Flow cytometry: a versatile tool for all phases of drug discovery

John P. Nolan, Sabine Lauer, Eric R. Prossnitz and Larry A. Sklar

The applications of flow cytometry are being extended beyond cells into molecular interactions and genomic analyses. The authors explain how instrumentation and reagent development are combining to improve flow cytometric throughput by orders of magnitude beyond that possible just a few years ago, such that the combination of sensitivity, throughput and versatility makes flow cytometry an analytical platform with applications at all stages of drug discovery.

Flow cytometry is a sensitive and quantitative platform for the measurement of particle fluorescence[1,2]. Traditionally, the particles studied are cells, especially blood cells, and it is used extensively in immunology. The applications of flow cytometry in pharmacodynamics and drug delivery have been reviewed recently[3].

In flow cytometry (Fig. 1), the particles in a sample flow in single file through a focused laser beam at rates of hundreds to thousands of particles per second. During the time each particle is in the laser beam, on the order of ten microseconds, one or more fluorescent dyes associated with that particle are excited. The fluorescence emitted from each particle is collected through a microscope objective, spectrally filtered through dichroic mirrors and bandpass filters, and detected with photomultiplier tubes. The fluorescence measurements are sensitive, with commercial instruments having detection limits of several hundred fluorophores per particle. With the use of appropriate standards and calibration protocols, the measurements can be made quantitative[4,5]. Because even the least expensive commercial instruments can detect three or four colors simultaneously, the analysis is intrinsically multiparameter. Because the flow cytometer can resolve free probe from that bound to particles, binding measurements often do not require a washing step. Particles can also be sorted on the basis of the fluorescence properties they exhibit.

Instrumentation advances

Several recent instrumentation advances promise to extend the sensitivity and range of flow cytometric measurements. By using pulsed-laser excitation and time-gated detection with avalanche photodiodes, the detection limit for fluorescence in a flowing sample stream has been pushed to a single fluorescent molecule[6]. Increases in sensitivity have allowed the detection of small biological entities including individual DNA fragments[7] and individual lipoproteins[8]. Modulated laser beams and phase-sensitive detection have enabled the measurement of fluorescence lifetimes on cells or particles[9,10]. The use of multiple lasers, detectors and dyes have allowed as many as ten different parameters to be used to characterize complex cell populations[11].

Sample handling

Another area of instrumentation development that is extending the capabilities of flow cytometry is in the automation of sample handling. The need for this in kinetic measurements and large-scale assays has been recognized for some years, but the capabilities of commercial instruments are limited essentially to less than two samples per minute. Recently, computer-controlled sample mixing and delivery systems have been adapted to provide flow cytometers with advanced sample-handling capabilities. A significant advance was the coupling of a stepper motor-driven mixing device to a flow cytometer to provide sample

John P. Nolan*, **Sabine Lauer** and **Larry A. Sklar**, National Flow Cytometry Resource, Life Sciences Division, LS-5, M-888, Los Alamos National Laboratory, Los Alamos, NM 87545, USA. **Eric R. Prossnitz** and **Larry A. Sklar**, Dept of Pathology, School of Medicine, University of New Mexico, Albuquerque, NM 87131, USA. *tel: +1 505 667 1623, fax: +1 505 665 3024, e-mail: nolan@telomere.lanl.gov

 1359-6446/99/$ – see front matter PII: S1359-6446(99)01320-3

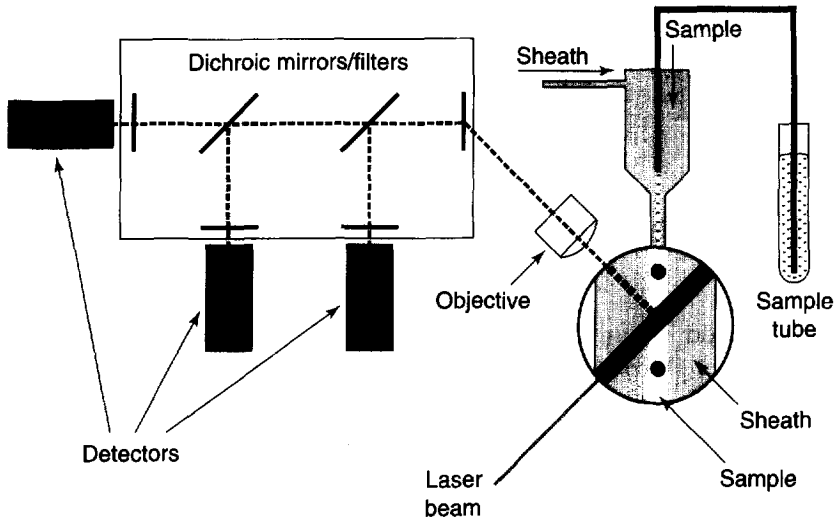

Figure 1. *In a flow cytometer, particles (cells or microspheres) flow in single file through a focused laser beam where one or more particle-associated fluorescent dyes are excited. Emitted fluorescence or light scatter from each particle is collected, spectrally filtered, and detected on photomultiplier tubes. Multiple optical signals can be detected without the need for a wash step, at rates of hundreds or thousands of particles per second. Resolution between free and bound probe is achieved because particles are illuminated in a very small volume (~20 pl) defined by the areas of intersection of the laser beam and the sample stream (magnified view). Under these conditions, the signal from free probe in this volume is small compared with the signal from fluorophore concentrated on the particle, and measurements can often be made without washing away free fluorophore.*

mixing and delivery to support continuous kinetic analysis with subsecond resolution[12]. With delivery times under 0.5 s and the ability to perform complex, multistep-mixing protocols[13], rapid-mix flow cytometry has made sensitive and quantitative real-time kinetic measurements of molecular interactions a reality. The adaptation of flow-injection analysis (FIA)-based sample handling[14] not only provides fast kinetic capabilities, but also enables the unattended execution of a series of experiments[13,15]. The development of robust automated sample handling compatible with laboratory automation systems can potentially provide sample throughput on the scale required for drug discovery applications.

Microsphere reagents

The development of new types of microspheres offers another avenue of opportunity. Just as solid-phase combinatorial chemistry plays an important role in drug discovery, bead chemistry will augment the display of targets, libraries and the analysis of their interactions by flow cytometry. A particularly promising direction, multiplexed assays, involves the use of microspheres stained with discrete levels of one or more fluorescent dyes[16,17]. Particle subpopulations associated with a particular target can be identified on the basis of fluorescence intensity in a manner analogous to a surface array (Fig. 2). Such 'soluble arrays' of microspheres are similar in concept to microarrays prepared on slides or microchips, except that the positions in the array are determined by fluorescence intensity, rather than by the physical location on a flat surface. This allows the array to be handled as a liquid and to be reconfigured readily by replacing individual microsphere elements.

Cellular screening assays

Multiparameter flow cytometry has, to a considerable extent, been developed and commercialized with immunophenotyping as a goal. Immunophenotyping consists of measuring several surface antigens and detecting them individually with antibodies in complex mixtures, such as white blood cells. The subsets of leukocytes from blood samples can readily be distinguished using a combination of specific antibodies against surface markers and the unique light-scattering properties of individual cell populations. In general, the side scatter contains information about cell granularity and the forward angle scatter contains information about cell size.

Flow cytometric instrumentation in its current form opens the door for many potential screening applications. For example, when leukocyte populations are studied, light scatter alone is sufficient to resolve the major cell types[18]. Lymphocytes (T and B cells) are typically the smallest cells, having few granules and, thus, show low scatter. Monocytes have few granules but are larger and, thus, show similar side scatter but higher forward scatter. Granulocytes (neutrophils, eosinophils and basophils), with increased granule content and size, show both increased side and forward angle scatter. Antibodies resolve the specific subsets within the major populations. This

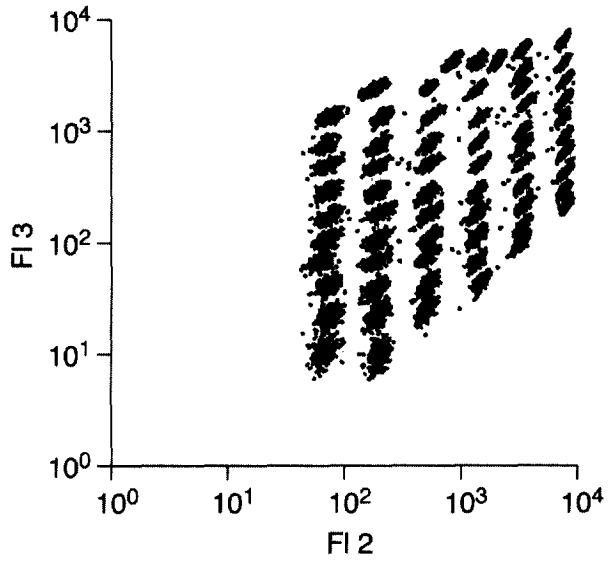

Figure 2. *Microsphere arrays. Polystyrene microspheres can be stained with one or more fluorescent dyes, such that individual microsphere subsets can be identified. In this example, microspheres are stained with different levels of an orange- and red-emitting dye, allowing the identification of 64 discrete subpopulations. Each subpopulation could display a different molecular target, each of which in turn could bind a different soluble ligand labeled with a green-emitting fluorophore. Fl 2 and Fl 3 are orange and red fluorescence channels, respectively. Reproduced with permission, from Ref. 17.*

means that distinct fluorescence parameters (spectral regions) can be used to distinguish ligand (FITC-labeled), antibody binding (for example, phycoerythrin-labeled antibodies) and intracellular calcium responses (with the chelating dye Fluo-3, for instance).

Investigators have also taken advantage of fluorescent nuclear stains to resolve nucleated cells in whole blood (the leukocytes) from the non-nucleated red cells and platelets[19]. In screening assays it is possible to identify the presence of an antagonist that, for example, blocks the calcium response to a stimulus, both in isolated leukocytes as well as a whole blood sample. In leukocyte preparations, the specific subset of cells responding to the stimulus can be characterized. An important further motivation for performing such experiments in blood samples is the ability to measure the presence of an antagonist in the circulation of an animal or a subject treated with the drug. Because the antagonists are likely to be washed away when the white blood cells are isolated from the other blood components, the whole-blood analysis provides the potential for analyzing the pharmacokinetics and the efficacy of the drug.

In addition to intracellular calcium and antibody binding to cell surface receptors, there are a remarkable number of relevant cell characteristics that are conveniently measured by flow cytometry. Many ligands for cell surface receptors have been labeled fluorescently, so that screening for the presence of drugs that block ligand binding is possible. For ligands that stimulate cellular responses, the presence of drugs that block the cell response can be measured in endpoint or kinetic assays. Endpoint assays include, but are not limited to, metabolic responses like oxidant production[20]; cell cycle[21]; activation of reporter genes[22]; apoptosis[23]; antigen upregulation or downregulation; and expression of neoantigens. In addition, several cell responses are short-lived and might require either a kinetic time-course or a measurement at a fixed, short time. These include responses such as calcium elevation[24], membrane potential[25] and pH changes[26]. Flow cytometry is also well suited for determining the toxicity of candidate drugs on cells.

The technique is now commonly used in the analysis of cell adhesion both in homotypic interactions, where the fluorescence and light scatter properties of a cell doublet are different from a singlet, and in heterotypic adhesion, where the aggregated conjugate is readily distinguished from the unaggregated cells[19]. Adhesion assays are likely to have implications in the analysis of ligands for adhesion receptors, cytokines that stimulate cell adhesion, and in diagnosing diseases that have altered cell adhesion as a component.

There are several situations where the use of cells in screening is less desirable. These might include cases where the assays require time periods that are longer than the cells are stable for or conditions where analysis of a specific intracellular molecular assembly is required. In cases like these, taking advantage of the display of cellular molecules in a variety of cellular and non-cellular conditions is possible. The cellular conditions could include cell preparations that were stabilized to retain ligand binding, or preparations using mutant receptors whose internalization was prevented by their molecular manipulation[27]. As described below, the non-cellular approaches include the display of purified proteins, cell membranes and other cell fragments on microspheres.

Analysis of molecular interactions *in vitro*

Much of modern biomedical research, including drug discovery, involves the analysis of molecular interactions. Important interactions include those between ligands and receptors, enzymes and substrates, and compounds and

their potential cellular targets. Traditionally, the methods used to study such interactions depended on the biological molecules being studied[28]. They were almost always discontinuous assays requiring a separation step. For instance, protein–nucleic acid interactions are often studied using gel electrophoresis or filtration, while interactions between ligands and membrane-bound receptors often involve centrifugation. Such approaches are often suitable for endpoint assays, but are not appropriate for kinetic analysis of rapidly equilibrating systems. Homogeneous assays involving fluorescence or other optical methods such as surface plasmon resonance[29] or evanescent waves[30] are more appropriate for kinetic characterization, but typically have limitations in sensitivity, throughput and quantification.

Recently, flow cytometry has begun to be recognized as a versatile platform for molecular interactions of all types. By immobilizing one species of biomolecule on the surface of microspheres and fluorescently labeling its interacting partner, the interaction between the two can be measured as a change in microsphere fluorescence. The resulting method has several beneficial characteristics. The overall sensitivity depends upon the number of particles (hundreds to thousands) and the number of molecules per particle (thousands), with an ultimate detection limit of attomoles (10^{-18}) of bound complex. For complexes that are stable over time frames of seconds or form with K_ds of no higher than tens of nM, the assays are homogeneous, require no wash steps and can provide continuous kinetic resolution. Because the analysis is multiparametric, it permits the simultaneous resolution of the interaction of multiple ligands with one target, or of multiple ligands with many targets. In addition, the approach can be employed with almost any biomolecule, provided that the appropriate immobilization and labeling strategies are available[28]. In addition to the traditional approach of probe conjugation, labeling using green fluorescent protein (GFP) chimeras is becoming popular[31]. A very recent approach involving the incorporation of a covalent labeling site into ligands or targets has been introduced[32].

Biomolecules bearing affinity tags can be immobilized on microspheres bearing the corresponding recognition unit. An example is the binding of biotinylated ligands to avidin-coated microspheres. Synthetic biotinylated, fluoresceinated DNA substrate bound to streptavidin microspheres allows the analysis of cleavage by the structure-specific nuclease human flap endonuclease-1 (FEN-1)[33,34]. The combination of low substrate concentrations and subsecond kinetic resolution allows the kinetic characterization of the cleavage mechanism as well as a structural analysis using site-directed mutant enzymes.

Proteins provide an especially difficult challenge for immobilization. Chemical biotinylation of the G protein β/γ subunit allows successful immobilization on streptavidin-coated microspheres for the analysis of binding by fluorescently labeled subunit[35,36]. A more generally useful approach might be to incorporate the affinity tag during protein expression. This allows proteins to be labeled at a specific site and to be immobilized on a surface with a homogeneous orientation. Examples include the binding of his-tagged FEN-1 to Ni^{2+} beads[37] and biotinylated, enhanced GFPs to avidin beads[38]. Another approach is to associate cell fragments with microspheres in a manner suggested by Sarvazyan *et al.*[35] More recently, we have observed that membrane fragments as well as solubilized receptors can be displayed on particle surfaces in a functional form (J. Vilven, S. Lauer, J.P. Nolan, E.R. Prossnitz and L.A. Sklar, unpublished).

Purified phospholipids can be assembled onto glass microspheres to form supported bilayer membranes. The resulting 'liposphere' have served as a platform for flow cytometric analysis of membrane binding by fluorescently labeled coagulation factors[39]. Glycolipid receptors can be incorporated into the bilayer to allow studies of ligand binding (S. Lauer and J.P. Nolan, unpublished) and receptor aggregation[40].

Beyond the sensitive and quantitative analysis of bimolecular interactions, the multiparameter measurement capabilities of flow cytometry enable more sophisticated analyses. By labeling different soluble ligands with different fluorescent dyes, it is possible to measure simultaneously the assembly of multimolecular complexes. Energy transfer between selected fluorophores can be used to measure distances between members of such a complex. Fluorescence energy transfer upon aggregation of mobile, lipid-linked receptors has been used for the sensitive detection of biological toxins in unknown samples[40]. Multiplexed analysis of binding to several targets is also possible using differently dyed or sized microspheres. Such approaches have been used in the design of multiplexed immunoassays[16,41], and can also be used to study the interaction of enzymes with multiple substrates (J.P. Nolan *et al.*, unpublished) or of ligands with multiple receptors. Such multiplexed analysis greatly increases the information obtained from a single experiment.

Flow cytometric genome analyses

As efforts to complete the sequencing of the human genome accelerate, increased attention is being focused on ways to use this new information. Two types of information that can be exploited to identify new drug targets

are differential gene expression and individual genetic variation. In gene expression analysis, cDNAs are arrayed to monitor the levels of hundreds or thousands of mRNAs, and each gene product is a potential drug target. Individual genetic variation, especially at the level of the single nucleotide polymorphism (SNP), provides landmarks to help extract critical information from the genome. Pharmacogenomic studies aim to use these genetic markers to aid the mapping of disease-related genes, the identification of new drug targets, the identification of optimal populations for clinical trials, and the screening of patients to determine the appropriate therapeutic course. For the analysis of gene expression and genetic variation, many genetic elements will need to be analyzed in parallel, in hundreds to thousands of individual samples. The much-publicized 'DNA chip' approach, involving two-dimensional microarrays of nucleic acids, holds great promise for highly parallel analysis of hundreds of thousands of genetic elements. The realization of this promise requires that several technical and practical limitations be addressed, namely relatively long sample preparation and analysis times and the large investments in set-up time and cost.

Microsphere arrays for flow cytometry offer several advantages. First, the preparation of microsphere-based nucleic acid arrays can be accomplished on the lab bench with well-known chemistries and commercially available reagents. Second, because each array element is prepared separately, arrays can be rapidly reconfigured by remaking individual elements, rather than the entire array, as would be required for a physical two-dimensional array. Third, while current microsphere arrays contain fewer elements than flat arrays (dozens or hundreds compared with thousands), analysis times can be much faster with flow cytometry (less than a minute compared with minutes to hours). Finally, microspheres represent a soluble surface that can be transported and mixed by conventional sample-handling technology. These advantages are especially important for genome analysis, where the sample numbers are especially large.

Flow cytometry is now being used in hybridization-based analysis for the detection of specific nucleic acid sequences[42]. This approach involves applications such as the detection of signature sequences from bacterial or viral pathogens and the detection of specific mRNAs. Individual genetic variation at the level of the SNP can be detected using enzymatic approaches involving DNA polymerases or ligases, both of which have been adapted to microspheres[42]. The discovery of new SNPs can be facilitated through the use of proteins that recognize the single base mismatches that result when heteroduplex DNA molecules are formed[43,44]; this approach is also amenable to flow cytometry[45]. The multiplexed analysis of many genomic sites with the high sample-analysis rates of flow cytometry represents a powerful combination for realizing the potential of the Human Genome Project in drug discovery and development.

Comparison of flow cytometry with other screening approaches

A comparison of flow cytometry and microplate-based assays is worthy of specific comment. Plate assays are commonplace in the analysis of cell physiology and molecular assemblies. For example, microplate-based detection formats show promise in endpoint assays that measure stable cellular responses to the presence of receptor ligands and antagonist drugs. Moreover, it is feasible to perform kinetic assays in 96- or 384-well plates, where the response, such as an elevation of intracellular calcium, is transient. The ability to use flow cytometry successfully in a parallel manner depends upon two elements: the effective use of automated, repetitive sample handling (ten or more samples per minute) and the potential for simultaneous analysis of multiple targets (ten or more) where the targets are distinguished by multiplex detection strategies.

Homogeneous binding assays

There is considerable interest in the development of homogeneous fluorescent assays for molecular interactions. Plate or suspension assays are heterogeneous when the reactants and products need to be separated. The need for a separation step is usually due to the fact that the products and the reactants have the same fluorescence characteristics. There are several strategies for distinguishing the fluorescence characteristics of reactant and products: for example, by using chromogenic substrates in enzyme assays or by using fluorescence polarization or resonance energy transfer in binding interactions. Flow cytometry is unique in that no spectral change is required for the distinction between free and particle-bound probe. Further, measurements can be made with very low concentrations of receptor, avoiding ligand depletion and enabling the accurate measurement of high-affinity interactions (Box 1).

Sample throughput

The multiparameter elements of flow cytometry can be put to further advantage. Not only can cell subpopulations and their responses be resolved, but also many combinations of cell response and binding interactions can be detected. There are, however, several commonly

Box 1. Binding assays by flow cytometry vs cuvettes

Continuous binding measurements in the bulk phase are possible when some spectroscopic feature of the ligand or receptor is altered upon binding. For example, if a fluorescent ligand is quenched or its polarization is changed upon binding to its receptor, the interaction can be measured continuously as a decrease or change in polarization of the ligand fluorescence. Generally speaking, in order to detect when ligand binds to a target, a significant fraction of the signal-generating component, the fluorescent ligand, needs to be associated with the target. This depends on the concentration of the reactants and the affinity of their interaction[46]. In the situation where the binding constant is 1 nM, the receptor density must be at least 1 nM in order for an appreciable fraction of ligand to be bound. Moreover, the ligand concentration cannot dramatically exceed the receptor concentration or the fractional change in the signal will be small. For cellular binding measurements, if the receptor density is 60,000 per cell, the particle density in the assay must be 10,000 per μl to produce 1 nM receptor concentration. The requirement for cell density increases as the K_d increases but decreases when the receptor expression is increased.

The considerations in flow cytometry are entirely different[5,46]. In the flow cytometric assay of ligand binding, there is no specific requirement that the bound ligand and the free ligand have distinct fluorescent characteristics. Receptor concentrations can be 100-fold lower than in cuvette measurements, avoiding conditions of ligand depletion that can lead to inaccurate affinity measurements. The homogeneous quality of the assay depends upon the fact that signal arises from the bound ligand when the particle passes through the laser beam. The signal associated with the free ligand is predominantly in the volume between the particles (Fig. 1).

Typically, only when the affinity of the binding interaction is low (K_d ~100 nM, requiring high ligand concentrations to saturate the receptor) or when the non-specific binding of the ligand is high, is the homogeneous character of the assay threatened[47]. The sensitivity of the assay depends upon the relative fluorescence emanating from a particle that has no bound ligand and a particle that has its receptor targets saturated with bound ligand. While microspheres typically have very low background signals, the 'autofluorescence' of unlabeled cells in a flow cytometer depends on the origin of the cell and can be affected by endogenous fluorophores or culture media, but is typically less than an equivalent of 10,000 molecules of fluorescein. A receptor density of ~10,000 per cell can be detected for ligands conjugated to small fluorescent molecules. However, as few as ~1000 receptors per cell can be detected with antibodies and very bright fluorophores, such as phycoerythrin.

perceived weaknesses in flow cytometry, especially for high-throughput screening of drug libraries. While commercially available sample handling for flow cytometry is largely limited to sample-tube carousels, sample delivery from 96-well plates is a feasible approach. The sampling format is sequential for end point assays in flow cytometry, with each sample generated individually and then individually delivered to the flow cytometer at rates of 1–2 samples per minute. Strategies that are likely to enable flow cytometry to achieve higher throughput include improved sample handling to allow on-line or continuous sampling and multiplexed analysis, which allows one drug to be screened against several targets simultaneously.

Because flow cytometers can analyze thousands of cells per second, but only several hundred are required to obtain adequate statistical information, individual assays require a second or less to perform. It has also been shown that samples can be delivered rapidly to a flow cytometer, that stable sample flow can be achieved in periods under a second[12,15] and that, in principle, repetitive samples can be delivered more or less continuously by separating biological samples with plugs of buffer (B. Edwards, F. Kuckuck and L.A. Sklar, patent pending). With the appropriate front end for sample handling and a computerized acquisition and analysis scheme, the possibility exists to analyze up to 60 samples per minute in conventional flow cytometry. This sampling capability could be potentially extended with microfluidic sample-handling systems.

Parallel analysis

Multiplex analysis is a familiar concept in flow cytometry as represented by the simultaneous analysis of several populations of cells or beads. In its most ambitious form, one could envision several populations of cells, with each population expressing individual target molecules. These target molecules could represent specific receptors for which fluorescent ligand molecules were available. If the ligand for each receptor were conjugated with the same fluorescent ligand, it would be necessary to distinguish the individual cell populations. One way of doing this would be to label each population with varying levels of two different dyes that could be detected separately. If five separate staining levels were achieved with each dye, 25 separate targets could be resolved. A 'hit' would represent any drug that blocked the binding of ligand to one of the target populations. A complementary approach would be to

mix the target cells in the presence of stimuli specific to each of them. Hits would be identified when a cell population in the mixed population had its response inhibited.

Because the ligand does not have to be depleted in these assays, the cell number in the assay only plays a role in the speed of signal acquisition. Accurate measurements can be made with 1000 particles, at rates of analysis ultimately approaching 100,000 particles per second. Data from a single target displayed on 1000 particles per μl is readily analyzed in a second. A sample volume as small as 1 μl is required if the appropriate microfluidic-handling capabilities were available for mixing the sample and delivering it to the sample line of the flow cytometer. By combining the best features of sample handling, multiplexing and data acquisition, one could envision a system with the following characteristics: samples would represent 100,000 cells in a volume of 10 μl; there would be 1000 cells for each of 100 different targets; each target would have a distinct ligand, but the targets would be resolved by a multiplex approach; each sample would screen 100 targets against a single component of the library; and a new sample would be delivered every few seconds.

Prospects for flow cytometry in drug discovery

The capabilities described above represent a unique combination of measurement possibilities that make flow cytometry an extremely flexible analytical platform. These features provide powerful assay potential at several stages in the drug discovery process.

Target identification

The process of drug discovery starts with the identification of appropriate drug targets. This process promises to be greatly facilitated by the availability of sequence from the Human Genome Project. New targets can be identified by either screening for mRNAs that are upregulated or downregulated in response to experimental stimuli, or by mapping phenotype- or disease-related genes using genetic markers, such as SNPs. The speed of both of these approaches can be facilitated by microarray-based methods, and flow cytometric analysis of microsphere-based microarrays provides experimental flexibility, sensitivity and throughput for large-scale genomic applications.

Cellular targets

Flow cytometry now plays a central role in the development and presentation of targets for drug discovery. Much of the detection technology for high-throughput screening requires cell lines expressing the appropriate molecular targets. While these targets are typically receptors expressed by native biological cell types, it is often not possible to obtain native cells in adequate quantity with the uniformity of target expression, the uniformity of response, and the stability required for screening assays. Thus, a crucial part of drug discovery now depends upon cell lines into which targets and/or cellular response pathways have been stably and uniformly transfected. Flow cytometry is widely used for sorting clonal cell lines of uniform populations of responsive, target-bearing cells, and it is uniquely capable of sorting cells whose responses are transient, lasting only for seconds.

Primary and secondary screening

The screening of large libraries of compounds requires high-throughput analysis. The targets might be purified molecules or transfected cells bearing a receptor of interest. Currently, initial library screens are most often performed as endpoint assays in a microwell-plate format, allowing the parallel analysis of hundreds or thousands of assays every few minutes. Flow cytometry does not presently approach this analysis throughput although, as described above, the combination of multiplexed analysis with automated sample handling could allow the analysis of hundreds of assays per minute. Because combinatorial libraries are often prepared on microsphere solid supports, the possibility of physically sorting microspheres that bound a labeled receptor offers the potential for extremely efficient primary screens[48,49]. Compounds scored as hits in a primary screen are typically subjected to secondary screens to profile target affinity, stability and efficacy in live cells. The ability of flow cytometry to make quantitative measurements on microspheres or cells with continuous kinetic resolution offers an attractive platform for more-detailed characterization of compounds of interest. For instance, it is possible to analyze receptor binding and cellular response in several cell types simultaneously.

Animal tests

Ramanathan has recently reviewed several applications of flow cytometry in pharmacodynamics and drug delivery[3]. However, even though flow cytometry is beginning to be used to determine the presence of drugs in the circulation of test subjects[50], several other applications have not been widely appreciated. It is already clear that physiological mediators can be detected by bead-based immunoassays[41]. Moreover, particle-based assays would appear to provide an approach for detecting drug molecules or their metabolites directly. The use of competitive assays would allow antibodies against drugs or small molecules on beads to detect circulating drug in the presence of a fluorescent

drug analog. Alternatively, by covalently attaching a drug to a bead, the drug in the test sample could be detected by competing for fluorescent antibody binding to the bead (T. Buranda and L.A. Sklar, unpublished).

Summary

Flow cytometry is uniquely capable of the sensitive and quantitative molecular analysis of genomic sequence information, interactions between purified biomolecules and cellular function. Especially powerful are the possibilities for multiplexed analysis of multiple factors or endpoints. Combined with automated sample handling for increased sample throughput, these features make flow cytometry a versatile platform with applications at many stages of drug discovery.

REFERENCES

1 Melamed, M., Lindmo, T. and Mendelson, M., eds (1990) *Flow Cytometry and Sorting*, Wiley–Liss
2 Shapiro, H.M. (1995) *Practical Flow Cytometry*, Wiley–Liss
3 Ramanathan, M. (1997) *Pharm. Res.* 14, 1106–1114
4 Schwartz, A. *et al.* (1996) *Cytometry* 26, 22–31
5 Nolan, J.P., Chambers, J.D. and Sklar, L.A. (1998) in *Cytometric Cellular Analysis* (Vol. 1) (Robinson, J.P. and Babcock, G., eds), pp. 19–46, John Wiley and Sons
6 Keller, R.A. *et al.* (1996) *Appl. Spectrosc.* 50, A12–A32
8 Robbins, D.L. *et al.* (1997) *Proc. SPIE* 2985, 213–222
9 Steinkamp, J.A., Yoshida, T.M. and Martin, J.C. (1993) *Rev. Sci. Instr.* 64, 3440–3450
10 Pinsky, B.G. *et al.* (1993) *Cytometry* 14, 123–135
11 Roederer, M. *et al.* (1997) *Cytometry* 29, 328–339
12 Nolan, J.P. *et al.* (1995) *Cytometry* 21, 223–229
13 Sklar, L.A. *et al.* (1998) *Proc. SPIE* 3256, 144–153
14 Lindberg, W., Ruzicka, J. and Christian, G.D. (1993) *Cytometry* 14, 230–236
15 Seamer, L.C., Kuckuck, F. and Sklar, L.A. *Cytometry* (in press)
16 Fulton, R.J. *et al.* (1997) *Clin. Chem.* 43, 1749–1756
17 Kettman, J.R. *et al.* (1998) *Cytometry* 33, 234–243
18 Stewart, C.C. and Stewart, S.J. (1994) *Methods Cell Biol.* 41, 61–80
19 Simon, S.I. *et al.* (1992) *J. Immunol.* 149, 2765–2771
20 Robinson, J.P., Carter, W.O. and Narayanan, P.K. (1994) *Methods Cell Biol.* 41, 437–447
21 Dressler, L.G. and Seamer, L.C. (1994) *Methods Cell Biol.* 41, 241–262
22 Ropp, J.D. *et al.* (1995) *Cytometry* 21, 309–317
23 Darzynkiewicz, Z. *et al.* (1997) *Cytometry* 27, 1–20
24 June, C.H. and Rabinovitch, P.S. (1994) *Methods Cell Biol.* 41, 149–174
25 Shapiro, H.M. (1994) *Methods Cell Biol.* 41, 121–133
26 Boyer, M.J. and Hedley, D.W. (1994) *Methods Cell Biol.* 41, 135–148
27 Hsu, M.H. *et al.* (1997) *J. Biol. Chem.* 272, 29426–29429
28 Nolan, J.P. and Sklar, L.A. (1998) *Nat. Biotechnol.* 16, 633–638
29 Jonsson, U. *et al.* (1991) *BioTechniques* 11, 620–627
30 Davies, R.D. *et al.* (1994) *Techniques Protein Chem.* V, 285–292
31 Mistelli, T. and Spector, D.L. (1997) *Nat. Biotechnol.* 15, 961–964
32 Griffin, B.A., Adams, S.R. and Tsien, R.Y. (1998) *Science* 281, 269–272
33 Nolan, J.P. *et al.* (1996) *Biochemistry* 35, 11668–11676
34 Shen, B. *et al.* (1997) *Nucleic Acids Res.* 25, 3332–3338
35 Sarvarzyan, N. and Neubig, R. (1998) *J. Biol. Chem.* 273, 7934–7940
36 Lan, K.L. *et al.* (1998) *J. Biol. Chem.* 273, 12794–12797
37 Nolan, J.P. *et al.* (1997) *Biophys. J.* 72, A383
38 Nolan, J.P. *et al.* (1998) *Proc. SPIE* 3256, 114–121
39 Gilbert, G.E. *et al.* (1992) *J. Biol. Chem.* 267, 15861–15868
40 Song, X., Nolan, J.P. and Swanson, B.I. (1998) *J. Am. Chem. Soc.* 120, 11514–11515
41 McHugh, T.M. (1995) *Methods Cell Biol.* 42, 575–595
42 Cai, H. *et al.* (1997) *Proc. SPIE* 3256, 171–177
43 Lishanski, A., Ostrander, E.A. and Rine, J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 2674–2678
44 Wagner, R., Debbie, P. and Radman, M. (1995) *Nucleic Acids Res.* 23, 3944–3948
45 Nolan, J.P. (1997) *Am. J. Hum. Genet.* 61, A241
46 Sklar, L.A. (1987) *Annu. Rev. Biophys. Biophys. Chem.* 16, 479–506
47 Murphy, R.F. (1990) in *Flow Cytometry and Sorting* (Melamed, M., Lindmo, T. and Mendelson, M., eds), pp. 355–356, Wiley–Liss
48 Needels, M.C. *et al.* (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 10700–10704
49 Muller, K. *et al.* (1996) *J. Biol. Chem.* 271, 16500–16506
50 Marder, P. *et al.* (1996) *Br. J. Clin. Pharmacol.* 42, 457–464

In short...

Corixa (Seattle, WA, USA) has acquired the biotechnology company **Anergen** (Redwood City, CA, USA) for approximately $8.1 million. The purchase was paid in ~1.1 million shares of Corixa common stock at a price of ~$7.30 per share.

Anergen develops drugs designed to interrupt antigen presentation selectively or inactivate the T cells that mediate the disease process. The acquisition thus adds to Corixa's research and clinical programmes in rheumatoid arthritis, diabetes and multiple sclerosis. 'We believe the acquisition of Anergen will extend the application of Corixa's immunotherapeutic expertise into multiple autoimmune disease fields,' said Steven Gillis, President and CEO of Corixa. 'With the addition of Anergen's programmes and technologies to our science platforms, we will be able to expand the breadth and depth of our product offerings to current and potential corporate partners.'