

Multiplexed Analysis of Beads and Cells With the ImageStream® Imaging Flow Cytometer

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BACKGROUND AND SUMMARY

The adoption of cytometric bead arrays (CBA) to simultaneously assess the concentrations of multiple cytokines in a single sample has been a significant technological advance in the field of cytokine biology. In this report we extend the use of CBA technology by using the ImageStream system to image cells in flow while at the same time measuring cytokine concentrations. Cytokine levels and cell classification can both be performed simultaneously using the ImageStream system.

The ImageStream system is operationally similar to a flow cytometer, but in addition it has the abil-

ity to capture six simultaneous multispectral images of each cell with a resolution similar to that seen through a fluorescent microscope. Each cell is represented by a brightfield image, a darkfield image and up to four different fluorescent images. The ImageStream can thus be used to provide quantitative information about the intensity of target molecules, their localization within the cell and statistically significant assessments of the morphologies of different cell populations. The combination of these capabilities offers a new level of integrated quantitative analysis – analysis both of the individual cell and of the cell population.

EXPERIMENT DESIGN AND RESULTS

For the purpose of multiplex analysis, a panel of cytokines was artificially added to the human peripheral blood sample. The cytokines included IL-2, IL-4, IL-6, IL-10, TNF α and IFN γ . In addition, cytokine capture beads and detection reagents arrays from a commercial CBA kit (Th1/Th2 CBA kit, BD Biosciences) were added to the sample. The blood was then stained with an anti-CD45 monoclonal antibody conjugated to the fluorochrome AlexaFluor® 488 (Invitrogen, Inc.), and red cells were lysed by a brief incubation in FACS lysing solution (BD Biosciences). After washing, DRAQ5, a DNA binding dye that can be excited with a 488 nm laser and emits in the red (BioStatus, Ltd, Leicestershire, UK) was added to the sample and analyzed on the ImageStream.

A bivariate dot plot (Figure 1) of CD45 fluorescent intensity (X-axis) versus darkfield intensity (Y-axis) reveals five distinct populations of cells. Since with ImageStream technology, each dot has an associated image, the identity and consistency of the populations can be determined. Examination of the imagery allows for the assignment of populations to the different clusters on the dot plot. This analysis allows for the identification of lymphocytes, neutrophils, monocytes, eosinophils and basophils. Representative composite images are shown from each population (Figure 2). Membrane staining in green represents CD45 and as expected, varies from population to population (lymphocytes are CD45 bright, neutrophils and basophils are CD45 dim).

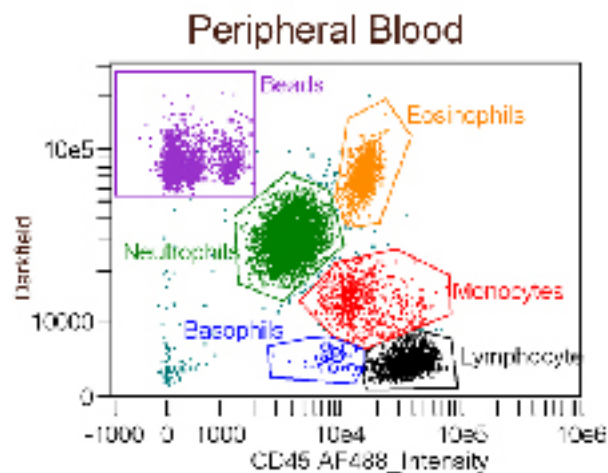


Figure 1. Bivariate plot of darkfield intensity (SSC) vs. CD45 intensity. Cytometric bead array analysis was performed in whole human peripheral blood. Blood was then stained with anti-CD45 mAb, red blood cells lysed, DRAQ5 added as a nuclear stain and cells run on the IS100. Beads from the CBA are in the upper left hand corner of the plot.

The cytometric bead array analysis kit used contains a set of 6 different beads each with a unique fluorescent intensity and each coated with a capture antibody specific for an individual cytokine. Quantitation of the cytokine concentration is achieved with a PE conjugated secondary antibody to the specific cytokine. In this experiment, samples of human peripheral blood were spiked with different concentrations of cytokines and processed as described above. A control sample not containing cytokines was also processed. After data acquisition on the ImageStream, the

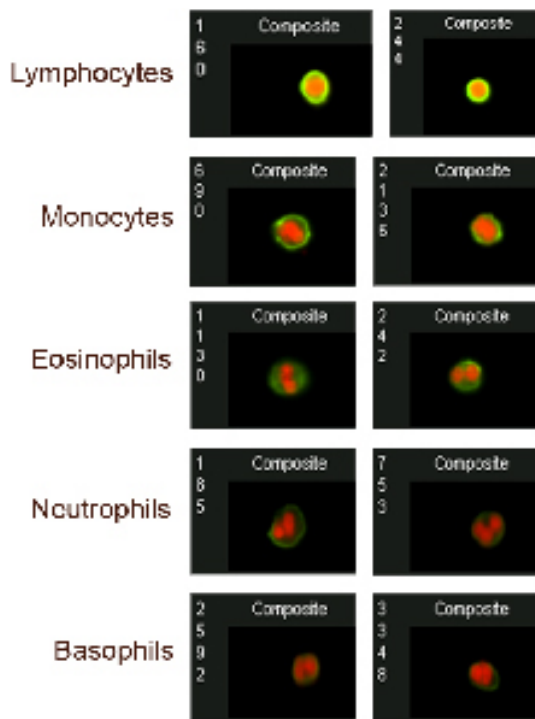


Figure 2. Representative composite imagery of peripheral blood leukocytes. Composite images were generated from the individual anti-CD45 and nuclear stain images. Imagery is utilized to determine gating and investigate cells not falling into traditional cell classifications.

events in the bead gate (Figure 1) were analyzed for the bead fluorescent intensity signature which is detected in channel 6 of the ImageStream and PE intensity which is detected in channel 4. Thus, the channel 6 bead intensity identifies the cytokine and the channel 4 intensity correlates with the cytokine concentration. The results of this analysis are shown in Figure 3. Bead separations comparable to those obtained on a conventional cytometer are clearly evident, demonstrating that the ImageStream was capable of resolving both the major populations of leukocytes as well as providing reliable and detailed data on cytokine concentrations as well. Comparison of standard curves of cytokine concentration between standard flow cytometers and the ImageStream demonstrated comparable sensitivity.

CONCLUSIONS

Multiplexed assays have great potential for providing significant advances in both research and medical diagnostics due to the increased information content obtained in less time and with less sample manipulation. Here we demonstrate the simultaneous assessment of serum cytokine levels and peripheral blood leukocyte differential classification from a blood sample using a single technology platform.

The instrumentation utilized was the ImageStream imaging cytometer, which has the capability to obtain brightfield and darkfield imagery plus four additional channels of fluorescent

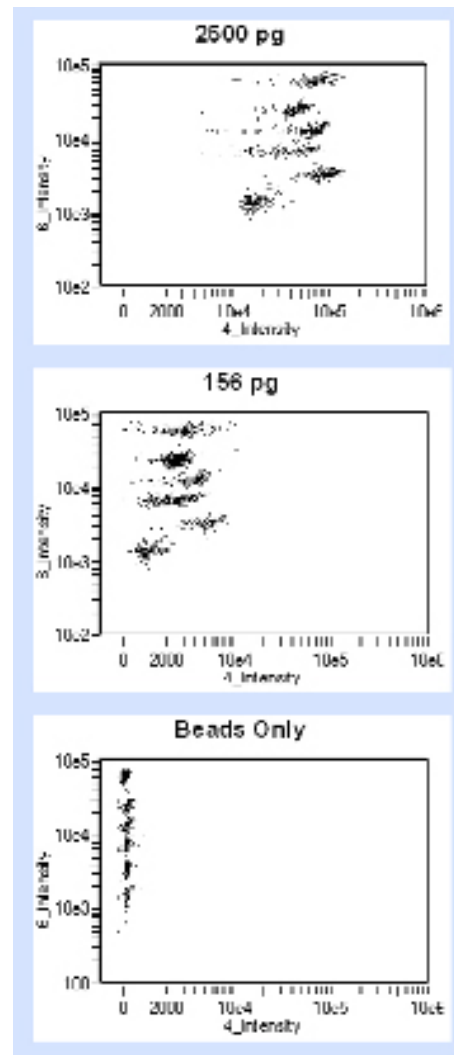


Figure 3 Cytometric Bead Array analysis of cytokines in whole blood. Cytokines were spiked into whole human blood and subject to analysis by a commercial CBA kit (Th1/Th2 CBA kit, BD Biosciences). The cytokines were IL-2, IL-4, IL-6, IL-10, TNF α and IFN γ . In the top 2 panels, cytokine concentrations of 2.5 ng/ml and 156 pg/ml were added to whole blood. Bead analysis and peripheral blood cell classification data were obtained from a single sample run on the ImageStream100 and analyzed with the IDEAS software package. The lower limit of sensitivity is in the 5 – 10 pg/ml range (data not shown).

imagery. The ImageStream was used to simultaneously image peripheral blood leukocytes and assess fluorescent intensity for the cytometric bead array assay. In an engineered experiment, cytokines were spiked into whole human peripheral blood and then subjected to a commercial CBA assay as well as staining with anti-CD45 and DRAQ5 as a nuclear stain. After data acquisition on the IS100, data analysis using the IDEAS data analysis package allowed the classification of human peripheral blood leukocyte subsets and assessment of the cytokine concentrations from a single analytical run. Thus, blood levels of 6 cytokines were obtained as well as a WBC differential in a single assay.