2011年1月11日・12日　KEK 4号館2階輪講室

PF研究会「X線トポグラフィの現状と展望」

タンパク質結晶のX線トポグラフィ ― これまでの研究と今後 ―

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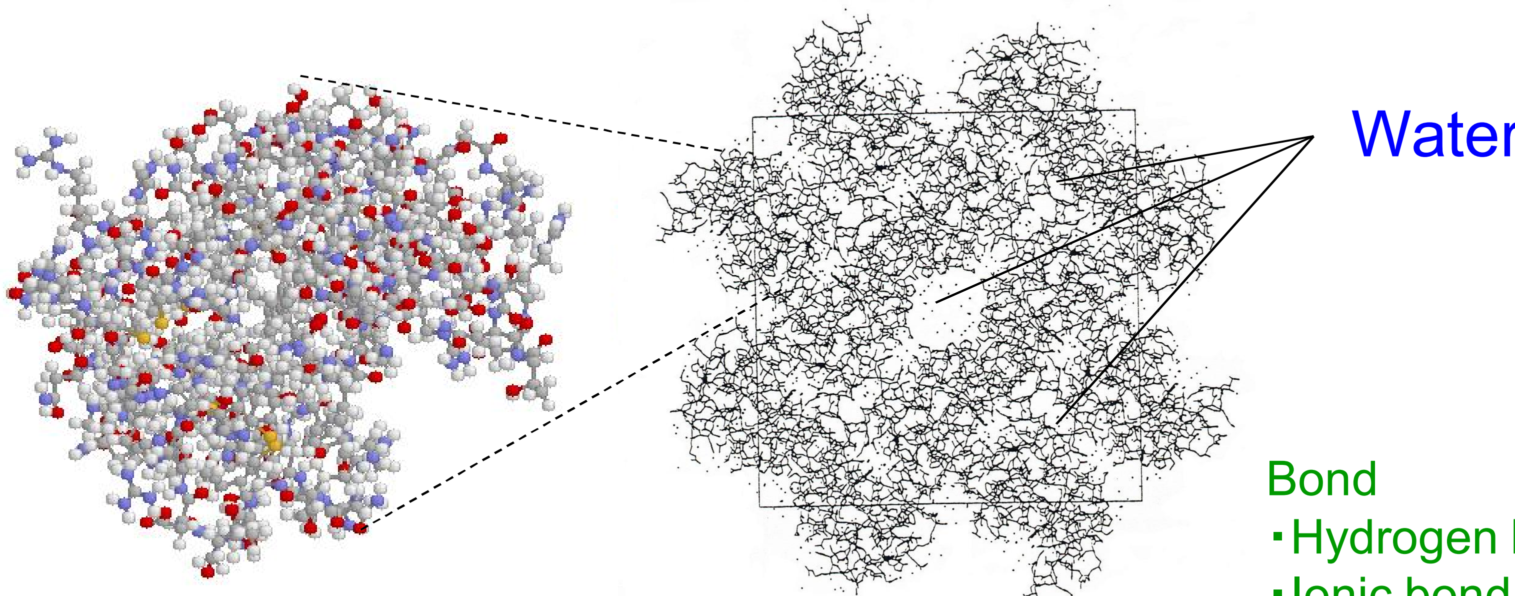
- Protein crystals
  - Crystallization
  - Assessment of crystal perfection
  - Characterization of crystal defects
- X-ray topography for protein crystals
  - Critical condition for direct images
  - X-ray topographs of protein crystals
  - Analysis of image width
- Digital topography for assessment of crystal perfection
  - Digital topographs
  - Locking curve analysis
  - Mapping of Local rocking curves
- Neutron topography
  - JRR-3 & J-PARC experiments

Protein crystals



Hen egg-white lysozyme (HEWL)

Tetragonal HEWL crystal

Bond
- Hydrogen bond
- Ionic bond
- Van der Waals force

Huge molecular weight > 10000

Huge molecular size > 1 nm

Huge lattice constant > 1 nm

A huge amount of water in the crystal : 30-80 vol.%

Sample conditions for X-ray topography

$$\xi = \frac{\pi V_C \cos\theta_B}{r_e \lambda |F_{hkl}|}$$

ξ: Extinction distance
V_C: Volume of unit cell
F_{hkl}: Struture factor

λ: Wavelength of X-ray
θB: Bragg angle
r_e: Radii of classical electron

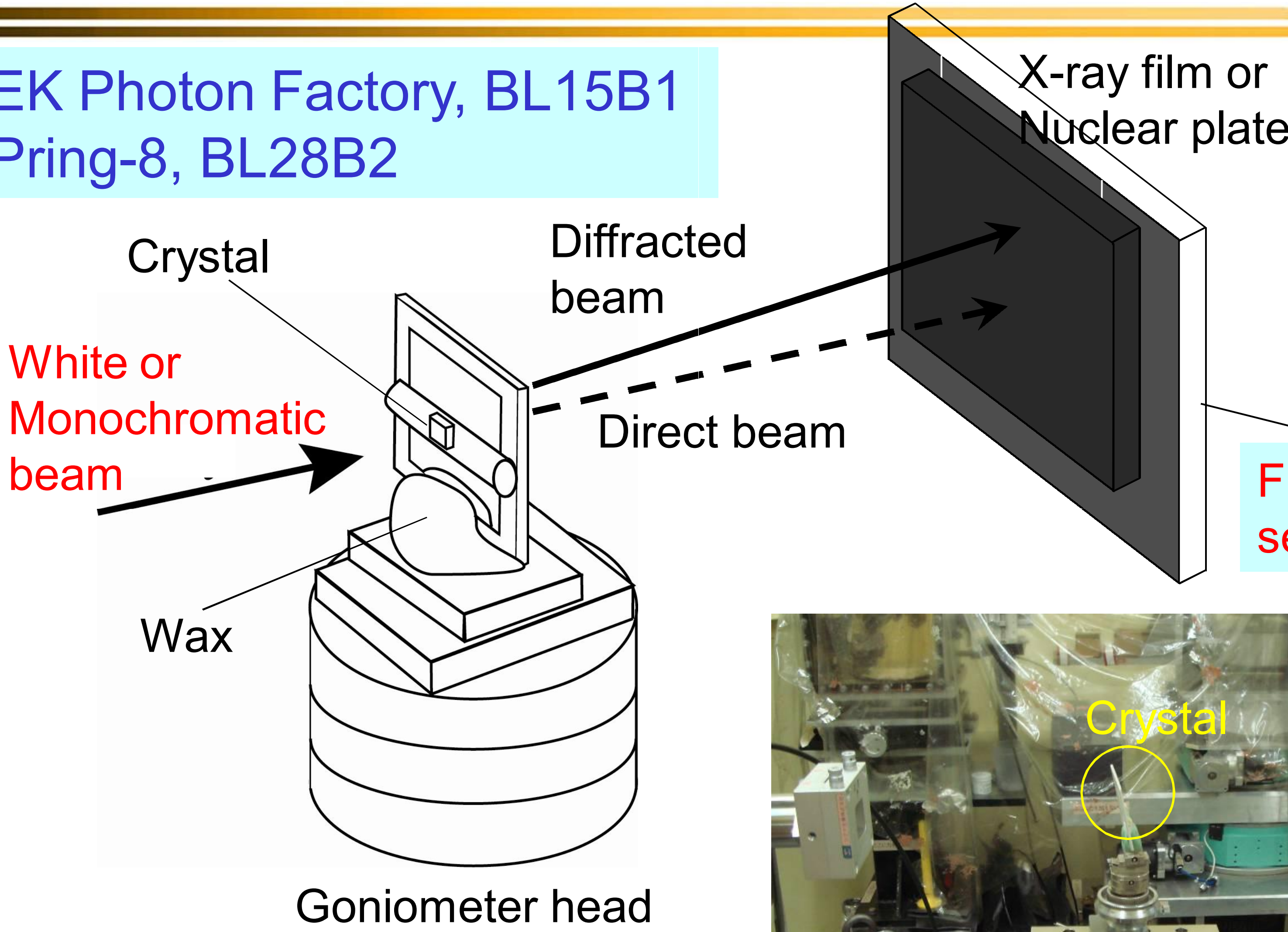
Crystal (Reflection)	V_C (Å ^{3})	$\|F_{hkl}\|$	μ (mm^{-1})	ξ (mm)	$0.4\xi < t < 1/\mu$ (mm)
Si (3,-3,3)	160	37.9	1.365	0.062	$0.024 < t < 0.732$
Benzil (2,-2,0,0)	416	61.9	0.7	0.096	$0.038 < t < 1.428$
Tetragonal hen egg-white lysozyme (800)	**237133**	**695**	**0.199**	**3.383**	**$1.35 < t < 5.02$**

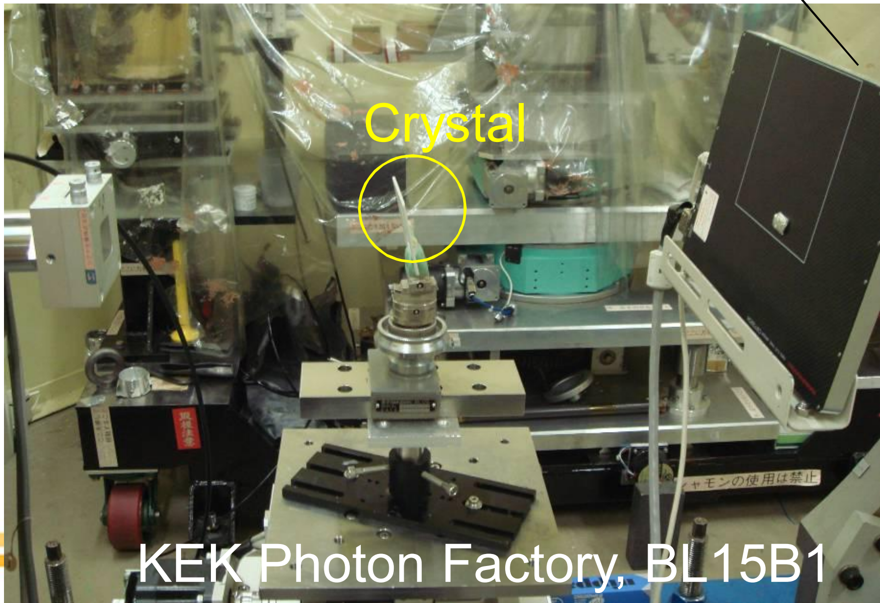
X-ray wavelegnths used in this calculation are 0.71 A, 1.54A and 1.41A for Si, benzil and tetragonal hen egg-white lysozyme. The Bragg angle are 20° , *** and 3.25° for Si, benzil and tetragonal hen egg-white lysozyme.

Large protein crystals of millimeter-size are needed !!

Synchrotron radiation X-ray topography

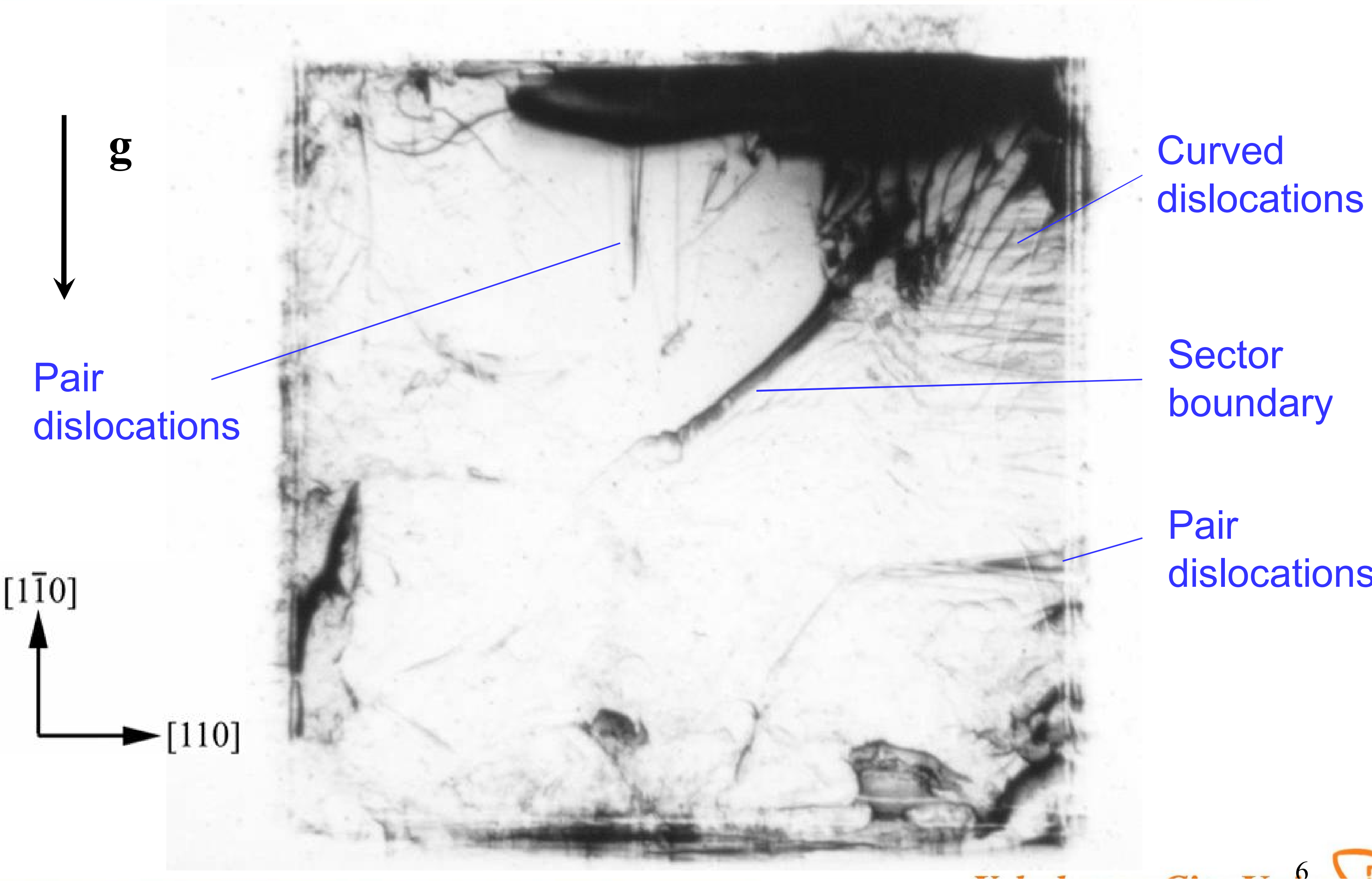
- KEK Photon Factory, BL15B1
- SPring-8, BL28B2





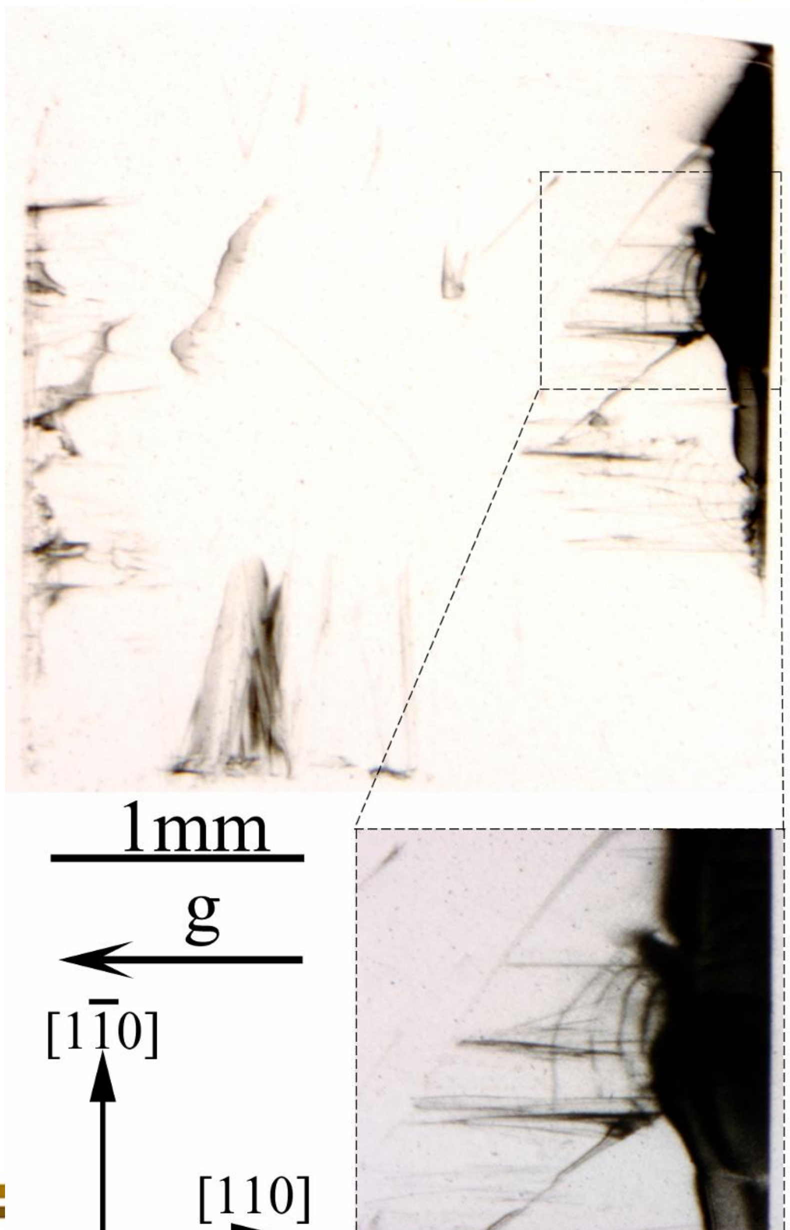
λ=1.2 Å in monochromatic case

Monochromatic-beam topograph for a tetragonal HEWL crystal

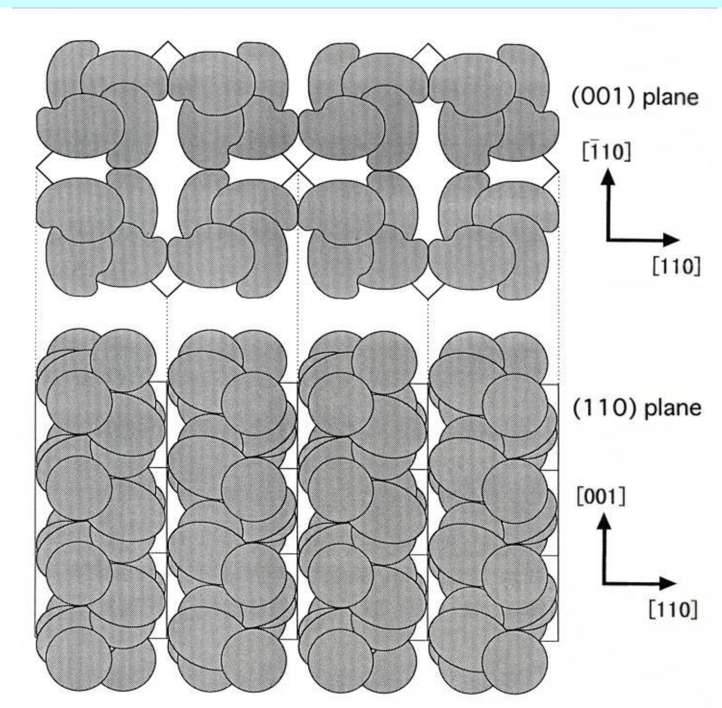


Koishi et al. Cryst. Growth & Des., 7, 2182 (2007)

Slip dislocations in a tetragonal HEWL crystal



Slip dislocations might be easily generated due to a stress

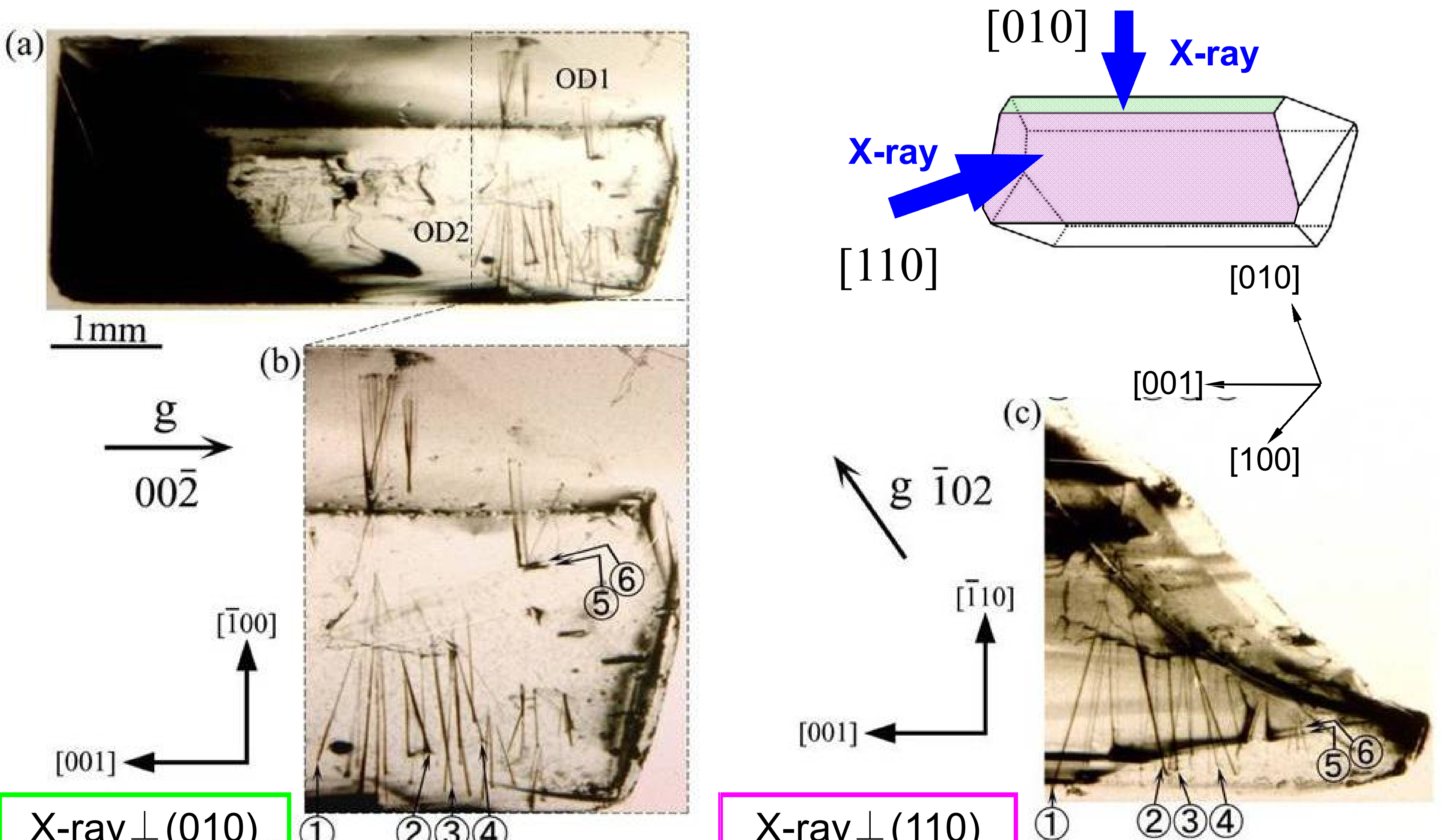


Slip system: {110}<001>

(001)<110>

Mukobayashi et al. Phys. Status Solidi A206,1825 (2009)

Dislocation images for a larger orthorhombic HEWL crystal

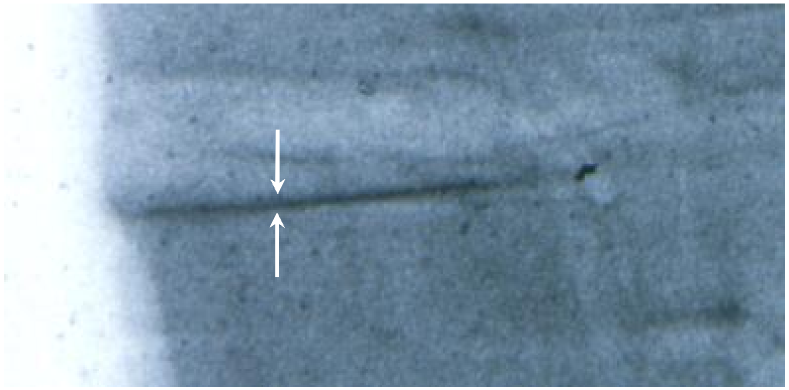


X-ray⊥(010)

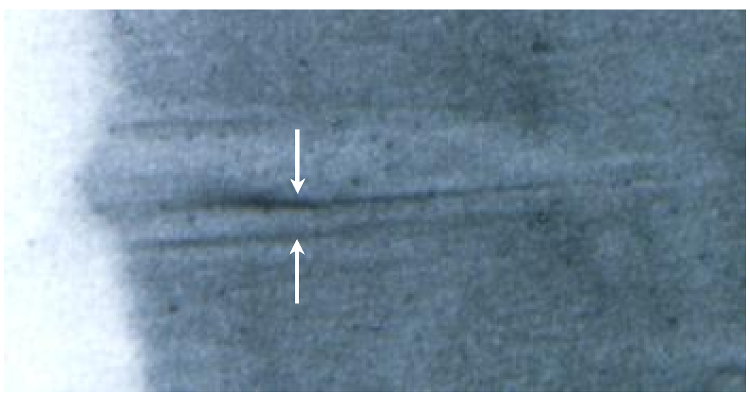
X-ray⊥(110)

Mukobayashi et al. Phys. Status Solidi A206,1825 (2009)

Observation of double images of dislocations



$0\bar{8}0$



$\bar{4}\bar{4}0$

Koizumi et al., Phil. Mag. 85, 3709 (2005)

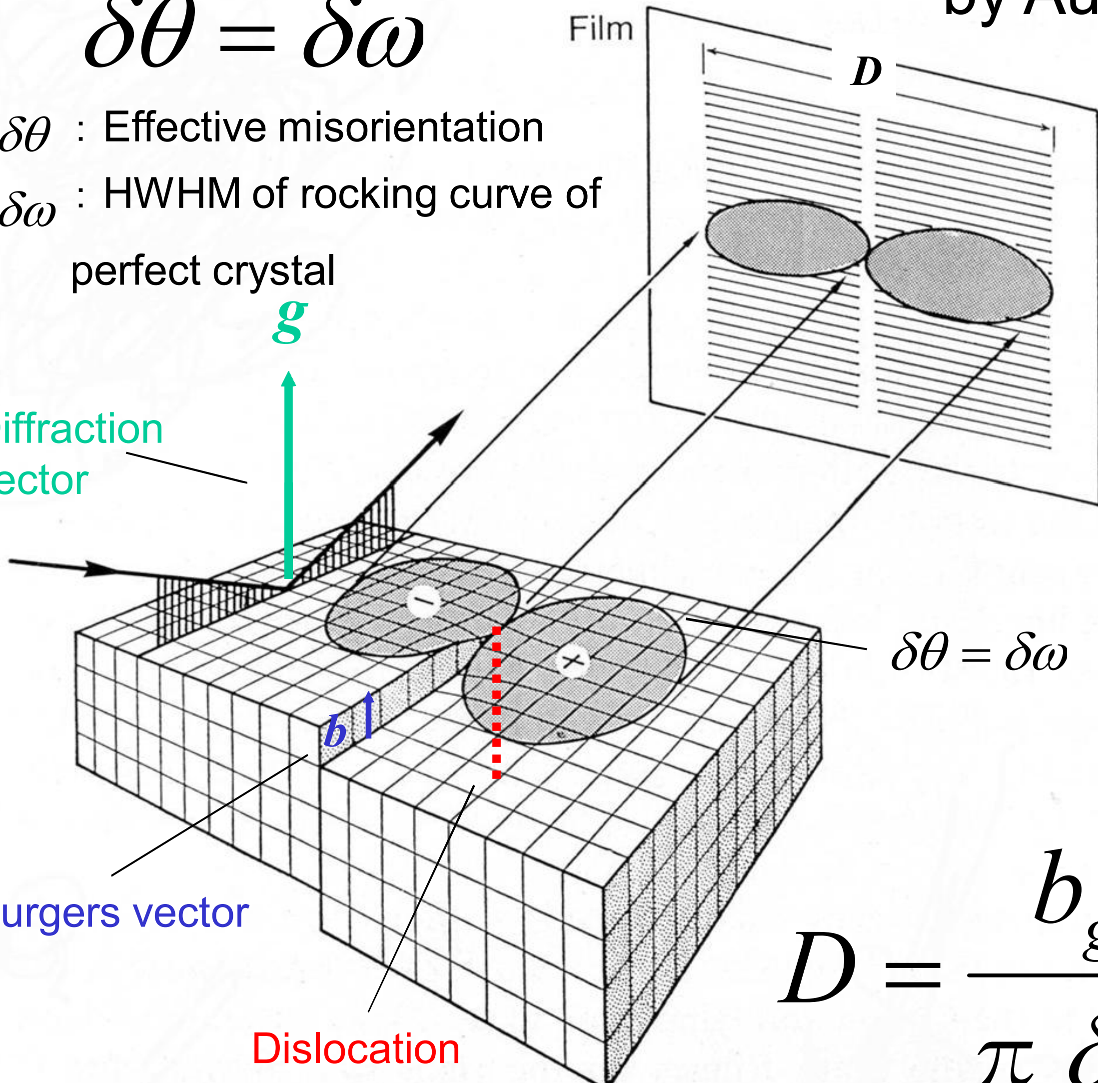
Theory of double images of dislocations

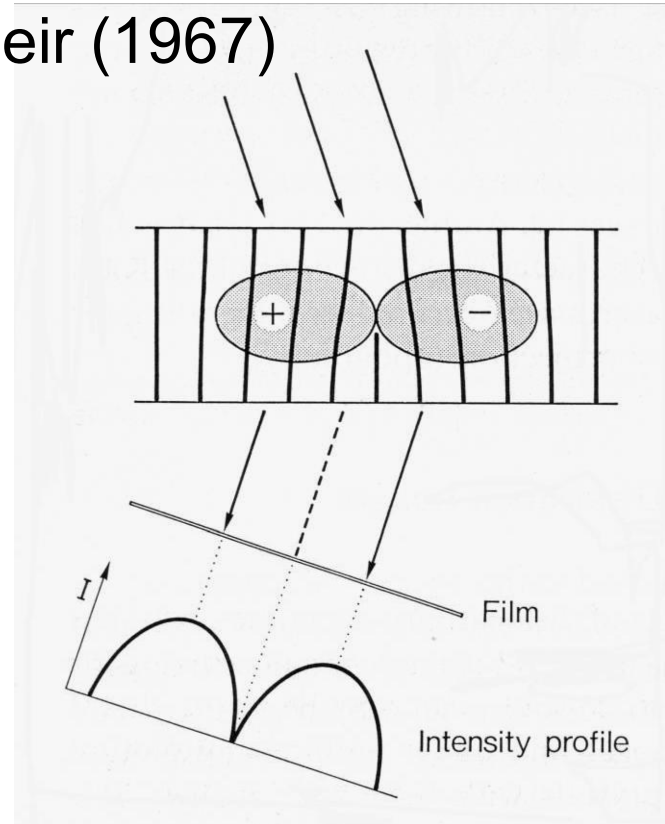
$$\delta\theta = \delta\omega$$

$\delta\theta$: Effective misorientation

$\delta\omega$: HWHM of rocking curve of perfect crystal

by Autheir (1967)





$$D = \frac{b_g}{\pi\ \delta\omega}$$

b_g: Component parallel to diffraction vector ***g*** for Burgers vector ***b***

Widths of double images (Theory & Observation)

$$D = \frac{b_g}{\pi\, \delta\omega}$$

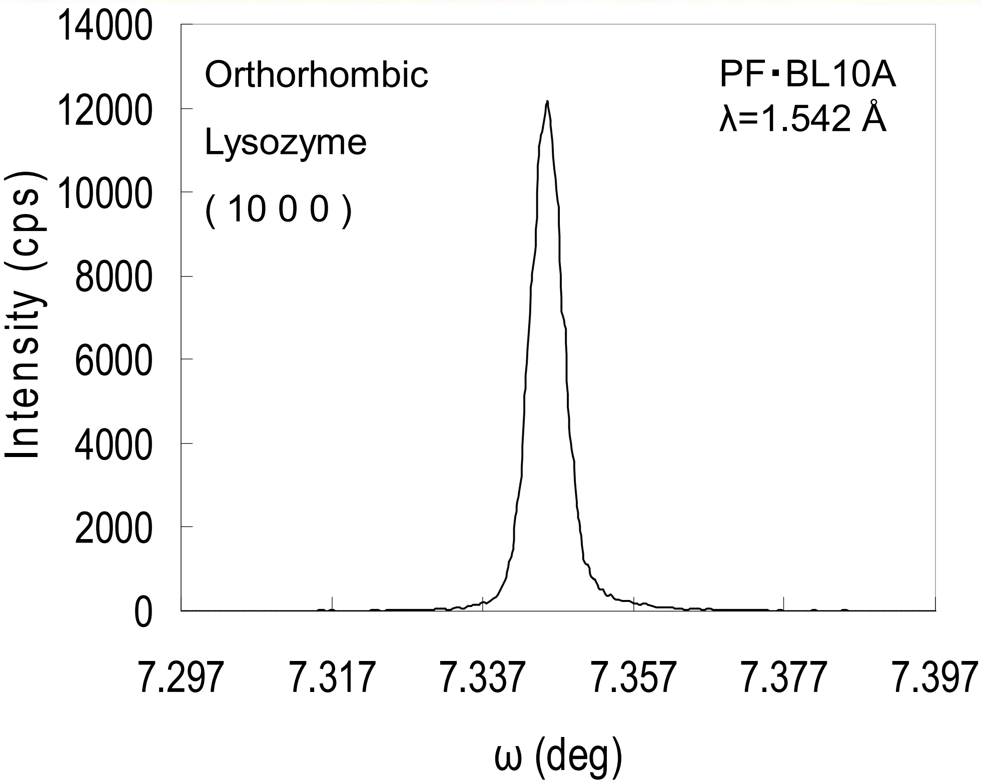
b_g: Component of parallel to diffraction vector ***g*** for Burgers vector ***b***
$\delta\omega$: Full width at half maximum of rocking curve of perfect crystal

Reflection	$\delta\omega$ (°)	Width of image (μm)	
		Theory	Observation
$0\bar{8}0$	5.37×10^{-5}	1.34×10^{3}	＜18 (single)
$\bar{4}\bar{4}0$	5.76×10^{-5}	1.77×10^{3}	50 (double)

The theoretical values are much larger than observed values by two-order !!

Koizumi et al., Phil. Mag. 85, 3709 (2005)

FWHMs of rocking curves (Theory & Measurement)



$$\delta\omega = \frac{r_e \lambda^2}{\pi \sin 2\theta} \cdot \frac{|F_{hkl}|}{V_c}$$

V_C: volume of unit cell
F_{hkl}: Structure Factor
λ: X-ray wavelengh
θ_B: Bragg angle
r_e: classical radius of electron

Measurement: 4.50×10^{-3} °
Theory: 7.47×10^{-6} °

The difference is also two order !!

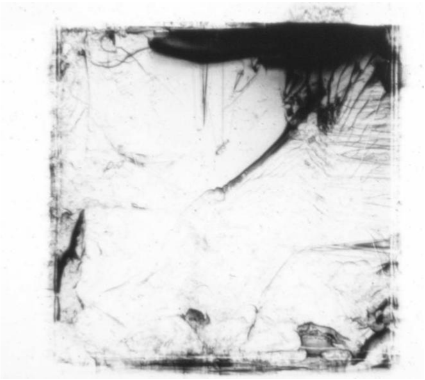
The difference in the dislocation image width can be explained by the larger rocking width of real protein crystals.

Why is digtal topogrphy

For exact assessment of crystal perfection, some methods should be used for “the same sample”.

However, protein crystals are easily damaged for some handling and X-ray irradiation. Thus, it is desired that some methods are simultaneously applied for “the same sample”.

Digital topograhy with CCD camera



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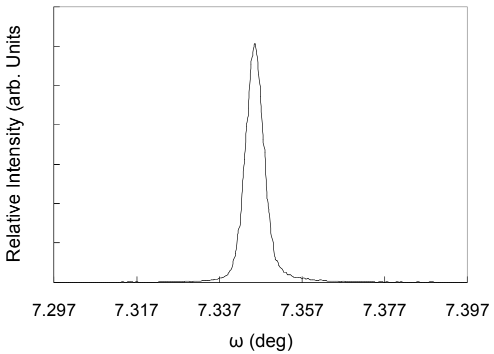
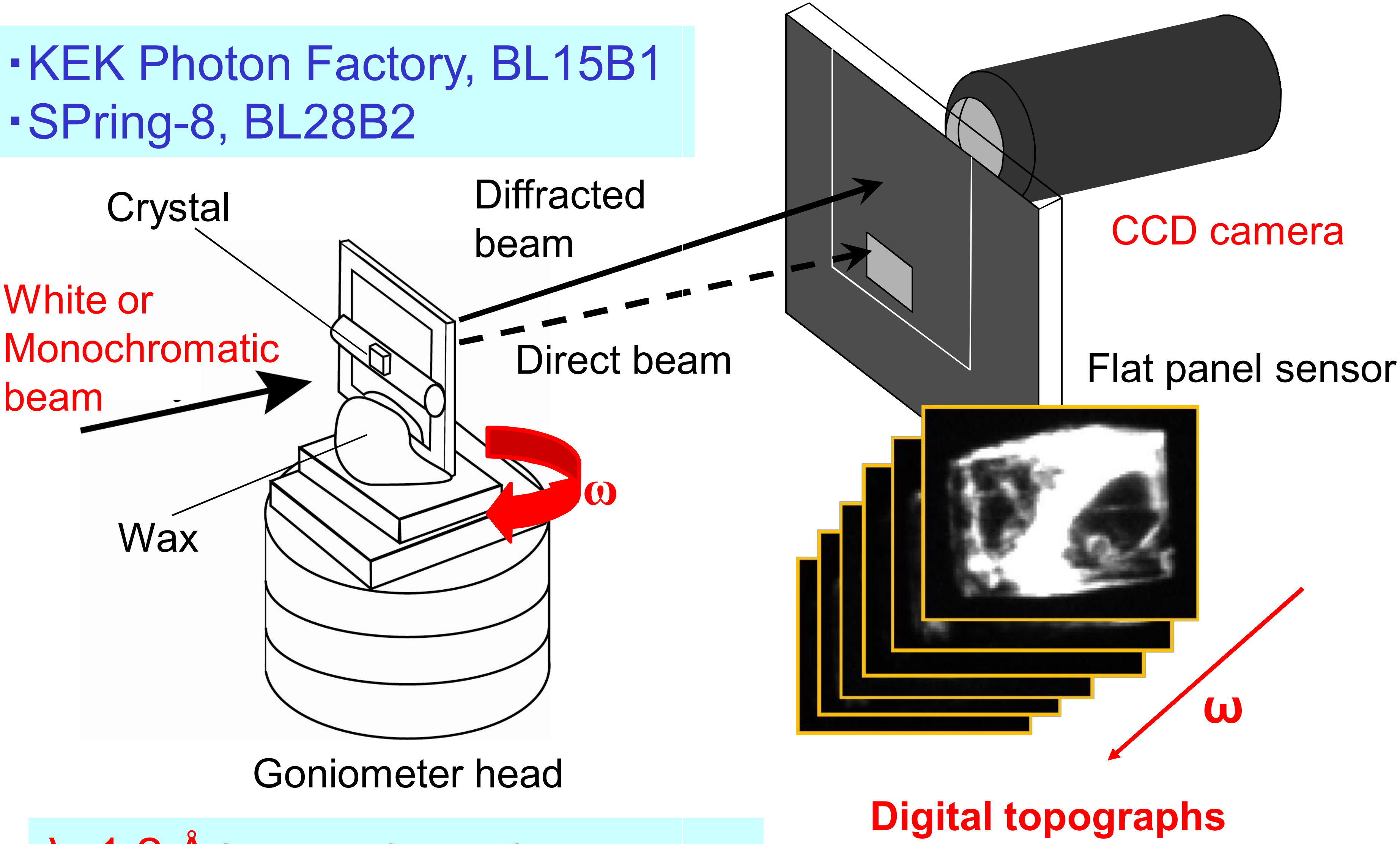


X-ray topograph
(Local distribution of defects)

Rocking curve
(Angular distribution of strain)

Digital topography with CCD camera

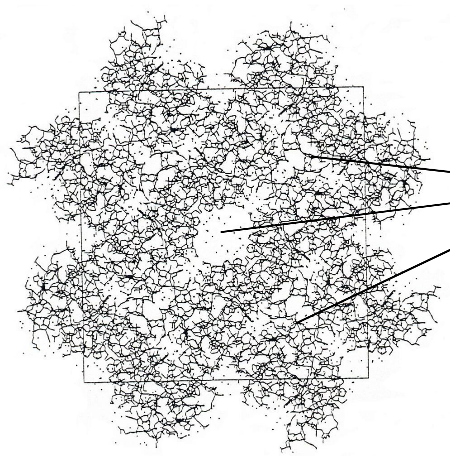
- KEK Photon Factory, BL15B1
- SPring-8, BL28B2



λ=1.2 Å in monochromatic case

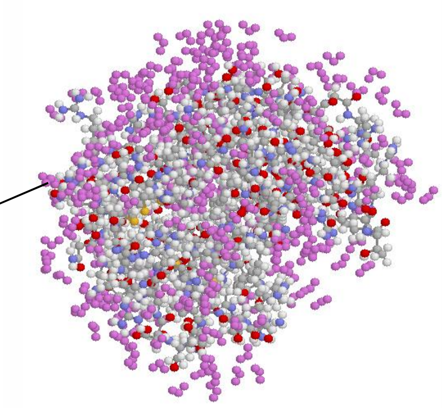
Why is neutron topography

Protein crystals include a huge amount of water: 30-80 vol.%



Mobile water

Bound water



Understanding the behavior of the water is very important

Neutron is very sensitive to water, or hydrogen atom

Neutron topography

Future works

- **More detailed understanding of dislocations in protein crystals**
  - More detailed analysis of topographic images of dislocations
  - Modeling of atomic structure of dislocations
  - In-situ observation of motion of dislocations
- **Development of digital topography with CCD camera including analyzing**
  - Assessment of perfection for various protein crystals
- **Combination of X-ray topography and neutron diffraction topography, and others**
  - Correlation between intra-crystalline water and dislocations in protein crystals