the nature of the crystal system. A preliminary examination under crossed polars will often show whether the crystal is isotropic, uniaxial or biaxial (see, for example, Hartshorne & Stuart, 1960; Bunn, 1961; Ladd & Palmer, 1985). Crystals that comprise two or more fragments will often be revealed by displaying both dark and light regions simultaneously. For uniaxial crystals, a birefringent orientation is always presented to the incident light beam if the unique axis is perpendicular to the microscope axis, and extinction will occur whenever the unique axis is parallel to the crosswires (assuming that the crosswires are parallel to the planes of polarization of the polarizer and analyser). If the unique axis is parallel to the microscope axis, a uniaxial crystal presents an isotropic cross section and will remain extinguished for all rotations of the crystal. Biaxial crystals have three principal refractive indices associated with light vibrating parallel to the three mutually perpendicular directions in the crystal. The two optic axes and their correspondingly isotropic cross sections that derive from this property are not directly related to the crystallographic axes. In the orthorhombic system, the three vibration directions are parallel to the crystallographic axes, often enabling identification of this crystal system. A monoclinic crystal lying with its unique axis parallel to the crosswires will always show straight extinction. If the crystal is oriented so that the unique axis lies along the microscope axis then, in general, the extinction directions will be oblique. In the triclinic case, the three mutually perpendicular vibration directions are arbitrarily related to the crystal axes. Even if it is not possible to discover the nature of the crystal system unequivocally, the extinction directions should at least enable the principal symmetry directions to be identified and therefore suggest how the crystals should be mounted for optimum data collection (see Chapter 3.4).

3.1.2.4. *Twinning (see Chapter 1.3)*

If at all possible, twinned crystals should *not* be used for structure analysis studies, but the recognition of twinning is critical, since unnoticed or misinterpreted twinning can prevent structure determination or lead to errors in the final structure solution. A distinction should be made between multiple crystal growth, whereby single crystals grow on or from the faces of a given single crystal, or from a common nucleation point, in non-specific orientations, and crystallographic twinning (see, for example, Phillips, 1971; Bishop, 1972). In the latter case, the relationship between the lattices of twinned crystals is normally that of rotation of 180° about a central lattice line, or reflection across a lattice plane. If the lattice is not geometrically symmetrical about the line or plane, two lattices with differing orientations will be produced, and the corresponding reciprocal lattices will be visible in the diffraction patterns. In ideal circumstances, the two patterns can be deconvoluted. If the lattice is geometrically symmetrical about the twin axis or plane, then the two reciprocal lattices will coincide and there may be no obvious signs of twinning in the diffraction pattern (merohedry). If the twins are present in almost equal amounts, the result will be an apparent mirror plane and perpendicular twofold axis in the Laue symmetry. It is therefore very important to examine carefully the Laue symmetry, preferably from a number of different crystals, if twinning is suspected. In some of these crystals, one twin component may be predominant, causing a breakdown in the pseudosymmetry.

Morphological evidence (a concave shape indicating an intersection between the two twin components) and optical examination under a polarizing microscope should also be employed to test for twinning. For lattices that are twinned in a geometrically nonsymmetrical manner, the different twin components will show extinction at different orientations. However, perfect optical extinction is not positive evidence of lack of twinning, since the geometrical symmetry plane (or axis) on which twinning takes place may be parallel to a symmetry plane (or axis) in the optical properties of the crystal.

Intensity statistics can also be used to detect twinning, particularly in the case of crystals twinned by merohedry (*e.g.* Britton, 1972; Fisher & Sweet, 1980). If crystallization conditions cannot be found that eliminate twinning, it is still possible, although difficult, to undertake structure analysis. Recent examples include Sr_3CuPtO_6 (Hodeau *et al.*, 1992), $RbLiCrO_4$ (Makarova, Verin & Aleksandrov, 1993), a serine protease from rat mast cells (Reynolds *et al.*, 1985) and plastocyanin from the green alga *Chlamydomonas reinhardtii* (Redinbo & Yeates, 1993).