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# A Small-Angle Optically Focusing X-Ray Diffraction Camera in Biological Research. Part I

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The small-angle optically focusing X-ray diffraction camera first described by Franks (*6–8*) has been modified for use with biological specimens. The camera as described is suitable for specimens of rather small natural line widths.

## THE FRANKS CAMERA

Low-angle X-ray diffraction studies run parallel to observations in the electron microscope and can provide information on intact (or living) biological specimens. Suitable cameras for this type of work usually require rotating anode X-ray tubes so that the exposure times are not too long.

The optically focusing small-angle X-ray diffraction camera of Franks (*6–8*) uses successive reflections, at a vertical and a horizontal optical flat (both slightly bent), to form on the X-ray film an image of the foreshortened focal spot of a microfocus X-ray tube. The camera is focused by bending the flats while observing the image (through a microscope) on a single-crystal fluorescent screen. At the critical angle for reflection of the characteristic $CuK_\alpha$ radiation only the characteristic and longer wavelengths are reflected, so the image is partially monochromatized. Although skillful experimenters can learn to focus this camera satisfactorily, the procedure is time consuming and imposes some strain on the experimenter. Franks' design has the disadvantage that no provision is made for extended exposures. Weakly diffracting protein fibers may require exposures of 2–3 weeks; during this time the X-ray beam should remain at peak intensity. Factors such as focal area movement, filament replacement, or the removal of target contamination all make it necessary to readjust the camera, and, since this can only be done optically, the specimen must be removed from the camera and the exposure ended. This limits the exposures to a very few days.

Nevertheless, Franks (*6*, *7*) and later Elliott (*2*) demonstrated the potentialities of this type of camera for biological materials. The present authors have modified the Franks camera for the study of biological specimens; many of the biological results have already been described (*2–5*, *10–12*).

## MODIFICATIONS OF FRANKS' DESIGN

A modification of the camera, which simplifies the adjustment procedure and permits extended exposure times, was first described by Worthington (*10*). Two movements are added which make it possible to change the direction of the camera axis with respect to the anode of the X-ray tube, thus making it possible to separate realigning the camera from refocusing the optical flats. The two movements are made by means of a vertical adjusting screw on the front of the camera table and a horizontal micrometer adjustment on the front of the camera base plate, the back of which is pivoted (see Fig. 1).

In the initial adjustment of the modified version, the two movements just described are first set so that the X-ray axis of the camera, judged by eye, appears to be in the required direction. The focusing of the glass flats then follows the routine given by Franks (*6*, *7*). Finally, the guard aperture and cassette are aligned using the techniques described by Worthington (*9*). To realign the camera without changing the adjustment of the optical flats, a counter tube is inserted between the specimen holder and the cassette, and the two camera-axis movements are adjusted for maximum response.

A photograph of one of our versions of the camera is shown in Fig. 1. Our cameras use glass plates 6 cm long; the focus-to-focus distance is 34 cm. The specimen-to-film distance can be up to 8 cm, depending on the type of specimen mount which is used to hold the specimen over the guard aperture. Franks (*6*), has shown that there is a theoretical best position for the guard aperture for maximum first-order horizontal resolution. This is, with our dimensions, about 6 cm in front of the film, but we find in practice that the effect of this variable is negligible and that with a given focus-to-focus distance it is better to have the longest possible specimen-to-film distance, and thus the largest possible distances to measure on the X-ray film.

The design of the optical movements calls for comment. In all four of our cameras the support and bending assemblies for the optical flats were built in our workshops, according to Franks' design for the camera built to his specifications in the workshops of the United Kingdom National Physical Laboratory. In our first two versions, the movements which position these assemblies with respect to the X-ray beam were

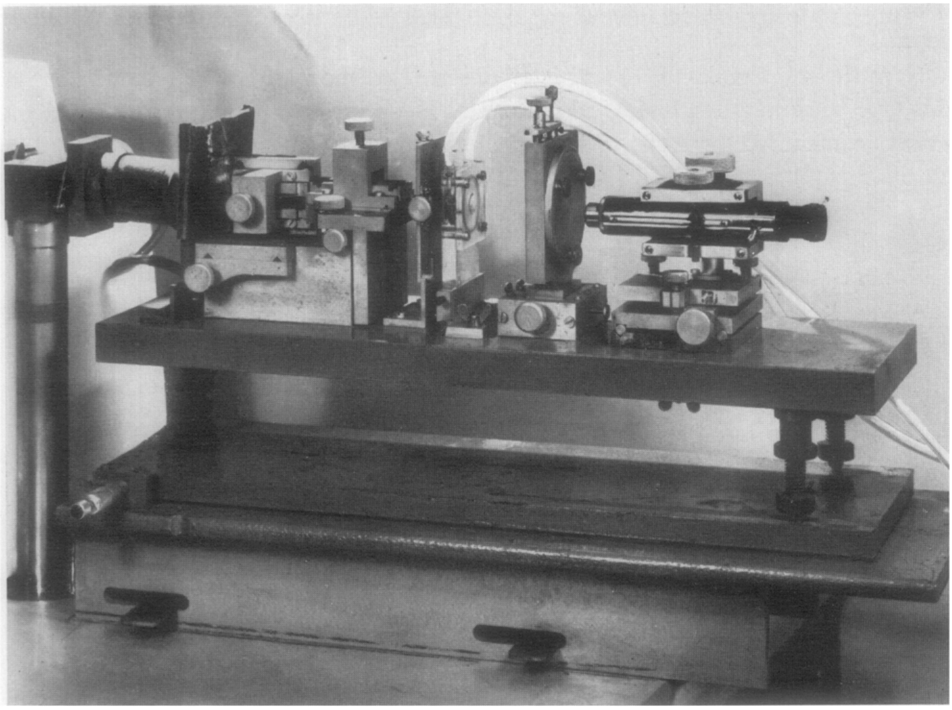

FIG. 1. Photograph of one of the latest versions of the camera in position on the microfocus set. This version has kinematic movements on the mirror mounts.

also constructed to Franks' specifications. We find that these movements frequently shift when the focusing or locking screws are adjusted. We therefore favor the use of either positive dovetails or positive kinematic movements in order to eliminate this motion during adjustment of the glass plates; we have used both these systems with success in our later versions of the camera.

## DISCUSSION

Our first two versions of the camera have given satisfactory performance over four to five years and have been found most reliable in operation. A few cameras following the design of our first two versions have been constructed in other laboratories. Many of the modifications described in this paper are to be incorporated in a commercial version of the Franks' camera.

The focusing of the optical flats is maintained over long periods, and in practice it is generally necessary only to readjust the direction of the camera axis: this takes

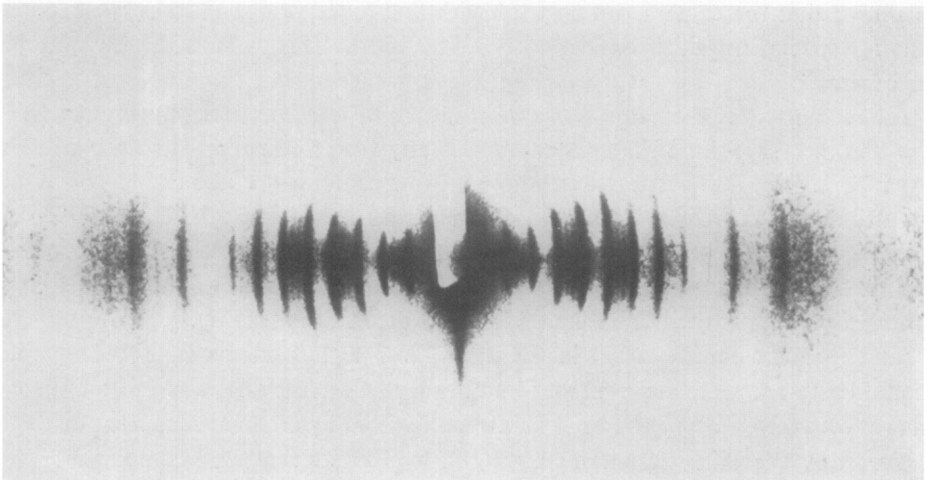

FIG. 2. X-ray diffraction pattern taken on point focus of rat-tail tendon stained with phosphotungstic acid. (Specimen prepared by Dr. A. C. T. North.) The fiber lay in the horizontal direction (direction of maximum resolution) and was exposed for 12 hours. The "thirding" between main orders of the 640 Å collagen spacing suggests a fundamental spacing of 1920 Å, the origin of which is unknown.

only a few minutes. When used with point focus of the microfocus X-ray tube, the X-ray beam has high intensity (quanta per unit area) at the fluorescent crystal, but the integrated intensity (total number of quanta) is comparatively small. Therefore there is little air scatter and the camera can be operated in air even for long exposures. The Perspex cover functions as a dust cover.

Theoretically, if the focal area has maximum loading per unit area, the X-ray beam viewed at the fluorescent crystal will have peak intensity as defined above. Good focusing of the microfocus on the X-ray tube (Ehrenberg and Spear, *1*) would thus seem to be important. We find empirically, however, that the focus in this type of camera is remarkably insensitive to the movement of the filament assembly along the tube axis, and it would appear that the focus in commercial versions of this tube is similarly insensitive to the position of the filament.

The focal spot on the tube anode is nominally 40 $\mu$ in diameter, but it is probably somewhat larger in fact. At the fluorescent crystal the focused X-ray beam should have the approximate dimensions of the projected focal area. As measured, the half-breadth is about $80\,\mu \times 25\,\mu$ (at a take-off angle of 6 degrees), showing that the focusing is not perfect (*3*). The camera is ideally suited for biological and high polymer specimens giving a natural line width of this order. For specimens with a large line width, such as liquids, the camera is not a good choice. In Part II of this paper it will be shown that the camera can be used with line focus, which reduces the exposure time for specimens which have moderate line width. This extends the usefulness of the camera.

The resolution of the camera has been discussed in some detail by Franks (*6*, *7*). The first-order resolution is greater horizontally than vertically. The practical first-order horizontal and vertical resolutions of our cameras are about 500–600 Å and about 400–450 Å, respectively. Higher first-order resolution can be obtained only with short exposures and strongly diffracting specimens, a situation which seldom occurs. The resolution between orders is very good; successive orders of a basic periodicity of approximately 2000 Å can easily be resolved (Fig. 2).

An important consideration in the use of low-angle cameras is that the camera should be able to view the focal area at a small angle to the anode surface; a range of angles from 0 to 6 degrees should be available. This requirement has been discussed at length by Worthington and Tomlin (*13*). It is a pity that with commercial X-ray tubes the important range of angles less than 6 degrees is often unattainable.

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