



Analysis of the raw data files of an automated hematology analyzer (Abbott CELL-DYN Sapphire) using stand alone flow cytometry data analysis software (FCS Express)

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ABSTRACT

Flow Cytometry is an important technique in the field of Hematology with a broad applicability for diagnosing and monitoring a wide variety of disorders. Traditional automated hematology analyzers have typically not been designed to be capable of multi-color immunofluorescence analysis. However, CELL-DYN[®] analyzers (Abbott Diagnostics, Santa Clara CA) which use blue (488nm) lasers have this capability and the raw data output is in the form of a readable raw data file. Data generated from these analyzers includes up to 4 angles of light scatter data (0°, 7°, 90°, 90° depolarizing) as well as three colors of fluorescence data (FL1, FL2, FL3). The newest of these systems (CELL-DYN Sapphire) makes this data available to the user using the Flow Cytometry Data Standard (FCS). The structure of traditional FCS files is relatively simple with a header section containing typical operational details of the instrument (flow rates, duration of collection, dilution etc.) followed by the scatter and fluorescence data on an individual cell basis. The FCS file structure of the CELL-DYN is more complex as it contains a variable number of sequential cellular analyses, including the optical analysis of leukocytes, RBC and Platelets as well as impedance analysis of the RBC and platelets. Most commercial flow cytometry data analysis packages cannot accommodate the CELL-DYN file structure, thus negating the utility of having flow cytometry capabilities within the analyzer.

FCS Express (De Novo Software, Ontario, Canada) is a flow cytometry software analysis suite which has been designed to be simple and intuitive to use, as well as having a comprehensive feature set. Using FCS Express, users are able to draw gates and define regional statistics. They can view histograms, two dimensional scattergrams and take advantage of extensive batch processing functionality. FCS Express has been extensively modified to accommodate the CELL-DYN file structure and is now the first flow cytometry analysis software that will allow users to comprehensively interrogate the raw data of an automated hematology analyzer. Examples of both normal and ambiguous blood analysis will be shown as well as an immuno-fluorescent assay performed using the CELL-DYN Sapphire. It is anticipated that users of the system will use the instrument capability for their own research and development. Analysis of raw data output from the instrument will allow users to confirm automated gate settings and proportionate counts associated with the automated differential and some of the immuno-fluorescent analyses performed by the instrument.

Introduction

Hematology analyzers rely on proprietary automated data analysis using software algorithms for data reduction and generation of blood count data. The process by which cell populations are classified and flagged for morphological analysis has essentially been closed to the instrument user. For the purposes of the majority of blood counting applications, this data has proven to be adequate. However, this approach of keeping the raw data closed to the user has limited the ability to scrutinize the analyzed data beyond review of instrument generated scatterplots and numerical data. Abbott Laboratories have included an option on their new CELL-DYN Sapphire analyzer to store the raw blood count data in Flow Cytometry Standard (FCS) files. The ability to get access to the instrument raw data could have a variety of interesting and novel applications.

CELL-DYN Sapphire Hematology Analyzer

The CELL-DYN Sapphire is essentially a fluorescence flow cytometer that has been developed for use as a routine hematology analyzer. The system uses a blue (488nm) diode laser as a light source. Data generated from these analyzers includes up to 4 angles of light scatter data collection (0°, 7°, 90°, 90°depolarizing) as well as three colors of fluorescence data (FL1, FL2, FL3). The different modes of analyzer operation and the data collected are shown in Table 1.

Table 1. Modes of operation and analysis as well as optical/ fluorescence data collected

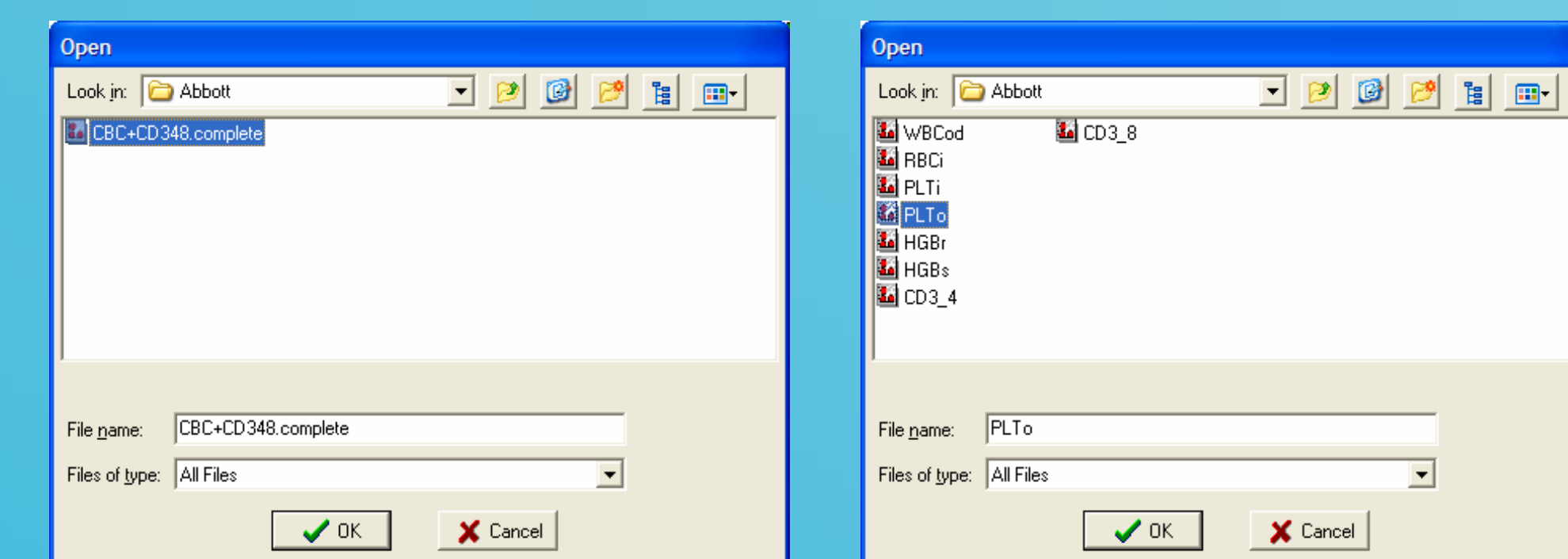
CD-Sapphire Procedure	Measurements Recorded
Routine blood count/WBC	Optical: 0°, 7°, 90°, 90°D
NRBC analysis	Fluorescent: FL3
Routine blood count/platelet analysis	Optical: 7°, 90°
Routine blood count/reticulocyte analysis	Optical: 7°
Automated CD4/CD8 Mode	Optical: 0° and 7° Fluorescent: FL1, FL2 & FL3
Automated CD61 Mode	Optical: 7° and 90°P Fluorescent: FL1
Manual SRP-CD3/4/8 Mode	Optical: 0° and 7° Fluorescent: FL1, FL2 & FL3
Manual SRP-Ret Mode	Optical: 7° Fluorescent: FL1

The raw data is then automatically analysed and used for enumeration of individual blood components including WBCs (differential and viability information) NRBCs, RBCs, Reticulocytes and Platelets. Additional automated immunofluorescence analyses are available for T lymphocyte subset (CD3/4/8) categorization as well as immunoplatelet (CD 61) counting. Supplementary opportunities for generation of raw data also exist in the standard reference particle (SRP) mode. This mode enables processing of reference particles by engineers for preliminary setup of the analyzer (Table 1). The data extraction process is easy and menu driven from within the CELL-DYN Sapphire software. The raw data is saved in the FCS format and output to DVD-RW. Scatter and fluorescence data is stored in 15 and 8 bit resolution, respectively. The raw data is stored as a multi-part FCS file, where each part consists of a individual component of the analyzed data (WBC analysis, RBC analysis, Platelet analysis etc.). The data can then be transferred to an IBM compatible PC.

FCS Express Software

FCS Express is a stand alone software application that has been developed as a flexible, comprehensive tool for analysis of flow cytometry data. The software includes the ability to open simple flow cytometry standard (FCS) files as well as those with complex file structures such as those generated by the CELL-DYN @ Sapphire which can include a series of individual data strings that comprise the blood count. When opening the CELL-DYN list mode data files, the individual components of the blood count are opened within the standard Windows® file dialog, as if they were standard separate files. Each part is appropriately named for easy recognition and selection (Figure 1b).

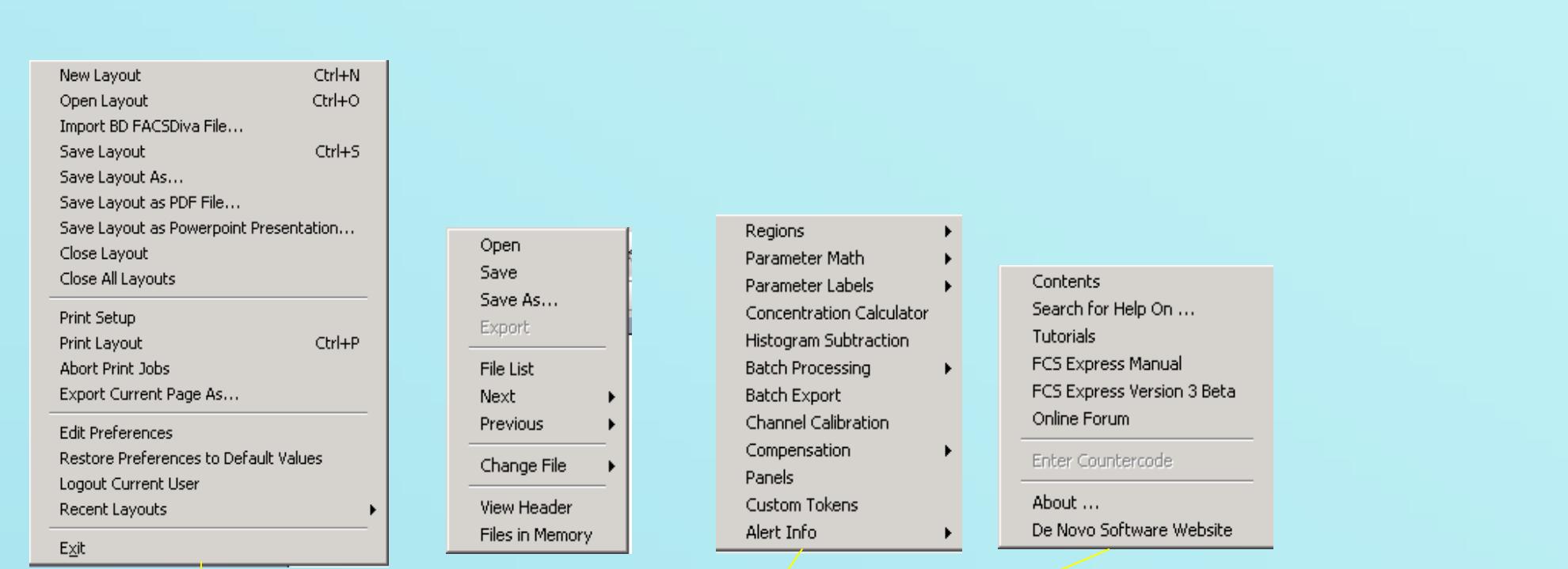
Figure 1. Standard Windows file dialogs modified for use with multi-part FCS files.



a. Standard windows file dialog displaying the actual file on disk created by the CELL-DYN @ Sapphire.
b. Modified dialog displaying the different parts of the CBC+CD348.complete data file as if each of the parts was an individual file.

FCS Express has been developed to be intuitive in use and the many functions are principally driven by an icon accessed toolbar (Figure 2). These functions include, histogram plotting, two dimension scatter plots as well as contour and density plots. Gating can be performed using quadrants, region selection tools (elliptical, rectangular, polygon and free form). Region statistics of gated cells are generated in an editable tabular form. Additional mathematical function tools are included that serve a variety of functions including final concentration calculation. The software can also perform fluorescence compensation of data in which fluorescence spillover from other fluorochromes in the experiment causes interference in the channel of interest. All of these tools can be set up for use in a batch processing function and the output can be in a variety of forms including Microsoft Powerpoint®, Adobe PDF® documents and report forms generated in Microsoft word.

Figure 2. FCS Express main menu system highlighting a wide variety of analysis options



Results

Figure 3 illustrates multi-dimensional analysis of optical/ fluorescence leukocyte data derived from a CELL-DYN® instrument. The figure illustrates the use of polygon gating of color dot plots as well as a density plot of the intermediate angle scatter data (7° or IAS) and axial light loss (0° or ALL) data. The user can adjust the regions within FCS Express, and obtain statistics on the new region shapes. In addition, the concentration (in cells/µL) of the cells in each region was automatically calculated by FCS Express using the total WBC concentration obtained from the CELL-DYN® instrument as a reference.

Figure 3. Multi-dimensional analysis of optical/ fluorescence leukocyte data derived from a CELL-DYN® instrument. Cells are color coded by cell type (region).

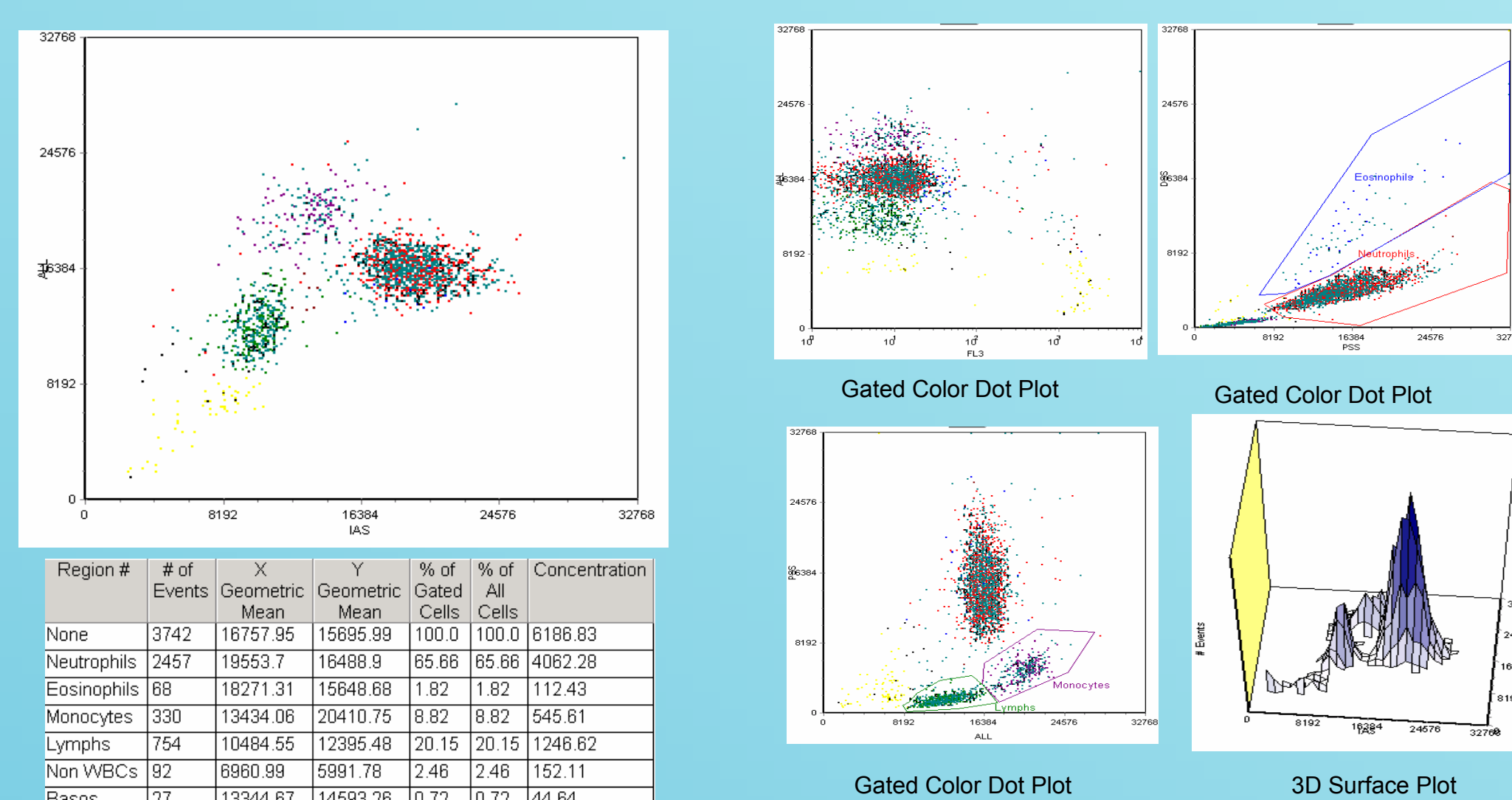


Figure 4. Multi-dimensional analysis of optical platelet data (7° or IAS vs 90° or PSS). These include gated scatter plots as well as density and contour plots.

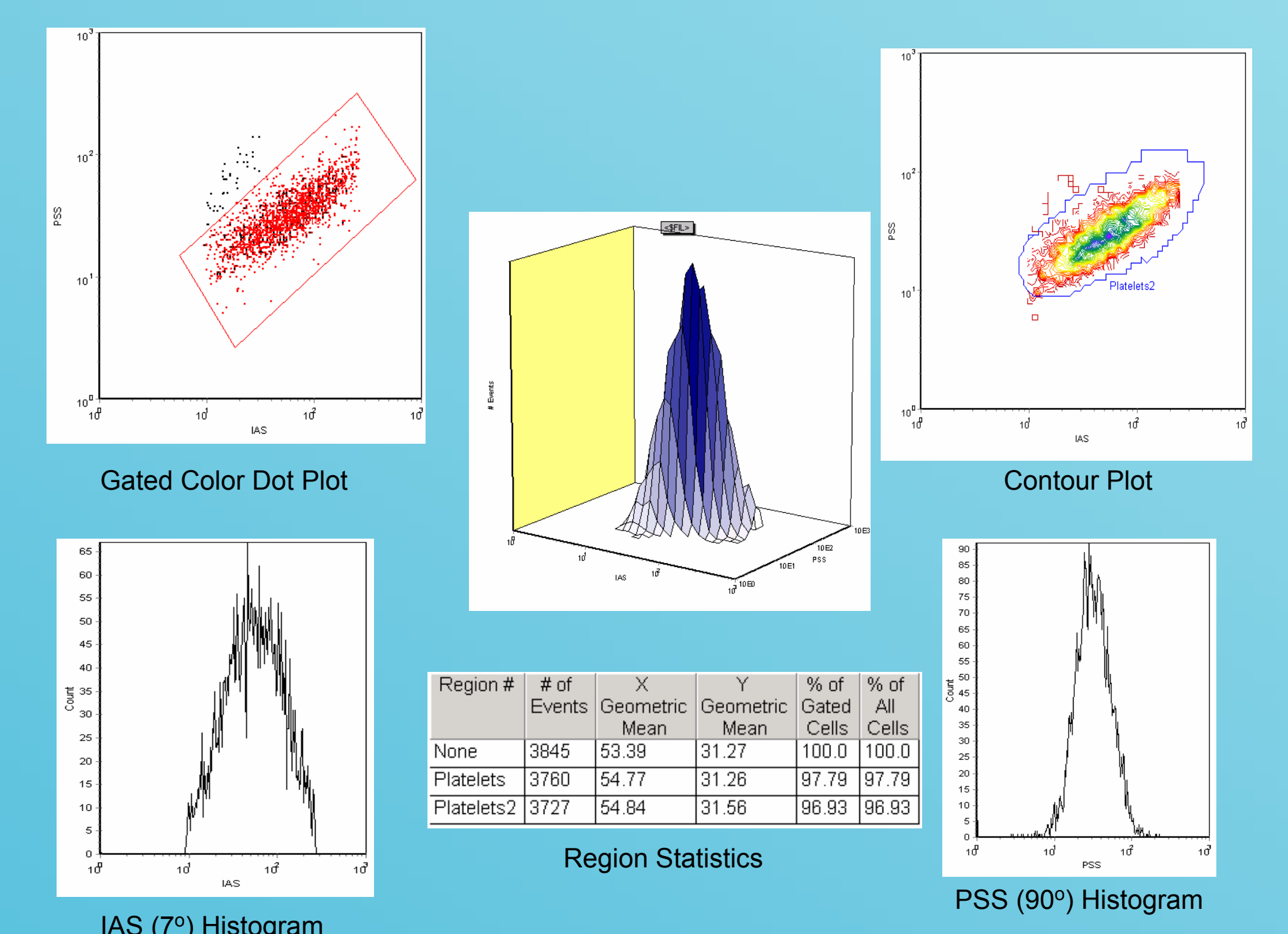
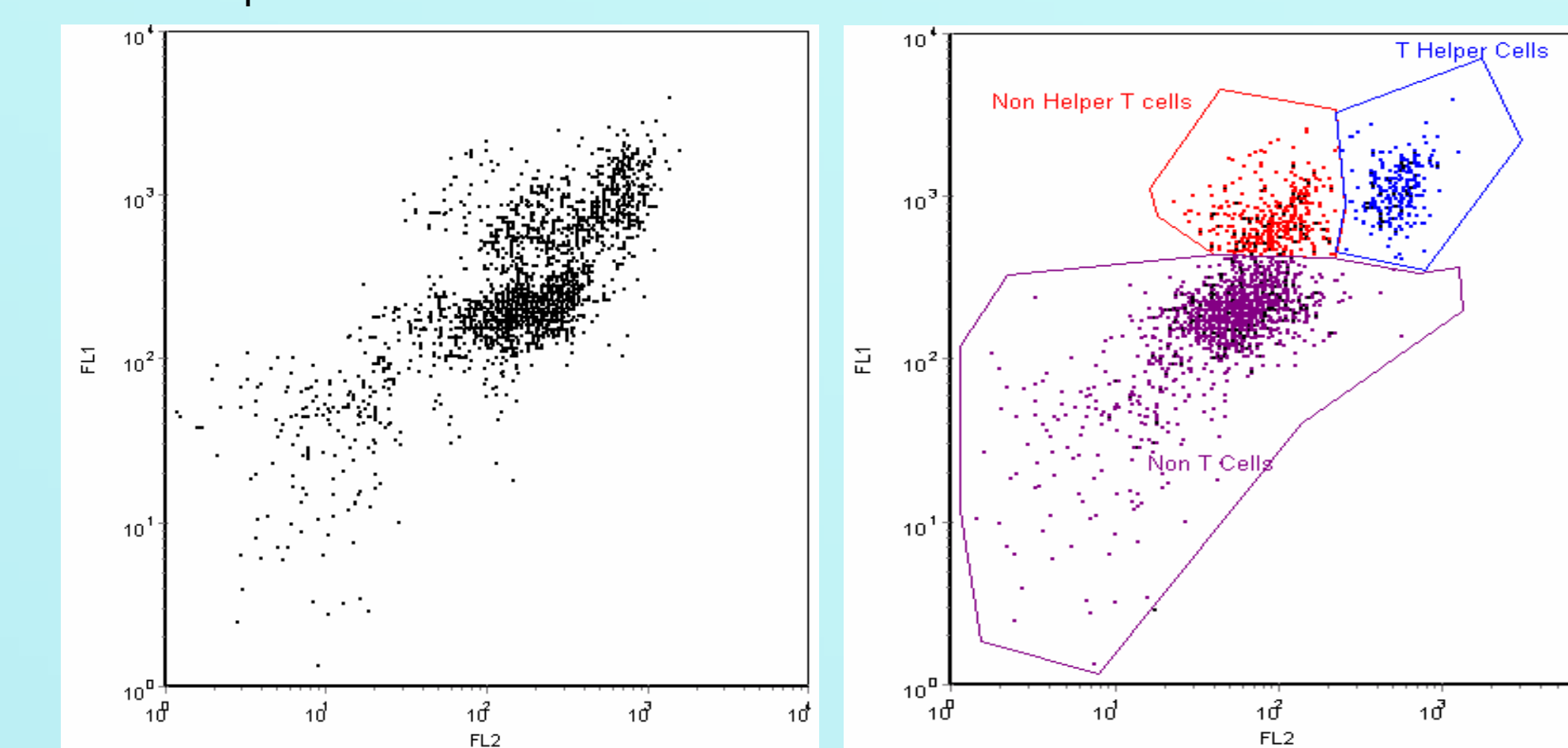


Figure 5 shows two dot plots from a CD3/4/8 analysis of a commercial quality control material. FITC labeled anti-CD3 is detected using FL1, whereas the phycoerythrin labeled anti-CD4 is detected in the FL2 channel. In the first plot, the CD3+4- cells are indistinguishable from the CD3+4+ cells due to red fluorescence from propidium iodide (primarily detected in FL3) spilling into the FL2 channel. The ability to perform compensation (FL3 on FL2) has permitted separation of the two cell clusters.

Figure 5. Applying compensation in FCS Express can resolve populations in spite of excessive spillover from other fluorochromes.

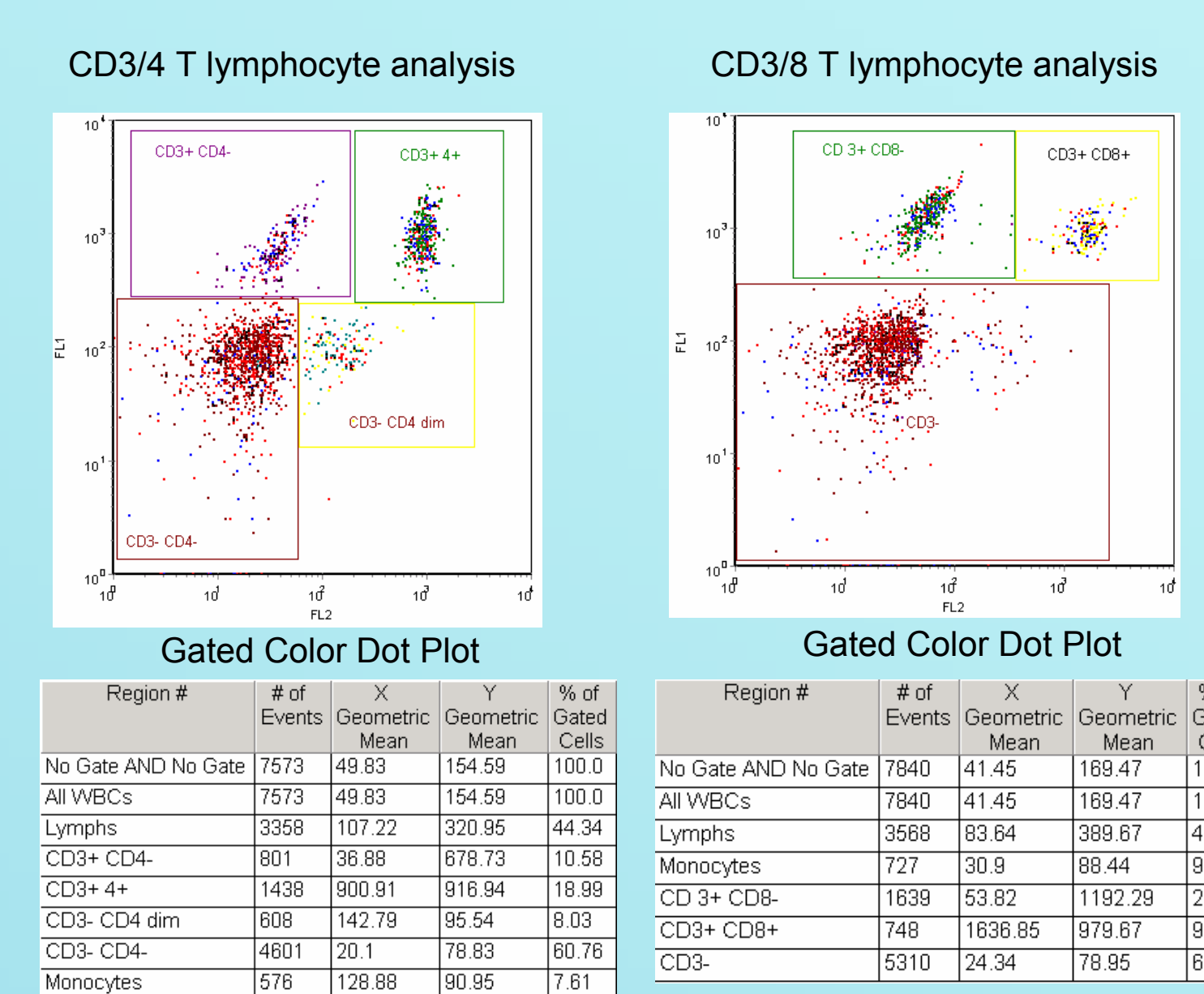


a. CD3 (FL1) vs CD4 (FL2) are indistinguishable due to spillover from PI.

b. After applying software compensation, the populations are easily resolvable.

Gated color dot plots from a CD3/4 and CD3/8 analysis of patient blood is shown in figure 6. The region statistics includes the percentage of T- helper and suppressor cells, as well as lymphocytes and monocytes

Figure 6. CD3/4 and CD3/8 T lymphocyte analysis



Conclusions

FCS Express provides users, for the first time, the ability to analyze the raw data from a commercial hematology analyzer. The ability to conveniently access and interpret the flow cytometric data output of a hematology analyzer is new and unique opportunity. FCS Express provides several key advantages over the standard software supplied with the CELL-DYN® instrument.

- 1) Users can create unlimited numbers of arbitrarily shaped gates.
- 2) Users can get visualize all dimensional representations of data.
- 3) A wider variety of plot types are available. Each plot type can be used with any of the dimensions of data.
- 4) Users can obtain any statistics they wish on the data.
- 5) There may be situations where the instrument cannot successfully gate populations but a human could. This allows users an understanding of which types of samples are more or less appropriate for the CELL-DYN® instrument.

Users of the instrument who are interested in interrogating the automatically generated results can now do so. For research and method development activities, the instrument user will no longer be limited to the instrument generated blood count parameters and flagging information. Instead they will be able to perform quantitative analysis of fluorescent and optical scatter data produced after processing of each of the blood elements. Excitingly, this opportunity also raises the possibility of developing some additional immunofluorescent methodologies which are applicable to routine hematological analysis