

NSRRC\_BL17B2 蛋白質結晶學實驗站之  
RAXIS-IV++ IP 面積偵測器性能測試報告

**Performance Test Report of Rigaku/MSR  
RAXIS-IV++ Image Plate Area Detector  
Used at NSRRC\_BL17B2 PX Station**

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## (一) 簡介

NSRRC\_BL12B2 蛋白質結晶學實驗站原配備一台 30 公分直徑，雙片 IP 的面積偵測器(Mac Science Dip2030k)，各項偵測參數性能良好，但是兩片 IP 交替至曝光位置的時間(以下稱 Dead Time，DT)太慢，需要 490 秒，造成大量的光束線時間被浪費，數據收集效率過低，用戶的實驗品質低落，用戶抱怨頻頻。因此 NSRRC 將此實驗站之面積偵測器升級至 Rigaku/MSK 之 RAXIS-IV++，其各項偵測參數性能與 Dip2030k 相當，但 Dead Time 只有 150 秒，理論上數據收集效率可提高三倍。本報告將利用一些標準樣本的量測，來檢驗這台新的面積偵測器性能是否正常。

## (二) 繞射儀系統

這台面積偵測器是裝置在 CrystalLogic 公司為此台偵測器所特別製造的垂直 Phi 軸繞射儀上。

下面列出此特繞射儀的規格：

### Specification for Goniometer Used in NSRRC\_BL17B2

#### **Beam Height:**

500 mm above base

#### **Omega Axis:**

Orientation: horizontal

Range: free rotation

Resolution: 0.0005 degree

Accuracy: +/- 0.005 degree

Reproducibility: 0.0005 degree

Error sphere: <10 um

Slew speed: 600 degrees/min

Scan speed: 0.01~60 degrees/min

Home position: +/- 0.005 degree

Distance from base of goniometer head to beam: 54 mm

Manual translation adjustment for goniometer head: +/-20 mm

Distance from top-face of omega ring to beam: 300 mm

Distance from exit aperture to centered crystal: 10~15 mm

#### **Motorized Detector Mount: (before reconfiguration)**

Range: 50~500 mm

Accuracy: 0.1 mm

Slew speed: 300 mm/min

Weight capacity: 200 kg

#### **Vertical Motorized Detector Mount: (before reconfiguration)**

Range: -5~300 mm

Accuracy: 0.1 mm

Slew speed: 120 mm/min

Weight capacity: 200 kg

#### **Motorized Detector Mount: (after reconfiguration)**

Range: 50~800 mm

Accuracy: 0.1 mm

Slew speed: 300 mm/min

Weight capacity: 200 kg

**Two-Theta Axis: (after reconfiguration)**

Orientation: horizontal

Range: 0~45 degrees

Accuracy: +/- 0.015 degrees

Reproducibility: 0.002 degree

Slew speed: 30 degrees/min

Weight capacity: 200 kg

**Collimator & Shutter Assembly:**

Collimator size: 0.1, 0.15, 0.25, 0.3, 0.5, 1.0 mm (x1), 0.2 mm (x2)

Collimator mounted on XY alignment device

Shutter latency: <10 msec

Shutter repeatability: <1 msec

Shutter material: >1 mm tungsten carbide

Shutter synchronization with motion: <1 msec

Exit aperture: 1 mm

Exit aperture material: SS or silver or gold

Exit aperture mounted on XY alignment device

Ion Chamber: one with complete electronics

**Beam Stop:**

Material: >2 mm tungsten

Diameter: 2 mm O.D., 1.4 mm I.D.

Mounted on XYZ alignment device

**Crystal Viewing System:**

Two high-sensitivity CCD camera with 7:1 zoom

Double crosshair generator

**Motor Controller:**

Compumotor 6K series, or VEXTA 5 phase stepping motor series if requested.

**Total Goniometer Weight:**

Less than 1000 kg, but the design should be strong enough to prevent any mechanical deformation. The influence of this mechanical deformation on the coincidence of beam reference with omega axis should less than 10 um.

下圖為此繞射儀之照片：

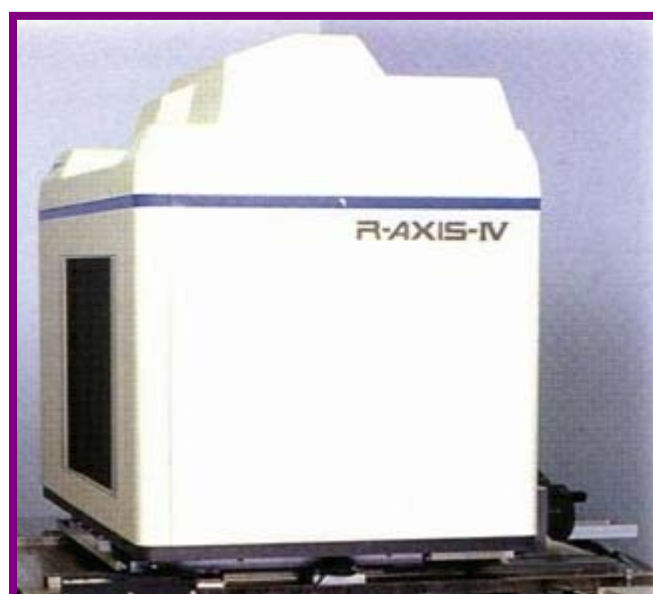


### (三) Rigaku/MSR RAXIS-IV++ 系統

下表為Rigaku/MSR RAXIS-IV++面積偵測器的一些特性參數：

R-AXIS IV++ enhancements		
		Comment
Imaging plate:	Fuji BAS MS	Better sensitivity
Laser:	50 mW, 658 nm	Faster, more efficient readout
Reading motor:	Servo motor (1800 rpm)	Faster readout
Number of pixel sizes:	3	Flexibility for readout
Read noise:	Less than 0.5 photon	Improves signal-to-noise
Pixel readout:		
200 $\mu\text{m}$ x 200 $\mu\text{m}$	50 seconds	
100 $\mu\text{m}$ x 100 $\mu\text{m}$	100 seconds	Flexibility in experimental design
50 $\mu\text{m}$ x 50 $\mu\text{m}$	225 seconds	

下圖為此面積偵測器之照片：

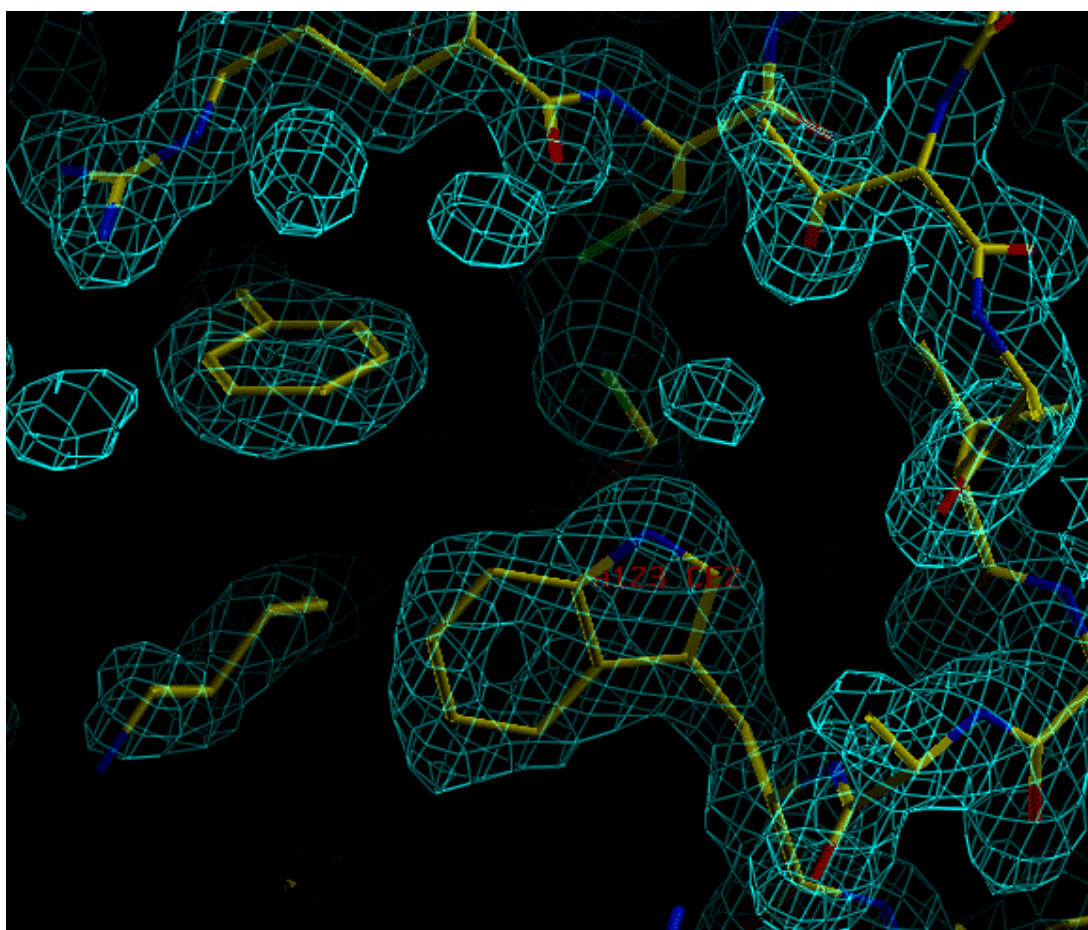


### (四) 性能測試結果(因專有名詞之故，此節將以英文撰寫)

#### 1. Lysozyme Crystal:

A total of 85 images of  $1^\circ$  oscillation angle and 100 second exposure time were collected and processed with HKL. The overall  $R_{\text{sym}}$  is 2.5% out to a maximum resolution of 1.80 Å (diffraction up to 1.4 Å) with an average redundancy of 6.62,  $\langle I/\sigma(I) \rangle$  of 24.6, completeness of 99.3% and  $\chi^2=1.10$ . These data were imported into CNS and rigid body refinement using the only the protein atoms of the lysozyme model from PDB. The preliminary refinement using only rigid-body refinement gave the R-factor of 21.3 % and R-free of 22.4% with all data 25-1.8 resolution. Below show the data statistics from HKL and the 2Fo-Fc electron density map (1.2 sigma cutoff).

Shell	Average			Norm. Chi**2	Linear R-fac	Square R-fac	
	I	error	stat.				
50.00	3.04	61607.5	1093.4	439.9	1.260	0.021	0.026
3.04	2.41	17943.3	330.1	159.5	1.295	0.023	0.025
2.41	2.11	9932.6	207.9	124.1	1.411	0.029	0.029
2.11	1.91	5356.4	138.6	101.3	1.451	0.039	0.037
1.91	1.78	2690.8	101.0	87.4	1.473	0.059	0.055
1.78	1.67	1608.3	110.7	104.5	1.483	0.083	0.076
1.67	1.59	1144.5	132.6	129.6	1.531	0.109	0.100
1.59	1.52	754.7	150.5	149.5	1.777	0.173	0.172
1.52	1.46	560.9	167.6	167.1	1.812	0.226	0.220
1.46	1.41	396.5	179.7	179.6	2.127	0.353	0.350
All reflections		13195.1	292.2	165.1	1.403	0.025	0.026



## 2. Insulin Crystal:

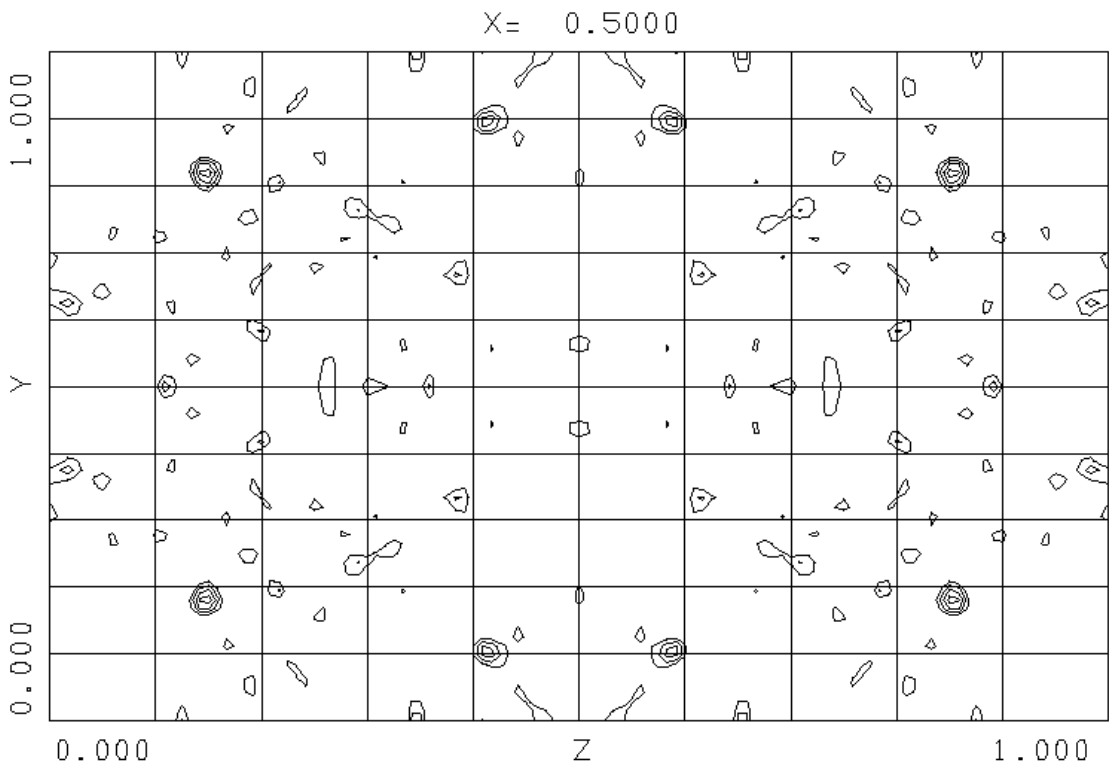
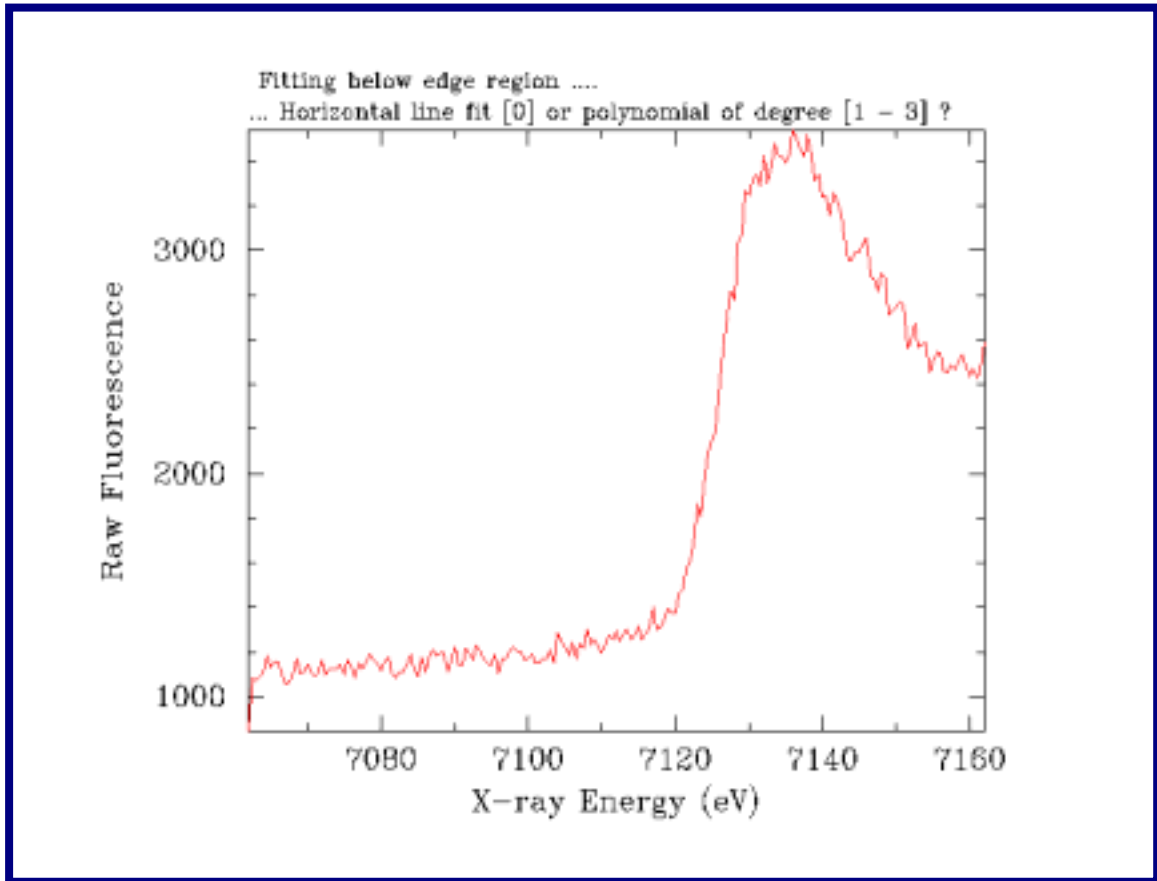
A total of 120 images of 1° oscillation angle and 300 second exposure time were collected and processed with HKL. The overall  $R_{\text{sym}}$  is 4.7% out to a maximum resolution of 1.80 Å (diffraction beyond 1.4 Å) with an average redundancy of 14.4,  $\langle I/\sigma(I) \rangle$  of 25.9, completeness of 100% and  $\chi^2=1.15$ .

	Shell	Average			Norm.	Linear	Square	
		<b>I</b>	<b>error</b>	<b>stat.</b>	<b>Chi**2</b>	<b>R-fac</b>	<b>R-fac</b>	
	30.00	3.88	41283.4	657.1	291.9	0.994	0.033	0.038
	3.88	3.08	34025.1	610.6	181.6	0.998	0.042	0.046
	3.08	2.69	15729.5	281.9	91.0	1.170	0.049	0.054
	2.69	2.44	11084.9	236.1	73.9	1.033	0.053	0.058
	2.44	2.27	8106.4	171.4	64.4	1.077	0.058	0.062
	2.27	2.13	7215.7	162.9	67.0	1.113	0.062	0.065
	2.13	2.03	5381.3	127.8	63.3	1.172	0.069	0.071
	2.03	1.94	3915.3	92.8	58.9	1.305	0.078	0.076
	1.94	1.86	3014.2	79.4	55.3	1.345	0.090	0.088
	1.86	1.80	2218.6	69.0	52.7	1.346	0.109	0.104
	All reflections		13473.5	253.0	101.7	1.154	0.047	0.045

### 3. Homoglobin Crystal:

The redish hemoglobin crystal with the size of 0.15 x 0.15 x 0.2 mm was first mounted in the glass loop and florescence-scanned to determine the heme Fe absorption edge. The spectrum is shown the X-ray at wavelength of 1.736 Å giving the maxium anomalous signal  $\delta f''$  as the below figure. A total of 152 images of 1° oscillation angle and 300 second exposure time were then collected at the X-ray wavelength of 1.736 Å and processed with HKL. The overall  $R_{\text{sym}}$  is 11.1% out to a maximum resolution of 3.00 Å (diffraction beyond 2.5 Å) with an average redundancy of 4.73,  $\langle I/\sigma(I) \rangle$  of 10.6, completeness of 98.4% and  $\chi^2=1.19$ . The anomalous difference Patterson for  $P2_12_12_1$  hemoglobin was calculated using the PHASES package.

Using the anomalous scattering signal of sulfur has been reported as a high potential method for solving the structures of stronger anomalous scattering macromolecules. A major prerequisite for using this method is data of high quality because the  $\delta f''$  term for sulfur is only 0.557 electrons. A strong set of difference Patterson vectors arising from the Fe atoms in the heme group with much signal become more widely used as a tool for checking the quality of data produced by modern area detectors, and, as a result, is a more demanding phasing method for metal-containing proteins. We have performed both of the above tests with our newly commissioned Raxis-VI++ system in order to assess the data quality. The resulting Patterson map (3.5 Å, 2 sigma cutoff, 1 sigma contour level) is clear and interpretable, and can be directly subjected to the Fe SAD phasing to structure determination.



## **(五) 結論**

由上列三個標準樣本的數據分析顯示，這台 RAXIS-IV++面積偵測器不論用來收集高解析度數據(Lysozyme Crystal and Insulin Crystal)，或是異常散射繞射數據(Homoglobin Crystal)，都能收集到品質良好的數據，性能優良可放心使用。