A protein biosensor using Geiger mode avalanche photodiodes

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Abstract. A compact optical sensor specifically designed for protein detection is introduced in this work. The sensor takes advantage of avalanche photodiode’s ultra-high sensitivity when operated in Geiger mode and is capable of detecting and quantifying very low light levels down to the single photons. The sensor has been tested with a luciferase gene reporter molecule detection system in Escherichia coli samples. The luciferase production is monitored via the APD and the luminescence amount detected is directly proportional to the amount of protein being produced. This reporter system will allow us to elucidate specific sources of proteins and to monitor the dynamics of protein activity within the cell in a real-time setting. The significant increase of photodiode breakdowns after the samples are applied to the sensor is the mechanism of detecting the bioluminescence. The degree of increase can be used to estimate the quantity of protein molecules. The sensor is packaged in a Teflon lightproof container to form a compact detection system.

1. Introduction
As the human genome project has been completed, there is a need to discover the proteins associated with each gene and consequently the function of these proteins. Proteomics is the study of the identity, function and interaction of proteins in living organisms. Our approach is to identify and quantify luminescent proteins by detection and quantification of the light signals they emit. A protein biosensor system that is capable of detecting and quantifying this low-level optical information is required for the proteomics sector.

Avalanche photodiode (APD), when working in the Geiger mode, which means the diode is biased higher than its breakdown level, is an excellent choice for detecting low-level optical signals such as the bioluminescence generated by proteins. Its internal amplification ability through avalanche multiplication of carriers gives the photodiode theoretically infinite gain to the photon-generated current. The introduction of thin-profile silicon avalanche photodiode allows the possibility of low operating voltage, high quantum efficiency, large wavelength coverage and cheaper detectors, which can be applied to portable, robust and low cost protein biosensors.

2. Geiger mode avalanche photodiode
Previously, silicon avalanche photodiodes of various structures have been developed [1][2][3]. Among those, the guard ring APDs receive special interest because of its relatively thinner profile and outstanding performance in Geiger mode operation [4]. The doping profile of a typical guard ring APD is shown in Figure 1.
Figure 1. Doping profile of Guard Ring APD

Figure 2. Photo of 20µm circular APD

Figure 2 shows the microscopic photograph of the detector used in our biosensor, a 20µm circular guard ring APD. It was manufactured by shallow n⁺ doping in p substrate using a CMOS compatible process so that statistical functions can be integrated into the detector chip in the future. A simple passive quenching circuit, including a ballast resistor to limit photocurrent avalanche and a fast voltage comparator to output the results, is applied to the APD anode [5][6]. This circuit enables the detector to work at room temperature (T=25°C) with a maximum working frequency of 0.2 MHz. The typical performance of the Geiger mode APD (GM-APD) used in this protein biosensor are given in Table 1 in comparison with the parameters from other major low-level light detectors, such as photomultiplier tube (PMT) and charge-coupled device (CCD).

Table 1. Parameters of GM - APD compared to other major low-level light detectors

|                  | Hamamatsu H7421-40 PMT | Andor Tech iXon 65 EMCCD | This work Geiger mode APD silicon diode |
|------------------|------------------------|--------------------------|---------------------------------------|
| Pixel diameter   | 5 mm                   | 20 µm x 30 µm            | 20 µm                                 |
| Num. of pixels   | 1                      | 576 x 288                | 1 – 10                                 |
| Spectrum range (nm) | 300-720            | 400 – 1000               | 400 – 850                             |
| Peak QE (%)      | 40 @ 500 nm            | 45 @ 700 nm              | 43 @ 650 nm                           |
| Dark counts (s⁻¹) | 40 - 100               | <1e⁻¹ s⁻¹                | 10 – 2000                             |
| Speed            | 300 ns                 | 10 ms                    | 5 µs¹                                 |
| Cooling          | TEC                    | TEC @ -50°C              | Air cooled                            |
| Op. voltage (V)  | 1000 - 2000            | -                        | 30 – 45                               |

¹ With passive quenching circuit, reduced to 50 ns by using active quenching circuit

3. Protein-generated luminescence
The gene expression system under investigation is based on the luciferase enzyme model. The luciferase-luciferin enzyme substrate reaction is a widely used gene reporter system [7]. Luciferin is the enzyme substrate, which produces light at a wavelength of 562 nm when it reacts with the enzyme luciferase in the presence of oxygen and ATP.

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\text{Luciferin + ATP + Oxygen} \rightarrow \text{Luciferase} + \text{Mg}^{2+} \rightarrow \text{Oxyluciferin + AMP + PPI + light}
\]

The gene for luciferase production can be inserted next to the gene of interest for protein production studies. The luciferase gene expression system can be monitored optically and the amount of luminescence detected is directly proportional to the amount of protein being produced by the gene under investigation. The culturing of an Escherichia coli strain (E. coli pSB401), which produces a
stably expressing luciferase gene from *Vibrio (Photobacterium) fischeri* has been performed [8]. The plasmid carrying the luciferase gene has been successfully isolated from the bacterial colonies. Upon lysis of the bacteria, light emission is produced by adding the luciferin substrate. Light production from the bacteria is then monitored by the GM-APD.

4. The biosensor

To detect the protein-generated luminescence with the GM-APD, a platform was custom designed to insulate it from ambient light as well as providing room for biological reactions to take place. This structure is illustrated in Figure 3 together with a picture of the prototype system. The APD is packaged in ceramic dual in line (DIL) package with the cavity window filled with transparent polydimethylsiloxane (PDMS), which is an insulator and protects the diode wire-bonds while allowing the light to pass through. A clear thin film is placed on top of the APD active area to prevent the detector chip from direct contact with the liquid from samples. The film is disposable to prevent against contamination from previous experiments. The DIL package is seated on a chip holder made of Teflon materials and roofed by another chip cover. A through hole is drilled on the chip cover, which forms a tube-shaped reaction well together with the protective film. There is a rubber ring around the bottom parameter of the through hole that seals the reaction well.

![Figure 3. Biosensor structure and prototype photo](image)

The increase of GM-APD counts against its dark count level is used as the biosensor reading, indicating the density of bioluminescence from the luciferase-luciferin reaction, as Figure 4 shows. This bioluminescence is directly proportional to the concentration of proteins being produced. The lower blue part in Figure 4 shows the results when inactive culture of *E. coli* is added to the reaction well. As no light is generated, these results reflect the GM-APD’s dark count level. The upper purple part shows the increase of GM-APD counts after luciferin substrate is applied to the sample and the oxidation of luciferin takes place. These readings are at least 200 counts/second higher than the base dark counts ($V_{ex} > 3V$), which is a strong evidence that proteins are being produced from the above reaction. The amount of increase is directly proportional to the concentration of oxyluciferin generated, which further reflects the concentration of *E. coli* bacteria in the culture.

5. Conclusion

The significant increase of biosensor readings proves the presence of protein generated bioluminescence. The high sensitivity of the APD allows minimum sample volume as well as very low protein concentration, which could hardly been detected by traditional luminescence detection systems. The presented system is very powerful for point of care analyses where portability and robustness are desirable. As research on proteins progresses, new methods will emerge to express specific proteins with unique luminescence information. By using GM-APD with specific peak
detection wavelengths corresponding to those proteins, this biosensor can be applied to a much wider field of proteomics.

Figure 4. GM-APD detects the presence of protein-generated bioluminescence

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