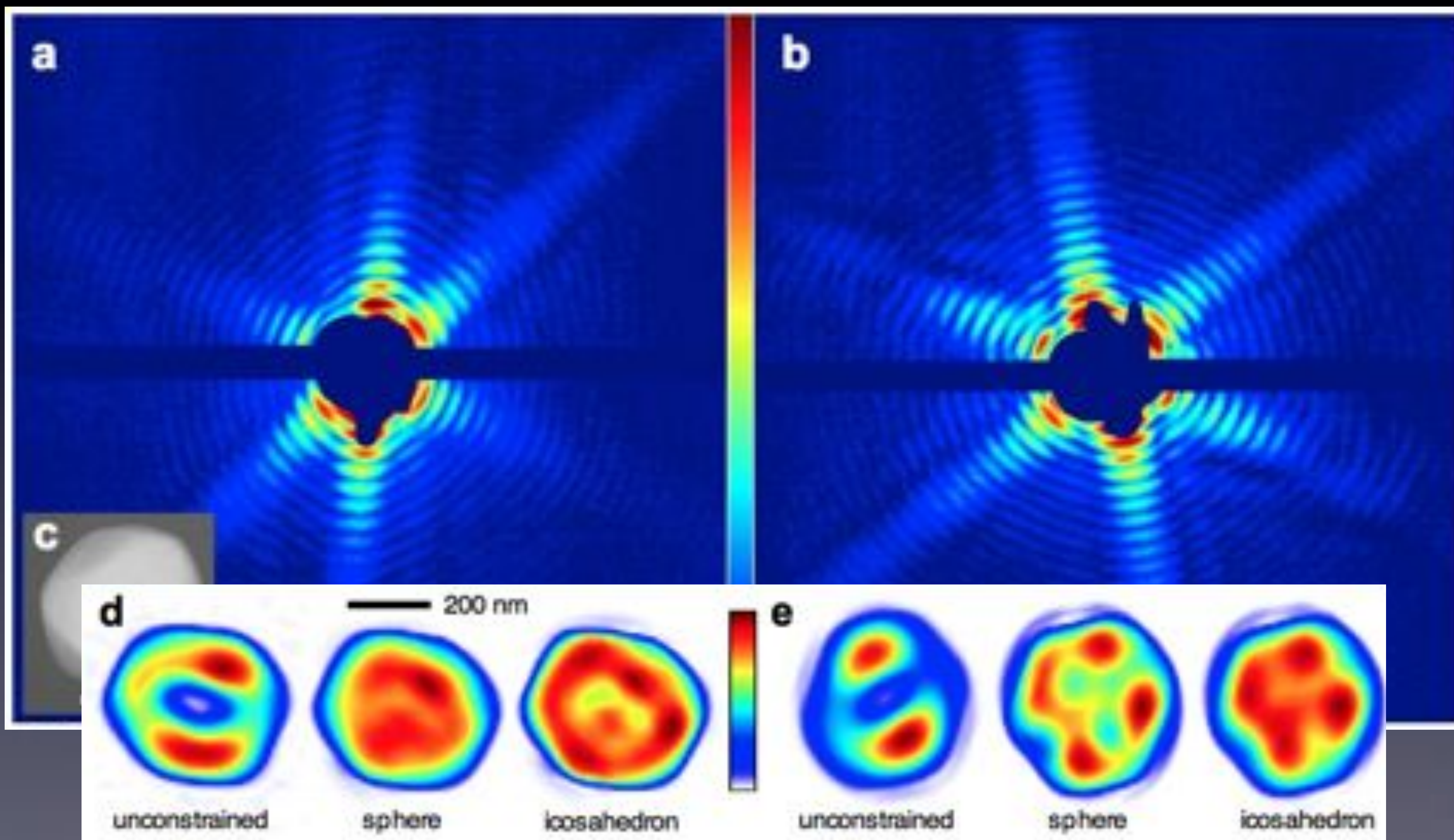
1.2 MILLION HITS on viruses in ~36 hours of beam time
(7.7% assuming 100% up-time)



Sample consumption: ~ 2 µl /min
Sample concentration: ≤10^{12} particles/ml

Janos Hajdu
Uppsala

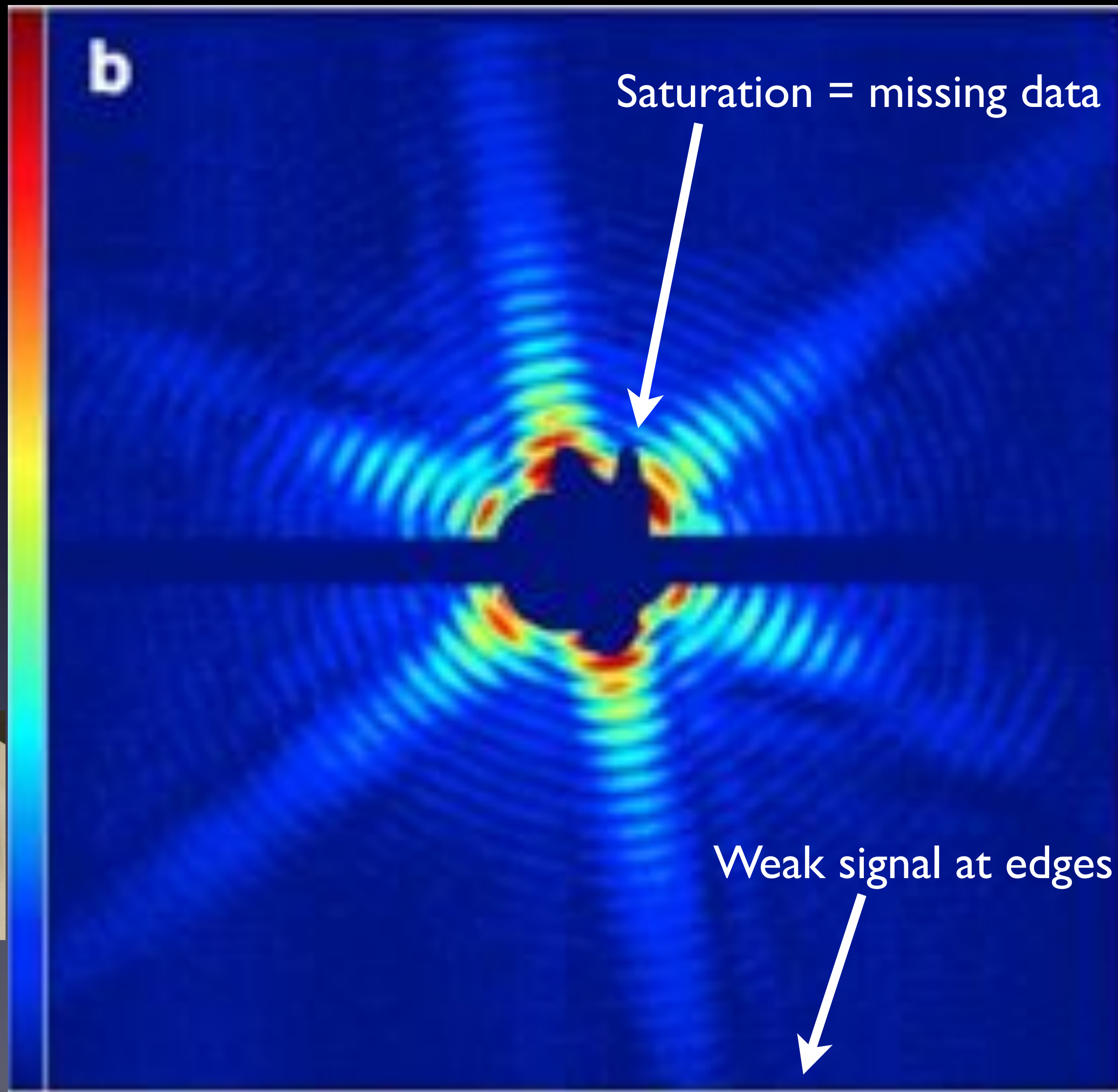
Mimivirus diffraction from LCLS reconstructs in 2D

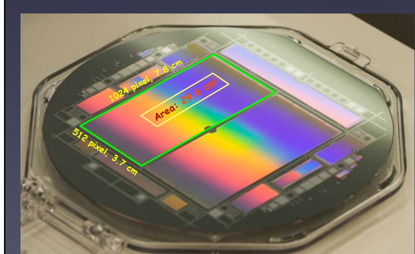


Seibert *et al.* Nature **470** 78–81 (2011)

CFEL SCIENCE

Diffraction from individual Mimivirus can easily saturate the detector at low resolution



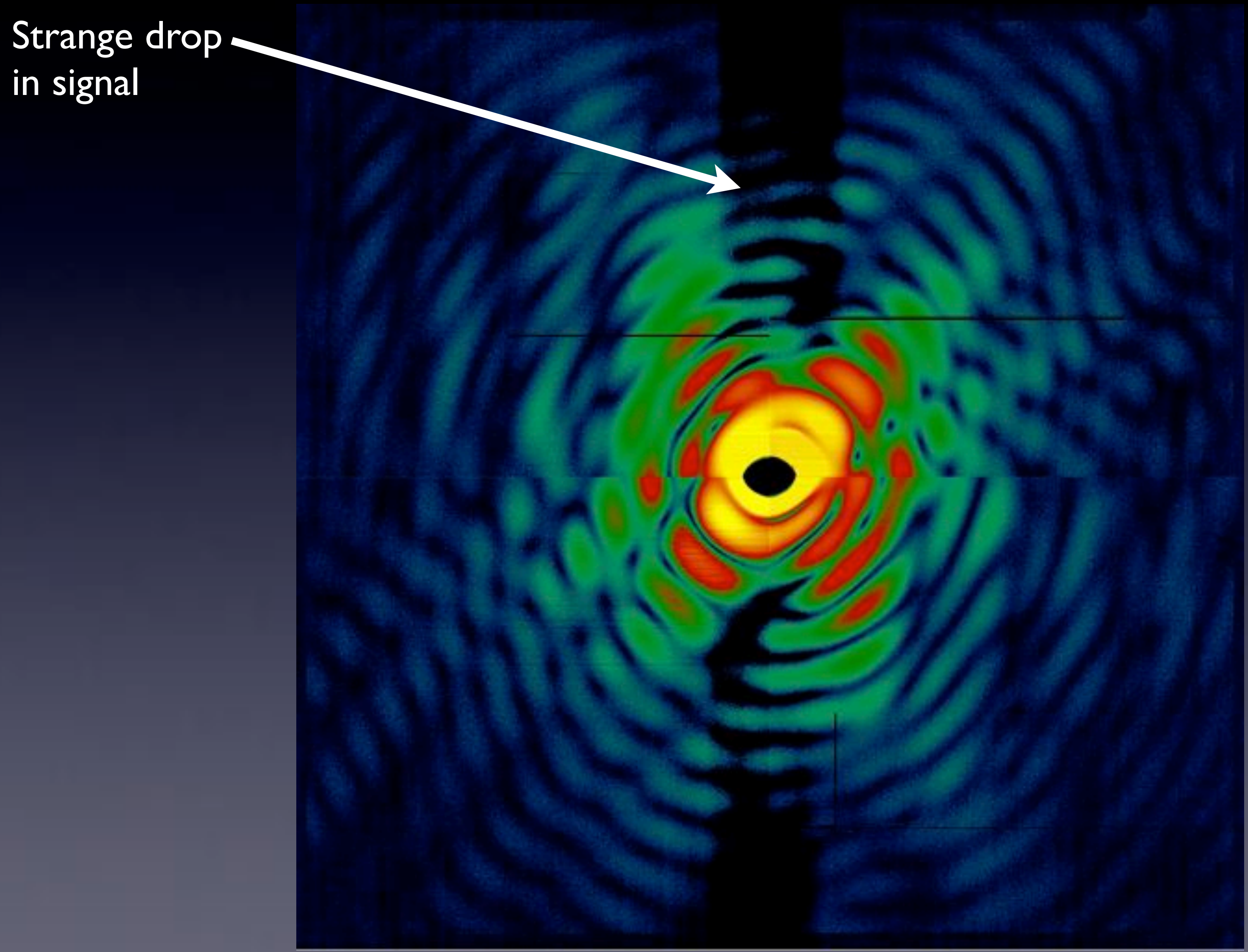


pnCCD

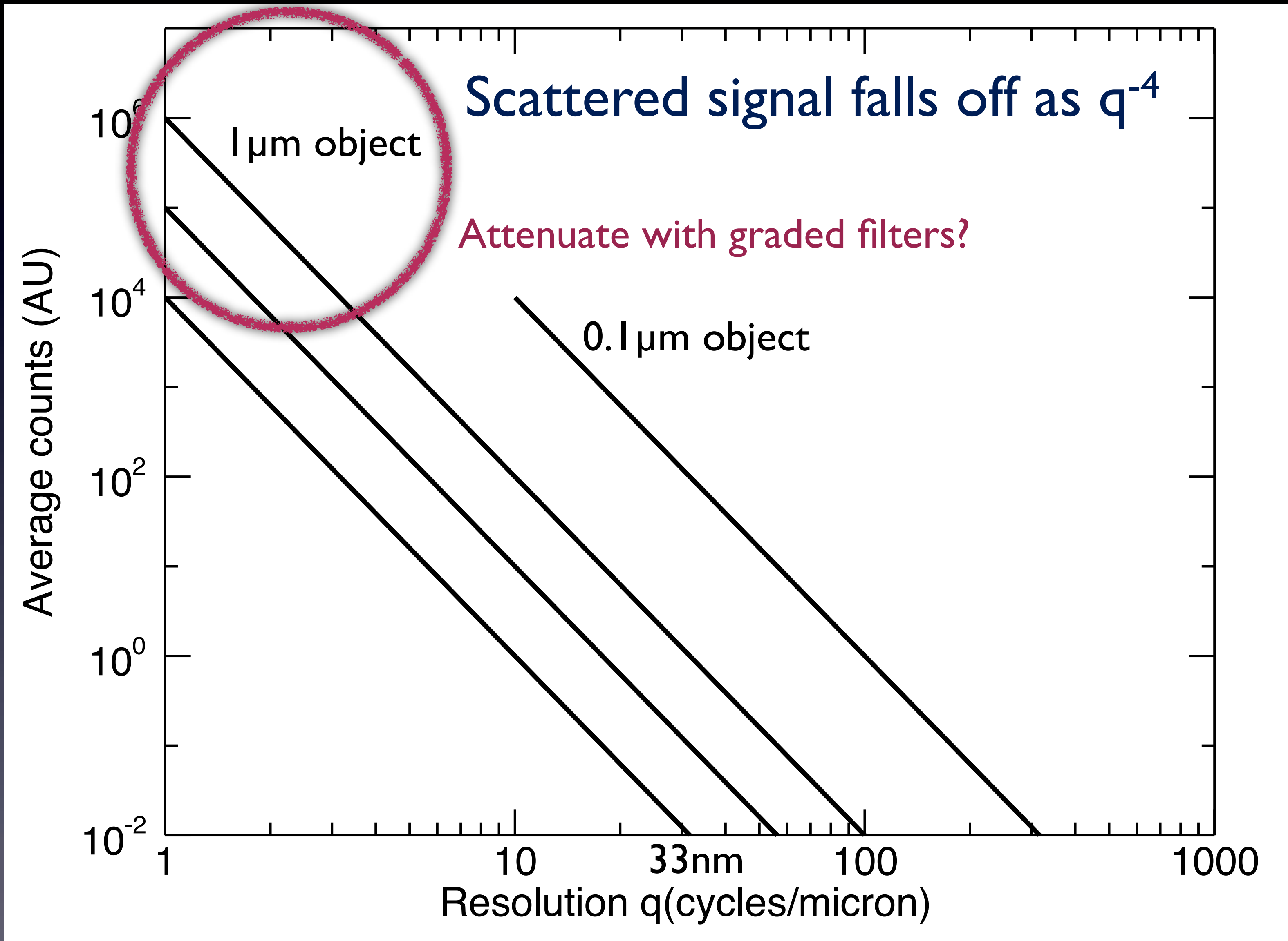
Janos Hajdu, Filipe Maia, Thomas Ekberg - Uppsala

CFEL SCIENCE

High intensity regions can overload readout channels



Resolution is limited by dynamic range when objects must be imaged with a single shot



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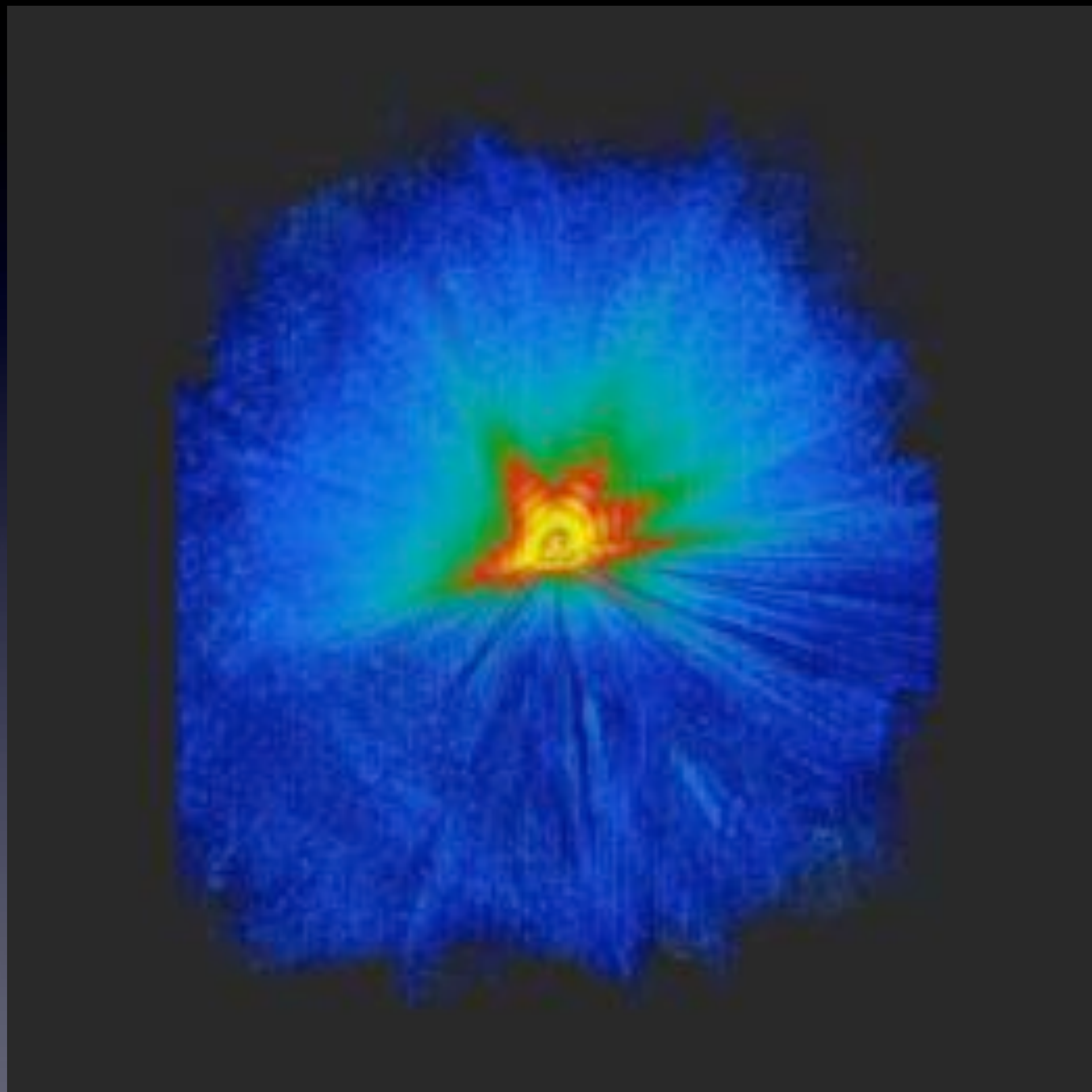
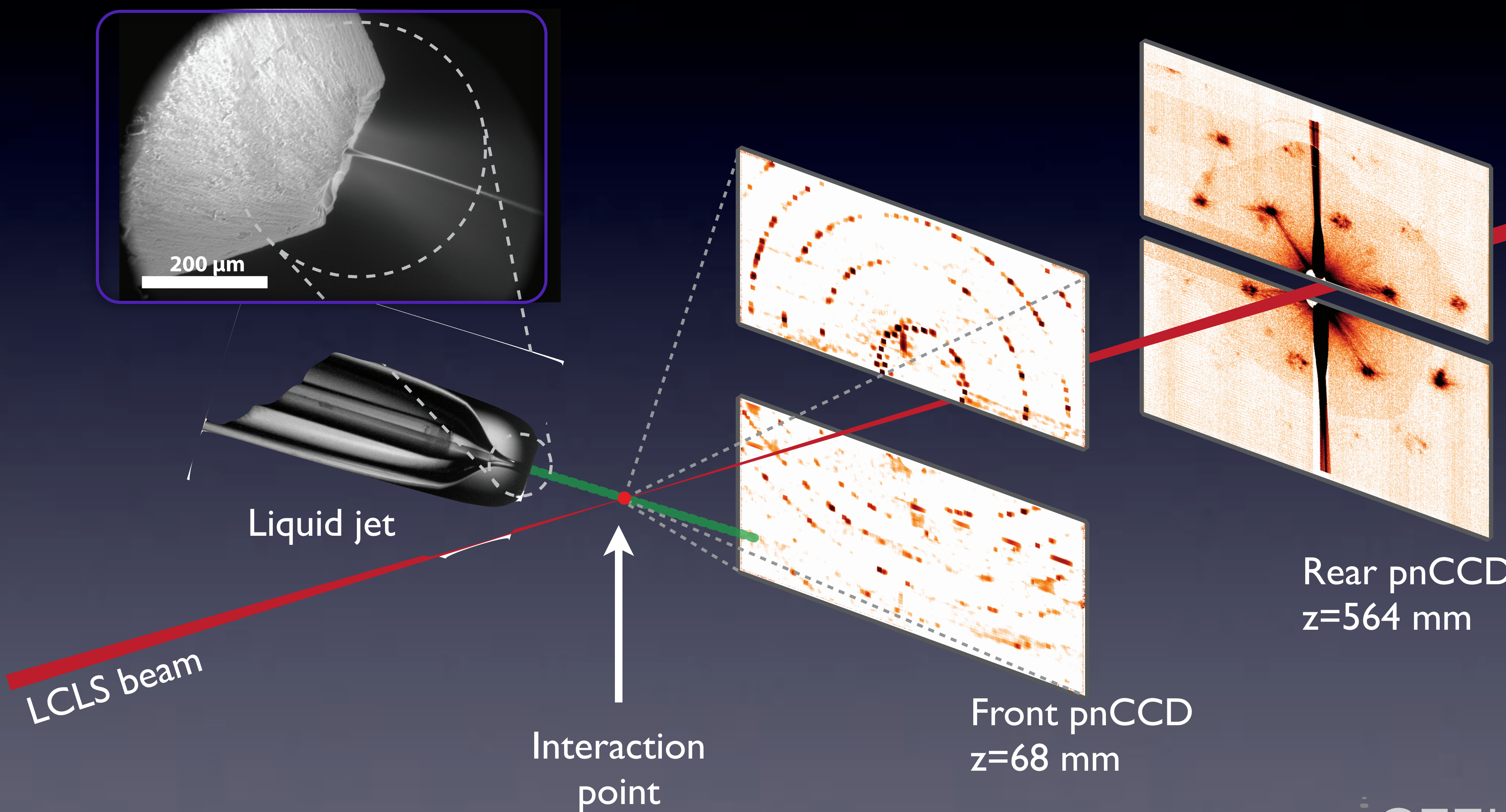
We can assemble individual snapshots in 3D



CFEL SCIENCE

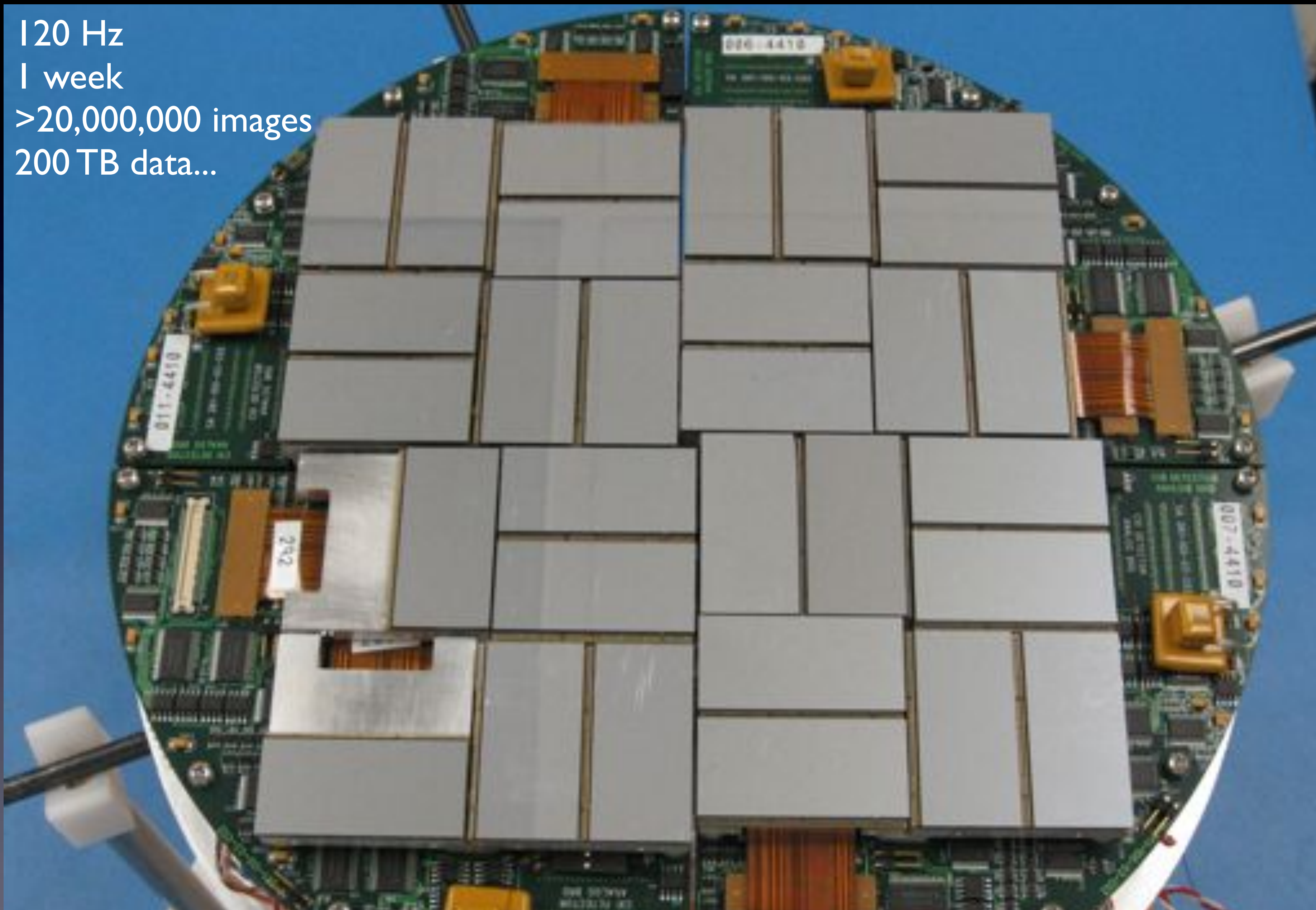
We performed protein nanocrystallography at room temperature in a flowing water microjet



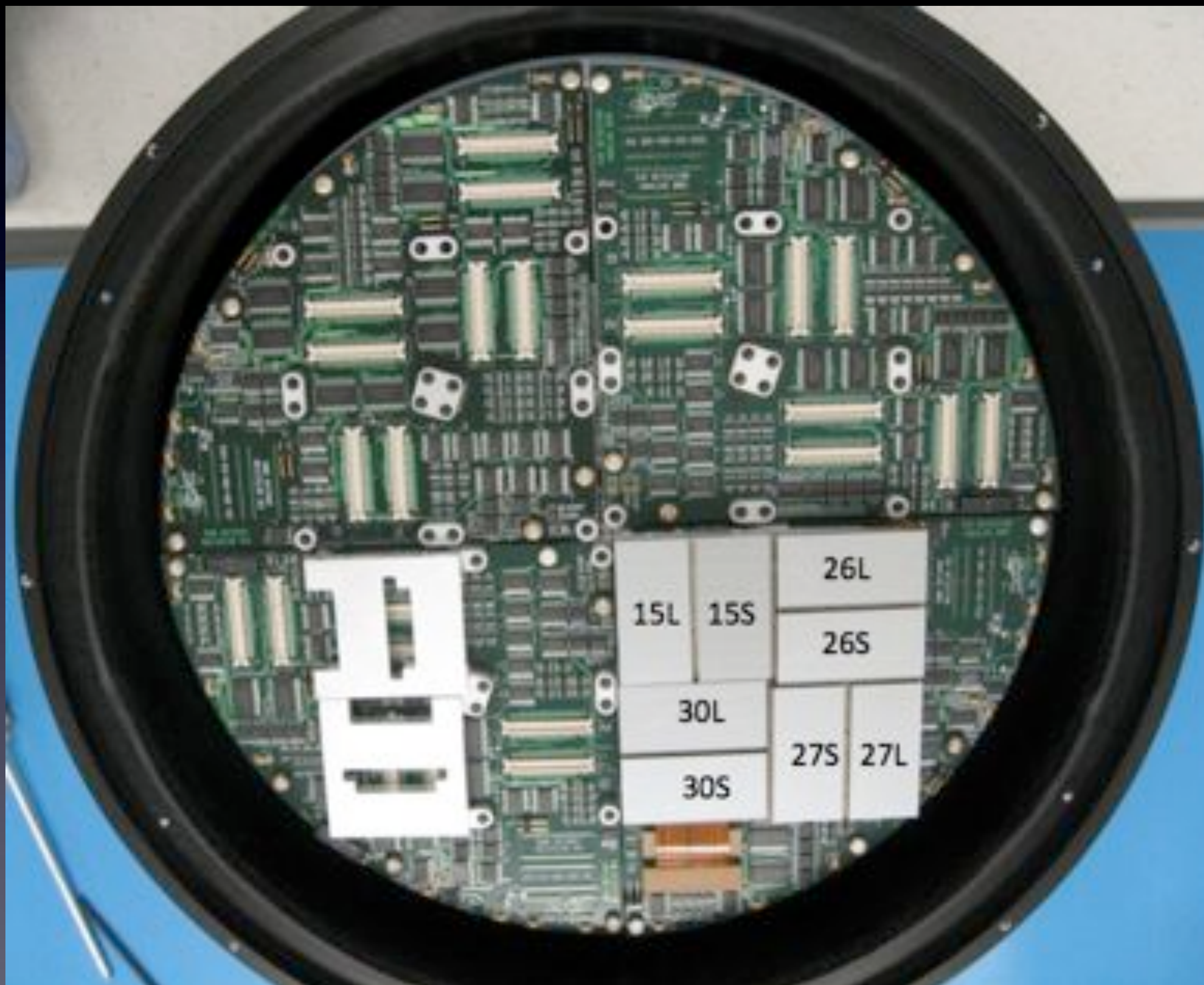
CFEL SCIENCE

The cspad pixel array detector was almost completely populated

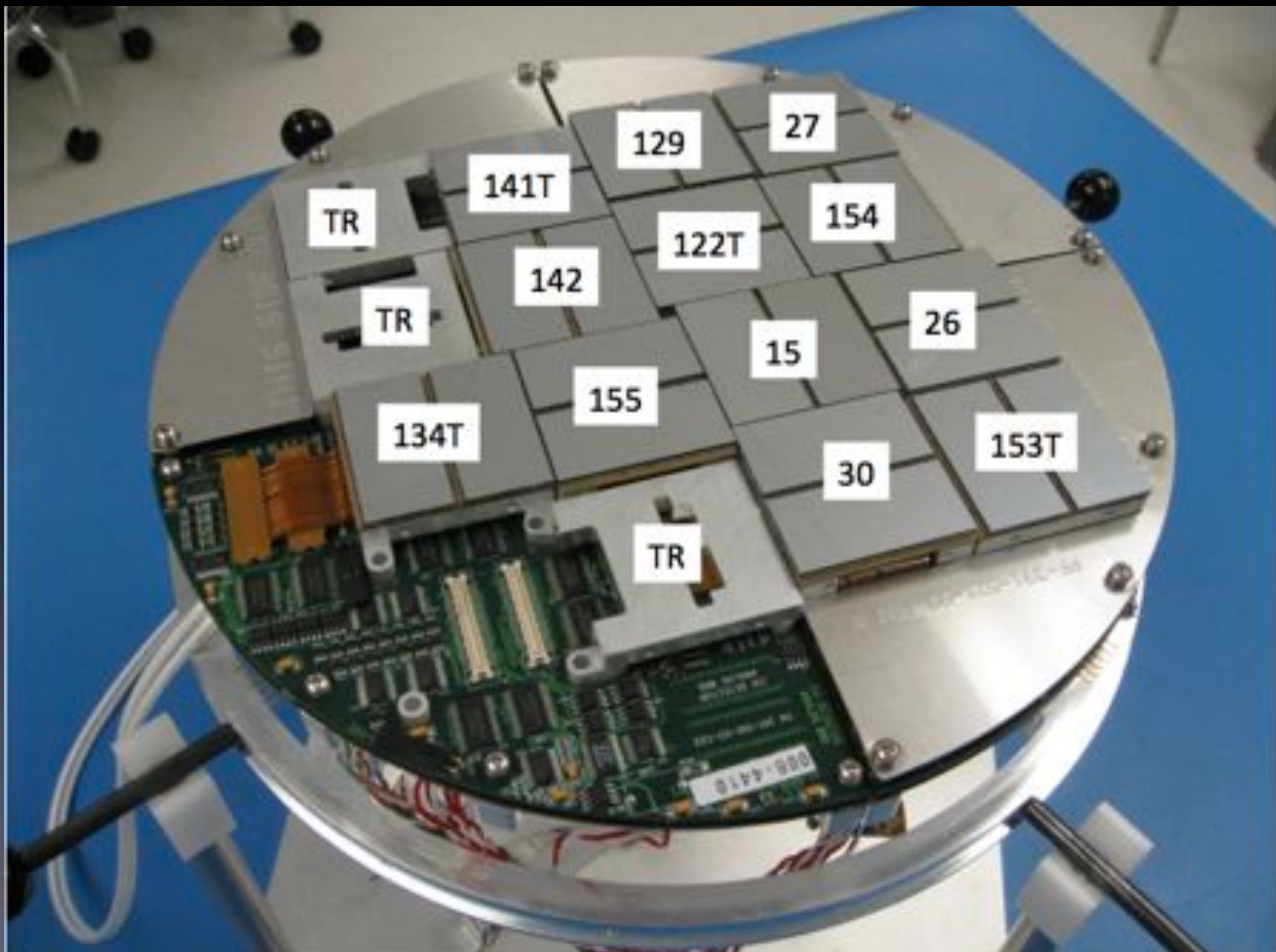
120 Hz
1 week
>20,000,000 images
200 TB data...



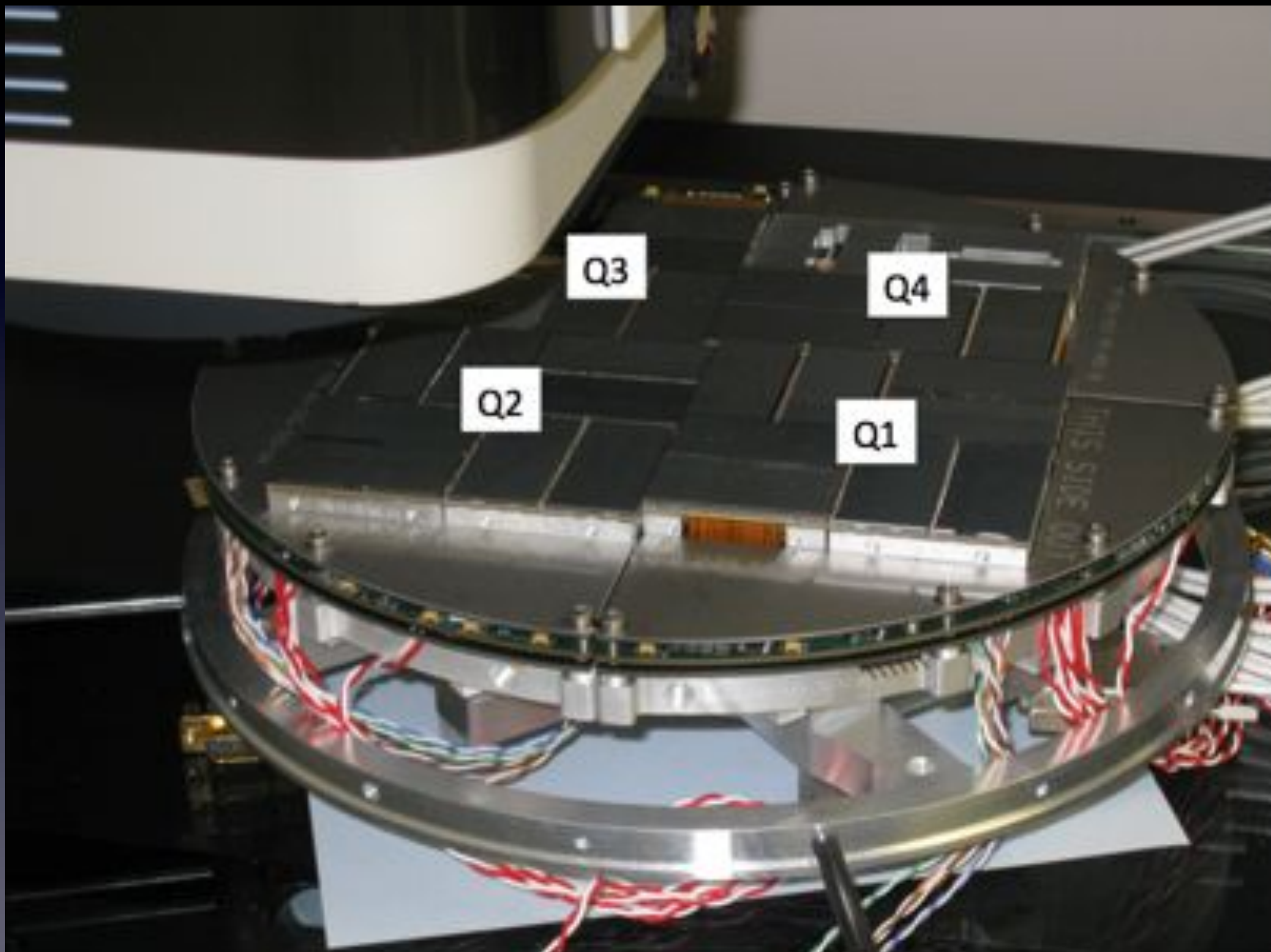
EL
SCIENCE



https://confluence.slac.stanford.edu/display/PCDS/CSPad+detector

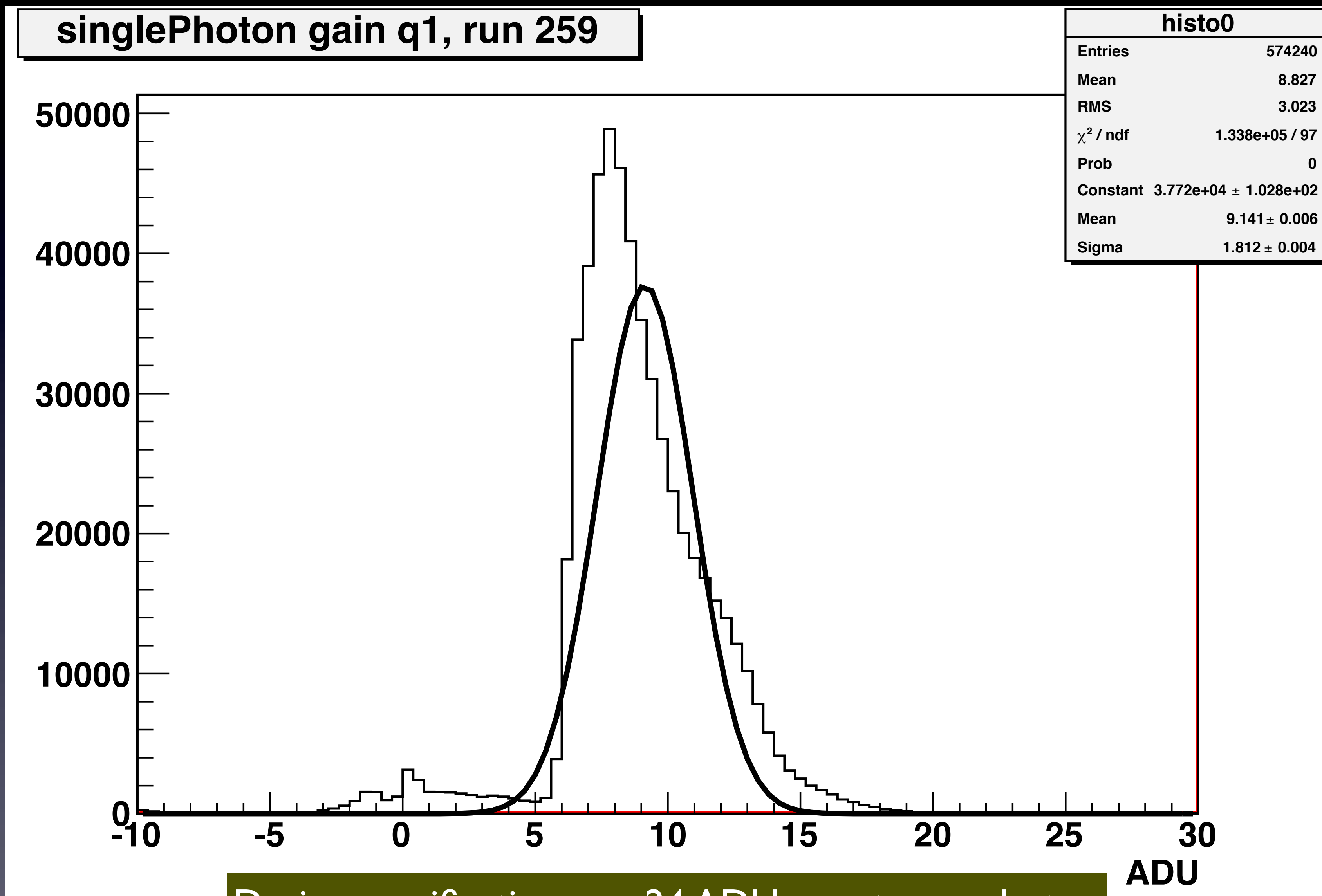


https://confluence.slac.stanford.edu/display/PCDS/CSPad+detector



https://confluence.slac.stanford.edu/display/PCDS/CSPad+detector

Single photon events produce about 8 ADU counts (at 8 keV)



Design specification was 24 ADU counts per photon

Philip Hart, SLAC

https://confluence.slac.stanford.edu/display/PCDS/CSPad+detector

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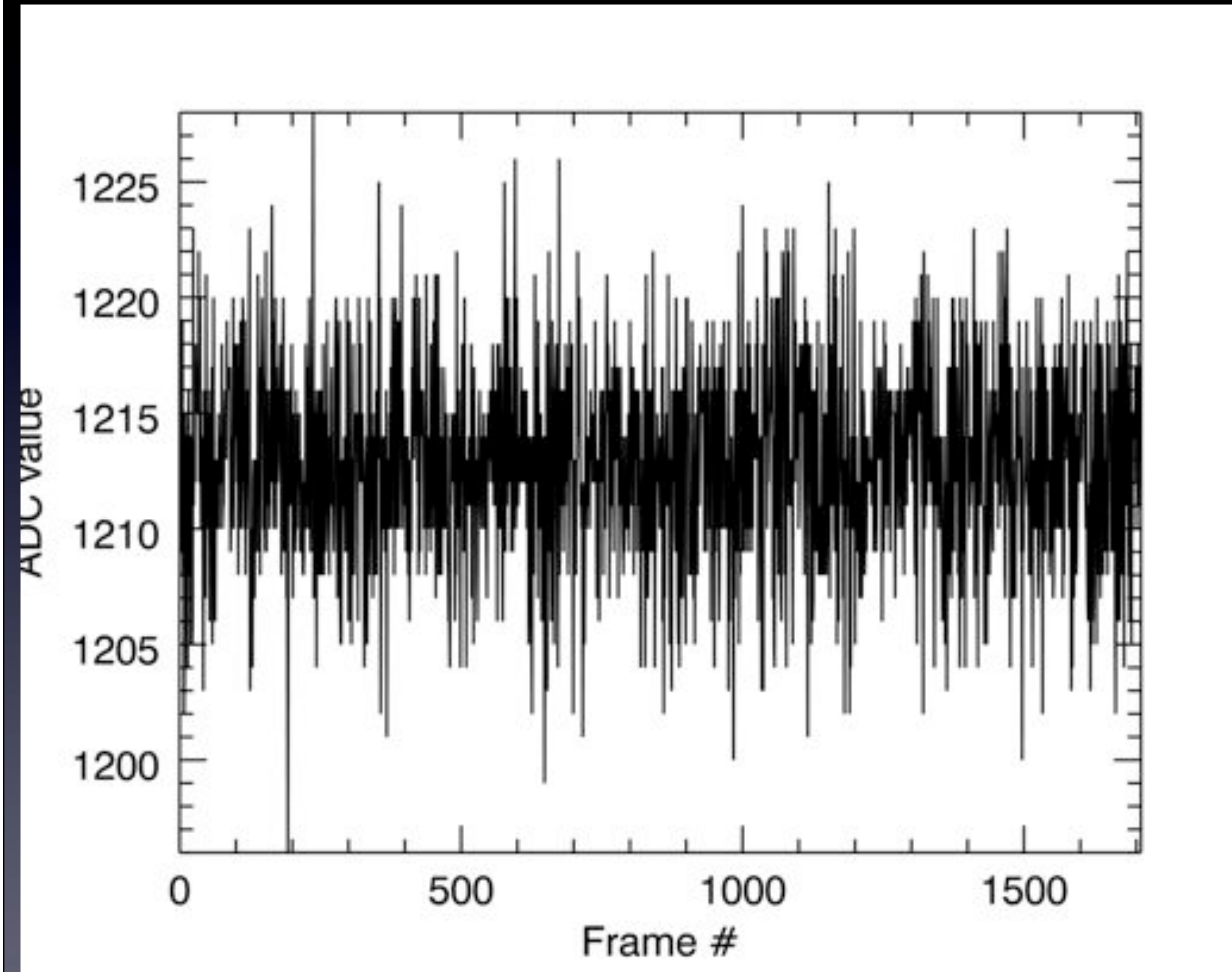
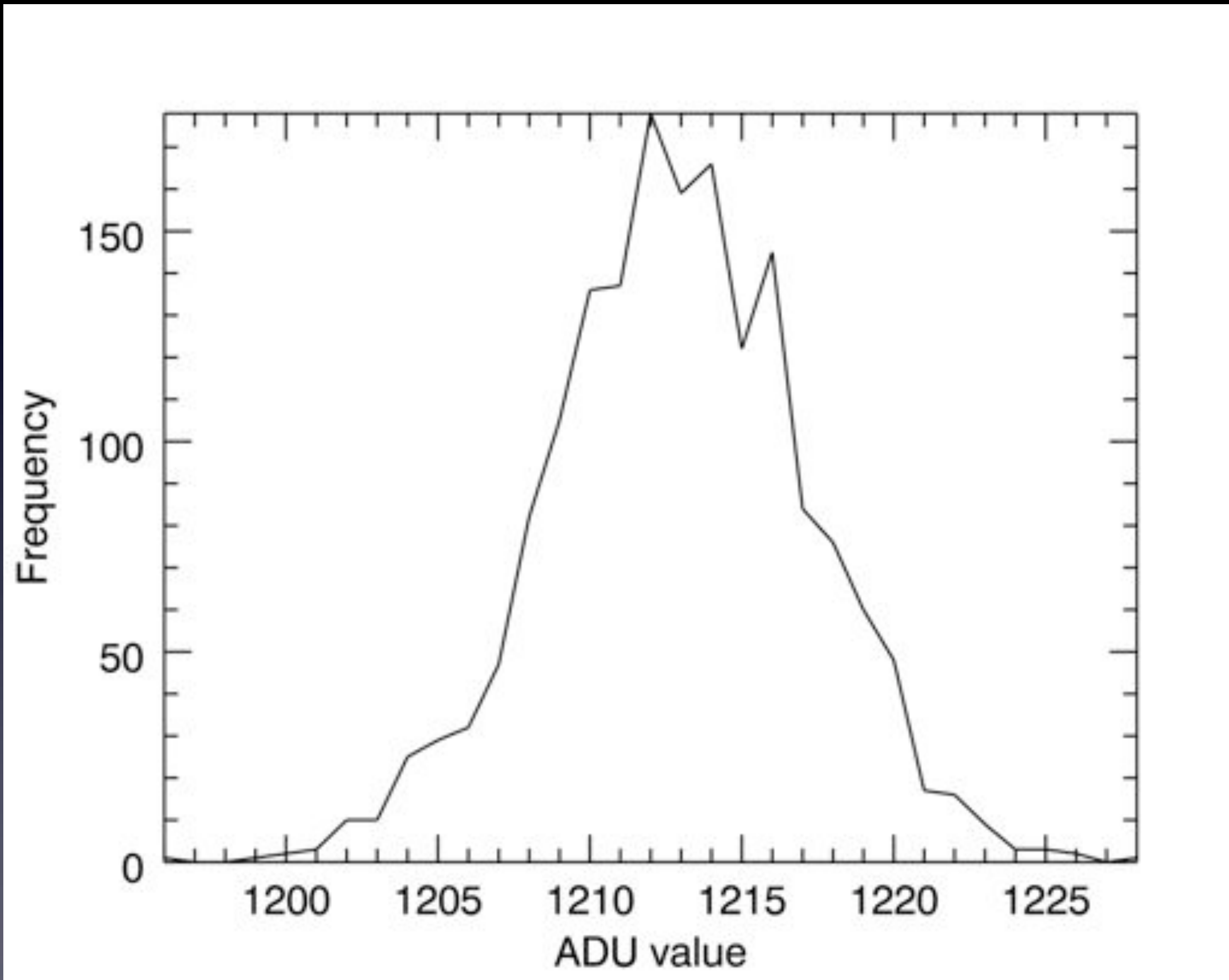
The offset on each pixel fluctuates over time



Mean = 1213 ADU
Std dev = 4.2 ADU
3-sigma = 12.7 ADU

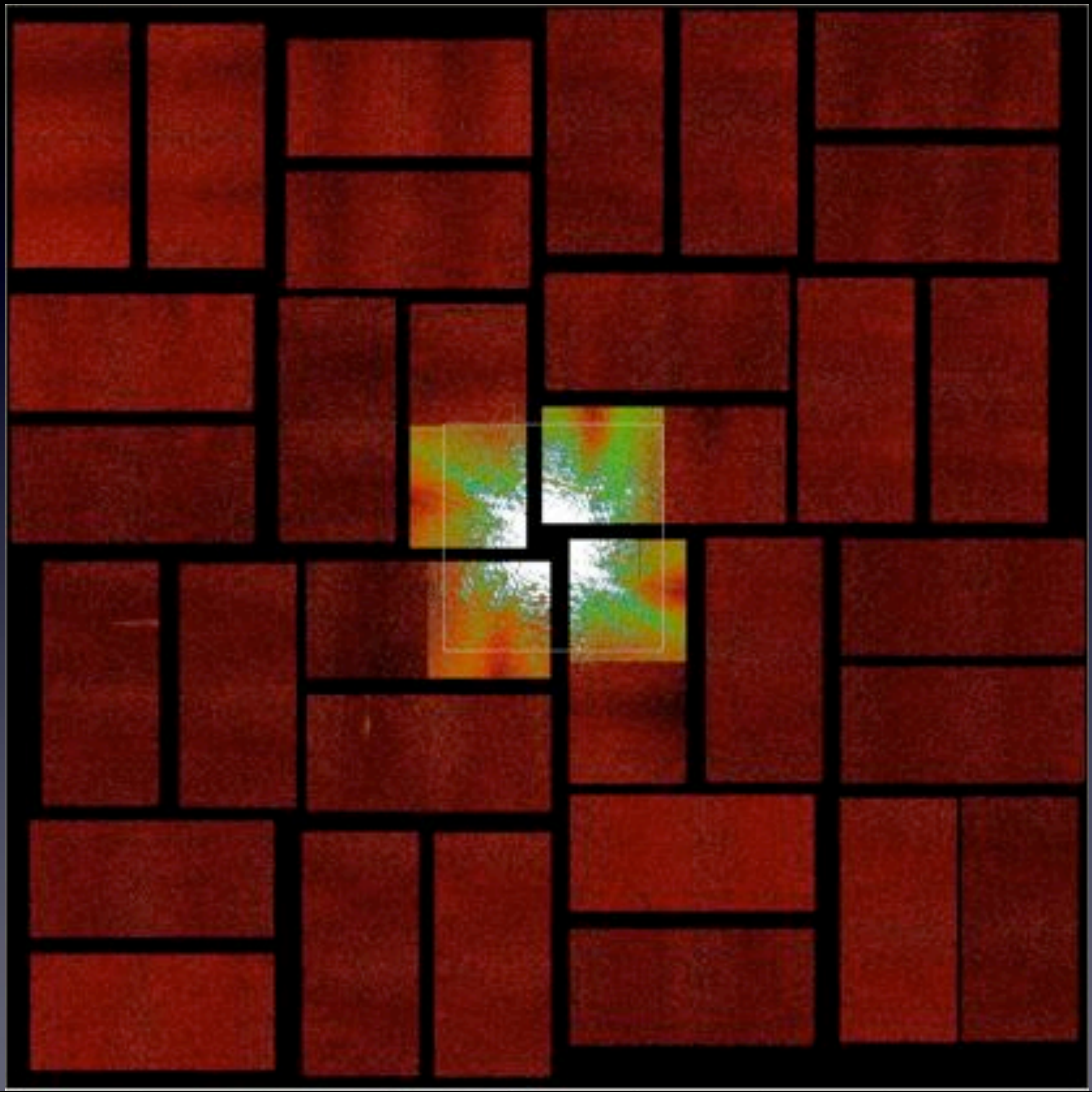
Distribution of dark pixel values looks roughly like a normal distribution



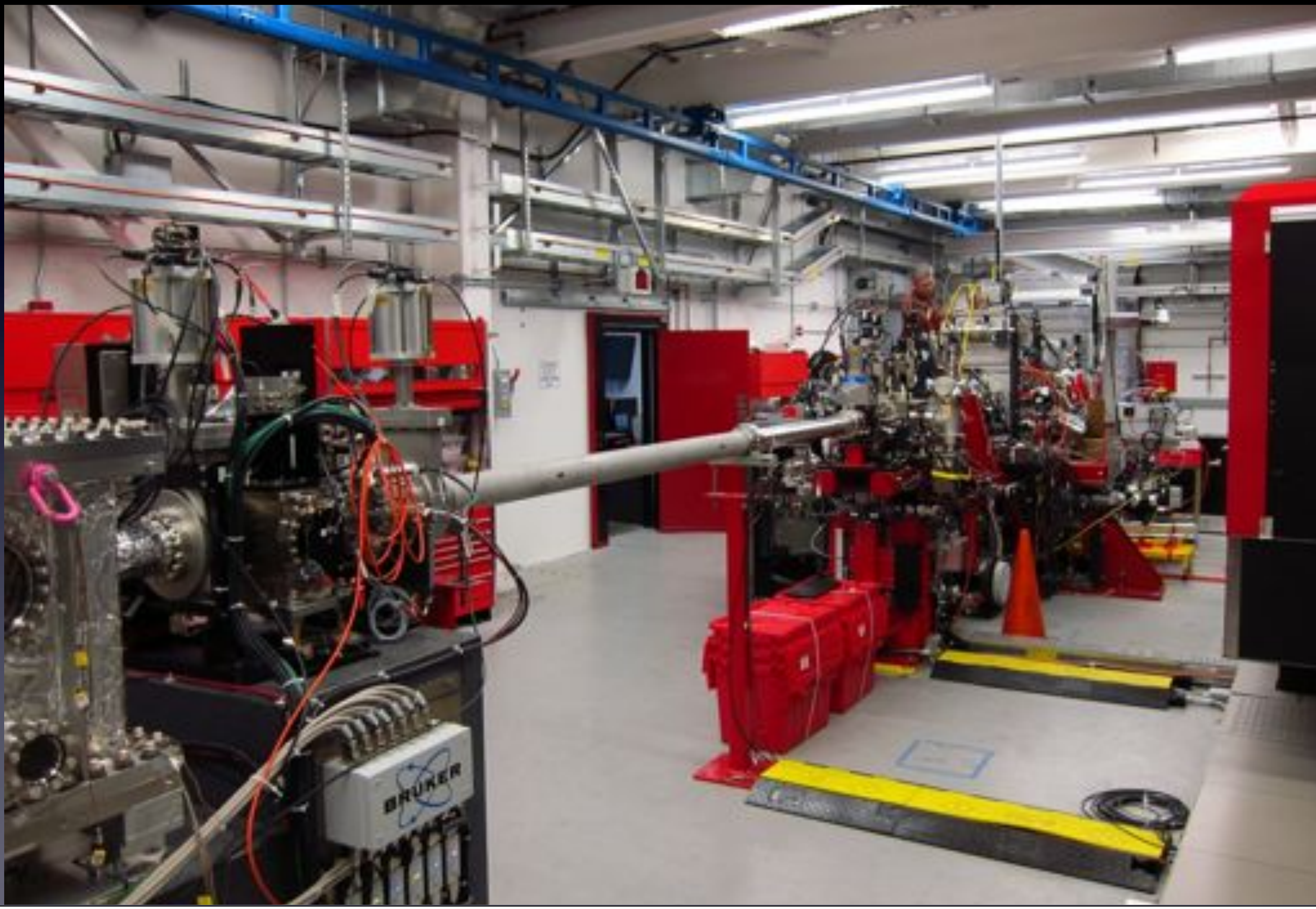
Mean = 1213 ADU
Std dev = 4.2 ADU
3-sigma = 12.7 ADU

CFEL SCIENCE

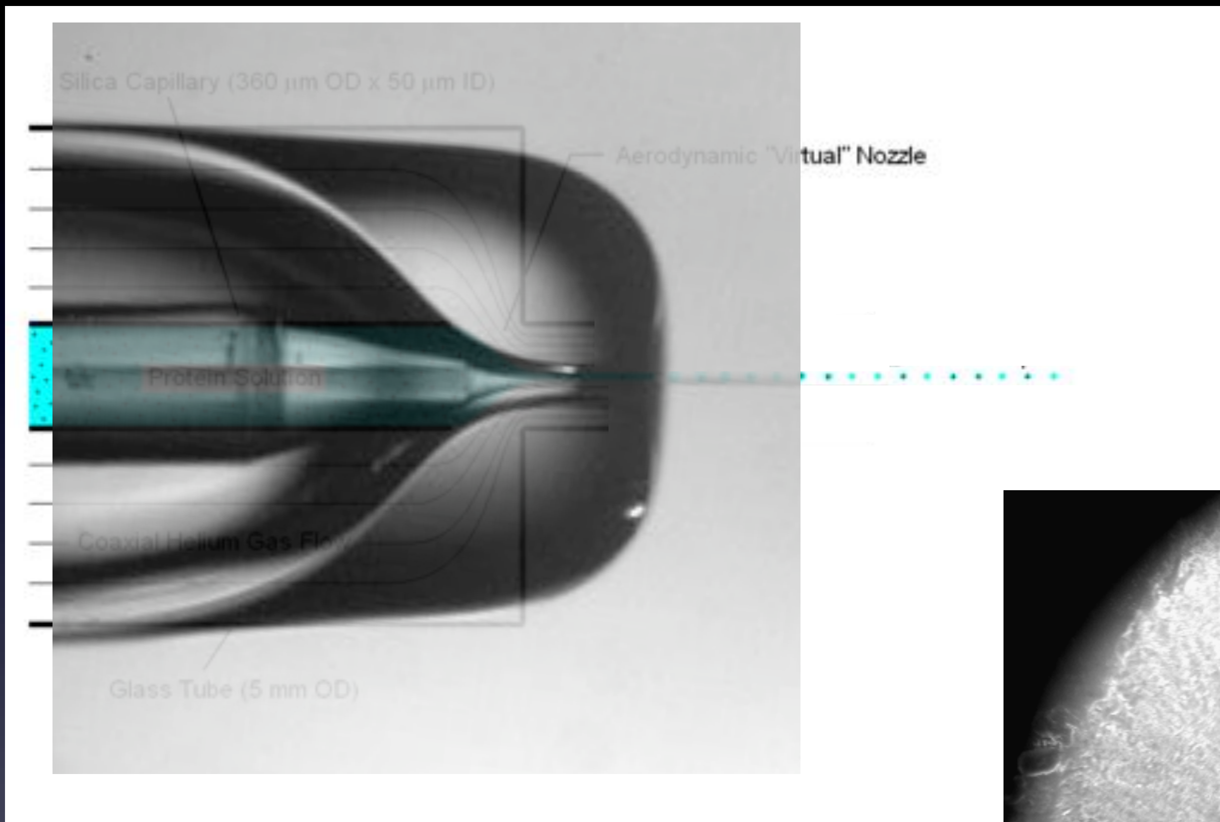
Offsets on individual ASICS vary with total signal (!)



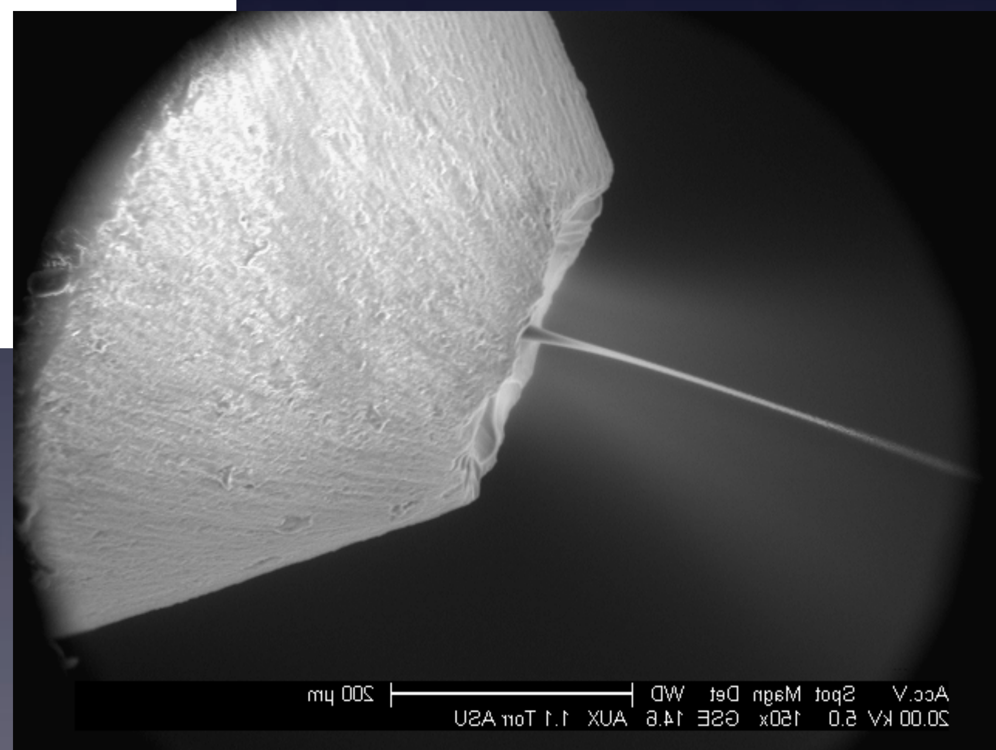
The first hard X-ray nanocrystal experiments were performed in the CXI instrument in February 2011



Submicron water jets are produced using a gas dynamic virtual nozzle



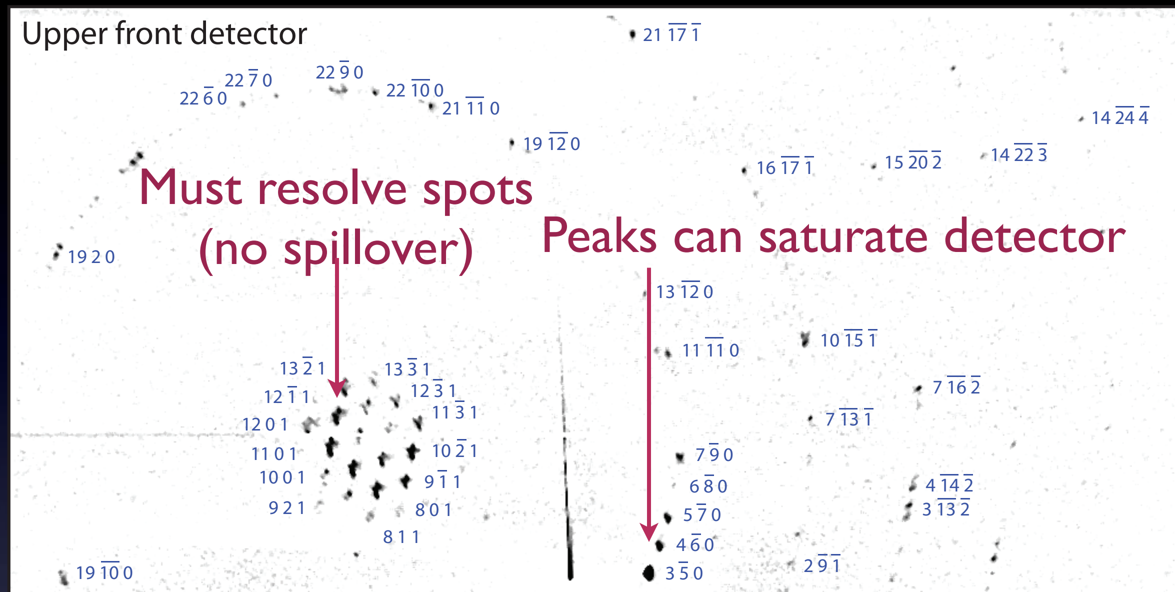
- Neutral drops do not disperse
- Droplets triggered by piezo
- Flow alignment possible
- Water acts as a tamper
- Sub-micron drops achievable

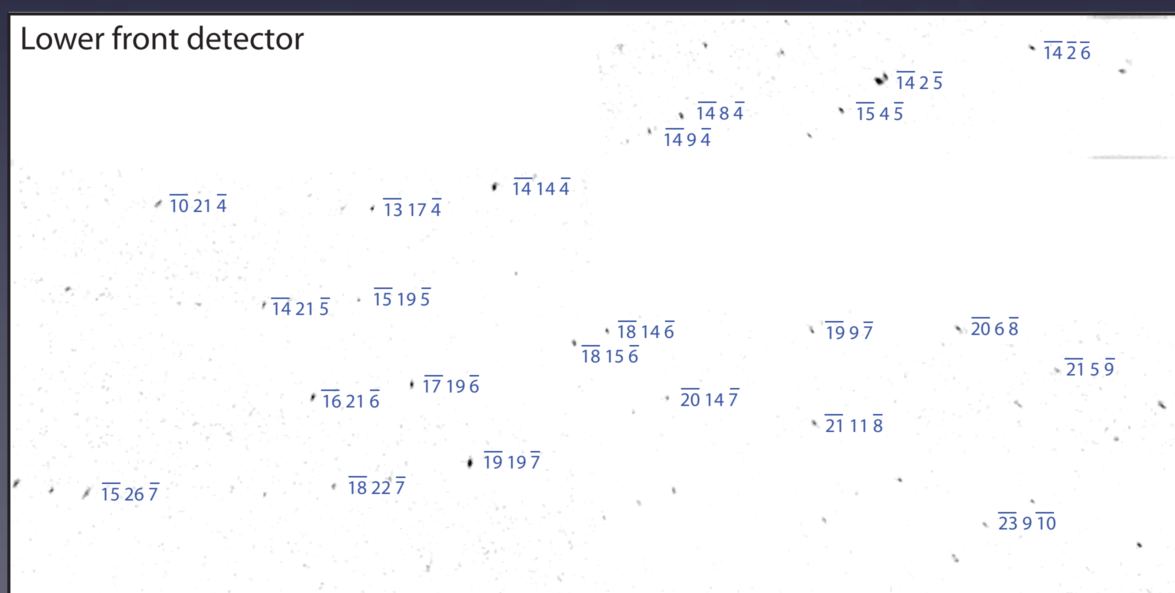


Dan DePonte (CFEL),
Bruce Doak, Uwe Weierstall, John Spence (ASU)

CFEL SCIENCE ASU

Nanocrystal diffraction gives rise to separated bright peaks, which must be distinguished and quantified





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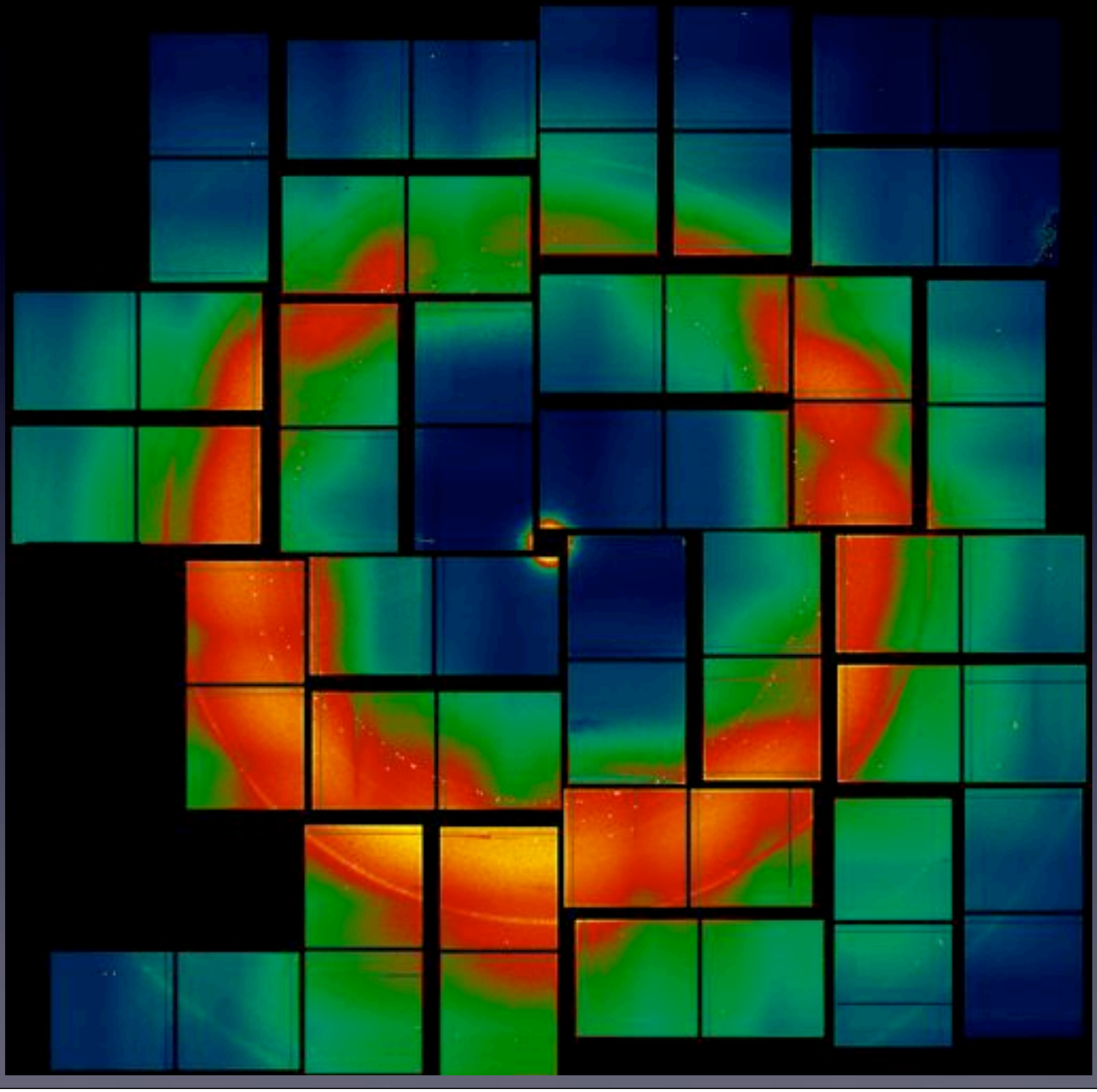
Sum of all frames is dominated by water ring background



LCLS pulses: 2,292,468

Acquisition time: 19,103 sec
(5 hr 18 min)

Photon energy: 9.4 keV

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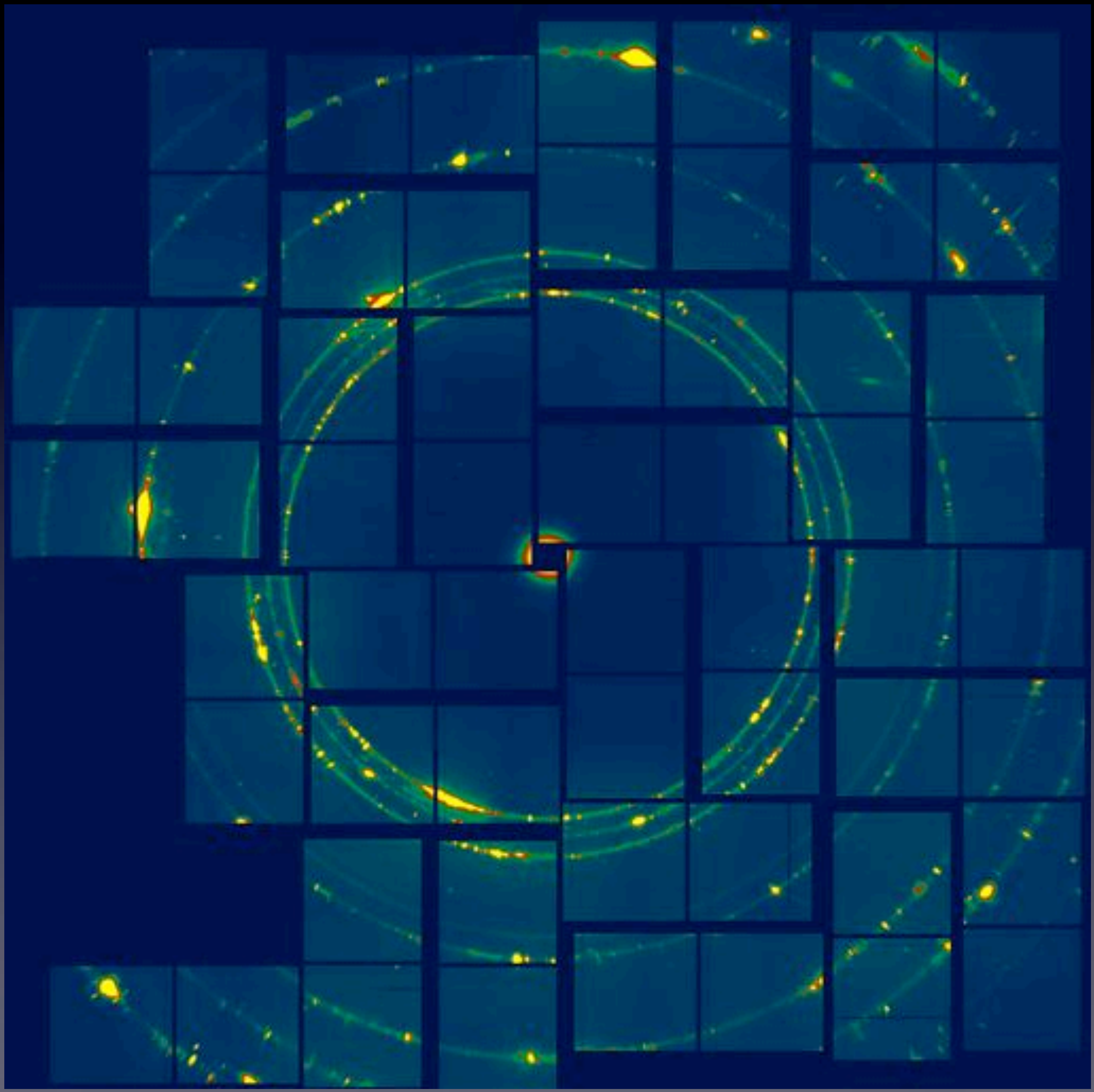
Ice gives rise to strong diffraction peaks on the detector



FEL pulses: 4,293

Acquisition time: 35 seconds

Photon energy: 9.4 keV

35 sec of ice delivered roughly the same local dose as 30 minutes of data collection

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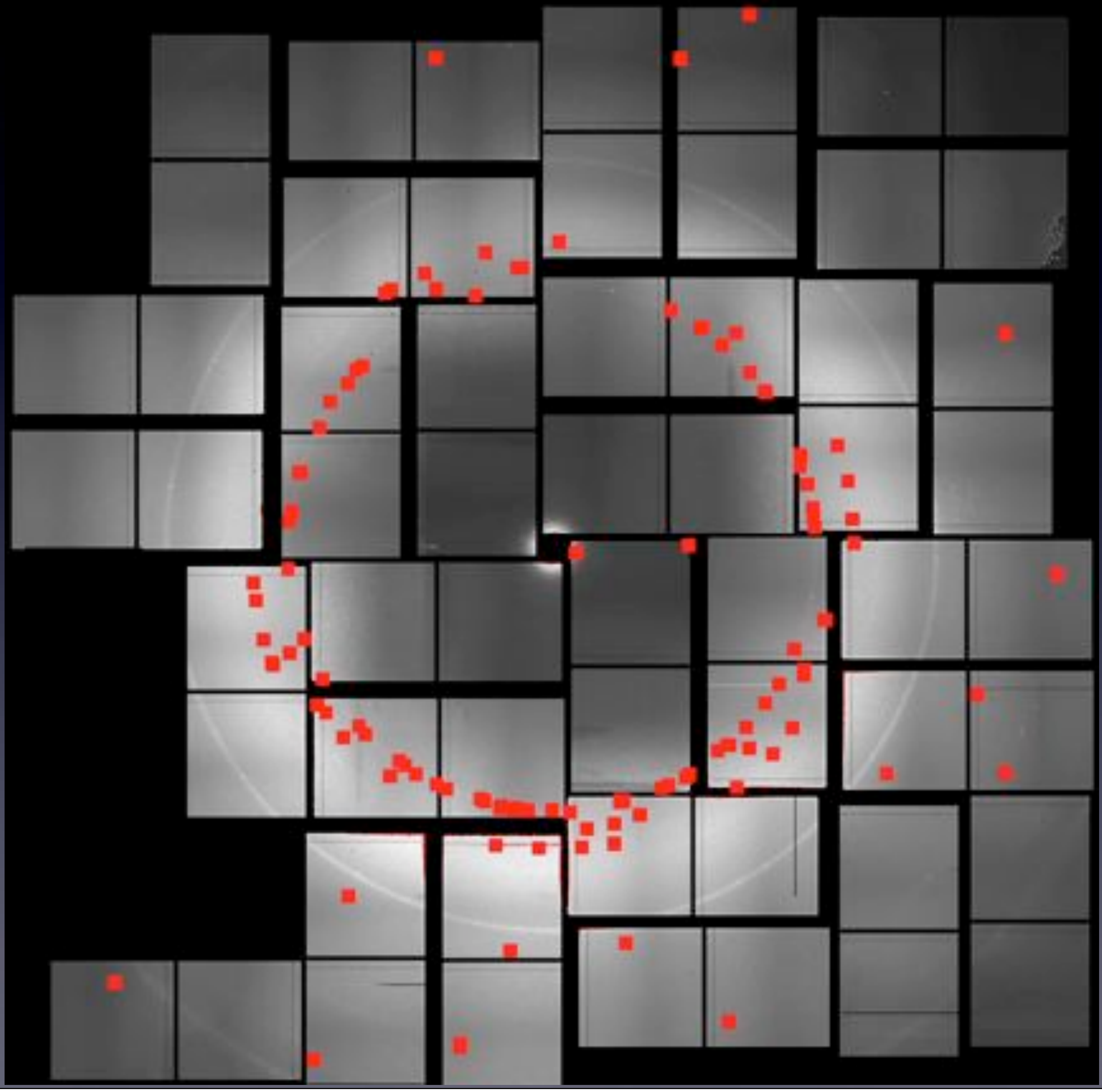
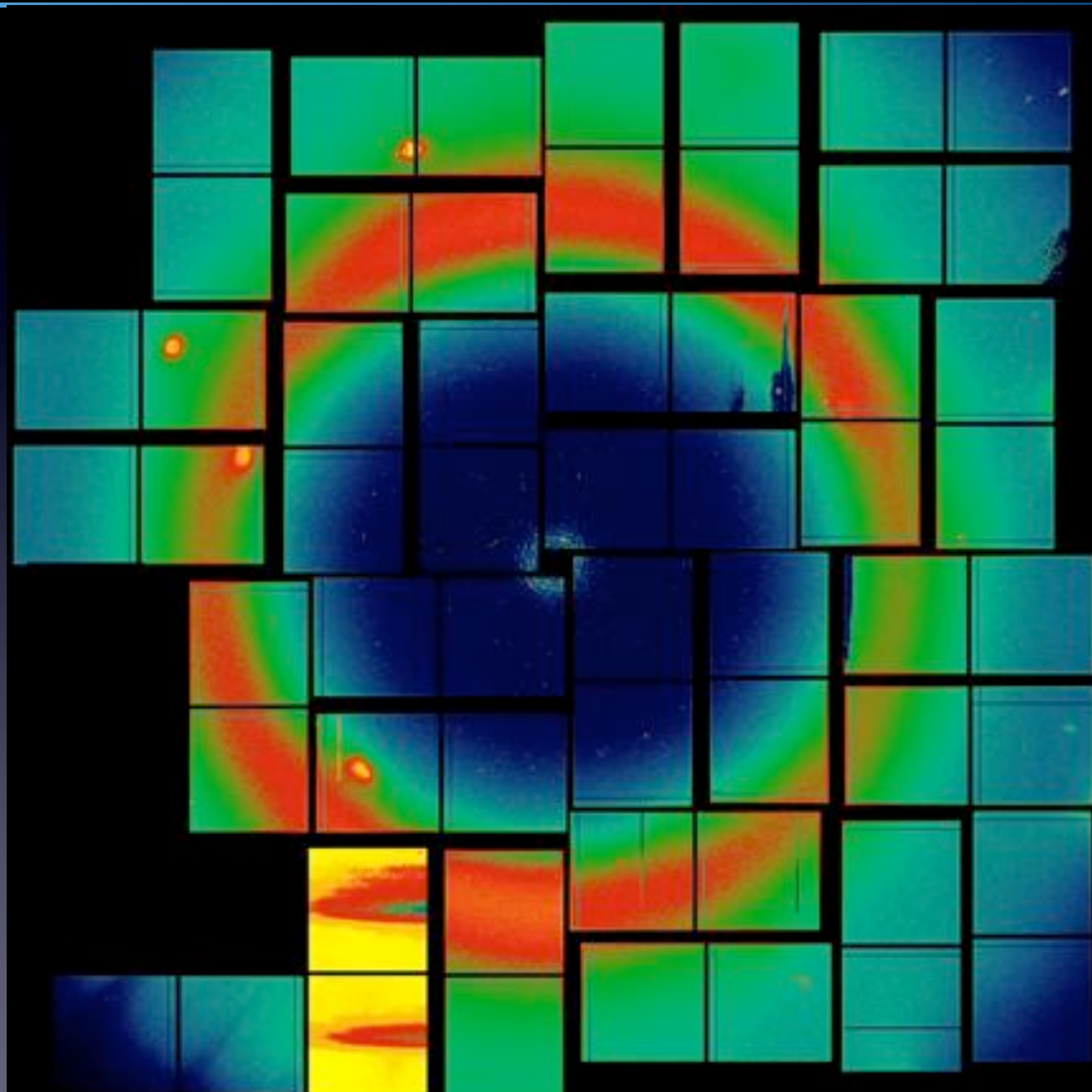
Dead pixels accumulate during the course of the experiment



Strong diffraction from accidentally forming ice can be very damaging to the detector



1/20 actual speed

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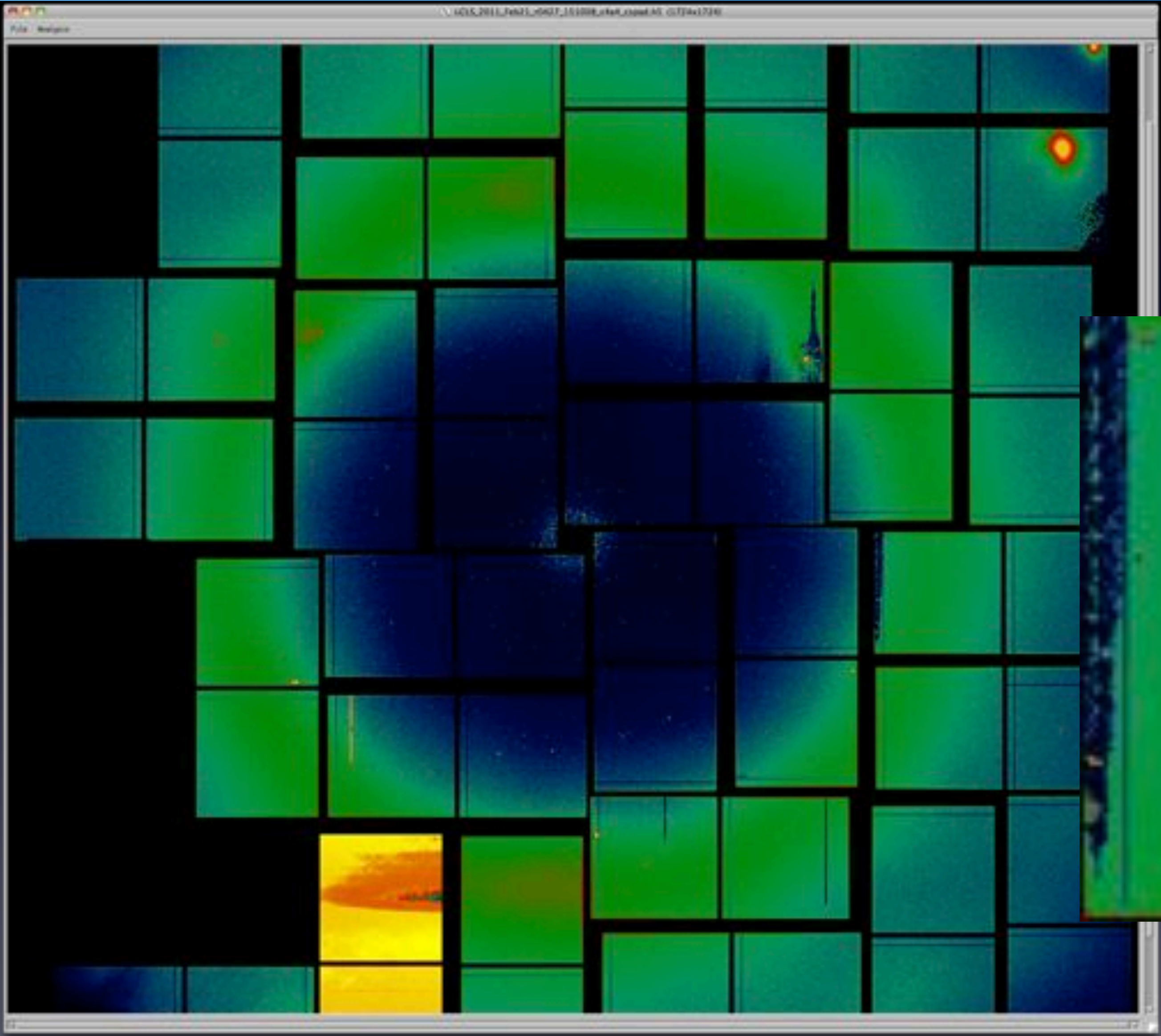
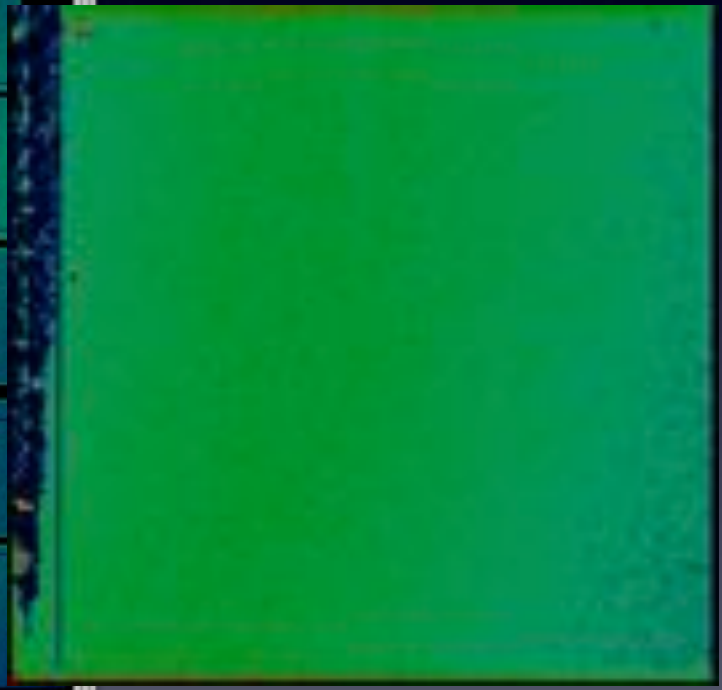
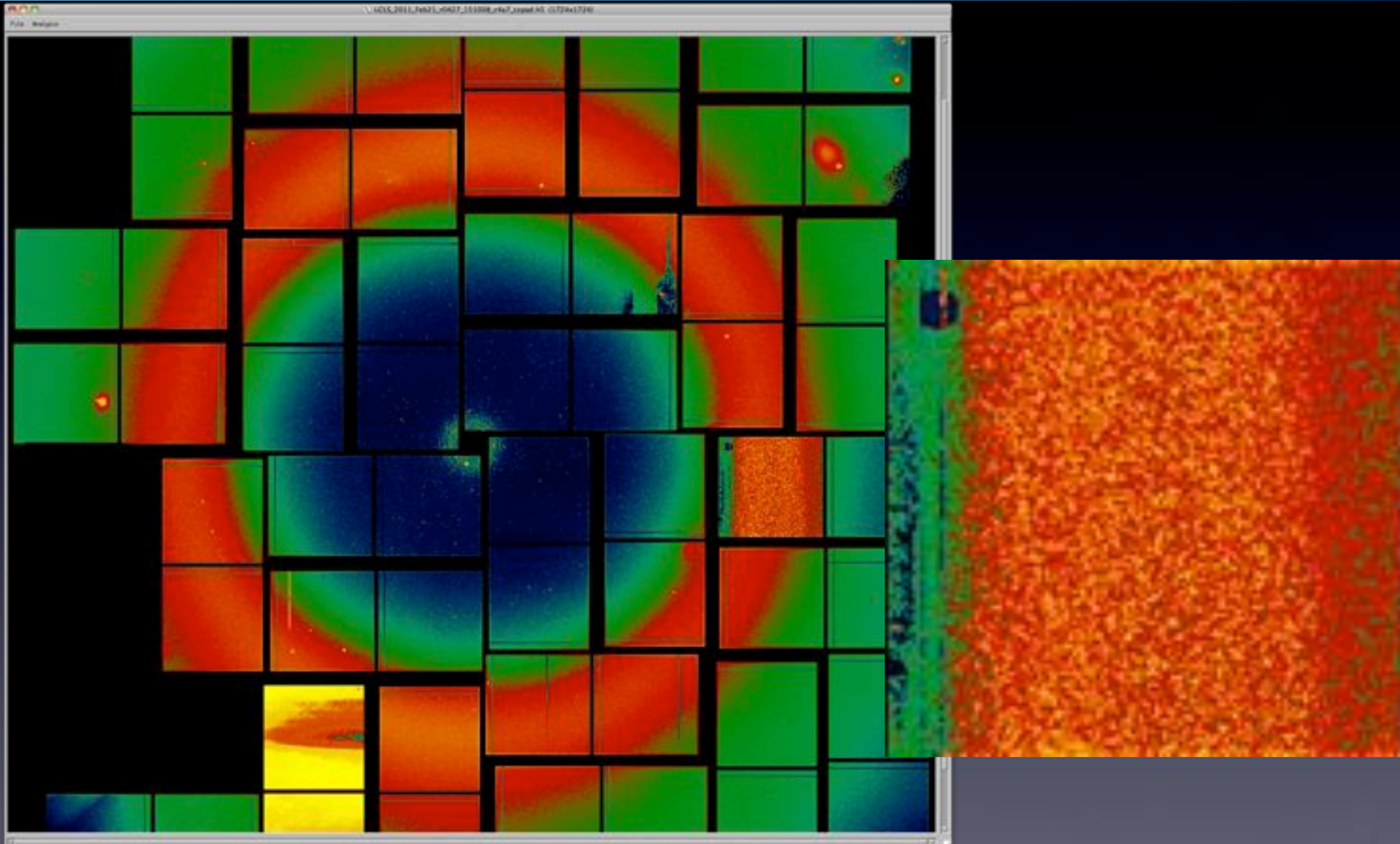
Death of an ASIC
Frame 1/4





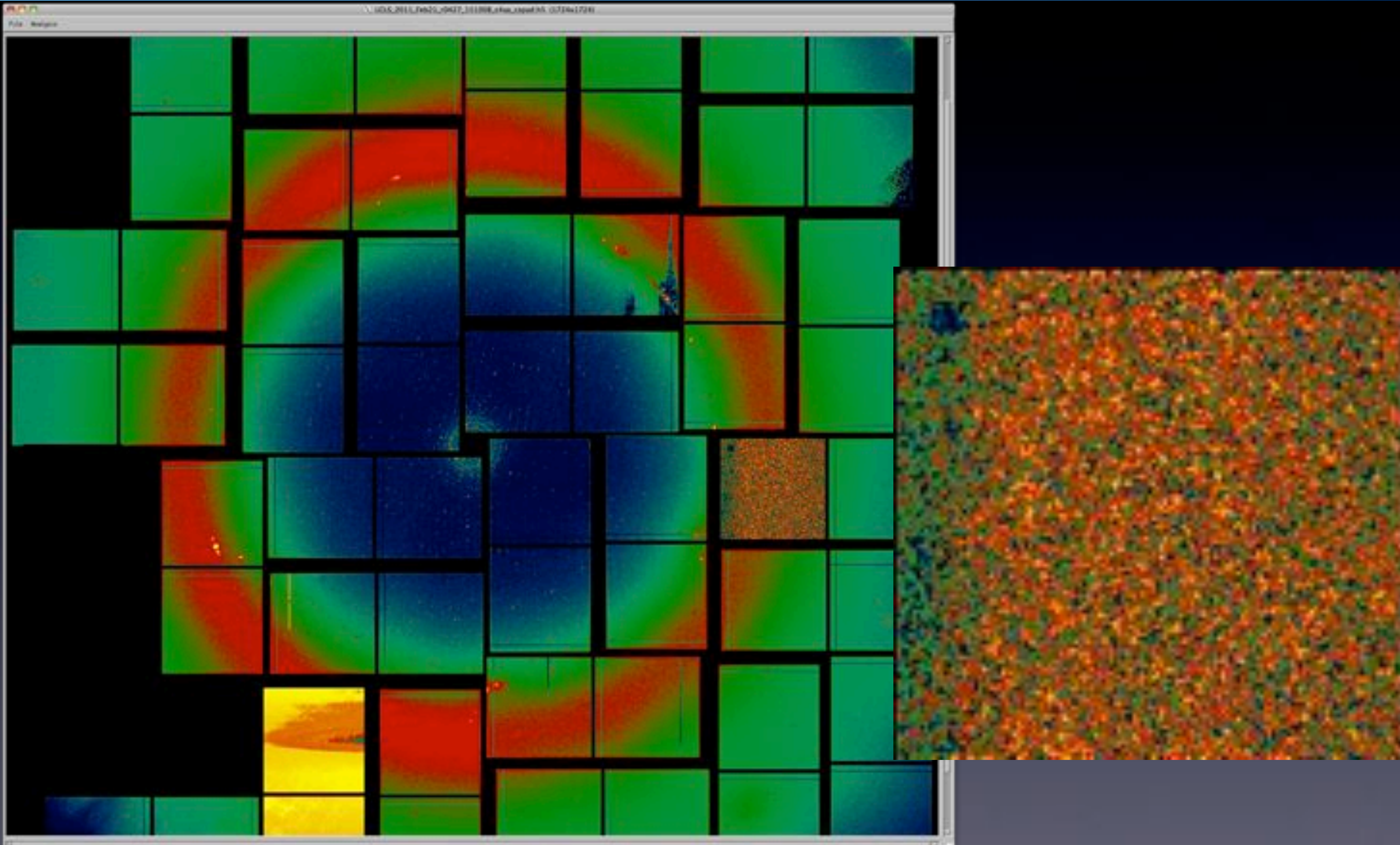
Frame 1:
Feb21_r0427_151008_c4a4

Death of an ASIC
Frame 2/4



Frame 2:
Feb21_r0427_151008_c4a7

Death of an ASIC
Frame 3/4



Frame 3:
Feb21_r0427_151008_c4aa

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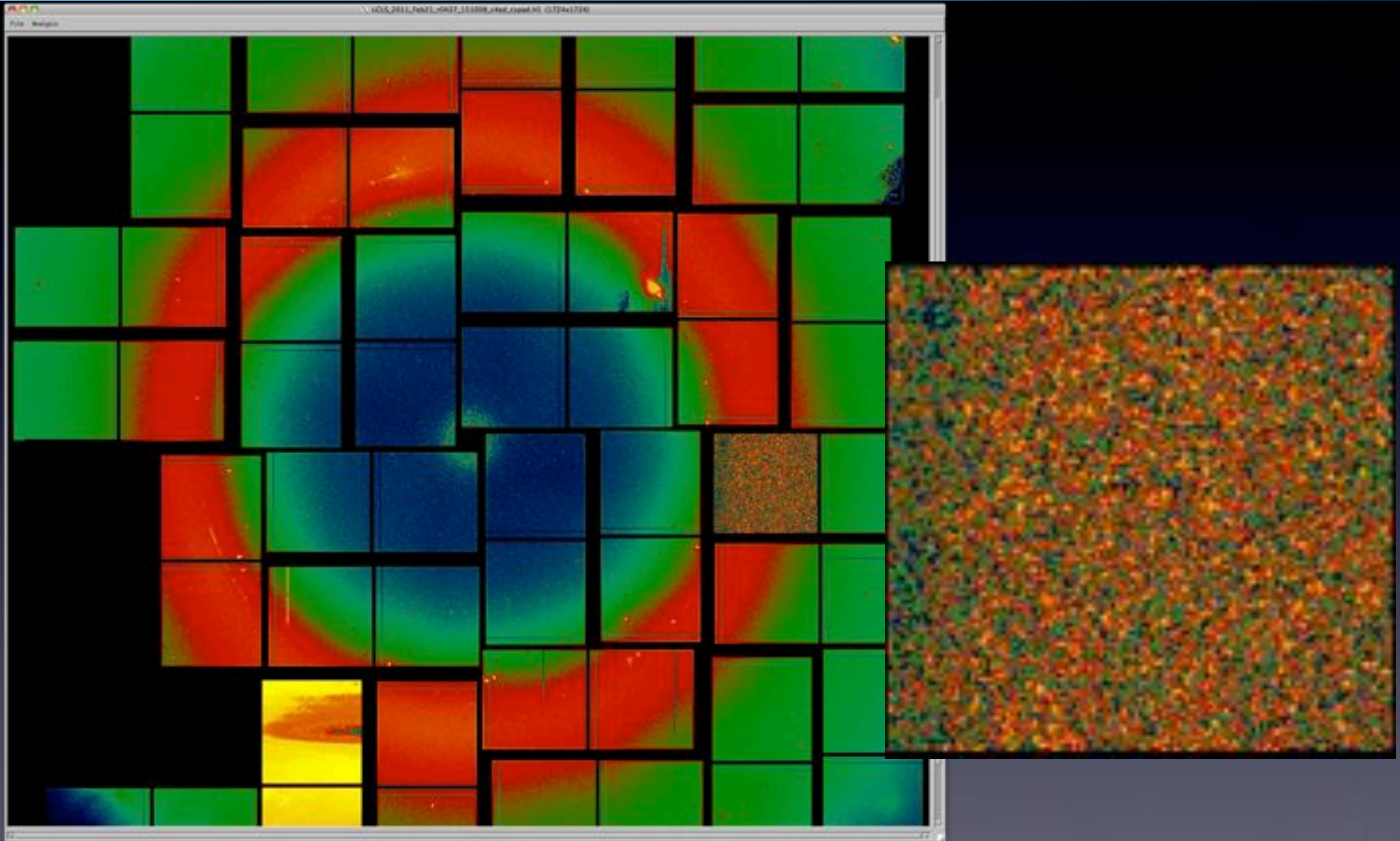
Death of an ASIC
Frame 4/4



Frame 4:
Feb21_r0427_151008_c4ad

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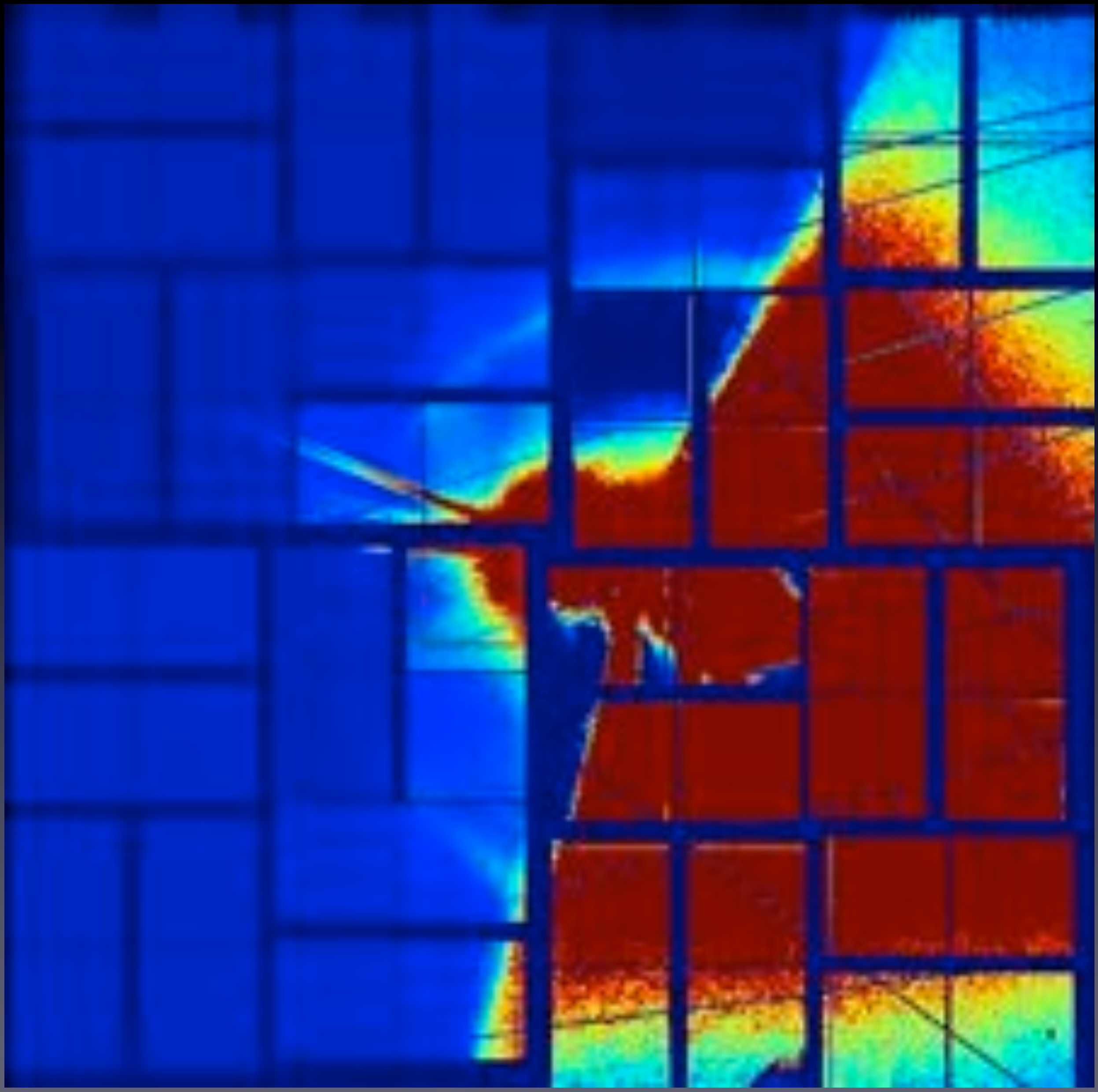
Accidents can happen: radiation dose event whilst moving hardware in the chamber



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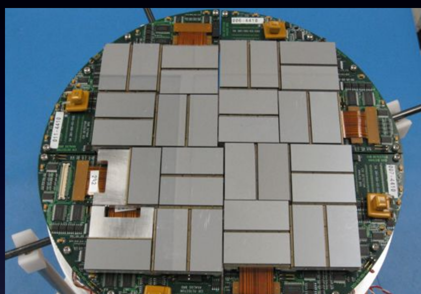
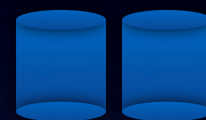
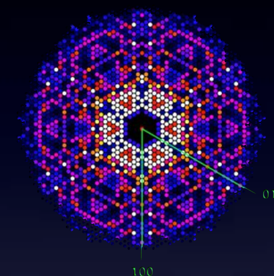
Our processing pipeline is an exercise in data volume reduction

Detector	Storage	Offline reduction	Analysis
			
120 Hz 4 MB/event 1.7 TB/hr 200TB/expt (5 days)	> 20,000,000 frames	< 1,000,000 frames	<100,000 frames
	Store all data (no corrections)	Retain only 'hits' (detector corrected)	Science analysis

Automated high volume image processing is essential
(reliable background correction, automatic identification of useful data)

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My data must be somewhere here....

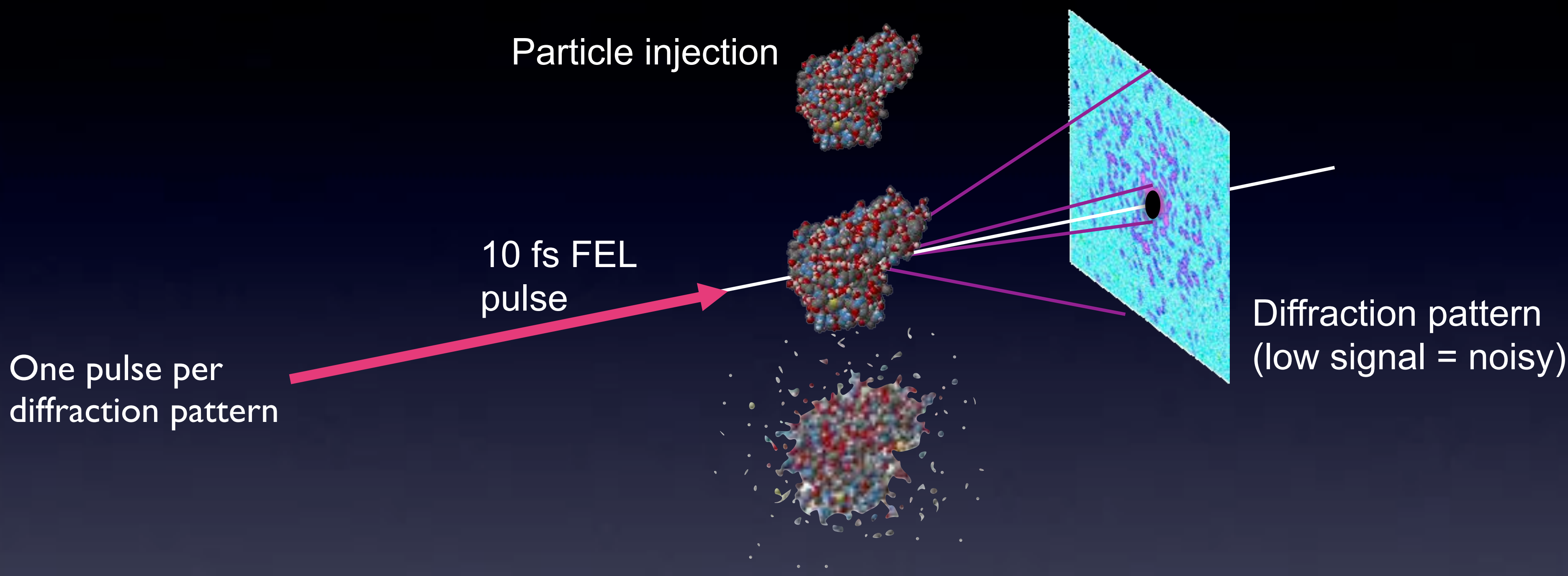
Data Direct Networks SFA10000
60-bay HDD enclosures in 4U format
~1.4 PB formatted per rack (600 x 3 TB HDDs)

600x 3TB hard drives

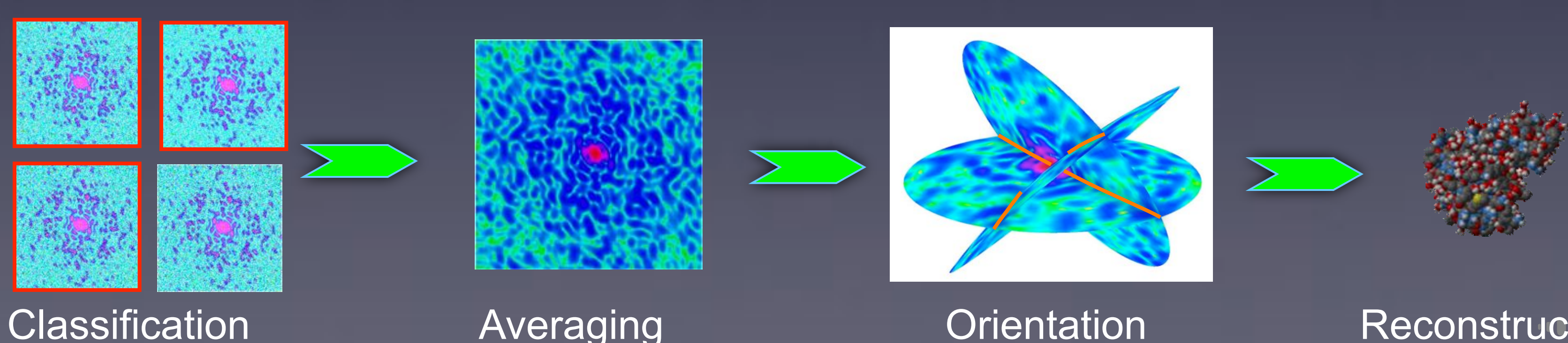
Scalable to over 13,440 HDDs
(over 10,000 TB formatted capacity)

CFEL SCIENCE

X-ray free-electron lasers may enable atomic-resolution imaging of macromolecules without the need to grow large crystals



Combine 10^5-10^7 measurements



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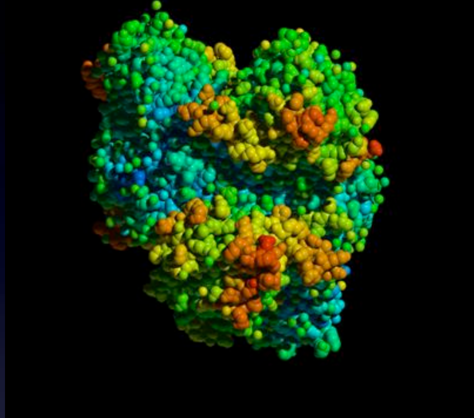
Moving to longer wavelengths increases the number of detected photons at the expense of spatial resolution

Anthrax lethal factor (1YQY)

12924 atoms, mol.wt. = 86,283 Da

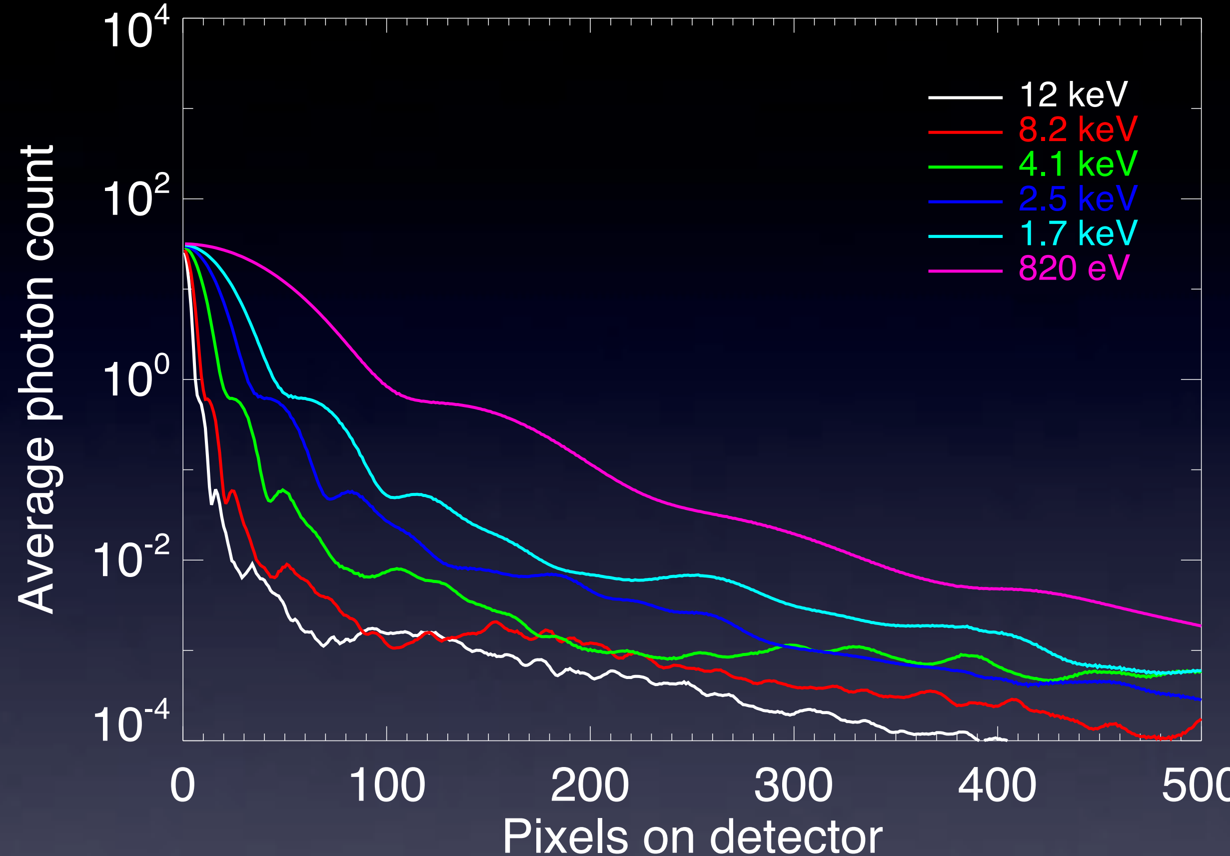


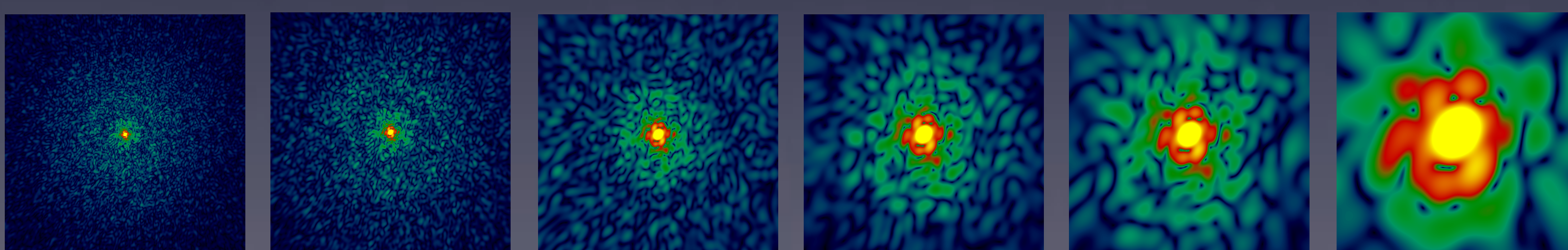
LCLS configuration

10^{12} X-rays in 100nm square

760x760x110μm pixel detector

placed 50mm from focus





12 keV (1 Å) (1,500 photons total)

8.2 keV (1.5 Å) (3,200 photons total)

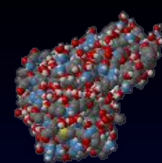
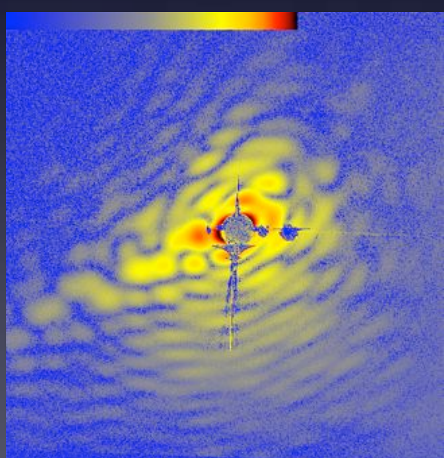
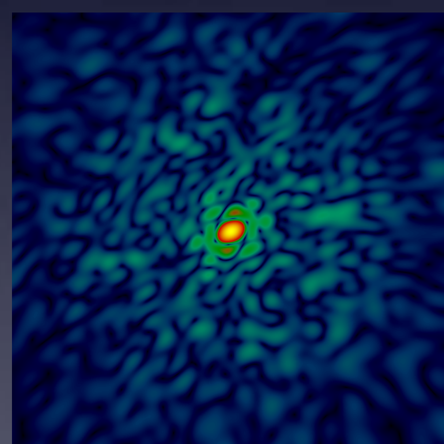
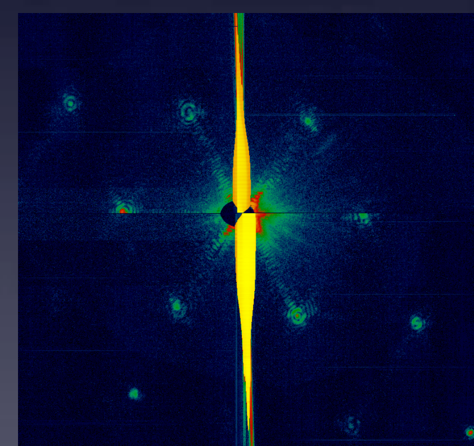
4.1 keV (3 Å) (12,000 photons total)

2.5 keV (5 Å) (33,500 photons total)

1.7 keV (7 Å) (66,500 photons total)

820 eV (15 Å) (299,000 photons total)

Ultrafast coherent imaging requires integrating detectors that can read out a full frame on each pulse

Heterogeneous objects	Single molecules viruses, etc	Protein nanocrystals
		
Reconstruct unique objects	Average weak signal	Index Bragg peaks
500 eV - 2 keV	2 - 8 keV	6-12 keV
		
No averaging: All data in a single shot High dynamic range	Very weak: Must average many shots Single photon discrimination	Bright, isolated peaks High dynamic range

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Our ideal detector must satisfy many constraints

Property	Why?
Pixel arrays 2k x 2k or more (1k x 1k minimum)	Need to resolve fine diffraction features
Actual pixel size not critical	Detector must fit in facility or vacuum vessel
Readout at facility repetition rate (LCLS: 120 Hz, XFEL: ~3000/bunch)	Each pulse creates a unique event
Dynamic range >10^4	Highly varying signal intensity
Low noise, single photon detection	Signals can be weak at high resolution
Stable pixel positions (<1/10 pixel)	Location of peaks need to be well defined
Photon integrating (not photon counting)	Multiple photons/pixel all come in <100 fs
Saturation is well controlled	Need to separate adjacent strong peaks
Correctable and well characterised artifacts	Robust background subtraction essential
Reliable, works when needed	Beamtime is very expensive
Replaceable modules	Radiation damage is a concern

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This work was the product of a large international team

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