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Highly Sensitive Filter-Less Fluorescence Detection Method Using an Avalanche Photodiode

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Abstract: Herein we report a highly sensitive filter-less fluorescence detection method using an APD (avalanche photodiode). Experimental measurements using the proposed APD-based highly sensitive fluorescence detection method exhibits the sensing capability to detect an excitation light and a fluorescence light without band pass filter or grating. The principle of this APD-based highly fluorescence detection method is used the varying multiplication ratio that is decided by wavelength. The wavelength controls running distance of photo-excited carrier by absorption coefficients, and this element decide multiplication ratio on fixed high electrical field. In fluorescence detection, they use two types of light: excitation light and fluorescence light. These lights have different wavelengths and make different multiplication ratio as well. Thus this method can separate two types of light easily by using multiplication ratios of APD without band pass filters/gratings. In this experiment, the excitation light is LED (light emitting diode) and fluorescence light occurs from FITC (fluorescein isothiocyanate) with ethanol. The FITC concentration changes from 0.1 μmol/L to 10 mmol/L. In this measurement circuit, we employ APD (S2385), power supply voltage, and pico ampere current meter. As a result, these lights are correctly separated by using multiplication ratio with calculation at every concentration FITCs.

Key words: Avalanche photodiode, filter-less, fluorescence.

1. Introduction

Conventionally, fluorescent labels and an expensive fluorescent scanner are used for DNA (deoxyribonucleic acid) analysis [1, 2]. The fluorescent scanner requires a special light source with a band pass filter [3, 4]. It is, in consequence, relatively bulky and low sensitivity by the filter. On the other hand, the biochemistry field has need of a straightforward space-saving and high sensitive fluorescence detection system [5-7].

To realize these requests, integration of the conventional system with low noise and high gain amplifier is required. The microchips possess following merits. Firstly, it is possible to miniaturize, so that it can incorporate with μ-TAS (micro total analysis system) easily. Furthermore, if fluorescent information may be obtained, without using a fluorescent scanner and optical microscope, fluorescence analysis would be easier and be high sensitive than in a conventional system. In addition, APDs (avalanche photodiodes) fabricated in CMOS (complementary metal oxide semiconductor) process make low noise and have amplifier gain [8]. Thus we propose APD-based highly sensitive fluorescence detection method. There is another merit: excitation light intensity with getting fluorescence light intensity detects at same time. The detecting excitation light intensity improves accuracy of fluorescence light intensity. For the first time, in this paper, we report an APD-based filter-less fluorescence detection method.

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2. Method

Our proposed method uses APD (S2385). APDs have multiplication ratio having incident light wavelength dependent properties. This means, when single light (excitation light or fluorescence light) radiates to the APD, the multiplication ratio (M_E or M_F) is controlled by the wavelength with 73 V power supply voltage. M_E and M_F are calculated as follows:

$$M_E = \left(\frac{Ip_E(73\ \text{V})}{Ip_E(50\ \text{V})}\right), \quad M_F = \left(\frac{Ip_F(73\ \text{V})}{Ip_F(50\ \text{V})}\right) \quad (1)$$

Ip_E (73 V) and Ip_E (50 V) are photocurrent of APD by excitation light with 73 V and 50 V, Ip_F (73 V) and Ip_F (50 V) are photocurrent of APD by fluorescence light with 73 V and 50 V. These photocurrent subtract dark current (I_d (50 V), I_d (73 V)) for accurate calculation. These photocurrents and dark current are measured by pico ampere meter in measurement circuit of Fig. 1. When mixed light (excitation light and fluorescent light) radiates to the APD, the mixed multiplication ratio (M_{Mixed}) is controlled by mixed ratios (X_E, X_F) of these lights (excitation light and fluorescent light) with 73 V power supply voltage. The M_{Mixed} is calculated as follows:

$$M_{Mixed} = \frac{Ip_{Mixed}(73\ \text{V})}{Ip_{Mixed}(50\ \text{V})} = \frac{Ip_E(73\ \text{V}) + Ip_F(73\ \text{V})}{Ip_E(50\ \text{V}) + Ip_F(50\ \text{V})}$$

$$= \frac{M_E \times Ip_E(50\ \text{V}) + M_F \times Ip_F(50\ \text{V})}{Ip_E(50\ \text{V}) + Ip_F(50\ \text{V})}$$

$$= \frac{M_E \times Ip_E(50\ \text{V})}{Ip_E(50\ \text{V}) + Ip_F(50\ \text{V})} + \frac{M_F \times Ip_F(50\ \text{V})}{Ip_E(50\ \text{V}) + Ip_F(50\ \text{V})}$$

$$= M_E \times X_E + M_F \times X_F \quad (2)$$

And X_E, X_F have Eq. (3):

$$X_E + X_F = \frac{Ip_E(50\ \text{V})}{Ip_E(50\ \text{V}) + Ip_F(50\ \text{V})} + \frac{Ip_F(50\ \text{V})}{Ip_E(50\ \text{V}) + Ip_F(50\ \text{V})} = 1 \quad (3)$$

Then, X_E, X_F calculate as follows:

$$X_E = \frac{M_{Mixed} - M_F}{M_E - M_F}, \quad X_F = \frac{M_{Mixed} - M_E}{M_F - M_E} \quad (4)$$

The quantum efficiency of excitation light (η_E) and fluorescent light (η_F) with the APD are 0.63 and 0.73, respectively. The η_E and η_F are the number of EHPs (electron-hole pairs) generated from each incident photon:

$$\eta_E = \left(\frac{X_E \times Ip_{Mixed}(50\ \text{V})}{q}\right) \cdot \left(\frac{W_E}{h\frac{c}{\lambda_E}}\right)^{-1}$$

$$\eta_F = \left(\frac{X_F \times Ip_{Mixed}(50\ \text{V})}{q}\right) \cdot \left(\frac{W_F}{h\frac{c}{\lambda_F}}\right)^{-1} \quad (5)$$

where, Ip_{Mixed} (50 V) is the photogenerated current from the absorption of incident optical power W_E and W_F at their wavelength λ_E (corresponding to a photon energy hc/λ_E) and λ_F (corresponding to a photon energy hc/λ_F). Then W_E and W_F are expressed as follows:

$$W_E = \left(\frac{X_E \times Ip_{Mixed}(50\ \text{V})}{q}\right) \cdot \left(\frac{\eta_E}{h\frac{c}{\lambda_E}}\right)^{-1}$$

$$W_F = \left(\frac{X_F \times Ip_{Mixed}(50\ \text{V})}{q}\right) \cdot \left(\frac{\eta_F}{h\frac{c}{\lambda_F}}\right)^{-1} \quad (6)$$

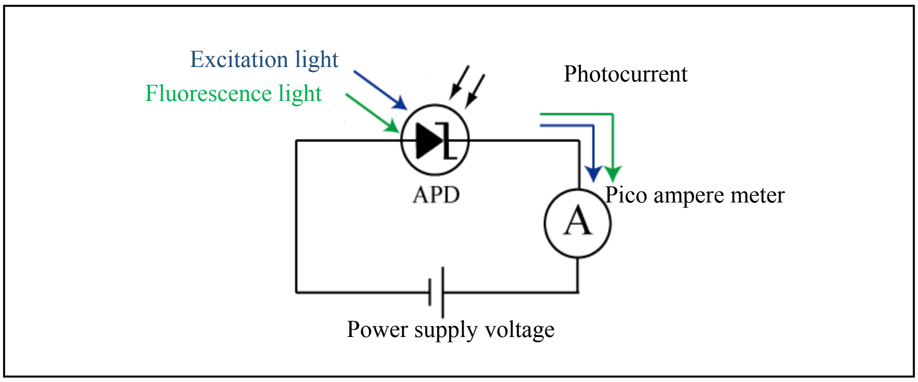
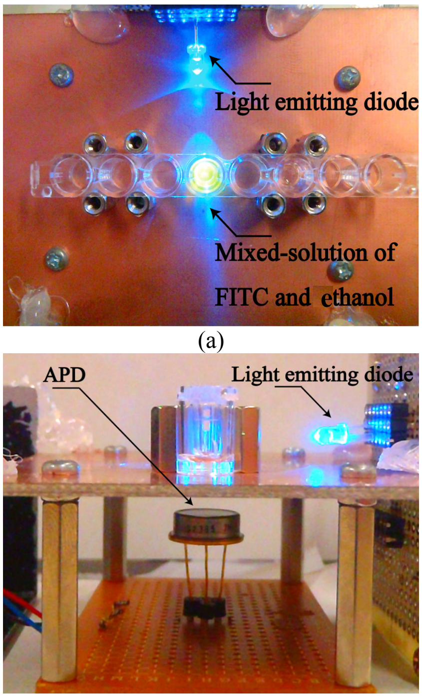


Fig. 1 Measurement circuit for measuring photocurrent and dark current.

From these equations, we can find W_E and W_F from measurement Ip_{Mixed} (50 V) and Ip_{Mixed} (73 V).

3. Experiment

Figs. 2a and 2b show measurement system from top side and cross side respectively. The LED is used as an excitation light that have 470 nm wavelength peak. The palette has solution mixed with ethanol and FITC (fluorescein isothiocyanate), and that total amount becomes 300 µL. We prepare seven patterns solutions for testing W_F detection by APD without filters. These FITC concentrations are 0 µmol/L (Solution 1), 0.1 µmol/L (Solution 2), 1 µmol/L (Solution 3), 10 µmol/L (Solution 4), 0.1 mmol/L (Solution 5), 1 mmol/L (Solution 6), 10 mmol/L (Solution 7), respectively. Set APD which is connected to power supply voltage and pico ampere meter under the palette in order to detect mixed light (W_E, W_F). After the measurement, spectroscope place under the palette instead of APD for comparing APD output (W_E, W_F) and spectroscope output.



(b)

Fig. 2 Measurement system from (a) top side and (b) cross side under the palette instead of APD for comparing APD output (W_E, W_F) and spectroscope output.

4. Measurement Flow

Measurement flow is separated a basic measurement and a target measurement. At first, we try to measure them using basic measurement as follows:

(1). Measure dark current (I_d (50 V), I_d (73 V)) of APD on measurement circuit (Fig. 3).

(2). Measure photocurrent (Ip_E (50 V), Ip_E (73 V)) by excitation light (Fig. 4). Then calculate multiplication ratio (M_E) from Ip_E (50 V) and Ip_E (73 V) using Eq. (1).

(3). Measure photocurrent (Ip_{Mixed} (50 V), Ip_{Mixed} (73 V)) by mixed light. Then, calculate photocurrent (Ip_F (50 V), Ip_F (73 V)) from differential Ip_{Mixed} (73 V) to Ip_E (73 V) (Fig. 5).

(4). Calculate multiplication ratio (M_F (73 V)) from Ip_F (50 V), Ip_F (73 V) using Eq. (1).

After finishing this basic measurement, target measurement is performed as follows:

(1). Measure mixed photocurrent (Ip_{Mixed} (50 V), Ip_{Mixed} (73 V)) by mixed light (Fig. 6).

(2). Calculate multiplication ratio (M_{Mixed}) using Eq. (2).

(3). Calculate mixed ratio (X_E, X_F) using Eq. (4).

(4). Calculate light intensity (W_E, W_F) using Eq. (6).

Through these calculations, W_E and W_F are detected.

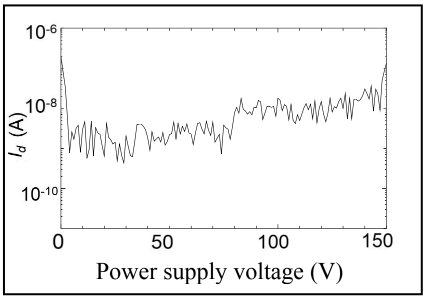


Fig. 3 Dark current.

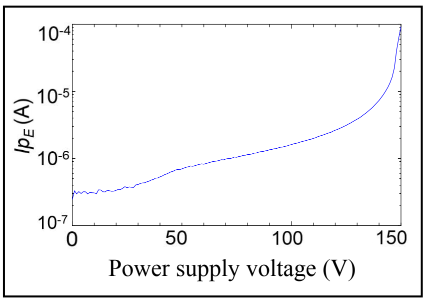


Fig. 4 Photocurrent by W_E.

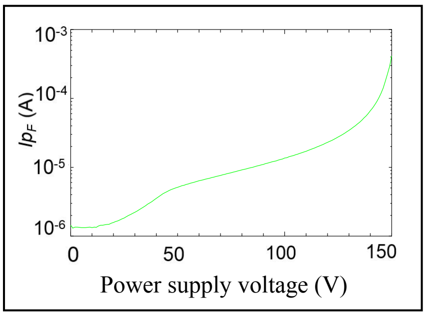


Fig. 5 Photocurrent by W_F.

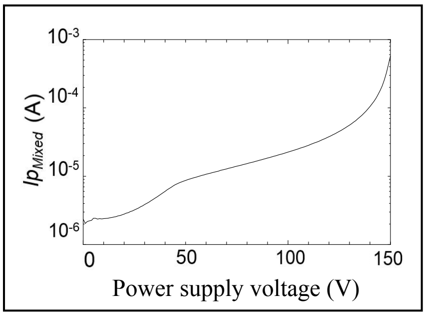


Fig. 6 Photocurrent by $W_E + W_F$.

5. Results

We acquired W_E and W_F from calculated APD output and light intensity spectrum from spectroscope with this measurement system (Fig. 2a). The light intensity spectrum has two peaks, one is excitation light peak and the other is fluorescence light peak. We call this amount of the peaks to P_E and P_F, respectively. Fig. 7 shows light intensity spectrum that has 470 nm peak (P_E) with Solution 1 by excitation light. There is not any other peak from fluorescence light because FITC concentration is 0 mol/L. In this time, W_E and W_F are, calculated APD output from Eq. (6), 4.28 μW and 0.0 W, respectively. Fig. 7a shows light intensity spectrum with Solution 2. There is one big peak and several small peaks; big one is P_E, several peaks are made by fluorescence light and peak has not yet cleared. The W_E and W_F, detected by calculated APD output are 3.17 μW and 0.09 μW, respectively. This means that, this method has more highly sensitive detection compared with the spectroscope on this system. Fig. 7b shows light intensity spectrum with Solution 3. There are two big peaks: P_E and P_F. This P_F is detected bigger than P_F of Fig. 7c. The W_E and W_F detected by calculated APD output are 3.73 μW and 1.16 μW, respectively. Fig. 7d shows light intensity spectrum with Solution 4. The P_F becomes bigger than P_E, and W_F becomes bigger than W_E. The spectroscope and APD provide similar output. Figs. 7e and 7f show light intensity spectrum with Solutions 5 and 6, respectively. The P_E is decreasing with FITC concentration, while the P_F is increasing with FITC concentration. Fig. 7g shows light intensity spectrum with Solution 7. P_E is very small compared with P_F. This shows P_E is difficult to separate other small peaks which are created by big fluorescence light. On the other hand, W_E and W_F detected by calculating APD output are 3.45 μW and 24.8 μW, respectively. This result means that, our method has more highly dynamic range compared with the spectroscope on the system.

Fig. 8 shows W_E and W_F characteristics of FITC concentrations: W_F is increased with the concentration, while the W_E is not depend on the concentrations. As a result, this method can separately sense W_E and W_F in any FITC concentrations.

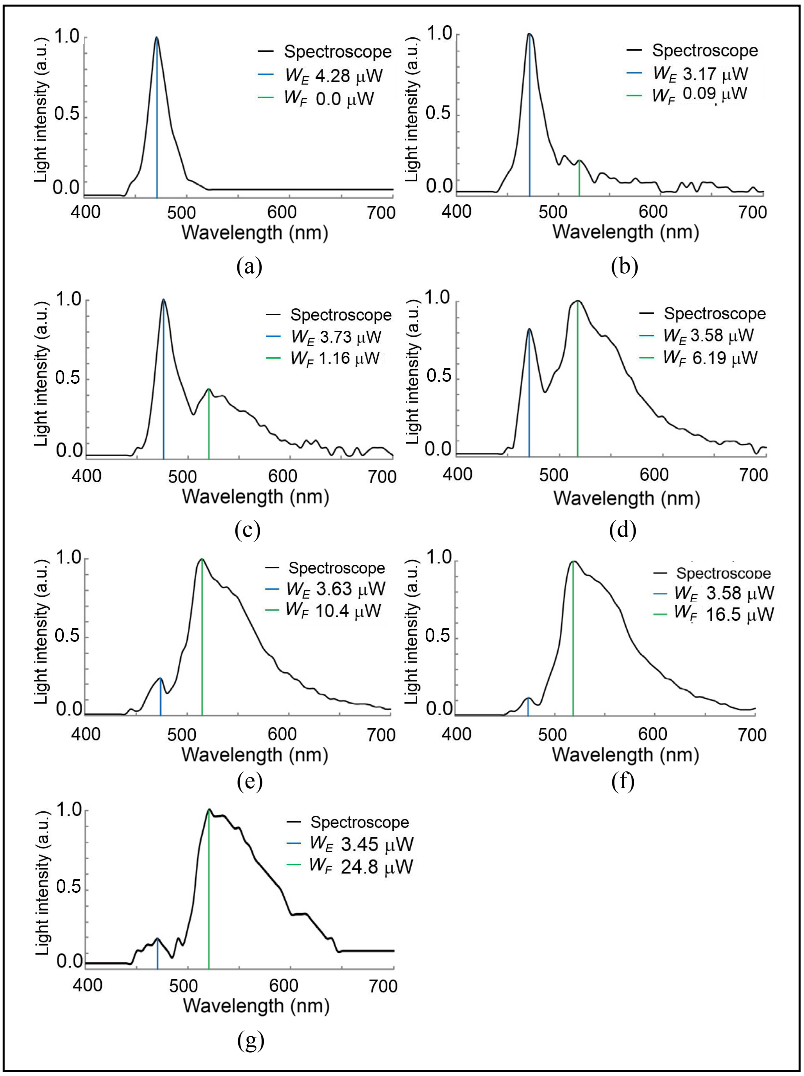


Fig. 7 Light intensity spectrum with (a) Solution 1, (b) Solution 2, (c) Solution 3, (d) Solution 4, (e) Solution 5, (f) Solution 6, (g) Solution 7.

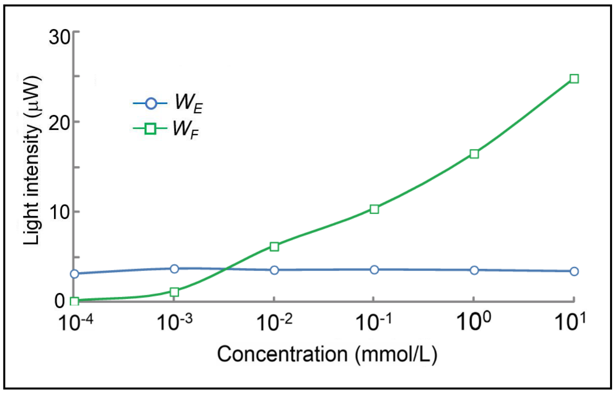


Fig. 8 W_E, W_F characteristics of FITC concentrations light.

6. Conclusions

These two light intensity values can be calculated from mixed multiplication ratio. Through these results, we can realize filter less W_F detection by APD, and we can achieve to make W_F detection system minimized on a chip with high gain, low noise and high dynamic range characteristics. Moreover, this method obtains both light intensity (W_E, W_F) at the same time. It also has a significant merit for improving accuracy of W_F value, because W_E is clearly effected by W_F value.

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