Suspension array technology: evolution of the flat-array paradigm

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Suspension arrays of microspheres analyzed using flow cytometry offer a new approach to multiplexed assays for large-scale screening applications. By optically encoding micron-sized polymer particles, suspension microarrays can be created to enable highly multiplexed analysis of complex samples. Each element in the array is comprised of a subpopulation of particles with distinct optical properties and each array element bears a different surface receptor. Nucleic acids, proteins, lipids or carbohydrates can serve as receptors to support the analysis of a wide range of biomolecular assemblies, and applications in genomic and proteomic research are being developed. Coupled with recent innovations for rapid serial analysis of samples, molecular analysis with microsphere arrays holds significant potential as a general analysis platform for both research and clinical applications.

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Biomedical research has evolved significantly over recent years, with the large-scale screening of whole genomes complementing focused studies on a few genes or proteins. This evolution has encompassed applications ranging from functional analysis of unknown genes to identification of disease-related genes, screening in drug discovery and clinical diagnostics. There has been a concurrent surge in technology development to facilitate large-scale biological analysis. In general, these technologies have two components: the assay chemistry and the detection platform. Perhaps the best-publicized detection platform of recent years is the flat microarray.

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Configured as 'DNA chips', these flat microarrays offer the promise of whole genome analysis of single samples. Each element or 'spot' on a flat-surface array contains a target-specific receptor, for example a DNA molecule to detect a specific DNA sequence, and the signal originating from that element reports the presence of a target molecule. A related detection platform that is proving to be compatible with a range of assay chemistries in a high-throughput format is the use of encoded microspheres in combination with flow cytometry, also known as suspension array technology (SAT). In this article we analyze the features of SAT and highlight current and potential applications in which SAT has unique advantages.

Microsphere arrays

SAT employs encoded microspheres as array elements that bear specific receptor molecules. Microspheres with distinct optical properties, such as light scatter or fluorescence from an internal dye, are used as solid supports for a variety of molecular analyses. By careful adjustment of these intrinsic optical properties, it is possible to prepare arrays of microspheres in which individual microsphere subsets can be identified and used to perform multiplexed analysis. Conceptually, microsphere arrays are similar to flat-surface microarrays, with distinct quanta of an intrinsic optical parameter substituting for physical location on a surface. Beyond this similarity, microsphere arrays and flat-surface arrays differ significantly in their implementation. The use of optically encoded microspheres as array elements affects issues such as array preparation, density and flexibility.

Array preparation

Microsphere arrays have a significant advantage over flat arrays in terms of preparation and use. Each array element (or spot) in each flat array is prepared individually, using spotting robots or photolithography. Although it is possible to attain some economy of scale with the simultaneous preparation of multiple arrays in parallel, there is a limit to the number of flat-surface arrays that can be prepared at one time. By contrast, with microsphere arrays each array element is prepared in bulk. A suspension of microspheres typically contains tens of millions of particles per milliliter that, when coupled with the appropriate receptor (antibody, other protein, nucleic acid or other molecule), can be used to prepare thousands of microsphere arrays. To reconfigure an array with new array elements, a new conjugation is performed on a particular microsphere subset and a new mixture of microspheres is prepared.

Array density

The ability to perform highly parallel measurements is important for maximizing the efficiency and speed of the analysis. First, by performing many analyses simultaneously on a single sample, the consumption of expensive reagents and valuable sample is minimized. Second, assay throughput is the product of parallel analysis capacity and the serial analysis rate. Typical spotted flat microarrays might contain array elements at a density of hundreds to thousands of array elements per square centimeter, depending

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Box 1. Encoding of microsphere arrays

Individual microsphere array elements are identified by one or more intrinsic optical properties of the particle. For example, the current generation of commercially available microsphere arrays (LabMap System, Luminex Corp) offers a 10×10 element array (ten different intensities for each of two fluorescent dyes contained within microspheres) to give a 100-element array. Whereas for many applications the ability to conduct a few dozen analyses simultaneously is entirely adequate, SAT has the potential to use much larger arrays. Given the ability to resolve ten discrete intensities per dye, the array size would increase as a power of the numbers of dyes used. Thus, if three fluorescent dyes were used to encode array elements, the array size could be 1000 and if six different dyes were used one million different array elements could be identified.

on the mode of preparation, providing a very high level of parallel analysis. However, the measurement of such an array might take several minutes, resulting in a fairly low serial analysis rate when many such arrays must be measured. For suspension arrays, the considerations and limitations are somewhat different. For example, array density is measured in terms of volume rather than of surface area, with a few microliters of microspheres typically containing hundreds of thousands of array elements. Thus each element in the array is represented by several hundred individual microspheres, and the flow-cytometric measurement represents a replicate analysis of each array element. The current generation of suspension arrays contain between a dozen and a hundred discrete array elements [1] but optical encoding approaches make very high-density arrays possible (Box 1). Furthermore, using flow cytometry as a measurement platform, particle analysis rates can be as high as 10 000 s^{-1}, making highly multiplexed analysis extremely rapid.

It should be noted that microspheres are being used as solid supports for analysis using a variety of assay platforms. For example, encoded microspheres are being used as array elements on the tips of optical fibers [2], which when bundled form an 'array of arrays'. Encoded microspheres are also being used in conjunction with imaging approaches to enable multiplexed analysis of protein and DNA [3,4]. However, we feel that flow cytometry offers an optimal platform for the implementation of high throughput applications for the widest range of users.

Flow cytometry

For the past two decades, flow cytometry has proven to be the method of choice for the quantitative analysis of cell populations. More recently, the ability to make sensitive and multiparameter fluorescence measurements at high speeds has been exploited for *in vitro* analysis of molecular interactions on microspheres [5,6] and for the analysis and sorting of biological libraries in single cells [7,8]. These features are also ideal for the analysis of microsphere populations in SAT.

Multiparameter detection capabilities

The most basic benchtop flow cytometers, with one or two lasers and four or five detectors, are capable of making sensitive (a few hundred to a few thousand molecules) and quantitative measurements of several different fluorescent probes simultaneously on individual particles. Specially configured research instruments can make simultaneous measurements of ten or more optical parameters [9]. In addition, because flow cytometry has the ability to distinguish free from particle-bound probe over a wide range of free-probe concentrations, measurements can often be made without a wash step, streamlining sample processing.

Recently, several flow cytometry instrument improvements have evolved to expand these capabilities for both cellular and microsphere-based analyses. One of these is the integration of stopped-flow mixing devices with flow cytometers to provide subsecond mixing and delivery for kinetic analysis [10]. Another is the implementation of novel thermoregulation capabilities to enable dynamic temperature control of samples being analyzed by the flow cytometer [11]. Perhaps the most exciting development for large-scale applications is the development of strategies for rapid serial-sample delivery from microwell plates for high-throughput screening applications.

High-throughput flow cytometry

Since its inception, flow cytometry has been known primarily as a tool that processed samples individually. Most commercial instruments required an individual sample tube to be manually fitted to a pressure-tight fitting and samples were pressure-driven to the point of analysis. It has recently become apparent that flow cytometers – with operating pressures typically between 5 and 50 PSI – were readily integrated with syringes, peristaltic pumps and programmable valves [12–14].

These sampling systems have begun to be automated and the potential throughput is worthy of consideration (Box 2). In the presently available soluble-array technology, a 100-plex assay can be performed approximately every 30 seconds. Running continuously, such as system could process 288 000 assays d^{-1}. One of us has recently reported on a high-throughput flow cytometry approach in which submicroliter samples can be delivered from multiwell plates at a rate approaching that of one

Box 2. What is the potential throughput of flow cytometry?

- In analysis with digital signal processing (DSP), raw analysis rates up to 100 000 sec^{-1} could be possible.
- In sorting with DSP, raw sorting rates of 50 000 sec^{-1} are claimed. If each cell is an assay, the rate is 4.32 billion assays d^{-1}.
- In multiplex analysis with microsphere arrays, a 100-plex assay can performed every 30 sec or 288 000 analyses d^{-1}.
- A commercial autosampler can sample a 96-well plate in ~60 sec or ~120 000 d^{-1}. With 1μl volume, a 100-plex assay could be completed in <1 sec or could perform 12 million analyses d^{-1}.

96-well plate min^{-1}[14]. By employing narrow-bore tubing and air bubbles to separate samples and reduce carryover, methods are now being implemented to enable the mixing of submicroliter samples of compounds from wells with particles at similar rates. Once fully automated, such a system could potentially deliver ~120 000 samples d^{-1}.

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In the context of SAT, throughput is not likely to be limited by digital signal processing (DSP; 100 000 events s^{-1}) or by well-to-well autosampling rates. Rather, we believe that particle density could turn out to be the rate-limiting factor. If the practical limit for particle density is 10 000 particles ml^{-1} and a sample of 2 ml can be delivered at optimal sampling rates, then 20 000 particles could represent a 100-plex assay in which each class is represented by 200 particles, a number large enough to provide adequate statistics for the behavior of each element of the array. Thus, the practical instrumental limit for SAT throughput is likely to be in the order of 100 000 arrays of 100 microspheres each per day per instrument.

The current capabilities of SAT, combined with these projected improvements in parallel and serial sample analysis rates, are driving the development of applications is several areas.

Applications of SAT: genomics and proteomics

The initial impact of SAT is being felt in two areas, immunoassays and genetic analysis. The approach of performing an ELISA-like sandwich assay on a microsphere rather than on a microwell plate has been around for more than two decades [15–17] but the commercial availability of encoded microsphere arrays has allowed these to be developed in a more highly multiplexed format [18–20] and commercial immunoassay kits will make this technology far more accessible [21].

Although not yet available in commercial form, DNA-bearing microspheres are being used for a variety of genomic analyses, with the scoring of single nucleotide polymorphisms attracting the largest efforts [22]. The assay chemistries for nucleic acid-based applications cover the range from fairly simple but difficult to generalize hybridization-based assays to specific and robust assay chemistries that employ solution-phase enzymatic reactions with capture-tagged primers and address-tagged microspheres [23–25]. The use of oligonucleotide address tags to create universal microsphere arrays is an important concept for maximizing the flexibility of the array.

Another field that is poised to benefit from the advantages of SAT is that of protein analysis. As attention shifts to encompass the proteome as well as the genome, efforts have begun to produce 'protein chips' analogous to DNA chips. Here, the advantages of SAT will be especially evident. First, a variety of protein-attachment chemistries are already in use on microspheres, ranging from physical adsorption or covalent coupling, such as are typically employed in the multiplexed immunoassays discussed above [17], to specific non-covalent attachment using affinity tags (poly-his, biotin, glutathione-S-transferase, etc.). Moreover, microspheres and flow cytometry are being used to perform functional and mechanistic analysis of molecular assembly in a variety of protein systems [26,27]. Thus, although the implementation of protein arrays faces several technical challenges, it is very likely that the best protein chips will not be chips at all but microsphere arrays of proteins.

Prospects

Long established as a key tool in the study of cells, flow cytometry is emerging as a major analysis platform for genomic and post-genomic applications. The development of suspension arrays that enable multiplexed analysis of many targets in small sample volumes makes suspension array technology a powerful alternative to less flexible flat surface microarrays. Whereas procedures using flat microarrays often require extensive washing to reduce high background signals, the ability of flow cytometry to resolve free and bound probe enables many assays to be performed without wash steps, streamlining sample processing. New approaches to rapid sample delivery from microwell plates will make flow cytometry sample handling compatible with existing laboratory automation hardware. The combination of highly parallel sample analysis and rapid serial delivery of samples makes suspension array technology an extremely attractive platform for a range of large-scale biomedical applications.

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