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REVIEW

Methods for counting particles in microfluidic applications

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Abstract Microfluidic particle counters are important tools in biomedical diagnostic applications such as flow cytometry analysis. Major methods of counting particles in microfluidic devices are reviewed in this paper. The microfluidic resistive pulse sensor advances in sensitivity over the traditional Coulter counter by improving signal amplification and noise reduction techniques. Nanopore-based methods are used for single DNA molecule analysis and the capacitance counter is useful in liquids of low electrical conductivity and in sensing the changes of cell contents. Light-scattering and light-blocking counters are better for detecting larger particles or concentrated particles. Methods of using fluorescence detection have the capability for differentiating particles of similar sizes but different types that are labeled with different fluorescent dyes. The micro particle image velocimetry method has also been used for detecting and analyzing particles in a flow field. The general limitation of microfluidic particle counters is the low throughput which needs to be improved in the future. The integration of two or more existing microfluidic particle counting techniques is required for many practical on-chip applications.

Keywords Particle counting · Microfluidics · Resistive pulse senor · Nanopore · Light scattering · Light blocking · Capacitance · Fluorescence · Micro PIV

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1 Introduction

Particle counting is important and widely used in various areas from environmental (Aalto et al. 2005) to biological applications (Smolen et al. 1983; Amann et al. 1990; Yarnell et al. 1991), such as the following: counting dust particles is required for clean room facility (Wu et al. 1989); counting debris particles is needed for studies of lubricating systems (Miller and Kitaljevich 2000); counting contaminant particles is the key factor in water purification system (Bundschuh et al. 2001; Judd and Hillis 2001); and counting white blood cells is essential for many biomedical diagnostic purposes such as detecting HIV infection (Yarnell et al. 1991; Kannel et al. 1992; Burnett et al. 1999; Vozarova et al. 2002). The particle counting techniques have been developed further to detect single DNA molecule (Akeson et al. 1999; Kasianowicz et al. 1996) and analyze DNA contents (Sohn et al. 2000). However, the conventional particle counting methods rely on costly, bulky, and complex instruments and require a large amount of samples and reagents. These are the barriers to many particle counting applications.

Microfluidic devices have been studied particularly in the past decade and have showed enormous potential for portable and low-cost applications, especially in medical diagnostics (Akeson et al. 1999; Burnett et al. 1999; Kannel et al. 1992; Sohn et al. 2000; Vozarova et al. 2002; Kasianowicz et al. 1996). Lithographic fabrication technique makes possible building inexpensive and small devices integrated with electrodes and sensors, and microfluidic control technologies such as electrokinetics (Li 2004) are able to control particles and liquid flow in micro- and nano-channels. In addition, microfluidic devices are particularly useful for applications where a very small quantity of samples is available or desired.

Microfluidics-based particle counting methods have great advantages over the conventional methods and allow the development of accurate, cheap, and portable particle counting devices.

In this paper, we review the major advancement of microfluidic particle counting techniques: microfluidic resistive repulse sensors, nanopore sensors, capacitance counters, light-scattering and light-blocking detectors, fluorescent detectors, and micro particle image velocimetry (PIV) counters. For each type of particle counters, we also explore various applications and examine the advantages and disadvantages.

2 Coulter counter

Coulter Counter perhaps is the most popular conventional method of particle counting. We review this method first for two reasons: it gives a general perspective of conventional particle counting methods, and its working principle of measuring electric resistive pulse is used in some microfluidic particle counting methods. Coulter Counter was first invented by Wallace H. Coulter during World War II and patented (Coulter 1953). When Coulter worked for US Navy, he used this technique to count the number of plankton particles that always caused large echoes on Sonar. In a Coulter counter, a small aperture on the wall is immersed into a container that has particles suspended in low concentration electrolyte solution. Two electrodes are placed: one in front and one behind the aperture, and a current path is provided by the electrolyte when an electric field is applied (Fig. 1) and the aperture creates a "sensing zone." As a particle passes through the aperture, a volume of electrolyte, equivalent to the immersed volume of the particle is displaced from the sensing zone. This causes a short-term change in the impedance across the aperture. This change can be measured as a voltage pulse or a current pulse. The pulse height is proportional to the volume of the sensed particle. If a constant particle density is assumed, the pulse height is also proportional to the particle mass. This technology thus is also called aperture technology.

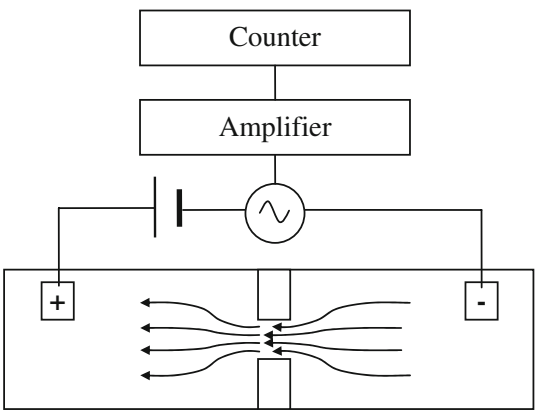

Fig. 1 Schematic of a Coulter counter

Because the Coulter counter is simple, highly sensitive, and reliable, it is widely applied in many areas including medical instruments such as counting and analysis of blood cells (Horne et al. 2005), protein (Kulp et al. 2004), and viruses (Wahl-Jensen et al. 2007) besides detecting fine particles and pollen (DeBlois and Bean 1970). Before the Coulter counter was invented, complete blood counts (CBCs) were carried out manually with a telescope and static samples, which is time-consuming and inaccurate. With the Coulter counter, CBCs can be done for a large amount of samples in a very short period of time. When Coulter first demonstrated the Coulter counter, it could count red blood cells at a high count rate of 6,000 particles/s, which revolutionized the science of hematology. Within a decade, literally every hospital laboratory in the US had Coulter counters, and today every modern hematology analyzer adopts the Coulter principle in some way. For example, Coulter counters may measure the change of impedance (Carbonaro and Sohn 2005; Jagtiani et al. 2006a, b; Zhe et al. 2007; Wu et al. 2008a, b), conductance (Sohn et al. 2000; Murali et al. 2009), and reflected radio frequency power (Wood et al. 2005) when a particle passes the aperture.

After many years of development, the modern Coulter counter is versatile and accurate in particle sizing and counting. Multisizer™ 4 COULTER COUNTER® (Beckman Coulter, Fullerton, CA, USA), for example, can provide size distribution in number, volume, and surface area in one measurement of particles ranging from 0.4 to 1,600 μm in diameter. Its aperture dynamic range can reach to 1:40 by diameter and reproducibility is about 1%. Although the Coulter counter detects and analyzes particles accurately and reliably, it has still many drawbacks: bulky size, heavy weight, complexity, high power consumption, high cost, and no-portability. Especially during outbreak of public diseases such as severe accurate respiratory syndrome (SARS) and Influenza, there is urgent need for simple, low power, low cost, and portable particle counters.

3 Microfluidic resistive pulse sensors

The microfluidic resistive pulse technique applies the basic working principle of the Coulter counter to microchannels for counting micro- and sub-micron particles. The resistive pulse sensor (RPS) was applied to detect submicron polystyrene beads of 90 nm inside a 0.4–0.5 mm diametric polycarbonate pore by DeBlois and Bean in 1970. DeBlois and Wesley (1977) utilized this technique in biological area

and succeeded in detecting viruses. After the technical advance of micro-fabrication (Rogers and Nuzzo 2005), the RPS method has been applied to count particles moving in microchannels. Many microfluidic RPS applications involve manipulating and transporting of particles by electrokinetic flow in microchannels (Jagtiani et al. 2006a, b) while some still use traditional flow control by hydraulic pressure.

The key advantages of the microfluidic RPS include label-free particle detection and simplicity without other peripheral complex instruments other than a simple electric circuit and a micro- or nano-scale sized channel. Therefore, it is mostly applicable for portable lab-on-a-chip (LOC) devices to detect biopolymers such as DNA, protein, and blood cells. However, the flow rate of the microfluidic RPS is small and the sensitivity of the microfluidic RPS is limited by its aperture size, resulting in poor throughput and sensitivity. To overcome these shortcomings, recent researches on microfluidic RPS focus on two main issues: improving of the sensitivity and enhancing of the throughput. The RPS throughput is evaluated by particle flow rate at the aperture or the number of the counted particles at a given time. Its sensitivity is determined directly by the volume ratio of the detected particles and the aperture and can be adjusted by controlling the amplification gain of the electronic circuit or instrument and the noise reduction from the fluidic network and the electronic sensing system.

DeBlois and Bean (1970) were able to detect 90 nm polystyrene spheres, which is equivalent to minimum volume ratio of 0.06% with submicron pores etched in irradiated plastic sheet. To improve the sensitivity, Xu et al. (2007) and Sridhar et al. (2008) used a relatively wide polydimethyl siloxane (PDMS) channel of 16 μm with metal-oxide-semiconductor-field-effect-transistor (MOSFET). They detected particles by monitoring the MOSFET drain current modulation instead of the modulation in the ionic current though the sensing channel and achieved ten times smaller minimum volume ratio of 0.006% than that of DeBlois and Bean. Recently, Wu et al. (2008a, b) developed a microfluidic RPS method utilizing a mirror symmetric channel structure and a two-stage differential amplifier (Fig. 2). They could significantly reduce the noise and achieve a much better signal-to-noise ratio. This sensing scheme detected 520-nm diameter polystyrene particles with a 20-μm sensing gate and improved a minimum volume ratio to as low as 0.0004%, which is about ten times more sensitive than the current commercial Coulter counter of 0.0037% (Beckman Counlter® Multisizer™ 4). The principle of the symmetric dual channel design is to make noise levels for the output signals (V_{D1} and V_{D2} as indicated in Fig. 2) from both gate branches identical and hence the noises can be canceled by a

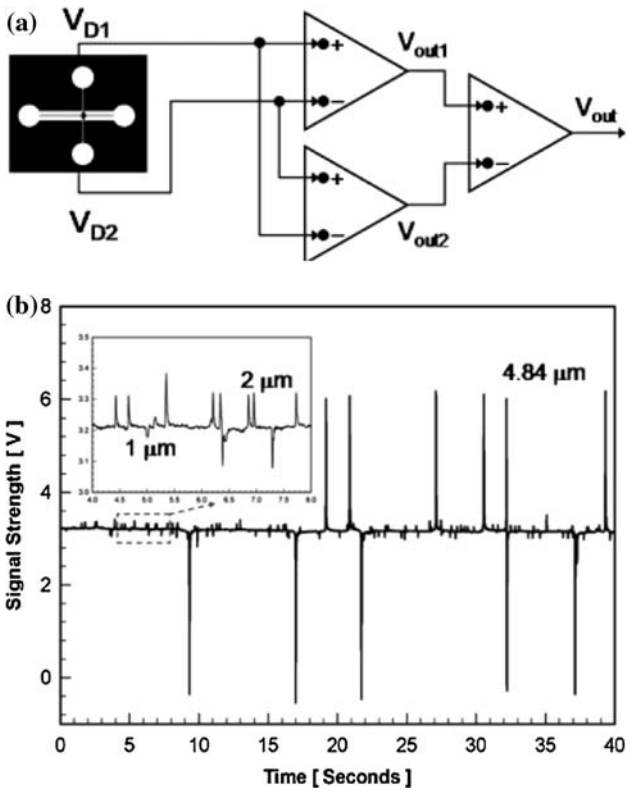

Fig. 2 The schematic drawing of dual-channel microfluidic differential RPS (**a**) and repulsive pulse signals of 1, 2, and 4.84 μm particles in 7.5 mM sodium borate buffer (**b**). The magnified *inset* shows the signal strength of 1 and 2 μm particles

subtraction electronic circuit. However, ideal noise subtraction is not possible due to realistic limitation to fabricate the identical dual channels. Furthermore, this dual channel method will not be able to detect particles when two particles pass the two apertures at the same time because the two signals with the similar amplitude will be subtracted by each other and cancelled at the second stage of differential amplifier.

To improve this microfluidic RPS method, Wu et al. (2008a, b) solved the signal cancelation problem as mentioned above by using a single sensing gate and two detecting arm channels next to the sensing gate at both ends (Fig. 3). They coupled the RPS with laser fiber-optic fluorescence technique to demonstrate a flow cytometer LOC that is able to detect fluorescent and non-fluorescent particles simultaneously, and the RPS signal-to-noise ratio is improved significantly. Two-stage differential amplification is also used to further increase the signal-to-noise ratio for fluorescent signals to detect 0.9 μm fluorescent particles. This flow cytometer chip showed comparable sensitivity for detecting fluorescent and non-fluorescent particles to commercial flow cytometers with simple, cheap, and compact system on a micro glass slide. Drawbacks of the method are the baseline drifting by multiple-stage differential

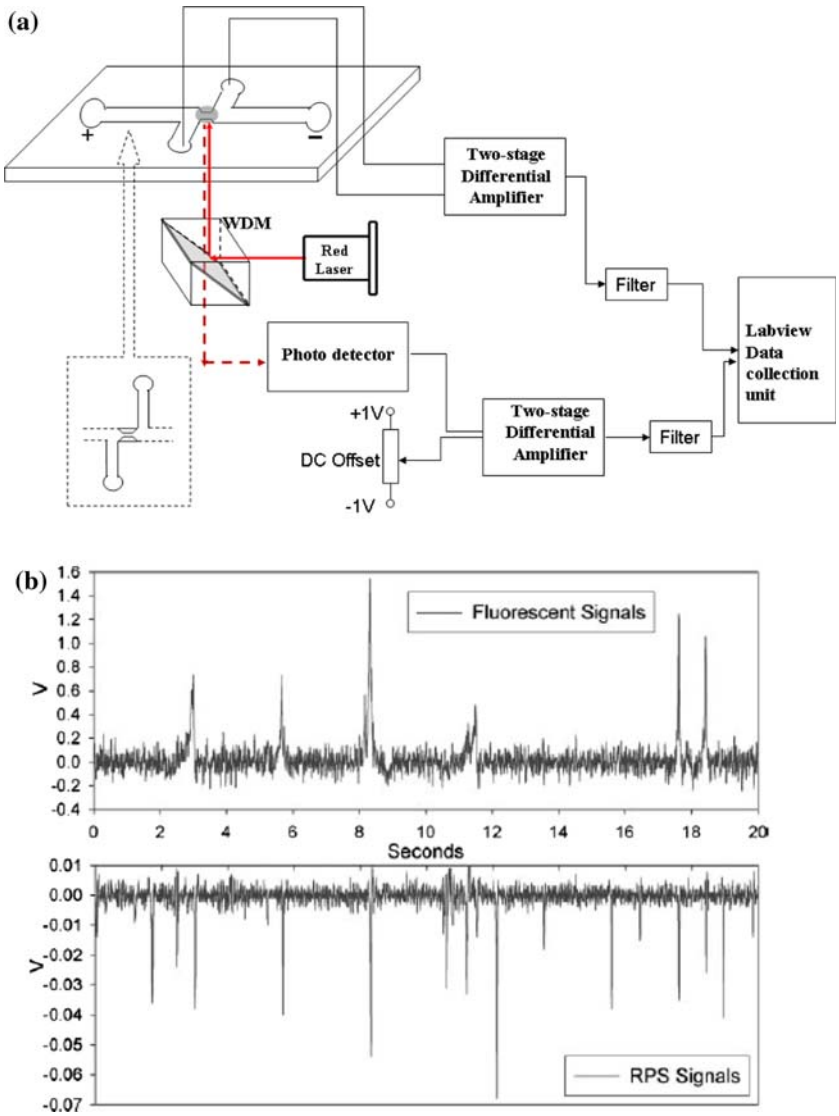

Fig. 3 The schematic diagram of single channel and two detecting arm channel microfluidic differential RPS (**a**) and the RPS counter and fluorescent signals for 0.9 μm Nile Blue particles mixed with the 0.99 μm Dragon Green particles (**b**). The greater RPS peaks are the signals of the 0.99 μm non-fluorescent particles

amplification and the low throughput of using single channel detection.

The throughput of a single-channel Coulter counter is proportional to the square of the diameter of the detecting aperture. When submicron-or nanometer-size particles are to be counted, the size of the aperture has to be scaled down to submicron or nanometer in order to maintain the sensitivity. Otherwise, the signal-to-noise ratio will be very low. While the sensitivity of the single-gate microfluidic RPS method is much higher, it requires long detecting time due to low flow rate in a microchannel and using of diluted samples to avoid multiple particles flowing together. To overcome this low throughput issue, multi-counting techniques are developed. Carbonaro and Sohn (2005) first demonstrated the simultaneous immunoassays of two different human antigens by integrate multiple artificial pores and the RPS technique on a single chip. Coulter and Hogg (1976) patented the particle analyzing apparatus and method with multiple sensing apertures. However, it is difficult to integrate the detection circuit and independent power supply of their systems on one chip. Zhe group (Jagtiani et al. 2006a, b) proposed a multi-aperture Coulter counter, which consists of four peripheral reservoirs and a central reservoir (Fig. 4a). Each peripheral reservoir is connected to the central reservoir through a miniature channel. Their results showed that the sensor can detect and count particles through its four sensing apertures simultaneously. However, the four apertures are the maximum number of apertures that can be built in a single chip due to the configuration limit. Furthermore, Zhe group (Zhe et al. 2007) proposed different high throughput single chip counter using multiple channels, operating in parallel with single common sample reservoir and a power source (Fig. 4b). This counter was capable of differentiating and counting polymethacrylate particles and Juniper pollen about three times faster than single channel counter. This

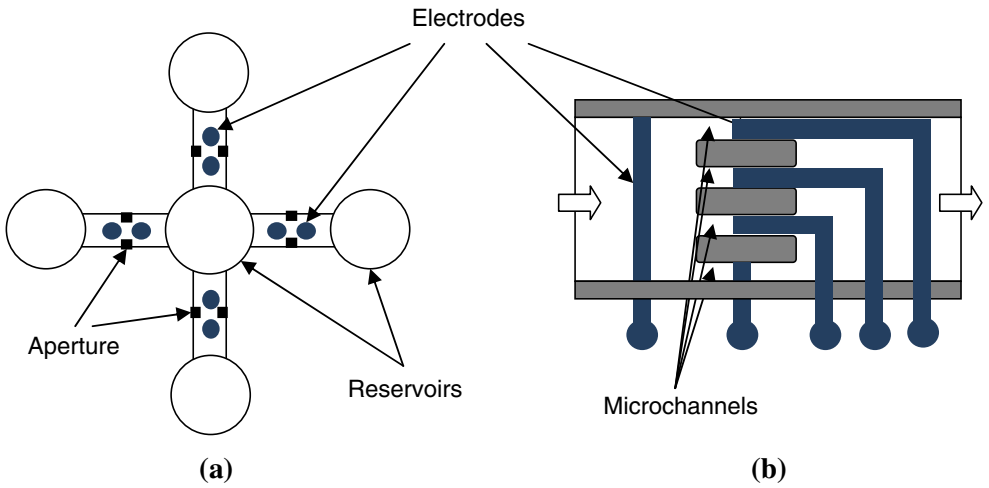

Fig. 4 The high throughput Coulter counters. A multi-aperture Coulter counter (**a**) and a multi-channel Coulter counter (**b**) for micro particle detection

concept could be extended to multi-channel microfluidic chips in the future to improve the counting efficiency.

4 Nanopore sensor

A nanopore here is referred to as a small pore of nanometer size in an electrically insulating membrane and a nanopore resistive sensor (NRS) is a nanometer-scale Coulter counter, which uses the nanopore as an aperture to detect single molecule. The NRS, the Coulter counter, and the RPS share the same principle for particle detection; however, the NRS has attracted many researchers since 1990s because the volumetric ratio of the NRS can be high enough to detect much smaller particles such as single molecule by the nanometer sized pores (Bezrukov et al. 1996; Kasianowicz et al. 1996; Akeson et al. 1999). There are two types of nanopores according to the nanopore materials: synthetic and natural materials. A typical natural nanopore is a biological protein channel in a lipid bilayer.

Bezrukov et al. (1996) demonstrated the counting of polymer molecules passing through a single alamethicin pore of 5 and 2 nm in length and diameter, respectively. Kasianowicz et al. (1996) showed that they were able to sense single-stranded RNA and DNA through a 2.6 nm diameter ion channel in a lipid bilayer membrane. This promising result sparked many studies of DNA translocation and dynamics in biological nanopores. Akeson et al. (1999) detected single DNA and RNA molecules in an α-hemolysin channel driven by an applied electric field (Fig. 5). Since the small-sized pore can hold only one strand of DNA or RNA at a time, nucleotides within the polynucleotide must pass through the channel/pore in sequential and single-file order during the translocation. In this process, not only counting but also discriminating

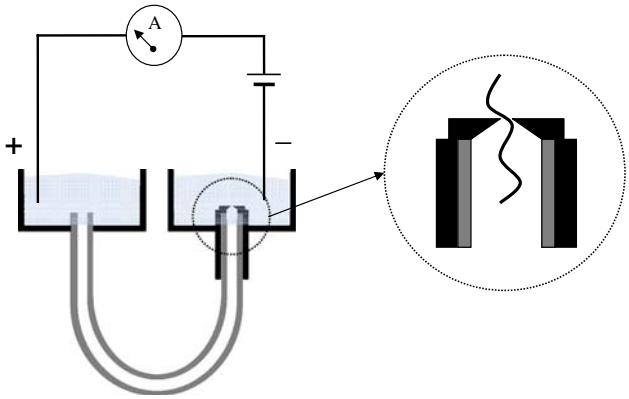

Fig. 5 Horizontal bilayer apparatus. A U-tube connects two baths and all are filled with KCl buffer. The baths are connected to an Axopatch 200B amplifier by Ag-AgCl electrodes. One end of the tube has a conical tip of a 25-μm aperture. Diphytanoyl phosphatidylcholine/hexadecane bilayers are formed across the aperture, and one or more α-hemolysin channels are inserted into the bilayer to detect single DNA or RNA molecule

between pyrimidine and purine segments along a DNA or RNA molecule can be accomplished. A very important application of this research is the direct sequencing of individual DNA and RNA molecules with a nanopore. In addition, nanopore method was used to measure small particles like metal ions, nucleic acids, and other types of polymers in a less than 10 nm channel (Biance et al. 2006).

The geometrical and chemical properties of biological nanopore can be reproducibly controlled by genetic engineering. However, the biological nanopores are not very robust and not size tunable. Even in laboratory environment it can last only several hours. Therefore, efforts have been made to fabricate artificial, solid-state nanopores to overcome this limitation. The nanopores based on synthetic

material are generally made in silicon compound membranes, such like silicon nitride. Manufacturing technique could be focused ion beam (FIB) sculpting or electron beam sculpting. Martin group did a series of research on synthetic conical nanopores for biosensing applications (Siwy et al. 2005; Harrell et al. 2006; Wharton et al. 2007; Sexton et al. 2007). They made a conical pore with a 1.5 mm base diameter and a 40-nm tip diameter and sensed a single-stranded phage DNA of 7,250 bp and a double-stranded plasmid DNA of 6,600 bp. They also detected protein in the same way. Alternatively, Ito et al. (2004) utilized a 132 nm multiwall carbon nanotube (MWNT) to detect 28–90 nm nano-particles. Synthetic nanopores are chemically and structurally stable; however, it is still hard to control the size of these nanochannels. Reproducing synthetic nanopores of the same size is hardly possible. Additionally, high manufacturing cost is another obstacle to be overcome for further development and applications of synthetic nanopore particle sensors.

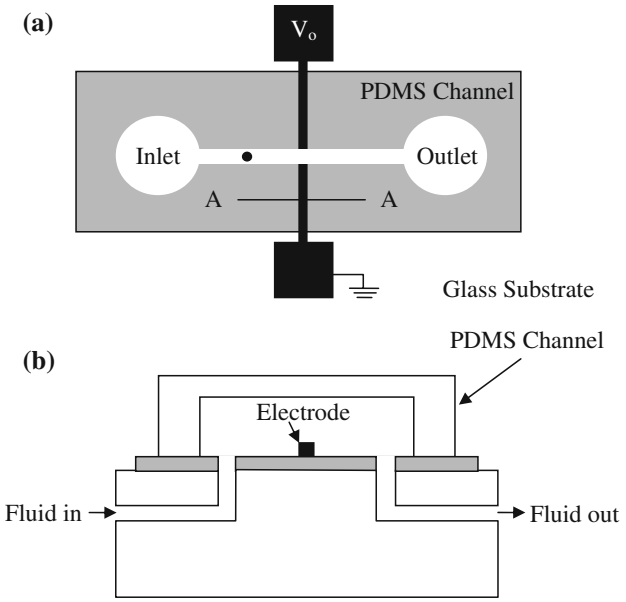

Fig. 6 Schematic illustration of the integrated microfluidic capacitance counter: top view (**a**) and side view along the line of A–A (**b**) of the capacitance counter

5 Microfluidic capacitance counter

The capacitance counter uses a similar principle to the Coulter counter: it measures the AC capacitance instead of DC resistance when a micron or sub-micron particle passes a sensing gate (aperture). The capacitance counter is particularly useful for detecting particles in liquids of low electrical conductance because the resistance change due to the passage of a particle is difficult to measure in a poor conducting liquid (Murali et al. 2009). In the past, the capacitance measurements have generally been used to identify bulk materials and to investigate ensembles of biological cells. However, Sohn et al. (2000) employed this technique to detect and quantify the polarization responses of DNA in the nucleus of single eukaryotic cells. They built an integrated microfluidic device (Fig. 6), and used a syringe pump to deliver the liquid to the device. A capacitance bridge at a frequency of 1 kHz across the device was used to detect the capacitance change to determine the DNA content of single eukaryotic cell. Additionally, they demonstrated the relationship between the capacitance and the DNA content of a cell. Recently, Murali et al. (2009) adopted the capacitance counter to monitor of the wear debris in lubrication oil to avoid catastrophic system failure of machine in real-time. Unlike bulk measurement methods, they could scan each individual particle and determine the size of particles without being affected by the change of oil properties. Particles from 10 to 25 μm were successfully detected.

Similar to a microfluidic RPS, the capacitance method has advantages in terms of simple sample preparation, cost, size, and robustness. In addition, the capacitance method is sensitive to probe the polarization response of a wide range of materials both organic and inorganic to an external electric field. Furthermore, it can monitor changes in DNA contents and cell-cycle kinetics so that it may serve as a medical diagnostic device to identify the presence of malignancy in very small quantities of tissue such as tumor cell and monitor in real-time of the effects of pharmacological agents on cell cycle and cell death. The capacitance method, however, has complications resulting from the charge-screening effects at the electrode-conductive liquid interface in an electronic measurement, which prevents the interpretation of the absolute capacitance value. Besides, due to AC voltage and frequency required to detect the capacitance, frequency modulation controller is necessary and external support such as a syringe pump is required to deliver a liquid in a channel. These restrictions in flow control and frequency modulation are some of the obstacles for developing compact microfluidic capacitance particle sensors.

6 Light-scattering and light-blocking counters

A light-scattering particle counter and a light-blocking particle counter are two similar types of light-based particle counters. They both use laser light sources such as laser diodes to illuminate individual particles that pass through the laser beam. The difference between these two counters lies in how the interaction between the light and the particle is measured for counting particles. When light strikes

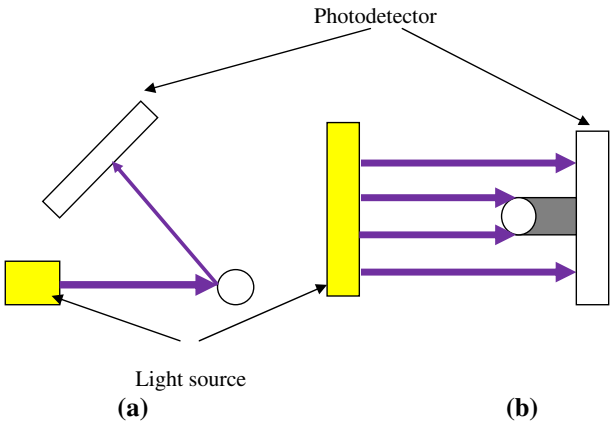

Fig. 7 Illustration of two types of light-based particle counters: a light-scattering counter (**a**) and a light-blocking counter (**b**)

an object generally it will be divided into three parts. Some light will pass through the object, some will be reflected, and the rest will be absorbed by the object. The portion of these three components is determined by optical properties of the material composition of the particle.

Light-scattering counter uses the reflected light of the particle. As shown in Fig. 7a, when the source light hits the particle, some of the light is reflected and the reflected light can be detected by a photo-detector positioned at a spot with a certain angle from the light path. In general, the detector is placed at the angle of 20°–40° from the light path to the particle. The strength of detected light signal corresponds to particle size, and the number of pulses of the detected light signal is proportional to number of particles. On the other hand, the light-blocking counter (Fig. 7b) measures the light absorbed or reflected away from the detector by the particle. In this arrangement, the light is focused directly onto the detector; when the particle passes between them, and the photo-detector senses the sudden change in the light intensity by a blocking particle. It is obvious that the larger the particle, the more light it will block. In principle, the light-scattering counter detects light while the light-blocking counter detects the darkness. The light-scattering method is more sensitive than the light-blocking method. This is the same principle that detecting a light in a darkroom is easier than detecting a dark spot in a bright room due to the diffuse reflectance caused by solid surfaces and particles in space. However, in order to focus and detect the scattered light at a specific angle, the light-scattering counter system normally contains more optical elements and complex electronic circuit, which makes the light-scattering counter more expensive than the light-blocking counter. The light-blocking counter is widely used in detecting particles in water (e.g., water treatment application) while the light-scattering counter is mostly used in detecting atmospheric particles. The key disadvantage of these light-scattering and light-blocking counters is the low sensitivity in comparison to the Coulter counter because the sensitivity of these light-based particle counters are determined by the particle's surface area while the sensitivity of the Coulter counter is decided by the volume of the particle.

Pamme et al. (2003) researched the counting and sizing of particles and particle agglomerates of C-reactive protein by laser light scattering method. They detected scattering light at two different angles of 15° and 45°. The experiment was carried out on a polymethyl methacrylate (PMMA) microchip which consists of three inlet channels of two buffers and one sample channel and one outlet channel. The fluid was driven by syringe pump generating a negative pressure with a flow rate of 1 μl/s to neglect the hydrostatic pressure in the inlet reservoirs. A beam splitter and an objective lens are applied to confine the He–Ne laser inside the channel to avoid scattering on the wall. The scattering light signals are sensed by two optical fibers and amplified by a photomultiplier tube (PMT). By this method, the authors realized the particle detection of 2–9 μm. In addition, size discrimination of particles with a diameter ratio of 1:2 was achieved. On the other hand, the standard deviation from the average scattering light intensity for a given particle population was high, up to 30%, and a wide laser beam led to overall lower scattering light intensity and higher background scattering from the channel walls. These cause problems for measurement of smaller particles due to the lower scattering intensity and the lower signal-to-noise ratio. Xiang et al. (2005) utilized the light-blocking technique and developed a multifunctional particle detection system with embedded optical fibers in a PDMS chip to detect moving micro particles in a microchannel. They developed a PDMS-glass microfluidic chip with two pairs of embedded optical fibers and removed the glass cladding layer of the input and receiving fibers. By filling the gap between the fiber and the fiber channel with PDMS, the light leakage from the fiber core and the light scattering from the fiber tips were minimized. By using the two-fiber detection method, particle velocity as well as particle counting and size identification were possible. They achieved counting of 10, 20, and 25 μm particles. To get a better signal, both input and receiving fibers must be perfectly aligned. They used larger size of the receiving fiber to improve this problem. However, in real applications, careful handling of the fiber-embedded chip and highly controlled fiber alignment are required.

Schafer et al. (2009) created an all silicon and glass microfluidic device using femto-second laser ablation and anodic bonding technique and applied it for cell counting. This microchannel fabrication needs just one-step process and shorter fabrication time by femtosecond pulse laser. The optical fibers for light-scattering signal detection directly contact the liquid, causing light focus on the

optical detector by removing the optical free space between the detecting fiber and the channel. The incident light was coupled to the fiber with a 20×, 0.4 NA objective, and the light was delivered through the fiber at the normal direction and collected through the fiber on the opposite side at 14° from the normal and then collimated by an objective and focused onto a photodiode. HeLa cell was detected by scattered light with the reported lowest power. Conclusively, the system achieved similar particle-detecting quality with lower laser power of 2 mW and a cheaper photodiode. However, this device has difficulty of controlling of the even depth and the alignment of the grooves used to guide the optic fibers, and has poorer re-productivity than PDMS chips.

Kummrow et al. (2009) developed a microfluidic flow cytometer combined light-scattering detection and fluorescence detection in a PMMA chip with integrated optical fibers, mirrors, and electrodes for flow cytometric analysis of blood cells. They used ultraprecision milling technique to fabricate different flow cells featuring single-stage and two-stage cascaded hydrodynamic focusing of particles in horizontal and vertical directions by a sheath flow. As shown in Fig. 8, the first stage decreased the diameter of the sample flow to about 30 mm, the second stage allowed to reduce the diameter down to 5 mm while maintaining stable operation for sample flow rates of up to 20 μl/min. Inserted optic fibers were used to excite fluorescence of stained cells and to detect the axial light loss and the light scatter. Integrated mirrors were used to image the sample flow in vertical direction, thus proving the efficiency of hydrodynamic focusing in two dimensions. After passing the optical sensor the particles enter the interaction zone

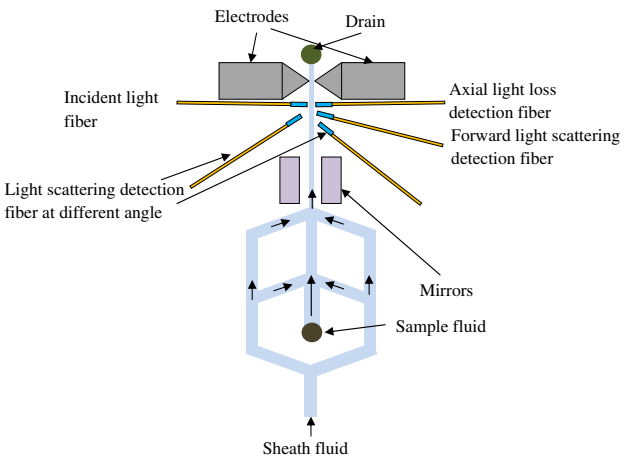

Fig. 8 Schematic diagram of a microfluidic system with two stage cascaded hydrodynamic focusing, integrated mirrors, optical fibers, and fluidic connection. Multimode fibers serve to detect the axial light loss or scattered light when particles pass the interaction region. Orthogonal light scatter and fluorescence is collected perpendicular to the joining plane by a microscope objective

for impedance measurements. Three multimode optic fibers with angles of 12°, 42°, and 145° from the incident light were used to detect the light scatter. The multimode optic fiber opposite to the incident light served to measure the axial light loss. An argon ion laser was used for forward light at 488 nm and for exciting the fluorescence of stained cells. T-helper lymphocytes labeled by monoclonal antibodies were identified by measuring side scatter and fluorescence. Using this cytometer, mono-disperse polystyrene spheres with diameters ranging from 2 to 22 μm were detected.

7 Fluorescence-based counter

Fluorescence is an optical phenomenon in which the molecular absorption of a photon triggers the emission of a photon with a longer and less energetic wavelength. The energy difference between the absorbed and emitted photons ends up as molecular rotations, vibrations, or heat. This fluorescence characteristic has been applied to count particles. Lin and Lee (2003) proposed a method of fluorescence detection in a micro flow cytometer without on-chip fibers. In this system a PDMS microchip is bonded to a 150-μm-thick glass substrate. Laser passes a filter cube and optical fiber to excite particles inside the micro-channel. The excited fluorescence is also detected by the same fiber and translated to electrical signals in the photo detector. The lock-in amplifier amplifies the electrical signal and transmits it to computer. Embedding the optic fiber into the chip adds difficulty and cost to manufacturing the chip. In this system, however, the fiber is separated by glass substrate and the microchip is disposable. In the experiments, the authors successfully detected and counted 5–20 μm fluorescent particles, white blood cells, and yeast cells. The major advantage of this method is no embedded optic fiber. The glass substrate works as the interface between the detection fiber and fluorescent particle. However, the detected fluorescent signals are not constant as they depend on the distance between the optic fiber detector and particles (the particles moving closer to the detector produce stronger signals). In addition, the precise alignment of the fiber and the channel is still difficult to realize.

Chen and Wang (2009) reported on-chip fluorescence detection and counting system in a PDMS microchip. The fluorescence and size information of particle were characterized by combining forward scattering signal and backward fluorescence signal. In the experiments, microparticles of four different sizes with diameters ranging from 3.2 to 10.2 μm were distinguished and counted. The relative percentage of the fluorescence-labeled particles can be analyzed by the ratio of the events of fluorescence signals to forward

scattered signals. Similar to Lin and Lee, this system also adopted the externally installed fiber system for disposable chips. However, without a bulk lock-in amplifier, they could detect 3.2–10.2 μm particle by using an avalanche photodetector and simple electronic filter circuit. In addition, the chip fabrication process is simpler and cheaper by only PDMS soft lithography. The same problems of inconsistent fluorescent signals caused by the distance between the photodetector and particles and the difficulty of fiber alignment still existed.

In general, fluorescence is the one of the most sensitive methods for particle detection; however, using this method, particles must have fluorescent characteristics and non-fluorescent particles have to be labeled with proper fluorophores. Therefore, this technique is widely used to distinguish specific target particles from other particles, and often used in combination with other particle counting techniques (Chen and Wang 2009; Kummrow et al. 2009; Murali et al. 2009).

8 Micro-PIV method

Particle image velocimetry is an optical method used to obtain instantaneous velocity measurements and related properties in fluids. When the small seeding particles flow inside a flowing liquid, the motion of these particles is traced and computed to show the fluid flow by taking sequential images of the positions of the tracing particles. Typical PIV apparatus consists of a digital camera, a high-power laser, and an optical arrangement to convert the laser output light to a light sheet. A fiber-optic cable often connects the laser to the cylindrical lens setup. The laser acts as a photographic flash for the digital camera, and the particles in the fluid scatter the light. The scattered light is detected by the camera. In the early twentieth century, German scientist Ludwig Prandtl first used particles to study fluids in a systematic manner (Goldstein 1996), and then the rapid development of lasers and camera technology enabled flow visualization and later on quantifying the whole flow field measurement. Modern PIV software continues to improve the performance of the PIV systems and their applicability to difficult flow measurements. Nowadays the PIV has become one of the most popular instruments for flow measurements in numerous applications.

Hirono group developed the image cytometry centrifugation (ICC) to count leukocytes (Yabusaki et al. 1999, 2000) and later extended their research to study theoretically and experimentally a microfluidic image cytometry by using micro-PIV flow visualization technique (Hirono et al. 2008). In their research, numbers and sizes of particles flowing through a microchannel were measured simultaneously by image sequence analysis. During the experiments, a dilution series of 2 μm polystyrene particle suspensions were measured and compared with the results obtained by conventional Burker–Turk hemocytometry for validation of the particle counting. For the particle diameter measurements, the diameters of 2, 5, 10, and 20 μm particles were measured and the results agreed well with the reference values. This method may be used to the quantitative study of platelet aggregation in blood flow and become a powerful diagnostic tool in the future. The image processing procedure demands complicated computational work and microscopic instrument, resulting in high cost, additional post processing, and large system.

9 Summary

For counting biological or non-biological particles, a traditional Coulter counter is still the most popular device because of its high sensitivity and throughput. However, due to the increasing needs for portability and low cost in wide biological applications such as a handheld flow cytometer and a single DNA analyzer, many recent researches have focused on micro- or nano- scale particle counting devices using advanced microfluidic technologies to realize portability, low cost, and simplicity.

In this paper, among many particle counting techniques, we reviewed only those applicable in microfluidic chips. Microfluidic RPS has been studied and demonstrated to have significantly higher sensitivity than the commercial Coulter counter with low cost. Furthermore, the RPS technique extends to nano-size by utilizing natural or synthetic nanopore to detect single DNA/RNA. For applications in liquids of low electrical conductance, microfluidic capacitance measurement has been used instead of resistance measurement for particle counting. The light-scattering and the light-blocking methods can also be applied in microfluidic chips and have a little lower sensitivity than RPS technique. However, they are more applicable in applications involving higher density particles. Fluorescent detection has an advantage in identifying specific particles among similar sized particles because the fluorescence method has higher specificity than any other electrical measurement. Micro PIV technique can perform particle counting and analyzing the flow filed simultaneously.

In addition to the disadvantages of the individual detecting techniques described in this paper, the low throughput is a major hindrance for the applications of microfluidic particle counting methods. A possible way to overcome this obstacle is to use multiple parallel channels; however, the number of parallel sensing microchannels can be built in single detecting chip is limited practically. Therefore, the enhancement of the counting speed as well

as stable and sensitive detection over a longer period of time is among the challenging issues. Furthermore, in order to meet the demand of the complex analytical detection in real applications, the integration of multiple particle counting methods in a single microfluidic system should be investigated.

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