

FCAP Array – Software to Harness Cytometric Bead Array Technology for Drug Discovery

a report by

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Cytomics, the science of cell-based analyses that integrates genomics, proteomics, traditional cytometry and microarray technologies, emerged with a radical improvement of the automated analytical capabilities by the end of the 1990s. The availability of cost-effective, multi-laser flow cytometry instruments equipped with digital signal processing and increased computing power for rapid data handling eliminated the obstacles blocking the introduction of the flow-cytometer-based simultaneous detection of multi-analytes with suspension array technology using fluorescent microbead particles. Since then, this quantitative, highly sensitive assay, first described as flow microsphere immunoassay (FMIA) in 1976, became one of the key tools in many disciplines of biomedical research and drug discovery.

The terminology describing microsphere-based assays has changed over time. It has been referred to by many names, including multiplexed bead assay (MBA), particle-based microarray technology (PBMT), microarray immunoassay (MAI), cytometric bead array (CBA), etc. For the purposes of this article, the CBA acronym will be used.

The future potential for CBA technology lies in its unique capacity to measure molecular interactions simultaneously on a solid phase surface, on microfluorospheres, in a multiplexed suspension environment. CBA performs numerous discrete homogeneous assays from a single 20µl to 250µl sample with the help of advanced flow cytometry. In order to assure progress that meets the requirements of research, diagnostic medicine and drug discovery of the future, Soft Flow has developed an innovative data analysis and management software – FCAP Array – for offline analysis of CBA data acquired with both the regular flow cytometers and the dedicated instruments for microspheres. CBA, with the power of the FCAP Array software, is a cost-effective assay platform that combines high throughput, sensitivity

and specificity, and also provides flexibility in rapidly rearranging assay combinations.

Features of FCAP Array

FCAP Array is compatible with the major legacy systems in that it is able to manage massive data volumes, supports a variety of data types and formats and provides powerful analytical capabilities in a format suitable for users in drug discovery, biomedical research and clinical diagnosis. The software is dedicated to both qualitative and quantitative assays of diverse assortments of fluorophores. It has been designed not only to support offline data processing but also to work out and record experimental protocols and report formats in accordance with the criteria for acceptance of electronic records and signatures and also supporting the use of electronic laboratory notebook concepts.^{1,2}

Three Key Elements

The first element of the FCAP Array software is the ‘assay specification file’ (ASF), a unified, integrated data repository of the CBA kit specifications. ASF stores all relevant data associated with the microbead array – including the cluster to analyte assignments, definitions of the suggested standards, mathematical models for the standard curve generation for each analyte and the comments/remarks to rapidly identify or filter out the results that are within or out of predetermined ‘normal’ values. The ASFs can either be provided by the manufacturers of the commercial assays or generated by the users. FCAP Array supports the development of versatile, laboratory-assembled assay kits.

The second element, the experiment design module, provides the tools for designing complex experiments combining any number of laboratory-assembled and/or commercial assay protocols and kits. Two assay protocols – ‘batch assay’ and ‘random assay’ – are

1. US Department of Health and Human Services, US Food and Drug Administration (FDA), “Guidance for Industry: Part 11, Electronic Records; Electronic Signatures – Scope and Application”, J:\GUIDANC\5667\fnl.doc, 28 August 2003.
2. J P Helfrich, A Process for Electronic Laboratory Notebooks, Westborough, Massachusetts: NuGenesis Technologies® Corporation, 2003.



supported, analogous to similar enzyme-linked immunosorbent assay (ELISA) protocols. The batch assay wizard provides the tools for the screening of a series of similar samples, which is a typical drug-screening application. In contrast, the random assay wizard helps to design the experiments in which the same samples need to be analysed with several assay protocols, such as the patient samples in a clinical diagnostic laboratory. Experiments can combine any number of batch assays and random assays.

Once the experiment has been designed, FCAP Array can generate printed laboratory instruction for physical preparation of the samples to be delivered to the flow cytometer in 96-well or 384-well microtiter plates or in tubes. The software can also generate BD CBA experiment files to be imported into the Experiment Wizard of the BD FACSAArray™ Bioanalyzer. Acquisition files save the steps that are required to process and run samples on the BD FACSAArray™ instrument.

The third element is a specialised analysis and reporting component specific to the unique needs of microbead array analysis.

Supported Instruments

FCAP Array reads the legal flow cytometry standard (FCS) 1.0, 2.0 and 3.0 file formats and also reads most of the non-standard FCS files produced by many instruments and programs.

BD FACSAArray™ Bioanalyzer

The FCAP Array software has dedicated interface elements for use with the BD FACSAArray™ Bioanalyzer from BD Biosciences, San José, California, a flexible, multifunctional system that supports both the multiplexed CBA technology and cellular analysis using dyes and single-colour antibody reagents. The BD FACSAArray™ instrument has two lasers (green: 532nm, red: 635nm), detects two scatter signals (forward-scatter width and side-scatter width) and four fluorescence signals (yellow, red, far-red, and near infrared). One or two of the fluorescence signals are dedicated to the microsphere classification. The samples should be delivered in 96-well microtiter plates to the instrument.

FCAP Array generates a BD CBA Experiment template that can be imported into the Experiment Wizard® of BD FACSAArray™. The template contains the standard and sample information, the plate – sample layout, the suggested acquisition settings and all other default settings that FACSAArray requires for the acquisition. After importing into the Experiment Wizard®, the settings can be modified and fine-tuned to the specific sample needs.

The BD FACSAArray™ software saves all data in a database. For offline analysis with the current version of FCAP Array, the acquired data should be exported in FCS2.0 or FCS3.0 format. FCAP Array is able to automatically process the exported list mode data files, extract the CBA results, print and save the reports. The raw data can also be exported into spreadsheet format.

Dedicated Flow Cytometers

Luminex® Corporation released the first flow cytometer designed specifically for multiplexed microbead analysis. The dual-laser instrument has a three-colour fluorescence signal-detection system. Two colours are dedicated to the microsphere classification; the third colour is used for measurement of the reporter fluorescence intensity. A matrix of the necessary microspheres and an XY platform designed for handling 96-well microtiter plates are offered.

FCAP Array supports the rapid, automatic off-line processing of data acquired with Luminex instruments. The software can handle both the flow cytometry standard (FCS) and the comma separated (.csv) file formats. If the sample acquisition is properly set, the subsequent data analysis and report generation are fully automatic processes. The software records the necessary details of the experimental design, sample preparation and handling, acquisition and data analysis for use in electronic notebooks.

Conventional Flow Cytometers

Microspheres that are suitable for suspension array technology using conventional flow cytometers are commercially available. The first BD CBA kits were also designed for use with the FACSCalibur™ and FACScan™ instruments. As most flow cytometers work with 125mm x 75mm tubes, the test-tube format of CBA should be used for these applications.

The FCAP Array software supports the CBA measurements on conventional flow cytometers as well. The acquisition and the data collection must be performed with the acquisition software of the instrument and the list mode data files should be saved into FCS2.0 or FCS3.0 format.

Potential Drug Discovery, Research and Clinical Applications

CBA, in its most neoteric version, is a solid-phase immunoassay performed in conjunction with assembled microspheres on a conventional flow cytometer. The technology in its current stage of development offers sensitive qualitative or

quantitative assay systems capable for detecting 20 to 30 or more analytes per sample. A series of beads with discrete fluorescence intensities are used to simultaneously detect multiple soluble proteins in a small sample volume. The beads serve to capture and quantify soluble analytes either when they are secreted or present in cell lysates. Some of the BD Biosciences CBA applications include cytokine and chemokine profiling, detection of inflammatory response, apoptosis and cell-signalling events. These applications are sandwich ELISA-type assays, in which the analytes in samples bind to a fluorescent microsphere coated with capture antibodies, and the captured molecules are detected with a reporter antibody conjugated with another fluorescent dye.

The cytokine family was the first choice for the commercial implementation of CBA.^{3,4} The role of cytokines/chemokines continues to be a challenge for clinical immunology. Due to the complex nature of the production of the cytokine network and the cytokine receptor expression, the multiparameter approach to monitor the kinetics of the cytokine secretion with CBA provides extremely useful information in the drug discovery experiments. The Batch Assay Wizard of the FCAP Array software has been designed for high-throughput drug screening experiments, and the Random Assay Wizard supports the typical, patient-oriented clinical applications.

The sandwich-type immunoassay is only one of the options of CBA applications. The microbead-based suspension array technology can be tuned for various types of other reactions, such as the polymerase chain reaction-based detection of genetic mutations.⁵ An assay system aiming at the development of a DNA binding protein test capable of profiling the transcriptional stage of cells or tissues by binding of fluorescent dye-conjugated transcription factors to a set of specific nucleotide motives anchored to microbeads has also been thoroughly tested. The great demand for this type of information in research, drug screening and clinical diagnosis makes it worthwhile to invest in the development of this promising technology.

Activity of hydrolytic enzymes such as proteases or nucleases, using fluorescent substrates immobilised on microbeads, appeared to be the candidates of choice for additional CBA applications.

Future Prospects

One of the goals of our future software development is to provide the background for microbead-based applications of a 'Lab on Beads™' project, which comprises the following elements:

- detection of gene-rearrangements;
- a procedure that is able to detect point mutations/deletions within any gene of interest; and
- development of very simple and sensitive tests for the measurement of the activity of certain hydrolytic enzymes in body fluids and cellular extracts.

The basic software tools such as the clustering algorithm(s) to identify and select microbead clusters from flow cytometry list mode data files have already been developed and thoroughly tested. On the other hand, it is believed that harnessing future suspension array technologies will require substantially new data processing solutions on longer run. In this context, we are planning to focus on the development of new data analysis and processing methods based on pattern recognition of the 'fingerprints' of the genomic, proteogenomic and proteomic status of cells and tissues.

The computing tasks relevant to processing the 'fingerprint pattern' of the CBA data are not as easy to specify as a conventional computer algorithm. This data is more complex, both in the relations between the features and in their interpretation. Subsequent to a sophisticated data analysis of flow cytometry list mode data files, the first step of the processing should provide the empirical fingerprint via advanced cluster analysis. Perhaps the most difficult task of the computation will be to develop the ability to classify the fingerprint data. The fingerprints may be considered as complex images formed by the individual CBA data points. ■

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4. F F Mandy, T Nakamura, M Bergeron and K Sekiguchi, "Overview and Application of Suspension Array Technology", *Clin. Lab. Med.*, 21 (2001), pp. 713–729.
5. J P Nolan and F F Mandy, "Suspension Array Technology: New Tools for Gene and Protein Analysis", *Cell. Mol. Biol.*, (Noisy-le-grand), 47 (2002), pp. 1,241–1,256.