A similar program was first written by the author in 1969 for the IBM 360/50 computer of the Centraal Rekeninstituut, Leiden University, the Netherlands, following a suggestion by the late Professor E. W. Gorter and Dr A. B. A. Schippers, but it has been revised and changed thoroughly to give the present program.

We thank Professor H. Burzlaff for a copy of his routine which interprets space-group symbols; Dr A. B. A. Schippers, who initially suggested the program, for helpful discussions, Mrs K. Cenzual for many useful suggestions, and Professor E. Parthé for reviving interest in the problem and for a critical reading of the manuscript.

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Laboratory Notes

J. Appl. Cryst. (1981). **14**, 153–154

A simple method for surveying macromolecular crystallization conditions by microdialysis

Growing crystals of a protein or macromolecular assembly often requires a large number of experiments and a considerable amount of material. Many of the techniques used have been reviewed by McPherson (1976). The most popular technique at present for screening crystallization conditions is the hanging-drop method. This technique has the advantage of using very small volumes of material (5–10 μl) per experiment, but suffers from the restriction that it is difficult to change accurately the pH of the protein solution during the crystallization experiment. In general, more material is lost during a preliminary dialysis than will be used in any single experiment.

The difficulties involved in changing pH may be overcome by using the microdialysis cell developed by Zeppenzauer (1971). This system consists of a short piece of capillary tubing containing a small volume of protein solution which is sealed at one end with a piece of dialysis tubing and at the other with some impermeable membrane. In principle, there is no limitation imposed upon the amount of material required for each experiment. However, the cells are somewhat tedious to construct and in case of small volumes, it is difficult to see whether crystals have grown because of the optical effects of the glass and surrounding solution.

A simple method for screening crystallization conditions by microdialysis which requires only small volumes (2–5 μl) and provides an unimpeded view of the results is described here. The technique involves placing a small volume of protein solution at the base of a conical tube sealed by a piece of dialysis tubing. The tip of the tube is held in contact with the equilibrating solution such that the protein solution may be viewed easily using a dissecting microscope.

The crystallization apparatus shown in Fig. 1 consists of an embedding capsule (Beem type 00) whose tip has been removed to leave a hole ~1 mm square. The aperture is covered by a piece of dialysis membrane which is held firmly in place by a short length of PVC tubing. This assembly is then inserted into a small straight-sided vial (12 mm diameter × 45 mm high) until the surface of the dialysis tubing contacts the precipitating solution (approximately 2 ml). The height of the dialysis cell is maintained by a short length of tubing which acts as a spacer between the cell and the vial. A small volume of the protein solution is placed in the base of the embedding capsule in contact with the dialysis membrane. The assembly is finally sealed with a piece of clear impermeable plastic film held in place by a short length of rubber tubing. Experiments using 'Saran Wrap' (Dow Chemical Co.) as the plastic film typically lost ~2 mg of water per week at room temperature. This loss is insufficient to alter significantly the equilibrium between the protein droplet and the precipitating solution. The results of an experiment may be observed using a dissecting microscope without disturbing the contents.

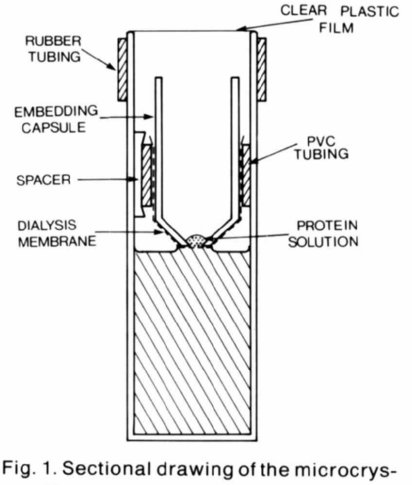

Fig. 1. Sectional drawing of the microcrystallization assembly.

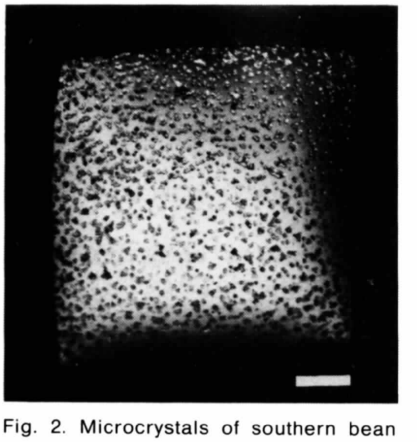

Fig. 2. Microcrystals of southern bean mosaic virus viewed by a dissecting microscope. The bar represents 200 μm.

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This procedure is most effective when the precipitant cannot pass through the dialysis membrane, *e.g.* polyethylene glycol (PEG), or for isoelectric crystallizations. In the first case the final concentration of protein is easily controlled by varying the initial PEG concentration in the protein solution, and may be used to concentrate a dilute protein solution. In experiments using salts or small molecules as precipitants, which can easily pass through the membrane, after the initial movement of water, the level of the solution in the dialysis cell will seek the level of the external solution. In this case crystallization may still occur, but the protein concentration will not be well defined because there is no constraint on the final volume occupied by the protein solution. This procedure was developed for crystallizing viral components of polyoma virus and has been tested using southern bean mosaic virus. An example is shown in Fig. 2; the experiment which used 2 μl of material clearly shows well-formed microcrystals of a form not previously characterized.

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Determination of Bragg peaks from a flat single crystal using a rotation photograph

It is often necessary to employ a flat single crystal for absolute X-ray intensity measurements involving diffuse scattering due to local order or thermal vibration, as well as for crystal structure determination. In the process of aligning such a specimen, locations of several Bragg reflections must be determined in order to obtain an orientation matrix which relates the crystallographic axes of the specimen to the diffractometer axes. This process was done principally by a back-reflection Laue technique in the past. We, however, have developed a method which utilizes a modified rotation photographic technique. This new approach allows for a fast search of the Bragg peaks without knowing the orientation of the crystal beforehand. Although a four-circle diffractometer is used in our discussion, the same method is also applicable to a three-circle diffractometer.

Fig. 1 shows the experimental arrangement for obtaining a rotation photograph while Fig. 2 illustrates the corresponding geometrical representation. An X-ray film which is mounted on the χ ring, a distance l from the specimen, is initially exposed to the direct beam at $\omega = 0$ in order to obtain a reference point. The χ ring, and therefore the film, is then rotated to a specific angle ω from zero and the crystal is continuously rotated about the φ axis. Fig. 3 illustrates the resulting rotation photograph, and distances x and y are measured from each pair of symmetry-related Bragg peaks.

In Fig. 2, point S is the location of the specimen, O is the location of the direct beam when $\omega = 0$, P is the location of a Bragg peak on the film and A is a point along the incident beam with $AS = SP$. Triangle $O'PP'$ represents the plane of the film while triangle $O'P'A$ is the horizontal plane. From the figure, it can be seen that $OP' = x$, $PP' = y$, $OS = l$, $\angle OSO' = \omega$ and $\angle O'SP = 2\theta$. From trigonometric relations, it can be seen that

$$\cos 2\theta = [(l')^2 + (SP)^2 - (O'P)^2]/[2(l')(SP)] \quad (1)$$

in which $l' = O'S$. Each term in the above expression can be further represented by measurable quantities of x, y, l and ω. Thus

$$\cos 2\theta = [l\cos\omega - x\sin\omega](x^2 + y^2 + l^2)^{-1/2}. \quad (2)$$

Again, from Fig. 2, the expression for angle χ ($= \angle KSQ$) can be obtained from the following equation

$$\cos\chi = (A^2 + B^2 - C^2)/2AB, \quad (3)$$

where

$$A = (x^2 + y^2 + l^2)^{1/2}\sin\theta,$$

$$B = \frac{(x + l\tan\omega)(x^2 + y^2 + l^2)^{1/2}}{l/\cos\omega + (x^2 + y^2 + l^2)^{1/2}},$$

$$C = \left\{\frac{y^2}{4} + \frac{y}{\tan\alpha}\left[\frac{1}{2} - \frac{(x^2 + y^2 + l^2)^{1/2}}{l/\cos\omega + (x^2 + y^2 + l^2)^{1/2}}\right]\right\}^{1/2}$$

and

$$\alpha = \angle PAP' = \sin^{-1}\{y/[2\cos\theta(x^2 + y^2 + l^2)^{1/2}]\}.$$

Once 2θ and χ have been determined, one can move the diffractometer to these calculated θ, 2θ and χ settings. A search for a Bragg peak is then made by rotating

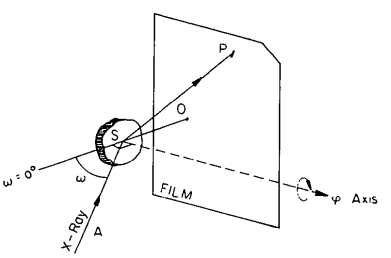

Fig. 1. Experimental arrangement for obtaining a rotation photograph.

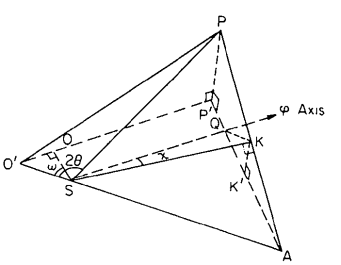

Fig. 2. Geometrical representation corresponding to experimental arrangement of Fig. 1.

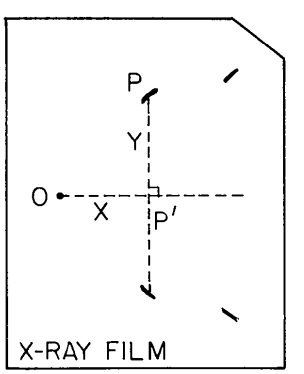

Fig. 3. Final rotation photograph.