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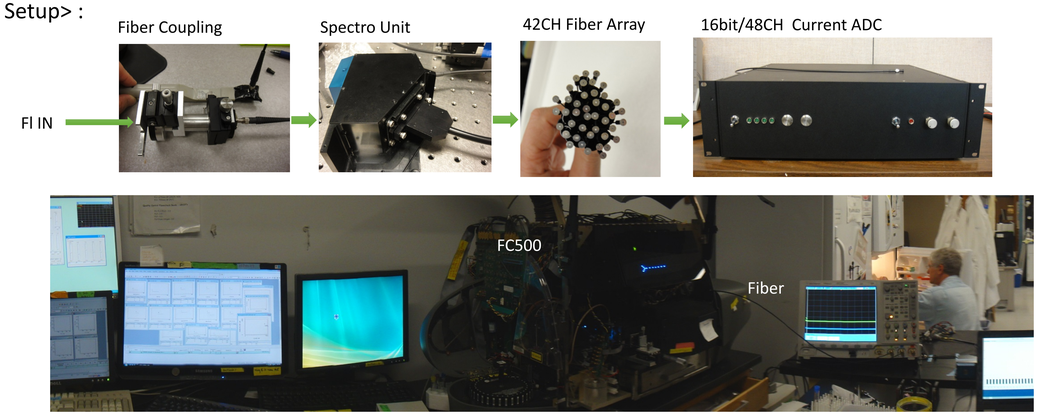
Abstract

Introduction & Background: Hyperspectral detection in flow cytometry is a powerful tool for over ten multi-color phenotypic analysis without compensation. There are many operating flow cytometers in the market place with only 4 or 5 color performance. An objective of this work is to provide a universal hyperspectral detection system to analyze fluorescence signals from a variety of flow cytometers. Using our prototype we were able to evaluate a wide wavelength range (340-800nm) with 42channels and 16 bit resolution.

Method and Results: In traditional flow cytometry, fluorescence and side scatter from the flow cell is collected by a high NA objective lens and collimated to detection optics including dichroic mirrors, bandpass filters and PMTs. By capturing the collimated fluorescence light with an achromatic lens and optical fiber, it is possible to analyze the entire visible light spectrum using a polychromator. We developed a custom polychromator, using an aberration corrected Toroidal Mirror Grating and 42CH linear fiber array. The advantages of the Toroidal mirror grating are 1) wide wavelength coverage from UV 340nm to IR 800nm, 2) high spectral resolution with equal bandwidth, 3) high coupling efficiency using just one reflected flat field. The focused spectral line "image" is connected to a 42CH fused silica fiber array with 0.5mm core and NA0.22. Once fluorescence light is distributed to the fiber, the signal can be detected by variety of sensors like APD, PMT or SiPM (Geiger mode APD Array). For conventional flow cytometry applications, we utilized 1mmϕAPDs (Hamamatsu). The amplified photo current from the APD was converted to digital signals by a custom developed 16bit current ADC. The developed system was coupled to a conventional flow cytometer (FC500, Beckman-Coulter) and we confirmed fluorescence signal from Rainbow beads. In combination with µPMT by Si-MEMS structure, the highest sensitivity is possible to apply flow analysis in the nanosecond domain. Due to smaller photocathode and dynode area, µPMT and low noise preamplifier with 70MHz bandwidth measured very low dark counts less than 10 (~aW level) at room temperature. It enable the analysis of the photon energy spectrum in nanoseconds, which we call "*Photon Spectroscopy*" flow cytometry. Challenges include how to reduce background counts in detection optics, but it may be possible to open new aspect in live cell analysis.

Conclusion : A hyperspectral detection system with toroidal mirror grating, 42CH fiber array and advanced electronics was developed. It is possible to evaluate spectrum range of UV340nm to IR800nm with 10nm resolution. In principal, any current commercial instrument can be updated with hyperspectral detection system. In addition, Si base photomultiplier and very high speed electronics enables the analysis of every photons energy level. Photon Spectroscopy for live cell analysis suggests "*analog to digital*" paradigm shift in cytomics.

<Experimental Setup> :

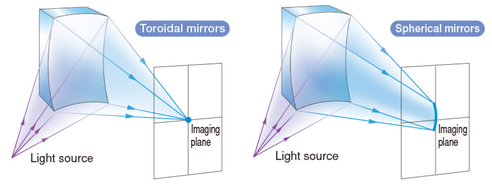


Motivation & Objectives

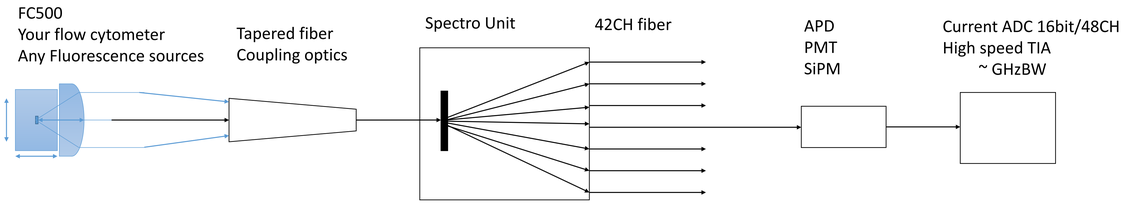
1. Develop wider Hyper-spectrum Unit (UV-IR)
2. Universal module for existing instruments
3. 42CH Quartz fiber array and tapered Input fiber
4. Flexible choice of photon detectors
5. 16bit Current ADC and 100M~1GHz TIA
6. Single photon detection & Photon Spectroscopy

Flat Field Toroidal Mirror Grating

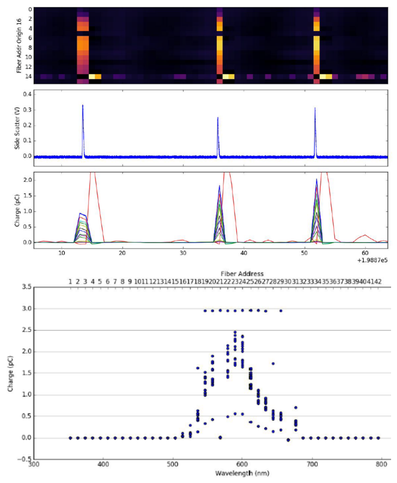
1. Min. aberration imaging spot to fiber array
2. Wider wavelength 340-800nm with equal resolution
3. High efficiency by one time reflection



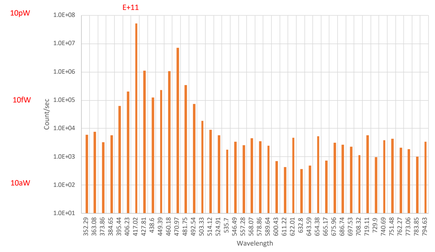
Method & Results



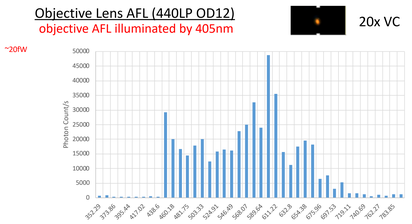
Fluorescence Bead signal from FC500 (Analog)



405nm Laser Background Photon Spectrum (Photon Counting)



Objective Lens AFL (440LP OD12)
objective AFL illuminated by 405nm



Discussion & Issues to be improved

1. Reset pulse for current ADC & Connectivity to existing instrument.
2. Coupling efficiency with separate optics.
3. Integration with FS and SS signal detection
4. Cost of many detectors and amplifier
5. Array detectors & Bias voltage supply
6. Temperature control
7. Integration as Hyper-spectrum module
8. Hyper-spectrum analysis software
9. Application for cell analysis

Conclusion

1. 42CH (340-800nm) Hyper-spectrum Unit is developed.
2. Resolution is 10.8nm/CH with equal bandwidth
3. Current ADC is useful for multi-channel detection.
4. Preliminary photon spectroscopy is confirmed.

Acknowledgement

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