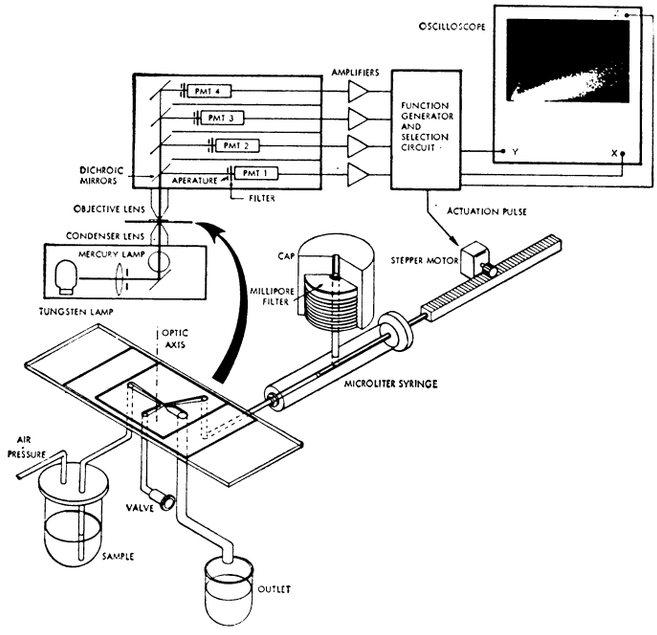
Spectrophotometric Cell Sorter

Abstract. *A new device can physically separate cells of predetermined optical properties from large populations of cells in suspension.*

A spectrophotometer has been described (*1*) for measuring certain optical properties of biological cells flowing in liquid suspension at rates up to 1000 cells per second. A few unusual cells in populations of 100,000 or more cells could be detected. The present report describes an extension of this work whereby cells may be physically separated according to functions of multiple simultaneous optical measurements on each cell.

Figure 1 shows both the optical and mechanical arrangements in the cell spectrophotometer and selector. The basic optical and electronic systems for measuring photometric properties have been described elsewhere (*1*). The present system has been modified to allow for simultaneous measurement at four different wavelengths in the ultraviolet and visible regions by the use of a series of dichroic mirrors to divert successively longer wavelengths to four photomultiplier tubes. We have used various combinations of wavelengths selected to study unstained or stained cells. The photomultiplier signals may be processed to obtain ratios or differences of measurements so that two functions of the photomultiplier signals for each cell may be represented by points on an oscilloscopic display as previously described. A selection circuit provides an activating signal whenever a specific cell exhibits photometric properties within a predetermined range. For example, nucleic acid content of each cell is estimated by total absorption at 2537 Å, and cellular size is estimated by visible scatter. If the ratio of absorption to scatter exceeds a specific value an actuation pulse is produced. The points



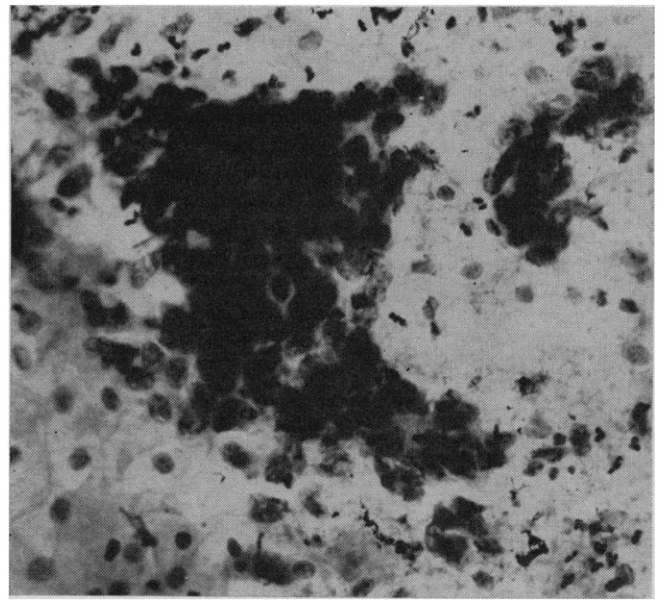


Fig. 1 (left). Diagram of the apparatus. Fig. 2 (right). Cells with high ultraviolet absorption selected from a vaginal wash specimen of a patient with carcinoma of the uterine cervix (*3*). This specimen was treated as described in Kamentsky *et al.* (*1*) and the separated cells were collected on a Millipore filter and stained with hematoxylin and eosin.

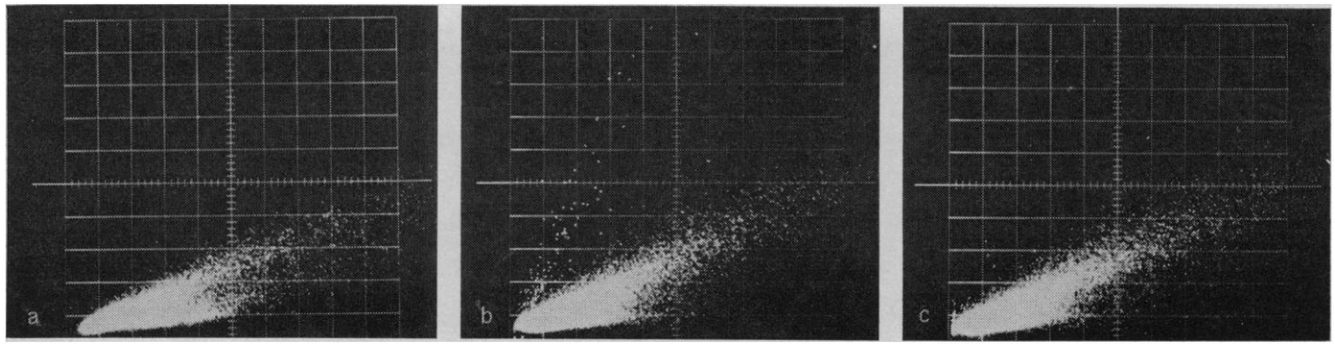


Fig. 3. Oscilloscopic display patterns of a test sample to illustrate the efficiency of separation. Total absorption at 2537 Å is shown on the vertical axis, and visible light scatter on the horizontal axis. The dots representing cells that will actuate separation are intensified. (a) Normal vaginal wash specimen with approximately 100,000 cells. (b) Vaginal wash specimen after seeding with cells, from a human embryonal rhabdomyosarcoma, that are highly absorbing in ultraviolet. (c) Same specimen as in (b), following separation of most of the highly absorbing cells.

representing these selected cells on the oscilloscope display screen can be intensified, resulting in a display photograph shown in Fig. 1, where the ordinate of each point is proportional to absorption and the abscissa to scatter. The actuation pulse also initiates a cell separation, as described below.

The cells suspended in a fluid medium are pumped by pressurization of the sample container at a flow rate of 0.5 ml/min through a flow channel (*2*) shaped as shown in Fig. 1. The cells are observed at the optical axis by the spectrophotometer and flow to the sorter junction in 2 msec. Unselected cells continue to flow out into the outlet container. If a specific cell has optical properties causing an actuation signal, the stepper motor is pulsed, causing the plunger in the syringe to draw 0.03 μl of fluid. This fluid pulse is propagated across the flow channel at the junction, causing fluid flow across the junction for about 3 msec. There is a delay of 2 msec from the time of detection until an appreciable flow occurs across the channel, matching the delay time of the cell to reach the junction from the observation point. In practice the cells flow at varying velocities, owing to the parabolic velocity profile across the channel. The velocity profile of the cells can be measured by imaging the cells upon a grating, and the actuation delay and displacement time can be matched to this profile. The actuation time is made longer than necessary to ensure that the desired cell has been captured in the side channel. Thus, several other cells will accompany the selected cell. We were successful in achieving a final concentration of selected cells of about 1 : 5 from initial concentrations in the range of 1 : 10,000. The selected cells are drawn far enough into the side channel to remain there for the duration of a run. Up to 300 selections can be made during a run of 100,000 or more cells.

After a sample has been processed the main channel is automatically flushed in reverse for cleaning. A Millipore filter is placed on a holder connected by tubing to a radial hole drilled into the barrel of the syringe as shown in Fig. 1. During the wash the plunger of the syringe is withdrawn to just beyond the radial hole so that there is a flow of clean water through the side channel, the syringe, and the Millipore filter. The separated cells are thus flushed out of the side channel and trapped on the Millipore filter. The Millipore filter is then removed, placed on a slide, and stained for visual observation of the cells. The cells are all visible in one low-powered microscopic field, as illustrated in Fig. 2.

The efficiency of separation can be demonstrated by comparing oscilloscopic display patterns of a test sample composed of two types of cells with different photometric properties before and after separation of one of the cell types. This is illustrated in Fig. 3.

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References and Notes

1. L. A. Kamentsky, M. R. Melamed, H. Derman, *Science* **150**, 630 (1965).
2. The fluid-switching flow channel was designed by H. H. Glattli.
3. This specimen was supplied by Herbert Derman.
4. We thank Herbert Derman and Leopold G. Koss for helpful suggestions and support, and H. H. Glattli, Issac Klinger, George A. Folchi, and Arnold Halperin for assistance in the design and testing of the apparatus.

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Saturation in Milk and Meat Fats

Abstract. *Meat and milk products from ruminants (cows, goats, sheep, and beef animals) contribute 35 to 40 percent of the fat in the average American diet. Such fat is highly saturated, containing less than about 4 percent polyunsaturated fatty acids. The unsaturated plant lipids (fats) ordinarily consumed by the ruminant are hydrogenated (saturated) in the rumen. Transport and incorporation of this hydrogenated fat into meat and milk follows. Rumen hydrogenation does not take place until the fat is broken down to free fatty acids, thus establishing the fact that lipolysis is an essential feature of the process. Circumvention of this lipolysis may lead to more-unsaturated meat and milk fat.*

In the course of the controversy concerning the relation between fats and heart disease, Americans have been advised "to eat less animal fat" (*1*) and "to increase the intake of unsaturated vegetable oils and other polyunsaturated fats, substituting them for saturated fats wherever possible." The proponents of this argument believe that polyunsaturated fats in the diet tend to establish lower amounts of cholesterol in the blood than the saturated fats do, and that high amounts of cholesterol in the blood are associated with damaging plaque formation in the arteries. The role of dietary fat in human health has been reviewed (*2*).

Why are fats saturated and can they be made less so? About 35 to 40 percent of the fat consumed in the average American diet is derived from meat and milk produced by ruminants (*3*); these products include milk, cream, butter, cheese, ice cream, and other dairy products (19.5 to 27 percent), as well as beef, veal, lamb, and mutton (14 percent). The polyunsaturated fatty acids in these foods seldom exceed 4 percent of the fat fraction, whereas in vegetable oils they predominate (*4*).

Lipids in plant materials fed to ruminants contain large quantities of polyunsaturated acids, and these acids become saturated in the rumen of the animal as a result of microbial action (*5*). This action changes the polyunsaturated fatty acids of the feed substantially into stearic acid which becomes a dominant factor in fat synthesis by the animal. Because the fatty acids in plant lipids occur mainly in esterified form and because the principal resultant of rumen digestion is free stearic acid, hydrolysis of the ester may be a prerequisite for hydrogenation of the fatty acid. Rumen hydrogenation of phytol is facilitated by its hydrolysis from the chlorophyll molecule (*6*). If hydrolysis is required, protection of fed lipids from rumen lipase should permit the polyunsaturated lipids to be metabolized from the lower gut in much the manner of a monogastric animal such as man. For example, Insull *et al.* (*7*) induced an increase in the linoleate (polyunsaturate) content of human milk fat from 5 to 45 percent by including corn oil in the mother's diet.

We analyzed the major lipid classes in rumen ingesta to determine their relative saturation. We extracted lipids (*6*) from ingesta recovered from the rumens of milking Holstein cows of the University herd. These lipids were then separated by chromatography on a silicic acid column into polar lipids (phospholipids and glycolipids), free fatty acids, and neutral lipids (glycerides and sterol esters). The purification steps were followed by thin-layer chromatography. Samples of the lipid classes were methylated and analyzed