

UAB CFAR Flow Cytometry Core

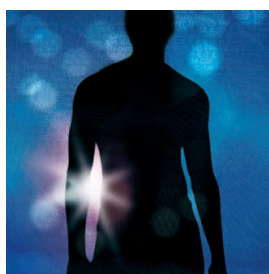
March 2008 - Volume 4

Introduction to the BioPlex Work Station at the UAB CFAR Flow Cytometry Core (May 2008)

The **Bio-Plex** system is a flexible, easy-to-use multiplex analysis system that permits the simultaneous analysis of up to 100 different biomolecules (**proteins, peptides, or nucleic acids**) in a single microplate well. By multiplexing with the Bio-Plex system, researchers can dramatically increase the amount of useful information from rare or volume-limited samples such as mouse or rat serum, and decipher complex interrelationships among proteins involved in signal transduction pathways. The microplate platform allows the automated analysis of 96-well plates with a throughput of more than 1,800 assay points (for a 23-plex assay) in 30 minutes. **BIO-RAD** has organized a Forum to introduce interested users to the technology.

April 8, 2008 8:30 a.m.
Finley-Compass Bank Genetics Conference Center (Kaul Building)

As soon as we have the Bio-Plex system up and running at the CFAR, we will organize a training session to introduce interested users to the technology. Attendance at this training session will be a requirement to utilize the machine. We will announce the training session in a timely manner. The Bio-Plex machine will be located in BBRB 530 (not in LHRB 610).



**Tuesday
April 8, 2008**

**UAB's
Finley-Compass
Bank Genetics
Conference Center
(Kaul Building)**

For more information,
please contact:

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Bio-Plex® Suspension Array Technology xMAP User Meeting

*Presented by Bio-Rad Laboratories
and Cell Signaling Technologies*

This meeting is for laboratories new to xMAP technology as well as those with an established research program. Join us in this open forum to learn about the most recent advances and approaches. Attend and hear experts discuss how they use suspension array technology to advance their research and engage in discussions with other researchers on their xMAP applications.

Bio-Plex Forum Schedule

8:30 – 9:00 AM Registration and continental breakfast.

9:15 AM Quantitative Analysis of Multiple Target Molecules from a Single Sample Using xMAP Technology, Marwan Alsarraj, Field Applications Specialist, Bio-Rad Laboratories

10:00 AM Luminex xMAP Technology: Past, Present and Future, Kathi Kellar, Ph.D., Biotechnology Core Facility, Division of Scientific Resources, National Center for Preparedness, Detection and Control of Infectious Diseases (CDC)

10:45 AM Break. Refreshments provided.

10:55 AM The Utilization of Phospho-specific Antibodies and Kinases in Basic Research and for Drug Discovery, A. Vicki McCulloch, Ph.D., Drug Discovery Specialist, Cell Signaling Technologies

11:30 AM Multiplex Assays Redefined with Magnetic Solutions, Deyrick Dean, Ph.D. Bio-Plex Program Manager, Bio-Rad Laboratories

12:15 PM Lunch Sponsored by Cell Signaling Technologies

1:00 PM Basic Science Applications of Bio-Plex Technology, Chad Steele, Ph.D., Associate Professor, Departments of Medicine and Microbiology, Division of Pulmonary, Allergy and Critical Care Medicine, UAB

1:50 PM Bio-Plex Manager™ 5.0: Control and analysis software for multiplex assay data acquisition and analysis using xMAP instrumentation, Marwan Alsarraj, Field Applications Specialist, Bio-Rad Laboratories

2:30 PM Roundtable discussion

About the Bio-Plex System

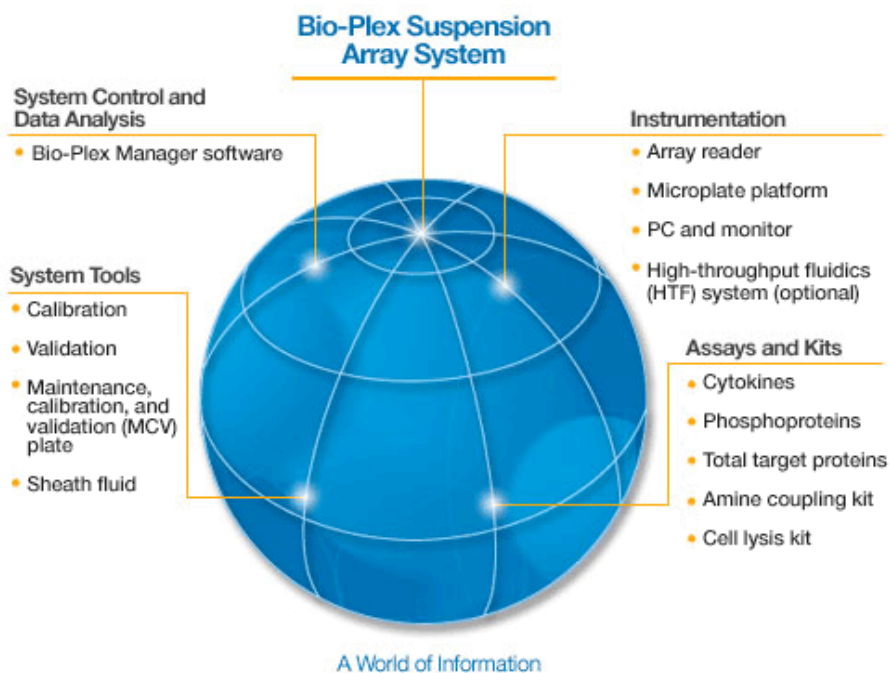
Based on xMAP technology licensed from Luminex corporation, the Bio-Plex system is a flexible multiplex analysis system that permits the simultaneous analysis of up to 100 different biomolecules (proteins, peptides, or nucleic acids) in a single microplate well. This approach allows researchers to dramatically increase the amount of useful information from rare or volume-limited samples and decipher complex relationships among proteins and nucleic acids.

Please RSVP by registering at www.regonline.com/bioplexforum40808 by April 3, 2008.

BIO-PLEX SYSTEM

The Bio-Plex suspension array is built around three core technologies. The first is the family of fluorescently dyed microspheres, or beads, from Luminex Corp. The second is a flow cytometer with two lasers and associated optics to measure biochemical reactions that occur on the surface of the microspheres, and the third is a high-speed digital signal processor to efficiently manage the fluorescent output. These technologies are brought together in the Bio-Plex workstation. Unique to Bio-Plex is the integration of assay kits, software, calibration and validation tools, and instrumentation into

a complete system. The convenience and consistency of the system approach is complemented by outstanding customer support.



Powerful Drug Discovery Tool

The Bio-Plex suspension array system helps to reveal a more complete view of the effects of candidate drugs on the biology of cells, research animals, and human subjects, and addresses your drug discovery and development needs by integrating hardware, powerful data reduction software, validation tools, and assays into a single platform. Potential applications include functional genomics, validation of drug leads, secondary screening, absorption, distribution, me-

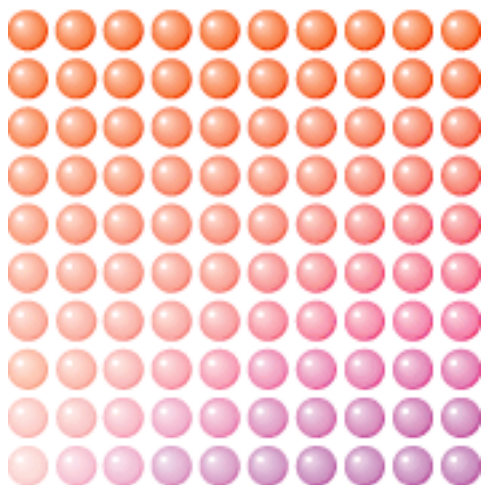
tabolism, excretion (ADME), and toxicology, as well as high-throughput ELISA and western blot assays.

Flexible Protein and Nucleic Acid Array System The Bio-Plex suspension array system uses patented multiplexing technology that uses up to 100 color-coded bead sets, each of which can be conjugated with a different specific reactant. Each reactant is specific for a different target molecule. Reactants can include enzyme substrates, DNA, receptors, antigens, or monoclonal antibodies. These reactants can be used to create, for example, a capture sandwich immunoassay as used in Bio-Plex assays. These assays and the antibodies used in these assays undergo rigorous optimization to ensure the highest degree of sensitivity, specificity, and reproducibility. To perform a multiplex assay, sample and reporter molecules are allowed to react with the conjugated bead mixture in microplate wells. The constituents of each well are drawn up into the flow-based Bio-Plex array reader, which identifies each specific reaction based on bead color and quantitates it. The magnitude of the reaction is measured using fluorescently labeled reporter molecules also specific for each target protein. Bio-Plex Manager software automates data analysis and generation of detailed summary reports. With the Bio-Plex suspension array system you can:

- Simultaneously quantitate up to 100 protein targets in culture media, sera, and other matrices
- Instantly customize your assays by mixing bead sets
- Automatically analyze up to 96 samples in under 35 minutes
- Dramatically increase the amount of useful data per sample

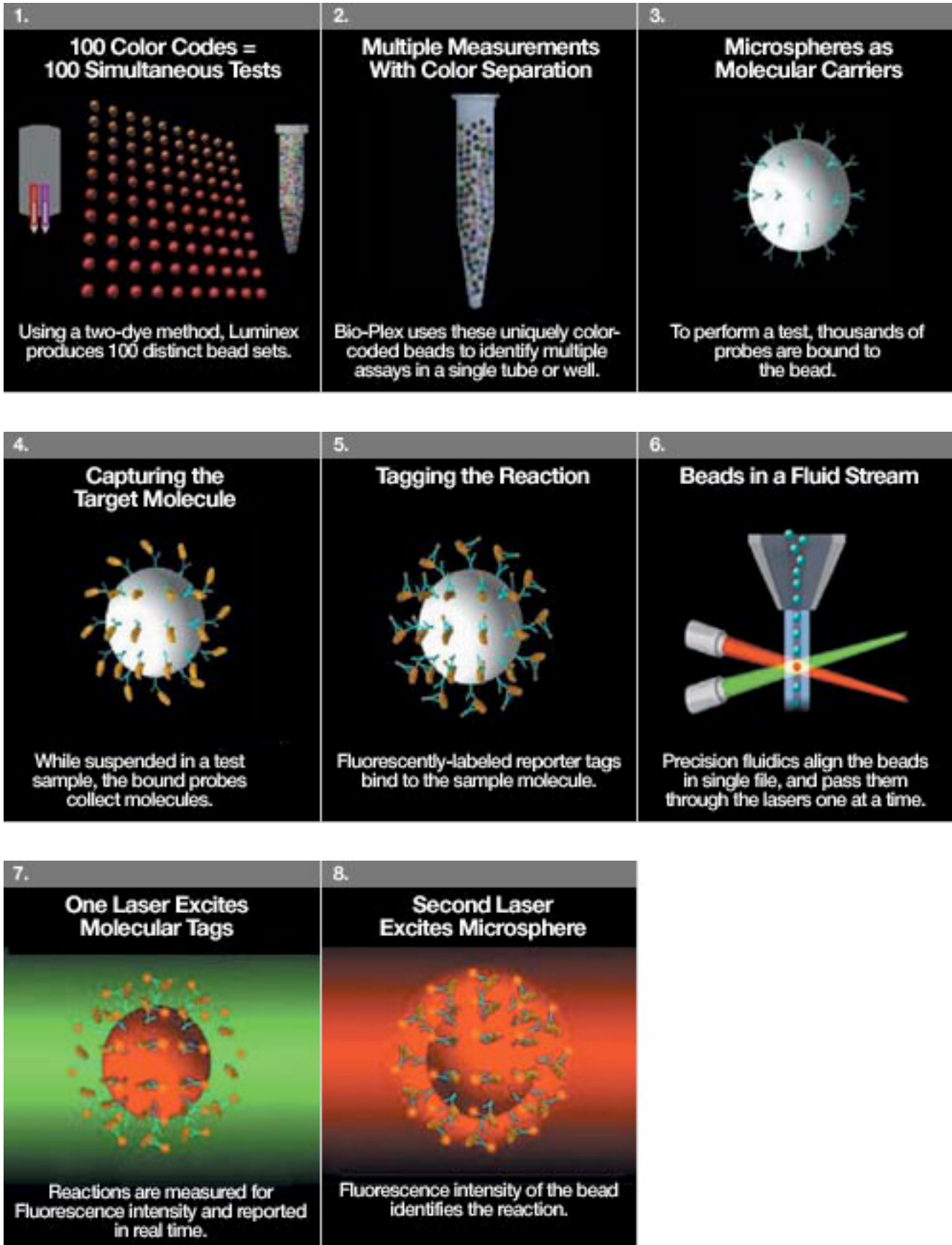
Multiplexing Bead Technology

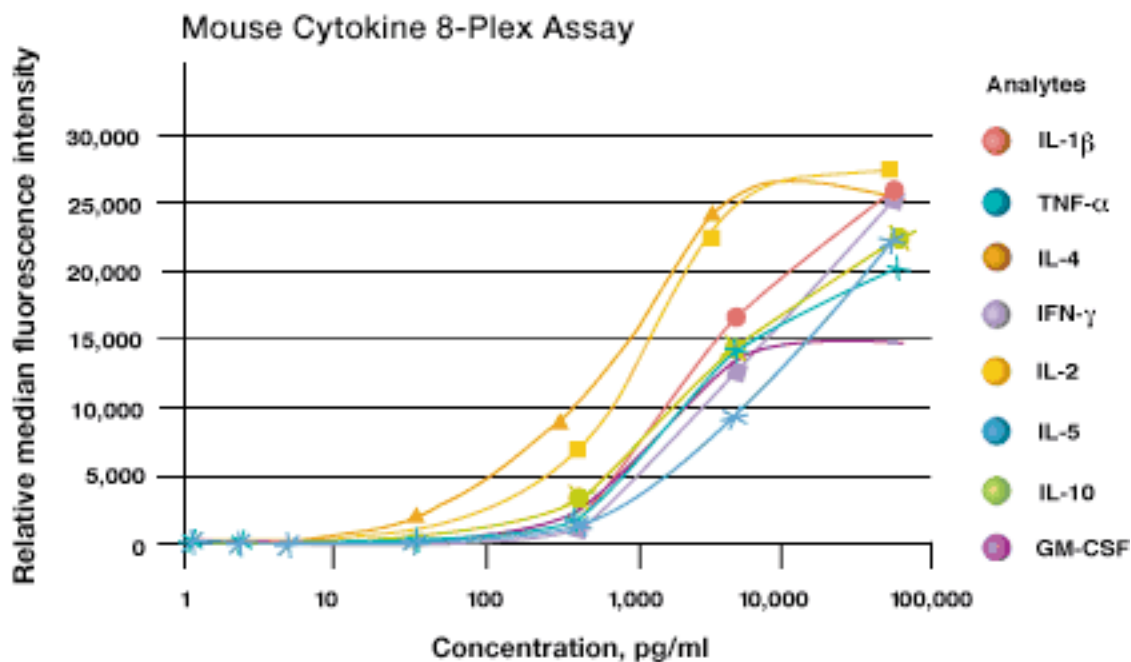
The Bio-Plex suspension array system uses the multiplexing technology of Luminex Corp. to enable the simultaneous quantitation of up to 100 analytes. This technology uses polystyrene beads internally dyed with differing ratios of two spectrally distinct fluorophores. Each fluorophore can have any of 10 possible levels of fluorescent intensity, thereby creating a family of 100 spectrally addressed bead sets as pictured below:



Bio-Plex assays contain dyed beads conjugated with monoclonal antibodies specific for a target protein or peptide such as a cytokine or a phosphoprotein. Each of the 100 spectrally addressed bead sets can contain a capture antibody specific for a unique target protein. The antibody-conjugated beads are allowed to react with sample and a secondary, or detection, antibody in a microplate well to form a capture sandwich immunoassay. Multiplex assays can be created by mixing bead sets with different conjugated antibodies to simultaneously test for many analytes in one sample.

The assay solution is drawn into the Bio-Plex array reader, which illuminates and reads the sample. When a red diode "classification" laser (635 nm) in the Bio-Plex array reader illuminates a dyed bead, the bead's fluorescent signature identifies it as a member of one of the 100 possible sets. Bio-Plex Manager software correlates each bead set to the assay reagent that has been coupled to it (for example, an IL-2 capture antibody coupled to bead #36). In this way the Bio-Plex system can distinguish between the different assays combined within a single microplate well. A green "reporter" laser (532 nm) in the array reader simultaneously excites a fluorescent reporter tag (phycoerythrin, or PE) bound to the detection antibody in the assay. The amount of green fluorescence is proportional to the amount of analyte captured in the immunoassay. Extrapolating to a standard curve allows quantitation of each analyte in the sample. Patented digital signal-processing algorithms provide simultaneous real-time acquisition of classification and reporter signal output from thousands of beads per second, supporting up to $100 \times 96 = 9,600$ analyte measurements from each 96-well plate. The Bio-Plex technology is summarized on the next page:





Powerful digital signal-processing algorithms accumulate fluorescent classification and reporter output from the beads. Bio-Plex Manager software then analyzes the data and presents them in meaningful form. Shown here is a standard curve from the simultaneous analysis of eight cytokines from mouse serum. Each data point represents the average of three individual samples.

Bio-Plex Assays and Reagents

Bio-Plex assays are multiplex bead-based assays based on xMAP technology and optimized for the Bio-Plex suspension array system. Bio-Rad offers a full line of assays and reagent kits, including:

- Cytokine assays
- High-sensitivity cytokine assays
- Angiogenesis assays
- Phosphoprotein assays
- Total target assays
- Isotyping assays
- Diabetes assays
- Acute phase assays
- Amine coupling kit
- xMAP COOH beads

Individual (singleplex) cytokine, phosphoprotein, and total target assays are designed to provide more flexibility. These assays can be used independently or combined to test for a specific set of proteins in a single sample. x-Plex assays are specially premixed multiplex assays that are quality tested at Bio-Rad and are in a convenient format for repeat sample testing. Catalog numbers for x-Plex assays in one or ten 96-well plate formats are available at www.bio-rad.com/bio-plex/x-plex/. Once the x-Plex assay catalog number has been identified, orders may be placed.

March 2008

All listed information was taken from the BioRad website, which is also a good place to obtain more detailed information:
http://www.bio-rad.com/B2B/BioRad/product/br_category.jsp?BV_SessionID=@@@@1382479410.1201282980@@@@&BV_EngineID=cccdadedefighjcfngcfkmdhkkdfm_0&divName=Life+Science+Research&loggedIn=false&serviceLevel=Lit+Request&lang=English&cse=HQ&catLevel=3&catOID=-24082&isPA=false&categoryPath=Catalogs%2fLife+Science+Research%2fMultiplex+Suspension+Array+System

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The CFAR Flow Cytometry Core is located on the sixth floor of the Lyons Harrison Research Building (LHRB); rooms 610A and 624.