TECHNICS OF PLANT HISTOCHEMISTRY AND VIROLOGY

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PREFACE

Plant histochemistry and the technics used in this field are subjects that have been somewhat neglected in America. The authors have been teaching plant histochemistry for some years and, during this period, have received reports from workers in the plant sciences which indicate that an earlier mimeographed edition of this book has been an aid as a laboratory reference book. We are accordingly having it published with the hope that others may find it useful.

Since most biologists and chemists have not been trained in the use of the polarizing microscope, they are not aware of its numerous applications. We have, accordingly, given considerable space to this subject. Detailed directions for identifying crystalline compounds are given in a simplified form so that one without training in micropetrography may identify crystalline compounds. The uses of the polarizing microscope in studying the fine structure of cell constituents are included also.

As is to be expected from the title, more space is devoted to the histochemical detection of plant constituents than to any other subject. An attempt has been made to give not only the histochemical methods, but also to include a brief treatment of the location and function of the various plant constituents in the cell.

The book has been used as a text for a course in advanced technic in the Plant Pathology Division at the University of California. We have, therefore, included those subjects needed by our students to give them a reasonably complete training in phytopathological technics.

Considerable space is given to modern virus technics because these have not been adequately covered in other books on phytopathological technic.

Short sections on statistics, photography, and photomicrography are included.

We are very grateful to Dr. A. Frey-Wyssling for permission to use form double refraction curves from the book Das Polarisationsmikroskop by Ambronn and Frey; to John Wiley and Sons, Inc., for permission to use certain illustrations; and to the National Fertilizer Association and the American

Society of Agronomy for permission to use information taken from the book Hunger Signs in Crops. We also wish to express our appreciation of the aid given by Dr. Robley C. Williams, who read certain sections of the book and made helpful suggestions.

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I. THE POLARIZING MICROSCOPE

REFRACTION

When light travels obliquely from one medium into another in which the speed is less, it is bent toward a perpendicular drawn into the second medium as shown in Figure 1. When it passes from one medium into another in which the speed is greater, it is bent away from a perpendicular drawn into the second medium. The angle between the incident beam and the normal to the surface is called the angle of incidence; that between the refracted beam and the normal to the surface is the angle of refraction. The bending decreases with a decrease in the angle of incidence until, at normal incidence, no bending occurs.

$$\frac{V = \text{velocity of light in air}}{V' = \text{velocity of light in a second medium}} = N$$

N = refractive index of second medium

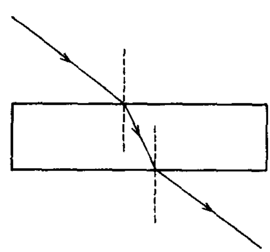

Fig. 1. Showing the refraction of light in passing through media having different refractive indices.

Each crystalline compound has one or more characteristic refractive indices which may be determined and which therefore serve in the identification of such compounds.

Following is given a derivation of the equation used in determining the refractive index of a substance:

In Figure 2 consider a plane wave AC incident obliquely on the smooth plane surface between air and a second transparent medium. Let us assume that the velocity of light in air is twice as great as in the second medium. A spherical wave will diverge from the point A into the second medium when the disturbance reaches that point. While the wave travels in air a distance CC′, the wave from A will travel one-half of this distance in the second medium, or a distance of AA′. Similarly, when the disturbance reaches the point J, a spherical wave will diverge from this point and will travel a distance JK in the second medium while the light is traveling twice this distance, IC′, in air. Thus the new wave front (A′C′) in the second medium is no longer parallel to the wave front AC occurring in air. The angle of refraction is the angle O and the angle of incidence is the angle Y.

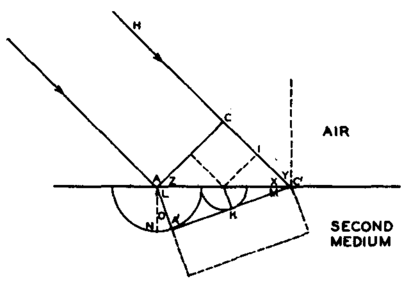

Fig. 2. **Showing the relation of the speed of light to refraction**

Since the sum of the angles in a triangle is 180°, the following conclusions may be drawn:

	angle X + angle Z = 90°
	angle X + angle Y = 90°
	therefore angle Y = angle Z
Similarly	angle L + angle M = 90°
	angle L + angle O = 90°
	therefore angle M = angle O

Now CC' = distance traveled by light in air during one unit of time

AA' = distance traveled by light in second medium during one unit of time

In other words,

CC' = velocity of light in air

and AA' = velocity of light in second medium

By definition, the refractive index

$$(N) = \frac{\text{velocity of light in air}}{\text{velocity of light in second medium}}$$

Therefore $$(N) = \frac{CC'}{AA'}$$

If we divide both the numerator and denominator by AC', we have

$$N = \frac{\frac{CC'}{AC'}}{\frac{AA'}{AC'}}$$

$\frac{CC'}{AC'}$ = sine of angles Z or Y = sine of angle of incidence

$\frac{AA'}{AC'}$ = sine of angles M or O = sine of angle of refraction

Therefore

$$(N) = \frac{\text{sine of angle of incidence}}{\text{sine of angle of refraction}}$$

By measuring the angles of incidence and refraction, and obtaining the value of the sines of these angles from a table of sines, one may determine the refractive index of a substance. This principle is applied in refractometers used for determining the refractive index of a liquid.

POLARIZED LIGHT

Light from the sun or from a lamp is unpolarized (vibrates in all directions perpendicular to the direction of transmission). When parallel unpolarized light passes through a nicol prism (polarizer), it is doubly refracted and resolved into two rays.

Each ray vibrates in a single direction (is polarized); the direction of vibration of one ray being perpendicular to that of the other. The slow ray is refracted more than the fast ray and is absorbed on the black wall of the polarizer (Fig. 3). The fast ray passes through the polarizer, vibrating in a single direction perpendicular to the direction of transmission. The plane containing the direction of vibration of this ray is usually indicated by a mark on the polarizer.

When polarized light strikes an isotropic crystal, it passes through with the plane of vibration unchanged (Fig. 3). The refractive index of an isotropic crystal is not dependent on the direction of vibration or the direction of transmission of the light passing through it, and such crystals exhibit the same refractive index in all positions.

When light from the polarizer is vibrating parallel to either of the vibration planes of an anisotropic crystal, it passes through such a crystal with the plane of vibration unchanged (Fig. 3). When vibrating in other directions, it is resolved into two rays which vibrate in directions which are normal to each other and which travel at different velocities while in the crystal (Fig. 4).

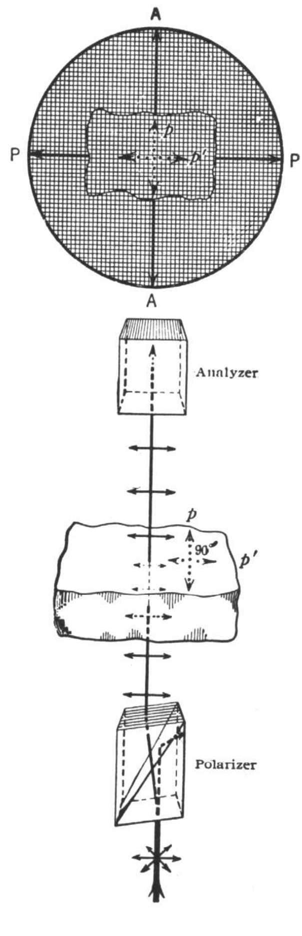

Fig. 3. Showing the behavior of light when an isotropic substance, or an anisotropic substance in an extinction position, is between crossed nicols. (Reproduced by permission from Handbook of Chemical Microscopy, First edition, by E. M. Chamot, and C. W. Mason, published by John Wiley and Sons, Inc., 1930.)

The analyzer is constructed similarly to the polarizer but is rotated 90° on its vertical axis to a "crossed" position. If polarized light vibrating parallel to the fast vibration plane of the analyzer strikes the analyzer from below, it passes through with its vibration plane unchanged; if vibrating parallel to the slow vibration plane of the analyzer, it is refracted to the side of the nicol where it is absorbed by the dark wall (Fig. 3).

If the two rays from a doubly refractive crystal enter the crossed analyzer (Fig. 4) and if the vibration directions of these two rays are not parallel to the vibration planes of the analyzer, each of the rays is resolved into a slow ray and a fast ray. The two slow rays vibrating in the slow vibration plane of the analyzer are refracted to the side of the analyzer, where they are absorbed, and the two fast rays vibrating in the fast vibration plane of the analyzer pass up through the analyzer. The crystal therefore appears bright in a dark field.

With this information in mind, one may explain the appearance of crystals when the polarizer, crystal, and analyzer are in the various possible positions. For example, one may explain why an anisotropic crystal becomes dark (shows extinction) four times when the stage is rotated through 360°.

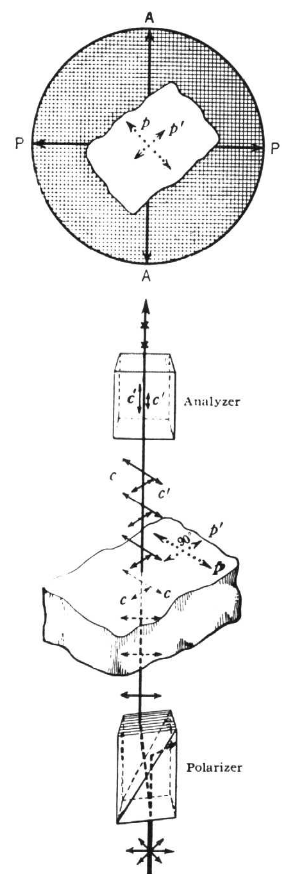

Fig. 4. Behavior of light when an anisotropic substance in a "bright" position is between crossed nicols. (Reproduced by permission from Handbook of Chemical Microscopy, First edition, by E. M. Chamot and C. W. Mason, published by John Wiley and Sons, Inc., 1930.)

RETARDATION

Since the slow ray is retarded more than the fast ray in passing through an anisotropic crystal, certain wave lengths are out of phase when they emerge from the top of the crystal. They then pass through air (each moving at the same velocity) and strike the analyzer, where each is resolved into a slow and a fast ray. The two fast rays pass through the analyzer vibrating in the same direction and therefore moving at the same velocity. Therefore, upon emerging from the analyzer, the retardation of the slower fast ray is the same as that of the slow ray after passing through the crystal.

If one car travels 10 miles per hour and another 20 miles per hour, the first will be retarded 10 miles behind the second at the end of the first hour and 20 miles behind it at the end of the second hour. Similarly, the retardation of the slow ray of polarized light is proportional to the thickness of the crystal.

Thus a given retardation produces a characteristic color, due to the interference of certain wave lengths. The colors

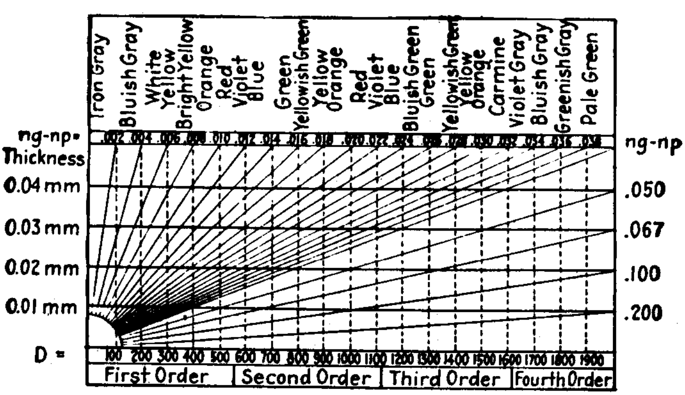

Fig. 5. Showing the relation of interference colors to retardation and birefringence. (Reproduced by permission from Elements of Optical Mineralogy, Fourth edition, by A. N. Winchell, published by John Wiley and Sons, Inc., 1931.)

produced by different amounts of retardation are shown in the polarization color chart (Fig. 5). For example, a retardation (D) of 530 millimicrons produces first-order red.

Since the speed of the two rays depends on the crystalline compound and the direction in which the light passes with reference to the optic axes, the retardation is dependent on these factors in addition to the thickness of the crystal.

If one crystal is placed above another in such a position that the slow ray in each vibrates in the same plane, the total retardation is equal to the sum of the retardations of the two crystals. This is to be expected, since the operation is essentially equivalent to increasing the thickness of one of the crystals. For example, if a thin crystal which produces slight retardation and is therefore white in color is placed with its slow ray parallel with that of the slow ray of a first-order red quartz plate, the crystal appears blue because the retardation produced by the crystal plus that produced by the first-order red plate gives a total retardation which produces a blue interference color of the second order.

If one crystal is placed over another so that the slow ray of one vibrates in a plane perpendicular to that in which the slow ray of the other vibrates, the total retardation is equal to that of the crystal causing the greater retardation less that of the crystal producing the lesser retardation. If the thin white crystal mentioned above were used, the retardation of the first-order red plate would be decreased by an amount equal to that produced by the white crystal and the total retardation would produce an orange interference color. If the crystal were thicker, so that it would produce the same retardation as the first-order red plate, the total retardation would be equal to zero and the crystal would appear dark. This process, whereby the retardation produced by one crystal is reduced to zero, is called compensation. Wedge-shaped quartz plates, called compensators, having a gradually increasing thickness and therefore producing different amounts of retardation according to the portion of the wedge used, may be used to compensate crystals and to measure the retardation produced by them. Methods for measuring low retardation are given by Schmitt and Bear (1937).