

ANALYTICAL APPLICATION NOTE

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SINGLE CRYSTAL DIFFRACTION

■ Single-crystal x-ray diffraction, commonly referred to as x-ray crystallography, is an analytical technique in which x-ray methods are employed to determine with certainty the actual arrangement of atoms within a crystalline specimen. The science of x-ray crystallography originated in 1912 with the discovery by Laue that crystals diffract x-rays. Since that time, single-crystal x-ray diffraction has developed into the most powerful method known for obtaining the atomic arrangement in the solid state.

X-ray crystallographic structure determination can be applied to a wide range of sizes of structures, from very small molecules and simple salts, to complex minerals, synthetically prepared inorganic and organometallic complexes, natural

products and to biological macromolecules, such as proteins and even viruses.

The precise knowledge of the molecular geometry is becoming increasingly important in nearly all fields of chemical and biological research. The three-dimensional atomic coordinates obtained from crystallographic studies, available in comprehensive crystallographic databases such as the Protein Data Bank and the Cambridge Structural Database, are often the starting point for most molecular modeling, drug design and molecular orbital calculations. Indeed, many of most significant advances in structural chemistry and structural biology are based upon results obtained from x-ray crystallographic analyses.

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The results of x-ray crystallographic analyses are complementary to other commonly used solid-state techniques, such as x-ray powder diffraction, solid-state NMR, EPR, FT-IR and Raman spectroscopy, and neutron diffraction. Chemists also routinely use such techniques as nuclear magnetic resonance, infrared and ultraviolet spectroscopy, mass spectrometry, x-ray fluorescence, and elemental analysis for the identification and characterization of compounds prepared and isolated in their laboratories. The experimental data obtained from these techniques may, after suitable analysis and interpretation, yield important information concerning the composition and structure of the compound. However, such information is often incomplete, fragmentary and ambiguous. There are many classes of chemical compounds such as natural products, organometallic complexes, inorganic salts, metal cluster systems, organic and inorganic reaction products for which the structure cannot be deduced even with all of the other methods combined. X-ray crystallography is uniquely capable of unambiguously determining the complete three-dimensional molecular structures (including the absolute stereochemistry) of chemical substances. Modern x-ray crystallographic data is often of sufficiently good quality to permit routine location and refinement of solvent molecules and hydrogen atoms.

A significant reason for the increased use of x-ray crystallographic methods is due to the remarkable improvements in instrument performance achieved through the introduction of two-dimensional detectors, especially CCD systems. The improvements in x-ray crystallographic instrumentation, along with associated advances in computer hardware and software have also resulted in enormous progress in efficiency and productivity. Crystal screening and identification tasks may now be performed in a matter of minutes, while analytical crystal structures may be completed in less than one hour and publication-quality structures are typically done in 3 to 13 hours, depending upon the size of the specimen. Crystallographic calculations that once required overnight execution on a main-frame computer are now easily carried out on a notebook computer. A single instrument may now be used to carry to screen and identify many thousands of samples and to determine several hundred structures per year, dramatically reducing the cost per structure.

X-ray crystallography, once regarded as an expensive and time-consuming technique used only by specialists, continues to gain new users in all branches

of chemistry and biochemistry. Modern commercial instruments feature easy-to-use graphical user interfaces and automated crystallographic routines that allow routine crystal structure analyses to be carried out quickly and easily by users with only minimal training in x-ray crystallography. The high throughput and low operating costs of modern single-crystal instruments and their ease-of-use now make them suitable for use in synthetic laboratories as routine analytical tools.

The improvements in speed of data collection and data quality offered by new instrument technology has also extended the use of x-ray crystallography to a further level. Charge-density studies, which were previously limited to very small molecules due to long data collection times (often 3 to 6 months on conventional instruments), may now be carried out in less than one week on much larger molecules. At this level, it becomes possible not only to determine with very high positional accuracy the three dimensional positions of atoms in molecules and hence their bond lengths and angles, but also a measure of bond strength, atomic charges, dipole moments and the electrostatic potential. All of these properties are extremely important in predicting the chemical behavior of molecules. (Figure 1).

The range of materials which may be studied by x-ray crystallography has recently been extended by the availability of higher flux x-ray sources (e.g., rotating anode generators, x-ray mirror optics, synchrotron

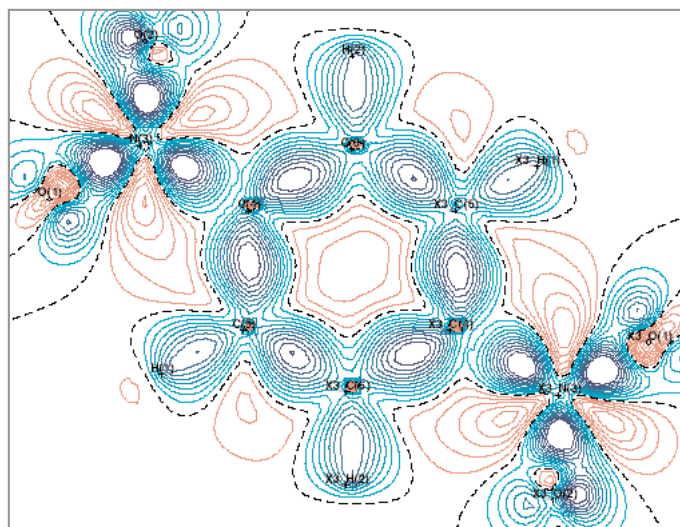


Figure 1. Electron density difference map for p-dinitrobenzene obtained by subtracting the pro-molecule electron density from the total electron density.

beam lines) and more sensitive x-ray detectors (e.g., image plates and CCD detectors), by the use of high speed computers with large amounts of mass storage and, the development of new algorithms for solving and refining large and problematic structures (e.g., twinned specimens, incommensurate structures). These advances, coupled with progress in the related fields of crystal growth, cryo-crystallography, and crystal mounting techniques now permit x-ray structure determinations to be carried out on very small specimens (minimum dimensions of a few microns), on materials with very large unit cells (maximum axis length 400Å) and on materials which are liquids at room temperature or which undergo solid-state phase changes.

Fundamentals

A perfect crystalline solid (single crystal) is made up of a large number of identical molecules which are arranged in a precisely regular way repeated in all directions to give a highly ordered structure. The basic building block (or motif) in a crystal is the unit cell. A crystal is made up of millions of identical unit cells arranged in a three-dimensional crystal lattice. Each crystalline substance has a unique set of lattice constants ($a, b, c, \alpha, \beta, \gamma$) which define the size and shape of the unit cell (Figure 2).

Based upon the lattice constants and the symmetry that the unit cells possess, the substance may be classified

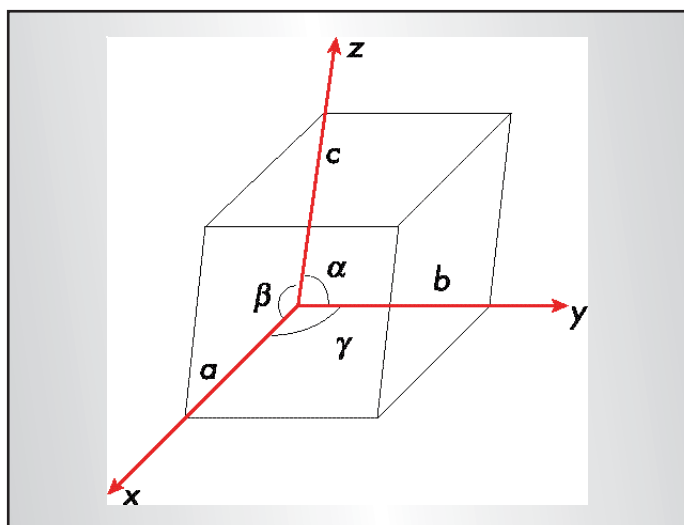


Figure 2. Unit-cell nomenclature: the reference axes x, y, z are right-handed, the length of the unit-cell edge parallel to each reference axis is a, b, c , respectively. And the interaxial angles are α, β, γ , respectively.

into one of seven crystal systems (i.e., triclinic, monoclinic, orthorhombic, tetragonal, cubic, trigonal or hexagonal). Finally, each substance may be further classified as belonging to one of 230 three-dimensional space groups. The lattice constants, crystal system, and space group are important physical constants for crystalline substances and may be used in conjunction with other physical measurements (e.g., density, conductivity, hardness) to explain the properties of solid-state materials.

When a beam of parallel monochromatic x-rays of approximately 1Å wavelength strikes a single crystal, the crystal acts as a three-dimensional diffraction grating and produces an x-ray diffraction pattern (Figure 3). This diffraction consists of a three-dimensional array of reflections that satisfy the conditions of Bragg's law:

$$n\lambda = 2d \sin\theta$$

where n is a small integer giving the order of diffraction, λ is the wavelength of the incident x-rays, d is the distance between a set of parallel lattice planes, and θ is the angle between the incident x-ray beam and the atomic lattice plane in the crystal (Figure 4). The diffraction pattern for a typical organic compound with 20 non-hydrogen atoms contains approximately 2,000 unique reflections. The spatial arrangement of the reflections in an x-ray diffraction pattern bears a reciprocal relationship to the

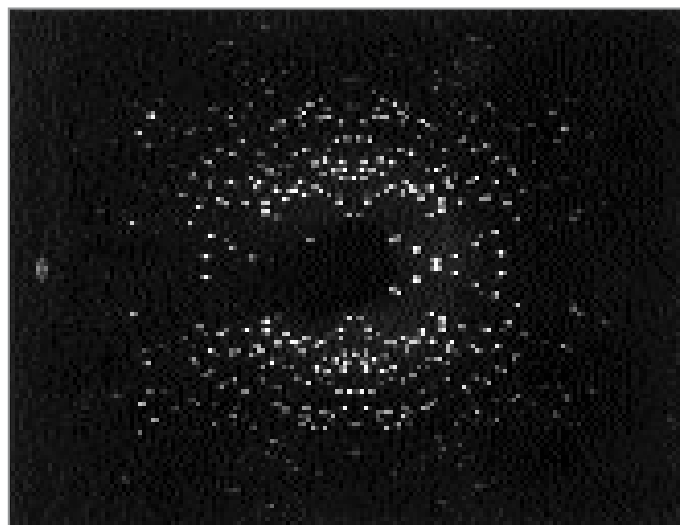


Figure 3. An example of an x-ray diffraction pattern (one-minute rotation image) produced by a randomly oriented single crystal.

dimensions of the unit cell in the crystal. Each reflection may be assigned a set of indices (hkl) which indicate its location in the diffraction pattern or reciprocal space. The diffraction pattern in reciprocal space has a Fourier transform relationship to the electron density in the unit cell in real space.

Experimentally, the unit-cell parameters for a crystalline specimen may be determined from an analysis of the spatial arrangement of the reflections in its x-ray diffraction pattern. On modern instruments, the measurement of reflection positions and the subsequent calculation of unit cell parameters is an automatic process which is carried out in a few minutes as part of the specimen screening process. There are now several commercial databases (e.g., NIST) that contain unit cell, space group, chemical composition and bibliographic data on thousands of compounds for which X-ray structure determinations have been carried out. Experimental unit cell data obtained in the crystal screening process and chemical composition information for the sample being studied may be used to search the databases. If an exact match is found, the compound has been identified and further single-crystal analysis may be unnecessary.

The analysis of the positional information for reflections, often called geometric data collection, may be used to provide direct information concerning the size and shape of the cell. In combination with

density and elemental analysis measurements, it may also yield information concerning the cell contents; however, it does not reveal the actual locations of the atoms within the unit cell.

The determination of the arrangement of atoms in the unit cell requires a very detailed analysis of the relative intensities of all unique reflections in the diffraction pattern. The precise measurement of the relative intensities of the reflection is termed intensity data collection. The collection of accurate intensity data requires a highly stable X-ray source, a precise mechanical goniometer for sample positioning and a very efficient counting system. An intensity data set consists of several thousand reflections indexed by h , k and l for which an integrated intensity $I(hkl)$ has been accurately measured.

The X-ray diffraction pattern consists of the superpositions of scattered waves of varying amplitude and phase. Each diffraction maximum or reflection has associated with it a structure factor $F(hkl)$ measured relative to the scattering by a single electron. The structure factor may be represented as a complex vector:

$$F(hkl) = A(hkl) + iB(hkl)$$

where $A(hkl)$ and $B(hkl)$ are the real and imaginary components of $F(hkl)$ (Figure 5). The magnitude or length of the vector $|F(hkl)|$ may then be represented as

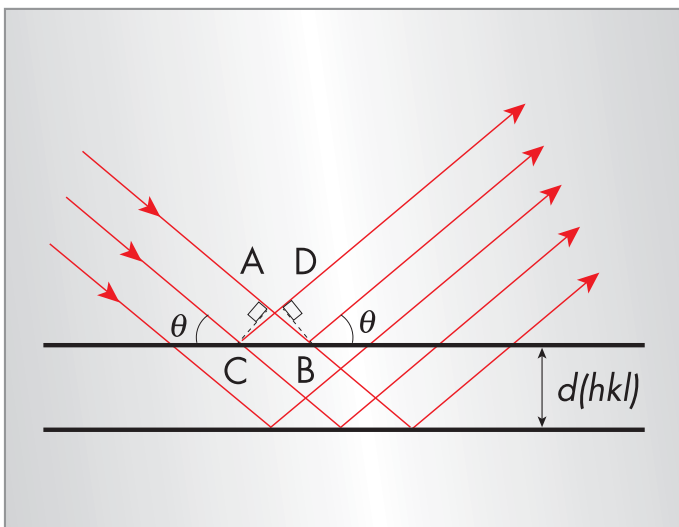


Figure 4. Diagrammatic representation of Bragg's Law showing the diffraction angle θ and the interplanar spacing $d(hkl)$. Two rays reflected from the same plane do not suffer any relative phase change or path difference ($AB = CD$)

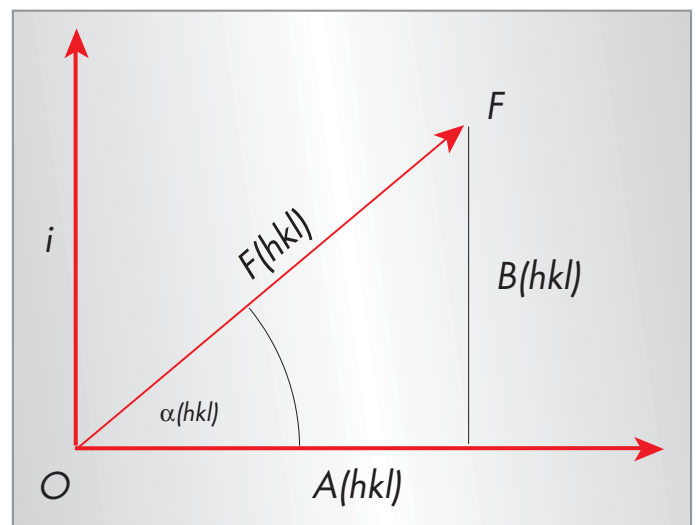


Figure 5. Structure factor $F(hkl)$ plotted on an Argand diagram. $a(hkl)$ is the phase angle and the amplitude is represented by OF

$$|F(hkl)| = \{[A(hkl) + iB(hkl)] \times [A(hkl) - iB(hkl)]\}^{1/2} \\ = [A(hkl)^2 + B(hkl)^2]^{1/2}$$

Alternatively, $F(hkl)$ may be expressed as an exponential quantity:

$$F(hkl) = |F(hkl)| \exp[i\alpha(hkl)]$$

where $|F(hkl)|$ is the amplitude of the scattered wave and $\alpha(hkl)$ is its phase angle. From Figure 5 it may be seen that

$$A(hkl) = |F(hkl)| \cos \alpha(hkl)$$

and

$$B(hkl) = |F(hkl)| \sin \alpha(hkl)$$

and that

$$\tan \alpha(hkl) = |B(hkl)| / |A(hkl)|$$

$|F(hkl)|$ may be calculated directly from the measured intensity $I(hkl)$ for a reflection, since

$$I(hkl) = K |F(hkl)|^2$$

where K is a constant. However, the phase angle $\alpha(hkl)$ cannot be measured experimentally and must therefore be obtained indirectly through a variety of numerical techniques.

The central problem in the solution of a crystal structure is the assignment of phase angles to each reflection in the data set. The solution of the phase problem is considerably simplified for crystals that possess crystallographic centers of symmetry, since, to a first approximation, the imaginary components $B(hkl)$ are zero for centrosymmetric space groups and the phase angles are therefore restricted to values of 0_j or 180_j . A structure is considered solved when a set of phase angles has been found that allows the atoms to be located and the experimental diffraction pattern to be matched to the calculated diffraction pattern.

Since the electron density in a crystal varies continuously and periodically in three-dimensional space, the electron density $\rho(xyz)$ at a point with fractional coordinates x, y, z in a unit cell of volume V may be expressed as a three-dimensional Fourier series:

$$\rho(xyz) = V \sum_h \sum_k \sum_l |F(hkl)| \cos[2\pi(hx+ky+lz)-\alpha(hkl)]$$

If both the amplitude $|F(hkl)|$ and the phase $\alpha(hkl)$ of each reflection are known, the electron density within the unit cell of the crystal can be calculated directly. On the other hand, if the positions of the atoms in the unit cell are known, both the structure factor and the phase for each reflection may be calculated from the structure factor equation:

$$F(hkl) = \sum_j f_j \exp[2\pi i(hx_j + ky_j + lz_j)]$$

where f_j is the atomic scattering factor for the atom j and x_j, y_j, z_j are its fractional coordinates. In an actual structure determination both forms of the Fourier transform equations are utilized to arrive at a model structure from which the observed diffraction pattern can be reproduced.

Instrumentation

The basic hardware components of typical automated single-crystal X-ray diffractometer system include:

- an X-ray source consisting of a high-stability X-ray generator, a copper or molybdenum target X-ray tube, a tube shield with associated shutters, attenuators and safety interlocks, a monochromator or X-ray mirror system, and an incident-beam collimator;
- a three- or four-circle goniometer system that allows the specimen to be precisely oriented in any position while remaining in the X-ray beam;
- a video camera or microscope for aligning the specimen and indexing crystal faces;
- a CCD-based two-dimensional X-ray detector system;
- a low-temperature attachment for cooling the specimen during data collection;
- a microprocessor-based interface module that receives commands from a host computer and carries out all real-time instrument control functions to drive goniometer motors, monitor the detector system, open and close the shutter and monitor collision sensors and safety interlocks;
- a host computer with a large hard disk mass storage device, a video monitor and keyboard, and diffractometer control programs to control the data collection strategy and to send commands to the microprocessor.

Structure determination calculations may be carried out on the computer used for data collection or they may be performed on a second computer linked to the diffractometer system. A large variety of hardware configurations are available, depending upon the requirements of the individual laboratory.

The most critical mechanical component in an X-ray diffractometer system is the goniometer assembly, which must be capable of keeping the specimen centered in the incident X-ray beam while at the same time changing its orientation in order to collect many thousands of frames of data in reciprocal space. The most commonly used type of goniometer is illustrated in Figures 6 and 7.

The most important and most expensive component of any modern single-crystal diffractometer system is its detector system. Most new instruments purchased since 1994 use a detector system based upon CCD (charge-coupled device) technology (Figure 8). These detectors include a square CCD-microprocessor chip with physical dimensions ranging from 24mm (1K chip) to 61 mm (4K chip) on each side. Each of these CCD chips contains from 1024x1024 (1K chip) to 4096x4096 (4K chip) independent pixels. The CCD chip is usually bonded to a fiber-optics taper to increase the effective size to 61mm on a side (Mo target chemical crystallography systems) or 90mm on a side (Cu target protein crystallography systems). The front end of the fiber-optics taper is attached to a phosphor, optimized for either Mo or Cu radiation, to convert X-ray photons to visible wavelength photons

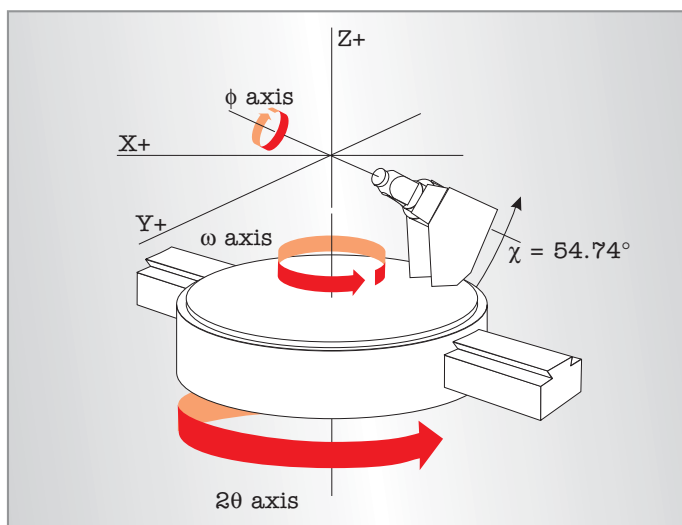


Figure 6. Diagram of axes for a three-circle goniometer (χ is fixed at 54.75°)

which can be transmitted to the CCD chip by the fiber-optics bundle. In order to reduce background noise and improve counting statistics, the CCD chip must be cooled to about -45°C using Peltier cooling methods. Typical CCD detector systems have counting efficiencies ranging from 10 to 90 electrons per X-ray photon.

Experimental Procedure

The first step in a crystal structure analysis is concerned with the selection and mounting of a suitable specimen. Ideally, a crystal whose structure is to be determined must be a single crystal of 0.1 mm to 0.5 mm size, not cracked and not twinned. The techniques required to obtain such crystals vary considerably depending upon the types of compounds to be analyzed. Stable crystals of typical organic, organometallic or coordination complexes can usually be grown by slow recrystallization from common solvents. Other types of compounds may require the use of sublimation, zone refinement, solvent diffusion, low-temperature and/or inert-atmosphere techniques in order to isolate suitable specimens.

- Once a suitable specimen has been selected, it is glued or otherwise securely attached to a goniometer head (sample holder) in an arbitrary orientation.
- The goniometer head is then placed on the base of the goniometer assembly and the crystal is optically aligned in the center of the incident X-ray

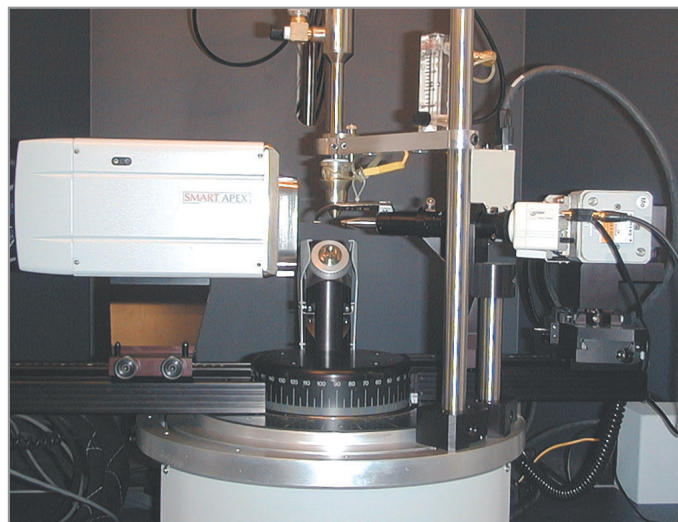


Figure 7. A commercial CCD-based single-crystal X-ray diffractometer system (courtesy of Bruker AXS Inc.)

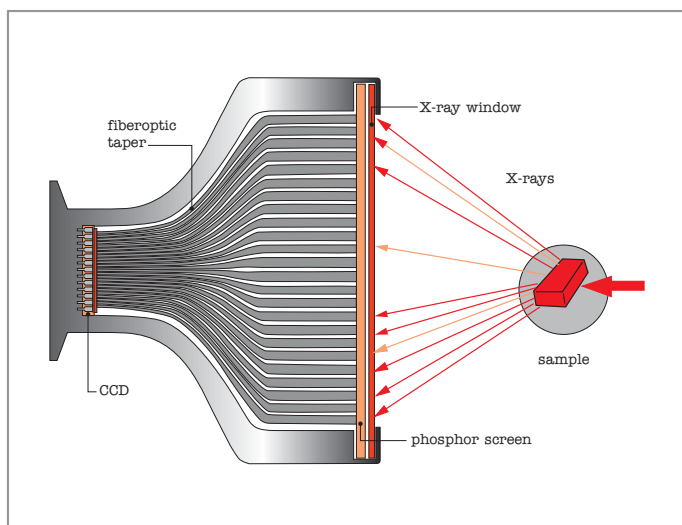


Figure 8. Diagram of a typical CCD detector used for X-ray diffraction.

beam using a video camera or microscope. The orthogonal X, Y, and Z translations on the goniometer head are adjusted until the specimen is centered on the cross hairs for all crystal orientations.

- A preliminary rotational image is then collected for one minute with the CCD detector to screen the specimen for analysis and to select suitable parameter values for subsequent steps.
- In order to determine the unit cell, a preliminary set of frames is measured using an automatic routine. For example, three sets of frames are collected in different parts of reciprocal space.
- These frames are then processed to locate spots on individual frames and to then determine the centers of reflections.
- An auto-indexing routine selects the appropriate reduced primitive unit cell and calculates the corresponding orientation matrix and lattice constants.
- This preliminary unit cell is then refined using a non-linear least-squares algorithm and converted automatically to the appropriate crystal system and Bravais lattice. This new cell is refined by the non-linear least-squares algorithm to yield an accurate orientation matrix which may be used to index crystal faces and to carry out integration calculations after intensity data collection.

After the above geometric data collection steps have been completed and an accurate orientation matrix has been calculated, intensity data collection is

carried out. Typically, a sphere or hemisphere of data is collected using a narrow-frame scan method in which several sets of frames (runs) are collected by scanning in 0.1° to 0.3° increments in the w and/or ϕ angle, while keeping all other instrument angles constant. There are options to limit data to a unique set of reflections, thereby reducing data collection times for high-symmetry crystal systems. Each two-dimensional frame is a two-dimensional array of independent pixels, each of which has an almost infinite dynamic range for sensitivity. Even though, the microprocessor chip in the CCD camera contains from 1024×1024 (1K) to 4096×4096 (4K) independent pixels, the software usually reads the data out in 'binned' mode as 512×512 or 1024×1024 frames. A typical data collection for such an instrument would place the detector at a distance of 5 cm from the crystal and a detector swing angle (2θ angle) of 28° , giving complete data to $2\theta \leq 55^\circ$ for molybdenum radiation. A complete data set may require anywhere from a few hours to overnight depending upon the size of the specimen and its diffracting power. Typical exposure times of 10 to 30 seconds per frame for a hemisphere of data require from 6 to 13 hours of total data collection time.

When the complete set of frames has been collected for a given specimen, the entire data set must be processed to obtain accurate integrated intensities for individual reflections. This process includes corrections for instrumental factors, polarization effects, X-ray absorption and possibly crystal decomposition. The integration process reduced the raw frame data, which require from 500 to 2000 megabytes of disk storage to a small set of individual integrated intensities for individual reflections. The final unit-cell constants are calculated from the centroids of many thousands of reflections selected from the entire data set and typically have relative errors of less than $1/10,000$.

Once the structure amplitudes are known, the phase problem must be solved to find a self-consistent set of phases that can be combined with the structure factor amplitudes to obtain the electron density and thereby determine the structure of the crystal. A number of crystallographic techniques exist for obtaining the phases of diffracted waves; the most widely utilized approaches to the solution of phase problem involve the use of either vector methods based on $|F(hkl)|^2$ or direct or statistical methods. Typically, the solution to the structure yields only a partial or approximate model, which must be improved by successive applications of Fourier-transform methods before the complete structure has been determined.

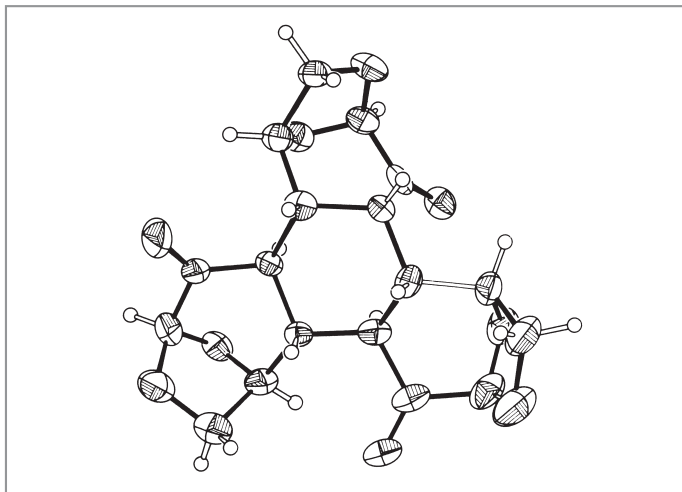


Figure 9. Thermal ellipsoid plot of the final structure of an organic compound ($C_{18}H_{18}O_9$).

After the entire molecular structure has been determined, the approximate positions of the atoms are refined by nonlinear least-squares techniques to give the best fit between the calculated and observed intensity data for the specimen. The refinement process yields very accurate values for atomic positions from which bond lengths, bond angles and other structural parameters may be calculated. Finally, upon completion of the X-ray diffraction analysis, the structure of the molecule or crystal lattice must be displayed or plotted (Figures 9 and 10).

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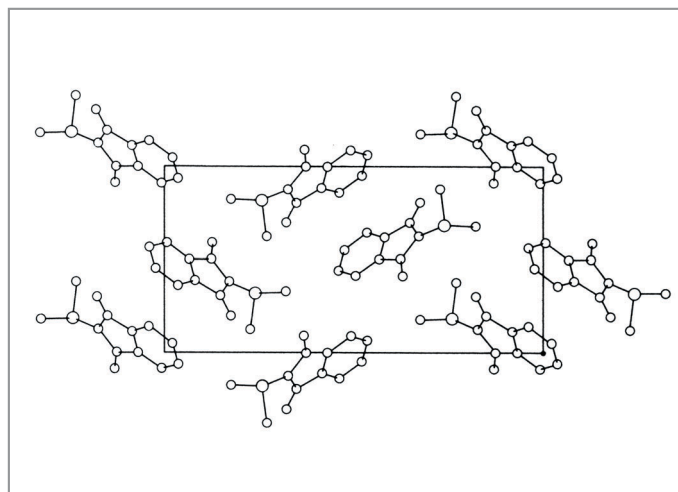


Figure 10. Unit-cell diagram showing the arrangement of molecules within the cell.

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