

PREFACE

X-ray Diffraction Data Collection



The techniques used for measuring the intensity and angle of diffracted X-rays have seesawed between “point” and “area” (originally film) detectors. The first X-ray diffraction experiment by Friedrich and Knipping [1] used film, but within the same year Bragg was using an ionization chamber mounted on a rotating arm [2]. The advantage of Bragg's X-ray detector was that it defined precisely the angle of the diffracted rays and, in general, gave more accurate intensity measurements. The advantage of film was that it readily recorded many reflections on one exposure. However, counter devices were tedious to use, particularly without automatic motorized drives, while film devices gave poor positional and intensity estimates.

The early days of structural crystallography, covering the period-1920-60, saw extensive use of film methods. This was a time when basic chemical formulas, such as the structure of benzene [3], were being verified and the relationship between bond order and bond lengths was being established [4]. The end of this period saw the gradual introduction of electronic computers. Previously, most structures had been solved by using principal projections only, thus greatly reducing the amount of data that had to be collected and the amount of computation that had to be accomplished. The data collection devices were analogue machines, such as the rotation and Weissenberg cameras and, eventually, the Buerger precession camera, designed to present the Ewald geometry in a simplified form.

The introduction of digital computers made it possible to control and program the diffractometer counter arm and crystal orientation, while measuring the intensity accurately with a scintillation counter. Use of these machines provided much higher quality data, without minute-by-minute manual assistance. Further improvements were made, for instance, by using multiple counters [5]. These diffractometers made feasible the study of far larger molecules, requiring the collection of larger data sets with more accuracy (e.g. lysozyme [6] and phosphorylase [7]). However, many biological macromolecules can be rapidly damaged by X-radiation. Thus, while an automated diffractometer is carefully measuring one reflection, there are thousands of other reflections, occurring at the same time, that are not monitored. This problem was recognized by Joseph Kraut and coworkers who proposed removing the layer line screens from a precession camera, but avoiding reflection overlap by using only very small precession angles [8]. Unfortunately, the screenless precession camera generally creates two spatially removed records of each reflection, thus increasing the chance of reflection overlap and reducing the intensity of each record. This problem was solved by Uli Arndt when he re-introduced the old oscillation camera [9], but in the era of computerized film scanners [10].

The screenless oscillation technique has held sway since the mid 70's, although the mode of detector has varied. Originally, the cameras were used with film. For the typical protein, one photograph might have taken several hours to one day. Thus, a carousel carrying eight successive film cassettes was usually plenty to make sure that the experimenter could obtain a good night's sleep without interruption during data collection. The greater availability of synchrotrons in the early 80's cut the film exposure time down to

Table I. X-ray diffraction data collection using fixed imaging plates or CCD detectors*.

I. Recording the diffraction pattern

1. Readout times are likely to be less than a minute for imaging plates or five seconds per 2000² pixels.
2. The detector is fixed in space. Every image has the same relationship to the camera axes.
3. Continuous display and automatic monitoring using feedback from processed images can be implemented.

II. Indexing

1. One-dimensional Fourier analyses and search procedure have already made auto-indexing a standard procedure [11, 12] and is likely to make possible the analysis of twinned or split crystals.
2. Re-refinement procedures based on partial reflections can become more powerful and automatic.
3. Feedback from previously processed images can be used to modify and improve the autoindexing.

III. Integration

1. Error analysis can be improved for difficult reflections in the event of overloaded, overlapped, damaged pixel, diffuse scattering or other crystal problems.
2. Profile fitting can be extended to partial reflections.

IV. Scaling

1. Scaling of data, when there are few, if any, whole reflections, can be rigorously implemented [13].
2. Post-refinement with anisotropic crystal properties for mosaicity and beam properties can be implemented.
3. Analysis of sequential scale factors in relation to the crystal properties and beam properties can be implemented.
4. Gradual variation in cell dimensions with exposure and radiation damage can be considered.
5. Computation of standard errors for each average reflection can include a record of problems encountered during processing.

*A number of new data processing packages are currently in development. This table reflects, to some extent, the work in progress in the author's laboratory.

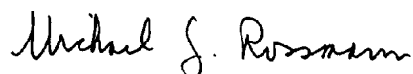
typically 5 to 20 minutes. This data collection rate required a large group of helpers working around the clock. The gradual introduction of multiwire 'area detectors' reduced the manual burden as readout could be automatic. In many ways, these detectors were a throwback to the point detector, except that there were now a larger number (often 512²) of detectors working simultaneously. Further improvement over the somewhat low efficiency and low dynamic range of film came with the introduction of imaging plates; however, these still required manual assistance for individual scanning of each image, although automatic devices, such as in the R-AXIS or MAR cameras, scan automatically after each exposure. The resulting optical densities can be stored directly on disk or tape, thus removing the tedious, time consuming and messy film developing operation. The imaging plate is most closely comparable with a film in its properties, although the use of merely a single or two imaging plates in automatic cameras removes much of the spatial uncertainty present in film measurements. Furthermore imaging plates have much higher sensitivity and dynamic range.

Most recently, charge-coupled devices (CCD) have become available with the number of pixels (~2000²) comparable to what is available for film or imaging plates. These allow rapid, direct readout after each exposure, with an accuracy and efficiency well above film and comparable to that of the diffractometer era.

Such devices are particularly useful at synchrotron beam lines where rapid readout of the scanned data is essential. For laboratory X-ray generators, imaging plates, which subtend a larger solid angle at the crystal than CCD's for the same number of pixels, probably have an advantage. Using a CCD detector at the Cornell High Energy Synchrotron Source (CHESS), it is now possible to collect one exposure every 15 seconds or so, even from crystals with unit-cell volumes of around 700^3 \AA . The readout time is only about four seconds; thus, the rate of accumulation of data is almost 1 megabyte per second. Such enormous rates of quality data collection at synchrotrons, as well as automatic imaging plate cameras in many laboratories, are revolutionizing the way data are processed and extending the range of feasible projects for the determination of biological structures (Table 1).

References

- [1] W. Friedrich, P. Knipping & M. von Laue: *Sitzber. math.-physik. Klasse Bayer. Akad. Wiss. München*, 1912, p. 303.
- [2] W. H. Bragg: *Nature* (London), **90** (1912), 219; *Nature* (London), **90** (1912), 360; *Nature* (London), **90** (1913), 572.
- [3] K. Lonsdale: *Nature* (London), **122** (1928), 810.
- [4] L. Pauling & L. O. Brockway: *J. Amer. Chem. Soc.*, **59** (1937), 1223.
- [5] U. W. Arndt & D. C. Phillips: *Acta Crystallogr.*, **14** (1961), 807.
- [6] C. C. F. Blake, D. F. Koenig, G. A. Mair, A. C. T. North, D. C. Phillips & V. R. Sarma: *Nature* (London), **206** (1965), 757.
- [7] R. J. Fletterick, J. Sygusch, N. Murray & N. B. Madsen: *J. Mol. Biol.*, **103** (1976), 1.
- [8] N. H. Xuong, J. Kraut, O. Seely, S. T. Freer & C. S. Wright: *Acta Crystallogr.*, **B24** (1968), 289.
- [9] U. W. Arndt, J. N. Champness, R. P. Phizackerley & A. J. Wonacott: *J. Appl. Cryst.*, **6** (1973), 457.
- [10] U. W. Arndt & A. J. Wonacott: *The Rotation Method in Crystallography* (Amsterdam: North Holland, 1977).
- [11] Z. Otwinoski & W. Minor: *Meth. Enzymol.*, **276** (1996), 307.
- [12] I. Steller, R. Bolotovskiy & M. G. Rossmann: *J. Appl. Cryst.*, (1997), in press.
- [13] R. Bolotovskiy, I. Steller & M. G. Rossmann: (1997), in preparation.



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