Automated, Multiplexed Biological Analysis using Renewable Surface Separations and Bead Suspension Arrays

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In this presentation we describe a new method of automated sample preparation for multiplexed biological analysis systems that use flow cytometry fluorescence detection. In this approach, color-encoded microspheres derivatized to capture particular biomolecules are temporarily trapped in a renewable surface separation column to enable perfusion with sample and reagents prior to delivery to the detector. This method provides for separation of the biomolecules of interest from other sample matrix components as well as from labeling solutions. After sample preparation, the beads can be released from the renewable surface column and delivered to a flow cytometer for direct on-bead analysis one bead at a time. Using mixtures of color-encoded beads derivatized for various analytes yields suspension arrays for multiplexed analysis. Development of this approach required a new technique for automated capture and release of the color-encoded microspheres within a fluidic system. We developed a method for forming a renewable filter and demonstrate its use for capturing microspheres that are too small to be easily captured in previous flow cells for renewable separation columns. The renewable filter is created by first trapping larger beads in the flow cell, and then smaller beads are captured either within or on top of the bed of larger beads. Both the selective microspheres and filter bed are automatically emplaced and discarded for each sample. A renewable filter created with 19.9 μm beads was used to trap 5.6 μm optically encoded beads with trapping efficiencies of 99%. The larger beads forming the renewable filter did not interfere with the detection of color-encoded 5.6 μm beads by the flow cytometer fluorescence detector. The use of this method was demonstrated with model reactions for a variety of bioanalytical assay types including a one-step capture of a biotinylated label on Lumaviden beads, a two-step sandwich immunoassay, and a one-step DNA binding assay. A preliminary demonstration of multiplexed detection of two analytes using color-encoded beads was also demonstrated. The renewable filter for creating separation columns containing optically encoded beads provides a general platform for coupling renewable surface methods for sample preparation and analyte labeling with flow cytometry detectors for suspension array multiplexed analyses.

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