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## Classification of experimental techniques

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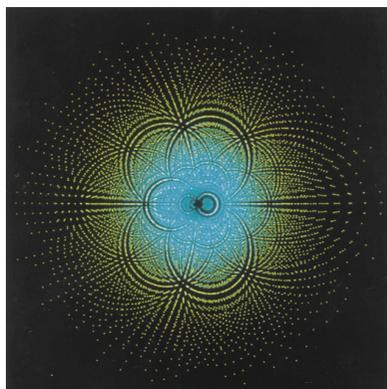
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In crystallography research directed at crystal structure analysis, the probes and methods span the use of X-rays, neutrons and electrons, and sample states that are in various degrees of order (*i.e.* single crystal, powder, fibre, surface, amorphous or liquid). Thus a summary of diffraction-based methods used in the study of the structure of matter is given in this chapter. Idealized states of matter and their idealized diffraction patterns do not necessarily apply, and this must also be considered.

The diffraction of a wave of characteristic length,  $\lambda$ , by a crystal sample requires that  $\lambda$  is of the same order in size as the interatomic separation. Beams of X-rays, neutrons and electrons can easily satisfy this requirement; for the latter two, the wavelength is determined by the de Broglie relationship  $\lambda = h/p$ , where  $h$  is Planck's constant and  $p$  is the momentum.

We can define 'diffraction geometry' as the description of the relationship between the beam and the sample orientation, and the subsequent interception of the diffracted rays by a detector of given geometry and imaging properties. Each diffracted ray represents successful, constructive interference. The full stimulation of a reflection is achieved either by using a continuum of values of  $\lambda$  incident on the crystal, as used originally by Friedrich, Knipping & von Laue (1912) (the Laue method) or alternatively by using a monochromatic beam and rotation or precession of a crystal (moving-crystal methods) or a set of randomly oriented crystallites (the powder method).

The analysis of single-crystal reflection intensities allows the three-dimensional architecture of molecules to be determined. However, a single crystal cannot always be obtained. Diffraction from noncrystalline samples, *i.e.* fibres, amorphous materials or solutions, yields less detailed, but often very valuable, molecular information. Another method, surface diffraction, involves the determination of the organization of atoms deposited on the surface of a crystal substrate; a surface of perfectly repeating identical units, in identical environments, on such a substrate is sometimes referred to as a two-dimensional crystal. Ordered two-dimensional arrangements of proteins in membranes are studied by X-ray and electron diffraction. Another experimental probe of the structure of matter is EXAFS (extended X-ray absorption fine structure). This technique yields details of the *local* environment of a specific atom whose X-ray absorption edge is stimulated; the atom absorbs an X-ray photon and yields up a photoelectron, which can be scattered by neighbouring atoms. Interpretation of EXAFS therefore closely follows low-energy electron-diffraction (LEED) theory. All these methods (Table 1) can be called methods of structure analysis. Techniques for examining the perfection of crystals are also very important. Defects in crystals represent irregularities in the growth of a perfect crystalline array. There are many types of defect. The



**Table 1**

Summary of main experimental techniques for structure analysis.

In addition to the techniques listed below, EXAFS can be performed on all types of sample whether crystalline or noncrystalline. It uses an X-ray beam that is tuned around an absorption edge and the transmitted intensity or the fluorescence emission is measured. The method of cryo-electron microscopy of biological macromolecules *via* single-particle imaging is increasingly providing molecular details at atomic resolution. There can be expected to be a close synergy with single-particle diffraction imaging using X-ray lasers.

Name of technique	Beam		Sample	Usual detectors or special features‡
	Usual type	Spectrum†		
<i>(a) Single crystal</i>				
Laue	X-ray or neutron	Polychromatic	Stationary single crystal	Film; image plate or storage phosphor; electronic area detector (e.g. CCD or pixel array detector); for spallation neutron source case, detector sensitive to time-of-flight and for neutron reactor Laue a cylindrical detector geometry
Still	X-ray or neutron or electron	Monochromatic or 'pink beam'§ (i.e. a narrow band of wavelengths)	Stationary single crystal	Film; image plate or storage phosphor; electronic area detector (e.g. MWPC, TV, CCD, pixel array detector)
Rotation/oscillation	X-ray or neutron or electron	Monochromatic	Single crystal rotating about a single axis	Film; image plate or storage phosphor; electronic area detector (e.g. MWPC, TV, CCD, pixel array detector)
Weissenberg	X-ray	Monochromatic	Single crystal rotating about a single axis coupled with detector translation	Film; image plate or storage phosphor
Precession	X-ray or electron	Monochromatic	Single crystal (the normal to a reciprocal-lattice plane precesses about X-ray or electron beam)	Flat film or device moving behind a screen coupled with crystal so as to be held parallel to a reciprocal-lattice plane
Diffraction	X-ray or neutron	Monochromatic	Single crystal rotated over a small angular range	Single counter, linear detector or area detector
Pair distribution function	X-ray or neutron	Monochromatic or time-of-flight	In between and beyond the Bragg diffraction reflections	High probe energy (short wavelength)
<i>(b) Polycrystalline powders¶</i>				
Monochromatic powder method	X-ray or neutron or electron	Monochromatic	Powder sample rotated to increase range of orientations presented to beam	Film or image plate; counter; 1D position-sensitive detector (linear or curved); pixel array detector
Energy-dispersive powder method	X-ray or neutron	Polychromatic	Powder sample	Energy-dispersive counter (for neutron case, detector sensitive to time-of-flight)
Pair distribution function	X-ray or neutron	Monochromatic or time-of-flight	In between and beyond the Bragg diffraction reflections	High probe energy (short wavelength)
<i>(c) Fibres, solutions, surfaces and membranes</i>				
Fibre method	X-ray or neutron	Monochromatic	Single fibre or a bundle of fibres; preferred orientation in a sample	Film or image plate; electronic area detector (e.g. MWPC, TV, CCD or pixel array device); records high-angle or low-angle diffraction data
Solution or 'small-angle method'	X-ray or neutron	Monochromatic	Dilute solutions of particles	Counter, MWPC, CCD or pixel array device
Surface diffraction	Electron or X-ray	Monochromatic	Atoms deposited or adsorbed onto a substrate	Phosphor or counter; pixel array detector
Membranes	Electron or X-ray	Monochromatic	Naturally occurring 2D ordered membrane protein	Film or image plate; CCD; pixel array detector
<i>(d) Single objects</i>				
Coherent diffraction imaging (CDI)	X-ray	Coherent monochromatic††	Single biological cells; single nanoparticles	Pixel array device

† Monochromatic: Typical value of spectral spread,  $\delta\lambda/\lambda$ , on a conventional X-ray source;  $K\alpha_1 - K\alpha_2$  line separation  $\sim 2.5 \times 10^{-3}$ ,  $K\alpha_1$  line width  $\sim 10^{-4}$ . On a synchrotron source a variable quantity dependent on type of monochromator; typical values  $\sim 10^{-3}$  or  $\sim 10^{-4}$  for the two common monochromator types [see Figs. 14 and 15, respectively, in Helliwell (2021)]. ‡ CCD = charge-coupled device; MWPC = multiwire proportional chamber detector; TV = television detector. 'Image plate' is a trade name of Fuji. 'Storage phosphor' is a trade name of Kodak. § A 'pink beam' is one derived from a multilayer optical element which provides a narrow band of wavelengths (e.g.  $\delta\lambda \approx 0.02$  Å), but which is broader than from a 'crystal monochromator' (e.g.  $\delta\lambda \approx 0.0001$  Å). ¶ A new theory of diffraction with particular relevance to powder diffraction has recently been offered and also includes an overview of the associated diffraction geometry and measured X-ray intensities (Fewster, 2014). †† Coherent X-rays are emitted in large fluxes by third-generation synchrotron sources. These fluxes have increased yet further, indeed substantially, with X-ray free-electron lasers (XFELs).

experimental technique of X-ray topography [see Chapter 2.7 of the previous edition of *International Tables for Crystallography* Vol. C (Lang, 2006)] is used to image irregularities in a crystal lattice.

Idealized states of matter and their idealized diffraction patterns do not necessarily apply to the real world of complex samples and their internal structure and dynamics. An increasingly applied technique involves analysis of the 'whole

diffraction pattern'. The extreme cases then of diffraction from a perfect single crystal on the one hand and a gas or liquid on the other hand respectively yield the atomic coordinates and their associated atomic displacement parameters in a perfect unit cell and a pair distribution function for the gas or liquid. The diffraction-geometry details of measuring from a 'real crystal or powder sample' involve the Bragg intensities and everything in between these and also beyond their measurable scattering. These 'non-Bragg diffraction data' can be weak compared with Bragg intensities, placing extra demands on the detector dynamic range, and also extend to higher diffraction angles, usually quantified in terms of their  $Q$  [ $= 4\pi(\sin \theta)/\lambda$ ] range, placing a requirement for use of higher energies (shorter wavelength) of the probe (usually X-ray or neutron) and in turn the need for the detector to have a high quantum efficiency at those high probe energies. For an up-to-date and extended description see Billinge (2019).

X-ray techniques expanded in the 1970s and 1980s with the utilization of synchrotron radiation. The methods based on the use of neutrons and electrons have also developed. Broadly speaking, the diffraction geometry is independent of the nature of the wave and depends only on its state, namely, the wavelength,  $\lambda$ , the spectral bandpass,  $\delta\lambda/\lambda$ , the convergence/divergence angles and the beam direction. The term monochromatic refers to the case where there is, practically speaking, a very small but finite wavelength spread. Similarly, the term polychromatic refers to the situation where the wavelength spread is of the same order as the mean wavelength. The technical means by which a given beam (of X-rays, neutrons or electrons) is conditioned vary, as do the means of detection. These methods are dealt with in Helliwell (2021) and in Part 2 of the previous edition of this volume (2006) as far as they relate to the geometry of diffraction.

In *International Tables for X-ray Crystallography* (1959, Part 4) and other editions of the present volume, various diffraction geometries were detailed and a variety of numerical tables were given. The numerical tables have mainly been dispensed with since the use of hand-held calculators and computers has rendered them obsolete.

In the case of the precession method with X-rays the method is now mainly of historical interest, but such 'photos' are useful and in the X-ray case can readily be achieved computationally by reconstruction from intensity data measured by e.g. the screenless rotation method. These are useful for looking for any systematically missing regions of the measured diffraction patterns. In the electron case the precession method is enjoying a resurgence for the purpose of considerably more accurate intensity recording (Vincent & Midgley, 1994).

Most recently 'single objects' such as biological cells and synthetic nanoparticles have become amenable to investigation using coherent X-ray diffraction (CDI) at third-generation sources, and this approach is expected to expand significantly at the new X-ray free-electron lasers (XFELs) (Miao *et al.*, 1999; Peele & Nugent, 2008). In addition,

microcrystals and sub-micron crystal diffraction ('nanocrystals') have become amenable to regular diffraction data set acquisition at X-ray laser sources, as well as using electron beams, and these XFEL source technologies are still developing rapidly. The recording of the 'still' diffraction patterns (Table 1) from nanocrystals, a description of the diffraction geometry used and a discussion of progress in refinement of reflection partiality from a single diffraction pattern is described in Helliwell (2021). The introduction of serial crystallography, with measurements from a stream of individual sub-micron crystals at XFELs, has now also been adopted at various synchrotron X-ray sources [for a review see Patterson (2014)].

As computer digital storage capacity has generally expanded there has been a growth of interest in archiving of raw diffraction data sets, in addition to the processed diffraction data and the derived molecular 'atomic coordinates' data. The scientific basis for such archiving is several fold and includes allowing the reader of a research publication to revisit decisions made by the authors about the diffraction data processing, such as choice of resolution limit and choice of unit cell and space group. In addition, as methods of analysis of the diffuse scattering between the Bragg diffraction intensities are developing, including computer calculations allowing 'molecular dynamics simulations' for extended periods (e.g. up to milliseconds, even for proteins), an archive of raw diffraction data images assumes a considerable significance. Suffice it to say that the expansion of computer digital storage capacity is also of considerable interest to other research communities with a long tradition of archiving of scientific data, such as the astronomers and particle physicists.

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