Technical Papers

Electronic Quantitation of Light Absorption and Nuclear Fluorescence in Living Cells

Charles N. Loeser[1] and Carl Berkley

Western Reserve University, Medical School, Cleveland, Ohio, and *Allen B. Du Mont Laboratories, Inc., Clifton, New Jersey*

This paper describes a study of the application of electronic methods to the observation and quantitation of the light absorption and nuclear fluorescence of living cells. This work followed upon development of a method of observing nuclear fluorescence in stained living animals by a quartz rod transillumination technique (*1*), modified for use with near ultraviolet light (*2*).

The amount of light emitted by the nuclei is, under controlled conditions, an estimate of dye uptake. Certain types of cancer cells, for example, take up more dye and can be distinguished by greater fluorescence on slides (*3, 4*). For this differential detection, Mellors and Silver (*4*) devised a microfluorometric scanner.

In the present investigation, for use in the living animal (in the moving blood stream, for example), it was felt that actual television methods, such as those discussed by Young and Roberts (*5*) and Zworykin and Flory (*6*) might prove adaptable to rapidly changing phenomena. Therefore, a program of investigation was undertaken of various ways to study nuclear fluorescence by television and other electronic means. Light absorption was soon discovered to be easier to study than fluorescence, especially *in vivo,* because of the higher signal to noise ratio and the consequent more accurate reading.

Observations were made in three ways. In living frogs, transillumination was carried out either by (*a*) a rod of fused quartz, which carries light from the source to the organ examined by the microscope, or (*b*) by suspending tissue over the condenser of an ordinary microscope; magnifications up to 440 × were used, and higher ones are certainly possible. (*c*) Living cells, buccal, vaginal, or cervical, were stained directly on slides and placed on the microscope stage.

For the elicitation of fluorescence in the living animal, acriflavine hydrochloride is relatively nontoxic. Rats survive and appear unaffected by intravenous doses of 12.5 mg/kg. Mitotic regeneration of cells with nuclei stained *in vivo* with comparable doses has been reported (*7*). Concentrations as low as 0.005 percent aqueous solution were used in frogs in the dorsal lymph sac. The smears were stained with 1:100,000 to 1:1000 acriflavine for 30 to 60 sec. Staining takes place without previous treatment.

[1] The senior author wishes to acknowledge his indebtedness to the staff of the Allen B. Du Mont Laboratories, for courteous cooperation and invaluable technical advice and for placing at his disposal equipment for this study. The portion of this study performed at Western Reserve University was supported by a grant from the Life Insurance Fund.

Specimens were examined by two television methods and by two lower frequency scanning methods using somewhat different electronic parts. The principle of examination was in each case the same. The object is studied by a photosensitive scanning device, which electronically displays the value of light and dark areas in respective degrees on an oscillograph, so that at the same time the picture is viewed by the observer a record is made of the light intensities that pass to the "eye" of the electronic observer. The oscillograph record is in pulses of variable height, depending on the amount of light transmitted, and of variable width, depending on the area of the specimen.

The most elaborate assemblage used consists of an Image Orthicon camera chain, into the pickup tube of which the microscopic image is focused without intermediate optics. A single scanning line across the television screen is selected from the picture by a Du Mont Type 280 oscillograph, which so interconnects television screen and oscillograph that the selected line is shown to the observer by a white "strobe" line. The pulses transferred to the oscillograph from this line are a direct measure of the light coming through the specimen to the television screen. The less elaborate Vidicon television camera was tested for similar work. Although it proved less sensitive for low light intensities and had lesser resolution, it should be adequate for most studies.

A scanning disk and multiplier phototube, approximating closely that discussed by Mellors and Silver (*4*), was also tried. The image from the microscope is sent partially to the eye of the observer by a beam splitter in the body of the microscope tube, and partially to the multiplier phototube mounted over the

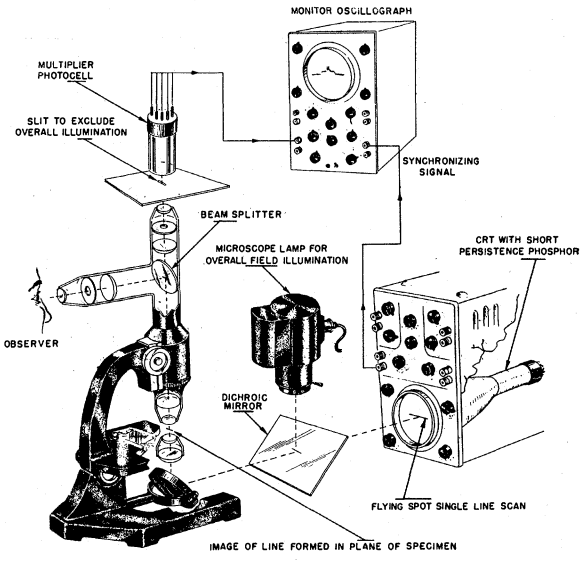

FIG. 1. Schematic diagram of optical system for single-line flying spot scanner.

ocular. Between the phototube and the ocular there rotates an opaque disk with a hole at one side, near the periphery. In rotating, the hole in the disk passes over a portion of the ocular field, permitting light to reach the photocell, and thus a portion of the field is scanned repetitively. Pulses registered by the photocell on an oscillograph indicated light transmitted. By proper manipulation, it is possible for an observer to know exactly which areas of observation are being indicated on the oscillograph.

One can substitute for the disk scanning a traveling spot of light from the fluorescent screen of a cathode-ray tube. Different fluorescence emission wavelengths are chosen for specific purposes (in the present experiments, the blue, for the absorption maximum of acriflavine). As shown in Fig. 1, the scanning source is directed at the mirror of the microscope, passes through the object, to the eye of the observer, and to the photocell. The eye sees the entire microscopic field with a single blue line. Pulse height on the oscillograph represents degree of absorption; pulse width represents size of the object interrupting the line.

By these methods, it was possible to study not only fluorescence from the stained smears but also absorption. Also, in living tissue—for example, mesentery—with rapidly moving cells in blood vessels, light absorption of any cells that passed the scanning line could be measured. Photographic methods are available to stop any such rapid measurement at any desired moment.

To make fluorescence studies, an exciting wavelength (4100 or 4350 A) was used with a yellow filter to eliminate transmitted blue light from the source but to permit yellow fluorescence to reach the "eye." For absorption studies, the yellow filter was removed.

Dye uptake was readily measured by studying increase in pulse height accompanying the gradually increasing amount of stain added to a specimen. With smears from known cancer patients, it was possible, since the pulses indicate not only amount of light but also the area supplying that light, to make simultaneous measurements of nuclear light absorption and nuclear size, a definite aid in picking up malignant cells present.

Readily visible *in vivo* nuclear fluorescence did not provide enough light for adequate oscillograph registration. With faint fluorescence, absorption studies proved satisfactory, the former being proportional to the latter under the conditions used.

Space does not permit more than a word of caution that such quantitation depends upon attention to details of careful absorption studies such as those described by Caspersson (*8*) and others. The instrumentation described here permits rapid measurement of absorption of light by morphologically identifiable microscopic portions of cells while the cells are alive and moving. The methods provide rapidity of determination, simultaneous measurement of size of object, density, and number of objects (by computers and counters, if desired), at any wavelength. Thus, other wavelengths, other dyes, and other tissues remain to be explored. The work invites further investigation and will be reported in greater detail.

References

1. M. H. Knisely. *Anat. Record* **64**, 499 (1936).
2. C. N. Loeser. *Anat. Record* **116**, 327 (1953).
3. H. P. Friedman, Jr. *Am. J. Obstet. and Gynecol.* **59**, 852 (1950).
4. R. C. Mellors, and R. Silver. *Science* **114**, 356 (1951).
5. J. Z. Young, and F. Roberts. *Proc. Inst. Elec. Engrs.* **99**, 747 (1952).
6. V. K. Zworykin, and L. E. Flory. *Elec. Eng.* **71**, 40 (1952).
7. P. P. H. Debruyn, R. C. Robertson, and R. S. Farr. *Anat. Record* **108**, 279 (1950).
8. T. O. Caspersson. *Cell Growth and Cell Function.* (W. W. Norton, New York, 1950), chap. II.

Received February 3, 1954.

Gallium Purification by Single Crystal Growth

W. Zimmerman, III

Crystal Branch, Solid State Division, Naval Research Laboratory, Washington, D.C.

In the course of an investigation of the preparation of intermetallic compounds of the Group III and Group V elements, it became necessary to obtain some high-purity gallium metal. A qualitative spectrographic analysis (*1*) of the available gallium indicated the presence of 12 elements as impurities (see Table 1, column A). The indicated range (*2*) of concentration of these impurities was from 0.1 percent down to less than 0.0001 percent. The total impurities indicated were about 0.3 to 0.5 percent.

Consideration of the various chemical means of purification (*3, 4, 5*) showed them to be complex and time consuming. In addition, many of the chemicals needed in the chemical procedures would have to be purified themselves before they could be used. Physical methods of purification were then considered. Distillation was immediately discarded because of the extreme liquid range and high boiling point of the metal. Recrystallization of the metal seemed to offer the best chance for success. Of the recrystallization methods available, zone purification and single crystal growth by the Kryropoulos technique (*6*) were considered to be the most promising.

It has been shown by previous work at this Laboratory (*7*) that the rejection of impurities during single crystal growth is extremely high. Hoffman (*3*) has shown that gallium grows easily. Therefore, despite the excellent results obtained in the zone purification of germanium, it was decided to try a modification of the Kyropoulos technique for gallium.

Since gallium melts at 29.7° C, a simple water bath held at a constant temperature of 38° C and controlled by means of a thermoregulator, was all that was required to keep the gallium molten. A lucite lid on the water bath held the container of gallium and the thermoregulator in position and prevented excess evaporation from the water bath. A thin-walled glass tube with a pointed tip and funnel top was filled with liquid nitrogen and used as a cold finger. Liquid nitrogen