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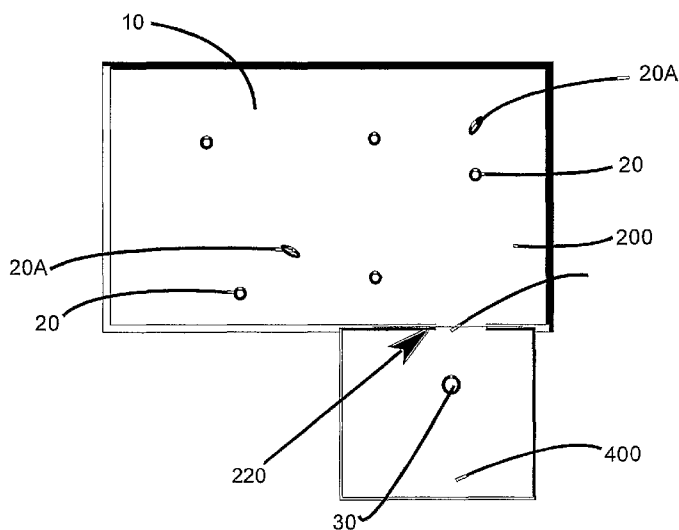
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(54) Title: CLASSIFIER FOR PARTICLES IN A LIQUID USING A LARGE-ORIFICE DROPLET EJECTOR



(57) Abstract: A classifier for particles in liquid for enumerating particles of interest in a liquid sample according to an optical property of the particles of interest, which includes: (a) a large-orifice droplet ejector that holds the liquid sample and ejects droplets of the liquid sample, wherein the droplets of the liquid sample ejected are at least several times smaller than any orifice surrounding the liquid sample at the liquid-air interface, so that clogging of the orifice by particles that are in the liquid sample is minimized, and (b) an airborne-droplet measurement subsystem that measures an optical property of each of these droplets of the liquid sample, so that the optical property of any particles of interest in the droplets of the liquid sample can be estimated from this measured optical property. Because the droplets of the liquid sample can be very small, the particles of interest can be measured with high sensitivity, one at a time, as in flow cytometry, with small chance of clogging the device even though no sheath flow is used.



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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

**CLASSIFIER FOR PARTICLES IN A LIQUID USING A LARGE-ORIFICE
DROPLET EJECTOR**

STATEMENT OF GOVERNMENT INTEREST

This application was supported by U.S. government grant. Accordingly the U.S. government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

1. FIELD OF THE INVENTION

[0001] This invention pertains generally to chemical analytical and immunological testing, and particularly to processes wherein samples are analyzed by using self-operated mechanisms or devices, and more particularly to processes wherein a continuously flowing stream of a sample or carrier fluid is formed and flows into and through analysis wherein the continuously flowing stream is segmented by alternately injecting a sample, reagent or any number of fluids into a common flow path.

2. DESCRIPTION OF THE RELATED ART

[0002] A need for improved instrumentation to identify biological particles carried in air exists in industry. Ideally the instrumentation should be able to run continuously and have a rapid response, but still use only a very small amount of consumable items such as liquids, reagents, or filters, so that the cost of running continuously is not prohibitive. Ideally, such instruments may be deployed in many locations, for example, public buildings, airports, subways, and military installations. Because it would

be useful to deploy many such instruments, the acquisition and operational costs should not be large.

[0003] We have submitted patent applications that have the goal of identifying biological particles collected from air, see Horn-Bond Lin and Steven Clyde Hill, "Aerosol Particle Analyzer for Measuring an Analyte in Airborne Particles," submitted to the USPTO, March 26, 2004; Steven Clyde Hill, Richard Kounai Chang and Jean-Pierre Wolf, "Aerosol Particle Analyzer for Measuring the Amount of Analyte in Airborne Particles," U.S. Application Serial Number 11/126,515, filed on May 9, 2005; and Steven Clyde Hill, "Aerosol Particle Analyzer for Measuring the Amount of Analyte in Airborne Particles," submitted to the USPTO, February 14, 2004, all three of which are herein incorporated by reference. These patent applications rely either primarily or exclusively on molecular aptamer beacons (MABs) for recognition of biological particles collected from air. Also, in another effort that relies primarily on MABs, Matt Hart has proposed using electrospray to generate microdroplets of a liquid that contains molecular-aptamer beacons mixed with the particles collected from air. The idea is to use electrospray to generate droplets and/or particles and to focus these using electrostatic or electrodynamic forces, and then to detect them using laser-induced fluorescence.

[0004] One problem with rapid implementation of MAB-based approaches to biodetection is that MABs technologies are relatively new, and the MABs that have been obtained so far for large proteins and bacterial cells do not appear to have the specificity of antibodies. Far more work has been done in selecting and characterizing antibodies of interest and in developing antibody-based technologies. Therefore, it appears that, for instruments that can be fielded relatively soon, antibody-based approaches are desirable.

[0005] A way to identify biological particles in air is to collect the particles into a water solution that contains fluorescent-tagged antibodies (F-Ab) that were chosen for their tendency to bind to a specific type of bacteria, spore, rickettsia, virus, or other particle that may be in the air sample. For simplicity we will typically discuss the problem in terms of a specific bacteria, with the understanding that the technique is general. If the particles collected from air include one or more of the specific bacteria to which the F-Ab bind preferentially, then the F-Ab diffuse to the specific bacteria and bind to them. Then if the sample's fluorescence is measured on a fine scale, the bacteria with the F-Ab bound to the surface appear as bright spots in a less bright background. The water solution itself retains some background fluorescence because it takes time for the F-Ab to diffuse to the surface of the bacteria, but also because the antibodies may bind to other particles in the sample, although they should bind far less well to these particles, and because some of the fluorophore may come unbound from the F-Ab, and because the amount of F-Ab may exceed the number of sites on the bacteria to which the F-Ab may bind, and for other less important reasons.

[0006] Typically the fluorescence of the fluorophore bound to the antibody does not change after a binding event. That is, whether the antibody is bound or not, the fluorescence of the fluorophore is unchanged. What changes is the local concentration of the fluorophore, so that when the liquid is broken into small droplets, the droplet containing a particle with many bound F-Ab will be brighter than droplets that contain only the background concentration of F-Ab.

[0007] Fluorescence microscopy and flow cytometry are two methods used to determine the numbers of F-Ab tagged bacteria in a sample.

Fluorescence microscopy is relatively slow and not simple to automate for a flow-through system that runs continuously.

[0008] Flow cytometers are used extensively to enumerate particles, especially biological cells, in liquid samples, and especially fluorescent-labeled cells. Flow cytometry is not used for routine, continuous monitoring and identification of particles collected from air samples, in part because it would be expensive to deploy and operate such instruments, even if they were automated and combined with highly efficient instruments that collect airborne particles into liquid. The reagents required to continuously operate existing flow cytometers to identify of biological agents in samples collected from air are expensive. Also, there has been concern that clogging of orifices in flow cytometers may be a problem, at least in applications of flow cytometry to identification of particles collected from air.

[0009] It appears that the ideal flow cytometric instrument most useful for continuously monitoring the air for harmful biological particles has different design parameters than instruments used in, for example, typical hospital or research laboratories. Some reasons are as follows: (1) It would be ideal if the instrument were small and could operate unattended for weeks, without replenishment of significant volumes of liquids. In a hospital or research laboratory, the volumes of liquids used for sheath flow are much less a major issue; (2) In continuously monitoring the air for potentially harmful particles, the collection and concentration of airborne particles into a small volume of liquid requires significant amounts of energy. It would require too much energy to collect 100,000 particles per second from air into a liquid volume small enough to analyze, for practical instruments. Therefore, an instrument that analyzes the liquid samples into which particles from air have been collected does not need to operate at 100,000

particles per second, as some commercial flow cytometers do. A rate of 10 to 1000 particles/second may be adequate; (3) Because collection of particles from air requires significant amounts of energy, then if flow cytometry is used to identify and enumerate the particles collected from air, it would be ideal if the flow cytometer detects every particle that is collected, i.e., not miss some particles because they do not flow through the detection volume. That can also be a priority requirement for more commonly used flow cytometers, but it may be a higher priority in the case of these expensive-to-obtain samples; and (4) It is most common for flow cytometers to be designed for mammalian cells, which are much larger than bacteria, although some flow cytometers have been designed primarily for detection of bacteria.

[0010] Typically, fluorescent-labeled mammalian cells have a much larger fluorescence than labeled bacteria, and can therefore be detected above the background fluorescence, i.e., the fluorescence from the unbound antibodies, in a larger volume of liquid. For a flow cytometer that has a relatively strong signal-to-background ratio the interrogation volume should be small. To obtain a small interrogation volume, while keeping the internal cross section of the cytometer tube not too small, in order to minimize clogging, while also minimizing the usage of sheath flow, is difficult.

SUMMARY OF THE INVENTION

[0011] The present invention provides a classifier for particles in liquid by enumerating particles of interest in a liquid sample according to an optical property of the particles of interest. The classifier includes a large-orifice droplet ejector that holds the liquid sample and ejects droplets of the liquid sample, wherein the ejected droplets of the liquid sample are at least

several times smaller than any orifice surrounding the liquid sample at the liquid-air interface, so that clogging of the orifice by particles that are in the liquid sample is minimized; and an airborne-droplet measurement subsystem that measures an optical property of each of the droplets of the liquid sample, so that the optical property of any particles of interest in the droplets of the liquid sample can be estimated from the measured optical property.

[0012] The present invention also provides a method of enumerating particles of interest in a liquid sample according to an optical property of the particles of interest. The method includes the steps of: ejecting droplets of liquid sample using a large-orifice droplet ejector that holds the liquid sample; and measuring an optical property of each droplet of the liquid sample by an airborne-droplet measurement subsystem so that the optical property of any particles of interest in the droplets of the liquid sample is be deduced from the measured optical property.

[0013] These and other features and advantages of the present classifier for particles in liquid by enumerating particles of interest in a liquid sample and the present method of enumerating particles of interest in a liquid sample according to an optical property of the particles of interest over the prior art will become apparent from the following detailed description and accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1 schematically illustrates a classifier for particles in a liquid.

[0015] FIG. 2 schematically illustrates a classifier for particles in a liquid with an acoustic wave liquid ejector (AWLE).

[0016] FIG. 3 schematically illustrates a classifier for particles which collects particles from air into a liquid.

[0017] FIG. 4 illustrates a classifier for particles in which the airborne particles are drawn through a nozzle and then directly impacted into the collection liquid into input well.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0018] In consideration of the problems stated above and the limitations in the partial solutions thereto, an object of the present invention is to provide a classifier for particles in liquid (CPL) 100 that can measure particles of interest (POI) 20A in a liquid sample (LS) 10, while circumventing several problems that occur in some types of flow cytometers. That is, the CPL 100 provided: (a) does not require a liquid sheath flow, (b) examines essentially all of the particles 20 in the LS 10, (c) has a very small interrogation volume, and (d) has a relatively low probability of clogging. In order to attain the objectives described above, according to an aspect of the present invention, there is provided a CPL 100 with large orifice droplet ejector (LODE) 200 for classifying POI 20A in an LS 10.

[0019] The main subsystems of this CPL are as follows: (1) large orifice liquid ejector (LODE) that holds LS, and ejects droplets of the liquid sample (DLS) 30 through an orifice 220, where the orifice 220 is large relative to the size of the DLS 30 ejected, and to any particles 20 in the LS 10; and
(2) an airborne-droplet measurement subsystem (ADMS) 400 that measures an optical property of the DLS 30 ejected by the LODE 200, so that the presence of POI 20 in the DLS 30 ejected can be determined from

this measured optical property.

[0020] Referring to FIG. 1, a classifier for particles in a liquid (CPL) 100 is illustrated schematically. The liquid sample (LS) 10 contains particles (20). Some of these particles 20 may be particles of interest (POI) 20A. The POI 20A have at least one optical property that is different from the particles 20 that are not POI 20A. In a preferred embodiment that optical property is the fluorescence in some wavelength band, and the POI 20A have a greater fluorescence in this wavelength band than do the particles 20 that are not POI 20A. In another preferred embodiment the POI 20A have a greater fluorescence in both of two different wavelength bands than do the particles 20 that are not POI 20A. There is no size restriction on the POI 20A. Although in many embodiments the POI 20A may be 0.5 micrometers or greater, a POI 20A can be as small as a single molecule that contains a single fluorescent molecule, as in the single-molecule in droplets work of Michael Barnes and Michael Ramsey et al., described above, or it may be a single quantum dot. It is the optical property, not the physical size of the particle 20, that determines whether a particle 20 is a POI 20A. In some embodiments particle-size can be a part of the criterion for what is or is not a POI 20A, because size can sometimes be estimated from the light scattering abilities of the particle 20 but it is not necessary in general. The LS 10 can contain multiple types of POI 20A where each of these multiple types of POI 20A differ from each other based on at least one optical property. The objective for the CPL 100 is to enumerate each type of POI 20A in the LS 10, that is, to classify the different types of POI 20A. For simplicity we describe this invention primarily in terms of enumerating a single type of POI 20A in one sample.

[0021] An example of the LS 10 is a water mixture which contains: (i) water, (ii) particles collected from air, and (iii) fluorescent-tagged

antibodies (F-Ab) that were chosen for their tendency to bind to a specific type of bacteria that may be present in the air sample. If the specific type of bacteria is in the water mixture, the F-Ab diffuse to and bind the surface of the specific bacteria. Once a sufficient number of F-Ab bind to the a particle, this particle becomes sufficiently more fluorescent than a similar volume of the LS 10 that does not contain that specific bacteria, and so this bacteria becomes a POI 20A.

[0022] In another example, the POI 20A could be as small as a single fluorescent molecule as in the single-molecule in droplets detection work described above. Unless the concentration of the specific bacteria in the LS 10 is extremely high, most of the DLS 30 ejected contain no bacterial particles, but only contain unbound F-Ab. This unbound F-Ab gives a background signal. The DLS 30 that contain the specific bacteria also contain unbound F-Ab, but in addition they contain the F-Ab that has bound to the specific bacteria, and so these DLS 30 have a significantly greater fluorescence. The concentration of the specific bacteria in the sample is determined from the number of droplets that have this significantly greater fluorescence. By choosing the DLS 30 to be small, for example 4 to 8 micrometers in diameter, the background signal from the unbound F-Ab, relative to the F-Ab bound to the specific bacteria, can be minimized, and so the detection sensitivity can be increased. A key advantage of the present invention is that it can eject small DLS 30, and thereby achieve high detection sensitivity, and it does this without a comparably small orifice 220.

[0023] In other examples the antibodies can be chosen to bind to specific spores, viruses, rickettsia, or other materials. Also, in other examples, the recognition elements need not be antibodies, but may be antibody fragments, aptamers, or other biomolecules, or may be dyes that bind to

specific types of molecules, such as acridine orange which binds to DNA and can indicate whether a cell is alive or dead. Howard Shapiro, Practical Flow Cytometry, 4th edition (John Wiley, 2003), chapter 7, p. 273-410), herein incorporated by reference, describes many of the possible options. In other examples, the LS 10 is not obtained by collecting particles directly from air, but is obtained by washing a surface that may be contaminated, or it may be from waste water or a blood sample.

[0024] Referring again to FIG. 1, the CPL 100 has two main subsystems: (a) a large-orifice droplet ejector (LODE) 200 that holds the LS 10, and ejects droplets of the LS (DLS) 30 through an orifice 220, where the orifice 220 is large relative to the size of the DLS 30 ejected and to any particles in the LS 10, and (b) an airborne-droplet measurement subsystem (ADMS) 400 that measures an optical property of the DLS 30 ejected by the LODE 200, so that the presence of POI 20 in the DLS 30 ejected can be determined from this measured optical property. The LODE 200 and the ADMS 400 may be connected with a gas-tight connection, but need not be. For example, when some types of commercially available or off-the-shelf ADMS 400 are used, it may be most convenient to not use a gas-tight-connection of the ADMS 400 to the LODE 200.

[0025] We assume that for most applications of the CPL 100, the majority of DLS 30 will not contain more than one POI 20A. In one preferred embodiment, and in the embodiments emphasized here, the humidity is high enough and the time before measurement is short enough that the DLS 30 evaporates relatively little before its fluorescence or other optical property is measured. An advantage of this embodiment is that the ejected DLS 30 will tend to move on the same trajectory of the other DLS 30 because all the DLS 30 ejected have a somewhat constant mass independent of the size of particles contained in the DLS 30. In another

preferred embodiment, the humidity is low enough that the liquid in the DLS 30 evaporates rapidly and so, at the time the DLS 30 is measured the DLS 30 may be primarily solid and, possibly very small. An advantage of this embodiment is that there is no additional liquid to complicate measurement of the particles that remain after the liquid evaporates.

[0026] The orifice 220 is large relative to the size of the DLS 30 ejected, and to any particles 20 in the LS 10, so that clogging of the orifice is unlikely even if particles that are larger than the DLS 30 are in the LS 10, while at the same time, small DLS still can be ejected and analyzed, so that the measured optical properties of DLS that do not contain POI are not such that they cannot be distinguished from DLS that do contain POI.

[0027] An advantage of the LODE is that it can repeatedly eject very small DLS without requiring a small orifice. For example, one type of LODE is the acoustic wave liquid ejector (AWLE) as described by Dia Huang and Eun Sok Kim, "Micromachined Acoustic-Wave Liquid Ejector," Journal of Electromechanical Systems, 10, 442-449 (2001), herein incorporated by reference. In this paper, droplets as small as 5 micrometers in diameter were ejected, even though the opening, which can be considered to be the "orifice" is a square with 1600 micrometer sides, which is many times larger than typical droplet sizes that would be useful for measuring optical properties of particles.

[0028] In other types of droplet generators, for example, a vibrating orifice droplet generator or a typical piezoelectric droplet generator, the ejected droplets have a diameter that is larger than the orifice. In our experience, repeatedly ejecting droplets that have diameters less than 25 micrometers and also contain significant amounts of bacteria or other particles that are one micrometer and larger is very difficult because the orifices tend to

clog. The solutions must be filtered in order to keep particles in the solution from clogging the orifice, but the filtering removes the particles.

[0029] In the Patent Publication US 2003/0027344, "DNA Probe Synthesis on Chip on Demand by MEMS Ejector Array," which is incorporated herein by reference, Eun Sok Kim and Jae Wan Kwan describe the use of an array of "ejectors" that may be "Self Focusing Acoustic Transducers," where the "ejector" elements of the array may be as those described as "acoustic wave liquid ejectors" in the paper by Huang and Kim, that was incorporated by reference above. In this patent application, Kim and Kwan further describe how the self focusing acoustic transducers can be modified to eject the droplets in specific directions.

[0030] The AWLE 240 in this present invention is not restricted to the type of "acoustic wave liquid ejector" described by Huang and Kim, or Kim and Kwan incorporated above. Any of a variety of acoustic wave liquid ejectors that do not need a small orifice to restrict the size of the ejected DLS 30 can be used for the present invention. One such acoustic wave liquid ejector is the "acoustic ejection device" as described by Richard N. Ellson, Mitchell W. Mutz, and Richard Michael Caprioli, US Patent Application Publication, 2005/0121537 A1, Publication Date, June 9, 2005, herein incorporated by reference, in which they also refer to several of their prior patents and patent applications using such devices.

[0031] Ellson et al., note that for their primary application, depositing fluids onto surfaces, especially for mass spectrometric analysis, that, "Optimally the volume of the ejected droplet is less than about 1 pL, e.g., in the range of about 0.025 pL to about 1 pL" (paragraph 15 of their application). For this range, 0.025 pL to about 1 pL in volume, the DLS 30 diameters are in the range 3 to 10 micrometers, a range that is useful for our primary goal

in this main embodiment, that of measuring small numbers of POI 20A that contain F-Ab tagged bacteria or other biological particles. Mitchell L. Mutz, Richard N. Ellson, and David Soong-Hua Lee, "Focused Acoustics for Detection and Sorting of Fluid Volumes," US 6,849,432 B2, herein incorporated by reference, describe an additional application of such an "acoustic ejection device."

[0032] Another such example of an AWLE 240 is the "liquid ejector" described by Hiroshi Fukumoto, Jyunichi Aizawa, and Hiromu Narumiya, US Patent, 6154,235, "Acoustic Liquid Ejector and Printer Apparatus Incorporating the Ejector," herein incorporated by reference.

[0033] The LODE 200 can also be the "piezoelectric droplet ejector" with "flextensional transducer" as described by Gokhan Percin and Butrus T. Khuri-Yakub, "Piezoelectric Droplet Ejector for Ink-jet Printing of Fluids and Solid Particles," Review of Scientific Instruments, 74, 1120-1127 (2003), herein incorporated by reference. This device requires an orifice 220, which in the paper by Percin and Khuri-Yakub, is 50 to 200 micrometers in diameter, a size that is large compared to the sizes of the particles 20 and DLS 30 that would be useful for many applications, for example, 5 or 10 micrometers in diameter. With this device, Percin and Khuri-Yakub ejected droplets with diameters as small as 5 micrometers.

[0034] In another embodiment the LODE 200 is the "Ultrafine Fluid Jet Apparatus," similar to that described by Kazuhiro Murata, Tsukuba-shi, and Ibaraki-ken, US Patent Application Publication US 2005/0116069 A1, herein incorporated by reference. The key difference is that in the present invention there is provided a hole in the counter electrode, positioned so that the ejected DLS 30 moves through this hole (instead of colliding with the counter electrode as in the Murata et al., invention), so that the optical

properties of the DLS 30 can be measured by the ADMS 400.

[0035] The purpose of the ADMS 400 is to measure the desired optical property of the droplets in some embodiments and particles in some embodiments that enter it. To give an idea of the large number of possible embodiments of the CPL 100 employing different types of ADMS 400, and to give an idea of how well developed some possible embodiments of the ADMS 400 are, we note that a large number of types of airborne-single-droplet and airborne-single-particle measurement systems that have been described in the literature, over at least 50 years for elastic scattering measurements and over 20 years for fluorescence and various types of nonlinear emission.

[0036] For one embodiment of the CPL 100 in which the optical property measured is elastic scattering the ADMS 400 is an example of a type of commercially available ADMS 400 that would be useful for measurement of the particles 20, including the POI 20A, that could remain after a droplet evaporated, for cases where the droplet does evaporate, the TSI Aerodynamic Particle Sizer (for example, the 3320) or any of a number of similar instruments that have been sold by TSI for the past 20 years, would be useful. Measurement of fluorescence, spontaneous Raman emission, lasing, stimulated Brillouin scattering, stimulated Raman scattering, sum-frequency generation, and other emission from airborne droplets are reviewed by Steven C. Hill and Richard K. Chang, "Nonlinear Optics in Droplets," in Ole Keller, ed., *Studies in Classical and Quantum Nonlinear Optics*, (Nova Science Publishers, Inc., 1995), pp. 171-242, herein incorporated by reference. The citations in the above chapter by Hill and Chang include a number of journal articles describing the optical arrangements used to detect the scattering and emission from the airborne droplets. Some of these measurements have been made with

continuous-wave lasers and others have been made with pulsed lasers.

[0037] Measurements of fluorescence from airborne droplets containing single fluorescent molecules using continuous wave lasers is described by N. Lerner, M. D. Barnes, C. Y. Kung, W. B. Whitten, and J. M. Ramsey, "High efficiency molecular counting in solution: Single-molecule detection in electrostatically focused microdroplet streams," *Analytical Chemistry*, 69, 2115-2121 (1997), and by C. Kung, M. D. Barnes, N. Lerner, W. B. Whitten, and J. M. Ramsey, "Single-molecule analysis of ultradilute solutions with guided streams of 1-micrometer water droplets," *Applied Optics*, 38, 1481-1487 (1999), both herein incorporated by reference.

[0038] Measurements of fluorescence from airborne particles using continuous wave lasers is also described in R. G. Pinnick, S. C. Hill, P. Nachman, J. D. Pendleton, G. L. Fernandez, M. W. Mayo and J. G. Bruno, "Fluorescence Particle Counter for Detecting Airborne Bacteria and other Biological Particles," *Aerosol Science and Technology*, 23, 653-664 (1995), herein incorporated by reference.

[0039] Measurement of airborne particle arrival times for triggering pulsed lasers and for gating on detectors are described by Richard K. Chang, Yong-Le Pan, Ronald G. Pinnick, and Steven C. Hill, US Patent 6,532,067, "Aerosol fluorescence spectrum analyzer for rapid measurement of single airborne particles," and Richard Chang, Yong-Le Pan, Ronald Gene Pinnick, and Steven Clyde Hill, "Method and instrumentation for measuring fluorescence spectra of individual airborne particles sampled from ambient air," U.S. Patent Application Publication 2004/0125371 A1, July 1, 2004, and US Patent 6,947,131 B2, both herein incorporated by reference, and by R. G. Pinnick, S. C. Hill, Y. Pan and R. K. Chang, "Fluorescence spectra of atmospheric aerosol at Adelphi,

Maryland, USA: measurement and classification of single particles containing organic carbon, *Atmospheric Environment*, 38,1657-1672 (2004), herein incorporated by reference.

[0040] This CPL 100 might be termed a “flow cytometer with acoustic-wave generation of droplets,” and that would fit with a broad definition of flow cytometer. For example, Gucker’s airborne-particle counter can be used as a flow cytometer (see Howard Shapiro, *Practical Flow Cytometry*, 4th edition (John Wiley, 2003), p. 10), and our fluorescence particle spectrum analyzer (Richard K. Chang et al., US Patent 6,532,067, and Richard Chang et al., US Patent Application Publication 2004/0125371 A1, July 1, 2004) could be termed a flow cytometer. However, we hesitate to call this present invention a “flow cytometer with large-orifice droplet ejector,” because that description calls to mind a commercial type of flow cytometer in which the cells are measured and classified in an unbroken liquid stream, and then the liquid can be broken into droplets for sorting. In the present invention, the liquid sample is broken into droplets before the fluorescence is measured, and these droplets are typically much smaller than the droplets used in typical cell sorters.

[0041] This CPL has similarities to the droplet-based single-molecule detection (SMD) methods in which the liquid sample is broken into a series of droplets, each of which contains either zero or one (or in the unlikely cases two or three) single molecules, as described by N. Lerner, M. D. Barnes, C. Y. Kung, W. B. Whitten, and J. M. Ramsey, “High efficiency molecular counting in solution: Single-molecule detection in electrostatically focused microdroplet streams,” *Analytical Chemistry*, 69, 2115-2121 (1997), and by C. Kung, M. D. Barnes, N. Lerner, W. B. Whitten, and J. M. Ramsey, “Single-molecule analysis of ultradilute solutions with guided streams of 1- μ m water droplets,” *Applied Optics*,

38, 1481-1487 (1999), both herein incorporated by reference. Such single-molecule in droplets methods can use an extremely small amount of liquid, but these methods have not been commercialized, partly because generating the required stream of droplets from the nozzle has been problematic, even when there are no particles in the liquid. Clogging of the orifice is a nontrivial issue, and to avoid clogging the liquid must be run through a filter that has a very small pore size.

[0042] Key differences between the CPL and the Single Molecule Detector (SMD) described above are as follows: (a) particles tend to clog the orifices used in the SMD, and so the SMD approach is not suitable for our primary emphasis, that of detecting fluorescent particles in a liquid sample, where the particle sizes need not be small, for example, much less than one micrometer; and (b) the droplets initially ejected from the droplet generator used in the SMD were large enough that usually the Raman signal from the liquid could exceed the fluorescence of the single molecule, and so the water from the droplet was allowed to evaporate (the droplet was made of a mixture of glycerol and water), and a linear quadrupole was used to keep the droplets to a line while they evaporated and while they slowed to a sufficiently low velocity, essentially the settling velocity, that they could be in the interrogating laser beam for a time as long as a 140 ms, while only traveling a very short distance.

[0043] The CPL 100 uses an LODE that can eject droplets of a size that is desirable for measurement, and so the droplet's fluorescence can be measured relatively soon after it is ejected, and this feature, along with the ability to eject droplets in a repeatable direction (several of the example LODE were developed in part for inkjet printing) mean that no linear quadrupole for focusing is needed, and that no low-vapor-pressure liquid is needed in the liquid sample, requirements that could be restrictive for

cases in which the speed of reaction, for example between antibodies and particles, is important, and where it is not desirable to use additional fluids such as glycerol.

[0044] FIG. 2 schematically illustrates a classifier for particles in a liquid with an acoustic wave liquid ejector (AWLE). In the embodiment described by FIG. 2, the LODE 200 is the acoustic-wave liquid ejector (AWLE) 240 as described by Dia Huang and Eun Sok Kim, "Micromachined Acoustic-Wave Liquid Ejector," Journal of Electromechanical Systems, 10, 442-449 (2001), which is incorporated herein by reference as fully set forth, where the AWLE 240 is illustrated in Figures 1, 3 and 11, of that paper and where Figures 15, 16 and 18 of that paper show photographs of ejected droplets.

[0045] In a preferred embodiment, the AWLE 240 further includes a "solid plate having a circular hole" (SPCH) 250 as described by Huang and Kim on p. 448. For embodiments that include the SPCH 250, the "circular hole" of the SPCH 250 defines the orifice 220. This SPCH 250 provides a means to keep the level of the LS 10 in the CPL 100 constant, so that the size of the DLS 30 ejected does not vary with time. Without the SPCH 250 the LS 10 level in the AWLE 240 may vary with time if nothing else is done to keep it constant, and if it does vary the size of the DLS 30 ejected may vary, or may not be ejected at all. Also, with the SPCH 250, capillary forces can continually draw LS 10 into the AWLE 240 through the input port 210, so that the AWLE 240 can run continuously, so long as LS 10 is available at the input port 210 to the AWLE 240. For embodiments that do not use a SPCH 250, the orifice 220 may be comparable in size to one side of the AWLE 240, and theoretically there is no upper limit to that dimension. In the AWLE 240 as described by Huang and Kim, 5-micrometer-diameter DLS 30 could be ejected at a rate of over 10 kHz.

[0046] This problem of detecting an increase in fluorescence in a volume of LS 10 that contains specific bacteria as compared with a volume that does not contain the specific bacteria also occurs with flow cytometers where the cells or particles are carried in a continuously flowing liquid. By minimizing the volume that is detected at any one time, it is possible to maximize the signal-to-background ratio, and the ability to clearly measure the specific bacteria desired. In a flow cytometer, if the volume that is measured is as small as the volume of a 4 micrometer diameter droplet, then either a significant volume of sheath fluids are required to focus this sample volume into the small region that is interrogated, and/or not all of the LS 10 is measured, and/or the cross section of the capillary through which the LS 10 flows is so small that clogging can be a problem.

[0047] In one preferred embodiment the ADMS 400 employs a laser 410 that emits light having a wavelength tuned to excite fluorescence in the fluorophore of the F-Ab. The fluorescence emitted from the DLS 30 is collected by a lens 420, then filtered by an optical filter 422 that blocks the excitation light and passes the fluorescence, and is then detected by a photodetector 424. In some embodiments the photodetector 424 is a photodiode. In another embodiment it is a photomultiplier tube.

[0048] In a preferred embodiment a second photodetector, not indicated in FIG. 2, measures the light that is elastically scattered from the droplet. From this scattered light intensity the size of the DLS 30 is estimated.

[0049] In FIG. 1 and FIG. 2 the DLS 30 is ejected downward. In other embodiments the DLS 30 is ejected in other directions. For embodiments in which the orifice 220 is so large that the LS 10 is not held in the LODE 200 by capillary forces, the DLS 30 is ejected upward or somewhat

upward and the ADMS is positioned substantially above the LODE 200.

[0050] In another embodiment a means is included to generate and control an airflow that entrains the droplets ejected from the LODE 200 and to carry these droplets to the region where the optical property is measured.

[0051] Although the measurement of the fluorescence of the ejected DLS 30 is emphasized, other optical properties are also measured, usually in addition to the fluorescence, in some embodiments of the CPL 100. Other such optical properties are fluorescence lifetime, fluorescence polarization, multiphoton-excited fluorescence, elastic scattering, two-dimensional angular optical scattering, absorption, laser-induced breakdown spectroscopy, and Raman emission. In some embodiments the light scattered at multiple excitation wavelengths is measured and used to estimate the absorption spectrum of the particle at the excitation wavelengths. Such scattering, absorption and emission throughout the optical range, from terahertz, to infrared through visible to ultraviolet. In some embodiments upconverting phosphors are used to label the antibodies (see, for example, J. Hampl, et al., "Upconverting phosphor reporters in immunochromatographic assays," *Analytical Biochemistry*, 288 (2), 176-187, January 15 (2001), herein incorporated by reference). In other embodiments upconverting phosphors are used to label the aptamers. In other embodiments upconverting phosphors are used to label RNA probes which bind to selected RNA or DNA molecules. In some embodiments quantum dots are used to label the antibodies. In other embodiments quantum dots are used to label the aptamers.

[0052] In some embodiments the liquid evaporates from the DLS 30 and then scattering is measured by several detectors and used to estimate the size of the DLS 30 that remains after the liquid has evaporated, and in

another embodiment, the two-dimensional angular optical scattering by the particle is measured so it can provide information about the shape of the DLS 30 that remains, as described in US Patent Application Publication 2003/0223063, by Steven Clyde Hill, Ronald Gene Pinnick, Yong-Le Pan, Kevin Aptowicz, Kristan P. Gurton, and Richard Kounai Chang, titled, "Method and Instrumentation for Determining Absorption and Morphology of Individual Airborne Particles," herein incorporated by reference.

[0053] In some embodiments the optical property of the DLS 30 and its residue is measured at multiple positions as the DLS 30 moves, so that these multiple measurements can be used to reduce uncertainties in the measured optical properties for each DLS 30.

[0054] Referring to FIG. 3, a classifier for particles which collects particles from air into a liquid is schematically illustrated. In other embodiments (see Fig. 3), the input port 210 of the LODE 200 is connected to the output of an airborne-particle into liquid collector (APLC) 500, which collects particles from air into a liquid. We mention three specific examples of types of APLC in the following paragraphs, but any of a large number of specific approaches could be used.

[0055] In yet other embodiments, the APLC is an impaction-based device, such as the one described by H. N. Phan and A. R. McFarland, "Aerosol-to- Hydrosol transfer stages for use in bioaerosol sampling," Aerosol Science and Technology, 38, 300-310, 2004, herein incorporated by reference. In another embodiment, the APLC is an impaction based APLC similar to that described by D. A. Masquelier, F. P. Milanovich and K. Willeke, "High Air Volume to Low Liquid Volume Aerosol Collector," US Patent 6,520,034 B1, herein incorporated by reference.

[0056] In another embodiment the collection liquid is in an input well of a microfluidic chip that is similar to one of the cell buffer wells of the microfluidic chips that are supplied in the "Cell Fluorescence LabChip Kit" sold by Agilent for their Bioanalyzer 2100.

[0057] Referring to FIG. 4, it is seen that the airborne particles are drawn through a nozzle and then directly impacted into the collection liquid in this input well. FIG. 4 schematically illustrates a classifier for particles in which the airborne particles are drawn through a nozzle and then directly impacted into the collection liquid into input well. This input well is directly connected through a microfluidic tube to the input port 210 of the LODE 200 of the CPL 100. For cases in which the airborne particles can combine with the collection liquid and rest on the surface of the collection liquid, and not enter the collection liquid because of surface tension, this APLC also includes a means to deposit an additional droplet of liquid onto the position where the particles collide with the collection liquid so that will be drawn into the collection liquid and be analyzed. For embodiments in which continuous operation is desired, any of a number of means to replenish the liquid in this microfluidic input well can be used. There are many more examples of impaction-based APLC. For embodiments where an impaction method is used to collect the particles into the liquid, and air-based puffer can impart the impaction force selectively onto particles flowing in a stream based on other criteria, allowing for presorting of the particles before processing by the CPL.

[0058] In a preferred embodiment the collection liquid held in the APLC includes labeled molecular recognition elements, which can be fluorescent-tagged antibodies, fluorescent-tagged antibody fragments, fluorescent-tagged aptamers or other biorecognition molecules which can bind to selected particles, so that the collection liquid into which the

airborne particles impact, becomes an LS 10, and need not later be mixed with labeled molecular recognition elements to become the LS 10.

[0059] In another preferred embodiment the collection liquid does not contain all the needed labeled molecular recognition elements, but contains other substances which prepare the collected airborne particles so that they can react with the labeled molecular recognition elements, which are added later. For example the collection liquid can contain a protein that can bind nonspecifically to a variety of materials, and thereby block some of these non-specific binding sites, so that the biorecognition molecules that are added later do not bind nonspecifically to these sites.

[0060] In some embodiments the APLC includes the aerosol deflection system (ADS) described by Richard K. Chang, Jean-Pierre Wolf, Veronique Boutou, and Yongle Pan, "Systems and Methods for Sorting Aerosols," US 2005/0028577 A1, published, February 10, 2005, and incorporated herein by reference. The ADS as claimed by Chang et al., deflects selected airborne particles into a "collection zone comprising one or more pathogen identification devices." The particles may be selected based on their laser-induced fluorescence being similar to that of biological aerosols, or based on other criteria. In the present invention, the deflected airborne particles (DAP) that were deflected by the ADS of Chang et al., are collected into the collection liquid by impacting them into the collection liquid. In a preferred embodiment, the deflected particles are impacted into the input well of a microfluidic chip which is connected by a short microfluidic channel to the input port 210 of the LODE 200. However, any means for collecting the deflected airborne particles into a collection liquid can be used.

[0061] In some embodiments the APLC is a shooting-droplet airborne-

particle collector having: (a) an airborne-particle detector, and (b) a droplet generator that shoots a collection droplet in a direction such that it collides with the airborne particle. Typically the collection droplet continues on a trajectory such that it moves into some type of system where it can be analyzed. This shooting-droplet airborne-particle collector is described in a patent application by Steven Clyde Hill, Richard Kounai Chang and Jean-Pierre Wolf, "Aerosol Particle Analyzer for Measuring the Amount of Analyte in Airborne Particles," USPTO Number, 11/126,515, submitted May 9, 2005, notice of allowance April 18, 2006, herein incorporated by reference. Typically the droplets in the shooting-droplet airborne-particle collector are shot only at particles that have laser-induced fluorescence and/or other properties that are similar to those of biological particles or other types of particles as desired. The liquid in the droplet that is shot at the airborne particle can include fluorescent-tagged antibodies or other labeled molecular recognition elements (biorecognition molecules) that can bind to specific particles. In the above patent application by Hill et al., MABs were emphasized as the labeled molecular recognition elements. In the present invention this emphasis on MABs is not needed.

[0062] In some embodiments the APLC includes of a means to impart a charge to the airborne particles drawn into it, and an oppositely charged volume of liquid held on the end of a capillary, as described in a patent application by Steven Clyde Hill and Horn-Bond Lin, "Aerosol into Liquid Collector for Depositing Particles from a Large Volume of Gas into a Small Volume of Liquid," submitted to the USPTO November 18, 2004, incorporated herein by reference, where the liquid held on the end of the capillary typically includes fluorescent-tagged antibodies or other biorecognition molecules. Other types of electrostatic-field enhanced aerosol-particle collectors may be used in other embodiments.

[0063] In some embodiments the CPL 100 also includes a container to collect the DLS 30 that DLS 30 that have had their optical property measured can be stored for later analysis by other means.

[0064] In some embodiments the CPL 100 also includes means to sorting the DLS 30 according the optical property measured, by applying a charge to the ejected DLS 30 and then deflecting these DLS 30 using voltage controlled electrodes toward one container or another. Such means are as described in H. M. Shapiro, Practical Flow Cytometry, 4th edition, herein incorporated by reference.

[0065] In some embodiments, more than one LODE 200 eject DLS 30 so that the sample rate can be increased. That is, arrays of LODE 200 and lasers 410 can be used to process samples in parallel for increased speed and capacity. For some of these embodiments, only one laser 410 is used but multiple photodetectors 424 are used.

[0066] In other embodiments the laser 410 is a diode laser. In some embodiments the laser 410 is replaced by a light emitting diode (LED).

[0067] As mentioned herein above, the present invention also provides a method of enumerating particles of interest in a liquid sample according to an optical property of the particles of interest. The method includes the steps of: ejecting droplets of liquid sample a large-orifice droplet ejector that holds the liquid sample; and measuring an optical property of each of droplets of the liquid sample by an airborne-droplet measurement subsystem so that the optical property of any particles of interest in the droplets of the liquid sample is be estimated from the measured optical property.

[0068] In the method of the present invention, the ejected droplets of the liquid sample are at least several times smaller than any orifice surrounding the liquid sample at the liquid-air interface to minimize clogging of the orifice by any particles that may be present in the liquid sample. The large-orifice droplet ejector can be an acoustic wave liquid ejector or a piezoelectric droplet ejector with flextensional transducer. The acoustic wave liquid ejector may include a solid plate having a circular hole to reduce evaporation from the liquid surface and to provide for capillary forces to draw liquid into the reservoir. In some embodiments, the large-orifice droplet ejector is positioned such that the droplets are ejected in a substantially downward direction so that the particles that are denser than the liquid are preferentially ejected.

[0069] The optical property can be fluorescence, fluorescence lifetime, fluorescence polarization, multiphoton-excited fluorescence, elastic scattering, including two-dimensional angular optical scattering, absorption, laser-induced breakdown spectroscopy, or Raman emission. In some embodiments, the optical property of the droplet and its residue are measured at multiple positions as the droplet moves to provide multiple measurements for improved accuracy of the measured optical property.

[0070] The method can further include one or more of the following steps:

(1) collecting airborne particles into a collection liquid through an airborne-particle into liquid collector (APLC) connected to the input of the classifier for particles in liquid; the collection liquid can contain labeled molecular recognition elements which selectively bind to specific particles collected from air into the liquid, whereby the specific particles become labeled and acquire different optical properties than particles that do not bind to the labeled molecular recognition elements to become particles of

interest;

- (2) shooting a collection-droplet through a collection-droplet generator in a direction such that it collides with the airborne particle;
- (3) receiving the airborne particle intercepted by the collection droplet into a collection-droplet receiver positioned to receive and move it to the large-orifice droplet ejector;
- (4) analyzing the airborne particle with an airborne-particle detector;
- (5) optically trapping the ejected droplets for additional control of the ejected;
- (6) sorting the droplets of the liquid sample according to the optical property measured; and/or
- (7) recycling the liquid of the liquid sample.

[0071] Preferably, the airborne-particle into liquid collector is an impaction based aerosol-particle collector and includes an aerosol deflection system (ADS), which deflects selected airborne particles into a collection liquid and a means for holding the collection liquid until it is drawn into the classifier for particles in liquid. The airborne-particle into liquid collector can further include means for depositing additional droplets of liquid onto where the particles collide with the collection liquid so that any particles resting on the surface of the collection liquid without entering the collection liquid because of surface tension are drawn into the collection liquid and analyzed.

[0072] Preferably, the labeled molecular recognition element is selected from fluorescent-labeled antibodies and fluorescent-labeled aptamers.

[0073] Although various preferred embodiments of the present invention have been described herein in detail to provide for complete and clear

disclosure, it will be appreciated by those skilled in the art that variations may be made thereto without departing from the spirit of the invention or the scope of the appended claims.

We Claim:

1. A classifier for particles in liquid for enumerating particles of interest in a liquid sample according to an optical property of the particles of interest, comprising:

a large-orifice droplet ejector that holds the liquid sample and ejects droplets of the liquid sample, wherein the ejected droplets of the liquid sample are at least several times smaller than any orifice surrounding the liquid sample at the liquid-air interface, so that clogging of the orifice by particles in the liquid sample is minimized; and

an airborne-droplet measurement subsystem that measures an optical property of each of the droplets of the liquid sample, so that the optical property of any particles of interest in the droplets of the liquid sample can be estimated from the measured optical property.

2. The classifier for particles in liquid of claim 1, wherein the large-orifice droplet ejector is an acoustic wave liquid ejector.

3. The classifier for particles in liquid of claim 2, wherein the acoustic wave liquid ejector comprises a solid plate having a circular hole to reduce evaporation from the liquid surface and to provide for capillary forces to draw liquid into the reservoir.

4. The classifier for particles in liquid of claim 1, wherein the large-orifice droplet ejector is a piezoelectric droplet ejector with flextensional transducer.

5. The classifier for particles in liquid of claim 1, further comprising:

an airtight container surrounding the airborne-droplet measurement

subsystem having an input port for accepting droplets ejected by the large-orifice droplet ejector; and

a gas-tight connection between the output of the large-orifice droplet ejector and the input to the container surrounding the airborne-droplet measurement subsystem.

6. The classifier for particles in liquid of claim 1, wherein the large-orifice droplet ejector is positioned so that the droplets are ejected in a substantially downward direction, so that the particles that are denser than the liquid are preferentially ejected.

7. The classifier for particles in liquid of claim 1, wherein the optical property is selected from the group consisting of:

fluorescence, fluorescence lifetime, fluorescence polarization, multiphoton-excited fluorescence, phosphorescence, single-angle elastic scattering, multi-angle elastic scattering, two-dimensional angular optical elastic scattering, multi-wavelength-excited elastic scattering, absorption, laser-induced breakdown spectroscopy, and Raman emission.

8. The classifier for particles in liquid of claim 1, wherein the optical property of the droplet and its residue are measured at multiple positions as the droplet moves to provide multiple measurements for improved accuracy of the measured optical property.

9. The classifier for particles in liquid of claim 1, further comprising:

an airborne-particle into liquid collector (APLC) connected to the input of the classifier for particles in liquid for collecting airborne particles into a collection liquid.

10. The classifier for particles in liquid of claim 9, wherein the collection liquid contains labeled molecular recognition elements which selectively bind to specific particles collected from air into the liquid, whereby the specific particles become labeled and acquire different optical properties than particles that do not bind to the labeled molecular recognition elements to become particles of interest.

11. The classifier for particles in liquid of claim 9, wherein the airborne-particle into liquid collector is an impaction based aerosol-particle collector.

12. The classifier for particles in liquid of claim 9, wherein the airborne-particle into liquid collector comprises:

an aerosol deflection system (ADS), which deflects selected airborne particles into a collection liquid; and

means for holding the collection liquid until it is drawn into the classifier for particles in liquid.

13. The classifier for particles in liquid of claim 11, wherein the airborne-particle into liquid collector further comprises:

means for depositing additional droplets of liquid onto where the particles collide with the collection liquid so that any particles resting on the surface of the collection liquid without entering the collection liquid because of surface tension will be drawn into the collection liquid and analyzed.

14. The classifier for particles in liquid of claim 9, wherein the airborne-particle into liquid collector further comprises:

an airborne-particle detector;

a collection-droplet generator for shooting a collection-droplet in a

direction such that it collides with the airborne particle; and

a collection-droplet receiver positioned to receive the airborne particle intercepted by the collection droplet and move it to the large-orifice droplet ejector where it can be analyzed.

15. The classifier for particles in liquid of claim 9, wherein the airborne particle into liquid collector further comprises:

means for charging airborne particles;

a capillary tube that contains a liquid that has a charge that is opposite to that of the charged airborne particles, and positioned so that the charged airborne particles collide with and are caught by this charged liquid in the capillary; and

a liquid-tight connection between the output of the capillary and an input to the large-orifice droplet ejector.

16. The classifier for particles in liquid of claim 9, wherein the airborne particle into liquid collector further comprises:

means for drawing fluid from the capillary tube into the liquid ejector for replenishing the liquid in the capillary tube and maintaining a constant fluid level.

17. The classifier for particles in liquid of claim 10, wherein the labeled molecular recognition elements are fluorescent-labeled antibodies.

18. The classifier for particles in liquid of claim 10, wherein the labeled molecular recognition elements are fluorescent-labeled aptamers.

19. The classifier for particles in liquid of claim 1, further comprising:

means for generating and controlling an airflow that entrains the

droplets of the liquid sample ejected from the large-orifice droplet ejector to the region where the optical property is measured.

20. The classifier for particles in liquid of claim 1, further comprising a container for collecting the particles that have had their optical property measured.

21. The classifier for particles in liquid of claim 1, further comprising:
means for sorting the droplets of the liquid sample according to the optical property measured.

22. A method of enumerating particles of interest in a liquid sample according to an optical property of the particles of interest, comprising the steps of:

ejecting droplets of the liquid sample using a large-orifice droplet ejector that holds the liquid sample; and

measuring an optical property of each of said droplets of the liquid sample by an airborne-droplet measurement subsystem so that the optical property of any particles of interest in the droplets of the liquid sample can be estimated from the measured optical property.

23. The method of claim 22, wherein the ejected droplets of the liquid sample are at least several times smaller than any orifice surrounding the liquid sample at the liquid-air interface to minimize clogging of the orifice by particles in the liquid sample.

24. The method of claim 22, wherein the large-orifice droplet ejector is an acoustic wave liquid ejector.

25. The method of claim 22, wherein the large-orifice droplet ejector is a piezoelectric droplet ejector with flextensional transducer.

26. The method of claim 24, wherein the acoustic wave liquid ejector comprises a solid plate having a circular hole to reduce evaporation from the liquid surface and to provide for capillary forces to draw liquid into the reservoir.

27. The method of claim 22, wherein the large-orifice droplet ejector is positioned so that the droplets are ejected in a substantially downward direction so that the particles that are denser than the liquid are preferentially ejected.

28. The method of claim 22, wherein the optical property is selected from the group consisting of:

fluorescence, fluorescence lifetime, fluorescence polarization, multiphoton-excited fluorescence, phosphorescence, single-angle elastic scattering, multi-angle elastic scattering, two-dimensional angular optical elastic scattering, multi-wavelength-excited elastic scattering, absorption, laser-induced breakdown spectroscopy, and Raman emission.

29. The method of claim 22, wherein the optical property of the droplet and its residue are measured at multiple positions as the droplet moves to provide multiple measurements for improved accuracy of the measured optical property.

30. The method of claim 22, further comprising the step of:
collecting airborne particles into a collection liquid through an airborne-particle into liquid collector (APLC) connected to the input of the classifier for particles in liquid.

31. The method of claim 30, wherein the collection liquid contains labeled molecular recognition elements which selectively bind to specific particles collected from air into the liquid, whereby the specific particles become labeled and acquire different optical properties than particles that do not bind to the labeled molecular recognition elements to become particles of interest.

32. The method of claim 30, wherein the airborne-particle into liquid collector is an impaction based aerosol-particle collector.

33. The method of claim 32, wherein the airborne-particle into liquid collector comprises:

an aerosol deflection system (ADS), which deflects selected airborne particles into a collection liquid; and

means for holding the collection liquid until it is drawn into the classifier for particles in liquid.

34. The method of claim 32, wherein the airborne-particle into liquid collector further comprises:

means for depositing additional droplets of liquid onto where the particles collide with the collection liquid so that any particles resting on the surface of the collection liquid without entering the collection liquid because of surface tension are drawn into the collection liquid and analyzed.

35. The method of claim 30, further comprising the steps of:
shooting a collection-droplet through a collection-droplet generator in a direction such that it collides with the airborne particle;
receiving the airborne particle intercepted by the collection droplet

into a collection-droplet receiver positioned to receive and move it to the large-orifice droplet ejector; and

analyzing the airborne particle with an airborne-particle detector.

36. The method of claim 31, wherein the labeled molecular recognition element is selected from the group consisting of:

fluorescent-labeled antibodies, fluorescent-labeled aptamers, fluorescent-labeled RNA probes, fluorescent-labeled nucleic acid probes, quantum-dot-labeled antibodies, quantum-dot-labeled aptamers, quantum-dot-labeled RNA probes, quantum-dot-labeled nucleic acid probes, upconverting-phosphor-labeled antibodies, upconverting-phosphor-labeled aptamers, upconverting-phosphor-labeled RNA probes, and upconverting-phosphor-labeled nucleic acid probes.

37. The method of claim 22, further comprising the step of:
sorting the droplets of the liquid sample according to the optical property measured.

38. The method of claim 22, further comprising the step of:
recycling the liquid of the liquid sample.

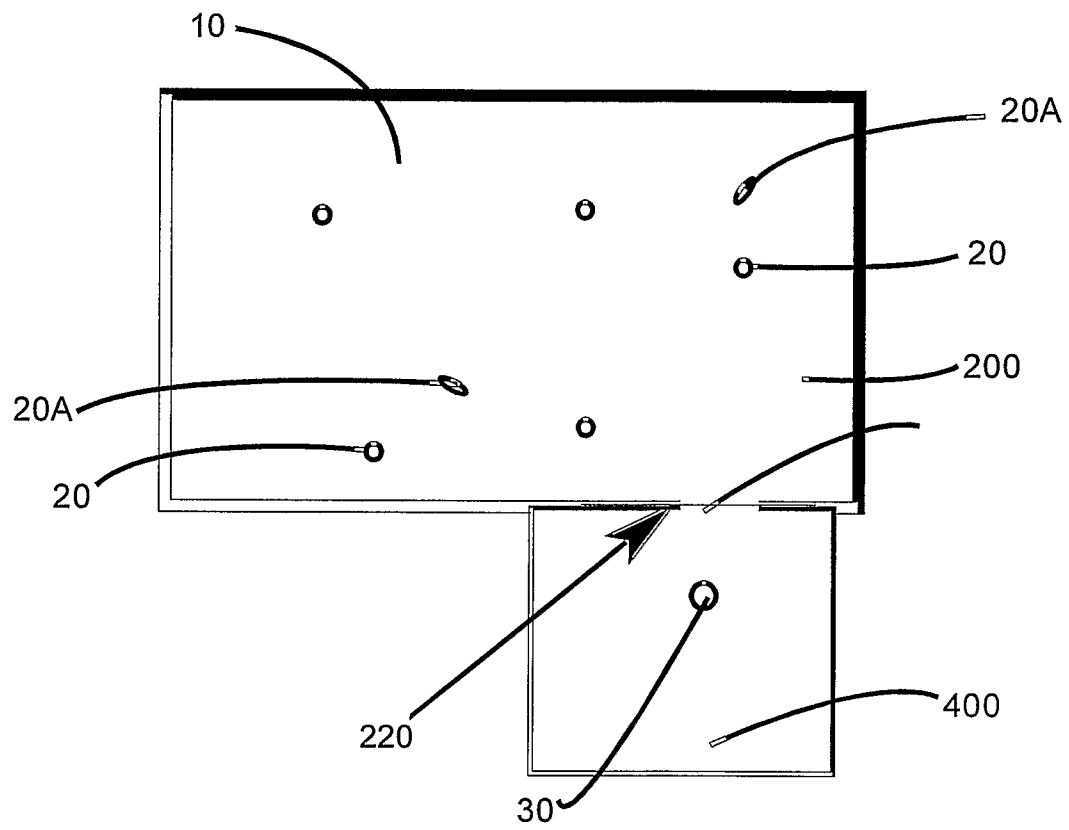


Fig. 1

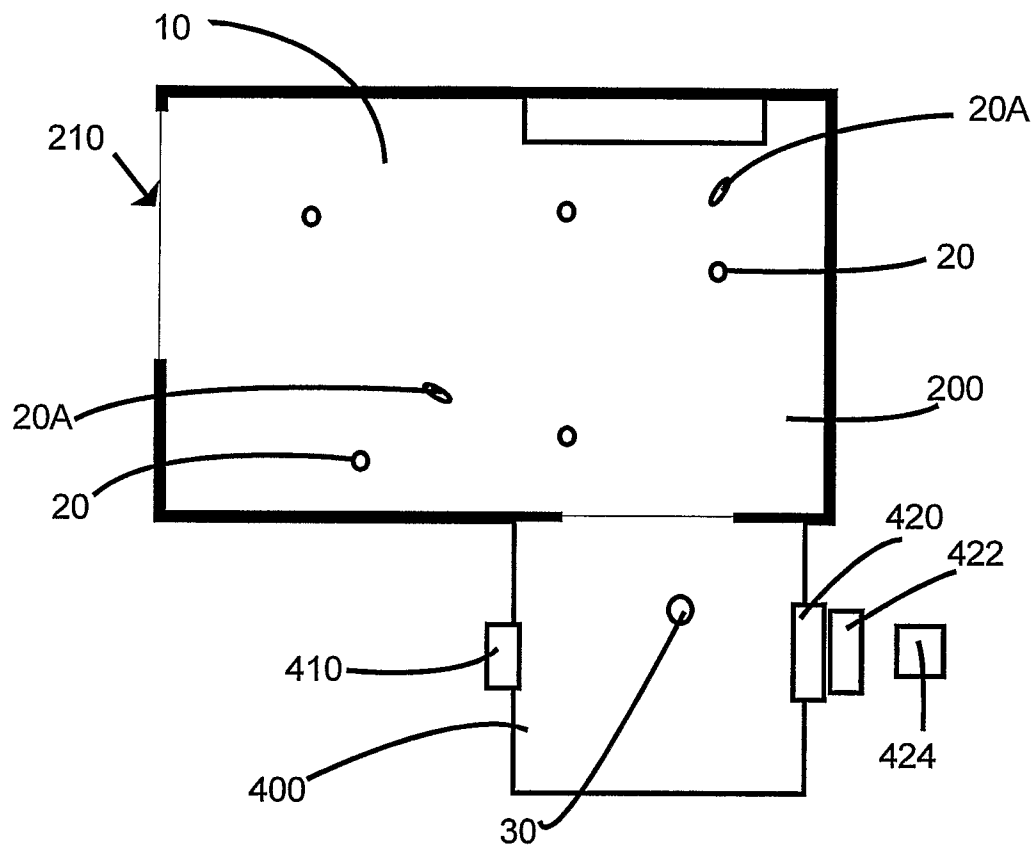


Fig. 2

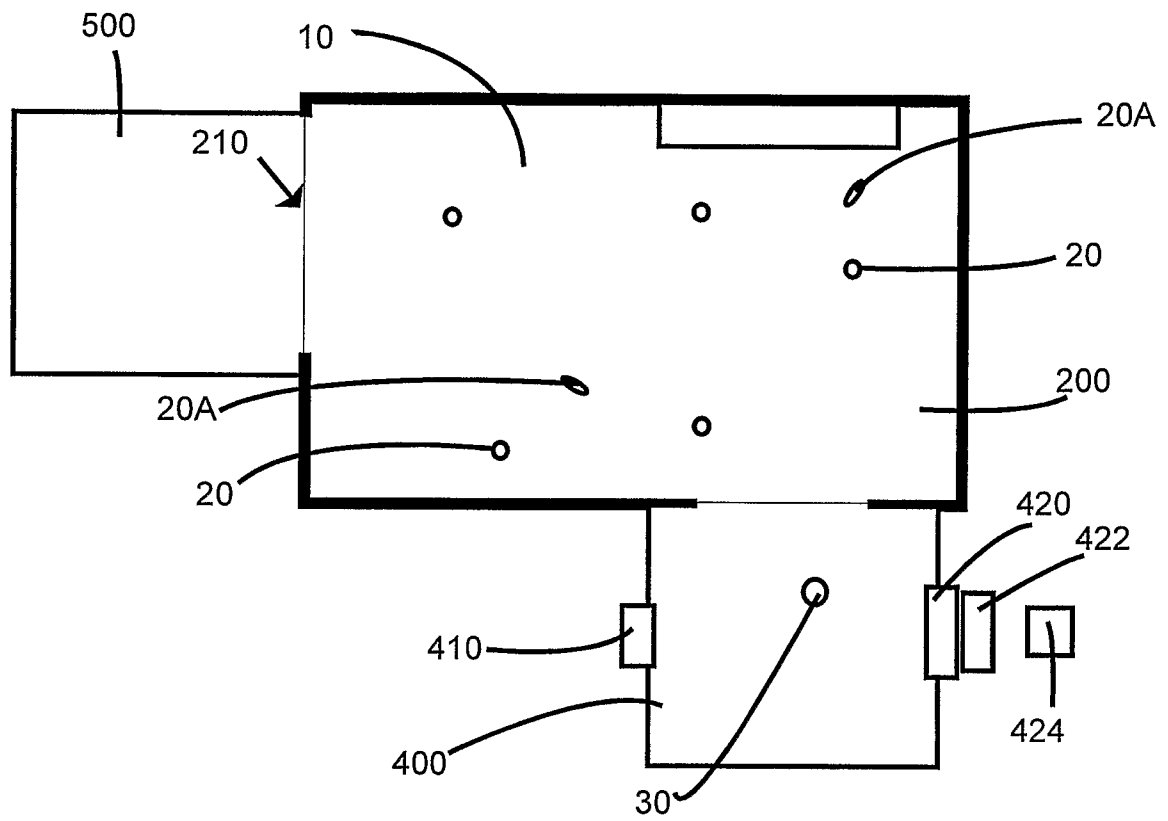


Fig. 3

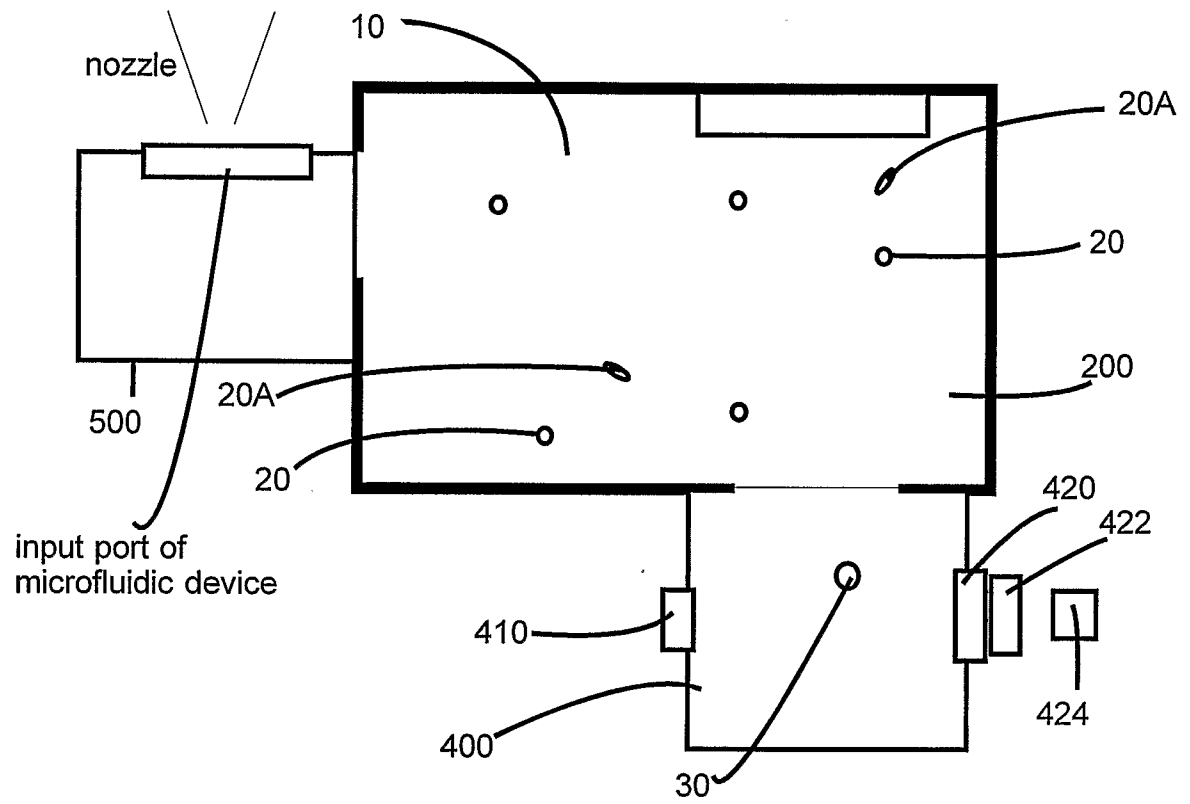


Fig. 4