

(51) International Patent Classification:

G01N 35/00 (2006.01) G01N 1/31 (2006.01)
G01N 15/14 (2006.01)

(21) International Application Number:

PCT/US2016/034435

(22) International Filing Date:

26 May 2016 (26.05.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/168,133 29 May 2015 (29.05.2015) US

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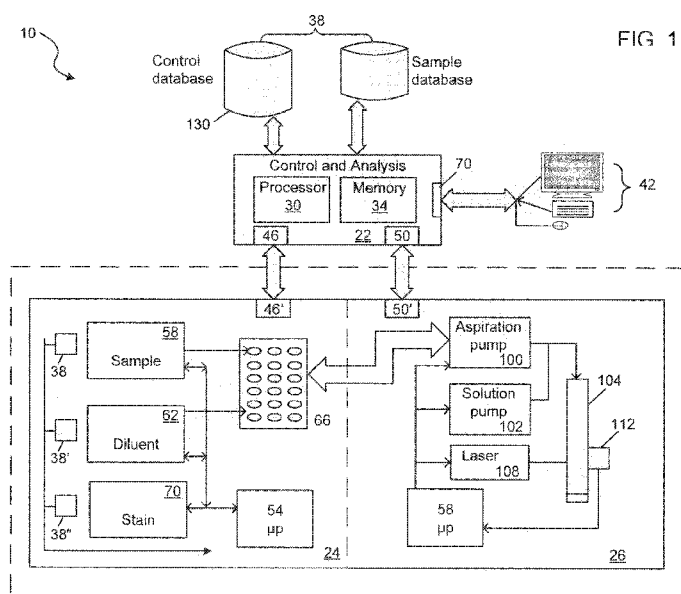
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LI, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: SYSTEM AND METHOD FOR DEFINING AND UTILIZING FLOW CYTOMETRY PROTOCOLS



(57) Abstract: An integrated sample analyzer. In one embodiment, the analyzer includes a sample preparation section; a cytometer section; and a controller. In another embodiment, the sample preparation section prepares the sample to form a prepared sample in response to a stored custom protocol provided by a user (a user-defined protocol). In still another embodiment, the sample preparation section provides the prepared sample to the cytometer section to measure sample parameters. In yet another embodiment, the sample preparation section and the cytometer section operate in response to instructions from the controller. In still yet another embodiment, the cytometer section provides the measured sample parameters to the controller for analysis and display.

SYSTEM AND METHOD FOR DEFINING AND UTILIZING FLOW CYTOMETRY PROTOCOLS

RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application 62/168,133, filed May 29, 2015, the contents of which are herein incorporated by reference.

FIELD OF THE INVENTION

[0002] The invention relates generally to the field of cytometry and more specifically to automated cell cytometers.

BACKGROUND OF THE INVENTION

[0003] Flow cytometry laboratories generally separate the activities of cell preparation and flow cytometry analysis, each activity using its own instrumentation. The cell preparation may be automated or semi-automated with the steps of aspirating a portion of the sample, for example blood, into a testing container, such as a well of a microtiter plate, to an appropriate dilution, staining or labeling the cells, for example white cells, with the desired cell stain or label, lysing the cells, for example red cells, that are not to be measured, and performing any other intermediate steps required, such as heating, incubating, agitating etc.

[0004] The prepared samples are then moved from the preparation instrument to the flow cytometer which makes the measurements on the sample and produces the results in graphical or statistical form. The flow cytometer draws the sample into a flow cell or measuring chamber and the sample is exposed to light that is scattered by the cells in the sample according to their staining and physical parameters. The flow cytometer measures the scattered light by intensity, wavelength and scattering angle and analyses the results, providing the numerical parameters of the cells and graphical representations of the cell distributions.

[0005] Laboratories typically use preparation and measuring protocols provided by the manufacturers of the instruments they use in preparing and making measurements on their samples. This process is fine for the usual parameters that are measured in most medical and industrial laboratories. However, if the user wishes to prepare or measure a sample using a protocol not contemplated by the manufacturers, the user must prepare and measure the sample manually. This is time consuming and can be error-prone if the user needs to examine many different samples using the new user defined protocol. This is

especially true for users who are researchers desiring to develop new ways of measuring samples. What is needed is a way for the user to designate a new protocol in terms of the preparation, measurement, and analysis steps.

[0006] Further, integrated analyzers may combine processes of sample preparation, sample measurement, and sample analysis. Protocols for integrated analyzers may also include instructions for each of these processes. An individual protocol may thus have a large number of instructions, each optimized for the individual requirements of the intended use. A specialized application is unlikely to operate optimally if constrained to use the same protocol instructions designed as part of another, manufacturer or factory -defined protocol. There is thus a need for an integrated analyzer to support entry of user-defined protocol instructions that may be optimized for an individual user's application.

[0007] When designing a protocol, it is possible to set up a process that is beyond the practical capabilities of an integrated analyzer. For example, a user may seek to add more liquid than a container is capable of holding, to lower a probe so that it pierces through the bottom of a container, or to over-agitate a container so that it sloshes or flings some of its contents as infectious aerosols. In extreme cases, a set of instructions could damage the instrument or injure the user. Factory-defined protocols avoid this by combining a thorough knowledge of system limitations with verification and validation testing of any factory-defined protocols. A user in entering his or her own protocols may lack intimate knowledge of system limitations and may not have time or patience for extensive protocol testing. There is thus a need for automatic verification of user-entered instructions to avoid these outcomes.

[0008] The present invention addresses these needs.

SUMMARY OF THE INVENTION

[0009] In one aspect, the invention relates to an integrated sample analyzer. In one embodiment, the analyzer includes a sample preparation section; a cytometer section; and a controller. In another embodiment, the sample preparation section prepares the sample to form a prepared sample in response to a stored custom protocol provided by a user (a user-defined protocol). In still another embodiment, the sample preparation section provides the prepared sample to the cytometer section to measure sample parameters. In yet another embodiment, the sample preparation section and the cytometer section operate in response to instructions from the controller. In still yet another embodiment, the cytometer section provides the measured sample parameters to the controller for analysis and display.

[0010] In one embodiment, the controller includes a scheduler for scheduling sample

preparation and prepared sample measurement for multiple samples concurrently. In another embodiment, the scheduler interleaves sample preparation steps and prepared sample measurement steps for more than one sample.

[0011] In another aspect, the invention relates to an integrated sample analyzer including a sample preparation section; a cytometer section; and a controller; the controller including a scheduler. In one embodiment, the sample preparation section prepares the sample to form a prepared sample in response to a stored custom protocol provided by a user. In another embodiment, the sample preparation section provides the prepared sample to the cytometer section to measure sample parameters. In yet another embodiment, the sample preparation section and the cytometer section operate in response to instructions from the controller. In still yet another embodiment, the cytometer section provides the measured sample parameters to the controller for analysis and display. In another embodiment, the scheduler schedules sample preparation and prepared sample measurement for multiple samples concurrently by interleaving sample preparation steps and prepared sample measurement steps for more than one sample.

[0012] In another aspect, the invention includes a method of processing cells from a sample. The method includes steps of loading the sample into an integrated analyzer that includes a preparation section to prepare the cells, a cytometer section to measure signals from the prepared cells, and a controller to analyze the measured signals of the cytometer section. The method includes steps of receiving a protocol from a user. This protocol includes a preparation instruction and an analysis instruction. The method includes preparing the cells according to the preparation instruction, measuring signals from the prepared cells in the cytometer section, and analyzing the measured signals according to the analysis instruction. The sample may be one of a blood sample, a white blood cell sample, a peripheral blood mononuclear cell sample, and a bone marrow sample.

[0013] The preparation instruction may include a labeling treatment as defined by the user. The cytometer section includes a plurality of detection channels, and the protocol includes a measurement instruction specifying signal measurement from one or more of the detection channels as defined by the user.

[0014] The method may also include comparing a parameter of the preparation instruction against a limiting value and verifying the preparation instruction if the parameter is less than the limiting value.

[0015] In yet another aspect, the invention includes an integrated analyzer to characterize cells. The integrated analyzer includes a preparation section to prepare cells from a sample, a cytometer section to receive the prepared cells from the preparation section and to measure signals from the prepared cells, and a controller. The controller coordinates actions of the preparation section and the cytometer section. The controller also analyzes the measured signals from the cytometer section and directs the preparation section to prepare the sample according to a preparation instruction defined by a user. The preparation instruction may include a labeling treatment such as contacting the cells with a labelled antibody. The analysis instruction may include a gating scheme as defined by the user. The cytometer system may include a preset instruction where at least one of the preparation instruction, the measurement instruction, and the analysis instruction includes the preset instruction as respective draft instructions for user editing.

[0016] In another embodiment, the invention includes an integrated analyzer to characterize cells. The integrated analyzer has a preparation section, a cytometer section in fluid communication with the preparation section, and a controller. The cytometer section includes a plurality of detection channels. The controller is configured to accept a test protocol from a user. The test protocol includes instructions specifying combination of the cells with a labeling reagent, selection of one of the plurality of detection channels, and a gating scheme. The controller directs the preparation section to contact the cells with the labeling reagent and directs the cytometer section to measure the prepared cells using the selected detection channel. The controller analyzes the measured values from the cells using the gating scheme.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The structure and function of the invention can be best understood from the description herein in conjunction with the accompanying figures. The figures are not necessarily to scale, emphasis instead generally being placed upon illustrative principles. The figures are to be considered illustrative in all aspects and are not intended to limit the invention, the scope of which is defined only by the claims.

[0018] Fig. 1 is a highly schematic block diagram of an embodiment of the hardware of a flow cytometry system constructed in accordance with the invention;

[0019] Fig. 2 is a block diagram of the functions of the embodiment of Fig. 1;

[0020] Fig. 3 is a high level flow chart of an embodiment of the steps of preparing,

measuring and analyzing a sample in accordance with the invention;

[0021] Fig. 4 is an embodiment of a display screen provided to permit a user to define a customized test;

[0022] Fig. 5 is an embodiment of a table correlating reagent names with reagent ID numbers;

[0023] Fig. 6 is a graphic depicting an embodiment of a custom reagent and its corresponding barcode;

[0024] Fig. 7 is an embodiment of a display screen provided to permit a user to define a customized protocol;

[0025] Fig. 8 is an example of a histogram of the results of a cytometry measurement;

[0026] Fig. 9 is a density plot of scattering angle versus number with a region selected for analysis;

[0027] Fig. 10 is an embodiment of a display screen provided to permit a user to define customized sample preparation;

[0028] Fig. 11 is a flow chart of an embodiment of the work flow by a user to create and use a custom test in the system;

[0029] Fig. 12 is an embodiment of a report generated by the system of the invention; and

[0030] Fig. 13 is an embodiment of the operation of the scheduler of the system.

DESCRIPTION OF A PREFERRED EMBODIMENT

[0031] Detailed embodiments of the invention are disclosed herein, however, it is to be understood that the disclosed embodiments are merely exemplary of the invention, which may be embodied in various forms. Therefore, specific functional details disclosed herein are not to be interpreted as limiting, but merely as a basis for the claims and as a representative basis for teaching one skilled in the art to variously employ the invention in virtually any appropriately detailed embodiment.

[0032] In brief overview and referring to Fig. 1, a flow cytometer preparation and analysis instrumentation system, also referred to as the instrument, constructed in accordance with the invention can be generally described as three subsystems or sections in one or more housings. When provided in a single housing, the instrumentation system occupies less lab space, is more automated and requires less human intervention than when it is constructed

as separate independent devices. For the purposes of this application, the instrumentation system is described as the unitary or integrated system in which the subsystems or sections are located in one housing, but this is done without any loss of generality and without limitation to systems constructed using individual devices.

[0033] The three subsystems of the instrument 10 are the instrument control and analysis subsystem or controller 22, sample preparation section 24, and cytometer section 26. The described subsystems or sections should be understood as functional divisions that may share components or resources rather than strictly distinct devices. For example, a pipetting probe may serve to transfer and mix reagents in the preparation subsystem 24 and may also serve to inject prepared cells into the flow cell of the cytometer section 26. Each section or subsystem is now considered individually.

[0034] The instrument controller section 22 includes a processor 30, working memory 34, a database 38, a user interface 42 and subsystem interfaces 46, 50. The processor 30 in various embodiments is a microprocessor or specialized controller. In one embodiment, the processor is an AMD Athlon dual core processor (AMD, Sunnyvale, CA). The operating system may be one of the currently available operating systems or a specially developed operating system. In one embodiment, the operating system is a 64 bit version of Windows 7 (Microsoft Corporation, Redmond, WA). The memory 34 is divided into program memory, containing the instructions and parameters to operate the system, and data memory to collect data from the sample. In one embodiment, the memory is in the form of 64GB of RAM. The database 38 holds sample data (sample database), protocols for preparing, measuring, and analyzing a sample and other instructions required to operate the instrument and access the data (control database). In one embodiment, data is accessed from the database 38 using a Microsoft SQL Server 2008 (Microsoft Corporation, Redmond, WA). In one embodiment, the user interface 42 provides input to and receives output from the processor through a mouse, keyboard, and monitor through USB connections 70.

[0035] The instrument 10, in many embodiments, includes multiple subsystem or section processors 54, 58 in communication with a main controller processor 30. The subsystem or section processors 54, 58 control the aspects of the subsystem or section of which they are a part, under instruction of the main processor 30. For example, the main processor 30 may provide a preparation protocol to the preparation section processor 54 that requires that a volume of sample 58 be aspirated and diluted in a volume of diluent 62. The sample preparation section processor 54 would then control the fluidics to aspirate a defined volume of diluent 62 into a testing container 66, in one embodiment a microtiter array of wells,

aspirate a defined volume of the sample 58 into the testing container 66, and agitate the diluted sample for uniformity. In this manner, the main processor 30 can proceed with other tasks while the subsystem processors are performing the required activities. In one embodiment, the main processor 30 provides the instructions to the section processors through the interfaces 46, 46', 50, 50'. If the instrument is constructed with only the single processor and not subsystem or section processors, the subsystem interface 46, 50 permits the processor to communicate with the pipetting probes, pumps, valves and other components of the preparation and measurement subsystems to control the components directly. For ease of explanation, this description will treat the instrument as having multiple or single controllers depending on the context, but it is to be remembered that this is done with no loss of generality or limitation of single or multiple controller systems.

[0036] In use, a user of the instrumentation system 10 enters, through a user interface 42, a request to instrumentation system 10 to perform a test on a sample 58. The request includes a sample identifier typically in the form of a barcode which indicates to which sample 58 a request refers, the protocol to be used, and in what form the results are to be presented, as discussed further below. In some embodiments, the protocol includes a description of the form of data analysis and results presentation. The barcode can be a standard barcode, a 2-D barcode, and RFID chip or any other identifier readable by the system. In some embodiments, the sample may be identified by the position of a sample in a cassette in queue or in a container, such as in a defined well of a multiwall plate. For the purposes of this explanation, such identification will be generally referred to as a barcode, without limitation or loss of generality.

[0037] Typically, the barcode encodes an identification number which can be read by the instrument 10 and laboratory records management system to point to an extensive record relating to the source of the sample. For example, if the sample is from a patient, the barcode would provide a pointer to the patient's medical record which in turn would contain the patient's personal information, the name of the doctor requesting the test, and other personal information.

[0038] In addition, the barcode may provide a link between a sample and the testing protocol to be used by the preparation subsystem 24 to prepare the cells, by the cytometer section 26 to measure the cells, and by the instrument controller 22 to analyze and present the data. The testing protocol may be an instrument supplied (or factory-defined) protocol, for example, that defines what stain 70 is to be used to label the biological cells; the volume of stain 70; the amount of time the stain is to be in contact with the cells; what lysing agent is

to be used to remove the cells not being measured; the volume of lysing agent; the time required to lyse the cells; at what temperature the lysing is to occur; any additional incubation period; and other parameters. If the testing protocol is a custom a user-defined protocol developed by the user and not supplied with the system, the user can enter the protocol steps and parameters for the purpose of testing the sample and if the protocol is successful, save the protocol as an additional protocol into the protocol database as described below.

[0039] The individual steps in a protocol are sometimes referred to herein as instructions, with instructions for different portions of the protocol referred to by the relevant portion of the process. For example, instructions relating to sample preparation, which are processed by preparation section 24, are preparation instructions; instructions relating to sample measurement, which are processed by cytometer section 24, are measurement instructions; instructions relating to sample analysis or display, which are processed by controller 22, are analysis instructions. Each of these instructions may include one or more parameters. Parameters are values or arguments required to execute an instruction. For example, a preparation instruction to label cells may include parameters identifying the stain or labelled antibodies to be used and define the volume of stain or labelled antibodies to be transferred and contacted with the cells.

[0040] The barcode may also be the link between the specific sample and measurement instructions of a protocol used by the measurement subsystem 26 for that sample. The measurement instructions define the settable measurement parameters, such as flow rate, triggering, measurement channels, gains, angular measurements, compensation, stop criteria etc. that are to be used by the sample measurement subsystem 26.

[0041] The sample identifier or barcode, in addition to providing links to the testing and measurement protocols, also provides a link to what data analyses are to be performed by the controller 22 on the sample measurement results. The analysis and display functions include what variables are to be plotted; in what form (histograms, scatter plots, 2D, 3-D, radar, among others); what graphing parameters that are to be used (axes selection, scale, color, region selection, etc.); and any statistical calculations to be included.

[0042] The analysis instructions may also define the gating scheme to be used to analyze the data. A gating scheme is the sequence of analytical comparisons to identify and characterize cells of interest using the measured values of the cells. The comparisons include comparing measured values from one or more measurement channels with gating

parameters or limits. This may be a multi-level comparison with cells identified from a first comparison further selected in a second or subsequent comparison. For example, a cytometer may measure each cell using a plurality of detectors 112. The detectors 112 may collect light at various angles, including a forward scatter angle and a side scatter angle, and at several different wavelengths, each corresponding to the color of fluorescent light emitted by labels attached to antibodies or other stains. A gating scheme may include, for example, a first comparison of the intensity of side-scattered light to a side scatter threshold and a second comparison of the intensity of forward-scattered light between upper and lower limits. The system may then further select among cells that “pass” both of these comparisons by comparing the intensity of light emitted at a first fluorescent wavelength (FL1) to a second threshold. Cells having at least this level of FL1 light may then be compared to a region defined on a two-dimensional plot of two other fluorescent wavelengths (FL2 and FL3). Cells falling within this region may be enumerated or characterized by their fluorescence at yet another fluorescent wavelength (FL4). An individual gating scheme may identify and characterize more than type of cell to more completely characterize the full sample. As may be apparent from the above example, gating schemes may be complex; repeated user entry of gating schemes may be error prone because of this complexity. The one-time entry of a gating scheme through entry of a user-defined protocol including analysis instructions thus reduces the likelihood of errors.

[0043] When a sample, such as whole blood, white blood cells, peripheral blood mononuclear cells, bone marrow cells, is obtained from a source such as, but not necessarily, a patient, the sample may be presented to the system as a single barcode labeled tube or as a barcode labeled tube in a cassette with other barcode labeled samples. In either case, the barcode of the next tube to be processed is read by a barcode reader 38, and the result is transmitted to the section processor 54 of the preparation section 24 for transmission to the controller processor 30.

[0044] The controller 22, upon receiving the identification of the sample, examines the current series of requests for tests which have been entered by the user and stored in memory 34, and determines that there is a sample 58 corresponding to the request in the system, that the required reagents 62, 70 (as may also be identified by barcode), such as stain, lysing agent, and so on, are also present in the system, and if everything that is required is present, the controller 22 schedules the testing as discussed below.

[0045] If the scheduling is successful, the scheduled actions, as defined by the preparation instructions and measurement instructions and as sequenced by the scheduler, are then

presented to the sample preparation section 24 and to the cytometer section 26, which then have all the information they need to prepare and test the sample. If anything that is required is missing or scheduling can not be completed for some other reason as discussed below, the controller places the sample number on the "incomplete" list which is accessible by the scheduler for subsequent rescheduling attempts. Any number of errors, such as missing reagents, missing samples and mechanical system errors can result in a configuration problem such that the request cannot be honored. An error message is also presented to the user through the user interface 42.

[0046] In more detail, and referring also to Fig. 2, the functions of the sample preparation section 24 and the cytometer section 26 can be further divided into sample handling components and reagent handling components. The sample handling component and the reagent handling component may include, for example, a turntable that supports multiple containers and presents them for fluid transfer. In the sample preparation section 24, the sample handling component determines what sample is being prepared 90, 90' as determined by the barcode and aspirates 94 the sample into the testing container 66 for dilution by adding a diluent 62. The reagent handling component 98 determines which reagent, again as determined by barcode, is to be applied to the sample and adds the prescribed amount of diluent reagent and subsequently, any labeling or staining reagents. The staining or labeling reagent and diluted sample then remain in contact for the prescribed amount of time. The reagent handling component 98 then determines which lysing agent is to be added, what amount, and for what amount of time, and instructs the fluidics 94 to add that amount of lysing agent to the testing container. Once the sample is prepared, it is then presented to the cytometer section 26.

[0047] Referring again to Fig. 1, the cytometer section 26 then aspirates 100 the sample from the testing container 66 and pumps 102 the sample through the measuring chamber 104. The cytometer section 26 probes the sample using one or more lasers 108 and measures the intensity of light scattered over various angles, as measured from the direction of the incident light of the laser, using a plurality of detectors 112. The cytometer section 26 also collects and measures the intensity of fluorescent light excited by one or more lasers 108. Fluorescent light may be produced by stains or labels attached to reagents (such as antibodies) exposed to the cells during the preparation steps. The collected intensity data 120 (Fig. 2) is then transmitted to the control and analysis subsystem 22 for storage in the sample database 38. The processor 30 of the controller 22 then analyses the data 124 (Fig. 2) according to the protocol specified in the request and displays the results to the user,

as specified in the request or according to analysis instructions of the protocol.

[0048] Such a process is generally shown in Fig. 3. A sample 58, either from a single tube or a cassette, is placed (Step 10) in the instrument system 10. A portion of the sample is aspirated and placed in the testing container, for example a microtiter well (Step 14). The sample is then labeled or stained (Step 16), and the sample and reagents allowed to incubate (Step 20). If it is necessary to lyse some cells of the sample to remove extraneous cells, a lysing agent is then added (Step 24). The testing container is then automatically moved to the sample measuring subsystem (Step 26) and the sample is measured. The data is then analyzed (Step 28) and the results are displayed (Step 32).

[0049] In some embodiments, the sample preparation process may also include steps of fixation or permeabilization. Fixation includes treatment with cross-linking reagents such as glutaraldehyde, formaldehyde, paraformaldehyde and similar materials. These fixation reagents serve to cross link proteins of the cells, physically binding them together so that the cells remain intact even with treatment by lysing or permeabilization reagents. Fixation may also serve to disable or degrade some enzymes that may further alter components of the cells or to ensure that measurements reflect the cells' condition at a fixed time in an experiment. Permeabilization reagents serve to allow access to the interior of cells by relatively large molecules such as labeled antibodies. This permits labeling of internal structures, such as signaling proteins or secondary messengers that form part of signaling cascades in some cells. Permeabilization reagents may also serve to expose antibody binding sites that are normally not accessible.

[0050] A key aspect of the invention is that not only can a user request tests using protocols supplied with the instrument, but a user can also define his or her protocols and the instrument will schedule and perform the tests based on the protocol specified. The ability to define protocols to be used by the instrument is made easier by a software test creation tool incorporated into the instrument. This software permits a user to create and modify protocols and store them in the control database portion 130 of the system database 38. In one embodiment, the control database 130 comprises two separate databases. One database is the standard control database that is supplied with the instrument and which contains the prepackaged tested protocols for performing various tests. The second database is a test creation database and is used to hold the protocols developed by the user. The separate databases make it easier to keep the supplied protocols from being damaged by the user in attempting to develop a new protocol. It should be noted that there are other methods of protecting the provided protocols while having a single control

database 130, including but not limited to providing a label or flag in the protocol data indicating that the protocol is in development.

[0051] In one embodiment, the test creation tool screen prompts the user for information regarding the new test information as a hierarchy of questions. Referring to Fig. 4, the user screen for defining a new test requests a list of containers or wells 140 that will hold the samples to be measured using the new protocol. The user indicates for each run 144, a protocol name 148, the preparation settings 152, and a list of reagent names that will be used by the protocol 156. The screen also requests that the user define Results 160 and how the results of the analysis are to be displayed 164 (for example, a histogram and statistics). Examples of requested results include numbers of cells within selected regions or gates and number or staining intensities of a certain type of cells present, as determined by a combination of signal intensities values indicating the presence or amount of labeled reagents in or on cells. Together these result definitions define a gating scheme and the way the system will display the collected data.

[0052] The reagent ID number is then entered into a table of reagents created for each reagent 156 entered into the test screen. The system uses the Reagent ID to determine various information 170 (Fig. 6) about the reagent, such as its lot number, expiration date, etc. and to generate a bar code 174 for the custom reagent bottle.

[0053] The next screen is the input screen for the user defined protocol name 148 (Figs. 4 and 7) and in one embodiment includes five windows for input data. In the first window 180, the user defines his or her display choices such as, but not limited to, histograms (Fig. 8), dot plots (Fig. 9), etc. In the second window 184, the user defines sample measurement parameters such as sample size and flow rate through the measurement column. In window three, the user further defines sample handling such as agitation, agitation time etc. In window four 192, the user determines when measurement is to be completed based on criteria including, but not limited to, the amount of fluid passing through the measurement column, the number of events measured, and time. In the fifth window, the user provides gating information, which may include thresholds, ranges, and a list of regions of interest within a plot of data to be analyzed. For example, the system allows the user to place a polygon or other closed boundary about the points of interest within a plot. In Fig. 9, the area in which lymphoid cells are found is shown surrounded by a polygon indicating the region for further analysis.

[0054] The next screen is the input screen for the user defined sample preparation 156

(Figs. 4 and 10), and in one embodiment includes three windows for input data. The first window 200 is used to determine the volume of sample to be placed in a testing container or well. The second window 204 is used to enter the list of reagents in order of use, the amounts to be added to the sample, how the sample and reagent are to be mixed, and the incubation period between steps, among many other cell treatments. The third window 208 is used to enter lysing information such as the lysing agent to be used, the volume, and amount of incubation time required.

[0055] Fig. 11 is a flow chart of the work flow performed by a user to create and use a custom test using the test creation function of the system. In the first step (STEP 50), the user uses the test definition screen (Fig. 4) to create the test and enter the Reagent information into the system. In the second step (STEP 54), the user enters the custom protocol information using the protocol screen (Fig. 7). In the next step (STEP 58), the user enters for each well or run the protocol to be used (Fig. 4) in that specific run or well. In (STEP 62), the user enters the sample preparation information in the sample preparation screen (Fig. 10), and in (STEP 66) enters the display information in the protocol screen (Fig. 7). Once this step is complete, the user, in one embodiment, saves the test information into the test database (STEP 70). With the data in the test database, the user begins the standard system functions, but using the test definition from the test database, and loads the system with the custom reagents with the custom barcodes (STEP 74). The newly created test and the custom reagents now appear in the system test menu and can be selected by the user. Now the user can begin to test samples with the customized protocol. An embodiment of a test results is shown in Fig. 12.

[0056] In some embodiments, the invention includes entry of a user-defined protocol based on another protocol stored in the system. The user selects a protocol as a base, and the system displays a duplicate of the selected protocol for user edit of instructions. In some embodiments, the system may limit the instructions that a user may edit. For example, a protocol may permit editing of measurement instructions and of analysis instructions but not of preparation instructions. This has the benefit of adding flexibility of operation but limiting risk of damage or injury. An example of changes of this type are measurement instructions that direct collection of additional signals from detectors corresponding to fluorescent wavelengths of user-selected stains or labeled reagents and analysis instructions that specify analysis using the existing or additional signals.

[0057] In other embodiments, the system may permit user edit or entry of a wider variety of instructions. Free editing might create protocols that are beyond the practical capabilities of

an integrated analyzer. For example, a user may seek to add more liquid than a container is capable of holding, or to pipette a smaller quantity than the system can reliably deliver. Further, over-agitation of a testing container that may hold infectious cells could create potentially infectious aerosols. In extreme cases, a set of instructions could damage the instrument or injure the user. Factory-defined protocols avoid this by combining a thorough knowledge of system limitations with verification and validation testing of any factory-defined protocols. A user in entering his or her own protocols may lack intimate knowledge of system limitations and may not have time or patience for extensive protocol testing. The invention thus includes automatic verification of user-edited or entered instructions to avoid these outcomes.

[0058] In general, verification of instructions includes setting of limits (such as single-ended thresholds or double-ended ranges) and comparison of instruction parameters against the limits. Some limits are simple and may be regarded as hard boundaries of the system. For example, a pipetting probe has maximum and minimum transfer volumes; a container has a maximal fill volume. These hard boundaries are determined by device design. Other limits depend on steps of the protocol in conjunction with hard boundaries. For example, a protocol may direct the preparation section to dispense to and aspirate from a single container multiple times during the course of a protocol run. Each of the individual fluid transfers may be within the hard boundaries of the pipetting probe and the container, but the sequence of steps may still overfill the container. The unused volume remaining in a container may be considered a soft boundary, as it varies during the course of the protocol. Both hard boundaries and soft boundaries are referred to herein as limits.

[0059] To verify an instruction or set of instructions, the system compares each instruction to then-current limits, including both hard boundaries and then-current soft boundaries, to determine whether the instruction can be verified. The system analyzes the instructions and their parameters (such as volume and direction of fluid transfer) and the resources used (such as containers) to determine (for example) a cumulative volume present in a container. If this cumulative volume at any point in the protocol exceeds the hard boundary of the container volume, then the system can flag at least the instruction that would have exceeded the limits. The system may also highlight each of the instructions that contribute to the identified problem (including, in the case of container overfill, each of the fluid transfer steps involving that container). The user interface may also display an appropriate error message (such as CONTAINER OVERFILLED: MAXIMUM FILL VOLUME OF CONTAINER X IS Y MICROLITERS; PROGRAMMED FILL VOLUME IS Z MICROLITERS, where X identifies the

container and Y and Z identify problematic volumes). The user may then edit the protocol instructions to stay within the limits. The above description uses container fill volumes as an example of instruction verification involving both hard and soft boundaries. The skilled practitioner will recognize that such verification is applicable to any of a variety of instruction parameters and is not limited to maximal volumes.

[0060] Another one of the important aspects of the invention is the ability of the system to perform or interleave multiple tasks during the same interval. This multitasking is performed by a scheduler function in the control and analysis processor. The scheduler is able to look at requests in the system and determine what functions can be performed simultaneously over multiple runs without interfering with the timing of any one run. In this context, a run is those time-constrained steps necessary to perform a single instance of a selected test protocol (including at least preparation and measurement steps) on a single sample. Analysis and display of results may be part of a protocol instance but not part of a run because these steps are not constrained to a particular time; analysis and display need merely occur after data collection.

[0061] Where tasks within a protocol use the same system resources (such as a pipetting probes, pumps, or reagent handling component 98 of sample preparation section 24, or the cytometer section 26) the scheduler “looks ahead” to assure that no required use of a resource in one instance of a protocol coincides with a required use of the same resource in either another instance of the same protocol or in an instance of another protocol. If a resource conflict is present, the scheduler delays the start of one instance so that such conflicts are avoided.

[0062] The scheduler looks at the global requirements of the samples and test requests. Referring to Fig. 13, when a user attempts to enter another run, the system first determines if a run can be added at the current time, Step 90. If the system determines that the system is operating at capacity such that no further runs can be added, the user is notified and waits for a few seconds (or looks ahead in the sequence of already scheduled operations), Step 94, and then checks availability again, Step 90.

[0063] Once system resources become available (or are not otherwise scheduled), Step 98, the run is added to the list of runs. The system can then order the operations, determine the resources required, etc., Step 102. The system then returns, Step 106, to await the next run. This ability to schedule functions when possible adds to the flexibility and throughput of the instrument.

[0064] Unless specifically stated otherwise as apparent from the discussion, it is appreciated that throughout the description, discussions utilizing terms such as "processing" or "computing" or "calculating" or "delaying" or "comparing", "generating" or "determining" or "forwarding" or "deferring" "committing" or "checkpointing" or "interrupting" or "handling" or "receiving" or "buffering" or "allocating" or "displaying" or "flagging" or Boolean logic or other set related operations or the like, refer to the action and processes of a computer system, or electronic device, that manipulates and transforms data represented as physical (electronic) quantities within the computer system's or electronic devices' registers and memories into other data similarly represented as physical quantities within electronic memories or registers or other such information storage, transmission or display devices.

[0065] Any algorithms presented herein are not inherently related to any particular computer or other apparatus. Various computers and operating systems may be used with programs in accordance with the teachings herein, or it may prove convenient to construct a more specialized apparatus to perform the required method steps. The required structure for a variety of these systems will be apparent from the description of the embodiment shown. In addition, the present invention is not described with reference to any particular programming language, and various embodiments may thus be implemented using a variety of programming languages.

[0066] The aspects, embodiments, features, and examples of the invention are to be considered illustrative in all respects and are not intended to limit the invention, the scope of which is defined only by the claims. Other embodiments, modifications, and usages will be apparent to those skilled in the art without departing from the spirit and scope of the claimed invention. Examples of values of numbers, unless specified otherwise, are to be assumed to include slight deviations from number. Such deviations may be 10-15% and still be within the value listed.

[0067] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components and can be selected from a group consisting of two or more of the recited elements or components. Further, it should be understood that elements and/or features of a composition, an apparatus, or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present teachings, whether explicit or implicit herein.

[0068] The use of the terms “include,” “includes,” “including,” “have,” “has,” or “having” should be generally understood as open-ended and non-limiting unless specifically stated otherwise.

[0069] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present teachings remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[0070] It is to be understood that the figures and descriptions of the invention have been simplified to illustrate elements that are relevant for a clear understanding of the invention, while eliminating, for purposes of clarity, other elements. Those of ordinary skill in the art will recognize, however, that these and other elements may be desirable. However, because such elements are well known in the art, and because they do not facilitate a better understanding of the invention, a discussion of such elements is not provided herein. It should be appreciated that the figures are presented for illustrative purposes and not as construction drawings. Omitted details and modifications or alternative embodiments are within the purview of persons of ordinary skill in the art.

[0071] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

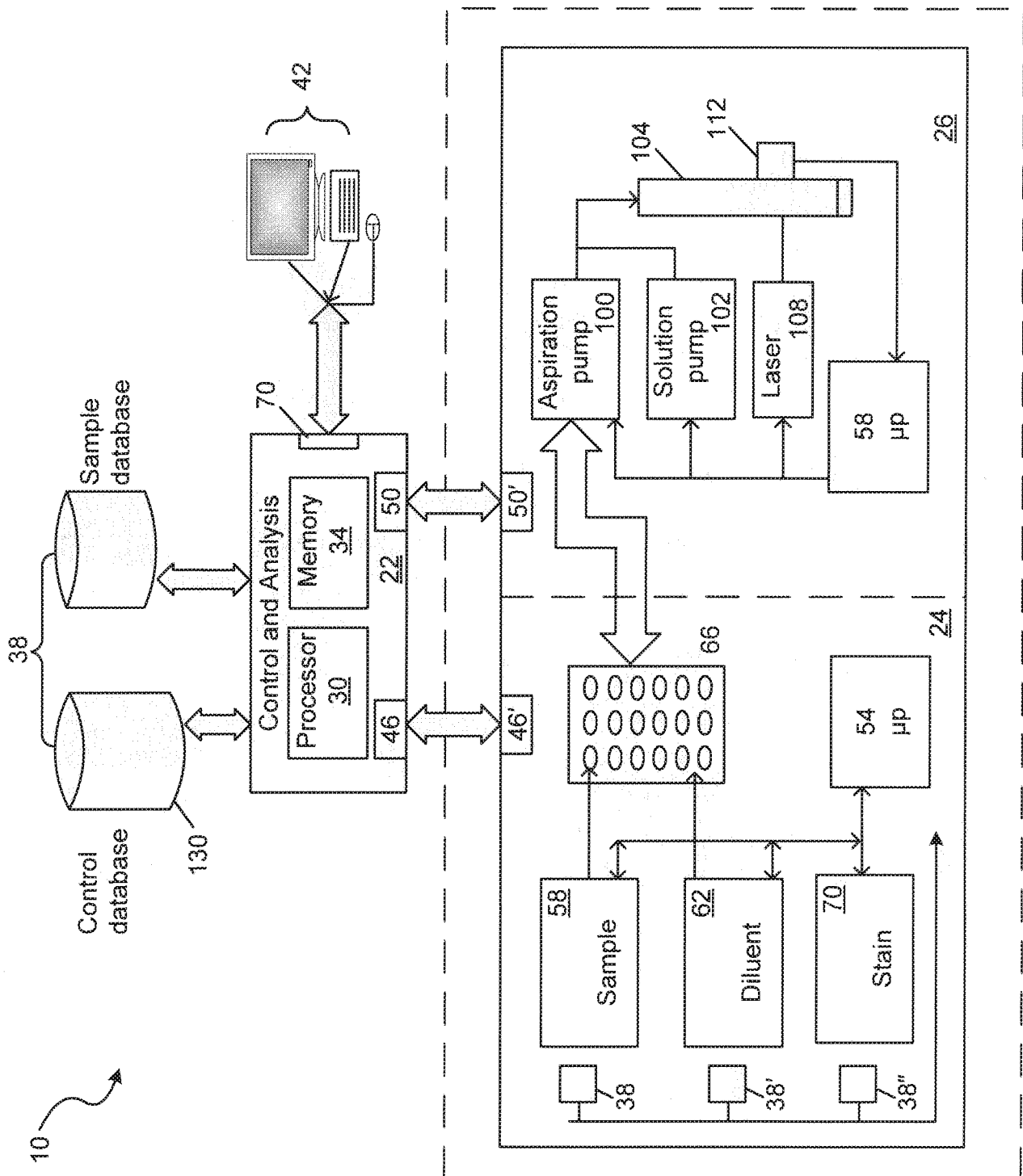
[0072] What is claimed is:

CLAIMS

1. A method of processing cells from a sample, the method comprising:
 - loading the sample into a cytometer system, the cytometer system including a preparation section to prepare the cells, a measurement section fluidically coupled to the preparation section to measure signals of the prepared cells, and a controller to analyze the measured signals of the measurement section;
 - receiving a first protocol from a user, the first protocol including a preparation instruction and an analysis instruction;
 - preparing the cells in the preparation section according to the preparation instruction;
 - measuring signals from the prepared cells in the measurement section; and
 - analyzing the measured signals according to the analysis instruction.
2. The method of claim 1, wherein the sample is one of a blood sample, a white blood cell sample, a peripheral blood mononuclear cell sample, and a bone marrow sample.
3. The method of claims 1 or 2, wherein the step of receiving a first protocol precedes the step of loading a sample.
4. The method of any of claims 1 through 3, wherein the preparation instruction includes a labeling treatment as defined by the user.
5. The method of any of claims 1 through 4, wherein the measurement section includes a plurality of detection channels and the first protocol includes a measurement instruction specifying signal measurement from one or more of the plurality of detection channels as defined by the user.
6. The method of any of claims 1 through 5, wherein the analysis instruction includes a gating scheme as defined by the user.
7. The method of any of claims 1 through 6, wherein the cytometer system includes a preset instruction and wherein at least one of the preparation instruction, the measurement instruction, and the analysis instruction includes the preset instruction.
8. The method of any of claims 1 through 7, further comprising the step of associating the sample to the first protocol.
9. The method of any of claims 1 through 8, further comprising the step of comparing a parameter of the preparation instruction against a limiting value and verifying the preparation instruction if the parameter is within the limiting value.

10. The method of claim 9, wherein the cytometer system includes a test container having a capacity, wherein the cytometer system tracks a fill level of the test container, wherein the parameter includes a volume to be transferred to the test container, and wherein the limiting value is the difference between the capacity of the test container and the fill level of the test container.
11. An integrated analyzer to characterize cells, the integrated analyzer comprising:
a preparation section to prepare cells from a sample;
a cytometer section to receive the prepared cells from the preparation section and to measure signals from the prepared cells and;
a controller to coordinate actions of the preparation section and the measurement section and to analyze the measured signals from the cytometer section, the controller configured to direct the preparation section to prepare the sample according to a preparation instruction defined by a user.
12. The integrated analyzer of claim 11, wherein the measurement section includes a plurality of detection channels, and wherein the test protocol includes a measurement instruction specifying signal measurement from one or more of the plurality of detection channels as defined by the user.
13. The integrated analyzer of claims 11 or 12, wherein the controller is further configured to analyze the measured signals using to a gating scheme according to an analysis instruction as defined by the user.
14. The integrated analyzer of any of claims 11 through 13, wherein the preparation instruction specifies combining the cells with a labeling reagent as defined by the user in the test protocol.
15. The integrated analyzer of any of claims 11 through 14, further comprising a preset preparation instruction, a preset measurement instruction, and a preset analysis instruction, wherein the controller is configured to replicate one or more of the preset preparation instruction, the preset measurement instruction, and the preset analysis instruction as respective draft instructions for user editing.
16. The instrument of claim 15, wherein one or more of the preparation instruction, the measurement instruction, and the analysis instruction include a default value from a factory-defined instruction.

17. The integrated analyzer of claim 15, wherein the controller is further configured to accept user entry associating the sample with one of the preparation instruction defined by a user and a factory-defined preparation instruction and to prepare the cells from the sample according to the associated one of the preparation instruction defined by a user and the factory-defined preparation instruction.
18. The instrument of claim 11, wherein the controller is further configured to compare a parameter of the preparation instruction against a preset bound and to verify the received preparation instruction if the parameter is within the preset bound.
19. The instrument of claim 18, wherein the parameter includes a volume to be transferred to a test container, and wherein the preset bound is an unfilled capacity of the test container.
20. An integrated analyzer to characterize cells, the integrated analyzer comprising:
a preparation section;
a cytometer section, in fluid communication with the preparation section,
including a plurality of detection channels;
a controller configured to accept a test protocol from a user, the test protocol including instructions specifying combination of the cells with a labeling reagent, selection of one of the plurality of detection channels, and a gating scheme,
wherein the controller directs the preparation section to contact the cells with the labeling reagent and directs the cytometer section to measure the prepared cells using the selected detection channel, and wherein the controller analyzes the measured values from the cells using the gating scheme.



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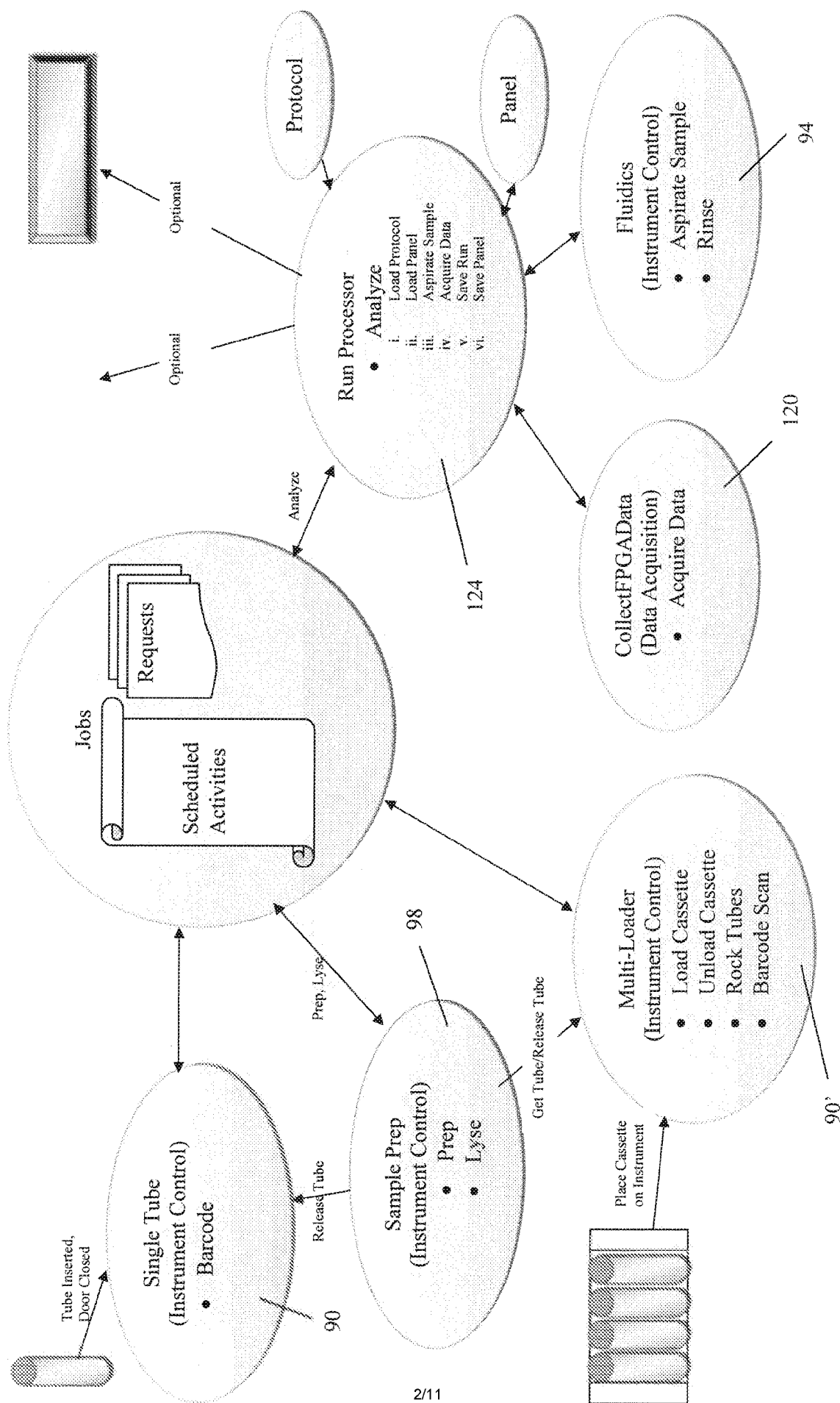


FIG. 2

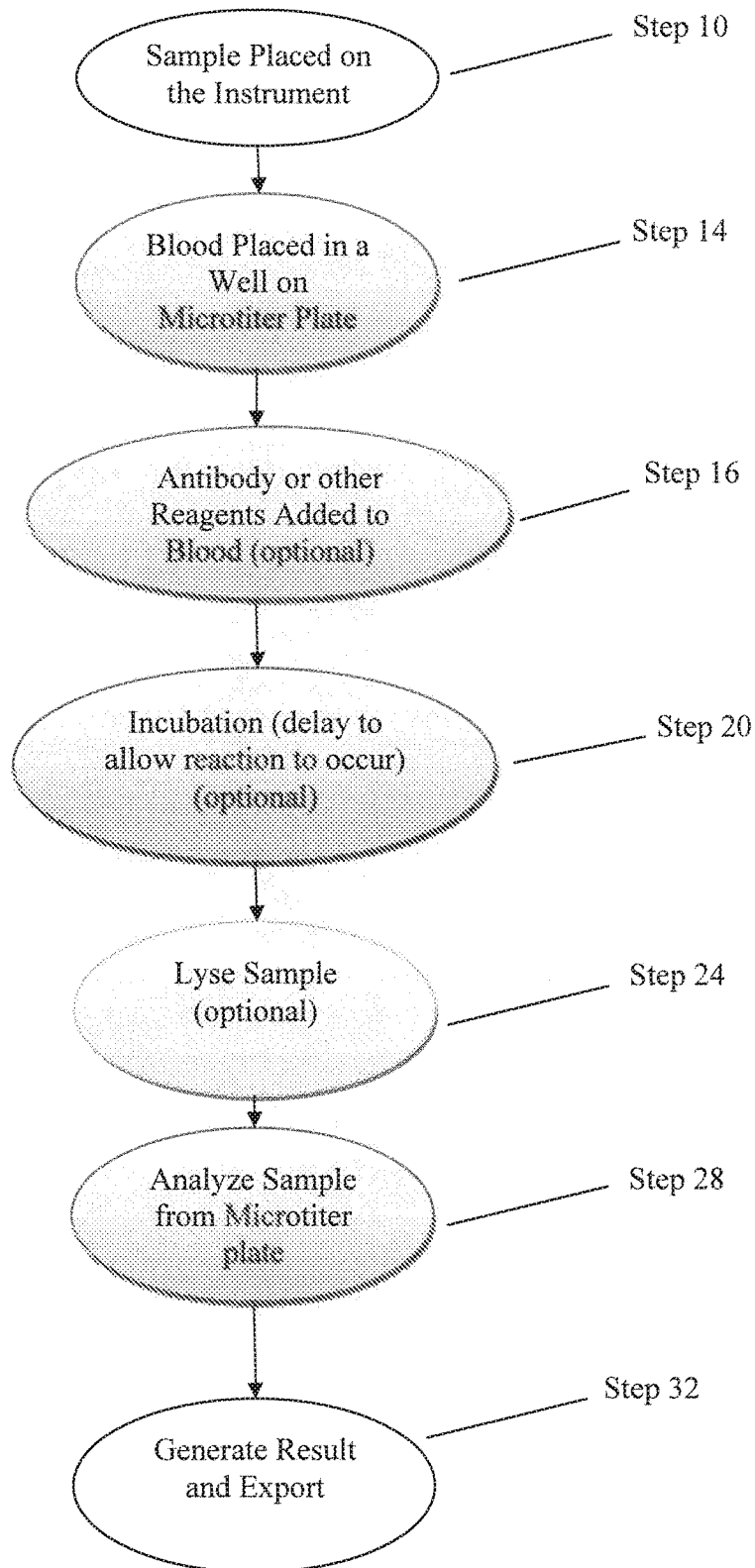


FIG. 3

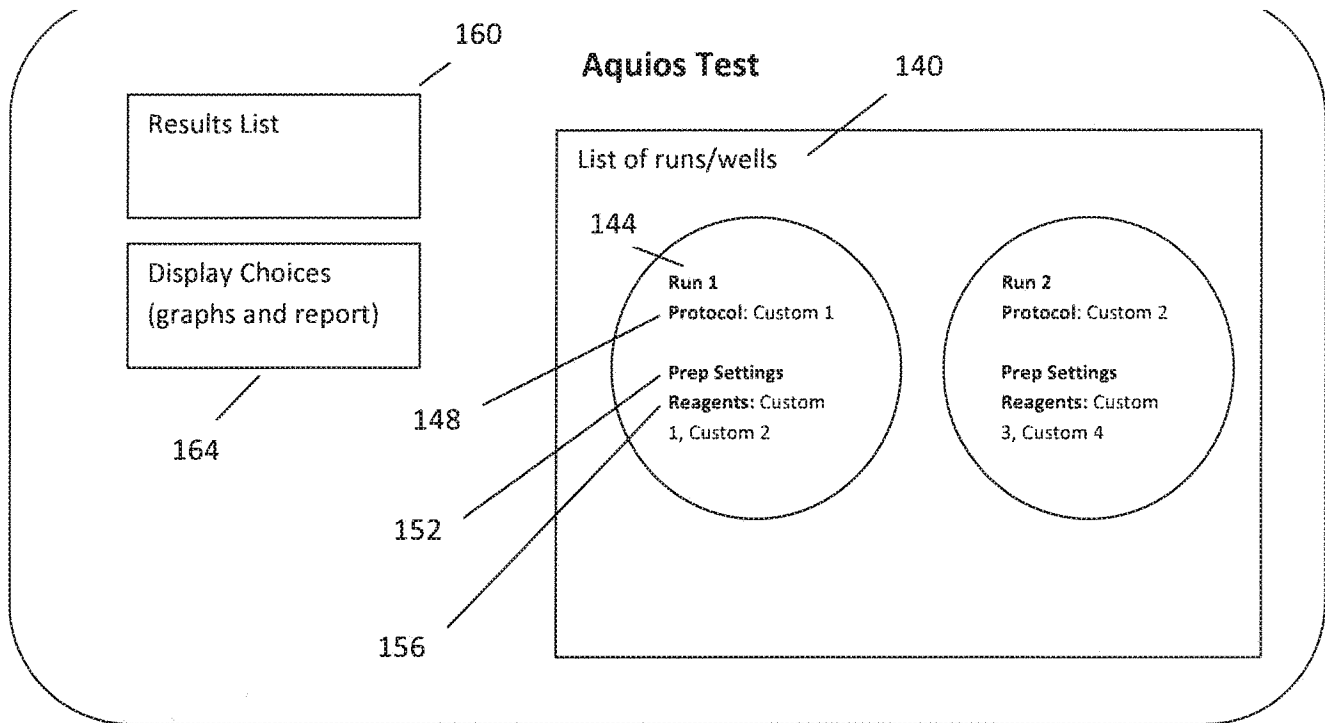


FIG. 4

Reagent Information

Reagent ID	Name
1001	Custom 1
1002	Custom 2
1003	Custom 3
1004	Custom 4

FIG. 5

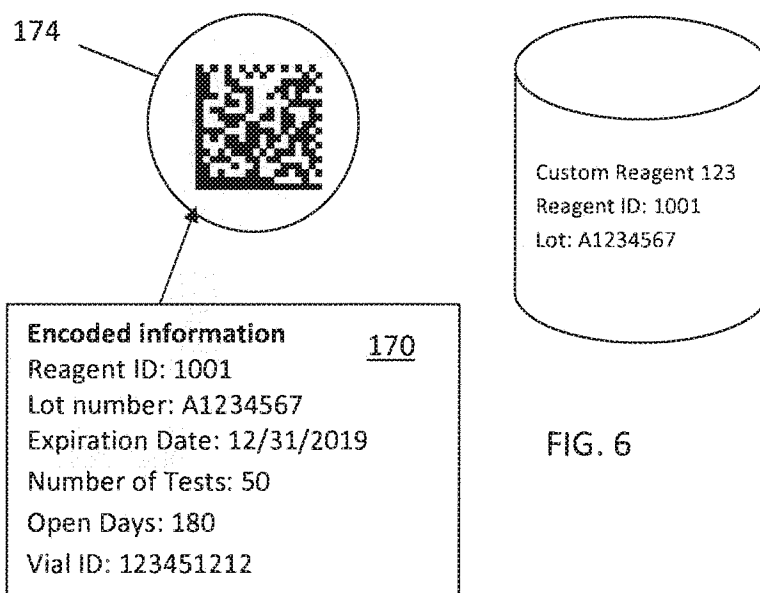


FIG. 6

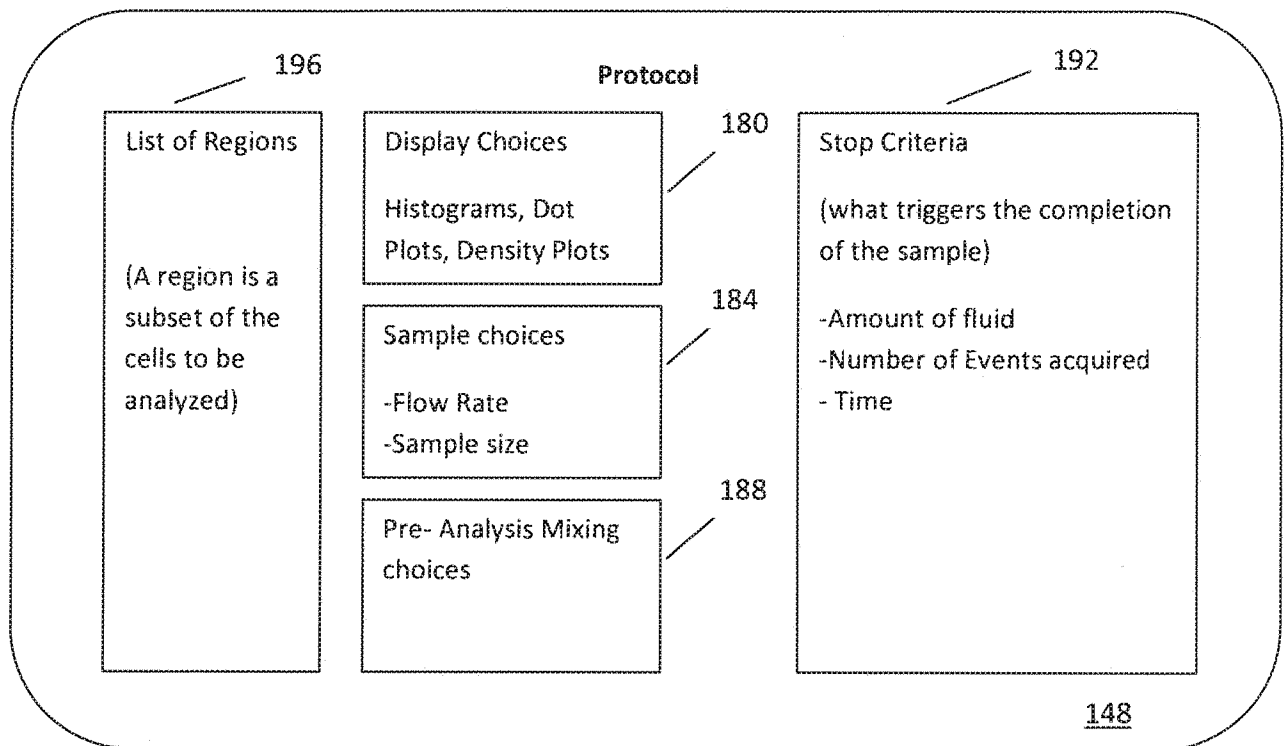


FIG. 7

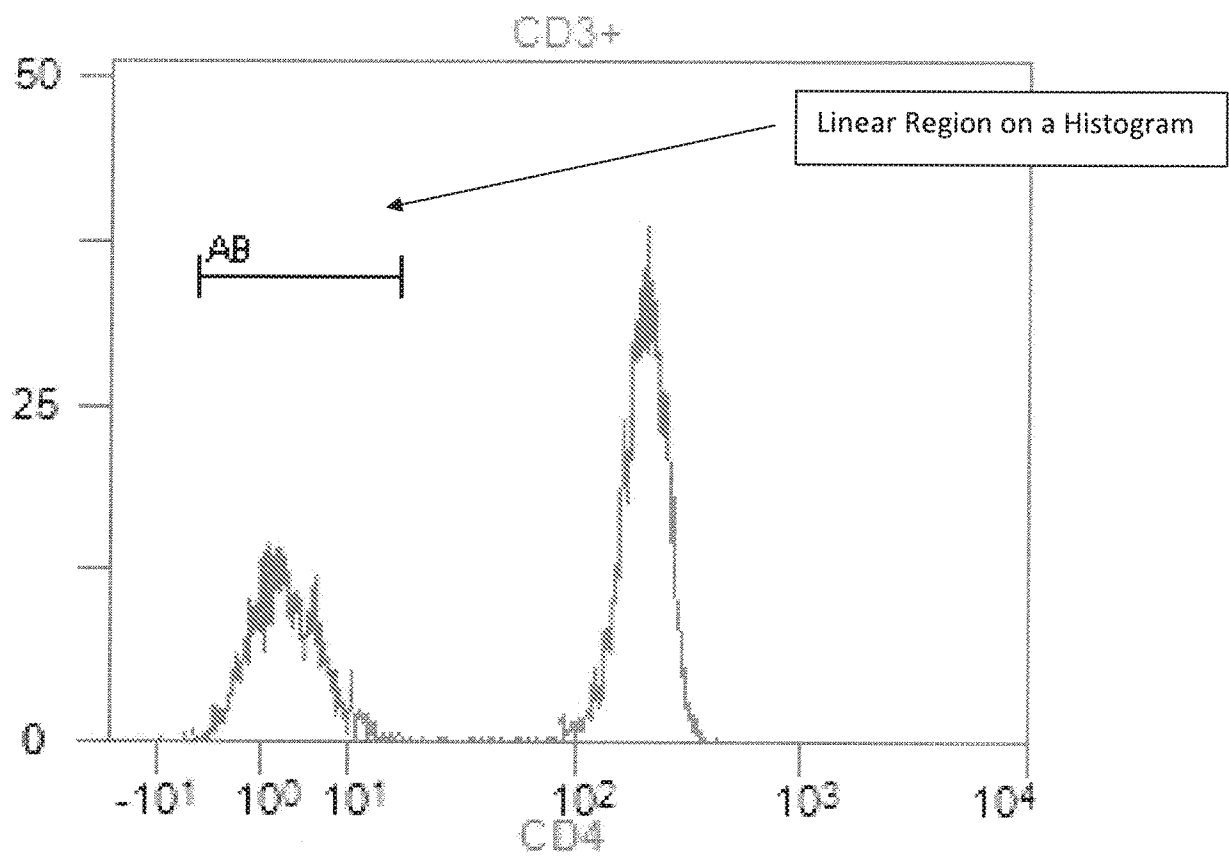


FIG. 8

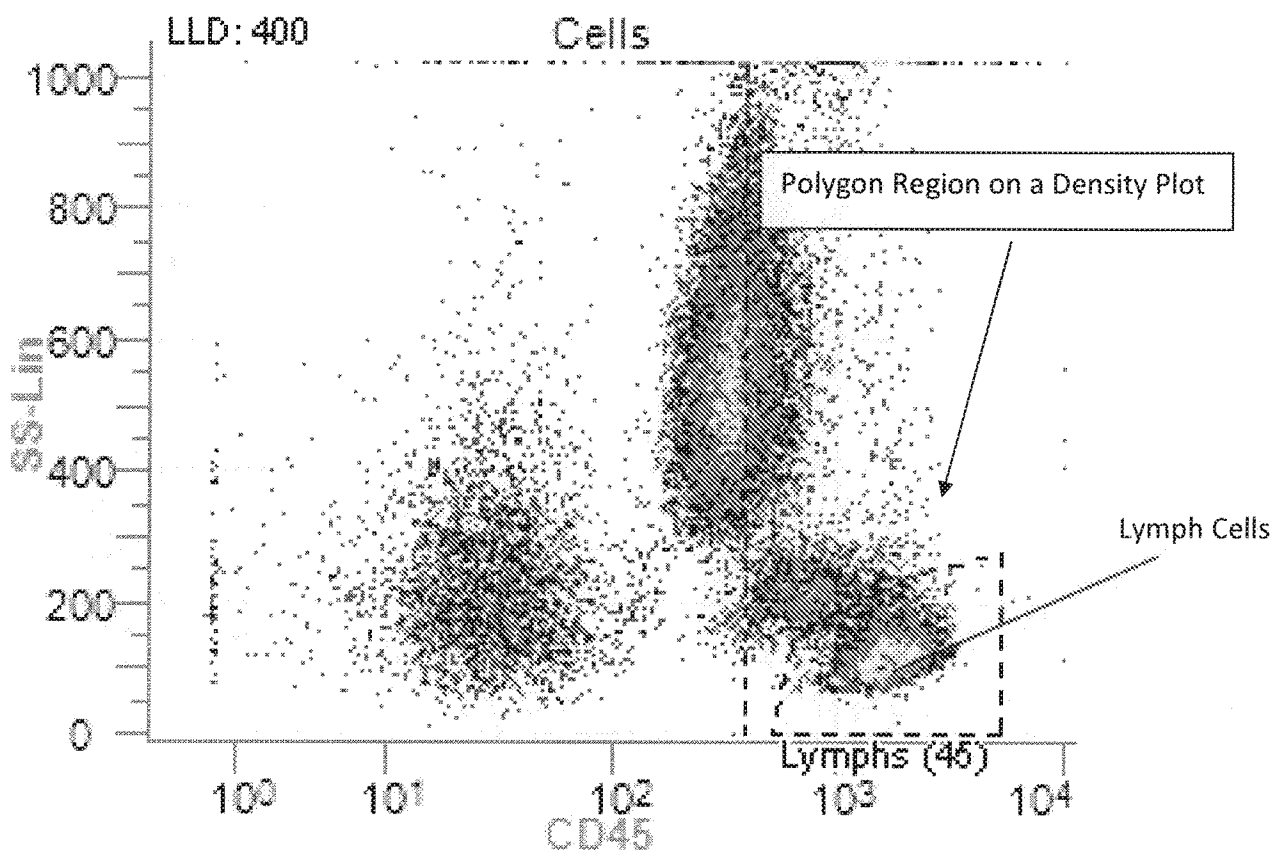


FIG. 9

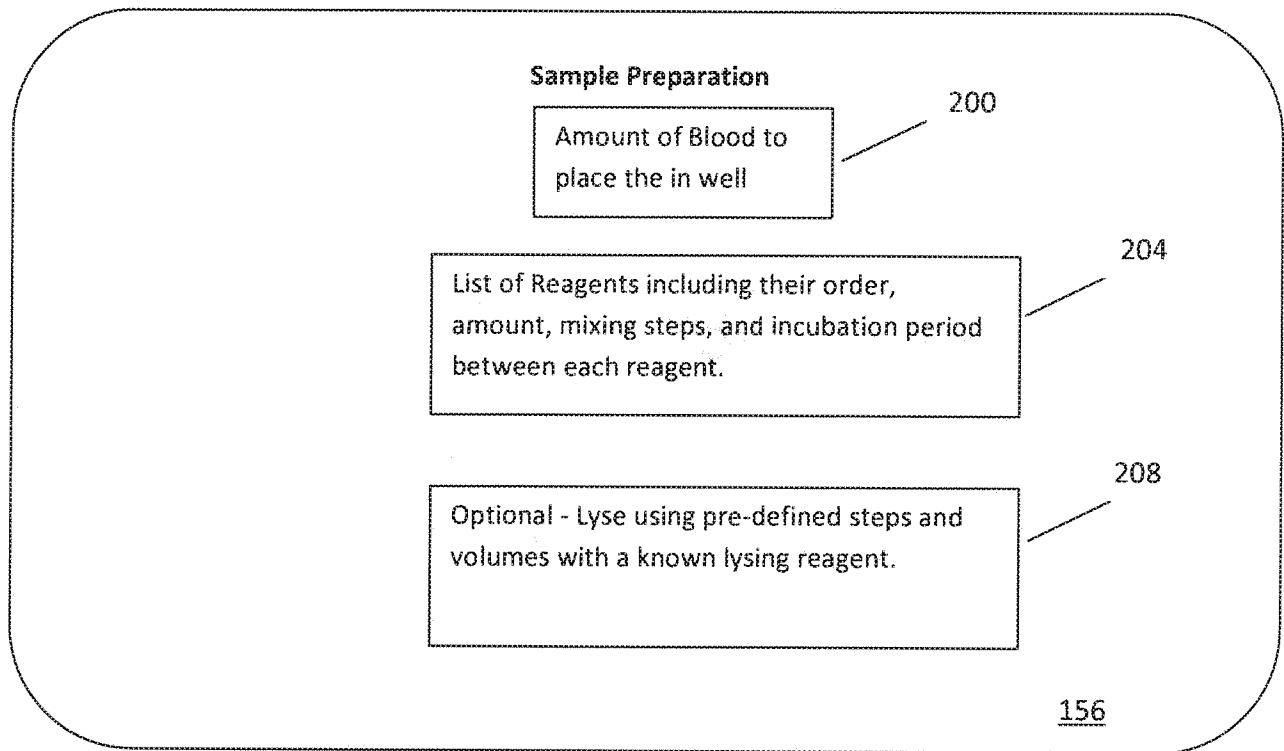


FIG. 10

Test Creation Software Workflow

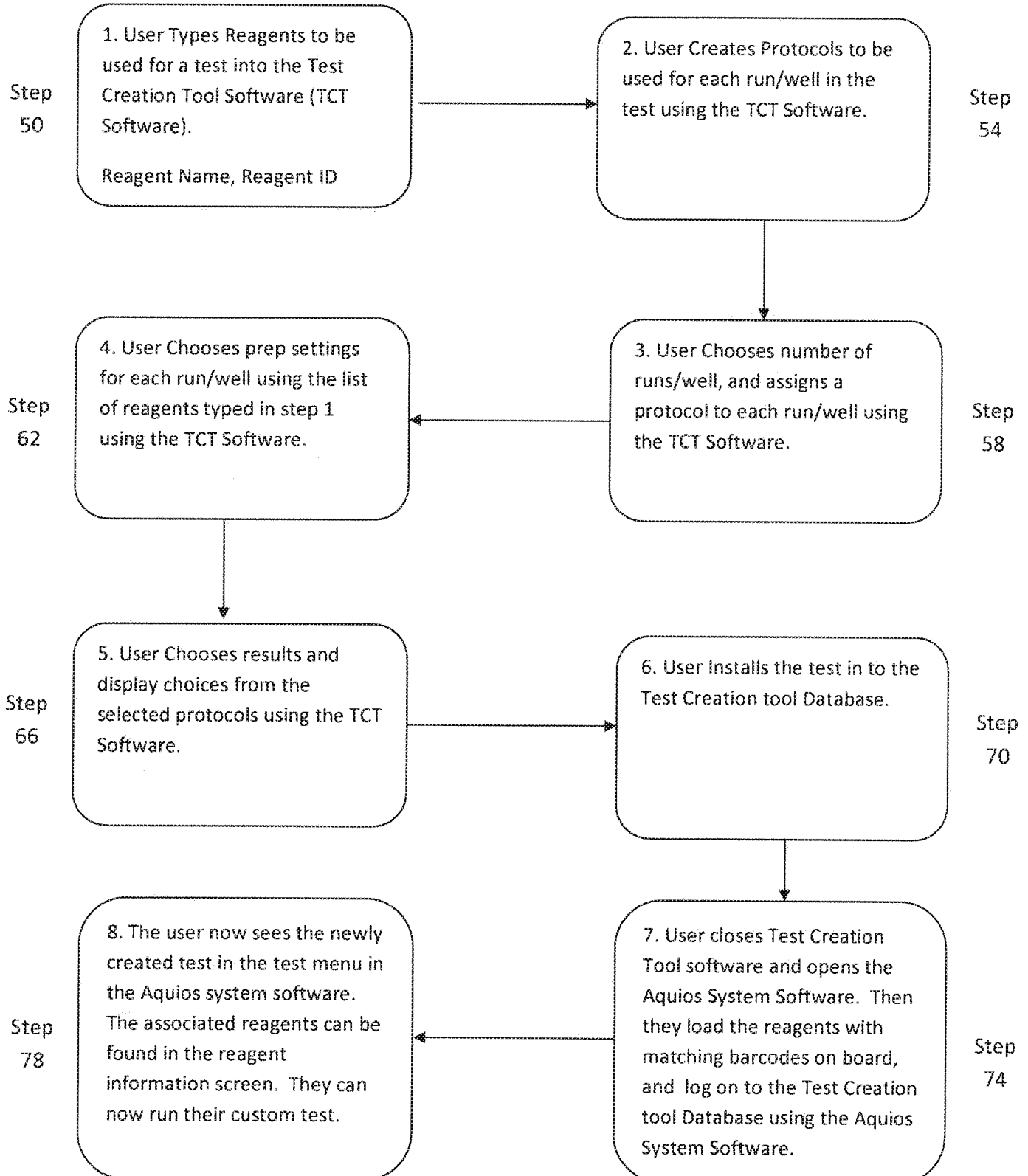


FIG. 11

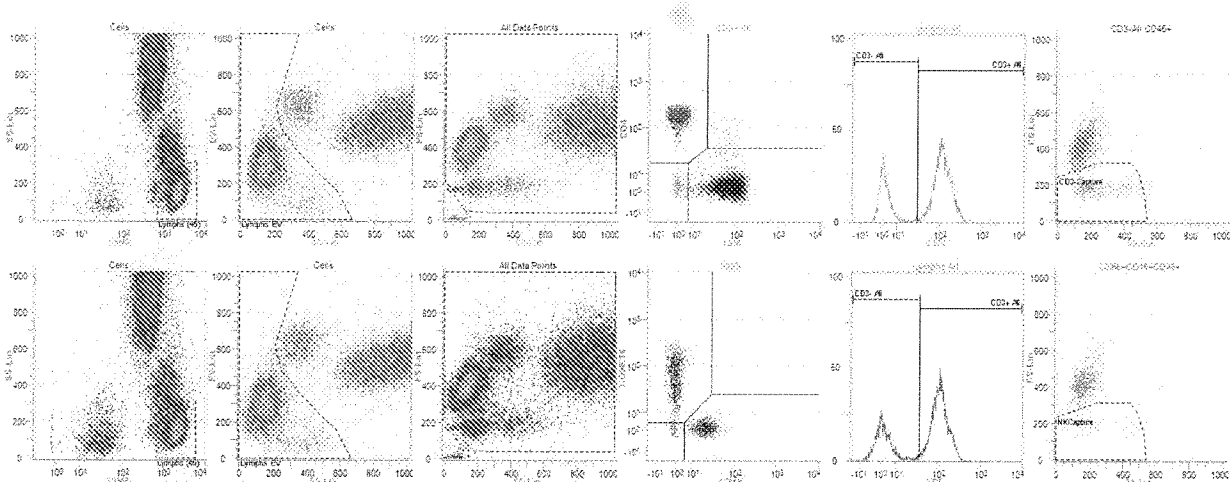
Sample ID: JMH0423003
 Run Date: 23 Apr 2014 14:22:49
 User: CQA1
 Specimen Type:
 Patient ID:
 Status:
 Gender:
 Physician:
 Instrument Serial #: AW13001

Test: Tetra Combo v1.0
 Analysis Date: 23 Apr 2014 14:24:39
 Collect Date:
 Patient Name:
 Gov. ID:
 Location:
 Date Of Birth:
 Physician Code:

Run Flags:

Run Notifications:

Comments:



Result	Value	Flag	Normal Range	Action Range
Average CD3+ (T Cells) Percent	70.35%			
Average CD3+ (T Cells) Count/uL	1,208			
CD3+/CD4+ (Helper T Cells) Percent	28.93%			
CD3+/CD4+ (Helper T Cells) Count/uL	497			
CD3+/CD8+ (Suppressor T Cells) Percent	39.23%			
CD3+/CD8+ (Suppressor T Cells) Count/uL	675			
CD3-/CD19+ (B Cells) Percent	14.50%			
CD3-/CD19+ (B Cells) Count/uL	249			
CD3-/CD56+CD16+ (NK Cells) Percent	14.19%			
CD3-/CD56+CD16+ (NK Cells) Count/uL	243			
CD4:CD8 Ratio	0.74			
*Total Lymphocytes (T+B+NK) Percent	98.55%			95.00%-105.00%
*CD3+ Reliability Check	2.69%			-5.00%-5.00%
*CD3+ Intrapanel Check	0.98%			-3.50%-3.50%

FIG. 12

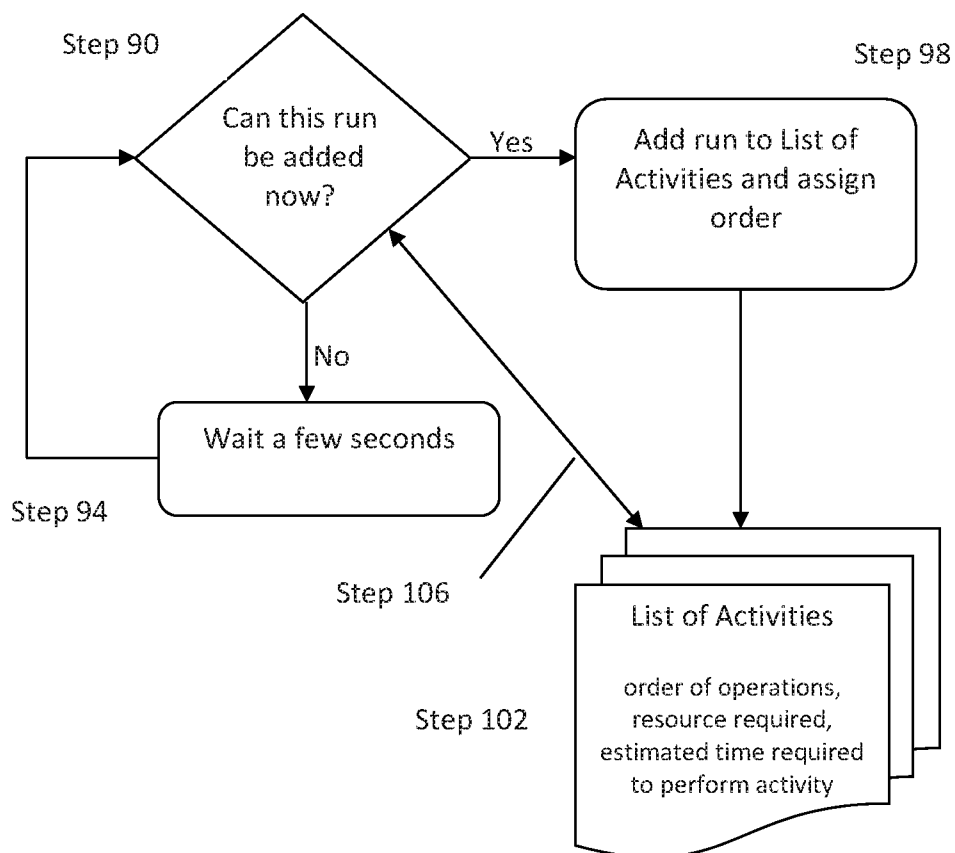


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/034435

A. CLASSIFICATION OF SUBJECT MATTER INV. G01N35/00 G01N15/14 G01N1/31 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/186228 A1 (DEKA CHIRANJIT [US]) 20 November 2014 (2014-11-20) paragraphs [0023], [0029], [0035], [0048], [0052] - paragraphs [0056], [0061] - [0064], [0066] - [0068]; figures 1a, 2, 5, 7, 8a, 8b	1-8, 11-15, 20
X	US 2005/044110 A1 (HERZENBERG LEONORE [US] ET AL) 24 February 2005 (2005-02-24) paragraph [0056] - paragraphs [0061], [0071], [0072], [0091], [0113] - [0115], [0119], [0125], [0127], [0130], [0135], [0136]; claim 20 ----- -/-	1, 4-7, 11-17, 20
☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 29 August 2016		Date of mailing of the international search report 06/09/2016
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016		Authorized officer Lokajova, Jana

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/034435

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/006303 A2 (NODALITY INC [US]; PUTTA SANTOSH K [US]) 14 January 2010 (2010-01-14) paragraphs [1026], [1033] - paragraphs [1038], [1086] - [1088], [1090]; figures 1,2 -----	1,9-11, 18,19
A	US 2013/224851 A1 (LJUNGMANN OYSTEIN HELGE [NO] ET AL) 29 August 2013 (2013-08-29) paragraph [0138] - paragraph [0157]; figures 11I,11J,11N,11O -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/034435

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014186228 A1	20-11-2014	EP 2997363 A1 US 2016061711 A1 WO 2014186228 A1	23-03-2016 03-03-2016 20-11-2014
US 2005044110 A1	24-02-2005	NONE	
WO 2010006303 A2	14-01-2010	GB 2474613 A US 2010042351 A1 US 2012215481 A1 WO 2010006303 A2	20-04-2011 18-02-2010 23-08-2012 14-01-2010
US 2013224851 A1	29-08-2013	AU 2012370530 A1 CN 104272083 A EP 2817606 A1 HK 1201917 A1 JP 2015508177 A US 2013224851 A1 WO 2013125959 A1	18-09-2014 07-01-2015 31-12-2014 11-09-2015 16-03-2015 29-08-2013 29-08-2013