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Siemens Smart CCD Detector Applied to Protein Crystallography with Synchrotron and Rotating Anode X-Ray Sources

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ABSTRACT

The performance of the Siemens CCD detectors with 1K and 2K chips is evaluated using radiation from bending magnet beamlines at SSRL and NSLS and from a rotating anode source with three type of optics. Structure solution quality data is obtainable in short times for macromolecular and small molecule crystallography. General experiments, such as time-resolved powder diffraction and overlay diffraction are also possible.

INTRODUCTION

The rapid success of the SMART system for small molecule crystallography using a Mo K α sealed tube source has prompted the investigation of its use for macromolecular crystallography. It was expected that SMART coupled to a high intensity, low divergence synchrotron radiation beam would perform well for this application. In particular, its advantages over other technologies presently used at synchrotron radiation sources are:

- short readout time (1.3s) improving data quality, observed resolution and data collection time (i.e. productivity of a beamline).
- better dynamic range and quantum efficiency
- "narrow data frames" giving better signal-to-background and allowing full 3D profiling.

To explore these possibilities tests were performed at SSRL beamline 1-5 AD and NSLS beamline X7B. These beamlines are both on bending magnets with tunable monochromators and focusing mirrors. Work has been performed on rotating anode sources coupled to three types of optics also and is reported here. This work is detailed in the summaries, tables and graphs below.

SSRL MEASUREMENTS SUMMARY

At SSRL a 1K CCD was tested with a thin phosphor suitable for detection of long wavelengths. Data was successfully collected from a small molecule at nine wavelengths (0.855 to 2.06 Å) which spanned the tuning range of the mirror/monochromator system. This showed that 1.1 Å would give the best intensities. A data set on a lysozyme crystal was obtained at 1.54 Å for a direct comparison with data from the same crystal collected using a rotating anode. This indicated improvements in intensities of 10 at 1.54 Å and 35 for 1.1 Å. Rocking curve widths were much narrower, an effect found with other samples and which can be exploited to give improved signal-to-background (Refs. 1-3). Data was collected at 1.1 Å on 231 Å axis crystal (β -2 Luciferase) with axis resolved at detector distance 100 mm and d-spacing at the edge of the detector of 1.88 Å. High resolution rocking curve measurements on Thaumutuin studied crystal growth in ground and space-grown crystals. The technique illustrates the parallelism of the synchrotron beam and the precision of the PLATFORM goniometer. Anomalous dispersion enhancement by choice of wavelengths close to the Fe K edge (1.74 Å) led to heavy atom location in a Patterson map. The sample was monoclinic Myoglobin and data collection took 2.7 hrs. Data to 1.9 Å and 3.3% R_{merge} were obtained.

The small molecule data sets showed that the SMART system can efficiently detect x-rays as low as 2.06 Å. A data set at 1.0 Å was solved by direct methods (SHELXTL) with a total exposure for the data set of only 3.5 minutes.

NSLS MEASUREMENTS SUMMARY

At NSLS the 2K CCD system was used exclusively. Also the goniometer was set up with the ω -2 θ axis horizontal, with a fast shutter to allow for short and accurate exposures and with provision for incident beam monitoring by the data collection software. Wavelengths close to 1 Å were used exclusively. Data on lysozyme with R_{sym} of 7.2% to 1.5 Å was obtained in 5.5 hrs. Data on $\alpha\beta$ luciferase, which has a 302 Å axis (Ref. 5) was obtained in 8.0 hrs with R_{sym} of 13.6% to 3.4 Å. The system was also used for small molecule crystallography, solving a porphyrin, a zeolite and a carborane structure previously unsolved because of poor or small crystals. The system was also used for time-resolved powder diffraction and diffraction by multilayers on a macroscopic single crystal substrate.

ROTATING ANODE MEASUREMENTS SUMMARY

On a rotating anode various sets of optics were tested with the 1K CCD. A 0.5x1 mm focus loaded to 15 kW and a graphite monochromator gave data on lysozyme of 4.0% R_{merge} to 1.8 Å resolution in 2hrs. With double Franks mirrors (one 60mm long, one 160mm) and a .3x3 mm focus loaded to 5 kW a similar data set gave an R_{merge} of 7.2% to 1.89 Å. With double graded multilayer monochromators (Ref. 4) and the same source a 2.9% R_{merge} to 1.7 Å was obtained in 4.8 hr. Data from these latter two measurements were used to calculate a 6-fold increase in diffracted intensities from the mirror to the multilayer optics. With the multilayers a 2K CCD was also tested giving data to 6.1% to 1.6 Å. Data on β -2 Luciferase was obtained with R_{sym} of 8.0% to 2.6 Å.

SOFTWARE

There have been recent attempts to standardize experimenter detector interfaces for protein crystallography at various synchrotron laboratories. The Siemens data collection software, with graphical user interface is demonstrated in the figures.

SMART 1K CCD Detector Characteristics

- 1K chip, 25x25 mm on a side
- 2.5:1 fiberoptic taper permanently bonded to chip
- Patented method of matching fiber to surface of chip to maintain 1 to 2 micron consistent distance, custom epoxy bonding
- 64 x 64 mm square active area, equal to 4096 mm²
- 90 mm diameter phosphor, several choices, changeable Siemens custom phosphors, gadolinium oxysulfide
- Cooled by closed-cycle refrigerant to about -50°
- Pixel resolution:
 - 25 microns at the chip
 - 60 microns at the detector face
- Readout 1024 x 1024 or 512 x 512 binned
- Readout time (512): 1.3 seconds; (1024): 5.2 seconds
- Digitization range, 19 bits (524,288:1)
- Total system noise < single x-ray photon
- Automatic gain, 4:1
- Spatial uniformity factory calibration (fiducial plate)
- Intensity uniformity factory calibration (flood field)
- < 3% R_{sym} on stable crystals demonstrates detector uniformity

PLATFORM Goniometer

3 axes		
Speed	2 θ	1000°/minute
	ω	1000°/minute
	ϕ	2000°/minute
Reproducibility		$\pm 0.0005^\circ$
Max range	2 θ	Full 360°
	ω	Full 360°
	ϕ	Full 360°
	χ	54.74° fixed

Note that the detector is on a 2 θ arm so that it can be positioned at different 2 θ positions and that it can be moved along a dovetail to obtain various detector to crystal distances (up to 30 cm for standard arm). With these adjustments it is possible to collect data to any resolution for small or large unit cells at a wide range of x-ray wavelengths. Because the c-value is fixed at 54.74 it is usually possible to collect a complete data set by rotating about ω at one or more positions of ϕ . Thus, no remounting of the crystal is necessary.

SMART 2K CCD Detector Characteristics

- 2K chip, 51x51 mm on a side
- 1.9:1 fiberoptic taper permanently bonded to chip
- Patented method of matching fiber to surface of chip to maintain 1 to 2 micron consistent distance, custom epoxy bonding
- 96.5 x 96.5 mm square active area equal to 9316 mm²
- 136 mm diameter phosphor, several choices, changeable Siemens custom phosphors, gadolinium oxysulfide
- Cooled by closed-cycle refrigerant to about -50°
- Pixel resolution:
 - 25 microns at the chip
 - 48 microns at the detector face
- Readout 1024 x 1024 or 512 x 512 binned
- Readout time (512): 1.3 seconds; (1024): 5.2 seconds
- Digitization range, 19 bits (524,288:1)
- Total system noise < single x-ray photon
- Automatic gain, 4:1
- Spatial uniformity factory calibration (fiducial plate)
- Intensity uniformity factory calibration (flood field)
- < 3% R_{sym} on stable crystals demonstrates detector uniformity

Software for single crystal experiments

SMART	Screen crystals via rotation Measure rocking curves Index reflections Determine Bravais lattice Refine unit cell parameters Collect and display data (automatic and hands-on)
ASTRO	Simulate data collection strategies Calculate percent completeness and redundancy Display 3D image of goniometer, detector, data, axes
SAINT	Integrate reflections with 3D profiling Sort and filter data Correct for crystal decay, x-ray absorption Average different data runs and/or different crystals Compute statistical analyses
SHELXTL	Verify cell dimensions, Laue class, space group Display data as reciprocal lattice layers Calculate and contour Patterson maps Full matrix least squares refinement, F^2 constraints and restraints from known protein and nucleic acid structures, 5000 atoms

Thaumatococcus

Unit cell dimensions $a=b=58.5$ $c=151.6$; Space group $P4_22_12$

This was a study on crystal growth by looking at rocking curves. Two crystals of thaumatococcus were used, one grown on the Space Shuttle and the second grown on the ground. The second crystal was considerably smaller than the first. Rocking curves were obtained for both crystals by measuring a series of 50 frames of 0.02° width for each crystal. The rocking curve peak widths (background to background) are about 0.2° for each crystal. Both crystals show the characteristic split peak.

NSLS Data SetsLysozyme

Unit cell dimensions $a=b=79.1$ $c=37.9$; Space group $P4_32_12$

Frame width= 0.15° Frame time=15 sec.; 667 frames

Total elapsed time= 5.5 hrs

Total number reflections= 80875

Total unique reflections= 19439

$R_{\text{sym}}=7.2\%$ to $1.5\text{\AA}$; 97.7% complete

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$\alpha\beta$ Luciferase

Unit cell dimensions $a=59.9$ $b=112.7$ $c=301.8$; Space group $P2_12_12_1$
Frame width= 0.2° Frame time=30 sec.; 660 frames
Total elapsed time=8.25 hrs
Total number reflections=53825 Total unique reflections=26128
 $R_{\text{sym}}=12.9\%$ to $2.7\text{\AA}$; 86% complete

$C_7N_1B_{10}H_{15}$

Unit cell dimensions $a=7.246$ $b=18.429$ $c=18.140$
 $\beta=92.482^\circ$; Space group $P2_1/n$
Frame width= 0.5° Frame time=15 sec.; 720 frames (hemisphere of data)
Total elapsed time=6 hrs
Total number reflections=5134 Total unique reflections=2153
 $R_{\text{sym}}=12.1\%$ to $0.86\text{\AA}$; 92.5% complete
Solved by SHELXTL direct methods and refined to $R1=0.068$

Sodalite

Unit cell dimensions $a=8.8465$ Space group $Im-3m$
Frame width= 0.5° Frame time=45 sec.; 150 frames
Total elapsed time=2.5 hrs
Total number reflections=278 Total unique reflections=77
 $R_{\text{sym}}=7.2\%$ to $0.891\text{\AA}$; 100% complete
Solved by SHELXTL direct methods and refined to $R1=0.041$

Fe Silicide on Si(111)

Sample with 10 overlayers shows diffraction peaks and diffuse scattering in a "powder scan" of 400 frames, 15s and 0.1° each.

Cu Rotating Anode Data Sets

A two hour data set for lysozyme and a 24 hour data set for β - 2 luciferase were collected using an 18kW Cu rotating anode generator with a graphite monochromator. The CCD detector was fitted with a phosphor designed to be efficient for a wavelength of 1.54Å. The results are shown below.

Lysozyme

Unit cell dimensions $a=b=79.1$ $c=37.9$; Space group $P4_32_12$
 Frame width= 0.3° Frame time=14.5 sec.; 334 frames
 Total elapsed time=2 hrs
 Total number reflections=27581 Total unique reflections=10872
 $R_{\text{sym}}=5.6\%$ to $1.77\text{\AA}$; 89% complete

β - 2 Luciferase

Unit cell dimensions $a=231.9$ $b=62.4$ $c=62.6$; Space group $P2_12_12_1$
 Frame width= 0.25° Frame time=182 sec.; 480 frames
 Total elapsed time=24 hrs
 Total unique reflections=24255
 $R_{\text{sym}}=12.9\%$ to $2.7\text{\AA}$; 86% complete

Cu Rotating Anode with Göbel Mirrors Data Sets

A two hour data set for lysozyme using a 1K SMART, a two hour data set for lysozyme using a 2K SMART, and a 2 hour data set for β - 2 luciferase using a 2K SMART were collected using an 18kW Cu rotating anode generator with cross coupled Göbel mirrors. The CCD detector was fitted with a phosphor designed to be efficient for a wavelength of 1.54Å. The results are shown below.

Lysozyme, SMART 1K

Unit cell dimensions $a=b=79.1$ $c=37.9$; Space group $P4_32_12$
 Frame width= 0.15° Frame time=21.9 sec.; 617 frames
 $R_{\text{sym}}=2.9\%$ to $1.7\text{\AA}$; 87.7% complete, Isotropic L.S. refinement $R_1 = 14.9\%$

Lysozyme, SMART 2K

Unit cell dimensions $a=b=79.1$ $c=37.9$; Space group $P4_32_12$
 Frame width= 0.15° Frame time=21.9 sec.; 617 frames
 $R_{\text{sym}}=6.1\%$ to $1.6\text{\AA}$; 98.6% complete

β - 2 Luciferase

Unit cell dimensions $a=62.4$ $b=62.6$, $c=231.9$; Space group $P2_12_12_1$
 Frame width= 0.30° Frame time=124 sec.; 400 frames
 $R_{\text{sym}}=8.0\%$ to $2.6\text{\AA}$; 85.0% complete

CONCLUSIONS

These results demonstrate that the Siemens SMART CCD system can be used to collect good protein data with rotating anode sources and with synchrotron radiation. Crystals can be screened and an optimised data collection strategy started and concluded rapidly due to the fast readout time of the detector and the associated software. This leads to improved utilization of synchrotron beam time. Other types of experiment highlight the versatility of the system. Data collection on axes up to 300Å was demonstrated

Several modifications were made between the SSRL and NSLS experiments which enhanced the performance. In particular, vertical mounting ensured good intensities at high resolution for the lysozyme, small molecule and powder diffraction experiments.

Cross coupled Göbel mirrors give superior results on a rotating anode due to increased beam flux and reduced divergence. Refinement of lysozyme data collected with SMART and the mirrors gives a R1 of 14.9%, and data collected for β -2 luciferase shows full resolution of the 231Å axis.

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