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STATUS QUO IN FLOW-THROUGH CYTOMETRY

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Flow-through systems of single cell analysis and sorting have been under development for several years. Fluorescence and volume sensors are well developed but light scatter sensors can be expected to improve. Cell sorters are in use in several laboratories and commercial versions are under development. The most serious problems in flow-through cytometry are in cell preparation and the greatest potential for advancement lies in development of new, specific stains and markers.

After several years of intensive development it is perhaps worthwhile to stop and examine where we stand with flow-through methods of cell analysis.

Flow-through systems of cytometry measure cell fluorescence, the light scattered by a cell traversing a light beam, the absorption of light by the cell and cell volume by the Coulter principle. Further, it is possible to electronically sort out (*i.e.*, physically isolate) cells selected according to information obtained with such sensors.

Fluorometry of a single cell, as it traverses a beam of exciting light, gives quantitative information on the amount of fluorescent dye bound to the cell. Currently, fluorometry is well developed with high resolution. The emitted fluorescent light can be divided into two or more color ranges which can be separately collected and measured. This permits the use of two or more dyes or the use of a single dye which emits at different colors depending on how it binds to different biopolymers.

Cell properties measurable by fluorometry using suitable staining procedures include relative deoxyribonucleic acid content, total cell protein, density of specific membrane binding sites, detection of specific cell enzymes and live versus dead cells. Limited differential counting of white blood cells is possible with acridine orange and other dyes. Using fluorescently labeled antigen or antibody, living cells, producing a specific antibody, can be identified and sorted permitting their function in the immunologic response to be determined.

In contrast to fluorescence sensors, the light scatter sensors have yet to develop the potential they promise. According to theoretical analysis, the light scatter pattern possesses information on intracellular structures including nuclei, granules, etc. To date, flow-through devices have been unable to extract this detailed information, the problem being spatial resolution of the scatter pattern.

Currently, light scatter measurements are used to distinguish large from small cells. Some workers have observed that living and dead cells scatter light differently as do infected and noninfected cells; however, these observations are preliminary and not yet well understood.

Although absorption (or removal) of light from the beam is easily understood in concept, quantitation of the amount of absorbing material in a cell is difficult because absorption depends on the average optical density of a cell which in turn depends on the spatial distribution of the absorbing material. A small very darkly stained structure will give a different signal than will a dispersed structure containing the same amount of absorbing material. Absorption measurement does make possible the use of a wide variety of dyes; further, spectrophotometric analysis may be possible.

Cell volume measurement by the Coulter principle has been around a long time. It is certainly the most widely used, reliable and well understood sensor in flow-through cytometry. Coulter volume sensors are in use which give coefficients of variation of 3% or less. Although we cannot expect much improvement in this sensor, clever design will be needed to incorporate a volume sensor into a photometric cell analyzer. A primary disadvantage of Coulter volume sensing is that the signal given by a cell

decreases after fixation, probably due to an increase in the permeability of the cell membrane.

The sensors I have discussed so far are often called zero resolution systems in that these sensors measure only gross cellular properties; *i.e.*, no morphology or other detailed information is obtained.

Low resolution systems able to give a limited amount of structural information are being developed. In a slit scan system the laser beam is focused into a thin (5 μm) ribbon of light through which the cell must move. By observing the time structure of the fluorescence pulse, passage of the leading and trailing edges of the cytoplasm and the nucleus can be observed and from these time intervals a ratio of nuclear diameter to cell diameter can be obtained. This nuclear-cell ratio should be very useful in cell discrimination.

Another system under development incorporates a scanning system in which a finely focused beam of light moves back and forth across the cell as it flows past. Thus, a coarse image is developed and analyzed yielding many cell measurements including nuclear diameter, cell diameter, nuclear area, cell area and others.

Cell sorting, *i.e.*, the ability to isolate physically cells of interest, greatly increases the utility of flow-through instruments. After measurement as described above, the cells exit the apparatus in a liquid jet which is broken into uniform droplets. Droplets, containing cells to be isolated, are electrically charged and subsequently electrostatically deflected into a collection vessel. Thus, it is possible to isolate pure classes of cells which may be studied in various ways. When prepared properly, the isolated cells remain viable permitting living functions to be studied.

The resolution of cell sorting (*i.e.*, the ability to pick out certain cells) is dependent on the resolution of the sensor system. If the cell can be uniquely identified, it can be sorted. The through-put of a sorter can be a limitation. For identification purposes where less than 10^5 cells are needed the speed of operation is adequate; however, for preparative purposes where 10^7 or more cells are required, considerable running time is necessary. Improvement will come in the area of reliability, simplification and throughput.

The major problems, impediments and opportunities are in the area of cell preparation, *i.e.*, dispersal, removal of debris and staining.

Flow-through machines have only limited capability to distinguish between single cells and clumps of two or more cells. Therefore, single cell dispersion is essential. Several groups are working on this problem but no generally applicable procedure has evolved, partially, I think, because the mechanisms by which cells stick together are not known.

Where do we go from here? Refinement of sensors will continue. Fluorescence sensors have sufficiently high resolution; the emitted fluorescent light can be split into more color ranges if needed but here again progress in biology and cell staining must provide guidance as to what is needed.

Light scatter will improve in sensitivity and discrimination; however, I do not anticipate much improvement in measurement of light absorption; nor can Coulter volume sensing be significantly improved.

In spite of their complexity and expense more laboratories will build cell sorters and several companies are developing commercial versions. Once these are available and proven, they will further stimulate interest in the flow-through method of cell analysis.

I feel that major advances will come in the area of fluorescent stain and marker development. Any cell property, biochemical, functional or otherwise, which can be fluorescently marked can be measured with a degree of speed and accuracy never before possible. Development of new markers can be expected to lead to new descriptions of cells in terms of functional, fundamental differences rather than differences in morphology. This reorientation of thinking will have great impact on cytology. I hope we will see a shift of emphasis to this less glamorous but vital area of automated cytology.