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Ultraviolet Television Color-Translating Microscope

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Every advance in microscope technique has been followed by comparable strides in biology and medicine. This has been true of the introduction of the light microscope, of the spectroscope, and of the electron microscope. We can thus hope that the ultraviolet television color-translating microscope, which adapts color vision to the viewing at high magnification of living cells illuminated by ultraviolet radiation, may also make a material contribution to biological research.

It is well know that the chemical constituents of unstained living cells, though practically transparent to visible light, exhibit strong characteristic absorptions in the ultraviolet, particularly in the range from 2200 to 3000 angstroms (*1*). Thus, the recognition of structure in cells and tissue specimens is greatly facilitated by studying them under ultraviolet illumination. In the past, ultraviolet microscopy has suffered from the drawback that photographic exposure and development was required to render the ultraviolet image visible. This difficulty was removed by the development of the ultraviolet television microscope, which permits the immediate and continuous observation of the specimen under ultraviolet illumination (*2, 3*).

The ultraviolet television microscope, like the photographic ultraviolet microscope, yields a simple monochromic representation of the specimen, in which the half tones correspond to the relative over-all absorption of different portions of the specimen for the illuminating radiation. Brumberg (*4*) recognized at an earlier date that considerably more useful information would be conveyed by a color representation of the object, in which the relative intensity of the three primary components—red, green, and blue—would be proportional to the transmission (or absorption) of the specimen at three selected ultraviolet wavelengths. If the wavelengths are chosen to correspond to the characteristic absorptions of specimen constituents of particular interest to the observer, this procedure makes optimum use of trichromatic vision to discriminate between the constituents.

Brumberg's idea was rendered practical for photography through the development of catadioptric microscope objectives (*5–7*) which were apochromatic over the entire range of the ultraviolet and visible spectrum (2200 to 8000 angstroms). With these objectives, Land and his coworkers (*8*) were able to construct a photographic color-translating microscope which yielded excellent color pictures of biological specimens under ultraviolet illumination. In Land's system, three film frames were exposed successively to the images of the object formed by ultraviolet radiation of three different wavelengths as delivered by a grating monochromator. These frames were developed, fixed, and washed in a high-speed developing system and then projected through the correlated red, green, and blue filters in superposition on a common viewing screen. Both negative representation, in which the intensity of a given color corresponded to the absorption of radiation of the correlated wavelength, and positive representation, in which the intensity corresponded to the transmission of the same radiation, were studied. The most effective correlations are shown in Table 1.

A color-translating ultraviolet microscope which follows Land's instrument in its main outlines has been marketed by the Scientific Specialties Corporation (*9*). Negative representation is employed. Photographic processing demands only 25 seconds; hence, the delay between exposure and examination of the picture is minimized. The exposure time for the three selected wavelengths is adjusted automatically to compensate for differences between the light output of the monochromator and that of the source, a water-cooled, high-pressure mercury arc.

As has already been indicated, some very good results have been obtained with the photographic method. Nevertheless, this method has certain obvious shortcomings. The most important of these is the impossibility of scanning large quantities of material at high speed for the detection, for example, of abnormalities or features of special interest. Under such circumstances, a delay of half a minute between the viewing of adjoining microscopic fields would seem to be prohibitively long. Furthermore, although the obtaining of a permanent photographic record of interesting specimen fields may be deemed an advantage, the necessity of obtaining such a record for every field examined is the opposite, both from the point of view of economy and from that of swamping the interesting material with records of little value.

It is the aim of the ultraviolet television color-translating microscope to overcome these drawbacks by providing an instantaneous color representation of the specimen. With it, the examination of specimens in the ultraviolet can be carried out with the same speed and simplicity as examination in the visual range. Whenever a permanent record is desired, it can be obtained by color photography of the receiver screen (*10*).

Construction

The basic plan of the several types of television color-translating microscopes which we have constructed is shown in Fig. 1. A microscope with reflective condenser and objective projects the image on the photosensitive target (or targets)

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of a television camera, whose video signal is employed for the reproduction of an image of the specimen on the screen of a color receiver. Illumination is provided by a pulsed light source incorporating one or several monochromators and so arranged that radiation of the three selected ultraviolet wavelengths falls on the specimen at successive pulses. The pulse source also controls the vertical deflection in the camera and receiver in such fashion that the pulses of illumination occur during vertical fly-back time, the picture signal stored by a radiation pulse of a particular ultraviolet wavelength being utilized for generating a component picture of the corresponding color in the succeeding frame period.

The feature of the sequential illumination of the specimen with ultraviolet radiations of different wavelength is common to the several instruments described in this article. Sequential illumination is to be preferred to the alternative of illuminating the specimen with unseparated, "white," ultraviolet radiation and effecting the separation subsequently by beam splitters or filters, or both, since it reduces the total exposure of the specimen to ultraviolet radiation and thus minimizes the chemical and biological changes effected by this exposure. Apart from this, there is considerable choice in the design of both the illuminating system and the camera.

The camera tube may, in principle, employ either a photoconductive or a photoemissive target. The photosensitive material must, of course, maintain high response throughout the portion of the ultraviolet spectrum utilized and must be deposited on a base which is transparent to ultraviolet radiation. The experimental ultraviolet-sensitive Vidicon, employing a photoconductive layer of amorphous selenium (*11*), which has been developed specifically for research applications, is particularly attractive since it combines a quantum yield of the order of 1 in the ultraviolet with simplicity of operation and compactness; it is interchangeable with the standard Vidicon employed in commercial industrial television equipment. Its spectral response is shown in Fig. 2.

Table 1. Most effective correlations between color and wavelength in negative and positive representation.

Ultraviolet wavelength (A)	Representation	
	Negative	Positive
2650	Green	Blue
2850	Red	Green
3150	Blue	Red

Table 2. Values of n, χ, and Δd at selected wavelengths for a lens of focal length of 4.5 inches.

λ (A)	n	χ	Δd (in.)
2537	1.5980		−0.210
3130	1.5737	69°4.5′	0
4358	1.5540		0.170

The ultraviolet-sensitive Vidicon exhibits a certain amount of lag—that is, the charge pattern on the target excited by exposure to radiation is not removed completely in a single scan, but persists for a fraction of a second, giving rise to after-images of rapidly decreasing intensity. Wherever this slight carry-over from one frame to the next is harmful, it becomes advantageous to employ a camera tube effectively free from lag, such as the image orthicon. Experimental image orthicons with ultraviolet-transmissive front ends and photocathodes of high sensitivity in the ultraviolet (Fig. 3) have been built to order for the television color-translating microscope. Their performance has been very satisfactory. At the same time, the higher cost and more complex auxiliary equipment demanded by these tubes makes it clearly desirable to use them only when freedom from lag and very high sensitivity are essential requirements.

The illuminating system of the color-translating microscope may employ either a single light source or three light sources. Similarly, the camera may operate on the field-sequential principle, employing a single camera tube, or on the simultaneous principle, employing three camera tubes in parallel. The first television color-translating microscope, constructed at the RCA Laboratories in Princeton, New Jersey (*3*), used a single pulsed light source and single Vidicon camera tube. A simple mechanical system was employed to select in turn radiation of three different wavelength ranges for illumination of the specimen.

The method of wavelength selection was based on the fact that the focal points for a prism monochromator with uncorrected collimator and telescope lenses made of the same material as the prism lie along a straight line which is oblique to the direction of the refracted pencils. If the entrance slit of the microscope illuminator is placed on this straight line, a plane mirror perpendicular to the line will project the focal point for radiation of different wavelengths onto the slit as it is displaced in a direction perpendicular to its surface.

Figure 4 shows the application of this principle. A medium-pressure mercury arc lamp illuminated the entrance pupil of a 60-degree quartz prism monochro-

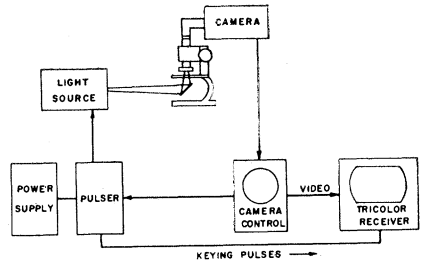

Fig. 1. General block diagram of television color-translating microscope.

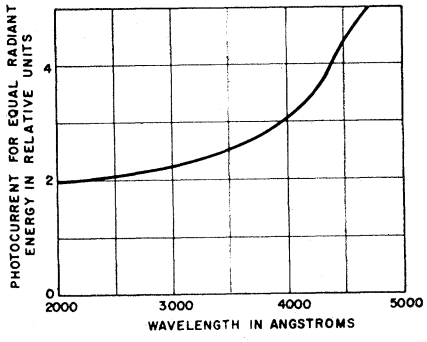

Fig. 2. Ultraviolet response of experimental ultraviolet-sensitive Vidicon.

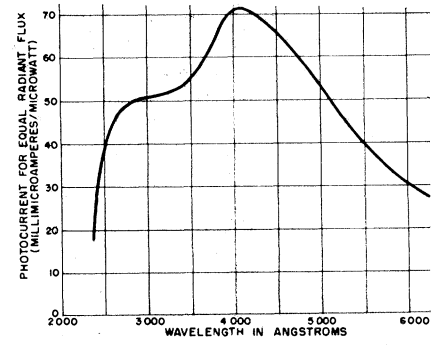

Fig. 3. Ultraviolet response of experimental ultraviolet-sensitive image orthicon.

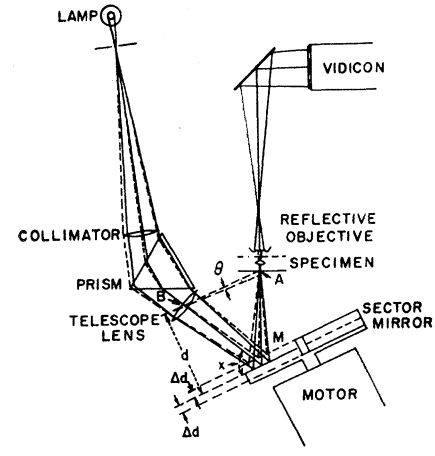

Fig. 4. Optical system of single-source television color-translating microscope.

mator provided with quartz collimator and telescope lenses, all cut perpendicular to the optic axis. The mirror M consists of a rotating disk with three sectors, recessed by different thickness differences Δd. For a 60-degree prism, it may be shown that the glancing angle χ formed at the mirror surface by the principal ray of radiation for which the index of quartz is n is given by

$$\tan \chi = \frac{\sqrt{4 - n^2}}{n - 1}$$

Furthermore, the relative displacement of the mirror surfaces is given by

$$\Delta d = -F \frac{n}{n-1} \sqrt{\frac{5 - 2n}{4 - n^2}}$$

where F is the focal length of the collimator and telescope lenses for the median wavelength. Thus, for a lens focal length of 4.5 inches, we obtain the values shown in Table 2 for a system employing 2537, 3130, and 4358 angstroms as the selected wavelengths.

The monochromator tube, synchronous drive motor, sector disk, and microscope are shown in Fig. 5. The disk was rotated at one-third the frame frequency of the television system. Automatic phasing of the television system could be achieved by deriving a triggering signal for the vertical deflection from the interruption of a light beam falling on a photocell by the rotating disk. The signal levels for the three wavelength ranges were balanced empirically by masking the mirror sectors.

Although the principle of color translation could be readily demonstrated with this instrument, it had several shortcomings. Perhaps the most obvious of these was the difficulty of changing the wavelength selection. Every change in wavelength demanded the insertion of a new sector mirror or plane parallel shim to adjust the height of the mirror surface. Furthermore, the fact that the angle of incidence of the illumination varied slightly with the wavelength made it very difficult to achieve color balance over the entire field. Finally, the lag of the experimental Vidicon employed in the instrument prevented the attainment of highly saturated colors.

These and other drawbacks were overcome in a more elaborate television color-translating microscope constructed at the Rockefeller Institute for Medical Research in New York. The illuminating system of this instrument is shown in Fig. 6. Three Farrand grating monochromators with individual pulsed light sources permit arbitrary adjustment of the illuminating wavelengths by means of the calibrated knobs on the front panel. The cover for one of the sources, a Hanovia 10B1 quartz mercury arc lamp, is removed in the illustration. The use of separate light sources has the incidental advantage that sources can be selected for high emission in particular wavelength ranges. Thus, for the shortest wavelengths, a Hanovia 250-watt xenon-mercury lamp is employed.

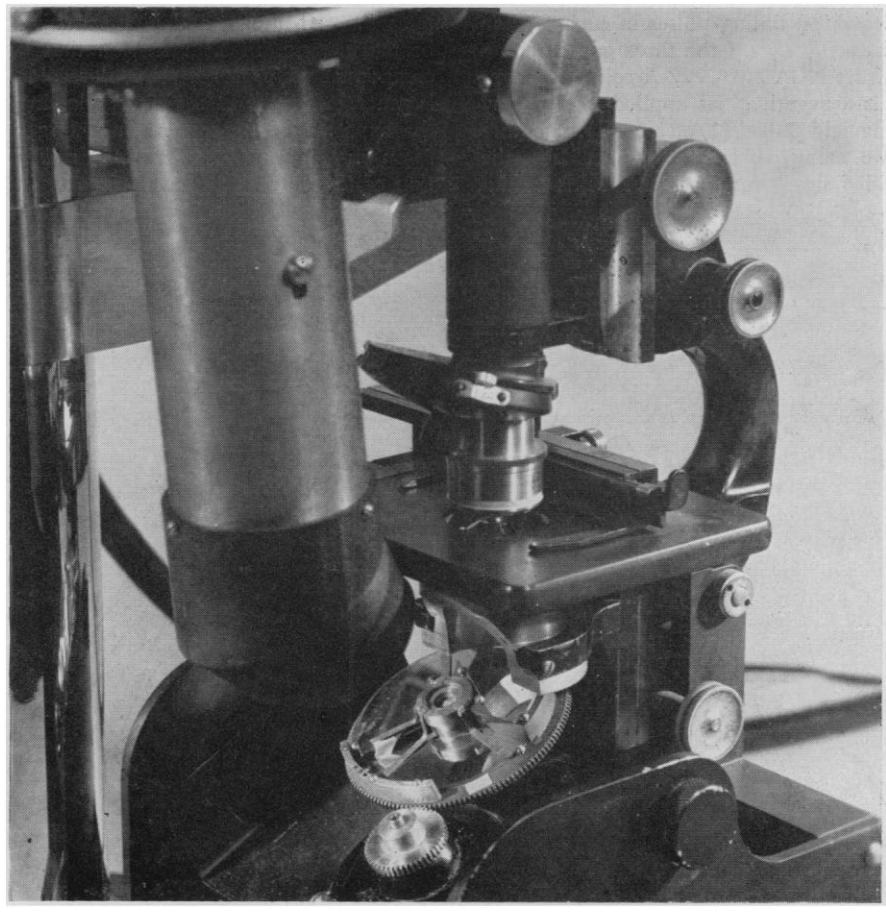

Fig. 5. Detail of single-source television color-translating microscope (RCA Laboratories).

The optical arrangement of the illuminator is more clearly evident from the diagram in Fig. 7. The source is imaged by a quartz lens on the entrance slit of the monochromator, and the exit slit is imaged by a second lens onto the center of a rotating 45-degree mirror which directs the illuminating radiation into the microscope substage, an $f/3.5$ aperture ratio being maintained throughout. The amplified vertical pulses from the synchronizing generator of the television system are applied through contact brushes on slip rings attached to the mirror stage to the three thyratron circuits feeding the light sources in succession, triggering a light flash at the instant when the principal ray from the monochromator and the normal to the rotating mirror surface lie in a common vertical plane. Synchronism between the mirror rotation and the synchronizing generator is assured by deriving control signals for the synchronizing generator from the mirror stage commutator. Fine adjustments of the monochromators make it possible to make the mechanical phase differences for the three illuminating units precisely 120 degrees.

The circuit arrangement for sequential camera operation, with a single camera tube, is shown in Fig. 8. The output signal of the camera is applied in parallel to the red, green, and blue video amplifiers of the color receiver. However, a set of three bistable multivibrators connected in a ring are driven by vertical-

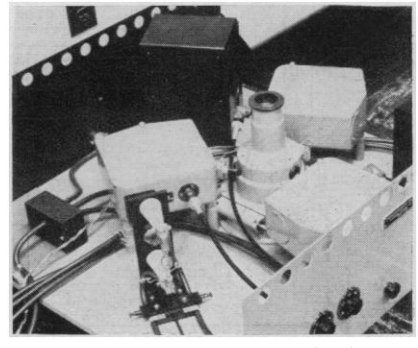

Fig. 6. Illuminating system of television color-translating microscope with three separate sources (Rockefeller Institute).

deflection timing pulses in such fashion that only one of the three gun cathodes of the RCA 21AXP22-A color kinescope has a negative bias applied to it in any one field period (1/60 second), the other two being driven to cut-off. Thus, the video signal for successive fields modulates the red, green, and blue scanning beams in turn, in such fashion that a component picture of a particular color is generated in the field period following the exposure of the camera tube target to an image formed by ultraviolet radiation of the correlated wavelength.

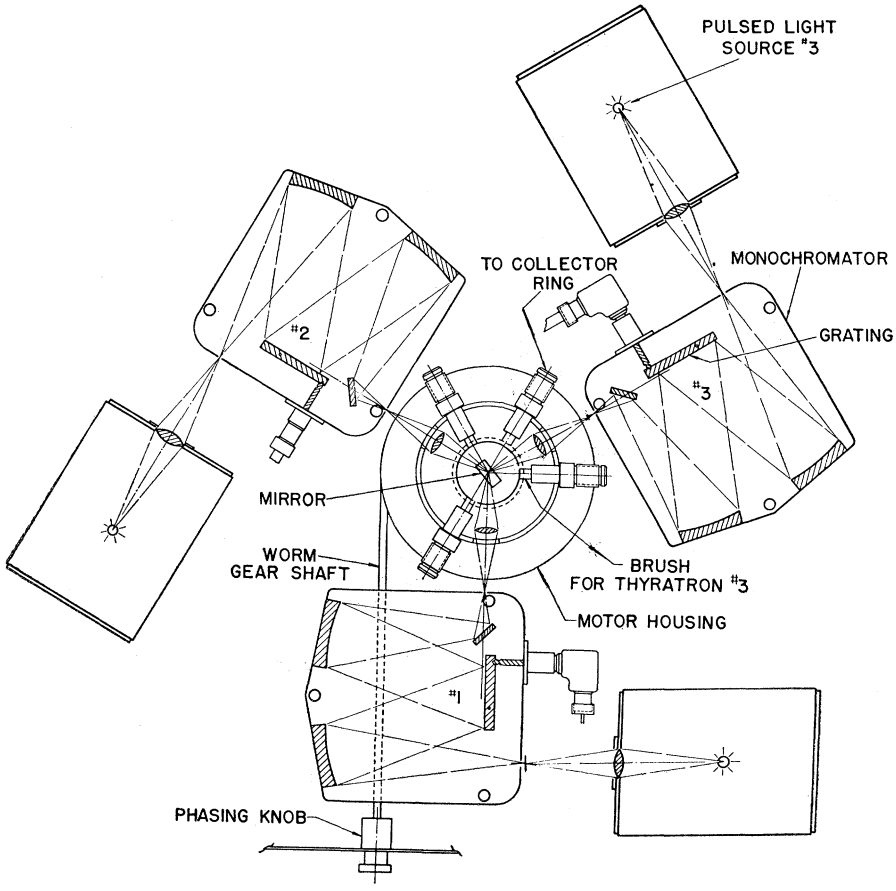

Fig. 7. Illuminating system.

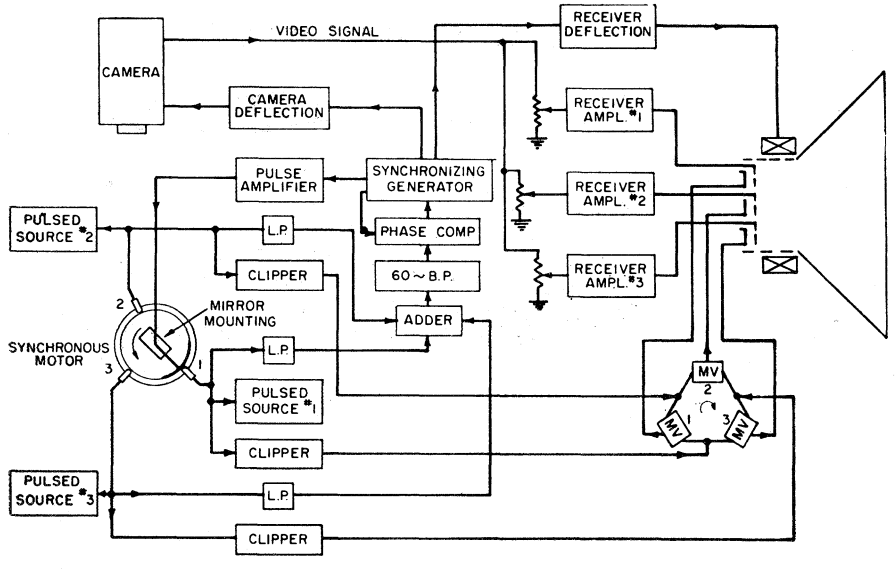

Fig. 8. Block diagram of television color-translating microscope at Rockefeller Institute for Medical Research.

Timing control for the entire system is provided by the commutator sector mounted on the rotating stage for the 45-degree mirror. This sector is connected both to a direct-current source and to a pulse amplifier deriving sharp timing pulses 1/60 second apart from the synchronizing generator. Thus, a narrow pulse riding on top of a broad square pulse is applied, in 1/20-second intervals, to each of the brushes. This signal is divided three ways. For timing control, the signal is passed through low-pass filters which remove the spike and smooth the square pulses, after which it is added to the similarly smoothed outputs of the other two brushes. A band-pass filter selects the 60-cycle component of the sum, which is phase-compared with the vertical synchronizing pulses delivered by the synchronizing generator. The output of the phase comparator is then employed to lock the synchronizing generator to the rotation of the 45-degree mirror.

A second current path from the brushes contains a clipper, which clips off the narrow timing pulses and applies them to the appropriate junction between a pair of the multivibrators, gating the beam currents in the color kinescope.

The third path provides the triggering pulses for the ultraviolet source circuit, which is shown schematically in Fig. 9. A 12-kilovolt pulse for starting the lamp is obtained from a sawtooth generator and an output tube feeding into an autotransformer. In addition, the synchronizing pulse is amplified and applied to a thyratron grid to provide a low-impedance path for a condenser discharge through the lamp. Finally, a low-voltage "keep-alive supply" maintains continuous current through the arc lamp. Thus the ionization within the arc lamp is maintained above a certain minimum level, thereby stabilizing the position of the arc and eliminating random delays between the discharge through the lamp and the application of the trigger pulse.

A view of the complete television color-translating microscope is shown in Fig. 10. The optical microscope, the image orthicon camera, and the black-and-white test monitor are mounted on a desk, whose central drawer space contains the illuminating system. Power supplies are housed in the drawer space to the left. The color monitor is a modified RCA color receiver.

The objective and the substage condenser of the microscope are identical catadioptric objectives corrected for different thicknesses of quartz; all the remaining lenses of the illuminating system are simple quartz lenses maintaining an $f/3.5$ aperture ratio. The slit image in the plane of the 45-degree mirror is imaged by a pair of these lenses into the

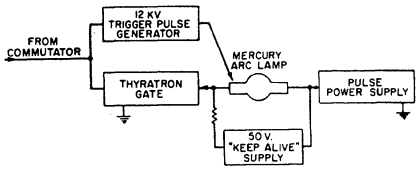

Fig. 9. Ultraviolet-source circuit.

entrance pupil of the substage condenser, which, in turn, images one of the lens surfaces in the specimen plane. With this arrangement, the illumination sweeps across the image plane as the 45-degree mirror is rotated. Thus the precise phasing between the light flashes and the mirror position afforded by the described circuits is essential for realizing color balance throughout the image field.

Performance

Within the short period during which the instrument has been in use, it has been applied to the study of a number of simple preparations, all of them unstained and unfixed. The possibility of studying living materials at high magnifications, without the introduction of artifacts resulting from chemical interference, may be regarded as one of its primary advantages. Protracted studies of tissue specimens—for example, mouse connective tissues—have revealed no visible structural changes resulting from the ultraviolet irradiation employed; paramecia, tetrahymena, and amebas, similarly, showed no change in behavior or motility, whereas ameboid slime-mold cells tended to be immobilized after about 2 minutes of irradiation at 2650 angstroms, and rotifers exhibited a definite negative tropism.

Cell preparations frequently reveal fine details, such as "chromatin" granules in protoplasm, which are not visible with illumination by visible light. The major absorptions are generally due to nuclear or protein materials, so that, in many preparations, only nuclei appear strongly colored. In addition, pigments such as hemoglobin or chlorophyll, present in too small concentrations to impart appreciable coloration to a specimen, give rise to striking color contrasts in the ultraviolet color-translating microscope; thus the streaming of protoplasm and cyclosis of chloroplasts become readily visible.

Particular chemical compounds with characteristic ultraviolet absorptions are readily detected by correlating critical ultraviolet wavelengths with the primary colors. Knowing the absorption coefficients of the materials in question, we can even obtain quantitative estimates of their concentrations at any point of the specimen. For this purpose we employ a line-selecter oscilloscope to select a particular horizontal scanning line and represent the variation of the video signal along it in the red, blue, and green channels on separate cathode-ray tubes. This is illustrated in Fig. 11, which shows a nucleated red cell of frog blood. In the original picture, photographed from the color monitor, the nucleus appears orange, the hemoglobin, yellow, and the white corpuscles, purple. A white line through the center of the red blood cell indicates the location of the selected line. The three oscillograms show the variation of the video signal for the three selected ultraviolet wavelengths and thus give an indication of the concentration of the principal absorbing materials along the selected line. Quantitative measurements by this means would of course require density step-wedge calibration.

The fact that, in the television color-translating microscope, practically any chemical differences can be translated

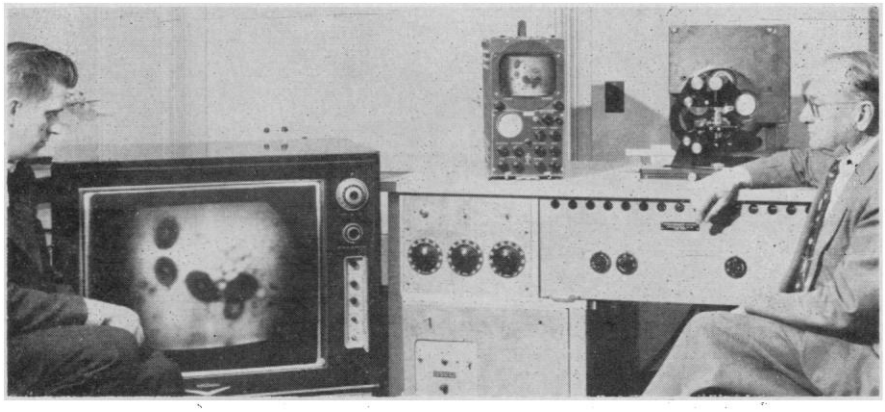

Fig. 10. Complete television color-translating microscope (Rockefeller Institute).

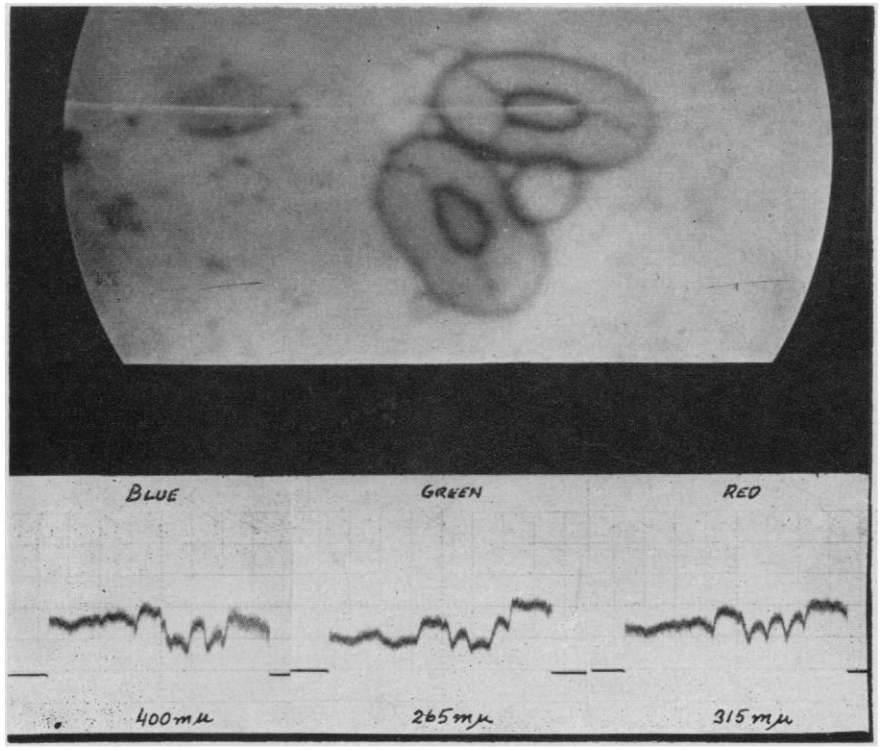

Fig. 11. Red cell of frog blood and oscillograms representing transmission at three ultraviolet wavelengths along selected scanning line.

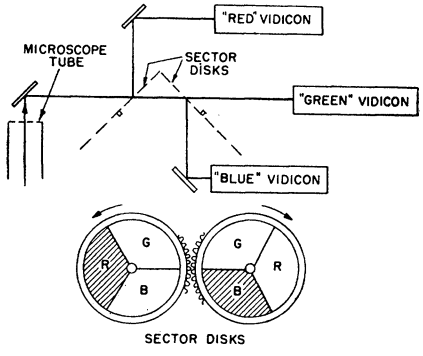

Fig. 12. Simultaneous color camera for television color-translating microscope.

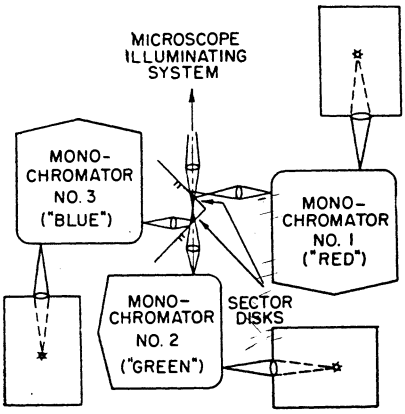

Fig. 13. Sector-disk illuminator for television color-translating microscope.

into color differences can make it a valuable tool in the rapid diagnosis of any disease whose onset is characterized by intracellular chemical changes. The detection of small quantities of a specific absorbing material can be further facilitated by modulating the gun cathode bias for the color channel correlated with the absorbed wavelength at a low frequency (of the order of 1 cycle per second), since the eye is very sensitive to the resulting flicker.

Modifications

An immediate improvement considered for the construction of the instrument is the replacement of the quartz lens in the illuminating system by quartz-fluorite achromats. This should eliminate the need for readjustment of the optical system of the illuminator with changes in wavelength selection and should lead to more nearly identical illumination at the three wavelengths.

A second change under consideration is the replacement of the single-image-orthicon camera by a three-Vidicon camera, as indicated schematically in Fig. 12. The beam of radiation from the microscope is directed successively to the "red," "green," and "blue" Vidicon—all of them, of course, of the experimental ultraviolet-sensitive type—by a pair of coupled sector disks synchronized with the vertical deflection; in Fig. 12, the shaded areas represent minor surfaces, while the clear areas transmit incident radiation unhindered. With such an arrangement, the camera becomes a simultaneous camera, and a standard simultaneous color monitor can be employed to view the picture. Vidicon lag no longer has an adverse effect on color purity (except in the viewing of objects in rapid motion), but merely serves to reduce flicker effects observed in the reproduced picture.

A similar system of sector disks (eventually rigidly coupled to those in the camera) may be employed in the illuminator to direct radiation from the three monochromators into the substage of the microscope (Fig. 13). This system possesses the advantage of rendering the timing of the pulsing of the light sources relatively uncritical and of achieving exact optical superposition of the three illuminating sources (*12*).

References and Notes

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10. If the three "separation negatives," corresponding to the three selected wavelengths, are preferred, they may be obtained either by photography of the monochrome monitor which is invariably employed for adjustment of the system or by using one color frame at a time.
11. P. K. Weimer and A. D. Cope, *RCA Rev.* 12, 314 (1951).
12. We are indebted to a number of workers at the Rockefeller Institute for Medical Research and at New York Hospital for supplying material for examination with the new instrument—in particular, to P. Weiss and A. C. Taylor for tissue cultures, M. A. Rudzinska for protozoans, E. L. Tatum for fungi, and G. N. Papanicolaou for cancer cells. The television equipment employed was made available on loan by RCA Laboratories. Carl Berkley made most of the preparations and contributed suggestions on the equipment. Other suggestions with respect to receiver modifications and the optical system were made by L. E. Flory and E. G. Ramberg of RCA Laboratories. The illuminating system was designed by P. Nolan of the Farrand Optical Company. The instrument represents an example of the effectiveness of cooperation between biologists and physical scientists in the development of new methods of biomedical investigation which could not have been achieved by either group working alone. It is now available at the Rockefeller Institute for Medical Research for cooperative investigations. Collaboration in the development of its uses and applications is welcomed.

The Maser

A Molecular Amplifier for Microwave Radiation

W. H. Culver

Electrical amplifiers in use today usually employ the motions of charged particles in electric or magnetic fields, as in vacuum tubes and transistors, or some nonlinear macroscopic property of matter, as in the case of the magnetic amplifier. An altogether different method of amplifying electrical signals is by stimulated emission of radiation. A device which uses this principle is called a maser (rhymes with razor). The word was coined by Townes and associates at Columbia University from the words *microwave amplification by stimulated emission of radiation* (*1*).

The maser principle was suggested by Weber in 1953 (*2*) and again independently by Bassov and Prokhorov in 1954 (*3*). The idea was used in a microwave spectrometer by Gordon, Zeiger, and Townes in 1954 (*4*). Within about the last year a large number of papers have been published on the subject, analyzing the theory and proposing new ways of incorporating the idea into practical devices (*5–17*).

Although the concept of this method of amplification is only a few years old, and although its successful embodiment into an instrument that can be used outside the laboratory awaits further development, its promise for future applications has aroused great interest in many technical fields. The main reason for this is that masers can operate with very low internal noise, much lower than the noise of the microwave amplifiers presently used. Thus, masers offer the possibility of greater ultimate sensitivities in the fields of radio astronomy, communications, and radar. Microwave spectrometers using the maser principle have already established their usefulness. Masers can be designed to oscillate at a very stable frequency, thus providing a basis for very accurate time standards.

The development of the maser was made possible by recent research in

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