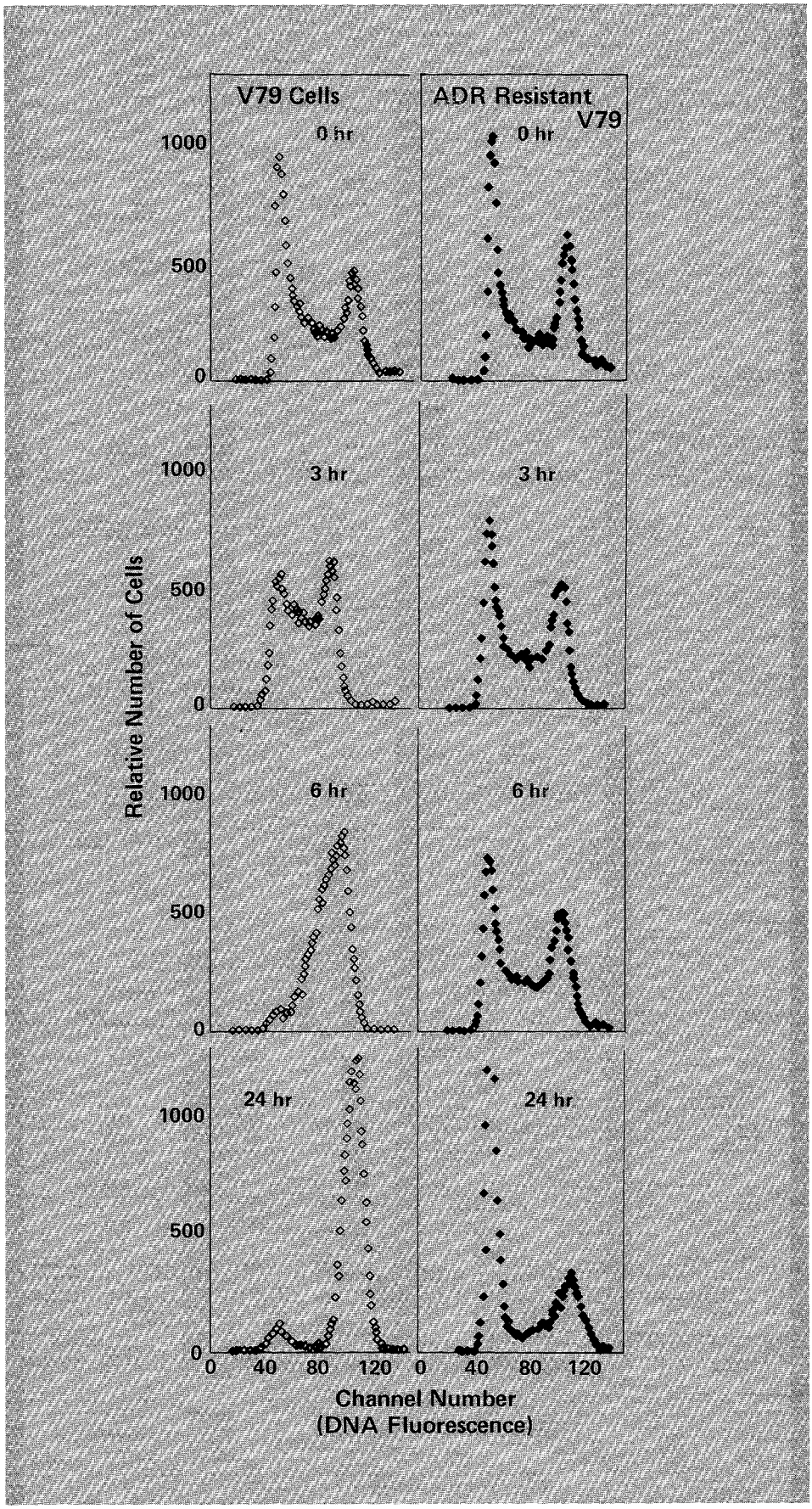

Fig. 16. Comparison of DNA distributions of normal cell line V79 and Adriamycin-resistant V79b cell line following exposures to Adriamycin (0.5 mg/ml, 1 h). (Courtesy of N. Tokita, LASL)

microscopic analysis by sorting cells from this area. Because some signals from normal cells and aggregates also appear in the area, we cannot use histogram analysis directly for automated diagnosis of cervical cancer. To increase the potential accuracy of automated diagnosis, a new independent marker correlated with cellular abnormality must be included as a third parameter.

Recently a group at the National Cancer Institute Division of Cancer Biology and Diagnosis used the LASL multivariable cell sorter in combination with the two-color DNA/protein staining protocol. A double-blind clinical trial was conducted so that experimenters were not informed of the clinical diagnosis of the samples. That is, each sample was divided into two fractions, one screened by cytopathology and the other screened by the machine. Upon completion of the trials, the instrument results were compared with the cytopathology results, which were assumed to be correct. With this parameter set the accuracy was 80%. Accuracy depends on normal variation and on how one sets the criteria for defining an abnormal cell. Because of variations between normal cells, it may be impossible to improve the accuracy by measuring only DNA and protein. It appears that additional variables must be measured to improve definition of a cancerous cell.

Errors are expected in any diagnosis. The two important errors are the false negative rate (rate of abnormal cases misclassified as normal) and the false positive rate (rate of normal cases classified as abnormal). In the trial just described, the false negative rate was 10%, a serious error because it means that disease is missed in 10% of the incidents. The false positive rate, 27% in the trial, is less serious. In any mass screening program, the false positive error increases both work and costs because sampling must be repeated. However, a 27% false positive rate would be acceptable, if the false negative rate could be brought down to about 5%. Thus, although these results do not justify a clinical technique at present, they are encouraging.

Perhaps as we learn more about the nature of cancer, we can identify more definitive parameters that will make

automated screening more accurate than the cytopathologist. Work continues with this goal in mind.

Monitoring Radiotherapy and Chemotherapy Effects

Flow cytometry is used extensively to study the effects of radiation and chemotherapeutic drugs on cultured cells, rodent tumors, and human tumors. Goals include an understanding of the damage in terms of cell-killing and the application of this information to radiotherapy and chemotherapy problems.

After cells are exposed to radiation or to chemotherapeutic drugs, the cell's DNA distributions and other cytometric parameters are measured at different times after exposure to complement the data obtained by other methods. For example, two cell lines of Chinese hamster origin differ considerably in their sensitivity to Adriamycin, a drug commonly used for the treatment of human cancer. When M. R. Raju (LASL) studied the DNA distributions for the two cell lines after treatment with 0.5 milligram per milliliter Adriamycin for 1 hour, he found that one cell line was more sensitive than the other (Fig. 16). Changes in DNA distributions in the normal (V79) cell line were dramatic, but the changes in the Adriamycin-resistant cell line (V79b) were small. Cell survival data obtained by colony formation indicated that cell survival of the normal (V79) cell line was 3%, but cell survival of the Adriamycin-resistant (V79b) cell line was 80%. Therefore, the magnitude of the cell cycle DNA distribution perturbations measured by flow cytometry was related to the cell killing. Since Adriamycin resistance is due to a decreased drug uptake, he measured the amount of Adriamycin per cell using flow cytometry. The measurements confirmed that the drug resistance of the V79b cell line was due to lower drug uptake. This study, together with others, indicates that DNA distributions can be used to measure and predict the drug sensitivity of tumor cells rapidly. DNA distributions also may be useful for studying drug-induced perturbations of cells from a patient biopsy as a prognostic test for cancer patients.

Search for Key Malignancy Parameters

Because it is likely that a single cell (a stem cell) is the origin of every cancer, the isolation and characterization of such a cell remains an important problem. A stem cell should be inherently different from other cells of the population in one or more characteristics. The differences probably originate in an altered expression of the cell's DNA. It is unrealistic to expect that initial changes in a cell's gene expression will be manifested in DNA changes large enough to be detected in the presence of the cell's total DNA content. In humans, the packaging of cellular DNA into chromosomes during a portion of the cell cycle (mitosis) provides a natural subdivision of cellular DNA into 46 chromosomes, which can be isolated and studied individually with flow cytometers and sorters. That chromosome changes are important in the etiology of tumors has long been recognized, but the number of chromosomes we can study using a microscope is too small for us to detect the rare chromosome changes that occur when a tumorigenic stem cell first begins to propagate.

Lawrence Livermore Laboratory and Los Alamos Scientific Laboratory in cooperation with the Max-Planck-Institut in Goettingen, West Germany, have demonstrated the capability to analyze isolated individual chromosomes by flow cytometry. Figure 17 is an example of such a high-resolution measurement in which all but one of the expected chromosome types isolated from Chinese hamster M3-1 cells were resolved. In addition, three "homologue" pairs (1, 1; 7, 7; and 9,9) had small differences in DNA content. Chromosome changes reflecting the appearance of a new stem cell in the population might be manifested by changes in the position or area of one of the peaks.

More subtle changes in chromosome morphology can be quantitated by using a unique chromosome-imaging flow sorter. In contrast to conventional flow cytometers that measure total fluorescence without regard to its spatial distribution, our chromosome-imaging sorter maintains the optical image as the object passes through the laser beam. The chromosome image is formed in front of a mechanical slit placed at the image plane; the slit scans the chromosome as it flows through the observation region. The intensity profile of a chromosome contains valuable additional information as illustrated in Fig. 18. A unique intensity profile is recorded for each chromosome at the rate of 1,000 per second. Now much more subtle features associated with chromosome aberrations can be sought, and when chromosomes with these features are found, they can be sorted for verification and further analysis. Chromosomes with aberrations such as breaks, some types of translocation of chromosome pieces, fragments, and dicentrics (two chromosomes joined at their ends) should be detectable. The search for additional parameters that will define chromosome types uniquely is continuing.

The role of chromosome changes in cancer is not well defined. In certain types of cancer, such as chronic myelocytic leukemia, a specific and fairly consistent chromosome abnormality has been identified. In advanced cancers, one observes a large variety of chromosome aberrations but not much consistency. These observations are in stark contrast with the remarkable process of DNA replication and cellular mitosis that results in exact maintenance of the amount of DNA per cell and the stability of chromosome number and

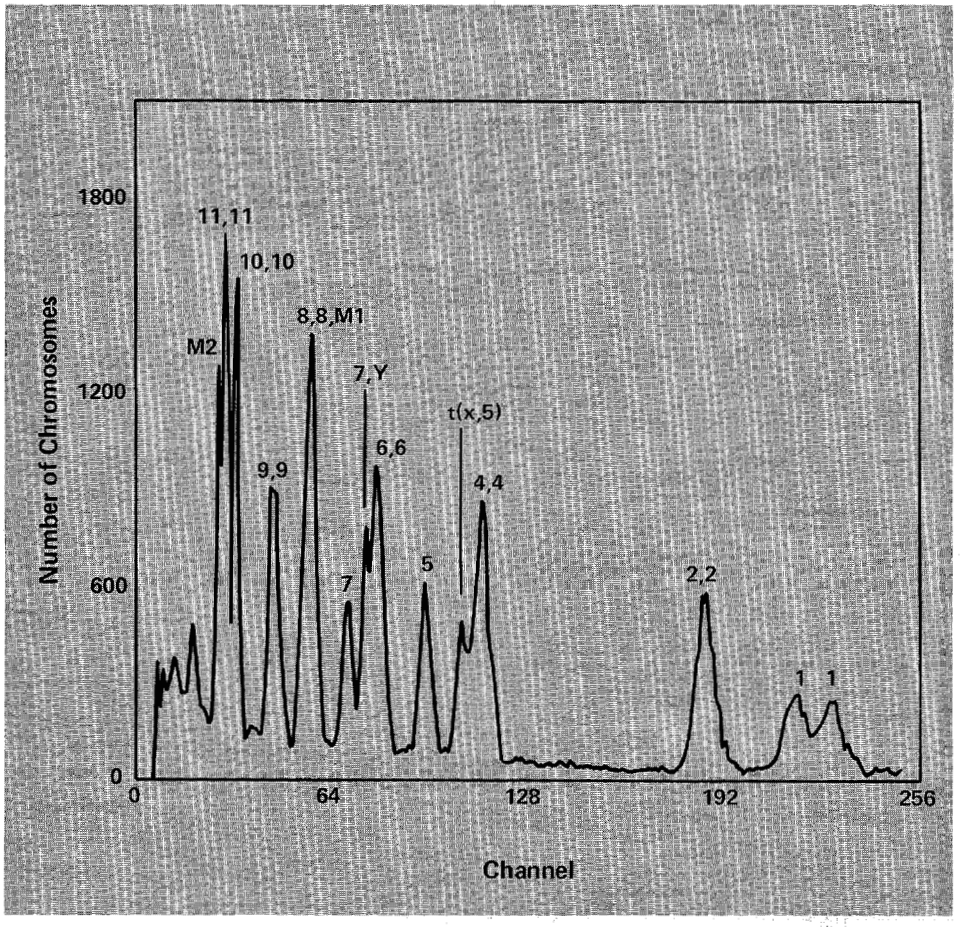

Fig. 17. The flow karyotype of chromosomes isolated from Chinese hamster M3-1 cells and stained with the bis-benzimadazole dye Hoechst 33342 (10 micromolar). The mean fluorescence intensity of a peak reflects stain content and hence DNA content for chromosomes of that type. The area of each peak is proportional to the number of chromosomes of that type. Approximately 30,000 chromosomes were analyzed. The type of chromosomes constituting each peak was verified by sorting and visual identification. The longest chromosomes (No. 1) have the most DNA, and the smallest chromosomes (M2 or markers) have the least. Each chromosome type and its corresponding peak are identified.

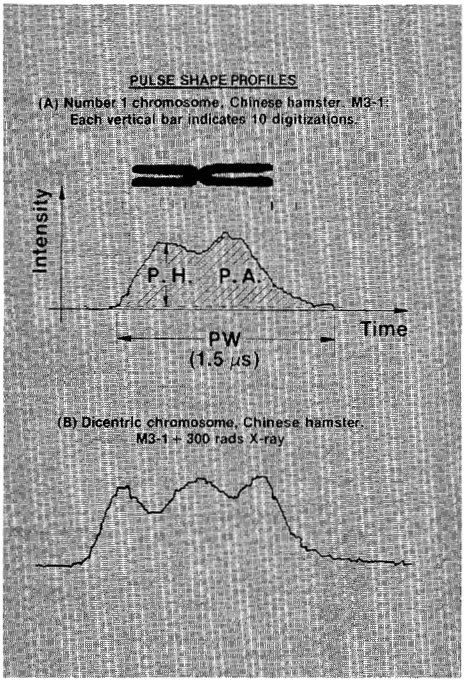

Fig. 18. Pulse shape profiles of individual chromosomes isolated from Chinese hamster M3-1 cells. (A) Pulse shape profile of a single No. 1 chromosome. The intensity of fluorescence across the length of each chromosome is analyzed by a waveform recorder, which rapidly digitizes the fluorescence intensity as a function of time. This information, recorded for each chromosome, can be used to make sorting decisions. Pulse shape profiles provide information on pulse area (P.A.) or total DNA content, pulse width (P.W.) or total chromosome length, and pulse height (P.H.) or fluorescence density per unit length. (B) Pulse shape profile of what appears to be a dicentric chromosome (two chromosomes attached end to end) isolated from the cells used in A but having received 300 rads of x ray 16 hours before chromosome isolation. The time axis is the same for both profiles.

morphology in all normal cells. It is this very contrast that makes chromosome stability appear to be of extraordinary importance. With full implementation of the imaging flow cytometer/sorter, we will be able to characterize 100,000 or more chromosomes—a truly remarkable accomplishment that has been equated to taking that first look at the back side of the moon.

The development of flow cytometry is due to the combined efforts of many people throughout the world. Many members of the LASL Life Sciences Division and groups at Stanford University, Lawrence Livermore Laboratory, Memorial Sloan-Kettering Cancer Center, and the University of Rochester have contributed to this work. The manufacture of commercial equipment is a strong indication that the technology has reached a fairly mature state. The instruments now can be used by a large variety of biologists throughout the world and the potential biological applications appear to be very great.

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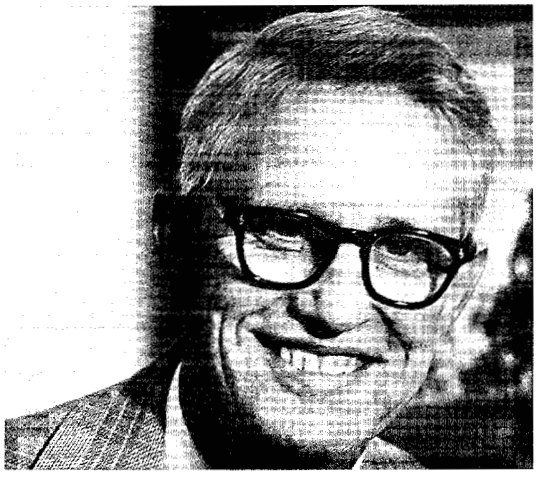
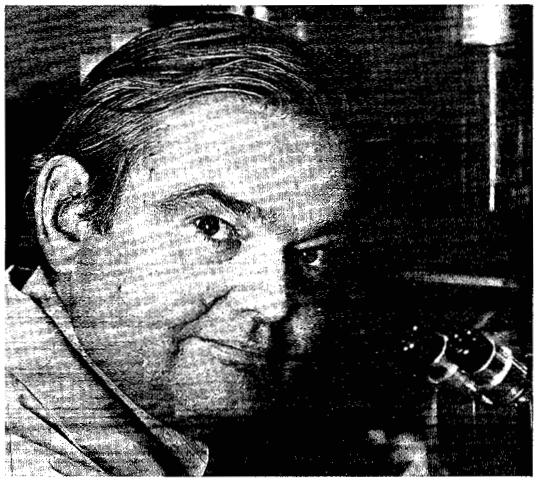
THE AUTHORS





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Paul F. Mullaney, Group Leader of LASL's Biophysics Group, received his bachelor of science degree in physics from Iona College in 1959 and his Ph.D. in physics and biophysics from the University of Delaware in 1965. He is president-elect of the Society for Analytical Cytology and serves on the editorial board of the Journal for Analytical Cytology. A Fellow of the Alexander von Humboldt Stiftung Foundation, Bad Godesberg-Bonn, FRG, Mullaney earned their Senior American Scientist prize in 1978. His work has resulted in patents issued for dual-parameter flow photometric apparatus and method, a potential-sensing cell analyzer, and an ellipsoidal cell flow system.