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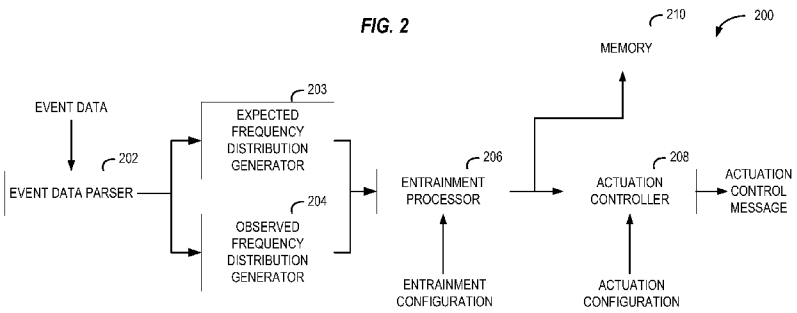
FIG. 2



(57) Abstract: Methods and systems are disclosed for generating an entrainment factor in a flow cytometry sample. The methods comprise flowing a sample with a series of particles through the flow cytometer, detecting events and calculating an expected frequency of those events based on a distribution, such as a Poisson distribution, and measuring an observed frequency of particle events. An entrainment factor may be generated from a ratio of observed event frequency to expected event frequency. Further adjustment to the flow cytometer maybe performed based on the indicated entrainment factor such as adjusted sorting bias.

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METHODS AND SYSTEMS FOR ASSESSING SAMPLE BEHAVIOR IN A FLOW CYTOMETER

CROSS-REFRENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit to U.S. Provisional Application No. 61/759,878, filed on February 1, 2013, entitled "Methods and Systems for Assessing Sample Behavior in a Flow Cytometer," the disclosure of which is hereby incorporated by reference in its entirety.

BACKGROUND

Technical Field

[0002] This disclosure relates to relates generally to the field of flow cytometry and more particularly to sample analysis methods.

Background

[0003] Particle analyzers, such as flow and scanning cytometers, are analytical tools that enable the characterization of particles on the basis of optical parameters such as light scatter and fluorescence. In a flow cytometer, for example, particles, such as molecules, analyte-bound beads, or individual cells, in a fluid suspension are passed by a detection region in which the particles are exposed to an excitation light, typically from one or more lasers, and the light scattering and fluorescence properties of the particles are measured. Particles or components thereof typically are labeled with fluorescent dyes to facilitate detection. A multiplicity of different particles or components may be simultaneously detected by using spectrally distinct fluorescent dyes to label the different particles or components. In some implementations, a multiplicity of photodetectors, one for each of the scatter parameters to be measured, and one for each of the distinct dyes to be detected are included in the analyzer. The data obtained comprise the signals measured for each of the light scatter parameters and the fluorescence emissions.

[0004] Cytometers may further comprise means for recording the measured data and analyzing the data. For example, data storage and analysis may be carried out using a computer connected to the detection electronics. For example, the data can be stored in tabular form, where each row corresponds to data for one particle, and the columns correspond to each of the measured parameters. The use of standard file

formats, such as an "FCS" file format, for storing data from a flow cytometer facilitates analyzing data using separate programs and/or machines. Using current analysis methods, the data typically are displayed in 2-dimensional (2D) plots for ease of visualization, but other methods may be used to visualize multidimensional data.

[0005] The parameters measured using a flow cytometer typically include the excitation light that is scattered by the particle along a mostly forward direction, referred to as forward scatter (FSC), the excitation light that is scattered by the particle in a mostly sideways direction, referred to as side scatter (SSC), and the light emitted from fluorescent molecules in one or more channels (range of frequencies) of the spectrum, referred to as FL1, FL2, etc., or by the fluorescent dye that is primarily detected in that channel. Different cell types can be identified by the scatter parameters and the fluorescence emissions resulting from labeling various cell proteins with dye-labeled antibodies.

[0006] Both flow and scanning cytometers are commercially available from, for example, BD Biosciences (San Jose, Calif.). Flow cytometry is described in, for example, Landy et al. (eds.), Clinical Flow Cytometry, Annals of the New York Academy of Sciences Volume 677 (1993); Bauer et al. (eds.), Clinical Flow Cytometry: Principles and Applications, Williams & Wilkins (1993); Ormerod (ed.), Flow Cytometry: A Practical Approach, Oxford Univ. Press (1997); Jaroszeski et al. (eds.), Flow Cytometry Protocols, Methods in Molecular Biology No. 91, Humana Press (1997); and Practical Shapiro, Flow Cytometry, 4th ed., Wiley-Liss (2003); all incorporated herein by reference. Fluorescence imaging microscopy is described in, for example, Pawley (ed.), Handbook of Biological Confocal Microscopy, 2nd Edition, Plenum Press (1989), incorporated herein by reference.

[0007] Fluorescence-activated cell sorting or particle sorting is a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of particles into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It records fluorescent signals from individual cells, and physically separates cells of particular interest. The acronym FACS is trademarked and owned by Becton Dickinson.

[0008] The particle suspension is placed near the center of a narrow, rapidly flowing stream of liquid. The flow is arranged so that on the average (Poisson distribution) there is a large separation between particles relative to their diameter. A vibrating mechanism causes the stream of particles to break into individual droplets.

The system is adjusted so that there is a low probability of more than one particle being in a droplet. Just before the stream breaks into droplets the flow passes through one or more laser intersects where the fluorescent character of interest of each particles are measured. If a particle is to be collected, a charge is applied to the flow cell during the period of time one or more drops form and break off from the stream. These charged droplets then fall through an electrostatic deflection system that diverts droplets into target containers based upon the charge applied to the droplet.

[0009] A sample can include thousands if not millions of cells. Cells may be sorted to purify a sample to the cells of interest. The sorting process can generally identify three varieties of cells: cells of interest, cells which are not of interest, and cells which cannot be identified. In order to sort cells with high purity (e.g., high concentration of cells of interest), droplet generating cell sorters typically abort cells that are too close to another unwanted cell electronically and thereby reduce contamination of the sorted populations. Highly discriminate sorting can lead to a reduced number of cells for analysis. In addition, the time needed to perform the detailed sorting may be higher than less strict sorting.

[0010] Furthermore, in samples of adherent cells, or antigen presenting cells such as monocytes and dendritic cells or those that have been processed in a manner that increases cell to cell interaction, this can often lead to poor recovery. In a well dispersed sample, the distribution of cells is random and follows the statistics of a Poisson distribution. As noted by Lindmo (1981) by measuring the time interval between events it is possible to evaluate the deviation from the ideal distribution. Accordingly there is a need to assess in 'real time' sample behavior to determine whether a sample is well distributed.

SUMMARY

[0011] The systems, methods, and devices of the disclosure each have several innovative aspects, no single one of which is solely responsible for the desirable attributes disclosed herein.

[0012] In one innovative aspect, a method for generating an entrainment factor for a flow cytometer sample is provided. The method includes flowing the sample comprising a series of particles through the flow cytometer wherein each particle is associated with a signal. The method includes detecting a series of events with a detection system in a first time interval from a stream formed from the flow

cytometer. The method further includes calculating an expected frequency of events in the first time interval based on a characterizing distribution such as a homogenous Poisson distribution. The method also includes measuring an observed frequency of events within a second time interval, wherein the second time interval is within the first time interval. The method includes generating the entrainment factor based at least in part on a ratio of the observed frequency to the expected frequency.

[0013] In another innovative aspect, a method for generating sample behavior information for a flow cytometer sample is provided. The method includes flowing the sample comprising a series of particles through the flow cytometer wherein each particle is associated with an event and the particles are distributed in a fluidic stream. The method includes detecting a series of events and a corresponding arrival time for each event. The method includes determining a series of inter-arrivals times relative to a previous event within the series and an associated probability of occurrence of the inter-arrival times relative to a length of a specified time interval. The method also includes calculating an expected frequency of events over the series of inter-arrival times for a portion of the fluidic stream having a length equal to the specified time interval, the calculating based on a predetermined distribution such as a Poisson distribution. The method includes measuring an observed frequency of events over the series of inter-arrival times within the portion of the fluidic stream. The method also includes generating the sample behavior information based at least in part on a ratio of the observed frequency to the expected frequency of events over the series of inter-arrival times for the portion of the fluidic stream.

[0014] In either of the innovative methods described, the stream may be comprised of regularly spaced droplets. In some implementations of the methods, the method may include comparing the entrainment factor to a predetermined value (e.g., greater than 1) and actuating a corrective action in a control system operationally connected to the flow cytometer based on a result of the comparing. An example of a corrective action includes halting the flow of the sample in the flow cytometer.

[0015] Some implementations of the method may include sorting particles in the stream. In such implementations, the corrective action may include adjusting the sorting based on the entrainment factor.

[0016] The innovative methods may generate the entrainment factor while the sample is flowing in the flow cytometer.

[0017] Time intervals in accordance with the innovative methods may be measured in a variety of ways. One example time interval unit is fractions of a droplet.

[0018] In some implementations, the entrainment factor is one of a plurality of entrainment factors calculated during successive time intervals for a flow cytometer sample.

[0019] The sample may include multiple labeled particles. For example, the sample may include peripheral blood cells wherein the peripheral blood cells as are comprised of a first and second component labeled with a first and second label. In implementations with multiple components, it may be desirable to generate an entrainment factor for each component. In such implementations, the method may include generating the entrainment factor for the first component and another entrainment factor for the second component.

[0020] In a further innovative aspect, a flow system for providing a sample entrainment factor is provided. The system includes a fluidics system. The fluidics system may include a sample tube and a moving fluid column within the sample tube in which particles of a sample move along a common sample path. The system includes a detection system for collecting a signal from each particle as it passes one or more detection stations along the common sample path. Each signal is assigned a signal value to form a data point for each particle. The detection system collects a succession of such data points in a first time interval. The system includes a control system operationally associated with the fluidics system. The control system is configured to generate a calculated signal frequency for at least a portion of the first time interval based on a Poisson distribution and the number of data points collected by the detection system during the first time interval. The control system is further configured to generate an experimental signal frequency based on the number of data points in the portion of the first time interval. The control system is further configured to compare the experimental signal frequency with that of a calculated signal frequency or a predetermined signal frequency. The control system is also configured to provide an entrainment factor for the sample, said entrainment factor based on said comparing.

[0021] The flow system may compare the experimental signal frequency by determining a ratio of the experimental signal frequency to that of the calculated signal frequency or the predetermined signal frequency.

[0022] In some implementations, the control system may, upon determining that the entrainment factor deviates from a predetermined value, actuate a corrective

action. Corrective actions may include interrupting collection of signals by the detection system, purging the sample tube by said fluidics system, altering collection of signals via acoustic focusing based on the entrainment factor, and resuming collection of the signals by the detection system. Resuming collection of the signals may occur after initiating dispersion of the sample.

[0023] In some implementations, the control system includes a first sorting configuration providing a high collection yield and a second sorting configuration providing a high collection purity. The control system may be configured to select one of the first sorting configuration or the second sorting configuration based on the entrainment factor.

[0024] In a further innovative aspect, a non-transitory computer readable medium including instructions executable by a processor of an electronic device may be provided. The instructions, upon execution by the processor, may cause the electronic device to perform one or more of the innovative methods described above.

BRIEF DESCRIPTION OF DRAWINGS

[0025] Several advantages of the present invention will be apparent upon consideration of the following detailed description taken in conjunction with the accompanying drawings, in which:

[0026] **FIG. 1** shows a functional block diagram for one example of an electronic device for processing cytometry data behavior information.

[0027] **FIG. 2** shows a functional block diagram for one example of a sample behavior analyzer.

[0028] **FIG. 3** shows a process flow diagram of an example method of sample behavior analysis.

[0029] **FIG. 4** shows plot diagrams for an example set of event data which does not exhibit clumpiness.

[0030] **FIG. 5** shows plot diagrams for an example set of event data which exhibits clumpiness.

[0031] **FIG. 6** shows a functional block diagram for one example of a sorting device including an entrainment factor feedback flow controller.

[0032] **FIG. 7** shows a reconstruction of event distribution in a portion of a stream which illustrates how the particles may be distributed within the portion of the stream.

[0033] **FIG. 8** shows a functional block diagram of a flow system for providing a sample entrainment factor.

DETAILED DESCRIPTION

[0034] The features described allow assessment, either automated or manually, of sample behavior using a simplified dispersion index. One aspect included to facility the assessment is an "entrainment factor." When analyzing different samples, the utility of the disclosed features provide a non-limiting advantage of distinguishing detected sample performance characteristics arising from sample behavior from those intrinsic to the sorter.

[0035] The present invention describes methods and systems for determining an entrainment factor in a flow cytometer sample comprising flowing a sample comprising a series of particles through the flow cytometer wherein each particle is associated with a signal and the particles are distributed in a fluidic stream that in some embodiments ultimately forms a series of regularly spaced droplets, detecting a series of signals with an inter-arrival time relative to the previous particle with the probability of occurrence relative to the length of a specified time interval, calculating the expected frequency of events in the time interval based on an Poisson (such as an ideal homogenous Poisson) distribution, measuring the observed frequency of events within a that time interval, and calculating a first entrainment factor wherein the first entrainment factor is determined from the ratio of observed frequency to expected frequency.

[0036] In some embodiments method further comprises actuating a corrective action in a control system operationally connected to the flow cytometer when the entrainment factor deviates from a predetermined value such as greater than 1. In some embodiments the corrective action may comprise halting the flow of the sample in the flow cytometer, purging the flow cytometer, initiating a user interface signal or the like. The entrainment factor may be calculated while the sample is flowing in the flow cytometer, or after the flow is completed. In some embodiments the time intervals are measured in fractions of a drop. In some embodiments the first entrainment factor is one of a plurality of entrainment factors calculated during successive time intervals for a

flow cytometer sample. The sample may comprise a biological sample such as peripheral blood cells.

[0037] Some implementations may include a fluidics system comprising a sample tube and a moving fluid column within the sample tube in which particles of a sample move along a common sample path. The fluidics system may also include a detection system for collecting a signal from each particle as it passes one or more detection stations along the common sample path, each signal being assigned a signal value to form a data point for each particle. The detection system may collect a succession of such data points in a first time interval. A control system may be operationally associated with the fluidics system. The control system may be configured to calculate at least a calculated signal frequency for a second time interval based on a Poisson distribution and the number of data points collected by the detection system during the first time interval and an experimental signal frequency based on the number of data points in the second time interval. The control system may be further capable of comparing the experimental signal frequency with that of a calculated signal frequency or a predetermined signal frequency in order to determine an entrainment value. The entrainment value may be determined based on the ratio of the experimental signal frequency to that of calculated signal frequency or the predetermined signal frequency wherein the second time interval is a subset of the first time interval. In some embodiments the entrainment value may deviates from a predetermined value and the control system may actuate a corrective action. The corrective action may be any action such as carrying out one or more cycles of interrupting collection of signals by the detection system, purging the sample tube by said fluidics system, and resuming collection of the signals by the detection system. In some embodiments resuming collection of the signals occurs after steps are taken to disperse the sample. Sample dispersion may be by any means such as acoustic focusing.

[0038] Aspects described herein provide systems and methods to assess sample behavior using a dispersion index and the concept of "entrainment factor" as a metric for sample quality. Systems and methods are demonstrated using different samples, and the utility of this tool in distinguishing performance issues arising from sample behavior from those intrinsic to the sorter is discussed. Implementations including one or more of the described features serve as an effective means to generally analyze data (e.g., cytometer data) to determine how "Poisson" a population actually is during a cell sorting or analysis process, thereby serving as a metric to aid in

improvement of sample preparation. Aspects described may also be used to inform a user on why a classified population may be exhibiting poor performance and contributing to a disappointing sort yield.

[0039] As used herein, the terms set forth with particularity below have the following definitions. If not otherwise defined in this section, all terms used herein have the meaning commonly understood by a person skilled in the arts to which this invention belongs.

[0040] As used herein, "system" and "instrument" and "apparatus" generally encompass both the hardware (e.g., mechanical and electronic) and associated software (e.g., computer programs) components.

[0041] As used herein, "Poisson distribution" refers to a discrete frequency distribution that indicates the probability of a number of independent events occurring in a fixed time.

[0042] As used herein, "entrain" identifies the degree of aggregation, clumping or other non-random association of particles.

[0043] As used herein, an "event" generally refers to the data measured from a single particle, such as cells or synthetic particles). Typically, the data measured from a single particle are include a number of parameters, including one or more light scattering parameters, and at least one fluorescence intensity parameters. Thus, each event is represented as a vector of parameter measurements, wherein each measured parameter corresponds to one dimension of the data space.

[0044] As used herein, a "population", or "subpopulation" of particles, such as cells or other particles, generally refers to a group of particles that possess optical properties with respect to one or more measured parameters such that measured parameter data form a cluster in the data space. Thus, populations are recognized as clusters in the data. Conversely, each data cluster generally is interpreted as corresponding to a population, although clusters that correspond to noise or background typically also are observed. A cluster may be defined in a subset of the dimensions, e.g., with respect to a subset of the measured parameters, which corresponds to populations that differ in only a subset of the measured parameters.

[0045] As used herein, a "gate" generally refers to a set of boundary points identifying a subset of data of interest. In cytometry, a gate may bound a group of events of particular interest. As used herein, "gating" generally refers to the process of classifying a population by defining a gate for a given set or subset of data.

[0046] As briefly discussed above, dot-plots are one way to visualize the data produced by the cytometer. When displaying and analyzing data, histogram, bin plot, 2D density, and/or contour plots may also be used.

[0047] The sorting of cells from a flow cytometer is a challenging task, made more so by the tendency of some cell preparations to result in the clumping or aggregation of cells or particles. Sorting efficiency in a series of droplets from a flow cytometer may show Poisson distribution in an ideal scenario, where the efficiency can be estimated by Equation (1) below:

$$Efficiency = e^{-(rate*(1-fraction)*\frac{d}{f})} \quad (1)$$

where: d is the drop packet defined as the time in drop units;

f is the frequency of the drop drive in drops per second;

rate is the sample rate in events per second; and

fraction represents the portion of all events for the population of interest.

[0048] Many samples processed through a flow cytometer do not show a Poisson distribution. Without being bound to a particular theory, a non-Poisson distribution may be the result of many factors such as the entrainment (or clumping) of cells. It is not uncommon for investigators to "lose" a larger portion of cells than had been expected or desired due to an undesirable entrainment of desired cells with unwanted cells. In some embodiments, measurement of how "Poisson" a sample may actually be can be achieved by determining a variance from homogenous Poisson behavior for a series of events detected by flow cytometry. In one implementation, where the variance to mean ratio is 1.0, then the sample may be identified as behaving in a Poisson manner.

[0049] In some embodiments a sample may contains two or more particle types (e.g., monocytes, lymphocytes or any other cellular component) and each particle type may be labeled in a manner that provides for a distinct detectable signal. The different cellular components of a sample may exhibit different aggregation behavior. The aspects described may provide for the determination of specific entrainment factors for each distinct signal generating particle. As one non-limiting benefit, these features

provide a way to understand of the behavior of distinct populations of particulates or cellular types in a sample.

[0050] In some embodiments the observed number of events within a given time interval is measured. The expected frequency of events in the same time interval is calculated based on an ideal Poisson distribution. By calculating the ratio of observed events to expected frequency of events for this or any other time interval we may obtain an "entrainment factor." This value identifies a measure of the clumpiness of the sample and the deviation from Poisson. The greater level of clumpiness indicated by the entrainment factor, the worse the sample behavior in flow.

[0051] Accordingly, the expression of the entrainment factor may be implemented in a variety of ways. For example, in one implementation a factor of 1.0 indicates Poisson samples while a factor greater than 1.0 identifies clumpy/entrained samples. In another implementation, the factor may be expressed with a zero base. In such an implementation a factor of 0 may indicate no clumps (e.g., Poisson) while positive values indicate a degree of clumpiness. In some implementations, level of clumpiness may be scaled, such as to 100. In such implementations, the factor may indicate a percent clumpiness for a sample. In some implementations, higher numerical values may indicate more Poisson samples and the lower the value the higher the clumpiness. The discussion here should highlight that the expression of the entrainment factor may be accomplished in a variety of ways without departing from the spirit of the entrainment factor which is to convey an indication of clumpiness.

[0052] Particle arrival time may be measured with a high degree of accuracy using intrinsic time stamps in firmware with a specified drop resolution. For example from $1/16^{th}$ of a drop, arrival time may be measured to 1.5625 microseconds at 40 KHz using BD Influx™ cell sorter available from Becton, Dickinson and Company, to 0.1 microseconds using BD FACSAria™ III cell sorter available from Becton, Dickinson and Company, or to 17.625 nanoseconds represented via a 48 bit time stamp using BD FACSJazz™ cell sorter also available from Becton, Dickinson and Company.

[0053] The arrival time may be included in the event frame of each processed event as a time stamp. In some implementations, such as in the case of the Influx™ or FACSJazz™, additional information may be provided such as the distance to the previous event in time. The data may be binned and fitted as a Poisson exponential distribution. The probability of another event occurring within time bins related to drop boundaries can then be obtained based on traditional Poisson estimates

(Pinkle and Stovel, 1985). A ratio of observed probability versus an expected probability for different relevant time spans provided an "entrainment factor" metric. As discussed, one example entrainment factor is such that the result would be 1.0 for true Poisson distributions and significantly greater for those particles that entrain in a non-random manner.

[0054] The behaviors of different sample/cell types, and of subpopulations within heterogeneous samples such as peripheral blood mononuclear cells (PBMCs), show entrainment behaviors in flow that range from true homogenous Poisson distributions to non-random entrainment significantly affecting sorting performance and reducing sort yield (e.g., number of cells for which a sorting decision was reached). Consider one sample which, in applying Equation (1), can have the efficiency expression shown in Equation (2).

$$p = 1.0 - e^{\frac{-rd}{f}} \qquad (2)$$

[0055] In the example sample the expected rate r is 714 events per second for a drop packet d of 1 at the frequency f of 40,400 Hz. In this example, there is only one population of interest and thus, the fraction term is not necessary. Event data for a sample can be acquired from the cytometer during a test. In one sample, we observed 829 events with delta times of 1 drop or less in sample of 10,000 events. The delta time represents a period of time for a given event to a previous event. In some implementations, the delta time measures the time to a previous event of the same type (e.g., same particle detection). In some implementations, the delta time measures the time to the previous event, irrespective of event type. In the example discussed above, the unit of time used is the number of drops. Units such as microseconds, absolute time, or other representations of time may be used within the methods and systems described. The resulting frequency for the observed sample is 0.0829. However, 175 events out of 10,000 (e.g., 0.0175 * 10000) with a delta time of 1 drop or less is expected based on a Poisson distribution of events and a sample rate of 714 per second.

[0056] By this example, the observed probability is 0.0829 while the expected probability for a Poisson distribution is 0.0175. In implementations where the entrainment factor is expressed as a ratio of the observed probability to the expected probability, the entrainment factor would be 0.0829 / 0.0175 or 4.73. If the sample exhibited the expected distribution, the ratio would yield an entrainment factor of 1.0

(e.g., 0.0175 / 0.0175). However, in the example sample discussed above, the entrainment factor is 4.73, nearly five-fold larger than if a homogenous Poisson process were in play.

[0057] **FIG. 1** shows a functional block diagram for one example of an electronic device for processing cytometry data behavior information. The electronic device **100** may be configured to implement one or more aspects of the processes described herein.

[0058] In some implementations, the electronic device **100** may be a cytometer. It will be appreciated that other elements included in such an implementation are not shown in **FIG. 1**. In some implementations, the electronic device **100** may be configured to communicate with a cytometer to provide behavior analysis and control messages based thereon.

[0059] To facilitate communication with a cytometer, the electronic device **100** includes a receiver **102**. The receiver **102** is configured to receive information for further processing by the electronic device **100** such as sample behavior analysis. The receiver **102** may receive raw flow cytometry data. The receiver **102** may also receive parameters to control the operational characteristics of the electronic device **100**. The receiver **102** may be configured to receive messages from another electronic device such as a tablet computer, a personal computer, or a smartphone.

[0060] The receiver **102** may be implemented as a wired or wireless receiver. In a wireless implementation, the receiver **102** may include an antenna and a signal processor. In a wired implementation, the receiver **102** may include one or more of a network interface, physical connections (e.g., Ethernet, USB, HDMI, telephone, etc.), and an application programming interface to the various features described.

[0061] To further facilitate communications with a cytometer and other electronic processing systems, the electronic device **100** may include a transmitter **104**. The transmitter **104** may be configured to transmit information generated or otherwise acquired by the electronic device **100**. One example of information that may be transmitted by the transmitter **104** is behavior analytics such as an entrainment factor and/or actuation messages based thereon.

[0062] The transmitter **104** may be configured to format the data for transmission. The formatting may include one or more of packetization, encapsulation (e.g., in a machine readable format such as XML; JSON; delimited text; binary format such as bitmap or other image file format; and the like), encryption, and compression.

[0063] The transmitter **104** may be configured for wired or wireless transmission. In a wireless implementation, the transmitter **104** may include a signal generator, amplifier, and antenna. In a wired implementation, the transmitter **104** may include one or more of a network interface and physical connections (e.g., Ethernet, USB, HDMI, telephone, etc.).

[0064] The electronic device **100** may include a memory **106**. The memory **106**, which may include read-only memory (ROM) and/or random access memory (RAM), may store information received by the electronic device **100**. The memory **106** may also store information generated by the electronic device **100**. A portion of the memory **106** may also include non-volatile random access memory (NVRAM).

[0065] The electronic device **100** may also include a processor **108**. The processor **108** may control and/or coordinate operation of the electronic device **100**. In some implementations, the processor **108** may be referred to as a central processing unit (CPU). The processor may be configured to transmit messages to one or more of the components of the electronic device **100**. The processor may also be configured to receive messages from one or more of the components of the electronic device **100**.

[0066] The processor **108** may be implemented with any combination of general or special purpose microprocessors, microcontrollers, digital signal processors (DSPs), field programmable gate array (FPGAs), programmable logic devices (PLDs), controllers, state machines, gated logic, discrete hardware components, dedicated hardware finite state machines, or any other suitable entities that can perform calculations or other manipulations of information.

[0067] The memory **106** may provide instructions and data to the processor **108**. The processor **108** may be configured to perform logical and arithmetic operations based on program instructions stored within the memory **106**. The instructions in the memory **106** may be executable to implement the methods described herein.

[0068] The electronic device **100** shown in **FIG. 1** includes a sample behavior analyzer **200**. The sample behavior analyzer **200** is shown as receiving sample event data. The sample event data may include event information such as mean fluorescent intensity for an event, event time relative to a point in time for the test (e.g., test start or drop interval), and a delta time. In some implementations, these inputs may be stored in the memory **106**. In some implementations, the input is received externally such as via the receiver **102** based on a received message.

[0069] The sample behavior analyzer **200** may be configured to generate behavior identification information for the sample or a portion of the sample. One example of behavior identification information is an entrainment factor. The behavior identifier may be based on the received event data where the actual event data is compared to an ideal statistical distribution for the event data. The sample behavior analyzer **200** may be configured to store the generated histogram in the memory **106**. Further details of the sample behavior analyzer **200** will be presented in reference to **FIG. 2** below.

[0070] In some implementations, the behavior identification information may include an actuation control message. The actuation control message may include information identifying the behavior and one or more flow adjustments to improve the sample test behavior.

[0071] The electronic device **100** includes a flow controller **600**. The flow controller **600** is configured to adjust the cytometric flow for a sample / test. Examples of the adjustments are described below with reference to **FIG. 6**. The flow controller **600** may receive initiation parameters as a first input. The initiation parameters indicate the initial flow configuration for a sample. The flow controller **600** shown also receives behavior identification information that it may use to adjust the initial flow configuration based on the behavior of the associated sample.

[0072] The above described elements of the electronic device **100** may be coupled by a bus **190**. The bus **190** may be a data bus, communication bus, or other bus mechanism to enable the various components of the electronic device **100** to exchange information. In some implementations, the bus **190** may facilitate the transfer of power among the elements shown. It will further be appreciated that while different elements have been shown, multiple elements shown may be combined into a single element.

[0073] **FIG. 2** shows a functional block diagram for one example of a sample behavior analyzer **200**. The sample behavior analyzer **200** received sample event data as an input. The event data collected by cytometers may vary. For example, some cytometers collect event data at a first level of resolution (e.g., number of bits) while others may collected at a higher resolution. As such, the event data may be more finely represented or include additional information from cytometer to cytometer. Because the sample behavior analyzer **200** may be analyzing event data obtained from various cytometers, the event data is provided to an event data parser **202**. Where the sample behavior analyzer 200 is included within a cytometer, the event data format can

more reliably be identified. In such implementations, the event data parser **202** may be omitted or combined in another element of the encompassing electronic device.

[0074] The event data parser **202** may be configured to determine the format of the even data and provide a standard set of event data for analytical purposes. In some implementations, the event data parser **202** may be configured to augment the received event data. For example, some cytometers may not report the so-called delta time. As such, the event parser **202** may be configured to process the received event data determine the delta time for one or more events included in the data set. The event data may further include the current flow configuration (e.g., rate, time period, etc.).

[0075] As shown in **FIG. 2**, the event data parser **202** may receive a parsing configuration as an input. The parsing configuration may include rules to indicate how to determine what parsing operation(s) are needed. This may be indicated using header fields, event data signatures, or other parsing method. The parsing configuration may further indicate the parsing operation(s) to be performed. For example, a paring configuration may include instructions which may be executed by a processor such as an executable script. The script may specify a series of transformations for the event data to generate the standardized output.

[0076] The standardized event data may then be provided to an expected frequency distribution generator **203**. The expected frequency distribution generator 203 may be configured to determine the characteristics of a set of events for a well-formed distribution such as a Poisson distribution. The type of distribution may be specified as an input to the expected frequency distribution generator **203** such as via an expectation configuration. The expectation frequency distribution generator **203** may analyze the event data to determine an expected event distribution for the rate and time period of the event data. It will be appreciated that the expected distribution may be identified for a portion of the event data, such as one drop, three drops, thirty drops, or one-sixteenth of a drop.

[0077] The standardized event data may also be provided to an observed frequency distribution generator **204**. The observed frequency distribution generator **204** may be configured to provide the actual frequency distribution for at least a portion of the event data.

[0078] The expected and observed distributions for the sample are then provided to an entrainment processor **206**. The entrainment processor **206** is configured to compare the expected and the observed distributions to generate behavior information

for the sample. In one implementation, the entrainment processor **206** may take the ratio of expected to observed as discussed above. The comparison implemented by the entrainment processor **206** may be specified using an entrainment configuration input to the entrainment processor **206**. The configuration may specify one or more aspects of behavior to analyze and include in the generated behavior information. The configuration may also specify an output format for the behavior information. For example, in an implementation where the sample behavior analyzer **200** is coupled with one or more cytometers, each cytometer may be configured to understand a specific format. Accordingly, the configuration may include static configurations (e.g., combination techniques) as well as dynamic configurations (e.g., target cytometer output format).

[0079] The entrainment processor **206** may be configured to then provide the behavior information for further processing. For example, the entrainment processor **206** may store the behavior information in a memory **210**. In some implementations, the entrainment processor **206** may utilize the memory **106** included in the electronic device **100** shown in **FIG. 1**.

[0080] The entrainment processor **206** may provide the behavior information to an actuation controller **208**. The actuation controller **208** may be configured to identify one or more adjustments to the cytometer based on the behavior information. For example, if the entrainment factor for the sample is high, it may be due to inclusion of a high purity tolerance for the test. The actuation controller **208** may provide a statistical indication of an improved yield for a given reduction in purity. In some implementations, the actuation controller **208** may be configured to automatically generate control messages, such as to reduce the flow rate for the test. The configurations may be specified as an actuation configuration input to the actuation controller **208**. The actuation configuration may include rules for adjusting the sorting logic for the test for the analyzed sample events. For example, a rule may specify that up to a 10% reduction in purity is acceptable if it results in a 20% or more increase in yield. In some implementations, the actuation configuration may increase the rate based on detection of an ordered flow and decrease the rate if a disordered flow is detected. Other examples of the adjustments are described below.

[0081] **FIG. 3** shows a process flow diagram of an example method of sample behavior analysis. The process shown in **FIG. 3** may be implemented by one or more of the devices described herein such as the electronic device **100** shown in **FIG. 1**

or the sample behavior analyzer **200** of **FIG. 2**. The method begins at node **302** where event data for a series of droplets in flow is received. The event data may be received from a flow cytometer during a test. Such provisioning proximate in time from the point of collection to the point of reception may, in some implementations, be referred to as "real-time." While the example shown in **FIG. 3** references a droplet as a unit of rate, another unit may be utilized in conjunction with the features described herein.

[0082] At node **304**, an expected event frequency for an assumed distribution is determined. One non-limiting example of an assumed distribution is a Poisson distribution. The determination may be based on the rate and timer period for at least a portion of the received event data.

[0083] At node **306**, an observed frequency for the event data is determined. The observed frequency may be determined, in one implementation, based on an applicaton of Equation (1) above.

[0084] At node **308**, an entrainment factor is generated. The entrainment factor generally describes the behavior of the sample based on the received event data. The entrainment factor may be generated based on a comparison of the expected frequency to the observed frequency. The comparison may be a ratio of the two frequencies. In some implementations, additional factors may be included such as flow configuration, originating cytometer information (e.g., type, model number, firmware version, configuration, and/or the like).

[0085] **FIG. 4** shows plot diagrams for an example set of event data which does not exhibit clumpiness. Using the example discussed above, the entrainment factor for the event data shown in **FIG. 4** would be near or at 1.0.

[0086] A first plot diagram **410** shows, on the x-axis, delta time in microseconds. The y-axis represents the number of events detected for the corresponding delta time. As shown in **FIG. 4**, very few events are detected with a delta time of zero. The most common delta time (e.g., peak point for the graph) is just under 300 microseconds. A second plot diagram **420** shows the same set of data but in log-scale form. It will be noted that this graph generally exhibits a downward slope as delta time increases.

[0087] **FIG. 5** shows plot diagrams for an example set of event data which exhibits clumpiness. The event data shown in **FIG. 5** represents a less well behaved sorted sample with an entrainment factor near 4.0.

[0088] A first plot diagram **510** shows, on the x-axis, delta time in microseconds. The y-axis represents the number of events detected for the corresponding delta time. As shown in **FIG. 5**, the most common delta time is about 225 microseconds. This shift in **FIG. 5** from the set shown in **FIG. 4** can be attributed to clumping in the set in **FIG. 5**.

[0089] The shift becomes more apparent when comparing a second plot diagram **520** to the second plot diagram **420**. As with the second plot diagram **420**, the second plot diagram **520** shows the event data of the first plot diagram **510** in log-scale form. An initial group of events **530** are detected with delta times near zero. This group **530** is missing from the set shown in **FIG. 4**.

[0090] **FIG. 6** shows a functional block diagram for one example of a sorting device including an entrainment factor feedback flow controller. The sorting device illustrated in **FIG. 6** may be referred to as stream-in-air sorting system. This and other sorting devices are described in U.S. Patent No. 8,140,300 which is commonly owned and assigned, the disclosure of which is incorporated by reference in its entirety.

[0091] Particles in sample stream **602** are shown. The sample stream **602** passes through an orifice **604** of a nozzle **606**. The sample stream **602**, upon exiting the nozzle **606** forms a jet **608**. A laser beam **610** is generated by a laser **611** to illuminate the particles that may be included in the jet **608**. The light is detected by a detection station **612** to generate multiple signals that are processed to generate a multiparameter event data point. The generated event data may be provided to a sample behavior analyzer. The providing may be direct, via communication intermediaries (e.g., network/cloud), via memory, or a hybrid configuration.

[0092] Based on the values of the multiple signals, prior to droplets leaving jet **608**, they are positively charged, negatively charged, or left neutral. Some droplets will include a particle of the sample as shown by a droplet **630** while other droplets will not include a particle of the sample as shown by a droplet **640**. Droplets pass through a deflection field **614**. The deflection field **614** includes two oppositely charged deflection plates **616a** and **616b**. The deflection plates **616a** and **616b** are configured to steer charged droplets in the jet **608** to their respective collection vessels **618a**, **618b**, or **618c**. As shown, vessel **618b** collects negatively charged droplets because the positive deflection plate **616a** will attract negatively charged droplets. Similarly, vessel **618c** will collect positively charged droplets because the negatively charged deflection plate **616b** will attract positively charged droplets.

[0093] In one collection scheme, the flow system identifies all particles of interest as they pass the detection station **612** based on the values of their signals, and then causes jet **608** to be charged or neutral at the instant the particle of interest leaves jet **608** as a droplet. In this way, particles of interest are caused to have the same charge. This allows collection of the particles in the same collection vessel.

[0094] Occasionally multiple particles pass the detection station **612** in close proximity (e.g., clumping). As discussed, entrained events produce signals that may not be distinguishable by the flow system. Such coincident events are generally undesirable and can lead to rejection of the droplet containing the particle of interest. Such rejection can impact the yield of the sort (e.g., number of cells positively identified as a cell of interest).

[0095] **FIG. 7** shows a reconstruction of event distribution in a portion of a stream which illustrates how the particles may be distributed within the portion of the stream. The stream **700** for the example test shown generally includes four types of particles, target classification particles, abort classification particles, unclassified particles, and particles which experienced an error during classification. The particles may appear alone or, in some circumstances, as clumps. In **FIG. 7**, a first set of particles **702** including just one particle of the target classification is shown. Continuing down the stream, a second set of particles **704** is shown including just one particle of the abort classification. A third set of particles **706** is shown including two unclassified particles and one error particle. The third set of particles **706** may be considered clumpy (e.g., entrained). A fourth set of particles **708** including one target particle is shown next in the stream **700**. As can be seen thus far, as the stream progresses so does time. The first portion of the stream **700** including sets **702**, **704**, **706**, and **708** may represent events appearing in a single droplet. The time between the arrival of the first target particle (e.g., set **702**) and the second target particle (e.g., set **704**) may be used to characterize the delta time.

[0096] The stream **700** continues with a fifth set of particles including two unclassified particles and one abort classification particle. A set **712** including a target classification particle and another set **714** including an error particle follow. A set **716** includes an abort particle and an error particle. In this case, the sorting may be configured to disregard the set **716** as it includes an error. In some implementations, where the sorting bias is adjusted such as based on the sample behavior analysis, the sorting may be configured to sort the set to the aborted collection container. A similar

logic may be applied to a final set shown in **FIG. 7**, set **718**. The set **718** includes one unclassified particle and one target particle. For high purity sorting, this set **718** may be directed away from the target collection vessel even though it includes the target particle. For high yield sorting, the set **718** may be directed to the target collection vessel. The stream **700** is simply an example of how particles may flow through the sorting device shown in **FIG. 6**. The space between sets may be greater or less than shown. Furthermore, there may be more or fewer classifications for particles depending on the test being performed.

[0097] The flow controller **600** receives one or more actuation messages. The flow controller **600** may receive one or more actuation control messages from the behavior analyzer. Based on a received actuation control message, the flow controller may adjust one or more elements of the cytometer. For example, the flow controller **600** may receive an actuation control message indicating low levels of clumpiness. In these circumstances, the sample may be aligning within the flow medium. In the event of such alignment, the rate of through put may be increased without sacrificing the purity of the sort. Accordingly, the flow controller **600** may adjust the cytometer to increase the pressure on the flow medium and/or sample to increase the rate at which the sample is introduced into a drop.

[0098] The actuation messages may be generated by the behavior analyzer based on the provided event data. As shown in **FIG. 6**, the flow controller **600** is in communication with various elements included in the sorting device. As such, the flow controller **600** may process an actuation message and adjust an operational characteristic of one or more elements. For example, the flow controller may adjust the laser **611** to change a property of the laser beam **612**. Examples of properties which may be adjusted include wavelength, timing of illumination, duration of illumination, angle of illumination, spread, and the like.

[0099] The flow controller **600** is shown in communication with the nozzle **606**. The flow controller may adjust one or more characteristic of the nozzle **606** such as the size of the orifice, the pressure of either or both of the sample particles or the fluid in which the particles will be included to form droplets, or the charge to be applied to droplets.

[0100] The flow controller **600** is shown in communication with a deflection processor **620**. The deflection processor **620** is configured to control the charge delivered by the deflection plates **616a** and **616b**. The flow controller **600** may be

configured to provide information to the deflection processor **620** to adjust the charge based on the sample behavior. For example, the strength of a charge may be adjusted such that one or both plates have higher or lower levels of attraction. In a test where purity is important, the strength may be precisely calibrated to attract only those particles of interest. However, if yield is important at the expense of a lower purity, the charge may be increased to allow the sorting more particles.

[0101] The flow controller **600** is shown in communication with the detection station **612**. The operation of the detection station **612** can be adjusted by the flow controller **600** in response to a received actuation control message. For example, the timing of detection, the resolution of detection, or the area of detection may be adjusted. By adjusting the detection to a less rigorous scheme, the speed at which the detection station **612** can operate may be increased due to the reduced complexity of information processing performed. This can provide another non-limiting advantage of reducing the power resources consumed during sample processing.

[0102] It will be understood that the flow controller **600** may adjust multiple elements concurrently. For example, the rate may be adjusted by actuating the nozzle **606** while also adjusting the deflection processor **620**. Adjusting the rate may also necessitate the adjustment of the detection station **612** to ensure the event data is captured for the new flow rate. It will also be understood that the flow controller **600** may adjust other elements included in the cytometer based on the actuation control message such as acoustic focusing.

[0103] The adjustments by the flow controller **600** based on an actuation control message provides a way for sample behavior as expressed, for example, in delta time to impact operational characteristics of the cytometer. Conditions such as within the instrument, for the sample, and for the flow medium, may vary during the test for a sample. As these conditions change, the delta time characteristics may change. Accordingly, the flow controller **600** may be configured to perform adjustments throughout the sample test. In one instance, the operational characteristics related to sorting may be dynamically adjusted for a sample as the sample is undergoing testing.

[0104] **FIG. 8** shows a functional block diagram of a flow system for providing a sample entrainment factor. The flow system **800** shown in **FIG. 8** may be configured to perform, in whole or in part, the methods described herein such as, for example, the method of **FIG. 3**. The flow system **800** includes a fluidics system **802**. The fluidics system **802** may include or be coupled with a sample tube **810** and a

moving fluid column within the sample tube in which particles **830** of a sample move along a common sample path **820**.

[0105] The flow system **800** includes a detection system **804** configured to collect a signal from each particle as it passes one or more detection stations along the common sample path. A detection station generally refers to a monitored area **840** of the common sample path. Detection may, in some implementations, include detecting light or other property of the particles **830** as they pass through the monitored area **840**. In **FIG. 8**, one detection station **808** with one monitored area **840** is shown. Some implementations of the flow system **800** may include multiple detection stations. Furthermore, it may be desirable for a detection station to monitor more than one area.

[0106] Each signal is assigned a signal value to form a data point for each particle. As described above, this data may be referred to as event data. The detection system **804** is configured to collect a succession of such data points in a first time interval.

[0107] The flow system **800** also includes a control system **806**. The control system **806** shown is operationally associated with the fluidics system **802**. The control system **806** configured to generate a calculated signal frequency for at least a portion of the first time interval based on a Poisson distribution and the number of data points collected by the detection system **804** during the first time interval. The control system **806** is further configured to generate an experimental signal frequency based on the number of data points in the portion of the first time interval. The control system **806** additionally compares the experimental signal frequency with that of a calculated signal frequency or a predetermined signal frequency. The control system **806** then provides an entrainment factor for the sample. The entrainment factor is based at least in part on a result of the comparing.

[0108] The behavior information (e.g., entrainment factor) can be used to further process cytometric event data. In one implementation, the entrainment factor may be used to adjust the resulting cell counts. For example, the entrainment factor may be combined with the clumpy cell count to provide an adjusted cell count which, statistically speaking, reflects an expected count for the sample.

[0109] In one implementation, the entrainment factor may be used to gate the events for a sample. For example, a subpopulation of interest having an entrainment factor may be provided identified. In some circumstances, the clumpiness may be used as an indicia of certain diagnostic conditions.

[0110] In a further implementation, the entrainment factors for tests for a cytometer may be tracked over time. Over time, the level of entrainment may change based on a variety of conditions which may be attributed to the cytometer. Where the entrainment information is tracked over time, a trend may be identified whereby the level of clumping in increasing for the cytometer. The increase may be an increase as compared to similar model cytometers, increase as compared to an average for the cytometer, or other comparison metric. Upon exceeding a configurable threshold, the cytometer may provide an indication that maintenance is needed to address the clumping. Such maintenance may include cleaning, recalibration, or other corrective action which can reduce the clumping for samples. In some implementations, the maintenance may be automatically performed such as by a processor configured to monitor entrainment over time and perform the above mentioned comparisons.

[0111] One implementation may include the features described to predict sorting performance for a population. For example, the inverse of the average population delta-time may be used as an average virtual rate estimate to predict sorting performance for that population and thereby provide information which can be used to optimize sorting logic for that population based upon its measured behavior. In an embodiment, a matrix may be generated, such as by the sample behavior analyzer **200** or the processor **108**, to assess yield (efficiency) and purity outcomes for a classified population *p*. The matrix may be generated based on average virtual rates computed from the inverse of the average delta-times. One example, which is a modification of Equation (1), is shown in Equation (3).

$$Efficiency_p = e^{-(\frac{1}{delta\ time_p} \times (1 - fraction_p) \times \frac{d}{f})} \quad (3)$$

[0112] Some implementations may include selective anti-coincidence logic. The sorting logic may adjust how the behavior is analyzed. For example, if with standard anti-coincidence logic enabled the logical drop packet *d* is 1.5 drops in "purity" mode, and 1.0 drops in a "yield" mode (to incrementally decrease the number of potential aborts). Some implementations may include an "enrich" mode with anti-coincidence disabled which can provide a 1.0 drops. The matrix may be generated to assess the sorting performance of multiple populations with differing average delta-times within a sample under the different sorting modes such as pure, yield, and enrich.

While the discussion uses pure, yield, and enrich as the example sorting logics, fewer or more sorting logics associated with different logical drop packets and/or other sorting parameters may be used to assess the sorting performance in conjunction with delta-times.

[0113] Furthermore, the estimates from measured delta-times may be used to compare predicted performance from average delta-times contrasted to that predicted if the population were homogenous Poisson in its behavior. This information is a further example of sample behavior information.

[0114] Consider a PBMC sample stained for CD4, CD19, and CD14 cell surface markers where the average rate is 6400 events per second and the drop rate is 39200 Hz. The four populations to be sorted are CD4+ (37.13%), CD4- (13.85%), CD19+ (10.97%), and CD14+ (1.83%). The average expected efficiency (yield) would be between approximately 79% and 85% yield in "purity" mode with a 1.5 drop purity check and between 85% and 90% in "yield" mode with a 1.0 drop purity check. Table 1 summarizes the estimated yield using standard method (e.g., not considering delta-time).

		estimated yield (standard method)		
Population	% Total	purity	yield	enrich
CD4+	37.13%	85.74%	90.25%	100.00%
CD4-	13.85%	80.99%	86.89%	100.00%
CD19+	10.97%	80.42%	86.48%	100.00%
CD14	1.83%	78.64%	85.20%	100.00%

TABLE 1

[0115] However, the observed yield from the CD14 population in purity mode during the sort was nearer to 33% as opposed to the expected 79%. The experimental results can be predicted by the average (median) delta-time of the CD14+ population. Table 2 summarizes the estimated yield using the delta-time method.

			estimated yield (delta time method)		
Population	delta time (μsec)	% Total	purity	yield	enrich
CD4+	168.4	37.13%	86.69%	90.92%	100.00%
CD4-	171.9	13.85%	82.55%	88.00%	100.00%

CD19+	174.5	10.97%	82.26%	87.80%	100.00%
CD14	33.2	1.83%	32.26%	47.03%	100.00%

TABLE 2

[0116] Similarly, the expected purity of the population may be estimated by estimating the probability of another particle being in the same drop packet as the classified cell being sorted. The expected purity is yet another example of sample behavior information. The measured variability in estimating particle location can be used as *d* in Equation (3) to estimate the frequency of error. For the example discussed above, the frequency of error was determined to be 0.2 drops. Using this information, the estimated purity may be generated. Table 3 shows estimated purities for various sorting modes for populations exhibiting a 0.2 drop frequency of error.

	estimated purity		
Population	purity	yield	enrich
CD4+	99.50%	97.97%	91.12%
CD4-	99.50%	97.23%	88.41%
CD19+	99.50%	97.14%	88.09%
CD14	99.50%	96.85%	87.11%

TABLE 3

[0117] In some implementations, behavior analysis may estimate the frequency of an unwanted passenger cell based on the drop rate. This frequency is a further example of sample behavior information. Equation (4) shows one expression of purity estimate which may be included in the devices and methods described.

$$Purity_{enrich} = 1.0 \Big/ \left(1.0 + 1.0 - e^{-(Rate \times \frac{1.0 - fraction}{f})}\right) \qquad (4)$$

[0118] As used herein, the terms "determine" or "determining" encompass a wide variety of actions. For example, "determining" may include calculating, computing, processing, deriving, investigating, looking up (e.g., looking up in a table, a database or another data structure), ascertaining and the like. Also, "determining" may include receiving (e.g., receiving information), accessing (e.g., accessing data in a memory) and the like. Also, "determining" may include resolving, selecting, choosing, establishing and the like.

[0119] As used herein, the terms "provide" or "providing" encompass a wide variety of actions. For example, "providing" may include storing a value in a location for subsequent retrieval, transmitting a value directly to the recipient, transmitting or storing a reference to a value, and the like. "Providing" may also include encoding, decoding, encrypting, decrypting, validating, verifying, and the like.

[0120] As used herein, a phrase referring to "at least one of" a list of items refers to any combination of those items, including single members. As an example, "at least one of: a, b, or c" is intended to cover: a, b, c, a-b, a-c, b-c, and a-b-c.

[0121] Those of skill in the art would understand that information and signals may be represented using any of a variety of different technologies and techniques. For example, data, instructions, commands, information, signals, bits, symbols, and chips that may be referenced throughout the above description may be represented by voltages, currents, electromagnetic waves, magnetic fields or particles, optical fields or particles, or any combination thereof.

[0122] Those of skill in the art would further appreciate that the various illustrative logical blocks, modules, circuits, and algorithm steps described in connection with the embodiments disclosed herein may be implemented as electronic hardware, computer software, or combinations of both. To clearly illustrate this interchangeability of hardware and software, various illustrative components, blocks, modules, circuits, and steps have been described above generally in terms of their functionality. Whether such functionality is implemented as hardware or software depends upon the particular application and design constraints imposed on the overall system. Skilled artisans may implement the described functionality in varying ways for each particular application, but such implementation decisions should not be interpreted as causing a departure from the scope of the present invention.

[0123] The techniques described herein may be implemented in hardware, software, firmware, or any combination thereof. Such techniques may be implemented in any of a variety of devices such as general purposes computers, wireless communication devices, or integrated circuit devices having multiple uses including application in wireless communication device handsets and other devices. Any features described as modules or components may be implemented together in an integrated logic device or separately as discrete but interoperable logic devices. If implemented in software, the techniques may be realized at least in part by a computer-readable data storage medium comprising program code including instructions that, when executed,

performs one or more of the methods described above. The computer-readable data storage medium may form part of a computer program product, which may include packaging materials. The computer-readable medium may comprise memory or data storage media, such as random access memory (RAM) such as synchronous dynamic random access memory (SDRAM), read-only memory (ROM), non-volatile random access memory (NVRAM), electrically erasable programmable read-only memory (EEPROM), FLASH memory, magnetic or optical data storage media, and the like. The computer-readable medium may be a non-transitory storage medium. The techniques additionally, or alternatively, may be realized at least in part by a computer-readable communication medium that carries or communicates program code in the form of instructions or data structures and that can be accessed, read, and/or executed by a computer, such as propagated signals or waves.

[0124] The program code may be executed by a processor, which may include one or more processors, such as one or more digital signal processors (DSPs), general purpose microprocessors, an application specific integrated circuits (ASICs), field programmable logic arrays (FPGAs), or other equivalent integrated or discrete logic circuitry. Such a processor may be configured to perform any of the techniques described in this disclosure. A general purpose processor may be a microprocessor; but in the alternative, the processor may be any conventional processor, controller, microcontroller, or state machine. A processor may also be implemented as a combination of computing devices, e.g., a combination of a DSP and a microprocessor, a plurality of microprocessors, one or more microprocessors in conjunction with a DSP core, or any other such configuration. Accordingly, the term "processor," as used herein may refer to any of the foregoing structure, any combination of the foregoing structure, or any other structure or apparatus suitable for implementation of the techniques described herein. In addition, in some aspects, the functionality described herein may be provided within dedicated software modules or hardware modules configured for encoding and decoding, or incorporated in a combined video encoder-decoder (CODEC).

[0125] The methods disclosed herein comprise one or more steps or actions for achieving the described method. The method steps and/or actions may be interchanged with one another without departing from the scope of the claims. In other words, unless a specific order of steps or actions is specified, the order and/or use of

specific steps and/or actions may be modified without departing from the scope of the claims.

[0126] Various embodiments of the invention have been described. These and other embodiments are within the scope of the following claims.

CLAIMS

What is claimed is:

1. A method for generating an entrainment factor for a flow cytometer sample, the method comprising:

flowing the sample comprising a series of particles through the flow cytometer wherein each particle is associated with a signal;

detecting a series of events with a detection system in a first time interval from a stream formed from the flow cytometer;

calculating an expected frequency of events in the first time interval based on a characterizing distribution;

measuring an observed frequency of events within a second time interval, wherein the second time interval is within the first time interval; and

generating the entrainment factor based at least in part on a ratio of the observed frequency to the expected frequency.

2. The method of Claim 1, wherein the characterizing distribution includes a Poisson distribution.

3. The method of Claim 1, further comprising generating at least one of an estimated yield or an estimated purity based on the entrainment factor and a sorting logic.

4. A method for generating sample behavior information for a flow cytometer sample, the method comprising:

flowing the sample comprising a series of particles through the flow cytometer wherein each particle is associated with an event and the particles are distributed in a fluidic stream;

detecting a series of events and a corresponding arrival time for each event;

determining a series of inter-arrivals times relative to a previous event within the series and an associated probability of occurrence of the inter-arrival times relative to a length of a specified time interval;

calculating an expected frequency of events over the series of inter-arrival times for a portion of the fluidic stream having a length equal to the specified time interval, said calculating based on a predetermined distribution;

measuring an observed frequency of events over the series of inter-arrival times within the portion of the fluidic stream; and

generating the sample behavior information based at least in part on a ratio of the observed frequency to the expected frequency of events over the series of inter-arrival times for the portion of the fluidic stream.

5. The method of Claim 4, wherein the predetermined distribution includes Poisson distribution.

6. The method of any one of Claims 1 or 4, wherein the stream is comprised of regularly spaced droplets.

7. The method of any one of Claims 1 or 4, further comprising:

comparing the entrainment factor to a predetermined value; and

actuating a corrective action in a control system operationally connected to the flow cytometer based on a result of the comparing.

8. The method of Claim 7, wherein the corrective action comprises halting the flow of the sample in the flow cytometer.

9. The method of Claim 7, further comprising sorting particles in the stream, and wherein the corrective action comprises adjusting the sorting based on the entrainment factor.

10. The method of Claim 1, wherein the entrainment factor is generated while the sample is flowing in the flow cytometer.

11. The method of Claim 6, wherein time intervals are measured in fractions of a droplet.

12. The method of Claim 1, wherein the entrainment factor is one of a plurality of entrainment factors calculated during successive time intervals for a flow cytometer sample.

13. The method of Claim 1, wherein the sample comprises peripheral blood cells wherein the peripheral blood cells as are comprised of a first and second component labeled with a first and second label.

14. The method of Claim 13, wherein the entrainment factor is generated for the first component and another entrainment factor is generated for the second component.

15. A flow system for providing a sample entrainment factor, the system comprising:

a fluidics system comprising a sample tube and a moving fluid column within the sample tube in which particles of a sample move along a common sample path;

a detection system for collecting a signal from each particle as it passes one or more detection stations along the common sample path, each signal being assigned a signal value to form a data point for each particle, the detection system collecting a succession of such data points in a first time interval; and

a control system operationally associated with the fluidics system, the control system configured to:

generate a calculated signal frequency for at least a portion of the first time interval based on a Poisson distribution and the number of data points collected by the detection system during the first time interval; and

generate an experimental signal frequency based on the number of data points in the portion of the first time interval;

compare the experimental signal frequency with that of a calculated signal frequency or a predetermined signal frequency; and

provide an entrainment factor for the sample, said entrainment factor based on said comparing.

16. The system of Claim 15, wherein comparing the experimental signal frequency comprising determining a ratio of the experimental signal frequency to that of the calculated signal frequency or the predetermined signal frequency.

17. The system of Claim 15, wherein upon determining that the entrainment factor deviates from a predetermined value, the control system actuates a corrective action.

18. The system of Claim 17, wherein the corrective action includes one or more of:

interrupting collection of signals by the detection system;

purging the sample tube by said fluidics system;

altering collection of signals via acoustic focusing based on the entrainment factor; and

resuming collection of the signals by the detection system.

19. The system of Claim 18, wherein resuming collection of the signals occurs after initiating dispersion of the sample.

20. The system of Claim 15, wherein the control system includes a first sorting configuration providing a high collection yield and a second sorting configuration providing a high collection purity, and wherein the control system is configured to select one of the first sorting configuration or the second sorting configuration based on the entrainment factor.

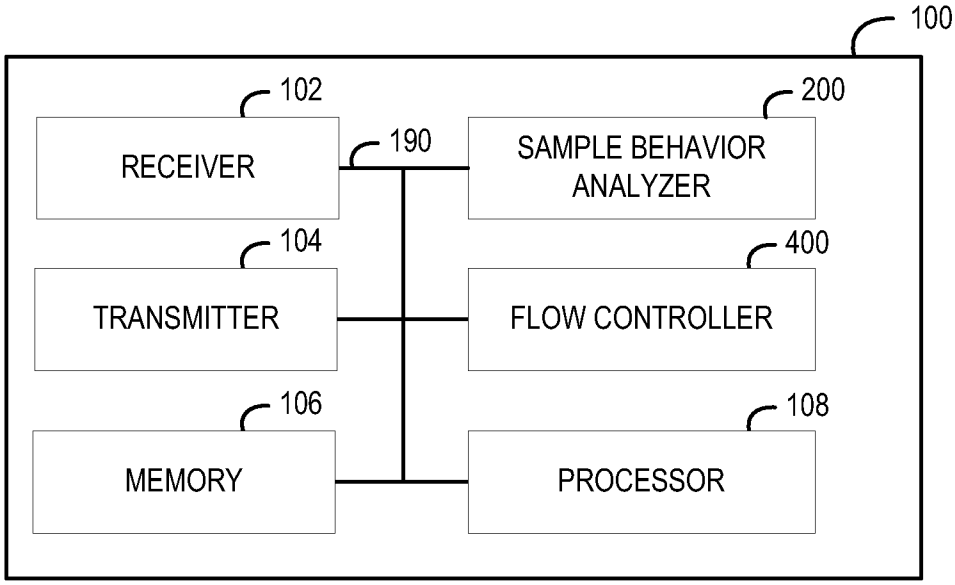
100
102
RECEIVER
190
200
SAMPLE BEHAVIOR ANALYZER
104
TRANSMITTER
400
FLOW CONTROLLER
106
MEMORY
108
PROCESSOR

FIG. 1

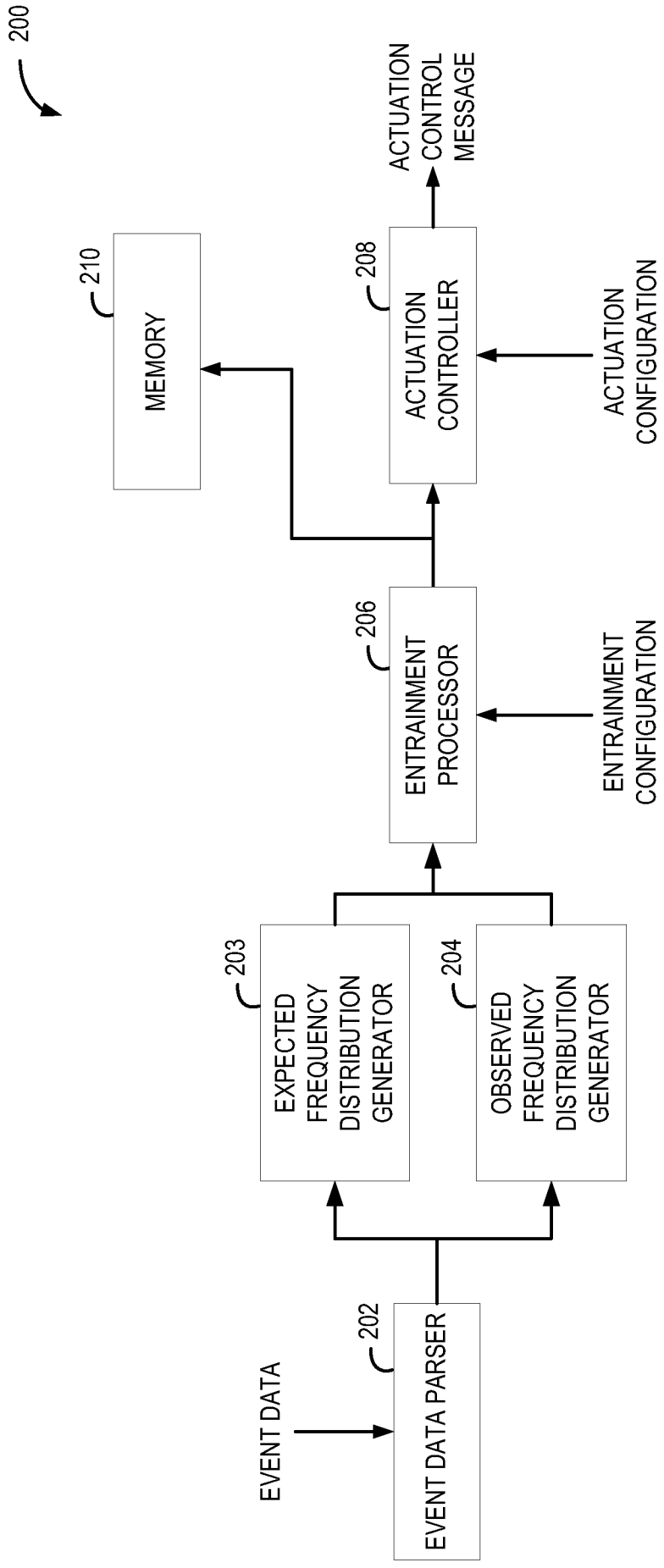
200
EVENT DATA
202
EVENT DATA PARSER
203
EXPECTED FREQUENCY DISTRIBUTION GENERATOR
204
OBSERVED FREQUENCY DISTRIBUTION GENERATOR
206
ENTRAINMENT PROCESSOR
ENTRAINMENT CONFIGURATION
208
ACTUATION CONTROLLER
ACTUATION CONFIGURATION
ACTUATION CONTROL MESSAGE
210
MEMORY

FIG. 2

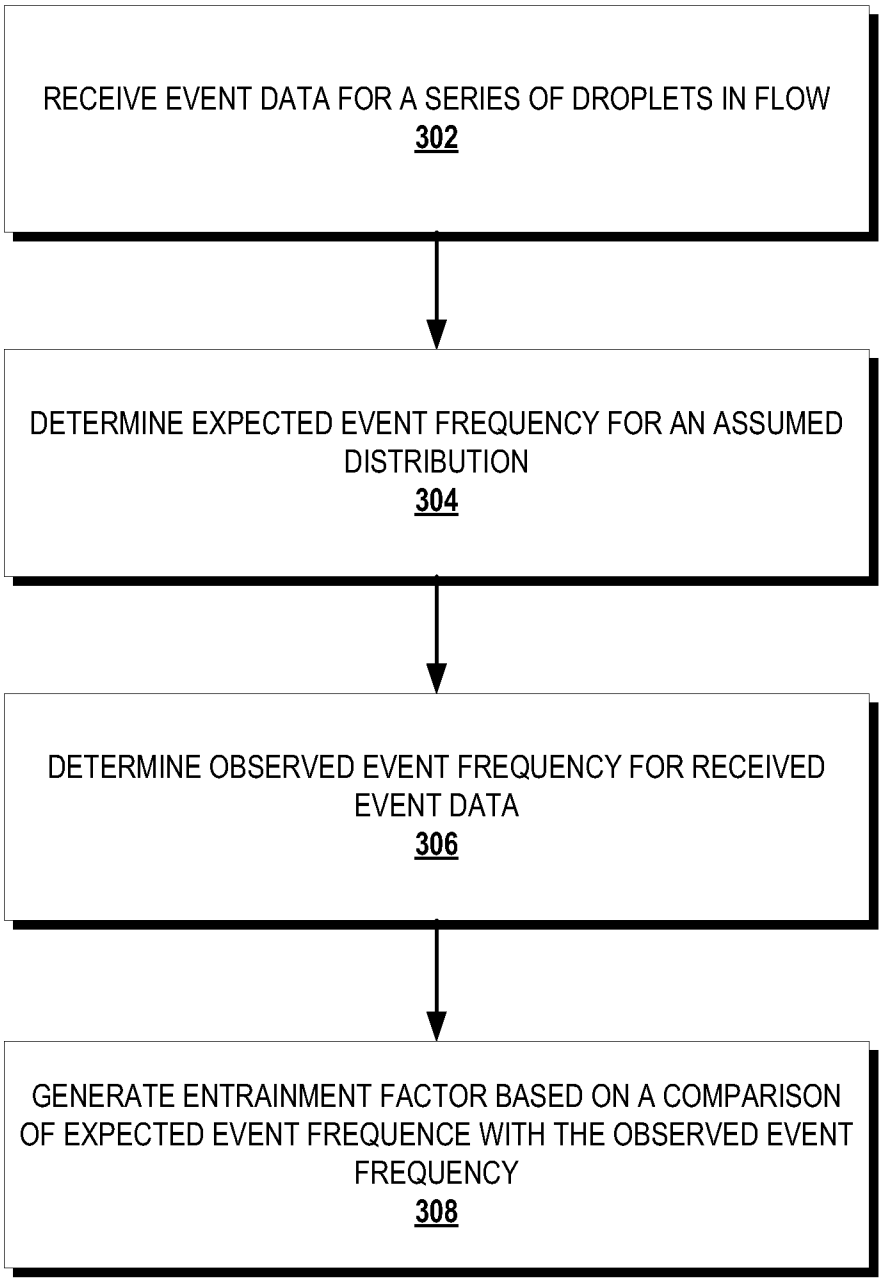
RECEIVE EVENT DATA FOR A SERIES OF DROPLETS IN FLOW
302
DETERMINE EXPECTED EVENT FREQUENCY FOR AN ASSUMED DISTRIBUTION
304
DETERMINE OBSERVED EVENT FREQUENCY FOR RECEIVED EVENT DATA
306
GENERATE ENTRAINMENT FACTOR BASED ON A COMPARISON OF EXPECTED EVENT FREQUENCE WITH THE OBSERVED EVENT FREQUENCY
308

FIG. 3

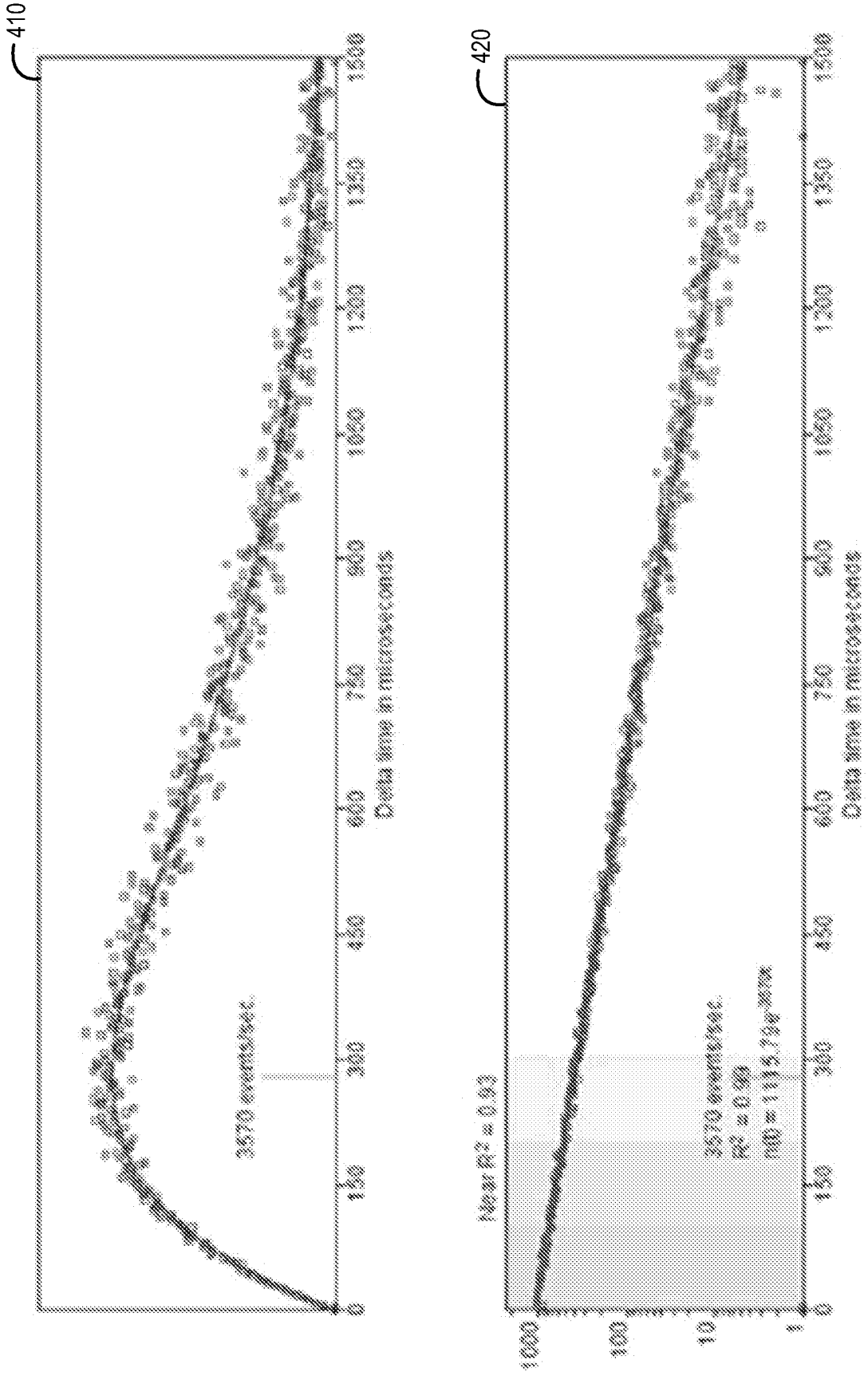
410
2570 events/sec
0
150
300
450
600
750
900
1050
1200
1350
1500
Delta time in microseconds
420
Mean R^2 = 0.93
2570 events/sec
R^2 = 0.99
MD = 1115.78 events/sec
1000
100
10
1
0
150
300
450
600
750
900
1050
1200
1350
1500
Delta time in microseconds

FIG. 4

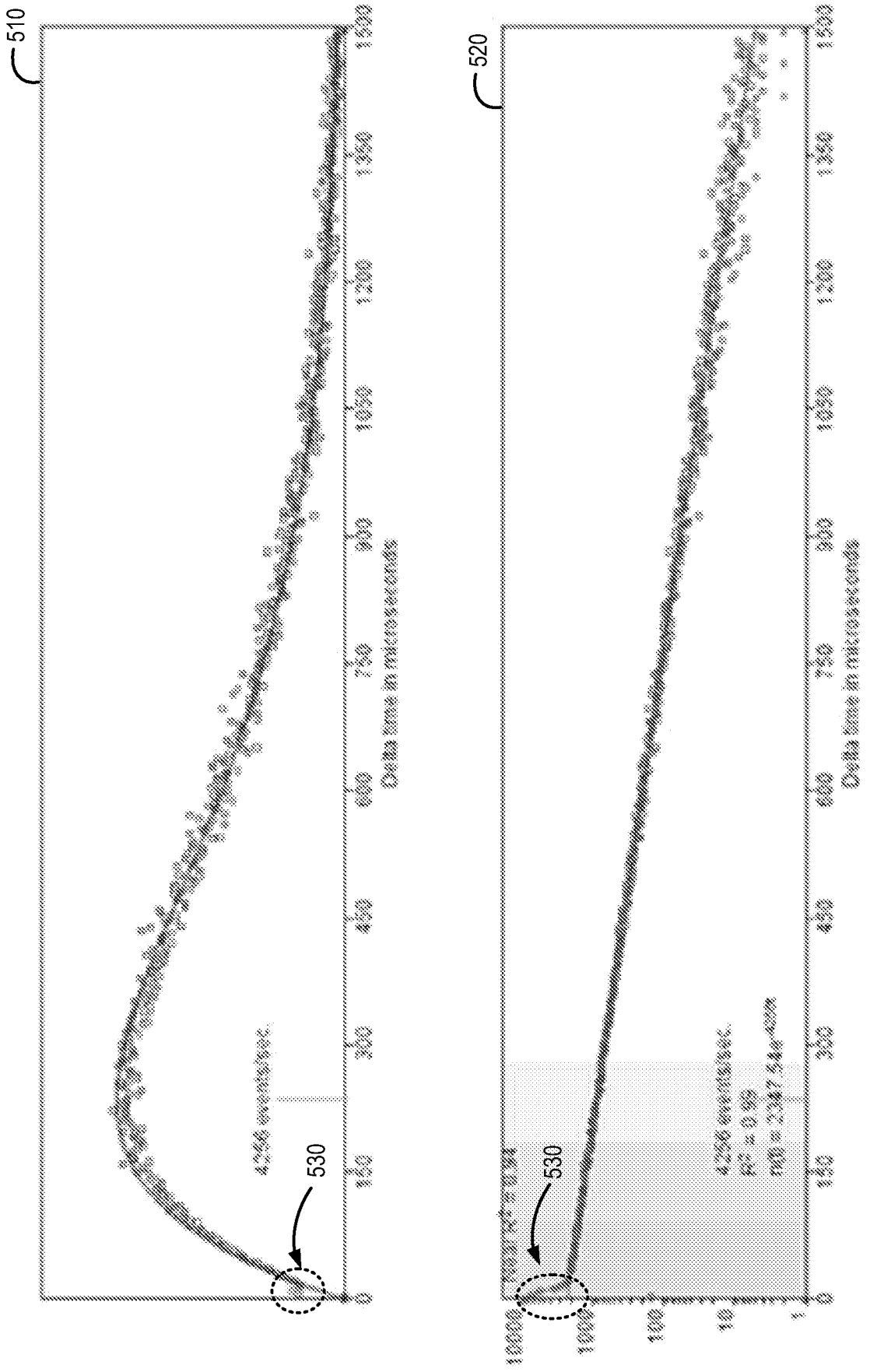
510
520
530
530
4256 events/sec
Delta time in microseconds
Delta time in microseconds

FIG. 5

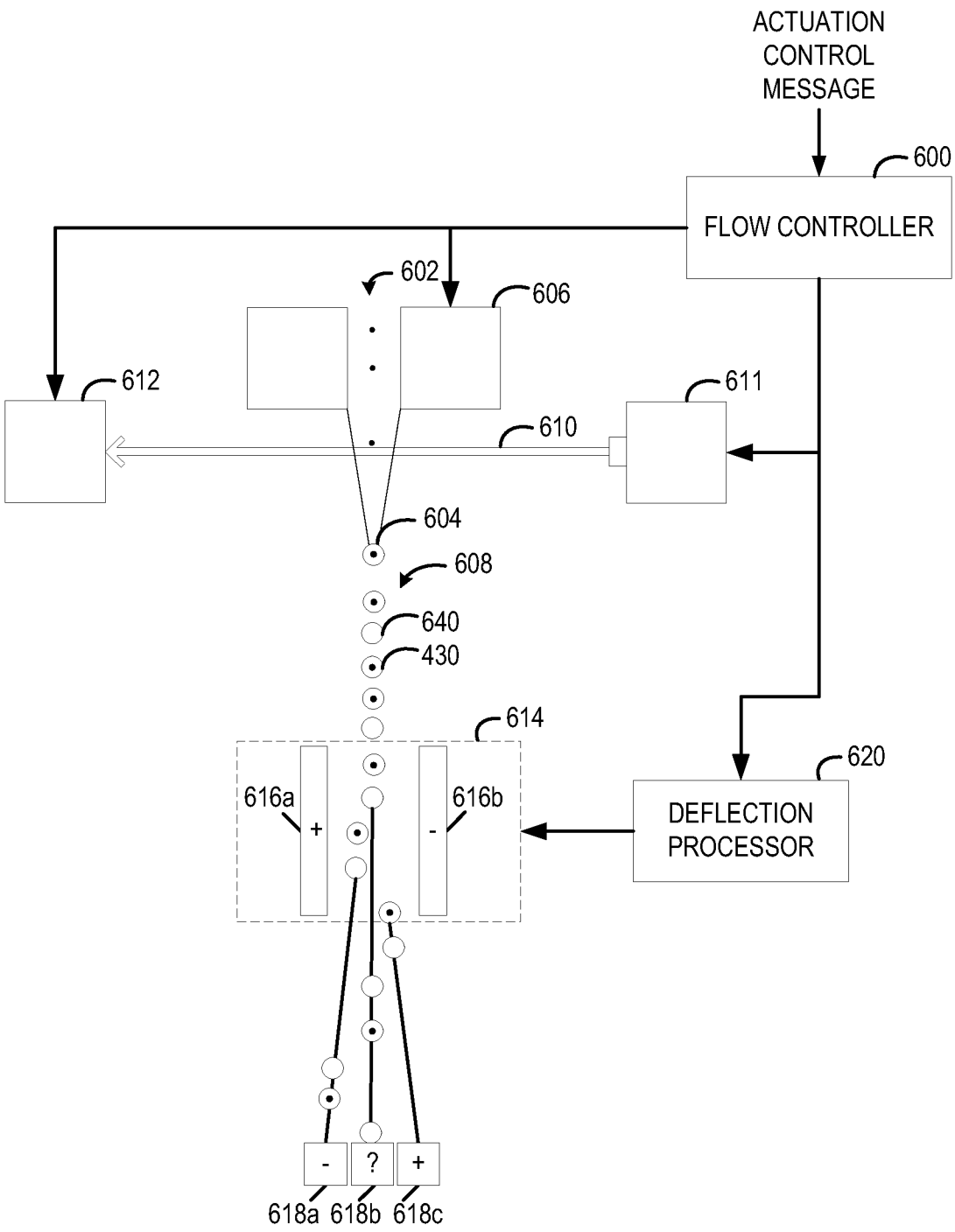
ACTUATION CONTROL MESSAGE
600
FLOW CONTROLLER
602
606
612
611
610
604
608
640
430
614
616a
+
-
616b
620
DEFLECTION PROCESSOR
-
?
+
618a
618b
618c

FIG. 6

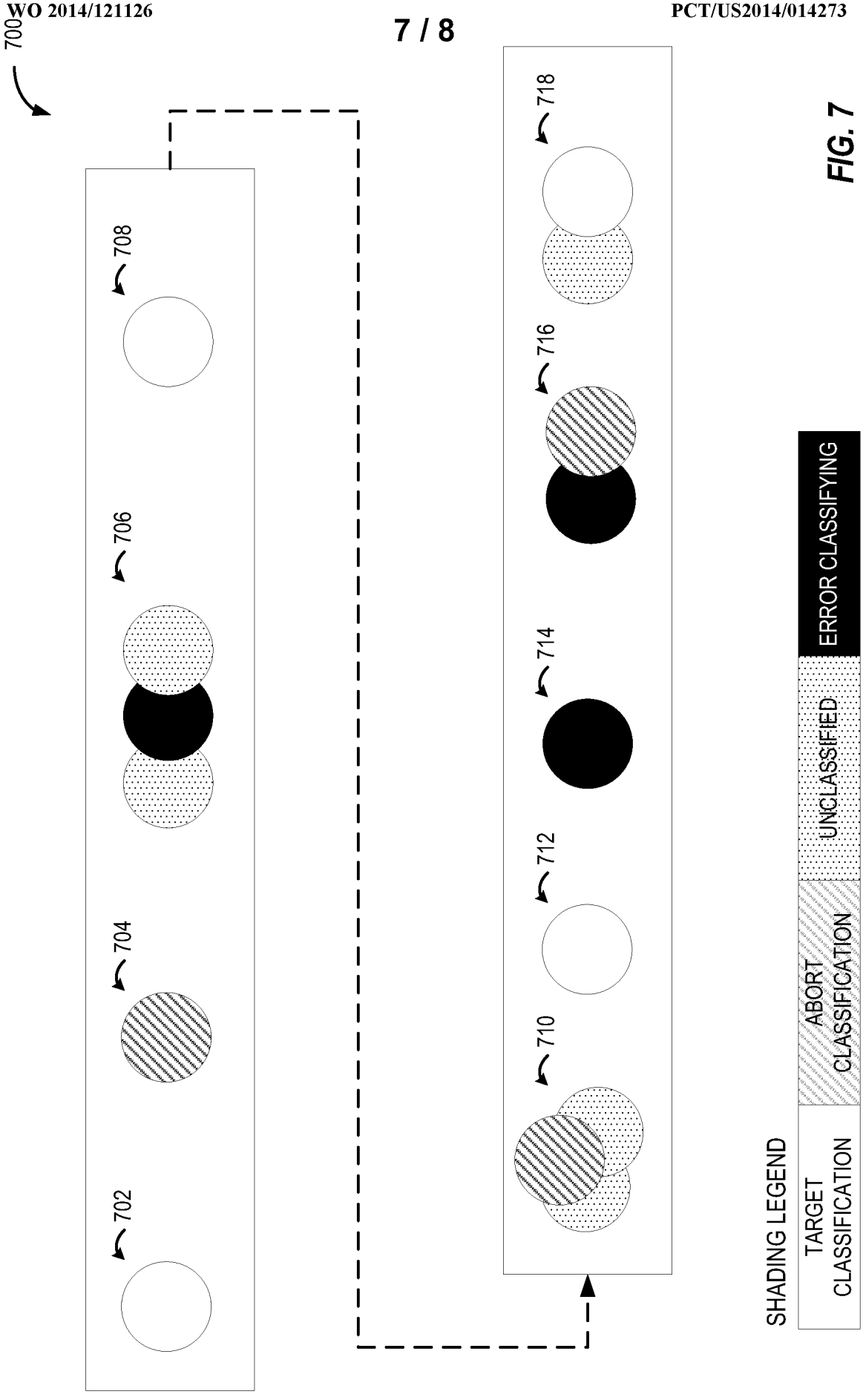
700
702
704
706
708
710
712
714
716
718
SHADING LEGEND
TARGET CLASSIFICATION
ABORT CLASSIFICATION
UNCLASSIFIED
ERROR CLASSIFYING

FIG. 7

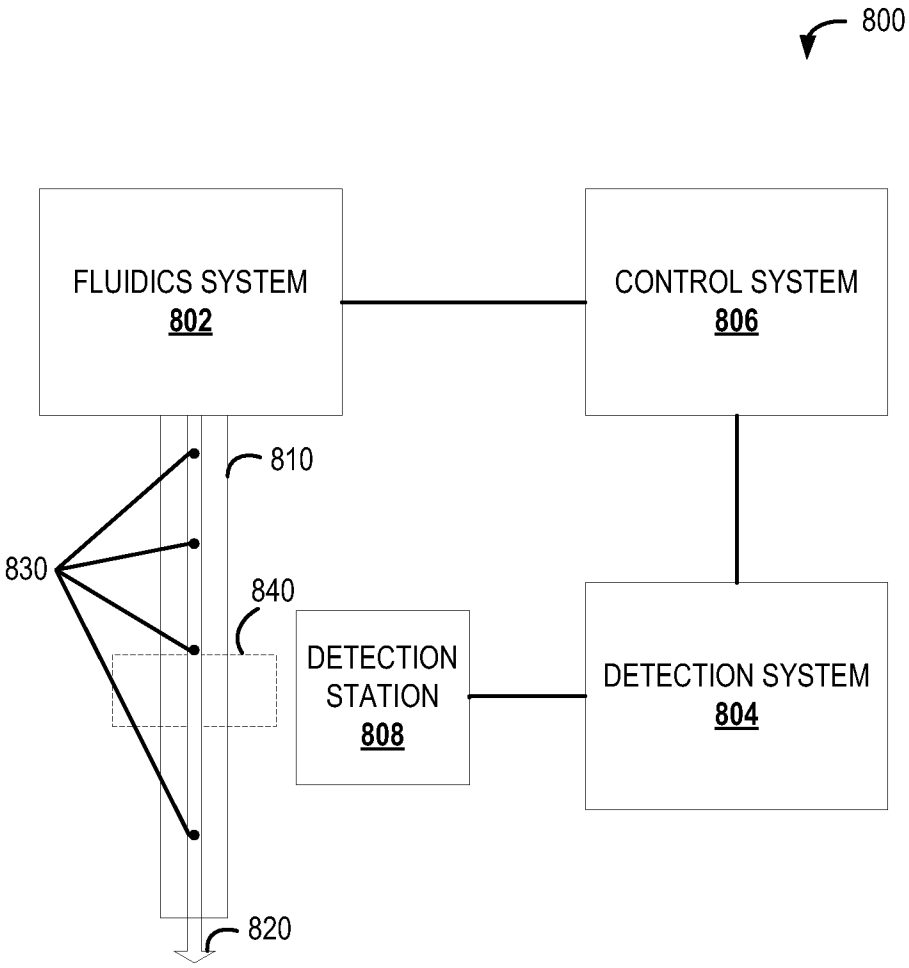
800
FLUIDICS SYSTEM
802
CONTROL SYSTEM
806
810
830
840
DETECTION
STATION
808
DETECTION SYSTEM
804
820

FIG. 8