Micro Flow Cytometry Utilizing a Magnetic Bead-based Immunoassay for Rapid Virus Detection

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The current study presents a new miniature microfluidic flow cytometer integrated with several functional micro-devices capable of viral sample purification and detection by utilizing a magnetic bead-based immunoassay. The magnetic beads were conjugated with specific antibodies, which can recognize and capture target viruses. Another dye-labelled anti-virus antibody was then used to mark the bead-bound virus for the subsequent optical detection. Several essential components were integrated onto a single chip including a sample incubation module, a micro flow cytometry module and an optical detection module. The sample incubation module consisting of pneumatic micropumps and a membrane-type, active micromixer was used for purifying and enriching the target virus-bound magnetic beads with the aid of a permanent magnet. The micro flow cytometry module and the optical detection module were used to perform the functions of virus counting and collection. Experimental results showed that virus samples with a concentration of 10³ PFU/ml can be automatically detected successfully by the developed system. In addition, the entire diagnosis procedure including sample incubation and virus detection took only about 40 minutes. Consequently, the proposed micro flow cytometry may provide a powerful platform for rapid diagnosis and future biological applications.

Conventional flow cytometry systems provide rapid and reliable cell counting capabilities, delicate optical components including focused laser beams, optical detection/filtering devices and complicated control circuits make the system relatively bulky and expensive. Besides, the operation of a flow cytometer still relies on well-trained personnel and a relatively high volume of the biological samples to be analyzed. The drawbacks of conventional flow cytometers have led to an increasing demand for a compact analysis system.

Figure 1 shows the experimental processes within the micro flow cytometry system. Superparamagnetic microspheres with a diameter of 6-μm are surface-coated with streptavidin (SA) and conjugated with biotinylated capture antibodies, were mixed with the bio-samples in the incubation chamber on a membrane-type micromixer (Fig. 1 a) for 10 minutes. The target viruses can be specifically captured and bound onto the antibody-conjugated magnetic beads. Then, a permanent magnet attached onto the bottom of the microfluidic chip generates the required magnetic field (about 430 mT) to trap the virus-bond magnetic complex. The non-bonded substances were removed by a stream of washing buffer propelled by microfluidic pumps. The target viruses were thus purified and separated from the bio-samples and can be concentrated by re-suspending the beads into a small volume of buffer solution. Next, the second set of developed antibodies labeled with fluorescent dye were mixed and incubated with the purified samples and used for identifying the target viruses (Fig. 1 c). The washing step was then repeated to flush out unbounded materials (Fig. 1 d). The purified virus-magnetic-bead complexes were then transported into a microfluidic focusing module which uses injected sheath flows into the microfluidic channel to focus the magnetic
beads into a narrow stream (Fig. 1 e). Subsequently, the fluorescent signal bound to the second antibodies on the target viruses can be excited and detected by an optical detection module consisting of a photo-multiplier tube (PMT) and a laser source (Fig. 1 e). Finally, active microvalves located downstream can be used to sort and collect the virus-bound magnetic beads into the collection chambers by using the feedback signals from the optical detection module (Fig. 1 f).

Figure 1 Schematic illustration of the integrated microfluidic chip. (a) Mixing bio-samples with magnetic beads; (b) Purifying and concentrating the target viruses through a washing process for purification of the target viruses; (c) Capture antibodies on the magnetic beads are conjugated with the custom developed antibodies; (d) Washing process for purification of the developed antibodies; (e) Fluorescent detection while the magnetic beads pass through optical detection region; (f) Sorting of magnetic beads utilizing microvalves.

Figure 2(a) illustrates schematically the micro flow cytometry system integrated with three functional modules for detection of bio-samples, namely, (1) a sample incubation module, (2) a micro flow cytometry module, and (3) an optical detection module. The sample incubation module comprises an incubation chamber, a membrane-type micromixer, two reagent loading chambers, a waste chamber, three pneumatic micropumps and a pneumatic microvalve. these two semi-circular PDMS membranes can be moved up and down sequentially by injecting compressed air into the cavities formed between the PDMS structures.
Figure 2 Schematic illustration of the integrated microfluidic chip. Several functional components including an incubation module and a flow cytometer module are integrated on a single chip.

A photograph of the assembled micro flow cytometer chip is shown in Figure 3(a). A master mold patterned with microstructures on the silicon wafer was formed by negative photoresist SU-8 using a standard lithography process, followed by a polydimethylsiloxane (PDMS) casting technique to form inverse images of the master mold. Finally, the PDMS structures and the glass substrate were bound together by utilizing an oxygen plasma treatment to form the micro flow cytometer chip. Figures 3(b) show scanning electron microscope (SEM) images of the master molds for the membrane-type micromixer, the pneumatic micropump, and microchannels with a hydrophobic valve, respectively.
The relationship between the flow pumping rate and the driving frequency of the EMV at three different air pressures of 10, 20 and 30 psi are shown in Figure 4(a). The maximum value of the fluid flow rate is measured to be 210 μl/min at a driving frequency of 30 Hz and starts to decrease due to the limitation of the frequency response of the whole system. As shown in Figure 4 (b), dengue virus serotype 2 (100 μl of 10^7 PFU/ml) was first mixed with the anti-dengue capture antibody-conjugated magnetic beads (10 μl of 10^3 beads/ml) in the incubation chambers, followed by incubating with the second custom developed, fluorescent-labeled antibodies under the optimal operating condition of the membrane-type micromixer. that the traditional method using a large-scale shaker would take more than 30 minutes for each incubation process.

Figure 4 (a) Relation between the pumping rate and the driving frequency of the EMV. (b) Relation between the mean fluorescent intensity and the incubation time.

Figure 5 (a) shows the experimental results for the optical detection of dengue viruses in the micro flow cytometry system. Each peak of the signal indicates the fluorescent intensity of the dengue viruses conjugated on the magnetic particles. A threshold line with an amplitude of 1 mV can be used to differentiate between signals and noise such that the signal-to-noise (S/N) ratio is measured to be higher than 17. Figure 5 (b) show the fluorescent intensity for viral samples with concentrations ranging from 10^7 to 10^2 PFU/ml. It was found that a virus concentration of 10^3 PFU/ml can be successfully detected by the developed system. Figure 5 (c) shows that the anti-dengue virus antibody conjugated to magnetic beads can only detect the dengue virus from a mixture of dengue virus and enterovirus 71. From the experimental results, it can be concluded that the high selectivity of anti-dengue antibodies can also purify the target dengue virus in the bio-samples.
Figure 5 (a) Fluorescent signals collected by a PMT module while the bead samples flow through the optical detection region. (b) Fluorescent intensity for different virus concentrations. (c) Specificity test of the developed protocol.