

DEPFET for structural biology

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“Construction of novel detector system for structural biology using
ultrafast fine pixel detector”

[Principle Investigator: WAKATSUKI, Soichi]

Needs of new detectors for protein xtallography/solution scattering experiments

Protein Crystallography

Now Efficient data collection

Issue How to measure intensities of very small spots from micro beam/micro xtals

Solution scattering

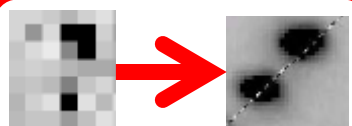
Now Protein folding & solution dynamics of protein-protein interactions

Issue fastest dynamics: ~msec

Targets: structure and dynamics of large complexes and membrane proteins

Required properties of X-ray detectors

- **High spatial resolution**
- High sensitivity
- Large # of pixels



- **Fast readout**
- Large dynamics range



CCD
(Quantum315r)

Detector	Effective pixel size	Readout speed	Dynamic range	Readout scheme
CCD	90 μm	300 msec	10^4	Integration
Hybrid PAD	170 μm	3 msec	10^6	Streamlined



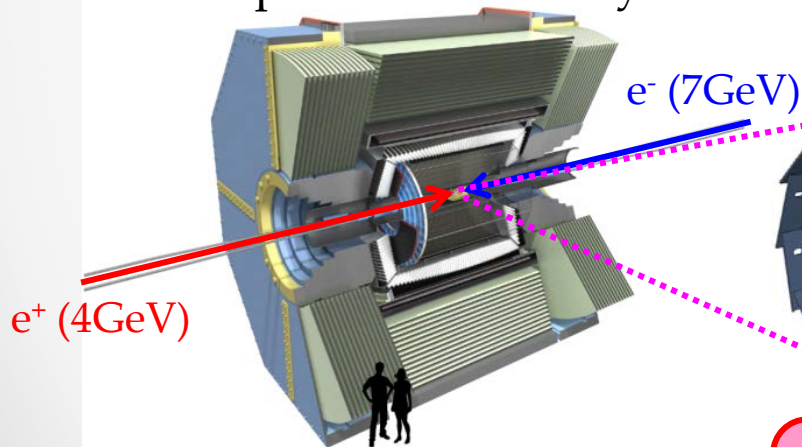
Hybrid PAD
(Pilatus 6M)

Needs for fast & high spatial resolution 2D detector for micro/nano beam

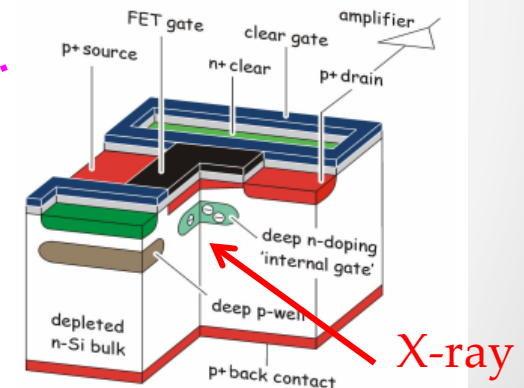
DEPFET (DEpleted P-channel FET)

Detector type	# of pixels	Eff. resolution	Readout speed	Dynamic range	Readout
C C D	16 M	90 μm	300 msec	10^4	Integration
Hybrid PAD	6 M	170 μm	3 msec	10^6	Continuous
DEPFET	8 M	<u>50 μm x 55 μm</u>	<u>20 μsec</u> (Continuous)	10^7 (Integration mode)	Switchable between integration / continuous

Belle II @ Super KEK B-factory



DEpleted P-channel FET



Upgrade of KEK-B, Nobel Physics Award to Kobayashi & Masukawa (Expected to start operation in 2015)

Major development for structural biology appl.

- ① smaller pixels large area
- ② Fast readout: 20 μs

Sensor chip development by Max Plank Institute, Munchen

① Prep for developing the DEPFET detector

Preliminary experiments to characterize DEPFET sensors for structural biology applications

① – I High spatial resolution images of diffraction patterns

Check of spatial resolution and peak shape with small beam matched for small xtals

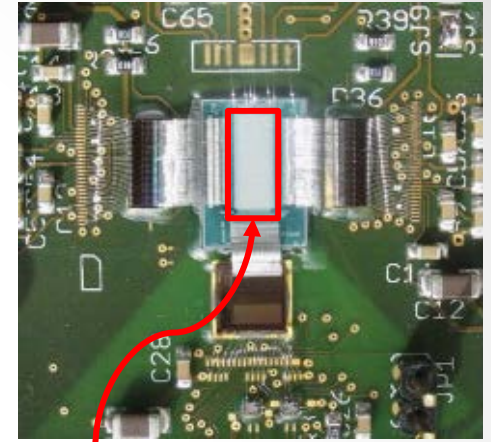
① – II Fast readout data acquisition for solution scattering experiments

Protein folding and photo excitation dynamics followed by time-resolved SAXS with 20 μsec time resolution

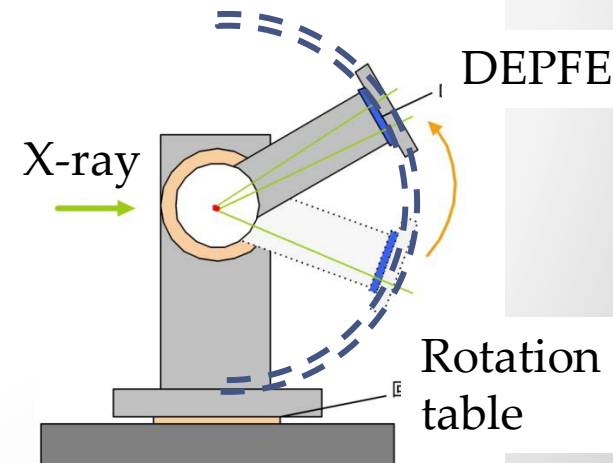


Design optimization of for large-area DEPFET detector system

- Spatial resolution, sensitivity, non-uniformity, dynamic range etc.
- Comparison with commercially available detectors



Small DEPFET sensor
256 × 64 pixels



Pseudo large solid angle data collection using a rotation stage

② Development of large area DEPFET detector

②— I Ultrafast readout system

**Crystallo-
graphic
analysis**

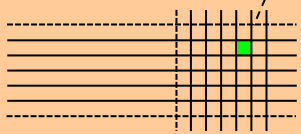
8 bit/pixel

**Integration
mode**

Large area sensor
 1536×256 pixels

On-the-fly integration of max
50,000 images (24 bit/pixel)

Software
for noise
reduction



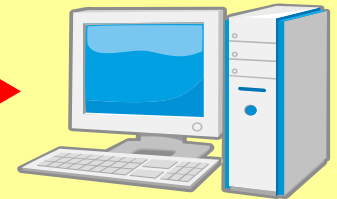
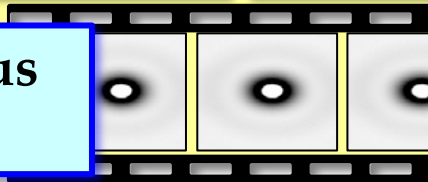
ADC

Fast noise
reduction

Max 1 Gbytes/sec

Fiber optics

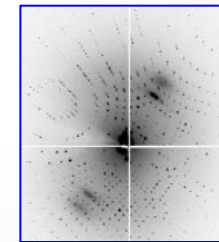
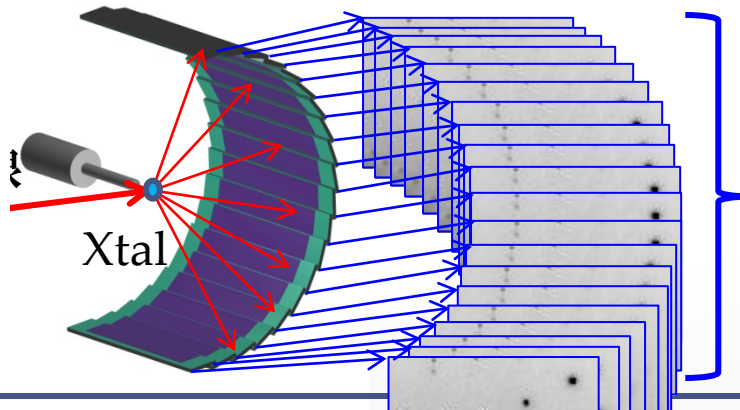
**Fast continuous
mode**



Protein dynamics

②— II 8M pixel DEPFET detector based structural/dynamics analysis system

8M pixels
with 20
DEPFET
sensors



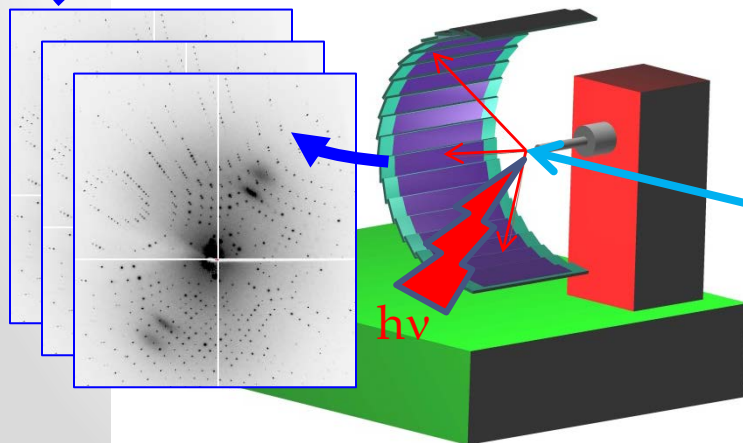
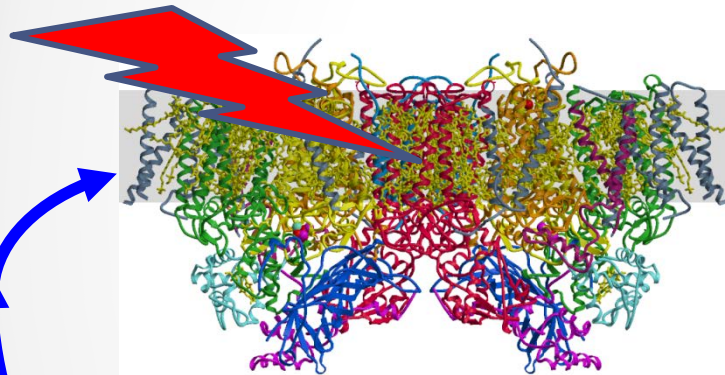
Data acquisition and analysis

③ Applications to challenging targets

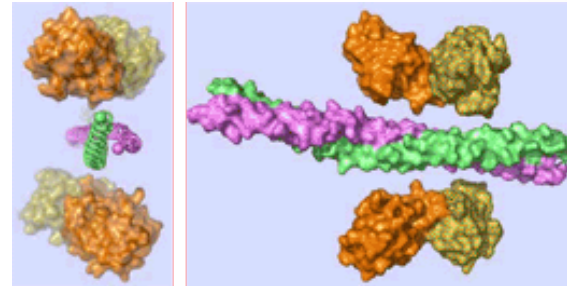
③— I Structural analysis and dynamics of membrane protein complexes & large complexes

Structure dynamics of complexes involved in photo synthesis and respiratory chain : photo excitation dynamics

Photo excitation

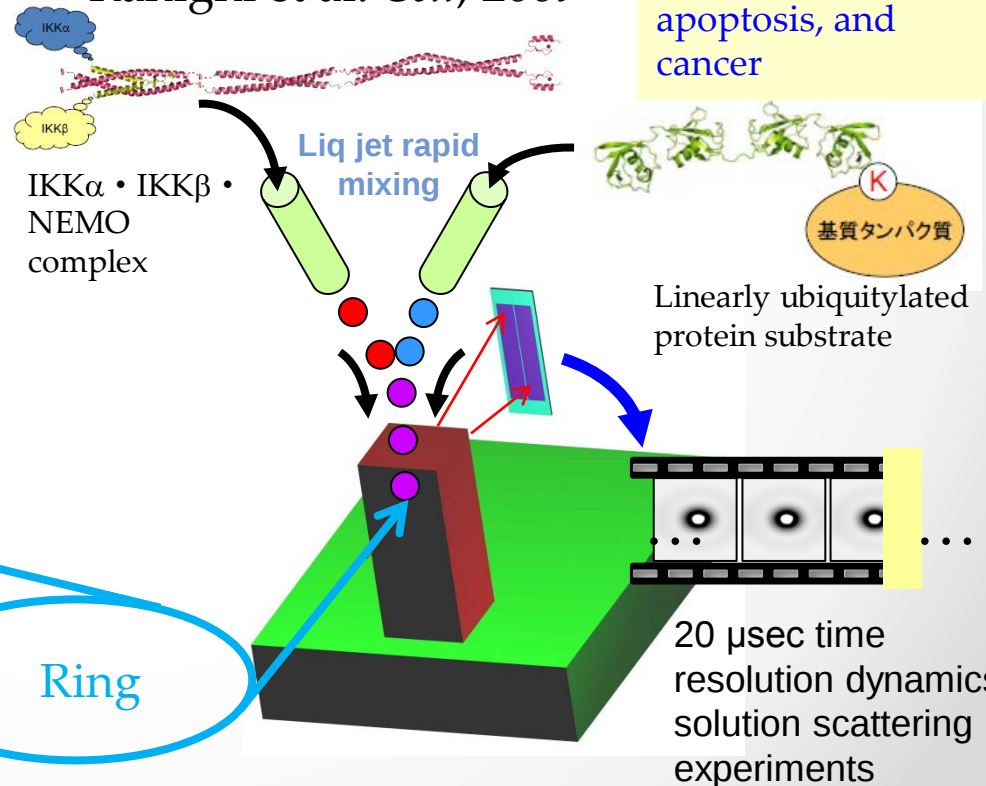


③— II Solution studies of domain association-dissociation dynamics and kinetics of signalosomes



Structural changes of NEMO in complex with linear ubiquitin chains: NF- κ B signal transduction pathway involved in inflammation, apoptosis, and cancer

Rahighi et al. *Cell*, 2009



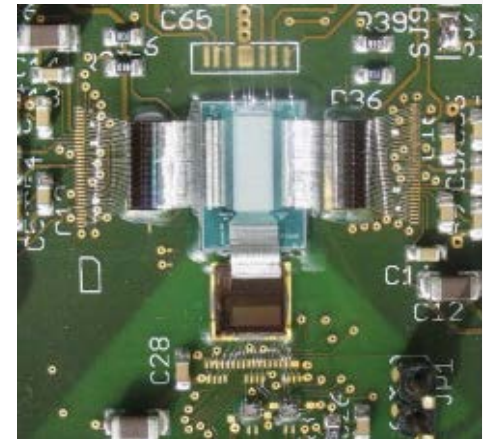
Current Studies

Preliminary experiments to characterize DEPFET sensors for structural biology applications

DEPFET early prototype sensor

Pixel size: $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$

- Sensor thickness: $450\text{ }\mu\text{m}$
- Sensor size : $5120\text{ }\mu\text{m} \times 1280\text{ }\mu\text{m}$
(256 x 64 pixels)
- Slow readout ($\sim 100\text{Hz}$)

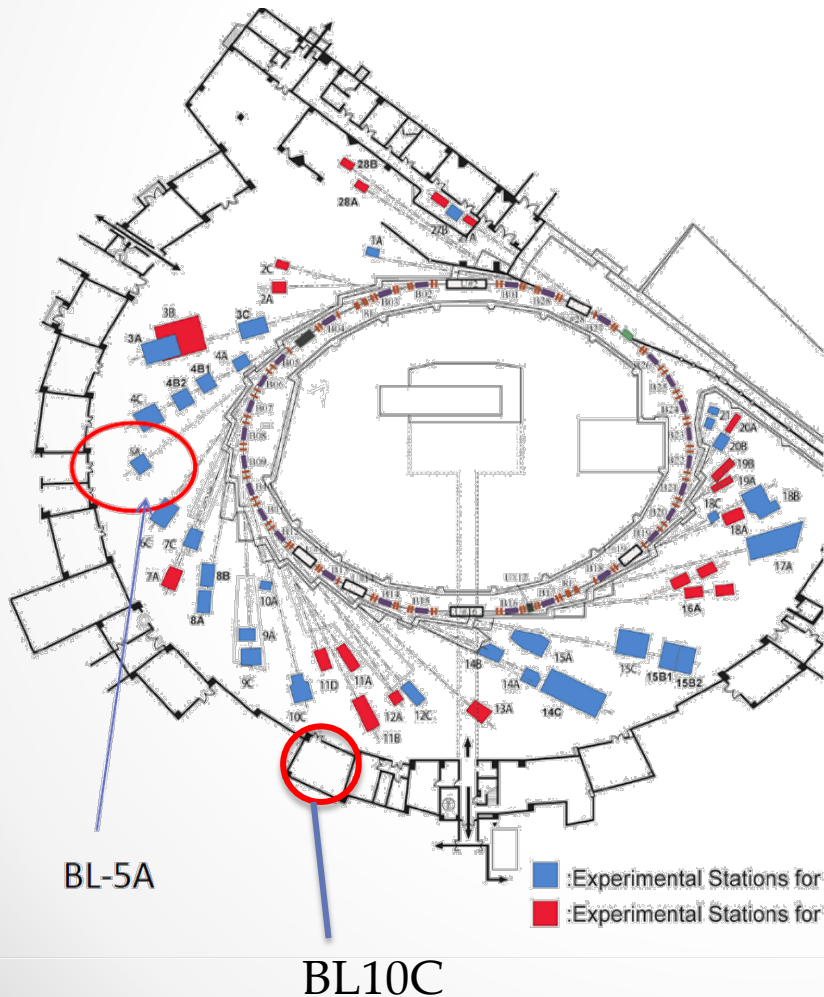


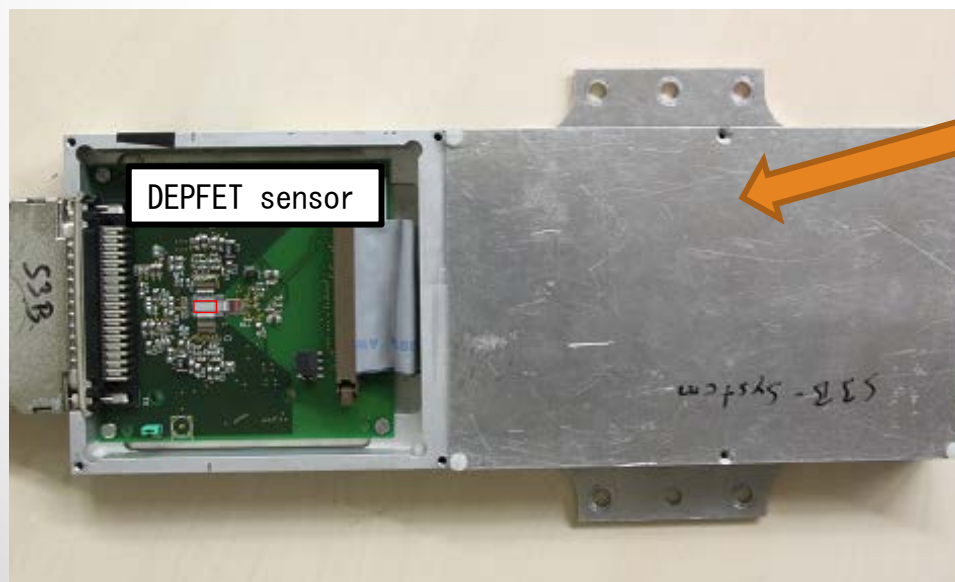
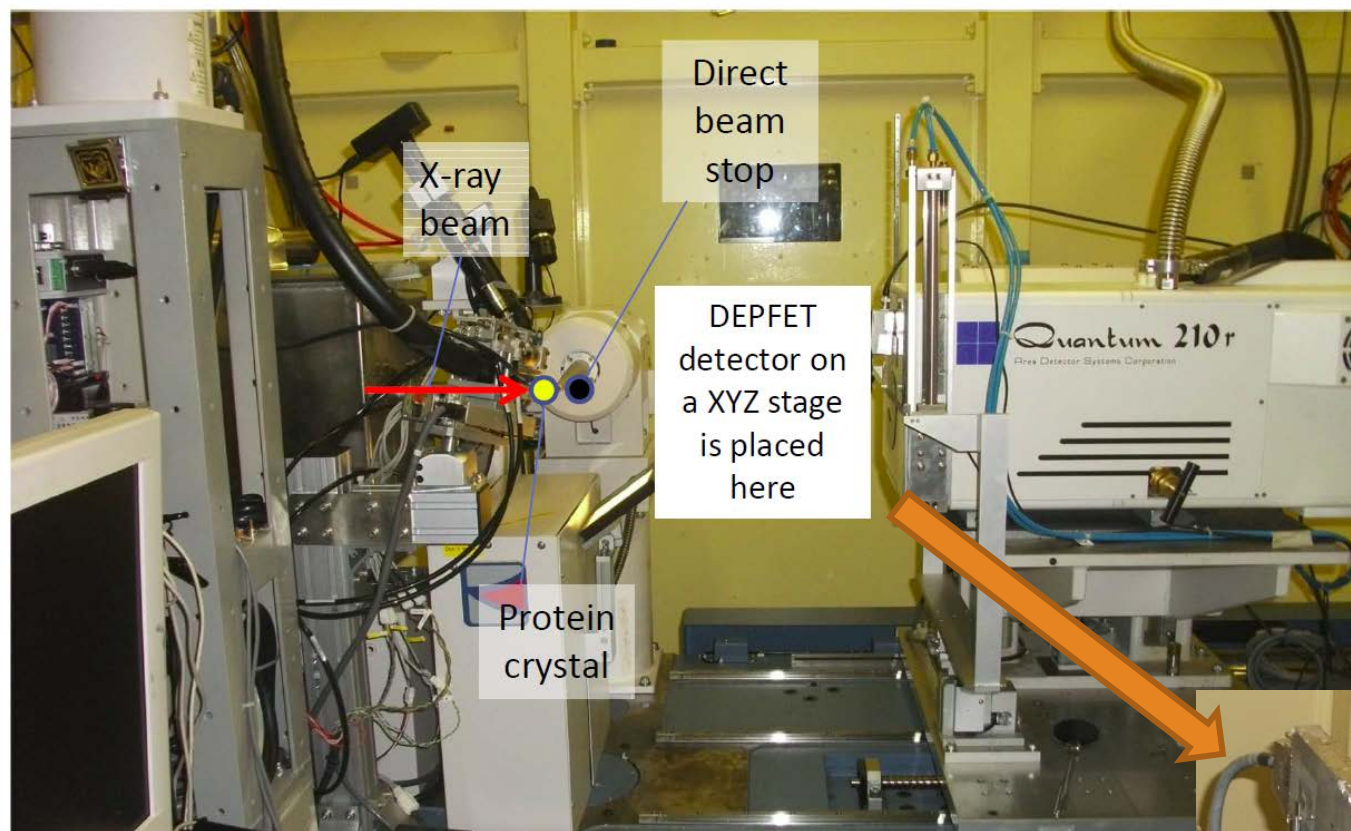
Confirm the response to the X-ray

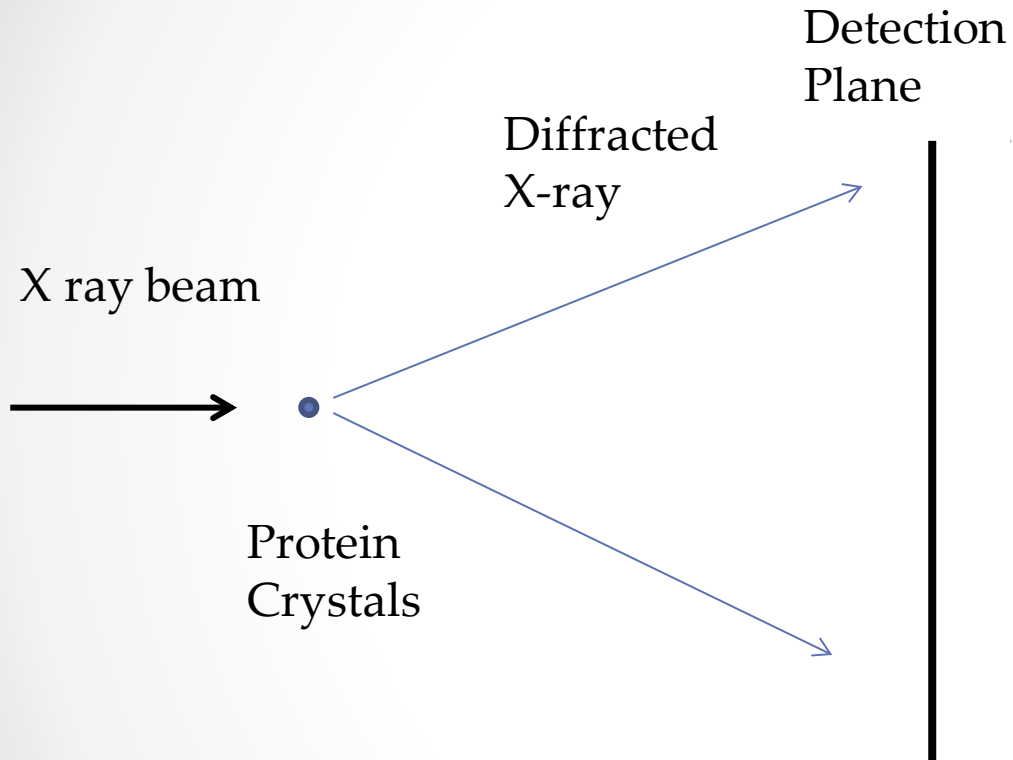
- Quality of image of diffracted X-ray from Protein
 - X-ray image for the protein crystal
 - X-ray image for the solution scattering
- Position resolution

Experimental apparatus

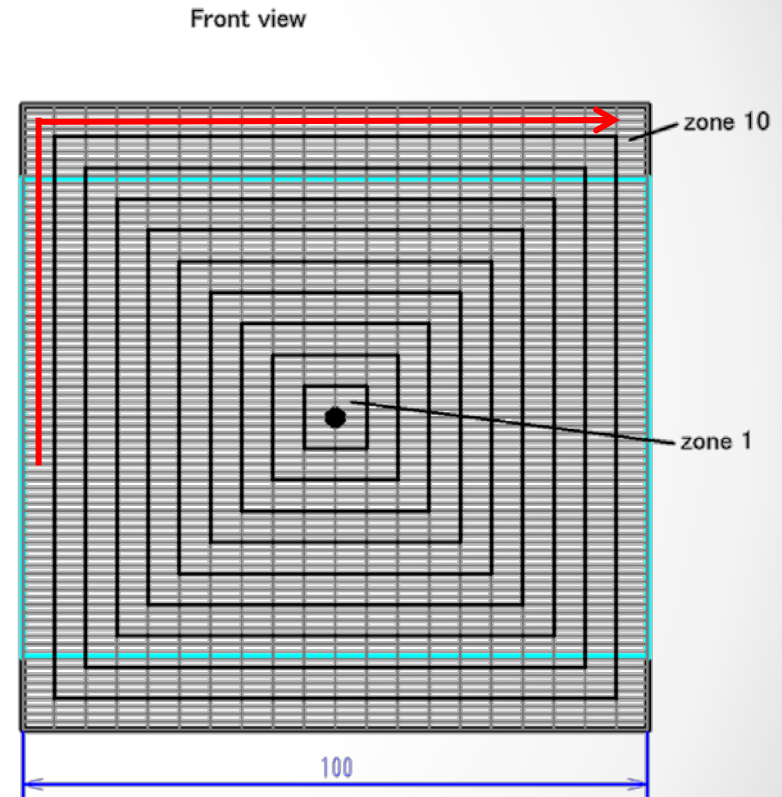
X-ray beam @
KEK Photon Factory





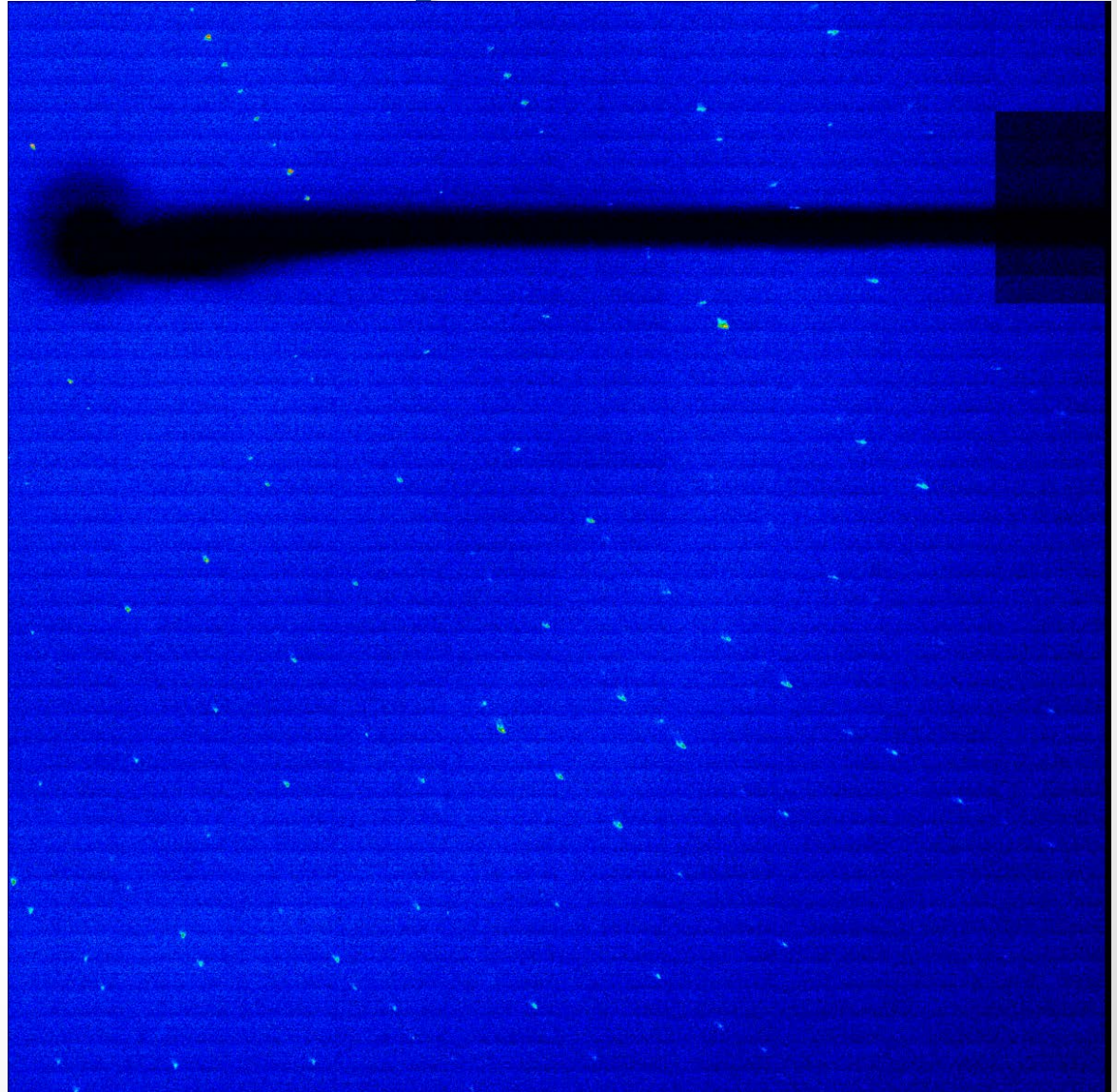


Scanned with XY-stage to make the image on the detection plane



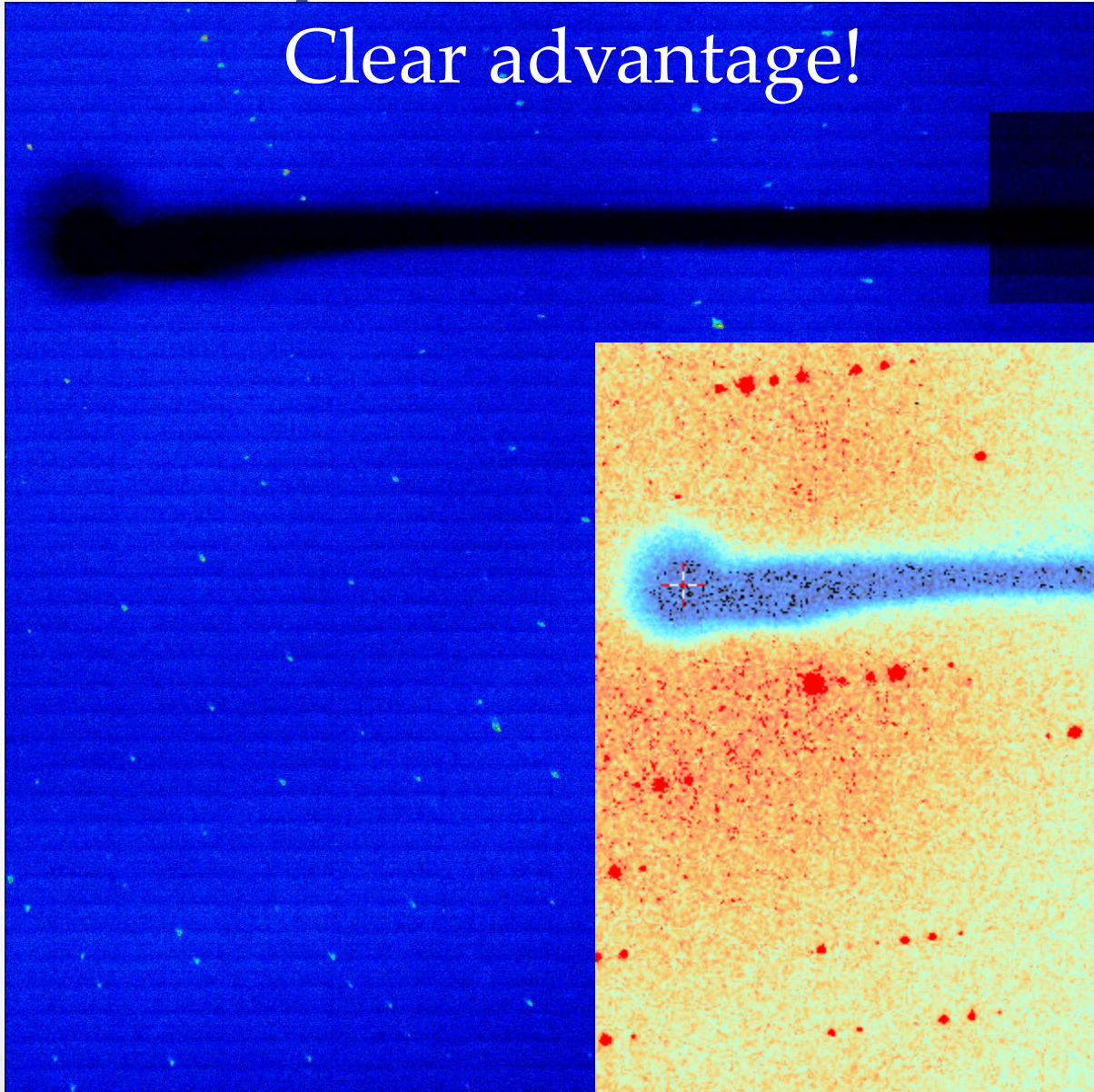
X-ray Diffraction Image from Protein Crystal

X-ray energy 12.4 keV
(wave length=1.0 Å)
@ BL-5A

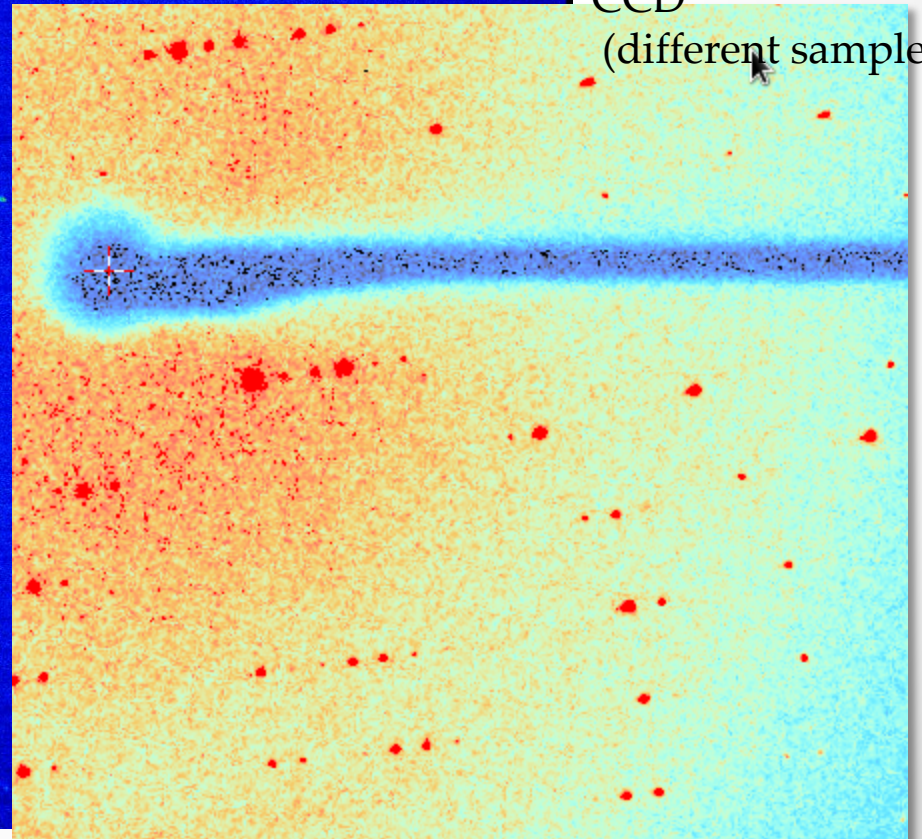


Comparison with CCD

Clear advantage!

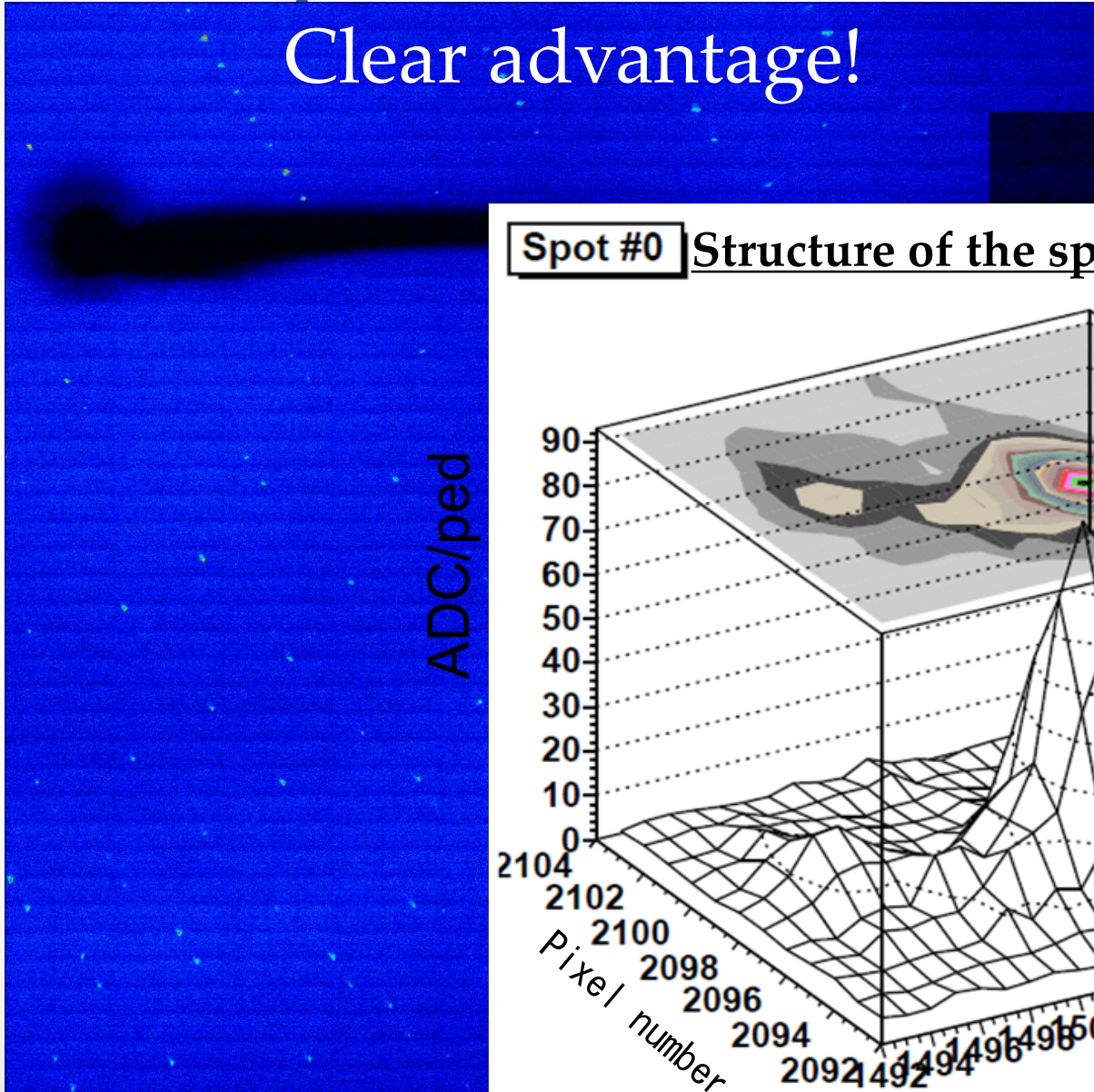


CCD
(different sample)



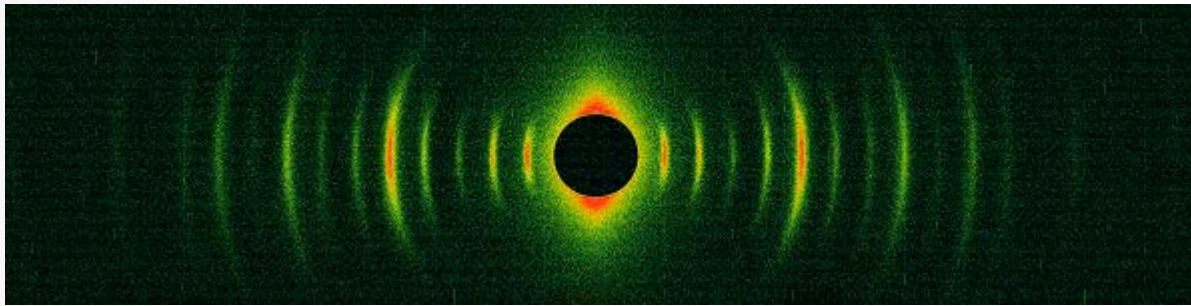
Comparison with CCD

Clear advantage!



X-ray solution scattering image

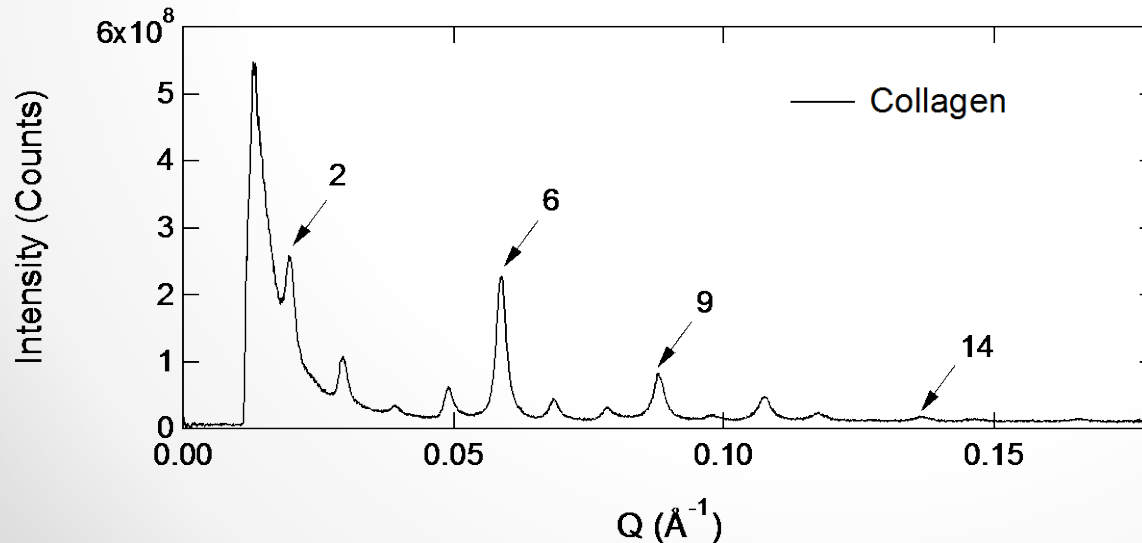
- Collagen from Chicken Achilles tendon
 - o X-ray energy 8.33 keV (wave length=1.488 Å) @ BL-10C
 - o 1-dimentional orientation
 - o Lattice spacing: $d=653\text{Å}$



Detection plane size:
20 x 20 sensors



1d conversion by Circular averaging

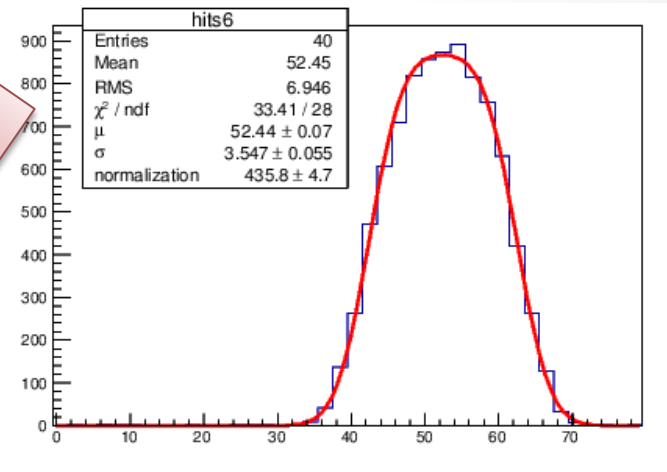
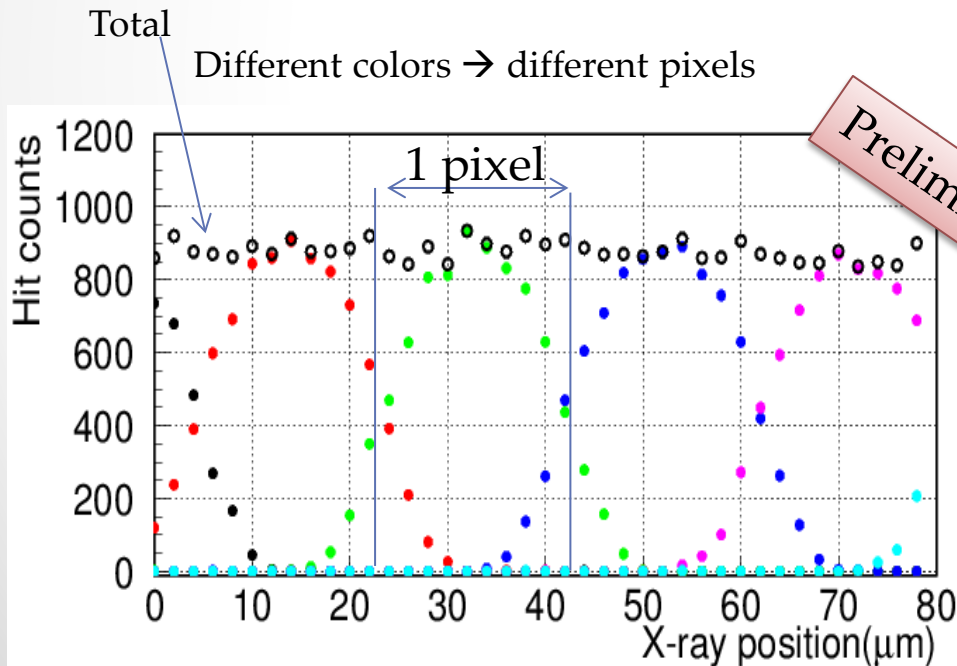
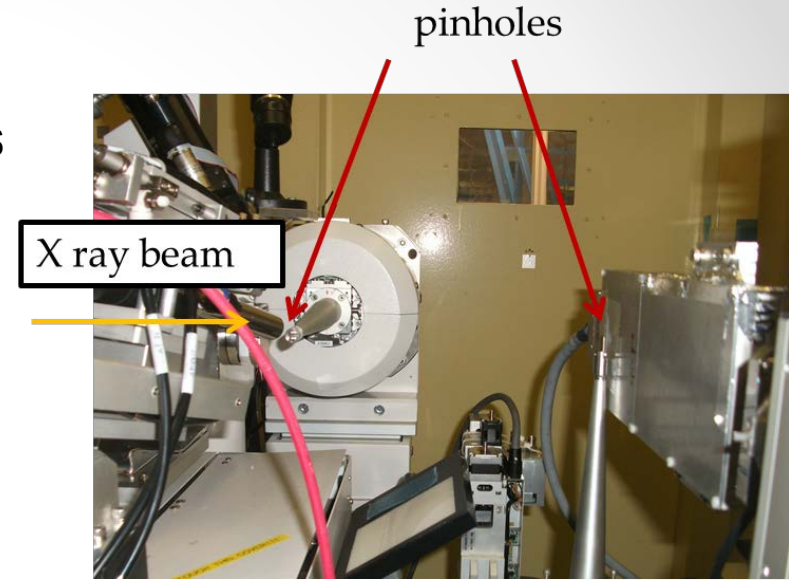


n: diffraction order

$$2d\sin\theta=n\lambda$$

Pixel Positional Response to X-ray photon

- KEK PF BL-5A
- X-ray energy : 12.4 keV, single photon signals
- Pencil beam created by two 10 μm pinhole (beam size not negligible compared to pixel)
- DEPFET sensor moved by XY-stage
- Count hits of pixels at each position



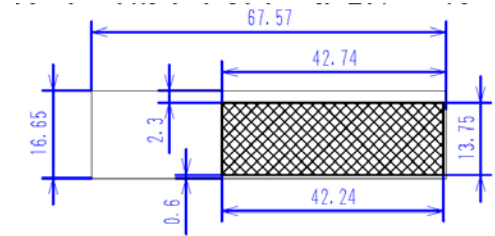
Smearing in pixel response: $\sim 3\mu\text{m}$
 $\leftarrow$ dominated by the beam size

Sensor parameters and layout

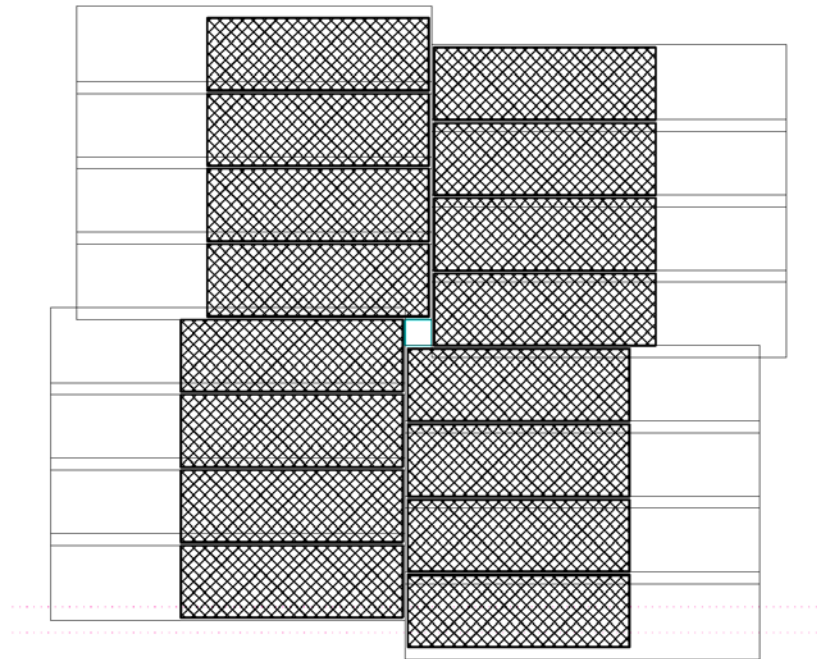
Pixel size: 55um x 55um

Number of rows and columns: 250x768 (same as Belle II)

Thickness: 450um (without thinning)



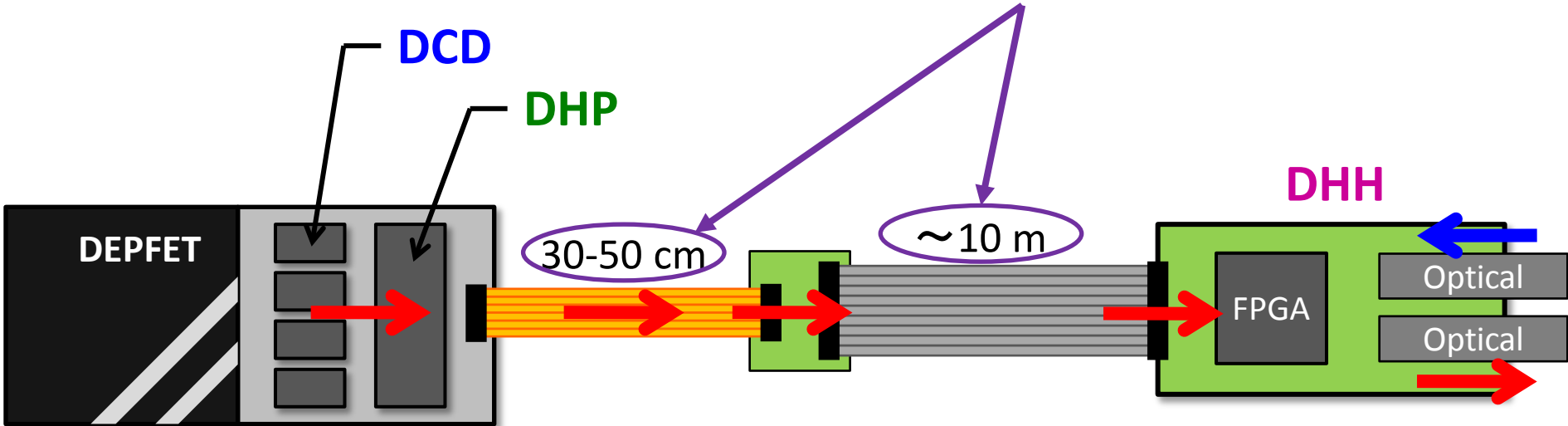
16 such sensors



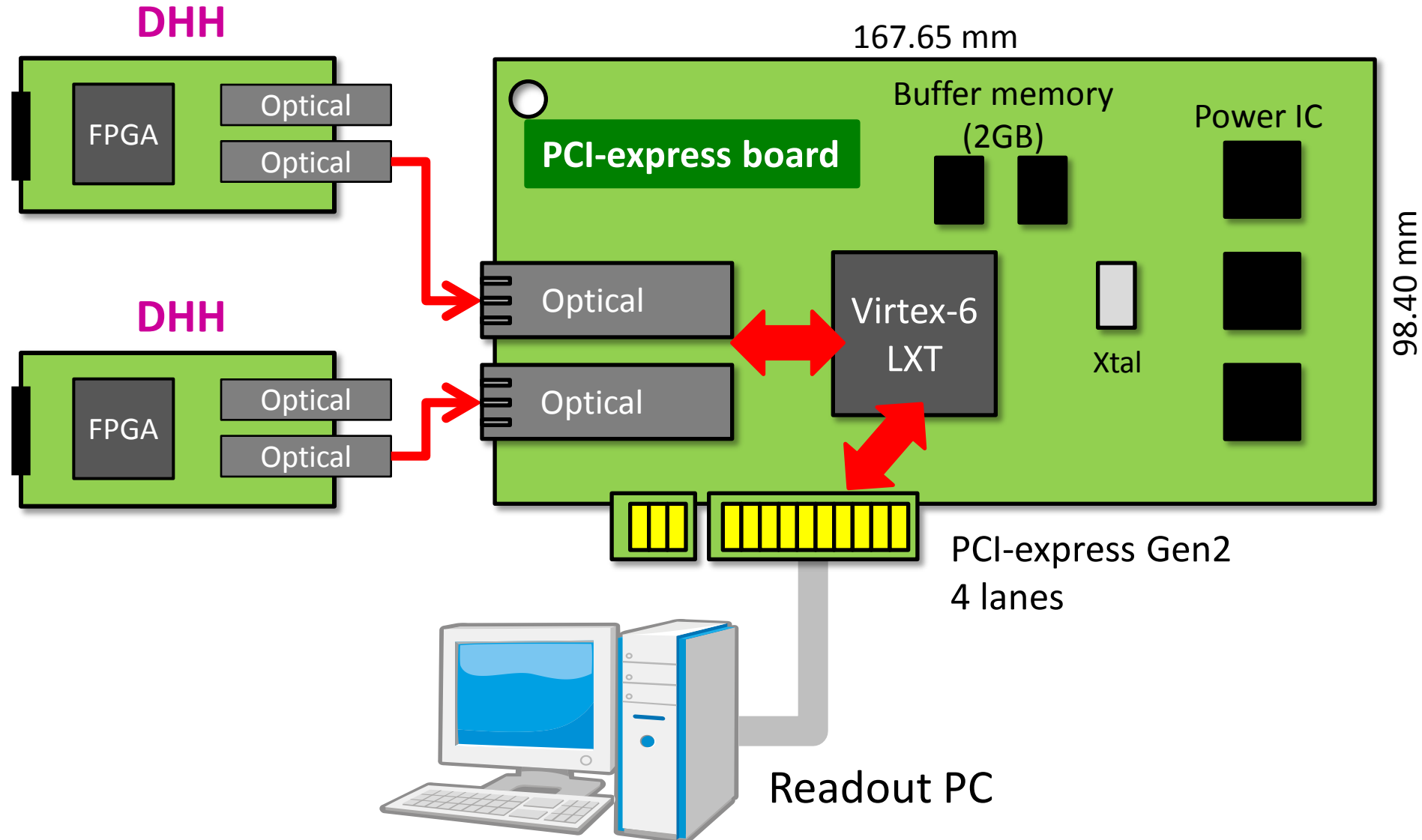
Readout

Belle II's configuration

Can be shorter for this application



Further downstream



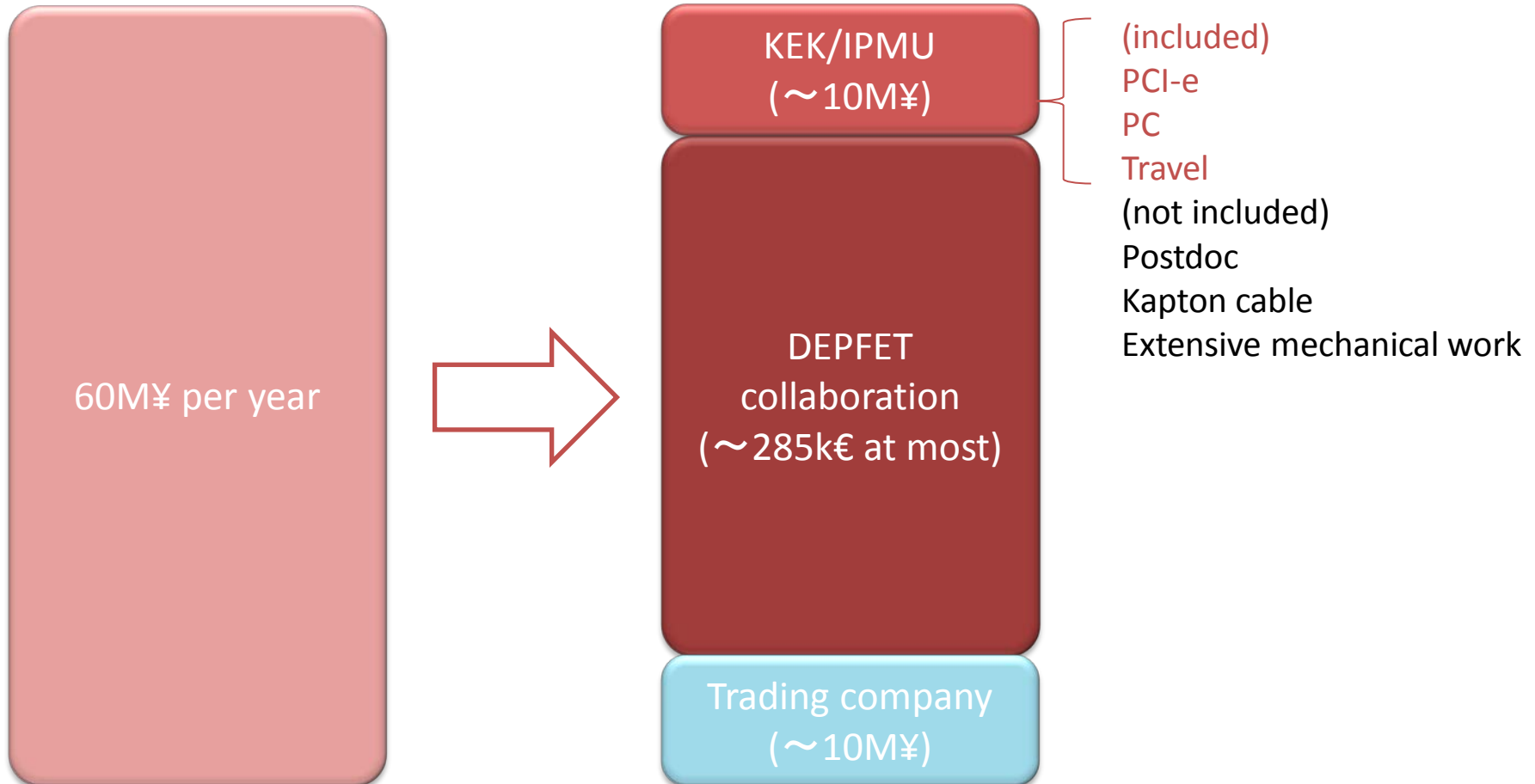
Work packages

1	Sensor production	HLL		
2	Testing of sensors			
3	ASICs			
4	Assembly			
5	Kapton cable			
6	Kapton attach, wire bond			
7a	DHH/FPGA			
7b	DAQ	KEK/IPMU		
8	Power Supplies			
9	Mechanics and cooling			
10	Project Leader			

Funding

Apr. 2014 – Mar. 2015: 60M¥

Apr. 2015 – Mar. 2016: 60M¥



Money transfer (contract) of >5M¥ requires an intermediate trading company, which takes about 20% of margin.

1€ = 140¥ (which was 100¥ when this project was approved)

Summary

- High spatial resolution and very fast readout is essential for protein structural analyses. DEPFET is one of very promising candidates.
- Basic properties has been successfully tested using a small prototype DEPFET sensor for ILC
 - Clear signal image of diffracted X-ray
 - Good position resolution for single photon
- We need 16 sensors + readout *similar* to Belle II PXD
- We will receive 60M¥+60M¥ in JFY2014 and 15, and can transfer some 285k€+285k€ to DEPFET collaboration
- We need your cooperation to go further