



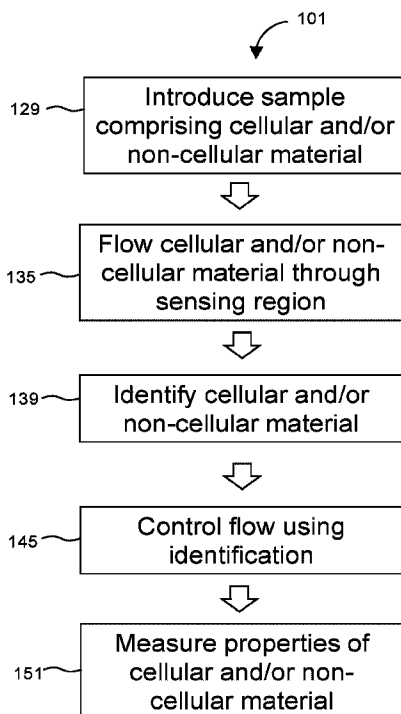
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(54) **Titre : ETALONNAGE ET CLASSIFICATION DE MESURES CELLULAIRES**  
(54) **Title: CELLULAR MEASUREMENT CALIBRATION AND CLASSIFICATION**



**FIG. 1**

(57) **Abrégé/Abstract:**

The invention provides devices and methods for optimized cellular measurements. The method comprises introducing cellular and/or non-cellular material into a measurement device, collecting data from the cellular and/or non-cellular material, providing the data to a classifier that utilizes the data to identify cellular and/or non-cellular material, and using the identification by the classifier to optimize cellular measurements made by the measurement device.

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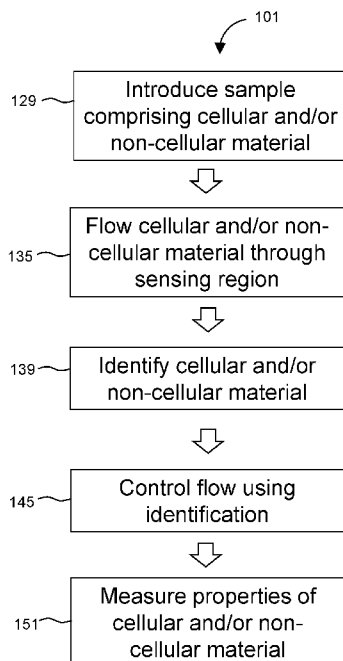


FIG. 1

(57) Abstract: The invention provides devices and methods for optimized cellular measurements. The method comprises introducing cellular and/or non-cellular material into a measurement device, collecting data from the cellular and/or non-cellular material, providing the data to a classifier that utilizes the data to identify cellular and/or non-cellular material, and using the identification by the classifier to optimize cellular measurements made by the measurement device.

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## **Cellular Measurement Calibration and Classification**

### **Technical Field**

[0001] The invention relates to methods of for optimizing cell measurements, particularly  
5 through use of a classifier in real time.

### **Background**

[0002] Cancer is a global health issue that causes millions of deaths worldwide every year. While a complete cure is the ultimate goal, a more practical goal is to manage control of the  
10 patient's cancer (i.e., the cancer is still present but not spreading over time). Other positive outcomes include complete or partial remission where cancer has responded to a treatment and is either significantly reduced (partial remission) or undetectable via radiological imaging or histological examination (complete remission).

[0003] Unfortunately, remission and control can be fleeting and cancer often recurs or  
15 progresses after initially responding to treatment and maintenance therapies. Due to the nature of cancer cells and tumors, drug targets can change through continued mutation and cancers can often develop resistance to previously effective therapies. While there is some effort to tailor treatment, there is limited ability to effectively predict how an individual patient will respond to a particular treatment. Moreover, traditional methods for measuring cancer biomarkers after  
20 treatment do not provide the requisite precision necessary to drive therapeutic choice, which may lead to extended periods of time in which a patient endures a treatment that simply isn't working as intended.

### **Summary**

[0004] The invention provides methods and devices for precisely measuring cancer biomarkers  
25 in patients with the accuracy necessary to monitor the efficacy of a cancer therapy. The precision achieved by the present invention allows for cancer treatments to be accurately tailored to individual patients and can be used to drive therapeutic choice. The invention can be used to optimize measurements from devices that measure functional biomarkers in cells, for example  
30 cell growth, mass accumulation, or phenotypic cell biomarkers. The invention achieves increased

precision by using a classifier that identifies cellular and non-cellular material in real time in order to optimize parameters of a measurement device for measuring cancer biomarkers. For example, the classifier can be trained to identify non-cellular reference materials, such as beads with a known property, in order to periodically load beads into the measurement device to  
5 recalibrate the device. Identification of cellular and non-cellular material by the classifier can also be used to optimize the flow rate at which cells and non-cellular material respectively flow through the measurement device. By increasing the precision at which cancer biomarkers can be measured, the invention can be used to ensure that a given cancer therapy is effective for an individual or can be used to assess the efficacy of a new cancer therapy.

10 **[0005]** A measurement device used in the present invention may comprise a sample channel, a secondary channel, and a sensor operating over a sensing region. Flow in the sample and secondary channels can be controlled using a classifier that utilizes data from the sensor to identify cellular and non-cellular material in real time. For example, flow in the sample and secondary channel may be controlled so as to flow a particle of cellular or non-cellular material  
15 once identified from the sample channel into the secondary channel.

**[0006]** The classifier may be based on a neural network architecture trained using the sensor data to classify cellular and non-cellular material. The classifier may be a convolutional neural network (CNN). Aspects of the invention may be accomplished by using an imaging sensor, including a lens-free imaging sensor. The imaging sensor provides image data to the classifier,  
20 which identifies cellular and non-cellular material based on the image data. By identifying cellular and non-cellular material, the classifier allows for particles of cellular and non-cellular material to be loaded at a specified ratio into the secondary channel. Particles of cellular and non-cellular material loaded into the secondary channel may be provided to a measurement device. The secondary channel may comprise the measurement device, such as a device  
25 comprising at least one suspended microchannel resonator (SMR). The designation of particles as cellular or non-cellular and the respective measurement(s) made by the suspended microchannel resonator may be paired.

**[0007]** Non-cellular material may be selected from the group consisting of synthetic particles, inorganic particles and debris. Non-cellular material may include reference material with a  
30 known property. The known property of the reference material may be size, mass, and/or density. The reference material may be a synthetic particle and the synthetic particle may be a bead, such

as a polystyrene bead. Debris, such as cell debris, may also be included in a sample. Debris once identified may be rejected from entering or removed from the measurement device.

**[0008]** Cellular material may be selected from the group consisting of cells, cell aggregates, exosomes, extracellular vesicles, cellular components, cellular fragments, organelles, organoids, proteins and protein aggregates, DNA, and RNA. Identification of cellular and non-cellular material by the classifier allows for cellular and non-cellular material to be introduced into the measurement device separately or simultaneously. Cellular and non-cellular material may be introduced in the same sample. Cellular material may comprise cancer cells and/or live cells. Accordingly, the method may further comprise obtaining cancer cells from a patient.

**[0009]** Further methods of the invention provide for optimized cellular measurement by introducing cellular and/or non-cellular particles with overlapping size and/or mass distributions to a measurement device comprising at least one suspended microchannel resonator, and identifying the sub-groups of particles in the mixture based on a classifier that utilizes data from the measurement device. The Identifying sub-groups of particles utilizing data from the measurement device may be based on a node-deviation signal of said suspended microchannel resonator and may be done in real-time. The classifier may be based on a neural network architecture trained using data from the measurement device previously obtained from different sub-groups of particles. The classifier may also discriminate between cells and non-cellular material based on surface stiffness.

**[0010]** Identification of cellular and non-cellular material by the classifier allows for cellular and non-cellular material to be introduced into the measurement device separately or in the same sample. The method may further comprise obtaining a density measurement of the fluidic sample containing cells and/or non-cellular material. The non-cellular material may be a bead having a known size, mass and/or density. Accordingly, the bead may be used as a reference material. The bead may be a polystyrene bead.

**[0011]** Optimized cellular measurements may comprise measurements of mass or mass accumulation rate (MAR) from a cell. The mass measurement from a bead with a known mass may be used to calibrate the mass accumulation rate measurement from a cell. The method may comprise introducing a bead with a known mass into the measurement device, obtaining a mass measurement of said bead, and calibrating mass measurements using the known mass of the bead. Measurements of mass accumulation rate from cells may then be collected by the

measurement device. Cells may comprise cancer cells and/or live cells. The method may further comprise obtaining cancer cells from a patient. The measurement of MAR in cells may provide a measure of cancer in the patient. Accordingly, the methods may comprise obtaining cancer cells from a patient being treated for the cancer, obtaining an optimized measurement of MAR from the cell, wherein the measurement of MAR is used to monitor the effectiveness of the cancer treatment.

**[0012]** Systems for optimized cellular measurement may comprise a measurement device comprising a sample channel, a secondary channel, and a sensor operating over a sensing region. The system may further comprises a classifier trained to identify cellular and non-cellular material utilizing data from the sensor in real-time and provide an output. The system also comprises a control system for controlling flow through the measurement device based on the output from the classifier. The classifier may be based on a neural network architecture trained to discriminate between cells and non-cellular material. The sensor may an imaging sensor, such as an imaging sensor with no lens.

**[0013]** The control system may be operable to load particles of cellular and non-cellular materials into the secondary channel at a specific ratio. The secondary channel may provide cellular and non-cellular material to a measurement device. The secondary channel may comprise at least one suspended microchannel resonator. The system may allow for cells and non-cellular material to be introduced to the measurement device separately or in the same sample.

**[0014]** Systems for optimized cell measurements may comprise a measurement device comprising a suspended microchannel resonator capable of loading cells and non-cellular particles with overlapping size and/or mass distributions, and a classifier trained to identify sub-groups of particles utilizing data from the measurement device. The classifier may identify sub-groups of particles in real-time. The classifier may be based on a neural network architecture trained using data from the measurement device previously obtained from different sub-groups of particles. The classifier may identify sub-groups of particles based on a node-deviation signal of said suspended microchannel resonator. The classifier may also discriminate between cells and non-cellular material based on surface stiffness.

### **Brief Description of the Drawings**

FIG. 1 diagrams a method of optimized cell measurement.

FIG. 2 shows a device for optimized cell measurement.

FIG. 3 shows a suspended microchannel resonator (SMR) device.

5 FIG. 4 shows a serial suspended microchannel resonator (sSMR) array.

FIG. 5 shows a cantilever of an SMR device.

FIG. 6 shows loading live cells into an instrument for an optimized cellular measurement.

FIG. 7 shows a system useful for performing methods of the disclosure.

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### **Detailed Description**

**[0015]** The present disclosure provides methods and devices for optimized cellular measurements in order to effectively measure cancer biomarkers in cells. In order to maintain throughput for cell measurements, the present disclosure adapts imaging and fluidic control software using a classifier to discriminate between non-cellular material and cells in real time.

15 For example, a convolution neural network (CCN) based classifier trained to identify cellular material such as cells and cell aggregates and non-cellular material such as polystyrene beads or debris in real time may be used to identify the cellular and non-cellular material. The results of this classification can be used to control the fluidic states of the measurement device to load cells and beads at different, independent rates to optimize system calibration and cell measurement  
20 throughput. In order to identify cells and beads, corresponding node deviation for resonant frequency peaks collected for each particle may be used to identify differences in stiffness between cells and beads in order to accurately classify each. The addition of polystyrene beads with cells in a single sample enables access to real time density estimates of the sample where the cells and beads flow together through the device. This additional information may be used for  
25 optimized cellular measurement at a great precision. Cellular measurement may include mass, growth rate, mass accumulation, or mass accumulation rate (MAR).

**[0016]** Methods for optimized cellular measurement may comprise introducing cells and/or non-cellular material into a measurement device comprising a sample channel, a secondary channel, and a sensor operating over a sensing region. Flow of fluids in the sample channel and secondary  
30 channels may be controlled using a classifier that utilizes data from the sensor to identify cellular and non-cellular material in real time. Cellular and non-cellular material may be introduced into

the sample channel and flow through the channel to the sensing region. The sensor operating over the sensing region may then collect and provide data to the classifier to identify cellular and non-cellular material in real time. The sensor operating over the sensing region may collect and provide data to the classifier in order to train the classifier. Identification of cellular and non-cellular material by the classifier may be used for calibrating the measurement device for future measurements.

**[0017]** Once identified, the flow of fluid in the sample and the secondary channels may be controlled in order to flow a particle of cellular and/or non-cellular material from the sample channel into the secondary channel. This allows for particles of cellular and non-cellular material to be introduced into the measurement device at different flow rates optimized for measuring cells or non-cellular material respectively. Fluid may flow from the secondary channel into a measurement device for cellular measurement and optimized measurements may be collected. The secondary channel may advantageously comprise a measurement device for collecting measurements from the cells or non-cellular material. Measurement devices according to the invention can make sensitive and precise measurements of properties of the cells and/or non-cellular material, such as mass or change in mass.

**[0018]** FIG. 1 diagrams an exemplary method for optimized cellular measurement 101. The method 101 includes introducing 129 particles of cellular and/or non-cellular material into a measurement device. The measurement device may comprise a sample channel which receives the sample and a secondary channel which can receive a particle from the sample. The measurement device also comprises a sensing region. The sample flows into the sensing region 135 which comprises a sensor operating over the sensing region. Data from the sensor is provided to a classifier which identifies cellular and/or non-cellular material 139 utilizing the data. Flow in the measurement device, including the sample channel and secondary channel, is controlled based on the identification provided by the classifier 145. Flow may be controlled so as to flow particles of cellular and/or non-cellular material from the sample channel into the secondary channel and/or from the sample channel into a measurement device. From the secondary channel and/or measurement device, optimized measurement of properties of the cellular and/or non-cellular material may be collected 151.

**[0019]** FIG. 2 shows an exemplary measurement device of the disclosure 201. The measurement device 201 comprises a sample channel 245 and a secondary channel 249. Cellular and/or non-

cellular material is introduced to the sample channel and flows through the sample channel 229 to a sensing region 235 comprising a sensor 239. The sensor may operate over the sensing region 235 and collects data from the cellular and/or non-cellular material that flows through the sensing region 235. The sensor then provides the data 259 to a classifier 255. The classifier  
5 utilizes the data from the sensor 259 to identify cellular and non-cellular material and provides the identification to the measurement device 265. The measurement device controls the flow 229 of cellular and/or non-cellular material through the sample channel 245 and the secondary channel 249 based on the identification provided by the classifier such that a particle of cellular and/or non-cellular material flows into 269 the secondary channel 249. The measurement device  
10 may comprise a control system for receiving the identification from the classifier 265 and control the flow of material through the sample channel 229 and the secondary channel 269. The secondary channel may comprise a measurement device, for example a suspended microchannel resonator (SMR) device 301, for making optimized cellular measurements.

**[0020]** The devices may comprise a suspended microchannel resonator (SMR) 301 or serial  
15 SMR (sSMR) for precisely making cellular measurements, such as mass and mass changes, of a materials flowing therethrough. The SMR device 301 comprises an exquisitely sensitive scale that detects minor weight change in cells. The SMR device 301 includes a structure such as a cantilever that contains a fluidic microchannel. Particles of cellular and/or non-cellular material are flowed through the structure, which is resonated and its frequency of resonance is measured.  
20 The frequency at which a structure resonates is dependent on its mass. By measuring the frequency at which the cantilever resonates when a particle of cellular or non-cellular material is at a first point along the cantilever, the instrument may compute a mass, or change in mass of the particle in the fluidic microchannel. By measuring the deviation of the resonant frequency at  
25 which the cantilever resonates when a particle of cellular or non-cellular material is at a second point along the cantilever, the instrument may compute structural properties of the particle in the fluidic microchannel, and the data may be used by a classifier to identify the particle as cellular or non-cellular material.

**[0021]** By flowing particles cellular and/or non-cellular material through such devices, properties of the particles can be observed. For example, by flowing cells through such devices,  
30 it can be determined whether or not the cells are growing and accumulating mass. By flowing non-cellular material through such devices, for example reference material with a known

property, measurements and devices can be calibrated. The mass accumulation or rate of mass accumulation is a clinically important property and is used to indicate the presence of cancer cells or the efficacy of a therapeutic on cancer cells. The speed and sensitivity of an SMR device 301 allows the SMR device 301 to detect a cancer cell's response to a cancer drug while the cell is still living. Suspended microchannel resonator devices 301 are described in Cermak, 2016, High-throughput measurement of single-cell growth rates using serial microfluidic mass sensor arrays, Nat Biotechnol, 34(10):1052-1059, incorporated by reference.

**[0022]** FIG. 3 shows a suspended microchannel resonator (SMR) device 301 of the disclosure. The SMR device 301 includes a microchannel 305 that runs through a cantilever 333. The cantilever 333 may be suspended between an upper bypass channel 309 and a lower bypass channel 313. The two bypass channels provide a control system to decrease flow resistance and accommodate the flow rate through the microchannel 305. The flow rate through the SMR device can be controlled based on the identification of particles in the SMR device as cellular or non-cellular material by a classifier 255.

**[0023]** Particles of cellular and/or non-cellular material in an eluate 317 flow through the upper bypass channel 309, wherein a portion of the eluate 317 collects in the upper bypass channel waste reservoir 321. The calibration method is being depicted. A particle of non-cellular material acting as a reference material 329 with known properties (and optionally a known mass) has been introduced into the channel 305. A portion of the eluate 317 including the reference material 329 flows through the suspended microchannel 305. The particle has previously been identified as non-cellular material by a classifier, and the flow rate through the suspended microchannel 305 has been adjusted by the pressure difference between its inlet and outlet to optimize measurement of the particle of non-cellular material. Since the flow cross section of the suspended microchannel is about 70 times smaller than that of the bypass channels, the linear flow rate can be much faster in the suspended microchannel than in the bypass channel, even though the pressure difference across the suspended microchannel is small. Therefore, at any given time, it is assumed that the SMR device 301 is measuring the eluate that is present at the inlet of the suspended microchannel.

**[0024]** The reference material 329 flows through the suspended microchannel 305. The suspended microchannel 305 extends through a cantilever 333 which sits between a light source 351 and a photodetector 363 connected to a chip 369 such as a field programmable gate array

(FPGA). The cantilever 333 is operated on by an actuator, or resonator 357. The resonator 357 may be a piezo-ceramic actuator seated underneath the cantilever 333 for actuation. After the reference material 329 is introduced to the lower bypass channel 313, the reference material 329 is collected in the lower bypass collection reservoir 345. A particle previously identified by the classifier as cellular material 329 flows from the upper bypass channel 309 to the inlet of the suspended microchannel 305, through the suspended microchannel 305, and to the outlet of the suspended microchannel 305 toward the lower bypass channel 313. A buffer 341 flows through the lower bypass channel towards a lower bypass channel collection reservoir 345.

**[0025]** By flowing the reference material 329 through the SMR device 301 a reading or measurement may be made and the readout of the measurement may be adjusted to converge on the known property of the reference material 329, thereby adjusting the readout for a cell or calibrating the measurement device 301 for subsequent readouts. Once the instrument is thus calibrated, it may be used for optimized cellular measurements such as optimized measurements of mass or mass accumulation rate (MAR). MAR measurements characterize heterogeneity in cell growth across cancer cell lines. Individual live cells are able to pass through the SMR device 301, wherein each cell has been previously identified by a classifier as cellular material, and parameters of the SMR device 301 have been adjusted to precisely weigh the cell multiple times over a defined interval. The SMR device 301 includes multiple sensors that are fluidically connected, such as in series, and separated by delay channels. Such a design enables a stream of cellular and/or non-cellular material to flow through the SMR device 301 such that different sensors can concurrently weigh flowing cellular and non-cellular material in the stream, revealing single-cell MARs. The SMR device 301 when used with a classifier provides real-time, high-throughput optimized monitoring of mass or mass change for cellular and/or non-cellular flowing therethrough. Therefore, the cellular measurements, including mass and/or mass changes (e.g., MAR), of a single cell can be precisely measured. Such data can be stored and used in subsequent analysis steps.

**[0026]** The measurement device may comprise an SMR device 301 comprising an array of SMRs with a fluidic channel passing 305 therethrough. For example, the measurement device may comprise a serial SMR (sSMR) in which fluid passes through an array of SMR devices, in which each successive pair of SMR devices is separated by a portion of the channel that provides a delay. The flow of fluid in each SMR may be controlled based on a classifier 255 that identifies

cellular and/or non-cellular material in real-time. The sSMR may include multiple sensors that are fluidically connected, such as in series, and separated by delay channels for optimized cellular measurements.

**[0027]** FIG. 4 shows a serial suspended microchannel resonator (sSMR) array 401. Instruments

5 may include one or more sSMR array 401 to make reliably sensitive and precise measurements of mass or change in mass with a classifier. Instead of a single suspended microchannel device 301, the instrument may include an sSMR array 401, which includes a plurality of cantilevers 449 and a plurality of delay channels 453. A live malignant cancer cell may be identified by a classifier as cellular material and then introduced into a first bypass channel 457 through the  
10 cantilevers 449 and delay channels 453 to the second bypass channel 461. Pressure differences in the first bypass channel 457 are indicated by P1 and P2, and pressure differences in the second bypass channel 461 are indicated by P3 and P4. The pressure differences in the first and second bypass channels may be controlled based on the identification of a particle in the sSMR as cellular or non-cellular by a classifier.

15 **[0028]** The live malignant cell flows through the sSMR array 401, which is resonated and its frequency of resonance is measured. In each cantilever in the array of cantilevers 449 the frequency at which a structure resonates when the cell is at a first point along the cantilever is dependent on its mass and by measuring the frequency at which the cantilever resonates, the instrument can compute a mass, or change in mass, of a living cell in the fluidic microchannel.

20 By flowing a live malignant cell through such devices, one may observe functions of those cells, such as whether they are growing and accumulating mass or not. The mass accumulation or rate of mass accumulation can be related to clinically important property such as the presence of cancer cells or the efficacy of a therapeutic on cancer cells. In each cantilever in the array of cantilevers 449 the deviation of the resonant frequency at which the structure resonates when the  
25 cell is at a second point along the cantilever is dependent on structural properties of the cell and can be used to identify the cell as cellular material.

**[0029]** Various embodiments of SMR devices 301 and sSMR instruments 401, as well as methods of use, include those instruments/devices manufactured by Innovative Micro Technology (Santa Barbara, CA) and described in U.S. Pat. 8,418,535 and U.S. Pat. 9,132,294,  
30 all incorporated by reference. Notably, SMR devices 301 and sSMR instruments 401 may be used together with a classifier 255 for optimized cellular measurements.

**[0030]** Cantilevers of an SMR device 301 of sSMR instrument 401 may be housed in an on-chip vacuum cavity, reducing damping and improving frequency (and thus mass) resolution for optimized measurements together with a classifier 255. As a particle of cellular or non-cellular material previously identified by a classifier flows through the interior of the cantilever, it transiently changes the resonant frequency of the cantilever in proportion to the buoyant mass of the particle. SMR devices 301 may weigh single mammalian cells with a resolution of 0.05 pg (0.1% of a cell's buoyant mass) or better. Where mass or MAR is measured, devices of the disclosure are provided that are capable of measuring the mass or MAR within certain valuable sensitivities or times from the particles identified by a classifier. For example, mass measurement instruments that use a suspended microchannel resonator (SMR) device 301 are capable of measuring mass, mass change, or MAR with a precision of at least about 0.01 % of a cell mass. SMR-based instruments are capable of measuring mass, mass change, or MAR with a precision of at least about 0.1% per hour. SMR-based instruments are capable of measuring mass, mass change, or MAR within a duration of measuring the MAR that is within about 20 minutes to about 3 hours. Embodiments of the technology use microchannel resonators to precisely measure mass and mass changes in individual living cells after identification of the cell as cellular material by a classifier. The sSMR array 401 includes an array of SMR devices fluidically connected in series and separated by delay channels between each cantilever 449. The delay channels give the cell time to grow as it flows between cantilevers.

**[0031]** SMR devices 301 to be used together with a classifier may be fabricated as described in Lee, 2011, Suspended microchannel resonators, Lab Chip 11:645 and/or Burg, 2007, Weighing of biomolecules, Nature 446:1066-1069, both incorporated by reference. Large-channel devices (e.g., useful for peripheral blood mononuclear cells (PBMC) measurements) may have cantilever 333 interior channels of 15 by 20  $\mu\text{m}$  in cross-section, and delay channels 20 by 30  $\mu\text{m}$  in cross-section. Small-channel devices (useful for a wide variety of cell types) may have cantilever 333 channels 3 by 5  $\mu\text{m}$  in cross-section, and delay channels 4 by 15  $\mu\text{m}$  in cross-section. The tips of the cantilevers 449 in the sSMR array 401 may be aligned so that a single line-shaped laser beam can be used for optical-lever readout. The cantilevers may be arrayed such that the shortest (and therefore most sensitive) cantilevers are at the ends of the array. Before use for measuring particles identified by the classifier, the sSMR array 401 may be cleaned with piranha (3:1 sulfuric acid to 50% hydrogen peroxide) and the channel walls may be passivated with

polyethylene glycol (PEG) grafted onto poly-L-lysine. In some embodiments, a piezo-ceramic actuator seated underneath the device is used for actuation. The SMR device 301 may include low-noise photodetector, Wheatstone bridge-based amplifier (for piezo-resistor readout), and high-current piezo-ceramic driver. To avoid the effects of optical interference between signals from different cantilevers (producing harmonics at the difference frequency), the instrument may include a low-coherence-length light source (675 nm super-luminescent diode, 7 nm full-width half maximum spectral width) as an optical lever. After the custom photodetector converts the optical signal to a voltage signal, that signal is fed into an FPGA board, in which an FPGA implements twelve parallel second-order phase-locked loops which each both demodulate and drive a single cantilever. The FPGA may be a Cyclone IV FPGA on a DE2-115 development board operating on a 100 MHz clock with I/O provided via a high-speed AD/DA card operating 14-bit analog-to-digital and digital-to-analog converters at 100 MHz.

**[0032]** To operate all cantilevers 449 in the sSMR array 401 in order to measure a particle identified by a classifier, the resonator array transfer function is first measured by sweeping the driving frequency and recording the amplitude and phase of the array response. Parameters for each phase-locked loop (PLL) are calculated such that each cantilever-PLL feedback loop has a 50 or 100 Hz FM-signal bandwidth. The phase-delay for each PLL may be adjusted to maximize the cantilever vibration amplitude. The FM-signal transfer function may be measured for each cantilever-PLL feedback loop to confirm sufficient measurement bandwidth (in case of errors in setting the parameters). That transfer function relates the measured cantilever-PLL oscillation frequency to a cantilever's time-dependent intrinsic resonant frequency. Frequency data for each cantilever may be collected at 500 Hz, and may be transmitted from the FPGA to a computer. The device may be placed on a copper heat sink/source connected to a heated water bath, maintained at 37 degrees C.

**[0033]** The sample is loaded into the device from vials pressurized under air or air with 5% CO<sub>2</sub> through 0.009 inch inner-diameter fluorinated ethylene propylene (FEP) tubing. The sample may comprise cellular and/or non-cellular material together. The pressurized vials may be seated in a temperature-controlled sample-holder throughout the measurement. FEP tubing allows the device to be flushed with piranha solution for cleaning, as piranha will damage most non-fluorinated plastics. To measure a sample of cells, the sSMR array 401 may initially flushed with filtered media. Particles of cellular and/or non-cellular material may be identified by a classifier

and then provided to the sSMR 401. The flow rate of particles through the sSMR 401 may be based on the identification of a particle as cellular or non-cellular material. On large-channel devices, between one and two psi may be applied across the entire array based on the identification of the particle by the classifier, yielding flow rates on the order of 0.5 nL/s (the array's calculated fluidic resistance is approximately  $3 \times 10^{16}$  Pa/(m<sup>3</sup>/s). For small-channel devices, 4–5 psi may be applied across the array, yielding flow rates around 0.1 nL/s based on the identification of the particle by the classifier. Additionally, every several minutes new sample may be flushed into the input bypass channel to prevent particles and cells from settling in the tubing and device. Between experiments, devices may be cleaned with filtered 10% bleach or piranha solution.

**[0034]** For the data analysis, the recorded frequency signals from each cantilever 449 are rescaled by applying a rough correction for the different sensitivities of the cantilevers. For example, particles of non-cellular reference material identified by the classifier may be used to calibrate the cantilevers of the device. Cantilevers differing in only their lengths should have mass sensitivities proportional to their resonant frequencies to the power three-halves. Therefore each frequency signal is divided by its carrier frequency to the power three-halves such that the signals are of similar magnitude. To detect peaks, the data are filtered with a low pass filter, followed by a nonlinear high pass filter (subtracting the results of a moving quantile filter from the data). Peak locations are found as local minima that occur below a user-defined threshold. After finding the peak locations, the peak heights may be estimated by fitting the surrounding baseline signal (to account for a possible slope in the baseline that was not rejected by the high pass filter), fitting the region surrounding the local minima with a fourth-order polynomial, and finding the maximum difference between the predicted baseline and the local minima polynomial fit. Identifying the peaks corresponding to non-cellular reference materials identified by the classifier allows one to estimate the mass sensitivity for each cantilever, such that the modal mass for the particles is equal to the expected modal mass. Peaks at different cantilevers 449 that originate from the same cell are matched up to extract single-cell growth information. The sSMR array 401 and can measure live cells.

Precision frequency detection following identification of particles by a classifier allows the SMR device 301 to measure resonant frequency and mass in single living cells, single nanoparticles, and adsorbed protein layers in fluid. Precision is the closeness of agreement between

independent test results. When determining SMR resonance frequency optically, the use of an external laser and photodiode are required and cannot be easily arrayed for multiplexed measurements. Electronic detection of SMR resonance frequency may be attained by fabricating piezo-resistive sensors using ion implantation into single crystal silicon resonators. The mass resolution achieved with piezo-resistive detection, such as 3.4 femtogram (fg) in a 1 kHz bandwidth, is comparable to what can be achieved by a conventional optical detector designed to weigh micron-sized particles and cells. The use of an SMR device 301 together with the classifier 255 provides the advantage of eliminating the need for expensive, delicate optical components and provides new uses for the SMR device 301 in multiplexed and field deployable applications. For example, piezo-resistive sensors eliminate the need for external components by measuring deflection through the resistance change of a sensing element integrated onto the cantilever. Microfluidic channels are incorporated inside a cantilever resonator, which significantly reduces viscous damping from fluid and allows buoyant mass to be measured with high resolution. Use of a classifier to identify particles before being introduced to the SMR device 301 allows for flow through microfluidic channels to be controlled to optimize measurements.

**[0035]** Methods for optimized cellular measurement may comprise introducing cells and/or non-cellular material into a measurement device comprising a sample channel, a secondary channel, and a sensor 239 operating over a sensing region 235. Cellular and non-cellular material may be introduced into the sample channel and flow through the channel to the sensing region 235. The sensor 239 operating over the sensing region 235 may then collect and provide data to the classifier to identify cellular and non-cellular material in real time. Once identified, the flow of fluid in the sample channel and the secondary channel may be controlled in order to control the flow of cellular and/or non-cellular material from the sample channel into the secondary channel. The secondary channel may advantageously comprise a measurement device, such as an SMR device 301, for collecting measurements from the cells or non-cellular material.

**[0036]** The classifier may identify particles of cellular and non-cellular material using signals from a sensor 239, frequency data from a resonator, or any other method for discriminating between particles.

**[0037]** When the classifier uses signals from a sensor 239, the sensor may be an imaging sensor, and may comprise an array of sensor elements. Sensor elements may include photoelectric sensor

elements. Imaging sensors collect data about light or diffraction patterns incident upon sensor elements from cellular or non-cellular materials in the sensing region 235. Upon receiving a signal to capture an image from the sensing region 235, incoming light from particles of cellular and non-cellular material reach an array of sensor elements of the imaging sensor. Each sensor element may collect and store photons from light as an electrical signal. By having an array of sensor elements configured to capture particles of cellular and non-cellular material, the imaging sensor can record a present state of the sensing region 235 for the classifier. When sensing color images, the imaging sensor may have a color filter array (CFA) that limits each sensor element to only collect incoming light for a particular color, for example each sensor element may capture light that corresponds to only one primary color.

**[0038]** After light exposure upon the array of sensor elements, the electrical signal from the individual sensor elements may then be used to reproduce the image of the sensing region 235 by configuring the color and brightness of matching pixels to the electrical signals. A computer may be provided to match pixels to recreate the image. In some instances, for every sensor element there may be a corresponding pixel within the recreated image that reflects the charge and color received at the sensor element from the sensing region 235. The classifier may identify cellular and non-cellular material based on the recreated image of the sensing region 235. The classifier may also identify cellular and non-cellular material directly from the electrical signals provided by the sensor.

**[0039]** The imaging sensor may comprise a lens and/or may comprise a camera such as a digital camera. The imaging sensor may be a charge-coupled device (CCD) or may be a complementary metal-oxide-semiconductor (CMOS) sensor. CCD and CMOS sensors can be arranged in a two-dimensional array to capture two-dimensional image signals. Sensor size and/or the number of sensor elements may be used to control the spatial resolution of the image captured. The resolution may be pixel resolution. Increasing the density of sensor elements increases spatial resolution. Increasing the size of sensors increases the amount of light incident on each sensor. Imaging detail may be limited by optics due to lens blurs, lens aberration effects, aperture diffractions, and optical blurring due to motion.

**[0040]** The imaging sensor may advantageously be a lens-free imaging sensor, for example an imaging sensor that does not comprise correction lenses or components. The lens-free imaging may be on chip imaging using a digital optoelectric sensor array, such as a CCD or CMOS chip.

Imaging chips and optical components provide the advantage when used with the classifier of capturing very high-resolution images. The chip may directly sample light transmitted through a source without the use of any imaging lenses between the source and the sensor planes. Lens-free imaging sensors can advantageously comprise more compact, lightweight, and simpler hardware than lens based sensors. Lens-free imaging sensors are described in Greenbaum, 2012, Imaging without lenses: achievements and remaining challenges of wide-field on-chip microscopy, Nat Methods, 9(9):889-895, incorporated by reference.

**[0041]** The classifier may identify cellular and non-cellular material based on an image of the sensing region 235. The image may have a pixel resolution. The classifier may also identify cellular and/or non-cellular material directly from the electrical signals provided by the sensor elements. The identification of cellular and non-cellular material by the classifier using data from an imaging sensor may be used to calibrate and optimize cellular measurements in real-time or may be used to calibrate and optimize future measurements from cellular or non-cellular materials.

**[0042]** A classifier may also identify cellular and non-cellular particles using data from a measurement device comprising at least one SMR device 301. Methods for optimized cellular measurement may also comprise the steps of introducing cellular and/or non-cellular particles with overlapping size and/or mass distributions to a measurement device comprising at least one suspended microchannel resonator (SMR) device 301 and identifying the sub-groups of particles in the mixture based on a classifier that utilizes data from said measurement device.

**[0043]** Classification of sub-groups of particles using an SMR device 301 may be based on a "node-deviation" signal from an SMR device 301. When measuring deviation of resonant frequency using an SMR device 301, the SMR device 301 acts as an acoustic energy source and scattered acoustic fields from particles provide a signal that is used to monitor mechanical properties of the particles. Vibration of the SMR device 301 varies along the length of a cantilever 333, with one local maximum near the center, referred to as an antinode, and a zero-minimum near the tip, referred to as a node. When cellular or non-cellular particles are at the antinode, the net change in mass of the particle corresponds to the change in kinetic energy of the system, and causes a shift in the resonant frequency of the SMR device 301. As described above, by measuring the frequency at which the cantilever 333 resonates, the instrument computes a mass, or change in mass, of a cellular and/or non-cellular particle in the fluidic microchannel

previously identified by a classifier. When the particle is at the node of the cantilever 333, a net change in mass had previously been theorized not to shift the frequency at which the cantilever 333 resonates because the vibration amplitude is zero and there is no change in kinetic energy. In practice, however, resonant frequency shifts may be consistently measured at the node, including  
5 when flowing cells and polystyrene beads through the microfluidic channel. This resonant frequency shift at the node is referred to as node-deviation and corresponds to an energy change due to acoustic scattering from a material's surface dependent on mechanical properties of the material. The SMR device 301 may collect resonant frequency data at the node of cantilever 333 for cellular and non-cellular flowing therethrough. The resonant frequency data from the SMR  
10 device 301 may be provided to the classifier and the classifier may identify sub-groups of particles based on a node-deviation of signal from the SMR device 301.

**[0044]** FIG. 5 shows a cantilever 333 of an SMR 301. When a cellular or non-cellular particle is at the antinode 505, the net change in mass of the particle corresponds to the change in kinetic energy of the system, and causes a shift in resonant frequency. By measuring frequency at the  
15 antinode 505 the instrument computes the buoyant mass of the cellular or non-cellular particle. When the particle is at the node of the cantilever 509 the resonant frequency shift at the node (the node deviation) corresponds to an energy change due to acoustic scattering from the material's surface dependent on mechanical properties of the cellular or non-cellular particle, such as surface stiffness. Node-deviation data from the SMR device 301 can be provided to a classifier  
20 that utilizes the data to identify subgroups of particles. The classifier may discriminate between cellular and non-cellular material based on surface stiffness. Non-cellular particles with a known size and/or mass may be used as a reference material to calibrate the measurement device in real-time or calibrate the measurement device for future measurements. Preferably, the reference material has an overlapping size and/or mass with cellular material.

**[0045]** Node-deviation can be measured independently of fluid velocity and vibration amplitude. Therefore, by measuring the resonant frequency shifts at the antinode and node as materials flow through the SMR device 301, one can simultaneously and independently quantify the buoyant mass of the material and the node deviation for the material. Node deviation may be influenced by a cellular or non-cellular material's volume. A volume correction may be applied to the  
25 measured node-deviation through size-normalized acoustic scattering, with the appropriate  
30 correction determined through, for example, finite element method (FEM) simulations for fluid-

structure acoustic interactions. Node-deviation may further be influenced by the cellular or non-cellular particle's mass distribution and/or orientation within a microfluidic channel. The mass distribution for a particle of cellular or non-cellular material may be acquired by bright-field images or may be known a priori, and a mass distribution correction may be applied to the measured node-deviation. Node-deviation can be used to determine one or more mechanical properties of particles of cellular or non-cellular material. For example, node deviation may be used to determine surface stiffness of the particle. When measuring node-deviation in a cell, the measurement may be used to determine cell surface stiffness or properties of the actomyosin cortex of the cell. For example, cell surface stiffness varies throughout cell mitosis and node deviation may be used to determine properties and stages of mitosis in the cell. The classifier may identify sub-groups of particles, such as cellular and/or non-cellular particles, based on surface stiffness and/or node-deviation data from an SMR device 301. The identification of sub-groups of particles by the classifier may be used to calibrate and optimize cellular measurements in real-time or may be used to calibrate and optimize future measurements of cellular or non-cellular particles by the measurement device.

**[0046]** The classifier may be based on any suitable machine learning system trained to discriminate between cellular and non-cellular material. For example, the machine learning system may learn in a supervised manner, an unsupervised manner, a semi-supervised manner, or through reinforcement learning.

**[0047]** In supervised learning models, the machine learning system is given training data categorized as input variables paired with output variables from which to learn patterns and make inferences in order to generate a prediction on previously unseen test data. Supervised models replicate an identified mapping system and recognize and respond to patterns in data without explicit instructions. Supervised models are advantageous for performing discrete classification tasks, in which data inputs are separated into categories. Supervised models are also advantageous for continuous regression tasks, in which the output variable is a real value, such as a price or a volume. The accuracy of a supervised model is easy to evaluate, because there is a known output variable to which the model is optimizing. Supervised models are advantageous for training a classifier to separate cellular and non-cellular material into respective categories when a suitable training data set for cellular and non-cellular materials is available. For example, a training set comprising labeled images of cellular particles and non-cellular particles may be

used by the classifier to identify cellular and non-cellular particles in imaging data provided by an imaging sensor.

**[0048]** In an unsupervised model or autonomous model, the machine learning system is only given input training data without paired output data from which to identify patterns

5 autonomously. Unsupervised models identify underlying patterns or structures in training data to make predictions for test data. Unsupervised models are advantageous for clustering data, anomaly detection, and for independently discovering rules for data. The accuracy of unsupervised models is harder to evaluate because there is no predefined output variable to which the system is optimizing. Autonomous models may employ periods of both supervised and  
10 unsupervised learning in order to optimize predictions. Unsupervised models are advantageous for training a classifier to cluster data into clusters when labeled training data is unavailable. The classifier may use additional data to identify each cluster as cellular or non-cellular material. For example, a classifier may identify clusters of data from a signal provided by an imaging sensor. The classifier may use previously collected node-deviation data from an SMR device 301 to  
15 identify which clusters identify cellular material and which clusters identify to non-cellular material.

**[0049]** In semi-supervised models, the machine learning system is given training data comprising input variables, with output variable pairs available for only a limited pool of the input variables. The model uses the input variables with output variable pairs and the remaining  
20 input training data to learn patterns and make inferences in order to generate a prediction on previously unseen test data. A semi-supervised model may advantageously query the user for additional paired output data based on unpaired data. Semi-supervised models are advantageous for training a classifier to separate cellular and non-cellular material into respective categories when an incomplete training data set for cellular and non-cellular materials is available. For  
25 example, a training set comprising labeled images for some cellular particles and some non-cellular particles may be used by the classifier to correctly identify those particles in an image provided by a sensor 239 while also identifying clusters of data from the image for particles it cannot identify from the training data set.

**[0050]** In a reinforcement learning model, the machine learning system is given neither input  
30 variables nor output variables. Rather, the model provides a "reward" condition and then seeks to maximize the cumulative reward condition by trial and error. A reinforcement learning model is

a Markov Decision Process. Supervised, unsupervised, semi-supervised, and reinforcement models are described in Jordan and Mirchell, 2015, Machine learning, Trends, perspectives, and prospects, Science 349(6245):255-260, incorporated by reference.

[0051] An example of a supervised learning model is a "decision tree." Decision trees are non-parametric supervised learning models that use simple decision rules to infer a classification for test data from the features in the test data. In classification trees, test data take a finite set of discrete values, or classes, whereas in regression trees, the test data can take continuous values, such as real numbers. Decision trees have some advantages in that they are simple to understand and can be visualized as a tree starting at the root (usually a single node) and repeatedly branch to the leaves (multiple nodes) that are associated with the classification. See Criminisi, 2012, Decision Forests: A unified framework for classification, regression, density estimation, manifold learning and semi-supervised learning, Foundations and Trends in Computer Graphics and Vision 7(2-3):81-227, incorporated by reference. Decision tree models can be advantageous for the classifier to identify particles of cellular or non-cellular material because the particles fall into a discrete set of classes or categories, i.e. cellular or non-cellular. For example, the classifier may identify that a particle is in an image provided by a sensor 239 and using training data to infer that the particle is cellular material. Once the particle is identified as cellular material, the classifier can use the training data to identify the cellular material as DNA.

[0052] Another supervised learning model is a "support-vector machine" (SVM) or "support-vector network." SVMs are supervised learning models for classification and regression problems. When used for classification of new data into one of two categories, such as whether a particle is cellular or non-cellular, an SVM creates a hyperplane in multidimensional space that separates data points into one category or the other. Although the original problem may be expressed in terms that require only finite dimensional space, linear separation of data between categories may not be possible in finite dimensional space. Consequently, multidimensional space is selected to allow construction of hyperplanes that afford clean separation of data points. See Press, W.H. et al., Section 16.5. Support Vector Machines. Numerical Recipes: The Art of Scientific Computing (3rd ed.). New York: Cambridge University (2007), incorporated herein by reference. Where output variable pairs are unavailable for input variables in the training data, SVMs can be designed as unsupervised or semi-supervised learning models using support vector clustering. See Ben-Hur, 2001, Support Vector Clustering, J Mach Learning Res 2:125-137,

incorporated by reference. SVM models can be advantageous for the classifier to identify particles of cellular or non-cellular material where the particles fall into a limited number of possible categories. Additionally, SVM models can be advantageous where only a limited set of training data is available for the classifier.

5 **[0053]** Logistic regression analysis is another statistical process that can be used by the classifier to find patterns in training and test data to make predictions. It includes techniques for modeling and analyzing relationships between a multiple variables. Specifically, regression analysis focuses on changes in a dependent variable in response to changes in single independent variables. Regression analysis can be used to estimate the conditional expectation of the  
10 dependent variable given the independent variables. The variation of the dependent variable may be characterized around a regression function and described by a probability distribution. Parameters of the regression model may be estimated using, for example, least squares methods, Bayesian methods, percentage regression, least absolute deviations, nonparametric regression, or distance metric learning. Like SVM models, regression models are also advantageous for the  
15 classifier to identify particles of cellular or non-cellular material where the particles fall into a limited number of possible categories. Regression models also provide the advantage of being effectively implemented by a variety tools and the model can be easily updated to identify new particles.

**[0054]** Bayesian algorithms can also be used to find patterns in training and test data to make  
20 predictions. Bayesian networks are probabilistic graphical models that represent a set of random variables and their conditional dependencies via directed acyclic graphs (DAGs). The DAGs have nodes that represent random variables that may be observable quantities, latent variables, node unknown parameters or hypotheses. Edges represent conditional dependencies; nodes that are not connected represent variables that are conditionally independent of each other. Each is  
25 associated with a probability function that takes, as input, a particular set of values for the node's parent variables, and gives (as output) the probability (or probability distribution, if applicable) of the variable represented by the node. Like SVM models and regression models, Bayesian models are also advantageous for the classifier to identify particles of cellular or non-cellular material where the particles fall into a limited number of possible categories. Bayesian models  
30 provide the advantage of generally requiring less training data than other models and can be used by the classifier to identify cellular and non-cellular material quickly.

[0055] Some models rely on clustering training data and test data to find patterns and make predictions. A "k-nearest neighbor" (k-NN) model is a supervised non-parametric learning model for classification and regression problems. A k-nearest neighbor model assumes that similar data exists in close proximity, and assigns a category or value to each data point based on the k nearest data points. k-NN models may be advantageous when the data has few outliers and can be defined by homogeneous features. k-NN models can be advantageous for the classifier to identify particles of cellular or non-cellular material because the particles fall into a discrete set of classes or categories, i.e. cellular or non-cellular. Moreover, k-NN models provide the advantage of continuously learning from test data and do not require a training period before identifying cellular or non-cellular material from training data.

[0056] An example of an unsupervised learning model that uses clustering is a "k-means" clustering model. A k-means model looks to find clusters of data in input data and test data. K-means models are advantageous when a defined number of clusters are known to exist in the data and are also advantageous when the test data has few outliers and can be defined homogeneous features. Additional models that cluster training data include, for example, farthest-neighbor, centroid, sum-of-squares, fuzzy k-means, and Jarvis-Patrick clustering. k-means and other unsupervised clustering models are advantageous for use by the classifier to identify cellular and non-cellular material when training data for cellular or non-cellular material is unavailable or limited.

[0057] Trained machine learning models can become "stable learners." A stable learner is a model that is less sensitive to perturbation of predictions based on new training data. Stable learners can be advantageous where test data is stable, but can be less advantageous where the system needs to continually improve performance to accurately predict new test data that may be less stable. Accordingly, a stable learning model may be advantageous for use by the classifier when the types of cellular and non-cellular material that may be introduced to the measurement device are known and are unlikely to change.

[0058] Several machine learning system types can be combined into a final predictive models known as ensembles. Ensembles can be divided into two types, homogenous ensembles and heterogeneous ensembles. Homogenous ensembles combine multiple machine learning models of the same type. Heterogeneous ensembles combine multiple machine learning models of different types. Ensembles can provide an advantage when used by the classifier to identify

particles of cellular and non-cellular material because they can be more accurate than any of the individual base member models ("members") in the ensemble. The number of members combined in an ensemble may impact the accuracy of a final prediction. Accordingly, it is advantageous to determine the optimal number of members when designing an ensemble system for use by the classifier.

**[0059]** Ensembles used by the classifier may combine or aggregate outputs from individual members by using "voting"-type methods for classification systems and "averaging"-type methods for regression systems. In a "majority voting" method, each member makes a prediction as to the identification for cellular and/or non-cellular material in test data and the prediction that receives more than half of the votes is the final output for the ensemble. If none of the predictions receives more than half of the votes, it may be determined that the ensemble is unable to make a stable prediction. In a "plurality voting" method the most voted prediction, even if receiving less than half of the votes, may be considered the final output for the ensemble. In a "weighted voting" method, the votes of more accurate members are multiplied by a weight afforded each member based on its accuracy. In a "simple averaging" method, each member makes a prediction for test data and the average of the outputs is calculated. This method reduces overfit and can be advantageous in creating smoother regression models. In a "weight averaging" method, the prediction output of each member is multiplied by a weight afforded each member based on its accuracy. Voting methods, averaging methods, and weighted methods can be combined to improve the accuracy of ensembles used by the classifier.

**[0060]** Members within an ensemble used by the classifier can each be trained independently or new members can be trained utilizing information from previously trained members. In a "parallel ensemble", the ensemble seeks to provide greater accuracy than individual members by exploiting the independence between members, for example, by training multiple members simultaneously to identify cellular and non-cellular material and aggregating the outputs from members. In "sequential ensemble systems", the ensemble seeks to provide greater accuracy than individual members by exploiting the dependence between members, for example, by utilizing information from a first member regarding the identification of cellular and non-cellular material to improve the training of a second member for identifying cellular and non-cellular material and weighting outputs from members.

[0061] Overall accuracy for ensembles used by the classifier can also be optimized by using ensemble meta-algorithms, for example a "bagging" algorithm to reduce variance, a "boosting" algorithm to reduce bias, or a "stacking" algorithm to improve predictions.

[0062] Boosting algorithms reduce bias and can be used to improve less accurate, or "weak

learning" models. A member may be considered a "weak learning" model if it has a substantial

error rate, but its performance is non-random, for example an error rate of 0.5 for classifying a

particle as cellular or non-cellular. Boosting algorithms incrementally build the ensemble by

training each member sequentially with the same training data set, examining prediction errors

for test data (i.e. labeling a cellular particle as a non-cellular particle), and assigning weights to

training data based on the difficulty for members to make an accurate prediction. In each

sequential member trained, the algorithm emphasizes training data that previous members found

difficult. Members are then weighted based on the accuracy of their prediction outputs in view of

the weight applied to the training data. The predictions from each member may be combined by

weighted voting-type or weighted averaging-type methods. Boosting algorithms are

advantageous when combining multiple weak learning models. Boosting algorithms may,

however, result in over-fitting test data to training data. Examples of boosting algorithms include

AdaBoost, gradient boosting, eXtreme Gradient Boost (XGBoost). See Freund, 1997, A

decision-theoretic generalization of on-line learning and an application to boosting, J Comp Sys

Sci 55:119; and Chen, 2016, XGBoost: A Scalable Tree Boosting System, arXiv:1603.02754,

both incorporated by reference.

[0063] Bagging algorithms or "bootstrap aggregation" algorithms reduce variance by averaging

together multiple estimates from members. Bagging algorithms provide each member with a

random sub-sample of a full training data set, with each random sub-sample known as a

"bootstrap" sample. In the bootstrap samples, some data from the training data set may appear

more than once and some data from the training data set may not be present. Because sub-

samples can be generated independently from one another, training can be done in parallel. The

predictions for test data from each member are then aggregated, such as by voting-type or

averaging-type methods.

[0064] An example of a bagging algorithm that may be used by the classifier to identify cellular

and non-cellular material is a "random forests". In a random forest the ensemble combines

multiple randomized decision tree models. Each decision tree model is trained from a bootstrap

sample from a training set for identifying cellular and non-cellular material. The training set itself may be a random subset of features from an even larger training set. By providing a random subset of the larger training set at each split in the learning process, spurious correlations that can results from the presence of individual features that are strong predictors for the output variable are reduced. By averaging predictions for test data, variance of the ensemble decreases resulting in an improved prediction to identify cellular and non-cellular material. Random forests may be autonomous models and may include periods of both supervised and unsupervised learning. Bagging may be less advantageous in optimizing an ensemble combining stable learning systems, since stable learning systems tend provide generalized outputs with less variability over the bootstrap samples. Random forests are advantageous for use by the classifier to identify cellular and non-cellular material by providing a great degree of versatility in identifying cellular and non-cellular material and reducing spurious identification by the classifier. See Breiman, 2001, Random Forests, Machine Learning 45:5-32, incorporated by reference.

**[0065]** Stacking algorithms or "stacked generalization" algorithms improve predictions by using a meta-machine learning model to combine and build the ensemble. In stacking algorithms, base member models are trained with a training dataset and generate as an output a new dataset. This new dataset is then used as a training dataset for the meta-machine learning model to build the ensemble. Stacking algorithms are generally advantageous for use by the classifier to identify cellular and non-cellular material when building heterogeneous ensembles. Ensembles are described in Villaverde et al., 2019, On the adaptability of ensemble methods for distribution classification systems: A comparative analysis, International Journal of Distributed Sensor Networks 15(7); and Heitor et al., 2017, A Survey of Ensemble Learning for Data Stream Classification, 50(2):Art. 23, each incorporated by reference.

**[0066]** Neural networks, modeled on the human brain, allow for processing of information and machine learning. The classifier for identifying cellular and non-cellular material may advantageously be based on a neural network. Neural networks include nodes that mimic the function of individual neurons, and the nodes are organized into layers. Neural networks include an input layer, an output layer, and one or more hidden layers that define connections from the input layer to the output layer. Systems and methods of the invention may include any neural network that facilitates machine learning. The system may include a known neural network

architecture, such as GoogLeNet (Szegedy, et al. Going deeper with convolutions, in CVPR 2015, 2015); AlexNet (Krizhevsky, et al. Imagenet classification with deep convolutional neural networks, in Pereira, et al. Eds., Advances in Neural Information Processing Systems 25, pages 1097–3105, Curran Associates, Inc., 2012); VGG16 (Simonyan & Zisserman, Very deep  
5 convolutional networks for large-scale image recognition, CoRR, abs/3409.1556, 2014); or FaceNet (Wang et al., Face Search at Scale: 80 Million Gallery, 2015), each of the aforementioned references are incorporated by reference. The advantage of using a classifier to identify cellular and non-cellular material based on a neural network architecture is that neural networks are able to learn patterns and correlations by themselves and produce outputs that are  
10 not limited to the training data provided to them. The neural network architecture allows the classifier to learn from examples of cellular and non-cellular particles and identify new particles in real-time. Additionally, the neural network architecture allows the classifier to identify multiple particles in parallel as they flow through a measurement device. For example, a classifier based on a neural network architecture may be provided image data from an image  
15 sensor 239 and identify each particle in the image data in real time with increasing accuracy as the number of images provided to the classifier increases.

**[0067]** Deep learning neural networks (also known as deep structured learning, hierarchical learning or deep machine learning) include a class of machine learning operations that may be used by the classifier that use a cascade of many layers of nonlinear processing units for feature  
20 extraction and transformation. Each successive layer uses the output from the previous layer as input. The algorithms may be supervised or unsupervised and applications include pattern analysis (unsupervised) and classification (supervised). Certain embodiments are based on unsupervised learning of multiple levels of features or representations of the data. Higher level features are derived from lower level features to form a hierarchical representation. Deep  
25 learning by the neural network includes learning multiple levels of representations that correspond to different levels of abstraction; the levels form a hierarchy of concepts. In some embodiments, the neural network includes at least 5 and preferably more than ten hidden layers. The many layers between the input and the output allow the system to operate via multiple processing layers. For example, a classifier based on a deep learning neural network may be  
30 provided image data from an imaging sensor. Earlier hidden layers in the network may identify the edges of particles and their location in the image with later hidden layers identifying the

brightness of each particle. The two features together may be used by a further hidden later to provide an output prediction for each particle in the image to the classifier.

**[0068]** Within a neural network that may be used by the classifier, nodes are connected in layers, and signals travel from the input layer to the output layer. Each node in the input layer may

5 correspond to a respective feature from the training data for cellular and non-cellular material.

The nodes of the hidden layer are calculated as a function of a bias term and a weighted sum of the nodes of the input layer, where a respective weight is assigned to each connection between a node of the input layer and a node in the hidden layer. The bias term and the weights between the input layer and the hidden layer are advantageously learned autonomously in the training of the

10 neural network. The network may include thousands or millions of nodes and connections. Typically, the signals and state of artificial neurons are real numbers, typically between 0 and 1.

Optionally, there may be a threshold function or limiting function on each connection and on the unit itself, such that the signal must surpass the limit before propagating. Back propagation is the use of forward stimulation to modify connection weights, and is sometimes done to train the

15 network using known correct outputs. See WO 2016/182551, U.S. Pub. 2016/0174902, U.S. Pat. 8,639,043, and U.S. Pub. 2017/0053398, each incorporated by reference.

**[0069]** An image from an imaging sensor provided to a classifier can be represented by a deep learning network in many ways, such as a vector of intensity values per pixel in the image, or in a more abstract way as a set of edges, regions of particular shape, etc. Those features are

20 represented at nodes in the network. Preferably, each feature is structured as numerical feature or vector that represents the image feature. This provides a numerical representation of objects in the image, since such representations facilitate processing and statistical analysis. Numerical features are often combined with weights using a dot product in order to construct a linear predictor function that is used to determine a score for making a prediction.

25 **[0070]** The vector space associated with those feature vectors may be referred to as the feature space. In order to reduce the dimensionality of the feature space, dimensionality reduction may be employed by networks used by the classifier. Higher-level features can be obtained from already available features and added to the feature vector, in a process referred to as feature construction. Feature construction is the application of a set of constructive operators to a set of

30 existing features resulting in construction of new features. For example, a classifier based on a neural network architecture may be provided image data from an image sensor. Early layers in

the neural network may identify horizontal lines and vertical lines in the image data. Later layers in the network may then use the lines identified to obtain edges, a higher-level feature, for particles in the image.

[0071] A convolutional neural network (CNN) is a class of deep neural network generally designed for two-dimensional image inputs in which a signal travels from the input layer through hidden layers comprising "convolutional layers" and "fully connected layers" to the output layer. Accordingly, a CNN is particularly advantageous for use by the classifier when provided image inputs, for example from an imaging sensor. In the input layer of a CNN, each pixel from an image is mapped. The input layer is connected to a convolutional layer. In a convolutional layer, each node is "sparsely connected", that is connected to only a sub-matrix of pixels or nodes from the previous layer. The connection between the submatrix of nodes and the convolutional layer is subject to a bias term as a set of weights designed detect a given feature in the input. The submatrix and weights together are known as a "filter," "kernel," or "feature detector". For a given convolutional layer, each filter is the same size and shape and applies the same set of weights. Each node in the convolutional layer is provided a summary of the weighted information from the filter as a scalar dot product. The filters are staggered from one another and may overlap such that each node in convolution layer provides a weighted summary for a different sub-matrix from the previous layer. A threshold function may be applied to each node in the convolution layer to determine whether the node will propagate the information from the filter, a function known as "squashing."

[0072] Sliding the filter systematically across the entire input allows the filter to discover a given feature anywhere in the input. This provides the advantage of allowing a classifier based on a CNN to identify particles anywhere in imaging data provided to the classifier. The function of sliding the filter over the entire image can be controlled by the number of nodes over which the filter passes, known as the "stride" of the convolutional layer. The stride determines the distance that each filter is staggered from adjacent filters and the degree of overlap between filters. The final two-dimensional array of dot products of the convolutional layer is known as the "convolved feature," "activation map," or "feature map."

[0073] Filters may also have a given depth. For example, color images have multiple channels, typically one for each color channel, such as red, green, and blue. This means that a single color image provided as an input to the input layer is, in fact, three images. A filter must always have

the same number of channels as the input, referred to as “depth“. If an input image has 3 channels then a filter applied to that image must also have 3 channels, resulting in, for example, each 2x2 filter becoming a 2x2x3 filter (length x width x depth). Regardless of the depth of the input and depth of the filter, the filter is applied to the input using a dot product operation which results in a single value. This means that if a convolutional layer has 32 filters, these 32 filters are not just two-dimensional for the two-dimensional image input, but are also three-dimensional, having specific filter weights for each of the three channels. Each filter contributes to a single feature map. Accordingly, a classifier based on a CNN may be advantageous where the data provided to the classifier comprises color images and/or inputs with multiple channels.

**[0074]** Different filters produce different feature maps. A convolutional layer may apply a different filter depending on the given input, with the types of filters available learned during training of the network. For example, the network may be trained to apply filters for a specific task the network is trained to resolve, such as detecting whether an input image contains a vertical line. The convolution layer may be trained to apply any number of possible filters to an input image.

**[0075]** In some instances it may also be convenient to “pad” an input to a convolutional layer with zero values around the border of the input, a process known as zero-padding. Zero-padding allows the size of feature maps to be controlled. This can allow for the feature map to remain the same size as the input through multiple layers of the CNN. The function of adding zero-padding is known as “wide-convolution” versus “narrow convolution” when no zero-padding is added.

**[0076]** The use of multiple convolutional layers in the network allows for hierarchical decomposition of the input. Convolutional filters that operate directly on input values may learn to extract low level features, such as lines. Convolutional filters that operate on the output from earlier convolution layers may learn to extract features that are combinations of lower-level features, such as features that comprise multiple lines to express shapes. The classifier can use multiple convolution layers to reconstruct particles from an input and thereafter identify the particles as cellular or non-cellular material.

**[0077]** A CNN used by a classifier may also comprise nonlinear layers (ReLU). A ReLU layer receives a feature map and replaces any negative values in the feature map with a zero. The purpose of the ReLU layer is to introduce non-linearity into the CNN and is advantageous when the input data that the CNN is expected to learn and identify is non-linear, including image

features such as particles. The non-linear output map from a ReLU is known as a "rectified" feature map. The CNN may also comprise pooling layers. A pooling layer reduces the size of the feature map or rectified feature map through dimensionality reduction in a process known as "spatial pooling," "subsampling," or "downsampling." For example, each node in a pooling layer may be sparsely connected to a sub-matrix of nodes from a convolution or ReLU layer. Each node in the pooling layer may then provide, for example, only the highest value, average of, or sum of the values in each submatrix. Pooling layers can be advantageous to make input representations smaller and more manageable, reduce the number of parameters and computations in the network, reduce the impact of distortions in the input image, and/or help scale representation of the image. This provides the advantage of reducing training time and controlling overfitting in the CNN used by the classifier to identify cellular and non-cellular material.

**[0078]** The final output from the convolutional, ReLU, and/or pooling layers, for example the extraction of particle features from imaging data, is provided to a fully connected layer. The fully connected layers operate under the same principles as a traditional neural network. In a fully connected layer each node in the layer is connected to all of the nodes in a previous layer and all of the nodes in a succeeding layer. The purpose of a fully connected layer is to classify the features extracted by the convolutional layers, for example using single vector machines (SVM) to classify the particle features extracted by the previous layers. Backpropagation in CNNs involves adjusting the weights of filters based on the error rate of the CNN, known as "loss." During backpropagation, the CNN determines the estimated loss at every node in each convolutional layer and adjusts filter weights accordingly to minimize loss. A CNN may be trained by multiple rounds of backpropagation. Convolutional Neural Networks are described in Haridas and Jyothi, 2019, Convolutional Neural Networks: A Comprehensive Survey, 14(3):780-789, incorporated by reference. CNNs are advantageous for use with the classifier for identifying cellular and non-cellular material because they provide automatic feature extraction from input data and autonomously learn the features necessary to allow the classifier to identify cellular and non-cellular material.

**[0079]** The classifier of the present invention may comprise a neural network architecture trained to use sensor 239 data to classify particles of cellular and non-cellular material or sub-groups of such particles. For example, the classifier may comprise a convolutional neural network (CNN).

Identification of cellular and non-cellular material or sub-groups of particles allows for control of flow of fluid through the measurement device, allowing for optimized cellular measurement, for example mass accumulation rate. The classifier may advantageously identify cellular and non-cellular material or sub-groups of particles in real time. The classifier may also identify cellular and non-cellular material or sub-groups of particles and the identification may be used to calibrate the measurement device for future measurements.

**[0080]** The classifier may be trained using data from a measurement device previously obtained from different sub-groups of particles. The sub-group of particles may comprise particles of cellular and/or non-cellular material. The classifier may be trained by backpropagation using data from a measurement device previously obtained from the sub-groups of particles. The classifier may be trained using a training data set comprising imaging data, for example from an imaging sensor. The classifier may be trained using resonant frequency data, for example node-deviation.

**[0081]** The identification of cellular and non-cellular material in the device allows for the flow of cellular and/or non-cellular material from the sample channel into the secondary channel. By controlling the flow of cellular and non-cellular material into the secondary channel, cellular and non-cellular materials can be loaded into the secondary channel at a specified ratio. Cellular and non-cellular materials may be loaded into the secondary channel at a ratio, for example, such that non-cellular reference material periodically flows into the second channel to recalibrate measurements for cellular material or to recalibrate the measurement device. Designation of cellular or non-cellular material may be paired with the respective measurements collected for cellular or non-cellular material. The measurements collected for cellular and non-cellular material may be mass or MAR. The measurements may be collected by an SMR device 301. The identification of particles of cellular and non-cellular materials or sub-groups of particles may be in real time, for example, where the particles flow through a device and data is collected from the particles and the classifier identifies the particles based on the data as the particles flow through the device. Data may also be collected from the particles and later provided to a classifier which identifies the particles, with the data used for training a classifier or for calibrating the measurement device.

**[0082]** Cellular material can include cellular material selected from the group consisting of cells, cell aggregates, exosomes, extracellular vesicles, cellular components, cellular fragments,

organelles, organoids, proteins and protein aggregates, DNA, and RNA. Cells can comprise any biological cells, such as bacterial cells or mammalian cells. Mammalian cells, for example, can include cancer cells, such as tumor cells, glioblastoma cells, or leukemia cells. Mammalian cells can also include immune cells and cancer related immune cells including T cells such as CD8<sup>+</sup> T cells. Cells can also be living cells. The classifier 255 may identify the cellular material as a specific type of cellular material, for example, cells, cell aggregates, exosomes, extracellular vesicles, cellular components, cellular fragments, organelles, organoids, proteins and protein aggregates, DNA, and RNA. Notably, methods of the invention analyze the sample without destroying the cells. The advantage of using living cells is that the cells are available for further analysis, such as genome sequencing, flow cytometry, or other measurements.

**[0083]** Non-cellular material can include material selected from the group consisting of synthetic particles, inorganic particles, and debris. The classifier 255 may identify the non-cellular material as a specific type of non-cellular material, for example synthetic particles, inorganic particles, or debris. Non-cellular material may include reference material with a known property.

The known property of the reference material may be size, mass, and/or density. The reference material may be a synthetic particle and the synthetic particle may be a bead. Beads may be microspheres and may have a known property, such as size, mass, and/or density for use as a reference material for calibrating measurements or measurement devices. For example, beads may have a known mass which can be used to calibrate a measurement device prior to taking measurements or may be used to adjust measurements that have been previously made. Beads may be selected to approximate the size, emission wavelength, and intensity of a biological sample. Beads may include polystyrene beads or silica beads. Debris, such as cell debris, may also be included in a sample. Debris once identified may be rejected from entering or removed from the measurement device. Debris may also be loaded with the sample into the measurement device and any measurements from debris excluded.

**[0084]** Cellular and non-cellular material may be introduced into the measurement device separately or together in the same sample. For example, the cellular and non-cellular material may be introduced together into the sample channel as a single fluid or as separate fluids. A sensor 239 operating over the sensing region 235 provides data to a classifier that utilizes the data to control flow in the sample and secondary channels based on the identification of cellular and/or non-cellular material in the fluid or fluids. For example, cells and polystyrene beads may

be introduced into the measurement device at the same time and the classifier may identify the cells as cells and the polystyrene beads as polystyrene beads. Loading cells together with polystyrene beads provides the advantage of allowing real-time density estimates of the fluid where cells and beads flow together through the device. Once identified, particles of cellular and/or non-cellular material may additionally be introduced into the secondary channel and/or into a measurement device at different flow rates optimized for measuring one or more properties of the cells and/or non-cellular material, for example mass or mass accumulation rate.

**[0085]** FIG. 6 shows introducing 129 a sample 601 comprising cellular and or non/cellular material 625 into an instrument 629 capable of making 651 optimized cell measurements 625.

The sample 601 may include one or more live cells, such as a cancer cell or an immune cell. Samples may be collected and stored in their own container 605, such as a tube or flask such as the 1.5 mL micro-centrifuge tube sold under the trademark EPPENDORF FLEX-TUBES by Eppendorf, Inc. (Enfield, CT). The instrument 629 is operable to make optimized cell measurements in the one or more live cells, such as single-cell biophysical properties, including, but not limited to, mass, growth rate, and mass accumulation of an individual living cell. The instrument 629 uses a classifier that utilizes data from a sensor or from a suspended microchannel resonator (SMR) device 301 to identify particles of cellular and non-cellular material and control flow of the sample through the instrument, such as through a sample channel 229 and a secondary channel 269. Particles of cellular and/or non-cellular material may be loaded into a secondary channel and/or a measurement device such as an SMR device 301. The SMR device 301 may be used to precisely measure biophysical properties, such as mass and mass changes, of a single cell flowing therethrough. The mass change may be mass accumulation rate (MAR). Upon passing through the instrument 629, single cells remain viable and can be isolated downstream from the instrument 629 and are available to undergo subsequent assays. As shown, a sample 609 of the one or more live cells having undergone the first assay (i.e., passing through the instrument 629) are collected in a suitable container 613 and are then available to undergo a second assay.

**[0086]** The mass accumulation or rate of mass accumulation can be a clinically important property that is used to indicate the presence of cancer cells or the efficacy of a therapeutic on cancer cells. Cancer cells may be obtained from a patient and introduced into the measurement device of the present invention for an optimized cell measurement. Cells may be from a

biological sample obtained from a patient by any suitable means. Examples of obtaining the sample include fine needle aspiration, blood draw, and biopsy.

**[0087]** Fine needle aspiration and bone marrow biopsy provide a solid biological sample from the patient, providing the ability to sample from pleural effusions and ascites. Accordingly, the sample does not need to be in liquid form. Solid biological samples, for example from fine needle aspiration, may preferably be disaggregated and/or added to a buffer prior to introduction to the instrument. Accordingly, optimized cellular measurements may be obtained from cells from a tissue sample obtained from a solid tumor and the tumor can be from one selected from the group consisting of a bone, bladder, brain, breast, colon, esophagus, gastrointestinal tract, urinary tract, kidney, liver, lung, nervous system, ovary, pancreas, prostate, retina, skin, stomach, testicles, and uterus of a subject. The methods may be used to obtain tumors or cancers of any suitable type. Methods may include accessing a tumor in a patient via fine needle aspirate to take a biological sample comprising cancer cells, disaggregating the biological sample to isolate at least one living cell. The solid biological sample may then be suspended in a media and introduced to the measurement instrument. Non-limiting examples of media include saline, nutrient broth, and agar medium. Examples of biopsies that may provide cells for optimized cellular measurement using systems and methods described herein can include, needle biopsy, bone biopsy, bone marrow biopsy, liver biopsy, kidney biopsy, aspiration biopsy, prostate biopsy, skin biopsy, or surgical biopsy.

**[0088]** A tissue sample may include a mass of connected cells and/or extracellular matrix material, e.g. skin tissue, hair, nails, nasal passage tissue, CNS tissue, neural tissue, eye tissue, liver tissue, kidney tissue, placental tissue, mammary gland tissue, placental tissue, mammary gland tissue, gastrointestinal tissue, musculoskeletal tissue, genitourinary tissue, bone marrow, and the like, derived from, for example, a human or other mammal and includes the connecting material and the liquid material in association with the cells and/or tissues.

**[0089]** Liquid material derived from, for example, a human or other mammal such as body fluids may also be utilized. Such body fluids include, but are not limited to, mucous, blood, plasma, serum, serum derivatives, bile, blood, maternal blood, phlegm, saliva, sputum, sweat, amniotic fluid, menstrual fluid, mammary fluid, follicular fluid of the ovary, fallopian tube fluid, peritoneal fluid, urine, semen, and cerebrospinal fluid (CSF), such as lumbar or ventricular CS. A sample also may be media containing cells or biological material. A sample may also be a

blood clot, for example, a blood clot that has been obtained from whole blood after the serum has been removed. In certain embodiments, the sample is blood, saliva, or semen collected from the subject.

[0090] Any suitable sample may be obtained for optimized cellular measurements by the methods and systems of the invention. For example, the sample may include immune cells or cancer cells. The sample may include tissue of any type including healthy tissue or bodily fluid of any type. In some embodiments, the tissue sample is obtained from a pleural effusion in a subject. A pleural effusion is excess fluid that accumulates in the pleural cavity, the fluid-filled space that surrounds the lungs. This excess fluid can impair breathing by limiting the expansion of the lungs. Various kinds of pleural effusion, depending on the nature of the fluid and what caused its entry into the pleural space, may be sampled. A pneumothorax is the accumulation of air in the pleural space, and is commonly called a "collapsed lung". In certain embodiments, the tissue sample is obtained from ascetic fluid in a subject. Ascites is the accumulation of fluid (usually serous fluid which is a pale yellow and clear fluid) that accumulates in the abdominal cavity. The abdominal cavity is located below the chest cavity, separated from it by the diaphragm. The accumulated fluid can have many sources such as liver disease, cancers, congestive heart failure, or kidney failure.

[0091] The biological sample may include a fine needle aspirate or a biopsy from a tissue known to be, or suspected of being, cancerous. The sample may include a bodily fluid from a patient either known to include, or suspected of including, cancer cells or cancer-related cells (i.e., immune cells).

[0092] Accordingly, the cancer cell may be from a patient having or suspected of having a cancer. Types of cancer are characterized by the cells from which they originate. Cancer types include carcinomas such as breast, prostate, lung, pancreatic, and colon cancers that arise from epithelial cells. Sarcomas are derived from connective tissue (e.g., bone, cartilage, fat, or nerve cells). Lymphoma and leukemia arise from hematopoietic cells and are found in the lymph nodes and blood of afflicted patients. Cancer of plasma cells (myeloma) is another cancer found in blood. Germ cell cancers derived from pluripotent cells and blastomas from precursor cells or embryonic tissue are other types of cancer. Cancers may be categorized by those detectable in body fluids, for example, lymphoma, leukemia, or multiple myeloma, as well as those detectable in solid tumors, for example carcinomas or sarcomas. Optimized measurements of the present

systems and methods may be used to measure cancers detectable in body fluids or cancers detectable in solid tumors. Accordingly, the cancer may be a leukemia, a lymphoma, a myeloma, a melanoma, a carcinoma, or a sarcoma. In certain embodiments, the cancer involves a solid tumor of, for example, the esophagus, kidneys, uterus, ovaries, thyroid, breast, liver, gallbladder, stomach, pancreas, or colon.

**[0093]** Optimized cellular measurement of properties, such as mass changes measured in cells, can reveal, for example, if the cells are growing, stationary, or atrophying. Those features of cellular life may be hallmarks of health, cancer, or drug response, and thus methods and devices of the disclosure are valuable tools for precision medicine. Precision Medicine refers to the tailoring of medical treatment to individual characteristics of a patient and the ability to classify individuals into subpopulations that differ in their susceptibility to a particular disease or treatment.

Precision medicine often involves genomic or molecular analysis of an individual patient's disease at the molecular level and the selection of targeted treatments to address that individual patient's disease process. In theory, therapeutic interventions are concentrated on those who will benefit, sparing expense and side effects for those who will not. Historically, next-generation sequencing (NGS) technologies make up the core of precision medicine. Clinicians use NGS technologies to screen for cancer-associated mutations or to study gene expression levels. Now, when coupled with existing approaches based on next-generation sequencing, functional measurements according to the invention provide for multi-dimensional precision medicine with benefits in disease areas such as oncology.

**[0094]** Methods and devices of the invention may be used to identify malignant cancer cells in a blood or tissue sample from a patient. Those tools may also be used as an *ex vivo* test of drug response, useful for therapeutic selection. For example, optimized measurement of MAR in cells provides a measure of cancer in a patient. After treatment of a patient, optimized cellular measurements may be used to monitor recurrence, remission, or relapse. Thus the invention provides for the improvement of patient care, greater chances of successful cancer treatment, and increased patient life spans. Cancer cells may be obtained from a patient treated for cancer, and the measurement of MAR by the methods and devices of the invention may be used to monitor the effectiveness of the cancer treatment.

**[0095]** Methods and devices of the disclosure are useful for precisely and rapidly measuring growth rates of living individual cells using a small amount of a sample. Only a small amount of

a sample may be used to observe and measure a single cell, as opposed to observing a population of cells in traditional methods. Therefore, a small amount of cells can be obtained directly from a subject, suspended in media, and then introduced to a measurement instrument without the need to add additional time-consuming steps, such as culturing the cells. In the invention, the cells  
5 from the biological sample are separated when flowing through a microfluidic channel of the measurement instrument and the growth rate of individual cells is measured.

**[0096]** A small sample size may be required as compared to sample sizes necessary in other measurement methods. For example the sample may comprise about 500 or fewer cells. A small amount of cells may be used because of the precision of the methods of measurement. Therefore,  
10 the optimized measurement of the present invention may be advantageous when limited tissue samples are available for testing and measurement. For example, a tissue sample may comprise about 10,000 cells. Such a tissue sample does not have enough cells present in the sample for traditional measurement methods, such as optics measurement methods. Therefore, because 500 or fewer cells may be used, if a sample of about 10,000 cells is provided 20 different test  
15 conditions may be tested. For example, 500 cells may be dosed with a first drug to determine the effects of the drug on mass accumulation rate of the cells. Therefore, as many as 20 different drugs may be tested with a sample containing 10,000 cells.

**[0097]** FIG. 7 shows an exemplary system 701 useful for performing methods of the disclosure. Preferably, the system provides an instrument 629 capable of making optimized cell

20 measurements and at least one computer 725. The system 701 also preferably includes at least one server 719. The instrument includes a sensor 239 and/or an SMR device 301 which provides data to a classifier 255. The classifier may operate in real-time, and the identification of cellular and/or non-cellular material may be used to control flow through the instrument 629. Either or both of the computer 725 and the server 719 may include and provide the classifier 255. The  
25 system 701 may optionally also include any one or more of a storage 713, a sequencing instrument 705, and any additional analysis instruments 709 for performing additional assays on the one or more cells downstream of the initial assay performed by instrument 301. Any of those elements may interoperate via a network 729. Any one of the instruments may include its own built-in or connected computer which may connect to the network 729 and/or the server 729. The  
30 instrument 629, for example, may have its own computer or server which provides the classifier 255. The computer 725 may include one or more processors and memory as well as an

input/output mechanism. Where methods of the invention employ a client/server architecture, steps of methods of the invention may be performed using the server 729, which includes one or more of processors and memory, capable of obtaining data, instructions, etc., or providing results via an interface module or providing results as a file. The server 719 may be provided by a single  
5 or multiple computer devices, such as the rack-mounted computers sold under the trademark BLADE by Hitachi. The server 719 may be provided as a set of servers located on or off-site or both. The server 719 may be owned or provided as a service. The server 719 or the storage 713 may be provided wholly or in-part as a cloud-based resources such as Amazon Web Services or Google. The inclusion of cloud resources may be beneficial as the available hardware scales up  
10 and down immediately with demand. The actual processors—the specific silicon chips—performing a computation task can change arbitrarily as information processing scales up or down. In an embodiment, the server 619 includes one or a plurality of local units working in conjunction with a cloud resource (where local means not-cloud and includes on or off-site). The server 719 may be engaged over the network 729 by the computer 725.

15 **[0098]** In the system 701, each computer preferably includes at least one processor coupled to a memory and at least one input/output (I/O) mechanism. A processor will generally include a chip, such as a single core or multi-core chip, to provide a central processing unit (CPU). A processor may be provided by a chip from Intel or AMD.

**[0099]** Memory can include one or more machine-readable devices on which is stored one or  
20 more sets of instructions (e.g., software) which, when executed by the processor(s) of any one of the disclosed computers can accomplish some or all of the methodologies or functions described herein. The software may also reside, completely or at least partially, within the main memory and/or within the processor during execution thereof by the computer system. Generally, each computer includes a non-transitory memory such as a solid state drive, flash drive, disk drive,  
25 hard drive, etc. While the machine-readable devices can in an exemplary embodiment be a single medium, the term “machine-readable device” should be taken to include a single medium or multiple media (e.g., a centralized or distributed database, and/or associated caches and servers) that store the one or more sets of instructions and/or data. These terms shall also be taken to include any medium or media that are capable of storing, encoding, or holding a set of  
30 instructions for execution by the machine and that cause the machine to perform any one or more of the methodologies of the present invention. These terms shall accordingly be taken to include,

but not be limited to one or more solid-state memories (e.g., subscriber identity module (SIM) card, secure digital card (SD card), micro SD card, or solid-state drive (SSD)), optical and magnetic media, and/or any other tangible storage medium or media.

[0100] A computer of the invention will generally include one or more I/O device such as, for example, one or more of a video display unit (e.g., a liquid crystal display (LCD) or a cathode ray tube (CRT)), an alphanumeric input device (e.g., a keyboard), a cursor control device (e.g., a mouse), a disk drive unit, a signal generation device (e.g., a speaker), a touchscreen, an accelerometer, a microphone, a cellular radio frequency antenna, and a network interface device, which can be, for example, a network interface card (NIC), Wi-Fi card, or cellular modem.

[0101] The system 701 or components of system 701 may be used to perform methods described herein. Instructions for any method step may be stored in memory and a processor may execute those instructions, including use and training of a classifier for identifying cellular and non-cellular material.

[0102] The system 701 thus includes at least one computer (and optionally one or more instruments) operable to obtain one or more live cells isolated from a sample of a patient, wherein the one or more live cells comprise at least one of a cancer cell and a cancer-related immune cell. The system 701 is further operable to perform a first assay on cellular and/or non-cellular material, wherein the first assay comprises making an optimized cellular measurement by the methods and systems of the invention. The system 701 is optionally further operable to perform a second assay on the one or more live cells having undergone the first assay. The system 701 is further operable to analyze data from the second assay and the optimized measurement from the first assay to determine at least a stage or progression of the cancer. Using the computer 701, the system is operable to provide a report comprising any suitable patient information including identity along with information related to the cancer evaluation, including, but not limited to, specific data associated with the first and second assays, a determination of a stage or progression of cancer, and personalized treatment tailored to an individual patient's cancer.

#### Incorporation by Reference

[0103] References and citations to other documents, such as patents, patent applications, patent publications, journals, books, papers, web contents, have been made throughout this disclosure. All such documents are hereby incorporated herein by reference in their entirety for all purposes.

Equivalents

[0104] 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  
5 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 therefore intended to be embraced therein.

What is claimed is:

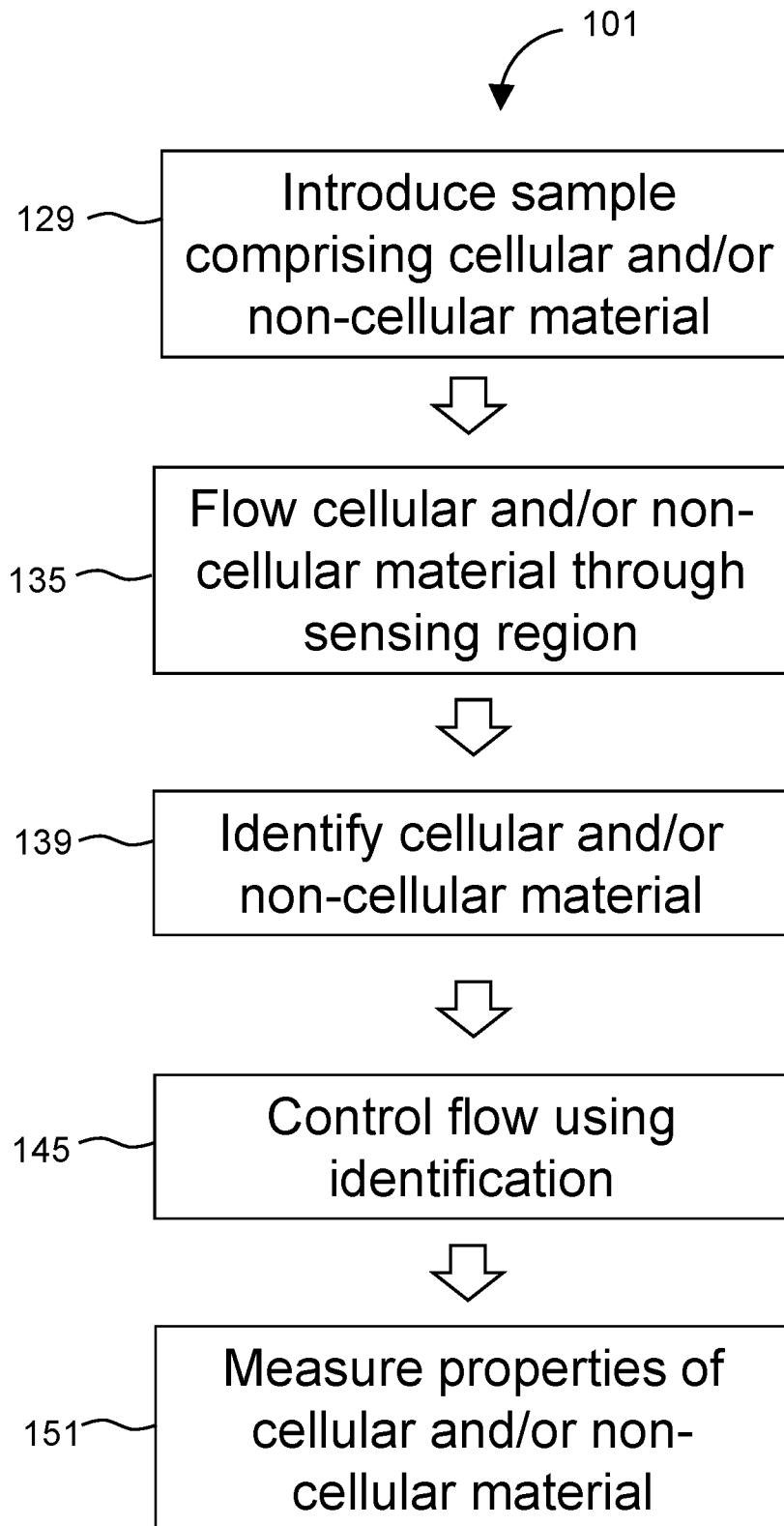
1. A method for optimized cellular measurement, the method comprising the steps of:  
introducing cellular and/or non-cellular material into a measurement device comprising a sample channel, a secondary channel, and a sensor operating over a sensing region; and  
controlling flow in the said sample and secondary channels using a classifier that utilizes data from said sensor to identify cellular and non-cellular material in real time.
2. The method of claim 1, wherein said classifier is based on a neural network architecture trained using the sensor data to classify cellular and non-cellular material.
3. The method of claim 1, wherein the sensor is an imaging sensor.
4. The method of claim 3, wherein the imaging sensor is an imaging sensor with no lens.
5. The method of claim 1, wherein particles of cellular and non-cellular material are loaded at a specified ratio into said secondary channel.
6. The method of claim 1, wherein the secondary channel comprises at least one suspended microchannel resonator.
7. The method of claim 6, wherein designation of cellular or non-cellular and the measurement(s) made by the said suspended microchannel resonator (SMR) are paired.
8. The method of claim 1, wherein said non-cellular material is selected from the group consisting of synthetic particles, inorganic particles and debris.
9. The method of claim 1, wherein said cellular material is selected from the group consisting of cells, cell aggregates, exosomes, extracellular vesicles, cellular components, cellular fragments, organelles, organoids, proteins and protein aggregates, DNA, and RNA.

10. The method of claim 1, wherein said cellular and said non-cellular material are introduced separately.
11. The method of claim 1, wherein said cellular and said non-cellular material are introduced simultaneously in the same sample.
12. The method of claim 1, wherein said cellular material comprises cancer cells.
13. The method of claim 1, wherein said cellular material comprises live cells.
14. The method of claim 10, further comprising obtaining said cancer cells from a patient.
15. A method for optimized cellular measurement, the method comprising the steps of;  
introducing cellular and/or non-cellular particles with overlapping size and/or mass distributions to a measurement device comprising at least one suspended microchannel resonator; and  
identifying the sub-groups of particles in the mixture based on a classifier that utilizes data from said measurement device.
16. The method of claim 15, wherein the identifying step is done in real-time.
17. The method of claim 15, where the classifier is based on a neural network architecture trained using data from said measurement device previously obtained from different sub-groups of particles.
18. The method of claim 15, wherein the classifier is based on a node-deviation signal of said suspended microchannel resonator.
19. The method of claim 15, wherein said classifier discriminates between cells and non-cellular material based on surface stiffness.

20. The method of claim 15, wherein said cells and said non-cellular material are introduced separately.
21. The method of claim 15, wherein said cells and said non-cellular material are introduced in the same sample.
22. The method of claim 15, further comprising obtaining a density measurement of a fluidic sample containing said cells and/or said non-cellular material.
23. The method of claim 15, wherein the non-cellular material is a bead.
24. The method of claim 15, wherein a bead has a known size, mass and/or density.
25. The method of claim 24, wherein the bead is a polystyrene bead.
26. The method of claim 15, further comprising obtaining a mass or mass accumulation rate (MAR) measurement of said cells and/or said non-cellular material.
27. The method of claim 27, further comprising using a mass measurement of a bead with a known mass to calibrate the mass measurement of a cell.
28. The method of claim 26, further comprising introducing a bead with a known mass into the measurement device, obtaining a mass measurement of said bead, and calibrating mass measurements using the known mass of the bead.
29. The method of claim 27, further comprising measuring a mass accumulation rate of said cells.
30. The method of claim 29, wherein said cells comprise cancer cells.
31. The method of claim 30, wherein said cells comprise live cells.

32. The method of claim 30, further comprising obtaining said cancer cells from a patient.
33. The method of claim 32, wherein a measurement of MAR in said cells provides a measure of cancer in the patient.
34. The method of claim 30, wherein said cancer cells are obtained from a patient being treated for the cancer, and wherein the measurement of MAR is used to monitor the effectiveness of the cancer treatment.
35. A system for optimizing cell measurement, the system comprising:  
a measurement device comprising a sample channel, a secondary channel, and a sensor operating over a sensing region;  
a classifier trained to identify cellular and non-cellular material utilizing data from the sensor in real-time and provide an output; and  
a control system for controlling flow through said measurement device based on the output from the classifier.
36. The system of claim 35, wherein said classifier is based on a neural network architecture trained to discriminate between cells and non-cellular material.
37. The system of claim 35, wherein the sensor is an imaging sensor.
38. The system of claim 37, wherein the sensor is an imaging sensor with no lens.
39. The system of claim 35, wherein the control system is operable to load cellular and non-cellular materials into the secondary channel at a specific ratio.
40. The system of claim 35, wherein the secondary channel comprises at least one suspended microchannel resonator.

41. The system of claim 39, wherein cells and non-cellular material may be introduced to the measurement device separately or in the same sample.
42. A system for optimizing cell measurement, the system comprising:  
a measurement device comprising a suspended microchannel resonator capable of loading cells and non-cellular particles with overlapping size and/or mass distributions;  
a classifier trained to identify sub-groups of particles utilizing data from the measurement device.
43. The system of claim 42, wherein the classifier identifies sub-groups of particles in real-time.
44. The system of claim 42, wherein said classifier is based on a neural network architecture trained using data from the measurement device previously obtained from different sub-groups of particles.
45. The system of claim 42, wherein the classifier identifies sub-groups of particles based on a node-deviation signal of said suspended microchannel resonator.
46. The system of claim 42, wherein the classifier discriminates between cells and non-cellular material based on surface stiffness.
47. The system of claim 42, wherein cells and non-cellular material may be introduced into the measurement device separately or in the same sample.

**FIG. 1**

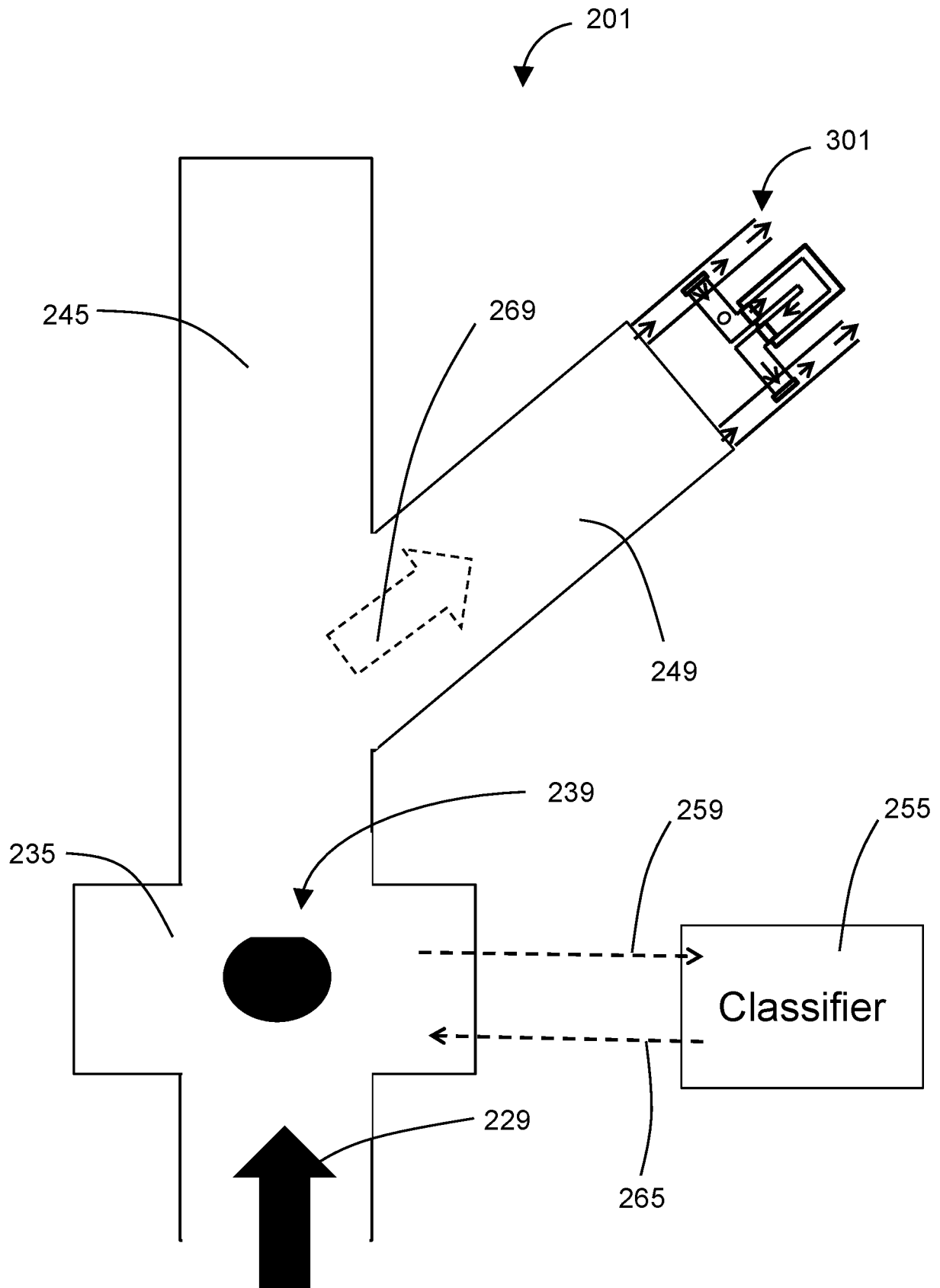


FIG. 2

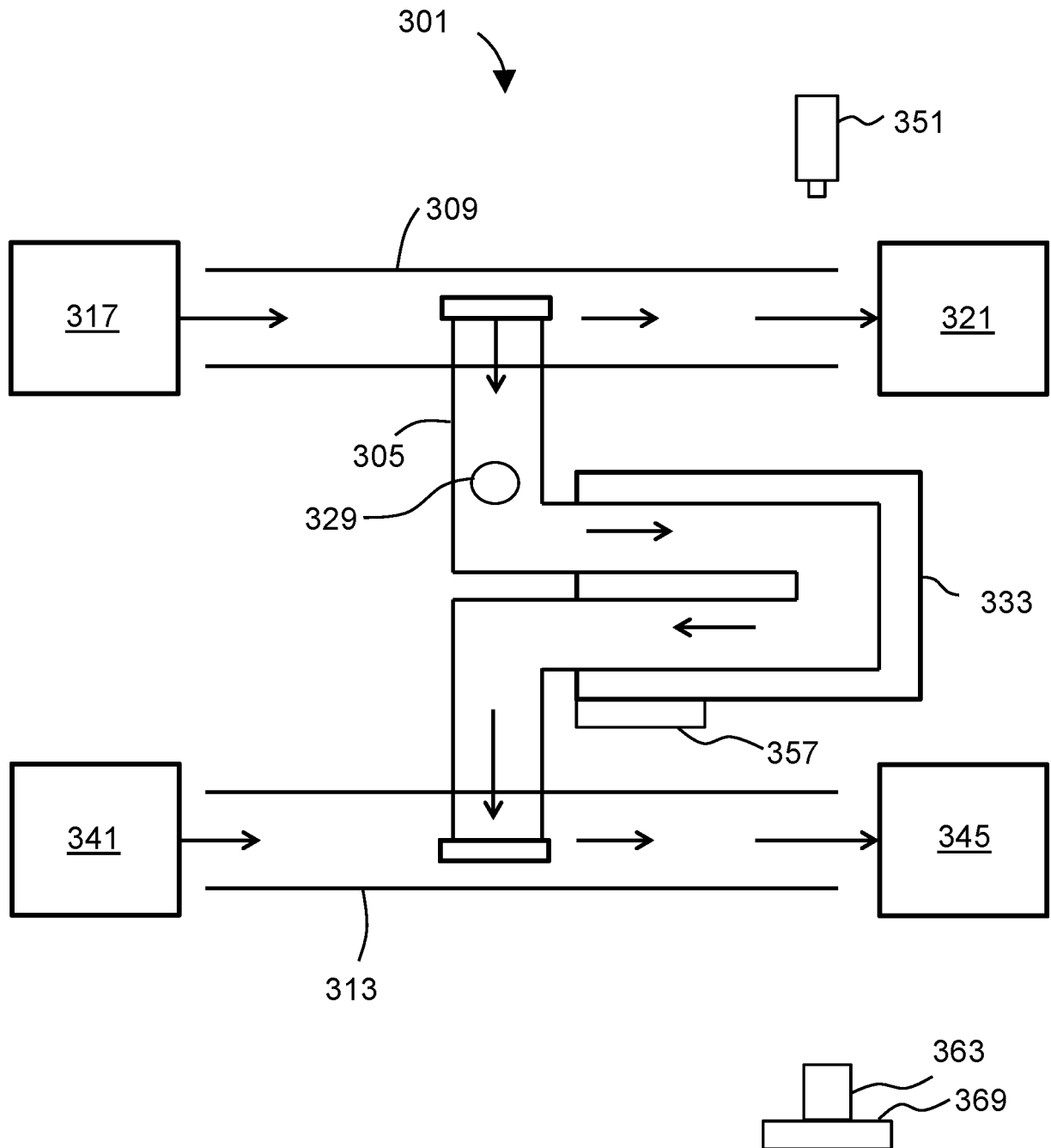


FIG. 3

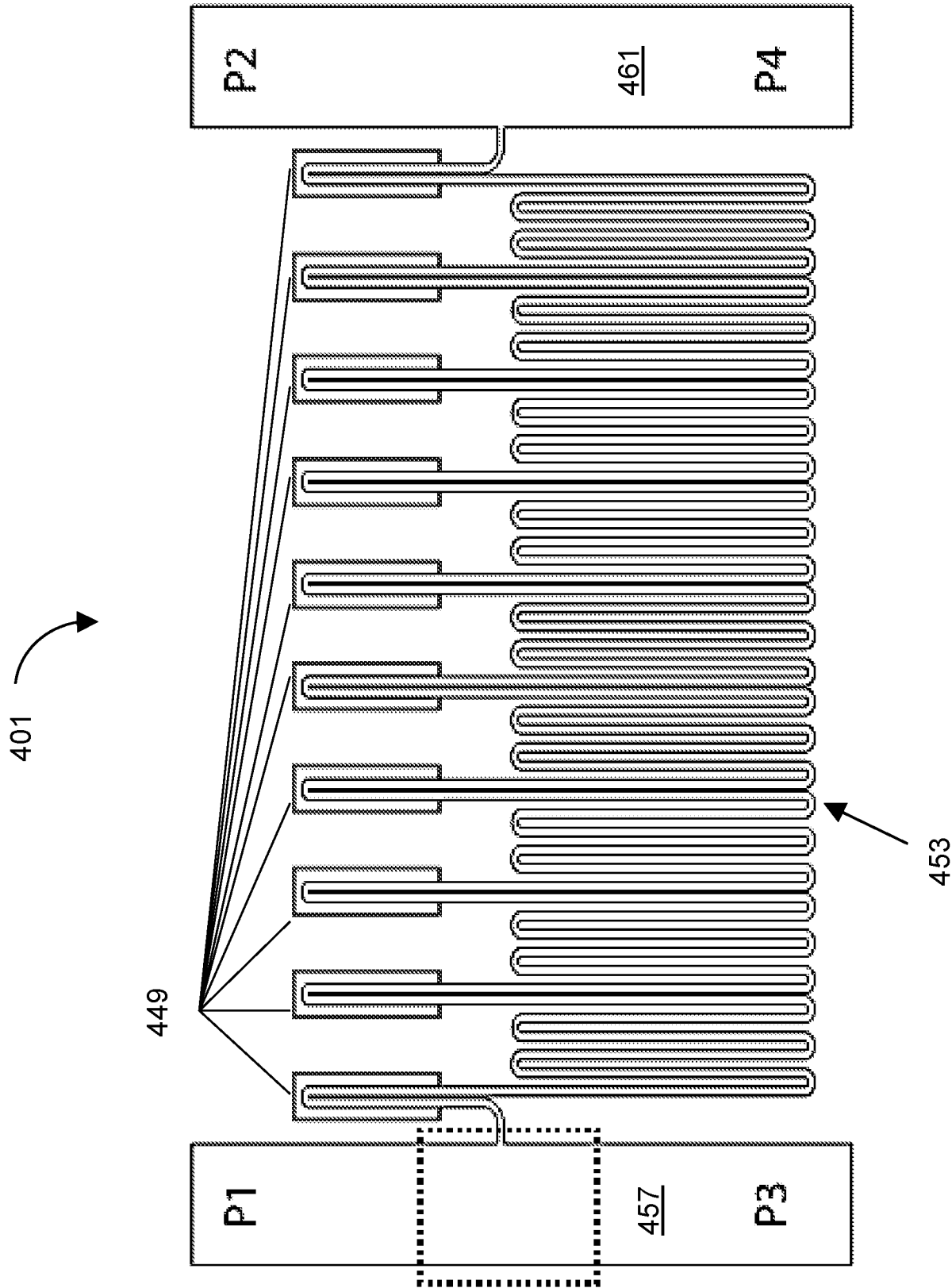


FIG. 4

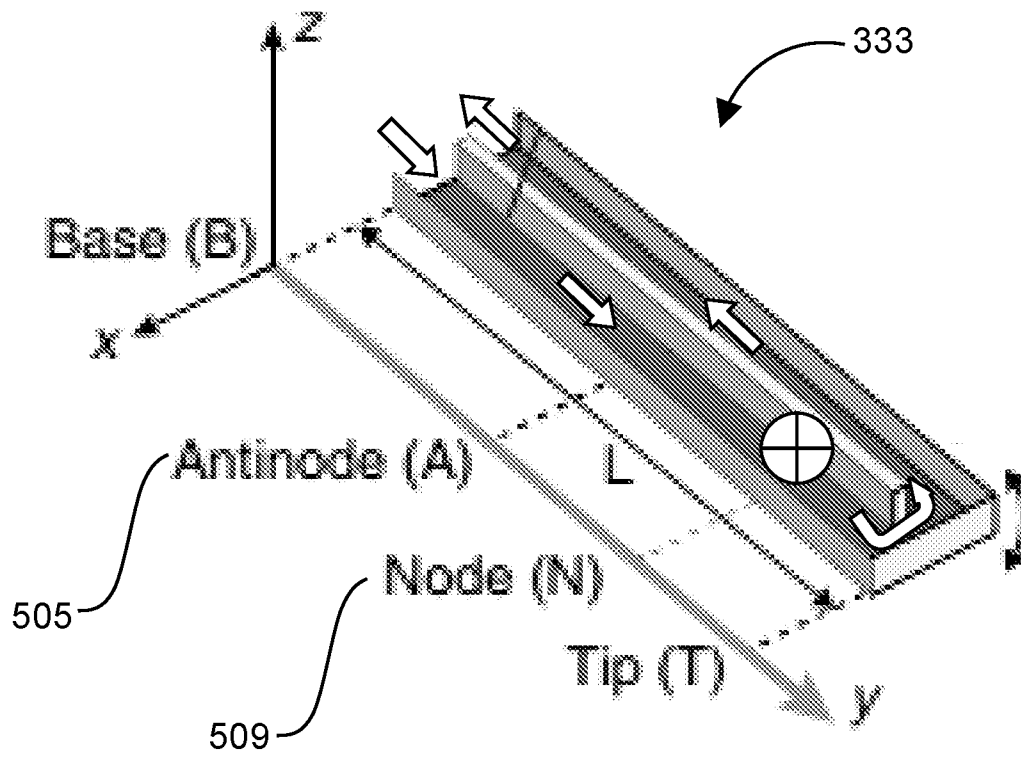
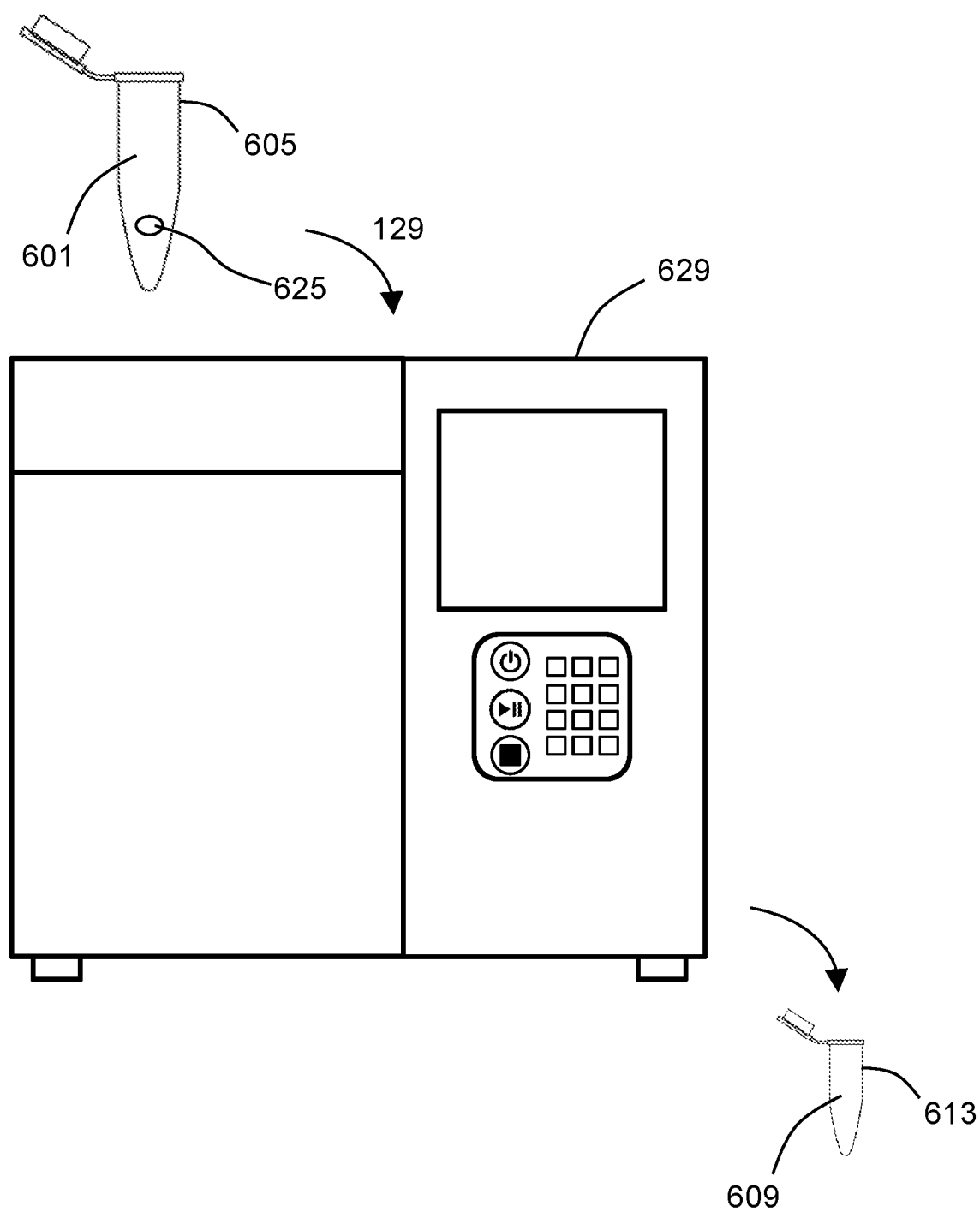
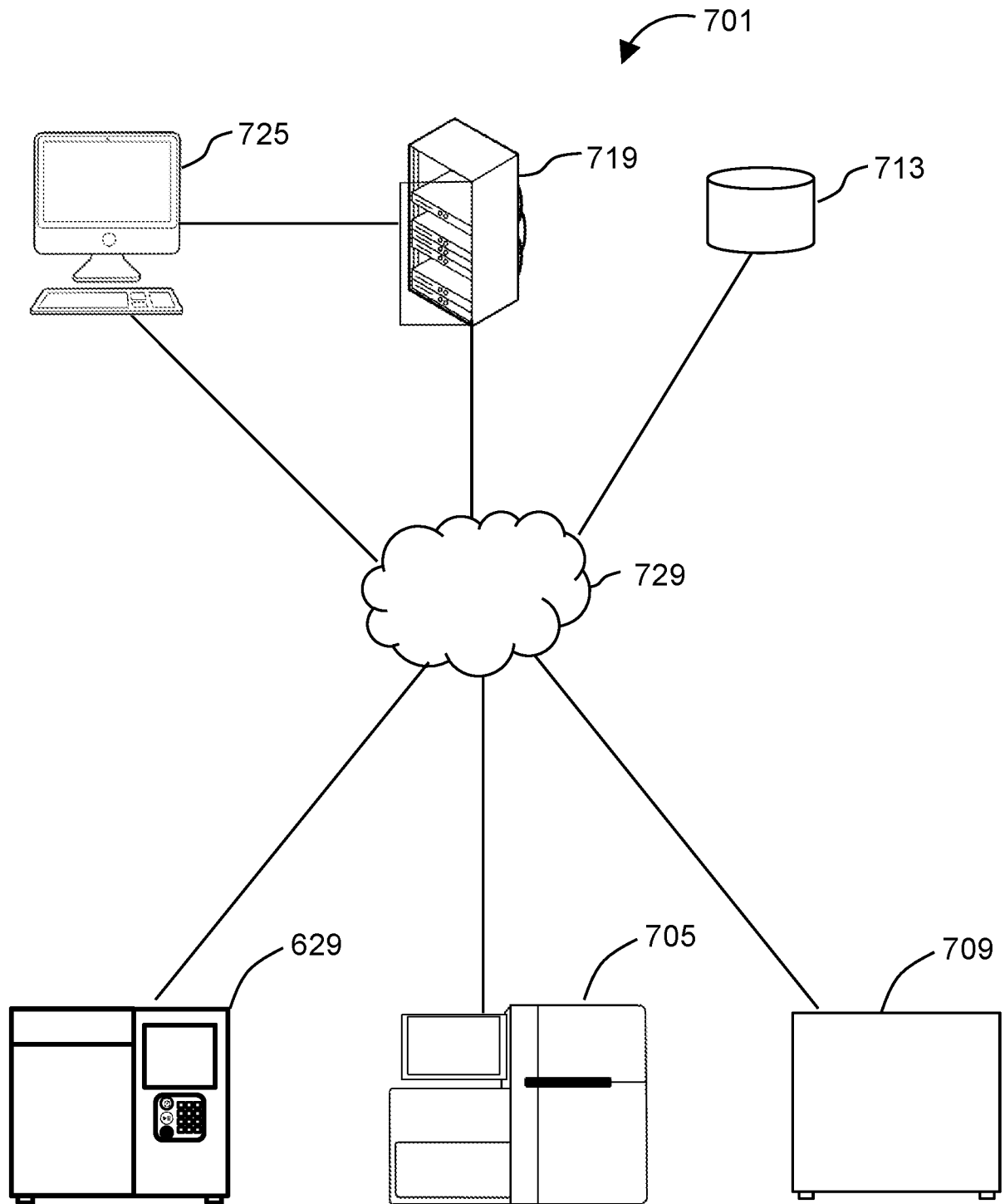
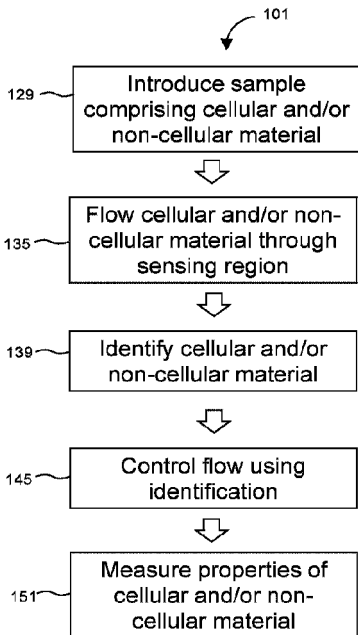


FIG. 5

**FIG. 6**

**FIG. 7**



**FIG. 1**