High gain avalanche photodiode (APD) arrays in flow cytometer opitical system

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***Abstract*—The basic principles of flow cytometer are introduced, on the basis of diode pump solid state laser (532 nm) as a excitation source and a high gain avalanche photodiode (APD) arrays as a light detector, a verification optical system is built. Then, Spherotech Rainbow Calibration Particle RCP-30-5A is used to evaluate the performance of the optical system, the fluorescence PE signal histogram of RCP-30-5A microshphere shows that the number of individual peaks is close to eight, which is close to the world advanced level.**

Keywords- flow cytometry; diode pump solid state laser; avalanche photodiode;forward scatter; fluorescence PE

I. Introduction

Flow cytometry is a technology that has impacted both basic cell biology and clinical medicine in a very significant manner[1-2]. The essential principle of flow cytometry is that single particles suspended within a stream of liquid are interrogated individually in a very short time as they pass through a light source focused at avery small region. The optical signals generated are mostly spectral bands of light in the visible spectrum, which represent the detection of various chemical or biological components, mostly fluorescence. A key aspect of flow cytometers is that they can analyze single particles/cells,and they also can be used to flow sorting which may be the best separation technique available when particles/cells differ from each other[3-4] .

Flow cytometry uses the principles of light scattering , light excitation, and emission of fluorochrome molecules to generate specific multi-parameter data from particles and cells in the size range of 0.5 μm to 40 μm diameter. Scattered and emitted light from cells and particles are converted to electrical pulses by optical detectors. Collimated (parallel light waveforms) light quanta is picked up by confocal lenses focused at the inters-ection point of cells and the light source. Light is send to different detectors by using optical filters. For example , a 532 nm band pass filter placed in the light path prior to the detector will only allow “green” light into the detector,such as PMTs or APDs. The electrical pulses originating from light detected by the PMTs or APDs are then processed by a series of linear and log amplifiers. After the different signals or pulses are amplified they are processed by an Analog to Digital Converter (ADC) which in turn allows for events to be plotted on a graphical scale.

II. EXPERIMENTS

On the basis of diode pump solid state laser (532 nm) as a excitation source and a high gain avalanche photodiode (APD) arrays as a light detector, a verification optical system is built.Then, RCP-30-5A is used to evaluate the performance . The results of experiment are as follows.

A. optical system with APD arrays

In most flow cytometers, fluorescent cells are illuminated with the light from a laser. Lasers are useful because they provide intense light in a narrow beam. In our verification optical system,a 5 mW green solid state laser (532nm) is used.The optical system can focus the beam from the green laser at a spot on the stream of the flowing particles. The spot where the laser beam intersects the stream of flowing particles is called the “analysis point”. The laser beam will have an elliptical cross-sectional area, brightest in the center and measuring approx 20×50 μm to the edges.The height of the laser beam marks the height of the analysis point and the dimension through which each particle will pass.

To evaluate the performance of the laboratory-built optical system, Spherotech Rainbow Calibration Particle RCP-30-5A and Suzhou nawei fluorescent particles are used. The fluorescent particles in a stream of fluid can move through light beam rapidly; under ideal circumstances, only one fluorescent particle will be illuminated at a time, and the illumination is bright enough to produce scattered light and fluorescence of detectable intensity.In the laboratory-built system, fluorescent particles flow through analysis point at velocity of 4m/s. They will, therefore, spend approx 5 μs in the beam.When the fluorescent particle passes through the laser beam, it scatters light and emits fluorescence,which are measured on detectors(APD1,APD2,APD3), and the resulting signal can provide information about the particles. The APD

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arrays is a new type of photon-counting device made up of multiple APD pixels operated in Geiger mode.The sum of the output from each APD pixels forms the APD arrays output,which allows the counting of single photons or the detection of pulses of multiple photons.The APD arrays is excellent photo-counting device,whose features include the following: excellent photon detection efficiency;high gain 10^5-10^6; room temperature operation;insensitive to magnetic fields;excellent time resolution,that are important for the laboratory-built optical system.

B. Forward scatter analysis

When the fluorescent particle passes through the laser beam, it scatters light. That light is measured on a detector and the resulting signal can provide information about the particles. Forward angle scatter (FS) is a measure of particles size and side scatter (90 ℃ scatting) is a measure of particles components or granularity[5].

RCP-30-5A is used in the experiment. When it passes through the green laser beam,it scatters light.Forward angle scatter is measured on the APD1 and Side scatter is measured on the APD2. Across the front of APD1 is a beam stop, approx 1mm wide,positioned so as to block the laser beam itself as it passes through the stream.Only light from the laser that has been refracted or scattered as it goes through a particle in the stream will be diverted enough from its original direction to avoid the beam stop and strike the APD1 behind it.The beam stop can prevent the light from the 532nm green laser beam entering the detector APD1 which forms a shield against the direct radiation from the laser, but which substantially does not impair the scattered light.

The forward scatter detection has been done using the RCP-30-5A particles, and the one-parameter histogram result has been gotten as showing in figure 1.

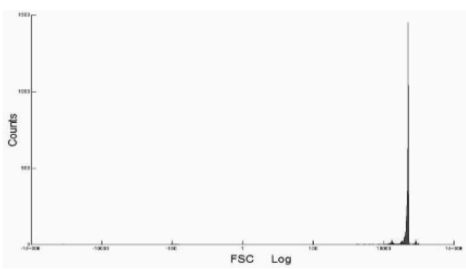

Figure 1. The FSC singal of RCP-30-5A

The FSC singal of RCP-30-5A is successfully detected by the laboratory-built system. As shown in the figure 1, a one-parameter histogram is a graph of particle count on the y-axis and the measurement parameter FSC on the x-axis.As priviously mentioned that the forward scattered light signal is sometimes referred to as a particles size signal. A particle with a large cross-sectional area will refract a large amount of light onto the detector.The RCP-30-5A used in the experiment contain a mixture of similar size particles which are about 3.2 μm in diameter ,so only one singal has been gotten.

In order to future study the relationship between the amount of light refracted past the beam stop and the size of the particle, the RCP-30-5A and Suzhou nawei fluorescent particles are used in the next experiment which are different size 3.2 μm and 5 μm in diameter.and the one-parameter histogram result of the two particles has been gotten as showing in figure 2.

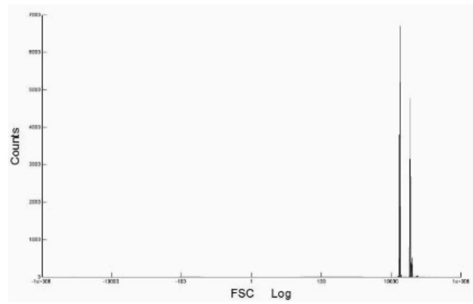

Figure 2. The FSC signals of the two different size particles

Because the forward scattered light signal is sometimes referred to as a particles size signal, the two different size particles 3.2 μm and 5 μm in diameter means the two different FSC singals. Based on the two different signals, the two different size particles are detected in turn. So the FSC singals can be used to distinguish the different size particles as they pass through the analysis point individually in a very short time, which is very useful in the flow cytometry .

C. Fluorescent light analysis

The spectrum-dividing system at right angles to the direction of the 532 nm green laser beam collects light that has been scattered to wide angles from the original direction. Light collected by this system is called the side sctter light (SSC),which is scattered to these angles primarily by irregularities or texture in the surface or cytoplasm of the particle. In addition to this scattered light, the fluorescent particle may also give off fluorescent light which is a relatively long wavelength that is emitted when the fluorescent particle absorbs high energy light and then emits the energy from that light as photons of somewhat lower energy.

Calibration particles RCP-30-5A contain a mixture of several similar size particles with different fluorescence intensities. Every particle contains a mixture of fluorophores that allows excitation at any wavelength from 365 to 650 nm. RCP-30-5A as a fluorescent particle and the 532 nm, 5mW green laser as a excitation source are both used in the laboratory-built system.

In the laboratory-built system, fluorescent particles RCP-30-5A flow through analysis point and are illuminated by the 532 nm green laser which is bright enough to produce scattered light and fluorescence PE(585 nm) of detectable intensity. The scattered light and fluorescence are collected by the spectrum-dividing system which contains wavelength-specific mirrors, filters and lens. These mirrors and filters are designed so that they transmit and reflect light of well-defined wavelengths. The light emitted to the side from an analysis point is focussed by the lens onto dichroic mirrors and bandpass filters that partition this multcolor light, according to its color, the SSC light onto the detector APD2 and the fluorescence PE onto the detector APD3. The electrical pulses originating from fluorescence PE detected by the APD3 are usually weak and they must be processed by a series of amplifiers. After the different signals or pulses are amplified they are processed by an ADC which in turn allows for events to be plotted on a graphical scale.

The one-parameter histogram result of the fluorescence PE has been gotten as the figure 3 showing,which is close to world advanced level as showing in the figure 4[6].

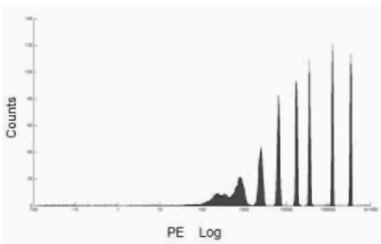

Figure 3. The fluorescence PE signal of the laboratory-built system

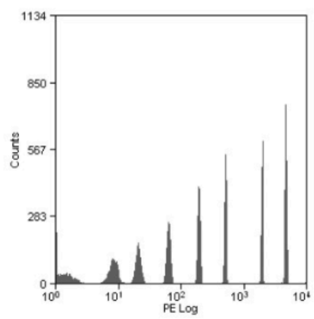

Figure 4. The fluorescence PE signal of the leading product

X-axis represents the intensity of the fluorescence PE from particles, and Y-axis repesents the RCP-30-5A cuonts detected by the APD3. Histogram shows individual peaks representing various fluorescence intensities in RCP-30-5A particles, which is close to the experimental result of the leading product as showing in the figure 4.

III. CONCLUTION

In the paper, the forward scatter and the fluorescent light analysis are carried out in the laboratory-built system,and good results have been gotten from the experiments.From the FSC singals the different size particles can be distinguished as they pass through the analysis point individually in a very short time. Fluorescence PE histogram shows close to eight individual peaks representing various eight fluorescence intensities in RCP-30-5A particles,which indicates the laboratory-built system has good sensitivity close to the world advanced level.

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