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INTRODUCTION

Flow microphotometry, a term we have coined to describe the unique flow systems developed over the last few years at the Los Alamos Scientific Laboratory (LASL), allows quantitative precision measurements to be made of the optical properties of individual mammalian cells. Mammalian cells in suspension are made to pass through a special flow chamber where they are lined up, one-at-a-time, for exposure to the blue light from an argon-ion laser. As each cell crosses the laser beam, it produces one or more optical pulses of a duration equal to cell transit time across the beam. These pulses are detected, amplified, and analyzed using essentially the techniques of gamma-ray spectroscopy.

Blue light from the argon laser is suitable for excitation of many fluorescent dyes of biological interest. Cells illuminated by this light will produce a light-scattering and fluorescence pulse if appropriately stained. Using various staining techniques, we have measured the (1) DNA content of various cell populations; (2) presence of virus in infected cells through the use of fluorescent antibody techniques; and (3) protein content of various tissue culture cells. Quantitative DNA distributions have also allowed us to distinguish tumor cells from normal cells in tumor material obtained from animals and humans, as well as to assay for radiation effects on tumor cells subjected to x and gamma irradiation. DNA measurements have also been used for life-cycle analysis, drug effects studies, and ploidy investigations on a number of mammalian cell types. Acridine orange staining has permitted verification of a crude white blood cell differential method and parasite identification in red blood cells.

This method of single-cell analysis is unique in several respects: (1) rapid quantitative measurements are possible on large numbers of cells (typically at

a rate of 5×10^4 cells/min); (2) as a result, statistical precision is very high; and (3) measurements not previously possible on individual cells within a population can be performed routinely with these methods. Commercial versions of this instrumentation are either on the market or will appear shortly. Through experience (sometimes bitter), we have learned that correct biological preparation is essential for successful exploitation of unique features by the instrumental methods discussed here. Our biology effort at the LASL places great emphasis on proper methods of cell dispersal, fixation, and use of specific staining procedures.

There have been many individuals who assisted in development at LASL of these techniques over the last few years. These include Dr. M. A. Van Dilla, the original project leader, currently with the Biomedical Division at the Lawrence Livermore Laboratory, University of California, Livermore, California; Dr. M. J. Fulwyler, formerly president of Particle Technology, Inc., Los Alamos, New Mexico; Dr. A. Brunsting, now with Coulter Electronics, Hialeah, Florida; and Dr. P. K. Horan, now at the University of Rochester, Rochester, New York. At Los Alamos, T. T. Trujillo, R. A. Tobey, P. M. Kraemer, H. A. Crissman, L. L. Deaven, and M. R. Raju, among others, have been responsible for developing interesting biological applications of the instrumentation described here.

HISTORICAL BACKGROUND

Modern flow-systems instrumentation has been under development since the middle 1960s. The earliest instrument in this category, of course, was the Coulter Cell Counter (1) which sensed cell volume and provided a means of cell enumeration. Further refinements on the technique (2) provided a method of

obtaining cell volume distributions. Other cell counters of the period included the Sanborn-Frommer and Vickers cell instruments which were optical devices based on light scattering. These instruments provided only enumeration capability. In these last two instruments, no attempt was made to correlate light-scattering signal with cellular characteristics such as the size of other morphological features. In the approach of Van Dilla et al. (2), quantization of cell-size information was made available. Essentially, the signal derived from a Coulter counter sensor was coupled to a pulse-height analysis electronics system common to gamma-ray spectroscopy, yielding pulse-height (volume) distributions. A further development of this technique resulted in the first working cell-sorting device which isolated single cells based on cell-volume differences (3).

Cell-volume spectrometry was initially employed in a large variety of studies of cell biology. Of interest here might be some of the studies performed on various white blood cell populations (2) and bone-marrow material (4) with the volume sorter. However, based on cell-kinetic studies, it became evident that cell volume by itself is not a very discriminating cell marker (5). Because of the lack of uniqueness of cell volume, many investigators became aware of the need to use some other cellular parameter with sufficient fine structure to reveal more subtle cellular differences. Because of the long experience with staining techniques for these purposes, these various investigators selected fluorescence detection as the next logical approach. In addition, it was recognized that light scattering could be related to cell size (6,7) and possibly to cell morphology. Hence, the measurement of optical parameters has been stressed in the last several years. Although the several optical flow instruments available today differ in detail, the basic

philosophical approach is common to all. Notable contributions, in terms of working systems used by biological investigators, can be credited to Van Dilla et al. (8), Kamentsky et al. (9), Mullaney et al. (10), Holm and Cram (11), Mullaney and West (12), Steinkamp et al. (13), Bonner et al. (14), and Dittrich and Göhde (15). Several of these instruments are capable of cell sorting as well as analysis. In this respect, there are two basic approaches: (1) those like in use at the LASL where cell measurements are made within an optical cuvette (13) or (2) where the measurements are made on cells in a stream in air (14,16). Sorting instruments based on the Stanford design have been reported more recently by other investigators (16,17).

Present-day commercial sources of instruments suitable for fluorescence single-cell analysis are available from (a) Biophysics Systems, Mahopac, New York; (b) Becton-Dickinson, Sunnyvale, California; and (d) Phywe, Göttingen, West Germany. Today major noncommercial research laboratories investigating flow-systems instrumentation and its applicability to cancer detection and other biological problems include the (a) Los Alamos Scientific Laboratory; (b) Lawrence Livermore Laboratory; (c) University of Rochester; (d) Stanford University; (e) Papanicolaou Institute of Miami, Florida; (f) Max-Planck Institute for Biophysical Chemistry, Göttingen, West Germany; and (g) Wilhelm Westphalia University, Münster, West Germany. Most of the above mentioned laboratories are interested in instrumentation development and applications. Many more institutes are investigating the application of this instrumentation, particularly as commercial instrumentation becomes available. It must be emphasized that the flow-systems instruments represent a new and novel approach to biomedical problems which ranks with electron microscopy, as an example, in potential.

At the present time, the availability of instrumentation for all interested investigators is limited. This is an important and expanding technology; the need for reliable, commercially available instrumentation cannot be stressed enough. From our own experience at the Los Alamos Scientific Laboratory, it is imperative that commercial instruments find their way into the hands of the many competent investigators who can advance the field by discovering applications not thought of today.

FLOW MICROPHOTOMETRY

The instrumentation described here is rather sophisticated and a far cry from the first cell flow photometer developed at LASL which used a compact arc mercury vapor lamp as the light source (18). This early unit permitted fluorescence measurements to be made on cells treated with fluorescein diacetate (19) or pollen grains stained with acridine orange. Although both of these samples are bright, it became apparent that this source of exciting light would not be sufficient for quantitative measurements on the typical fluorescently stained mammalian cell. In early 1969, a 2-watt Spectra-Physics Model 140 argon-ion laser was obtained and substituted as the light source. An increase of about two orders of magnitude in signal strength was achieved immediately. Today, all our flow microphotometers or cell-sorting instruments employ argon lasers (Coherent Radiation Laboratory Model 54 or Model 52, Spectra-Physics Model 164) as the excitation source. Since all the light is contained in a beam of about 1 mm diameter, simple optics can be used to collect the light and to shape the beam. The narrow beam diameter and small divergence angle permit scattering measurements to be made at very small angles from the optical axis of the system, where most of the scattering from particles of cell size (10 μm diameter) occurs.

Light from the laser is collected and focused to a small spot at the center of a special laminar flow chamber through which the cells pass. Fluorescent measurements are made at 90° to the optical axis of the system; light-scattering measurements are generally made at angles as small as 0.5° from the optical axis in the forward direction. The basic principles outlined in this section apply to all our instruments of either the cell-analysis or cell-sorting type.

The crucial part of the flow system of the cell-analysis instruments is the flow chamber, which is shown schematically in Fig. 1. Several versions of this chamber have been built, but all operate on essentially the same principles. The chamber is a variation of a flow chamber originally developed for aerosol counting (20). Although other designs were tried (21), this one was selected for the reasons given below.

A laminar flow of any convenient liquid (water, saline, buffer solution, etc.) is established within the central bore of the chamber (B, Fig. 1) which is 3.2 mm in diameter. This "sheath" fluid is introduced at the bottom of the chamber through the ports (A). The flow then enters a smooth transition region (D) where the flow diameter decreases to about 300 μm. The fine hypodermic tubing (C) of 300 μm inside diameter on the axis of the main cylindrical bore of the chamber serves to introduce the cell suspension smoothly into the faster flowing "sheath" fluid. The Reynolds number in region B of the chamber is typically 200, well below the critical value of about 2000 which is the "boundary" between laminar and turbulent flow. In the small bore region (D), the Reynolds number is about 1000 and, thus, is closer to the value at which turbulence can develop. The effect of constriction D is to speed up the flow and to decrease the total flow from 3.2 mm to 3000 μm in diameter. There is a similar decrease in cell stream diameter from 300 to about 20 μm. At a typical cell concentration of 50,000 cells/ml, the average separation between

cells is about 3 cm, and the chance of two cells passing a given point simultaneously is quite small. Thus, the cells have been lined up, one-at-a-time, for exposure to the light beam.

The flow jets out of the nozzle (D) across a relatively quiescent region where laminar flow is maintained (E) and exits through the tube (F) of the same diameter as D. The flow chamber is of square cross section (22 mm on a side) with four windows (W), allowing a perpendicular view of the fluid flow. Exciting laser light enters through one window, allowing the use of f/1 light collection optics for the fluorescence signal. Light scattering is viewed through the exit window for the main laser beam. A pressure differential of about 5 in. of mercury is maintained across the chamber, producing a sheath flow rate of 30 ml/min. The sample flow rate is 1 ml/min or less and is controlled by adjusting the differential pressure between the sheath and sample streams. A schematic diagram of the integrated cell-analysis system is shown in Fig. 2. The actual flow chamber shown in Fig. 1 is used. The fluorescence signals are detected at 90° to the optical axis of the system; light-scattering measurements are made in the forward direction.

Instrumentation has also been devised to permit physical separation of the cells based on light-scattering, fluorescence, or volume (Coulter principle) measurements. Laminar flow is established in a flow cell (Fig. 3) somewhat similar to that described above. Once a cell of interest has been sensed, an electronic time delay is activated for the length of time required for the cell to cover the distance from the point of cell detection to the point of droplet formation. Droplet formation is accomplished by vibrating the flow chamber at the resonant frequency of the stream (about 40,000 cycles/sec); this causes droplet formation at the same rate. When a cell of interest arrives at the droplet formation point, a charging pulse is applied to the column and six

droplets are charged. When these droplets enter the dc field, they are deflected to one side (Fig. 4); about one droplet in 70 contains a cell of interest. Therefore, a sample is collected in which there is a high purity in the cell of interest.

The signals generated by the cells are processed using the techniques of gamma-ray spectroscopy. After amplification, the signals are subjected to pulse-height analysis using either a hardwired analyzer or a small computer (PDP-11/20). The data are displayed as a frequency distribution histogram of the parameter of interest.

RESULTS

A large effort has been devoted to using DNA as a cellular marker to do a number of basic studies with tissue-culture cells, and these have been summarized in considerable detail elsewhere. As an example of the usefulness of cell sorting, we have considered the identification of tumor cells by elevated DNA content. The DNA frequency distributions for (1) normal mouse spleen cells and (2) cells obtained from a MCA-1 tumor growing in the same mouse are shown in Fig. 5. In each of these displays, the vertical axis is the number of cells measured, and the horizontal axis is the channel number directly proportional to signal strength. Frame A is the DNA distribution obtained when the cells are stained with acriflavine using the Feulgen procedure (22). The main peak near channel 20 contains cells in G_1 phase, the peak near channel 40 cells in G_2 + M phase, and the area between the two peaks cells in S phase synthesizing DNA. The peak near channel 20 is due to normal cells in G_1, and the other two peaks near channels 42 and 85 are thought to be cycling tumor cells. The sorter was adjusted to sort the cells into two groups, as shown in Fig. 5, and the results of the sort are shown in Fig. 6. Frame A is a photomicrograph of the

mixture obtained from the tumor; in frame B are normal leukocytes from circulation at the tumor site, and in frame C are the larger tumor cells with enlarged nuclei.

A similar expriment was performed for several cultured tumor lines, and these data are summarized in Table 1. For the hyperploid lines, the DNA content observed by the flow microfluorometer (FMF) method ranges from 1.5 to 1.71 times normal. In addition, the chromosome number predicted from these data agrees well with the measured chromosome number. These data show the usefulness of this method for detection of some tumors due to elevated DNA content.

In addition to fluorescence measurements, considerable attention has been placed on measuring the light scattered by cells as they traverse the laser beam. Light is scattered by small particles into patterns which are indicative of size (13,23), internal structure (7,24,25), and surface detail. Small-angle light scattering has been combined with fluorescence and selective absorption to stained human blood to obtain various levels of leukocyte differentiation (26-31). We describe here a flow-systems technique for separating cells into different morphological classes based only on measurements of the light scattered from each unfixed, unstained cell at two angles with respect to the laser beam. To demonstrate the utility of the method, we have shown that it can be used to distinguish three types of unstained human leukocytes: lymphocytes, monocytes, and granulocytes. The value of this technique is that only minimal cell preparation is required and that neither fixation nor staining with their associated risks of artifact introduction is required. Hence, viable individual cell types can be separated for use in other experiments. The cell types were separated using a flow-systems cell sorter similar to that described above. Type identification was made after sorting by staining the cells with Wright-Giemsa stain and examination under a microscope.

A two-parameter pulse-height distribution giving a histogram of the number of cells (z axis) vs the two coincident light-scatter signals (x and y axes) is shown in Fig. 7. The upper half is a contour map displaying the intensified regions containing counts above a threshold which is shown in the isometric view in the lower half. Peaks 1-3 were found to contain mostly neutrophils, monocytes, and lymphocytes, respectively. Between 5000 and 20,000 cells were separated for each of the groups in Fig. 7 for each individual. The cells in a peak were separated by setting a pulse-height window on each of the two light-scatter signals to define a rectangular region around the peak and sorting all those cells whose signals fell within the rectangle.

The separated cells were placed on microscope slides using a Shandon Scientific Company (Sewickley, Pennsylvania 15143; catalog No. 0025) cytocentrifuge, fixed in methanol, and stained with Wright-Giemsa stain for cytological examination. The morphological classifications of the sorted populations are shown in Table 2. The leukocyte types used in the differential counts are shown in the first five columns, and the number of cells examined for each group is given in the sixth column. The differential percentages for each sorted group for each individual are presented in the labeled rows. Because normal human blood contains low percentages of both eosinophils and basophils, these cell types were not isolated from human blood.

Unstained leukocytes can be classified into distinct morphological types based only on a measurement of light scattered by each cell at two different angles. Thus, unstained human leukocytes were classified into three distinct types. Similar analysis of irradiated monkey blood indicates that eosinophils may be separable from neutrophils with increased system resolution. All results were obtained with unfixed, unstained cells. We anticipate that a more complete leukocyte differential may be possible by detecting the scattered

light intensity with greater spatial resolution, followed by computer analysis using conventional pattern recognition techniques. Thus, multiangle light scattering from unstained cells may be a useful tool for separating cells of different morphology.

An instrument is presently under development which will permit simultaneous measurement of the light scattered at 32 different angles. The scatter detector is a photoconducting array consisting of 32 elements as shown in Fig. 8. As a cell crosses through the laser beam, the scattered light is detected between 0° and 20°. The information is strobed into a PDP-11/45 computer where it is stored on a disk. Essentially, the intensity vs angle curve for each cell is recorded. After each experiment, a mathematical cluster analysis code is used to divide the gross population into subpopulations based on the number of classes of light-scattering intensity curves that are generated. Using this technique, we have been able to identify several types of blood cells, as well as algae. In all these cases, the objects identified by light scattering are unfixed, unstained, "virgin" cells. This has several potential advantages. These cells can be sorted and used in future experiments since they have not been altered in any way by fixation, staining, etc. In addition, sorted cells in the virgin state can be presented to a cytotechnician for eventual staining and microscopic verification. The light-scattering approach might be a more suitable approach to the question of acceptability than staining.

DISCUSSION AND SUMMARY

We have only begun to scratch the surface of this new technology. There are many technical innovations that are possible, as well as interesting biological applications. A few of these will be outlined below. We are

currently expanding our capability for making several simultaneous scattering measurements on single cells. This might permit another method of cell identification, as suggested above. Measurements at several angles can be used also to relieve difficulties associated with scattering measurements on low refractive index cells (23).

Absorption measurements on single cells are also possible, although these have not been pursued to as great an extent as fluorescence measurements. Direct measurements on live cells are made attractive, now that ultraviolet laser systems are becoming available. Some higher power argon lasers have usable power levels at 350.0 and 363.8 nm.

In the area of fluorescence measurements, there are several innovations possible. Our present bi-color sensor can be replaced with a more sophisticated optical system to permit some spectra analysis on fluorescence emission. Analysis of lifetimes of the fluorescent states should yield information on the nature of binding between the appropriate cellular constituents and the fluorescent dyes. Excitation at more than one wavelength will be possible in the near future. This advance, coupled with the multi-color sensor, should allow use of several dyes on each cell.

The sensitivity for optical signal detection can be increased by more efficient light-collecting systems. An increase in sensitivity should permit measurements on smaller objects such as bacteria. Following the method suggested by Thomas and Leif (32), we have designed an electronic cell sensor which detects the potential difference as a cell traverses a Coulter orifice. This unit is capable of signal-to-noise ratios two to three times superior to that obtained with standard Coulter sensing (33).

Again there are many areas of application that we have only begun to explore. Using the FMF principles outlined, it should be possible (at least

in principle) to detect any cellular property that can be labeled with a fluorescent dye. In this respect, we are exploring other stains for cellular proteins and enzymes. In addition to the fluorescence antibody work cited above, preliminary studies are under way here on other immunofluorescent systems.

Further extension of detection sensitivity will allow measurements of natural fluorescence of biological systems. Extensions of our studies on life-cycle analysis methods will permit kinetic studies on some tumor cells. We have begun studies on animal tumor cells for which x irradiation is therapeutic. An analysis of the DNA distributions on irradiated cells from the tumor can give an indication of the mechanism of cure (34). This method is being developed as a tool for basic radiobiology, as well as a possible diagnostic for treatment evaluation.

It has been shown recently that live tissue-culture cells can survive cell sorting and go on to reproduce. Thus, live cells with interesting immunofluorescence properties can be separated and collected for further studies. The ability to sort cells is important as new methods of cell analysis are developed. Cells identified instrumentally as different can be separated and subjected to human cytological examination for verification.

The topics for further study listed in this section are just a few of the many areas in which there is work to be done. The technology pioneered at LASL is sure to find many varied and interesting applications in the near future, particularly as other investigators enter the field and reliable commercial instruments become available.

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TABLE 1. Summary of Nucleic Acid Data for Several Cultured Tumor Lines

Cell type (strain)	Passage	Relative DNA by FMF ± σ (average CV)	Predicted chromosome number from FMF (mean ± σ)	Observed mean chromosome number (mean ± σ)
Hyperdiploid Lines				
Epidermal carcinoma (A-253)	28-32	1.70 ± 0.06 (6.9)	78.2 ± 2.8	66.0 ± 3.7
	26-33	1.71 ± 0.09 (6.1)	78.7 ± 4.1	
Pharyngeal carcinoma (Det 562) (pleural effusion)	114-115	1.71 ± 0.09 (6.1)	78.7 ± 4.1	63.1 ± 1.9
Malignant melanoma (A101D)	15-38	1.45 ± 0.08 (7.8)	66.7 ± 3.7	59.4 ± 5.1
	17-26	1.51 ± 0.12 (6.3)	69.5 ± 5.5	
Choriocarcinoma (BeWo)	198-204	1.66 ± 0.05 (7.5)	76.4 ± 2.3	73.3 ± 11.6
Breast carcinoma (734B/C) (pleural effusion)	48-51	1.66 ± 0.04 (7.7)	76.4 ± 1.8	72.2 ± 10.1
Diploid or Near-Diploid Lines				
Rhabdomyosarcoma (A-204)	48-49	1.00 ± 0.04 (6.8)	46.0 ± 1.8	46.4 ± 4.1
	58-59	1.08 ± 0.04 (5.9)	49.7 ± 2.0	
Rhabdomyosarcoma (A-673)	24-25	1.00 ± 0.04 (6.6)	46.0 ± 1.8	46.6 ± 1.2
Rhabdomyosarcoma (130T)	unknown	1.04 ± 0.05 (6.3)	47.8 ± 2.3	51.0 ± 12.3
Breast carcinoma (Levine III) (pleural effusion)	unknown	1.05 ± 0.05 (5.9)	48.3 ± 2.3	48.5 ± 7.1
Burkett lymphoma (P3J)	unknown	1.00 ± 0.05 (5.8)	46.0 ± 2.3	47.3 ± 7.4
Control Cells				
Chinese hamster ovary	unknown	1.00 ± 0.04 (4.5)	23.0 ±*	23.0 ±*
WI38	19-24	1.00 ± 0.05 (5.0)	46.0 ± 2.3	46.6 ± 2.0
FL	unknown	1.60 ± 0.09 (5.0)	73.6 ± 6.6	70.0 ± 6.0

*Although Chinese hamster ovary cells contain only 23 chromosomes, all normal mammalian cells have the same amount of DNA per cell.

Table 2. Leukocyte Differential Counts for Each of the Separated Populations

Composition of Sorted Peak (%)		Leukocyte Type					Number of Cells Examined Microscopically
		Neutrophil (segmented)	Neutrophil (band)	Monocyte	Lymphocyte	Eosinophil	
Human A	Peak 1	88	5	3	4	0	400
	Peak 2	9	2	80	9	0	300
	Peak 3	0	0	5	95	0	400
Human B	Peak 1	83	12	1	4	0	100
	Peak 2	2	0	77	21	0	100
	Peak 3	1	0	6	93	0	100
Human C	Peak 1	84	6	5	4	1	200
	Peak 2	2	0	83	15	0	200
	Peak 3	1	0	1	98	0	300

Fig. 1. The flow chamber (scale 4 times actual size): (A) sheath entry ports; (B) central bore of chamber; (C) sample injection tube; (D) nozzle; (E) quiescent (viewing) region; (F) exit port; and (W) viewing windows. The sample injection tube and exit port are sealed with O-rings. The chamber is mounted on a micro-manipulator to allow precision alignment of the sample stream and laser beam.

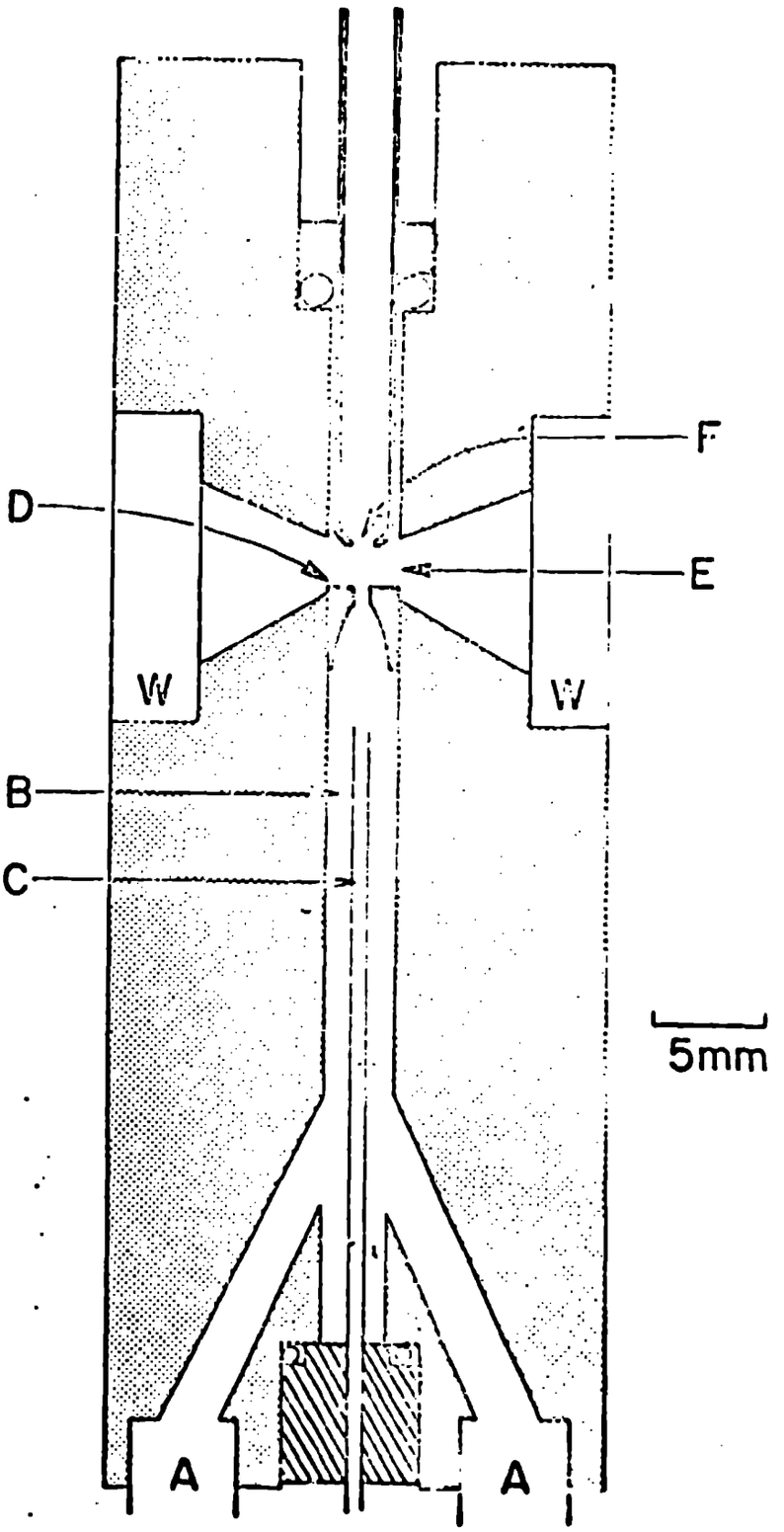

D
F
E
W
W
B
C
5mm
A
A

Fig. 2. Schematic diagram of the dual-parameter flow microfluorometer. The argon-ion laser beam (lower right corner) is focused with an 18-cm lens to a 50-μm spot at the center of the flow chamber. Fluorescent light is detected at 90° to the incident direction. The light scattered in the forward direction between the central beam stop and outer stop is collected with a 10-cm lens and focused onto a photodiode.

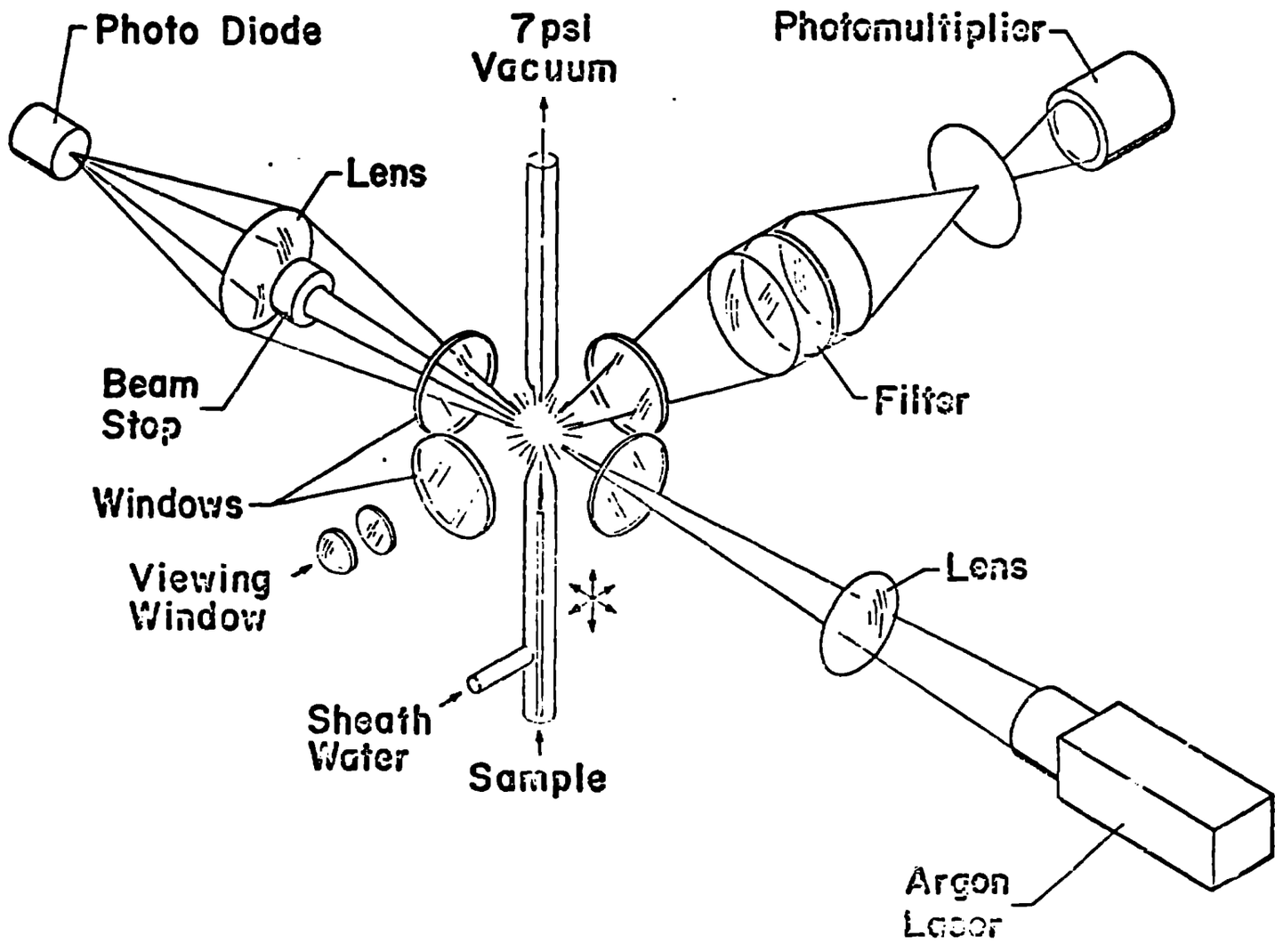


Photo Diode
7 psi
Vacuum
Photomultiplier
Lens
Beam
Stop
Filter
Windows
Viewing
Window
Lens
Sheath
Water
Sample
Argon
Laser

Fig. 3. Cutaway view of the multiparameter cell sorter flow chamber, showing sample and sheath fluids, Coulter sensor, and laser illumination.

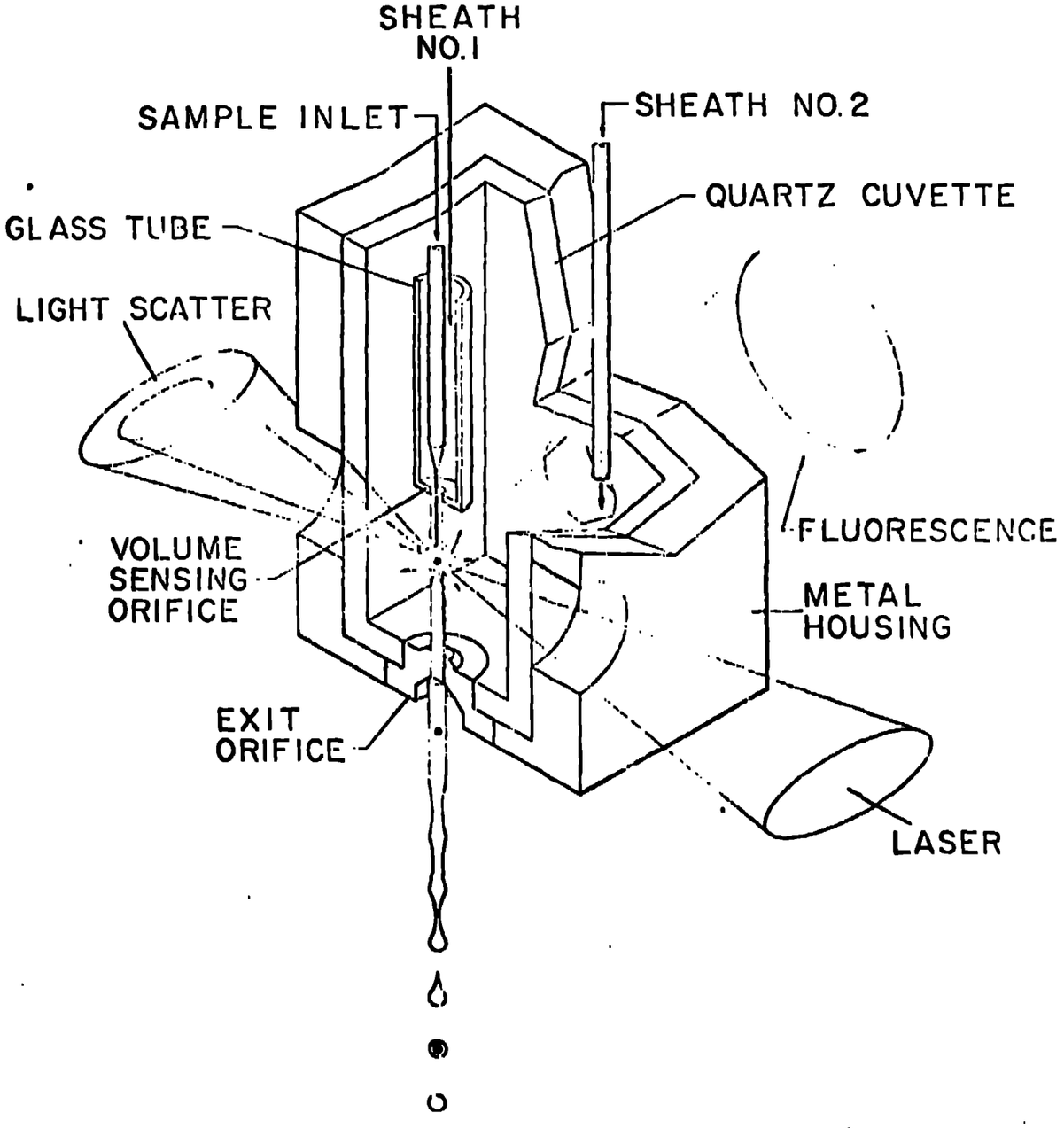

SHEATH
NO.1
SAMPLE INLET
SHEATH NO.2
QUARTZ CUVETTE
GLASS TUBE
LIGHT SCATTER
FLUORESCENCE
VOLUME
SENSING
ORIFICE
METAL
HOUSING
EXIT
ORIFICE
LASER

Fig. 4. The multiparameter cell sorter. The charging and deflection electrodes are located below the chamber. Droplets are formed by ultrasonic vibration of the chamber.

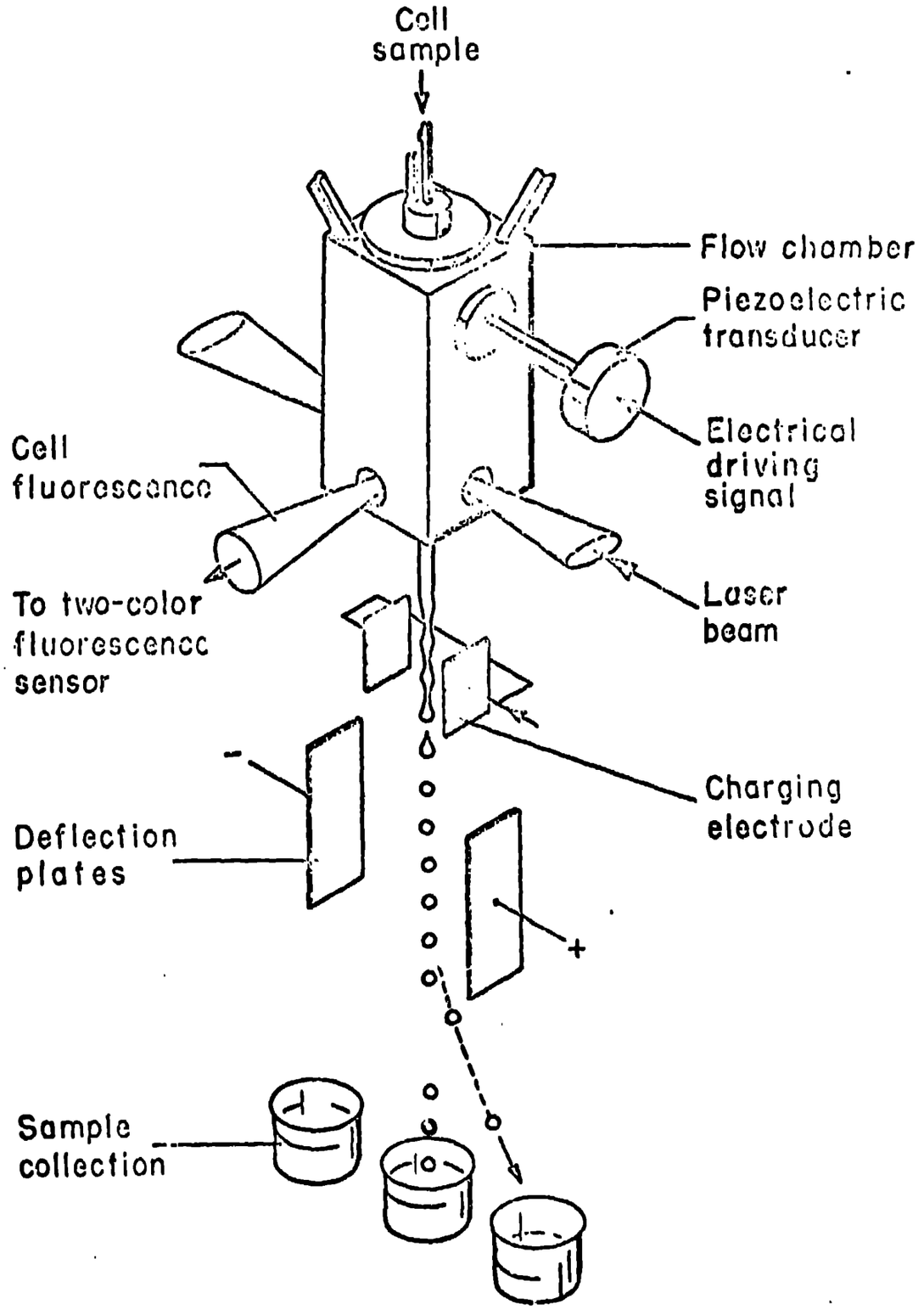

Cell sample
Flow chamber
Piezoelectric transducer
Electrical driving signal
Cell fluorescence
To two-color fluorescence sensor
Laser beam
Charging electrode
Deflection plates
Sample collection

Fig. 5. Feulgen-DNA distribution obtained for (A) normal mouse spleen cells and (B) cells from MCA-1 induced tumor in a mouse. The first peak (1) corresponds to normal diploid mouse cells. The elevated DNA peaks (2) and (3) correspond to tumor cells.

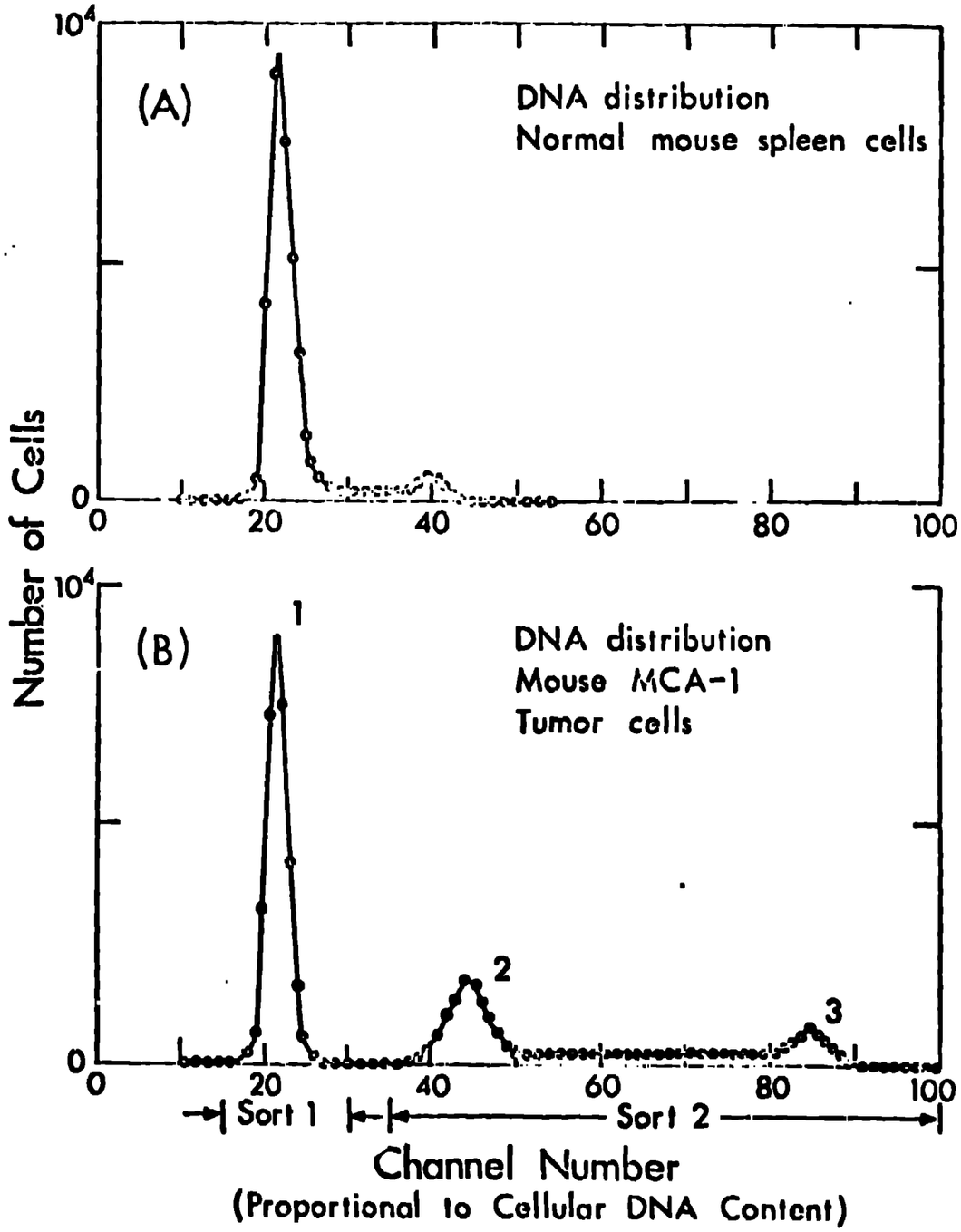

(A)
DNA distribution
Normal mouse spleen cells
(B)
DNA distribution
Mouse MCA-1
Tumor cells
1
2
3
Sort 1
Sort 2
Number of Cells
Channel Number
(Proportional to Cellular DNA Content)

Fig. 6. Photomicrograph of cells showing (A) mixture prior to sorting; (B) Sort I region, normal mouse leukocytes; and (C) Sort II region, tumor cells.

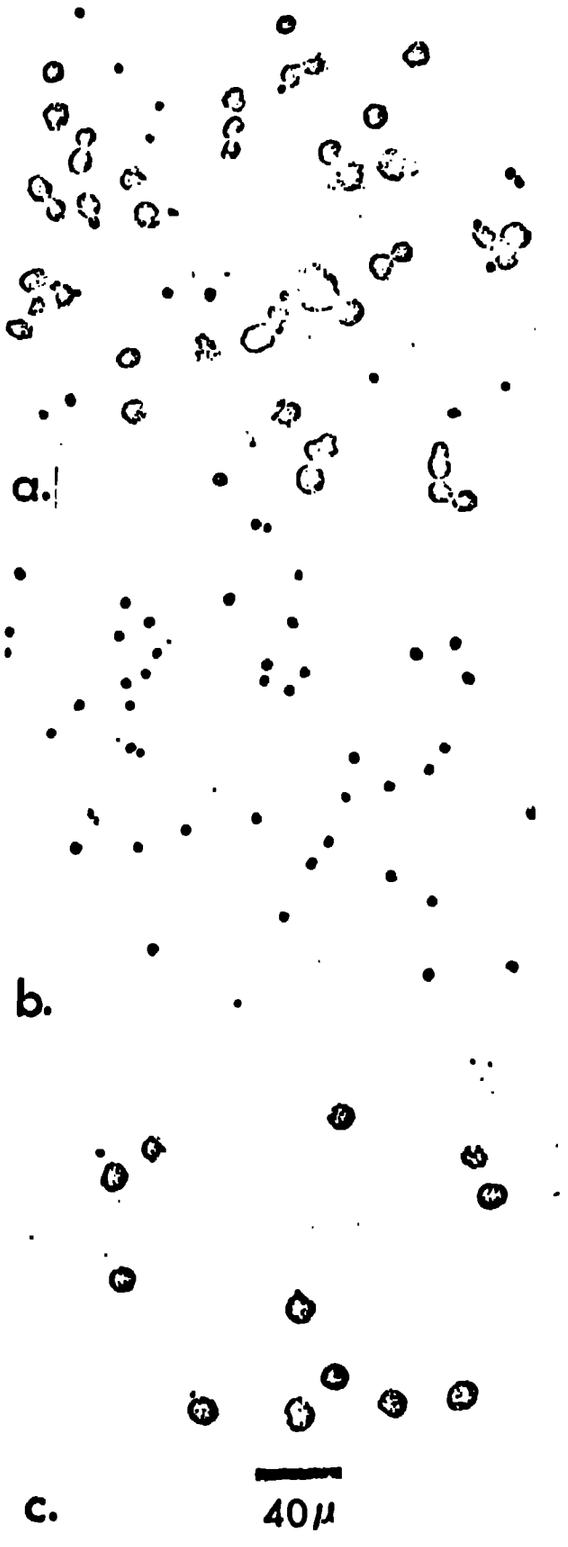

a.
b.
c.
40μ

Fig. 7. Two-parameter plot of the light-scattering intensity obtained from normal human leukocytes at 1° and 90° from the optical axis. The top frame is a contour plot and the bottom frame an isometric plot. Cells in regions 1, 2, and 3 were sorted (see details in Table 2).

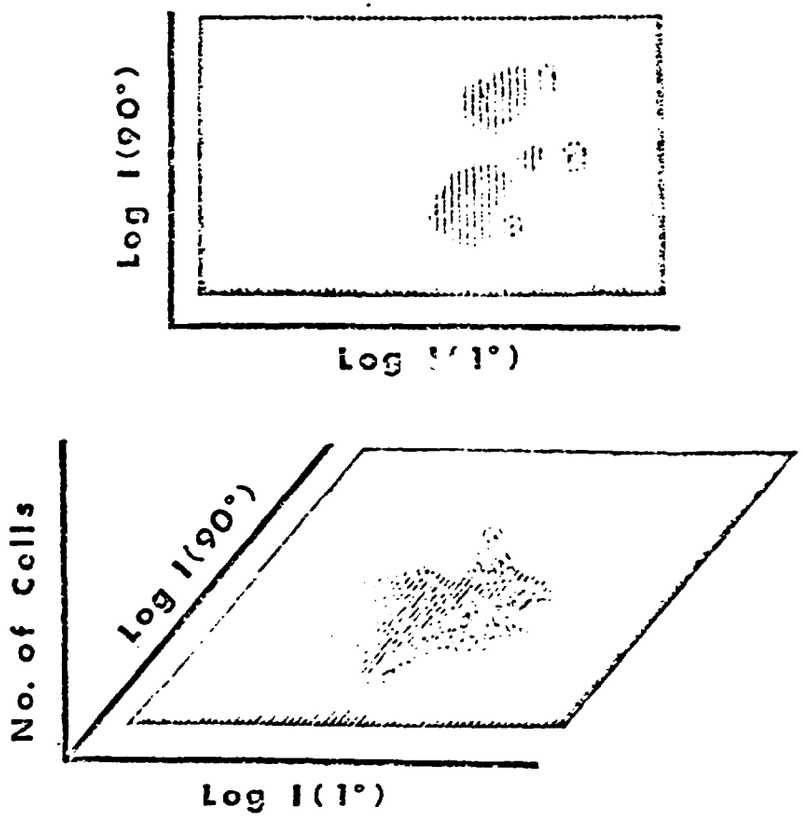
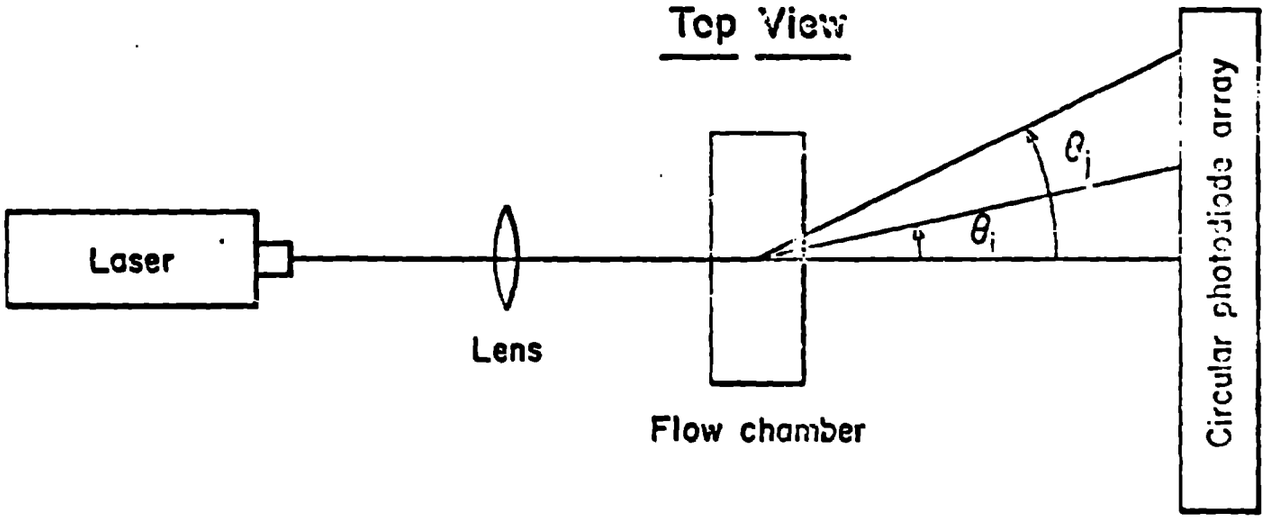

Log I(90°)
Log I(1°)
No. of Cells
Log I(90°)
Log I(1°)

Fig. 8. Schematic diagram of the 32-angle light-scattering flow photometer. Only the ring sections of the detector are used in this application.


Top View
Laser
Lens
Flow chamber
Circular photodiode array
θ_j
θ_i