

## New CCD detector for macromolecular diffraction data

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Higher intensity beamlines at synchrotron sources have driven the development of new x-ray sensitive detectors with enhanced performance capabilities for the collection of macromolecular diffraction data. Desired characteristics include improved sensitivity, dynamic range, count rate, and efficiency of data collection. To address this, a new integrating detector based upon a silicon chip charge-coupled device, or CCD, has been developed and successfully applied to the collection of macromolecular diffraction data at CHESS.

This detector is currently being made available to the macromolecular crystallographic user community as part of the upgraded data collection capabilities of the newly recommissioned A1 line. Dan Thiel and I have helped users collect their first data on the detector. In its early applications, the detector has proven exceptional for the collection of macromolecular diffraction data.

The CCD detector has been developed as a collaboration between Don Bilderback of CHESS, the laboratory of Dr. Steven Ealick at Cornell, the group of Dr. Sol Gruner of Princeton University (including Sandor L. Barna, Michael E. Wall) and Dr. Eric Eikenberry of the Robert Wood Johnson Medical Center and Dr. John Lowrance of Princeton Scientific Instruments. Both a schematic and a photograph of the detector are shown.

Operationally, x-ray photons are converted to visible photons by a  $Gd_2O_3:Tb$  phosphor. The pixel size at the phosphor end is  $50\ \mu\text{m} \times 50\ \mu\text{m}$ . This signal is transferred from the phosphor by a 2.6:1 fiber optic taper to a  $1024 \times 1024$  pixel CCD chip ( $\sim 20$  mm on an edge). Signal is stored in the chip by the generation of about 11 electrons per 13.6 keV x-ray absorbed, which is then maintained by an imposed voltage until readout. At

readout, charge stored in a pixel row is sequentially shifted horizontally to the single readout amplifier. Subsequently, the charge in each row of pixels above is shifted downward and read out in like manner. In this way, a stationary controller converts the horizontal and vertical position of each pixel and its stored charge to a digitized x,y coordinate on the detector face and a raw intensity. These data are then written to some sort of storage device (currently a magnetic disk or tape) and displayed on a monitor for inspection and limited manipulation.

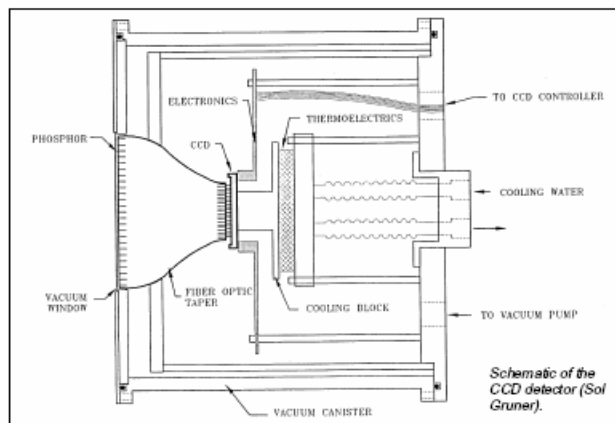
In practice, this detector has provided several advantages for the collection of macromolecular diffraction data. To begin, the CCD chip pixel size is  $19.46\ \mu\text{m}$  which translates roughly into  $50\ \mu\text{m}$  square pixels at the detector face. With a bloomed point spread function at full width half maximum of  $80\ \mu\text{m}$ , the detector truly gives a spatial resolution limited by the pixel size. Further, detector sensitivity at wavelengths from 8 keV to 14 keV, as measured by fraction of x-rays stopped, ranges from 99% to 70% efficiency, respectively. Finally, the readout dynamic range of the detector is 36,000 x-rays. In its current configuration, a 20 second CCD readout time is used. While this is comparatively slow for a chip of this size, it was chosen as a compromise between minimizing readout noise from the single amplifier and decreasing the duty cycle for each macromolecular diffraction image. (This time will be reduced in future detectors.) In addition to such



Photograph of the CCD detector in place at the CHESS A1 station. A protective metal screen (shown open at left) protects the phosphor when not collecting data.

performance capabilities, each image is immediately available for inspection and limited manipulation which includes image zoom and spot intensity integrating and profiling capabilities, among others. While the active area of the detector is rather small, its spatial resolution allows crystal to detector distances as close as 35 mm to be used (giving  $1.4\ \text{\AA}$  data on an edge with  $0.91\ \text{\AA}$  wavelength x-rays in a symmetric configuration). For medium to slightly larger unit cell dimensions (high resolution data for cells with axes up to  $180\ \text{\AA}$  have been successfully collected), the detector can be offset and pulled back to give more reasonable overlap while still recording high resolution data ( $< 2.0\ \text{\AA}$ ).

Relative to crystallographic results, an example of a diffraction im-



age from data collected using this detector on A1 is shown at right. During a three day experimental run in September, seventeen complete data sets were collected for five different protein crystallography projects. The diffraction image is from a frozen crystal of a complex between the enzyme nucleoside deoxyribosyl transferase and a substrate, deoxyuridine (cubic,  $I2_3$  crystals with unit cell axes of  $148.2\text{\AA}$ ). The  $R_{\text{sym}}$  for all data was 5.0% to 2.3 $\text{\AA}$ , and it is currently being used for high resolution refinement and active site analysis. Because of detector sensitivity and speed and flash freezing of the crystals (see page 44), a complete (97%), high quality, high resolution data set was possible from a single crystal. This was previously impossible with these extremely radiation sensitive crystals.

A second example produced an electron density difference for the active site of the enzyme bovine purine nucleoside phosphorylase in complex with a non-deavable substrate

*(Right) Diffraction image from a crystal of a complex between the enzyme nucleoside deoxyribosyl transferase and deoxyuridine, a substrate. Data was usable to 2.3 $\text{\AA}$  in this data set. The image is a 20 second, 0.75° oscillation with a crystal to detector distance of 60 mm (this detector configuration allowed 2.2 $\text{\AA}$  data on the edges and 1.7 $\text{\AA}$  data in the corners of the detector).*

analogue, 9-deazainosine (cubic,  $P2_3$  crystals with unit cell axes of  $93.3\text{\AA}$ ). Again, high quality, high resolution data with an  $R_{\text{sym}}$  of 5.2% for all data to 1.7 $\text{\AA}$  (94% complete) were obtained using the CCD detector in

conjunction with flash freezing. These data are currently being used to investigate the mechanism of the enzyme. Such results were typical of the data collected during this run. Since then, the detector has been used to collect thousands of images during a month and a half long MAD phasing run on the F2 beamline and is currently being applied to the collection of macromolecular data for outside users on A1.

At CHESS, the development of beamlines and improved x-ray detectors, such as the CCD, is going hand in hand. Plans for continued improvement in detector development are already in place. These include doubling the active area and decreasing the readout time to five seconds in the current design and a planned mosaic detector design. The CCD detector and planned improvements to it will play a role in keeping CHESS at the forefront of macromolecular crystallography for the foreseeable future.

