Multispectral Flow Cytometry with a Single-Element Detector

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Abstract. Omega Optical has recently developed a multispectral laser-scanning confocal system which employs pulsed laser excitation with a serial array of fibers in the detection path. The face of the fiber tip acts as the confocal pinhole, while spectral bins are defined by reflecting elements at the end of each sequential fiber tip, such that the blue light reaches the detector first, followed by up to 10 spectral bins of increasingly red light. This system is easily adapted for use in a flow-cytometer arrangement where one or both scanning mirrors are removed. Our system can nearly simultaneously detect backscattering, orthogonal scattering and fluorescence. We will present a proof-of-concept of this design and results.

1. Introduction

Omega Optical has been working on a fast multispectral confocal imaging system for the past several years. The original concept was to develop a system with extremely high sensitivity for the imaging of intrinsic fluorescence in tissues for the identification of cancer and other diseases in-vivo via micro-endoscopy or ex-vivo via microscopy. The literature has many examples of using intrinsic fluorescence as a diagnostic tool. [1-4] The system was designed for near-video rate data display and processing (10 fps) for use in an operating room or clinic. [5-6]

The speed and sensitivity of the system makes it a good fit to flow-cytometry. To that end, we performed some proof-of-principle experiments to demonstrate its effectiveness in this area.

2. Single-point Flow Cytometry Methods and Results

Figure 1 describes the layout and spectral response of our system. Because we were adapting an imaging system to do this work, we focused on the sample using confocal scanning, then centered and disabled the scanning mirrors to do single-point measurements of beads flowing through glass capillary tubing. Both 405 nm and 488 nm pulsed lasers were used for this work. A 100 ns laser pulse is focused onto the sample and the resulting fluorescence is directed onto the face of a 62.5 μm core fiber which acts as a confocal pinhole. The light passes through a 50/50 splitter into blue and red fiber arrays containing increasingly red reflecting elements. Spectral bands return to the detector at defined time points, thus indexing wavelength into the time domain. Instead of using the imaging software for these experiments, we detected raw signals from an oscilloscope (Figure 2). The data appears as a series of peaks in the time domain corresponding to each of the wavelength bins as they reach the detector. Noise can easily be distinguished from signal in this configuration because noise typically appears as a single spike and not as a series of equally-spaced peaks. The entire spectrum of 10 bins is collected in 2.5 μs using a single PMT.

In Figure 2 we show the detection of multicolored 6 μm Spherotech beads of varying intensities at a linear flow rate of 1 m/s. The inset is an expanded view of the brightest bead. It clearly shows

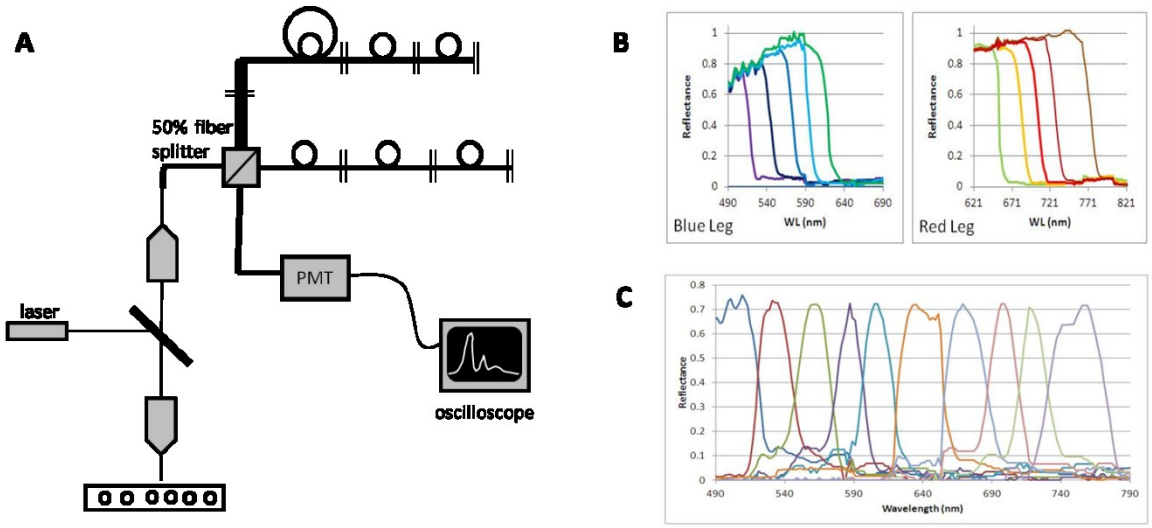

Figure 1. a. Schematic of the fast multispectral detection system in single point mode. The sample flows past the detector through a glass capillary tube by manually compressing a syringe. **b.** Spectral response of the individual coated fiber tips. **c.** Scaled spectral response of the entire fiber array. The shape of each bandpass is formed by the 2 edges surrounding it.

interactions with 3 laser pulses. The size of the bead or the linear flow-rate can be determined by analyzing the FWHM of the envelope of the response. Slower flow rates generate wider responses, as do larger particles. To detect 6 µm beads, a minimum of one laser pulse will have to hit it as it traverses the focal point, which leads to a maximum flow rate of about 2 m/s and a detection speed of over 150,000 particles/s for this size. State of the art sorters can reach over 100,000 cells/s. [7]

Figure 3 shows a mixture of beads of different sizes and colors. Yellow astericks indicate multicolor Spherotech beads, red astericks indicate 2 µm red fluorescent beads and green astericks indicate 1 µm green fluorescent beads. The top panel is most zoomed out in time to show all beads on a single trace. The middle panel shows the envelope of one bead of each type. The smaller beads have a proportionately smaller signal envelope. The bottom panel illustrates the spectral distribution of the different bead types. They are spectrally quite different and easy to differentiate. The large first peaks are due to rejection of blue light from the red leg of the system (Figure 1a) and are not typically used for analysis.

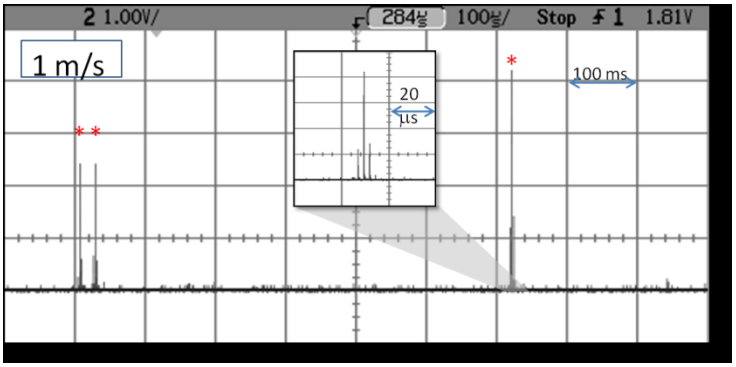

Figure 2. 6 µm Spherotech beads flowing at a rate of 1 m/s. These beads vary in intensity and are indicated with red astericks. At this speed, 3-5 laser pulses interact with the bead (inset). We predict a maximum linear flow rate of about 2 m/s for beads of this size.

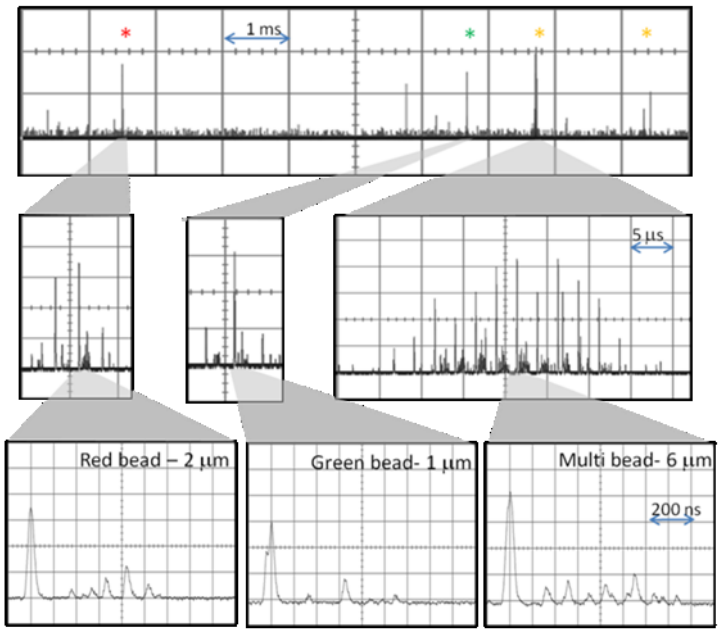

Figure 3. A mixture of 3 bead sizes and colors (2 µm red beads, 1 µm green beads and 6 µm Spherotech beads indicated with astericks). Each row is displayed at a different timescale (top = 1 ms per division, middle = 5 µs, bottom = 100 ns)

3. Flow-Cytometry Single-Axis Imaging Methods and Results

To illustrate imaging capabilities of this system, we disabled the y-mirror and moved the sample (beads on a slide) underneath the focus of the microscope lens using a motorized stage as illustrated in Figure 3a. By using a static sample slide, we were able to compare images of identical beads at different rates (Figure 3b). Our current software does not allow for different numbers of pixels in the

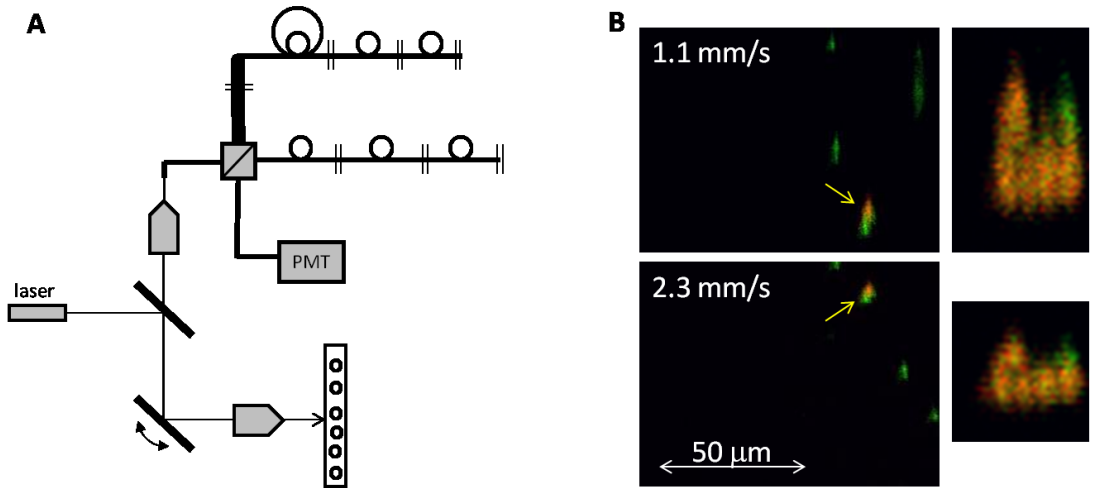

Figure 4. a. Schematic of imaging flow-cytometer. The y-mirror is disabled in this arrangement while the sample is moving past the focus. In this case, we imaged beads on slides (the same beads as above) to compare imaging rates. Using the current software, the x-mirror scans 256 pixels per line (100 µm long). **b.** Still images using this method- 2 µm red beads and 1 µm green beads. The lateral resolution of the system is just under 2 µm. Yellow arrows identify identical bead clusters at 2 different scan speeds (full-scale). On the left are zoomed in images of bead clusters at 2 speeds.

x-direction- only the step size. This severely limits the maximal flow rate of the system because each line takes 640 μs to acquire. Given this limitation, we are able to acquire good images at 2.3 mm/s linear speed. At slower speeds, the images become elongated in the y-direction and when stationary, the beads become stripes. Matching the flow rate to the x-scan rate is crucial for good imaging in this implementation.

4. Conclusions and Future Directions

Herein we demonstrated a fast multi-spectral detection scheme for flow-cytomtery that uses a single PMT detector. This system can be used at linear flow rates of up to 2 m/s for particle detection and has been shown to generate images with decent resolution at 2 mm/s. Further developments include changes to the hardware and software to reduce the number of pixels in the x-axis to more closely match the particle size and improvements in the flow-system to include hydrodynamic or acoustic focusing, pumps, etc. Optical enhancements include adding scattering channels (forward and side) with associated fiber delays and tips. This approach to detection holds great promise for reducing the size and cost of flow cytometers.

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