Agilent PX Scanner: Improved Source and Detector for Enhanced Protein Crystal Screening

Application Note

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Figure 1. The new and improved PX Scanner.

Abstract

The new Agilent PX Scanner features a brighter Nova source with more than a **2-fold** increase in X-ray intensity. In combination with greater CCD sensitivity, it provides **3.3-fold** stronger diffraction images, as demonstrated by comparisons between images generated using 1st and 2nd generation systems.

Introduction

Selecting protein crystals for X-ray diffraction analysis can be time consuming because picking the right crystal is not always a certainty. The new PX Scanner is a revolutionary product from Agilent Technologies, which has been designed specifically for the identification and X-ray diffraction analysis of protein crystals *in situ* within multiwell crystallization plates.

The PX Scanner is unique in being both an optical and an X-ray imager in one, compact, self-contained unit. The optical imaging component allows the user to visually locate and select objects, which can then be studied by X-ray diffraction using the built-in microfocus X-ray source, optics, and CCD detector. The 2nd generation Agilent PX Scanner is equipped with a significantly brighter Nova X-ray source and a CCD detector with greatly improved sensitivity. These enhancements make it even more effective at screening protein crystals undisturbed in crystallization plates, saving time by increasing throughput and improving the detection of weakly diffracting crystals.

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Experimental

To assess the performance of the enhanced PX Scanner compared to the original design, X-ray images were collected from sets of lysozyme, glucose isomerase, and Ylid crystals using the same exposure times and oscillation ranges for both instruments. The CrystalEyes program was used to count diffraction peaks on X-ray images, while CrysAlis^{Pro} was used to analyze the intensities of peaks in the raw images.

Three different SBS plate-types were used for these measurements: Greiner X plates, Innovadyne SD2 plates, and Greiner 3sq plates. Of these, the new Greiner X plate has a significantly lower profile (thinner plastic) and therefore provides a greatly reduced background signal. This makes it of particular use for very weakly diffracting samples.

Figure 2. Inserting a crystallization plate into the PX Scanner.

Results and Discussion

X-ray Images from Glucose Isomerase Crystals in a Greiner X Plate

Two diffraction images were obtained from the same crystal, using the same exposure time. Two-dimensional plots of the image cross-sections (from the center point to the resolution edge) were generated in CrysAlis^{Pro}, in order to compare the strength of the signal from both instruments.

Images collected with the new PX Scanner are visibly stronger and have significantly more diffraction peaks than those collected with its predecessor. The cross-sections in Figure 3 illustrate that the image from the new PX Scanner is 3.34 times stronger than results achieved with the original PX Scanner.

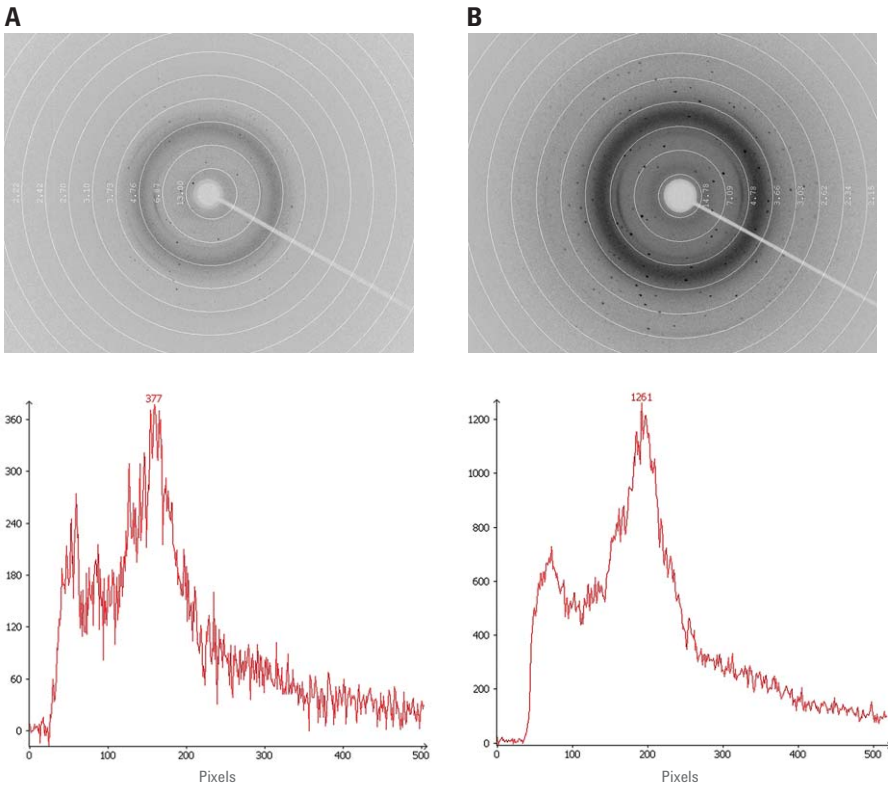


Figure 3. X-ray images and cross-section plots from an original (A) and a new (B) PX Scanner, using a glucose isomerase crystal (6 x 10s x 0.5°).

Peak Counting

Several crystals of lysozyme and glucose isomerase were screened to determine the number of peaks observed. The same exposure times were used, and standard routines in the CrystalEyes software package were used for peak counting.

1. Lysozyme crystals in Greiner 3sq plates

	Number of Peaks Obtained	
Crystal	Original PX Scanner	New PX Scanner
1	153	264
2	390	595
3	44	124
4	81	160

2. Glucose isomerase crystals in Innovadyne SD2 plates

	Number of Peaks Obtained	
Crystal	Original PX Scanner	New PX Scanner
1	136	245
2	16	65
3	18	42

The images obtained from the new PX Scanner experiments show consistently higher numbers of peaks, indicating that for the same experimental parameters it can achieve a significantly higher signal-to-noise ratio.

X-ray Images for a Ylid crystal in an Innovadyne SD2 Plate

A standard ylid calibration crystal was screened in a single well of an Innovadyne plate. The same exposure time and scan width were used, and a plot of the image cross-section (from the center to the resolution edge) was obtained from CrysAlis^{Pro}.

The highest peak on the cross-section corresponding to the plate scattering on the X-ray image is 2787 for the original PX Scanner and 9565 for the new PX Scanner, a 3.43-fold increase.

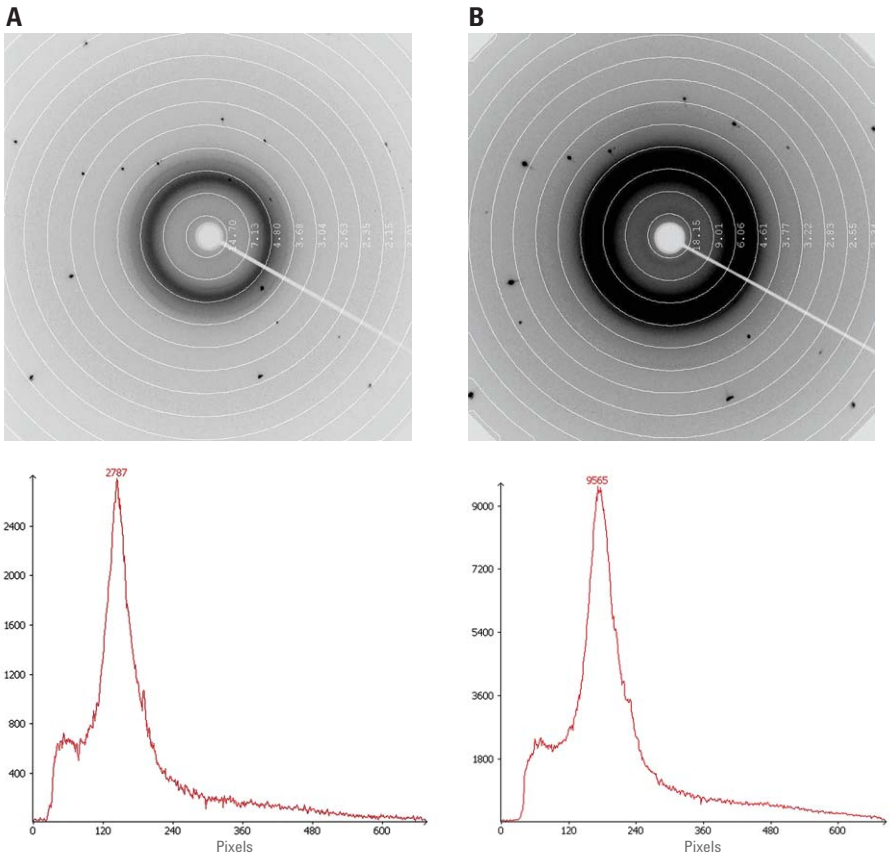


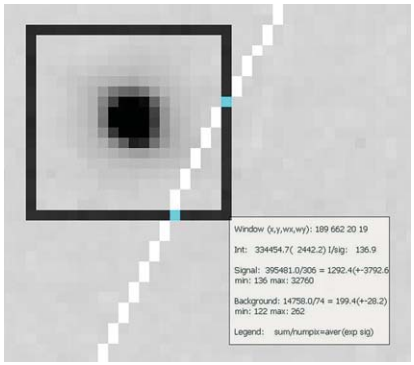
Figure 4. X-ray images and cross-section plots from an original (A) and a new (B) PX Scanner, using a Ylid crystal (1 x 30s x 6°).

Ylid Data Peak Analysis

The relative signal-to-noise (I/sig) values of two equivalent diffraction peaks were compared in CrysAlis^{Pro}.

Diffraction peaks on X-ray images obtained with the new PX Scanner are significantly stronger for equivalent peaks as compared with the 1st generation system.

A



B

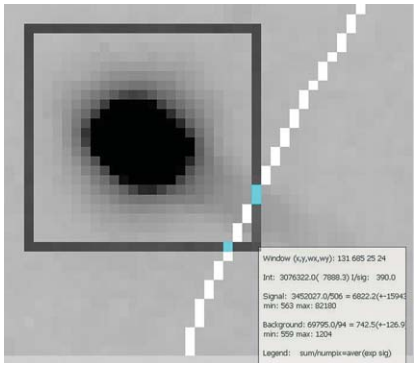


Figure 5. Relative signal to noise with an original (A) PX Scanner: I/sig=136.9, and a new (B) PX Scanner: I/sig=390.0.

Conclusions

The new and improved PX Scanner produces X-ray diffraction images that are more than 3.3 times stronger than those obtained with the 1st generation system. The higher number of peaks demonstrates that the new PX Scanner is capable of achieving a much higher signal-to-noise ratio for the same exposure time. This is confirmed by comparing integrated peak intensities from identical experiments on the two instruments.

Significant increases in X-ray source intensity and CCD sensitivity make the 2nd generation PX Scanner an even more powerful tool for protein crystal screening, with higher sample throughput and improved detection of small, weakly diffracting crystals.

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