

A PIEZOELECTRIC THIN-SECTIONING  
ATTACHMENT FOR A STANDARD MICROTOME

by

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INTRODUCTION

The development of the electron microscope to the point of practicality has opened up many fields of sub-microscopic research previously unrevealed. Electron microscopy is a relatively new science since commercial instruments have been made available in only the last ten years.

Because of the physical and electronic nature of the electron microscope, severe restrictions are imposed on the types of specimens that can be viewed directly in their original form. They are subjected to the bombardment of 50 Kev electrons, pressures of  $10^{-5}$  mm Hg., and can not be over 0.1 micron in thickness. It would seem that this would limit the usefulness of the instrument, but microscopists have developed numerous techniques in specimen preparation, including freeze drying, shadow casting, replica technique, and staining, that circumvent most limitations. It has been repeatedly demonstrated that the versatility of the instrument is limited only by the ingenuity of the operator. Much has been written on electron microscopy but each day brings new problems in technique which require new methods in their solution.

Electron microscopists are repeatedly confronted with the problem of rendering the specimen thin enough to enable the electron beam to pass through it. The art of cutting thin sections for use

with the light microscope has become a routine procedure through the years by the development of relatively inexpensive commercial microtomes. The thinnest sections available, however, from the methods developed here are much too thick for electron microscopy, the thinnest being in the order of one to five microns. Commercial microtomes especially suited to electron microscopy have recently been made available but they are costly due principally to the high degree of precision machining required in their manufacture.

It is the purpose of this paper to describe an inexpensive device for adapting a standard microtome to produce thin sections suitable for electron microscopy. Basically the unit replaces the mechanical feed by the calibrated piezoelectric expansion of a rochelle salt crystal. The thickness of the sections is determined by the magnitude of the applied voltage increments.



## THEORY

Certain insulating solids change shape when placed in an electric field, and conversely, produce an electric field when subjected to mechanical stress. The former is called the converse piezoelectric effect and the latter, the direct piezoelectric effect. These phenomena were discovered experimentally by Pierre and Jacques Curie, in such crystals as quartz and rochelle salt, in the year 1880.

It was the converse effect that was used to produce a calibrated feed mechanism for determining the thickness of the slices produced in a microtome. This device has an advantage over the thermal expander used on some commercial microtomes, in that it is time independent.

The dielectric properties of rochelle salt have been compared to the ferromagnetic properties of iron. It is ferroelectric in the temperature range from  $-18^{\circ}\text{C}$  to  $+24^{\circ}\text{C}$ , known as Curie points, which accounts for the strong piezoelectric properties at room temperature (2, p.8). Rochelle salt is unique in that it has both an upper and a lower Curie point.

The equations for the converse effect in terms of strain are:

$$X_x = d_{11}E_x + d_{21}E_y + d_{31}E_z$$

$$Y_y = d_{12}E_x + d_{22}E_y + d_{32}E_z$$

$$Z_z = d_{13}E_x + d_{23}E_y + d_{33}E_z$$

$$Y_z = d_{14}E_x + d_{24}E_y + d_{34}E_z$$

$$Z_x = d_{15} E_x + d_{25} E_y + d_{35} E_z$$

$$X_y = d_{16} E_x + d_{26} E_y + d_{36} E_z$$

where  $X_x$  = normal strain in X direction

$E_x$  = electric field in X direction

In rochelle salt, only  $d_{14}$ ,  $d_{25}$ , and  $d_{36}$  differ from zero. The piezoelectric equations then reduce to:

$$Y_z = d_{14} E_x$$

$$Z_x = d_{25} E_y$$

$$X_y = d_{36} E_z$$

For X-cut crystals we need consider only the constant  $d_{14}$  since an electric field applied in the x-direction will produce only a shear in the x-z plane. To obtain a unit that would expand only along one axis, i.e. a normal strain, a  $45^\circ$  X-cut crystal was used in the expander unit. The effect in the normal direction is effectively halved; thus the equation becomes:

$$s = \frac{d_{14} E_x}{2}$$

where  $s$  = linear expansion (normal strain)

The value of  $d_{14}$  is a function of temperature and stress so that the unit must be calibrated for the particular temperature and installation in which it is to be used.



## DESIGN CONSIDERATIONS

Numerous factors were considered in the preliminary design. To be of practical use it was felt that certain requirements would have to be satisfied. It was essential that the accuracy of the feed mechanism be independent of machined surfaces or bearings which would impose expensive if not impossible requirements in machining, since the tolerances would be in the order of 0.01 micron. The crystal had to be rigidly mounted to minimize errors due to vibration but also free to expand as the voltage is applied. It was desired to use the light microtome to provide the cutting action without modification. To minimize power supply stabilization problems, the control voltage must be kept below 200 volts.

One unfortunate characteristic of rochelle salt is that the piezoelectric effect is dependent upon temperature with the effect dropping off sharply above 24°C. It was at first thought that perhaps a less temperature dependent crystal could be substituted but it was found that the piezoelectric effect is too small in all other crystals to be of use here. Rochelle salt has a piezoelectric constant in the order of 100 times its closest rival and therefore is the logical choice for the expansion unit.

It will be shown later when the calibration curves are considered, that temperature variations can be essentially ignored below a temperature of 22°C. This is not an unreasonable temperature requirement and should be well within the range of an air-conditioned

laboratory. Since it is recommended that the electron microscope be operated under these same temperature conditions, there should be little difficulty from this standpoint.

## DESCRIPTION OF MECHANICAL PARTS

As a result of preliminary design considerations, the device shown in Figure 1 was constructed. The rochelle salt crystal is 1" x 1/2" x 5" and is shown full size in the figure. It was chosen as large as practical to insure maximum strength and maximum piezo-electric effect and it could still be mounted easily on the microtome.

The X-cut crystal as obtained was coated with thin metallic foils on opposite faces which make close contact with the surface of the crystal. The crystal was initially coated on all areas not protected by the conductive coating with ceresin wax. This was to reduce the tendency for the crystal to lose or accept moisture which could destroy it. Before assembly the ceresin wax and conductive coating were removed from the portion of the crystal that was to be cemented into the turned brass holder. The various components before assembly are shown in Figure 2. Leads were cemented to the metal foil with silver paint and the crystal was cemented into the holder with Eutectic cement. The exposed end of the crystal is free to expand when a potential is applied.

A brass plate fitted with a tapped brass plug was cemented to the free end of the crystal. Small specimen holders and a mirror for calibration were made to screw into this plug. A water jacket was fitted over the crystal and fastened to the brass holder with small screws. This allowed the temperature of the crystal to be accurately controlled and it also afforded a protective sheath to the crystal.

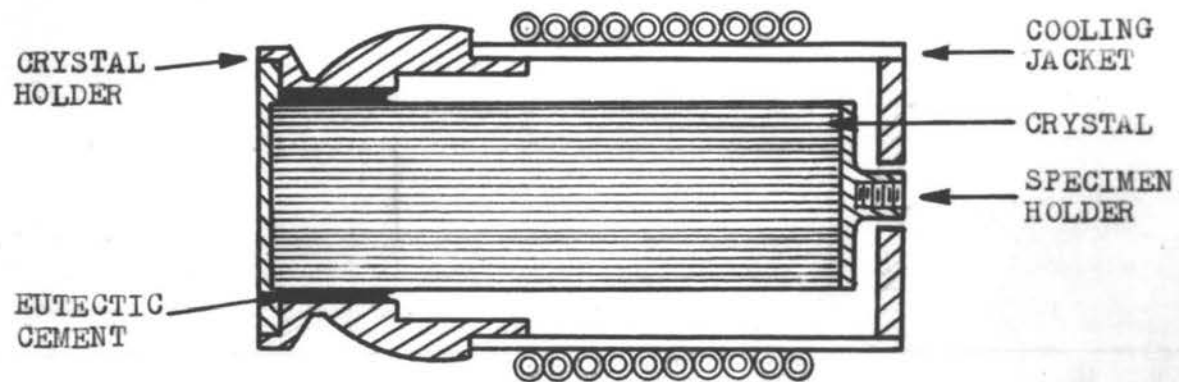


FIG. 1 WATER COOLED THIN SECTIONING ATTACHMENT



FIG. 2 COMPONENTS OF SECTIONING ATTACHMENT

By mounting the specimen holders directly on the crystal, the need for precision machining was eliminated and it was felt that since the specimens were of the order of one mm. in cross section, the stresses imposed on the crystal would be negligible. The crystal was then calibrated for various temperatures. The results of this will be discussed later.

The strength of the crystal was checked by deliberately breaking it in a testing machine. A force was applied to the specimen plug at right angles to the principal axes of the crystal which corresponds to the direction of forces encountered in slicing specimens. The long moment arm of the unsupported end of the crystal caused it to fracture at the cemented joint under a 1 $\frac{1}{4}$  lb. load. The possibility of getting an impulse of several pounds when cutting collodion impregnated specimens is not too remote. It became apparent that the crystal would have to be eliminated from the cutting shear to reduce the possibility of crystal breakage.

A second crystal was mounted as before for further calibration. When a potential was first applied to the crystal, it shattered approximately one-fourth inch below the surface of the cemented joint. It was then discovered that the Eutectic cement used to fasten the crystal in the brass holder expanded appreciably upon setting and exerted a tremendous pressure. The slight transverse motion caused by the expanding crystal caused it to break.

The crystal holder was redesigned and is shown in Figure 3. It is made to clamp directly into the original specimen holder



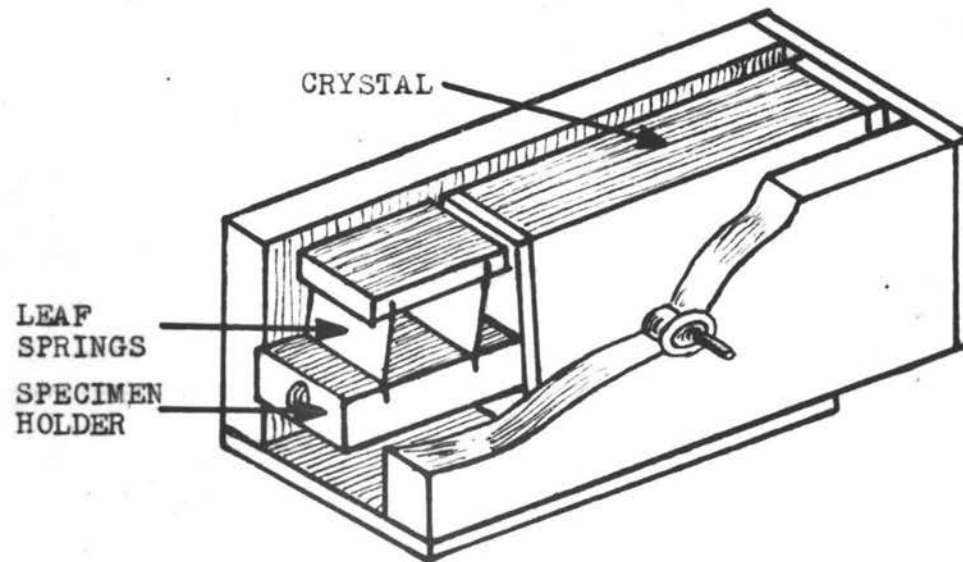


FIG. 3 SECTIONING ATTACHMENT, FINAL DESIGN

supplied with the light microtome. The heart of the unit which transmits the motion of the crystal to the specimen consists of two small brass blocks connected by two phosphor bronze springs. As seen in the figure, one block is rigidly fastened to the frame of the holder and the other rests against the end of the crystal. A small brass plate is cemented to the front end of the crystal to protect it and also to provide a rigid flat surface. The lower block exerts a slight tension upon the crystal through the leaf springs, causing it to follow the motion of the crystal exactly. It is drilled and tapped to take the calibration mirror and specimen holders. All of the shearing forces are absorbed by the mechanism without restricting the crystal in any way. The back end of the crystal is cemented to a brass plate which is screwed to the frame of the holder. Leads are brought out through insulating terminals soldered into the frame members. The unit is shown mounted in a light microtome in Figure 4.



FIG. 4 SECTIONING ATTACHMENT INSTALLED IN MICROTOME

## ELECTRONIC EQUIPMENT

A well regulated 200 volt power supply was used to calibrate the expander unit. The output potential was applied to two Helipot connected in series as indicated in Figure 5. One was then used as a voltage divider supplying a potential to the crystal that was variable from zero to 100 volts. The applied voltage was read directly from the vernier attached to the Helipot in steps of 0.1 volt. A one megohm resistor was connected in series with the crystal to protect it from excessive charging currents.

One of the crystals was accidentally damaged by over-heating which lowered the resistance of the crystal to approximately 100,000 ohms. Since this was not discovered immediately, erroneous data was taken, which was later discarded. A 0-5 milliammeter was connected in series with the crystal so that any tendency for current to flow could be detected.

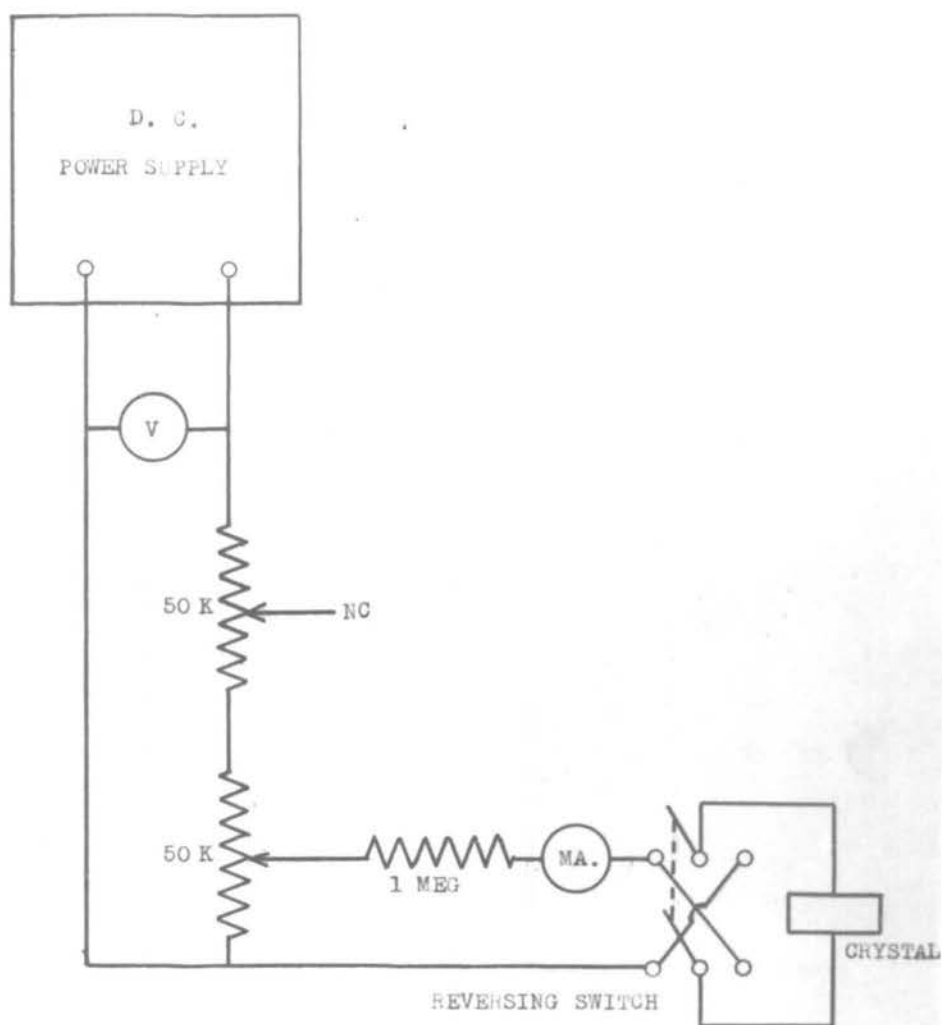


FIG. 5 ELECTRONIC EQUIPMENT

## OPTICAL SYSTEM

The expansion unit was calibrated by an optical interferometer using a mercury lamp and filter to obtain monochromatic light of 5460A wavelength. The system is shown in Figure 6. An optical flat was cemented to threaded brass stock which fit the threads of the specimen holder. The flat was given a thick silver coating. A second flat with semi-transparent coating of silver was cemented to an aluminum holder which was constructed to fit the knife holder on the microtome. The apparatus is shown in Figure 7.

The fringes resulting from the constructive and destructive interference of light reflected between the two silvered surfaces are observed with a microscope. A shift of one fringe indicates a change in the length of the crystal of one-half wavelength of the light that is used. It was found that changes in length could be read with an accuracy of approximately 0.1 fringe. Alignment of the optical flats was facilitated by the three standard mounting screws that hold the expansion unit in the microtome.



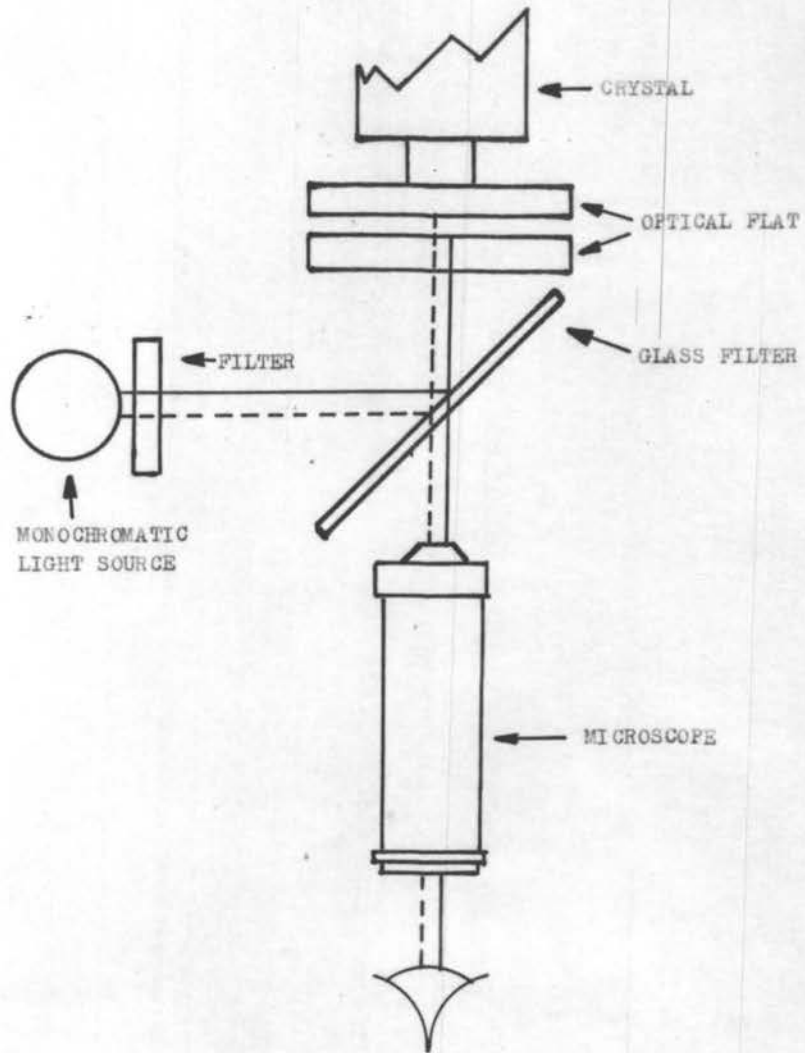


FIG. 6 OPTICAL SYSTEM



FIG. 7 CALIBRATION EQUIPMENT

## CALIBRATION

Measurements were made to determine the dependence of the calibration upon temperature and crystal history. It took approximately one-half hour for the unit to come to temperature equilibrium.

The crystal was first given a history by increasing the potential to 60 volts, reducing it to -60 volts and then bringing it back to zero. This voltage cycle was then repeated for a temperature of 16°C and then 24°C with readings taken in one volt variations. The results are shown in Figure 8. The result is a hysteresis loop analogous to that found for ferromagnetic substances.

In practice the voltage will not be reversed during a cutting cycle. It was found that this could be done without hampering the calibration if the voltage was first increased to 60 volts and then brought back to zero. The effect of this operation is to bring the crystal to point "A" on the graph in Figure 8. Increasing the potential then causes the deformation to follow the curve shown in dotted lines to point "B".

In calibrating the crystal expansion at a particular temperature, readings were estimated to one-fourth of a fringe. The maximum possible error in reading was therefore one-eighth of a fringe. In checking the reproducibility of the calibration curves, it was found that in no instance did the total deviation from the curves in Figure 9 exceed one fringe. For this reason it is assumed that the variations were primarily due to errors in reading.

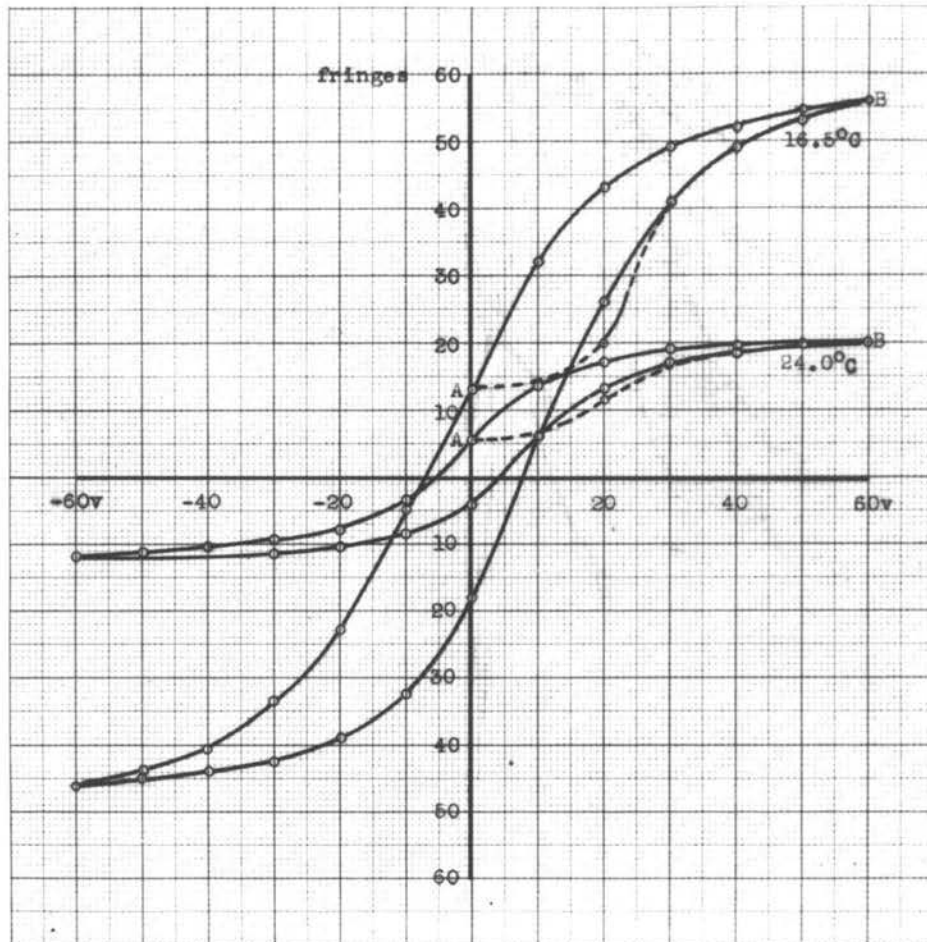


FIG. 8 HYSTERESIS EFFECT

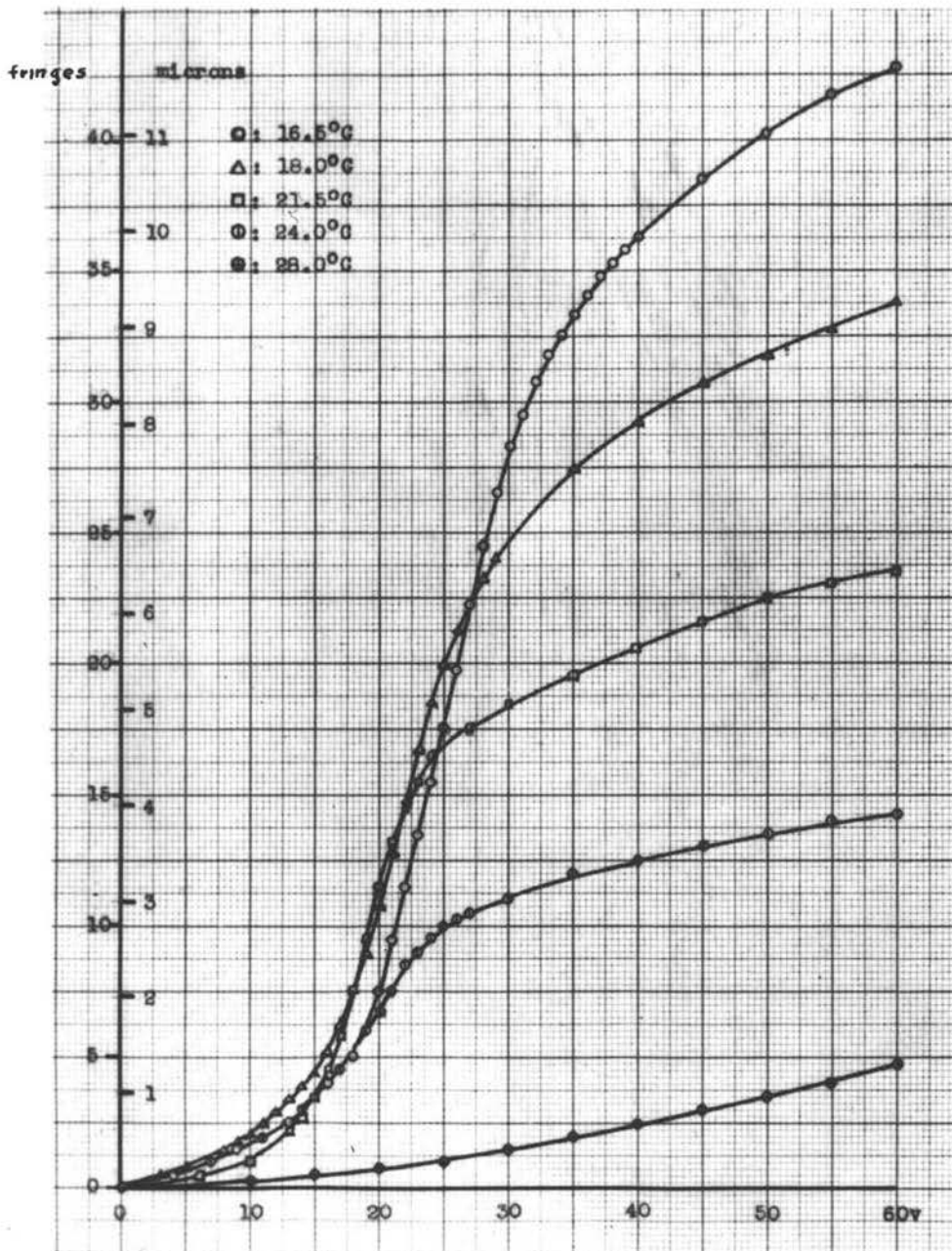


FIG. 9 CALIBRATION CURVES

The linearity of the Helipot is within 0.5%. A vernier adjustment allowed the voltage to be read accurately to 0.1 volt. Errors caused by voltage readings can therefore be ignored.

It takes a finite time for the crystal to expand and come to equilibrium after the voltage is varied. It was found that when the potential was varied in steps of one volt, it took from 10 to 15 seconds for the crystal to reach equilibrium. When using the unit to produce thin slices, therefore, it will be necessary to wait approximately 15 seconds between operations. This time interval is independent of temperature variations within the range investigated.



## CONCLUSIONS

The unit appears to fulfill the requirements that were initially set, viz., inexpensive, simple, rugged, and easily used in conjunction with a standard light microtome.

The slopes of the calibration curves shown in Figure 9 are nearly equal for temperatures below 22°C in the lower 25 volt range. Between 17 and 25 volts, each curve has a linear portion which has a slope of  $1.9 \pm 0.1$  fringe per volt. Since the curves do not coincide, these linear portions of equal slope do not occur for the same range of voltages.

A curve representing the mean of the calibration curves was drawn and is shown in Figure 10. The slope of the linear portion of this curve between 17 and 30 volts is 1.9 fringe per volt. Each calibration curve has a linear portion which coincides with a portion of the mean curve within 10%. The curve favors the higher operating temperatures in the low voltage range and favors the lower operating temperatures in the high voltage range. This curve is independent of temperature in the range specified and can be used to determine specimen thickness when great accuracy is not required. In the range represented by this curve it is possible to obtain from 30 to 100 slices depending upon the thickness required. In practice one need only sort out the slices that are suitable for electron microscopy. In most instances the actual thickness of the slices is unimportant. In cases where great accuracy is desired, the original calibration

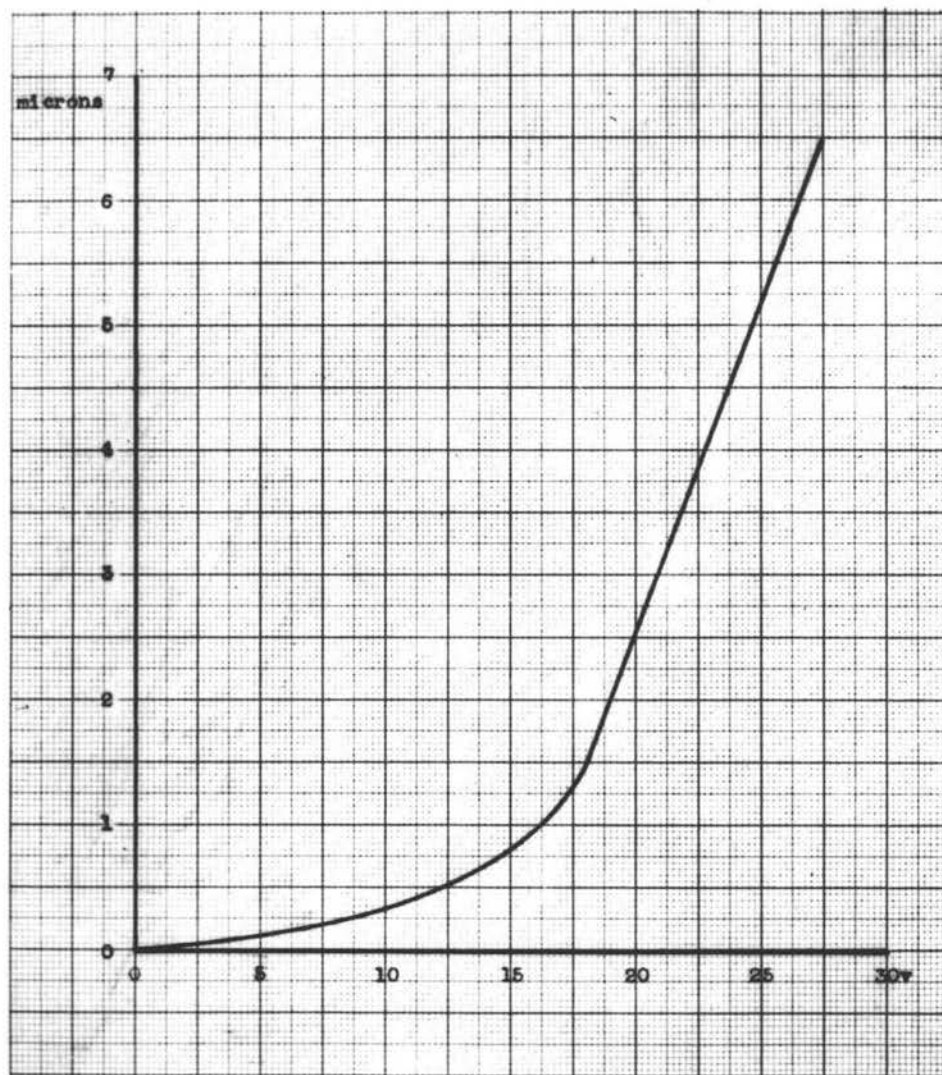


FIG. 10 TEMPERATURE INDEPENDENT CALIBRATION CURVE

curves can be used. With reasonable care, tolerances should be within 10% providing temperature remains constant.

The effect of temperature variations on any one calibration curve can be approximately determined from the calibration curves. Since the thickness of the slice is determined by the slope of the curve, the errors due to temperature variation will be directly proportional to the deviation of slope from the calibration curve. This can be illustrated by assuming a temperature change from 16.5°C to 18°C during a slicing operation. For a particular voltage setting, the slopes of these two curves can be compared to determine the maximum possible variation. Errors from zero to 100% occur in the 15 volt to 25 volt range with an average of about 20%. In practice large variation in temperature during a slicing operation is rather remote.

Figure 11 shows the light microtome modified for ultra-thin sectioning. The expander unit is clamped into the specimen holder and a glass knife is installed in the modified knife holder. A small tray containing a 50% alcohol solution is cemented to the glass knife for catching the sections as they are cut.

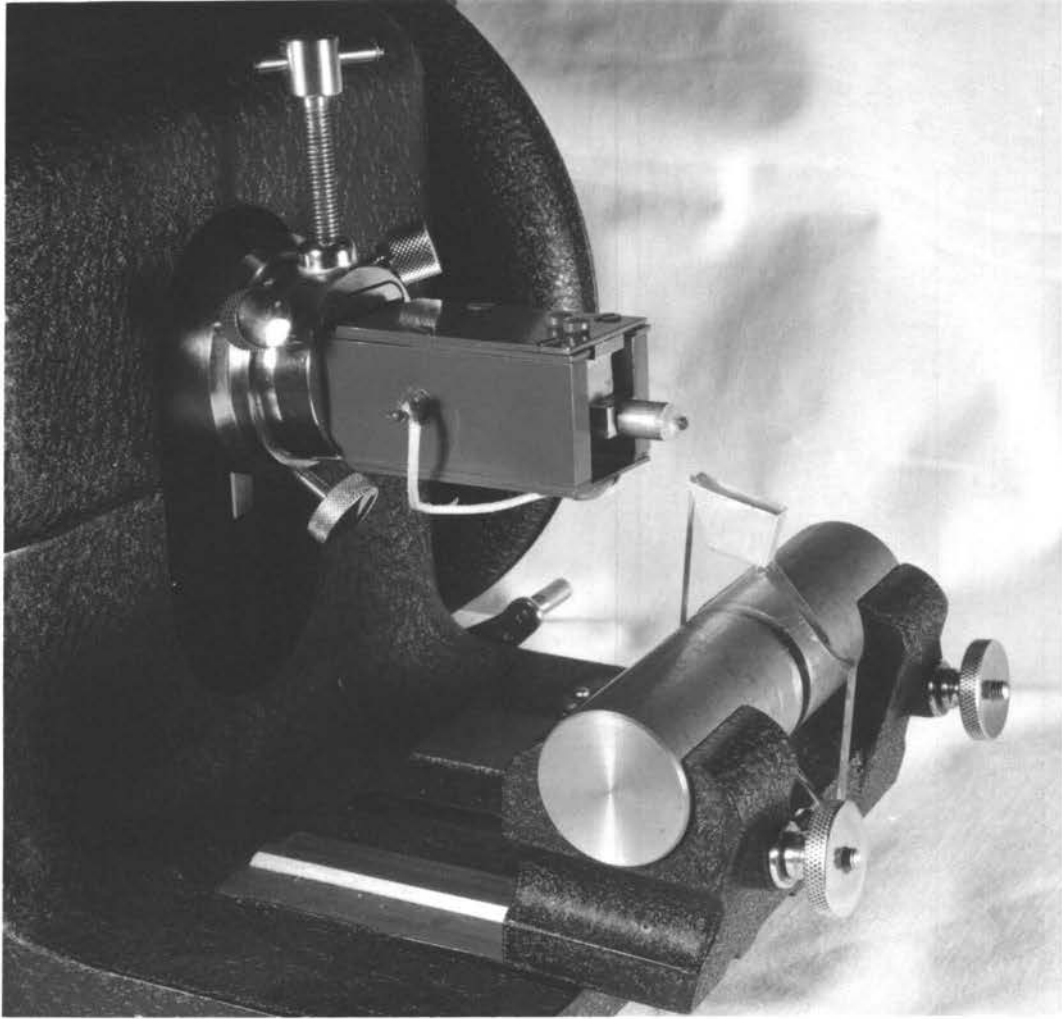


FIG. 11 MICROTOME SET UP FOR SLICING

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