B259 Multispectral flow cytometry using serial fiber arrays and a single-element detector

Sarah Locknar, John Barton and Gary Carver - Omega Optical, Inc. Brattleboro, VT 05301

1

Abstract

Omega Optical has recently adapted our multispectral fluorescence detection system to flow-cytometry. The hardware was originally developed for confocal applications and consists of pulsed laser excitation with a serial array of reflecting fiber tips separated by fiber delay lines. The proximal tip of the fiber array acts as the confocal pinhole. Each fiber tip in the series reflects a sequentially longer wavelength into the PMT detector, generating a time-indexed spectrum. Up to 10 spectral bins are defined by the wavelength intervals between the reflecting elements. This strategy enables the acquisition of a multispectral dataset within 2.5 microseconds using a single PMT, which can be spectrally unmixed or further processed. A sequence of excitation lasers with matched fiber arrays and detectors can be placed in the flow path to further multiply the number of possible labels. The fiber-based strategy can simultaneously be used to detect forward and side scattering in the cells as well. We anticipate that with a non-imaging application we will be able to sort up to 80,000 cells per second, and should be able to image up to 400 cells per second using our current hardware. The imaging rate could be further increased by switching to a resonant-scanning mirror instead of the galvo mirror in current use. Spatial resolution can be adjusted by focusing and defocusing. We will present preliminary results of both sorting and imaging modes. This approach will lead to compact, light weight flow systems with multiple channels.

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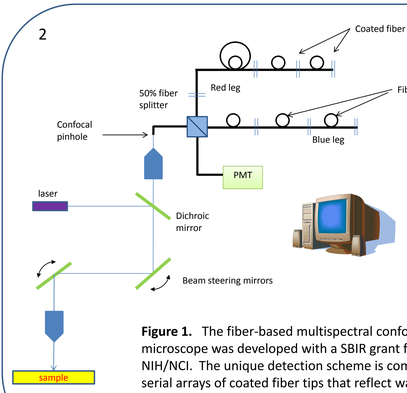

Figure 1. The fiber-based multispectral confocal microscope was developed with a SBIR grant from the NIH/NCI. The unique detection scheme is composed of 2 serial arrays of coated fiber tips that reflect wavelength bins back to the detector at sequential timepoints, building a spectrum of each pixel within 2.5 µs.

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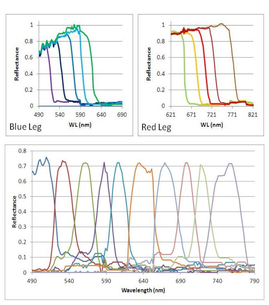

Figure 2. Top, spectral response of the individual fiber tips. The blue wavelengths exhibit fiber loss. Bottom- Scaled spectral response of the entire fiber array. The light reaching the PMT is a time-indexed series of band-passed light centered at progressively longer wavelengths

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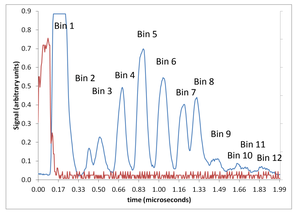
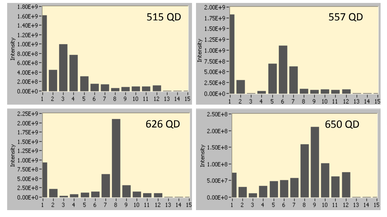
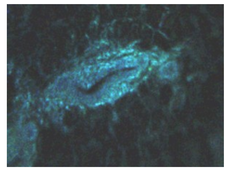
Figure 3. The data acquisition and display process



The "go" (red) pulse is sent to the laser and a 100 ns laser pulse is sent to the sample. The wavelength bins return to the detector sequentially in time. Raw data is detector-limited in speed. The first bin is the rejection of "blue leg" light from the red leg. Bin 2 is empty, bins 3-12 represent wavelength bins illustrated above. The delay lines are spaced to allow for maximal detector response and recovery between the pulses.



Displayed data is processed to remove small amounts of cross-talks between the channels and to account for non-centrosymmetricity of the sequential fiber arrays. These are example histograms extracted from the GUI illustrating the emission spectra of a number of quantum dots excited at 488 nm. All bins are acquired and processed for each pixel.



Data can be displayed in various ways in real-time, including monochrome (max pixel intensity), monochrome single-channel, 3-channels simultaneously (RGB, above), multi-channel math and spectral angle mapping.

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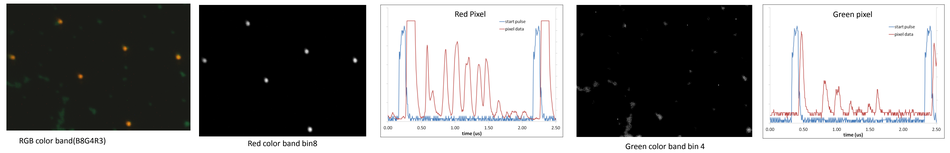
Preliminary Results. Our system was originally designed to do imaging, so we started using current capabilities. Because we do not have independent control of the mirrors at this time, we proceeded with single-point scanning to ascertain the speed limits of the system.



RGB color band(B8G4R3)

Red color band bin8

Green color band bin 4

Figure 4 illustrates mixed beads and the raw data traces that correspond to the red and green pixels from the oscilloscope. Red pixels were 4µm and green beads were 2µm in diameter. These were colorized in real time by assigning RGB colors to bins x,y,z respectively

~ 0.04 mm/s

~0.2 mm/s

Figure 5. Overlay of sequential images while moving the stage. Images are displayed with RGB. We manually moved the stage while acquiring sequential images to illustrate the speed of the system. Note that the shapes are not perturbed by the motion at these speeds.

6 **Methods- Single Point Measurements**

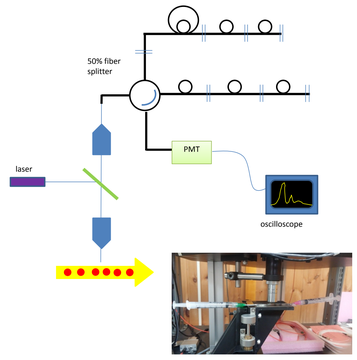
We used either a 100 mm ID square quartz tubing or a pulled round glass tubing to simulate the flow cytometer's detection chamber. This was epoxied onto syringe needles and onto a microscope slide for stability during the experiments.

The syringes were manually compressed to induce flow of beads through the tubing. The system was focused on the beads in the tubing and then the scanning mirrors were centered and disabled.

To bypass the image processing portion of the software, we used direct oscilloscope output to determine the speed of the system. Oscilloscope output (TIFF) was analyzed for FWHM of the time responses using ImageJ to calculate the linear speed of the beads in the system.

Initial experiments utilized 6 mm diameter 6-color Spherotech beads for a uniform signal and speed estimates. We also performed experiments with a mixture of 2 mm green, 4 mm red and 6 mm Spherotech beads.

Figure 6 – (top) Schematic of Single-point measurements, (inset) photo of the flow cell installed on the stage



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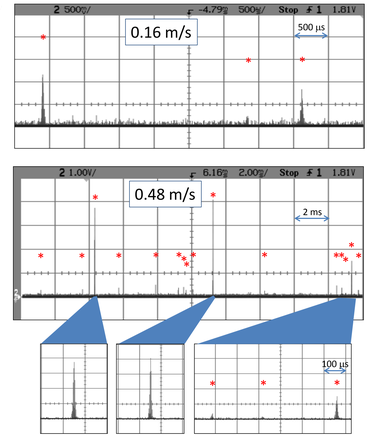
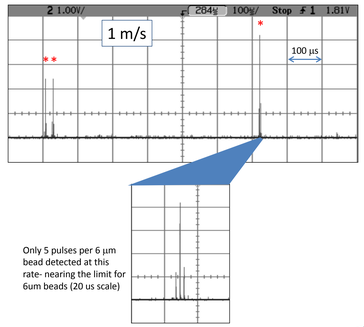


Figure 7

These are 6 mm Spherotech beads that exhibit a variety of intensity levels.

Theoretical limit for 6 mm beads is 2.4m/s which would give one pulse per particle. Electronic noise does not show up in all bins, but rather as random spikes. This allows for filtering.



Only 5 pulses per 6 µm bead detected at this rate- nearing the limit for 6um beads (20 us scale)

8

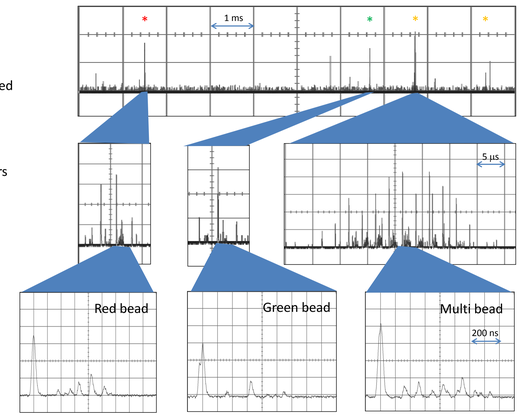
Figure 8- Single-point detection of mixed beads

flow rate ~ 0.5 m/s

A mixture of beads with different colors and sizes

1 µm green beads
2 µm red beads
6 µm multi-colored beads

The system can easily determine the bead type based on size and spectral composition



9 **Conclusions and Future Directions**

Herein we demonstrated a single-point, 10-channel fluorescence flow-cytometer detection scheme capable of linear flow rates of 1 m/s. Depending on the particle size, faster rates can be accomplished. This system can be modified to include front and side-scatter capabilities as illustrated in Figure 9.

With minor modifications to our system, we anticipate that 10-color **imaging** detection can also be accomplished at rates up to 400 cells per second (6 µm diameter). This would require altering the mirror-control software. Figure 10.

Both approaches would require rewrites of the user interface optimized for flow. We welcome collaborators for further development of this system.

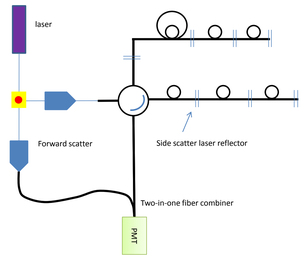


Figure 9. Forward and side scatter channels can be added to the detection array by adding another reflector and a fiber combiner

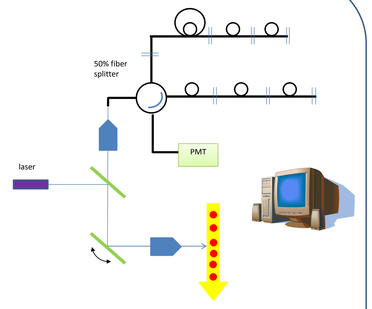


Figure 10. In imaging mode, a single x-mirror can be used to raster across the cells while detecting in the back-scatter direction

10

References

1. Carver, G.E., Locknar, S.A., Morrison, W.A., Ramanujan, V.K. and Farkas, D.L. *High-speed multispectral confocal biomedical imaging*", J. Biomed. Opt. 19(3), 036016 (Mar 21, 2014).; http://dx.doi.org/10.1117/1.JBO.19.3.036016

2. Carver, G.E., Chanda, S.K., Morrison, W.A., Locknar, S.A., and Johnson, R.L. *System for Multispectral Imaging of Fluorescence* US Patent 9,155,474 (Oct 13, 2015)

Acknowledgements

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