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Ultra-Violet Flying Spot Television Microscopy. Study of Living HeLa Cells.* (22772)

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In another communication the authors have described a technic for obtaining relatively monochromatic U-V television images by means of the flying spot microscope(1). This system is in operation, and the purpose of this paper will be to report results of preliminary experiments designed to establish the period of time over which unchanged images can be obtained with the flying spot system.

Methods. The light source is a U-V emitting cathode ray scanner tube on the face of which is formed a 240 line raster. This raster is traced by a spot deflected horizontally at 60 cycles/second and vertically at one cycle every 4 seconds. These sweep rates were arbitrarily chosen to investigate potentialities of the system. A minified image of this raster is formed on the specimen by a Bausch and Lomb .75 NA Grey reflecting objective. Transmitted radiant energy is collected by a reflecting condenser optically matched to the objective and is focused on the entrance slit of a Bausch and Lomb grating monochromator. An ultra-violet-sensitive photomultiplier tube is located at the exit slit of the monochromator. The monochromator is adjusted to 2680 Angstroms with a 100 Angstrom band width. The electrical output of the multiplier tube is amplified and modulates a monitor display tube. The time bases of this monitor tube are locked in synchronism with those of the scanner tube. The screen has a very long persistency, of the order commonly employed for radar tubes. This long persistency screen is necessary because of the long vertical sweep cycle. Thus, the entire system becomes an

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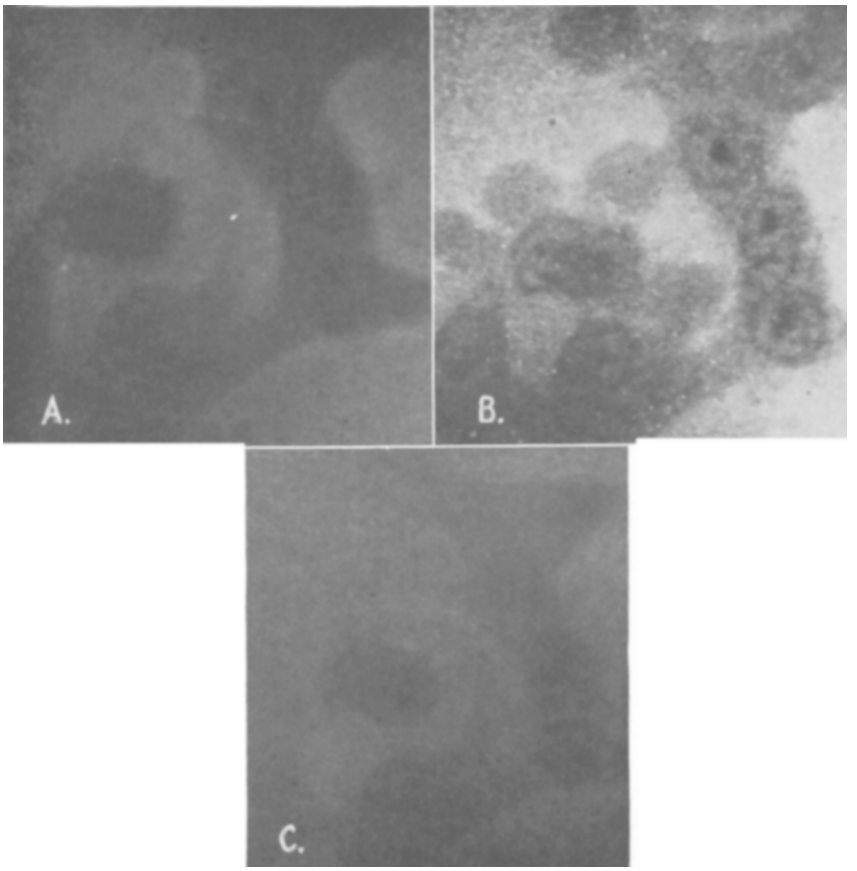

FIG. 1. Living HeLa cells at 2680 Å (667×). A. Original appearance. B. presence of cytoplasmic appearance at the end of 6 hr of continuous scanning. C. Return to normal after 12 hr interruption in scanning.

image converter and a specimen scanned in ultra-violet is displayed in visible light. The image on the monitor screen is photographed by a Polaroid Land camera. The signal to noise ratio of the display at these low radiation levels requires that the images of several consecutive frames be stored in the film emulsion. The signal to noise ratio then becomes a function of the square root of the number of frames stored. During the entire course of these experiments the microscopic field was continually irradiated by the scanner tube and photographs were obtained at periodic intervals from 15 minutes to 1 hour. In the experiments described, living HeLa strain cells were prepared on Vycor cover slips and sealed to Vycor slides by means of a wax vaseline ring. The cells were suspended in their own media composed of chick embryo extract, human ascitic fluid and Hank's balanced salt solution or in ascitic fluid alone, or in balanced salt alone. We were unable to detect any significant differences in the images obtained attributable to the nature of the media employed. Once the preparation was mounted on the microscope it was allowed to remain at room temperature (72°F) for the duration of the experiment.

Results. Fig. 1A shows a typical absorption photograph of living HeLa cells at 2680 Å as obtained with the flying spot technic. This field was then continuously irradiated and intermittent photographs of the monitor tube were obtained. The photographic images remained the same for a period of approximately 6 hours. At this time cytoplasmic bubbles began to form about the periphery of some of the cells in the field. These bubbles

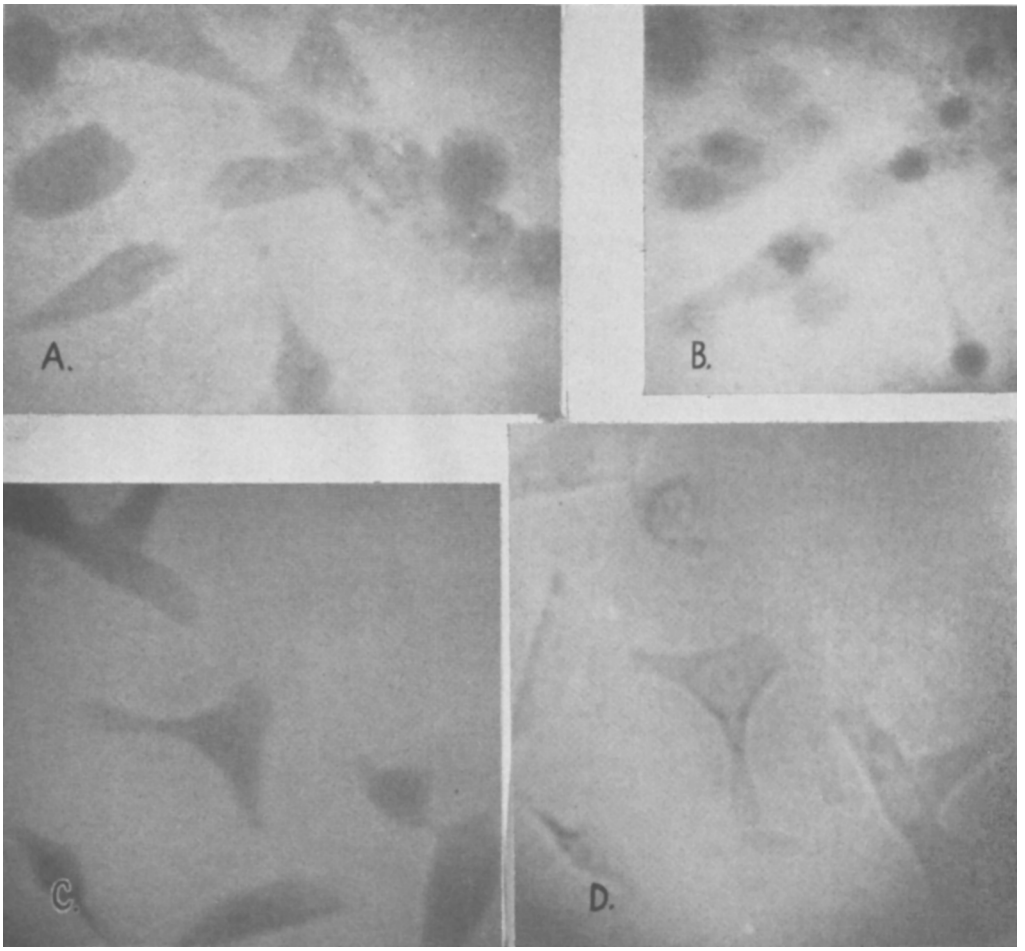

FIG. 2. Living HeLa cells at 2680 Å (533×). A. Original appearance. B. Following 6 hr of continuous scanning showing marked nuclear opacity and cytoplasmic transmission. C. Living HeLa cells at 2680 Å (533×). D. Living HeLa cells at zero orders showing lack of typical U-V absorption.

showed absorption at 2680 Å as may be seen in Fig. 1B but they were clear when viewed with visible light. In this experiment the phenomenon was reversible following a 12 hour respite from the continual irradiation, and the bubbles did not reappear upon subsequent irradiation of 3 hours duration as illustrated by Fig. 1C. One notes that the cytoplasmic absorption, nuclear lack of absorption, nuclear membrane and nucleolus all remain constant in their absorption appearance during this prolonged exposure to U-V irradiation. These 3 photographs do not show identical background densities of the photographic plates in each case and the density of the absorption is thus not quantitatively comparable. The present instrumentation does not permit a constant monitor screen brightness over 64 frames, thus variations in the amount of light reaching the photographic plate are inevitable. In a longer experiment consisting of 7 hours of continuous irradiation marked changes in the absorption pattern of the cells occurred at the end of the 7 hour period. These changes consisted of a complete opacity of the nucleus with lack of definition of the nucleolus, and a relative lack of the normal cytoplasmic absorption. The contrast between the appearance of these cells at the end of 2 hours and at the end of 7 hours is illustrated by Fig. 2A and 2B. In this experiment the cytoplasmic bubbles

described in the previous experiment appeared at the end of 5 hours and disappeared just before the appearance of the nuclear opacity.

In a third experiment continuous irradiation was applied to the cells for 6 hours. During this time the photographic images of the monitor screen remained the same. In this experiment 2 photographs were obtained at each time interval. Fig. 2C is a photograph obtained at the end of 2 hours. In this photograph the monochromator is set at 2680 Å. Fig. 2D is a photograph obtained immediately after Fig. 2C, the monochromator in this case is adjusted to the zero orders. Fig. 2C is a typical absorption photograph while Fig. 2D illustrates an image formed principally by light scattering. The latter image shows the characteristic clarity of the living nucleus when viewed with visible light.

Discussion. The principle of the flying spot microscope as described by Roberts and Young(2) has been adapted for use in the deep ultra-violet. The purpose of this adaptation was to find if possible a method which would permit the continuous or nearly continuous recording of ultra-violet absorption images of living cells without producing severe irradiation damage in the process. It would appear from these preliminary studies that this technic will yield just such results.

The failure of each group of cells to respond in an identical fashion to roughly equivalent time intervals of continuous scanning is not explained. It may represent differences in culture technic as the cells were transferred from stock cultures at irregular intervals, or it may represent variations in amount of irradiation. Complete regularity of U-V emission from the scanner tube is not possible in the present state of tube and phosphor development. Furthermore, day to day variations in the room temperature and instruments settings could not be rigidly controlled and it is conceivable that minor uncontrolled variations contributed to the experimental variations observed.

The data presented here are in agreement with those presented by Brumberg and Larionow(3). These authors describe the undamaged nucleus to show little or no absorption at 2680 Å. This is in marked contrast to Caspersson's observations(4), and may perhaps be accounted for by the lack of irradiation damage in this and Brumberg's work. Thus, lack of nuclear absorption by visible or ultra-violet light in normal living cells is one criterion of the absence of significant irradiation damage. As irradiation damage occurs the nuclear absorption increases until the nucleus becomes homogeneously opaque. The apparent increasing density of the nucleus in contrast to its normal transmissiveness may represent an alteration in the physical state of the DNA of the chromosomes. Such alterations have been produced by Ris and Mirsky (5) by physiological saline or fixation procedures. They consider the appearance of structure in the nucleus in visible light to be good evidence for cellular damage.

It appears that the flying spot scanning system of U-V microscopy is capable of producing continuous absorption images of living cells without producing significant irradiation damage. The system may be further improved by several technics, the most important of which will be to make the spot monochromatic before it reaches the specimen.

Summary. 1. Preliminary experiments with the continuous production of monochromatic ultra-violet absorption images of living cells indicate that this may be accomplished by the flying spot television microscope technic.

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