

A Color Translating Ultraviolet Microscope¹

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THE DESIGN of monochromatic ultraviolet microscope objectives by Köhler and von Rohr (13, 14), their commercial production by Zeiss, and their use in conjunction with photographic film marked an important step forward in man's ability to see very small structures in spectral regions where his eyes are not sensitive. Many investigators (cf. 7, 8, 9, 16, 17, 21) have used this ultraviolet microscope in the examination of plant and animal tissues. Ultraviolet microscopy of such substances has been useful because the inherent absorption of biological materials in the ultraviolet region makes possible photography without the use of dyes or stains, and because the shorter wavelengths of the incident radiation allow approximately twice the resolving power that can be obtained with visible light.

The well-known Zeiss ultraviolet microscope objectives, because they are monochromats, are restricted to use at one wavelength for a given focal setting. On the other hand, the microscope described here is not only achromatic, but also apochromatic from 220 to 800 m μ . This makes possible a new conception of the method of using an ultraviolet microscope; namely, to use simultaneously or 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 superposed, a visible image in full color is obtained. This procedure was earlier suggested by Brumberg and others (4, 5, 23). The colored image which is seen might be thought of as what the eye would see in the microscope if its receptors had their peak sensitivities at the ultraviolet wavelengths in question.

We now wish to present a preliminary description of a system of ultraviolet microscopy and an instrument which employs this procedure. The system employs photography with rapid development and fixation to convert the ultraviolet image into a visible image. The procedure involves image formation with an apochromatic ultraviolet microscope objective (11),

exposure of successive areas of the film using three different wavelength bands of ultraviolet light, rapid development and fixation of the three latent photographic images, and the simultaneous projection of the three fixed images through three additive color filters to give a full-color representation of the object in terms of its ultraviolet absorption. Although other means of wavelength translation such as fluorescent screens and television methods have been considered (12), it was decided on the basis of sensitivity, availability, and particularly retention of a permanent record, first to develop an instrument using photographic techniques.

The instrument (Fig. 1) employs a superhigh pressure, water-cooled mercury arc (Type A-H6) as a light source (A), a Wadsworth-type grating monochromator (B), a microscope (C) using apochromatic objective lenses and identical lenses as condensers, the camera (D) which employs 35-mm motion picture film, the film-processing station (E), the three-beam projector (F), and the viewing screen (G). The instrument will be described in detail in a subsequent publication, but it is perhaps worth while to mention at this time a few pertinent characteristics of the components now in use.

A.) The characteristics of the superhigh pressure mercury arc have been described (1, 2, 10, 22). This source was found to be the brightest of a great variety of sources examined in the region 260 to 400 m μ . Exposure times required vary from 0.01 to 30 seconds, depending not only on the wavelength region, the film type, and developing conditions, but also to a considerable extent on the age of the arc. The achievement of proper exposure will be facilitated by an automatic photoelectric exposure control device now under construction.

B.) The monochromator was designed and constructed in this laboratory and employs a Baird Associates 15,000 line per inch grating (B₁) approximately 3 by 1.5 inches in area. Settings for wavelength are made reproducible by means of micrometer-controlled stops. Band widths are controlled by slit size.

C.) The optical system of the microscope will be described in more detail in a subsequent communication.

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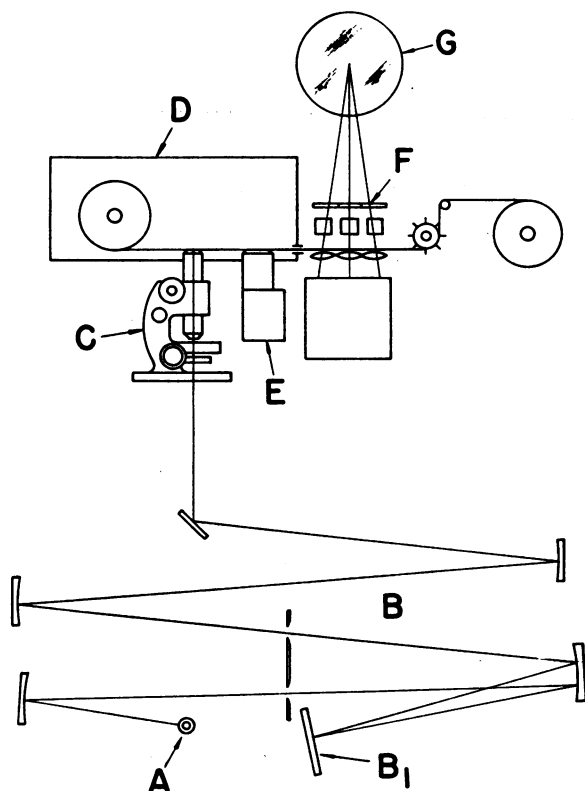


FIG. 1. Schematic diagram of color translating ultra-violet microscope: A—light source, B—monochromator, B₁—diffraction grating, C—microscope, D—camera, E—film processing station, F—three-beam projector, G—viewing screen.

tion (11). The objective and condenser are apochromatic over the wavelength range 220–800 mμ. This of course means that it is now possible to focus the microscope for ultraviolet light by focusing it with visible light; this eliminates what has previously been one of the most difficult aspects of ultraviolet photomicroscopy. Two objectives, one of a reflecting type (6, 18) and one of a combined reflecting-refracting type, having numerical apertures of 0.4 and 0.72 respectively, have been designed and constructed. These lenses fit into a standard microscope stand (Fig. 2) and may be used as condensers as well as objectives, although an achromatic quartz-fluorite ultraviolet condenser has also been made. The objectives may be used without eyepieces and provide magnifications of 77× and 200× before projection. Lenses of higher numerical aperture and with similar apochromatic properties are now being developed.

D.) The main requirement of the camera is that successive frames be indexed very accurately (to at least 0.001 inch), so that the subsequent triple projection is in register. Approximately 400 feet of 35-mm film can be used in one loading.

E.) After the film is exposed, it moves on automatically to the film processing station, where successive treatment of the emulsion with hot alkaline developer, hot fixer, water, and air produces within 10 seconds a stable negative suitable for projection (3). The useful diameter of the processing cup is 0.7 inch, and therefore this is also the diameter of the developed area. The principal mechanical-chemical problems of fast processing, such as time cycle, temperature control, and homogeneity of processing, have been solved.

F.) After the development and fixation of the negative image, the film is moved on to a projector, where three images which had been taken successively at three different ultraviolet bands are projected simultaneously through three additive color filters (red, green, blue) upon a 4½-inch circular translucent screen. The magnification from the film to the screen is eight times. The resulting single, full-color image is reasonably sharp and bright, and may be viewed by several persons simultaneously.

The relationship between the visible and ultraviolet bands may be chosen for maximum color contrast, since there are no “natural” colors. With three ultraviolet bands and three visible colors, there are six possible combinations of the images and filters. In addition there is the possibility of processing the ultraviolet image to yield a positive rather than a negative. We have examined the six negative and six positive color combinations, using ultraviolet photo-

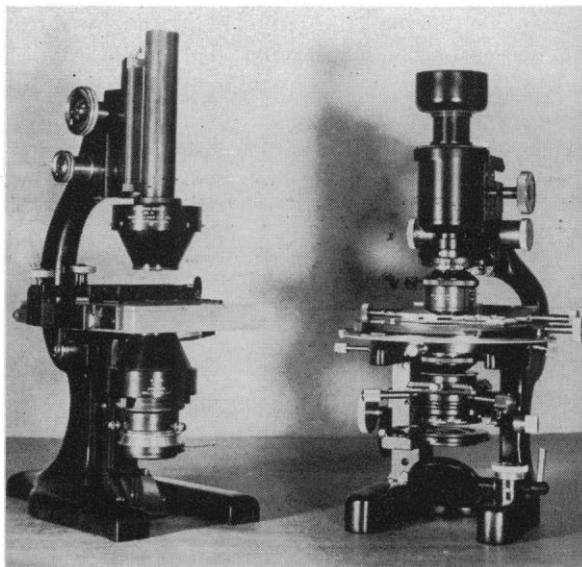


FIG. 2. Apochromatic reflecting-type microscope lenses in conventional stands: 0.4 NA objective lens (left); 0.72 NA objective lens (right); identical lenses used as condensers.

micrographs of tissue sections, and, on the basis of using equal gammas and equal background densities in the negatives, have selected the following as showing the greatest color contrasts:

<i>Ultraviolet Wavelength</i>	<i>Visible Wavelength</i>	
	<i>Negative</i>	<i>Positive</i>
Short ($\sim 265 \text{ m}\mu$)	Green	Blue
Medium ($\sim 285 \text{ m}\mu$)	Red	Green
Long ($\sim 315 \text{ m}\mu$)	Blue	Red

We are not unmindful that with the use of other ultraviolet wavelengths or the use of other photographic procedures, different color combinations may show better color contrast.

In the mechanical arrangement employed in the instrument, the visible color filters are segments of a rotatable disc arranged so that as each new image is developed and moves into the projector, an image that had been projected through the same color filter moves out of the projector; and the two remaining images are advanced one frame, along with their respective filters. Thus the desired correspondence of ultraviolet and visible wavelengths is maintained once it is set.

G.) The clear screen brightness obtained through the three filters is about 100 foot-lamberts. Use is made of a special plastic-in-plastic screen (15) which gives the maximum screen brilliance, maximum evenness of illumination, and maximum definition of any of the many different types of translucent screens we have investigated. The addition of a plastic Fresnel condensing lens (20), placed directly back of and

in contact with the screen, aids in achieving even illumination.

The device is called a color translating microscope, rather than a micrograph, because the manipulative operations involved and the general time requirements are rather similar to those involved in ordinary visual microscopy. One sits adjacent to the microscope, moves the slide about on the microscope stage, changes the focus as desired, and—without significant delay—studies the magnified, full-color image. The instrument is different from an ordinary microscope in that it permits several persons to view the image simultaneously, provides permanent records of what is seen, and of course permits observation of specimens in terms of their ultraviolet absorption.

In Fig. 3 we have reproduced three black-and-white ultraviolet photomicrographs of the type used for color projection. Unfortunately, since full-color printing is not used in this journal, we are unable to reproduce a color photomicrograph with this article.

Finally, it should be pointed out that the 35-mm black-and-white film, with its groups of three images, each corresponding to a different ultraviolet band, provides a permanent photographic record of each experiment, which can be examined at leisure, or each group of three images may be processed into a single full-color photograph. For example, a picture may be taken of the image on the projection screen with color film such as Kodachrome or Ansco color, or the black-and-white negatives may be used as color-separation negatives in the Technicolor or Polacolor processes. This color film may then be viewed in an ordinary 35-mm motion picture or slide projector, thus

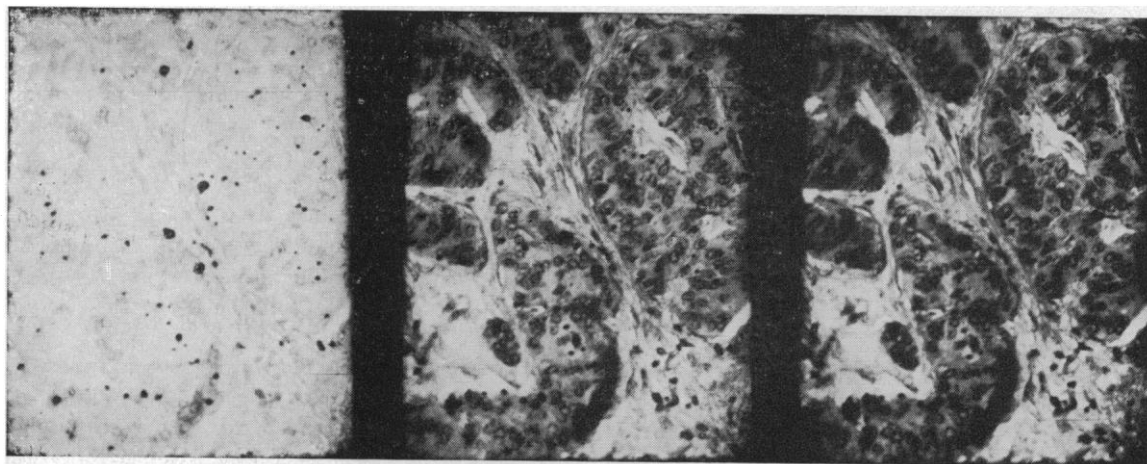


FIG. 3. Positive print from a 35-mm film strip showing three pictures taken at peak transmissions of 315 $\text{m}\mu$ (left), 285 $\text{m}\mu$ (center), and 265 $\text{m}\mu$ (right); band width approximately 10 $\text{m}\mu$ in each case; photographed with 0.4 NA apochromatic reflecting lenses used as objective and condenser; subject, unstained intestinal carcinoid showing argentaffine cells; specimen (furnished by Dr. Maurice L. Pechet, Children's Hospital, Boston, Massachusetts) prepared by formalin fixation, embedded in paraffin, cut at 4 μ thickness, and mounted in glycerin on Vycor slide.

making possible careful, prolonged study of subjects of ultraviolet color-translation photomicrographs.

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Examination of the Target Theory by Deuteron Bombardment of T-1 Phage

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THE BIOLOGICAL ACTION OF IONIZING RADIATIONS is of great current importance. The effects produced are often readily measured and a clear understanding of the process involved is therefore capable of yielding structural information about biological systems.

Lea (2) has examined carefully the target theory of biological action according to which a localized sensitive volume of definite size exists and within which there must be considerable liberation of energy for biological action to take place. This direct action is certainly not the only action and Lea has pointed out that indirect action by free radicals and by hydrogen peroxide will occur, particularly at low concentrations. According to his views a specimen ir-

radiated in high concentration, and in the presence of a high concentration of large molecules, should have a low efficiency for indirect action and the direct, target type action should predominate.

There is today some question as to whether direct action ever occurs, since the similarity of action of mutagenic agents like nitrogen mustard and of X radiation is very striking, and certainly mutagenic agents must operate by collision rather than by penetration and ionization.

In view of this present uncertainty it appeared to us to be worth while to examine the target theory carefully in bombardments where it should certainly be valid. For this we chose T-1 bacteriophage (1) which acts on *E. coli*, strain B. This phage has a spherical head, diameter 50 mμ and a long tail, length 120 mμ of diameter 10 mμ. No complex genetic structure has been reported for this phage. We found it to be

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