Introduction
The differential analysis of leukocytes in peripheral blood is traditionally performed either by manual microscopic analysis of a slide-based blood smear stained with Wright’s-Giemsa dyes or with automated clinical instruments that correlate cell volume and impedance (Coulter principle) with other measures such as light scatter and patterns of cellular staining.

Evaluations using automated clinical instruments can accurately analyze and report on many samples per hour. The principal limitation of the automated approach is its difficulty in handling samples with atypical profiles. Such samples are generally subjected to additional procedures, usually including manual slide based microscopic analysis. Difficulties associated with analysis of blood samples is not limited to automated systems. For example, problems associated with
variability in sample preparation, analytical subjectivity and the low numbers of cells that can be analyzed by visual means (which limits the identification and accurate quantitation of “rare events”) represent significant problems for standard methods of analysis. Finally, tracking and archiving glass slides used for the visual analysis is frequently a significant logistical problem.

The ImageStream 100 cell analysis system offers significant improvements over these standard procedures. Using cell morphology and a small number of reproducible stains, the ImageStream system can collect statistically significant data with increased reliability and less subjectivity. The capabilities of the system also allow extending the standard five-part differential analysis to include other cell physiological factors that may be of interest to the investigator.

**ImageStream technology**
The ImageStream 100 is operationally similar to a flow cytometer, simultaneously capturing multispectral images of each cell with a resolution similar to that seen through a fluorescent microscope. As many as six independent images (including brightfield, darkfield and up to four independent fluorescent images) may be collected from each cell at a rate of approximately 100 cells per second. The ImageStream 100 can thus be used to provide detailed morphological information about each individual cell as well as statistically significant assessments of sub-populations of cells. The combination of these capabilities allows a new level of integrated quantitative analysis – analysis both of the individual cell and of the cell population.

**Experimental Design and Results**
Human peripheral blood was stained with a FITC conjugated monoclonal antibody to CD45 and red cells lysed by a brief incubation in FACS lysing solution (Becton Dickinson). After washing, DRAQ5 (a red DNA binding dye; BioStatus, Ltd, Leicestershire, UK) was added to the sample. A 10,000 event file was then collected and analyzed on an ImageStream 100.

A bivariate dot plot (Figure 1) of CD45 fluorescence intensity (X-axis) versus darkfield intensity (Y-axis) reveals 5 distinct populations of cells. One of the unique features of the ImageStream technology is that each dot in the bivariate plot has an associated set of cellular images, and examination of the imagery facilitates the assignment of sub-populations of cells to 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. Membrane staining in green represents CD45 and, as expected, varies in intensity from one sub-population of cells to another (e.g., lymphocytes are CD45 bright, whereas neutrophils and basophils are CD45 dim). Thus, using the unique capabilities provided by this new class of instrumentation we were able to perform a 5-part differential classification of peripheral blood leukocytes, based solely on intensities of CD45 expression and light scatter.

**Conclusions**
The ImageStream 100 cell analysis system provides high resolution, high sensitivity image analysis of cells in flow. The classification presented here is based on analysis of a large number of cells (10,000) and any atypical events can be examined directly without additional procedures. This significantly reduces the subjectivity of the analysis while increasing reliability and reducing test to test variability. The ability to accumulate large data files of thousands of cells also allows greater statistical power when comparing differences between samples as well as discerning the validity of ‘rare events’.