High Throughput Microfluidic Electrical Impedance Flow Cytometry for Assay of Micro Particles

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Abstract: Recent advances in microfluidics and microfabrication techniques have led to a variety of portable and inexpensive lab-on-a-chip devices to make quantitative assays of microscale and nanoscale bioparticles. Among them, electrical impedance flow cytometers have become an indispensable tool in clinical and research laboratories for analysis of micro/nano bio-objects. Because of their simplicity and capability of single cell analysis, electrical impedance flow cytometers have been used widely to detect and characterize latex beads, pollen, biological cells, bacteria, viruses and DNA. One long standing drawback of the electrical impedance flow cytometers is their low throughput, namely they can only process a small amount of analyte at one time.

To enable rapid analysis and real time detection of micro and nano objects, high throughput microfluidic electrical impedance flow cytometers have been developed to analyze a large volume of sample in a reasonable time. These devices are especially useful for rapid detection of bio-objects present in ultra low concentrations without a need for tedious preconcentration. In this article, we will review the state-of-the-art high throughput microfluidic electric impedance sensors for rapid analysis of micro bioparticles, including 1) multi-channel Coulter counters, 2) multiplexed resistive pulse sensors, 3) electrical impedance spectroscopy sensors and 4) radio frequency high bandwidth particle counters. Advantages and limitations of each type of impedance sensors are discussed.

Keywords: Flow cytometry, Coulter counter, High throughput, Impedance spectroscopy, Signal multiplexation, Radio frequency sensor, Resistive pulse sensor.

1. INTRODUCTION

Bioactive particles, including bacteria, viruses and pollen, are an important class of environmental threat to public health and national security. It is of utmost importance to be able to make rapid quantitative assays of microscale and nanoscale bioparticles with portable and inexpensive devices for various applications ranging from health care to environmental monitoring. For example, quantification of blood cells including red blood cells, white blood cells (WBC) and platelets can provide important information for early warning and diagnosis of diseases and conditions such as neutropenia, anemia, thrombocytopenia, immune disorders, viral or bacterial infection, leukemia and lymphoma [1-3]. In addition, there is a need for early detection bacteria such as E. coli and Salmonella responsible for food contamination. Conventional particle detection methods in laboratory such as pyrolysis-GC-MS [4-5], Immunoassay [6-7], FACS [8] rely on costly, bulky, and complex instruments, requiring large amounts of samples and reagents, and a long analysis time. It is urgent to develop new analytical devices that are portable, require no complex set-up, and sample preparations for field applications. Further, the devices should be inexpensive, robust, and mass-producible.

Recent advances in microfluidics and microfabrication techniques have led to a variety of lab-on-a-chip sensors to detect, analyze and quantify microscale and nanoscale particles such as optical flow cytometers [9-10], electrical flow cytometers such as coulter counters [11-12], optical spectroscopy [13], light scattering [14], blocking counters [15], fluorescence counters [16-17] and micro PIV counters [18-19]. These devices and their sensing mechanism are discussed in detail in a review article [20]. Among them optical and fluorescence detection methods require complicated optical setup and fluorescence labeling, which limit device portability; [21-22] the detection is susceptible to background noise, and often requires complicated electronics. In comparison, electrical impedance flow cytometers have shown promise as a reliable, inexpensive tool for assay of particles and have become indispensable in clinical and research laboratories for label-free detection of micro/nano bio-objects suspended in a solution.A typical impedance flow cytometer utilizes a single pore or microfluidic channel with electrodes located on two sides of the pore. Translocation of an object through pore causes an impedance change of the pore, which can be related to particle size, shape, mobility and surface properties. The advantages of an electrical impedance flow cytometer include simplicity, low cost, low power consumption and ease of miniaturization [23]. Because of these advantages and its capability of single cell detection, electrical impedance flow cytometers have been used widely to detect and characterize latex beads [11,12,24,25], oil debris [26-27], pollen [28-31], bacteria [32-34], viruses [32] and DNA [35].

One long standing disadvantage of single-channel microfluidic electrical impedance sensors is their low throughput, i.e., these sensors can process only a very small volume of sample at a time [29-31]. This is a significant limitation when detecting bioactive particles particularly in low concentrations such as rare malignant cells which are predictors of early onset of cancer [36]. Thus, to analyze a relatively large volume of particle solution in low concentration using a traditional impedance sensor would take an impractically long time. In the past decade, high throughput microfluidic electrical impedance flow cytometers have been developed to analyze a large volume of sample in a reasonable amount of time. In this article, we review the major advances in high-throughout microfluidic electrical impedance flow cy-

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tometers, including multi-channel Coulter counters, multiplexed resistive pulse sensors, electrical impedance spectroscopy sensors and radio frequency high bandwidth particle counters. For each type of sensor, the merits and disadvantages are discussed.

2. MULTI-CHANNEL COULTER COUNTERS

Coulter counters (or called Resistive pulse sensors), invented in 1953 [37], are a well-developed impedance sensing technology used to measure the size and concentration of biological cells and colloidal particles suspended in an electrolyte solution [11]. A typical single channel Coulter counter device consists of a microchannel or pore that separates two chambers. When a particle flows through the microchannel, it causes a change in the electrical resistance of the microchannel. The change in resistance can be measured in terms of current or voltage pulses, which can be correlated various attributes of the particles such as size, shape, mobility, surface charge and concentration. Because of their simplicity, high sensitivity and reliability, Coulter type devices have been used for an extensive number of applications, from the analysis of blood cells to the detection and counting of colloidal beads [11-12], pollen [28-31], and viruses [32]. More recently, the use of Coulter-type devices has been extended to the detection of nano-scale particles, including single molecules [38], DNA [35,39,40] and antibody–antigen binding [41].

For Coulter counters with a single aperture, throughput is proportional to the square of the diameter of the aperture. When such a device is adapted to count particles at micrometer or nanometer scale, the diameter of the detector must be made proportionately small, so that the presence of a particle affects the channel's resistivity in a significant and measurable way; otherwise, the sensitivity of the device is compromised. The result is that single aperture microfluidic devices for counting nanoscale particles can process only a very small volume of sample at a time, and therefore have low throughput and low counting efficiency. Analyzing a relatively large volume of sample with such a device would take an impractically long time. Throughput can be improved by using a large channel to increase the volume of sample flowing through the channel in a given time. Li and colleagues [42-46] have achieved certain success in improving counting throughput with this approach. However, increasing channel size causes a reduction in sensitivity, and loss of the pulse information needed to determine particle shape; additionally sophisticated electronics for signal amplification and noise reduction are then needed to improve detection sensitivity so as to be able to detect smaller particles.

Alternatively the throughput can be improved by using a Coulter counter with multiple apertures, all operating simultaneously to improve counting efficiency and throughput [29-31]. A schematic of such a four-aperture Coulter counter for counting and differentiation of polymethacrylate and pollen particles reported by Zhe's group in 2006 [29-30] is shown in Fig. (**1**). This device has a central reservoir with four peripheral reservoirs. Each peripheral reservoir was connected to the central reservoir through a micro scale aperture fabricated on a polymer membrane such as PDMS approximately 100 μm in diameter and 100 μm thicknesses. Ag/AgCl electrodes were placed on either side of the aperture to allow DC measurements to monitor the change in resistance of the channel in real time. Testing was done by introducing particle solution in the central reservoir and allowing them to flow into the peripheral reservoirs [29] or by introducing different particle solutions in the peripheral reservoir and allowing them to flow into the central reservoir [30]. The device was tested with polymethacrylate particles, Cottonwood pollen and Juniper Scopulorum pollen particles. Results demonstrated the use of four sensing apertures improved throughput approximately 300% over a single channel device with the same channel geometry. The throughput can be increased further by increasing the number of channels without loss in device sensitivity and accuracy. However, there is a limitation in the total number of peripheral channels and reservoirs that can be incorporated into this device using the radial arrangement.

To overcome the limitation, Zhe's group developed a micro fabricated Coulter Counter with parallel sensing channels [31]. The multi-channel device shown in Fig. (**2**) used four parallel sensing channels to demonstrate the sensing principle and improved throughput over the single channel sensor. The parallel sensing channels were micro fabricated using soft lithography on PDMS. A major electrode was placed at the entrance of the four channels and four central electrodes placed in the middle of each channel. The central electrodes divided the channels into two half channels. The first half of the channel was used to sense the change in resistance as the particle passes through the channel. The second half of the channel is used to create an isolation resistance, which helps to reduce crosstalk resulting from the particle passing in the neighboring channel since all channels are electrically connected due to the electrolyte. The device was demonstrated to sense particles flowing through the multiple channels simultaneously, while differentiating latex beads and polymer particles owing to the difference in surface charge of the particles. Therefore, the throughput was improved. Compared to the multichannel sensor using radial arrangement, a large number of sensing channels can be added to this device to significantly increase the throughput.

While both device designs using the radial and parallel arrangement of channel have demonstrated the principle of parallel sample analysis, each channel can essentially be considered an individual instrument. As the number of channels is increased further to significantly improve throughput, it becomes impractical to implement detection electronics for each channel; further, the amount of data that needs to be acquired becomes impractically large.

3. MULTIPLEXED RESISTIVE PULSE SENSORS

The key to high throughput is the use of high density parallel sensing arrays, with a large number of parallel microfluidic channels. As the number of channels grows, it becomes impossible to monitor them all individually, and multiplexed detection becomes necessary. If the individual channel impedances are correctly modulated, a multiplexed signal representing a number of channels can be acquired, and then demodulated to recover the individual channel signals. Zhe's group reported a multiplexed micro resistive pulse sensor for high speed counting and accurate particle sizing of micro

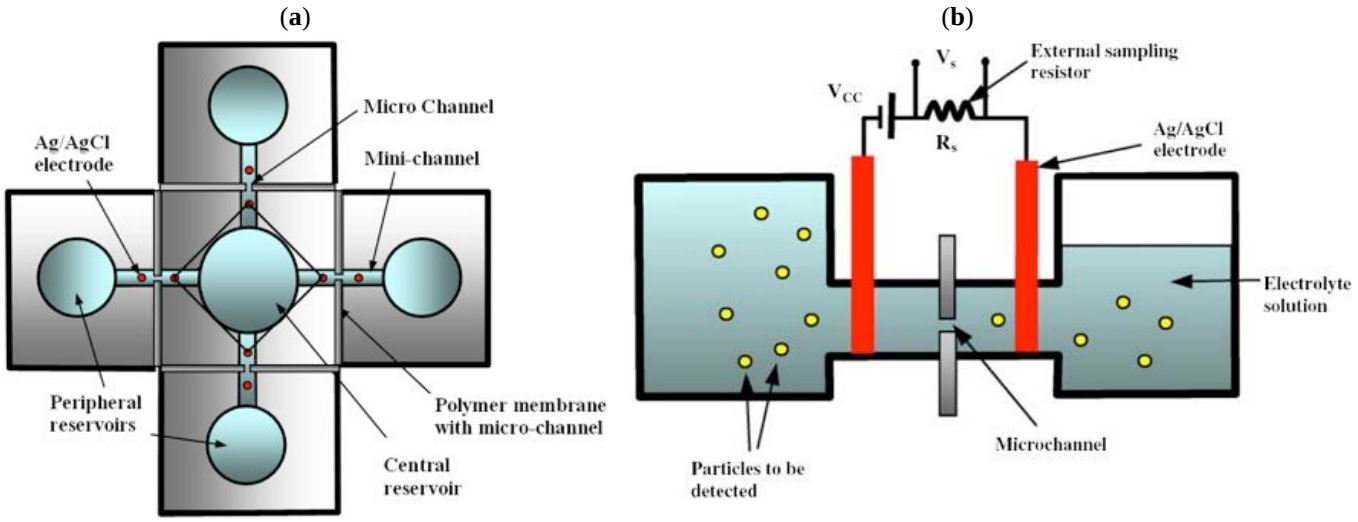


Fig. (1). Schematic of a four aperture multi-channel Coulter counter for detecting and differentiation micro scale particles (**a**) top view (**b**) front view of a single channel [29-30]. (Reprinted with permission from the Institute of Physics).

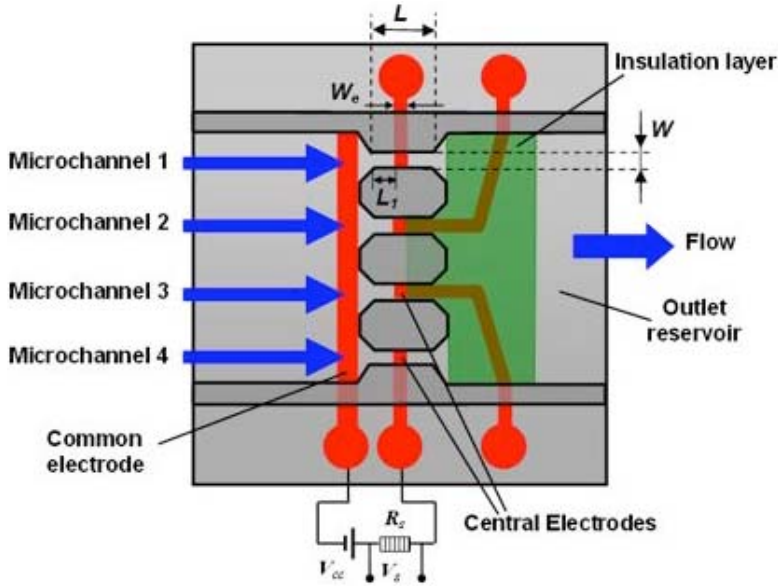


Fig. (2). Schematic of the micro fabricated multi-channel Coulter counted with four parallel sensing electrodes. The measurement setup for a single channel is shown. V_{CC} is the applied DC voltage, R_S is the sampling resistor across with the voltage V_S is measured [31] (Reprinted with permission from the Institute of Physics).

particles using a single set of detection electronics [47]. Unlike other multi-channel devices [29-31] that measure individual signals for each channel, this device measures a combined signal for all four channels, with each channel encoded at a different frequency. The combined response, measured using a single data acquisition channel, is then demodulated digitally to obtain the signals for each individual channel. A schematic of the multiplexed multi-channel device along with the measurement setup is shown in Fig. (**3a**). The device consists of two reservoirs to load analytes, parallel micro channels for counting particles, a pair of major electrodes on both sides of the micro channels to apply a DC bias, and central electrodes in each micro channel to apply AC modulation signals. Each central electrode divides the micro channel into two half micro channels. The modulation frequencies were experimentally determined at which the resistive behavior of the electrolyte-filled micro channels dominates over capacitive effects. The signal in each channel was modulated by an AC sine wave of known and unique frequency (25 kHz, 40 kHz, 55 kHz, and 70 kHz). The current through each micro channel flows into an inverting summing amplifier, the combined response was detected at a sampling rate of 2.5 MHz and demodulated by filtering out a band around the modulation frequency for each channel. Then, envelope detection was used to recover the signal for each individual channel. The combined signal is demodulated by using a set of four band pass filters. A band of ±5kHz, centered at the modulation frequency of the corresponding channel, was determined to be wide enough to pass information about the pulse shape in that channel, but not so wide that significant information from neighboring channels could leak in. An electrical equivalent circuit of the device along

with the measurement setup is shown in Fig. (**3b**). Testing results using polystyrene particles demonstrate that the throughput of the multiplexed device is improved 300% over a single channel device using a single set of detection electronics. Demodulated data for each channel at 25 kHz, 40 kHz, 55 kHz and 70 kHz are shown in Fig. (**3c**), with each pulse representing a particle passing through a corresponding sensing channel. In addition, the AC modulation method used in this paper reduced the polarization effect on the microelectrodes, thereby allowing measurement of the actual particle sizes. Note that the multiplexed detection principle can be extended to a larger number of channels to further improve the throughput, without increasing the external detection electronics.

One limitation of this multiplexed resistive pulse sensor is that the multiplexation technique is constrained by the

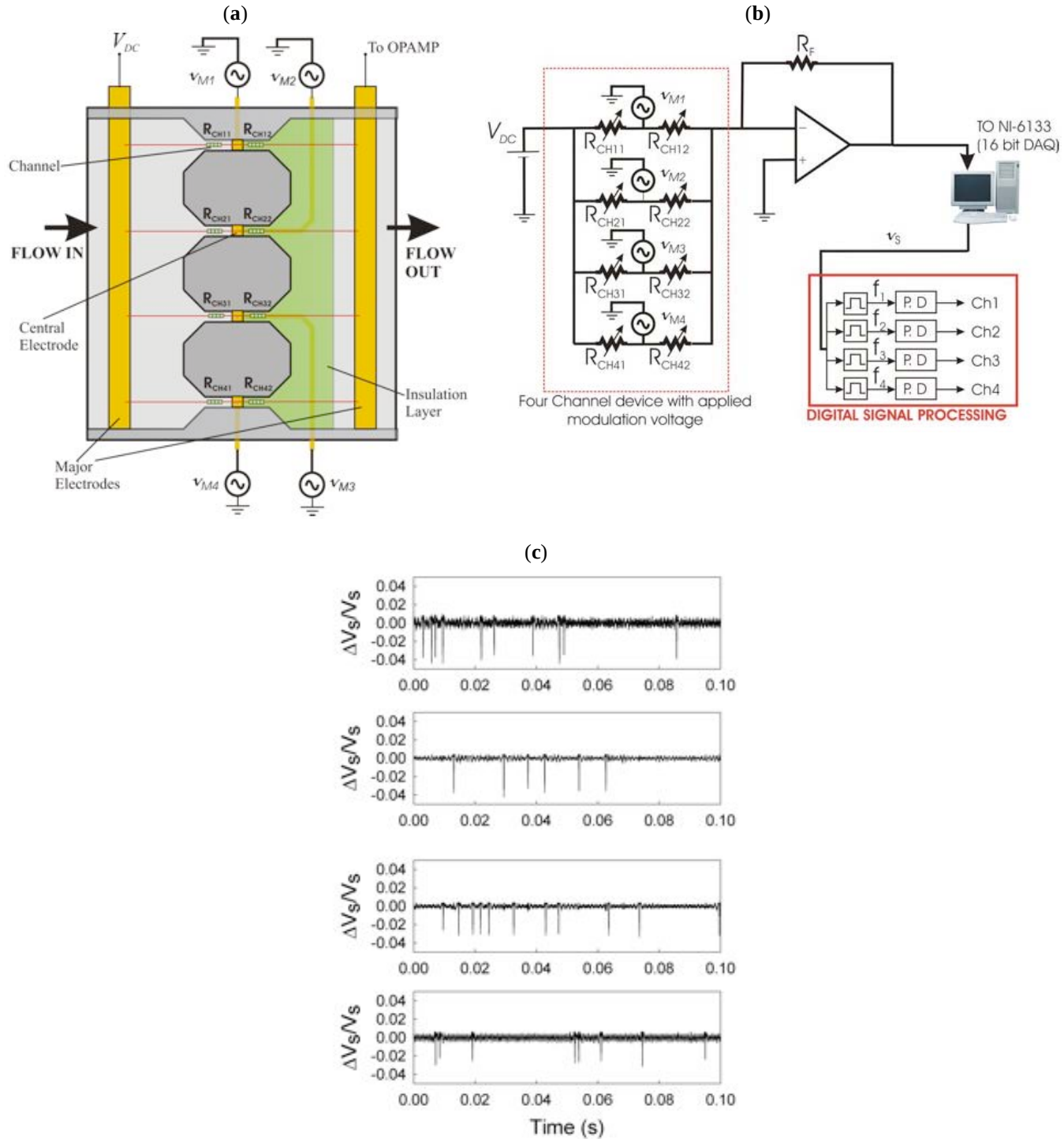


Fig. (3). (**a**) Schematic of a four channel resistive pulse sensor used for multiplexed detection (**b**) Electrical equivalent circuit along with the measurement setup of the multiplexed detection scheme (**c**) Demodulated data from the multiplexed sensor at (top to bottom) 25kHz, 40kHz, 55kHz and 70kHz for Channels 1, 2, 3, and 4 respectively [47].

sampling rate of data acquisition board. If the modulation frequencies are high, the number of detection channels is limited One possible solution is to extend the resistive region to lower frequency range using electrodes with large surface areas [48-49]; this makes the impedance of double layers formed on the electrode-electrolyte interface negligible. If signals from detection channels can be modulated at lower frequencies, a large number of detection channels can be used, and the throughput can be significantly improved [47].

4. ELECTRO IMPEDANCE SPECTROSCOPY SENSORS

Electrical impedance spectroscopy is an established technique to measure the electrical properties of particles suspended in electrolyte solution using a frequency sweep. Microparticles such as biological cells are polarized in alternate-current electric fields. Impedance characteristics of particles at a wide frequency range are indicative of particle size, dielectric properties and conductivity [24, 50]; this information allows identification and differentiation of particles including polystyrene beads [24], red blood cells [24], white blood cells [51], bacteria [34], HeLa cells [52-53] and CD4+ cells [54]. Similar to single channel Coulter counters, traditional electrical impedance spectroscopy sensors usually have low throughput. The throughput can be improved by increasing the number of particles flowing through the sensing channel. This can be achieved by increasing the flow rate of the particle-laden solution. However, with a large flow rate, the particles move fast and the translocation time in the channel is short. Within the short period of time when a particle passes through the sensing channel, it is impossible to measure impedance characteristics of the particle by a sweep measurement over a wide frequency range.

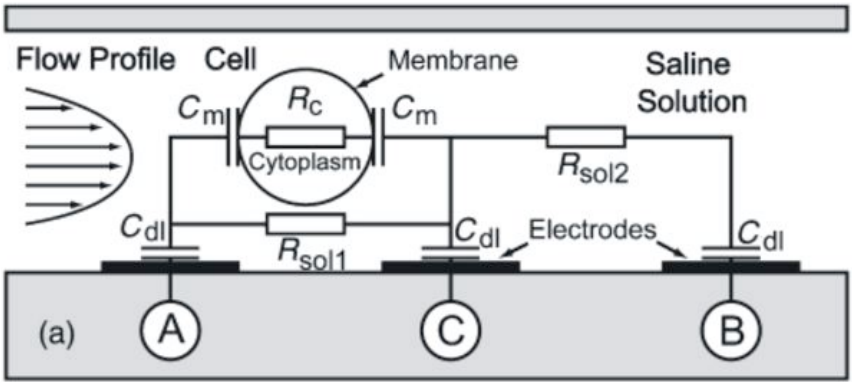
To enable the high throughput particle detection using electro impedance spectroscopy, particles can be characterized at a few selected fixed frequencies representative of particles' properties such as size, permittivity and conductivity. Impedance measurement at these selected fixed frequencies can be made simultaneously to significantly reduce the measurement time in comparison to a frequency sweep measurement; this allows the use of a high flow rate of particle-laden sample solution and therefore enables a high throughput measurement. Fuller *et al*. [55] demonstrated such a device using three selected frequencies ranging from low (100 kHz) to high (10 MHz). The detection rate reached 100 particles s^{-1}. By incorporating hardware for real time analysis, the detection rate could be increased to 1000 particles s^{-1}.

Gawad *et al*. [50] reported a similar micromachined device to analyze and count cells such as latex beads, erythrocytes and ghost cells at a rate of 100 cells s^{-1} using simultaneous impedance measurements at multiple fixed frequencies ranging from 100 kHz to 15 MHz. A schematic of the device is shown in Fig. (**4a**). The device uses three coplanar electrodes; the central electrode divides the sensing region into two successive channel segments; a differential measurement of impedance was made between the two segments to circumvent environment noise and parasitic effect. One drawback of this design is that the electric field is non-uniform because of the use of co-planar electrodes; as a result, similar particles passing the sensing channel at different heights would generate different impedance responses. While hydrodynamic focusing techniques can be used to keep particles at the central plane of the channel, doing so would increase device complexity. To overcome this problem Cheung *et al*. [24] presented an improved device using parallel facing electrodes on the top and bottom surfaces of the channel (see Fig. **4b**) to generate a more uniform electric field. Two pairs of electrodes were fabricated within the channel, one pair of the electrodes acts as a reference whereas the other pair was used to detect the impedance change due to the presence of the particle (see Fig. **4b**); differential measurements were made between the two pairs of electrodes. Impedance measurements at a low frequency and high frequency were made simultaneously at both electrodes. The detection setup for the electrical impedance spectroscopy measurement is shown in Fig. (**4c**). A detection rate of ~17 cells s^{-1} was reported. The throughput of this device can be further improved to 100 cells s^{-1} by increasing the flow rate. Additionally, the testing demonstrated that the device can differentiate polystyrene beads, red blood cells, ghost cells and fixed RBCs in terms of the difference in impedance amplitude and phase angle at specific frequencies.

For electrical impedance spectroscopy sensors using constant amplitude sine wave excitation [24,50,55], a separate set of hardware such as signal generator, mixers, filters, quadrature demodulators, lock-in amplifiers are needed for multi- frequency excitation and to obtain the impedance response at each discrete frequency. This limits the total number of excitation frequencies that can be used in these devices. Broadband spectroscopy can be used to overcome this disadvantage while achieving high detection rates. Broadband spectroscopy uses a single short electrical pulse as excitation signal to generate an impulse response. Coupled with Fast Fourier Transform (FFT) the fast changing impedance response at multiple frequencies can be detected within a short time [56-57]. Rectangular, Dirac, Gaussian, sinc or chirp pulses can be used as excitation sources. Among them, sinc and chirp pulses were found to be more efficient as only a small percentage of energy (approximately 2% to 3%) falls outside the target bandwidth while the percentage is 40% for rectangular pulse excitation; this results in higher signal to noise ratio (SNR) for measurements using sinc and chirp pulses. In addition, the duration of chirp pulses can be reduced to achieve faster response time. Min *et al*. used both Gaussian and chirp functions to characterize cells passing through a micro channel with a translocation time less than 1ms [57]. The main drawback using a single pulse broadband excitation is that the energy is spread across a wide frequency band resulting in a low signal to noise ratio compared to a single frequency excitation. Although energy distribution can be improved by increasing the amplitude of the excitation voltage, doing so often causes damages to electrodes [58].

To circumvent the problem, broadband spectroscopy can be implemented using maximum length sequences [58-60]. A maximum length sequence (MLS) is a pseudorandom binary sequence which has a power spectrum similar to white noise with uniform distribution of energy over a large bandwidth. The pseudorandom sequence has higher input energy in comparison to a single pulse excitation with the same

(**a**)



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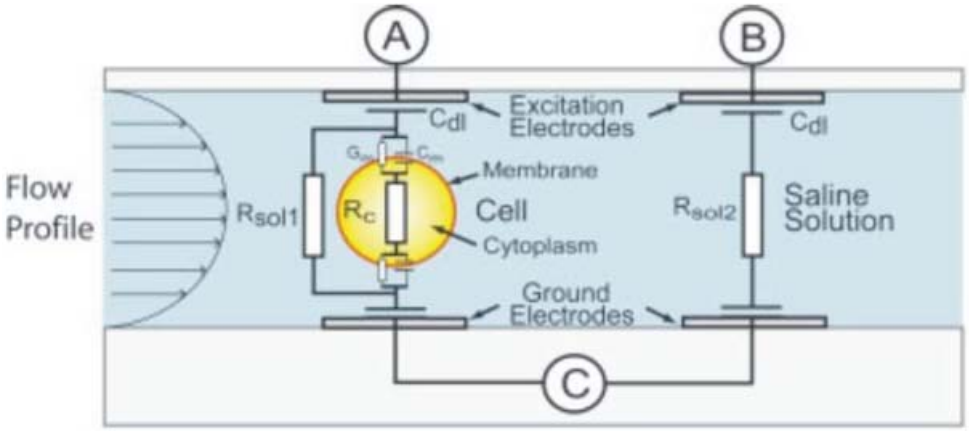

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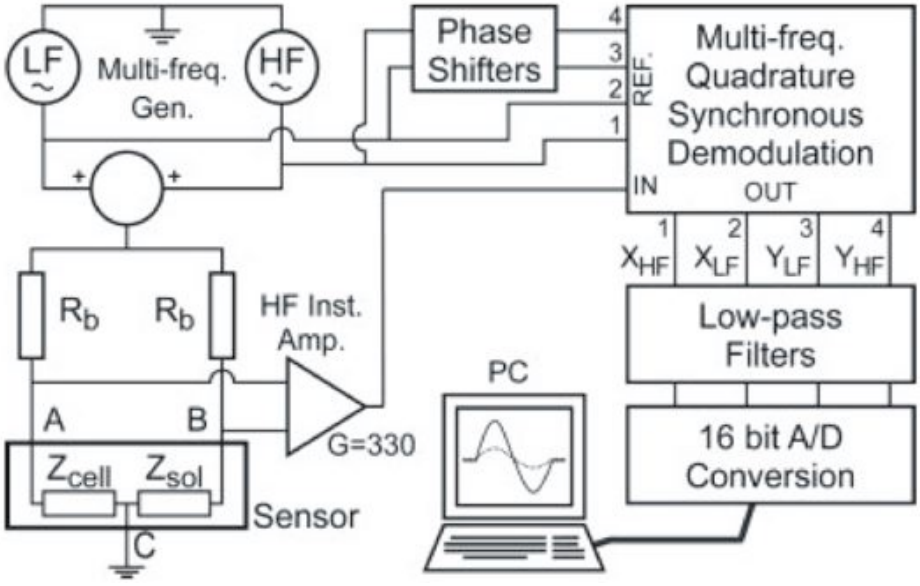

Fig. (4). Schematic of Electro impedance spectroscopy sensors using (**a**) coplanar electrodes [50] (**b**) parallel top down electrodes [24] with the electrical equivalent models and (**c**) detection setup for the impedance spectroscopy measurement (Reprinted with permission from the Royal Society of Chemistry and John Wiley and Sons).

amplitude due to the lower crest ratio, resulting in improved signal to noise ratio [58-61]. A multi-frequency measurement using this technique was demonstrated [58-60] to detect particle with a translocation time less than 1ms. A schematic of the measurement setup is shown in Fig. (**5**). The impedance spectrum of the device can be obtained from the output periodic impulse response using a fast M-sequence transform (FMT) coupled with Fast Fourier Transformation (FFT). Gawad *et al*. [58-60] reported that this technique can be used to analyze impedance response of the device at 512

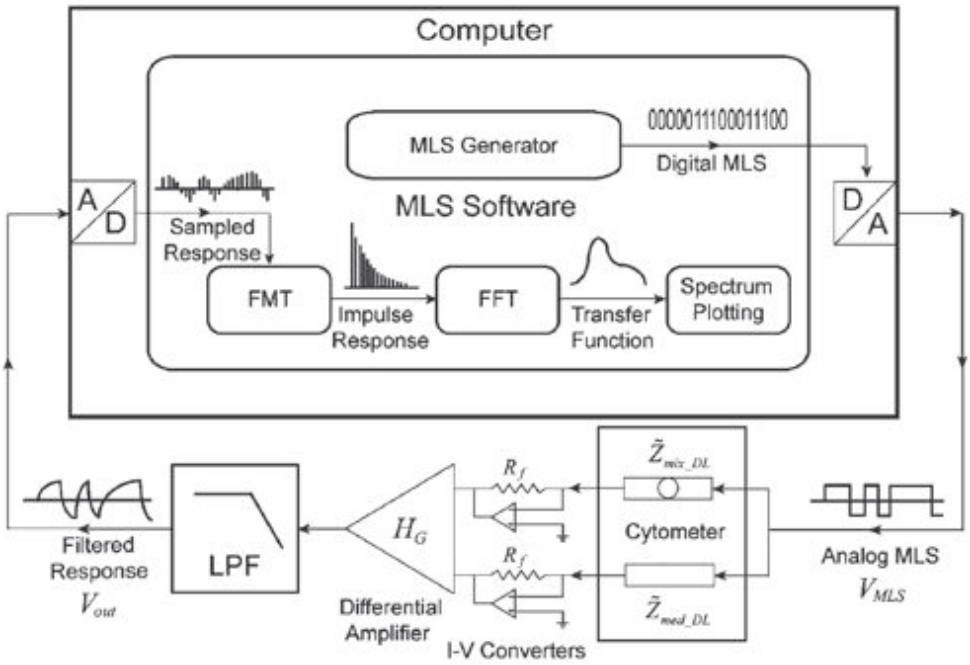

Fig. (5). Schematic showing the MLS impedance measurement setup along with the signal processing steps required to determine the impedance characteristics at multiple frequencies [58-60]. (Reprinted with permission from the Royal Society of Chemistry).

discrete frequencies simultaneously between 976 Hz to 500 kHz in less than 1ms with improved signal to noise ratio (SNR). Nevertheless, the detection rate, throughput and frequency range of the MLS measurement are limited by the sequence and the period of the MLS.

In summary, while electrical impedance spectroscopy sensors enable high throughput detection and counting of micro particles using a single sensing channel, the measurement usually requires sophisticated hardware and signal processing algorithms. This may cause difficulties in integrating all components into portable lab-on-a-chip devices for practical applications.

5. RADIO FREQUENCY HIGH BANDWIDTH PARTICLE COUNTERS

One additional limitation of electrical impedance spectroscopy sensors is that the sensitivity and detection rate are usually limited by the large impedance of double layer capacitance, electrolyte-filled microchannel capacitance and the stray capacitance [25]. At low frequencies, the impedance of the double layer capacitance at the electrode-electrolyte interface is large; making it difficult to measure small resistance due to the presence of a micro particle. At high excitation frequencies, the high impedance of the electrolyte-filled channel capacitance and the stray capacitance shunts the detection current, severely degrading the detection sensitivity and limiting the bandwidth, and in turn limiting the detection rate [25,50]. Radio frequency (RF) resonance detection was reported to circumvent the above problems and achieve higher throughput without sacrificing the sensitivity [25,62,63]. By using an external tuned circuit to eliminate the channel capacitance and stray capacitance effects, the impedance change of the device is dominated by resistance change caused by a particle entered the sensing channel; when properly impedance tuned, maximum sensitivity to small impedance change can be achieved. The use of measurement frequency in RF range (40MHz~240MHz) enables a large measurement frequency bandwidth, allowing detection of impedance pulses with extremely short duration; so that very high detection throughput can be achieved.

Wood *et al*. [62] presented a micromachined RF particle counter for high throughput detection of latex beads shown in Fig. (**6a**). An electrical equivalent of the device and the measurement setup are shown in Fig. (**6b**). In this device, the impedance change was detected by monitoring the RF reflectance with three coplanar electrodes when the particle passes through a microchannel. The device was connected to a tuned circuit; by properly tuning the circuit most of the incident RF power was absorbed by the fluid. Therefore at resonance frequency, very small impedance changes can be detected by measuring the RF reflectance change. With a resonance frequency of 169 MHz, Wood *et al.* reported a detection rate of 30,000 beads s^{-1} for polystyrene particles.

A limitation of the above device is that the coplanar electrodes generates non-uniform electric field, so that identical particles passing through the channel at different channel heights resulted in large difference in output signals, causing errors in estimating the particle size. By contrast, in a uniform electrical field, the detected signal does not depend on the particle's height and lateral position. Wood *et al*. [62] improved the device by using a pair of parallel electrodes fabricated on the top and bottom surfaces of the channel shown in Fig. (**7**) to generate a more uniform electrical field. Measurements were made with an excitation signal in the range of 105-108MHz to discriminate latex beads ranging from 4.4 μm to 9 μm. The testing results showed that the measurement error in particle sizes was significantly reduced, ranging from 8% to 16% for particles with different sizes.

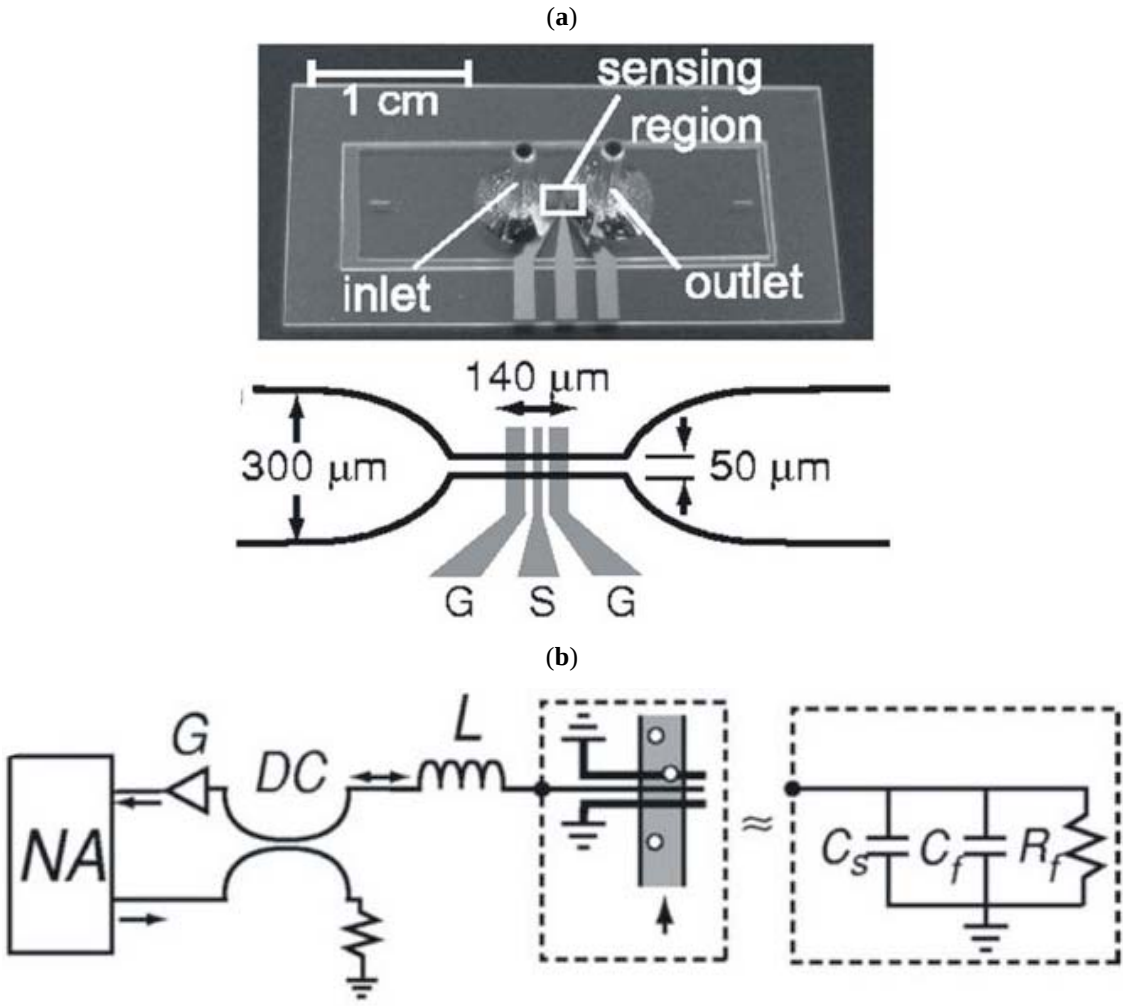

Fig. (6). (**a**) Device picture and schematic of a single channel radio frequency high bandwidth particle detector using coplanar electrodes (**b**) electrical equivalent circuit with the measurement setup used to detect the impedance change [25]. (Reprinted with permission from the American Institute of Physics).

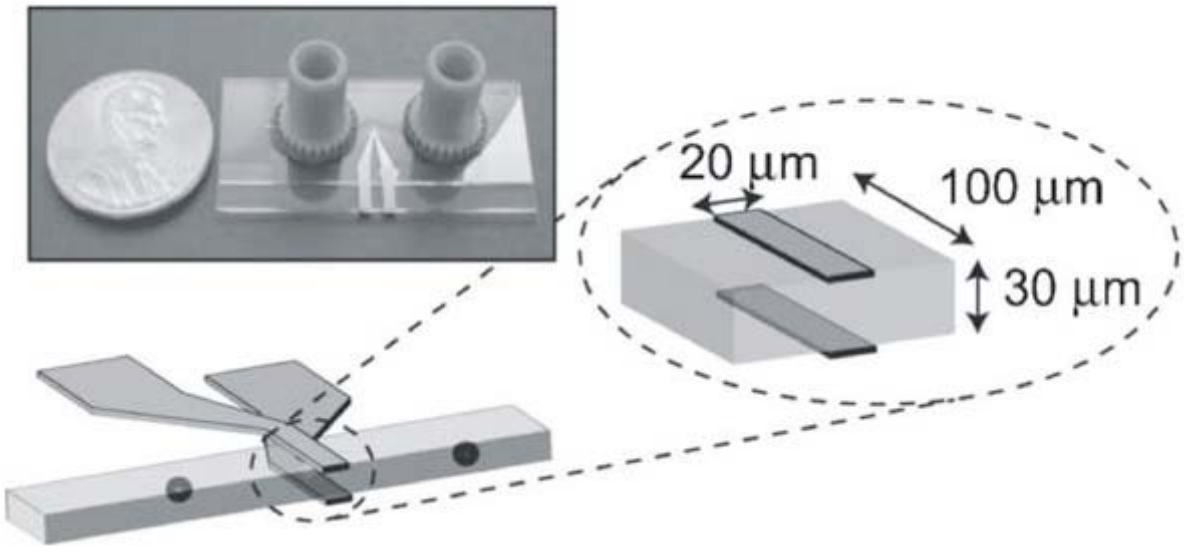

Fig. (7). Schematic of a parallel plate radio frequency high bandwidth particle sensor [62-63] (Reprinted with permission from the American Institute of Physics).

The measurement bandwidth and thus the detection rate can be improved by increasing the measurement frequency for RF counters because the measurement bandwidth is approximately 5% to 15% of the excitation frequency. Oh *et al*. [63] and Wood *et al*. [62] reported a detection rate up to 12×10^6 particles per second by increasing the resonance frequency to 240 MHz, a high measurement bandwidth of 25MHz was achieved which allowed the detection of impedance pulses with duration of 80 ns.

Later on, Wood *et al*. [64] reported a high throughput RF particle counter that utilized microfabricated digital barcodes linked to the cells to identify and sort cells. With functionalization of barcodes using biotin-streptavidin as well as human CD4 antibody, unique barcodes can be attached to specific cell types that allow the identification of each cell *via* an electronic barcode readout scheme. Thus, with the digital labeling method, the RF particle counter provides a compact, inexpensive tool for cell identification and sorting.

In comparison to electro impedance spectroscopy sensors, radio frequency particle counters can achieve a higher throughput. However, similar to electrical impedance spectroscopy flow cytometers, RF measurement requires sophisticated hardware and signal processing algorithm. Additionally, RF particle counters typically have a complicated transfer function between the amplitude of the output signal and particle size; a calibration is usually needed to accurately measure the size of particles.

6. DISCUSSION AND CONCLUSION

Electrical impedance flow cytometers are important tools for detection and analysis of a large variety of micro and nanoscale objects because of their simplicity, low cost and capability of single cell analysis. However, because of their low throughput, traditional single channel electrical flow cytometers can process only a very small volume of sample at a time. High throughput electrical impedance flow cytometers that are able to make rapid quantitative assays of bioparticles are urgently needed for biomedical research, environmental monitoring and health care.

Recent advances in microfluidics have resulted in many high throughput electrical impedance flow cytometers that are inexpensive, require no complex set-up and sample preparations. Among them, electrical impedance spectroscopy sensors and Radio frequency high bandwidth particle counters have achieved high throughout detection rate using a single microfluidic channel by increasing the flow velocity of particle-laden solution, so that a large number of particles can pass through the channel within a short time. Electrical impedance spectroscopy sensors can differentiate bio-objects by taking impedance measurement at selected fixed frequencies, but its sensitivity and throughput is limited by the effects of double layer capacitance, microchannel capacitance and stray capacitance. RF high bandwidth particle counters use an external turning circuit to eliminate these effects and can achieve a very high detection rate. However, because the particle passes through the sensing region very fast, this approach usually requires sophisticated hardware and signal processing algorithms, which may cause difficulties in integrating all components into portable lab-on-a-chip devices for practical applications. More importantly, increasing the particle velocity may reduce the amount of information in the sensed impedance pulses, so that the acquired signal might lack sufficient information for label-free bioassay of particles.

An alternative way to improve the throughput is to use multiple parallel detection channels. Multichannel resistive pulse sensors allow a larger amount of particle-laden samples to be analyzed in parallel. While the throughput is improved by parallel sample processing, each detection channel is fundamentally an individual instrument. If the number of channels is large, it becomes impossible to monitor them all individually. Multiplexed resistive pulse sensors have been developed to solve this problem; the multiplexed device modulate the individual channel impedance using unique frequencies, so that a multiplexed signal representing a number of channels can be acquired, and then demodulated to recover the individual channel signals using a single set of detection electronics. Therefore, a large number of detection channels can be integrated in one chip to significantly increase the throughput without sacrificing sensitivity and without much increasing the device complexity. The use of multiple channels also allows a low flow velocity, so that the shape of the measured pulses should provide more detailed information about the particles. Signal multiplexation also improves the signal-to-noise-ratio because the measured combined signal is significantly larger than it would be for a single channel, and is thereby easier to distinguish from instrumentation noise.

While multiplexed resistive pulse sensors are promising for high throughput assay of bioparticles, one hindrance is that the sensitivity and increase in throughput is usually constrained by the sampling rate of the data acquisition board. This problem can be possibly solved by using electrodes with large surface areas so that modulating frequencies can be set at lower frequency band where double layer impedance is negligible [48-49]. In addition, AC impedance measurement at a few representative frequencies can be incorporated into the multiplexed resistive pulse sensor for bioparticle differentiation. It is also worthwhile to note that here that the concept of using parallel detection channels could be used for AC impedance spectroscopy sensors and RF particle high bandwidth counters to further improve the throughput.

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