

放射光タンパク質結晶構造解析と検出器

山本 雅貴

理研 / JASRI / SPring - 8

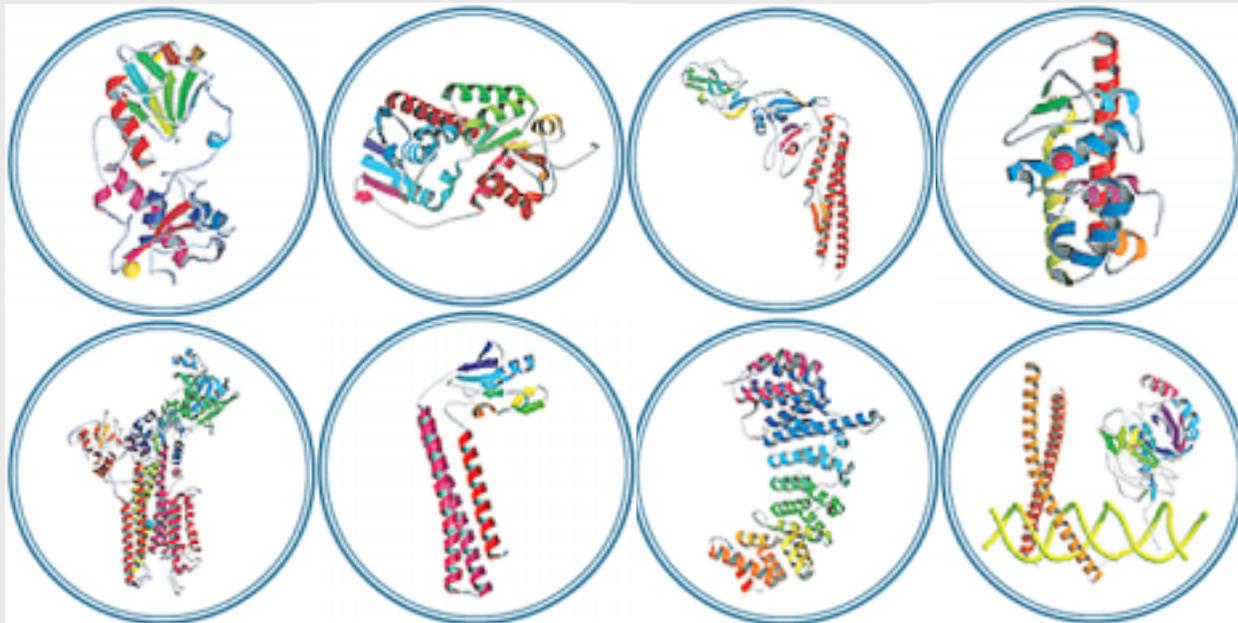
内容

- ・タンパク質と構造解析
- ・検出器の現状
- ・将来の検出器

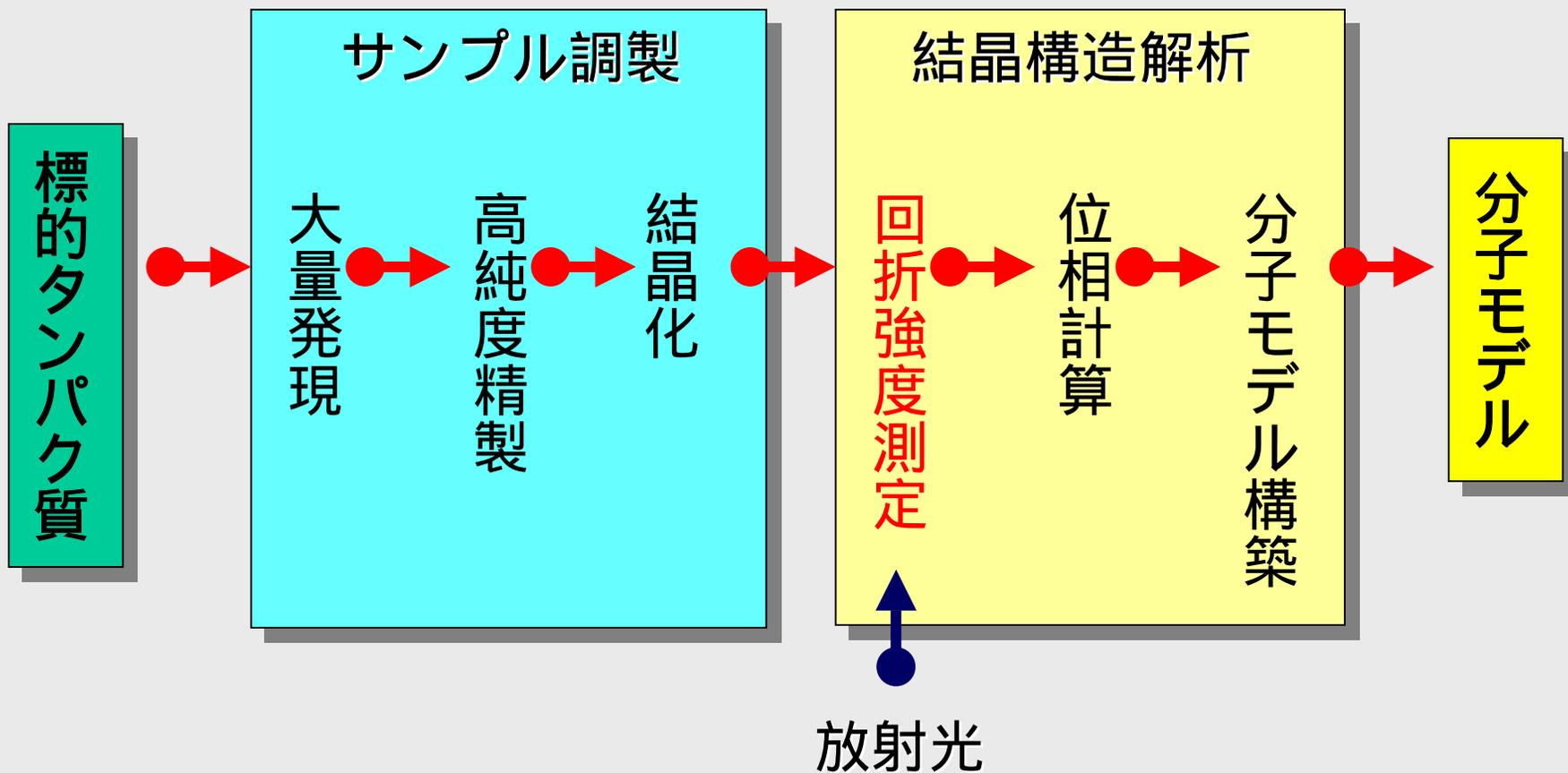
タンパク質と立体構造

- ・ タンパク質は遺伝子情報（1次元）をもとに作られた生命現象の基本装置（分子機械）
- ・ 生命現象を理解するためにタンパク質（分子機械）の3次元立体構造情報が必要である

⇒ タンパク質結晶構造解析



タンパク質 X 線結晶構造解析の流れ



タンパク質X線結晶構造析解 1 タンパク質結晶

0



透明

1



沈殿(茶色)

2



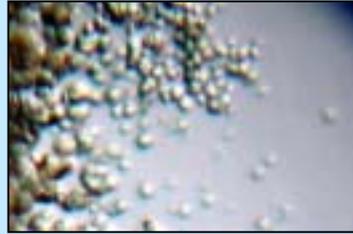
沈殿(白色)

3



沈殿(透明)

4



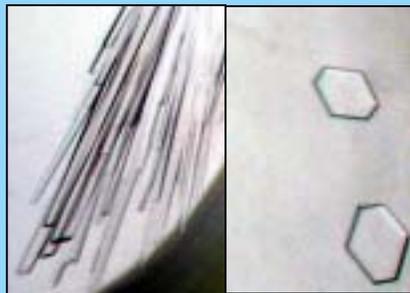
非晶質の粒

5



微結晶

6



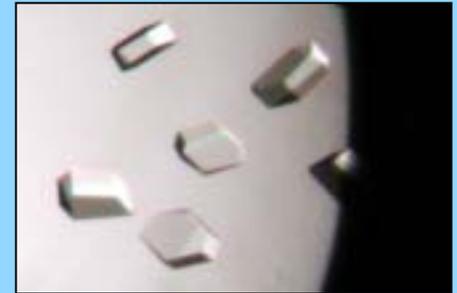
針状結晶 板状結晶

7



結晶のクラスター

8



3次元単結晶(0.05-0.2mm)

9



3次元単結晶(0.2mm以上)

放射光ビームライン
(実験ステーション)

放射光

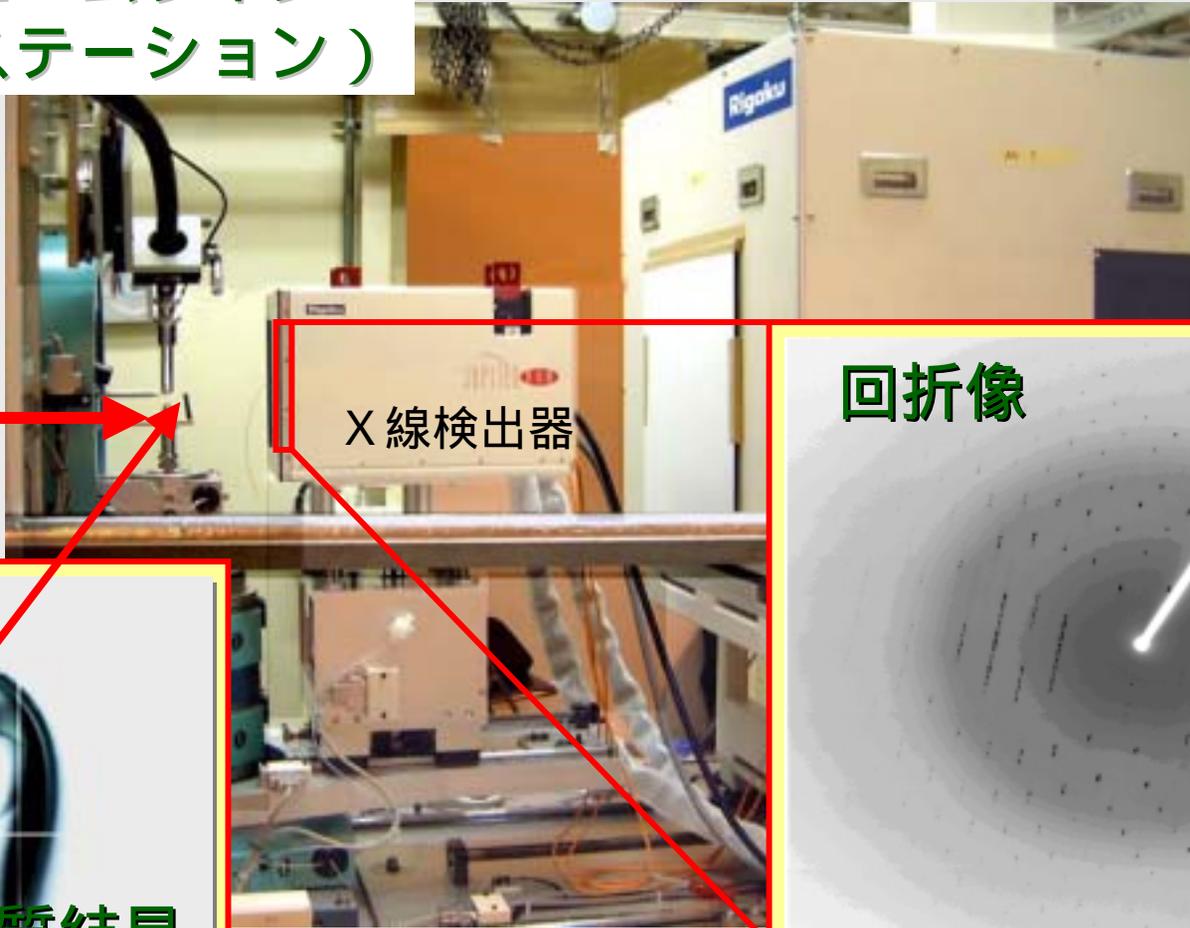
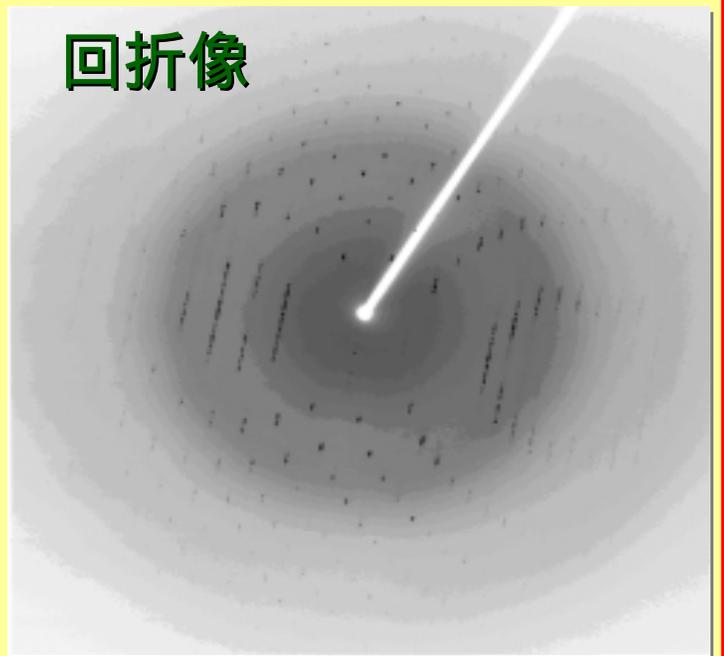


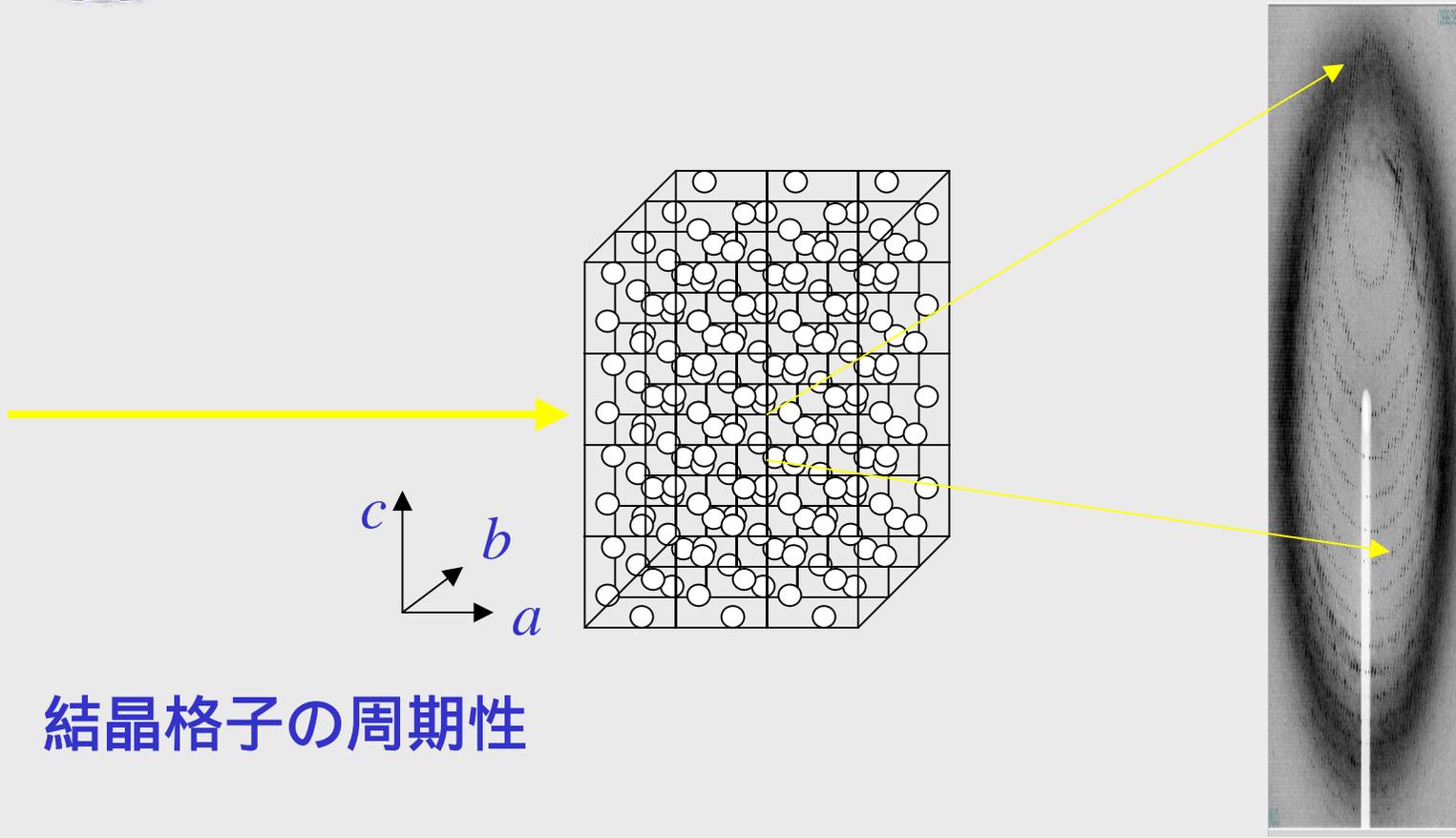
X線検出器

タンパク質結晶



回折像



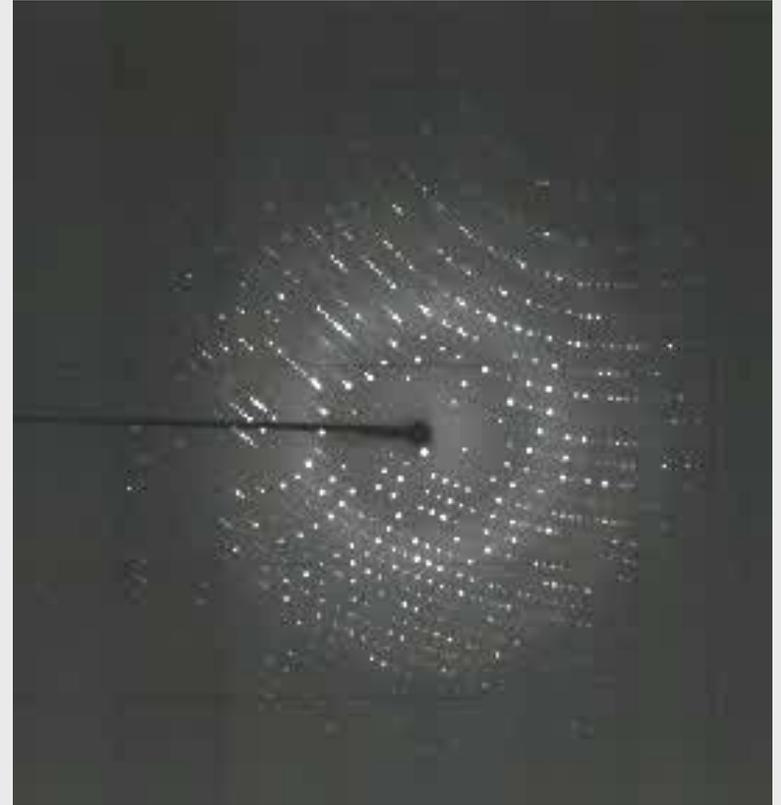
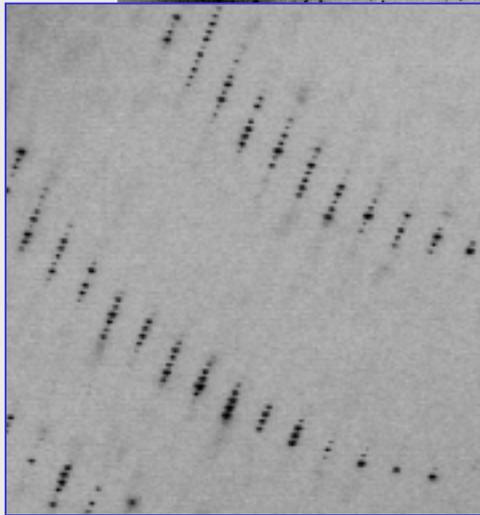
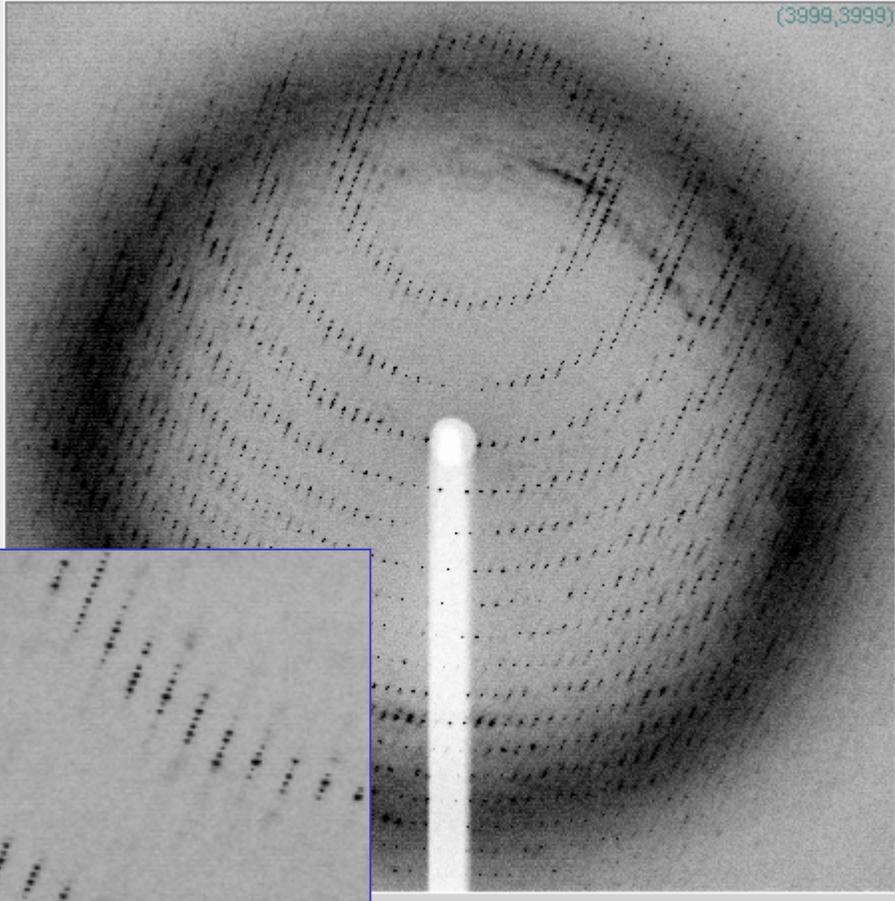


結晶格子の周期性

$$F(\mathbf{S}) = \sum_t \sum_u \sum_v g(\mathbf{S}) \exp[2\pi i \mathbf{S} (t \mathbf{a})] \exp [2\pi i \mathbf{S} (u \mathbf{b})] \exp [2\pi i \mathbf{S} (v \mathbf{c})]$$

$$\mathbf{S} \mathbf{a} = h, \mathbf{S} \mathbf{b} = k, \mathbf{S} \mathbf{c} = l$$

結晶格子による散乱：回折

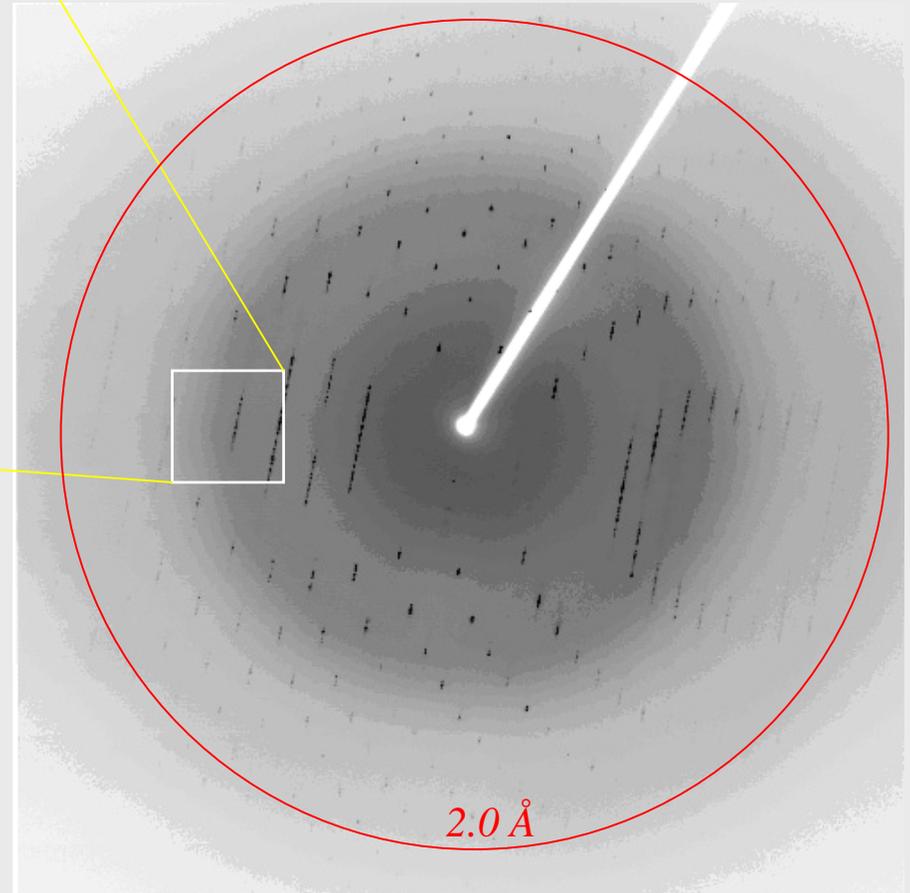
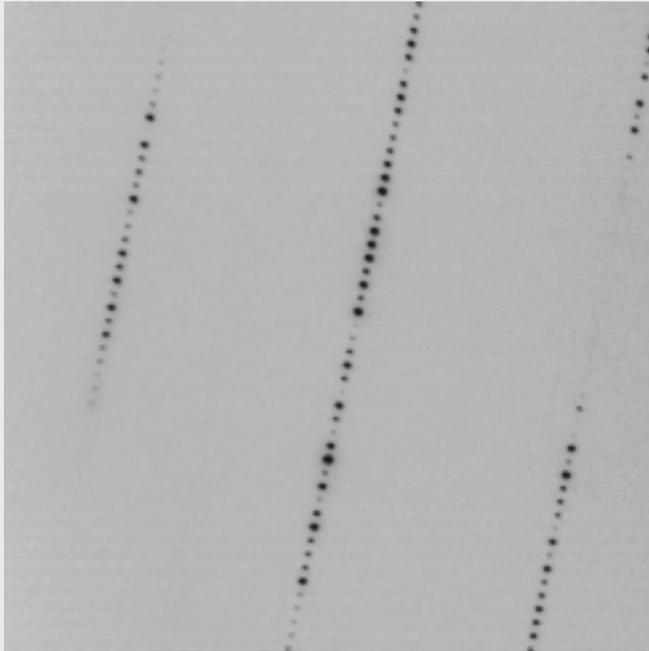


Long unit-cell & High resolution Calmodulin & binding-protein Complex

Crystallized by E.Yamauchi (RIKEN/MD/Taniguchi-Team)

Unit cell $a=b=40.5\text{\AA}$, $c=343.5\text{\AA}$ (Space Group P6122)

Resolution limit Higher than 2.0\AA



Osc. Angle = 1.2 degree & Exp. Time = 20 Sec.
Camera length = 350 mm & Wavelength = 1.02 Å

Resolution (Å)	No.of ref. / Comp. (%)	I/sigma	Rmerge
50.00 - 4.31	1117 / 74.8%	22.3	0.053
4.31 - 3.42	1136 / 88.0%	21.5	0.063
3.42 - 2.99	1198 / 94.1%	18.8	0.069
2.99 - 2.71	1174 / 95.6%	16.6	0.074
2.71 - 2.52	1171 / 97.8%	15.1	0.085
2.52 - 2.37	1194 / 98.1%	11.1	0.103
2.37 - 2.25	1175 / 97.8%	9.8	0.117
2.25 - 2.15	1196 / 98.2%	8.1	0.144
2.15 - 2.07	1154 / 98.2%	6.1	0.193
2.07 - 2.00	1148 / 98.0%	5.2	0.243
50.00 - 2.00	11663 / 93.6%	18.1	0.070

Electron Density at 1.3 Å Resolution

Stereum purpureum Endopolygalacturonase I

by T.Shimizu (RIKEN/MD/Kato-Team)

Space Group *P1*

Unit cell $a = 37.2 \text{ \AA}$, $b = 46.5 \text{ \AA}$, $c = 52.1 \text{ \AA}$
 $a = 70.4$, $b = 72.5$, $c = 69.0$

Camera length = 200 mm, Wavelength = 1.02 Å

Osc. Angle = 1.5 degree, Exp. Time = 15 sec.

Total. Exp. 240 images

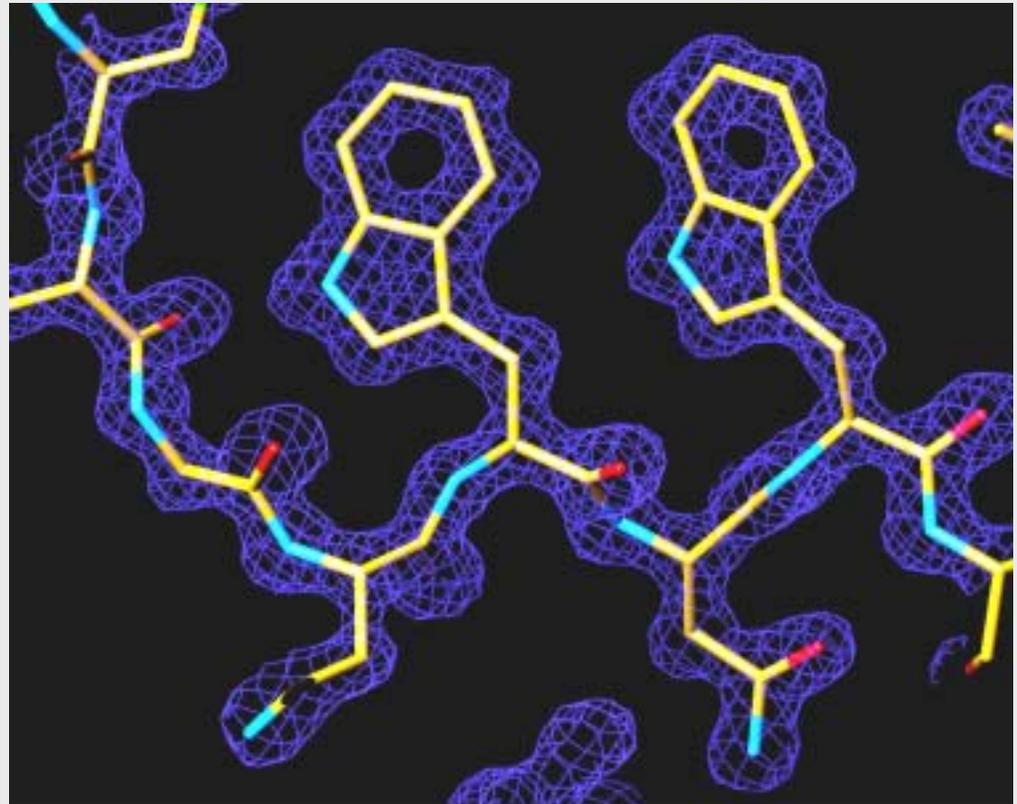
Resolution limit 1.3Å (Rmerge = 5.6%)

MR with MolRep

Rigid body refinement with Refmac

Refinement and auto-model building
with ARP./wARP

R-factor = 0.178 Rfree = 0.211



超高分解能構造解析

doi:10.1016/j.jmb.2004.02.007 J. Mol. Biol. (2004) 338, 403-408

JMB Available online at www.sciencedirect.com
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The PDZ2 Domain of Syntenin at Ultra-high Resolution: Bridging the Gap Between Macromolecular and Small Molecule Crystallography

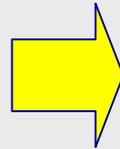
Beom Silk Kang, Yancho Devedjiev, Urszula Derewenda and Zygmunt S. Derewenda*

Department of Molecular Physics and Biological Physics, University of Virginia Charlottesville, VA 22908-0736 (USA)

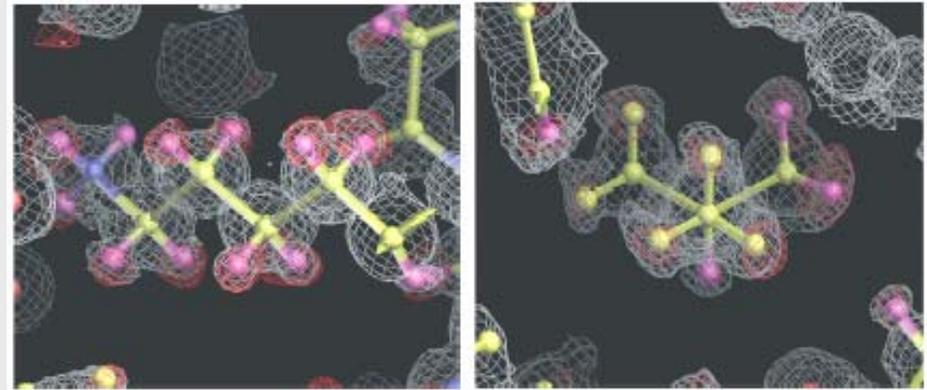
The crystal structure of the second PDZ2 domain of the scaffolding protein syntenin was solved using data extending to 0.73 Å resolution. The crystallographic model, including the hydrogen atoms and the anisotropic displacement parameters, was refined to a conventional R -factor of 7.2% and R_{free} of 8.7%, making it the most precise crystallographic model of a protein molecule to date. The model reveals discrete disorder in several places in the molecule, and significant plasticity of the peptide bond, with some ω angles deviating by nearly 20° from planarity. Most hydrogen atoms are easily identifiable in the electron density and weak hydrogen bonds of the C-H...O type are clearly visible between the β -strands. The study sets a new standard for high-resolution protein crystallography.

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*Corresponding author. Keywords: PDZ; syntenin; ultra-high resolution; crystallography



0.73 resolution
X8C beamline of the NSLS (Brookhaven)



Hydrogen atoms

Available online at www.sciencedirect.com
SCIENCE @ DIRECT® BBRC
Biochemical and Biophysical Research Communications 366 (2007) 644-648
www.elsevier.com/locate/ybbrc

Preparation and X-ray crystallographic analysis of rubredoxin crystals from *Desulfohalobium gigas* to beyond ultra-high 0.68 Å resolution^{a,c}

Chun-Jung Chen,^{a,*} Ming-Yih Liu,^b Yi-Ting Chen,^a and Jean LeGall^b

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^b *Department of Biochemistry and Molecular Biology, University of Georgia, Athens GA 30602-2229, USA*

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0.68 resolution
BW7A line of the DORIS (Hamburg)

CNRS, CNRS, Mod. 546-96-01 (2004) 774-782
1429-0254/04/000774-09
DOI: 10.1016/j.jmb.2004.02.007
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CMLS Cellular and Molecular Life Sciences

Ultra-high-resolution X-ray structure of proteins

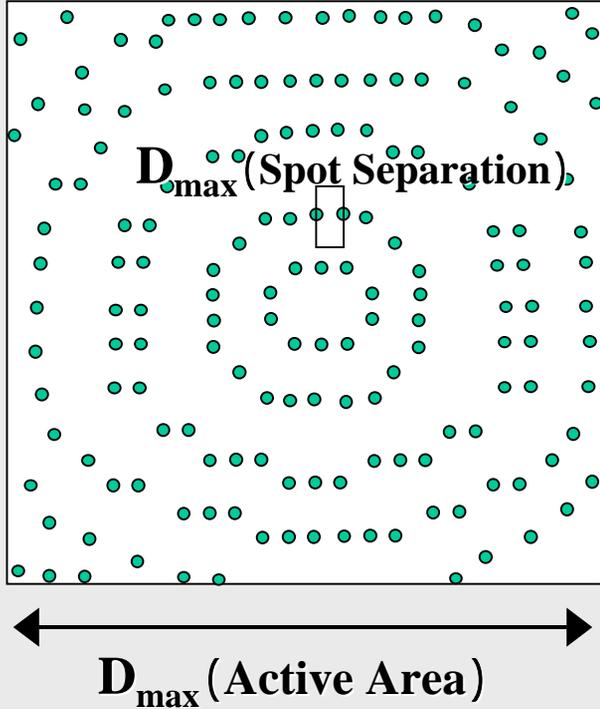
C. Lecroq^{a,*}, B. Gault^a, N. Meent^b, V. Pichon-Prieux^c and C. Achery^d

^a *LCM38, UMR CNRS 7036, Faculté des Sciences et Techniques, Université Henri Poincaré, Nancy 1, BP 239, 54506 Vandœuvre-lès-Nancy (France), e-mail: c.lecroq@cmis.uhp-nancy.fr*
^b *Sanofi-Synthelabo Recherche, Drug Design, 10 rue J. A. Aron, 67081 Strasbourg (France)*

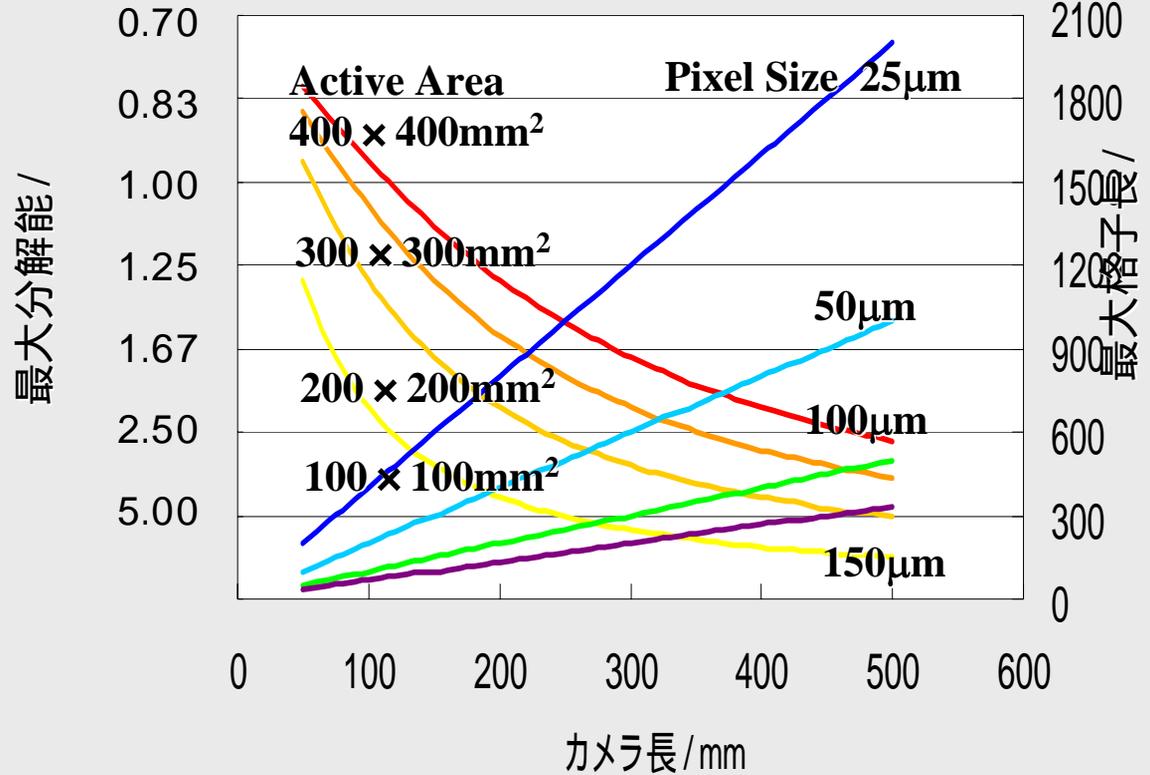
0.68 resolution
BL12B2 of the SPRING-8 (Harima)

2次元検出器のパラメータ (Dimension)

Diffraction Pattern



最大分解能
 $2D_{max} \sin \theta = \lambda$



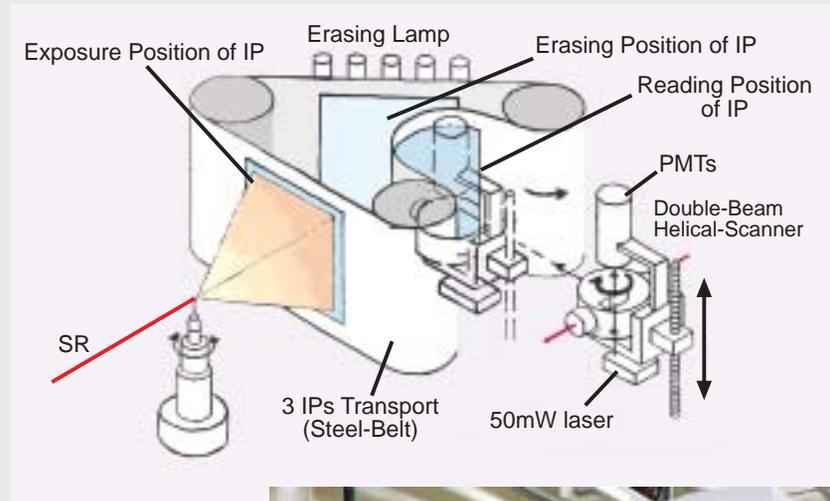
要求性能

最大分解能 1.0
 最大格子長 1000

RIKEN Structural Genomics Beamline (BL26B1&B2)

RIGAKU R-AXIS V

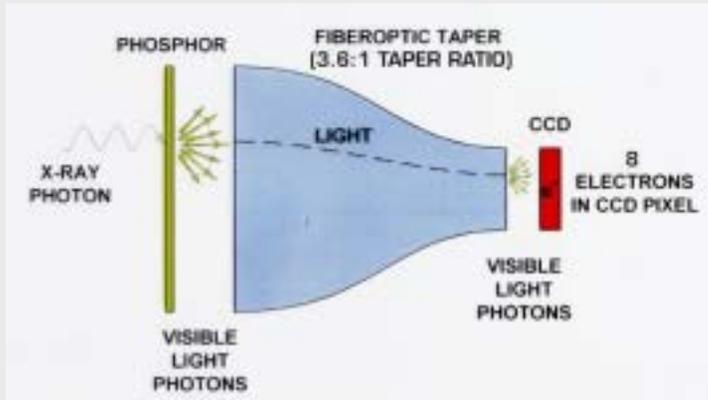
- Detector area : $400 \times 400 \text{ mm}^2$
- CCD size : **4000 × 4000** pixel
- Pixel size : $100 \times 100 \text{ }\mu\text{m}^2$
- ADC : 20 bit
- Readout - time : 50 Sec.



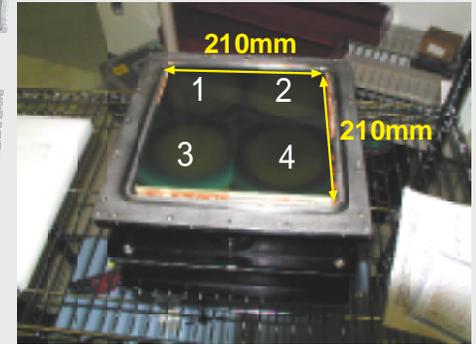
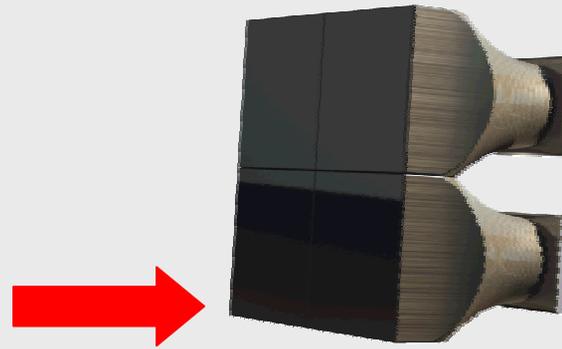
Large IP Detector
Mosaic CCD



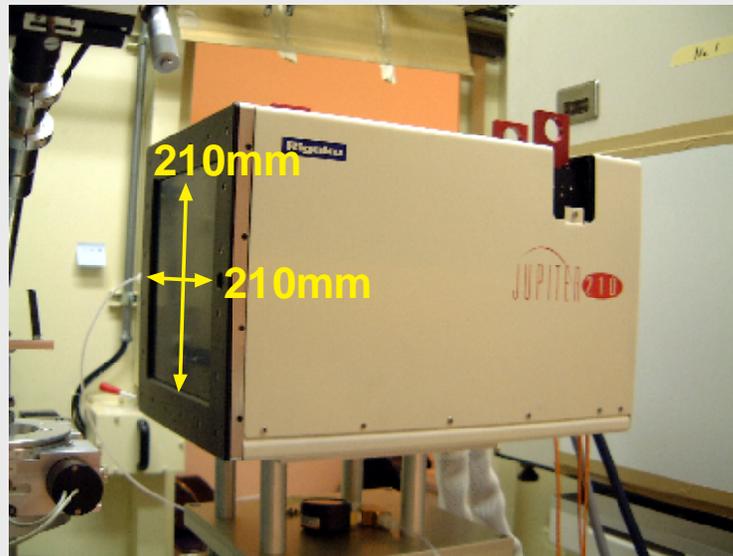
Mosaic CCD 検出器



CCD&FOT Single Module



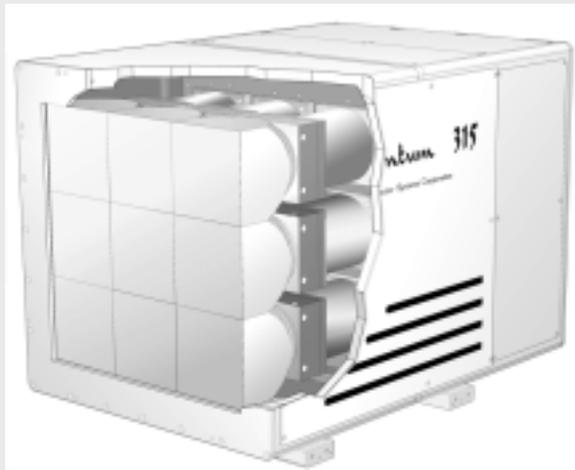
2x2 Mosaic Module



RIGAKU/MSM Jupiter210

- Detector area : $210 \times 210 \text{ mm}^2$
- CCD size : 4096×4096 pixel
- Data size : 32MB
- Pixel size : $51 \times 51 \mu\text{m}^2$
- ADC : 16 bit

ADSC Quntum315 @BL41XU



3x3 Mosaic Module

Spec

- Detector area : $315 \times 315 \text{ mm}^2$
- CCD size : 6072×6072 pixel
 3036×3036 pixel (2×2 Bin)
- Data size : $72\text{MB}(\text{Full}) / 18\text{MB}(\mathbf{2 \times 2\text{Bin}})$
- Pixel size : $0.051 \times 0.051 \text{ mm}^2$
 $0.102 \times 0.102 \text{ mm}^2 (\mathbf{2 \times 2\text{Bin}})$
- ADC : 16 bit
- Read-out time : 1.2 sec.
- Turn-over time : 3 sec.
(without exposure time)
- Camera length : 155 ~ 900 mm

放射光

極めて明るい光

細く平行性が高い光

広い波長領域の光

(X線から赤外線まで)

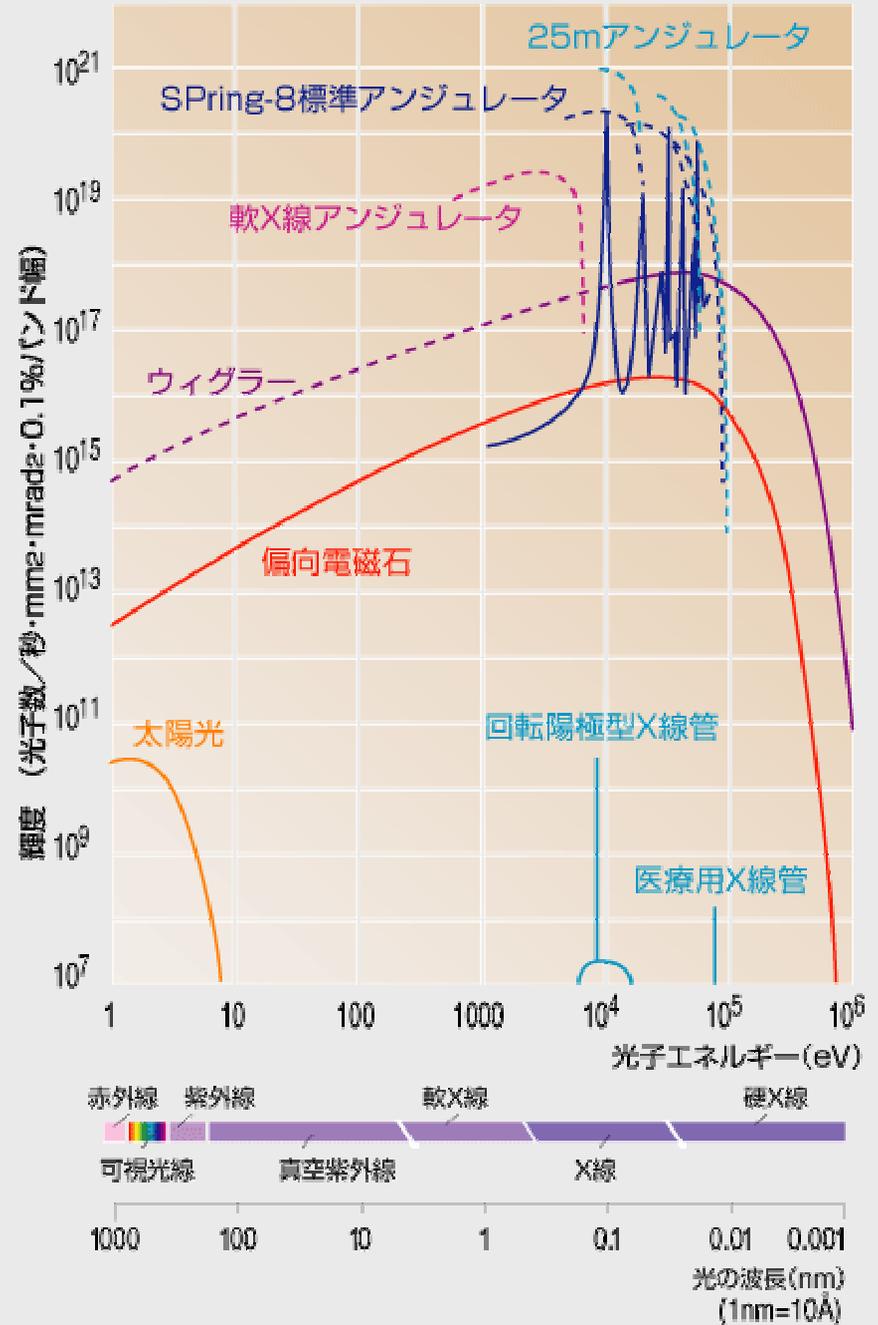
偏光した光

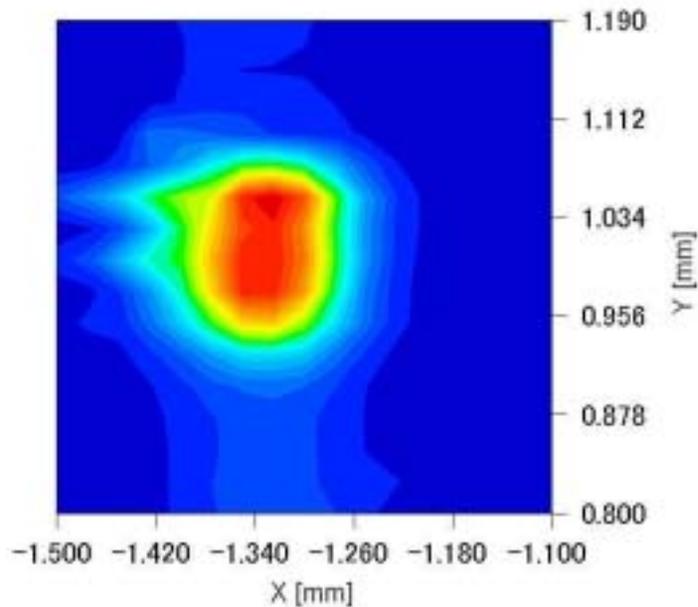
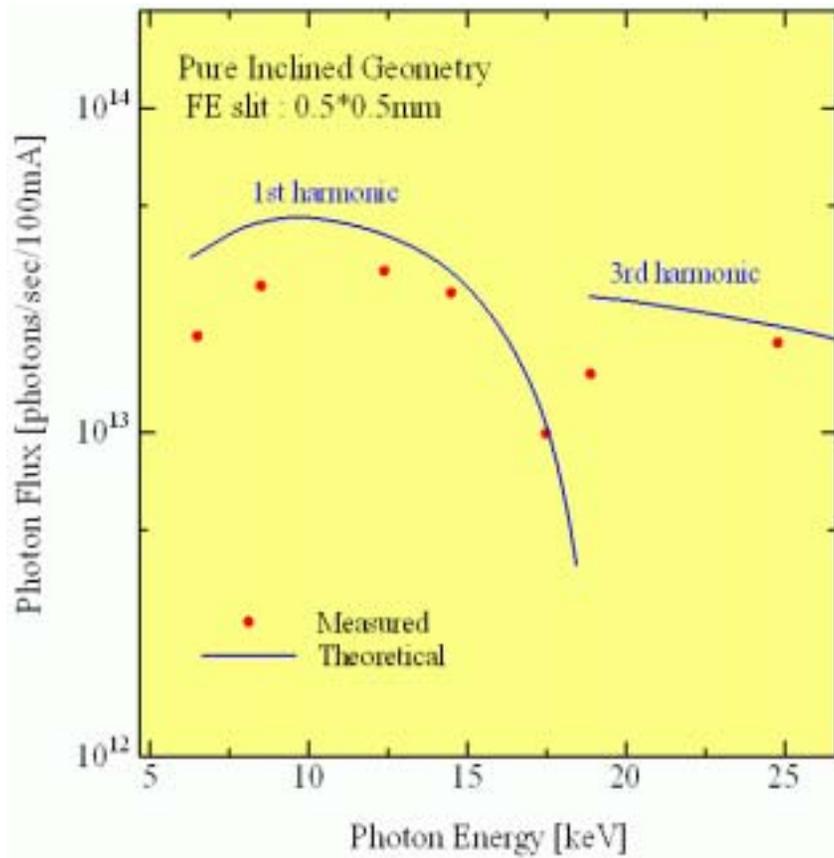
短いパルス光の繰り返し

バンチ長 34 pSec.

バンチ間隔 24 nSec(42.4MHz)

積分型検出器





ビームサイズ $120 \times 100 \mu\text{m}^2$

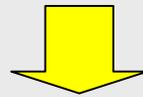
サンプル位置での光子数
光子密度

10^{12} photons/sec
 10^{14} photons/sec/mm²

BL41XUでのデータ収集

一般的な測定条件

- ビーム強度 1/10減衰 ($<10^{13}$ photons/sec/mm²)
- 露出時間 (シャッター制御) 1 秒
- 振動角 1 度
- 振動範囲 180 度 (全 180 イメージ)



CCD検出器のダイナミックレンジ (16 Bit) と同等



高速 & 広ダイナミックレンジ検出器

10Hz & 16bit

(or >100Hz & 14bit)

CMOSフラットパネル検出器

目標

より高速 (>100Hz) & 狭いダイナミックレンジ (14bit)



Shad-o-Box™1024

X-Ray Camera



	Rad-ikon Shad-o-Box 1024	Hamamatsu C7942	ADSC Quantum 4R
Number of pixels	1024×1024	2240×2368	2304×2304 (4 CCD)
Pixel size (μm)	48	50	82
Readout time (sec)	0.44 (2 channels)	0.44 (8 channels)	9
Dark current (e ⁻ /pixel/sec)	4000	5900	0.03
Readout noise (RMS, e ⁻)	< 500	1100	10@150kHz
AD converter (bits)	12	12	16
Maximum charge (e ⁻ /pixel)	ca. 2,000,000	2,200,000	440,000
Electrons/ADU	500	500	7
Electrons/x-ray photon	125@15keV [§]	165@12.4keV*	8.5@12.4keV
Resolution (line-pairs/mm)	10	8	90 μm (FWHM)
Phosphor	Gd ₂ O ₂ S:Tb	CsI:Tl	Gd ₂ O ₂ S:Tb
Operating temperature	Room temp.	Room temp.	-50 °C
Size (mm)	124×127×23	200×198×28	330 × 356 × 610

まとめ

放射光タンパク質結晶構造解析の理想の検出器

- ・ より大きな検出面積 **> 250 × 250 mm²**
- ・ より小さな画素サイズ **< 50 × 50 μm²**
> 5000 × 5000 pixels
- ・ より高速な読み出し速度 **> 100 Hz**
- ・ 広いダイナミックレンジ **> 14 bits**
Image Size > 50MB
Data Rate > 5GB/Sec. ???