© 1991 Wiley-Liss, Inc. Cytometry 12:82–90 (1991)

An Innovation in Flow Cytometry Data Collection and Analysis Producing a Correlated Multiple Sample Analysis in a Single File[1]

J. Paul Robinson,[2] Gary Durack, and Steve Kelley
Purdue University Cytometry Laboratories and Department of Veterinary Physiology and Pharmacology, Purdue University, West Lafayette, Indiana 47907

Received for publication April 17, 1990; accepted August 26, 1990

The problems associated with rapid analysis and interpretation of data from multicolor immunofluorescence panels have been a formidable barrier to their routine use. Using present flow cytometry concepts, a panel of 11 tubes each containing multiple phenotypic markers or controls requires postdata acquisition manipulation of many multiparameter histogram and listmode files. We have developed a method that compresses all of the information from such a panel into a single listmode data file during run time. A single data file is used to record the entire phenotypic analysis for a particular patient or series within an experiment. This is accomplished by the incorporation of a *tube identifier parameter* (TIP) as well as the fluorescence and light scatter parameters normally collected. The TIP can then be used for gating discrimination of any tube or set of tubes within a panel. When the TIP is correlated with the PRISM parameter the entire patient phenotypic image can be represented within a single two-parameter histogram we have called a *phenogram.* This phenogram can be generated in real time, providing on-line preprocessing of a complex multicolor experiment. By examining the image created by the phenogram it is possible to rapidly flag abnormalities such as incorrect gating. This procedure was carried out on an EPICS Elite flow cytometer in its standard configuration with the addition of hardware to provide an input for the TIP.

Key terms: Multiparametric analysis, tube identifier parameter, pattern recognition, data analysis, automated immunophenotyping, phenogram

The collection and analysis of flow cytometry data have become a routine procedure; however, attempts to make these analyses efficient have been less successful. Historically, flow cytometry involved analysis of single parameter histograms showing fluorescence intensity. It is standard procedure in clinical laboratories to use panels of phenotypic markers in single or dual color whereby results are obtained by analyzing and printing these histograms individually or as coplots on the same page. It is increasingly more difficult to present multicolor data in a useful and understandable form. This has been termed the "multiparameter problem" (1), and, with a few exceptions (7), solutions have not been forthcoming. Attempts have been made by commercial manufacturers to provide software that addresses this question [examples are Lysis (Becton-Dickinson Immunocytometry Systems), Gateway (Coulter Cytometry), multi-2D (Phoenix Software), and IsoContour (Verity Software House)]. Recent publications describing state-of-the-art analytical methods include simple two and three-parameter graphical and analytical solutions but fail to demonstrate effective methods for routine comparative analysis of data or data management (10)

The problems of data management, data analysis, interpretation, and report generation have become more significant with the increase in usage of cytome-

[1]This work was supported by NIH grants GM38877, ES04911, and CA23168. Additional instrumentation support was provided by Coulter Cytometry. Parts of this study were presented at the International Society for Cytology Meeting in Asheville NC, March 18–22, 1990.

[2]Address reprint requests to J. Paul Robinson, Purdue University Cytometry Laboratories, Lynn Hall, West Lafayette, IN 47907.

try. Some laboratories have developed complex procedures requiring additional personnel and computer systems to address these problems. We propose a simple procedure, adaptable to most commercial flow cytometers, that dramatically simplifies data analysis and management. With this procedure, an entire multicolor phenotypic analysis panel can be compressed into a single data file as the tubes are run on the flow cytometer.

MATERIALS AND METHODS

Hardware

All flow cytometric data were collected on an EPICS Elite flow cytometer (Coulter Cytometry, Hialeah, FL) using instrument standard computer, optics, and electronics. The Elite was configured with three lasers: an air-cooled 15 mW argon (Cyonics Model 2201, San Jose, CA), an air-cooled 12 mW HeNe (Uniphase Model 106-1, Mantica, CA), and a water-cooled argon-ion Coherent Innova 90-5 laser. The water-cooled laser was not used in the present study. The computer system on the Elite consisted of an Intel 16 MHz 80386 computer containing 4 megabytes of random access memory (RAM). Software used for this study was the standard Elite cytometry software. This instrument was capable of correlating six cell measurements, the ratio of any two of the six cell measurements, elapsed time, and PRISM as parameters. Printouts were made on a Hewlett-Packard Laserjet printer.

Tube Identifier Hardware

The only modification of the instrument was the addition of a DC voltage source. This voltage source could be varied in discrete increments of 500 mV over a range of 0–10 V. The output of the voltage source was connected to the input of one of the ADC channels on the instrument. The DC voltage level was successively incremented for each tube in the panel. This provided a distinct voltage reference for each tube, which was recorded by the ADC and correlated with all events from that tube. This reference level became an additional parameter in the listmode file. The parameter created by this reference voltage is the *tube identifier parameter* (TIP). The TIP was then used as a gating parameter to discriminate any desired set of tubes in the panel. Listmode files containing a TIP were referred to as TIP files.

Immunophenotyping

Studies were carried out on blood obtained from normal human volunteers. Ten milliliters of heparinized blood was taken and 100 μl was pipetted into each of several tubes. Five microliters of the appropriate monoclonal antibodies or controls were added to the tubes and each tube processed on a Q-Prep (Coulter Cytometry). After preparation, tubes were run on the cytometer as a set.

Table 1
Marker Combinations Selected for a Two-Color Phenotypic Analysis of Human Blood[a]

Tube No.	Letter	Markers
1	**A**	**MsIgM-PE/MsIgG$_1$-FITC**
2	B	CD14-PE/CD45-FITC
3	**C**	**MsIgG$_1$-PE/MsIgG$_1$-FITC**
4	D	CD4-PE/CD8-FITC
5	E	CD56-PE/CD8-FITC
6	F	CD45R-PE/CD4-FITC
7	I	CD29-PE/CD4-FITC
8	J	CD2-PE/CD19-FITC
9	**K**	**MsIgG$_1$-PE/MsIgG$_{2a}$-FITC**
10	L	CD3-PE/I3-FITC
11	M	CD2-PE/CD20-FITC

[a]In boldface are the isotypic controls and following the appropriate markers. The tube letter associated with each row becomes synonymous with the spike letter on the tube identifier parameter. Any combination of one, two, three, or four color marker combinations can be handled using this system.

RESULTS

Data Analysis Solution

To demonstrate the procedure, a panel of leukocyte markers conjugated to multiple fluorescent probes was run on the flow cytometer. In the example, 11 tubes were used to measure 12 different markers (tubes B, D–J, L, M) and three isotypic controls (tubes A, C, K), as is shown in Table 1.

Using classical methods, it would be necessary to save histogram data files, listmode data files, or a combination of both for each tube (assuming it was necessary to save any data at all). These files would be retrieved and manipulated by analysis software to obtain the appropriate results for each tube. These results would then be correlated as a data set to produce a report for the panel. This entire process could involve manipulation of 10–20 separate data files and take a considerable amount of time. The TIP procedure reduces these tasks to rapid analysis of a single data file.

Table 2 shows the parameters acquired in a two-color panel. Before samples were run, appropriate quality control and instrument alignment, including setting color compensation correction, were performed. The tubes in the panel were run in the order shown in Table 1. The reference voltage for the TIP was set at 500 mV, and 5,000 lymphocytes were collected from the first tube. Acquisition was paused, the reference voltage stepped up to 1 V, and acquisition continued for the second tube collecting 5,000 lymphocytes. The process of incrementing the tube identifier voltage and sampling a new tube was repeated for each of the tubes in the panel. As each tube was placed onto the instrument, the TIP tube number was displayed on a diode for reference. Appropriate PRISM and quadstat analysis boundaries were set after running each control. Real-time acquisition was monitored using the oscilloscope and graphics displays on the cytometer. After the last tube from the panel was run, the standard phenotypic analysis for percent positive for each marker com-

Table 2
Parameters Collected for All Samples[a]

No.	Parameters collected
1	FALS
2	90° Light scatter
3	FITC
4	PE
5	Not used
6	Tube identifier parameter
7	PRISM
8	Time

[a]Parameter 5 would normally be used for APC if three colors were collected. Parameter 6 is the tube identifier parameter, which is generated using a voltage source. PRISM and time are also collected on the Elite. If four colors were required, the sixth parameter would be used for this color, and the tube identifier parameter would be sent through the ratio circuitry of the Elite, allowing collection of nine parameters overall.

bination was complete, and the results were printed out, together with any combinations of histograms required. At this point the single listmode data file, which was in RAM memory, was then archived to disk. This file contained all the multiparameter data for the complete phenotypic panel. The instrument was then available for the panel from the next patient. The complete panel, including flow cytometry, printing of results, data storage, and final report generation, required less than 15 min to complete.

Analysis of the Files

The instrument is controlled by the EPICS Elite work station software version 2.0. Analysis in these experiments is accomplished during real-time acquisition; in addition, the software is capable of identical off-line analysis of TIP files collected from other flow cytometers. To demonstrate the versatility of the TIP concept, four examples of analysis options are shown in Figure 1, which portrays the options described in detail in the following sections. Any one of these options could produce the standard phenotypic results from the panel. All these options are available for presentation immediately after the last tube is run on the cytometer.

Option 1. Figure 2 shows the classical approach to multicolor immunofluorescence analysis, which represents most combinations of antigenic markers using two parameter dot plots. The traditional light scatter histogram is also shown but in this case represents the composite of all the tubes in the panel. Each tube is gated using the gating regions A–K. The spikes are separated by 500 mV, but any reasonable separation distance can be used. Each spike in the single parameter TIP histogram represents an individual tube in the panel. Tubes are incremented from left to right on this histogram. Each of the 11 dual-parameter fluorescence histograms shown in Figure 2 represents an individual tube from the panel. The fluorescence histograms were derived by gating on the TIP spike and the appropriate light scatter population (in this case lymphocytes). The software allows simultaneous location of separate quadstat regions on each of the fluorescence histograms. Figure 2 is a composite of two screens from the Elite software, which is limited to eight simultaneously displayed histograms. This presentation is a composite quadstat, since all the histograms are generated at once.

Option 2. The second stage of analysis is to reduce the complex multiparametric array within the large listmode file into a more concise form. Figure 3 is a representation of all the information in Figure 2 but in a single histogram. This becomes a "picture" or pattern for the entire set of tubes in the assay. We have termed this display the *phenogram*. This was accomplished using the PRISM parameter on the instrument. PRISM reduces the information from a multicolor tube to a quantitative bar chart that represents all possible combinations of positive and negative marker conditions. In this case, since only two markers were used in each tube, there are four PRISM bars for each tube. The dual-parameter histogram shows the correlation of the PRISM and TIP parameters. This two-parameter array contains the same information as all 11 dual-parameter fluorescence histograms from Figure 2.

Each PRISM vs. TIP element can be related to a specific quadrant from the quadstat analysis shown in Figure 2. Phenotypic results for the entire panel can be obtained by measuring the total number of events in each PRISM vs. TIP element. This method could extend to three, four, or even five markers per tube by adjusting the number of PRISM combinations to eight, 16, and 32, respectively. Since the X,Y channel intercepts for each PRISM vs. TIP element are determined by the TIP procedure and the PRISM electronics, the position will always remain constant for a given panel of markers. With the TIP procedure, automated analysis using pattern recognition techniques becomes much more feasible. Specific recognizable patterns can be defined using this system, providing a rapid means of complex data reduction. Because this two-parameter histogram is created in real time, phenotypic information from the entire panel can be viewed immediately upon removing the last tube from the flow cytometer. This summary histogram can be saved or easily recreated from the TIP listmode data. The size of this file is very small (9,000 bytes), yet it contains the quadstat information for each marker in the entire panel.

Option 3. An alternative option with the TIP procedure can produce the display shown in Figure 4, which we have termed the *overview* or *boilerplate*. In Figure 4, each probe is shown independently in the two dual-parameter fluorescence histograms. All PE histograms are shown on the left box, and all FITC histograms are shown in the right box. Total positives for each marker were determined from the total count in each boxed region. The left side of each box corresponds to the location of both the quadstat and PRISM bound-

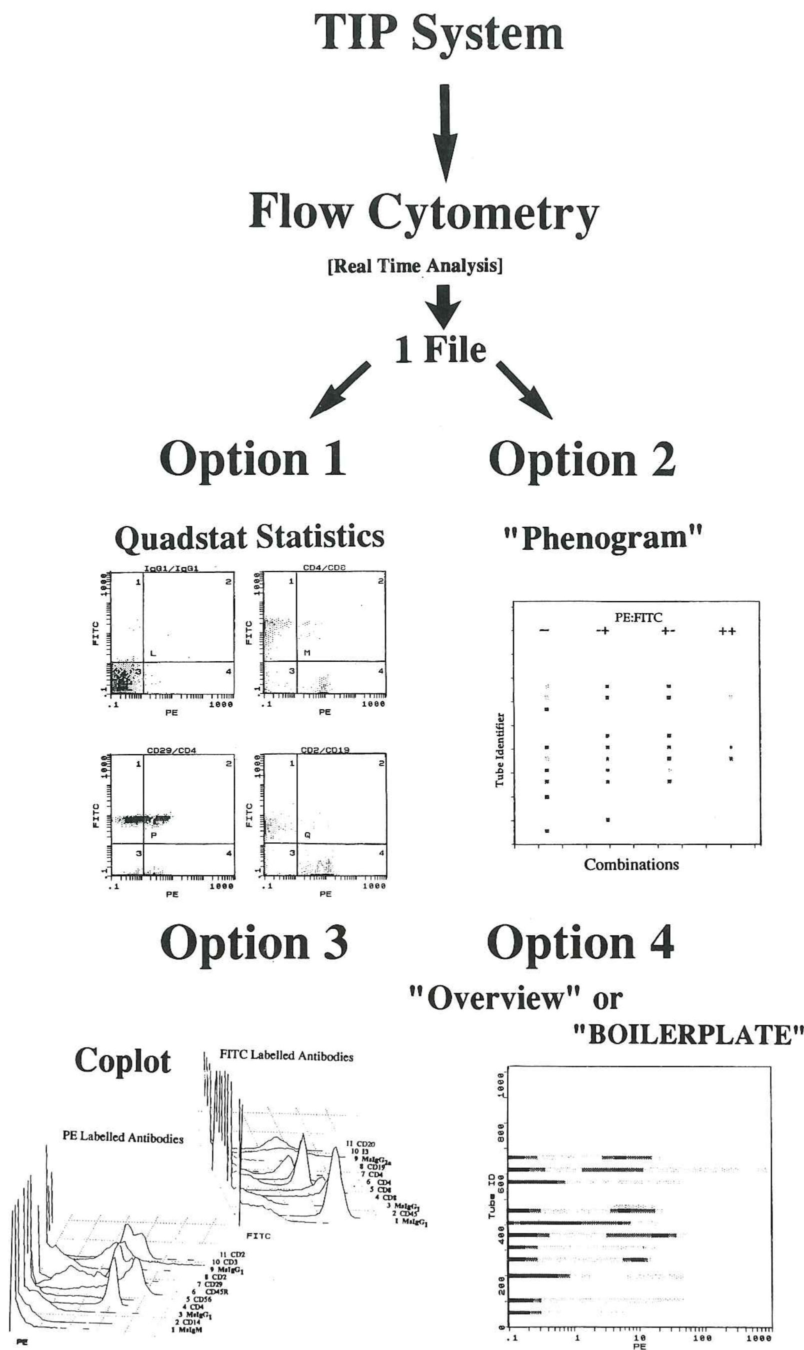

FIG. 1. Versatility of the tube identifier parameter (TIP) system for flow cytometry. Each tube is collected on the flow cytometer as previously; however, a single listmode file is created by pausing acquisition software between each tube. A special parameter, the TIP is inserted as each tube is run, allowing individual identification of the tubes later. Shown are four of the many possible analytical options available using the TIP system. Option 1 shows the classical two-parameter dot plots from which quadstat statistics are calculated. Option 2 demonstrates a new concept, the phenogram, which provides a multiparametric presentation in the form of a "pattern" display. Option 3 shows a more traditional coplot. Since all the tubes in the assay are in a single file, it is trivial to plot out the histogram. Option 4 shows another new display option we have termed the "overview" or "boilerplate" display, which provides a more analytical display than coplot.

aries in Figures 2 and 3, respectively. This is a useful display of the data in that it allows quick viewing of the entire panel. It is also possible that with experience an observer will collect quantitative and qualitative information from this display more rapidly than with traditional displays. Figure 5 is a *coplot* display of the

same two histograms shown in Figure 4. This provides the familiar summary display of the entire panel routinely used to *overview* histograms. Table 3 shows a comparison of the statistical analysis results obtained from the three examples by comparing single-parameter, quadstat, and PRISM statistics from the same data set.

DISCUSSION

Many flow cytometric assays involve panels, batches or groups of samples that require intergroup comparison. The TIP concept departs from traditional methods in that it establishes the group relationship on the instrument at run time. The concept of multiple samples in a single listmode file is simple, but its effect is significant. The TIP concept reduces both the time and the effort required to produce multicolor phenotypic analysis results. Rapid production of accurate results after tubes are run on the cytometer would be an ideal situation. Experiences from many facilities demonstrate that complex analysis of data, such as immunophenotyping, requires significant analysis time.

The proposed system has benefits that extend beyond immunophenotyping. Data management is simplified by reducing the number of files stored or archived by a factor of 10–20 in most cases. If histogram data are saved rather than listmode files, this number may be as high as 20–30 files per patient. Using TIP, when a particular experiment or patient panel needs to be retrieved from the archive storage, a single file is all that is required. Furthermore, if a histogram from a complex experiment is required, it is a trivial task to extract the information and plot the data. This is achieved from within a single analysis program analyzing a "single" if somewhat large listmode file. It takes less than 30 sec to perform any of the analyses described herein. Additionally, the size of the listmode file is directly related to the available RAM memory. Increasing the RAM memory to 6 or even 8 megabytes would allow collection of a significantly greater number of tubes and cells than in the present study.

For functional assays that are regularly run in this laboratory, 60–90 tubes are often collected in a single listmode file, allowing correlatable information to be accumulated over the entire set of tubes. The listmode file is significantly larger than the histogram files and can be as large as 2 or 3 megabytes. However, management of a single 2 megabyte file is considerably less complex than management of 60–90 smaller ones and the additional information derived is considerable (Robinson et al., submitted).

We have used the PRISM components on EPICS cytometers to achieve our objectives. Although this is a convenient technique, other methods can be employed to achieve similar results on other instruments. While this may be perceived as a disadvantage, the PRISM concept could be easily programmed into software, achieving the same utility. This would also allow solution of another potential problem with the techniques, the orthogonal boundaries between clusters. Although it may appear to be easier to place orthogonal boundaries, arguments exist showing that it is both simple and more useful to place them according to population (5). If a PRISM concept were developed in software, both of these potential problems would be solved. Additionally, there are potential difficulties in subjective boundary placement for PRISM values. Further consideration must be given to solutions to this, such as using an automatic calculation based on a control tube. Similar problems arise in attempts to set automatic color correction. Alteration of the PRISM boundaries can be made at appropriate times during the data collections if necessary.

The incorporation of a unique TIP as a parameter attached to information collected with each cell is a significant change from accepted flow cytometry practice. We initially used a voltage source to place a specific voltage level representing each tube. We are pursuing development of a bar code system that will identify each tube from the bar code printed on the label. This code will represent a binary number, which will carry with it unique information about that tube, eliminating the possibility of errors of identification when running or analyzing data. Additional uses could be made of this concept with other data, such as time, sample temperature, or other parameters, automatically encoded into the file, as has been proposed by others (3,8). Watson (9) has also proposed routine collection of time with data as a quality control parameter, and strong support has come from other quarters (5). Steinkamp (6) has used time as a parameter for gating changes in fluorescence intensity, as we have previously (4).

The production of the phenogram as an electronic file leads to possibilities for pattern recognition algorithms for diagnostic purposes. Using expert systems to analyze flow cytometry data is attractive. One approach has been described in the experimental design area for flow cytometry assays, but this failed to address the analysis question (2). Presently, the ability to display such complex information requires analysis of many individual files, running several different programs and consuming a tremendous amount of time. With the TIP system, appropriate data are already correlated within the file. The time taken for analysis of each listmode file is small. For an 11 tube assay where 5,000 lymphocytes are collected for each tube (total of 185,000 events), 40–50 s are required to provide each of the three major reports generated for this paper, complete with all statistics and printouts. Archiving the 2 megabyte files takes 18–20 s using a Novell network. Saving relatively large listmode files for routine clinical analysis is an extension of present practices of listmode acquisition. These are significantly larger files than most cytometrists are comfortable with. However, we have demonstrated that management of these large files is far simpler than handling vast numbers of smaller listmode or histogram files. Well de-

COMPOSITE QUADSTATS

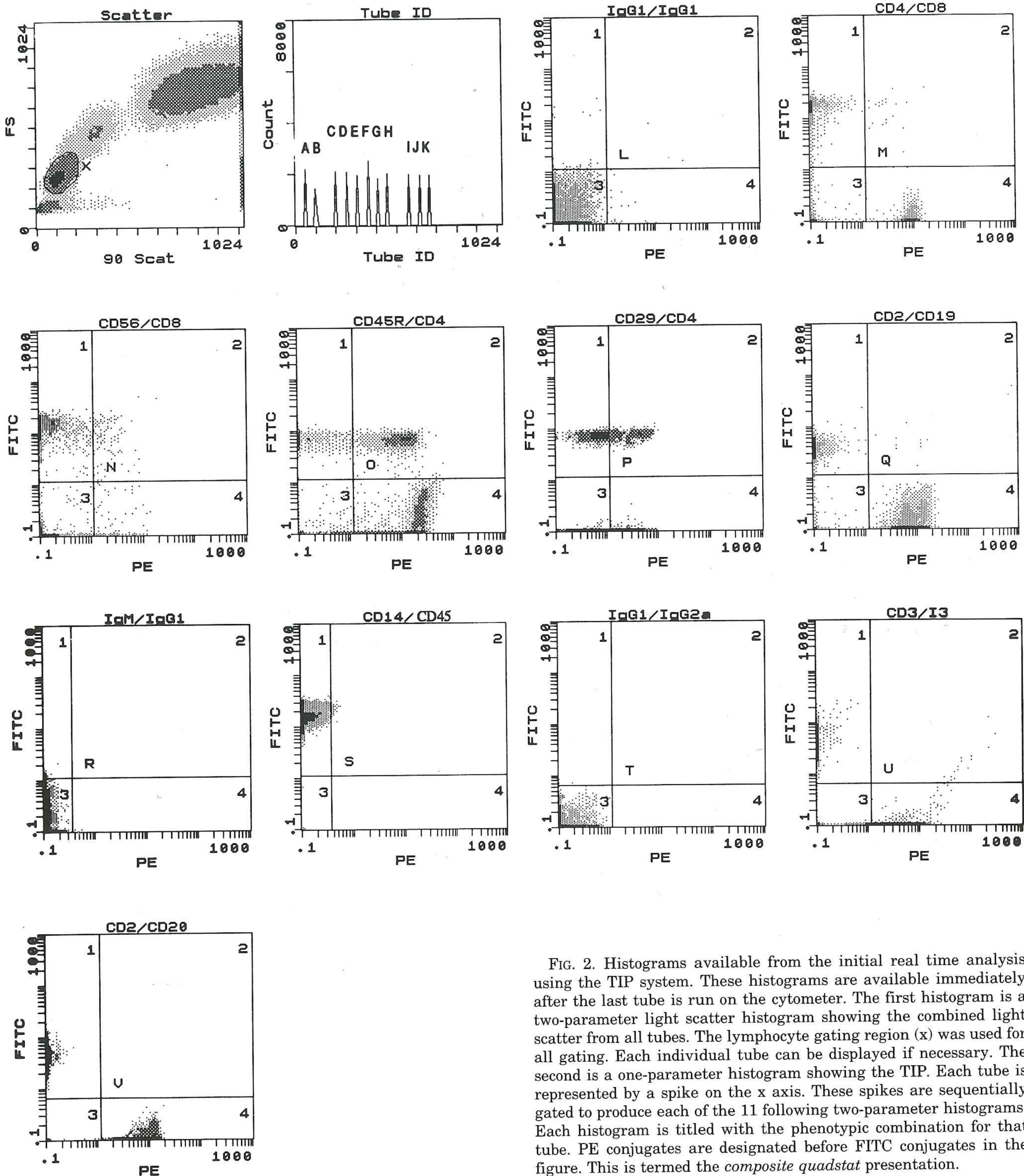

FIG. 2. Histograms available from the initial real time analysis using the TIP system. These histograms are available immediately after the last tube is run on the cytometer. The first histogram is a two-parameter light scatter histogram showing the combined light scatter from all tubes. The lymphocyte gating region (x) was used for all gating. Each individual tube can be displayed if necessary. The second is a one-parameter histogram showing the TIP. Each tube is represented by a spike on the x axis. These spikes are sequentially gated to produce each of the 11 following two-parameter histograms. Each histogram is titled with the phenotypic combination for that tube. PE conjugates are designated before FITC conjugates in the figure. This is termed the *composite quadstat* presentation.

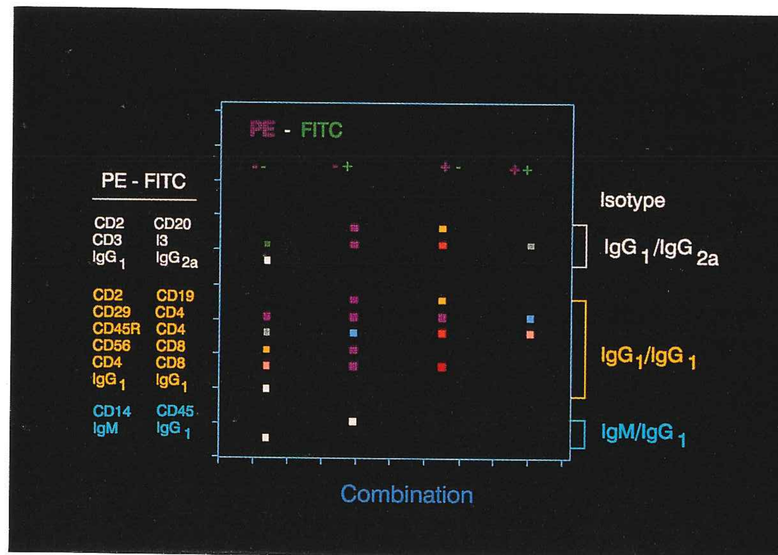

FIG. 3. "Phenogram" representation of the entire phenotypic analysis in a single two-parameter histogram as represented on the computer screen. Gating regions can be placed around each box to provide statistics. The number of cells in each box relates to the color bar shown in Figure 4. Percentages are calculated from each row (representing a single tube). All statistical information relating each "cell" to another is contained within this histogram. Controls can be easily identified by the patterns they produce. For example, the isotypic controls clearly mark the dual negative populations. Similarly, a clear pattern exists for the gating control tube CD14/CD45. For lymphocytes there must only be cells in the $CD14^-/CD45^+$ box as shown in this phenogram. Because the TIP spikes can be in fixed locations for any given profile of tubes, it is possible to predict specific patterns for combinations of tubes. Furthermore, by substituting particular reagents such as known number of beads in control tubes, it is possible to include checks of technical accuracy. Specific patterns within the phenogram may be recognizable as being diagnostic information. If three colors or four colors are used, the x axis combination (PRISM) parameter would simply increase to eight and 16 levels, respectively. Although this would create a tremendous amount of information on a single histogram, automated analysis programs could be developed to identify diagnostic patterns.

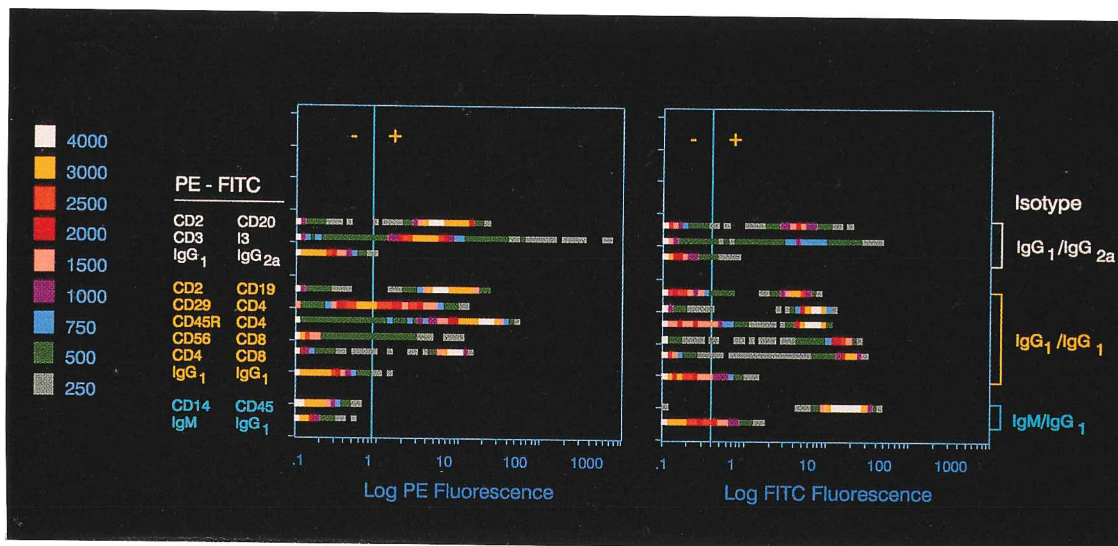

FIG. 4. "Overview" presentation showing two two-parameter dot plot histograms in an alternative representation of a phenotypic panel as seen on the computer screen during acquisition. As each tube is run, the data appear on the histogram on the cytometer computer screen. The left histogram shows histogramic data from PE-conjugated antibodies and the right from FITC-conjugated antibodies. On the left is the color bar representing numbers of cells. Beside the histogram is the phenotypic information showing the PE followed by the FITC antibodies. On the right, the isotypes are clustered so that appropriate isotype controls appear with the corresponding test antibodies. It is particularly easy to view the entire panel using this presentation technique. The data are identical in Figures 2–5.

COPLOT

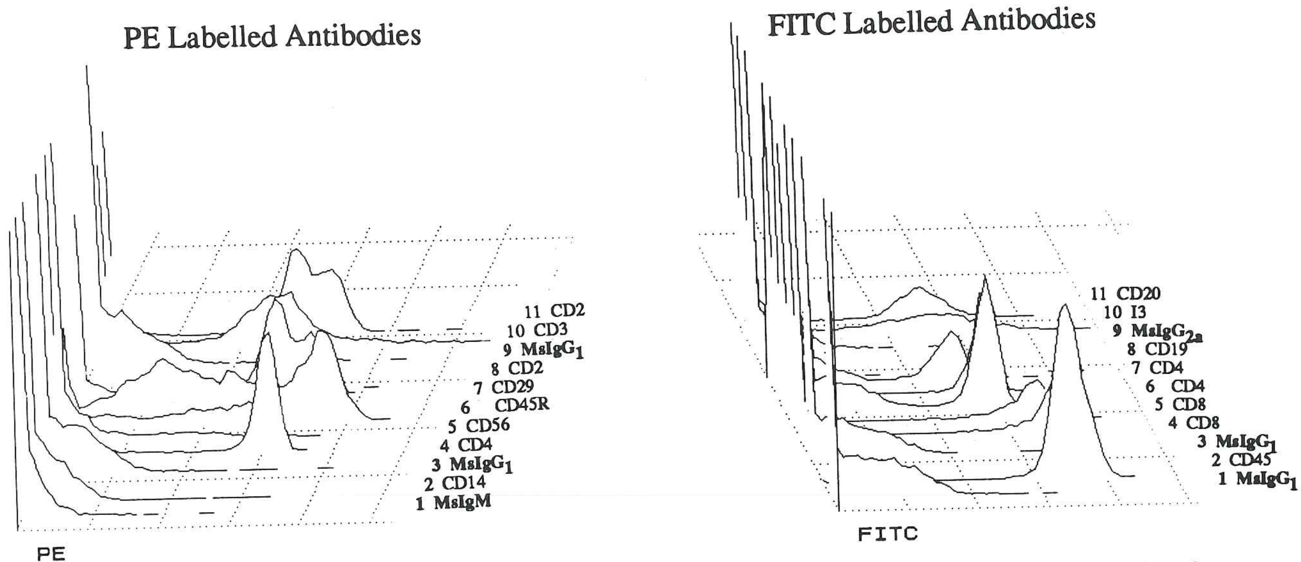

FIG. 5. TIP generated coplot of each tube for both PE (**left**)- and FITC (**right**)-conjugated antibodies. This is a three-dimensional version of data presented in Figure 4. Although little quantitative analysis is available from this type of histogram, it is commonly used as a composite of several markers. By gating on particular tubes, any combination of the available markers can be drawn together. Because all the marker combinations are in the same file, production of this type of histogram is trivial with TIP files.

fined Ethernet file transfer capability from cytometers is an important consideration. If it is not necessary to save the listmode data, the phenogram histogram can be saved instead. This phenogram contains all the statistical information of the entire set of two-parameter histograms while being no larger than any other two-parameter histogram. Saving the phenogram would be equivalent to the present practice of saving all the histogram data on each phenotypic marker, but in a single file.

Although there may be some concern about implications of blockages in the flow cell using this system, we have found that blockages rarely occur when using the 250 μm square channel SortSense tip on the cytometer. However, using the time parameter as a quality control parameter, data could be gated out from the analysis. This repair mechanism is a useful concept for all flow cytometry. Since data are collected into a RAM (volatile) memory on the cytometer, a power outage during a run could result in loss of all data run in that particular set. Addition of an uninterruptible power supply to the computer on the cytometer would prevent data loss in that situation. Other potential problems such as instrument failure could result in an incomplete TIP set. If this were to occur, no significant disadvantage would arise, since individual tubes could still be collected. Any tubes already collected in a partial TIP set could still be analyzed normally. If the instrument failure was serious enough to cause a lengthy delay, the entire set

Table 3
Single Parameter Data[a]

Marker	Single parameter	Quadstat	PRISM
PE markers			
$CD14^+$	130 (2)	130 (2)	136 (2)
$CD4^+$	2210 (44)	2210 (44)	2210 (44)
$CD56^+$	275 (5)	275 (5)	276 (5)
$CD45R^+$	4414 (88)	4414 (88)	4420 (88)
$CD29^+$	2003 (40)	2003 (40)	2019 (40)
$CD2^+$	3574 (71)	3574 (71)	3568 (71)
$CD3^+$	3207 (64)	3207 (64)	3212 (64)
$CD2^+$	3532 (70)	3532 (70)	3532 (70)
IgG1-PE^+	28 (<1)	26 (<1)	25 (<1)
IgM-PE^+	28 (<1)	28 (<1)	26 (<1)
FITC markers			
$CD45^+$	4777 (97)	4777 (97)	4777 (97)
$CD8^+$	1203 (24)	1203 (24)	1203 (24)
$CD8^+$	1137 (23)	1137 (23)	1138 (23)
$CD4^+$	2568 (51)	2568 (51)	2570 (51)
$CD4^+$	1958 (39)	1958 (39)	1959 (39)
$CD19^+$	1169 (23)	1169 (23)	1166 (23)
$I3^+$	1497 (30)	1497 (30)	1499 (30)
IgG1-$FITC^+$	63 (2)	63 (2)	62 (2)
IgG2a-$FITC^+$	21 (<1)	21 (<1)	20 (<1)
$CD20^+$	1237 (25)	1237 (25)	1237 (25)
IgG1-$FITC^+$	33 (<1)	33 (<1)	33 (<1)

[a]Comparison of three sets of data accumulated using the TIP system, the first using the single-parameter histograms, the second using the quadstat regions from two-parameter histograms, and the last using PRISM. No significant difference were observed in the data derived using the three methods. Numbers in parentheses indicate the percentage positive for that marker.

would probably have to be rerun. This would be no different from using conventional procedures. The major source of problems in our experience is in training staff to consider an entire experiment as a single file rather than many individual files. Once this is accomplished, the assay is a simple and effective technique.

In short, we have reduced the time taken for most procedures to minutes rather than hours, while producing a more comprehensive analysis of multiparametric information. A large amount of information is provided in orders of magnitude less time and energy with this system. Additional measurements that could be made include DNA vs. multicolor phenotypic markers or multiple sets of DNA comparisons, analysis of cross-reactive antibodies, or cross matching. We have demonstrated that the system is advantageous for assays in which repeat measurements on sets of tubes must be made and for assays involving large numbers of replicate tubes. The potential for pattern recognition and artificial intelligence programs to assist in the diagnostic process is greater with this system.

LITERATURE CITED

1. Bagwell CB: Clinical data analysis for flow cytometry. In: Flow Cytometry in Clinical Diagnosis, Keren DF (ed). ASCP Press, Chicago, 1989, pp 310–330.
2. Barsalou T: An object-based architecture for biomedical expert database systems. Comput Methods Programs Biomed 30:157–168, 1989.
3. Bender JG, Van Epps.D.E., Steinkamp JA: Characterization of human mononuclear cells using reduced pyridine nucleotide fluorescence and flow cytometry. J Leukocyte Biol 38:603–611, 1985.
4. Robinson J Paul, Bruner LH, Bassoe C-F, Hudson JL, Ward PA, Phan SH: Measurement of intracellular fluorescence of human monocytes indicative of oxidative metabolism. J Leukocyte Biol 43:304–310, 1988.
5. Shapiro HM: Practical Flow Cytometry. Alan R. Liss, Inc., New York, 1988.
6. Steinkamp JA: Flow cytometry. Rev Sci Instrument 55:1375–1400, 1984.
7. Sychra JJ, Bartels PH, Bibbo M, Wied GL: Dimensionality reducing displays in cell image analysis. Acta Cytol 21:747–752, 1977.
8. Van den Engh G, Stokdijk W: Parallel processing data acquisition system for multilaser flow cytometry and cell sorting. Cytometry 10:282–293, 1989.
9. Watson JV: Time, a quality-control parameter in flow cytometry. Cytometry 8:646–648, 1987.
10. Wood JC, Horton AF, Byrne JD, Pedroso RI, Bisnow M, Auer RE: Dual laser excitation flow cytometry: The state-of-the-art. In: Flow Cytometry: Advanced Research and Clinical Applications, Volume 1. Yen A (ed). CRC Press, Boca Raton, FL, 1989, pp 5–62.