Refractive index determination using the central focal masking technique with dispersion colors

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Abstract

The central focal masking ("dispersion staining") technique is convenient and effective for determining the refractive index of a microfragment by the immersion method and for distinguishing between minerals in an immersion mount. For most microscopes the only modification needed is the installation of a small opaque dot at or near the focal point of the medium power objective. White light illumination, stopped down to the angular aperture of the opaque dot, produces a dark field on which the image of the fragment is outlined in diagnostic dispersion color.

Precision of refractive index determination by this technique, about \( \pm 0.001 \) under routine controlled conditions, is similar to that of the conventional Becke line technique using monochromatic yellow illumination. However, it has the advantages that (1) near the match the direction and approximate amount of mismatch may be inferred from the dispersion color of the image alone without the need for manipulation of the focus, (2) at the match the microfragment is clearly visible, and (3) results are obtainable even in the presence of an appreciable amount of inclusions or specific absorption (body color) in the fragment.

Besides providing a useful means for refractive index determination, focal masking permits rapid distinction among constituents in a mixture and an estimation of their proportions. As a teaching aid the focal masking technique provides a convincing demonstration of the manner of image formation and resolution in the microscope.

Introduction

The general term "focal masking" is applied to those techniques of microscope illumination in which selected wavelengths of the image-forming light rays are removed or modified by insertion of a pair of conjugate masks, one in the focal plane of the objective and the other in the focal plane of the substage condensing system. Included here are such well known techniques as dark-field microscopy and phase-contrast microscopy. The chief form dealt with in this paper, called central focal masking, has been used with success for two decades in laboratories of the U.S. Geological Survey for routine mineral identification by the immersion method.

The central focal masking technique as applied to immersion methods is one variant of the "focal screening" techniques of Cherkasov (1955a, 1955b, 1957, 1969; see also Wilcox, 1962; Feklichev, 1963; Hartshorne and Stuart, 1970, p. 311 ff). The same technique is termed "dispersion staining" by Brown and McCrone (1963; also Brown et al., 1963; McCrone et al., 1978). All these techniques have the advantage over conventional Becke line techniques in that no manipulation of focus is required. Rather, the difference between refractive index of fragment and liquid is shown at once for all grains in the field of view by the dispersion colors of their images. The technique is useful further in revealing variations of refractive index within grains, the extent of which may be translated into terms of zoning of chemical composition.

In the immersion method by focal masking, advantage is taken of the fact that the wavelength dispersions of refractive index of the common organic immersion liquids are appreciably greater than those of most inorganic solids of similar index. A typical example is illustrated graphically on Figure 1 for a glass that, at a given temperature, matches an immersion liquid for the orange-yellow light of the standard Fraunhofer D-line wavelength (589.3 nm) at refractive index 1.534. It is seen that for shorter wavelengths (the greens, blues, and violets of the spectrum) the refractive index of the liquid is higher, and for longer wavelengths (the oranges and reds) it is lower than that of the glass. It may be noted also on Figure 1 that the higher liquid (\( n_D = 1.538 \)) matches this glass at wavelength near 650 nm, whereas the lower liquid (\( n_D = 1.530 \)) matches near 520 nm, and that the match for liquid \( n_D = 1.522 \) falls far outside the visible spectrum.

Figure 2 represents the essential behavior of axially parallel rays of white light passing through a fragment
Immersed in such a “matching” liquid. Because upper and lower surfaces in the central part of the fragment are largely at right angles to the incident light, rays of all wavelengths pass without deviation. Near the edge of the fragment, however, the solid/liquid interface is steeply inclined, with the result that the rays of shorter wavelength (the blue and green colors, for which the index of liquid is higher than that of the glass) are refracted toward the normal to the interface. In contrast, those of longer wavelength (orange and red) are refracted away from the normal. (Note that here the sequence of colors of the resulting spectrum is the reverse of that in the commonly illustrated prism dispersion of white light at a solid/air interface, in which case the dispersion of the solid is the greater.) The yellow rays of wavelength near the D-line, pass through without significant deviation, because the refractive indices of solid and liquid are essentially matched for those wavelengths. Those light rays that do not pass through the fragment are not refracted of course and continue on to the objective without change in direction.

All light rays that were undeviated in the immersion mount are gathered by the objective and made to pass through the focal point, whereas the deviated rays pass to the side of the focal point. In central focal masking, an opaque dot at the focal point (Fig. 3), blocks the undeviated rays to produce a dark field. (Specific directions for modification of the microscope are given in Appendix 1.) Superposed on this dark field is a sharp image formed by the rays that are deviated by the edges and surface irregularities of the fragment and passed to the side of the opaque mask at the focal point. Because the rays of color (wavelength) of the match are blocked by the opaque dot, the image of the fragment is seen in the combined color of the remaining parts of the spectrum—essentially the complement of the wavelength band of the match. Thus,
Appendix 2. These include apertural, unilateral, annular, and strip focal masking, the latter two especially well suited for refractive index determination of very fine grained fragments requiring high magnification.

**Determination of refractive index**

**Isotropic substances**

Fragments of an isotropic substance mounted in an immersion liquid and illuminated as described above will be seen outlined on a dark field. If refractive indices of fragments and liquid are far apart, the fragments appear dark with bright outlines. In such case the direction of mismatch is revealed by the conventional Becke line test. Based on this test, successive immersion liquids are chosen until one is found in which the fragment outlines take on a pellucid color, indicating the approach to a match in index. With the fragments in focus, and remembering that a match is being sought for the index at the wavelength of the D-line (Fig. 1), the following may be inferred: If the edges and surface irregularities of the fragments appear light-blue or blue-green, the refractive index of the fragments is slightly lower than the liquid; if purple, red, or orange, refractive index of the fragments is slightly higher; if deep violet, refractive index of the fragments is very close to a match with the liquid. The colors observed at focus in representative situations are described in Figure 4, column 2 and serve as a general guide. Because of the possible different connotations of these descriptors to different people, however, and because of different spectral compositions of light sources of different microscopes, each person should standardize his or her own color judgment and microscope system, especially in the immediate vicinity of the match of indices. For this a procedure is outlined in the section “Calibration of color perception” (Appendix 3).

Fig. 4. Dispersion colors observed in focal masking, modified from Cherkasov (1957). Colors may vary somewhat depending on spectral composition of the illumination.
measured temperature variation, one may change the temperature to achieve the final match of index, then make the appropriate correction to the nominal index of the liquid.

Anisotropic substances

The procedure outlined in most textbooks for determining the principal refractive indices, $\epsilon$ and $\omega$ of uniaxial substances and $\alpha$, $\beta$, and $\gamma$ of biaxial substances, involves the use of random multiple grain mounts in a succession of liquids of different refractive indices (see Stoiber and Morse, 1972; Bloss, 1961). By observation of interference figures, it is possible in many mounts to find a fragment close to the correct orientation for a particular principal index, which then may be compared to the liquid by focal masking and the liquid for the next random grain mount chosen accordingly. The method, however, is time consuming and requires experience, patience, and perseverance as well as careful cross checking to obtain reliable values of principal refractive indices. Further, it may be greatly complicated and even misleading when the fragmented sample contains two or more anisotropic minerals of similar or overlapping optical properties, or when the fragmented sample comes from a chemically-zoned crystal belonging to an isomorphous mineral series.

For maximum definitive information gained with minimum time and effort, the spindle stage offers by far the most effective method for determination of the optical properties of an anisotropic material, because all principal refractive indices as well as other diagnostic properties may be obtained on a single fragment (Wilcox, 1959; Hartshorne and Stuart, 1970; Bloss and Light, 1973; Bloss, 1981). This at once eliminates the uncertainties and complexities of random grain mounts of a powdered sample composed of more than one substance or of a range of compositions. (A number of fragments, of course, should be so examined to ascertain the range of properties or the presence of more than one substance.) Orientation of the fragment for each principal index is carried out conoscopically by interference figures, where-upon the illumination is converted to central focal masking for determination of that principal refractive index following the procedure described above for isotropic fragments. Lacking favorable conditions for conoscopic orientation, the values of $\alpha$, $\beta$, and $\gamma$ of a biaxial fragment can be determined as maxima and minima of index encountered along the two series of extinction positions (Wilcox 1959, p. 1282; Bloss, 1981), changing liquids as necessary and using the criteria of dispersion colors.

Interpolation and extrapolation

In determining the refractive index of an unknown solid by mounting its fragments successively in liquids of a regularly graduated set of immersion liquids, one arrives finally either at the liquid that matches the solid, or at two adjacent liquids that bracket the index of the solid. In the latter case if the spacing of liquids in the set is small, for example 0.002, one may interpolate the index of the solid directly with sufficient precision for routine work. If the spacing is appreciable, one may use a Hartmann net (Stoiber and Morse, 1974, p. 74), on which the dispersion curves of the liquids are drawn as straight lines in accordance with the dispersion data for the liquids. This is illustrated by Figure 5 for the case of two adjacent liquids of $n_D$ indices 1.570 and 1.580, having dispersion $n_F - n_C$ of 0.0190 and 0.0200, respectively. Judging from dispersion colors (as listed in Fig. 4, column 2), the $\beta$-index of the anhydrite fragment matches the 1.570 liquid at a wavelength about 500 nm, and the 1.580 liquid at about 650 nm. The line between these respective points of intersection with the liquid curves represents the dispersion curve of the solid, and it intersects the wavelength of the D-line, (589.3 nm) at $n_D = 1.577$.

Should the dispersion of the solid as well as that of the liquid be known already, as in dealing with a member of a known solid solution series (plagioclase, olivine, certain glasses, etc.), an extrapolation to the refractive index of the solid for the standard D-line could be made from the results of only one immersion mount, in which the solid has been observed in a diagnostic clear dispersion color. Thus in the Hartmann net of Figure 6, a liquid $n_D = 1.540$...
and dispersion \( n_F - n_C = 0.0164 \) matches the \( \alpha \)-index of a plagioclase of dispersion \( n_F - n_C = 0.0083 \) at wavelength about 520 nm, as inferred from the reddish-orange dispersion color under central focal masking (Fig. 4, column 2). The solid’s dispersion curve through this match point intersects the D-line at 1.543, which may be taken then as the \( \alpha \)-index of the plagioclase.

Figure 7 relates index difference, dispersion difference, and observed color. For the previous example the dispersion difference between solid and liquid, \( (n_F - n_C)_{\text{liq}} - (n_F - n_C)_{\text{sol}} = 0.0081 \), may be projected vertically from 0.0081 on the abscissa to intersect the inclined line for the match at 520 nm, thence horizontally to read the index difference as \((-) 0.003\) on the ordinate scale.

Substituting in the expression

\[
\text{Index difference} = n_{D(\text{liq})} - n_{D(\text{sol})} = -0.003 = 1.540 - n_{D(\text{sol})}
\]

then

\[
n_{D(\text{sol})} = 1.543
\]

It is obvious that accuracy will be poorer the greater the distance of extrapolation to the D-line and the less certain the estimate of the wavelength of match.

**Precision and limitations of the technique**

For refractive index determination by the central focal masking technique with calibrated immersion liquids at intervals of 0.002, a precision of about \( \pm 0.001 \) can be obtained when careful attention is paid to corrections for temperatures above or below the temperature at which the immersion liquid was calibrated. This is similar to the precision attainable on favorable material by the conventional Becke line ("central illumination") method using monochromatic sodium light. The precision with central
focal masking stands up well for less-than-ideal natural substances, whereas that of the conventional Becke line method deteriorates in several common situations: for instance, when abundant foreign inclusions are present in the substance under investigation, edges of the fragment do not approach wedge- or lens-shape, or the mineral has appreciable color or absorption. As compared to the method of "Becke line colors" of Emmons and Gates (1948), the central focal masking method has somewhat greater precision, probably because the diagnostic colors are not diluted by the white light of the field and therefore are more definite and easily recognized. (If the immersion liquid is itself highly colored, however, account must also be taken of this color added to that of the dispersion color at the wedge edge of the fragment.)

A factor affecting precision in central focal masking is the amount of difference in dispersion between the solid and the near-matching liquid. The greater this difference, the greater is the range of mismatch over which the dispersion colors are seen. Precision will therefore fall off somewhat for greater differences in dispersion, as may be inferred by examination of Figure 7. It might appear that, conversely, the precision should be better for very low differences in dispersion between solid and liquid, but a practical limit is soon reached because the angular aperture of the spectrum produced becomes so small that most of it is blocked by the opaque dot. In this circumstance, the index of the liquid must be very close to that of the solid before any dispersion color is seen, and the color itself is muddy because now only the colors at opposite ends of the spectrum come through.

Thus, taking the expression \((n_F - n_C)\) for the dispersion color of the solid and \((n_F - n_C)\) for the dispersion color of the liquid, we have

\[
\Delta n = \frac{\sum (n_F - n_C)}{N}
\]

where \(N\) is the number of substances. This expression gives the average difference in dispersion between the solid and liquid.

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Fig. 8. Plot of dispersion vs. refractive index for selected organic immersion liquids and inorganic solids. Values for solids taken from Winchell (1929, table V) and Winchell and Winchell (1964, p. 137.)
as a measure of dispersion differences, values about 0.010 to 0.020 appear to be well suited for routine work with most minerals. Examination of Figure 8 shows, however, that for the commonly available immersion sets (Cargille “Certified”; Butler, 1933) dispersion differences are smaller than desired for work with many low-index minerals and higher than desired for many high-index minerals. To improve the situation in the low-index range a special set of liquids was formulated (Wilcox, 1964) from mixtures of ethyl cinnamate (nD = 1.558, nF - nC = 0.028) and glyceryl triacetate (nD = 1.429, nF - nC = 0.007). This series can be extended to higher refractive index by mixture with α-iodonaphthalene (nD = 1.700, nF - nC = 0.037). A few inorganic solids (for instance, certain chromates, vanadates, and HgS) and probably quite a number of organic solids have dispersions greater than that of the matching organic immersion liquid. In such rarely encountered cases the movements of the colored Becke lines (Fig. 4, columns 3 and 4) are reversed, as are the combined colors above and below the match for the D-line. Nevertheless, at the match the dispersion color is the same deep violet.

Additional applications

Some other applications of focal masking techniques, mainly utilizing the difference in dispersion colors for different degrees of index mismatch, are worth mention here. To estimate the proportions of quartz, microcline, and oligoclase in a granite, for example, a sample of the pulverized rock may be mounted in a liquid of index and oligoclase in a granite, for example, a sample of the main utilizing the difference in dispersion colors for orange and can be counted in comparison to the oligoclase grains (nD = 1.534 to 1.545), which shows colors from purple to strong blue, and the microcline (nD = 1.519 to 1.525), which shows cold bright blue color. ( Conceivably, the choice of counting the frequency distribution of the mineral constituents might be taken over by an automated microscope scanner fitted with appropriate color filters.) A related important problem in industrial hygiene is the determination of the amount of quartz particles in the aspirable fraction of dust, such as in a quarry or mine operation (Crossmon, 1966). In control of a beneficiation process, the product may be mounted in an immersion liquid that gives a distinctive coloration to the unwanted constituent, from which the degree of remaining contamination may be estimated.

For a rough estimate of average and range of refractive index of an isotropic substance, such as volcanic glass, one may make multi-grain mounts in a series of liquids at closely-spaced index intervals. With focal masking illumination, one then takes as the average (really the mode) the index of the liquid in which about half the grains are above and half below the liquid index. If a chart of composition vs. refractive index is available, this value can be used further to infer the average chemical composition.

This approach can be extended to estimate the “average” composition of a sample of an anisotropic mineral of low birefringence in an isomorphous series. An example would be a powdered concentrate of rock-forming plagioclase. Here again one makes a series of immersion mounts and chooses as an “average” the index of the liquid in which, under focal masking illumination, about as many grains of plagioclase are of greater as are of lesser index. Entering this value on a chart of anorthite-content vs. principal refractive indices, a rough “average” anorthite content may be read off from the intersection with an imaginary line of “average” refractive index along the trend of the α, β, and γ lines. This rests on the assumptions that the distribution of compositions is unimodal and preferential orientation due to cleavages do not appreciable affect the results.

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References

Appendix I
Modification of the microscope

A conventional petrographic microscope may be converted to central focal masking by (1) installing an opaque stop of small diameter at the focal point of the medium power objective and (2) restricting the aperture of the substage iris diaphragm to pass only axially parallel or nearly parallel rays.

The opaque stop may consist of a 2 to 3 mm diameter dot of opaque ink—e.g., India ink or “Lab-ink”—centered on a thin glass disc, such as a cover glass, of proper size to fit in the barrel of the objective. This glass disc, which should be unstrained and should have plane-parallel surfaces, is then cemented at or near the level of the focal point of the objective. (The presence of the dot does not noticeably handicap the use of this objective for ordinary observational work.) For the medium power objectives of Zeiss and Leitz, the focal point is within the barrel of the objective, requiring disassembly to reach the correct placement by experiment. For the medium power objective of Unitron (10×, 0.25 N.A. for MPS model petrographic microscope) disassembly is not necessary; a 15-mm diameter dot-bearing cover-glass may be cemented on the apertural collar at the rear of the objective barrel. This inexpensive objective may be used on the Leitz Dialux Pol microscopes directly and on other polarizing microscopes with an adapter and centering collar. McCrone Associates (2820 S. Michigan Ave., Chicago, Ill. 60616) provide a “Dispersion Staining Objective” with appropriately built-in masks.

The required narrow pencil of nearly parallel light rays may be produced in the substage by closing down the iris of the substage apertural diaphragm until its image, as viewed with the Bertrand lens, is just slightly smaller in diameter than that of the opaque dot in the objective (Fig. A1a). With the immersion mount in place on the stage of the microscope, the substage centering screws are adjusted to shift the image of the iris so that it lies wholly behind that of the opaque dot of the objective (Fig. A1b). Then with the Bertrand lens withdrawn, the images of the fragments in the immersion mount are displayed on the dark field.

If, as is sometimes the case, the coverglass of the immersion mount does not lie strictly perpendicular to the microscope axis, rotation of the microscope stage to a new setting may result in partial illumination of the field, whereupon insertion of the Bertrand lens reveals that the image of the substage opening has shifted from behind the opaque dot. This may be corrected in the new setting by readjusting the centering screws of the substage to once more shift the image of the substage iris opening behind the opaque dot. (It is of course necessary to recenter the substage prior to resumption of work with conventional illumination.)

A more convenient arrangement for repeated repositioning of the substage opening employs a separate apertural stop, fashioned by hand from thin metal stock for insertion into the substage near the level of the iris diaphragm. A protruding handle on this stop permits it to be shifted laterally as the occasion requires. Starting with metal foil, a small hole is punched with a needle and enlarged in successive trials until the size of its image is correct for the opaque dot. If desired, a more
substantial stop may then be constructed by drilling a hole of this size in heavier stock. (The design of the Leitz Dialux Pol microscope permits yet another position for the constructed stop—it may be laid directly on the illuminator window in the base of the microscope, apparently a conjugate focal plane of the lens system, where it is easily accessible for lateral shifting as needed to compensate for ray deflection.)

Appendix 2

Alternative modes of focal masking

While not as easily adapted as central focal masking for use on most petrographic microscopes, alternative configurations, known as apertural focal masking, unilateral focal masking, annular focal masking, and strip focal masking, may also be useful in certain situations and may be helpful in demonstrating the manner of image formation in the microscope.

Apertural focal masking

The term apertural masking is preferred here as being more descriptive than "annular masking", used for this configuration by Cherkasov (1957; see also Brown and McCrone, 1963). The term annular masking is here reserved for the true ring-shaped configuration (see below).

In apertural masking (Cherkasov, 1957; Wilcox, 1962; Harshorne and Stuart, 1970) a constricted apertural stop is substituted for the opaque dot of central masking at the focal point of the objective, as represented diagramatically in Figure A2. The apertural mask may consist of a thin metal disk in the center of which a hole of appropriate size has been drilled or punched. Alternatively, a universal stage objective, such as Leitz No. UM-3, may be modified to enable drastically constricting the iris diaphragm. The apertural mask is also available as part of the "Dispersion Staining Objective" of McCrone Associates.

The apertural mask passes the rays of wavelength for which indices of solid and liquid match, but blocks those for nonmatching wavelengths, thus it is just the converse of central focal masking. The image is displayed on a dimly lit field in the color (wavelength) for which the indices match. A match near the wavelength of the D-line, 589.3 nm, is indicated when the image of the edges of the fragment is deep orangish-yellow, as listed in Figure 4, column 5; on the other hand, if the image is green or blue, the index of the fragment is greater than that of the liquid; if orange or red, it is less than that of the liquid. Liquids may be changed to obtain or to bracket the diagnostic deep orangish-yellow color of the D-line. If the borders of the fragment are dark and without apparent color, the mismatch is large, and the direction of mismatch may be determined by converting to ordinary illumination for the Becke line test.

Apertural focal masking has the advantage of simplicity, in that it displays the image directly in the color of the wavelength of refractive index match. Yet a sharp focus of the image cannot be obtained due to the very small angular aperture. This is critical for small fragments, because their diagnostic dispersion colors are lost in the background of the light field. As a teaching aid, however, apertural masking provides a convincing demonstration of dispersion phenomena and also "empty magnification", that is, the loss of resolving power when the angular aperture of the lens system is drastically reduced.

Unilateral focal masking

In unilateral focal masking (Cherkasov, 1957; Wilcox, 1962) a circular mask of about a third of the full aperture is placed in the objective, and the much smaller apertural mask in the substage is shifted laterally until, with the Bertrand lens in place, its image may be seen to be partly hidden behind the edge of the objective mask. The bundle of parallel light rays is then at an angle to the microscope axis, as illustrated in Figure A3.

This arrangement is analogous to the well known method of oblique illumination (method of Schroeder van der Kolk, 1906), and the colors appearing on opposite edges of the fragment are interpreted in the same manner. If fragment and liquid differ widely in refractive index, the fragment is seen to have high relief without color. If solid and liquid are at or near a match, paired colors appear on opposite sides of the fragment and are to be used as listed in Figure 4, columns 6 and 7. Upon shifting the substage aperture a bit farther off center so that it is completely behind the objective mask, the orthoscopic field becomes dark, and the paired colors change to those as listed in columns 8 and 9. Whereas the image definition is as sharp as in central focal masking, the color effects are complex and somewhat more difficult to standardize in terms of index match.
Fig. A3. Representation of the formation of the dispersion color image using unilateral focal masking when refractive indices of fragment and liquid match in the region of the yellow wavelengths.

**Annular focal masking**

Where particles are so small that a high magnification objective is required, the pinhole light source of central focal masking, described above, may not provide sufficient intensity from the usual microscope illuminator to produce readable dispersion colors. A solution suggested by H. Piller (written comm., December 1963; see also Correns and Piller, 1974, p. 407; Schmidling, 1981) employs the same arrangement as in phase contrast microscopy, except that in the objective an opaque ring takes the place of the phase ring. A complementary annular opening in the substage admits much more light and provides brilliant dispersion colors, even with high-N.A. oil immersion objectives.

The question arises whether an illuminating system of such high angular aperture permits accurate determination of a principal refractive index of an anisotropic fragment, which necessarily must be oriented so that its corresponding privileged vibration direction is (1) parallel with the plane of the polarizer and (2) perpendicular to the microscope axis. It is seen from Figure A4 that the vibration directions of the strongly convergent light rays from sectors A and C of the annular opening in the substage will be inclined to the privileged direction of the oriented crystal. It would therefore be expected that the observed dispersion color will have been modified to some extent by the addition of components representing non-principal refractive indices, and thus that one might be led to infer an incorrect refractive index. Similar color contamination is to be expected in the phase contrast technique, as pointed out by Saylor (1966), and in the dark-field technique of Dodge (1948) and Crossmon (1948), in which the maximum angular aperture of the substage is used.

An actual test shows, however, that the error of the inferred principal index for routine work may only be significant for crystals of extreme birefringence. Using an opaque ring objective (modified Zeiss Ph 2, 40×, 0.75 N.A.) and complementary annular opening in the substage, a fragment of aragonite ($\gamma - \alpha = 0.155$) was oriented with its acute bisectrix sensibly parallel to the microscope axis and with $\alpha$ perpendicular to the microscope axis in the plane of the polarizer. From the “match” dispersion color $\alpha$ was inferred to be 1.534, but using an opaque dot objective the inferred correct index was 1.530, a difference of 0.004. Further tests indicated that the error is much smaller for crystals of only “strong” birefringence and is undetectable in crystals of moderate or low birefringence.

**Strip focal masking**

That the source of the color contamination in the above test of the annular mask is indeed in sectors A and C of Figure A4 is demonstrated by inserting masks in the substage to lie over these sectors, whereupon the dispersion colors became normal and the true index can be inferred. One should therefore be able to avoid the contamination entirely by use of a straight illuminating slit across the center in the substage (Fig. A5a) and a complementary strip mask in the objective, as suggested by Saylor (1966, p. 67) for the analogous problem in phase contrast methods. Narrow flared strips (Fig. A5b) conceivably would provide still greater illuminating intensity without introducing noticeable color contamination.

Fig. A4. Substage condensing system for annular focal masking: (a) vertical section in vibration plane of polarizer, (b) bottom view of substage annular opening.

Fig. A5. Alternative forms of substage opening for strip focal masking: (a) simple strip opening, (b) flared opening for increased illumination.
Appendix 3

Calibration of color perception

The success of the method requires correct interpretation of the color effects observed when fragment and liquid are at or near a match in refractive index. Rather than depending on Figure 4, each observer should "calibrate" his or her own color perception for a particular microscope under known conditions of match and mismatch. For this the following procedure is suggested:

1. Adjust the microscope for conventional illumination with light of the D-line wavelength. This may be obtained from the well-known NaCl fragment on a Bunsen burner, a sodium vapor lamp, or more conveniently, a graduated interference filter, or appropriate monochromatic interference filter, such as Schott-Jena, Type "IL" or "PL".

2. Mount fragments of a homogeneous isotropic substance (for instance, crushed fragments of coverglass or object slide) in successive immersion liquids until a precise match is obtained as judged by the Beckeline test, or more sensitively by inclined illumination.

3. Without otherwise changing the mount or the temperature, convert to central focal masking illumination, and at focus observe and fix mentally the shade of dispersion color seen at the edges and surface irregularities of the fragment (the "deep violet" of Fig. 4, column 2). Also observe the colors of the oppositely moving Becke lines as the focus is raised slightly (Fig. 4, columns 3 and 4).

4. In mounts 0.001 or 0.002 above and below that of the index match, observe and fix mentally the dispersion colors at focus and with slightly raised focus.

In carrying out the above procedure, it will be noted that the steeper the margin of a fragment the greater is the intensity of the color. With an index match at the D-line, the steep edge may appear brighter and more bluish than the "deep violet" of the less steep edges. This is because a larger proportion of the visible spectrum is enabled to pass the dot mask, even though it is centered about the same wavelength of true match. In practice any possible confusion of the wavelength of match may be resolved by also observing the fragments in liquids of index just above and just below that of the match. In the liquid of lower index, the color of the fragment edge takes on a definitely purplish or reddish hue, whereas in the liquid of higher index, the image is definitely blue or greenish blue. The refractive index of the fragment for the D-line may therefore be taken with confidence to lie between those of the two liquids.

With very thick fragments, it is not possible to bring the entire height of a steep edge into focus at the same time, and the two colored Becke lines persist at all levels of focus. Here it may be possible to obtain the combination of the two Becke lines at minor irregularities of the surface of the fragment. Otherwise one may use the colors of the deliberately separated Becke lines, as listed in Figure 4, column 3 and 4.