WHAT IS FLOW CYTOMETRY?

by Sue Herms

One of the most frequent uses of flow cytometry in medical diagnosis is to distinguish among the various types of leukemias and lymphomas. Flow cytometry may be particularly helpful in cases where analysis of cancer cells under the microscope is not adequate to determine the specific type of tumor present.

The modern flow cytometer consists of a light source, collection optics, electronics, and a computer to translate signals to data. In most modern flow cytometers, the light source of choice is a laser that emits light at a specified wavelength. Scattered and emitted light is collected by two detector lenses (one set in front of the light source and one set at right angles).

In the diagnosis of leukemias and lymphomas, usually a portion of the bone marrow or other tissue, such as a lymph node, is broken up into single cells and placed in a liquid— the cells in the liquid then flow through a chamber, one at a time very quickly, up to several thousand cells per second. At this point, the laser beam hits the cells as they pass. The way the laser beam light bounces (scatters) off each cell is picked up by the detectors and provides information about the cell's characteristics.

Light that bounces forward (called forward scatter or FSC) gives information about the cell size. Light that bounces off the side (called side scatter or SSC) provides information about the inner structure of the cell, such as the shape of its nucleus or the amount and kind of granules present. Each type of cell in the immune system (lymphocytes, monocytes, neutrophils, etc.) has a unique combination of forward and side scatter measurements, allowing the instrument to count the number of each type of cell present in the sample.

Flow cytometers can also use antibodies tagged with fluorochrome stains that bind to specific antigens on the cell surfaces. In the case of leukemias and lymphomas, these fluorochrome-tagged antibodies bind with and identify protein surface markers on the immune cells called cluster differentiation (CD) markers. When a fluorochrome stain is added to the cell sample, a laser beam excites the cells so that they fluoresce and emit a specific color of light, depending on the type of fluorochrome stain used. Color detectors collect the different colors of light and send the data to the computer.

Modern flow cytometers usually have multiple lasers and fluorescence detectors. Increasing the numbers of lasers and detectors allows for multiple antibody labeling and can more precisely identify a target population of cells.

The computer attached to the flow cytometer tabulates the data from the light scatter measurements and the fluorescing cells and can plot the data in several ways. The most common plot is a graph called a histogram, where fluorescence intensity or the degree of scattered light (x-axis) is plotted against the frequency at which this type of event occurs (y-axis). From such a histogram, the population distribution

for the particular cell(s) of interest can be determined. Other types of plots are called dot, density, or contour plots. The operator of the flow cytometer can select certain cell populations based on their characteristics in order to refine and further analyze the data—a process called gating.

Flow cytometers can also be configured as sorting instruments. As the cells pass through, they can be electrically charged selectively and on their exit can be deflected into separate paths of flow. It is therefore possible to separate several defined populations of cells from an original mixed sample with a high degree of accuracy and speed. Sorted cells can be further examined microscopically, re-stained for additional analysis, or put into tissue culture for research purposes.

IWMF Board Member Sue Herms is chair of the Publications Committee, member of the Research Committee, and Associate Editor of the Torch. Her column Medical News Roundup appears in every issue of the IWMF newsletter. Sue Herms holds degrees in Zoology and in Medical Technology from Ohio University and from the Medical University of South Carolina, respectively, and is affiliated with Roper Hospital in Charleston, South Carolina.

This article was published in the IWMF *Torch*, (Fall 2007) pages 3, 20.