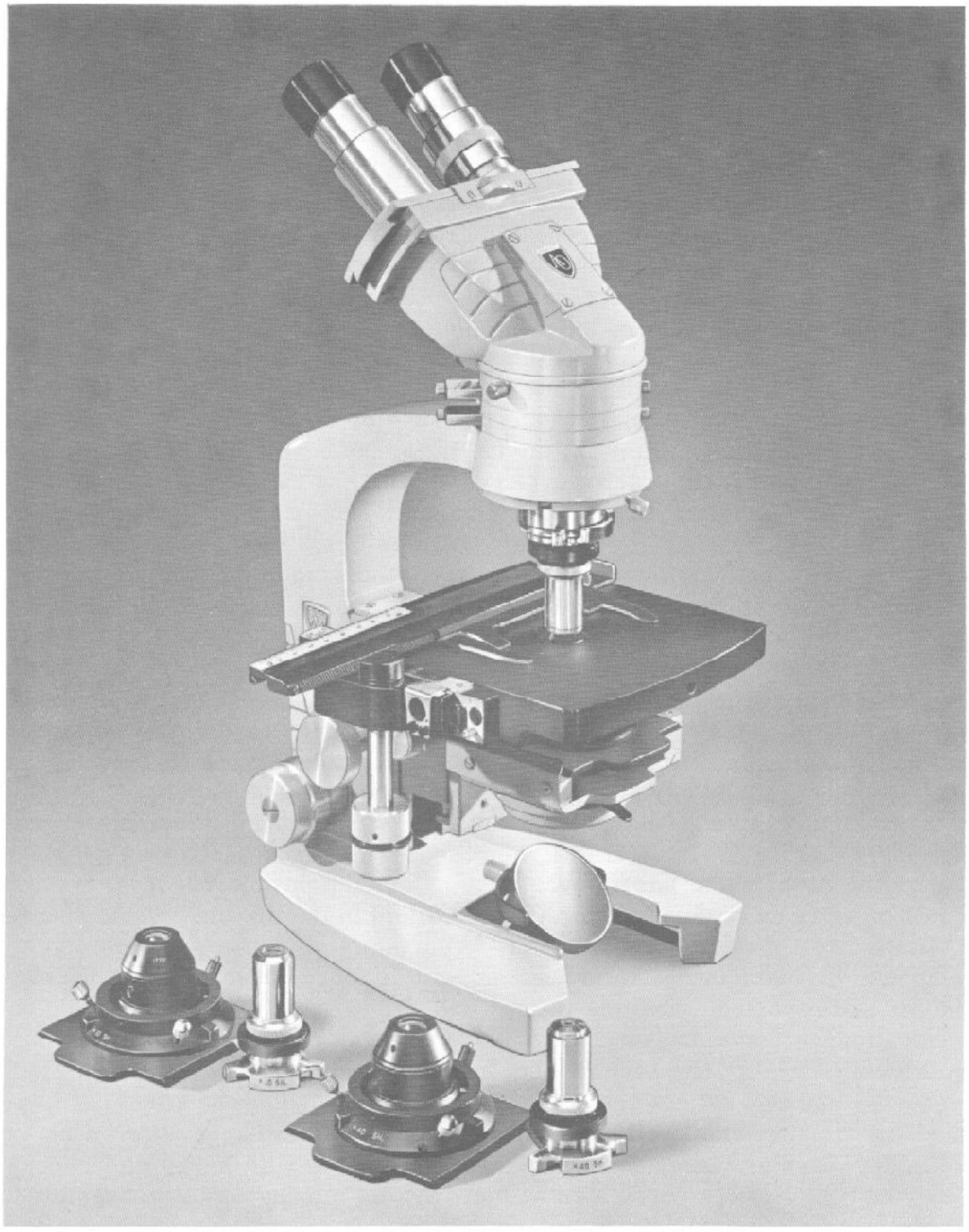


AO Baker Interference Microscope Model 7BG-QSW

TABLE OF CONTENTS

	PAGE
I. Introduction	4
II. Phase and Interference Microscopy	5
III. The AO Baker Microscope	5
IV. Microscope Adjustment - Variable Contrast	8
V. Specimen Preparation	10
VI. Interference Colors	10
VII. Quantitative Interference Microscopy	11
VIII. Selected References	20
IX. Set-Up Check List	27

I. INTRODUCTION

Interferometry, while well established in other fields, has only recently been applied in microscopy. An early device measured optical thickness*[77] and the first biological application[87] may be the work of Frederikse[62]. Multiple beam methods are extensively used for the examination of surfaces of opaque materials having good reflection[64, 93] and Merton's work[87-89] stimulated the application to transparent materials[32]. Two beam interference microscopes[15] appeared shortly thereafter.

In the multiple beam method, the specimen is mounted between two flat metalized reflecting surfaces and illuminated with parallel monochromatic light. The recombinations from repeated reflections of the light through the specimen produce fringes which are used to measure the optical path differences within reasonably transparent specimens. Optical path is the product of thickness and the refractive index of the specimen.

In the two beam interference microscope coherent illumination (from a single source) is divided so that part of the light passes in focus through the specimen and the rest of the light passes to one side of the specimen, or is out of focus at the specimen. On reuniting the light, the beams interfere to produce measurable patterns from which one can determine the path differences of the specimen. The beam separation can be accomplished by reflection, as in the Cooke-Dyson[30] microscope, or by polarization as in the Baker-Smith[29] microscope. The Françon Interference eyepiece is a shearing interferometer.[31]

Interference microscopes reveal the optical path difference detail within the specimen in variable colors with white light, or of intensity with monochromatic light. When the thickness of the specimen is known, the refractive index can be measured and in some cases, when the specimen can be placed in media of different known refractive indices, both thickness and index can be measured. When the path difference is due to known substances their concentrations can be estimated. The wet and dry mass can be determined without damage to the cells of some living organisms. Barer[1-5], Davies[7, 48], Mitchison[24] and others have pioneered in this field. Interference microscopes have increased vertical resolution, but have the same lateral resolution as other light microscopes.

Changes in mass during growth[25], of colloid in the thyroid[65] from changed diet, in the liver of fasting and fed mice[74], during fertilization and embryonic development[24], and from calcium accumulation in blood vessels[54] have been measured and reported. Enzyme action[20b, 37a, 43], hemolysis[81] and hemaglobin concentration[21, 78] are measurable. Tissue cultures can be examined[18, 42]. Muscle structure[39, 73] has been studied including its damage by virus[17]. The nebenkern[27] and cell details are being investigated including the effects of fixatives[22, 42, 67]. Bacterial cell size is measurable[28, 63]. Large differences were found in dry mass of cancer cords[46]. Good correlation with X-ray microscopy is reported[43, 47]. When correlated with ultraviolet microscopy it is possible to measure protein and nucleic acid concentrations. Interference microscopy and chemical methods correlate closely in dry weight of isolated thymus nuclei[66]. Refractive index and protein concentration of cytological components are measured[5, 27]. Other transparent materials, as glass, plastics, emulsions, etc. can be examined. Hydrolysis, partial solution and changing concentration may be measured. Commercial applications are not as promptly published although some are known to be under way.

*Superscripts refer to citations in the reference list.

II. PHASE AND INTERFERENCE MICROSCOPY

Nearly transparent materials having optical path differences can be seen with both interference and phase microscopes[8, 38]. Phase contrast is accomplished in the phase microscope by the recombination in the image of the direct light with the light deviated by the specimen after modification by a diffraction plate. Optical path differences and small absorption differences may be involved and a more correct term, densiphase contrast, has been proposed (38 p. 11). As the phase microscope redistributes the light in the image, haloes are often seen around the observed details; although a proper choice of diffraction plate lessens the haloes. The phase microscope makes visible the optical path differences, but not their numerical magnitude. Phase microscopes have been modified to provide variable contrast, but not to measure densiphase detail.

Interference contrast is accomplished by the recombination in the image of two beams of coherent light (from the same source), one having been modified by passing through the specimen. Most interference microscopes do not produce haloes around the details. The interference microscope provides variable color contrast with white light illumination, intensity variation in the color of the monochromatic light when monochromatic light is used, and with monochromatic light measurement of the optical path differences in the specimen. Differences in absorption are not directly measurable with present interference microscopes.

Gradients within a specimen are shown to advantage with phase while larger regions of the same path size are revealed better by the interference microscope. Both methods may be used for examination of unstained, living cells and tissues.

III. THE AO BAKER INTERFERENCE MICROSCOPE (Figure 1.)

This microscope is fundamentally a polarizing microscope modified into a two-beam interferometer. The condenser has a birefringent plate which divides the light into two beams and the objective has a corresponding plate which recombines the beams after one of them has passed through the specimen. Above the objective is a quarter-wave compensator and an analyzer. Various eyepieces may be used to obtain different magnifications with the Shearing or Double Focus types of 10X, 40X and 100X objectives.

The polarizer, figure 2, underneath the condenser polarizes the light in a plane at 45° to the axis of the birefringent plate. The birefringent plate at the top of the condenser separates the polarized light into two beams which are plane-polarized at right angles to each other. One beam passes through the specimen, and the other passes to one side of the specimen in the Shearing system. In the Double Focus system one beam focuses at the specimen and the other spreads around the specimen to focus above it. The phase of the beam passing through the specimen is changed by the local variations in optical thickness in each portion of the specimen; while the changes in the reference beam depend on the average optical thickness of the specimen and the region around it in the Double Focus system; or the region to one side of the specimen in the Shearing system, figure 3.

The birefringent plate on the front of the objective unites the two beams and the quarter-wave plate changes the two oppositely polarized beams into left

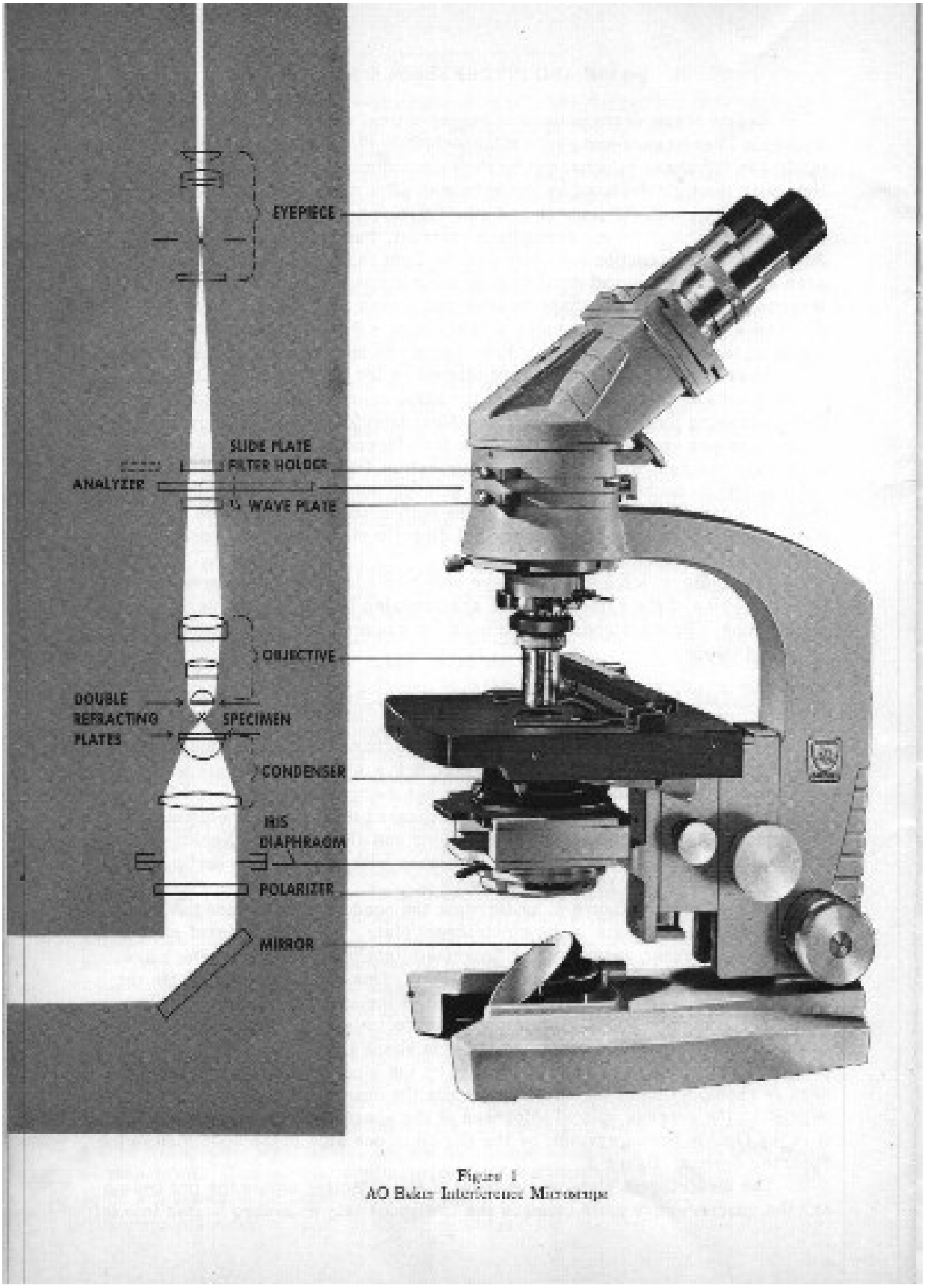
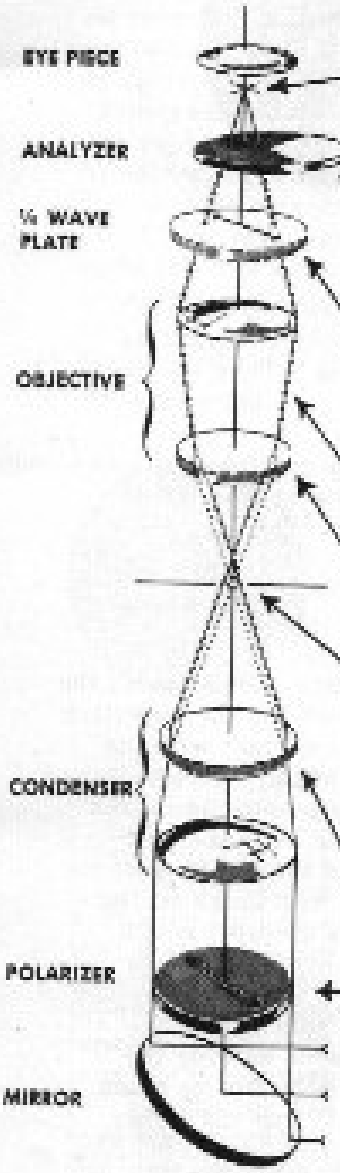


Figure 1
AO Baker Interference Microscope



The specimen detail is darker or lighter relative to the background depending on the analyzer position, and in color when white light is used. The angle difference between the minimum brightness setting for the background and for the specimen is 1/2 the phase shift introduced by the specimen.

The analyzer measures the angle of the plane of the polarized light.

The ordinary and extraordinary plane polarized beams are converted into counter-rotating circularly polarized beams which combine into plane polarized light.

The ordinary and the extraordinary rays travel along common ray paths.

The separated beams are recombined by the doubly refracting plate at the front of the objective.

The ordinary and extraordinary rays converge through different levels of object space. The specimen detail at the focus of the ordinary rays introduces an optical path difference, or phase shift, of the ordinary relative to the out of focus extraordinary rays.

The polarized light is separated into the ordinary and extraordinary beams by a doubly refracting plate at the top of the condenser.

The light is plane polarized at 45° to the optic axes of the doubly refracting plates.

Figure 2
Optical System of AO Baker Interference Microscope (double focus system)

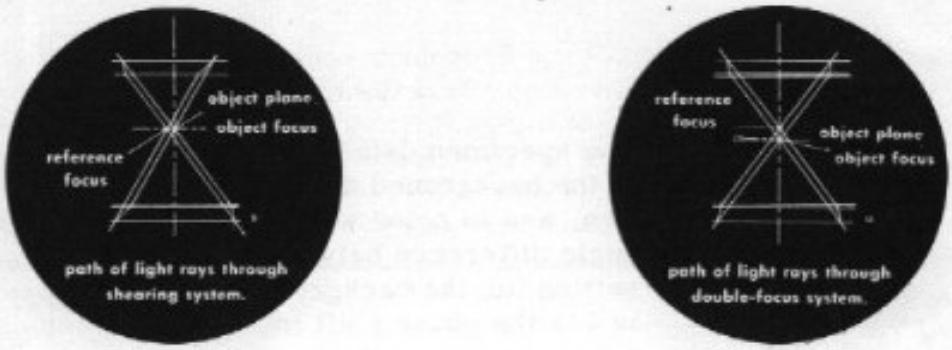


Figure 3

and right-handed circularly polarized light. The resultant of two circularly polarized beams is plane polarized light, the direction of the plane depending on the phase difference between the circularly polarized beams. Thus the phase differences in the specimen can be determined by turning the analyser to the position of minimum luminance, or extinction, in the image.

A vector theory[3, 5] and an integration theory[13] are proposed for the mathematical analysis of the performance of the microscope. Some knowledge of polarization microscopy is helpful in interference microscopy.[10, 11].

IV. MICROSCOPE ADJUSTMENT - VARIABLE CONTRAST

(For a brief check-list of procedures see page 27, Section IX)

Each objective has a matched condenser. To use the microscope insert the proper condenser and objective; be certain the condenser mount and the objective mount (if microscope has revolving nosepiece) come into firm contact with the stand. The Köhler method of illumination is preferable and readily accomplished by focusing the image of the source of a No. 735C or 390B lamp onto the closed iris diaphragm of the microscope condenser. The lamp should be placed far enough from the microscope that the image fills the opening of the condenser being used. With mercury arcs the image is not as sharp as with tungsten filament, but usually the edge or center part of the arc stream is visible enough for the focusing. Closing the iris diaphragm of the illuminator will improve visibility of the source.

Then focus the microscope condenser so that the iris diaphragm of the lamp is in focus with the specimen. With transparent specimens closing the microscope condenser may aid this preliminary focusing. Any diffusing glass should be removed from the lamp filter holder. It is essential that the light beam be centered on the microscope mirror.

Turn the control lever of the polarizer at the lower side of the microscope condenser to "Int.", insert the quarter-wave plate and turn the analyzer to about 110° if you are using a Double Focus condenser and objective, or with a Shearing (SH) condenser and objective turn the analyzer to the setting indicated on the card accompanying the condensers.

Remove the ocular, place the telescope in the ocular tube and focus the telescope on the back aperture of the objective. A ground glass should be placed between the lamp and the microscope and any other filters removed. A uniform band of color or a series of bands (fringes) should be seen, figure 4. Turning

the tilting screws at the front of the condenser-mount in opposite directions moves the fringes over the aperture. Find the middle darkest fringe (zero order) and try spreading the next fringe to it as uniformly over the aperture as possible by turning the tilting screws in the same direction. Should it not spread over the aperture, try the other first order fringe on the opposite side of darkest fringe. Use the one that spreads best over the aperture. If neither of the center one spreads well, see instruction 10, p. 28. Remove the telescope and replace the ocular and the specimen with slight refocusing should be visible in colors. Readjust the mirror, if necessary, so that the field is evenly lighted. Variable color contrast occurs as the analyzer is rotated.

For ordinary observation, a tungsten light source may be used. The instrument is most sensitive when the zero order, central darkest, fringe is spread over the aperture, but more brilliant colors result when the first order fringes are used. Should more intensity be required, a more powerful lamp will be necessary and the mercury arcs are used, even though there is less red in the mercury light.

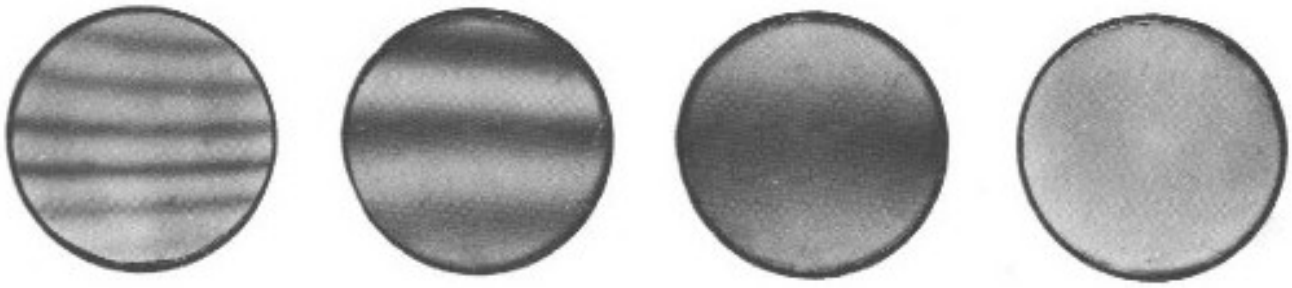


Figure 4
Back aperture of objective as seen with the telescope, showing the centering and spreading of a fringe.

In the Double Focus system, the reference beam passes around the specimen and the specimen should be much smaller than the area occupied by the reference beam. This area varies with different objectives. If the reference beam does pass through part of the specimen, then the combined effect depends on the sum of the optical paths of the specimen and the reference medium. However, when it is used only to see detail in variable contrast it is possible to use large tissue sections as the haloes are averaged out.

No haloes are formed with the Shearing objectives and condensers. The specimen must be smaller than the amount of shearing or overlapping will occur and interpretation of the colors will be made difficult. For specimen size, see Section VII B on measurement.

Turning the polarizer to the position marked "Off", sliding the quarter-wave plate out and turning the analyzer to the ungraduated part of the scale "Out" converts the Interference Microscope into a useful bright field microscope. The imagery may not be quite as good as a regular bright field microscope, because the other extra optical elements are still in the microscope even though the interference is made inoperative and the specimen is examined in plane polarized light.

V. SPECIMEN PREPARATION

The Interference Microscopes reveal the total optical path which includes the slide, mounting medium, specimen and coverglass. Any waviness, defects in the glass, or inhomogeneity in the mounting medium will produce artifacts and errors. Even with better than average quality cover glasses and slides and with care in mounting the specimen, some wedge usually is present in the slide-medium-cover glass combination. This will show as a change in luminance as the stage is turned, or in different readings depending on the orientation of the sample. A 2μ difference in thickness of a slide 25mm wide produces a measurable wedge error.

Only good grade selected slides and coverglasses, free from scratches and other defects should be used. The objectives are corrected for 0.18mm thick cover glasses. Purchase of No. 1-1/2 covers will give a better yield of this thickness than No. 1's or No. 2's. When greater working distance is required, thinner covers may be used when the added spherical aberration is unimportant. The tilting of the condenser reduces the working distance so that thinner slides may be useful, when of good quality glass.

Specimen preparation otherwise is much the same as for phase microscopy (Ref. 38. pp. 172-182). Mounting fluids should be filtered or centrifuged to be particle free for interference microscopy. Wet preparations should be sealed and the cover glass kept as nearly parallel to the slide as possible.

VI. INTERFERENCE COLORS

Light passes through an optically denser medium more slowly than in a less dense medium and is retarded with respect to light through the less dense medium. The amount of retardation (phase difference) is proportional to the difference in refractive index for the particular wavelength considered.

For example, should the denser regions of a specimen illuminated with light from a mercury arc retard the blue light exactly one-half wavelength and the analyzer be set to extinguish the blue light, the specimen then would be seen only in the remaining yellow-green light.

With tuhgsten light the blue is not limited to such a single wavelength as the mercury arc, but is a band of light ($\pm$440 - 490mμ). A single particle cannot retard exactly to a half wavelength all of these blue wavelengths, therefore some blue will be lost and some transmitted and the particle will appear more or less yellow depending on the amount of blue lost.

Phase changes affect other colors in a similar manner and the actual interference colors depend on the composition of the light from the illuminator

Table I. VISUAL EFFICIENCY OF ISOLATING FILTERS
FOR THE H100-A4 MERCURY ARC
(Based on nominal values from manufacturer's literature)

Mercury line	Blue, 0.436μ		Green, 0.546μ		Yellow 0.577μ	
Relative energy	80%		100%		88%	
Eye Relative luminosity (100 at 0.555μ)	1.8		98.4		89.8	
Filter	% Trans.#	Rel. Vis.*	% Trans.#	Rel. Vis.*	% Trans.#	Rel. Vis.*
Corning CS4-120	0	—	44	43	0	—
Corning CS-584	22	3	0	—	0	—
Ilford 625	0	—	35	34	8	6
Wratten 50	6.4	0.9	0	0	0	0
Wratten 62	0	—	10	9.8	0.5	0.4
Wratten 74	0	—	10	9.8	0.2	0.2
Wratten 77A	0	—	68	67	0	0
Wratten 77A + 58	0	—	29	28	0	0
Wratten 77	0	—	74	73	0.5	0.4
Wratten 77 + 58	0	—	31	31	0.06	—
Wratten 58	0	—	42	31	11	9
Wratten 22	0	—	0	—	71	62

*Relative luminosity = Relative energy × filter transmission × relative luminosity of the ICI Standard Observer.

#Trans. = transmittance.

and on how the optical paths in the specimen retard or advance each wavelength (color). The relative amount of each wavelength passing through the analyzer determines the color of the particle.

Light is radiation to which the eye is sensitive (380-740Mμ) and can be seen. Interference microscopy is possible with invisible, infrared and ultraviolet radiation with receptors sensitive to the radiation used. Some instruments will be more useful than others. The AO-Baker should be used with light as its polarizing system is not designed for other radiation.

VII. QUANTITATIVE INTERFERENCE MICROSCOPY

A. Illumination and filters

Measurement of the optical path requires the use of monochromatic light to avoid the color interferences mentioned in section VI. Monochromatic light is light of a single wavelength and is usually obtained from a single line of a spectral source. As the mercury arc has most of its radiation concentrated in a few lines it is the usual source for such illumination. The mercury arc has the further advantage in that the green line of 546mμ wavelength is quite close to the maximum sensitivity of the human eye (555mμ). Sodium light is suitable, although not as comfortable for visual use.

To isolate the light from a single line in the mercury spectrum a filter is used that transmits the light from the desired line and absorbs the light from the other bright lines in the spectrum of this source.

Some filters for use with mercury arcs are listed in Table I. The least expensive is the Wratten 62 or 74, but these transmit only about 10% of the green light and do not exclude the light from the yellow line. The Corning CS4-120 transmits more of the green and no yellow. The Wratten 77 and 77A also transmit more green light than the 62 or 74, but for monochromatic light need to be combined with the 58 filter which reduces the light correspondingly. Filters for isolating the blue and yellow mercury lines are included in the table.

For some measurement, where the highest precision is not required, approximately monochromatic light is adequate and may be obtained with tungsten lamps and "narrow band" filters or with "interference" filters. The latter often have the disadvantage of low transmission.

The H85-C3 or H100-A4 (formerly AH3 and AH4) mercury arcs are satisfactory for many visual applications, but require long exposure when photomicrographs are to be made. More intense mercury arc sources such as the B-T-H 250 and the Osram HB0200 give more light, especially when monochromatic light is used, and are desirable for photography.

Table I. VISUAL EFFICIENCY OF ISOLATING FILTERS
FOR THE H100-A4 MERCURY ARC
(Based on nominal values from manufacturer's literature)

Mercury line	Blue, 0.436μ		Green, 0.546μ		Yellow 0.577μ	
Relative energy	80%		100%		88%	
Eye Relative luminosity (100 at 0.555μ)	1.8		98.4		89.8	
Filter	% Trans.#	Rel. Vis.*	% Trans.#	Rel. Vis.*	% Trans.#	Rel. Vis.*
Corning CS4-120	0	—	44	43	0	—
Corning CS-584	22	3	0	—	0	—
Ilford 625	0	—	35	34	8	6
Wratten 50	6.4	0.9	0	0	0	0
Wratten 62	0	—	10	9.8	0.5	0.4
Wratten 74	0	—	10	9.8	0.2	0.2
Wratten 77A	0	—	68	67	0	0
Wratten 77A + 58	0	—	29	28	0	0
Wratten 77	0	—	74	73	0.5	0.4
Wratten 77 + 58	0	—	31	31	0.06	—
Wratten 58	0	—	42	31	11	9
Wratten 22	0	—	0	—	71	62

*Relative luminosity = Relative energy × filter transmission × relative luminosity of the ICI Standard Observer.

#Trans. = transmittance.

Light from the mercury arc without a color filter can be used for variable color contrast microscopy although it will be seen that the mercury light has very little orange and red as compared to tungsten or daylight. When used with a filter the path differences in the specimen are seen only in the color of the filter, but with variable intensity.

B. Measurement with the AO Baker Interference Microscope

Light from the lamp is made monochromatic with an appropriate filter and the microscope is adjusted as described in sections IV or IX.

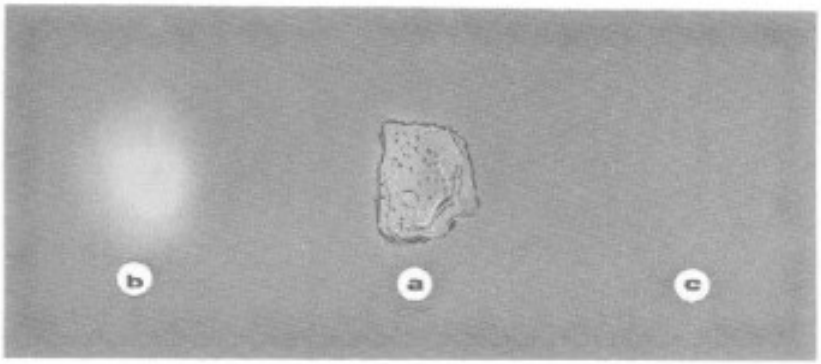


Figure 5. (a) Epithelial Cell
(b) Out-of-focus image of epithelial cell (c) Clear area

The Shearing Objectives and Condensers are preferred for measurement, because the greater displacement of the reference beam permits the use of larger specimens. With these optics an out-of-focus, astigmatic image of the specimen will be seen to one side of the specimen, figure 5, (left side when microscope arm is between the lens system and the operator and in color complimentary to that of the specimen with white light illumination). The corresponding area on the opposite side of the specimen should also be clean and free from materials that alter the reference beam, or shear over onto the specimen, otherwise the measurement of the specimen will be in error by the amount of the overlapping interference. The specimen should not be larger than the central 330μ of the field for the 10X objective, 160μ for the 40X and 27μ for the 100X objective.

The area available for the specimen with the Double Focus Objective is less: 90μ for the 10X and 40X objectives and 20μ for the 100X objective. The reference beam in the Double Focus system is out-of-focus in the plane of the specimen and less affected by an inhomogeneous surround than the Shearing system. This is an advantage for the Double Focus system when used for variable contrast microscopy. The Double Focus system is not recommended for quantitative measurements because of uncertainty in optical path encountered by the reference beam.

More care in specimen preparation is called for in quantitative interference microscopy. The immersion fluid for the 100X objective should be distilled water, free from any inclusions, and centrifugation of the immersion fluid and of the medium to remove foreign materials is desirable.

The measurement is made by rotating the analyzer until the background area close to the specimen is as dark as possible and reading the position in degrees on the analyser scale. Then the specimen, or region of the specimen to be measured, is made as dark as possible and the position of the analyzer is noted. The path difference in degrees is twice the difference between the two readings.

When there are a number of regions within a specimen, e.g. parts of an individual cell, that are to be measured, and when it can be established that the largest phase difference to be measured is less than a half wavelength of the monochromatic light used, then the following matching method can be used.

Determine the position of the analyzer for the extinction of the surround (darkest). The analyzer is then turned until the surround and the outer portion of the specimen being measured appear equally dark, or light, and match in intensity. The optical path is then four times the difference between the readings. There is another match point where both surround and specimen are bright. For measurement use the position where they are equally luminous, but dark.

The matching process may be continued, e.g. with a cell the cytoplasm can be measured with reference to the surround, then the nucleus with reference to the cytoplasm, etc.

Enough measurements should be made to make sure that the reference area is homogeneous and free from gradients. Rotating the specimen and measuring will indicate the presence or absence of any wedge or gradients. Slight variations may be averaged out.

The correct direction to rotate the analyzer in transferring the extinction from the surround to the specimen is determined by the optical path of the specimen. The microscope is adjusted, figure 6, so that a retardation in the object beam requires a clockwise turning of the analyzer (as one looks down on the scale) with the 10X and 40X objectives; counterclockwise for the Double Focus 100X.

When it is known that the specimen advances or retards the light of the in-focus beam, the direction to turn the anlayzer is determined. When this is not known, or the specimen is too weak for the Becke line test, the analyzer should be turned in the direction which causes the detail to darken as soon as rotation starts.

c. Close condenser iris to exclude non-uniform outer area. At least 2/3rds of the aperture should be filled.

E. Double focus system
Turn analyzer so it is in the light path (on the graduated part of the scale) and then spread either fringe next to the central darkest one over the aperture. One screw can be used to spread the fringe and the other to check any rotation of the fringe pattern. Do not close the iris very much as the full aperture is needed for the passage of the reference beam.

7. Replace eyepiece and the specimen should be seen in color, which may be varied by rotating the analyzer. Open and close the iris of the condenser slightly to obtain the best contrast. (Note 6E above).

8. Immersion 100X objectives
   A. Handle with extreme care as the front lens is mounted flush and easily damaged.
   B. Use only distilled water that is free from any suspended particles. Centrifuging may be necessary to remove particles.
   C. Immerse the objective to the cover glass. Do NOT immerse the condenser.
   D. Do not use immersion oil, nor organic solvents to clean the lens.

9. Measuring (See also Section VII B, C & D)
   A. Place the isolating filter on the Hg arc.
   B. Recheck back aperture with telescope for uniformity.
   C. i. Determine the position of the analyzer that makes the background near the specimen darkest and read scale value on the analyzer.
      ii. Turn analyzer to position making detail to be measured darkest and read analyzer scale.
      iii. The optical path difference in degrees is two times the difference between the readings.
   D. Alternative matching method. Find darkest position for the reference region and then adjust the analyzer until the reference region and detail match in intensity, i.e. are equally dark. The path in degrees with this method is four times the difference of the two readings.
   E. Make several measurements of the reference region to make sure it is homogeneous. Also make a measurement with the specimen rotated 90^{o}, 180^{o} to determine whether there is any error from a wedge in the specimen or mount.
   F. Be sure there is no dust on the polarizer; or in the immersion fluid when the 100X objectives are used.
   G. If the extinction occurs in the ungraduated part of the analyzer, turn it 180^{o} to get back onto the scale.

10. A. Centration. The iris of the lamp should be concentric with the field when the lamp is in line with the microscope and the light is carefully centered in the mirror.

B. The microscope condenser iris should be concentric with the back aperture of the objective (as seen with the telescope) when the best fringe is spread over the aperture. Usually only one fringe can be so spread. The instrument is most sensitive when the central darkest zero order fringe is used. For variable contrast, especially in color, either of the first order fringes beside the central darkest fringe is preferred and the microscopes are so adjusted at the factory.

C. When no fringe can be well spread, it may be necessary to loosen the upper front screw, figure 7, on the condenser and to rotate the condenser slightly until the best fringe spreads most uniformly. This rotates one calcite plate with respect to the other. Should the zero order fringe be used, it may be necessary to rotate the condenser.

Figure 7

D. Tilting the condenser moves the image of the microscope iris. When this is too far from centered, the 3 centering screws of the condenser should be loosened and the condenser moved to center. Then the fringe spread should be checked and the field stop centered by moving the mirror. Sometimes several approximations are required to get good contrast.

E. Do not move the objective or else all this centering will have to be redone! The objectives are locked into the nosepiece and should not be removed.

11. Quarter waveplate. With no objective or condenser on the stand, turn the analyzer until the field appears darkest. Pushing the quarter waveplate in and out should not noticeably change the darkness. Also examine a specimen known to have greater optical path than its surround, e. g. section VII C-2 and note whether its extinction follows the diagram of figure 5. Failure to meet these tests indicates improper orientation of the quarter waveplate.

12. Slide Plate filter holder. The AO Baker Microscope is equipped with a slide plate containing one clear aperture and a green filter, figure 1. The green filter may be used with a mercury arc lamp or a tungsten lamp to provide filtered or approximately monochromatic light suitable for variable intensity contrast. Precise measurements require the use of filters with greater transmission selectivity; see section VII, A.

13. More than usual cleanliness of mirror, polarizer, condenser, objective and analyzer surfaces, slides and cover glasses, is necessary in interference microscopy. This is a polarizing instrument utilizing strain-free optics. Avoid excessive heat from the illuminator and any rough handling of the optics.

c. Close condenser iris to exclude non-uniform outer area. At least 2/3rds of the aperture should be filled.

E. Double focus system
Turn analyzer so it is in the light path (on the graduated part of the scale) and then spread either fringe next to the central darkest one over the aperture. One screw can be used to spread the fringe and the other to check any rotation of the fringe pattern. Do not close the iris very much as the full aperture is needed for the passage of the reference beam.

7. Replace eyepiece and the specimen should be seen in color, which may be varied by rotating the analyzer. Open and close the iris of the condenser slightly to obtain the best contrast. (Note 6E above).

8. Immersion 100X objectives
   A. Handle with extreme care as the front lens is mounted flush and easily damaged.
   B. Use only distilled water that is free from any suspended particles. Centrifuging may be necessary to remove particles.
   C. Immerse the objective to the cover glass. Do NOT immerse the condenser.
   D. Do not use immersion oil, nor organic solvents to clean the lens.

9. Measuring (See also Section VII B, C & D)
   A. Place the isolating filter on the Hg arc.
   B. Recheck back aperture with telescope for uniformity.
   C. i. Determine the position of the analyzer that makes the background near the specimen darkest and read scale value on the analyzer.
      ii. Turn analyzer to position making detail to be measured darkest and read analyzer scale.
      iii. The optical path difference in degrees is two times the difference between the readings.
   D. Alternative matching method. Find darkest position for the reference region and then adjust the analyzer until the reference region and detail match in intensity, i.e. are equally dark. The path in degrees with this method is four times the difference of the two readings.
   E. Make several measurements of the reference region to make sure it is homogeneous. Also make a measurement with the specimen rotated 90°, 180° to determine whether there is any error from a wedge in the specimen or mount.
   F. Be sure there is no dust on the polarizer; or in the immersion fluid when the 100X objectives are used.
   G. If the extinction occurs in the ungraduated part of the analyzer, turn it 180° to get back onto the scale.

10. A. Centration. The iris of the lamp should be concentric with the field when the lamp is in line with the microscope and the light is carefully centered in the mirror.

100. Talmon, F. R., & J. G. Wood, 1956. Fringe spacing in interference microscopes. J. Sci. Inst. 33:236-238.

IX. SET-UP CHECK LIST

1. Place the corresponding objective and condenser on the microscope! Be sure condenser and nosepiece slides (if revolving nosepiece is supplied) are pushed completely in to the end of their slides.

2. Arrange illumination:
   - A. 735-type.
     - a. Center the filaments and intermesh the filament and its reflected image by adjusting the mirror.
     - b. Remove ground or blue glasses.
   - B. 390B-type, or mercury arcs. Use only with proper reactance transformer on alternating current.
     - a. Center the arc to the center of the lenses of the illuminator.
     - b. Remove filters.
   - C. Set lamp at such distance from the microscope that its filament or arc image fills the aperture in the microscope condenser diaphragm, and that the light is centered onto the mirror. Focus the filament or arc image on the condenser iris diaphragm.

3. Place a specimen on the stage and focus on it. With very transparent specimens closing the condenser, as necessary, aids seeing. Move the specimen so that the reference beam is in a clear area, figure 4.

4. Focus the microscope condenser so that the image of the lamp iris is in focus with the specimen.

5. Put the telescope in the ocular tube in place of the eyepiece.

6. Bands of colored light should be seen across the aperture of the objective, figure 3. Moving the specimen to one side or out of the field viewed is helpful while spreading the fringes when the specimen has much structure.
   - A. Open the microscope condenser iris to fill the aperture.
   - B. Insert the $\lambda/4$ plate and turn the polarizer to the on position (Graduated part of the scale).
   - C. Place a ground glass on the lamp.
   - D. Shearing system.
     - a. Set the analyzer at the position indicated on the card accompanying the objectives.
     - b. Turn the tilting screws in opposite directions, or turn only one screw, to bring the central darkest band of the fringe system to the center of the aperture, move the fringes until the next fringe centers in the aperture, and then turn both screws in the same direction to spread the fringe so as to fill as much of the aperture as uniformly as possible. Holding the fringes in the horizontal position is best. If this fringe does not spread well try the one on the opposite side of the central darkest fringe. If no fringe appears to spread properly see item 10C.