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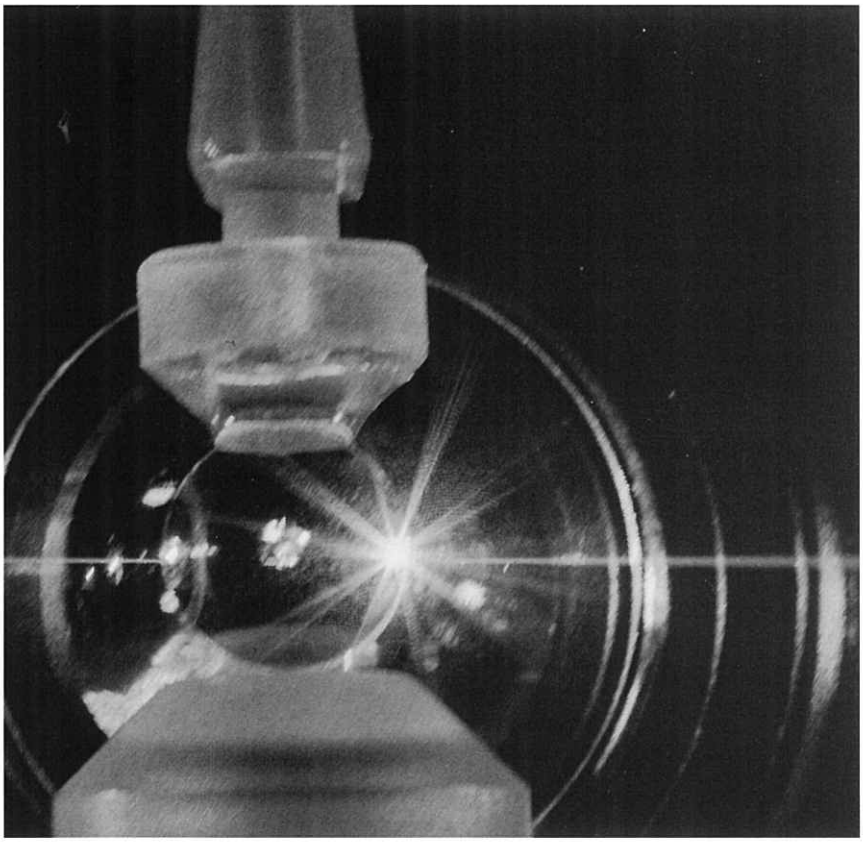

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The flow chamber of the Phase-Sensitive Flow Cytometer is illuminated by an intensity-modulated laser beam, which is used to excite cells and subcellular components labeled with fluorescent probes. The fluorescence emissions provide detailed information on cells' structural, functional, and biochemical properties. The flow chamber was donated by the Coulter Corporation (Hialeah, Florida).

Phase-Sensitive Flow Cytometer

John A. Steinkamp and Harry A. Crissman, Life Sciences Division, Los Alamos National Laboratory

An important tool for use in clinical and biomedical research laboratories, flow cytometry quickly measures differences between cells and provides a way to distinguish individual cells within populations or subpopulations. Developed at Los Alamos in the late 1960s, flow cytometry uses lasers to excite cells or subcellular components, such as chromosomes, that have been stained with fluorescent dyes. The fluorescence emissions are then analyzed to provide detailed information on such properties as DNA, protein, enzymes, calcium, and cell-surface receptors. On the basis of these properties, cells or chromosomes can be characterized and then physically isolated for further study.

Commercial flow cytometers generally measure light scatter, which provides information on cell size, and multicolor fluorescence within specified spectral regions. Using advanced fluorescence spectroscopy techniques combined with conventional flow cytometry principles, the Phase-Sensitive Flow Cytometer can obtain these conventional measurements and more. Through the use of an intensity-modulated laser excitation source and phase-sensitive detection electronics, we can also measure the phase shift of the emissions, which enables us to determine fluorescence lifetimes. We can then use this information to separate electronically the emissions from multiple fluorescent probes.

Clinical tests and biological experiments often require that cells and subcellular components be labeled with multiple fluorescent probes so that different properties may be analyzed together. In the past, many such procedures were limited by the availability of dyes with both common excitation regions (so that a single excitation source could be used) and emission spectra that were sufficiently distinct to allow them to be separated using optical filters.

Our cytometer can resolve signals from different dyes electronically so that multiple probes can more readily be used on the same sample. Because dyes bind to specific targets, this capability increases the number of potential probes and thus the number of properties that can be studied together and correlated.

Our Phase-Sensitive Flow Cytometer won a 1993 R&D 100 Award, an honor given annually by *R&D Magazine* to the one hundred most significant technical innovations of the year.

The Invention—Characteristics and Advantages

Unlike commercial flow cytometers, which use direct, unmodulated laser beams to illuminate stained samples as they flow past electrical and optical sensors, the Phase-Sensitive Flow Cytometer uses a high-frequency, intensity-modulated (sinusoidal) laser excitation source. This excitation source, combined with phase-sensitive detection electronics, gives our cytometer its unique capabilities.

After the fluorescence emissions are picked up by a detector, they are processed in one of two ways. The signals may be passed through a filter that removes the high-frequency component, thereby converting the signals to low-frequency, conventional flow cytometry signals. Alternatively, the signals may be processed through phase-sensitive detection electronics, which resolve the individual signals based on the amount by which their phases have shifted from the phase of the excitation reference signal. Because the phase shift is a function of the fluorescence lifetime, we can use these phase-resolved signals to directly calculate lifetimes of individual dyes. Our cytometer can then use differences in fluorescence lifetimes to resolve signals from multiple dyes.

The Phase-Sensitive Flow Cytometer offers the increased measurement sensitivity and precision

necessary for improved clinical and research flow cytometry. By separating signals electronically, our cytometer eliminates light loss—and hence, data loss—caused by optical filtering. Furthermore, phase-sensitive measurement capabilities can readily be added to existing commercial flow cytometry systems.

Applications

The Phase-Sensitive Flow Cytometer can be used for virtually any clinical or research application involving the analysis of cells (including plant cells and microorganisms), cell functions, or subcellular components through the use of fluorescent dyes directed to specific targets. For example, it might be used to track the minute changes taking place as cells change from normal to abnormal or to analyze changes in subpopulations of cells, such as the shifts in a patient's immune cells as AIDS progresses.

In addition, the reduced background interference and improved sensitivity offered by our cytometer enable it to quantitatively and qualitatively analyze whole chromosomes or even specific regions on chromosomes. By improving researchers' understanding of biological processes at the cellular, subcellular, and even molecular level and through its direct clinical applications, our cytometer will contribute to improving the diagnosis and treatment of a wide variety of human diseases.

The cytometer can also use fluorescence lifetime itself as a spectroscopic probe to study the interaction of dyes with their targets, as in structural biology studies. Additional potential applications include characterizing microorganisms; analyzing plant genetics, diseases, and nutritional requirements; studying cellular physiology and pharmacology; and assessing quality control for drug and biotechnology companies. ■

Both developers of the Phase-Sensitive Flow Cytometer are staff members in the Cell Growth, Damage, and Repair Group of the Laboratory's Life Sciences Division.

John Steinkamp earned a B.S. in electrical engineering from Purdue University and served in the Air Force for three years before attending graduate school. After earning a Ph.D. in electrical and biomedical engineering from Iowa State University, he came to Los Alamos in 1970 as a postdoctoral fellow. He became a staff member the following year. His research interests are in automated biophysical instrumentation methods for making rapid measurements on single cells and particles.

Harry Crissman served in the Army for two years before graduating with a B.S. from Lock Haven State College in Pennsylvania. He later earned a Ph.D. in zoology from Pennsylvania State University. He came to Los Alamos in 1971 as a postdoctoral fellow and became a staff member in 1973. His research interests are in cell cycle regulation and in using his biology and cytochemistry background to improve the instumentation available for use in cell biology research. He was a developer of the Optical Microrobot Single-Cell Manipulator/Analysis System, which won a 1988 R&D 100 Award.

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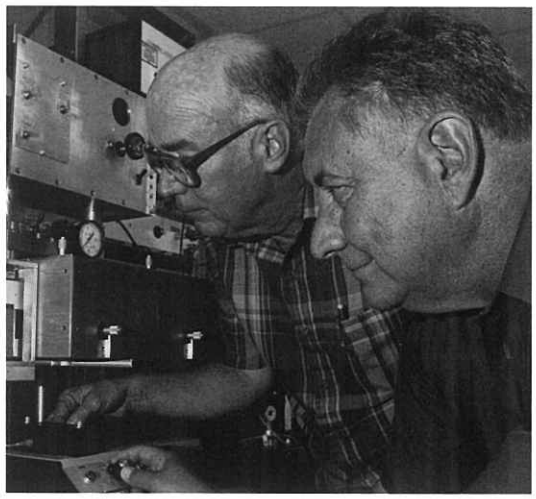

John Steinkamp (left) and Harry Crissman, inventors of the Phase-Sensitive Flow Cytometer, calibrate the instrument before an experiment.

For more information about the Phase-Sensitive Flow Cytometer, please contact Kay Adams, Industrial Partnership Center, Los Alamos National Laboratory, P.O. Box 1663, Mail Stop M899, Los Alamos, NM 87545.
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