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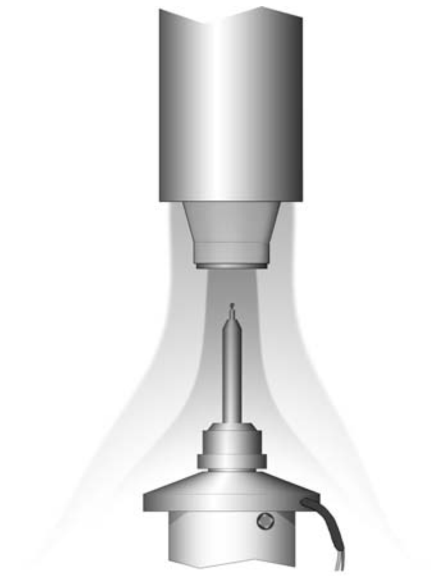

Figure 10.1.4.1
Schematic drawing of a dual-stream setup with the streams parallel to the diffractometer φ axis. The top part represents the outlet end of the stream delivery device. The outer stream (lighter grey) is dry, warm air. The goniometer head is protected by a shield.

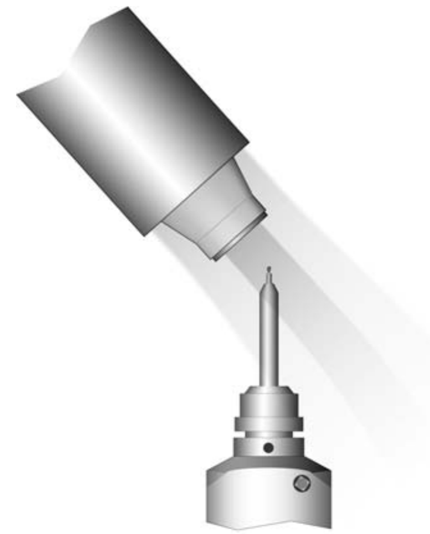

Figure 10.1.4.2
Schematic drawing of a dual-stream setup with the streams angled relative to the diffractometer φ axis. Stream representations are the same as in Fig. 10.1.4.1. The cold stream misses the goniometer head, so no shield is required.

For a given setting of the flow control valve, the flow rate depends on the tank pressure. For a constant temperature, a constant pressure is required. The pressure is controlled with a pressure regulator that can reduce the tank pressure by releasing helium gas or raise it by adding helium gas from an external helium supply source. A typical delivery tank pressure is around 20 kPa. It is very important that the pressure is constant. The precise value attained is less important.

At atmospheric pressure, helium at around 40 K has the same density as room-temperature air. This means that very cold helium will rise in air, and has the capacity to seriously cool instrument parts in its way. Goniometer heads, beam stops and beam collimators are particularly vulnerable. A simple remedy is to use a small fan to mix the cold helium with room air.

10.1.3.3. Frost prevention

Three areas must be kept frost-free: the crystal, the crystal mount and the delivery end of the transfer tube. The first successful solution to this problem was the dual-stream design of Post *et al*. (1951). It provides for a cold stream surrounded by a concentric warm stream. If the warm stream is sufficiently dry, this will prevent frost around the outlet. The crystal will remain frost-free only if mixing of the two flows occurs downstream from the crystal. For a stream aligned with the axis of the goniometer head, an additional shield is needed to keep the goniometer head frost-free.

10.1.4. Operational considerations

10.1.4.1. Dual-stream instruments

Fig. 10.1.4.1 shows a schematic drawing of the region around the crystal in a traditional dual-stream apparatus, first described by Post *et al*. (1951). The device provides for a cold stream surrounded by a concentric warm stream. The diameter of the cold stream is typically around 7 mm with a shield stream of 2–3 mm. The two streams flow parallel to the axis of the crystal mount. In a properly functioning apparatus, the warm stream supplies enough heat to keep the tip of the cold-stream tube above the dew point. It is important that the streams do not mix, or the crystal temperature will not be stable. This is achieved by careful balancing of flow rates to minimize turbulence. (Absence of turbulence can be judged by the shape of the shadow of the cold stream in a parallel beam of bright light.) In a laminar cold stream, the crystal is well protected and no special precautions are needed. The region of constant, minimum temperature will typically have a diameter of about 3 mm. Turbulent flow will result in the absence of any constant-temperature region, so it is vitally important to verify the stream quality.

The cold stream has sufficient heat capacity to cool down the goniometer head, and sometimes other adjacent equipment parts as well. A simple solution consists of an aluminium cone equipped with a heating coil on the back. A shield that functions well has been described by Bellamy *et al*. (1994).

Fig. 10.1.4.2 illustrates a situation where the stream direction deviates substantially from the head-on direction in Fig. 10.1.4.1. An angle of 35–55° will give good results. An advantage of an angled delivery is that the cold stream will not touch the goniometer head, and therefore the heated stream deflector is not needed, resulting in simplified installation and operation.

Analysis of the dual-stream apparatus reveals a twofold function of the outer stream: it keeps the nozzle frost-free and it supplies heat to the mounting pin. Protection of the crystal is, in reality, already provided by the laminar cold stream. The nozzle can be kept frost-free simply with an electric heater. Ice formation on the crystal mount can be easily suppressed by appropriate design of the mounting pin and mounting fibre, and attention to their interaction with the cold stream. A successful solution is sketched in Figs. 10.1.4.3 and 10.1.4.4.

10.1.4.2. Electrically heated nozzle

Fig. 10.1.4.3 shows the functional equivalent of Fig. 10.1.4.1. Instead of the warm stream, an electrical heating element is used to keep the tip of the delivery tube ice free. An actual construction will usually consist of a nozzle that can be attached to the delivery tube. The heating element is made from standard resistance wire (*e.g.* Nichrome). About 5 W will usually be

Figure 10.1.4.3
Schematic drawing of a single-stream setup with the stream parallel to the diffractometer φ axis. The cold stream is represented by a grey region. The nozzle is heated above the dew point with a heating coil. The goniometer head is protected by a shield.

Figure 10.1.4.4
Schematic drawing of a single-stream setup with the stream angled relative to the diffractometer φ axis. The cold stream is represented by a grey region. The crystal mounting pin protrudes 1–2 mm into the cold stream. This prevents frost from forming on the mounting fibre. The nozzle is heated above the dew point with a heating coil. The cold stream misses the goniometer head, so no shield is required. In general, the simplest operation is attained with a setup similar to that shown here.

enough to prevent frost or condensation. The head-on direction results in reliable frost protection for the crystal, but requires that the goniometer head is equipped with a heated stream deflector.

Fig. 10.1.4.4 shows the functional equivalent of Fig. 10.1.4.2. This arrangement leads to the simplest design, although some precautions are needed. The mounting pin itself supplies the heat needed to prevent ice formation on the pin. The tip of the pin should be smooth in order to prevent turbulence. About 1–2 mm (but not more) of the tip must protrude into the cold stream. If too much of the pin is in the cold stream, the rest of the pin can become too cold and ice up. If the pin is too far out of the stream frost prevention will also fail, because glass or other insulating mounting materials will invariably collect ice at the cold/warm interface. As frost prevention depends on heat conducted from the rest of the pin, it must be made from copper (a requirement not strictly necessary for the dual-stream design). This design has been extensively tested and has been used for many years for the collection of a large number of data sets. Results are uniformly good, with simple operation and reliable frost prevention even in high humidity.

Fig. 10.1.4.5 is a photograph of a laboratory implementation of the setup of Fig. 10.1.4.4, with helium as the coolant. Operation is as simple as with nitrogen cooling.

Omission of the warm stream results in significant design simplification. The entire apparatus for production of the outer stream is left out, resulting in real savings in manufacture, operation and maintenance. There is no obvious disadvantage, as ice protection is as good as with the dual stream. The main cost to the user is in the requirement that the mounting system be constructed within somewhat narrower limits, including the requirement of a copper-shafted mounting pin. The fact that operator errors tend to become apparent through frost formation can actually be an advantage. With a dual-stream device, an improperly positioned cold stream or an improperly prepared crystal mount may not produce overt signs, even though the crystal temperature is ill-defined.

In all configurations shown, correct positioning of the cold stream is essential. The centre of the stream should not miss the centre of the diffractometer (and hence the crystal) by more than 0.5 mm.

With helium, the single-stream technique represents the best solution and reliable frost prevention at temperatures down to around 5 K is easily attained. Crystal mounting is simple and the sample is always visible, simplifying centring. In the authors' experience, liquid-helium cooling is as simple as liquid-nitrogen cooling.

10.1.4.3. *Temperature calibration*

Measurement of the temperature at the crystal site with sensing devices that require attached leads is very difficult, mainly because of heat conduction along the leads. It is usually necessary to loop the leads into the delivery nozzle.

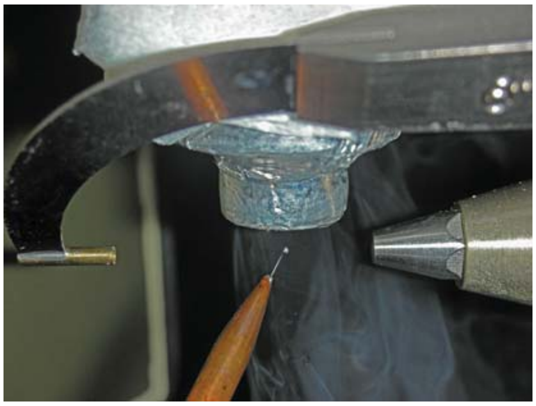

Figure 10.1.4.5
A crystal in a helium stream at 8 K in a setup corresponding to Fig. 10.1.4.4. A thin layer of fog forms at the helium–air interface. The cold stream breaks up well below the crystal position. Rising cold gas (visible as fog to the right of the cold stream) has been mixed with air to prevent cooling of diffractometer parts. The nozzle temperature is 295 K. The crystal is attached to a tapered glass fibre. The light-coloured region at top is not ice; it is part of the insulation. This photograph was taken after the end of data collection.

Both Si diodes and Pt resistance sensors have become sufficiently miniaturized to make them preferred choices at the lowest temperatures. Thermocouples are acceptable above about 80 K. A reliable method of calibration makes use of the known temperature of a phase transition of a crystal in the normal data-collection position. For example, KH_2PO_4 (often referred to as KDP) has a sharp transition at 123 K from tetragonal to orthorhombic, and is commonly used. Another possibility is KH_2AsO_4, which has a corresponding phase transition at 95 K.

Two readout temperatures suffice, one at room temperature and one at the phase transition. The difference between readout temperature and crystal-site temperature can be assumed to vary linearly with T, so interpolation or extrapolation is simple.

10.1.4.4. Transfer of the crystal to the diffractometer

Inspection of Figs. 10.1.4.1–10.1.4.4 reveals that the mounting of a crystal on a mounting pin *via* the traditional placement of the pin in the hole of a standard goniometer head is not simple, because the cooling nozzle is in the way. The solution to the problem is a design that allows side entry. This is most commonly achieved with a magnetic platform on the goniometer head and a corresponding magnetic base on the mounting pin, but an alternative means of side entry employs a slot on a modified goniometer head; the slot is equipped with a spring-loaded catch that allows a very smooth, but stable, catch of the pin.

The use of liquid-N_2 cooling and side entry, and the requirement of reproducible knowledge of crystal temperature at all times, led to the development of a set of tools for crystal mounting as described by Parkin & Hope (1998). The tools include special transfer tongs used for moving crystals from liquid N_2 to the goniometer head. The temperature of the crystal is maintained by the heat capacity and low heat conductance of the tongs. The operation is independent of the orientation of the goniometer head because there is no liquid to contain.

10.1.4.5. Automated robotic crystal handling

The era of structural genomics has necessitated a move to high throughput at high-intensity synchrotron sources. To meet this goal, a number of robotic crystal-handling devices have been developed. These in turn have had the beneficial effect of increased standardization in mounting-pin geometry.

10.1.5. Concluding note

With correctly functioning low-temperature equipment and appropriate techniques, a crystal can be maintained frost-free for the duration of a data-collection run. Formation of frost on the crystal indicates malfunction of the equipment, or operator error. The most likely cause is operator error, but faulty equipment cannot be ruled out. The techniques described here have been used for collecting thousands of data sets from ice-free crystals and crystal mounts. There is no reason to accept frost problems as an unavoidable part of cryocrystallography.

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