Spectrophotometer: New Instrument for Ultrarapid Cell Analysis

Abstract. *A new device has been developed for measuring and displaying multiple spectrophotometric properties of biological cells at rates exceeding 500 cells per second. Preliminary observations of human cells from different sites in the body were made at wavelengths of 2537 and 4100 angstroms to estimate cellular nucleic acid per unit volume of individual cells of large populations of cells. Display patterns were obtained which were consistent, and characteristically different for certain of the cell populations studied.*

Spectrophotometry provides a means for measuring nucleic acids and certain other constituents within the intact cell, as in studies of cell maturation and function (*1*) or in comparison of normal and neoplastic cells (*2*). We now present a new, automatic, rapid means of determining and displaying spectrophotometric properties of large populations of individual biological cells, whereby studies of the huge numbers of cells encountered in many clinical and research problems are facilitated.

As an alternative to mimicking the complex scanning methods of the human microscopist, we have developed an apparatus to determine spectrophotometric properties of cells flowing in liquid suspension through a beam of measuring radiant energies (Fig. 1). All the cells pass through the apparatus without selection at rates exceeding 500 cells per second; samples containing 100,000 cells or more can be examined in a few minutes. Values are recorded for absorption or scattering of light in such a way that a few unusual cells can be defined within very large populations. The apparatus is adaptable to the study of various types of cell populations—human, animal, or tissue culture—and for different multiple, simultaneous, spectrophotometric determinations. We describe the apparatus as applied to a study of two parameters, absorption at 2537 Å and scattering at 4100 Å; we include some preliminary observations on differences between different types of human cells.

The suspension of cells to be studied is screened with a Buckbee Mears micromesh (250 lines per inch) to remove tissue fragments and very large clusters of cells. It is then made to flow at a rate of 0.5 ml/min through a bow-tie shaped channel with a 100-μ by 100-μ constriction; the channel was cut into a quartz microscope slide with an ultrasonic cutter. A quartz cover was sealed to the slide over the channel, and polyethylene tubing was cemented into each of holes drilled in the slide near each end of the bow tie.

The slide is mounted on the stage of a microscope, with a Zeiss Ultrafluar, 100/1.25, glycerin-immersion objective imagining a 100-μ section of the flow channel onto an aperture. A Zeiss 0.85 NA immersion condenser images a Hanovia low-pressure mercury source on the channel; the lamp radiates much of its energy at 2537Å and near 4100 Å. A quartz lens and dichroic mirror are arranged to image light of wavelengths greater than 4000 Å from the back plane of the objective lens on to the face of a photomultiplier and to reflect shorter wavelengths at a 90-deg angle. The reflected beam is imaged on to a solar-blind photomultiplier after passing through a 3-mm quartz cell containing 1,4-diphenylbutadiene dissolved in ethanol. The combination of photomultiplier and filter was effective in isolating the 2537 Å line.

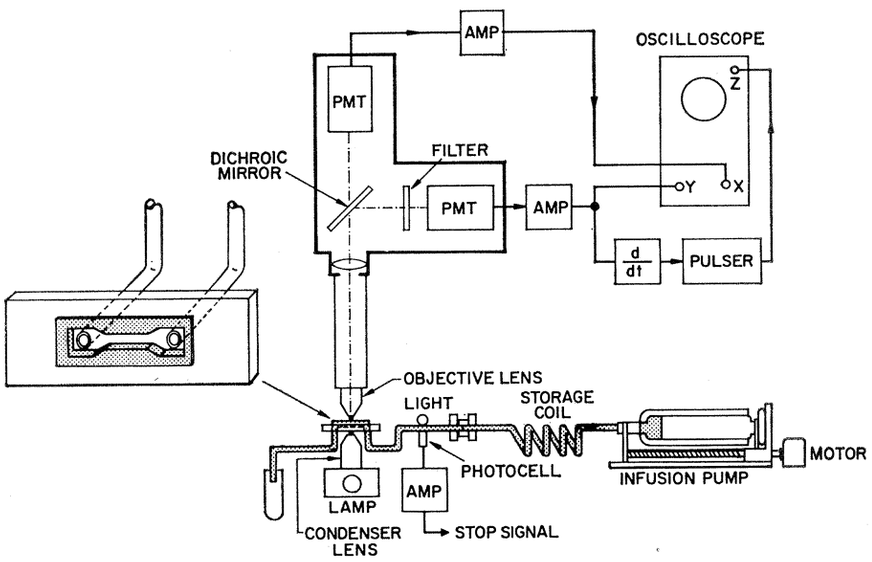

Fig. 1. Diagram of the apparatus. *AMP*, amplifier; *PMT*, photomultiplier; *d/dt*, differentiator circuit; *X* and *Y*, signal inputs; *Z*, intensity modulator.

The cells, flowing at 500 cells per second, produce pulses of 200-μsec duration at the output of each photomultiplier. The wavelength of 2537 Å is near the absorption maximum of nucleic acids. The numerical apertures of the condenser and objective lenses were selected to minimize scattered light in order to make the magnitude of the absorption pulse at 2537 Å a good estimate of the nucleic acid content of each cell. The other photomultiplier measures the light backscattered by each cell at wavelengths near 4100 Å. Although the scatter pulse is 30 times smaller than the 2537-Å absorption pulse, with equal incident energies, and although a higher gain is required in the scatter-signal amplifier, sufficient visible energy is present to give good signal-to-noise ratios for the signal pulses from both photomultipliers. The magnitude of the scatter signal proved to be the best of several methods tested for estimating cell size or mass (*3*).

The photomultiplier signals are band-limited from 300 cy/sec to 5 kcy/sec, amplified, and clamped to zero. The absorption-signal line is connected to the vertical deflection plates of an oscilloscope; the scatter-signal line, to the horizontal deflection plates. Each cell thus produces a line on the face of the oscilloscope, the end point of which line has coordinates given by the magnitudes of the two signals. The absorption signal is differentiated; the zero crossing of this signal initiates a 1-μsec pulse which is used to intensify the end of the oscilloscope trace. The beam intensity of the oscilloscope is set so that only a single dot appears on the screen for each cell at coordinates given by the estimates of cell size and nucleic acid content; all such dots produced by a sample are photographed.

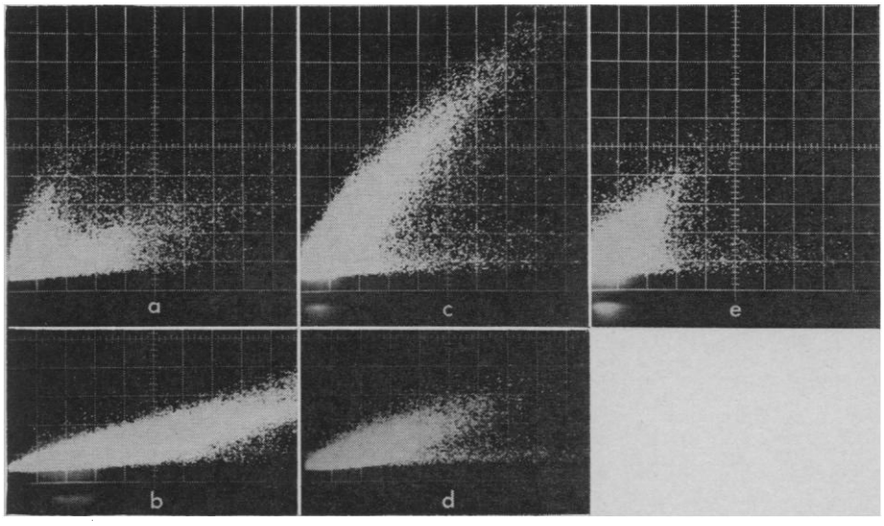

Fig. 2. Patterns of metastatic, keratinizing, epidermoid carcinoma at *p*H 2.1 (*a*) and at *p*H 3.8 (*b*); normal colonic epithelium (*c*), showing high absorption at 2537 Å compared with normal epidermoid epithelium (*d*); normal epidermoid epithelium (*d*) obtained by vaginal wash; epidermoid carcinoma of cervix (*e*), obtained by vaginal wash.

Other studies of ultraviolet absorption had required fixed cells on a quartz slide; there was limited information regarding the kind of fixation best suited for these measurements on cells in suspension. It was important to preserve cellular integrity, to minimize leaching, particularly of nucleic acids, and to standardize any denaturation of protein as well as ultraviolet absorption characteristics. Many fixative solutions were tested, as well as nonfixative salt solutions. The procedure finally adopted is as follows: a 4 : 1 mixture of isotonic saline and ethanol is used to collect cells which are subsequently fixed by resuspension in a mixture of ethanol, glacial acetic acid, and water (50 : 2 : 48). Cells stored in this fixative medium for 3 months or longer show little change in display patterns.

Differences in oscilloscope display patterns among varying types of cells are brought out best at low *p*H. For example, the oscilloscope display generated by cells of a metastatic, keratinizing, epidermoid carcinoma (obtained by scraping the cut surface of a cervical lymph node replaced by the cancer) is shown measured in an isotonic solution of sodium acetate and acetic acid at *p*H 2.1 (Fig. 2*a*) and in a mixture of ethanol, glacial acetic acid, and water at *p*H 3.8 (50 : 2 : 48) (Fig. 2*b*). These differences may result in part from the known effect of *p*H on absorption by nucleic acids (*4*).

The oscilloscope display patterns obtained for any one specimen are consistent and reproducible for at least four or five measurements; some deterioration appears after a dozen or more runs.

Cell suspensions prepared from a variety of surgically resected tumors were examined by the method just described, and their oscilloscope display patterns were compared with those from suspensions of corresponding nonneoplastic cells whenever possible. There was considerable variation in the oscilloscope patterns generated by benign epithelium of various types. For example, normal colonic epithelial cells (Fig. 2*c*) have relatively higher absorption than benign squamous cells (Fig. 2*d*); benign lymphocytes are intermediate. Some variation in the oscilloscope display patterns of cell suspensions obtained by vaginal irrigation depends on the phase of the menstrual cycle.

Among tumors examined were epidermoid carcinomas of lung and of uterine cervix, keratinizing squamous carcinomas from mouth and pharynx, adenocarcinomas of endometrium, colon, breast, and ovary, and malignant lymphomas. Cancer-cell suspensions from tumors so far studied have consistently shown cells (or cell clusters) with higher absorption at 2537 Å, when plotted against the light scatter signal, than the corresponding benign-cell suspensions.

Vaginal irrigation specimens from women with known invasive (Fig. 2*e*) and *in situ* epidermoid carcinomas of the cervix also contained varying numbers of cells with relatively high absorption at 2537 Å; typically, though, these cells were few compared with those obtainable by direct scraping of the cut surface of a carcinoma in a surgical specimen.

Several very abnormal patterns came from what are called "borderline lesions" of the cervix, or "dysplasia"—epithelial lesions that may precede the appearance of *in situ* carcinoma in the course of carcinogenesis (*5*). Specimens obtained by vaginal irrigation from a few patients with known cancer yielded patterns within the normal range. This was probably because of poor sampling; other specimens from the same patients contained significant numbers of abnormally absorbing cells.

From random observations of vaginal wash specimens from women outside the narrow group who are clinically healthy, nonpregnant, and of childbearing age, we know that abnormally high-absorption patterns may sometimes be expected in the absence of clinical cancer. We know that highly absorbing cells may be found after x-ray therapy; they may be present after apparently successful surgical resection of cervix carcinoma and in patients with ovarian carcinoma or with atrophic vaginal mucosa. To define these highly absorbing cells more fully in various clinical settings, it is necessary to correlate their absorption properties with cell size and form.

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