ISAC XXIII International Congress May 20--24, 2006

A 16-Channel APD Array for Visible and Near-IR Flow Cytometry

William G. Lawrence[*‡], *Christopher Stapels*[*], *Joseph D. Tario*[†], *Edward Podniesinski*[†], *Paul K. Wallace*[†], *and James F. Christian*[*]

* Radiation Monitoring Devices, Inc., 44 Hunt Street, Watertown, MA 02472-4699; [†]Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo NY 14263

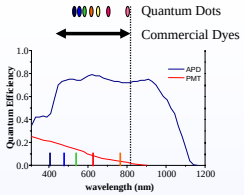
I. Abstract

We report on the development of a flow cytometer based on a 16-channel avalanche photodiode (APD) linear detector array. The array, configured with a dispersive grating, simultaneously records emission over a broad wavelength range using the 16 APD channels. The APD detector elements have a peak quantum efficiency of 80% and have at least 40% quantum efficiency over the 400-nm to 1000-nm wavelength range. The extended red sensitivity of the detector array facilitates the use of Near-IR emitting dyes or quantum dots. This extended range can be used to monitor additional fluorescent channels and can reduce the impact of autofluorescence. The wide wavelength sensitivity of the APD array permits the use of multiple excitation sources and many different fluorescent labels to maximize the number of independent parameters in a given experiment.

We compare the performance of a single APD detector to a red enhanced PMT at 585 nm and 820 nm. We present results where a system with a single APD detector monitors lymphocyte proliferation with a 834-nm dye. Initial results for the flow cytometer with the 16-element APD array and the 16-channel readout ASIC (application specific integrated circuit) are also presented.

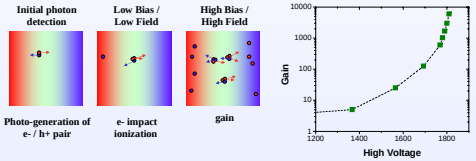
II. Introduction & Background

a) Spectral Response and Noise of APD and PMT



- APD detectors have high Quantum Efficiency
- APD detectors have an extended Near-IR spectral response
- There are a limited number of commercially available fluorescent markers in the NIR

b) Avalanche Photodiode Provide Internal Gain



c) Signal-to-Noise Considerations

$$SNR = \frac{\bar{G}^2\eta^2\phi^2}{\bar{G}^2 F\eta\phi + \bar{G}^2\bar{F}\bar{d} + \sigma^2_{readout}}$$

No dark noise ⇒ No readout noise: $SNR = \frac{\eta\phi}{F}$

F ~ 2 for APD

F ~ 1 for PMT

- APDs need low readout noise

$\bar{G}$ ≡ mean gain

$\bar{d}$ ≡ mean dark current

F ≡ excess noise factor

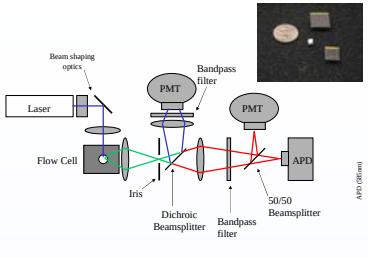
η ≡ quantum efficiency

ϕ ≡ incident photon flux

$\sigma^2_{readout}$ ≡ readout noise

III. Single Element Detector Results

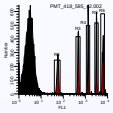
a) Single Element APD Test Bed



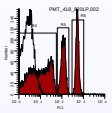
- 488-nm excitation
- 585/42-nm Bandpass filter
- 2.5-micron AlignFlow beads
- Calibrated detection intensity

b) Comparison of APD & PMT Performance in the Visible & Near-IR spectral regions

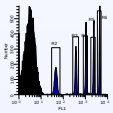
PMT: 585/42 nm



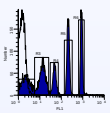
PMT: 820 nm LP



APD: 585/42 nm



APD: 820 nm LP



RMD temperature stabilized APD compared to Hamamatsu R-3896 PMT

Spherotech Ultra Rainbow calibration beads (URCP-38-2K)

488-nm Excitation (13.5 mW)

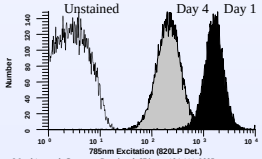
Top: 585/42-nm Detection

Bottom: 820-nm Long Pass Detection

c) Near-IR Proliferation Studies

Excitation: 44 mW at 785 nm

Emission: 834 nm (820 nm long pass)



Woodring et al, *Cytometry Part A*, vol. 67A, pp. 104-111, 2005.

PTI CellVue Tracking Dye

Track proliferation of U937 cells

Cells were labeled with CellVue NIR Tracking dye from PTI Research

785-nm Excitation

834-nm Emission

Day 1: MFI = 1696 : Cell Count = 4.9 x 10^5

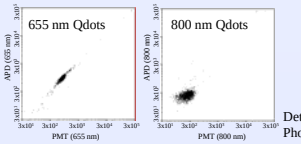
Day 4: MFI = 223 : Cell Count = 3.6 x 10^6

MFI ratio : 7.6

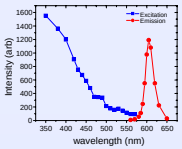
Cell Count ration : 7.3

d) Quantum Dots on Latex Microspheres

- 2.2-µm Biotin coated microsphere
- Streptavidin conjugated Qdots
- 5000 Qdots / microsphere

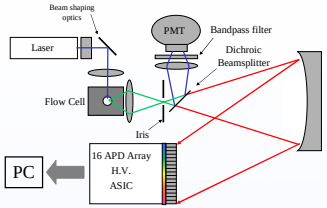


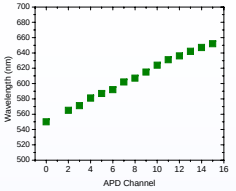
Detected Photons



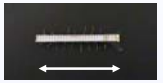
IV. APD Array Results

a) APD Array Layout and Wavelength Response





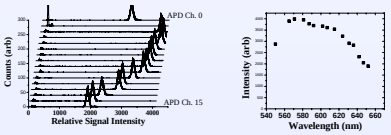
A 16-Channel ASIC (application specific integrated circuit) from RMD contains the preamplifier, trigger, and peak-hold circuits



50 mm

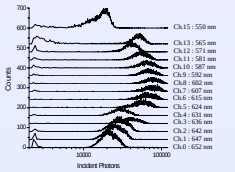
b) 16-Channel LED Spectrum

- Pulsed white LED (2 microsecond pulse width)
- Trigger provided by PMT
- 16 Channels recorded simultaneously by the ASIC



Reconstructed Spectrum

c) 6-micron AlignFlow Bead Spectrum



- 6-micron AlignFlow bead spectrum recorded using the grating and the 16-element APD array
- Each histogram was recorded sequentially using the analog output of the ASIC
- The fluorescence intensity of the beads was calibrated using an iron-55 x-ray source

VII. Conclusion

- The results demonstrate the performance of the APD sensitivity in the visible and Near - IR spectral regions
- The APD performance is comparable to the PMT performance in the visible and superior to the PMT at wavelengths longer than 830 nm.
- The Near-IR sensitivity of the APD and new CellVue Tracking dyes enable proliferation studies without interfering with conventional flow cytometry measurements
- Multiple APD elements can be coupled together to do multi-color flow cytometry in a compact form factor
- Low noise pre-amplification important for low-intensity light measurements with APD detectors

Acknowledgements

RMD would like to thank the National Cancer Institute of NIH for their partial support of this work under Grant No. 2R44 RR017126-02.

The Flow Cytometry Lab at RPCI was established in part by funds from NIH Shared Instrument Program and receives support from Core Grant (5 P30 CA016056-01)

[‡]Corresponding Author: William G. Lawrence, Ph.D.

RMD
Radiation Monitoring Devices, Inc.
44 Hunt Street
Watertown, MA 02472-4699, U.S.A.
http://www. rmdinc .com
Phone: (617) 926-1167
Fax: (617) 926-9980