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The Reflecting Microscope[1]

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THE USE OF REFLECTING SYSTEMS of mirror-pairs in microscope objectives extends the range of achromatism of the microscope through the entire optical spectrum—the infrared, visible, ultraviolet, and vacuum ultraviolet regions. This constitutes the most significant advance since the microscope was designed for use in the ultraviolet region by Köhler (*38*) and combined with quantitative spectroscopic techniques by Caspersson (*16*). The reflecting microscope, which historically dates back about three centuries, has been developed in England, Russia, and the United States. Burch (*14*) in 1939 designed a reflecting objective of numerical aperture (N.A.) 0.65 with an aspheric mirror-pair of Schwarzschild (*52*) aplanats, spherically corrected and coma-free. In 1940 Gershgorin, Radchenko, and Brumberg (*13*) extended the system of Maksutov (*43*) and designed a reflecting objective with a spheric mirror-pair and N.A. 0.5. The combination of reflection and refraction was used in objectives designed by Linfoot (*41*) in 1938 and by Johnson (*32*) in 1941. Grey (*28, 29*) in 1949 described a series of microscope objectives of N.A. 0.4 to 1.0 in which Schwarzschild pairs of spheric mirrors are combined with refracting components. Objectives with spheric mirror-pairs have been designed by Seeds and Wilkins and by Kavanagh (*55, 37*). Drew (*22*) has described a solid reflecting objective.

Specifications for various designs of reflecting objectives are given in Table 1. The linear obscuring ratio of numerical aperture has a maximum permissible value of about 0.4 (*23*), beyond which a deterioration of the image may occur. Burch (*15*) has used aspheric mirror-pairs to reduce the fraction of the numerical aperture obstructed by the convex mirror to 0.2 or less and has added a normal-incidence immersion lens, the surface of which is spherical and concentric with the axial object point, to achieve N.A. 0.98. The transmission of all-mirror systems is limited only by the reflectivity of surfaces and extends through the infrared, visible, and ultraviolet regions. The transmission limits of combined reflection and refraction systems are determined by the elements in the refracting component, which include quartz and fluorite.

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TABLE 1

EXAMPLES OF REFLECTING OBJECTIVES

Designer	Type	N.A.	E.F.L.	M.N. O.R.	Made by
Burch (*14, 15*)	A	0.65	3.0	0.2	
	AI	.98	3.0	< 0.2	
Brumberg *et al.* (*13*)	S	.50	6.0		
Grey (*28, 29*)	S	.4	2.4	0.4	BL
	SR	.72	2.8	0.3	BL
Kavanagh (*37*)	S	.56	3.2	0.5	AO
	SI	.85	2.8	< 0.5	AO
Seeds and Wilkins (*55*)	S	0.65	2.6	0.4	RJB

S, spheric, *A*, aspheric mirror systems.
R, refracting, *I*, immersion components.
O.R., linear obscuring ratio of numerical aperture.
BL, Bausch & Lomb Optical Co.; *AO*, American Optical Co.; *RJB*, R. & J. Beck, Ltd.

Optical systems for the application of the reflecting microscope to absorption microspectroscopy in the infrared, visible, ultraviolet and vacuum ultraviolet regions, and to fluorescence and emission microspectroscopy are described herewith.

INSTRUMENTATION

I. *Monochromator, reflecting microscope, and radiation detector.* This system (Fig. 1) is used for microscopy and photomicrography: ultraviolet (*12, 39, 40, 47*), visible, fluorescence (*46*), polarized light (*47*), and color translating (*13, 40, 7, 45*), and for microphotometry and absorption microspectroscopy (*42, 47, 46, 54*) in the ultraviolet and the visible regions, with photographic or photoelectric detectors, and in the infrared region with thermal detectors. There are two principal methods, which are the equivalents of Köhler and critical illumination, for illuminating a reflecting microscope with a monochromator. In the usual method (*a*) the prism or the grating of the monochromator is imaged in the plane of the object, the field of the objective but not the aperture may be filled with light as the slit width is decreased, and all portions of the field are illuminated with the same intensity and spectral distribution of light. In the alternative method (*b*) the exit slit of the monochromator is imaged in the plane of the object, the dimensions of the field but not the aperture decrease with the slit width, and there may be variation in the intensity and the spectral distribution of light in the field. Method (*a*) is preferred for microscopy and photomicrography, whereas method (*b*) is useful for absorption microspectroscopy at high spectral resolution. The general equation relating the optics of the microscope and the monochromator is as follows (*42*):

$$\frac{fc}{d_c} \div \frac{fo}{d_o} = \frac{d_s}{d_f}\left(1 + \frac{1}{M}\right), \qquad (1)$$

where $\frac{fc}{d_c}$ and $\frac{fo}{d_o}$ are, respectively, the aperture numbers (focal lengths divided by linear apertures) of the monochromator collimator and microscope objective, d_s is the monochromator slit width, and d_f and M are, respectively, the field of view and the magnification of the microscope objective.

An example of this system is illustrated in Fig. 2.

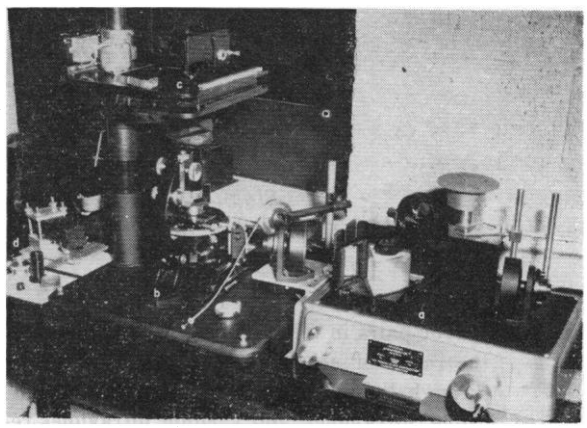

FIG. 2. Achromatic monochromator (*a*); reflecting microscope (*b*); plate board (*c*), with rotating sector and 35-mm camera; and (*d*) objectives, polarizer and analyzer, focusing ground glass, and photomultiplier tube.

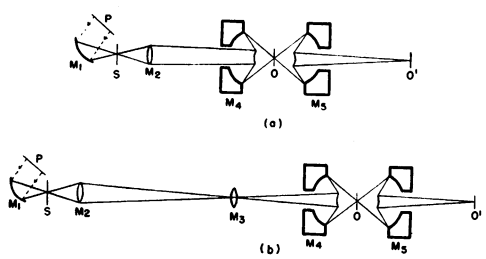

FIG. 1. Achromatic monochromator, reflecting microscope, and detector. Two methods of illumination: (*a*) the grating or prism, *P*, is imaged in the object plane, *O*; (*b*) the exit slit, *S*, is imaged in the object plane, *O*. Suitable detector is placed in the image plane, *O'*.

The monochromator (Perkin-Elmer) is achromatic throughout the optical spectrum, may employ either a prism (quartz, calcium or lithium fluoride, sodium chloride) and Littrow mirror or a grating, and has effective aperture numbers, 4.5 and 6.0, respectively, in the axes of slit height and width. Accessory reflecting optics designed by Scott (*53*) permit illumination in accordance with method (*b*), Fig. 1. The reflecting microscope has a condenser and objective with N.A. 0.72, aperture number 0.5, and design by Grey (*29*). A rotating step sector is placed at the image plane for plate calibration in photographic photometry (*18*). A photomultiplier tube (RCA 1P28) is used for photoelectric photometry (*8*). Investigations with plane polarized ultraviolet light are carried out with a modified (*1*) Glen-Foucault prism polarizer and analyzer. For color-translating ultraviolet microscopy (*40*) a 35-mm camera is used, which very accurately indexes successive frames. A system for

phase contrast observations in visible light employs an accessory illuminator (Bausch and Lomb) which projects an optical annular diaphragm into the back focal plane of the reflecting condenser.

If the values for the aperture numbers of the monochromator collimator and microscope objective, together with that for the field of view, 0.15 mm, are substituted in equation (1), the slit width, d_s, is found to be 1.8 mm. At 250 mμ the linear dispersion at the exit slit of the monochromator is 2.0 mμ per mm for a fused quartz prism, so that the required band pass is 3.6 mμ. When the microscope is illuminated by method (*b*), Fig. 1, the objective aperture and a useful, although not a complete, field of view are filled with a band pass of 1.2 mμ.

An achromatic monochromator (Bausch and Lomb), especially designed by Foster (*26*) for the illumination of a reflecting microscope in accordance with method (*a*), Fig. 1, has an effective aperture number, 4.4, and a Wadsworth mounting of a 50-mm replica grating, which is blazed for first-order ultraviolet light so that there is a very high efficiency in the region of 230–260 mμ. The linear dispersion in the first order is 6.6 mμ per mm, and the band pass required to fill both field and aperture of the reflecting objective with N.A. 0.72 is 10 mμ.

Recent advances in instrumentation which utilize system (I) include a continuous recording ultraviolet and visible microspectrophotometer designed by Sinsheimer (*56*) under the supervision of Loofbourow and a color-translating ultraviolet microscope designed by Land *et al.* (*40*). The recording spectrophotometer has a hydrogen arc source, a Wadsworth mounted concave grating monochromator, a reflecting microscope, and a photomultiplier tube as a radiation detector. A comparison beam from the monochromator output and the exit beam from the microscope are recombined, after being chopped at different frequencies, and are superimposed on the same aspect of a photomultiplier tube. The signals are amplified and separated, the comparison beam signal is used to control the voltage applied to the photomultiplier tube, and the compensated signal is then sent through a logarithmic amplifier into a recording potentiometer.

The color-translating principle first suggested by Brumberg (*7*) has been utilized in a microscope designed by Land *et al.* (*40*), which makes it possible to use sequentially three different ultraviolet wavelengths and to convert the ultraviolet images so obtained into visible images in three primary additive colors. When these three images are superimposed on a viewing screen a visible image in full color is obtained. The complete instrument includes the following components: a source of ultraviolet radiation; a Wadsworth-type grating monochromator; an automatic exposure control system; a reflecting microscope with optics designed by Grey (*29*); a 35-mm camera; a film-processing station for developing, fixing, and drying 3 adjacent frames in 10–20 seconds; and a triple-beam projector and viewing screen. Other means of ultraviolet image conversion have been considered (*34*) and are under investigation.

II. *Illuminator, reflecting microscope, and spectrograph.* This system (Fig. 3) is used for absorption (*46, 42, 2, 35, 48*) and fluorescence (*46, 48*) microspectrography of cells, solids, and solutions in the ultraviolet, visible and, potentially, the vacuum ultraviolet regions, and for microemission spectrography (*42*). A reflecting condenser focuses the spectrum of the source on the object, and a reflecting objective images the object on the slit of a prism or grating spectrograph. The images are dispersed in the spectrograph and refocused on the photographic plate as a series of discrete or overlapping monochromatic images. A condition that is to be met in order to fill the aperture of the spectrograph with light is as follows (*42*):

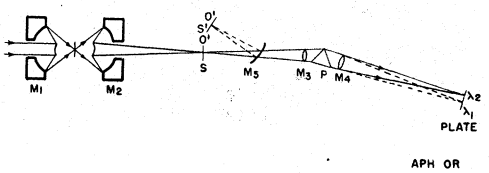

FIG. 3. Illuminator, reflecting microscope, and spectrograph, or spectrometer. Reflecting condenser, M_1, focuses spectrum of source on object. Reflecting objective, M_2, images object, O, on slit, S, of spectrograph. Images, O^1, are dispersed by grating or prism, P, and refocused by lens, M_4, on photographic plate. In alternative method, M_5 reimages O^1 at entrance slit, S^1, of spectrometer. For fluorescence spectrography suitable filter is inserted proximal to M_1.

$$\frac{fc}{d_c} \div \frac{fo}{d_o} \geqq M + 1, \qquad (2)$$

where $\frac{fc}{d_c}$ and $\frac{fo}{d_o}$ are, respectively, the aperture numbers of the spectrograph collimator and the microscope objective, and M is the objective magnification.

In absorption microspectrography a comparison is made between the photographic density of the spectral images of the source with and without the object imaged in the slit. For fluorescence microspectrography all but the exciting radiations are filtered from the source. Microemission spectroscopy (*42*) requires the use of a simple reflecting objective which images the emission spectra in the slit of the spectrograph.

A working example of the system is illustrated in Fig. 4. The apparatus consists of interchangeable sources of illumination for the region 230–650 mμ (Type AH-4 mercury arc and Hanovia hydrogen arc), as well as an auxiliary visible phase system designed

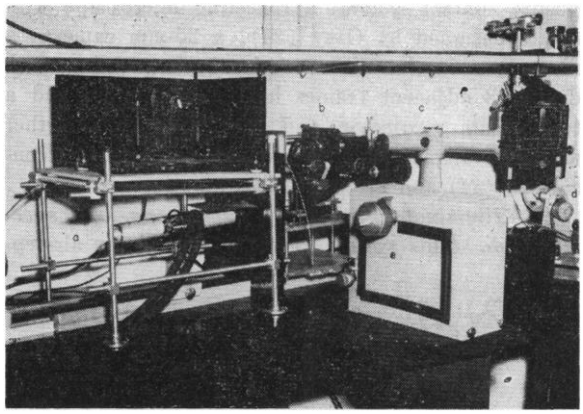

FIG. 4. Illuminator (*a*) with mercury arc above and hydrogen are below: reflecting microscope (*b*), rotating sector and spectrograph (*c*), logarithmic cam (*d*), and grating monochromator (*e*).

by Kavanagh (*37*); a microscope with a reflecting condenser and objective of N.A. 0.56, aperture number 0.7, and design by Kavanagh (*37*) and a small quartz spectrograph (Hilger) with aperture number 11. It is found from equation (2) that under these conditions the objective magnification required to fill the aperture of the spectrograph with light should not exceed 15. In actual practice the objective magnification is 50, so that less than the full aperture, resolving power, and speed of the spectrograph is utilized. Aside from theoretical considerations the minimum practical slit width employed in absorption spectrography and determined by the sensitivity of the recording material (Kodak Spectrum Analysis No. 1, 103-O-UV, SWR, and Tri X Pan) and by the densitometer scanning beam is 15 μ, which corresponds to a linear dimension of 0.3 μ in the object. A rotating step sector is placed at the entrance slit of the spectrograph for plate calibration (*6*). For the detection of finer detail in the absorption or the fluorescence spectrum the photographic plate can be moved during exposure by means of a logarithmic cam in accordance with the method of Holiday (*31*). In fluorescence spectrography a set of chemical and glass filters (*5, 36*) is used to limit the exciting radiations of the mercury are to band widths of 15 μ in the ultraviolet region from 245 to 365 mμ.

System (II) has been used by Jope (*35*) and by Barer (*2*) for microabsorption spectrography of the red blood cell, employing reflecting objectives designed by Burch (*15*). Loofbourow (*42*) has made use of the method in microabsorption spectrography of single crystals, thin solid films, and minute quantities of solutions, and in microemission spectrography.

III. *Illuminator, reflecting microscope, monochromator, and radiation detector.* This system has been used in infrared microspectroscopy by Barer, Cole, and Thompson (*3*), by Blout, Bird, and Grey (*4*), and by Gore (*27*) and is indicated in the alternative method of Fig. 3. The spectrum of the infrared source is focused on the object by the reflecting condenser. The image formed by the objective is relayed by the mirror, M_5, to the entrance slit of the monochromator in a single beam recording infrared spectrometer (Perkin-Elmer). Barer, Cole, and Thompson, using reflecting objectives of N.A. 0.65 designed by Burch, obtained infrared spectra over the region of 2–15 μ with objects (crystals, fibers, tissues) that were 20–50 μ in diameter. Blout, Bird, and Grey, using reflecting objectives of N.A. 0.4 designed by Grey (*40*), have recorded infrared spectra of objects as small as 50 μ and compared the spectral data with those obtained for macro-samples of the same material. In a detailed theoretical treatment of the factors involved, it was shown, as can be less rigorously derived from equation (2), that the useful magnification of a microscope objective when associated with a spectrometer is equal to the ratio of the numerical apertures, respectively, of the objective and the spectrometer. A consideration of diffraction theory gave a value of 6 μ as the minimum for two linear dimensions of an object whose spectrum is to be obtained with an ideal infrared microspectrometer having an objective numerical aperture of 1.5, and recording out to wavelength of 15 μ. The minimum value of the third dimension of the object, the sample thickness, is determined by the absorption coefficients and the signal-to-noise ratio of the spectrometer.

A potential hazard in the use of either system (II) or (III) is that the object is exposed to the entire spectral radiation of the source throughout the recording. In order to avoid this an infrared microspectrometer based upon system (I) with an infrared monochromator, followed by a reflecting microscope and a radiation detector, is currently being designed (Perkin-Elmer).

IV. *Reflecting microscope with oblique illumination.* Two methods for the oblique illumination (*17*) of a reflecting microscope (*a*), by transmitted light and (*b*) by reflected light, are given in Fig. 5. An annular diaphragm in (*a*) is placed at the back focal plane of an Abbe-type refracting quartz condenser of N.A. 1.25 and is dimensionally equivalent to the image of the objective aperture at that plane. No direct rays from the condenser enter the objective, because the rays in the central cone are stopped by the convex mirror of the objective, those in the middle of the hollow cone are stopped by the annular diaphragm, and the rays in the outer hollow cone are too oblique. For oblique illumination with reflected light (*b*), the source is imaged onto the plane of the object by a spherical mirror in such a manner that the sum of the

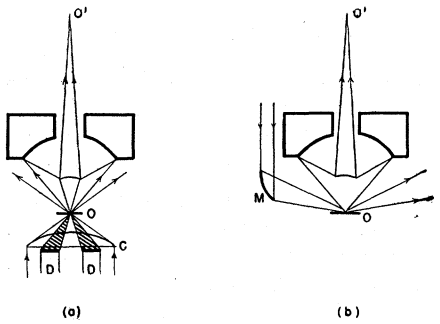

FIG. 5. Reflecting objective with oblique illumination (dark field) : (*a*) by transmitted light from refracting condenser, *C*, fitted with annular diaphragm, *D* ; (*b*) by reflected light from spherical mirror, *M*.

angles of incidence and reflection relative to the optic axis exceeds the angular aperture of the objective. When light scattered from the object with unaltered frequency is imaged in the slit of a spectrograph, either Tyndall or Rayleigh scattering occurs, whereas if the frequency is changed, either fluorescence or the Raman effect is operative.

APPLICATIONS OF THE REFLECTING MICROSCOPE

Chemistry and physics. The potential usefulness of the reflecting microscope in systems for absorption microspectroscopy in the infrared, visible, ultraviolet, and vacuum ultraviolet regions lies in the small order of magnitude of the analytic sample. In accordance with diffraction theory the resolving power, d, of a microscope is given by

$$d = \frac{1.2\,\lambda}{NA_o + NA_c} = \frac{0.6\,\lambda}{NA_o}, \qquad (3)$$

when NA_o, the numerical aperture of the objective, is the same as that of the condenser, NA_c, and λ is the wavelength. Thus, the theoretical minimum transverse diameter of the analytic sample as set by the resolving power of an objective with N.A. 1.5 and computed from equation (3) is 6 μ (*4*) in the infrared region at λ, 15μ, and 0.1 μ in the ultraviolet region at λ, 0.25 μ. The minimum cross-sectional area of the sample as estimated from the square of the linear resolving power of the microscope may not always be capable of achievement because of other limiting factors, such as the sensitivity of the spectrometer and the absorption coefficient of the sample. For this reason Blout, Bird, and Grey (*4*) have discussed the ultimate performance characteristics of an infrared microspectrometer in terms not of the minimum cross-sectional area of sample but of the smallest volume, a product of the area and thickness, which can be observed with a satisfactory signal-to-noise ratio in the recorded spectrum.

Microabsorption spectra in the infrared region from 2 to 15 μ have been recorded on single crystals, fibers, and particles with minimum cross-sectional diameters of the order of 20 (*3*) by 100 μ (*4*), thickness of 1–100 μ, and mass of the order of 10^{-7} to 10^{-8} g (*3*). The infrared spectra of minute samples of vitamins, antibiotics, hormones, and fibrous proteins mounted on disks of silver or sodium chloride have been studied by Barer, Cole, and Thompson (*3*), by Gore (*27*), and by Blout, Bird, and Grey (*4*). An additional application of the reflecting microscope has been made by Barer, Cole, and Thompson (*3*) in the use of polarized infrared radiation (*20, 24, 33, 44*) for the investigation of the internal structure of single crystals and fibers. These preliminary studies indicate that the reflecting microscope in systems for infrared microspectroscopy will be useful in determining both the presence and the spatial orientation of atomic groupings and will contribute to the understanding of the physical and the chemical structure of organic molecules.

Microabsorption spectra in the ultraviolet region from 230 to 370 mμ have been determined on optically homogeneous samples with cross-sectional diameters of the order of 0.3 × 1 μ (*46, 48*) to 10 × 100 μ (*42*), thickness of 1–1000 μ, and mass of the order of 10^{-8}–10^{-10} g (*2*). A sensitive densitometer (Ansco), fitted with a circular aperture of diameter 20–50 μ and used with a high-speed recording potentiometer (Leeds and Northrup), permits a photometric analysis at a slit height of 50 μ, which is equivalent to an object dimension of 1 μ (*46*) in a system of 50 times magnification. Loofbourow (*42*) has recorded the ultraviolet absorption spectra of minute quantities of amino acids, purines, and pyrimidines, either mounted on quartz slides in the form of single crystals and vacuum-evaporated solid films or placed in solution in microcuvettes with capacities of 0.003 ml. Finally, if the ultraviolet light is plane-polarized, information can be obtained concerning the spatial configuration of absorbing groups in relation to the axis of a crystal or a fiber.

Additional applications of the reflecting microscope to physics and chemistry aside from those related to spectrochemical microabsorption analysis include emission and fluorescence microspectroscopy. For microemission spectroscopy, a reflecting objective has been used by Loofbourow (*42*) to form a magnified image of a minute area (166 μ²) of an iron arc on the entrance slit of a spectrograph. The fluorescence spectra of single crystals of organic compounds in view of the low intensities that are often involved are preferably photographed at magnifications that are just sufficient to fill the spectrograph aperture with light. The relation between the spectral distribution of the energy of excitation and of emission can be studied by simultaneously photographing the absorp-

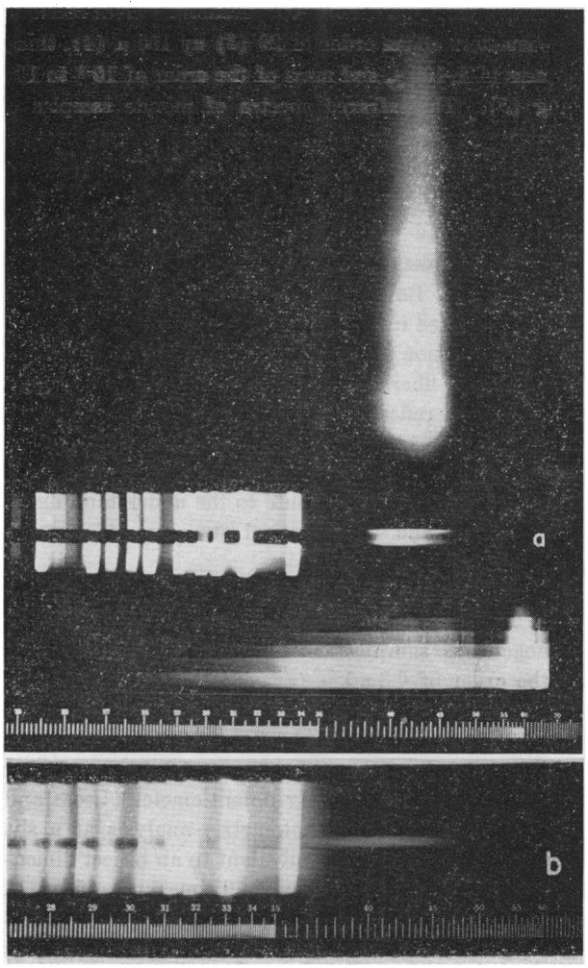

FIG. 6. Fluorescence spectra of (*a*) 20 μ crystal of β-naphthylamine with stationary and moving plate; and (*b*) human red blood cell treated with formalin. λ scale, mμ/10.

tion and the fluorescence spectra (Fig. 6). Oblique (dark-field) illumination with transmitted light is in some instances preferred for fluorescence microspectrography and is useful for the investigation of scattered radiations of the Tyndall and the Rayleigh type. A microdensitometric tracing is made of the experimental spectrum, which is photographed on a calibrated plate and compared with a reference spectrum obtained from the directly transmitted radiations of the source. By this means the difference between the relative spectral distribution of the incident and the scattered or the emitted radiations is determined.

Biology and medicine. The reflecting microscope can be used for microscopy in the visible region with bright-, dark-field, phase contrast, and fluorescence illumination and unpolarized or plane-polarized light and in the ultraviolet or infrared region with a suitable means of image-conversion. The methods as outlined by Jones (*34*) for converting an image of one wavelength into an image in another wavelength employ the photographic plate or film, the fluorescent screen, and electronic devices—the image tube, the flying spot scanner, and the image orthicon—developed for use in television. In the ultraviolet region from 0.2 to 0.4 μ and in the infrared region from 0.8 to 1.2 μ, the photographic method has been conventionally used; the infrared image tube (*57*) (1P25, RCA) is useful (*25*) for radiations in the neighborhood of 0.8 μ. A photographic method of image-conversion from the ultraviolet to visible full color has been ingeniously developed by Land *et al.* (*40*).

In microscopy, in order to meet the requirements of image contrast and resolution, it is necessary to fulfill the usual conditions of Köhler and Abbe that: (1) the access of the illuminating beam to the condenser be controlled by an aperture diaphragm, (2) the area of illumination in the object plane be limited to the field of view, and (3) the aperture of the condenser and the objective be uniformly filled with light. The peculiarity of design of reflecting microscope optics is such that at the customary position the substage diaphragm is not likely to serve adequately as the sole aperture stop, and stray radiations may pass the central mirror of the condenser and be transmitted directly to the field of view. It is generally desirable, therefore, to illuminate the central mirror with a circular beam of light that is nearly collimated and dimensionally comparable in cross section to the central mirror.

The reflecting microscope may be used in biology and medicine for the observation, and for the physical and chemical analysis, of tissues, cells, and cellular constituents. Analytic systems include microscopy with image-conversion from the invisible to the visible spectrum; absorption microspectroscopy in the vacuum ultraviolet, ultraviolet, visible, and infrared regions with unpolarized and plane-polarized light; and fluorescence microspectroscopy, together with other means of studying the transformation of absorbed radiation.

The natural appearance of the living mammalian cell in ultraviolet light was first photographed with a reflecting microscope and found to contrast with that of an injured or a dead cell (*39*). Microspectroscopic studies (*47*) of single living cells in tissue culture were carried out at a few wavelengths in the ultraviolet region and within limits of exposure that were not injurious. Spectrographic recordings (*48*) of the ultraviolet absorption of living cells were made simultaneously at many wave bands from 240 to 365 mμ, but not without eventual injury to the cell.

When the reflecting microscope is used for the absorption spectrography of a cell in accordance with system (II), the images of the cell in many wave-

lengths of light are brought to focus in the plane of the slit, and the light that passes through the slit is dispersed and focused on the photographic plate. Upon the images of the lines or of the continuum emitted by the source are superimposed the absorption patterns of the cell. The portion of the cell through which the incident light passes in order to gain entrance to the slit is a volume bounded by two parallel planes, *ab* and *cd* in Fig. 7, and by the upper and lower surfaces of the cell. The separation of the two planes is 6 μ for the bright line source and 2 μ for the continuous source. The spectromicrograph obtained in this manner has two components: one in the spectral, or horizontal, axis which relates the light absorption and wavelength for any morphologic component; and the other in the morphologic, or slit height, axis which indicates the absorption of various morphologic constituents at a particular wavelength. In collaboration with G. N. Papanicolaou, this analytic method was used to study fixed and unfixed squamous, parabasal, and abnormal cells of the cervical mucosa designated by Papanicolaou (*49*) as classes III, IV, and V, respectively, with features suggestive of, fairly conclusive for, and conclusive for cancer. The ultraviolet spectromicrographs of portions of these cells cells were recorded and the extinctions $\left(E\lambda = \text{Log}\ \frac{I_o}{I}\right)$ were calculated over the region of 240–350 mμ. Frequency distribution data for the maximum extinctions at 260 mμ in the nuclei of cells are given in Fig. 8. There is both a greater average magnitude and a broader range of values for the extinction in the abnormal cells. Since the optical path length when estimated from the nuclear dimensions was not alone sufficient to account for the differences in the extinctions, and the influence of fixation was not a determining factor—the relative differences were comparable for cells treated with formalin or glycerin—it was concluded that an average increase of both the totality and the density of factors that account for optical opacity had occurred in the abnormal cell.

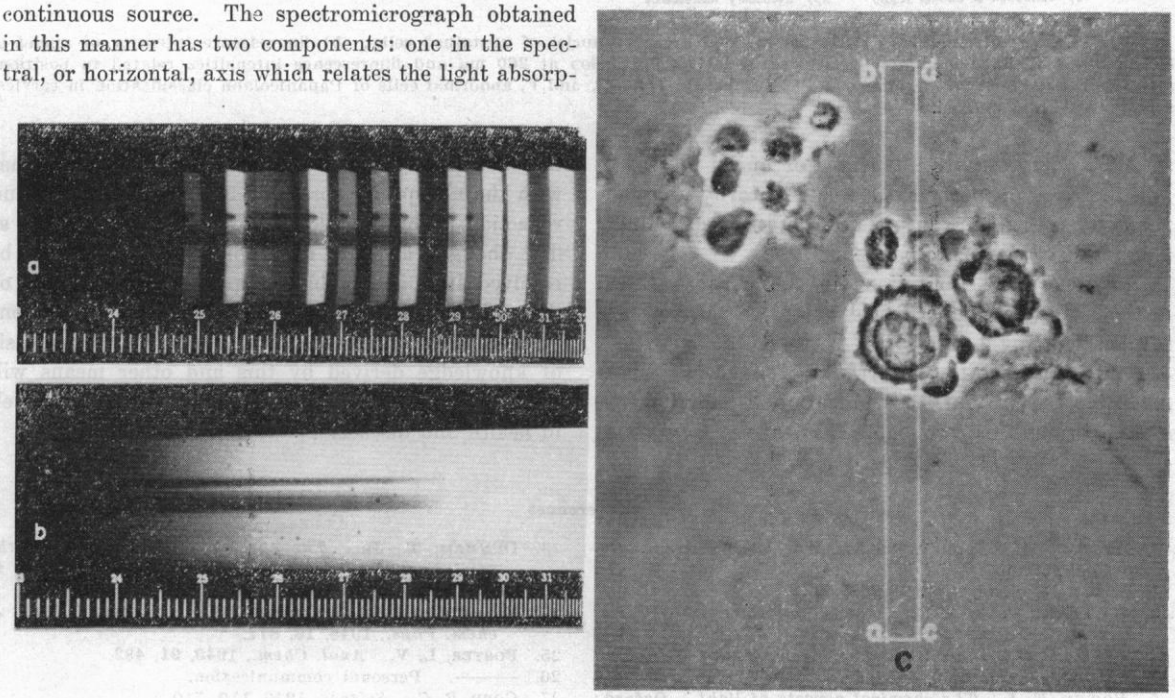

FIG. 7. Spectromicrographs, (*a*) with mercury arc and (*b*) with hydrogen arc, of field indicated in phase photomicrograph (*c*) by the area *abdc*. Class IV cell of cervical smear, formalin-fixed. λ scale, mμ/10.

These observations were then considered from the point of view of developing a quantitative, physical, and ultimately automatic method for scanning and processing smears of cells in clinical oncology. The use of light absorption in a scanning method is precluded by the frequent occurrence of stratification of cells and the summation of effects, whereas with a light-emission method, such as secondary fluorescence photometry, the effects of stratification are not necessarily additive. For this reason, the fluorescence intensities at 365 mμ (Fig. 8 [*b*] were determined for cell nuclei stained with a basic fluorochrome such as berberine sulfate (*30*), under specified conditions (*46*) which limit the dye principally to nuclear structures. It was found that, as the morphologic appearance of the cell became more significantly abnormal, both the average and the range of relative values for the

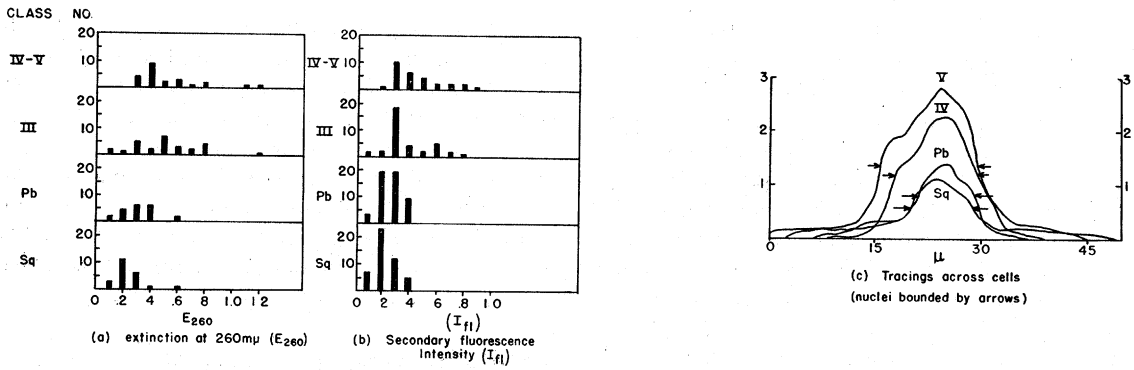

FIG. 8. (*a*) Extinctions at 260 mμ in 0.3μ² areas of nuclei of unstained cells; (*b*) fluorescence intensity of nuclei of cells stained with basic fluorochrome; (*c*) relative absorption at 260 mμ and fluorescence intensities related to positions along cell diameters. *Sq*, squamos; *Pb*, parabasal; *III, IV,* and *V*, abnormal cells of Papanicolaou classification in cervical smears, fixed and unfixed.

secondary fluorescence intensities of nuclei increased. Whether this property can be satisfactorily and conveniently utilized in the physical detection of cellular abnormality remains to be seen.

The theoretical limitations in the use of optical methods for the quantitative study of the cell are emphasized by the number of factors (*16, 19, 21, 47, 51*) that influence the absorption of light in cellular material: the amount, the volume of distribution, the spatial orientation, the photochemical reactivity, and the multiplicity of the absorbing material, together with the nonspecific losses of light by scattering and reflection. The complexity of the problem that at first thought appears incapable of solution may be resolved into analyzable components by the use of the reflecting microscope in various or all portions of the optical spectrum. It is hoped that a synthesis of knowledge derived by this and other means will then contribute to a further understanding of the cell in health and disease.

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Amplifying and Intensifying the Fluoroscopic Image by Means of a Scanning X-Ray Tube[1]

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THE X-RAY TUBE OF TODAY represents a considerable improvement over that of Roentgen at the time he discovered x-rays. The development of a detector for the x-ray shadow image has not progressed nearly so far, however, in spite of the fact that techniques that are applicable to this process have been known for some time. The commonest device for the direct observation of an x-ray shadow image is the fluoroscope, which is not fundamentally very different from devices developed before the turn of the century. In a general way, the basic problem is this: to find a means whereby the maximum amount of intelligence as to the structure of an object under observation may be extracted from the x-ray photons that have penetrated it and to create a readable shadow image that may be viewed directly, and that is bright enough and large enough to show the desired detail. Such an ideal system would necessarily reduce the x-ray dosage to an object, which is important especially if it is a living organism.

It was Paul C. Hodges, of the Department of Roentgenology at the University of Chicago, who vividly pointed out to me in a casual conversation the necessity for intensifying the fluoroscopic image without increasing the x-ray dosage to a patient, in particular for the fluoroscopic examination of the human abdominal region. At that time, my thoughts were concerned with the construction of a high-energy electron-Bremsstrahlung scanning microscope, and a search was being conducted for a suitable and fast detector for the Bremsstrahlung. The success that I. Broser and H. Kallman (*1*) had achieved with anthracene for the detection of beta and gamma rays suggested the possibility of inorganic fluorescent crystals as a means for detecting high-energy quanta. A search was made of several inorganic crystals, and a few, such as calcium fluorite, calcium tungstate, and lead barium sulfate, showed extremely promising possibilities. These crystals had high density, a short period of fluorescence—of the order of a fraction of a microsecond—and were transparent to their fluorescent radiation. The realization that such crystals existed immediately pointed to the possibility of a solution to Dr. Hodges' problem.

First, just what is the magnitude of the problem? A photographic film may be used to record a shadow image, although it is rather insensitive and not suitable for direct viewing. After exposure and development, the film is generally observed at a brightness level of some 30 ml, at which brightness level the eye is capable of separating contours of 100 percent contrast spaced .001 inch apart. On the other hand, if a more sensitive fluoroscope is used in observing the human abdomen, the brightness level will be of the order of 100 μml, a level such that an eye well adapted to the dark will just separate 2 contours of 100 percent contrast spaced approximately 2.5 mm apart. There is also another difference. At a brightness level of 30 ml, the difference in contrast that may be detected by the eye is of the order of 1 or 2 percent, whereas at a brightness of 100 μml the difference in contrast level that the eye may detect is of the order of 20–40 per-

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