ACOUSTIC CONCENTRATION, TRANSFER AND ANALYSIS OF SAMPLES CONTAINING PARTICLES

BACKGROUND

[0001] In life science research and clinical diagnostics, analytical detectors are often used to analyze the size of cells and/or particles disposed within a fluid sample. In view of increasing demand for analysis of the size of cells and/or particles disposed within a fluid, high-throughput approaches and related systems for analyzing the size of cells and/or particles are of interest. Analyzing particles within a fluid sample may require the sample to be loaded into a container and diluted to a particle density suitable for analysis or to have the bulk fluid properties be compatible with the counting and sizing method. Often, in particle sizing within fluid samples, the Coulter Principle is applied as it is effective for biological materials such as cells and viruses as well as non-conductive particles in general. For example, methods of utilizing the Coulter principle for particle counting are disclosed with reference to a Coulter counter (U.S. Patent 2,656,508) to discriminate the presence of non-conducting cells or particles in a conductive fluid.

[0002] Coulter counting employs a conductive solution that forms a conductive path between electrodes where the presence or absence of non-conductive materials (e.g., non-conductive cells and/or particles) varies the resistance of the conductive path between the electrodes. If a particle is moving past the electrodes, the variation in the resistance can be used to determine the particle size. Commercial systems such as those from Beckman Coulter Inc. (Brea, California) can analyze conductive fluid samples by detecting changes in electrical impedance due to the presence of particles in the fluid as they pass through a measurement region positioned between two electrical terminals.

[0003] However, with Coulter counting, as with other methods of particle counting, sample transfer and sample dilution are potential sources of error or contamination. Therefore, improved methods of sample transfer and dilution of particle-containing samples are desired. Sample transfer methods using acoustic radiation (i.e., acoustic pressure waves) have been described in, e.g., US Patent No. 10,156,499. However, reliable transfer of particle-containing fluid for analysis of suspended particles has not previously been achieved.

BRIEF SUMMARY

[0003a] According to a first aspect of the present invention, there is provided a method for acoustic transfer of particle-containing fluid droplets, the method comprising: positioning a fluid sample reservoir containing a fluid sample having suspended cells or particles that is acoustically

coupled with an acoustic radiation generator in alignment with an inlet of an analytical device configured for counting cells or particles; concentrating the cells or particles at a target location within the fluid sample prior to applying the ejection tone burst by applying a concentrating tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample, the concentrating tone burst configured to move the cells or particles toward the target location within the fluid sample without causing droplet ejection; and applying an ejection tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample within the fluid sample reservoir to eject a droplet from the fluid sample reservoir such that the droplet contacts the inlet of the analytical device, the droplet containing at least one cell or particle.

[0003b] According to a second aspect of the present invention, there is provided a system for acoustic ejection of particle-containing droplets, comprising: an acoustic radiation generator; a fluid sample reservoir containing a fluid sample, the fluid sample reservoir acoustically coupled with the acoustic radiation generator by an acoustic coupling medium; an analytical device configured for counting cells or particles, the analytical device comprising an inlet positioned in alignment with at least one of the acoustic radiation generator and fluid sample reservoir; and a controller comprising one or more processors and a memory device containing executable instructions that, when executed by the one or more processors, configure the controller to: concentrate the cells or particles at the target location within the fluid sample prior to applying the ejection tone burst by applying a concentrating tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample within the fluid sample reservoir to concentrate cells or particles within the fluid sample at the target location without causing droplet ejection; and apply an ejection tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample at a target location to eject a droplet containing at least one cell or particle from the fluid sample reservoir such that the droplet contacts the inlet of the analytical device.

[0003c] According to a third aspect of the present invention, there is provided a method for acoustic transfer of particle-containing fluid droplets, the method comprising: applying a first tone burst of focused acoustic radiation by an acoustic radiation generator to a fluid sample within a fluid sample reservoir containing suspended cells or particles such that the first tone burst concentrates the cells or particles at a target location within the fluid sample; and applying a second tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample at the target location corresponding to the concentrated cells or particles to eject a droplet from the fluid sample reservoir containing at least one cell or particle.

[0003d] According to a fourth aspect of the present invention, there is provided a system for acoustic ejection of particle-containing droplets, comprising: an acoustic radiation generator; a

fluid sample reservoir containing a fluid sample, the fluid sample reservoir acoustically coupled with the acoustic radiation generator by an acoustic coupling medium; and a controller comprising one or more processors and a memory device containing executable instructions that, when executed by the one or more processors, configure the controller to: apply a first tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample within the fluid sample reservoir to concentrate cells or particles within the fluid sample; and apply a second tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample at a target location corresponding to the concentrated cells or particles, to eject a droplet containing at least one cell or particle from the fluid sample reservoir.

[0004] Embodiments disclosed herein include a system having an acoustic radiation generator, a fluid sample reservoir containing a fluid sample, and an analytical instrument with an inlet that

WHAT IS CLAIMED IS:

1. A method for acoustic transfer of particle-containing fluid droplets, the method comprising:

positioning a fluid sample reservoir containing a fluid sample having suspended cells or particles that is acoustically coupled with an acoustic radiation generator in alignment with an inlet of an analytical device configured for counting cells or particles;

concentrating the cells or particles at a target location within the fluid sample prior to applying the ejection tone burst by applying a concentrating tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample, the concentrating tone burst configured to move the cells or particles toward the target location within the fluid sample without causing droplet ejection; and

applying an ejection tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample within the fluid sample reservoir to eject a droplet from the fluid sample reservoir such that the droplet contacts the inlet of the analytical device, the droplet containing at least one cell or particle.

2. The method of claim 1, wherein the concentrating tone burst and the ejection tone burst are applied sequentially.

3. The method of claim 1, further comprising:

repeatedly applying the concentrating tone burst to maintain an increased concentration of the cells or particles at the target location within the fluid sample.

4. The method of claim 1, wherein the concentrating tone burst comprises one of a continuous waveform or a long tone burst configured to produce a standing acoustic wave in the fluid sample to trap the cells or particles in a resonant or anti-resonant node.

5. The method of claim 1, wherein the concentrating tone burst comprises a swept frequency configured to push the cells or particles toward the target location within the fluid sample.

6. The method of claim 1, wherein:

the acoustic radiation generator comprises an annular first acoustic radiation generator and a disk-shaped second acoustic radiation generator positioned concentric with the first acoustic radiation generator;

the concentrating tone burst is generated by the first acoustic radiation generator; and

the ejection tone burst is generated by the second acoustic radiation generator.

7. The method of claim 1, further comprising:

subsequent to the ejection of the droplet from the fluid sample reservoir, position a second fluid sample reservoir containing a second fluid sample in alignment with the acoustic radiation generator;

apply a second concentrating tone burst of focused acoustic radiation by the acoustic radiation generator to the second fluid sample to concentrate cells or particles within the second fluid sample; and

apply a second ejection tone burst of focused acoustic radiation by the acoustic radiation generator to the second sample fluid within the second fluid sample reservoir at a second target location corresponding to the concentrated cells or particles, to eject a second droplet from the second fluid sample reservoir containing at least one cell or particle.

8. The method of any one of claims 1 through 7, further comprising:

adjusting a vertical position of the fluid sample reservoir to align a focal region of the ejection tone burst with an upper surface of the fluid sample.

9. The method of any one of claims 1 through 7, further comprising:

performing a particle count by the analytical device on the droplet contacting the inlet of the analytical device.

10. The method of claim 9, wherein:

the analytical device comprises an electrolytic particle counter configured to count or measure the suspended cells or particles in the droplet contacting the inlet of the analytical device by measuring electrical impedance of fluid drawn from the droplet across an aperture of the analytical device fluidly coupled with the inlet; and

performing the particle count comprises electrically sensing passage of the cells or particles through the aperture from the droplet contacting the inlet.

11. The method of claim 9, further comprising:

ejecting additional subsequent droplets from the fluid sample contained in the fluid sample reservoir to supplement the droplet contacting the inlet of the analytical device; and

cease ejecting the additional subsequent droplets from the fluid sample based on an attribute of the particle count.

12. The method of claim 11, wherein the attribute of the particle count is one of a number of cells or particles, a size of cells or particles, a number of cells or particles per unit time, a number of cells or particles within a predetermined range of size, or a total volume passed through the analytical device.

13. A system for acoustic ejection of particle-containing droplets, comprising:

an acoustic radiation generator;

a fluid sample reservoir containing a fluid sample, the fluid sample reservoir acoustically coupled with the acoustic radiation generator by an acoustic coupling medium;

an analytical device configured for counting cells or particles, the analytical device comprising an inlet positioned in alignment with at least one of the acoustic radiation generator and fluid sample reservoir; and

a controller comprising one or more processors and a memory device containing executable instructions that, when executed by the one or more processors, configure the controller to:

concentrate the cells or particles at the target location within the fluid sample prior to applying the ejection tone burst by applying a concentrating tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample within the fluid sample reservoir to concentrate cells or particles within the fluid sample at the target location without causing droplet ejection; and

apply an ejection tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample at a target location to eject a droplet containing at least one cell or particle from the fluid sample reservoir such that the droplet contacts the inlet of the analytical device.

14. The system of claim 13, wherein the acoustic radiation generator comprises an annular element configured to focus the concentrating tone burst to concentrate the cells or particles, and a disk-shaped element concentric within the annular element configured to focus the ejection tone burst to eject the droplet, wherein:

the concentrating tone burst is applied via the annular element; and

the ejection tone burst is applied via the disk-shaped element.

15. The system of any one of claims 13 through 14, further comprising a second fluid sample reservoir containing a second fluid sample, and an actuator configured to

move the second fluid sample reservoir relative to the acoustic radiation generator, wherein the executable instructions, when executed by the one or more processors, further configure the controller to:

subsequent to the ejection of the droplet from the fluid sample reservoir, cause the actuator to position the second fluid sample reservoir in alignment with the acoustic radiation generator; and

apply a second ejection tone burst of focused acoustic radiation by the acoustic radiation generator to the second sample fluid within the second fluid sample reservoir to eject a second droplet containing from the second fluid sample reservoir such that the second droplet contacts the inlet of the analytical device.

16. The system of claim 15, wherein:

the second fluid sample comprises additional cells or particles; and

the executable instructions, when executed by the one or more processors, further configure the controller to:

concentrate the additional cells or particles by applying a second concentrating tone burst of focused acoustic radiation by the acoustic radiation generator to the second fluid sample within the second fluid sample reservoir.

17. The system of any one of claims 13 through 14, wherein the executable instructions, when executed by the one or more processors, further configure the controller to:

apply subsequent additional ejection tone bursts to eject additional subsequent droplets from the fluid sample contained in the fluid sample reservoir to supplement the droplet contacting the inlet of the analytical device;

cause the analytical device to obtain a particle count of cells or particles contained in the droplet contacting the inlet of the analytical device; and

cease ejecting the additional subsequent droplets based on an attribute of the particle count.

18. The system of claim 17, wherein the attribute of the particle count is one of a number of cells or particles, a size of cells or particles, a number of cells or particles per unit time, a number of cells or particles within a predetermined range of size, or a total volume passed through the analytical device.

19. The system of any one of claims 13 through 14, wherein the analytical device comprises one of an electrolytic particle counter configured to count or measure suspended cells or particles by measuring electrical impedance of particle-containing fluid across an aperture fluidly coupled with the inlet or a visual particle counter configured to count or measure suspended cells or particles by optically identifying the at least one cell or particle contained in the ejected droplet contacting the inlet.

20. A method for acoustic transfer of particle-containing fluid droplets, the method comprising:

applying a first tone burst of focused acoustic radiation by an acoustic radiation generator to a fluid sample within a fluid sample reservoir containing suspended cells or particles such that the first tone burst concentrates the cells or particles at a target location within the fluid sample; and

applying a second tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample at the target location corresponding to the concentrated cells or particles to eject a droplet from the fluid sample reservoir containing at least one cell or particle.

21. A system for acoustic ejection of particle-containing droplets, comprising:

an acoustic radiation generator;

a fluid sample reservoir containing a fluid sample, the fluid sample reservoir acoustically coupled with the acoustic radiation generator by an acoustic coupling medium; and

a controller comprising one or more processors and a memory device containing executable instructions that, when executed by the one or more processors, configure the controller to:

apply a first tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample within the fluid sample reservoir to concentrate cells or particles within the fluid sample; and

apply a second tone burst of focused acoustic radiation by the acoustic radiation generator to the fluid sample at a target location corresponding to the concentrated cells or particles, to eject a droplet containing at least one cell or particle from the fluid sample reservoir.