cell axes in the crystal (Hartshorne & Stuart, 1960). Examination of a crystal under crossed Nicol prisms can also provide information on crystal quality. For example, sometimes the components of a twinned crystal extinguish plane-polarized light independently. Other methods of examining crystals include Raman spectroscopy (Kudryavtsev *et al.*, 1998).

#### *5.1.1.3.2. Refractive indices and what they tell us about structure*

The refractive index of a crystal can be measured by immersing it in a mixture of liquids of a known refractive index in which the crystal is insoluble. The liquid composition is then varied until the crystal appears invisible. At this point, the refractive indices of the crystal and the liquid are the same. If the refractive index is the same in all directions, the crystal is optically isotropic, but most protein crystals are optically anisotropic and have more than one refractive index. For example, tetragonal crystals have different refractive indices for light vibrating parallel to the fourfold axis and for light vibrating perpendicular to it. These refractive indices are measured by the use of plane-polarized light.

### *5.1.1.4. Packing of molecules in crystals*

Growth kinetics of the different faces should be correlated with the structural anisotropy of the intermolecular contacts. It has been found that a judicious mutation of a single surface residue of a protein can markedly affect its solubility and hence crystallizability. This method has been used with great success for crystallizing a retroviral integrase (Dyda *et al.*, 1994).

The relationship between crystal morphology and internal crystal structure was examined in the mid-1950s (Hartman & Perdok, 1955*a*,*b*,*c*). It was shown that the morphology of a crystal is determined by 'chains' of strong intermolecular interactions (hydrogen bonding, van der Waals contacts, molecular stacking) running through the entire crystal. For a crystal to grow in the direction of a strong interaction ('bond'), these bonds must form an uninterrupted chain through the structure, giving rise to the periodic bond chain theory. The stronger the interaction between molecules, the more likely the crystal is to be elongated in that direction. If a bond chain contains interactions of different kinds, its influence on the shape of the crystal is determined by the weakest interaction present in a particular chain. Prominent faces are parallel to at least two high-energy bond chains. This enables a correlation to be made between the crystal lattice and the crystal morphology, based on the fact that direct protein–protein contacts, reinforced by well ordered solvent molecules, are important in determining crystal packing (Frey *et al.*, 1988). Studies of the morphology of tetragonal lysozyme (Nadarajah & Pusey, 1996; Nadarajah *et al.*, 1997) showed that the crystallizing unit is a helical tetramer (centred on the $4_3$ crystallographic axes).

## 5.1.2. Crystal mounting

### *5.1.2.1. Introduction to crystal mounting*

Once crystals have been obtained and visually characterized, the next procedure involves the transfer of a selected crystal to an appropriate mounting device so that the crystal may be characterized using X-rays. Macromolecular crystals are generally obtained from and stored in a solution containing the precipitant or precipitants and other substances such as uncrystallized protein or other macromolecules. The object is to mount the crystal in such a way that it is undamaged by cracking, drying out, dissolving *etc.* during this operation. In some cases, the crystal may have been stored in a solution containing volatile solvents. Alternatively, the crystals may have been grown at a temperature lower than room temperature and therefore may require special handling in order to avoid crystal deterioration. In other cases, it may be desirable to prepare the crystal for study at cryogenic temperatures. This section deals with the mounting of crystals for all these conditions and concentrates on the mounting of crystals for diffraction experiments at or just below room temperature. Procedures such as 'flash cooling' are used to reduce radiation damage. Crystal-mounting techniques for cryogenic experiments are covered in detail in Part 10 and are only mentioned briefly here. In general, the most difficult part of mounting macromolecular crystals is the transfer of the crystal from a holding solution to a suitable mount. A capillary or, if cryogenic experiments are to be carried out, a cryoloop should be used.

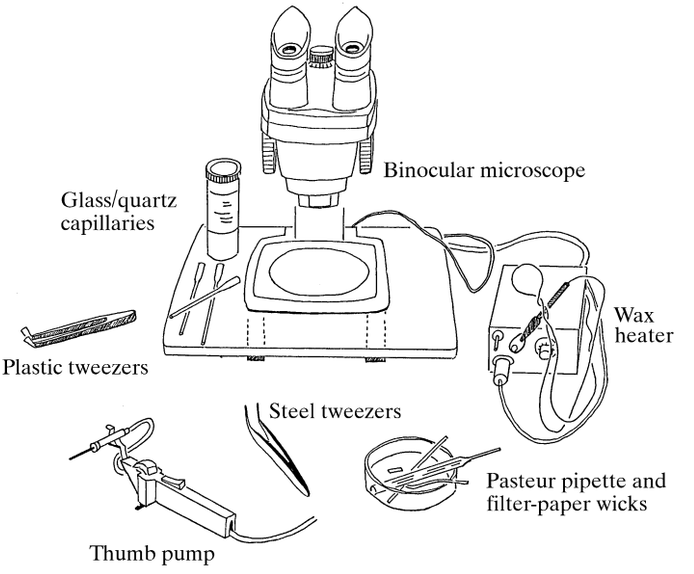


**Figure 5.1.2.1**
Tools commonly used for mounting crystals.

### *5.1.2.2. Tools for crystal mounting*

In order to facilitate the process of mounting macromolecular crystals for X-ray diffraction experiments, it is necessary to have the appropriate tools for the task. Fig. 5.1.2.1 shows a collection of some useful tools for the mounting of crystals. These include a binocular microscope, tweezers (two types), thin glass capillaries, Pasteur pipettes, a heater, paper wicks, and a thumb pump. Other useful tools and supplies include surgical scissors, dental wax, latex tubing, light vacuum oil, a cryogenic mounting loop, Plasticine, mounting platforms, mounting pins, absorbent dental points and micropipettes with plastic tips. There are many other items that might be useful, and several variations are found in different laboratories. An important factor in the transfer of crystals from a holding solution to a capillary is that the experimenter needs to feel at ease with the process. The method that will be detailed here has evolved over time and has proved to be a relatively anxiety-free process. Other methods for crystal mounting may be found in the literature (Rayment, 1985; Sawyer & Turner, 1992; McRee, 1993). All of the methods outlined here and in the literature have the same goal, namely, the successful transfer of a macromolecular single crystal to a suitable mount for X-ray data collection.

*5.1.2.2.1. Microscope*

Perhaps the single most important piece of equipment for examining and mounting crystals is a binocular dissection microscope. This should have variable zoom capabilities, and there should be sufficient distance (*e.g.* 5–10 cm) between the objective lens of the microscope and the microscope stage to accommodate the necessary equipment and allow manipulation of the crystals and solutions. A magnification of between 10 and 40 times is probably best in practice. It is also important to ensure that the light source of the microscope is not so intense that it heats the microscope stage, thereby damaging the macromolecular crystals. If the microscope is fitted with crossed polarizers, the quality of the crystals can be assessed.

*5.1.2.2.2. Capillaries*

The capillaries used for crystal mounting are made of thin-walled glass. These capillaries range from 0.1 to 2.0 mm in diameter and have a stated wall thickness of 0.01 mm. In practice, however, the larger the diameter of the capillary, the thinner the glass wall. Therefore, handling of the larger-diameter capillaries is generally very difficult because they are so fragile. Capillaries made of fused quartz are also available, but are not recommended for general use because they produce a higher background with X-rays. Quartz capillaries are not as fragile as the thin-walled glass capillaries, however, and may be useful in experiments where the tensile strength of the capillary is important, for example, when a diffraction flow-cell experiment is planned (Petsko, 1985). In addition, small-diameter capillaries (produced in the laboratory by drawing out glass tubing or Pasteur pipettes) will be needed to aid in the removal of excess liquid around the crystal after the transfer from the crystallization dish to a capillary has been completed.

*5.1.2.2.3. Thumb pump*

The thumb pump is a simple micropipetting device for transferring very small amounts of liquid in a highly controlled manner, making it an extremely useful tool for directly transferring protein crystals from solution to capillary, thus minimizing the chance of crystal damage. This simple device allows the experimenter to have much more control over volume transfers than any other device we have tried. The mechanism is simple and easy to operate. The device shown in the illustration can be held and manipulated with one hand. The capillary is held firmly in place and the pipetting action is controlled by a thumb wheel (part of the thumb pump), which affords a great deal of control over the volume of liquid being transferred with the crystal.

*5.1.2.2.4. Heater*

The heater illustrated here consists of a variable rheostat and a heating element. The latter is a short piece of Nichrome wire which has been coiled and attached to the rheostat *via* wires that run through a ball-point pen barrel. This permits a fine temperature control for melting dental wax and for controlling where the heat is applied.

*5.1.2.3. Capillary mounting*

One must first select a capillary for mounting the crystal. A general rule to follow is to select a capillary that has a diameter that is approximately twice the size of the crystal dimension to be placed along the breadth of the capillary. Thus, to mount an elongated parallelepiped with the longest crystal dimension parallel to the capillary, it is necessary to take into account the cross section of the crystal perpendicular to the longest dimension. This rule is only a guide and is probably broken most of the time. Indeed, for 'chunky' crystals, it may be advantageous to use a capillary only slightly larger than the crystal so that the crystal may be in contact with the capillary wall in more than one place, thereby making the mount more stable. The object is to have enough of the crystal in contact with the capillary wall to allow the crystal to be held in place with a small amount of mother liquor. One possible problem that occurs with very thin crystals is that the crystal may bend to conform to the shape of the capillary wall. In this case, the crystal is rendered unsuitable for X-ray diffraction experiments.

The capillary is prepared by first removing the flame-sealed tip. This is done with surgical scissors or by pinching with surgical tweezers and then gently tapping the capillary tip against a smooth hard surface to remove the jagged edges which may have resulted from this cutting. The removal of the jagged edges at the broken end of the capillary will simplify the transfer of the crystal from the holding solution to the capillary. The large flared end of the capillary is left intact, and, at this time, a rubber transfer tube with a mouthpiece can be fitted over the flared end of the capillary. The capillary can then be rinsed with distilled deionized water, or it may even be desirable to treat the capillary with some other solution, such as EDTA, or perhaps with a solution identical to that surrounding the crystal. The rinse solution is gently drawn up into the capillary, and the solution is then blown out into a waste container. This can be accomplished fairly rapidly, and then the capillary can be dried by gently drawing air in through the capillary tip. Only the excess liquid should be removed at this point if the rinse solution contains salts.

The crystal must now be transferred to a capillary from the storage location, which may be a shallow well in a depression slide, a droplet on a cover slip, or perhaps a vial containing crystals. Direct transfer from a droplet on a cover slip or from a shallow well of a depression slide to the final capillary is possible, but can be complicated if several crystals are present in the drop. The easiest way to set up the final crystal transfer is to first remove it from the original drop or vial using a micropipette with a tip that has been enlarged so that it will accommodate the desired crystal. The crystal is then transferred together with a few microlitres of solution to a siliconized cover slip or depression well using the micropipette. It may even be easier to place 5–10 µl of solution on a siliconized cover slip or depression well and then use a cryoloop to capture the crystal and deposit it in the solution. The crystal can now be easily drawn up into the capillary with the aid of the thumb pump. It will be accompanied by a small column of mother liquor, and the thumb pump can be used to position the crystal with its liquid at the desired location in the capillary. The excess mother liquor can now be removed by using a capillary that is much smaller than the data-collection capillary, as well as smaller than the crystal. A final drying can be accomplished using appropriately sized filter-paper wicks or absorbent dental points. A very small amount of liquid should be left behind to keep the crystal moist and to 'glue' the crystal to the capillary wall *via* surface tension. A crystal that is too dry will probably deteriorate and be useless for diffraction experiments, while a crystal that has too much liquid can slip during data collection. On the other hand, moderate drying has been found, in certain cases, to give a crystal with improved diffraction.

After the crystal is safely in position in the capillary, the capillary must be sealed in order to maintain the moisture necessary to prevent crystal deterioration. If desired, a short column of mother liquor may be placed in the capillary a few

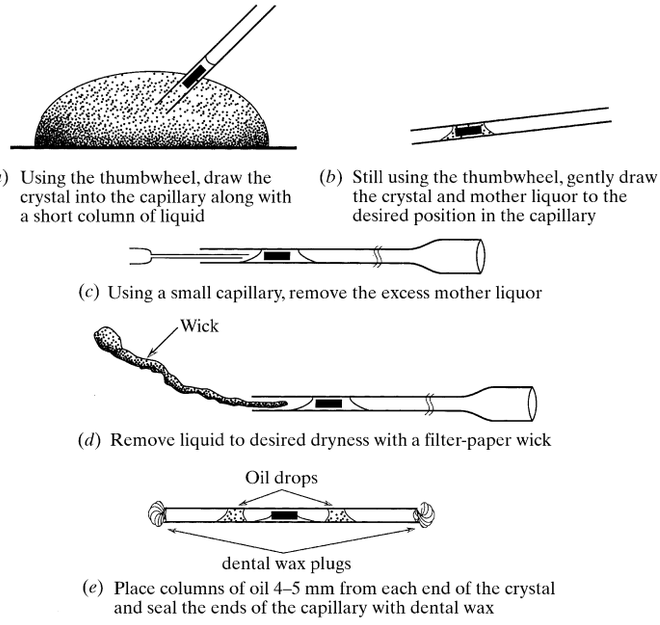


**Figure 5.1.2.2**
Mounting a crystal in a capillary.

millimetres away from the crystal. This is usually necessary if capillaries larger than 1 mm in diameter are used. A small strip of filter paper may also be placed in the capillary and then dampened with mother liquor. Both methods allow the moisture level in the crystal to be maintained. A reasonably good first seal may consist of a short column of light vacuum oil on both sides of the crystal, again, a few millimetres away from it. At this time, a ring of molten dental wax is placed along the capillary beyond the oil drop nearest the flared end of the capillary, and the capillary is then cut or broken just beyond the wax. The final seal may then be accomplished using molten dental wax or perhaps even epoxy at each end of the capillary. The diffraction equipment and arrangement will dictate the position of the crystal in the capillary, and this should be accommodated before the final seals are put in place. The geometry of the capillary could aid in preventing slippage of the wedged crystal during data collection (Åkervall & Strandberg, 1971). Alternatively, a specific crystal coating which effectively glues the crystal to the interior of the capillary can be used (Rayment *et al.*, 1977). The capillary with its crystal is now ready to be placed on the platform of choice for placement on the goniometer head in final preparation for diffraction experiments. Fig. 5.1.2.2 illustrates the steps in mounting a crystal in a capillary in preparation for the X-ray experiment.

The above method deals only with crystals which are to be mounted at or near room temperature for experiments at or near room temperature. An alternative approach is to grow a crystal in a capillary (Åkervall & Strandberg, 1971), which could eliminate the need to manipulate the crystal manually. When crystals have been grown in the presence of detergents or gels, specific methods may be required for mounting (McRee, 1993). The appropriate procedure for flash cooling of crystals is detailed in Part 10.

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